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Six novel genes necessary for pre-mRNA splicing in *Saccharomyces cerevisiae*

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ABSTRACT

We have identified six new genes whose products are necessary for the splicing of nuclear pre-mRNA in the yeast *Saccharomyces cerevisiae*. A collection of 426 temperature-sensitive yeast strains was generated by EMS mutagenesis. These mutants were screened for pre-mRNA splicing defects by an RNA gel blot assay, using the intron-containing *CRY1* and *ACT1* genes as hybridization probes. We identified 20 temperature-sensitive mutants defective in pre-mRNA splicing. Twelve appear to be allelic to the previously identified *prp2, prp3, prp6, prp16/prp23, prp18, prp19* or *prp26* mutations that cause defects in spliceosome assembly or the first or second step of splicing. One is allelic to *SNR14* encoding U4 snRNA. Six new complementation groups, *prp29–prp34*, were identified. Each of these mutants accumulates unspliced pre-mRNA at 37°C and thus is blocked in spliceosome assembly or early steps of pre-mRNA splicing before the first cleavage and ligation reaction. The *prp29* mutation is suppressed by multicopy *PRP2* and displays incomplete patterns of complementation with *prp2* alleles, suggesting that the *PRP29* gene product may interact with that of *PRP2*. There are now at least 42 different gene products, including the five spliceosomal snRNAs and 37 different proteins that are necessary for pre-mRNA splicing in *Saccharomyces cerevisiae*. However, the number of yeast genes identifiable by this approach has not yet been exhausted.

INTRODUCTION

Pre-mRNA splicing proceeds by assembly of splicing factors on pre-mRNA molecules to form spliceosomes, followed by intron excision and exon joining. These complicated processes require numerous *trans*-acting protein factors and snRNAs (reviewed in 1–4). The *cis*-acting sequences within pre-mRNAs required for splicing have been studied in detail (reviewed in 3 and 5), as have the roles of the U1, U2, U4, U5 and U6 snRNAs (3,6). Protein factors necessary for splicing have been identified by their capacity to bind to pre-mRNA or by fractionation and reconstitution of active extracts from mammalian cells (reviewed in 3). snRNP particles have been purified and their protein constituents identified (7). Mammalian spliceosomes purified by affinity chromatography using biotinylated pre-mRNA contain at least 30 distinct proteins, including some proteins that had previously been identified (8).

The power of genetic techniques in *Saccharomyces cerevisiae* provides a tool orthogonal to biochemical approaches to identify splicing factors and to study their functions. Since spliceosome assembly and the *trans*-esterification reactions of splicing occur by similar steps in yeast and metazoans, results obtained from the study of yeast splicing factors are of general relevance. The first identification of yeast protein splicing factors emerged from analysis of temperature-sensitive mutants defective in RNA synthesis (9,10). It was subsequently established that the primary defect in these *rna* mutants is lack of splicing of nuclear pre-mRNAs (11,12, reviewed in 13). Thus these genes were renamed *prp* (pre-mRNA processing) mutants, *prp2–prp10/11* (*prp10* and *prp11* are identical). Cloning of the *PRP2–PRP11* genes and subsequent characterization of *PRP* gene products and *prp* mutant extracts revealed that these Prp proteins are directly involved in splicing (reviewed in 1).

Additional yeast splicing factors have been discovered by searching for pseudorevertants or multicopy suppressors of mutations in either *PRP* genes or introns, or for mutations synthetically lethal with mutations in genes encoding small nuclear RNAs, or by identification of sequences in the genome homologous to metazoan splicing factors (reviewed in 1,2,4). Identification of mammalian homologues for several of these yeast proteins confirms that the structure or function of these splicing factors is conserved across species (14–19).

Analysis of the *prp2–prp11* mutants led to the identification of *bona fide* splicing factors. However, the search for *prp* mutants clearly was not saturated. Therefore, several laboratories initiated additional screens of banks of temperature- or cold-sensitive mutants for those defective in pre-mRNA splicing. The *prp17–28, prp38–39* and *brr1–brr5* mutants were identified in *S. cerevisiae* and *prp1–3* in *S.pombe* (20–24; S. Noble and C. Guthrie, personal communication). We have carried out a similar

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Table 1. Yeast strains used in this study

Strain	Relevant Genotype	Background Genotype	Source
DS94	wildtype	*MATα his3-11,15 leu2-3,112 lys2 lys1 trp1Δ ura3-52*	E. Craig
JWY70	wildtype	*MATα his3-11,15 cry2::LEU2 leu2-3,112 lys2 lys1 trp1Δ ura3-52*	Derived from DS94 by disruption of *CRY2*
DBY1034	wildtype	*MATa his4-519 lys2-801 ura3-52* Gal$^+$	D. Botstein
JWY627	*prp2-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY630	*prp3-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY633	*prp4-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY636	*prp5-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY638	*prp6-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY639	*prp7-2*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY640	*prp8-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY643	*prp9-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY644	*prp11-1*	*MATa ade1 ade2 gal1 his7 lys2 tyr1 ura1*	A. Lustig
JWY792	*prp17*	*MATa ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY793	*prp17*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY794	*prp18*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY795	*prp18*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY796	*prp19*	*MATα ade2-101 his3-Δ200 leu2 lys2-801 ura3-52*	J. Abelson
JWY797	*prp19*	*MATa ade2-101 his3-Δ200 leu2 lys2-801 ura3-52*	J. Abelson
JWY798	*prp20*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY799	*prp20*	*MATα ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY800	*prp21*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY801	*prp21*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY802	*prp22*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY803	*prp22*	*MATα ade2-101 his3-Δ200 lys2-80 ura3-52*	J. Abelson
JWY804	*prp23*	*MATα ade2-101 his3-Δ200 lys2-801 tyr1 ura3-52*	J. Abelson
JWY805	*prp23*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY806	*prp24*	*MATa ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY807	*prp24*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY808	*prp25*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY809	*prp25*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY810	*prp26*	*MATa ade2-101 his3-Δ200 tyr1 ura3-52*	J. Abelson
JWY811	*prp27*	*MATa ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY812	*prp27*	*MATα ade2-101 his3-Δ200 lys2-801 ura3-52*	J. Abelson
JWY780	*prp29-1*	*MATa his4-519 ura3-53 lys2-801*	Ts$^-$ spore from hs140 x DBY1034
JWY781	*prp30-1*	*MATa his4-519 ura3-52 lys2-801*	Ts$^-$ spore from hs283 x DBY1034
JWY771	*prp31-1*	*MATa his4-519 ura3-52 lys2-801 trp1Δ*	Ts$^-$ spore from hs29 x DBY1034
JWY777	*prp32-1*	*MATa his4-519 ura3-52 lys2 leu2-3,112*	Ts$^-$ spore from hs337 x DBY1034
JWY778	*prp33-1*	*MATa his4-519 ura3-52 lys2*	Ts$^-$ spore from hs68 x DBY1034
JWY786	*prp34-1*	*MATa his4-519 ura3-52 lys2*	Ts$^-$ spore from hs87 x DBY1034

screen and identified temperature-sensitive mutants defective in pre-mRNA splicing that define six new genes, *PRP29–PRP34*.

MATERIALS AND METHODS

Strains, media and plasmids

JWY yeast strains were derived from strain A364A or from strains congenic to A364A. These and other yeast strains used in this study are described in Table 1. Yeast strains were grown in YEPD or defined synthetic medium lacking appropriate supplements as described in (25). Diploids were sporulated on 1.5% agar medium containing either 0.1% dextrose, 0.25% yeast extract and 1.5% potassium acetate or 0.8% nutrient broth, 1% yeast extract and 1% potassium acetate. Plasmids containing *PRP2*, *PRP3*, *SPP2* or *CRY1* were previously described (26–28). Plasmid pYACT1 (29) was kindly provided by John Abelson.

Genetic procedures

Methods for yeast mating, sporulation and tetrad analysis are described in (30). The temperature-sensitive mutants were scored by lack of growth on YEPD medium at 32 or 37°C. To determine complementation patterns for *prp* mutants, diploids produced by pair-wise mating with other putative splicing mutants or with *prp2–prp11*, *prp17–prp27* and *prp38–prp39* strains were tested for growth on YEPD at 37°C. Complementation with *prp38* and *prp39* (20–21) was tested by Brian Rymond (personal communication). In some cases, noncomplementing diploids were sporulated and tetrad progeny examined for segregation of temperature sensitivity. Complementation between splicing-defective mutants that were not temperature sensitive for splicing defects was tested by RNA blotting. Yeast cells were transformed with DNA by the spheroplast method (31) or by the LiAc protocol of ref. 32.

Isolation of temperature-sensitive mutants

Wild-type yeast strain JWY70 was mutagenized with ethylmethane sulfonate (EMS), as described in (33). Single colonies were patched onto YEPD plates and replica-plated to YEPD, and the plates incubated at 13, 23, 30, 32 or 37°C. Patches that grew slowly or failed to grow at 13 or 37°C were retained and frozen in 15% glycerol to create collections of cold-sensitive and heat-sensitive mutants, respectively.

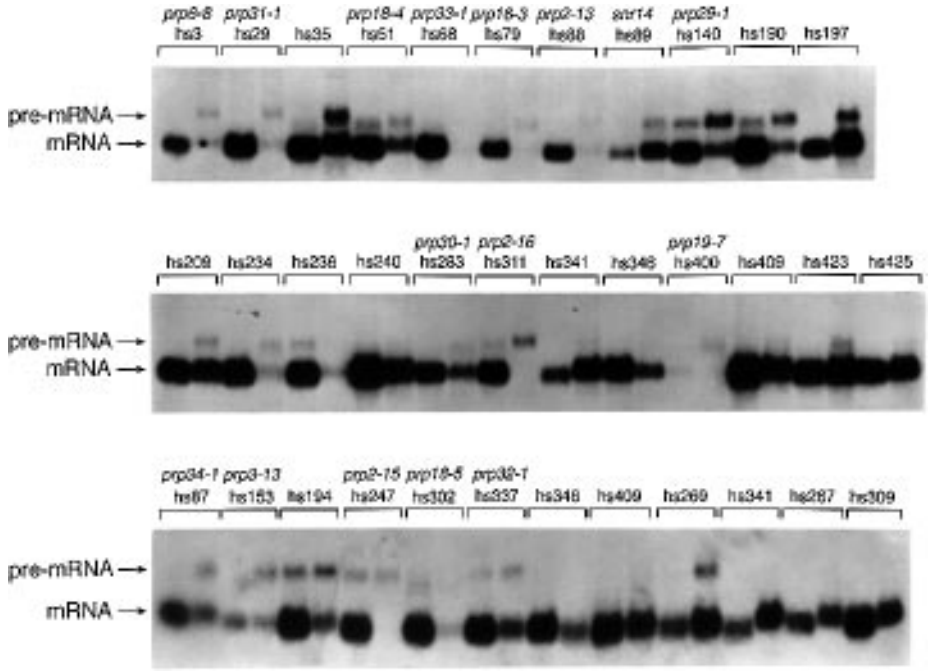

Figure 1. Identification of splicing mutants by RNA blot hybridization. Mutants were grown to mid-logarithmic phase at 23°C; half of each culture was maintained at 23°C, and half shifted to 37°C for 1–2 h. RNA was extracted from each culture, resolved by agarose gel electrophoresis, blotted to Nytran and hybridized with the intron-containing *CRY1* DNA labeled with ^{32}P. Each hs mutant strain is indicated by a bracket above two lanes. Mutants containing alleles of previously identified *PRP* genes or novel *PRP* mutants are designated with *PRP*. A mutant containing an allele of *SNR14* encoding U4 snRNA is also indicated. Those mutants not labeled with *PRP* are not defective in splicing or were subsequently discarded for other reasons. For each pair of lanes, RNA from cells grown at 23°C is on the left and that from cells shifted to 37°C is on the right. The positions of unspliced *CRY1* pre-mRNA and spliced *CRY1* mRNA are indicated. The differences in absolute amounts of RNA among samples most likely reflect differential yield of RNA, assayed by measuring ethidium bromide stained rRNA.

Isolation of RNA

RNA was isolated from yeast by a modification of the method of ref. 34. A 3 ml culture of cells was grown in YEPD at 23°C to early log phase (1–2 × 10^7 cells/ml). For temperature shift experiments, half of each culture was shifted to 37°C for 1–2 h, prior to extraction of RNA. Cells were pelleted by centrifugation, suspended in 0.2 ml Kirby salts (35), and lysed by addition of 0.2 ml Kirby phenol plus one half volume glass beads and subsequent vortexing. Organic and aqueous phases were separated by centrifugation and the aqueous layer removed to a new Eppendorf tube. The organic phase was extracted again with Kirby salts, and the two aqueous phases pooled and extracted once more with phenol. The RNA was precipitated by ethanol overnight at –20°C, collected by centrifugation in an Eppendorf microcentrifuge, washed in 70% ethanol, dried *in vacuo*, and suspended in 30 µl H_2O.

Gel electrophoresis, blotting and hybridization of RNAs

Total RNA (~5 µg) from each sample was subjected to gel electrophoresis and RNA blot analysis as described in ref. 26. Two intron-containing yeast genes, *CRY1* and *ACT1*, were used as hybridization probes. The 2.2 kb *Hin*dIII genomic DNA fragment containing *CRY1* (26) and the 2.2 kb *Eco*RI–*Hin*dIII genomic DNA fragment containing *ACT1* (29) were purified from plasmids pCRY1 and pYACT1, respectively, by restriction enzyme digestion, gel electrophoresis and extraction with Gene Clean (Bio 101). These DNA probes were radioactively labeled with ^{32}P by the random oligonucleotide primer method (36).

RESULTS

Isolation of temperature-sensitive lethal mutants

Fourteen pools of wild-type yeast strain JWY70 were mutagenized with EMS under conditions that resulted in 50–80% killing. Approximately 20 000 independent colonies were picked and screened for acquisition of heat sensitivity (slow growth or no growth at 32 or 37°C) or cold sensitivity (slow or no growth at 13°C). The wild-type parental strain grew at all of these temperatures. 426 heat-sensitive (Ts^-) mutants (hs1–hs426) and 194 cold-sensitive (Cs^-) mutants (cs1–cs194) were identified among the different batches of mutagenized cells. Because the mutagenized populations of cells were allowed to recover for 2 h after mutagenesis, any two mutants within one pool could contain identical mutations.

Identification of temperature sensitive mutants defective for pre-mRNA splicing

We screened the temperature-sensitive mutants for defects in pre-mRNA processing by an RNA blot hybridization assay using the *CRY1* or *ACT1* genes as probes. *CRY1* and *ACT1* each contain a single intron and encode abundant transcripts that are efficiently spliced in wild-type cells (26,37). Unspliced *CRY1* or *ACT1* pre-mRNAs are readily detected by Northern blot assays of RNA extracted from the *prp2–prp11* mutants grown at 23°C and

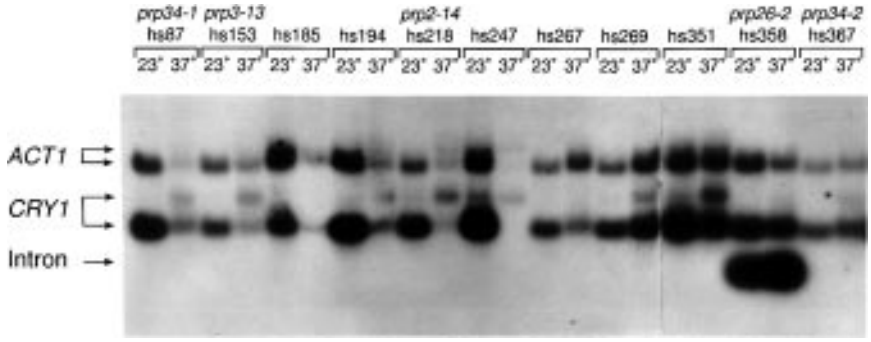

Figure 2. Assaying splicing defects using both *CRY1* and *ACT1* probes. Cells were grown and RNA prepared and assayed as described in Figure 1. Mutant strains and *CRY1* and *ACT1* pre-mRNA and mRNA are indicated.

shifted to 37°C for 1–2 h (5,12,26). To expedite screening of the 426 hs mutants, we first assayed only *CRY1* RNA from cultures grown at 23°C to mid-log phase and from cells shifted to 37°C for 1–2 h, using a rapid protocol for extracting RNA from small volumes of culture. RNA blot data from a representative subset of the 426 mutants screened are shown in Figure 1. Clearly, unspliced pre-mRNA or lariat intermediate accumulated and mRNA was diminished in many of the mutants shifted to 37°C. In some cases, defects in splicing were less clear, due to underloading or loading differences between 23 and 37°C samples, e.g. hs 68 or hs283. Other gels of these mutants clearly demonstrate a splicing defect (data not shown). Those mutants in which *CRY1* mRNA splicing was defective at 37°C (Fig. 1) were rescreened, using both *ACT1* and *CRY1* DNAs to probe RNA from cells grown at 23°C and from cells shifted to 37°C (Fig. 2). Probes containing two different mosaic genes were used to increase the likelihood of detecting mutants defective in the splicing machinery rather than mutants in which the splicing of one transcript is specifically blocked (38,39). Mutants exhibiting no splicing phenotypes in either screen were discarded, e.g. hs185, hs267, hs309, hs341, hs346, hs409 and hs425.

Of the 426 hs mutants screened, three phenotypic classes were observed in which splicing of both *CRY1* and *ACT1* mRNA was defective compared to wild-type cells. Most of these mutants accumulated *CRY1* or *ACT1* RNA that co-migrated on agarose gels with unspliced *CRY1* or *ACT1* pre-mRNA. Two mutants (hs51 and hs302) accumulated *ACT1* or *CRY1* RNA with electrophoretic mobilities intermediate to those of pre-mRNA and mRNA (Fig. 1). Primer extension analysis using oligonucleotides complementary to the 3′ exon and to the 5′ end of the intron of *ACT1* and *CRY1* demonstrated that these RNAs were intron-lariat 3′ exon splicing intermediates (data not shown). Mutant hs358 contained wild-type amounts of mRNA and no pre-mRNA after 1–2 h at 37°C, but accumulated a faster-migrating RNA species that was shown by primer extension using oligonucleotides complementary to the *CRY1* and *ACT1* introns to be the spliced intron-lariat product (Fig. 2 and data not shown).

The ratio of unspliced pre-mRNA to spliced mRNA varied considerably in mutants shifted to 37°C for 2 h. Although many of the mutants that accumulated pre-mRNA at 37°C contained diminished amounts of mRNA compared to cells grown at 23°C, several mutants contained near wild-type levels of spliced mRNA, e.g. hs35, hs197, hs209 and hs269 (Fig. 1). These may be particularly leaky mutants that are only partially defective in

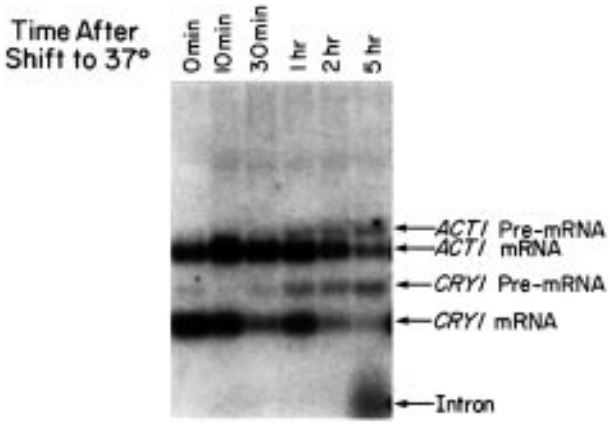

Figure 3. Time course of accumulation of unspliced *CRY1* and *ACT1* pre-mRNA and diminution of spliced *CRY1* and *ACT1* mRNA in the hs337 (*prp32*) mutant. The hs337 (*prp32*) strain was grown to mid-log phase at 23°C and shifted to 37°C. At the indicated times, aliquots of cells were removed from the culture, and RNA was extracted, subjected to electrophoresis, blotted to Nytran and hybridized with ^{32}P-labeled *CRY1* and *ACT1* DNAs. The positions of the *CRY1* and *ACT1* unspliced pre-mRNAs, spliced mRNAs and intron-lariat products are indicated.

mRNA splicing. Upon testing some of these mutants, we observed that the splicing defect was exaggerated upon prolonged incubation at the nonpermissive temperature. One example (hs337) is shown in Figure 3. For this mutant, we also observed accumulation of intron lariat at later times after shift, suggesting a second defect in intron turnover or spliceosome disassembly. Several of the temperature-sensitive mutants that grew well at 23°C nevertheless exhibited modest defects in splicing at 23°C as well as a more severe splicing defect at 37°C. This phenotype is shown in Figure 1 for mutants hs51, hs140, hs190, hs194, hs236, hs247, hs302 and hs311.

Genetic analysis

Each of the Ts$^-$ mutants defective in pre-mRNA splicing was mated to a wild type strain DBY1034 to create diploids. Each of these diploids heterozygous for hs mutations grew well at 37°C and was wild type for pre-mRNA splicing, indicating that each mutation is recessive. The resulting diploids were sporulated, and tetrads analyzed. Temperature sensitivity and the splicing defects

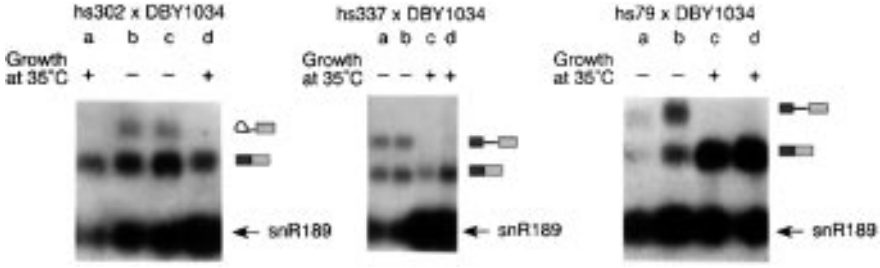

Figure 4. Co-segregation of pre-mRNA splicing defects and temperature sensitivity of the hs mutants in a cross to wild-type yeast. Ts⁻ mutants hs302, (*prp18*), hs337 (*prp32*) and hs79 (*prp16*) were mated to wild-type strain DBY1034, diploids were sporulated, and tetrads dissected. RNA was extracted from cultures of each spore clone grown to mid-log phase at 23°C and shifted to 35°C for 2 h, and assayed for splicing defects by RNA blotting and hybridization with ^{32}P-labeled *CRY1* DNA. Unspliced *CRY1* pre-mRNA and spliced *CRY1* mRNA are indicated, as is the snRNA snR189 that was used as a loading control.

each segregated 2^+:2^- and co-segregated, indicating that the Ts⁻and splicing defects are, in each case, due to a mutation in a single nuclear gene. Results for crosses of strains hs79, hs302 and hs337 to wild-type are shown in Figure 4.

To determine whether the hs mutants contain mutations in novel *PRP* genes or are new alleles of previously identified *PRP* loci, complementation tests were performed (Tables 2 and 3). In most cases where noncomplementation was observed, the diploids were sporulated and tetrads were analyzed to determine whether the noncomplementing mutations were linked. The five mutants hs283, hs29, hs337, hs68 and hs89 complemented each other, all of the other hs mutants, and the previously identified *prp* mutants *prp2–prp11*, *prp16–prp24*, *prp27* and *prp38–prp39*. hs87 and hs367 complemented all other mutants, but failed to complement each other. Crosses indicate hs87 and hs367 contain tightly linked or identical mutations conferring a Ts⁻ phenotype. We conclude that each of the hs283, hs29, hs337, hs68 and hs87/hs367 strains contains a mutation in a single nuclear gene and defines one of five new genes necessary for pre-mRNA splicing, which we designate *prp30–prp34*, respectively. A Northern blot showing the splicing defect of each of these mutants shifted from 23 to 37°C for 2 h is shown in Figure 5.

Table 2. Complementation tests between hs mutants and *prp* mutants[1]

MATα hs strains	MATa prp mutants																			
	2	3	4	5	6	7	8	9	11[2]	17	18	19	20	21	22	23[3]	24	27	38	39
3	+	+	+	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
29	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
51	+	+	+	+	+	nd	+	+	+	+	–	nd	+	+	+	+	+	+	+	+
68	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
79	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+
87	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
88	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
89	+	+	+	+	+	+	+	+	+	nd	+	+	+	+	nd	+	+	+	+	+
140	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
153	+	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
218	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
247	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
283	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
302	+	+	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
311	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
337	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
367	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
400	+	+	+	+	+	+	+	+	+	+	nd	–	+	+	+	+	+	+	+	+
420	+	–	+	+	+	nd	+	+	+	+	+	nd	+	+	+	+	+	+	+	+

[1]Diploids were produced by pairwise matings between the original *MAT*α hs strains and *MATa prp* mutants, and tested for growth on YEPD medium at 37°C. nd, diploids were not obtained for every pairwise combination due to inefficient mating. In particular, *prp7* and *prp19* mutants mated poorly.
[2]*prp10* = *prp11*.
[3]*prp23* = *prp16*.

Table 3. Complementation tests between hs mutants defective in pre-mRNA splicing

MATα hs mutants	*MATa* hs mutants											
	3	29	68	79	87	89	153	283	302	337	140	311
3	–	+	+	+	+	+	+	+	+	+	+	+
29	+	–	+	+	+	+	+	+	+	+	+	+
68	+	+	–	+	+	+	+	+	+	+	+	+
79	+	+	+	–	+	+	+	+	+	+	+	+
87	+	+	+	+	–	+	+	+	+	+	+	+
89	+	+	+	+	+	–	+	+	+	+	+	+
153	+	+	+	+	+	+	–	+	+	+	+	+
283	+	+	+	+	+	+	+	–	+	+	+	+
302	+	+	+	+	+	+	+	+	–	+	+	+
337	+	+	+	+	+	+	+	+	+	–	+	+
400	+	+	+	+	+	+	+	+	+	+	+	+
88	+	+	+	+	+	+	+	+	+	+	±	–
140	+	+	+	+	+	+	+	+	+	+	–	±
218	+	+	+	+	+	+	+	+	+	+	–/+	–
247	+	+	+	+	+	+	+	+	+	+	–	–
311	+	+	+	+	+	+	+	+	+	+	–/+	–

Pairwise matings were between hs mutants of opposite mating types, derived by backcrossing to wild type strain DBY1034. Resulting diploids were tested for growth on YEPD medium at 37°C.

The wild-type gene corresponding to the hs89 mutant allele was cloned by transformation and complementation of the Ts⁻ phenotype. We confirmed that the cloned DNA originated from the same genomic locus as the Ts⁻ mutation by integrating the cloned DNA at its homologous locus and analyzing linkage of the integrated *URA3* marker to the Ts⁻ phenotype (40, data not shown). The smallest complementing subclone derived from the original plasmid contains a 0.9 kb *Cla*I–*Eco*RV fragment bearing the *SNR14* gene encoding U4 snRNA (41). Thus hs89 may contain a Ts⁻ allele of U4 snRNA. Consistent with this finding, we identified the *SNR6* gene encoding U6 snRNA as a multicopy suppressor of hs89 (40).

The temperature-sensitive phenotype of the hs88, hs218, hs247 and hs311 strains was complemented in crosses to all of the other hs mutants and to *prp3–prp11*, *prp16–prp24*, *prp27* and *prp38–prp39* mutants, but was not complemented in crosses to *prp2* mutants (Tables 2 and 3). In addition, hs311 failed to complement hs88, hs218 or hs247 (Table 3). The temperature sensitivity of these four hs mutants was specifically complemented upon transformation with YCp50-borne *PRP2* (28) and was efficiently suppressed by introduction of one or a few extra copies of *SPP2*, a gene dosage suppressor of *prp2* (42). Analysis of crosses of each of these hs mutants to a *prp2* strain indicated that each mutation conferring a Ts⁻ phenotype was tightly linked to *prp2*. We conclude that hs88, hs218, hs247 and hs311 contain Ts⁻ alleles of *prp2*.

Crosses of hs140 to all of the *prp* mutants except *prp2* revealed efficient complementation of the Ts⁻ phenotype at 37°C. Complementation between hs140 and *prp2* was more complex; hs140/*prp2* diploids did not grow as well at 37°C as wild-type controls or diploids formed between hs140 and other *prp* mutants. To determine whether the weak complementation between hs140 and *prp2* was specific to certain alleles of *prp2*, seven other previously identified *prp2* isolates (9) as well as hs88, hs218, hs247 and hs311 were mated to hs140. Some combinations of hs140/*prp2* diploids grew well at 37°C while others did not (43; Tables 2 and 3). Analysis of the meiotic segregation of Ts⁻ phenotypes of hs140 and several different *prp2* isolates indicated that the mutation in hs140 conferring a Ts⁻ phenotype is linked to *prp2* but is in a different gene. Fourteen parental ditype, one nonparental ditype and six tetratype tetrads were recovered from 21 four-spored tetrads, indicating that the mutation in hs140 is 29 cM from *prp2*. The Ts⁻ phenotype of hs140 was efficiently suppressed at 37°C upon transformation with Ycp50-borne *PRP2*. However, extra copies of *SPP2*, a multicopy suppressor of *prp2* (27,42), did not suppress the Ts⁻ phenotype of hs140. We conclude that hs140 contains a mutation in a novel gene, which we designate *prp29*, that fails to efficiently complement the Ts⁻ phenotype of some alleles of *prp2*.

We observed complementation between mutants hs153 or hs420 and all of the other hs mutants and *prp* mutants except *prp3* (Tables 2 and 3). The Ts⁻ phenotype of hs153 was complemented by transformation of YCp50 containing *PRP3* (28); hs420 was not tested. Thus hs153 contains a mutation most likely allelic to *prp3*, and hs420 may as well. hs3 was identified as a possible allele of *prp6* by both complementation tests and molecular cloning. hs51 and hs302 specifically did not complement *prp18*, and hs400 did not complement *prp19*. hs79 failed to complement *prp16* and *prp23*, which are identical to each other (44).

Both the intron-accumulation and the temperature-sensitive phenotypes of mutant hs358 segregated $2^+:2^-$ in crosses to wild-type, but did not cosegregate (data not shown). Thus a nonlethal mutation caused accumulation of spliced intron RNA in strain hs358 and was unlinked to the temperature-sensitive mutation in that strain. For this reason, complementation tests were done

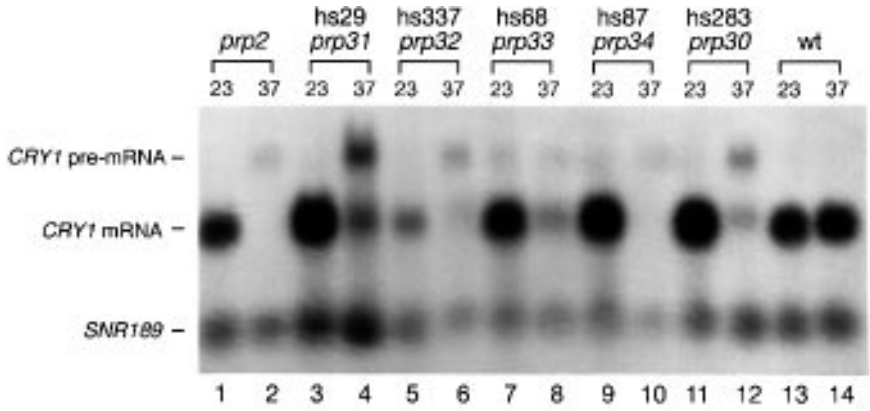

Figure 5. Each of the temperature-sensitive mutants *prp30–prp34* accumulates unspliced pre-mRNA when shifted to the nonpermissive temperature for 2 h. Strains hs283 (*prp30*), hs29 (*prp31*), hs337 (*prp32*), hs68 (*prp33*), hs87 (*prp34*), as well as wild type and *prp2* strains were grown to mid-log phase at 23°C. Half of each culture was shifted to 37°C for 2 h. RNA was extracted from each, subjected to electrophoresis, blotted and probed with ^{32}P-labeled *CRY1* DNA. *CRY1* pre-mRNA and mRNA, as well as snR189 used as a loading control, are indicated.

between hs358 and the intron-accumulating *prp25* and *prp26* mutants by assaying phenotypes of the diploids on RNA blots. Mutant hs358 failed to complement *prp26* but did complement *prp25*. Linkage analysis will be necessary to distinguish whether hs358 is an allele of *prp26* or exhibits unlinked noncomplementation with *prp26*.

DISCUSSION

Screening collections of conditional lethal yeast mutants for those defective in pre-mRNA splicing continues to yield novel genes. It is straightforward and efficient: 3–5% of the Ts$^-$ mutants that have been screened in five independent collections are defective in splicing (10,20,22,24, this work). Most (but not all) yeast splicing factors thus far identified are essential, as one might expect. Thus screens of conditional lethal mutants could yield many but not all potential yeast splicing factor genes. Some genes may not be mutable to temperature or cold-sensitivity (45,46), or might be so small that the probability of identifying mutant alleles is low. However, we did recover in our screen a mutant allele of *SNR14* encoding the 160 nucleotide long U4 snRNA.

Among the mutants we identified, 12 are apparently allelic to previously identified *prp* mutants, one is allelic to *SNR14* encoding U4 snRNA, and seven are novel (Table 4). As observed in previous screens, more alleles of *prp2* were found than for any other gene (10,24; Table 4). The continued isolation of additional complementation groups and of only one or two isolates of each suggests that these screens are not exhausted for Ts$^-$ splicing-defective mutants.

Some mutants may be only indirectly affected in splicing; e.g. the expression, post-translational modification, or intracellular localization of splicing factors may be deficient, rather than their function. Analysis of splicing extracts derived from the Ts$^-$ mutants identifies those gene products directly involved in splicing. Like most of the previously identified Ts$^-$ *prp* mutants, extracts prepared from *prp31* or *prp33* mutants can be specifically heat-inactivated *in vitro*, indicating that Prp31p and Prp33p are likely to be directly involved in splicing (50; J. Roy, unpublished). Cloning and sequencing of *PRP31* and *PRP33* has verified that they are novel splicing factors (50; V. Lay, J. Roy, J. Woolford and J. Friesen, manuscript in preparation).

Table 4. hs mutants that correspond to previously identified *prp* mutants

prp2	hs88	*prp2-13*
	hs218	*prp2-14*
	hs247	*prp2-15*
	hs311	*prp2-16*
prp3	hs153	*prp3-13*
	hs420	*prp3-14*
prp6	hs3	*prp6-8*
prp16	hs79	*prp16-3*
prp18	hs51	*prp18-4*
	hs302	*prp18-5*
prp19	hs400	*prp19-7*
prp26	hs358	*prp26-2*

The majority of mutants we identified, including all those defining novel genes, accumulate unspliced pre-mRNA at the nonpermissive temperature. These mutants may be blocked either in different steps of spliceosome assembly or in splicing functions prior to the first cleavage and ligation reaction. Mutants blocked in both early and late steps of splicing might also accumulate pre-mRNA. Two mutants we identified, hs51 and hs302, are specifically blocked in the second step of splicing and contain alleles of *prp18*. The hs358 mutant is blocked in intron turnover and may contain a mutant allele of *prp26*. One potential class of splicing mutants not unambiguously distinguishable by our RNA blot assay are those that fail to produce spliced mRNA, yet do not accumulate unspliced pre-mRNA or splicing intermediates. Such might occur if splicing were blocked at any early step prior to assembly of pre-mRNA with any splicing factors, such that unspliced pre-mRNA were degraded. Alternatively, pre-mRNA or splicing intermediates might be degraded in mutants in which splicing complexes form but are disassembled.

Two results suggest that *prp29* (or its product) may interact with *PRP2*: (i) diploids formed between the *prp29* mutant (hs140) and some *prp2* strains grow poorly at 37°C. Noncomplementation of nonallelic genes is in some cases correlated with functional

or physical interactions between the gene products (47–48). (ii) Extra copies of *PRP2* suppress the Ts⁻ phenotype of *prp29*. The *spp2*⁻ mutation is also suppressed by extra copies of *PRP2* (27). We have recently shown that Spp2p interacts with Prp2p and is necessary for association of Prp2p with the spliceosome (42). Identification of other molecules that interact with Prp2p is of interest; Prp2p is an RNA-dependent ATPase thought to be responsible for causing important changes in the active site of the spliceosome prior to the first catalytic reaction of splicing (49).

A wide array of splicing factors has been identified by genetic screens in yeast, including snRNP proteins stably associated with the snRNAs and spliceosomes, and proteins only transiently or weakly associated with spliceosomes or snRNPs (reviewed in 1). Many of these proteins might have been difficult to identify by conventional biochemical approaches but can now be analyzed using the mutant strains. Likewise, a different set of splicing factors has been identified by fractionation of mammalian splicing extracts. Just as the similarities of the basic mechanisms of pre-mRNA splicing in yeast and metazoans are becoming more apparent, so too are the two sets of splicing proteins beginning to converge as more homologues are identified. Nevertheless, new factors continue to be discovered.

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