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Isolation of an essential *Schizosaccharomyces pombe* gene, *prp31*$^+$, that links splicing and meiosis

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ABSTRACT

We carried out a screen for mutants that arrest prior to premeiotic S phase. One of the strains we isolated contains a temperature-sensitive allele mutation in the fission yeast *prp31*$^+$ gene. The *prp31-E1* mutant is defective in vegetative cell growth and in meiotic progression. It is synthetically lethal with *prp6* and displays a pre-mRNA splicing defect at the restrictive temperature. We cloned the wild-type gene by complementation of the temperature-sensitive mutant phenotype. Prp31p is closely related to human and budding yeast PRP31 homologs and is likely to function as a general splicing factor in both vegetative growth and sexual differentiation.

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* enters a sexual differentiation cycle in response to nutrient limitation (reviewed in 1,2). Cells of the opposite mating type conjugate with one another to form a transient diploid, which then proceeds directly to meiosis and sporulation. Meiotic progression in the fission yeast is regulated by a number of specific factors, most importantly the Mei2 protein. This RNA-binding protein is essential for the initiation of meiotic S phase and the subsequent meiotic divisions (3). Its activity is mediated by association with a small RNA molecule called meiRNA, encoded by the *sme2*$^+$ gene (3). Mei2p is inhibited by the Pat1p protein kinase (4). During nitrogen starvation, expression of *mei2*$^+$ is strongly induced (5). If the cells are diploid (determined by the presence of both mating pheromones), the *mei3*$^+$ gene is also induced (6). Mei3p then inhibits Pat1p repression of Mei2p and the cells proceed into meiosis (2,7). However, if Pat1p is inactivated by a thermosensitive mutation in vegetative cells, cells enter meiosis inappropriately due to activation of Mei2p (7,8). This ectopic meiosis is independent of ploidy or nutrient conditions. Strikingly, the cells complete two abnormal meiotic divisions and form spores even from the haploid state, although the spores are inviable. *pat1*-induced meiosis has been used extensively to model the events of normal sexual development (9–12).

Meiotic progression also requires many vegetative cell cycle genes, including the p34^{cdc2} kinase (9,13). This is not unexpected, since many of the events of meiosis are similar to those in the mitotic cycle. Surprisingly, however, we have found that meiotic DNA replication occurs in the absence of several vegetative initiation proteins, including the MCM proteins and Cdc18p (14). We therefore carried out a screen for mutants defective in initiation of meiotic DNA replication. Our screen also isolated mutants required early in meiosis. In this paper we describe isolation of one such mutant and cloning of the cognate gene.

The fission yeast *prp31-E1* mutant is temperature-sensitive for growth in vegetative cells, arresting with a *cdc*-like phenotype. A number of other *prp* (pre-mRNA processing) mutants identified in fission yeast also exhibit cell cycle defects (15–17). *prp31*$^+$ encodes the fission yeast homolog of *Saccharomyces cerevisiae* PRP31, which is involved in assembly of the spliceosome by recruiting the U4/U6×U5 tri-snRNP to prespliceosome complexes (18). We show that Prp31p is essential for normal mRNA splicing. Although this mutant was isolated in a screen for meiotic factors, our data suggest that *prp31*$^+$ is a general splicing factor required throughout the fission yeast life cycle.

MATERIALS AND METHODS

Strains and media

The *S.pombe* strains used in this study are listed in Table 1. Yeast were grown in YES medium or Edinburgh minimal medium (EMM) supplemented with adenine, leucine and uracil as required (19). Strains were constructed using standard genetic techniques (19).

Mutagenesis and screen

The *pat1-114* strain FY903 was plated on YES at a density of ~2000 cells/plate and mutagenized with 100 J/m^2 UV in a Stratalinker to obtain a 50% survival rate. Plates were incubated in the dark at 25°C for 5 days to allow survivors to form colonies and replica-plated onto EMM plates. Plates were incubated overnight at 34°C to inactivate *pat1-114* and induce a haploid meiosis. Cells were exposed to iodine vapor to determine sporulation efficiency, revealed by dark staining. We identified 114 mutant strains with reduced iodine staining out of ~20 000 colonies sampled. Cell morphology of the mutants was observed by microscopy and 46 strains which contained no visible spores were identified. These strains were tested for temperature sensitivity on YES at 34°C and reconfirmed for

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Table 1. Strains used in this study

Strain	Genotype	Source
FY254	*h⁻ can1-1 leu1-32 ade6-M210 ura4-D18*	(36)
FY255	*h⁺ can1-1 leu1-32 ade6-M210 ura4-D18*	(36)
FY261	*h⁺ can1-1 leu1-32 ade6-M216 ura4-D18*	(36)
FY527	*h⁻ his3-D1 leu1-32 ade6-M216 ura4-D18*	(26)
FY528	*h⁺ his3-D1 leu1-32 ade6-M210 ura4-D18*	(26)
FY903	*h⁺ pat1-114 Δcdc19*::[*cdc19HA leu1⁺*] *ura4-D18 leu1-32 ade6-M210*	(14)
FY1138	*h⁻ prp31-E1 ura4-D18 leu1-32 ade6-M210*	This study
FY1150	*h⁺ cdc5-120*	KGY162
FY1162	*h⁺ pat1-114 cdc5-120 ura4-D18 ade6-M210*	This study
FY1182	*h⁺ prp31-E1 ura4-D18 leu1-32 ade6-M216*	This study
FY1183	*h⁻ prp31-E1*::[*prp31⁺ura4⁺*] *leu1-32 ade6-M210*	This study
FY1184	*h⁻ ura4-D18 leu1-32 ade6-M210 his3-D1 prp31⁺*	
	h⁺ ura4-D18 leu1-32 ade6-M216 his3-D1 Δprp31::*his3⁺*	This study
FY1185	*h⁺ pat1-114 prp31-E1 ura4-D18 ade6-M210*	This study
KGY246	*h⁻ leu1-32 ura4-D18 ade6-M210*	P. Nurse
KGY2457	*h⁻ prp2-1 leu1-32*	(33)
KGY2457	*h⁻ prp6-1 leu1-32*	(33)

reduced iodine staining. A meiotic time course was performed (described below) and samples were collected for FACS analysis to analyze DNA content. Cells fixed for flow cytometry and rehydrated in 50 mM sodium citrate were stained with DAPI to assess nuclear divisions. Single gene mutations were isolated by backcrossing to the parent strain and performing a random spore analysis.

Physiological analysis (time courses)

For vegetative time courses, cultures were grown in EMM supplemented with uracil, leucine and adenine overnight at 25°C to mid-exponential phase (OD_{600} ~0.4). The cultures were split and incubated at 25 and 36°C and sampled every 2 h for FACS and DAPI staining. Samples were harvested by centrifugation and fixed for flow cytometry as described (20), except that we stained the cells with 1 μM Sytox Green (Molecular Probes). Data were collected on a Becton Dickinson FACScan and analyzed using Cell Quest software for the Macintosh.

For meiotic time courses, haploid strains containing the *pat1-114* allele were grown to mid-log phase in EMM with uracil, leucine and adenine, then starved overnight in EMM lacking nitrogen to arrest in G_1. Cultures were re-fed with an equal volume of preheated EMM-N supplemented with 1 g/l NH_4Cl and 70 μg/ml required supplements at 36°C to release into meiosis. For meiotic time courses in diploids, appropriate strains were starved overnight in EMM without nitrogen and glucose to arrest in G_2. We added glycerol to 1% and glucose to 0.1% to induce meiosis.

Cloning of *prp31⁺*

Yeast strain FY1138 containing the *prp31-E1* mutation was transformed with a pUR19 genomic library (21). Cells were plated onto EMM supplemented with uracil, adenine and leucine at 25°C for 48 h to allow phenotypic expression, then shifted to 36°C to select for complementation. Plasmids were isolated from the ts⁺ candidates, transformed by electroporation into *Escherichia coli* and prepared for restriction analysis (22). All clones isolated contained overlapping restriction fragments representing two different plasmids. Approximately 700 nt at either end of the smallest plasmid (pDB8) were sequenced using plasmid primers (Salk Institute Sequencing Facility), which revealed two open reading frames (ORFs) (Fig. 3A). We subcloned each ORF. pDB2, containing ORF1 (the *prp31⁺* gene), was generated by cleaving out a 2.2 kb *Sph*I fragment from pDB8. pDB3, containing ORF2, was constructed by cleaving out a 2.3 kb *Spe*I–*Bam*HI fragment. Each subclone was transformed into FY1138 to determine which ORF was responsible for complementing activity.

A 2.4 kb *Ssp*I–*Sac*I fragment from pDB2 containing the *prp31* gene was subcloned into the 2.9 kb *Eco*RV–*Sac*I fragment of pBluescript II SK(+) to generate pDB4. A 2.4 *Sac*I–*Kpn*I fragment from this construct was subcloned into pJK210 (23) to generate *prp31-E1*::[*prp31⁺ura4⁺*]. This new construct (pDB7) was linearized with *Pst*I and transformed into FY1138 to create FY1183. Integration at the *prp31-E1* locus was verified by crossing against a wild-type strain and verifying that no temperature-sensitive segregants were recovered.

prp31 disruption

The Δ*prp31*::*his3⁺* disruption construct was generated by digesting pDB4 with *Eco*RI to release a 0.9 kb fragment within the ORF of *prp31*. A *Bgl*II linker was added following an end filling reaction and the *his3⁺* cassette from pAF1 (24) was inserted, creating pDB6. The disruption cassette was excised with *Kpn*I and *Sac*I and transformed into a diploid strain

(FY527/FY528) to create FY1184. Transformants were then sporulated and analyzed by tetrad dissection and random spore analysis.

Spore germination

We analyzed strain FY1184 (Δ*prp31-E1*::*his3*$^+$/*prp31*$^+$ *his3-D1*/*his3-D1*) and a diploid heterozygous at the *his3* locus (*his3*$^+$/*his3-D1*) by spore germination (25,26). Spores were washed and partially purified by centrifugation through a 25% glycerol cushion. For the spore germination time course, spores were inoculated into EMM plus adenine, leucine and uracil to a final concentration of 1×10^8 spores/ml. Cultures were grown, with shaking, at 32°C. Samples were collected at 0, 8 and 26 h for FACS analysis and nuclear counts as previously described.

Splicing assays

Total RNA was isolated as described (19). Aliquots of 15 µg of total RNA were separated using formaldehyde–agarose gel electrophoresis (27), transferred to Duralon-UV™ membranes (Stratagene, La Jolla, CA) by capillary action and UV crosslinked by exposure to a Stratalinker™ UV source (Stratagene). Oligonucleotides were ^{32}P-labeled by the StarFire method (Integrated DNA Technologies) according to the manufacturer's directions and used to probe for precursor (TF2D intron, 5′-CTTGTTCCCTATCCAAAGAGGCACG-ACxxxxxx-3′) or mature (TF2D exon, 5′-GGAGTCATC-CTCGGATTTGCCACCCxxxxxx-3′) *tf2d*$^+$ RNA. The filter was prehybridized in 10 ml of Perfecthyb™ hybridization buffer (Sigma, St Louis, MO) and hybridized overnight at 60°C in 10 ml of Perfecthyb™ hybridization buffer with 10^7 c.p.m. of StarFire labeled probe (10^6 c.p.m./ml). The filter was washed at 25°C for 10 min twice in 2× SSC (0.3 M NaCl, 0.03 M Na citrate), 0.5% SDS and twice in 0.5× SSC, 0.5% SDS. To detect *his3*$^+$ RNAs, a 484 bp fragment was excised from pKG594 (28) using the restriction endonucleases *Eco*RV and *Bam*HI. This DNA fragment was labeled with ^{32}P using the Prime It II kit (Stratagene) according to the manufacturer's instructions. The filter was prehybridized in 10 ml of ExpressHyb™ hybridization buffer (Clontech) and hybridized for 1 h at 68°C in 10 ml of ExpressHyb™ hybridization buffer with 10^7 c.p.m. of probe (10^6 c.p.m./ml). Filters were washed twice at 50°C for 10 min in 2× SSC, 0.5% SDS. After exposure, the filters were visualized on a PhosphoImager™ with ImageQuant™ v.3.3 software (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Isolation of mutants defective in meiotic S phase

Recently, we found that several vegetative DNA replication initiation proteins are not required for meiotic progression (14). Therefore, we designed a screen for mutants that block premeiotic S phase to identify any meiosis-specific factors. Wild-type cells that complete meiosis and sporulation are stained dark brown by iodine vapor. Colonies that do not complete meiosis or sporulation have reduced iodine staining. The Pat1p kinase represses meiotic progression; the temperature-sensitive *pat1-114* mutation allows even haploid cells to enter meiosis at 34°C, where they complete sporulation and stain dark with iodine (8). Therefore, we employed a *pat1-114* mutant to induce meiosis in response to a shift in temperature. We mutagenized *pat1* mutant cells by UV treatment (see Materials and Methods) and isolated colonies that showed reduced iodine staining at the restrictive temperature. These iodine-minus mutants were further screened for those that maintained temperature sensitivity, to ensure that the *pat1* lesion was not suppressed, and by FACS analysis, to determine whether they entered meiotic S phase. We isolated four mutants that were iodine minus, still temperature sensitive and blocked meiotic progression prior to bulk DNA synthesis. This paper will discuss the first of the four mutants, which we designate *prp31-E1* for reasons that will be explained below.

We characterized the meiotic defect of *prp31-E1* by carrying out a meiotic time course. We arrested *pat1-114 prp31-E1* cells in the G_1 phase of the cell cycle by nitrogen starvation, then released them directly to meiosis by re-feeding and shifting to the restrictive temperature. This method is specific for meiotic phenotypes and is unaffected by any vegetative phenotype of the gene under study. We harvested and fixed samples at each time point. For each time point we determined DNA content by FACS analysis and monitored progression through both meiotic divisions by counting the number of nuclei per cell. The parental *pat1-114* strain usually completes DNA replication after 4 h at the restrictive temperature; it completes the second meiotic division with 60% efficiency at the end of 8 h, as determined by the fraction of cells with three or more nuclei (Fig. 1A–C). The *pat1-114 prp31-E1* mutant did not undergo normal meiotic DNA replication and arrested with a single nucleus, indicating a block prior to meiotic S phase (Fig. 1A–C).

To verify that these results reflected a role for *prp31* in meiosis and were not an artifact of inducing meiosis using the *pat1* mutant, we constructed a homozygous *prp31* mutant diploid using complementation of the *ade6-M210* and *ade6-M216* markers (19). The cells were arrested in G_2 of the vegetative cell cycle by starvation for glucose and nitrogen, then released to meiosis at 34°C by addition of glucose and glycerol (29). Again, this protocol separates vegetative effects from meiotic requirements by inducing meiosis at the same time as the *prp31* mutant protein is inactivated. Completion of meiosis was monitored by counting the percentage of asci formed after a day long incubation at restrictive versus permissive temperatures (Fig. 1D). Whereas the wild-type diploid was able to complete meiosis efficiently at both temperatures (ratio ≥1), the mutant diploid was severely defective at the restrictive temperature. We conclude that *prp31* is required for normal meiotic progression.

prp31-E1 has a vegetative phenotype and encodes a putative splicing factor

To analyze the phenotype of *prp31-E1* in vegetative cells, we crossed the *pat1 prp31-E1* strain to wild-type and dissected tetrads to segregate it from the *pat1* mutation. We found that *prp31-E1* in a wild-type background was temperature sensitive for growth at 36°C (Fig. 2A). FACS analysis showed that *prp31-E1* arrests with a 2C DNA content and somewhat heterogeneously elongated cells (Fig. 2B and C). This indicates that *prp31* is essential for growth in vegetative cells and suggests that it may have a cell cycle defect. There was no evidence for a specific defect in S phase in these asynchronous cultures.

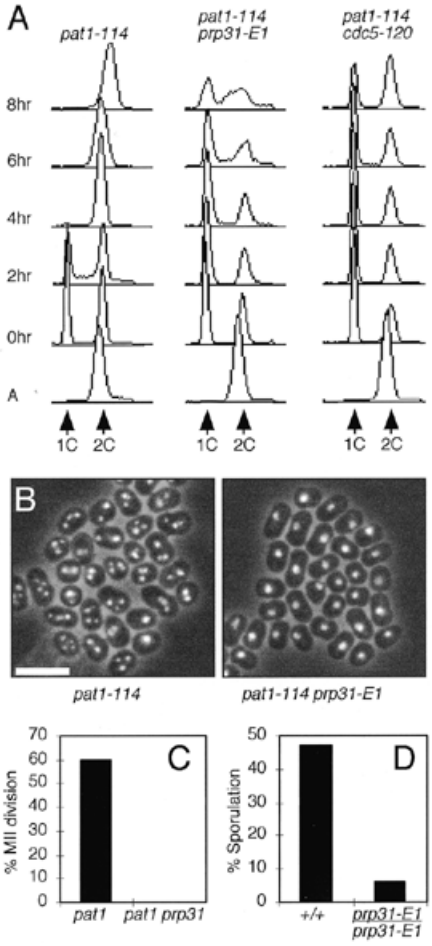

Figure 1. *prp31-E1* is defective in meiotic S phase. (**A**) *pat1-114*, *pat1-114 prp31-E1* and *pat1-114 cdc5-120* strains were arrested in G_1 and released to meiosis as described in Materials and Methods. Samples were harvested every 2 h for flow cytometry. Positions of 1C (unreplicated) and 2C (replicated) DNA content are indicated. A, asynchronous. (**B**) Nuclear morphology of cells in (A) after 8 h. Cells were stained with DAPI to visualize nuclei. Cells with two nuclei have completed the first meiotic division and cells with more than three nuclei have completed both meiotic divisions. Bar, 10 µm. (**C**) The percentage of cells in (B) that completed the second meiotic (MII) division after 8 h was determined by counting cells with more than three nuclei. (**D**) Percentage of diploid cells completing sporulation was determined by counting asci under phase contrast microscopy, following release of diploid strains to meiosis as described in Materials and Methods.

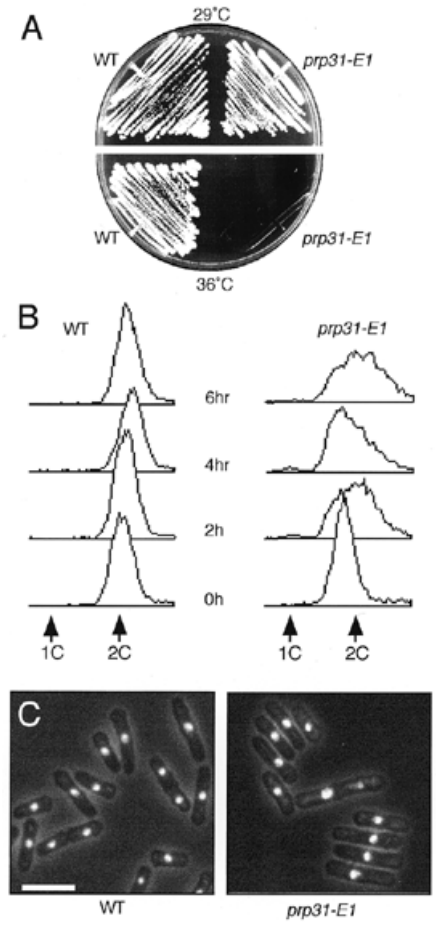

Figure 2. *prp31-E1* has a vegetative phenotype. (**A**) Wild-type (FY261) and *prp31-E1* strains were streaked onto YES plates and incubated for 3 days at 29 and 36°C. (**B**) Wild-type and *prp31-E1* strains were grown overnight at the permissive temperature of 25°C and shifted to 36°C as described in Materials and Methods. Samples were harvested every 2 h for flow cytometry. Positions of 1C (unreplicated) and 2C (replicated) DNA content are indicated. (**C**) Nuclear morphology of cells in (B) after 6 h. Cells were stained with DAPI to visualize the nuclei. Bar, 10 µm.

We cloned the *prp31*$^+$ gene by complementation of the temperature-sensitive phenotype. We transformed the mutant *prp31-E1* strain with a genomic library and isolated clones that supported growth at 36°C (see Materials and Methods). Two independent plasmids with overlapping fragments were isolated. The smallest fragment was partially sequenced and compared to the fission yeast genome database BLAST server (http://www.sanger.ac.uk/Projects/S_pombe/). This revealed two ORFs on the insert (Fig. 3A). These were separately subcloned and each subclone was transformed back into the original *prp31-E1* mutant and tested for complementation at 36°C. Only the subclone containing the *prp31*$^+$ ORF complemented the mutant.

We verified that the sequence cloned corresponded to the mutant locus by targeting a plasmid containing *prp31*$^+$ and *ura4*$^+$ for integration at *prp31-E1*. The resulting strain *prp31-E1*::[*prp31*$^+$*ura4*$^+$] was no longer temperature sensitive. When crossed against the wild-type, no temperature-sensitive segregants were observed. When crossed against *prp31-E1*, 50% of the segregants were wild-type for growth and all these were *ura4*$^+$. This indicates that the cloned sequence is tightly linked to the original mutant allele.

The sequence of this ORF was used for a GenBank BLAST search (http://www.ncbi.nlm.nih.gov/Genbank/) and found to be homologous to a number of sequences, including proteins from humans and *Arabidopsis* and the *S.cerevisiae* gene *PRP31*, encoding a pre-mRNA splicing factor (Fig. 4). *PRP31* is required for assembly of the spliceosome, specifically in recruitment of the U4/U6×U5 tri-snRNP to prespliceosome complexes (18). Interestingly, fission yeast Prp31p showed

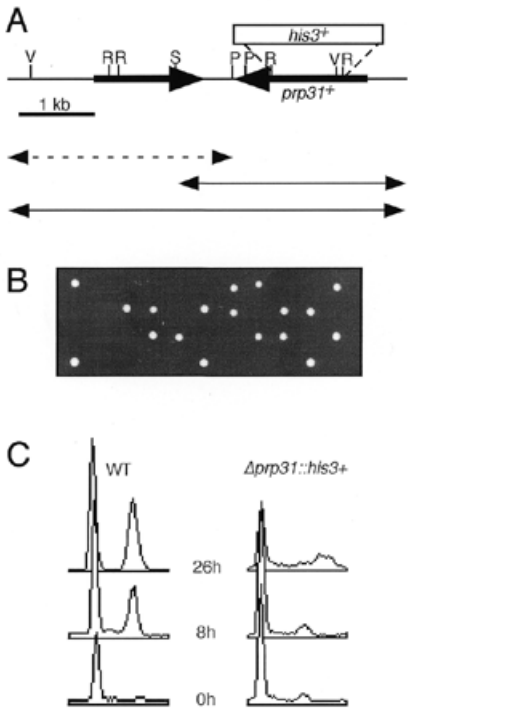

Figure 3. Analysis of the *prp31*$^+$ locus and disruption phenotype. (**A**) Schematic of the genomic clone containing the *prp31*$^+$ gene. Double-headed arrows represent subclones that were transformed into the *prp31-E1* mutant strain FY1138 to test for complementation activity scored by the ability to form colonies at 36°C. Continuous lines, subclones that were able to complement at 36°C; broken line, subclone that failed to complement at 36°C. The box above the line indicates the location of the disruption/deletion with the *his3*$^+$ marker. V, *Eco*RV; R, *Eco*RI; S, *Sph*I; P, *Spe*I. (**B**) Tetrads dissected from the diploid *prp31*::*his3*$^+$/*prp31*$^+$ were grown on YES at 32°C for 2 days. All growing spore clones were His$^-$ (data not shown). (**C**) Flow cytometry of germinating wild-type (WT) and Δ*prp31*::*his3*$^+$. Samples were taken at 0, 8 and 26 h from cells growing in selective medium.

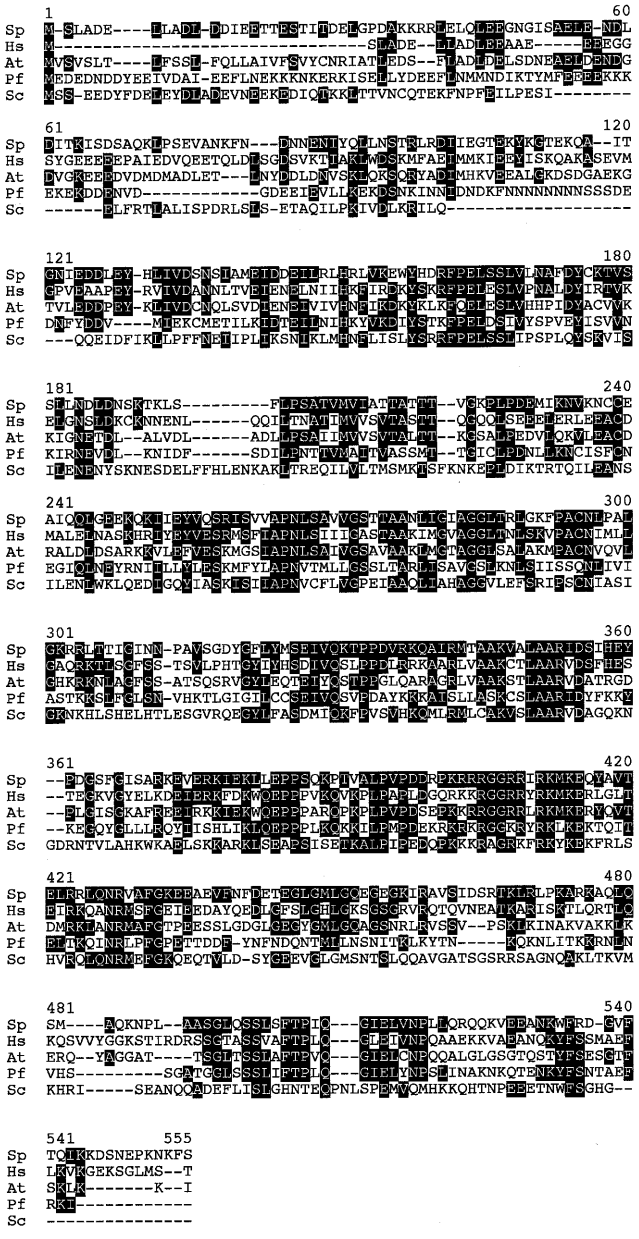

Figure 4. Alignment of *prp31*$^+$-related protein sequences. Sequences were aligned using MEGALINE by DNASTAR. Identical residues are shown in white text with a black background. Sp, *S.pombe* (accession no. CAA17928); Hs, human (accession no. CAB43677); At, *Arabidopsis thaliana* (accession no. 3249066); Pf, *Plasmodium falciparum* (accession no. CAB62868); Sc, *S.cerevisiae* (accession no. S64386).

significant similarity (34%) across the entire sequence to both the human and *Arabidopsis* genes, while sharing a smaller core of homology (28%) with the budding yeast homolog. Based on this comparison, we named our fission yeast gene *prp31*$^+$.

A number of *prp* mutants with potential cell cycle defects have been previously characterized (16,30). These mutants affect mRNA processing and often express *cdc*-like phenotypes. We tested for genetic interactions between *prp31-E1* and alleles of previously identified *prp* genes, *prp1-prp7* and *prp11*, as well as *cdc5* (16,24). *prp31-E1* showed a significant genetic interaction with only one mutant that was tested, *prp6-1*. In this case, the two mutations were synthetically lethal. Of the 12 tetrads examined, two were parental ditypes, one was a non-parental ditype and nine were tetratypes. Although the identity of *prp6*$^+$ has not yet been determined, these data indicate that Prp6p and Prp31p may be involved in the same biochemical step of pre-mRNA splicing. *prp6*$^+$ may also be involved in spliceosome assembly or may interact physically with *prp31*$^+$. Importantly, these data also indicate that *prp31*$^+$ is not allelic to other *prp* mutants already defined in fission yeast.

prp31$^+$ is essential

We determined the phenotype of cells lacking *prp31*$^+$ by constructing a deletion/disruption. We replaced the majority of the *prp31*$^+$ ORF with a fragment containing *his3*$^+$ (Fig. 3A; Materials and Methods). A linear fragment containing this construct was integrated into a diploid strain and His$^+$ transformants were isolated. Upon sporulation, we found that no viable His$^+$ haploids were recovered. Upon tetrad dissection,

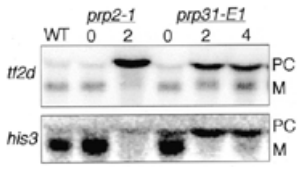

Figure 5. *prp31*$^+$ is required for pre-mRNA splicing *in vivo*. RNA was prepared from wild-type (WT), *prp2-1* cells at either the permissive temperature of 25°C (0) or after 2 h at the restrictive temperature of 36°C (2) and *prp31-E1* cells at the permissive temperature of 25°C (0) or after 2 (2) and 4 (4) h at the restrictive temperature of 36°C. Northern blots were probed for the intron-containing RNAs, *tf2d*$^+$ and *his3*$^+$. Precursor RNAs (PC) and the mature messages (M) are indicated.

we isolated eight complete tetrads which showed 2:2 segregation of viable:inviable spores (Fig. 3B); all viable spores were His$^-$. We transformed the parent diploid with a plasmid containing the cloned *prp31*$^+$ and tested whether the plasmid could rescue the lethal phenotype. Random spore analysis of this strain showed that His$^+$ segregants were recovered if they also contained the *prp31*$^+$*ura4*$^+$ plasmid. Microscopic analysis revealed that spores which did not form colonies underwent one division.

We analyzed the phenotype of the Δ*prp31* spores using a spore germination procedure. We prepared spores from the Δ*prp31*/*prp31*$^+$ diploid and inoculated them into medium lacking histidine. Under these conditions, only the spores containing the gene disruption are able to germinate. We sampled this preparation for DNA content, by FACS, and cell morphology (Fig. 3C). The Δ*prp31* cells have a mixed phenotype, with a large number of elongated cells. A fraction of the cells produce a 2C DNA content, although this appears to be far less than half.

Prp31p is involved in pre-mRNA splicing

To investigate the role of *prp31*$^+$ in pre-mRNA splicing, we determined whether *prp31-E1* mutant cells accumulate unspliced messages. We prepared total RNA from *prp31-E1* grown at the permissive temperature or at the restrictive temperature for 2 and 4 h. Total RNA prepared from the known pre-mRNA splicing mutant *prp2-1* and wild-type cells served as controls. We probed northern blots for the intron-containing transcripts *tf2d*$^+$ and *his3*$^+$. In both cases, we observed an accumulation of unspliced precursor RNA and a reduction in mature transcript in the *prp31-E1* mutant cells at the restrictive temperature (Fig. 5). These data indicate that *S.pombe prp31*$^+$ function is required for pre-mRNA splicing *in vivo*.

Other splicing factors are also required for meiosis

The phenotype of *prp31-E1* suggests that there may be a general requirement for mRNA splicing factors in meiotic progression as well as in the vegetative cell cycle. We tested this by analyzing the requirement for *cdc5*$^+$, which encodes a Myb-related splicing factor (24,31,32). *cdc5ts* mutants also show a strong cell cycle phenotype (24). We constructed a *pat1-114 cdc5-120* double mutant strain and carried out the same analysis done previously, using FACS analysis to monitor meiotic S phase and visual inspection to determine meiotic divisions. Similarly to *prp31-E1* in the *pat1-114* background, the *cdc5-120* mutation blocked meiotic DNA replication and meiotic divisions at a high temperature (Fig. 1A), suggesting that there is a general requirement for mRNA splicing in meiosis.

DISCUSSION

In this report, we describe a new mutant isolated in a screen for *S.pombe* mutants that block an early stage of meiotic progression. We cloned the corresponding gene *prp31*$^+$, which encodes a protein homologous to proteins in *S.cerevisiae* (PRP31), human, *Arabidopsis* and *Plasmodium*. Budding yeast *PRP31* has been shown to be involved in assembly of the spliceosome by recruiting the U4/U6×U5 tri-snRNP to prespliceosome complexes (18). *Schizosaccharomyces pombe prp31-E1* cells are defective in normal vegetative growth and *prp31-E1* is synthetically lethal in combination with *prp6*, another presumed splicing factor. The *prp31-E1* mutant shows a splicing defect in vegetative fission yeast cells comparable to that of *prp2-1* (33). Both *prp2* and *prp6* were isolated from a screen for temperature-sensitive mutants exhibiting pre-mRNA splicing defects (16,33).

prp31$^+$ is essential for viability of vegetative cells and Δ*prp31*::*his3*$^+$ results in a *cdc*-like arrest with limited accumulation of DNA. In meiosis, *prp31* blocks cells prior to completion of S phase. Similar effects on meiotic progression were observed for another splicing mutant, *cdc5*. We conclude that other general splicing factors are likely to be required early in meiosis. However, *prp31-E1* does not show an S phase defect in vegetative cells when asynchronously growing cells are shifted to the restricted temperature. Therefore we conclude that its arrest prior to meiotic S phase and the S phase defects of the disruption merely reflect the assay used. We anticipate that *prp31*$^+$ probably affects multiple targets throughout the cell cycle in both vegetative and meiotic cells.

Interestingly, there is evidence that fission yeast undergoes meiosis-specific splicing of the *mes1*$^+$ gene, required for the MII division (34). However, because the arrest point of *prp31-E1* is significantly earlier in meiosis than that of *mes1* and because *prp31*$^+$ is required for vegetative growth as well, it is probable that Prp31p is required for splicing of other meiotic substrates. Whether the general splicing apparatus is also required for *mes1*$^+$ splicing remains to be determined. Meiosis-specific splicing has also been reported for budding yeast and does require general splicing genes (35). The interplay of general splicing factors with meiosis-specific elements may provide a general method of regulating differentiation.

What are the likely targets of the splicing apparatus in meiosis? A number of vegetative genes are required for premeiotic DNA synthesis (9,14); in addition, there are meiosis-specific factors (reviewed in 1). Recognizable introns have been identified in several genes required for meiotic S phase, including *res2*$^+$ (transcription factor; accession no. D1776), *cdc17*$^+$ (DNA ligase; accession no. X05107), *pol1*$^+$ (catalytic subunit of DNA polymerase α; accession no. X69673), *cdc22*$^+$ (large subunit of ribonucleotide reductase; accession no. X65116), *rpa1*$^+$ (large subunit of RPA; accession no. U75446) and *pol3*$^+$ (catalytic subunit of DNA polymerase δ; accession no. X59278). Mutations in *res2* and *cdc22* in particular block cells prior to meiotic S phase and bulk DNA

synthesis (9,13). These introns may explain the requirement for general splicing factors early in the meiotic pathway. It is likely that genes required later in meiosis will also require the activity of the cellular splicing machinery, which is thus ubiquitously required in the life cycle of fission yeast.

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