An enhancer screen identifies a gene that encodes the yeast U1 snRNP A protein: implications for snRNP protein function in pre-mRNA splicing

Xiaoling C. Liao,¹ Jie Tang, and Michael Rosbash,²

Howard Hughes Medical Institute, Department of Biology, Brandeis University, Waltham, Massachusetts 02254 USA

In an enhancer screen for yeast mutants that may interact with U1 small nuclear RNA (snRNA), we identified a gene that encodes the apparent yeast homolog of the well-studied human U1A protein. Both in vitro and in vivo, the absence of the protein has a dramatic effect on the activity of U1 snRNP containing the mutant U1 snRNA used in the screen. Surprisingly, the U1A gene is inessential in a wild-type U1 RNA background, as growth rate and the splicing of endogenous pre-mRNA transcripts are normal in these strains that lack the U1A protein. Even in vitro, the absence of the protein has little effect on splicing. On the basis of these observations, we suggest that a principal role of the U1A protein is to help fold or maintain U1 RNA in an active configuration.

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Pre-mRNA splicing is characterized by a pair of highly conserved transesterification reactions that remove intervening sequences from primary transcripts. In both yeast (*Saccharomyces cerevisiae*) and mammalian extracts, these reactions take place in a large complex called the spliceosome. Small nuclear ribonucleoprotein particles (snRNPs), as well as many protein factors, participate in the spliceosome assembly process and are required for pre-mRNA splicing. Each of the four splicing snRNPs (U1, U2, U4/U6, and U5) contains one or two small nuclear RNAs (snRNAs). In mammalian systems, where they are better characterized, each snRNP also contains 7–10 proteins. These include core snRNP proteins, common to all four snRNPs (e.g., Sm proteins), and snRNP-specific proteins (for reviews, see Maniatis and Reed 1987; Steitz et al. 1988; Green 1991; Guthrie 1991; Rosbash and Séraphin 1991; Ruby and Abelson 1991).

Accumulating evidence indicates that in mammals as well as in yeast, U1 is the first snRNP to interact with pre-mRNA and that this interaction is required for the subsequent binding of U2 snRNP and the U4/U6/U5 tri-snRNP (Legrain et al. 1988; Ruby and Abelson 1988; Bartkiewicz et al. 1989; Barabino et al. 1990; Lamm et al. 1991; Séraphin et al. 1991). In yeast extracts this interaction has been shown to commit the pre-mRNA to the splicing pathway (Séraphin and Rosbash 1989), and re-

cent evidence indicates that a similar situation is likely to obtain in mammalian systems (Michaud and Reed 1991; Jamison et al. 1992). All of these results suggest that U1 snRNP plays a prominent role in the early interactions between the pre-mRNA substrate and the splicing machinery. Many proteins, both snRNP and non-snRNP factors, undoubtedly mediate these interactions.

Mutant screens for yeast splicing factors have enjoyed considerable success (Ruby and Abelson 1991), yet the one identified gene that might contribute to these early assembly-recognition events (encoding a putative U1 70K protein) was identified through random sequencing of the yeast genome (Smith and Barrell 1991). To ameliorate this deficiency, we have exploited a synthetic lethal or enhancer strategy to screen for mutants that would make a viable strain containing a mutant U1 RNA die (thus, the name *mud* for *mutant-u-die*).

This general strategy has been exploited previously by other investigators (Huffaker et al. 1987; Kranz and Holm 1990; Simon et al. 1991; Frank et al. 1992) and was dictated here by several considerations. First, we were concerned that certain splicing functions might not be easily revealed in traditional mutant screens. Some factors might mutate only rarely to temperature sensitivity, and the function of others might be buffered by redundant activities. In addition, growth rate is an insensitive indicator of splicing activity (Pikielny and Rosbash 1985), and this is likely to be equally true for mutations targeted to U1 snRNP (Séraphin and Rosbash 1989). Sec-

¹Present address: Department of Microbiology and Immunology, University of California, San Francisco, California 94143.

²Corresponding author.

ond, the synthetic lethality was dependent on a mutant U1 snRNA, hopefully making it more focused on early splicing events and U1 snRNP function than more general screens for splicing defective mutants (Vijayraghavan et al. 1989). The genetic screen was augmented by an in vitro, biochemical approach; extracts were prepared from candidate mutant strains and assayed for U1 snRNP function. Among the first mutants examined, one strain was dramatically defective in U1 snRNP complex formation. Cloning and sequencing of the mutant gene identified the apparent yeast homolog of the well-studied human U1 snRNP protein, U1A. Characterization of strains deleted for the U1A gene indicated that the gene is inessential. Even in vitro, splicing and U1 snRNP activity in the absence of the U1A protein were indistinguishable from what was observed in wild-type extracts. Taken together with the synthetic lethality, these observations suggest that the U1A protein and part of the U1 RNA have overlapping functions to help fold or maintain the universally conserved structural elements of U1 RNA in an active configuration.

Results

Because the initial strain subjected to mutagenesis contained a U1 RNA gene with a deletion of the yeast core region, we hoped that this strategy might also provide some insight into the function of this domain of the yeast U1 molecule. Although neither present in vertebrates nor essential for yeast, these structural elements are conserved among U1 snRNAs from different yeast species (Kretzner et al. 1990) and contribute to yeast U1 snRNP function (Liao et al. 1990). In addition to a deletion of the yeast core region, we included a point mutation in the conserved A loop in the strain subjected to mutagenesis so as not to restrict the mutant search to proteins that might interact exclusively with the yeast core region (Fig. 1A).

In the scheme for the genetic screening of *mud* mutants (Fig. 1B), the chromosomal U1 gene was deleted. The essential U1 function is provided by two plasmids: One contains the URA3 marker and the GAL-U1 gene, and the other contains the TRP1 marker and a mutant U1 gene carrying a deletion of the yeast core region (Δ YC), combined with a point mutation of the universally conserved A loop (Fig. 1B; Liao et al. 1990). In the presence of glucose, which represses expression from the GAL-U1 gene, this merodiploid strain is strictly temperature sensitive, consistent with the previously described properties of the mutant U1 gene (Liao et al. 1990). Similarly, this strain grows at 30°C but not at 37°C when replicaplated to 5-fluoro-orotic acid (5-FOA) plates (to select for loss of the GAL-U1 plasmid) (Boeke et al. 1984).

The starting strains were mutagenized by UV or EMS and the viable colonies were replica-plated to selective medium (5-FOA). The *mud* mutants were defined by their inability to grow in the absence of the wild-type U1-containing plasmid, even at the permissive temperature (30°C). These strains were collected from the mas-

ter plates and checked for their inability to grow on glucose. From 34,000 viable colonies, 178 putative *mud* mutants, comprising at least 16 complementation groups, were defined by this strategy.

The *mud1-1* mutant

To assay for effects of the *mud* mutants on in vitro splicing, we made extracts from many of the putative mutant strains after glucose depletion of the wild-type U1 snRNA. Although incubation in glucose ultimately leads to cell death, overnight incubation, which allows 5–10 rounds of cell division, dilutes the wild-type U1 snRNPs, and promotes the in vivo assembly and accumulation of U1 snRNPs that contain only the mutant U1 snRNA. The cells then contain an effectively homogeneous mutant U1 snRNP population whose function can be assayed in vitro after extract preparation (Sérafin and Rosbash 1989; Liao et al. 1992).

Although several mutations affected U1 snRNP activity qualitatively or quantitatively, one strain (*mud1-1*) showed a striking decrease in splicing (data not shown) and in U1 snRNP-pre-mRNA complex formation (Fig. 2A). In contrast, extracts derived from the control strain showed a single major band, characteristic of strains containing the mutant U1 Δ YC (Liao et al. 1992). The lack of in vitro U1 snRNP activity was the result of the *mud1-1* mutation because the phenotype was rescued by transformation with the *MUD1* gene (Fig. 2A and see below). As the mutant U1 snRNA accumulated nearly normally during the overnight incubation in glucose in the *mud1-1* strain (Fig. 2B), we interpret the mutation as affecting U1 snRNP activity rather than U1 snRNP assembly or accumulation. This interpretation is supported by the observation that the mutant U1 snRNA is still immunoprecipitated normally by antibodies directed against the U1 70K protein (data not shown).

To assay for in vivo splicing defects, we examined several intron-containing transcripts from *mud1-1* strains after glucose depletion of the wild-type U1 snRNA population. Primer extension analysis of pre-U3A and pre-U3B RNAs (Myslinski et al. 1990; Zavanelli and Ares 1991) showed a dramatic increase in level that was *mud1-1* specific (Fig. 3, lane 2), that is, glucose depletion in other *mud* mutant backgrounds (e.g., *mud3-1*) showed no comparable pre-mRNA increase (Fig. 3, lane 1). The accumulation of pre-U3 RNA, like all of the in vitro and in vivo phenotypes of the *mud1-1* strain, was reversed by the cloned *MUD1* gene (data not shown). This indicates that the splicing defects observed after depletion of the wild-type U1 snRNA were linked to the lethal phenotype of *mud1-1*; in all likelihood, the combination of the *mud1-1* allele and the mutant U1 snRNA leads to in vivo splicing defects responsible for the lethality.

The *mud1* gene encodes the yeast U1 snRNP-specific A protein

The wild-type *MUD1* gene was cloned by rescuing the growth defect of the original *mud1-1* mutant strain (con-

Figure 1. Scheme of the mammalian and yeast U1 snRNAs. (A) The thick lines represent double-stranded RNA, the thin lines single-stranded RNA, and the solid ovals represent the RNA loops. The mutation $\Delta\text{YC}+\text{LII26A}$ is a combination of the yeast core deletion (ΔYC) and a point mutation in loop II (A loop) of yeast U1 snRNA (formerly LII-27A) (Liao et al. 1990). The deleted portion is shaded, and the point mutant is indicated with an arrow and a small open circle. This mutant U1 gene is carried in plasmid pXL18-84. (B) Schematic of the genetic screening. Two plasmids, one carrying the wild-type U1 gene under the control of GAL-UAS (GAL-U1) and the other carrying the mutant U1 gene, are shown with their selectable markers. The interactions between the gene product of the *MUD* (or *mud* after mutagenesis) locus and the two types of U1 snRNPs are represented by the two-way arrows pointing to the corresponding loci. (+) Interactions sufficient for cell growth (LIVE); (–) a failure of interaction or aberrant interactions that result in cell lethality (DIE). Extracts can be made from the strain expressing the *mud* mutation after overnight growth in glucose-containing medium, and these extracts have a nearly homogeneous mutant U1 snRNP population (see text), the composition and activity of which is under the influence of the *mud* mutation.

taining the mutant U1 RNA) with a centromere-based library. Only two sets of plasmids were recovered: one contained overlapping sequences that encoded the wild-type U1 RNA gene, and the other contained different overlapping sequences that encoded the *MUD1* gene. Subclones were generated, and a 1.5-kb *HindIII*–*HindIII* fragment was able to rescue the *mud1-1* mutation. In parallel, a linker insertion strategy was used to locate the relevant open reading frame (ORF) (Legrain et al. 1991). These linkers later served as primers to sequence double-stranded DNA in opposite directions. The entire 1.5-kb subclone of the *MUD1* gene was sequenced in both directions. The internal region of the 1.5-kb subclone contained a single long ORF with a functional intron at its 5' end (Fig. 4A).

The initiating methionine codon is located in the proper context for translation initiation (Donahue and Cigan 1990). Computer-aided searches of protein se-

quence data bases identified the human U1A protein as the only close relative of Mud1. The relationship is highly significant, and both proteins contain two RNA-binding domains (RBDs) separated by spacer regions of similar lengths (115 amino acids for yeast vs. 120 amino acids for human). In addition, the monoclonal antibody 12CA5 specifically immunoprecipitated U1 RNA from extracts derived from strains that contain a hemagglutinin (HA) epitope-tagged version of the yeast U1A protein (Wilson et al. 1984; Abovich et al. 1990). The 12CA5 antibody recognizes the 9-amino acid epitope derived from the influenza virus HA protein. The results were comparable with those obtained with an HA epitope-tagged version of the U1 70K protein (Fig. 4B).

The homology between the yeast and the human proteins begins within the $\beta 2$ region of the amino-terminal RBD and continues until the end of the protein (Fig. 4C). The second RBD is the most highly conserved portion of

Figure 2. *mud1-1* abolishes U1 snRNP activity in combination with the mutant U1 snRNA. (A) Extracts from strain Ym8 (XLY219 with the *mud1-1* mutation) and the control strains (XLY219 or Ym8 plus the cloned *MUD1* gene) were grown in glucose-containing medium for 16 hr and assayed for commitment complex assembly after U2 killing (Liao et al. 1992). The position of the commitment complexes (CC) is marked with a bracket. (+MUD1) The *mud1-1* strain transformed with the wild-type *MUD1* gene. (B) The mutant U1 snRNA accumulates normally in the *mud1-1* strain. The mutant and control strains were grown in glucose-containing medium for 16 hr before RNAs were prepared and analyzed by primer extension. The positions of the wild-type U1 snRNA (WT U1) and the mutant U1 snRNA (mutant U1) are indicated by arrows.

the protein, and there is 36% identity and 64% similarity between the yeast and the human proteins in this region. The computer searches failed to identify the very similar U2 snRNP protein U2B''. This is likely the result of the much different spacing between the two RBDs in U2B'' (66 amino acids) and the three small regions of Mud1 that are conserved with human U1A but not with U2B'' (identified in boldface type in Fig. 4C). There are no comparable regions that are more conserved with U2B'' than with U1A. On the basis of this conservation and the biochemical assays, we propose that *MUD1* encodes the yeast U1A protein.

The human U1A protein has been an object of intense study. Its amino-terminal RBD has been shown to bind to the B loop of human U1 snRNA, and the structure of this RBD has been established by X-ray crystallography (Nagai et al. 1990) and by nuclear magnetic resonance (NMR) spectroscopy (Hoffman et al. 1991). The loop 3 subregion of this RBD contributes to specificity and is substantially different in amino acid sequence between U1A and U2B'' (Scherly et al. 1990); this region of the yeast protein is more similar to U1A than to U2B'' (Fig. 4C; SRS at amino acids 68–70). There is also much more

conservation between the amino-terminal RBDs of the two proteins when amino acids known or suspected to contact the human B loop RNA sequence are examined (Scherly et al. 1990, 1991; Jessen et al. 1991); of these 13 amino acids, 8 are identical in Mud1, and 2 more have conservative amino acid substitutions. This contrasts with an overall 24% sequence identity and 47% sequence similarity between the two RBDs. These considerations suggest that the as yet undefined B loop equivalent in yeast U1 RNA may bear some resemblance to its mammalian counterpart.

Yet an optimal alignment of the two proteins generated a substantial insertion in the loop 2 region of this RBD (Fig. 4C). As no well-defined stem-loop is apparent in the general region of the yeast molecule that corresponds to the location of the metazoan B loop and as the precise B loop sequence does not exist in the yeast U1

Figure 3. *mud1-1* increases the U3 primary transcript levels in combination with the mutant U1 snRNA. The RNA samples from strains grown in glucose-containing medium for 16 hr were analyzed by primer extension using oligonucleotide DT1967: 5'-CCAAGTTGGATTCAGTGGCTC-3'. The mutant strains were XLY219, with the *mud1-1* mutation and XLY216 with a less well-characterized *mud3* mutation as shown above the lanes. The control strain is XLY219. The positions of the precursor U3 RNA (pre-U3A and pre-U3B) and mature U3 snRNA (U3) are indicated with arrows. A lower exposure indicated that there was no detectable difference in the levels of mature U3 mRNA among the three lanes.

Figure 4. Sequence analyses of the *MUD1* gene. (A) Sequence of the *MUD1* gene. The *MUD1* gene contains a functional intron with conserved splicing signals (indicated by lowercase characters). The nucleotides that are mutated in four *mud1* alleles are underlined; the mutant nucleotides are shown above the line of sequence. The mutations of *mud1-1* and *mud1-2* are identical and create a stop codon at amino acid 84 (indicated as *mud1-1*). The mutation of *mud1-3* changes the initiation codon to an isoleucine, and that of *mud1-4* changes amino acid 33 from a tyrosine to an asparagine. The bracket above nucleotides 993–1000 defines the distal breakpoint on chromosome II of the *lys2-Δ201* strains. The breakpoint cannot be defined precisely because of a direct repeat of these 7 bp in the *LYS2* gene where they mark the proximal breakpoint of *lys2-Δ201*. The broken line extending to the left of the bracket indicates that the 5' half of the *MUD1* gene is deleted in the *lys2-Δ201* strains. The numbering of *MUD1* starts from the 5' *HindIII* site. (B) The *MUD1* gene product interacts specifically with U1 snRNA. An extract containing an HA-tagged *MUD1* gene product and an extract containing an HA-tagged U1 70K gene were immunoprecipitated with the anti-HA monoclonal antibody 12CA5 as described in Materials and methods. (Lanes 1–4) Tagged U1 70K protein; (lanes 5–8) tagged Mud1 protein; (lanes 1,5) total RNA extracted from the extracts; (lanes 2,6) immunoprecipitation done at 100 mM salt; (lanes 3,7) 150 mM salt; (lanes 4,8) 200 mM salt. (C) The amino acid sequence comparison of Mud1 with the human U1A protein. The substructure of both RBDs is indicated as described previously (Nagai et al. 1990; Hoffman et al. 1991). Three groups of amino acids that are identical to human U1A but different from U2B' are highlighted with bold characters. (D) The *MUD1* gene is located in chromosome II, adjacent to the *LYS2*, *TEF2*, and *CBP6* genes. The arrows indicate the orientations of the genes. The region deleted in the *lys2-Δ201* strains is marked by vertical broken lines at the deletion boundaries. Abbreviations for restriction enzymes are (H) *HindIII*; (Ba) *BamHI*; (X) *XhoI*; (B) *BglII*; (E) *EcoRI*; (P) *PstI*.

RNA molecule, the yeast binding sequence must be different from the metazoan B loop. A different binding site is likely to account for some of the differences in sequence and structure (e.g., the large loop II) between the yeast and the human amino-terminal RBDs.

Although the regions between the two RBDs are very similar in length in both organisms, their amino acid contents appear quite different. The yeast protein is rich in positively charged amino acids, whereas the human protein is proline rich in this region. As the function of this domain is unknown, the significance of this difference is impossible to evaluate.

The high degree of conservation of the carboxy-terminal RBD is impressive and may reflect the fact that this is the most important region of the protein and that the RNA sequence to which it binds—as yet unknown—is similar in yeast and humans. This RBD may interact with a universally conserved region of U1 RNA, another snRNA, or pre-mRNA. Attempts to define its *in vitro* binding sequence have been unsuccessful, and the entire human U1A protein shows RNA-binding properties indistinguishable from its amino-terminal RBD (Lutz-Freyermuth et al. 1990; Scherly et al. 1990; Tsai et al. 1991).

To verify that the *MUD1* gene is the wild-type equivalent of the *mud1-1* locus, we sequenced the *mud1-1* gene and compared it with the *MUD1* sequence. A single-nucleotide difference was identified, creating a stop codon at amino acid 84 (Fig. 4A). As this mutation should lead to the synthesis of little or no full-length U1A protein (and given that the *mud1-1* strain grows normally in combination with a wild-type U1 gene), we considered the possibility that the gene is inessential. This possibility was supported by an analysis of several mutant strains missing chromosomal material near the yeast U1A-coding region (Schirmaier and Philippsen 1984): The sequence of the 1.5-kb U1A-containing subclone identified the yeast translation elongation factor 1 α -B gene (*TEF2*) at one end and at the other the yeast nuclear gene *CBP6* required for mitochondrial cytochrome *b* expression. This information mapped *MUD1* and *CBP6* to chromosome II, next to *LYS2* and *TEF2* (Fig. 4D). A previously reported deletion of *LYS2* (*lys2-Δ201*) was shown to remove ~7 kb of DNA, including the *TEF2* gene, that lies between *LYS2* and *MUD1*. To map the distal breakpoint precisely, we cloned the relevant DNA from several *lys2-Δ201* strains and identified the breakpoint by DNA sequencing. The data showed that the deletion removed all of the *MUD1* 5'-flanking DNA as well as the amino-terminal half of the coding region (Fig. 4A,D). In rich media, these strains grow normally (Schirmaier and Philippsen 1984), as does a deletion of the entire *MUD1*-coding region (data not shown). These results support the notion that the *MUD1* gene is inessential in the presence of a wild-type U1 gene and suggest that the *mud1-1* stop codon eliminates much and perhaps all Mud1 activity.

Consistent with the lack of an obvious growth defect of the *mud1* mutant strains containing a wild-type U1 gene, the splicing of several introns (e.g., *rp51A*, *actin*,

and U3) was not detectably affected by the *mud1-1* mutation in this background (data not shown). However, by adopting a more sensitive assay for *in vivo* splicing efficiency, we could demonstrate that U1 snRNP function was nonetheless compromised. We introduced constructs encoding intron-containing β -galactosidase genes in which enzyme activity requires precise intron removal. Two different intron-containing reporter constructs were compared: One contained an efficiently spliced intron (HZ18; Pikielny and Rosbash 1985), and the other an inefficiently spliced artificial intron (Acc; Legrain and Rosbash 1989). Although the *mud1-1* mutation had no detectable effect on the splicing of the efficient HZ18 intron (consistent with the lack of an effect on the splicing of several endogenous intron-containing transcripts; data not shown), it did reduce the splicing efficiency of the inefficient Acc intron by almost 10-fold (Table 1). Presumably, the artificial intron present in the Acc construct is able to detect a deficit in splicing activity that does not impact on efficiently spliced primary transcripts required for vegetative growth. We conclude that the absence of the U1A protein has an adverse effect on U1 snRNP function, even in concert with wild-type U1 RNA, but that this activity is not rate-limiting for the splicing of most normal pre-mRNA substrates.

To test for an effect of the absence of the U1A protein on *in vitro* splicing, extracts were made from the *mud1-1* strains that contained wild-type U1 RNA. With standard pre-mRNA substrates, splicing was indistinguishable from that of the control *MUD1* extracts, indicating that the U1A protein is not required for *in vitro* splicing (data not shown). Although U1 snRNP complex formation was also robust, examination of splicing complexes indicated that there was an assembly phenotype. The early U1 snRNP-containing complexes from a *mud1-1* background migrated with a notably faster mobility than those derived from a wild-type background (Fig. 5, lane 2). Identical results were obtained with extracts derived from *MUD1* deletion strains (data not shown). These faster-migrating commitment complexes maintained specificity for different mutant substrates, that is, the effects of 5' splice site and branch point mutations were indistinguishable from their effects on complexes derived from wild-type extracts (data not shown). The mobility difference is rescued by a Mud1-encoding plasmid, as an extract from this strain (*mud1-1* + *MUD1*) was indistinguishable from wild-type (Fig. 5, lane 1). A comparable mobility difference was apparent in U1 snRNP

Table 1. Assay of reporter gene splicing in *mud1* or *MUD1* strains that carry a wild-type U1 snRNA gene

Construct	SD5 (%)	Acc/SD5 (%)	HZ18/SD5 (%)
Wild type	100	13.0	41
<i>mud1-1</i>	100	1.4	41

The absolute values of β -galactosidase for SD5 (non-intron-containing parent plasmid) were identical for both strains (1700 units) (Legrain and Rosbash 1989) and were defined as 100%.

Figure 5. Assembly phenotype of *mud1* strain. Whole-cell extracts and a pellet fraction from the strain that carries the *mud1-1* mutation in a wild-type U1 background were assayed for commitment complex formation after U2 killing as in Fig. 2A (lanes 2,3). The extract and pellet derived from the wild-type strain were assayed in parallel (lanes 3,4), as was the extract derived from the *mud1-1* strain transformed with the cloned *mud1* gene (lane 1). The position of the commitment complexes (CC) is marked with a bracket.

complexes derived from a 0.5 M KCl pellet fraction, which contains active U1 snRNPs but no loosely associated proteins (Fig. 5, lane 5). As a direct assay of U1 snRNPs (by blotting snRNP gels with a U1 snRNA probe) gave rise to a similar mobility difference (data not shown), all of these observations are most easily accounted for by the absence of a core U1 snRNP protein in the *mud1-1* extracts.

Discussion

The first gene identified in this synthetic lethal search was the yeast U1A protein. Surprisingly, the gene is inessential in a wild-type U1 RNA background, in vitro as well as in vivo. This finding validates the synthetic lethal strategy for identifying U1 snRNP (or U1 snRNP-interacting) proteins, as it is very unlikely that this gene would have been identified with a more traditional mutant screen in a wild-type background.

Taken together, our observations suggest either that yeast U1A is not essential for splicing or that another as yet unidentified gene provides a redundant function. We view the latter possibility as less likely based on the

following reasons. First, we have been unable to detect the presence of another related gene by low stringency Southern hybridization to the cloned U1A gene (data not shown). Second, the *mud1-1* strain (with wild-type U1 snRNA) had a splicing defect as assayed with the inefficiently spliced Acc reporter gene construct (Table 1); the presence of a truly redundant function would provide for wild-type splicing activity in a U1A-deletion strain. Third, the U1 snRNPs from the *mud1-1* strain display a uniform mobility shift (Fig. 5) that is easily explained by the absence of one or more snRNP proteins. Identical data were obtained from all of the other deletion strains. We cannot, however, rule out the possibility that there is a loosely associated protein that substitutes for U1A. In any case, the observation that a well-characterized and conserved snRNP protein is inessential suggests that many factors important for splicing may prove to be inessential as defined genetically or biochemically.

Paradoxically, the issue of functional redundancy may be relevant to the synthetic lethal interaction that originally defined the yeast U1A gene. On this basis, it is possible that the U1A protein and the U1 snRNA gene provide somewhat overlapping functions. For example, regions of the U1 RNA and the U1A protein may both function to help fold or maintain the U1 snRNA molecule in an active conformation.

The original U1 snRNA mutant used to identify *mud1-1* contained a yeast core deletion as well as an A loop point mutation (Fig. 1A). The lethal interaction with *mud1-1* did not require both U1 snRNA mutations and was seen with the yeast core deletion alone, that is, *mud1-1* was lethal in combination with a U1 snRNA that only carried the yeast core deletion and was viable in combination with a U1 snRNA that only carried the A loop point mutation (data not shown). On this basis, we propose that a major function of the “extra” information in the 568-nucleotide yeast U1 RNA is to help fold or maintain the universally conserved elements of the molecule in an active configuration. Although structural information will be required to support this suggestion, the reasoning suggests that the large yeast U1 RNA molecule might be somewhat less dependent on protein factors than its smaller vertebrate counterpart. A consistent, but not compelling, argument comes from experiments in *Xenopus* showing that the U1 snRNA B loop sequence (i.e., the binding site for the U1A protein) is critical for U1 snRNP function (Hamm et al. 1990). In any case, the inessential nature of the yeast U1A protein and its synthetic lethal interaction with the yeast core deletion suggest that a function of the U1A protein is to help the U1 snRNA achieve an active configuration.

The proposed function implies that snRNPs will follow principles established from some better understood systems of RNA–protein interactions such as ribosomes and group I introns. The data are now quite compelling that one function of ribosomal proteins is to aid the formation of a particular structure in a region of rRNA (Noller et al. 1992). Moreover, several *Escherichia coli* and yeast ribosomal proteins are inessential; in their absence, cell growth is normal with only subtle phenotypic

effects (Dabbs 1986; Woolford and Warner 1991). In the case of different group I introns, there is a common core structure but often different peripheral structural elements. The role of at least some of these peripheral elements is to help fold the catalytic core into an active configuration (Celander and Cech 1991; Jaeger et al. 1991; van der Horst et al. 1991; Michel et al. 1992). Proteins also assist; for example, the *Neurospora* CYT-18 protein helps to stabilize the core in a conformation required for catalytic activity (Guo and Lambowitz 1992; Mohr et al. 1992). In this view, one can imagine that both the U1A protein and the "extra" RNA of yeast U1 snRNA perform analogous functions to stabilize the conserved regions of U1 snRNA in an optimal configuration.

Materials and methods

Strains used in mutagenesis

The *mud1-4* allele was obtained from an early pilot experiment after UV mutagenesis of the strain XLY140 (*MAT α* , *trp1-289*, *ura3-52*, *leu2-3*, *leu2-112*, *his3- Δ 1*, *cyh^R*, *snr19::LEU2*, pXL46, pXL18-84), which differs from XLY216 by the configuration of the genes at the *snr19* locus. The strains used in the mutagenesis were XLY219 [*MAT α* , *trp1-289*, *ura3-52*, *leu2-3*, *112*, *arg4* (*RV⁻*), *ade2*, *snr19 Δ ::ADE2*, pXL46, pXL18-84] and XLY216 [*MAT α* , *trp1-289*, *ura3-52*, *leu2-3*, *112*, *his3- Δ 1*, *cyh^R*, *snr19 Δ ::ADE2*, pXL46, pXL18-84], where pXL46 is the URA3-GAL-U1 plasmid.

Mutagenesis and genetic screen

The starting strains were grown in Trp⁻Ura⁻ + 3% galactose + 1% sucrose medium before mutagenesis. UV mutagenesis was carried out with a UV lamp (UV Stratalinker 1800, Stratagene) on individual cells plated on the Trp⁻Ura⁻ + galactose plates. Ethylmethane sulfonate (EMS) mutagenesis was carried out in cell suspension according to standard protocols (Lawrence 1991), and mutagenized cells were plated immediately on the plates described above. The viable colonies (5–15% of the mutagenized cells) on the master plates were replica-plated to 5-FOA plates and grown at 30°C. The master plates and replica plates were compared, and the missing colonies on the replica plates were identified as having candidate mutations that synergize with the mutant U1 snRNA to cause a lethal phenotype. The corresponding mutant colonies (called *mud* mutants) were collected from the master plates and examined for their auxotrophic markers and their inability to grow in medium containing glucose. The *mud1-1* and *mud1-2* alleles were obtained after UV mutagenesis of the strain XLY219, whereas the *mud1-3* allele was recovered from EMS mutagenesis of the same strain.

In vitro spliceosome and commitment complex assembly

The strains were grown in medium containing 4% glucose for 16 hr before RNA or extracts were prepared. The extracts were prepared using a modified miniextract protocol (Séraphin and Rosbash 1989; Abovich et al. 1990). In vitro spliceosome assembly was performed according to standard protocols (Séraphin and Rosbash 1991), and the U2 killing of extracts and pellet fractions was as described (Liao et al. 1992). For gel assays, each figure comes from a single gel and autoradiographic exposure. The primer used for reverse transcription of U1 snRNA is DT13, which is complementary to nucleotides 535–551 of U1 snRNA.

Cloning of the wild-type MUD1 gene and mapping of *mud1* mutants

The original *mud1-1* mutant (Ym8) was used as the host for transformation with a wild-type yeast library based on the LEU2-CEN vector p366. Transformants were selected on Leu⁻ plates containing glucose and confirmed by replica-plating to 5-FOA. Total RNA was made from independent transformants after 5-FOA selection, and primer extension of U1 snRNA was employed to distinguish the strains that were rescued by the *MUD1* clones, which would express only mutant U1 snRNA, from those rescued by the wild-type U1 snRNA gene, which would express both the full-length U1 snRNA and the mutant U1 snRNA. The *MUD1* clones were recovered from yeast by transforming *E. coli*. To prove the existence of a functional intron and to map the precise splice sites, total RNA from a wild-type strain DBY745- Δ A was reverse transcribed and PCR amplified with primers DT2325 and DT2311, which are identical to nucleotides 425–444 and complementary to nucleotides 706–723, respectively (Fig. 4A). For the sequencing of the four *mud1* alleles, yeast DNA from these *mud1* mutant strains was amplified and sequenced by PCR using primers DT2312 and DT2313, which are identical to the first 18 nucleotides and complementary to the last 18 nucleotides of the 1.5-kb *HindIII* fragment (see Fig. 4A), respectively. The *lys2- Δ 201* and the control *LYS2* strains were a kind gift from Cora Ann Styles in G. Fink's laboratory (Whitehead Institute, MIT, Cambridge, MA). These strains are L983 (*LYS2*), L1535 (*lys2- Δ 201*), L3282 (*lys2- Δ 201*), and L4576 (*lys2- Δ 201*). The deletion junction of *lys2- Δ 201* was cloned from the three *lys2- Δ 201* strains by PCR using primer DT2428 for the *LYS2* gene (5'-CCAGCAGGCATGACATCT-3') and DT2323, which is complementary to nucleotides 1190–1213 of *MUD1*. DT2323 was also used for sequencing the deletion junction.

Fractionation of extracts

Extracts were fractionated by centrifugation, as in Abovich et al. (1990), except (1) the KCl was at either 50 mM (the normal concentration in the extract, called low salt) or 500 mM (called high salt); (2) the centrifugation was for 4 hr; and (3) buffer D contained 0.05% NP-40 for resuspending the pellets.

Immunoprecipitation

Strains were constructed containing HA-tagged versions of the U1 70K gene and the *MUD1* gene essentially as described previously for the *PRP6* gene (Abovich et al. 1990). In both cases, the epitope was placed at the carboxyl terminus of the protein, and the tagged gene was used to complement a gene knockout. Extracts were made from these two strains, and the 12CA5 antibody was used to immunoprecipitate the tagged proteins from the extracts. RNA was extracted from the precipitates and subjected to primer extension using the primer DT58 for U2 snRNA and DT586 for U1 snRNA (Liao et al. 1990). Extract preparation, immunoprecipitation, RNA extraction, and primer extension were as described (Abovich et al. 1990).

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X C Liao, J Tang and M Rosbash

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