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Synthetic Lethal Mutations Suggest Interactions between U5 Small Nuclear RNA and Four Proteins Required for the Second Step of Splicing

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To investigate the function of the U5 small nuclear ribonucleoprotein (snRNP) in pre-mRNA splicing, we have screened for factors that genetically interact with *Saccharomyces cerevisiae* U5 snRNA. We isolated *trans*-acting mutations that exacerbate the phenotypes of conditional alleles of the U5 snRNA and named these genes *SLU*, for synergistically lethal with U5 snRNA. *SLU1* and *SLU2* are essential for the first catalytic step of splicing, while *SLU7* and *SLU4* (an allele of *PRP17* [U. Vijayraghavan, M. Company, and J. Abelson, Genes Dev. 3:1206–1216, 1989]) are required only for the second step of splicing. Furthermore, *slu4-1* and *slu7-1* are lethal in combination with mutations in *PRP16* and *PRP18*, which also function in the second step, but not with mutations in factors required for the first catalytic step, such as *PRP8* and *PRP4*. We infer from these data that *SLU4*, *SLU7*, *PRP18*, *PRP16*, and the U5 snRNA interact functionally and that a major role of the U5 snRNP is to coordinate a set of factors that are required for the completion of the second catalytic step of splicing.

The discovery that small nuclear RNAs (snRNAs) are functional components of the spliceosome allowed major advances in our understanding of the mechanism of pre-mRNA splicing. First was the elucidation of the role of the U1 snRNA in identifying the 5′ splice site, via direct Watson-Crick base pairing with the pre-mRNA (40, 42, 47). This intermolecular RNA interaction provided a powerful paradigm, which has been extended to the identification of the branchpoint by the U2 snRNA (33, 46, 48). Roles for the U4/U6 and U5 snRNAs have been harder to assign, since no extensive snRNA–pre-mRNA complementarity is apparent. It has been suggested that U6 may play a role in the catalysis of the transesterification reactions, while U4 may be a regulated antisense inhibitor of U6 (10, 19).

The U5 snRNP appears to join the spliceosome simultaneously with U4/U6 in a tripartite complex (12, 22). This occurs after the binding of U1 and U2 to the substrate. In the absence of the U5 snRNA, pre-mRNA splicing is, in most instances, blocked prior to the first transesterification reaction both in vitro and in vivo (24, 34, 39, 45). The U5 snRNP has been indirectly implicated in binding of the polypyrimidine stretch in mammalian systems (11, 17, 43). This interaction with the polypyrimidine tract, in conjunction with in vivo and in vitro evidence demonstrating a requirement for U5 in the second catalytic step of splicing (34, 45), has led us to suggest that the U5 snRNP plays a role in 3′ splice site identification and utilization (32). Strong support for this interpretation has recently been provided by Newman and Norman (30), who demonstrated that point mutations in the conserved loop I of the U5 snRNA (Fig. 1) (19) can suppress point mutations in the 3′ splice site-3′ exon region of the *CYH2* gene. Since point mutations in other loop I positions can suppress mutations at the first position of the *CYH2* intron, the U5 snRNA is also implicated in the process of 5′ splice site selection (29). Although the sequence of the U5 snRNA clearly influences the use of both 5′ and 3′ splice sites in the case of the *cyh2* mutants tested, it remains to be established whether the structure of the U5 loop also influences the splicing of wild-type genes or of mutant introns other than *cyh2*. The lack of conservation in yeast 3′ exon sequences clearly suggests that any such U5 snRNA–pre-mRNA interaction is not mediated solely by loop I of the U5 snRNA. Indeed, the low level of sequence conservation observed among U5 snRNAs from different species (19), the large number of proteins associated with the U5 snRNP (4), and the number of proteins already implicated in specific 3′ splice site interactions (13, 16, 17, 37, 43) suggest that any U5-splice site interaction is mediated extensively by proteins.

To better define the role of the U5 snRNP in splicing, we have used a genetic screen with the goal of identifying gene products with functions related to that of the U5 snRNA. We have searched for mutations in genes that synergize with partially functional alleles of U5 snRNA to produce a lethal phenotype. In addition, we have demanded that the synergistic lethal mutations confer a temperature-sensitive phenotype of their own, i.e., in the presence of the wild-type U5 allele. Such a conditional lethal phenotype greatly facilitates the further analysis of new genes by allowing their role in splicing to be analyzed directly. The isolation of synergistic lethals offers several potential advantages over a classical suppressor hunt (see reference 20 for a general discussion of these genetic strategies). In particular, the range of mutations that would exacerbate a given phenotype should be broader than the range of mutations that would improve it. For this reason, a synergistic lethal approach is an especially promising one for isolating components of a multisubunit complex such as an snRNP or the spliceosome. Indeed, the identification of synergistic lethal interactions has proven to be helpful in the characterization of such complex systems as the secretory pathway (21), the cytoskeleton (reviewed in references 1, 20, and 31), the translation apparatus (15), and the transcription machinery (3).

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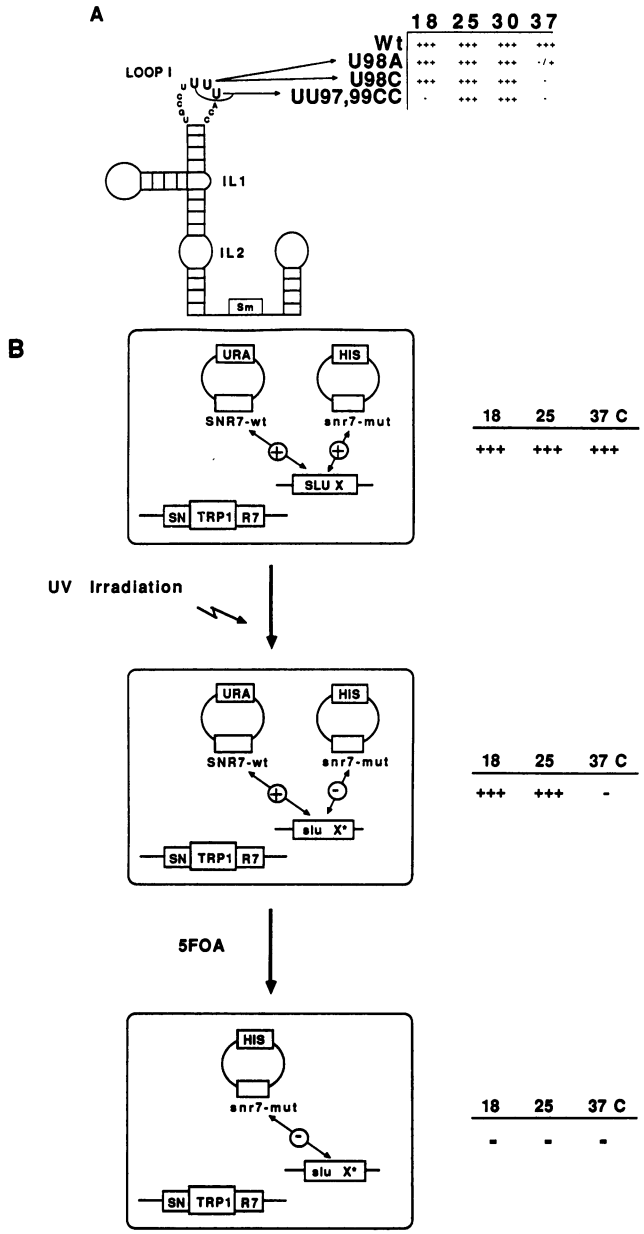

FIG. 1. U5 mutant phenotypes and strategy for isolation of synergistic lethal loci. (A) Schematic of the structure of the yeast U5 snRNA (15a, 19). The sequence of the loop I domain is shown, and the alterations in the three U5 mutants used in this study are indicated. The growth phenotypes of the wild-type (Wt) and three mutant strains are indicated for the temperatures used in this work. IL1 and IL2, internal loops; Sm, Sm protein binding site (19). (B) One of the strategies used for isolation of synergistic loci. The first panel shows the starting strain containing a chromosomal disruption of the *SNR7* gene (which encodes the U5 snRNA), a wild-type copy of the *SNR7* gene on a *URA3*$^+$ plasmid, and the mutant U5 allele being studied on a *HIS3*$^+$ plasmid. The *SLU* locus is wild type and thus interacts productively with both U5 alleles. In the second panel, UV-induced mutagenesis of the *SLU* locus has produced the *slu*X mutation, and the *slu*X gene product no longer interacts productively with the mutant U5 allele. The strain is viable because of the presence of the wild-type U5 allele; however, it is now temperature sensitive because of the defect in the *slu*X gene product. In the third panel, the *URA3* plasmid has been lost (this event is selectively recovered by the inclusion of 5-FOA in the medium), leaving only the U5 mutant allele. Since this allele does not interact productively with the *slu*X mutant, this strain is inviable under all conditions.

MATERIALS AND METHODS

Plasmids and strains. Mutagenesis was performed on *Saccharomyces cerevisiae SNR7* gene disruption strains generated previously (34). The *URA3*$^+$ plasmid bearing wild-type *SNR7* was created by putting a 600-nucleotide *Hin*dIII-*Cla*I fragment encoding *SNR7* (34) into *Hin*dIII-*Cla*I-digested YCp50 to generate YCp50-WT. This same fragment was used to create pBWHis-WT by cloning into pBWHis3 (34). To create the *SNR7* mutants, the wild-type fragment was cloned into M13, and oligonucleotide-directed mutagenesis was performed by standard methods (23). These mutant *SNR7* alleles were also cloned into YCp50 and pBWHis3. Construction of the pG1-*ACT1-CUP1* fusion is described by Lesser and Guthrie (24a).

Yeast genetic methods. All yeast genetic techniques, including transformations, media, diploid selection, sporulation, dissection, and the plasmid shuffle, were performed as described previously (18). For the mutagenesis, mid-log-phase cells were pelleted, washed with sterile deionized H_2O, and then mutagenized by irradiation with UV light for either 15 or 30 s (this resulted in 30 or 90% lethality, respectively). Cultures were then plated on selective media and grown at 25°C. All plates were wrapped in tin foil to prevent exposure to visible light.

Allele specificity. To determine growth characteristics of strains containing *slu* mutations and the various U5 mutant alleles, strains containing YCp50-WT and mutant U5 alleles on *HIS* plasmids were streaked from histidine- and uracil-deficient media to 5-fluoro-orotic acid (5-FOA) plates and placed at 25°C [*slu7-1*(U98A) was also scored at 30°C]. We scored the time for single colonies to reach an average diameter of 1 mm.

RNA analysis. Cells were grown at the temperature indicated in the text and on the appropriate selective media to an optical density at 600 nm of 0.8 to 1.0 and then harvested. For temperature shift experiments, cells were initially grown at a permissive temperature (25°C), pelleted, and then resuspended in prewarmed media. Dilutions were established to produce an optical density at 600 nm of 0.8 to 1.0 at harvest time. RNA was then extracted by the method of Wise et al. (45a).

Primer extensions were performed as follows. Thirteen micrograms of RNA was mixed with 1 ng of ^{32}P-kinased oligonucleotide in a volume of 6.4 μl and then added to 1.6 μl of annealing buffer (250 mM Tris-Cl [pH 8.3], 300 mM NaCl, 50 mM dithiothreitol). After heating for 3 min at 68°C, this mixture was frozen in dry ice-ethanol and then allowed to thaw on ice. To this mixture was added 11.3 μl of reverse transcriptase buffer (18 mM Tris-HCl [pH 8.3], 21 mM NaCl, 3.6 mM dithiothreitol, 11 mM magnesium acetate, 0.72 mM each deoxynucleoside triphosphate) and 14 U of avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc.). The reaction mixtures were incubated at 37°C for 5 min and then at 42°C for 20 min. Reactions were stopped by adding 10 μl of formamide dyes before heating for 3 min at 90°C. Primer extension products were resolved on 6% polyacrylamide–7 M urea gels, which were subsequently dried for 1 h. Quantitation of primer extension products was accomplished via phosphorimaging (Molecular Dynamics). The sequences of oligonucleotides used in this study, as well as the positions in the mRNA with which they anneal, are as follows: *MATa1*, 5′-GAATTTATTTAGATCTCATACG TTT (nucleotides 380 to 405); *ACT-CUP1*, 5′-CTTCATTT TGGAAGTTAATTAATT (nucleotides 74 to 97 of *CUP1*);

and *SNR19*, 5'-CAATGACTTCAATGAACAATTAT (nucleotides 97 to 119).

In vitro splicing. Whole cell splicing extracts were prepared according to the protocol of Lin et al. (25). Splicing assays were performed at both 15 and 23°C as described by Lustig et al. (27). For the splicing time course experiments, splicing reaction mixtures were incubated at 15°C for 20, 40, 60, or 80 min. Reactions were quenched on ice by the addition of 0.2 ml of stop solution (50 mM sodium acetate, 1 mM EDTA, 0.1% sodium dodecyl sulfate, 33 μg of *Escherichia coli* tRNA per ml). Similar time courses were also performed at 23°C for 15, 30, 45, or 60 min.

RESULTS

Identification of synergistic lethal mutations. We first generated several point mutations in the highly conserved loop I sequence of the yeast U5 snRNA. Several of these U5 alleles (Fig. 1A) confer significant growth impairment to the cell when provided as the sole source of U5 activity. Point mutations at position 98 (U98C and U98A), for instance, cause severe defects in growth at 37°C. The double mutant UU97,99CC confers both cold sensitivity (at 18°C) and heat sensitivity (at 37°C) (we have not yet determined how each of the two point mutations in UU97,99CC contributes to these phenotypes). Examination of several spliced messages under nonpermissive conditions failed to reveal a striking defect in splicing (35a). Nonetheless, these defective U5 alleles provide genetic tools for identifying new gene products which function in conjunction with the U5 snRNA.

Cells containing both wild-type and mutant U5 genes were mutagenized and screened for second-site mutations which supported growth in the presence of the wild-type U5 allele but were inviable when synthesis of the wild-type U5 allele was abolished. We used two different means to achieve conditional expression of the wild-type U5 allele. In strategy A (diagrammed in Fig. 1B), the wild-type U5 allele (the *SNR7* gene) resides on a yeast centromere plasmid bearing the *URA3* gene. In the absence of a wild-type chromosomal *URA3* gene, this plasmid allows selection of cells which either contain the plasmid (by selecting for uracil prototrophy) or have lost the plasmid as a result of its low-level mitotic instability (by selecting on media containing 5-FOA [8]). In strategy B, conditional expression of U5 is achieved by fusing it to the yeast *GAL1* promoter; U5 RNA is transcribed on galactose-containing, but not glucose-containing, media (34).

After screening approximately 4,000 mutagenized colonies, we recovered 13 mutants which were viable when wild-type U5 was provided but failed to grow (or grew extremely poorly) when only a mutant U5 allele was present. We designate these mutants *SLU* genes, for synergistic lethal with U5 snRNA. *slu1-1*, *slu2-1*, *slu3-1*, and *slu13-1* were isolated by using strategy B and the U5-UU97,99CC allele, while *slu4-1* through *slu10-1* were isolated by using strategy A and the U5-U98A allele. *slu11-1* and *slu12-1* were isolated by using strategy A and the U5-C98A allele. For clarity, we will include the name of the U5 allele against which the *slu* allele was originally isolated, e.g., *slu1-1* (UU97,99CC).

Since we were specifically interested in conditional lethal alleles of *slu* genes, we examined the ability of the mutants to grow at 18, 30, and 37°C. This screening was done under conditions in which the wild-type U5 allele was expressed, and thus the growth data reflect the defect of the *slu* allele rather than of the recessive U5 mutant allele. As shown in Table 1, *slu1-1*, *-2-1*, *-3-1*, *-4-1*, *-5-1*, *-6-1*, and *-7-1* all displayed a temperature-sensitive growth phenotype at 37°C. In addition, *slu7-1* exhibited a cold-sensitive growth phenotype at 18°C. These findings were subsequently repeated after introduction of the *slu* alleles into strains with a wild-type chromosomal U5 gene, and in all cases the growth characteristics were the same. In contrast, *slu8-1*, *-9-1*, *-10-1*, *-11-1*, *-12-1*, and *-13-1* did not display conditional growth phenotypes at 18, 30, or 37°C. For the remainder of this report, we will focus on the temperature-sensitive mutations indicated in Table 1.

TABLE 1. Phenotypes of temperature-sensitive *slu* alleles

Strain	U5 allele[a]	Growth[b]			Splicing defect[c]	Known complementation group
		18°C	25°C	37°C		
Wild type		+++	+++	+++		
slu1-1	UU97,99CC	+++	+++	−	Step 1	
slu2-1	UU97,99CC	+++	+++	−	Step 1	
slu3-1	UU97,99CC	+++	+++	−	?	
slu4-1	U98A	+++	+++	−	Step2	*PRP17*
slu5-1	U98A	+++	+++	−	?	
slu6-1	U98A	+++	+++	−	?	
slu7-1	U98A	−	+++	−	Step2	

[a] Allele against which the mutants were identified.
[b] Effects of different temperatures on the growth of strains carrying each allele in the presence of the wild-type U5 snRNA.
[c] The catalytic step of splicing that appears to be defective in each mutant.

Genetic characterization. To determine whether the synergistic lethal phenotype cosegregated with the temperature-sensitive phenotype, we backcrossed *slu1-1* through *slu7-1* to the unmutagenized parental strain. The resulting diploids were sporulated and dissected, and the segregation patterns of the synergistic lethal and temperature-sensitive phenotypes were examined. For each of the mutants tested (*slu1* through *slu7*), we found that the temperature-sensitive and synergistic lethal phenotypes cosegregated in all spores that could be scored (at least 32 spores were scored in each case). This result indicates that a single genetic defect is responsible for both phenotypes in each of the mutant strains. We also determined that all mutations segregated independently of the plasmids being used and the *SNR7* locus and therefore do not represent alterations of the U5 snRNA.

We next tested whether the *slu* mutations were allelic with each other or with any of the previously identified temperature-sensitive splicing factors (*prp2* through *prp27* [reviewed in reference 36]). To do so, we performed complementation tests by crossing the *slu* alleles to one another and to the *prp* alleles. From all possible combinations, only the *slu4-1* (U98A)/*prp17-1* diploid failed to grow at the nonpermissive temperature. Dissection of this diploid yielded only temperature-sensitive spores, indicating that these two mutants are indeed allelic (data not shown). Interestingly, *prp17-1* was isolated in a general screen for splicing mutants and confers a temperature-sensitive block in the second transesterification reaction (44). The formal designation for *slu4-1*(U98A) is *prp17-2*, but for clarity in this report, we will continue to refer to it by its *slu* designation. Finally, *slu1-1*, *slu2-1*, *slu4-1*, and *slu7-1* are not allelic to any of the splicing snRNAs (tests of *slu3-1*, *slu5-1*, and *slu6-1* are in progress).

Biochemical characterization. We next analyzed the ability of *slu1* through *slu7* to perform mRNA splicing at the nonpermissive temperature. For these experiments, we used *slu* alleles that had been outcrossed into an otherwise wild-type genetic background. In each case, RNA was

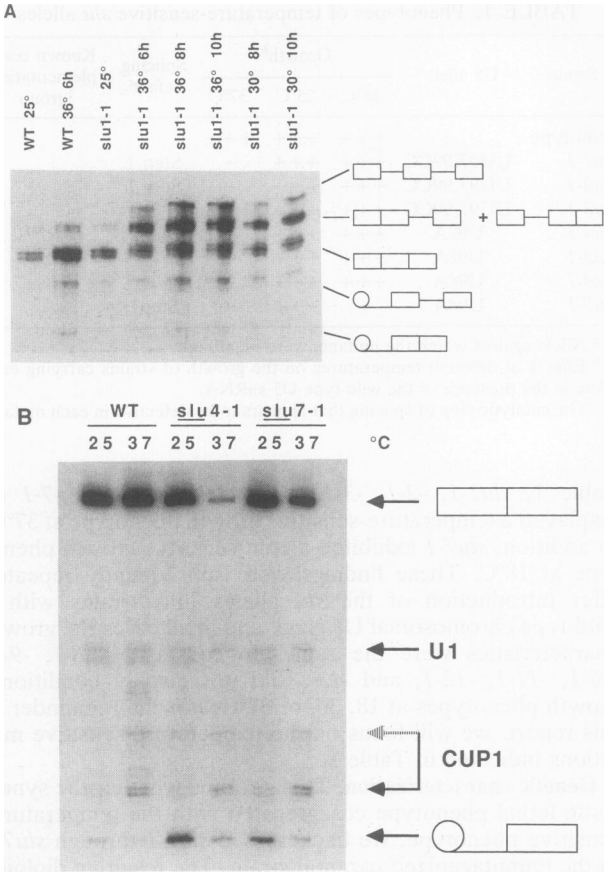

FIG. 2. In vivo splicing defects of *slu* mutants. (A) Primer extensions of the *MATa1* transcript in wild-type (WT) and *slu1-1*(UU97,99CC) strains under a variety of temperature shift regimens. In each case, the strain was shifted to a nonpermissive temperature (30 or 37°C, as indicated) for the amount of time shown prior to RNA extraction. The positions of the primer extension products derived from spliced (mature), unspliced (precursor), and hemispliced (one intron removed) *MATa1* transcripts are indicated. (B) Primer extensions of the *GPD-ACT1-CUP1* fusion transcript, which contains the actin intron (see Materials and Methods). The positions of mature mRNA and lariat intermediate extension products are as indicated. The arrowheads indicate the positions of endogenous *CUP1* and U1 snRNA primer extension products, included as internal controls for RNA levels.

prepared from cells that had been shifted to 37°C for 4 to 6 h (two to three generations). This RNA was analyzed by primer extension to ascertain the effects on splicing of specific mRNA precursors; several different mRNAs were examined in each case.

In the case of *slu1-1*(UU97,99CC), we observed an accumulation of *MATa1* precursors at the nonpermissive temperature (Fig. 2A). Interestingly, while the *MATa1* pre-mRNA contains two introns, our primer extension data indicated that the predominant accumulated product contained one intron but not both introns. We have not ascertained whether only one of the two introns is always unspliced or whether either is spliced randomly, but both are rarely removed from the same transcript.

For *slu2-1*(UU97,99CC), there was also a first-step defect in the splicing of *MATa1* transcript (data not shown), but this defect appeared to be nonconditional. In the case of both *slu1-1*(UU97,99CC) and *slu2-1*(UU97,99CC), accumulation of precursor was not accompanied by accumulation of lariat intermediate (occurrence of the latter intermediate would provide evidence that the alleles were only partially defective in the first step); the *slu1-1*(UU97,99CC) and *slu2-1* (UU97,99CC) alleles are primarily defective in the first step of splicing.

The splicing defects of *slu4-1*(U98A) and *slu7-1*(U98A) were very similar to one another with respect to both pre-mRNA substrates affected and defects observed. In each case, the most striking defect was in the splicing of the actin intron (the actual transcript analyzed was an *ACT1-CUP1* fusion construct containing the actin intron; see Materials and Methods). In contrast to *slu1-1*(UU97,99CC) and *slu2-1* (UU97,99CC), precursor levels were unchanged in *slu4-1* (U98A) and *slu7-1*(U98A) (data not shown). Instead, as shown in Fig. 2B, we observed a defect in the second step of splicing that resulted in the accumulation of the lariat intermediate at both a permissive (25°C) and a nonpermissive (37°C) temperature. The lariat/mature ratio provides a measure of the extent to which the second step is blocked. Phosphorimaging analysis indicated that this ratio was increased 7-fold for *slu4-1*(U98A) and 2.5-fold for *slu7-1* (U98A) at 25°C. At the nonpermissive temperature, the increases in this ratio were 40-fold for *slu4*(U98A) and 6-fold for *slu7-1*(U98A). Our observation of a second-step defect for *slu4-1* (an allele of *PRP17*) thus corroborates the results of Vijayraghavan et al. (44).

In the cases of *slu3-1*(UU97,99CC), *slu5-1*(U98A), and *slu6-1*(U98A), we were unable to observe any splicing defects.

For *slu4-1*(U98A), we also noted a dramatic decrease in the absolute levels of mRNA without a commensurate increase in the level of the lariat intermediate. In Fig. 2b, this is clearly evidenced by the disappearance of the spliced *ACT1-CUP1* fusion relative to the internal control, U1 snRNA. As further controls for general RNA loss, we examined the intronless *URA3* mRNA as well as the U5 snRNA (data not shown); in neither case did we observe a decreased level of these RNAs at the nonpermissive temperature. In contrast, mRNA levels were greatly reduced in *slu4-1* for the spliced transcripts of *CYH2*, *MATa1*, and the actin gene. We have also observed this reduction in *CYH2* mRNA with *slu7-1*(U98A), *prp17-1*, *slu5-1*(U98A), *slu6-1* (U98A), and to a lesser extent *slu1-1*(UU97,99CC) (data not shown).

We interpret this loss-of-mature phenotype to be a secondary effect of the splicing defects caused by the *slu/prp* mutants. Such a phenotype can arise if the splicing mutations cause an increase in the degradation rates of the splicing intermediates (precursor or lariat intermediate) in addition to their primary effects on splicing. Thus, the accumulation of intermediates would be partially countered by a greatly increased rate of their degradation. The net effect of this process would be a decrease in spliced mRNA without a commensurate increase in the levels of splicing intermediates. Since this phenotype results from destabilization of either precursor or lariat intermediate, however, it is not an informative means of differentiating first-step from second-step mutants. Nonetheless, the loss-of-mature phenotype represents a novel splicing phenotype that could potentially be exploited in future genetic screens.

While the in vivo splicing phenotypes of *slu1-1*(UU97, 99CC), *slu2-1*(UU97,99CC), *slu4-1*(U98A), and *slu7-1*(U98A) suggest that these genes function in the splicing process, they do not reveal whether the gene products act directly (i.e., are spliceosomal components) or indirectly (i.e., are

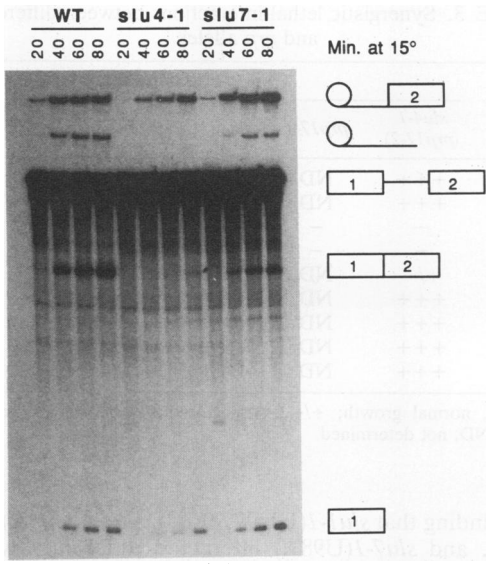

FIG. 3. In vitro splicing of the actin intron in extracts of *slu4-1* and *slu7-1*. Products from in vitro splicing reactions performed at 15°C by using extracts prepared from wild-type (WT), *slu4-1*(U98A), and *slu7-1*(U98A) strains are shown. Splicing reaction mixtures were incubated for the designated times and then quenched on ice (see Materials and Methods for details).

biosynthetic components) in splicing. To differentiate direct from indirect effects of the *slu* mutations on splicing, we prepared whole cell extracts (25, 27) from *slu1*, *slu4*, *slu5*, *slu6*, and *slu7* strains grown at a permissive temperature (25°C) and tested their in vitro splicing activities. In vitro splicing reactions were carried out at either 15 or 23°C, the standard temperatures for assaying temperature-sensitive splicing mutants (27).

Extracts made from *slu1-1*(UU97,99CC), *slu5-1*(U98A), and *slu6-1*(U98A) exhibited no splicing defects at 15 or 23°C, even after prior heat inactivation treatment of the extracts at 32°C (data not shown). In contrast, splicing time course experiments carried out at 15°C revealed that extracts made from *slu4-1*(U98A) and *slu7-1*(U98A) strains were defective, relative to a wild-type extract, in the conversion of lariat intermediate to mature RNA (Fig. 3). The failure of the mutant extracts to efficiently complete the second step of splicing is clearly evidenced by the accumulation of lariat intermediate and free exon 1, with little or no subsequent production of mRNA. The kinetics of lariat intermediate and exon 1 production were identical in the *slu7-1*(U98A) and wild-type extracts, indicating that the *slu7-1*(U98A) allele is defective solely in the second step of splicing. Lariat intermediate and exon 1 formation in the *slu4-1*(U98A) extract lagged behind the rates observed for wild-type and *slu7-1* (U98A), suggesting that the *slu4-1*(U98A) mutation causes some inhibition of the first step. The first-step lag observed in the *slu4-1*(U98A) extract is minor, however, compared with the almost complete inhibition of mRNA formation. We interpret this result to mean that the *slu4-1*(U98A) allele is primarily defective in the second step of splicing in vitro. Splicing time course experiments carried out at 23°C produced results similar to those at 15°C for both *slu4-1*(U98A) and *slu7-1*(U98A) extracts. Thus, both of these mutant extracts are constitutively defective in the second step of splicing in vitro. The in vitro splicing phenotypes of *slu4-1* (U98A) and *slu7-1*(U98A) were not exacerbated by heat treatment at 32°C. Finally, *slu4-1*(U98A) and *slu7-1*(U98A) extracts prepared independently of those assayed in Fig. 3 produced results identical to those shown here.

While the results of the in vitro experiments do not precisely parallel those of the in vivo experiments, we attribute these differences to the dissimilarities between the two assay systems that have been observed for other splicing mutants (27). Nonetheless, for both *slu4-1*(U98A) and *slu7-1*(U98A), we observed strong support for the hypothesis that these alleles are specifically defective in the second step of splicing. Preliminary experiments in which splicing extracts have been immunodepleted of SLU4 or SLU7 protein support these conclusions (19a).

Finally, U5 snRNA levels were measured for *slu1-1* (UU97,99CC), *slu2-1*(UU97,99CC), *slu4-1*(U98A), *slu5-1* (U98A), *slu6-1*(U98A), and *slu7-1*(U98A) strains upon shifting cultures to a nonpermissive temperature (conditions were the same as those used for the primer extension analysis). In no case did we observe an appreciable change in the cellular level of U5 snRNA (data not shown). Thus, these alleles probably do not influence the expression or stability of the U5 snRNA.

Genetic interactions. A major concern with the synergistic lethal approach arises from uncertainty regarding the relatedness of the new mutation to the starting mutation with which it synergizes. The splicing defects seen in *slu1-1*(UU97,99CC), *slu2-1*(UU97,99CC), *slu4-1*(U98A), and *slu7-1*(U98A) argued for such a link. We had no evidence, however, that these genes were directly involved in U5 function. Similarly, we wanted to test the hypothesis that the other *slu* mutants were related in function to U5, even though we had been unable to discern a splicing defect. We addressed this problem by examining the interaction of each of the *slu* alleles with each of the conditional U5 alleles used in the initial screen (Fig. 1). We reasoned that mutations in a factor only distantly related to U5 function, such as a transcriptional regulator, would show linear effects when combined with the mutant U5 alleles; i.e., all of the U5 mutant phenotypes would be exacerbated to a similar degree by a given *slu* mutant. The double-mutant phenotypes would therefore display the same relative severities, with respect to the U5 alleles, as in the presence of the wild-type *SLU* allele. Alternatively, if a *slu* allele showed a much stronger genetic interaction with a specific U5 allele, we could infer that the *slu* allele either interacts physically with the U5 snRNA or acts closely to it in time or space.

To characterize the allele specificity of the synergistic lethal interactions, each of the *slu* mutations was introduced into a genetic background in which the only wild-type U5 allele was carried on a centromeric plasmid bearing the *URA3* gene. These strains were then transformed with *HIS3* centromeric plasmids bearing one of the mutant U5 alleles (U98A, U98C, and UU97,99CC) or wild-type U5. We then selected for cells on 5-FOA (which is lethal to *URA3*$^+$ cells), thus revealing the viability of cells expressing only the mutant U5 allele.

The results (Table 2) represent the actual growth rates of the haploid double mutants relative to their expected growth rates. That is, a double mutant that grew more than twice as slowly as would be predicted from the behavior of the single mutants was scored as having a synergistically lethal phenotype. In an otherwise wild-type background, the relative health of the U5 alleles at either 25 or 30°C is wild type (U98) > U98A > U98C = UU97,99CC. Clearly, the different *slu* alleles showed different spectra of responses to the three U5 alleles examined. Thus, *slu1-1*(UU97,99CC) was primarily

TABLE 2. Allele specificity of *slu*-U5 interactions

Strain	Growth with U5 allele[a]:			
	U98	U98A	U98C	UU97,99CC
Wild type	+++	+++	+++	+++
slu1-1(UU97,99CC)	+++	+++	++	−
slu2-1(UU97,99CC)	+++	+++	−	−
slu3-1(UU97,99CC)	+++	ND[b]	ND	ND
slu4-1(U98A)	+++	−	+++	++
slu5-1(U98A)	+++	−	++	−
slu6-1(U98A)	+++	++	−	−
slu7-1(U98A)	+++	−	+++	+++
prp8-1	+++	−	−	−
prp18-1	+++	−	−	−
prp16-2	+++	+++	ND	ND
prp24-1	+++	+++	+++	ND

[a] Wild-type cells bearing the wild-type U5 allele produce a 1-mm colony in 4 days at room temperature. For each U5 mutant and each *slu* allele, the extent of phenotype was assessed as the factor by which 4 days was multiplied to give the observed time for colony appearance. For double mutants, the expected value was derived by multiplying 4 days by both factors. If the observed time was less than or equal to this value, growth is represented as +++; ++ and + designate 1.25 and 1.5 times slower growth, respectively, than anticipated; − signifies more than twice the predicted time. ND, not determined. In an otherwise wild-type background, the relative health of the U5 alleles at either 25 or 30°C is wild type (U98) > U98A > U98C = UU97,99CC. Note that *slu7* was assayed at 30°C on YEPD rather than 5-FOA–SD (see text for details).

[b] ND, no data.

TABLE 3. Synergistic lethal interactions between different *slu* and *prp* alleles

Strain	Growth at room temp[a]					
	slu4-1 (*prp17-2*)	*prp17-1*	*slu5-1*	*slu6-1*	*slu7-1*	*prp16-2*
slu5-1	+++	ND				
slu6-1	+++	ND	+++			
slu7-1	−	−	+++	+++		
prp18-1	−	−	+++	+++	−	+++
prp16-2	+/−	ND	+++	+++	+/−	ND
slu1-1	+++	ND	+++	+++	+++	ND
prp4-1	+++	ND	+++	+++	+++	ND
prp8-1	+++	ND	+++	+++	+++	ND
prp27-1	+++	ND	+++	+++	+++	ND

[a] +++, normal growth; +/−, slow growth; −, the double mutant is inviable; ND, not determined.

affected by U5-UU97,99CC, was only marginally affected by U5-U98C, and showed no apparent synergistic effect with U5-U98A. *slu2-1*(UU97,99CC) was lethal in combination with U98C and UU97,99CC, but was viable with U98A. In contrast, *slu4-1*(U98A) synergized strongly with U5-U98A (>2× doubling time) and slightly with U5-UU97,99CC (>1.25× doubling time). *slu5-1*(U98A) was markedly less perturbed by U98C than by the other alleles, while *slu6-1*(U98A) was relatively allele nonspecific, synergizing to some degree with each U5 allele tested.

Finally, *slu7-1*(U98A) did not display a synergistic interaction with any of the U5 alleles at 25°C, but at 30°C we observed synergistic interactions with both U98A and UU97,99CC. We could not score the interaction with U98C, since otherwise wild-type cells bearing only U98C are unable to grow at 30°C on 5-FOA. To alleviate this problem, we examined the allele specificity of *slu7-1*(U98A), using the inducible *GAL*-U5 construct. In this system, *slu7-1*(U98A) was lethal in combination with U98A but not U98C or UU97,99CC (data not shown). Note that the *slu7-1*: UU97,99CC mutant grew on YEPD (rich medium) but not 5-FOA (minimal medium) at 30°C, a result that we attribute to the different stringencies of the growth media.

As a control for this analysis, we examined the interaction of several previously characterized temperature-sensitive splicing mutants (pre-mRNA processing, or *prp* [36]) with the mutant U5 alleles. We included in this analysis an allele of the previously characterized U5 snRNP protein gene *prp8-1* (26), two mutants that have second-step splicing defects (*prp18-1* [44] and *prp*16-2 [38]), and *prp24*, a U6 snRNP protein (41). Only *prp8-1* and *prp18-1* showed synergistic interactions. In both cases, the resultant lethality was allele nonspecific with respect to the U5 mutations. Since *prp16-2* was not lethal in combination with the U5 alleles tested, we conclude that synergistic lethality with U5 mutants is not a general property of second-step defective splicing mutants.

The finding that *slu1-1*(UU97,99CC), *slu4-1*(U98A), *slu5-1* (U98A), and *slu7-1*(U98A) interacted differently with the three alleles of U5 tested (i.e., altered the relative severity of the U5 alleles) is important for several reasons. First, the allele specificity suggests that these *slu* genes are related functionally to U5 snRNA, since they are sensitive to the sequence of U5 snRNA. The different synergistic interactions also suggest that the different U5 snRNA mutants are defective in different ways; interactions or functions which are perturbed in U5-U98A may be with different proteins or at different stages of the splicing reaction from those perturbed in U5-UU97,99CC. This possibility is bolstered by the observation that U5-UU97,99CC is the only U5 allele that confers a significant cold-sensitive phenotype.

Genetic interactions among second-step mutants. To determine whether the second-step-defective alleles interact genetically with one another and with other splicing factor mutants, we generated double-mutant strains and then tested their viability and growth characteristics. Included in this analysis were the second-step-defective *slu* alleles *slu4-1* (U98A) and *slu7-1*(U98A) as well as *slu1-1*(UU97,99CC) and the two U98A-derived alleles for which we have been unable to identify splicing defects [*slu5-1*(U98A) and *slu6-1*(U98A)]. From the previously characterized *prp* alleles, we chose *prp16-2*, which has been shown to be required in vitro for the second but not the first step of splicing (38); *prp17-1*, which is allelic to *slu4-1*(U98A); *prp18-1*, which shows a second step defect in vivo; and *prp27-1*, which accumulates excised lariat in vivo (*prp17*, *prp18*, and *prp27* are described in reference 44). We also included *prp4-1* and *prp8-1* because they demonstrate first-step splicing defects and are both constituents of the U4/5/6 tri-snRNP; PRP4 is a U4 snRNP protein (5, 6), and PRP8 is a U5 protein (26). The growth phenotypes of the double-mutant haploid strains are summarized in Table 3.

Our most striking finding was that the *slu* alleles required for the second step of splicing [*slu4-1*(U98A) and *slu7-1* (U98A)] exhibited the strongest interaction with each other as well as with other alleles required for the second step (*prp16-2* and *prp18-1*). Indeed, we were unable to recover viable double-mutant combinations for any of the pairwise combinations of *slu4-1*(U98A), *slu7-1*(U98A), and *prp18-1*. Similarly, *prp16-2* was lethal in combination with both *slu4-1*(U98A) and *slu7-1*(U98A) but not *prp18-1*. The failure of *prp16-2* to synergize with *prp18-1* indicates that involvement in the second step of splicing is not itself sufficient to give rise to synergistic lethality. Nonetheless, the interac-

tions were not necessarily allele specific, because *prp17-1* (an independently isolated allele of *slu4-1*(U98A) [44]) showed the same interactions as did *slu4-1*(U98A).

DISCUSSION

Identification of genes encoding products functionally related to U5 snRNA. One caveat of screens that identify new gene products on the basis of phenotypic enhancement is that alleles isolated by using such screens may exert their effects indirectly and thus be functionally unrelated to the defective starting allele (see, for example, reference 28). However, we reasoned that a search for synergistic lethal phenotypes would offer the potential of recovering a broader spectrum of mutants than would a suppressor hunt and might also enrich for conditional defects. Furthermore, the lack of clear splicing defects exhibited by the starting U5 alleles prompted us to search for conditions (i.e., synergistic lethality) that would exacerbate the starting phenotypes. An analysis of the resulting synergistic phenotypes could thus provide useful information about the functions of the starting U5 alleles, specifically, how the various alleles disrupt splicing.

By starting with defective alleles of U5, we identified 13 synergizing alleles which are unlinked to the U5 gene. Each of these alleles defines a distinct complementation group, since all pairwise combinations of mutants complemented one another. Of these 13 mutants, 7 are temperature sensitive for growth. To date, we have been able to identify splicing defects in vitro and/or in vivo for four of them [*slu1-1*(UU97,99CC), *slu2-1*(UU97,99CC), *slu4-1*(U98A), and *slu7-1*(U98A)]. Indeed *slu4-1*(U98A) is allelic to *prp17-1*, a mutation previously isolated on the basis of its splicing defect (44). Furthermore, we have shown that most of these mutants exhibit allele-specific synergistic lethality with different mutant U5 alleles, suggesting that they associate, either physically or functionally, with the U5 snRNA. Indeed, several of the *slu* mutant strains reverse the relative severities of the U5 mutations examined; while U5-U98A is the least severe U5 allele in a wild-type background, it is the most severe in the *slu4-1*(U98A), *slu5-1*(U98A), and *slu7-1* (U98A) backgrounds. Such nonlinear responses are clearly inconsistent with models that explain synergistic lethality in terms of simple additive mutational effects (28). Finally, two of the alleles that we identified in this screen [*slu4-1*(*U98A*) and *slu7-1*(*U98A*)] are specifically defective in the second step of splicing and show synergistic interactions with one another as well as with other genes involved in the second step of splicing (*prp16-2* and *prp18-1*; see below). For these reasons, we conclude that *slu1-1*, *slu2-1*, *slu4-1*, and *slu7-1* are likely to be defective in functions directly related to those perturbed in the starting U5 alleles. Clearly, the strength of this conclusion is limited by the analysis of single-mutant alleles; we are now attempting to isolate additional alleles of these genes in order to further test this hypothesis.

While synthetic lethal screens have long been used to identify genes with overlapping functions (14), our results indicate that screening for synergistic lethality can be a powerful approach for identifying factors that are functionally related. As described in the introduction, multisubunit complexes, such as the cytoskeleton and the transcription machinery, are especially good targets for this type of analysis. In a number of cases, these genetically interacting factors are known, by other criteria, to physically interact (2, 3), thus further validating the utility of this genetic approach.

Roles of U5 snRNA and *slu* mutations in splicing. Although the precise role of the U5 snRNP in splicing remains somewhat enigmatic, it has been implicated in several aspects of spliceosomal function. On the basis of in vitro splicing complex studies, it has been concluded that the U5 snRNP can form a tri-snRNP complex along with the U4 and U6 snRNPs (7, 9, 12, 22). It is in this form that these snRNPs are thought to join the committed complex, prior to the first catalytic step. Hence, the depletion of the U5 snRNA in vivo or the U5 snRNP in vitro severely impedes the first step of splicing (24, 34, 39, 45). Furthermore, the genetic data of Newman and Norman (29) suggest that the U5 snRNP may play a functionally active, rather than simply structural, role in the first step (see the introduction for details). Finally, some transcripts undergo the first transesterification reaction but, upon depletion of U5, fail to undergo the second (34, 45). From these latter results, we and others have inferred that the U5 snRNP also plays a key role in the second step of splicing (32, 34, 45). As discussed above, strong genetic evidence in support of this idea has recently been provided by Newman and Norman (30).

Since *slu1-1* and *slu2-1* cause the accumulation of unspliced precursors in vivo, they are candidates for factors that affect the first step of splicing, by blocking, for instance, the formation of the tri-snRNP complex or its subsequent incorporation into the spliceosome. We have not been able to reproduce the *slu1-1* splicing defect in vitro, so it is unclear whether the effect that we observed in vivo is a direct or indirect effect of the loss of *SLU1* activity. For instance, mutations in factors required for the synthesis, modification, or stability of bona fide splicing factors might also result in synergistic lethality. We are continuing the biochemical analysis of *slu1-1*(UU97,99CC) and *slu2-1* (UU97,99CC) in order to address this question. In particular, we are interested in determining what, if any, effect these mutations might have on either U4/U5/U6 formation or 5′ splice site selection.

Identification of a genetically related group of factors required for the second step of splicing. Since we believe that the U5 snRNP plays a key role in the second step of splicing, we further analyzed the two second-step mutants, *slu4-1* (U98A) and *slu7-1*(U98A). We found that *slu4-1 slu7-1* double-mutant haploids were lethal, as indeed were combinations of either of these alleles with alleles of *prp16* and *prp18*, which are also defective in the second step of splicing. The *prp16-2* and *prp18-1* alleles, however, were not synergistically lethal when combined with one another; thus, lethality is not caused simply by the combination of any two second-step mutants. Finally, we have shown that *prp18-1* shows synergistic lethality with U5 mutations. These relationships are shown graphically in Fig. 4, in which solid connecting lines specify synergistic lethal interactions.

While we acknowledge that synergistic phenotypes cannot be interpreted as strongly as allele-specific suppressor mutations, we suggest that Fig. 4 represents a provisional relatedness map of these particular alleles. The most powerful argument for the specificity of the genetic interactions derives from two data sets: (i) none of the *slu* mutants that have first-step defects exhibit synergistic lethality with those that show second-step defects, and (ii) two previously isolated splicing mutants with defects in the first step, *prp4-1* and *prp8-1*, were also tested against *slu4-1*(U98A) and *slu7-1* (U98A) and did not show synergistic interactions. This is of particular interest with regard to *prp8-1*, which both shows synergistic lethality with the three U5 alleles discussed in this report (Table 2) and is known to be a component of the

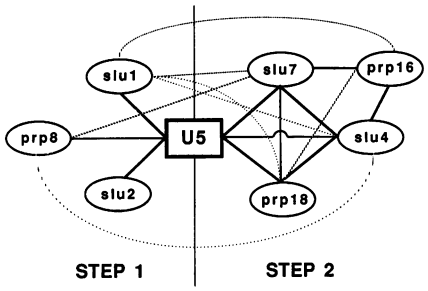

FIG. 4. Genetic interactions between U5 snRNA and *prp* and *slu* mutants. Mutants that show defects in the first step of splicing are shown on the left of the dividing line, while those that show second-step defects are shown on the right. Wide, solid lines indicate synergistic lethal interactions observed between certain alleles of the connected genes. Thinner, lighter lines indicate cases in which double-mutant strains have been constructed but in which no synergisms have been observed. Not shown are *slu5* and *slu6* (which synergize only with U5) and *prp4* and *prp27* (for which no synergisms have been detected).

U5 snRNP (26). The fact that a group of genes, required for the second step of splicing, exhibits synergistic lethality with the U5 snRNA and with one another lends credence to the idea that these factors represent multiple components of a single unit of function. Whether this unit represents a discrete physical entity or is rather a manifestation of involvement in a common process awaits biochemical tests.

What roles might these second-step factors play in splicing? Earlier evidence from other systems, indicating a role for the U5 snRNP in 3′ splice site binding (11, 17, 43), lead us to propose that the U5 snRNP plays a key role in the identification and utilization of 3′ splice acceptor sites prior to the second transesterification reaction (32). In addition, the provocative work of Newman and Norman (30) suggests that the U5 snRNA itself may play an instructive role in 3′ splice site specification. In light of the low information content of the 3′ exon sequences that are predicted by Newman and Norman (30) to interact with the U5 snRNA, it is likely that additional factors are required to enhance or stabilize this snRNA–pre-mRNA interaction. If this hypothesis is correct, then *slu* or *prp* alleles that show defects in the second step of splicing may represent participants in this specific aspect of U5 function (i.e., interaction with the 3′ splice site). As an initial step in assigning a specific role to these factors, we have developed a series of constructs that allow us to assess the effects of these mutations on 3′ splice site choice (35). This work (15b) indicates that *SLU7* is involved in the selection of 3′ splice sites, while *SLU4*, *PRP16*, and *PRP18* perform a generic second-step function.

REFERENCES

1. **Adams, A. E. M., and D. Botstein.** 1989. Dominant suppressors of yeast actin mutations that are reciprocally suppressed. Genetics **121**:675–683.
2. **Adams, A. E. M., D. Botstein, and D. G. Drubin.** 1989. A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. Science **243**:231–233.
3. **Arndt, K. T., C. A. Styles, and G. R. Fink.** 1989. A suppressor of a HIS4 transcriptional defect encodes a protein with homology to the catalytic subunit of protein phosphatases. Cell **56**:527–537.
4. **Bach, M., G. Winkelmann, and R. Lührmann.** 1989. 20S small nuclear ribonucleoprotein U5 shows a surprisingly complex protein composition. Proc. Natl. Acad. Sci. USA **86**:6038–6042.
5. **Banroques, J., and J. Abelson.** 1989. A protein of the yeast U4/U6 small nuclear ribonucleoprotein particle. Mol. Cell. Biol. **9**:3710–3719.
6. **Bjorn, S. P., A. Soltyk, J. D. Beggs, and J. D. Friesen.** 1989. *PRP4* (*RNA4*) from *Saccharomyces cerevisiae*: its gene product is associated with the U4/U6 small nuclear ribonucleoprotein particle. Mol. Cell. Biol. **9**:3698–3709.
7. **Black, D. L., and A. L. Pinto.** 1989. U5 small nuclear ribonucleoprotein: RNA structure analysis and ATP-dependent interaction with U4/U6. Mol. Cell. Biol. **9**:3350–3359.
8. **Boeke, J. D., J. Truehart, G. Natsoulis, and G. R. Fink.** 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol. **154**:164–175.
9. **Bordonné, R., J. Banroques, J. Abelson, and C. Guthrie.** 1990. Domains of yeast U4 spliceosomal RNA required for PRP4 protein binding, snRNP-snRNP interactions, and pre-mRNA splicing in vivo. Genes Dev. **4**:1185–1196.
10. **Brow, D., and C. Guthrie.** 1989. Splicing a spliceosomal RNA. Nature (London) **337**:14–15.
11. **Chabot, B., D. L. Black, D. M. LeMaster, and J. A. Steitz.** 1985. The 3′ splice site of pre-messenger RNA is recognized by a small nuclear ribonucleoprotein. Science **230**:1344–1349.
12. **Cheng, S. C., and J. Abelson.** 1987. Spliceosome assembly in yeast. Genes Dev. **1**:1014–1027.
13. **Choi, Y. D., P. J. Grabowski, P. A. Sharp, and G. Dreyfuss.** 1986. Heterogeneous nuclear ribonucleoproteins: role in RNA splicing. Science **231**:1534–1540.
14. **Dobzhansky, T.** 1946. Genetics of natural populations. XIII. Recombination and variability in populations of Drosophila pseudoobscura. Genetics **31**:269–290.
15. **Donahue, T. F., A. M. Cigan, E. K. Pabich, and B. C. Valavicius.** 1988. Mutations at a Zn(II) finger motif in the yeast eIF-2B gene alter ribosomal start-site selection during the scanning process. Cell **54**:621–632.

15a. **Frank, D.** An essential splicing factor, *SLU7*, mediates 3′ splice-site choice in yeast. Genes Dev., in press.

15b. **Frank, D., and C. Guthrie.** Submitted for publication.

16. **Garcia-Blanco, M. A., S. F. Jamison, and P. A. Sharp.** 1989. Identification and purification of a 62,000-dalton protein that binds specifically to the polypyrimidine tract of introns. Genes Dev. **3**:1874–1886.
17. **Gerke, V., and J. A. Steitz.** 1986. A protein associated with small nuclear ribonucleoprotein particles recognizes the 3′ splice site of premessenger RNA. Cell **47**:973–984.
18. **Guthrie, C., and G. R. Fink (ed.).** 1991. Guide to yeast genetics and molecular biology. Academic Press, Inc., San Diego, Calif.
19. **Guthrie, C., and B. Patterson.** 1988. Spliceosomal snRNAs. Annu. Rev. Genet. **23**:387–419.

19a. **Haltiner-Jones, M., and D. N. Frank.** Unpublished data.

20. **Huffaker, T. C., M. A. Hoyt, and D. Botstein.** 1987. Genetic analysis of the yeast cytoskeleton. Annu. Rev. Genet. **21**:259–284.
21. **Kaiser, C. A., and R. Schekman.** 1990. Distinct sets of SEC genes govern transport vesicle formation and fusion early in the secretory pathway. Cell **61**:723–733.
22. **Konarska, M. M., and P. A. Sharp.** 1987. Interaction between small nuclear ribonucleoprotein particles in formation of spliceosomes. Cell **49**:763–764.
23. **Kunkel, T. A., J. D. Roberts, and R. A. Zakour.** 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. Methods Enzymol. **154**:367–382.
24. **Lamm, G. M., B. J. Blencowe, B. S. Sproat, A. M. Iribarren, U. Ryder, and A. I. Lamond.** 1991. Antisense probes containing 2-aminoadenosine allow efficient depletion of U5 snRNP from HeLa splicing extracts. Nucleic Acids Res. **19**:3193–3198.

24a. **Lesser, C., and C. Guthrie.** Unpublished data.

25. **Lin, R. J., A. J. Newman, S. C. Cheng, and J. Abelson.** 1985. Yeast mRNA splicing *in vitro*. J. Biol. Chem. **260**:14780–14792.
26. **Lossky, M., G. J. Anderson, S. P. Jackson, and J. Beggs.** 1987. Identification of a yeast snRNP protein and detection of snRNP-snRNP interactions. Cell **51**:1019–1026.
27. **Lustig, A. J., R. Lin, and J. Abelson.** 1986. The yeast RNA gene

products are essential for mRNA splicing in vitro. Cell **47**:953–963.

28. **Munn, A. L., L. Silveira, M. Elgort, and G. S. Payne.** 1991. Viability of clathrin heavy-chain-deficient *Saccharomyces cerevisiae* is compromised by mutations at numerous loci: implications for the suppression hypothesis. Mol. Cell. Biol. **11**:3868–3878.
29. **Newman, A., and C. Norman.** 1991. Mutations in yeast U5 snRNA alter the specificity of 5′ splice-site cleavage. Cell **65**:115–123.
30. **Newman, A. J., and C. Norman.** 1992. U5 snRNA interacts with exon sequences at 5′ and 3′ splice sites. Cell **68**:743–754.
31. **Novick, P., B. C. Osmond, and D. Botstein.** 1989. Suppressors of yeast actin mutations. Genetics **121**:659–674.
32. **Parker, R., and B. Patterson.** 1987. Architecture of fungal introns: implications for spliceosome assembly, p. 133–149. *In* M. Inouye and B. S. Dudock (ed.), Molecular biology of RNA: new perspectives. Academic Press, Inc., San Diego, Calif.
33. **Parker, R., P. Siliciano, and C. Guthrie.** 1987. Recognition of the TACTAAC box during mRNA splicing in yeast involves base pairing to the U2-like snRNA. Cell **49**:220–239.
34. **Patterson, B., and C. Guthrie.** 1987. An essential yeast snRNA with a U5-like domain is required for splicing in vivo. Cell **49**:613–624.
35. **Patterson, B., and C. Guthrie.** 1991. A U-rich tract enhances usage of an alternative 3′ splice site in yeast. Cell **64**:181–187.

35a. **Patterson, B., and C. Guthrie.** Unpublished data.

36. **Ruby, S. W., and J. Abelson.** 1991. Pre-mRNA splicing in yeast. Trends Genet. **7**:79–85.
37. **Ruskin, B., P. D. Zamore, and M. R. Green.** 1988. A factor, U2AF, is required for U2 snRNP binding and splicing complex assembly. Cell **52**:207–219.
38. **Schwer, B., and C. Guthrie.** 1991. PRP16 is an RNA-dependent ATPase that interacts transiently with the spliceosome. Nature (London) **349**:494–499.
39. **Séraphin, B., N. Abovich, and M. Rosbash.** 1991. Genetic depletion indicates a late role for U5 snRNP during in vitro spliceosome assembly. Nucleic Acids Res. **19**:3857–3860.
40. **Séraphin, B., L. Kretzner, and M. Rosbash.** 1988. A U1 snRNA: pre-mRNA base-pairing interaction is required early in yeast spliceosome assembly but does not uniquely define the 5′ cleavage site. EMBO J. **7**:2533–2538.
41. **Shannon, K. W., and C. Guthrie.** 1991. Suppressors of a U4 snRNA mutation define a novel U6 snRNP protein with RNA-binding motifs. Genes Dev. **5**:773–785.
42. **Siliciano, P. G., and C. Guthrie.** 1988. 5′ splice site selection in yeast: genetic alterations in base-pairing with U1 reveal additional requirements. Genes Dev. **2**:1258–1267.
43. **Tazi, J., C. Alibert, J. Temsamani, I. Reveillaud, G. Cathala, C. Brunel, and P. Jeanteur.** 1986. A protein that specifically recognizes the 3′ splice site of mammalian pre-mRNA introns is associated with a small nuclear ribonucleoprotein. Cell **47**:755–766.
44. **Vijayraghavan, U., M. Company, and J. Abelson.** 1989. Isolation and characterization of pre-mRNA splicing mutants of *Saccharomyces cerevisiae*. Genes Dev. **3**:1206–1216.
45. **Winkelmann, G., M. Bach, and R. Lührmann.** 1989. Evidence from complementation assays in vitro that U5 snRNP is required for both steps of mRNA splicing. EMBO J. **8**:3105–3112.

45a. **Wise, J.** 1991. Preparation and analysis of low molecular weight RNAs and small ribonucleoproteins, p. 405–415. *In* C. Guthrie and G. R. Fink (ed.), Guide to yeast genetics and molecular biology. Academic Press, Inc., San Diego, Calif.

46. **Wu, J., and J. L. Manley.** 1989. Mammalian pre-mRNA branch site selection by U2 snRNP involves base-pairing. Genes Dev. **3**:1553–1561.
47. **Zhuang, Y., and A. M. Weiner.** 1986. A compensatory base change in U1 snRNA suppresses a 5′ splice site mutation. Cell **46**:827–835.
48. **Zhuang, Y., and A. M. Weiner.** 1989. A compensatory base change in human U2 snRNA can suppress a branch site mutation. Genes Dev. **3**:1545–1552.