spp42, Identified as a Classical Suppressor of *prp4-73*, Which Encodes a Kinase Involved in Pre-mRNA Splicing in Fission Yeast, Is a Homologue of the Splicing Factor Prp8p

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ABSTRACT

We have identified two classical extragenic suppressors, *spp41* and *spp42*, of the temperature sensitive (ts) allele *prp4-73*. The *prp4*$^+$ gene of *Schizosaccharomyces pombe* encodes a protein kinase. Mutations in both suppressor genes suppress the growth and the pre-mRNA splicing defect of *prp4-73*^{ts} at the restrictive temperature (36°). *spp41* and *spp42* are synthetically lethal with each other in the presence of *prp4-73*^{ts}, indicating a functional relationship between *spp41* and *spp42*. The suppressor genes were mapped on the left arm of chromosome I proximal to the *his6* gene. Based on our mapping data we isolated *spp42* by screening PCR fragments for functional complementation of the *prp4-73*^{ts} mutant at the restrictive temperature. *spp42* encodes a large protein (p275), which is the homologue of Prp8p. This protein has been shown in budding yeast and mammalian cells to be a *bona fide* pre-mRNA splicing factor. Taken together with other recent genetic and biochemical data, our results suggest that Prp4 kinase plays an important role in the formation of catalytic spliceosomes.

INTRONS of pre-mRNA in eukaryotes are removed in a spliceosome, a large complex consisting of the small nuclear ribonucleoprotein particles (snRNP's) U1, U2, U4/U6, and U5 and many so-called non-snRNP proteins. It is the dynamic interaction of the spliceosome components, including RNA-RNA, RNA-protein, and protein-protein interactions, which accurately recognize introns and form a catalytic complex leading to the release of spliced mRNA and the excised intron sequences (Will and Lührmann 1997; Staley and Guthrie 1998). The basic biochemistry of the splicing machinery has been worked out *in vitro* using the mammalian system (Manley and Tacke 1996; Wang and Manley 1997; Will and Lührmann 1997) and with a genetic approach combined with biochemistry using the budding yeast *Saccharomyces cerevisiae*. Many pre-mRNA splicing factors have been identified as conditional *prp* (*p*re-m*R*NA *p*rocessing) mutants in *S. cerevisiae* (Vijayraghavan *et al.* 1989). Cloning and characterization of these factors revealed a wealth of information about the formation and disassembly of a catalytic spliceosome (Guthrie 1991; Ruby and Abelson 1991; Staley and Guthrie 1998).

We compared sequences of components that have been determined as *bona fide* splicing factors in both systems. This comparison revealed that some essential splicing factors in the mammalian system, for example, U2AF^{65}, have counterparts in the budding yeast, but show very weak or no sequence homology (Abovich and Rosbash 1997). In contrast, Prp8p, also an essential splicing component in both systems, reveals >60% identical amino acids between budding yeast and mammals (Hodges *et al.* 1995). Moreover, typical SR splicing factors, involved in intron recognition in constitutive and alternative splicing, appear to be absent in the budding yeast (Manley and Tacke 1996).

Prp8p homologues have been found in budding yeast, trypanosomes, nematodes, plants, and mammals. The Prp8p protein is large (>200 kD) and shows 40 to 80% identical amino acids among these eukaryotes with no significant homology to other proteins (Hodges *et al.* 1995). In mammals, yeast, and trypanosomes the protein has been shown to be a snRNP U5 protein, appears to be involved in the recognition of introns, and might play an important role at the active site of the spliceosome (Beggs *et al.* 1995; Teigelkamp *et al.* 1995; Umen and Guthrie 1996; Lucke *et al.* 1997; Sha *et al.* 1998; Reyes *et al.* 1999).

We discovered that the fission yeast *Schizosaccharomyces pombe* is capable of correctly splicing a mammalian virus transcript (Käufer *et al.* 1985). On the basis of investigations of the architecture of artificial and natural *S. pombe* introns, we know now that this finding was fortuitous. The small t SV40 intron is spliced in *S. pombe* because it has the architecture of a typical fission yeast intron. It is conceivable that *S. pombe* introns might represent

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archetypal introns; however, this is mere speculation (Gatermann *et al.* 1989; Prabhala *et al.* 1992).

In the fission yeast 14 temperature- and cold-sensitive *prp* mutants (*prp1-prp14*) have been identified (Potashkin *et al.* 1989; Rosenberg *et al.* 1991; Urushiyama *et al.* 1996). All the *prp* mutants show the common characteristic of accumulating pre-mRNA at the restrictive temperature, whereas some produce, in addition, elongated cells and arrest with a 2C DNA content (Lundgren *et al.* 1996; Urushiyama *et al.* 1996).

We isolated and characterized *prp4*$^{+}$ encoding a protein kinase. This was the first kinase gene isolated whose molecular phenotype suggested a direct or indirect involvement in pre-mRNA splicing (Alahari *et al.* 1993). Sequences similar to Prp4p have been isolated from human and mouse (Gross *et al.* 1997). The genome of *S. cerevisiae* does not contain a sequence bearing similarity to Prp4p. Prp4p kinase of fission yeast is capable of phosphorylating *in vitro* the RS domain of the mammalian SR protein splicing factor ASF/SF2 (Gross *et al.* 1997).

Recently, we have also identified and characterized *srp1* of fission yeast. Srp1p is the first protein in a unicellular organism closely related to the family of mammalian SR protein splicing factors. Srp1p contains a typical SR-RNA binding domain (RBD) and an RS domain (Gross *et al.* 1998). Overexpression of Srp1p in a *prp4-73*^{ts} genetic background suppresses the splicing defect of these cells, resulting in the production of mRNA at the restrictive temperature; however, the growth defect is not suppressed (Gross *et al.* 1998). We do not yet have any evidence whether Srp1p is an *in vivo* or an *in vitro* substrate of Prp4p.

prp2^{+} of fission yeast encodes the homologue of the mammalian U2AF^{65} splicing factor (Potashkin *et al.* 1993) and *prp10*$^{+}$ encodes the homologue of the mammalian splicing factor SAP155 (Habara *et al.* 1998). Furthermore, *prp1*$^{+}$ encodes a protein containing 19 tetratrico peptide repeats (TPR) and has been shown to be involved in pre-mRNA splicing, in poly(A)$^{+}$ RNA transport, and possibly in cell cycle regulation (Urushiyama *et al.* 1997). The protein shows ~30% amino acid identity to Prp6p of *S. cerevisiae*, which has been shown to be a component of the snRNP U4/U6 (Abovich *et al.* 1990). Interestingly, the double mutant *prp1 prp4* is not viable, indicating a functional relationship between Prp1p and Prp4p kinase (Urushiyama *et al.* 1997).

In this study we describe the identification and genetic characterization of two independent classical extragenic suppressors of the *prp4-73*^{ts} mutation. Both suppressors, *spp41* and *spp42*, are located on the left arm of chromosome I. The observation that *spp41* and *spp42* are synthetically lethal suggests that both proteins are functionally related. Based on our mapping data, *spp42* was isolated by screening candidate genes in a *prp4-73*^{ts} background for function. Computational analysis of the isolated gene sequence strongly suggests that *spp42* encodes a homologue of the splicing factor Prp8p.

MATERIALS AND METHODS

Yeast strains, media, and genetic methods: The *S. pombe* strains used were derived from those originally isolated by Leupold (1950). All the strains used in this study are listed in Table 1. The standard genetic techniques and the media are described by Gutz *et al.* (1974). The long-range mapping procedure was developed by Schmidt (1993). Yeast-extract agar (YEA), malt-extract agar (MEA), and minimal medium agar (MMA) were supplemented with adenine (75 mg/liter), leucine (150 mg/liter), lysine (50 mg/liter), uracil (100 mg/liter), histidine (100 mg/liter), and 0.68 mg/liter thiamine when needed. All crosses were performed on MEA plates and incubated at 25° for 3 days. Tetrads were dissected using the Singer MSM 200 micromanipulator (Singer Instruments, Somerset, England). The spore clones were grown on YEA master plates and then replica plated on MMA with appropriate supplements.

Suppressor screen: A total of 5×10^7 cells of the strain HE622 (Table 1) were suspended in 1 ml minimal medium (MM) and 10^5 cells were plated on YEA. Plates were irradiated with UV-light (366 nm) for 0 (control), 15, 30, and 60 sec, respectively, with a UV-radiation lamp (366 nm). With this approach we obtained 2800 colonies growing at 36°. These revertants were first screened for cold sensitivity at 15°. Several candidates, which appeared to grow slower than the control strain, were collected. These strains were named the CS (cold-sensitive) series. Finally, tetrad analysis after crossing the CS strains to wild type revealed that the three mutants CS1, CS2, and CS3 were true extragenic suppressors (Table 2); however, the cs phenotype of these three strains is leaky at best.

Molecular biological methods: Standard molecular genetic procedures used in this study have been described by Moreno *et al.* (1991). Isolation of DNA for PCR templates and transformation of *S. pombe* with shuttle vectors were performed as previously described by Gatermann *et al.* (1989).

Oligonucleotides and PCR procedure: The following oligonucleotides containing the restriction sites *Bal*I and *Xma*I, respectively, were used as primers. The sequence for the primer pair was derived from the open reading frame (ORF) on cosmid SPAC4F8.c12 stored under GenBank accession no. Z98530. The PCR fragment with these primers is expected to be 7 kb.

*Bal*I primer: 5′-CCATGGCCATGGCGTCGTTACCACCGGGGAATCCTCC-3′;
*Xma*I primer: 5′-GGGCCCGGGAAAACTATAATCTAACGCATATGTACTTGATG-3′.

In the PCR, Expand high fidelity polymerase (Boehringer, Indianapolis) was used in 35 cycles. In each cycle the DNA was denatured for 30 sec at 94°, followed by 45 sec annealing time and 10 min synthesis at 68°. The PCR products were cloned in the *Escherichia coli* pCR2.1 vector using the TOPO TA cloning kit (Invitrogen, San Diego) following the manufacturer's protocol.

Subcloning of the PCR products in the expression vector pREP3: The plasmid pCR2.1 containing the 7-kb PCR fragments were digested with *Bal*I and *Xma*I, separated from the vector sequences, purified, and subcloned in pREP3. This cloning step brings the 7-kb fragments under the control of the thiamine repressible promoter (Maundrell 1993).

RNA analysis: RNA isolation and Northern analysis was performed as described by Nischt *et al.* (1986). Total RNA was fractionated on a 1.2% agarose gel, subsequently transferred

TABLE 1

***S. pombe* strains used in this study**

Strain	Genotype	Source
L 968	h^{90} (wild type)	Leupold (1950)
L 972	h^- (wild type)	Leupold (1950)
L 975	h^+ (wild type)	Leupold (1950)
HE622	*h⁻ leu1-32 ura4-D18 prp4-73ts*	This study
CS 1	*h⁻ leu1-32 ura4-D18 prp4-73tsspp41-1*	This study
CS 2	*h⁻ leu1-32 ura4-D18 prp4-73tsspp42-1*	This study
CS 3	*h⁻ leu1-32 ura4-D18 prp4-73tsspp42-2*	This study
HE300	*h⁺ leu1-32*	This study
HE691	*h⁺ leu1-32 ura4-D18 prp4-73tsspp41-1*	This study
HE692	*h⁺ leu1-32 ura4-D18 prp4-73tsspp42-1*	This study
SL28	*h⁺ lys1 ade4-31 prp4-73ts swi5-39*	This study
SL30	*h⁻ ade6-M210 prp4-73ts spp41-1 swi5-39*	This study
SL31	*h⁻ ade6-M210 prp4-73ts spp42-2 swi5-39*	This study
SL41	*h⁺ura1-171 his6-365 prp4-73ts swi5-39*	This study
SL42	*h⁻ prp4-73ts spp41-1 swi5-39*	This study
SL44	*h⁺lys1 prp4-73ts swi5-39*	This study
KR1	*h⁻ prp4-73ts spp42-2 swi5-39*	This study
KR2	*h⁻ his6-365 prp4-73ts spp41-1*	This study
KR4	*h⁻ his6-365 prp4-73ts spp42-2*	This study

onto nitrocellulose, and probed with the *dis2* gene, including the 395-bp intron sequence. The *dis2* gene encodes a phosphatase (Ohkura *et al.* 1989). The probe was isolated from a plasmid that was a gift from Dr. M. Yanagida (Japan). The probe was labeled with a random priming kit using [α-^{32}P]dCTP (Amersham, Buckinghamshire, U.K.).

RESULTS

Screen for extragenic suppressors of *prp4-73*: The *prp4-73ts* allele was previously isolated from a temperature-sensitive strain (HE622, Table 1) following 5′-nitroso-guanidine mutagenesis (Rosenberg *et al.* 1991). The *prp4-73ts* allele conferring temperature sensitivity harbors a mutation in kinase subdomain IV. A tyrosine residue, instead of a cysteine, was found at position 235 (Gross *et al.* 1997). When cells containing the temperature-sensitive allele are shifted to the restrictive temperature (36°), cell division stops immediately and all genes containing introns accumulate pre-mRNA, whereas processed mRNA is rapidly degraded (Figure 1, Rosenberg *et al.* 1991). *prp4*$^+$ is located on chromosome III (Alahari *et al.* 1993). We mutagenized HE622 (Table 1) using UV-light as mutagen and screened for growth at 36°. Growing colonies were crossed with the strain HE300 containing *prp4*$^+$ (Table 1) and tetrad analysis was performed to analyze the nature of the reversion. The results of this analysis are depicted in Table 2. Two strains, called CS1 and CS2, respectively, revealed ratios of the three classes of tetrads (PD, NPD, and T) that indicate independent assortment of a locus suppres-

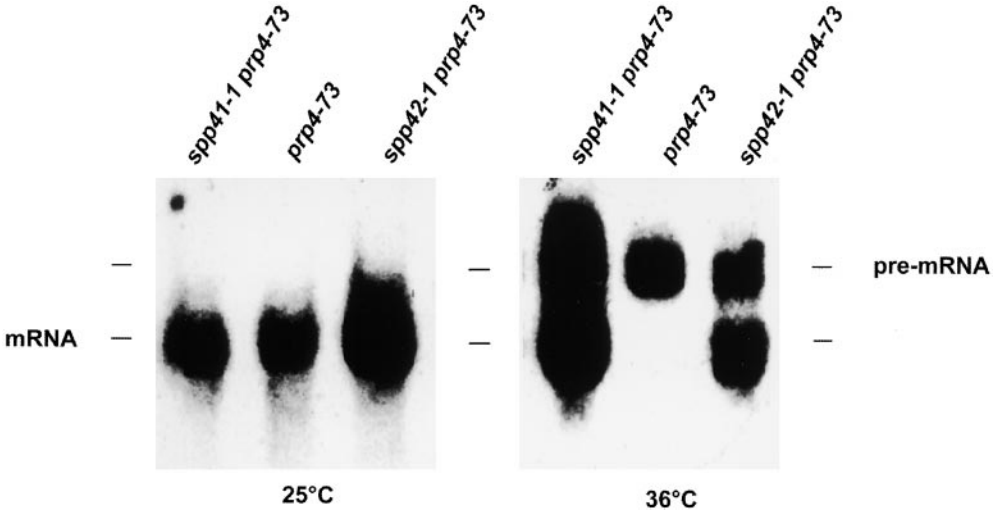


Figure 1.—Northern analysis of the suppressor strains CS1 (*spp41-1*) and CS2 (*spp42-1*) to test for suppression of the splicing defect. The two CS strains and the control strain HE622 (*prp4-73ts*) were transformed with a plasmid carrying the intron containing *dis2* gene. Total RNA was isolated from cells grown at 25° and from cells shifted to 36° for 3 hr as indicated. Each lane contains 25 μg RNA. The blot was probed with a radioactive labeled intron containing *dis2* fragment.

TABLE 2

Tetrad analysis of extragenic suppressors of *prp4-73^{ts}*

Cross	Gene pair	No. of tetrads[a] PD	NPD	T
HE300 × CS1	*prp4-spp*[b]	2	1	4
HE300 × CS2	*prp4-spp*[b]	1	1	11
HE300 × CS3	*prp4-spp*[b]	13	0	16

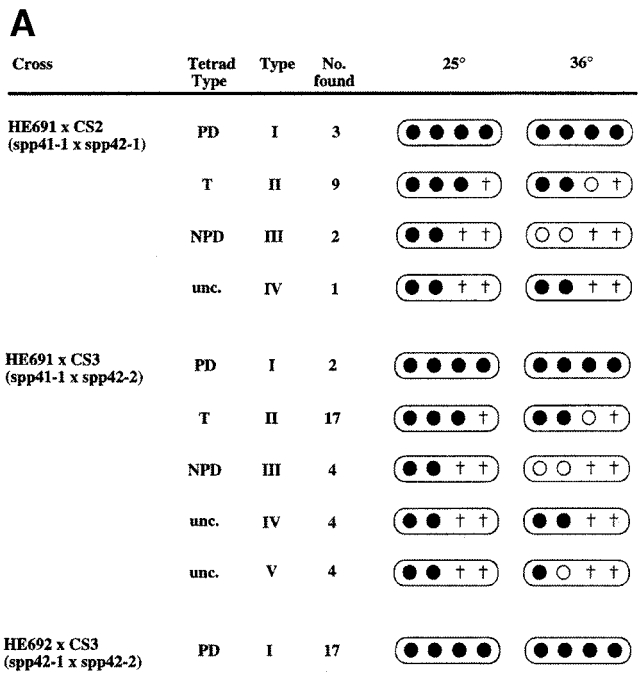
[a] PD, parental ditype: four growing colonies; NPD, nonparental ditype: two growing colonies; T, tetratype: three growing colonies when incubated at 36°.

[b] spp, suppressor gene. After the dissection of tetrads, spores were incubated at 25° on YEA, then replicated and incubated at 36°.

sing the *prp4-73^{ts}* allele at 36°, thus representing a classical extragenic suppressor. Although the cross between HS300 and CS3 indicates linkage between suppressor and *prp4*, further analysis unambiguously shows that the suppressor is located on chromosome I.

Identification of two different suppressor loci: To test the three CS strains for allelism of the extragenic suppressor, we constructed strains with opposite mating types, called HE691 and HE692 (Table 1). Then we crossed the appropriate strains to each other. The crosses of strains HE691 × CS2 and HE691 × CS3 revealed a ratio of the three tetrad classes, which indicates the independent assortment of two different suppressor loci (Figure 2A), whereas 17 asci of the cross between HE692 and CS3 produced at 36° four growing colonies, clearly suggesting that the suppressor mutations of these two strains are allelic (Figure 2A). Furthermore, in Figure 2B we show results of a tetrad analysis after a cross of HE691 to CS3 that was incubated at the permissive temperature (25°) for *prp4-73^{ts}*. The results suggest that spores containing *prp4-73^{ts}* and both suppressor mutations are not viable. Thus, we have identified two extragenic suppressors of *prp4-73^{ts}*, which we call *spp41-1* (CS1), *spp42-1* (CS2), and *spp42-2* (CS3) (suppressor of *p*recursor mRNA *p*rocessing). The results also suggest that the double mutant *spp41 spp42* (*prp4-73^{ts}*) is synthetically lethal, indicating a functional interaction between Prp4p, Spp41p, and Spp42p. Figure 1 demonstrates by Northern analysis, probing with the intron-containing *dis2* gene (Ohkura *et al.* 1989), that both suppressors are also capable of suppressing the pre-mRNA splicing defect of the *prp4-73^{ts}* allele at 36°.

Genetic mapping of *spp41* and *spp42*: We used the long-range mapping procedure developed by Schmidt (1993), testing in the first step for linkage of *spp41* and *spp42* to known markers of the three chromosomes. The two genes were mapped in a *swi5* genetic background, reducing recombination frequency ~10-fold (Schmidt 1993). As markers we used *lys1* for chromosome I, *mat1* for chromosome II, and *ade6* for chromosome III. In Table 3 the results of the tetrad analyses for both genes and the indicated chromosomal markers are listed. Based on the numbers obtained for the three classes of tetrads, it appears that *spp41* as well as *spp42* are linked to the marker *lys1* on chromosome I. To determine the approximate position of *spp41* and *spp42* on chromosome I, we used the markers *ura1*, *his6*, *lys1*, and *ade4*; *ura1* and *his6* are located on the long left arm of chromosome I and *lys1* and *ade4* on the short right arm of chromosome I. The markers *his6* and *lys1* are proximal to the centromere region and *ura1* and *ade4* are markers toward the ends of chromosome I. The results of the



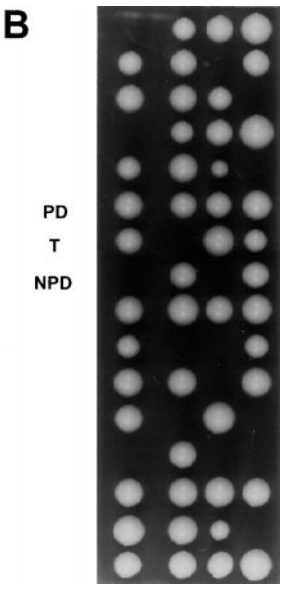


Figure 2.—(A) Test for allelism of the suppressor locus in the strains CS1, CS2, and CS3. Strains were crossed as indicated. Tetrads were dissected and spores were grown on YEA at 25° (column 25°); subsequently they were replica plated and incubated at 36° (column 36°). ●, growing colonies; †, dead spores; ○, not growing after incubation at 36°. unc., unclassified asci. (B) *spp41* and *spp42* are synthetically lethal with each other. HE691 was crossed to CS2. Tetrads were dissected and spores were grown at 25°. Spores containing the double mutant do not form colonies.

TABLE 3

Linkage analysis of *spp41* and *spp42*

Gene pairs	No. of tetrads[a] PD	NPD	T	Ch.[b]
spp41–lys1	34	0	9	I
spp41–mat1	16	7	18	II
spp41–ade6	18	14	10	III
spp42–lys1	20	4	7	I
spp42–mat1	9	11	19	II
spp42–ade6	12	9	14	III

[a] PD, parental ditype; NPD, nonparental ditype; T, tetratype.
[b] Ch., chromosome number of the marker. The strains used for linkage analysis were *swi5*$^-$ (Schmidt 1993).

TABLE 4

Long-range mapping of *spp41* and *spp42* on chromosome I

Gene pairs	No. of tetrads[a] PD	NPD	T	Recomb. fr.[b]
spp41–ura1	130	1	32	11.5
spp41–his6	164	0	4	1.5
ura1–his6	111	1	29	12.4
spp41–lys1	141	1	35	12.4
spp41–ade4	39	5	63	43.5
ade4–lys1	45	4	59	38.5
spp42–ura1	32	0	5	6.7
spp42–his6	33	0	6	8.4
ura1–his6	26	0	12	15.7
spp42–lys1	24	0	10	14.7
spp42–ade4	11	1	22	41.2
ade4–lys1	15	0	22	29.7

[a] PD, parental ditype; NPD, nonparental ditype; T, tetratype.
[b] Recombination frequencies determined from crosses homozygous for *swi5*$^-$ where *swi5*$^-$ reduces the recombination frequency about 10-fold (Schmidt 1993). The formula of Perkins (1949) was used for calculation: 50 (T + 6NPD)/(PD + T + NPD). The following schematic drawing shows the relative position of *spp41* and *spp42* on the left arm of chromosome I:

ura1 *spp42* *spp41* *his6*

tetrad analyses of these crosses are shown in Table 4. Both suppressor loci, *spp41* and *spp42*, are located on the left arm of chromosome I proximal to the *his6* marker. However, *spp41* and *spp42* are not closely linked.

Further genetic analysis: We constructed diploids homozygous for *prp4-73*^{ts}/*prp4-73*^{ts} and heterozygous for *spp41*$^+$/*spp41*$^-$ and *spp42*$^+$/*spp42*$^-$, respectively. In both cases the diploid cells grew at 36°, suggesting that in this genetic background the suppressor locus is dominant over wild type. Based on these observations we decided to screen a *prp4-73*^{ts} strain for growth at 36° using genomic libraries of the suppressor strains. Therefore, we constructed genomic libraries of the suppressor strains *spp41* and *spp42*, respectively. We used the vector pUR19 and constructed the libraries as suggested by Barbet *et al.* (1992). In addition, the partially digested genomic DNA was separated on sucrose gradients and DNA fragments of 3–5 kb in size were collected and cloned into the vector. Both libraries contained ~100,000 independent clones. However, with this approach neither *spp41* nor *spp42* was isolated, possibly for either of two reasons. First, the vector pUR19 is a high-copy plasmid; overexpression of the suppressors might have a dominant negative effect on growth. Second, it is conceivable that the suppressor genes encode large proteins not represented in the libraries, since we selected for 3- to 5-kb DNA fragments.

Isolation of *spp42*: The *S. pombe* genome is currently being sequenced by the Sanger Center (Cambridge, U.K.) and 12 other European laboratories (http://www.sanger.ac.uk/Projects/S_pombe/). We searched the *S. pombe* sequences of chromosome I at the Sanger Center for the *his6* gene and found it located on a cosmid called SPAC3F10. The sequence of this cosmid is also available in the data bank. We "walked" from the *his6* gene on the left arm of chromosome I toward the chromosome end. Unfortunately, next to the cosmid containing *his6* is a huge sequence gap of unknown size; therefore, our "walk" was directed, but without guidance. In any case, on cosmid SPAC4F8 we identified a gene sequence whose derived ORF (2363 amino acids) is >60% identical to a large protein, called Prp8p, of the budding yeast *S. cerevisiae*. Prp8p has been shown to be a *bona fide* pre-mRNA splicing factor associated with the snRNP U5 and the protein appears to be highly conserved from yeast to humans (Table 5; Hodges *et al.* 1995; Teigelkamp *et al.* 1995). Since *spp41* and *spp42* map in this region, we decided to test whether this locus may encode one of the suppressors.

The *S. pombe* sequence revealing this large ORF appears not to be interrupted by putative introns. We designed a pair of primers, including the putative start codon, to produce PCR products from genomic DNA. According to the sequence on cosmid SPAC4F8, we would expect a PCR product of 7 kb. As template DNA for this primer pair we isolated DNA from a wild-type strain and from the suppressor strains *spp41* and *spp42*, respectively. As shown in Figure 3A, the size of the PCR products of the three strains is consistent with the predicted size of ~7 kb. Restriction analysis showed that all three PCR fragments contained the same restriction sites found in the database sequence (Figure 3B). The three PCR products were cloned into the plasmid pCR2.1 for amplification in *E. coli*. In the next step we subcloned the 7-kb DNA fragments into the fission yeast expression vector pREP3, placing the ORFs under the control of the thiamine repressible *nmt1* promoter. pREP3 contains *LEU2* as selectable marker (Maundrell 1993). The plasmids containing the 7-kb DNA

TABLE 5

Comparison of the ORF derived from *spp42* with the data bank

	spp42 ORF	hPrp8p	cPrp8p	scPrp8p	tPrp8p
Length aa	2363	2335	2329	2413	2403
% Identity[a]	100	73	73	61	40

Length aa, number of amino acids found in the indicated amino acid sequence. hPrp8p, *Homo sapiens* (GenBank accession no. AB007510); cPrp8p, *Caenorhabditis elegans* (GenBank accession no. L14433); scPrp8p, *S. cerevisiae* (EMBL accession no. Z24732); tPrp8p, *T. brucei* (EMBL accession no. Y12638).

[a] Percentage overall amino acid identity with *spp42* ORF.

fragment of the three different strains were transformed into the strain HE622 containing *prp4-73^{ts}* (Table 1) and screened at the permissive temperature (25°) on minimal medium (MM) containing thiamine for leucine prototrophic colonies. All three plasmids produced colonies (Figure 4A). Growing colonies were replated on MM plates containing thiamine and incubated at 36°, again selecting for leucine prototrophy. Under these conditions only the cells carrying the plasmid-borne 7-kb fragment of the *spp42* strain produced colonies (Figure 4A). This suggests that the 7-kb PCR fragment of the *spp42* suppressor strain carries the extragenic suppressor of *prp4-73^{ts}*. This result was confirmed when we replated the cells transformed with the three different plasmids onto nonselective medium. Again, only the cells that were transformed with the plasmid carrying the 7-kb DNA fragment derived from the *spp42* strain produced colonies at 36° (Figure 4B). Furthermore, in these cells the suppressor can be overproduced without any negative effect on the cells; however, overproduction of the wild-type (*spp42^{+}*) gene in the *spp42* suppressor background has a dominant negative effect on growth (results not shown).

DISCUSSION

Suppressor phenotypes of *spp41* and *spp42* at 36°: We isolated two classical extragenic suppressors of the *prp4-73^{ts}* allele. Mutations in both loci suppress the growth and the splicing defect caused by *prp4-73^{ts}*. The results of Northern analysis suggest that both suppressor strains efficiently produce mature mRNA at 36°. However, since the cells were transformed with a plasmid-borne reporter gene, splicing activity cannot be quantified (Figure 1).

***spp42* encodes a homologue of Prp8p:** The sequence on cosmid SPAC4F8 revealed a noninterrupted ORF of 2363 amino acids with an estimated molecular mass of 275 kD. The gene for this ORF, including promoter and 3′ control region, must be contained on at least an 8-kb DNA fragment. This might have been the reason that our approach to screen for the suppressor genes with the genomic libraries failed. Both libraries were made with fragments between 3 and 5 kb. Possibly, *spp41* also encodes a large protein. According to our mapping data, *spp41* might lie in a chromosomal region that has not been sequenced yet. For this reason, we have started to construct a library of the *spp41* suppressor strain with partial *Sau*3A fragments >7 kb.

For further analysis of the isolated suppressor gene *spp42*, we compared the ORF of 2363 amino acids with the data bank and found an overall amino acid identity of 73% between the fission yeast sequence and the human hPrp8p (also named p220, Table 5). The overall identity between the fission yeast sequence and Prp8p of *S. cerevisiae* is 61% (Table 5). On the basis of these results, we suggest that *spp42* of fission yeast encodes a homologue of Prp8p (Table 5). In the fission yeast the gene named *prp8/cdc28* encodes a putative helicase, which is possibly involved in pre-mRNA splicing and cell cycle regulation (Lundgren *et al.* 1996); to avoid confusion we will call the gene *spp42* and its product Spp42p.

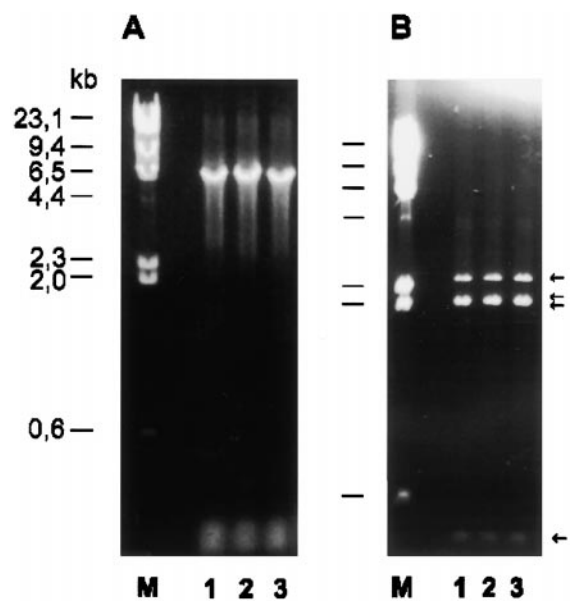


Figure 3.—(A) PCR fragments produced using a primer pair designed to isolate the putative Prp8p ORF on cosmid SPAC4F8.12c. As templates genomic DNA was isolated from the following: lane 1, wild-type strain L968; lane 2, suppressor strain CS1(*spp41-1)*; and lane 3, suppressor strain CS2 (*spp42-1*). M, *Hin*dIII digested λ DNA. A 1% agarose gel was used for electrophoresis. (B) PCR fragments digested with *Eco*RI. According to the database sequence four fragments of 2.465, 2.161, 2.159, and 0.215 kb were expected (arrows).

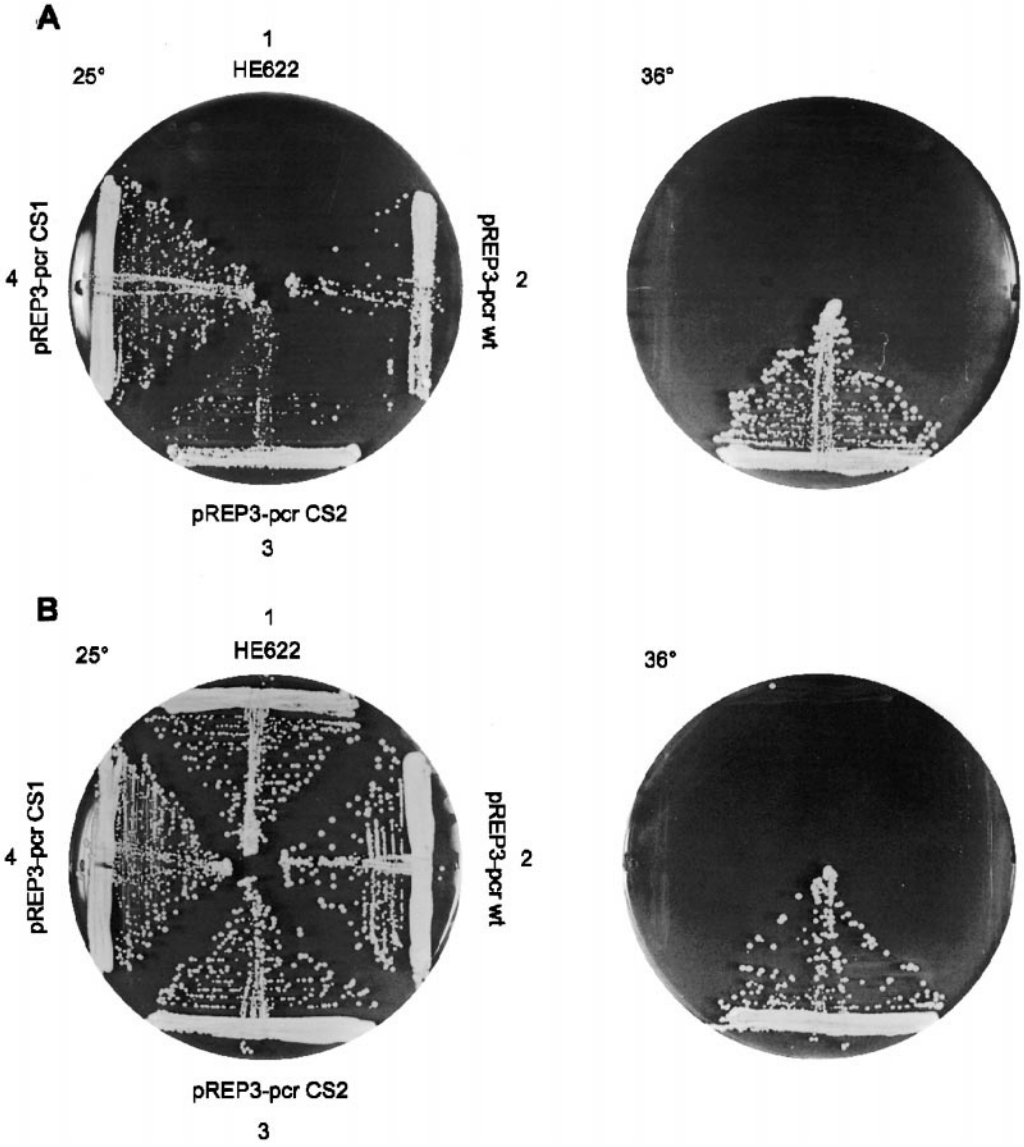


Figure 4.—Test for suppressor function of the candidate gene. (A) The strain H622 containing *prp4-73*^{ts} was transformed with the expression plasmid pREP3 containing the 7-kb PCR fragment of the wild-type strain (2); with pREP3 containing the 7-kb PCR fragment of the suppressor strain CS2 (*ssp42-1*) (3); with pREP3 containing the 7-kb PCR fragment of the suppressor strain CS1 (*ssp41-1*) (4) and grown at 25° on plates without leucine to select for the plasmids. (1) HE622 (without plasmid). Growing colonies were replated and incubated at 36° under selective conditions. The plates contained thiamine to keep expression of the cloned gene low. (B) The transformed cells were plated on nonselective medium (YEA) and incubated at 25° and 36°. (1) HE622; (2), (3), (4) as in A.

It has been shown *in vitro* that Prp8p of *S. cerevisiae* and hPrp8p contact pre-mRNA. Both proteins form crosslinks that indicate that this protein is always in close proximity to the splice sites during the splice reaction (Teigelkamp *et al.* 1995; Sha *et al.* 1998). In addition, with a genetic approach it has been shown that Prp8p in *S. cerevisiae* is involved in 3′ splice site selection, possibly required for specificity and fidelity of utilization of the splice site (Umen and Guthrie 1996). And more recently, extensive cross-linking studies with human hPrp8 (p220) revealed that it cross-links specifically with the conserved GU at the 5′ splice site (Reyes *et al.* 1999). On the basis of these genetic and biochemical data it was suggested that Prp8p might play an important role in the formation of the catalytic center of the spliceosome.

Genetic interactions: The genetic interaction of *prp4* with *spp41* and *spp42*, respectively, suggests a functional relationship of Prp4p with the products of the suppressor genes. Furthermore, *spp41* and *spp42* are synthetically lethal (in the presence of *prp4-73*^{ts}), which also defines a functional interaction between *spp41* and *spp42*. Based on these observations, it is possible that Spp41p and Spp42p encode substrates and/or regulators of Prp4p kinase that might function in a complex.

In addition, Urushiyama *et al.* (1997) have shown a genetic interaction between Prp4p kinase and Prp1p. Prp4p *in vitro* is an active kinase. Therefore, we wanted to use bacterially produced Spp42p and Prp1p to test the proteins as *in vitro* substrates of immunoprecipitated Prp4p kinase (Gross *et al.* 1997). Bacterially produced Prp1p was phosphorylated by immunoprecipitated Prp4p (W. Schmidt and N. F. Käufer, unpublished results). However, so far we have not been able to produce in bacteria the whole Spp42 protein in appreciable amounts.

What is the function of Prp4p? Prp4p is essential for growth located in the nucleus and kinase activity is required for pre-mRNA splicing. An extensive mutational analysis of *prp4* revealed alleles that, when overex-

pressed, have a dominant negative effect leading to morphological and molecular phenotypes that suggest impairment in a variety of intracellular processes (Alahari *et al.* 1993; Gross *et al.* 1997). Taking into consideration the genetic and biochemical data available, we propose here the following working hypothesis for the function of Prp4p kinase:

The genetic interaction of Prp4p kinase with Spp42p and Prp1p suggests a functional relationship. Prp4p kinase might be involved in targeting the Spp42p and Prp1p molecules to the location of spliceosome assembly and/or Prp4p kinase activity might be involved directly in the control of the formation of active spliceosomes.

The mammalian protein kinases Clk/Sty and SRPK1 phosphorylate *in vitro* the mammalian SR splicing factors (Gui *et al.* 1994; Colwill *et al.* 1996). The SR splicing factors are found in subnuclear structures, called speckles. It has been suggested that the kinases Clk/Sty and SRPK1 are involved in targeting the SR proteins from the speckles to the location of spliceosome assembly (Colwill *et al.* 1996; Wang and Manley 1997; Duncan *et al.* 1998). However, whether these kinases are directly involved in the formation of active spliceosomes and phosphorylate splicing components other than SR proteins is not known (Wang and Manley 1997; Will and Lührmann 1997). To the best of our knowledge, Prp4p kinase is the first kinase showing functional interaction with non-SR-splicing factors. On the basis of our results discussed in this article and in previous studies (Gross *et al.* 1997, 1998), we suggest that Prp4p is an excellent candidate for a kinase directly controlling the assembly of catalytic spliceosomes. Further genetic and biochemical analysis will reveal the specific role of this protein kinase.

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