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The fission yeast *prp10⁺* gene involved in pre-mRNA splicing encodes a homologue of highly conserved splicing factor, SAP155

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ABSTRACT

In the fission yeast *Schizosaccharomyces pombe*, 14 *prp* (pre-mRNA processing) mutants have been isolated to date. We cloned the *prp10⁺* gene by complementation of the temperature-sensitive growth of *prp10*. Five types of transcripts were found that were alternatively spliced with respect to two possible introns located in the 5′-terminal region. Three of them are probably functional and code for putative proteins of ~1200 amino acids. Proteins highly homologous to Prp10p are present in other organisms, one of which is a human spliceosome-associated protein SAP155, a subunit of the splicing factor complex SF3. The C-terminal two-thirds of Prp10p is highly conserved among species, and contains consensus repeats for the regulatory subunit A of protein phosphatase PP2A. A gene disruption experiment indicated that the *prp10⁺* gene is essential for viability in *S.pombe*. Prp10p tagged with GFP is predominantly localized in the nuclear DNA region. A series of deletions showed that the less conserved N-terminal region of ~300 amino acids in Prp10p is dispensable, although the corresponding region was thought to play important roles in the mammalian splicing system.

INTRODUCTION

In eukaryotic cells, removal of an intron from a pre-mRNA, a process known as pre-mRNA splicing, is one of the essential steps for gene expression. All introns within nuclear pre-mRNAs are thought to be removed by the same two-step mechanism. In the first step, cleavage at a 5′ splice site, and ligation of the 5′ end of the cleaved intron to a branch site within the intron occur simultaneously. In the second step, cleavage at a 3′ splice site occurs at the same time as two exons are ligated. These dynamic reactions take place in a large complex called a spliceosome. Major components of the spliceosome are the small nuclear ribonucleoprotein particles (U1, U2, U4/U6 and U5 snRNPs) and a series of non-snRNP proteins. Recent works revealed that non-snRNP proteins play important roles in pre-mRNA splicing as do the snRNPs. Some of the non-snRNP proteins were identified in biochemical assays using a mammalian *in vitro* splicing system, and others were identified by genetic analyses in yeast (1–4).

We have been investigating the mechanism of pre-mRNA splicing in the fission yeast *Schizosaccharomyces pombe*. In contrast to the genes in *Saccharomyces cerevisiae*, about half of the genes have introns in *S.pombe*. The *S.pombe* splicing system seems to be closer to that of mammals than is the system of *S.cerevisiae*. An SV40 small T antigen transcript with a typical mammalian intron was spliced in the fission yeast (5). Although mammalian introns are not always excised in *S.pombe*, it remains a good model organism for genetic studies of pre-mRNA splicing.

So far, 14 *prp* mutants (*prp1–prp14*) have been isolated in *S.pombe* (6–9). At the non-permissive temperature, these mutants are defective in pre-mRNA splicing and accumulate pre-mRNAs in the cells. The wild type genes that rescue the mutation have been cloned in four of them. The *prp2⁺* gene encodes spU2AF^{59}, which is a fission yeast homologue of the human U2AF large subunit, U2AF^{65} (10,11). The *prp4⁺* gene product is predicted to be a serine/threonine kinase (12,13). *prp8⁺* was found to be identical with *cdc28⁺* encoding a DEAH-box RNA helicase (14). The *prp1⁺* gene encodes a protein with a TPR-motif (15).

SAP155 is one of the spliceosome-associated proteins (SAPs) originally identified by Bennett *et al*. (16). They isolated 20 novel SAPs by two-dimensional gel electrophoresis. SAP155 was isolated as one of the 3′ splice site-specific SAPs. The splicing factor complex SF3 was identified from HeLa nuclear extracts by a traditional biochemical method, through several steps of fractionation (17). SF3 was then shown to be a U2 snRNP-associated complex and to consist of two subcomplexes, SF3a and SF3b (18,19). SF3a contains three subunits (SAP61/SF3a^{60}, SAP62/SF3a^{66} and SAP114/SF3a^{120}) (20–22) and homologues corresponding to these subunits were identified in *S.cerevisiae* (PRP9, PRP11 and PRP21, respectively) (23–25). SF3b contains four subunits (SAP49/SF3b^{50}, SAP130/SF3b^{130}, SAP145/SF3b^{145} and SAP155/SF3b^{155}), and cDNAs for these subunits, except for SAP130/SF3b^{130}, have been cloned (26–28). *Saccharomyces cerevisiae* homologues for SAP49 and SAP145 (HSH49 and CUS1, respectively) and *Xenopus* homologue for SAP155/SF3b^{155} were identified (29,30). By using UV cross-linking and a yeast

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two-hybrid assay, it was recently shown that SAP155/SF3b^{155} binds to pre-mRNA on both sides of a branch site in the spliceosome complex A and interacts directly with U2AF (31). Interestingly, SAP155/SF3b^{155} was found to associate with cyclin E and to be efficiently phosphorylated *in vitro* by cyclin-cdk2, a critical regulator of cell cycle progression from G_1 to S, suggesting that pre-mRNA splicing is linked to the cell cycle machinery in mammalian cells (32).

Here, we report cloning and characterization of the *prp10*$^+$ gene. The *prp10-1* mutant was originally isolated from a ts$^-$ mutant bank generated by 1-methyl-3-nitro-1-nitrosoguanidine treatment and on the basis of pre-U6 snRNA accumulation at the non-permissive temperature (8). We found that the *prp10*$^+$ gene encodes a protein of ~1200 amino acids. Homology analysis revealed that the amino acid sequence of Prp10p is highly conserved among species and it is an *S.pombe* homologue of the SF3b subunit, SAP155.

MATERIALS AND METHODS

Yeast strains and general methods

The *S.pombe* strains used in this study are listed in Table 1. We used the standard genetical procedures for *S.pombe* described by Moreno *et al*. (33) and Alfa *et al*. (34). The transformation of *S.pombe* with a cosmid library was done as described previously (35). Isolation of the haploids with double mutations was done as described in Urushiyama *et al*. (15).

Table 1. List of *S.pombe* strains

Strains	Genotypes
972	*h*$^-$
UR230	*h*$^-$, *prp10-1*
SU59-1D	*h*$^-$, *leu1-32*
YH01	*h*$^-$, *prp10-1*, *leu1-32*
YH03	*h*$^-$, *prp10-1*, *leu1-32*
UR104	*h*$^+$, *prp1-4*
SU35-3A	*h*$^+$, *prp2-1*
SU50-4D	*h*$^+$, *prp2-2*
SU13-2C	*h*$^+$, *prp3-3*
YH10	*h*$^-$, *prp2-2*, *leu1-32*
UDP6	*h*$^+$/*h*$^-$, *ade6-M216*/*ade6-M210*, *ura4-D18*/*ura4-D18*, *leu1-32*/*leu1-32*

YH03 is a strain obtained from YH01 during culturing. YH03 grows faster than YH01, but the extent of temperature sensitivity in growth and splicing defect is the same as for YH01.

Gap repair experiment and genetic mapping

Each of the four different gapped plasmids shown in Figure 1 was introduced into YH03, and Leu$^+$ transformants were obtained. One hundred Leu$^+$ transformants were selected randomly, grown at 26°C and then replica-plated. One plate was incubated at 26°C, and the other at 36°C. The number of Ts$^+$ colonies was then counted. We confirmed that the ratio of Ts$^+$ to Ts$^-$ transformants was much the same when we used the original *prp10-1* strain, YH01, as a recipient. The rescue activity of a gapped plasmid depends on the location of the mutation and the distance between the gapped region and the mutation site on the genome (36). With this experiment, a mutation site was predicted to locate in a C-terminal region of the *prp10* ORF (Fig. 1). The gap-repaired plasmid (pGR20) was recovered from the transformants. Sequencing from *Hin*dIII site to stop codon 1.7 kb region of two plasmids recovered from YH03, one from YH01 and one from WT cells revealed that the *prp10-1* has two mutation sites. Identification of mutation sites in the cloned gene suggested that the isolated gene is an authentic *prp10*$^+$ gene.

Preparation of RNA and northern blot analysis

Preparation of total RNA and northern blot analysis were carried out as described in Urushiyama *et al*. (8). The oligonucleotide probes used are complementary to the first intron (U6-IN1) and the second exon (U6-EX2) of *S.pombe* pre-U6 snRNA (37,38), and the first intron (TFII-IN1) and the third exon (TFII-EX3) of *S.pombe* TFIID mRNA (39), respectively. Nucleotide sequences of the probes are as follows. U6-IN1: 5′-TCGAACCTTGGTAA-ATATTG-3′; U6-EX2: 5′-CAGTGTCATCCTTGTGCAGG-3′; TFII-IN1: 5′-GAAATCTCGTGACATGGTAG-3′; TFII-EX3: 5′-GAGCTTGGAGTCATCCTCGG-3′.

Disruption of the *prp10*$^+$ gene

An *Xho*I–*Bam*HI fragment containing the *prp10*$^+$ gene was subcloned in pUC18. Then a 3416 bp *Eco*RI–*Kpn*I fragment of the *prp10*$^+$ gene was removed and replaced with the *ura4*$^+$ gene. The resulting plasmid was linearized by *Bam*HI/*Nco*I digestion and introduced into the diploid strain, UDP6. Stable Ura$^+$ diploid transformants were isolated and replacement of the *prp10*$^+$ gene with the disrupted construct was verified by PCR.

Localization of the GFP-tagged Prp10p

The *prp10*$^+$ gene subcloned in pSP1 (40) was digested by *Eco*105I and ligated with an oligonucleotide linker 5′-GCTAGCTCCGGAAGGCCTATGTCAACTGGTAC-3′. Then, the resultant plasmid was digested with *Mro*I and *Nhe*I, and ligated with the *Mro*I–*Nhe*I fragment from pEGFP-C1(CLONTECH) containing the GFP gene. The fusion plasmid was introduced into YH03, YH01 or SU59-1D strains. The transformants were viable at 37°C, indicating that the fusion protein is functional in *S.pombe*. After culturing at 26°C, the transformants were adhered on a poly-L-lysine coated slide, stained with 0.5 μg/ml Hoechst 33342 (Sigma) for 15 min and observed under a Zeiss Axioplan 2 fluorescence microscope with a Photometrics Quantix cold CCD camera to determine localization of Prp10p tagged with GFP.

cDNA cloning

Schizosaccharomyces pombe total RNA was prepared using an mRNA purification Kit (Pharmacia). Poly(A)$^+$ RNA was reverse transcribed with You-Prime First-Strand Beads (Pharmacia) and a primer pp10-10 (5′-TCCTGATCTTCCAGTGTTCG-3′). The *prp10*$^+$ cDNA was then amplified by PCR using primers pp10-F (5′-GACGCGCAACATGAAAGCAT-3′) and pp10-9 (5′-AAT-GCACTCTTACGCATGGG-3′). The amplified fragment was cloned into the pGEM-T vector (Promega), and sequenced with an ABI PRISM 310 Genetic Analyzer (Perkin-Elmer).

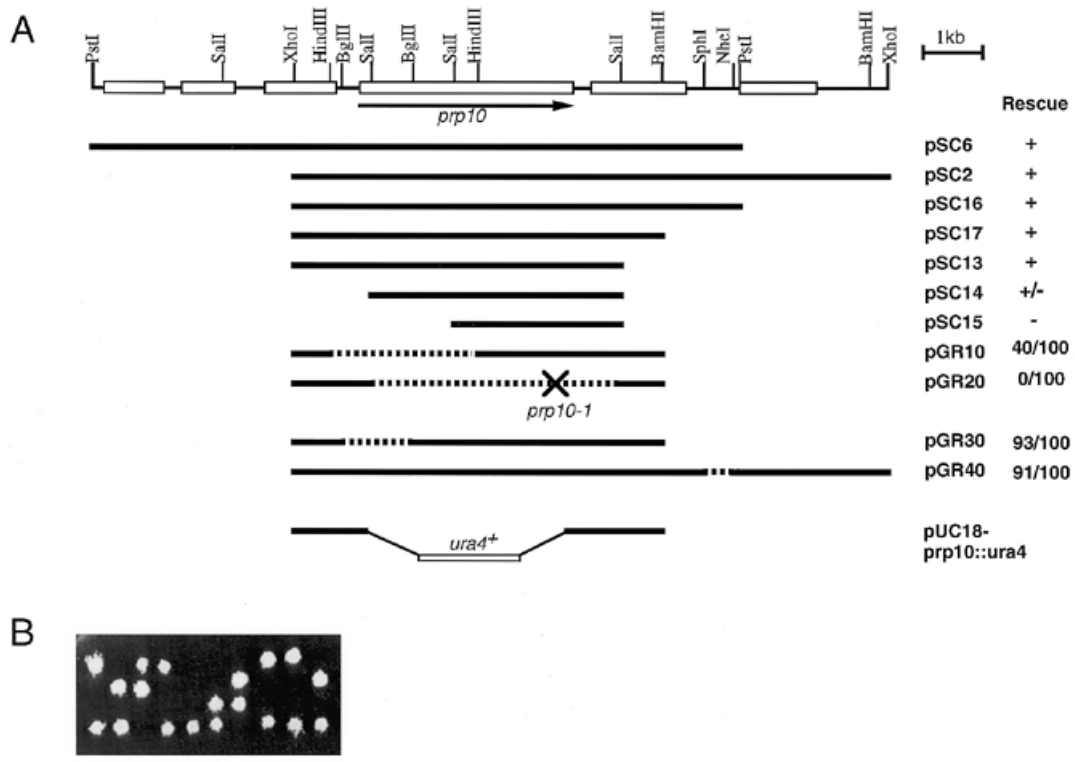

Figure 1. (**A**) Restriction map of the region containing the *prp10*$^+$ gene and complementing activities of the subcloned genomic fragments. Open boxes indicate putative open reading frames. pSC14 clone showed partial rescue activity, although the insert has no promoter region and no initiation methionine codon. pGR10–40 are gapped plasmids. The × mark indicates *prp10-1* mutation sites. Position of the *ura4*$^+$ insertion for gene disruption is also shown. (**B**) Tetrad analysis for the *prp10::ura4*$^+$ strain.

Deletion mutants of the *prp10* gene

To generate a series of N-terminal deletion mutants of the *prp10* gene, a plasmid pSC16 containing the *prp10*$^+$ gene was digested with a restriction enzyme: *Eco*RV, *Aat*II, *Bgl*II, *Pvu*II or *Nde*I. The linearized plasmid was blunt-ended with a Klenow enzyme or mung bean nuclease, followed by digestion with *Bam*HI. The expression vector pREP3 (41) digested with *Sal*I was also blunt-ended and digested further with *Bam*HI. The resultant two fragments were then ligated to generate the mutant *prp10* gene with an N-terminal deletion. Partial sequences of the resultant plasmids were determined to confirm the in-frame ligation with the coding sequence of *nmt1* in pREP3.

RESULTS

Cloning of the *prp10*$^+$ gene

To clone the *prp10*$^+$ gene, an *S.pombe* genomic cosmid library constructed in pSS10 (42) was introduced into YH03. Six Ts$^+$ transformants were isolated. Two cosmids recovered from the transformants were reintroduced into YH03 and YH01 to confirm their ability to complement the growth defects of *prp10-1* at the restrictive temperature. Each cosmid had an insert of ~40 kb in length. The inserts were subcloned into pSP1, an *S.pombe ars1* multicopy vector (40). In the course of subcloning, we found that the two cosmids contained an overlapping region, sharing a 13 kb *Xho*I–*Pst*I fragment with a rescue activity for *prp10* (Fig. 1, pSC2 and pSC6). After several steps of subcloning, we identified a 5.6 kb *Xho*I–*Sal*I fragment complementing the temperature-sensitive growth of *prp10-1* (Fig. 1, pSC13). Complementation activity for pre-mRNA splicing with this fragment was also verified by northern blot analysis (Fig. 2).

Partial sequencing of the region essential for complementation revealed that the isolated fragment was a part of the *S.pombe* cosmid clone c27F1 (43). The 5.6 kb fragment described above contains a single ORF (SPAC27F1.09c) (44). As subcloned fragments deleting a part of this ORF had no rescue activity, we concluded that a protein encoded by this ORF complemented the *prp10* mutation.

To identify a mutation site, *prp10* was transformed with each gapped plasmid shown in Figure 1. The plasmid with the *Sal*I–*Sal*I gapped gene (pGR20) generated no Ts$^+$ transformants, suggesting that the gapped region contains a mutation site. Sequencing the 1.7 kb region between a *Hin*dIII site and the stop codon revealed two mutation sites. One is present at position 3266 and results in replacement of alanine with valine. The other mutation is located at position 3290, leading to a change from serine to phenylalanine. Identification of the mutation sites in the corresponding gene from the *prp10-1* demonstrated that the cloned gene is not a suppressor gene for *prp10*, but the authentic *prp10*$^+$ gene.

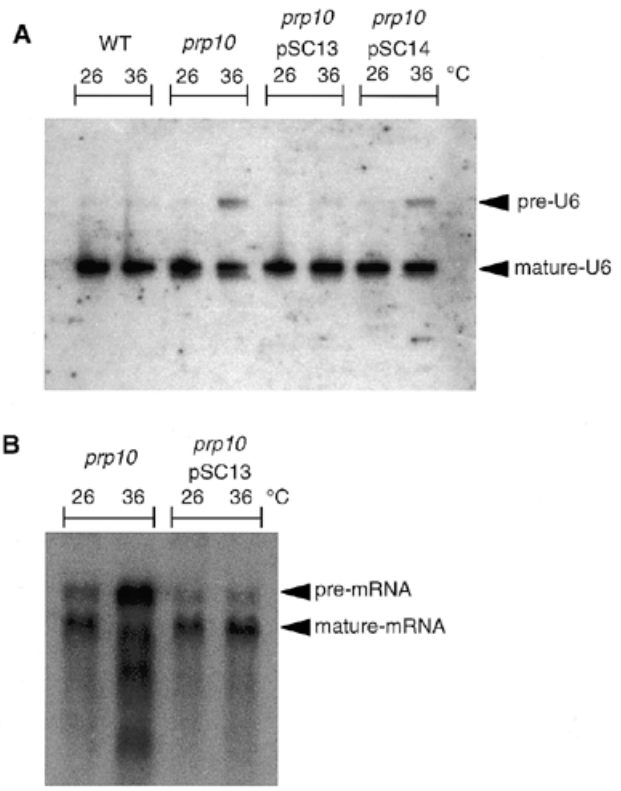

Figure 2. Defects in pre-mRNA splicing in the *prp10* mutant and complementation by the isolated genomic fragment. (**A**) Northern blot analysis of U6 snRNA. Total RNA was isolated from WT, *prp10* or *prp10* transformed with pSC13 or pSC14 shown in Figure 1, which had been grown at 26°C or shifted to 36°C for 2 h. Five μg of total RNA was fractionated on an 8% polyacrylamide–8.3 M urea gel and blotted to a membrane. The blot was probed with the mixture of the U6-IN1 and U6-EX2 probes. (**B**) Northern blot analysis of TFIID (TBP) mRNA. Total RNA (30 μg) from each strain was fractionated on a 1% formaldehyde agarose gel and blotted to a membrane. The membrane was then probed with the mixture of the TFIID-IN1 and TFIID-EX3 probes.

prp10$^+$ is essential for viability

To examine if the *prp10*$^+$ gene is required for cell viability in *S.pombe*, we constructed a null mutant of *prp10* (Materials and Methods). The *prp10::ura4*$^+$ diploid strain was sporulated and asci were dissected. In all tetrads, only one or two viable spores were obtained and all surviving progeny were Ura$^-$, suggesting that the *prp10*$^+$ gene is essential for viability in *S.pombe* (Fig. 1B).

prp10$^+$ encodes a highly conserved protein

Searching the database for homology with the obtained ORF sequence revealed proteins highly homologous with Prp10p in *S.cerevisiae*, *Caenorhabditis elegans*, *Plasmodium falciparum* and *Arabidopsis thaliana*. These proteins with unknown functions consist of 971, 1303, 1386 and 1269 amino acid residues (45–48). We also found that a human spliceosome associated protein, SAP155 (28), and a novel nuclear protein from *Xenopus laevis* (30), of which cDNAs were recently reported, were highly homologous with Prp10p.

Structural comparison of Prp10p with its putative homologues is shown in Figure 3. C-terminal two-thirds of the proteins are highly conserved among all seven putative homologues. In contrast, N-terminal regions are less conserved among species in sequence and in length. The highly conserved region in SAP155 was found to contain consensus repeats for the regulatory subunit A of phosphatase PP2A (28). Similar repeat sequences are also present in Prp10p and other putative homologues. SAP155 contains seven RWDETP motifs in the N-terminal region (28). This motif is conserved in the four multicellular eukaryotes. In the three unicellular eukaryotes, *P.falciparum*, *S.cerevisiae* and *S.pombe*, however, only one or two RWDETP-like sequences are present. TP dipeptide motifs clustered in the N-terminal region of SAP155 were recently shown to be possible phosphorylation sites for cyclin E-cdk2 (32). Such dipeptide motifs are conserved in higher eukaryotes, but not in yeasts (Fig. 3).

The *prp10*$^+$ transcripts show several splicing patterns

The ORF for Prp10p was predicted to contain one possible intron (43). To determine if the predicted intron is actually spliced out and to determine the precise splice sites, we performed RT–PCR analysis using poly(A)$^+$ RNA from wild type cells. The cDNAs of *prp10*$^+$ mRNA were cloned and sequenced. Surprisingly, we obtained five types of cDNAs that were derived from four types of alternatively spliced products and an unspliced transcript (Fig. 4). These results suggested that there are two possible introns in the N-terminal region of the *prp10*$^+$ gene, and that each intron has two possible 3′ splice sites. These two possible introns have no in-frame termination codon and their length is a multiple of three, except when the proximal 3′ splice site of the second intron was used. Thus, unspliced mRNA (form A in Fig. 4) will be translated to make the Prp10p protein of 1205 amino acids. B and E RNAs will generate proteins of 1188 and 1166 amino acids, respectively. On the other hand, C and D RNAs, which were spliced at the proximal 3′ splice site of the second intron, are probably unable to make a functional Prp10p protein because of the frameshift and a termination codon. Of 12 cDNA clones sequenced, seven represented the unspliced mRNA, suggesting that form A is the major *prp10*$^+$ mRNA. We analyzed 22 cDNA clones derived from poly(A)$^+$ RNA by digestion with a restriction enzyme (data not shown). Judging from the mobility of the DNAs in agarose gels, it seems that 10 cDNAs are derived from the unspliced mRNA (form A), six are from form B in which one intron was removed and six are from the RNAs in which both introns were removed (forms C, D or E).

The N-terminal region of Prp10p is not essential for growth

The N-terminal region of the Prp10p varies among species in amino acid sequence and length. In addition, we found several splicing patterns in the N-terminal region of Prp10p in *S.pombe*. To determine if the N-terminal region of this protein is essential for its function, we constructed a series of the mutant gene truncated in the N-terminal region (Fig. 5). We used the *nmt1* promoter to test the rescue activity of N-terminal deletion series of Prp10p. Expression of the intact *prp10*$^+$ gene by the *nmt1* promoter complemented *prp10-1* at 36°C, but growth of the carrier was slow at 26 and 36°C (data not shown). Deletion of 200 amino acids from the N-terminus, which contains a TP dipeptide motif, had no apparent effect on complementing activity of the cloned *prp10*$^+$ gene, suggesting that the deleted region is not essential for the function of Prp10p. The mutant gene with a deletion of N-terminal 279 amino acids showed partial rescue activity (Fig. 5). The deleted region contained RWDETP motifs

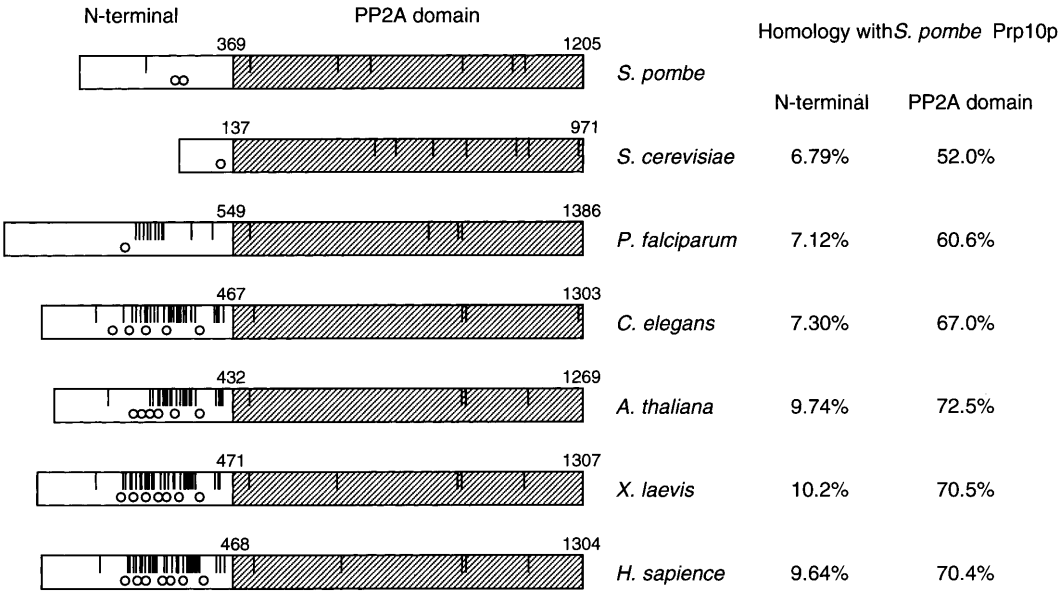

Figure 3. Structural comparison of Prp10p with its homologues. The hatched boxes represent the highly conserved C-terminal two-thirds regions. Vertical bars and open circles indicate the TP dipeptide motifs (putative phosphorylation sites) and the RWDETP motifs, respectively. Amino acid sequence identity with Prp10p in each domain was calculated by the GENETYX-MAC program (Software Development Co., Ltd). Numbers above the boxes denote amino acid positions.

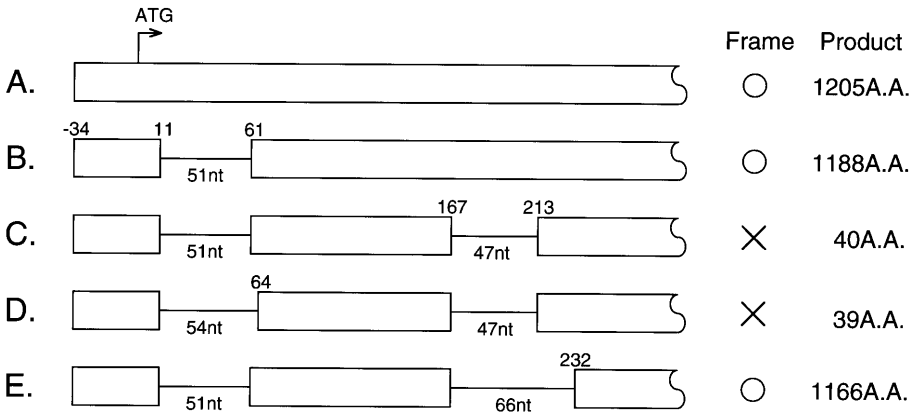

Figure 4. Splicing patterns of the *prp10*$^{+}$ pre-mRNA. The numbers indicate nucleotide positions of the end of introns taking residue A in the first ATG codon as +1. The numbers of amino acid residues of proteins translated from the first ATG are also shown in the Product column. In forms C and D, excision of the second intron results in a frameshift indicated by ×, and generates a termination codon downstream of the 3′ splice site of the second intron.

which, in SAP155, are likely to be involved in interaction with U2AF. The mutant gene with a deletion of 430 amino acids, which includes ~60 amino acids of the conserved PP2A domain, could not rescue temperature-sensitive phenotype of *prp10* (Fig. 5).

Prp10p is localized in the nucleus

To examine subcellular localization of Prp10p, we constructed a GFP–Prp10p fusion gene, and introduced it into wild type cells or the *prp10* mutant. The fusion gene could complement the temperature-sensitive phenotype of the *prp10* mutant, suggesting that Prp10p tagged with GFP is functional in *S.pombe* (data not shown). Observations using a fluorescence microscope showed that GFP-tagged Prp10p is located predominantly in the DNA region of the nucleus (Fig. 6).

Genetic interaction between *prp10* and *prp2*

Recently, SAP155 was shown to crosslink to pre-mRNA at the branch site in the A complex (27). Direct interaction between SAP155 and U2AF was also reported (31). U2AF consists of subunits of 65 and 35 kDa in humans, and is required for association of U2 snRNP with the branch site in pre-mRNA.

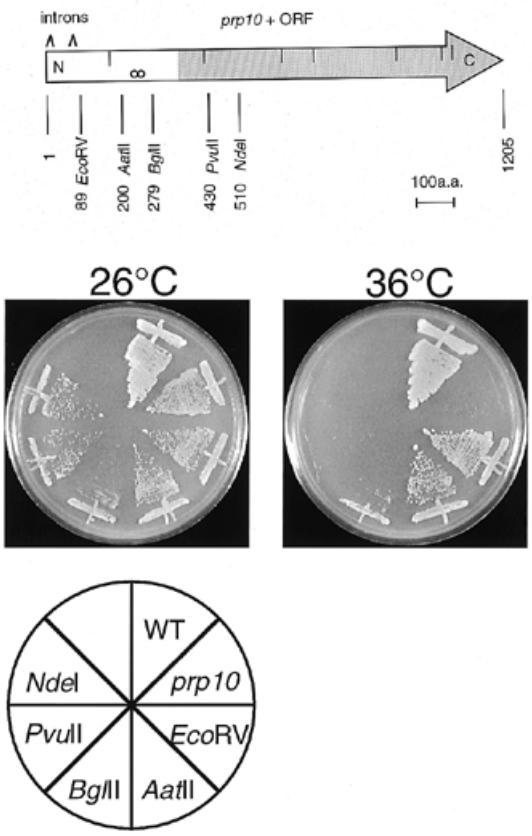

Figure 5. The N-terminal region of Prp10p is not essential for growth. The plasmid containing each truncated *prp10* gene was introduced into YH01 strain. The transformants were streaked on MMA plates and then tested for their temperature sensitivity. Wild type cells, 972, were used as a positive control and UR230 cells were used as a negative control. The plate was incubated for 7 days at 26°C, or 6 days at 36°C. The shaded area in the *prp10*$^+$ ORF indicates the region containing PP2A-like repeats conserved amoung species. Vertical bars and open circles indicate TP dipeptide motifs and RWDETP motifs, respectively.

In *S.pombe*, the *prp2*$^+$ gene encodes a large subunit of U2AF, spU2AF^{59}, which is an *S.pombe* homologue of human U2AF^{65} (10). To examine if Prp10p interacts with Prp2p/spU2AF^{59} in *S.pombe* like SAP155, we generated double mutants carrying *prp2* and *prp10* mutations and examined synthetic effects. There are three alleles of the *prp2* mutation, *prp2-1* (6), *prp2-2* (8) and *mis11-453* (49). We constructed double mutants with all combinations between *prp10-1* and each of these three alleles. We also made *prp1 prp10* and *prp3 prp10* double mutants. The *prp1*$^+$ gene encodes a protein homologous with *S.cerevisiae* PRP6, a component of U4/U6 snRNP (15). The results are shown in Figure 7. Both *prp2-2* and *prp10-1* could proliferate at 26°C, but *prp2-2 prp10-1* double mutant was unable to grow at 26°C. In contrast, *prp1-4 prp10-1* and *prp3-3 prp10-1* double mutants grew at 26°C. Such synthetic lethality of *prp2-2* with *prp10-1* at 26°C suggests that Prp10p is also interacting with Prp2p/spU2AF^{59} *in S.pombe*. The *prp2-1 prp10-1* and *mis11-453 prp10-1* double mutants did not show a strong synthetic effect.

DISCUSSION

We have cloned the *prp10*$^+$ gene of *S.pombe*. This gene codes for proteins of ~1200 amino acids. Prp10p is a putative homologue

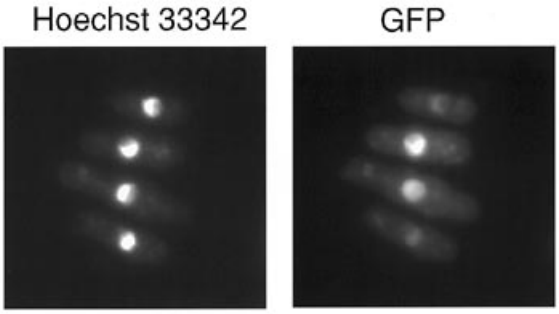

Figure 6. Prp10p protein is present in the DNA region of the nucleus. The plasmid containing the GFP–Prp10p fusion gene was introduced into SU59-1D strain. Cells were cultured at 26°C, and stained with Hoechst 33342. About 10–20% of the cells showed bright signals in the nucleus, particularly in the DNA region of the nucleus (two center cells in this figure). Weak GFP signals were detected in the DNA region in other cells (top and bottom cells).

of human splicing factor SAP155 (28). SAP155 is the largest subunit of the splicing factor complex SF3 that consists of seven subunits (3). The cDNAs for all subunits of SF3, except for SAP130 were cloned in humans, and their homologues were identified in budding yeast (20–29). Prp10p/SAP155 shows the highest conservation in amino acid sequence among the SF3 subunits, suggesting that Prp10p/SAP155 plays a central role in the function of SF3.

Northern blot analysis showed accumulation of pre-mRNA in *prp10* at the non-permissive temperature. No accumulation of intermediate products was apparent (Fig. 2), suggesting that *prp10* has a defect in the first step of the splicing reaction. This is compatible with the finding that Prp10p/SAP155 is required for recognition of the branch site by the U2 snRNP (27,28).

An unexpected finding is that the *prp10*$^+$ gene generates four kinds of alternatively spliced products, in addition to the unspliced mRNA (Fig. 4). Of those, forms C and D for which the proximal 3′ splice site in the second intron was used in the splicing reaction, will produce short polypeptides, which seem to be non-functional. On the other hand, forms A, B and E will generate Prp10p of 1205, 1188 and 1166 amino acids in length, respectively. The deletion experiments showed that the N-terminal region of Prp10p, in which there is a phylogenetic variation, is dispensable for complementing activity of the temperature-sensitive phenotype of *prp10-1*. This result means that alternative splicing related to the two introns near the N-terminus has no evident influence on the function of Prp10p. On the other hand, mutation sites were found near the C-terminus in the corresponding gene of *prp10-1* mutant, indicating functional importance of a C-terminal region. Therefore, all three forms A, B and E probably have complementing activities for *prp10-1*. Whether or not these three proteins encoded by the alternative splicing products have distinct biological functions remains to be investigated.

As the GFP–Prp10p fusion protein is localized in the DNA region of the nucleus, Prp10p may function there. Subnuclear localization of splicing factors has been extensively studied in mammalian cells. In the mammalian nucleus, splicing factors such as SC35 and ASF/SF2 are localized in 20–40 distinct domains called speckles (50). Those splicing factors are recruited to transcription sites when the transcripts have introns (51,52). Some splicing factors, such as U2AF and U1 snRNP, were found not only in speckles but also diffusely in the nucleus (53).

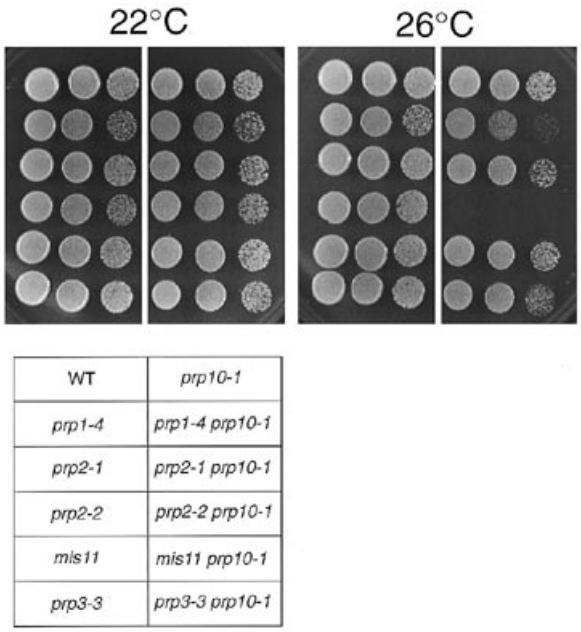

Figure 7. The *prp2-2 prp10-1* double mutant shows a synthetic effect on viability. Each strain was suspended in water, adjusted to 2×10^6 cells/ml and diluted twice, 10 times each. Dilutions (4.5 µl) were spotted on a YPD plate to contain ~9000, 900 or 90 cells in each spot. The plate was incubated for 7 days at 22°C or for 5 days at 26°C.

Schmidt-Zachmann *et al*. (30) reported recently that a *X.laevis* homologue of the Prp10p co-localized with Sm proteins and SF3a[66], with a speckled pattern. In yeasts, there are contradictory results on the localization of factors involved in splicing. Potashkin *et al*. (54) found that snRNAs are enriched in the so-called nucleolar region. In contrast, non-snRNP splicing factor PRP6 was localized in the DNA region of the *S.cerevisiae* nucleus (55). Prp10p was localized uniformly in the DNA region, and no speckled pattern was observed in the distribution of Prp10p. Localization of splicing factors seems to differ between higher eukaryotes and yeasts.

We found genetic interaction between *prp2* and *prp10*, suggesting that Prp10p interacts with Prp2p/spU2AF[59] in *S.pombe*. This finding supports the notion that Prp10p is a functional homologue of human SAP155. Gozani *et al*. (31) showed that the N-terminal region in SAP155 containing the RWDETP motif (amino acids 267–369) and the C-terminal region in human U2AF[65] containing the third RRM (amino acids 334–475) are responsible for the interaction with each other. Unexpectedly, *prp2-1 prp10-1* and *mis11-453 prp10-1* double mutants showed only a weak synthetic effect, although *prp2-2 prp10-1* double mutant exhibited an apparent synthetic lethal effect at 26°C. The mutation site in *prp2-2* is located in the first RRM (amino acids 259), whereas those in *prp2-1* (10) and *mis11-453* are out of the RRM (amino acids 387 and 307, respectively; Y.Habara *et al*., unpublished data). This allele-specific synthetic effect indicates that the first RRM in Prp2p might play an important role in the interaction with Prp10p, in addition to its role in the recognition of RNA in *S.pombe*. In contrast, the corresponding RRM in human U2AF[65] is dispensable for the interaction with SAP155 in the yeast two-hybrid assay (31). In human U2AF[65], the third RRM and its flanking sequence are essential for the interaction with SAP155 (31). We do not know if the third RRM in Prp2p/spU2AF[59] is also essential. Determination of the Prp10p interacting domain in Prp2p/spU2AF[59] will be necessary to clarify the discrepancy in the RRM requirement between human U2AF[65] and Prp2p/spU2AF[59].

SAP155 was found to associate with cyclin E-cdk2 *in vivo* (32) and to be phosphorylated concomitant with the splicing reaction (28). The N-terminal region of SAP155 contains a cluster of the TP dipeptide motifs, possible phosphorylation sites for cdk (32), and a domain required for the interaction with U2AF described above (31). It has been proposed that phosphorylation of the TP dipeptide motifs in the N-terminus of SAP155 disrupts SAP155–U2AF interaction, leading to the replacement of U2AF with U5 snRNP on the 3′ splice site (28). Therefore, the N-terminal region of SAP155 is assumed to play important roles in the association with U2AF and in the regulation of splicing reactions in mammalian cells. In contrast, deletion of the N-terminal region in Prp10p (1–279) corresponding to the U2AF[65] interaction domain of SAP155 resulted in partial rescue of the temperature-sensitive phenotype of *prp10-1*, suggesting that this N-terminal region of Prp10p is not essential for its function in yeast. Although human SAP155 interacts with both the large and small subunits of U2AF, *S.pombe* Prp10p interacts with only a large subunit of Prp2p/spU2AF[59] (31). In *S.pombe*, a direct interaction between Prp10p and Prp2p/spU2AF[59] might be dispensable for recruitment of U2 snRNP to the branch point sequence, or the U2AF interaction region might not be localized to the N-terminal region in Prp10p. It is noteworthy that the U2AF binding site, a polypyrimidine tract at the 3′ splice site, is less conserved in yeasts and that the U2AF homologue MUD2 in *S.cerevisiae* is dispensable as pointed out by Abovich *et al*. (56). Mechanisms for the recognition of the branch site in yeast might be slightly different from those functioning in mammals. Further investigation of Prp10p is expected to provide an insight into regulatory mechanisms of branch site recognition in yeast.

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