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Extensive Genetic Interactions Between *PRP8* and *PRP17/CDC40*, Two Yeast Genes Involved in Pre-mRNA Splicing and Cell Cycle Progression

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ABSTRACT

Biochemical and genetic experiments have shown that the *PRP17* gene of the yeast *Saccharomyces cerevisiae* encodes a protein that plays a role during the second catalytic step of the splicing reaction. It was found recently that *PRP17* is identical to the cell division cycle *CDC40* gene. *cdc40* mutants arrest at the restrictive temperature after the completion of DNA replication. Although the *PRP17/CDC40* gene product is essential only at elevated temperatures, splicing intermediates accumulate in *prp17* mutants even at the permissive temperature. In this report we describe extensive genetic interactions between *PRP17/CDC40* and the *PRP8* gene. *PRP8* encodes a highly conserved U5 snRNP protein required for spliceosome assembly and for both catalytic steps of the splicing reaction. We show that mutations in the *PRP8* gene are able to suppress the temperature-sensitive growth phenotype and the splicing defect conferred by the absence of the Prp17 protein. In addition, these mutations are capable of suppressing certain alterations in the conserved PyAG trinucleotide at the 3′ splice junction, as detected by an *ACT1-CUP1* splicing reporter system. Moreover, other *PRP8* alleles exhibit synthetic lethality with the absence of Prp17p and show a reduced ability to splice an intron bearing an altered 3′ splice junction. On the basis of these findings, we propose a model for the mode of interaction between the Prp8 and Prp17 proteins during the second catalytic step of the splicing reaction.

PRE-mRNA splicing takes place by two consecutive *trans*-esterification reactions. In the first step the border between the 5′ exon and the intron is cleaved, yielding the 5′ exon and lariat intron-exon intermediates. In the second step, the 3′ splice site is cleaved and the two exons are joined, creating a mature RNA and the lariat intron (Madhani and Guthrie 1994a). Accurate splicing requires conserved sequences within the introns of yeast pre-mRNAs at the 5′ (GUAUGAGU) and at the branchpoint (UACUAACA). Recognition of the 3′ splice site requires a specific trinucleotide (PyAG) usually preceded by a pyrimidine-rich tract. Spliceosomal small nuclear ribonucleoprotein particles (snRNPs) U1, U2, U4/6, and U5 recognize these elements and assemble onto the pre-mRNA substrate in a stepwise fashion. Initially the U1 snRNP interacts with the pre-mRNA at the 5′ splice site, followed by the U2 snRNP contacting the branchpoint. The U4/U6 and U5 snRNPs then join, as a tri-snRNP, thus forming the mature spliceosome (Madhani and Guthrie 1994a; Kramer 1996; Nilsen 1998). Much knowledge has been gathered about the RNA interactions responsible for recognizing and aligning the 5′ splice site and branch site before the first catalytic step; in contrast, much less is known about the events that lead to 3′ splice site selection and the second catalytic step of splicing (reviewed in Umen and Guthrie 1995c).

Pre-mRNA splicing requires the activity of a large number of *trans*-acting factors (Kramer 1996; Wang and Manley 1997; Will and Luhrmann 1997). Many of the proteins involved in the process of splicing have been identified by genetic screens for conditional mutations in yeast (Vijayraghavan *et al.* 1989; Woolford and Peebles 1992; Beggs 1995). These *prp* (*p*re-m*R*NA *p*rocessing) mutants are partially or completely defective in the removal of intervening sequences from pre-mRNAs.

Four genes that are required specifically for the second catalytic step in the yeast *Saccharomyces cerevisiae* have been identified: *PRP16*, *SLU7*, *PRP17*, and *PRP18* (Vijayraghavan *et al.* 1989; Schwer and Guthrie 1991; Frank and Guthrie 1992). These genes share a unique set of genetic interactions with each other, suggesting a physical or functional association of the encoded proteins (Frank *et al.* 1992; Jones *et al.* 1995; Xu *et al.* 1998). Previous work has shown that the four proteins act in concert in the second step of the splicing reaction. This step could be further separated into an ATP-dependent stage, which requires the activity of Prp16p and Prp17p, and a subsequent ATP-independent stage at which the Slu7 and Prp18 proteins participate (Jones *et al.* 1995). *PRP16* encodes an RNA-dependent ATPase of the DEAH-box family, which has been shown recently to unwind RNA duplexes *in vitro* (Wang

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et al. 1998). *SLU7* encodes a protein with a cysteine-rich zinc knuckle element, a motif that has been implicated in RNA binding (Frank and Guthrie 1992). It has been shown that the requirement for Slu7p during the second step of splicing increases with the distance between the branchpoint and the 3′ splice site (Brys and Schwer 1996). *PRP18* is a nonessential gene that encodes a small (29-kD) protein. The absence of Prp18p confers a temperature-sensitive phenotype; in addition, in *prp18* mutants the second step of the splicing reaction is inhibited, although not abolished (Horowitz and Abelson 1993). A physical interaction between Slu7p and Prp18p has been demonstrated using the two-hybrid assay (Zhang and Schwer 1997).

Mutations in the *PRP17* gene cause accumulation of splicing intermediates (Vijayraghavan *et al.* 1989; Ben-Yehuda *et al.* 1998). Functional interactions between Prp17p and the U2 and U5 snRNAs were suggested by the synergistic lethality of alleles of *PRP17* in combination with specific U2 or U5 snRNA mutations (Frank *et al.* 1992; Xu *et al.* 1998). It has been found recently that *PRP17* is allelic to the *CDC40* gene, which was characterized previously as a gene involved in cell cycle progression (Kassir and Simchen 1978). The *cdc40-1* mutation affects both the mitotic and meiotic cell cycles (Kassir and Simchen 1978). For the sake of clarity, in this article we refer to the *PRP17/CDC40* gene as *PRP17.* A full deletion allele of the gene shows a temperature-sensitive phenotype, and a cell cycle arrest at the G2 phase of the cell cycle, after the completion of DNA replication (Vaisman *et al.* 1995; Seshadri *et al.* 1996). In addition, the Prp17 protein is needed for the maintenance of the mitotic spindle after the cell cycle arrest at the restrictive temperature (Vaisman *et al.* 1995). The *PRP17* gene codes for a protein with several copies of the WD repeat (Vaisman *et al.* 1995; Ben-Yehuda *et al.* 1998; Zhou and Reed 1998). This repeated motif is found in a large family of proteins that play important roles in signal transduction, cell cycle progression, splicing, transcription, and development (for review see Neer *et al.* 1994).

In addition to the four mentioned genes involved exclusively in the second step of the splicing reaction, a major role in executing this step was demonstrated for the *PRP8* gene. Prp8p interacts with both splice sites, contacting the 3′ splice site after the first step is concluded (Teigelkamp *et al.* 1995a,b; Umen and Guthrie 1995a). Mutational analysis of *PRP8* has revealed that it plays a role in governing the specificity and the fidelity of 3′ splice site selection (Umen and Guthrie 1995b, 1996).

Here we report extensive genetic interactions between the *PRP17* and the *PRP8* genes. We show that some mutations in a confined region of the *PRP8* gene suppress the temperature-sensitive phenotype conferred by the *PRP17*Δ allele, while others show synthetic lethality with the absence of the Prp17 protein. We propose that the Prp17 and Prp8 proteins cooperate in the recognition of the 3′ splice site during the second catalytic step of the splicing reaction.

MATERIALS AND METHODS

Yeast strains and plasmids: The yeast strains used in this study are listed in Table 1. Plasmids pJU225 and pJU255 carry the *PRP8* and *prp8-122* alleles, respectively, on a high-copy-number *TRP1*-marked vector (pRS424). pJU169 bears *PRP8* on a centromeric *URA3*-marked plasmid (Umen and Guthrie 1996). pJU97, pJU98, pJU143, pJU146, pSB30, pSB33, pSB38, pSB47, and pCC71 are 2μ, *LEU2*-marked *CUP1-ACT1* reporter plasmids (Burgess and Guthrie 1993; Lesser and Guthrie 1993a,b; Umen and Guthrie 1995a,b, 1996; C. Collins and C. Guthrie, unpublished data). These plasmids were generously provided by C. Collins. Deletion of the *PRP17* gene was carried out by a one-step replacement method using plasmid pSBY15 (*PRP17::LEU2*) (Vaisman *et al.* 1995). Plasmids pSBY18, pSBY19, and p1426 carry the *PRP17* gene on centromeric vectors and are marked with *TRP1*, *TRP1*, and *ADE3*, or *URA3* and *ADE3*, respectively. pSBY55 is YEp24 (*URA3*, 2μ), carrying the *PRP8* gene.

Media, growth conditions, and general procedures: Standard molecular biology procedures such as restriction enzyme analysis and Southern blot analysis were carried out as described in Sambrook *et al.* (1989). Yeast media and molecular biology procedures (transformations, DNA preparations, etc.) were as described in Sherman *et al.* (1986). Plasmid DNA extraction from yeast cells was as described by Robzyk and Kassir (1992). Yeast cells were grown at 25°, 30°, or 34° in YEPD (1% yeast extract, 2% Bacto peptone, 2% dextrose) or SD media (0.67% yeast nitrogen base, 2% dextrose, and the appropriate nutrients added). Bacto agar (1.8%) was added for solid media. Selective media lacking one nutrient are designated SD-nutrient (*e.g.*, SD-Ura). Ura^- colonies were selected on SD complete medium with uracil (50 mg/liter) and 5-fluoroorotic acid (5-FOA, 0.8 g/liter) (Boeke *et al.* 1987). Sporulation was carried out in SPO medium (1% K acetate, 0.1% yeast extract, 0.05% dextrose).

Isolation of *prp8-scf* alleles: Yeast strains D103 and D110 (*PRP17*Δ) were plated on YEPD plates (1×10^7 cells/plate) and subjected to UV irradiation to yield a survival rate of 10%. The cells were incubated for 3 hr at the permissive temperature (25°) and were transferred to the restrictive temperature (34°). After 3 days of incubation, 20 independent temperature-resistant colonies were isolated. The mutants (SCF strains) were then crossed to the parental strains. The diploids thus obtained were sporulated and the 2:2 segregation of the temperature-sensitive phenotype was verified. Derivatives of all the mutants were crossed in all possible combinations and were subjected to complementation and allelism tests.

Cloning of the *SCF* gene: SCF103A was transformed with a YEp24(*URA3*)-based genomic library. The transformants were screened for the inability to grow at 34°. Out of 30,000 transformants that were screened, only one colony exhibited a temperature-sensitive phenotype that correlated with the presence of the plasmid. The plasmid was isolated from this transformant, subjected to partial DNA sequencing, and compared to the yeast complete sequence in the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces). The complementing genomic fragment contained a single complete open reading frame (ORF) (YHR165c) corresponding to the *PRP8* gene. pSBY57, carrying the *Hpa*I-*Xho*I fragment of the *PRP8* gene on an integrative vector (YIplac204); (Gietz and Sugino 1988), was integrated in the genome of

TABLE 1

Strains used in this study

Strain	Genotype	Source
YJU75	*MAT***a** *ade2 cup1Δ::ura3 his3 leu2 lys2 prp8Δ::LYS2 trp1* (*PRP8-TRP1*-2μ)	Umen and Guthrie (1996)
prp8-122	YJU75 (*prp8-122 TRP1*-2μ)	Umen and Guthrie (1996)
SB87	*MAT***a** *PRP17Δ::LEU2 prp8Δ::LYS2 his3 leu2 lys2 trp1 ura3 pJU225* (*PRP8 TRP1*-2μ)	This study
SB90	*MATα PRP17Δ::HIS3 cup1Δ::ura3 his3 leu2 lys2 trp1 ura3*	This study
D103	*MATα PRP17Δ::LEU2 his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
SCF103A	*MATα PRP17Δ::LEU2 prp8-scf103A his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
SCF103C	*MATα PRP17Δ::LEU2 prp8-scf103C his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
SCF103E	*MATα PRP17Δ::LEU2 prp8-scf103E his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
D110	*MAT***a** *PRP17Δ::LEU2 his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
SCF110F	*MAT***a** *PRP17Δ::LEU2 prp8-scf-110F his3 leu2Δ1 lys2-801a trp1Δ1 ura3-52*	This study
YH2	*MAT***a** *PRP17Δ::LEU2 ade2 ade3 leu2 ura3 trp1 lys2*	This study
SYF14	*MAT***a** *PRP17Δ::LEU2 prp8-syf14 ade2 ade3 leu2 lys2 trp1 ura3 pSBY19 (PRP17 TRP1 ADE3)*	This study
SYF77	*MAT***a** *PRP17Δ::LEU2 prp8-syf77 ade2 ade3 leu2 lys2 trp1 ura3 pSBY19 (PRP17 TRP1 ADE3)*	This study
SYF102	*MAT***a** *PRP17Δ::LEU2 prp8-syf102 ade2 ade3 leu2 lys2 trp1 ura3 pSBY19 (PRP17 TRP1 ADE3)*	This study

Parentheses represent episomal plasmids.

a wild-type strain and crossed to the *scf* mutants. Tetrad analysis confirmed allelism between the integrated plasmid at the *PRP8* locus (scored as Trp^+ spores) and the original *scf* mutation.

Mutagenesis, screening, and genetic characterization of *prp8-syf* alleles: Strain YH2 (*PRP17Δ ura3 trp1 ade2 ade3*) carrying the centromeric plasmid p1426 (*PRP17*, *ADE3*, *URA3*) was subjected to UV irradiation to yield a survival rate of 10%. The surviving cells were allowed to form colonies on YEPD plates and were screened for red, nonsectoring colonies. After restreak, the colonies were tested for sensitivity to 5-FOA. All the nonsectoring, 5-FOA^s colonies were transformed with plasmids pSBY18 (*PRP17*, *TRP1*) or the vector YCplac22 (*TRP1*, Gietz and Sugino 1988). The presence of *PRP17* on pSBY18 allows the loss of p1426, conferring red/white sectoring and resistance to 5-FOA. Only strains that showed sectoring and 5-FOA resistance with pSBY18 but not with YCplac22 were taken for further studies.

Each putative *syf* mutant was crossed to YH3 (*MAT***a**, isogenic to YH2), and the 2:2 segregation of the sectoring/nonsectoring phenotype was verified. All the diploids exhibited red/white sectoring, demonstrating that the mutations were recessive. Derivatives of all the mutants were crossed in all possible combinations, and complementation groups were established. One member of each complementation group was chosen for cloning of the complementing gene.

Cloning of the *SYF* genes: Nonsectoring *syf* mutants carrying plasmids p1426 (*PRP17*, *URA3*, *ADE3*) or pSBY19 (*PRP17*, *TRP1*, *ADE3*) were transformed with a YEp24(*URA3*)-based genomic library. The transformants were screened for the ability to form red/white sectors on rich medium and tested for resistance to 5-FOA. The plasmids were isolated from sectoring or white transformants, subjected to partial DNA sequencing, and compared to the yeast complete sequence in the *Saccharomyces* Genome Database (http://genome-www.stanford.edu/Saccharomyces). Three different, overlapping plasmids complemented the synthetic lethality of strain SYF14. The three plasmids overlapped in a region containing a single ORF, YHR165c, corresponding to the *PRP8* gene. Allelism between this gene and the alleles present in *prp8-syf* mutants was confirmed using the integrative plasmid pSBY57, as described above.

Mapping of the *prp8-scf* and *prp8-syf* alleles: The *prp8-scf* or *prp8-syf* alleles were cloned by a gene conversion strategy. Yeast strains bearing the relevant mutations in the *PRP8* locus were transformed with plasmid pSBY55 (*PRP8*, 2μ) linearized with the *Sac*I, *Asp*718, or *Sna*BI restriction enzymes. Transformants of the SCF strains (*PRP17Δ prp8-scf*) were screened for the ability to grow at the restrictive temperature in the presence of the plasmid, indicating a gene conversion event in which the genomic *prp8* mutation was transferred to the plasmid during its repair. In a similar way, SYF transformants were screened for the inability to form sectors in the presence of the plasmid (red colonies). The plasmids were isolated from each mutant strain and were subjected to restriction analysis to rule out the possibility of plasmid deletions. Each plasmid was then retransformed to the mutant parental strain to confirm the mutant phenotype.

The mutations in each allele were mapped by cotransformation. Each plasmid was linearized and cotransformed to its parental mutant strain together with one of a series of overlapping fragments obtained from the wild-type *PRP8* gene (ratio 1:10 plasmid/fragment). The following fragments were used (numbers reflect nucleotides in the *PRP8* ORF): full length (1–7242); fragment I (1–5723); fragment II (2510–5723); fragment III (3711–7242). A recombination event between the mutant *prp8* allele on the plasmid and the wild-type information on the fragment can restore *PRP8* activity, provided the fragment information overlaps the location of the mutation on the *prp8* allele. The transformants obtained in each cotransformation reaction were tested for their ability to complement the mutations as indicated by the inability to grow at the restrictive temperature (SCF strains) or by the ability to segregate sectored or white colonies (SYF strains).

Copper-resistance assay: Yeast strains transformed with wild-type, UAG 3′ splice site, pJU97 (3′ U*G*G) or pJU98 (3′ U*U*G), containing the *ACT1* intron fused to the *CUP1* gene (Umen and Guthrie 1996), or pJU143 (+TPyDOWN), or pJU146 (+A WT) bearing the *CUP1* gene with an intron containing duplicated 3′ splice sites, or pSB30 (5′ G1A), or pSB33 (5′ G5A), or pCC71 (5′ U2A), or pSB38 (branchpoint A6C), or pSB47 (branchpoint C3A; Burgess and Guthrie 1993; Lesser and Guthrie 1993a,b; Umen and Guthrie 1995a,b, 1996) were tested for copper sensitivity as follows: 1×10^7 and 1×10^6 cells of the tested strains were spotted on SD-

Leu plates containing different copper concentrations. Plates were made to a chosen copper concentration (0.025–2 mm) by adding to SD-Leu media a dilution of filtered 1 m $CuSO_4$ after autoclaving.

RNA analysis: RNA was extracted by a hot phenol method (Schmitt *et al.* 1990) from cells grown to an OD_{600} of 0.4–0.6 in SD-Leu-Trp medium at 30°. Primer extension analysis was performed essentially as described by Boorstein and Craig (1989) (but omitting actinomycin D), using the *ACT1-CUP1* primer as in Lesser and Guthrie (1993a) and, as a control, a U1 snRNA primer (CACGCCTTCCGCGCCGT).

RESULTS

Mutations in the *PRP8* gene are able to suppress the temperature-sensitive phenotype conferred by the *PRP17*Δ allele: The *PRP17* gene encodes a protein that is essential only at elevated temperatures and affects two major processes: pre-mRNA splicing and cell cycle progression (Vaisman *et al.* 1995; Ben-Yehuda *et al.* 1998; Boger-Nadjar *et al.* 1998). To identify genes that may interact with *PRP17*, we performed a screen for suppressor mutants that are able to grow at the restrictive temperature in the absence of the *PRP17* gene product.

Yeast strains (D103, D110; both *PRP17*Δ) were mutagenized using UV irradiation and incubated at the restrictive temperature (34°). Twenty independent temperature-resistant colonies were isolated. When the mutants were crossed to the parental strains (*PRP17*Δ), all the obtained diploids failed to grow at the restrictive temperature, indicating that the mutations were recessive. Tetrad analysis of each diploid revealed a Mendelian segregation of the temperature-sensitive phenotype, implying that each mutant bears a mutation in a single gene. When mutants were then crossed with each other in all possible combinations, the resulting diploids were able to grow at the restrictive temperature, indicating that they fall into a single complementation group. Tetrad analysis of individual diploids failed to produce temperature-sensitive recombinant spores, confirming that the 20 mutants represent alleles of the same gene. The mutations were designated *scf* (*s*uppressors of *c*dc *f*orty).

Since all the *scf* mutations were recessive, the complementing gene from a yeast genomic library was cloned by transforming a *PRP17*Δ *scf* strain (SCF-103A) and screening for transformants unable to grow at the restrictive temperature. Out of 30,000 transformants that were tested, only one colony exhibited a temperature-sensitive phenotype that correlated with the presence of the plasmid. The complementing genomic fragment contained a single complete ORF (YHR165c) corresponding to the *PRP8* splicing factor gene. Allelism tests confirmed that the *scf* mutations were allelic to the *PRP8* gene (see materials and methods). *PRP8* encodes a large, evolutionarily conserved protein that is an essential component of the spliceosome during both steps of the splicing reaction (Lossky *et al.* 1987; Whittaker *et al.* 1990; Hodges *et al.* 1995; Teigelkamp *et al.* 1995a,b). It is thought that Prp8p anchors the exons in the catalytic center of the spliceosome and stabilizes the fragile interactions between the U5 snRNA and the nonconserved exon sequences (Beggs 1995; Teigelkamp *et al.* 1995a,b; Dix *et al.* 1998). In addition, similarly to *PRP17*, the *PRP8* gene has been found to play a role in cell cycle progression. Certain mutant alleles of *PRP8* (*dbf3*, *dna39*) were isolated as cell-cycle-specific mutants with phenotypes very similar to those of *PRP17* mutants (Dumas *et al.* 1992; Shea *et al.* 1994). Thus, the finding that mutations in *PRP8* can rescue the cell cycle defect conferred by deletion of the *PRP17* gene suggests that Prp8p and Prp17p participate in a common cellular process or processes involving pre-mRNA splicing and cell cycle progression.

The *PRP17* mutants are sensitive to DNA-damaging agents, such as methylmethane sulfonate (MMS), and are unable to undergo sporulation and meiosis (Kassir and Simchen 1978; Kassir *et al.* 1985; Kupiec and Simchen 1986). The *prp8-scf* mutations suppressed these phenotypes, too (data not shown). Thus, the *prp8-scf* mutations, isolated by their ability to allow growth at the restrictive temperature, suppress all the tested phenotypes conferred by the deletion of the *PRP17* gene. In a $Prp17^+$ background the *prp8-scf* alleles showed no detectable growth differences nor any sensitivity to DNA-damaging agents, in comparison to the isogenic wild-type strain, at several temperatures. Meiosis and sporulation in *prp8-scf*/ *prp8-scf* strains were also normal (data not shown).

***syf* alleles of *PRP8* exhibit synthetic lethality with the *PRP17*Δ allele:** In an effort to identify genes that interact with *PRP17*, we have performed recently a synthetic lethality screen seeking mutants unable to survive even at the permissive temperature in the absence of the Prp17/Cdc40 protein. The mutants thus obtained (*syf*: *sy*nthetic lethal with cdc *f*orty) were separated into complementation groups by genetic analysis (see materials and methods). The largest complementation group was found to contain eight alleles of the *PRP8* gene. Thus, some mutations in *PRP8* could suppress the temperature-sensitive phenotype conferred by the deletion of the *PRP17* gene (*prp8-scf* alleles), while others cause lethality in the absence of Prp17p (*prp8-syf* alleles).

***prp8* alleles act in a dosage-dependent fashion:** The *prp8-scf* mutations were recessive in diploids, and the *PRP8* gene on a high-copy-number plasmid prevented a haploid *PRP17*Δ *prp8-scf* strain from growing at the restrictive temperature. However, the same haploid strain bearing a single-copy plasmid carrying the wild-type *PRP8* gene was able to grow, albeit slowly, at 34°. This indicates that under these conditions the *prp8-scf* allele was semidominant over the wild-type *PRP8* allele. Hence, the suppression of the cell cycle phenotype is a dosage-dependent trait, with *prp8-scf* alleles being reces-

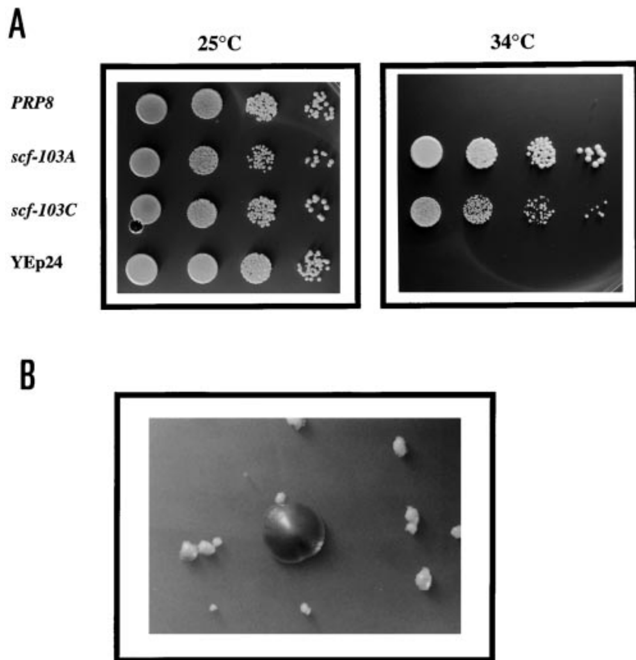

Figure 1.—(A) Overexpression of *prp8-scf* alleles is dominant. Strain D110 (*PRP17Δ PRP8*) was transformed with a 2μ-*URA3*-marked plasmid carrying the mutant *prp8-scf103A* or *prp8-scf103C* alleles, or with a plasmid bearing the *PRP8* gene (pSBY55), or with the vector alone (YEp24). The transformants were diluted and spotted on SD-Ura plates and assayed for viability at either the permissive (25°) or the restrictive (34°) temperature. (B) Overexpression of *prp8-syf* alleles is dominant. Strain D110 (*PRP17Δ PRP8*) was transformed with a 2μ-*URA3*-marked plasmid carrying the mutant *prp8-syf77* allele, or with a similar plasmid bearing the *PRP8* gene (big colony). Growth after 4 days is shown.

sive in diploids, but semidominant or dominant in haploid cells, depending on the expression level. Consistent with these results, the mutant *prp8-scf* alleles were dominant over the wild-type allele when expressed from high-copy-number plasmids (Figure 1A).

In a similar way, although the synthetic lethal phenotype of *prp8-syf* mutants was recessive in diploids, high-copy-number plasmids carrying different *prp8-syf* alleles resulted in semilethality in *PRP17Δ PRP8* yeast cells: upon transformation very small colonies were obtained, which displayed many death sectors (Figure 1B). This phenotype was not observed in Prp17^{+} cells; thus, the *prp8-syf* alleles behave as semidominant when present in high copy number.

These results suggest that the phenotypes displayed by the different *prp8* alleles are affected by their dosage. The wild-type and mutant proteins may compete in the creation of spliceosomal complexes: the proportion of complexes carrying wild-type Prp8p *vs.* those carrying Prp8 mutant proteins determines the cell phenotype. A similar effect has been shown for the *PRP16* gene: overexpression of nonviable alleles of *PRP16* impaired the growth of wild-type *PRP16* cells (Hans-Rudolf and Schwer 1998). The mutant proteins, therefore, may be able to assemble into the spliceosomal complexes, but impair their function.

Cloning and identification of the *prp8-scf* and *prp8-syf* alleles: Since the *PRP8* gene encodes one of the largest proteins (2413 residues, a molecular weight of 280 kD) in *S. cerevisiae*, it was interesting to determine whether the genetic interactions with the *PRP17* gene are restricted to specific regions of *PRP8.* Four different *prp8-scf* alleles and three different *prp8-syf* alleles were cloned by a gene conversion strategy, and their mutations mapped using a cotransformation assay (see materials and methods). The mutations in all seven *prp8* alleles mapped to the same 2-kb region of the *PRP8* gene (*Hpa*I-*Msc*I fragment, encoding amino acids 1237–1908). Thus, mutations that suppress the phenotypes conferred by the deletion of *PRP17*, or mutations that result in a synthetic lethal phenotype with the absence of Prp17p, map to the same region of *PRP8.* In a previous study, mutations in this region were found to affect 3′ splice site fidelity (region C, Umen and Guthrie 1996).

Sequence analysis of two of the *prp8-scf* alleles revealed that *scf-103A* carries three amino acid changes, W1575C, E1576K, and G1636S, whereas the *scf-103C* allele has a single amino acid change, K1563I. Similarly, the *prp8-syf77* allele encodes a protein containing phenylalanine instead of leucine at position 1557, only a few amino acids upstream of the *prp8-scf* mutations. Thus, the mutations were clustered to a small region of the Prp8 protein, a region highly conserved in evolution from yeast to humans (Hodges *et al.* 1995; Umen and Guthrie 1996; Luo *et al.* 1999).

***prp8-scf* alleles affect the fidelity of 3′ splice site utilization:** The mutations W1575C and E1576K of the *prp8-scf103A* allele affect the same residues as those in the *prp8-122* (W1575R) and *prp8-123* (E1576V) mutant alleles, described by Umen and Guthrie (1996). These mutant alleles were isolated in a screen for *PRP8* alleles that affect the fidelity of 3′ splice site utilization. The *prp8-122* and the *prp8-123* alleles were able to suppress the splicing defect caused by a single base alteration in the conserved PyAG motif located at the 3′ splice site of an *ACT1-CUP1* reporter gene. *CUP1* is a nonessential gene that allows cells to grow in the presence of copper in a dosage-dependent manner; the intron-containing *ACT1-CUP1* construct thus enables quantitative analysis of pre-mRNA splicing (Lesser and Guthrie 1993a; Umen and Guthrie 1996). Whereas the wild-type *PRP8* strain was unable to efficiently splice pre-mRNA molecules with a modified U*U*G sequence at the 3′ splice junction, *prp8-122* and *prp8-123* strains were able to do so, providing a copper-resistant phenotype (Umen and Guthrie 1996).

Using the *ACT1-CUP1* reporter system, we tested whether the *prp8-scf* alleles identified in our screen affect 3′ splice site fidelity. Yeast strain YJU75 (*prp8Δ*) bearing the *PRP8* gene on a *TRP1*-marked plasmid (Umen and Guthrie 1996) was transformed with *URA3*-marked

TABLE 2

The effect of different *prp8-scf* alleles on 3′ splice site fidelity

Allele	Copper concentration (mm)									
	0.00	0.05	0.10	0.15	0.20	0.25	0.35	0.50	1.00	2.00
PRP8	+	+	−/+	−	−	−	−	−	−	−
prp8-scf103A	+	+	+	+	+	+	+	+	+	−/+
prp8-scf103C	+	+	+	+	+	−/+	−/+	−/+	−	−
prp8-scf103E	+	+	+	+	−/+	−/+	−/+	−	−	−
prp8-scf110F	+	+	+	+	−/+	−/+	−/+	−	−	−
prp8-122	+	+	+	+	+	+	+	−/+	−	−

A *prp8*Δ strain (YJU75) carrying an *ACT1-CUP1* 3′ U*U*G reporter plasmid was tested for the ability to grow on different copper concentrations in the presence of different *PRP8* alleles on *URA3*-marked plasmids.

plasmids carrying each of the four *prp8-scf* alleles. Following plasmid shuffling, the strains harboring the mutant *prp8-scf* alleles or the wild-type *PRP8* gene were transformed with either the *ACT1-CUP1* wild-type 3′ UAG reporter or the *ACT1-CUP1* 3′ U*U*G reporter. The resistance to various copper concentrations was measured. In strains bearing the *prp8-scf* alleles and the wild-type 3′ UAG reporter, no effect on copper resistance was seen, in comparison to the control strain bearing the wild-type *PRP8* gene (data not shown).

The results obtained with the different *prp8* alleles in the presence of the *ACT1-CUP1* 3′ U*U*G reporter are presented in Table 2 and Figure 2. As for the *prp8-122* allele (Umen and Guthrie 1996), all four *prp8-scf* alleles enabled growth in the presence of this reporter at higher copper concentrations than those allowed by the wild-type *PRP8* allele. Thus, as for *prp8-122*, the *prp8-scf* alleles suppressed the splicing defect caused by a mutation in the 3′ splice site. The degree of suppression varied among the alleles and showed a good correlation with the ability to suppress the temperature sensitivity of *PRP17*Δ mutants, as assayed by generation time and plating efficiency at the restrictive temperature. Accordingly, the strongest *prp8-scf* allele, *scf-103A*, exhibited resistance to the highest copper concentrations; in fact, yeast strains carrying this allele were able to grow in the presence of 2 mm copper, a concentration at which even *prp8-122* strains cannot grow (Table 2).

To confirm that the increased resistance to copper in the *prp8-scf* mutants was really due to higher splicing efficiency, the RNA was analyzed by primer extension. As shown in Figure 3, the U*U*G 3′ splice site reporter RNA was indeed spliced significantly better (at least fourfold) in the strain carrying the *prp8-scf103A* allele, in comparison to the *PRP8* control. The effect of the *prp8-scf103C* allele was less obvious, indicating that the copper-resistance test is more sensitive than the primer extension assay. Moreover, splicing of the wild-type *ACT1-CUP1* RNA was also elevated in the *prp8-scf103A* strain. Presumably, this effect was not seen with the

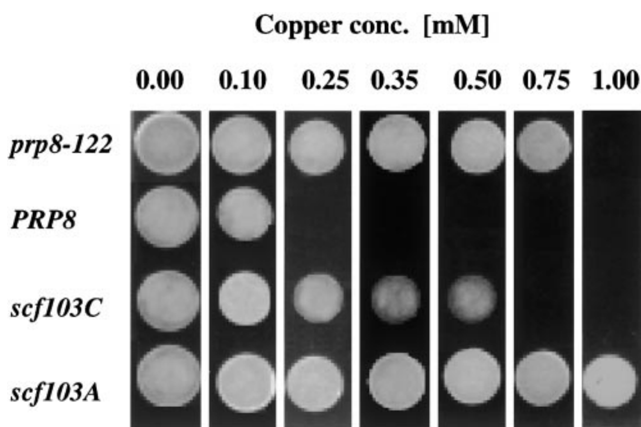

Figure 2.—The effect of different *prp8-scf* alleles on 3′ splice site fidelity. A *prp8*Δ strain (YJU75) carrying different *PRP8* alleles on *URA3*-marked plasmids was tested for the ability to grow on different copper concentrations in the presence of the *LEU2*-marked plasmid bearing a *ACT1-CUP1* 3′ U*U*G reporter.

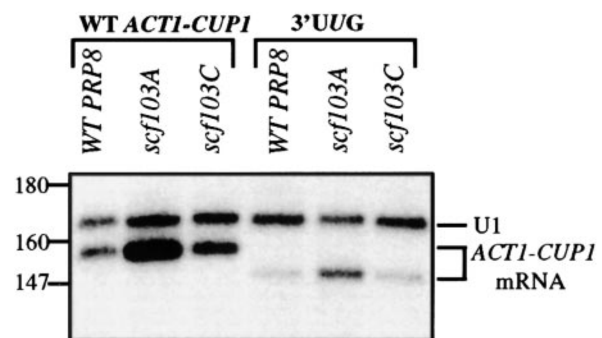

Figure 3.—Primer extension analysis of the splicing of *ACT1-CUP1* reporter transcripts in *PRP8* or *prp8-scf* strains. RNA was extracted from YJU75 (*prp8*) cells carrying wild-type (WT) or mutant (U*U*G) 3′ splice site *ACT1-CUP1* reporter plasmids and plasmids bearing wild-type *PRP8*, *prp8-scf103A*, or *prp8-scf103C* alleles. Primer extension products are shown for the *ACT1-CUP1* reporter mRNAs and (as a loading control) for U1 snRNA. The wild-type and 3′ splice site reporter mRNAs differ slightly in length due to minor differences in the *ACT1-CUP1* junction sequence, but are comparable between *PRP8* alleles.

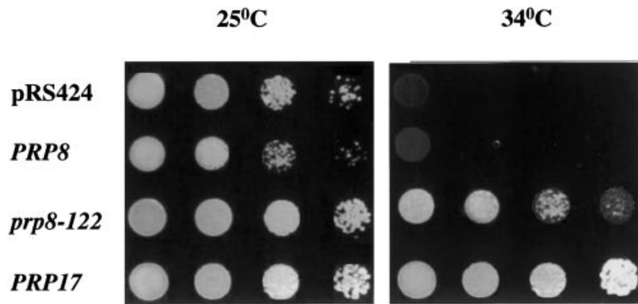

Figure 4.—The 3′ splice site fidelity mutant *prp8-122* is able to suppress the temperaturesensitivity conferred by the *PRP17*Δ mutation. A *PRP17*Δ*PRP8* strain (D110) carrying a *TRP1*-marked plasmid bearing the *prp8-122* allele was tested for the ability to grow at either the permissive (25°) or the restrictive (34°) temperature. Plasmids bearing *PRP17*, *PRP8*, or the vector alone (pRS424) were used as control.

growth assay because the level of copper resistance was already maximal with the wild-type reporter and wild-type *PRP8*.

Since the *prp8-scf* alleles showed an effect on the fidelity of 3′ splice site utilization (the activity for which *prp8-122* allele was isolated), the reciprocal experiment was performed, testing whether the *prp8-122* fidelity mutant allele was able to suppress the temperature sensitivity of a *PRP17*Δ strain. The results, shown in Figure 4, demonstrate that *prp8-122* was able to partially suppress the temperature-sensitive phenotype. Hence, a correlation was established between the ability to suppress the temperature sensitivity of the *PRP17*Δ allele and the ability to suppress the 3′ splice site mutation.

The *prp8-scf* alleles specifically affect efficient splicing of reporters harboring 3′ splice site alterations: The suppression by *prp8-scf* alleles could be due to a higher efficiency of splicing in general, rather than to a specific effect on 3′ splice site fidelity. To rule out this possibility, we have tested the ability of the *prp8-scf* alleles to splice reporter constructs carrying introns with different alterations. *ACT1-CUP1* reporter plasmids bearing mutations in the conserved 5′ splice site sequence (G1A, U2A, and G5A; Lesser and Guthrie 1993b; C. Collins and C. Guthrie, unpublished observations), or in the conserved $UAC_{256}UAA_{259}C$ branchpoint sequence (C256A and A259C; Burgess and Guthrie 1993) were introduced into strains carrying either *PRP8* or *prp8-scf* alleles. The ability of the *prp8-scf* strains to grow with increasing copper concentrations was tested and was found to be identical to that of the wild-type *PRP8* control strain for all the mutant constructs.

In addition, the ability of the *prp8-scf* mutants to affect the recognition of the pyrimidine tract, an additional feature of the 3′ splice site, was tested. Although the pyrimidine residues in yeast 3′ splice sites are generally less conserved than those in mammalian introns, they play an important role in efficient 3′ splice site utilization. Two *ACT1-CUP1* reporter plasmids were tested: +TPyDOWN detects splicing of an intron containing duplicated 3′ splice sites, one of which is uridine rich (proximal) and the other adenosine rich (distal), whereas +A WT carries the 3′ splice sites in the reverse order: the proximal site is adenosine rich, and the distal site is uridine rich. Loss of pyrimidine recognition generates a higher level of in-frame message and Cup1 protein (Umen and Guthrie 1995b). None of the *prp8-scf* alleles showed any alteration of pyrimidine tract recognition (data not shown).

Therefore, we conclude that the *prp8-scf* alleles preferentially affect recognition of the conserved PyAG sequence at the 3′ splice site and do not affect recognition of other sequence elements important for splicing. These results also suggest that the *prp8-scf* alleles may suppress the temperature sensitivity of *PRP17*Δ strains by increasing the efficiency of 3′ splice site recognition of certain intron(s).

***prp8-syf* strains show defects in 3′ splice site utilization:** As the *PRP17*Δ mutations can be suppressed by *prp8* alleles that allow splicing of altered 3′ splice sites, the *prp8-syf* alleles, which are lethal in the absence of Prp17p, may exhibit the opposite effect. Therefore, we tested the resistance of strains bearing the *prp8-syf* alleles to increasing copper concentrations in the presence of the reporter genes previously described. The results, shown in Table 3, demonstrate that the *prp8-syf* alleles confer hypersensitivity to low copper concentrations in the presence of the 3′ U*U*G alteration. Whereas wild-type *PRP8* supports growth in up to 0.125 mm copper, the *prp8-syf* strains show growth defects in the presence of 0.05 mm copper. The *prp8-syf* alleles did not affect the splicing of the wild-type *ACT1-CUP1* reporter or the splicing of reporter genes carrying alterations in the 5′ splice site or in the branchpoint. In addition, they did not show any alteration of pyrimidine tract recognition (data not shown). Therefore, we propose that the *prp8-syf* alleles affect 3′ splice site usage antagonistically to the *prp8-scf* alleles, reducing splicing in the presence of the 3′ U*U*G alteration.

DISCUSSION

The results described here demonstrate extensive genetic interactions between the *PRP17* and the *PRP8* splicing factor genes. The involvement of both genes in the second step of the splicing reaction has been shown previously (Vijayraghavan *et al.* 1989; Umen and Guthrie 1995a,b, 1996; Ben-Yehuda *et al.* 1998). Here we report that in the absence of Prp17p, mutations in *PRP8* can either suppress the temperature-sensitive phenotype (*prp8-scf* mutations) or confer synthetic lethality (*prp8-syf* mutations). In addition, we demonstrate a strong correlation between the ability of *prp8* alleles to suppress the phenotype of the *PRP17*Δ allele and their ability to recognize mutant 3′ splice sites. Conversely, we show that the previously described *prp8-122*

TABLE 3

The effect of different *prp8-syf* alleles on 3′ splice site fidelity

	Copper concentration (mm)						
Allele	0.00	0.025	0.05	0.075	0.10	0.125	0.15
PRP8	+	+	+	+	−/+	−/+	−
prp8-syf77	+	+	−	−	−	−	−
prp8-syf14	+	+	−/+	−/+	−	−	−
prp8-syf102	+	+	−/+	−/+	−	−	−

A *prp8*Δ strain (YJU75) carrying an *ACT1-CUP1* 3′ U*U*G reporter plasmid was tested for the ability to grow on different copper concentrations in the presence of different *PRP8* alleles on *URA3*-marked plasmids.

3′ fidelity mutant allele (Umen and Guthrie 1996) is able to suppress the temperature sensitivity of a *PRP17*Δ strain. The correlation between *PRP17* suppression and 3′ splice site recognition is strengthened by the observation that the *prp8-syf* mutations, which are synthetic lethal with the *PRP17*Δ allele, reduce the ability of the cells to recognize mutant 3′ splice sites. Neither the *prp8-scf* nor the *prp8-syf* alleles show any effect on splicing of introns bearing alterations in the 5′ splice site, in the branchpoint conserved sequences, or in the uridine tract, suggesting that the *prp8* mutations specifically affect 3′ splice site recognition.

On the basis of these observations we propose a model for the mechanism of interaction between the Prp8 and the Prp17 proteins. According to this model, the two proteins cooperate during the second step of splicing. Whereas Prp8p plays a role in recognizing the conserved PyAG motif, Prp17p serves as an accessory protein that enhances the efficiency of this recognition. Prp17p may act by helping to position Prp8p at the correct site on the pre-mRNA. Alternatively, the Prp17 protein might act by producing a conformational change in Prp8p, which enhances the recognition step. In the absence of Prp17p, Prp8p is still able to identify the proper splice site, albeit less efficiently, and it is therefore proficient enough to enable growth at the permissive temperature (25°). At the restrictive temperature, the interaction of Prp8p with the PyAG motif becomes unstable, and the assistance of the Prp17 protein becomes essential. The requirement for Prp17p may be critical for the splicing of specific introns. Failure to splice these introns could account for the cell cycle arrest phenotype seen in the absence of Prp17p or in the presence of certain Prp8 mutant proteins (Dumas *et al.* 1992; Shea *et al.* 1994; see below).

This hypothesis is supported by the following observations:

1. Cross-linking studies have demonstrated that Prp8p interacts directly with the pre-mRNA during both steps of the splicing reaction (Teigelkamp *et al.* 1995a,b; Umen and Guthrie 1995a,b). During the second step of splicing, an interaction occurs between Prp8p and the 3′ splice site after ATP hydrolysis by the RNA-dependent ATPase Prp16p. This interaction is enhanced by the presence of Prp17p (Umen and Guthrie 1995a).
2. *PRP17*Δ strains show enhanced defects in 3′ splice site recognition. We have found that the splicing defect of *PRP17*Δ strains at the permissive temperature is stronger for a reporter gene carrying the 3′ U*U*G splice site mutation; this effect is even more pronounced at the semipermissive temperature (data not shown). These results suggest that the *PRP17*Δ strain is defective in 3′ splice site recognition in a way similar to that seen in strains bearing the synthetic lethal *prp8-syf* alleles.
3. The *prp8-101* allele was isolated in a search for mutants with altered uridine tract recognition (Umen and Guthrie 1995b). This strain was later shown to exhibit a decreased level of cross-linking of Prp8p to the 3′ splice site *in vitro* and to be synthetically lethal with mutations in the *PRP17* gene (Umen and Guthrie 1995a). We have found that, as for the other synthetic lethal *prp8-syf* alleles, this mutation exhibits a strong defect in the ability to splice the U*U*G 3′ splice site mutation (Umen and Guthrie 1995b; data not shown). Hence, all the tested *prp8* alleles, which are synthetic lethal with *PRP17*Δ, show greater discrimination in 3′ splice recognition. Conversely, all the *PRP17*Δ suppressor mutations found in our work were alleles of *PRP8* that are less discriminating in recognition of the 3′ PyAG motif.

In addition to the Prp8 and Prp17 proteins, genetic data point to the involvement of several snRNAs in 3′ splice site recognition. Mutations in two spliceosomal snRNAs, U2 and U6, were able to suppress PyAG alterations, similar to the *prp8-scf* alleles (Lesser and Guthrie 1993b; Madhani and Guthrie 1994b). Interestingly, mutations in U2 snRNA that perturb the U2-U6 snRNA helix II interactions were found to be synthetic lethal with mutations in either *PRP17* or *PRP8* (Xu *et al.* 1998), and one of the *syf* mutants obtained in our synthetic lethality screen also maps to the U2 snRNA (S. Ben-Yehuda, unpublished data). It has been shown that the RNA-RNA interactions between U2 and U6 snRNAs form an active site involved for the second step

of the splicing reaction (Madhani and Guthrie 1994b). Therefore, the Prp17 and Prp8 proteins may assist in bringing the active site of the spliceosome to the appropriate conformation during the second step of the splicing reaction.

Other biochemical and genetic observations provide links between yeast Prp8p and various factors in or near the catalytic centers of the spliceosome: (1) Prp8p has been proposed to trigger the unwinding of the U4/U6 duplex prior to the interaction of U6 with U2 to form part of the reaction center in the spliceosome (Kuhan *et al.* 1999); (2) before the second catalytic step, Prp8p binds the ends of both the exons that are to be joined, possibly being responsible for anchoring these in the catalytic center (Teigelkamp *et al.* 1995a,b; Umen and Guthrie 1995a,b); and (3) Prp8p binds to the invariant loop of U5 snRNA that is critical for precisely aligning the end of exon 1 with the 3′ splice site for the second step of splicing (Dix *et al.* 1998). Thus, Prp8p may serve as an anchor in the spliceosome, not only of the ends of the exons, but also to position snRNAs appropriately to form the reaction centers for each step, while Prp17 may be involved only in the second-step interactions that are mediated by Prp8p.

Why are the *prp8-scf* and the *prp8-syf* alleles more and less permissive, respectively, for changes in the PyAG motif? One possible mechanism is that the *prp8-scf* mutants encode proteins with a more flexible conformation. This increased flexibility may allow a higher efficiency of 3′ splice site recognition at the expense of accuracy and thus could bypass the need for Prp17p that is required for 3′ splice site recognition at the restrictive temperature. Conversely, *prp8-syf* mutations have the opposite effect: they cause a conformational change in Prp8p that reduces its flexibility and thereby increases the stringency of 3′ splice recognition, such that the activity of Prp17p becomes essential even at the permissive conditions. A similar model was proposed to explain the ability of mutations in the U2 and U6 snRNAs to partially suppress the splicing defect caused by mutations in the 3′ PyAG motif. It has been suggested that specific mutations in these snRNAs change the fit of the 3′ splice site in the spliceosomal active site such that noncanonical 3′ splice sites can be accommodated (Madhani and Guthrie 1994b). Examples of mutations that act through such a mechanism have been reported previously in other systems. For example, mutants encoding a bacterial serine protease with a broader range of substrate recognition were isolated. Crystallographic analysis showed that the decreased specificity is due to a greater flexibility of the active site (Bone *et al.* 1991). Similarly, a specific mutation in DNA polymerase β of *Escherichia coli* decreases accuracy during DNA synthesis, resulting in an increase in both base substitutions and frameshift errors. X-ray crystallographic studies suggest that the mutation causes a conformational change that increases the flexibility of the polymerase (Pelletier *et al.* 1996; Opresko *et al.* 1998). Not all of the 3′ splice site alterations are equally affected by the *prp8-scf* mutations. For example, no increased copper resistance was observed in the *prp8-scf* strains with the reporter gene carrying a U*G*G mutation at the 3′ splice site (pJU97). The only exception was the strong *prp8-scf103A* allele, which was resistant to higher copper concentrations than the wild-type allele in the presence of the 3′ U*G*G reporter. This latter 3′ splice site was more difficult to recognize than the 3′ U*U*G reporter, by both mutants and wild-type *PRP8* alleles (data not shown).

An alternative hypothesis to explain the effect of the *prp8-scf* mutations is based on the mechanism proposed for the role of the Prp16 protein. Mutations in the *PRP16* gene that produce a protein with reduced ATPase activity are able to splice pre-mRNA molecules carrying altered branch site sequences. The mutations slow down the reaction, and thus may allow more time for aberrant lariats to proceed to productive splicing (Burgess and Guthrie 1993). Similarly, it is possible that the reduced accuracy seen with the *prp8-scf* mutations results from a conformational change in Prp8p that reduces the kinetics of the second step of the splicing reaction. The slow reaction provides more time for both aberrant and optimal 3′ splice sites to be processed in the splicing pathway. On the other hand, according to this proposal, one has to speculate that Prp17p exerts its function by slowing down the second step of the splicing reaction and that *prp8-syf* alleles encode proteins that increase the pace of this step, possibilities that are less plausible, but nonetheless should be tested.

Although it is tempting to speculate that the Prp17 protein could interact directly with the PyAG motif during 3′ splice site recognition, Prp17p does not contain any known RNA-binding motif that may justify this assumption. The Prp17 protein contains several copies of the WD repeat (Vaisman *et al.* 1995; Ben-Yehuda *et al.* 1998; Zhou and Reed 1998), which probably serves in protein-protein recognition (Sikorski *et al.* 1990; Williams *et al.* 1991). However, we were unable to detect physical interactions between Prp8p and Prp17p using the two-hybrid methodology (unpublished data). Thus, the interaction between Prp17p and Prp8p, which allows efficient and accurate recognition of the PyAG motif, may take place indirectly through other proteins. The clustering of *scf* and *syf* alleles to a small region of Prp8p defines the region that mediates the interactions between this protein and Prp17p. A possible candidate for a protein that may serve as a link between Prp8p, Prp17p, and the 3′ splice site is Slu7p. Slu7p has been shown to interact with both Prp8p and Prp17p during the second step of the splicing reaction (Frank and Guthrie 1992; Frank *et al.* 1992; Umen and Guthrie 1995a). It has been suggested that Slu7p plays a role in the recognition of the 3′ splice site, since its requirement increases with the distance between the branchpoint and the 3′ splice site (Brys and Schwer 1996).

The *PRP17* gene was identified originally through the temperature-sensitive mutation *PRP17-1*, which affects both the mitotic and meiotic cell cycles (Kassir and Simchen 1978). Interestingly, some mutant alleles of *PRP8* (*dbf3*, *dna39*) were also isolated as cell-cycle-specific mutants (Dumas *et al.* 1992; Shea *et al.* 1994). The phenotype of *dbf3-1* strongly resembles that of *PRP17* strains: *dbf3-1* cells held at the restrictive temperature show a cell cycle arrest at the G2/M transition, a delayed entry into the S-phase, and sensitivity to hydroxyurea (HU), a well-characterized inhibitor of DNA synthesis. These phenotypes were absent from other tested mutant alleles of *PRP8* (Shea *et al.* 1994). The extensive genetic interactions between the *PRP17* and *PRP8* genes presented here suggest that the phenotypes of the *dbf3-1* allele originate from the loss of interaction between these proteins; in the *dbf3-1* allele of *PRP8*, Prp17p cannot exert its function, resulting in similar phenotypes to those seen in the absence of the Prp17 protein.

How could mutations in the second step of the splicing reaction account for the associated cell cycle arrest? The yeast *S. cerevisiae* has highly conserved 5′ splice site and branch site sequences, but it shows limited conservation of 3′ splice site sequences (Guthrie 1991). This variability could be a target of regulation, allowing the controlled splicing of specific RNA molecules. One possible mechanism for splicing regulation is that a small number of cell-cycle-specific, intron-containing genes may require special splicing factors for their correct expression. Therefore, it is possible that Prp17p is essential for the efficient splicing of genes involved in cell cycle progression, which contain some unique features at the 3′ splice site of the introns. A search in the databases reveals many intron-containing yeast genes that may affect cell cycle progression. The identity of the genes potentially involved in such a mechanism, however, remains to be determined, since there are many possible variations in 3′ sequences/environments that could be important for the recognition process. In an alternative mechanism, mutations in some splicing genes may disrupt the normal process of pre-mRNA splicing, eliciting a checkpoint response that arrests the cell cycle, similar to the one observed when the integrity of other cell components, such as the spindle or the DNA, is compromised (Elledge 1996).

The connection between pre-mRNA splicing and cell cycle regulation was strengthened recently by the finding of a physical association in mammalian cells between cyclin E-Cdk2 and components of the U2 snRNA-associated proteins: SAP114, SAP145, and SAP155. The splicing proteins are phosphorylated, and inhibitors of Cdk activity, such as p21, inhibit their phosphorylation (Seghezzi *et al.* 1998). Therefore, it is possible that certain introns may be removed at a specific stage of the cell cycle, at which splicing factors become activated via phosphorylation and dephosphorylation activities. The conservation of the Prp17 and Prp8 proteins throughout the evolutionary scale (Anderson *et al.* 1989; Pinto and Steitz 1989; Hodges *et al.* 1995; Ben-Yehuda *et al.* 1998; Zhou and Reed 1998) suggests that a similar cell cycle control through splicing may exist in other organisms and it will be interesting to determine whether phosphorylation plays a role in this process.

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