PRP38 Encodes a Yeast Protein Required for Pre-mRNA Splicing and Maintenance of Stable U6 Small Nuclear RNA Levels

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PRP38 encodes a cellular polypeptide that is thought to act in conjunction with the other known snRNP factors and U1 snRNA to mediate nuclear pre-mRNA splicing. In vitro splicing inactivation and complementation analyses reveal that PRP38 inhibits 5' splice site cleavage and 3' splice site ligation by altering snRNP structure and composition. PRP38 appears to act in the intracellular processing of U6 snRNA. The details of PRP38's in vitro splicing function suggest that the PRP38-encoded polypeptide plays an additional role in the stability of U6 snRNA.
creases in U6 snRNA abundance. In light of this observation, we propose that snRNA instability is a hallmark of mutations that perturb snRNP structure. Our data suggest that PRP19 and the newly defined PRP38 gene encode proteins that interact (directly or indirectly) with at least one of the U6 snRNA-containing snRNP complexes required for pre-mRNA splicing.

**MATERIALS AND METHODS**

**Yeast strains.** Genotypes of the strains used are shown in Table 1. Strains MGD353 46D and 13D were obtained from B. Seraphin and M. Rosbash, prp-211 and prp17-27 strains were provided by J. Woolford and J. Abelson, respectively.

**Genetic studies.** (i) **Mutagenesis of yeast strains and selection for temperature sensitivity.** A stationary-phase culture of MGD353 46D was mutagenized to 10% survival in phosphate buffer containing methanesulfonate as previously described (49). The mutagenesis was quenched with 5% sodium thioglycolate, and the cultures were immediately frozen in aliquots at −80°C. Cells from the mutagenized culture were grown under nonselective conditions (23°C, YEPD broth) for one to two generations (3 h) before plating on a master plate of YEPD agar (23°C). Temperature-sensitive mutants were identified as colonies from replica transfers that failed to grow at the restrictive temperature (37°C).

Complementation analysis, sporulation, and tetrad dissection were performed by standard techniques (49). Diploids were selected by scoring for nutritional markers (see above), and complementation of the splicing defect was assayed by monitoring the growth of diploid strains on YEPD media at 23 and 37°C. Ascii were digested for 30 min in 0.5 mg of Zymolyase 100T (ICN) per ml-M sorbitol-0.1 mM EDTA and dissected on YEPD agarose.

(ii) **Isolation of PRP38.** Yeast strains 192 were transformed by the lithium acetate procedure of Ito et al. (20) as previously modified (49) with 10 μg of a yeast DNA library on the centromeric plasmid vector YCP50 (39). The yeast cells were plated on complete medium lacking uracil for 24 h at room temperature (to allow expression of the plasmid-encoded genes) and then shifted to 37°C to identify transformants with complementing DNA segments. Plasmids were recovered from these cultures, amplified in *Escherichia coli*, and reintroduced into ts192 and other mutant prp cultures to demonstrate plasmid-dependent, gene-specific complementation.

Linkage of the cloned 5.0-kbp **PvuII** fragment with **PRP38** was established by inserting the fragment into the HindIII and EcoRI-cleaved, Klenow enzyme-blunted (43) sites of the **URA3**-containing integrating vector Yip21 (16). This DNA was then cleaved with HindIII and targeted to its corresponding chromosomal location in ts192 cells by transformation. The fidelity of the integration event was confirmed by Southern blot. Several yeast transformants were mated with MGD353 13D, the diploids were sporulated, and the segregation pattern of the temperature-sensitive and splicing phenotypes was monitored.

Disruption of the **PRP38** open reading frame was accomplished by inserting the 2.2-kb **SalI**-**XhoI** LEU2 fragment (6) into the **KpnI** site of an XbaI-FseI **PRP38** subclone on plasmid pTZ19R (U.S. Biochemical [USB]). Both the vector and insert DNAs were rendered blunt ended by treatment with mung bean nuclease as instructed by the manufacturer (USB) prior to joining with T4 DNA ligase. The **LEU2**-disrupted **PRP38** gene was excised with HindIII and PsI and used to transform diploid yeast strain MGD407. Leu+ transformants were screened for the presence of the correct insertion event by Southern analysis.

**DNA and RNA manipulations.** DNA sequencing was performed by the dideoxy-chain-termination method (44) with Sequenase DNA polymerase (USB). Single-stranded DNA templates were prepared from the **pTZ18** and **pTZ19** plasmid vector series as instructed by the manufacturer (USB). Both strands of **PRP38** were sequenced by using subclones derived from the available restriction sites (**HindIII**, **KpnI**, and **PstI**), using synthetic DNA oligonucleotides. M13 reverse and −40 DNA sequencing primers were purchased from USB; all other oligonucleotides used for DNA sequencing and mutagenesis (24) were prepared by the University of Kentucky DNA synthesis facility. The sequences (‘5’ to ‘3’) of the **PRP38**-specific sequencing primers were as follows: (i) **TTTGGTGTATACATATTCAAGA**, (ii) **TACTGCGACGTGAATATA**, (iii) **GGGACTCACGTCCTGGAATGC**, (iv) **CTTAAGAGATCGGTCAAA**, and (v) **TGAAGTGTATACGGAATCGCAG**. The mutagenesis oligonucleotide [**GTGTAGGAG(T/C)(G/A)AGCAGAG(T/C)(G/A)GTCTACTTG**] was also used for sequence analysis.

Total yeast RNA was isolated from mechanically disrupted cell lysates according to previously published procedures (36) and fractionated on a 5% polyacrylamide–8 M urea gel or on a 1% agarose–formaldehyde gel. snRNA probes were prepared by random priming DNA fragments containing the following snRNA genes: U1 (polymerase chain reaction fragment of coding nucleotides 1 to 569 of **SNR19**), (23, 51; U2 (Clal fragment of **LSR1**), (2); U4 (**EcoRI-EcoRV** fragment of **SNR4**), (50), U5, (**HpaI-NcoI** fragment of **SNR7**), (34), and U6 (**TaqI** fragment of **SNR6**). (7). Plasmid **SPRP51A** (41) was labeled as an intron-plus-exon DNA probe. Autoradiograms were quantified by using a LKB 2400 gel scanner.

In vitro splicing. Yeast whole cell lysates were prepared according to the method of Lin et al. (28). In vitro splicing reactions were assembled, and the results were analyzed as described previously (37), using −1 ng of **RP51a**-derived pT7Δ2 RNA (42).

**TABLE 1. Yeast strains**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
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<tr>
<td>MGD353 46D</td>
<td>MATα ura-3-52 leu2-3,112 his3 cyh1</td>
</tr>
<tr>
<td>MGD353 13D</td>
<td>MATα ura-3-52 ade2 leu2-3,112 arg4 trpl-289</td>
</tr>
<tr>
<td>MGD407</td>
<td>MATα/a ura-3-52 leu2-3,112</td>
</tr>
</tbody>
</table>

| ts192     | MATα prp38-1 ura3-52 leu2-3,112 his3 cyh1 |
| ts146     | MATα prp4 ura3-52 leu2-3,112 his3 cyh1 |
| ts368     | MATα prp2-1 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts125     | MATα prp-3 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts308     | MATα prp4 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts108     | MATα prp5-1 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts166     | MATα prp6-1 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts226     | MATα prp7-2 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts219     | MATα prp8-1 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts237     | MATα prp9-1 ade2 ade2 ura1 tyr7 his7 lys2 gal1 |
| ts514     | MATα prp16-2 ade2-101 his3 200 ura3-52 tyr1 |
| ts365     | MATα prp17 ade2-101 his3 200 ura3-52 lys2-801 |
| ts503     | MATα prp18 ade2-101 his3 200 ura3-52 lys2-801 |
| ts87c     | MATα prp19 ade2-101 his3 200 ura3-52 lys2-801 |
| ts319     | MATα prp20 ade2-101 his3 200 ura3-52 tyr1 |
| ts47      | MATα prp21 ade2-101 his3 200 ura3-52 tyr1 |
| ts107     | MATα prp22 ade2-101 his3 200 ura3-52 tyr1 |
| ts344     | MATα prp24 ade2-101 his3 200 ura3-52 lys2-801 |
| ts397     | MATα prp25 ade2-101 his3 200 ura3-52 lys2-801 |
Heat inactivation of the temperature-sensitive extracts was performed by incubating 4 µl of extract in dialysis buffer (28) for 20 min at 34°C. Control reaction mixtures were assembled and incubated at 23°C. Micrococcal nuclease (MN) digestions (10) and N-ethylmaleimide (NEM) treatment (22) were performed as previously described, using MGD353 46D whole cell extracts. Complementation studies were performed by mixing ~40 µg (protein) of MN-, NEM-, or heat-treated (10 min, 50°C) MGD353 46D extract with an equal amount of heat-inactivated ts192 extract prior to addition of pre-mRNA. All splicing reactions were performed at 23°C.

**Nucleotide sequence accession number.** The PRP38 DNA sequence has been deposited in the GenBank data base and assigned accession number M95921.

**RESULTS**

Identification of a conditional lethal splicing mutant. The ts192 mutant was isolated from a bank of yeast mutants temperature sensitive for growth and for the correct processing of RP51a pre-mRNA. When assayed at 23°C, the ts192 and wild-type strains exhibited comparable growth characteristics and RP51a RNA profiles (Fig. 1A and B). In contrast, at 37°C, the ts192 cells selectively stopped growing, RP51a mRNA levels dropped, and an RP51a RNA form of lesser electrophoretic mobility accumulated. This new RNA species comigrated with RP51a pre-mRNA isolated from a prp2 splicing mutant under the same conditions. Primer extension analysis of RNA from ts192 cells transformed with the well-characterized RP51a-lacZ fusion gene HZ18A2 (36) confirmed that the novel RP51a RNA accumulating at 37°C is unspliced precursor (Fig. 1C). The extent of the change in RNA pattern varied somewhat between experiments (compare Fig. 1B and C) but was consistently observed and limited to polymerase II genes having introns. Transcripts from the intron-containing RP51a, ACT1, CYH2, and RPS10 genes each displayed the characteristic shift in electrophoretic mobility associated with increased pre-mRNA levels, while no changes were noted for the intronless CYC1, ADE3, and SS rRNA transcripts (data not shown). Thus, at the restrictive temperature, ts192 cells are specifically inhibited in the conversion of pre-mRNA into splicing intermediates and products.

The ts192 strain defines a new gene, PRP38. The simplest explanation of the conditional lethal phenotype of ts192 cells is that growth arrest at 37°C is a direct consequence of the defect in pre-mRNA splicing. Consistent with this interpretation, the temperature-sensitive growth and splicing deficiencies meiotically cosegregated in eight dissected ascii (data not shown). Furthermore, a single cloned DNA segment simultaneously relieved both defects when introduced by transformation (Fig. 1A and data not shown). Complementation analysis performed between the ts192 mutant and the previously defined splicing mutants (prp2-11 and prpl7-24) demonstrated that the ts192 defect resides in a distinct chromosomal locus which we have named PRP38.

**PRP38 encodes a yeast splicing factor.** The PRP38 gene product may influence pre-mRNA splicing directly by controlling the assembly or function of the splicesosome or indirectly by regulating the expression of other splicing factors, e.g., by modulating the transcription or translation of genes encoding components of the splicing apparatus. Proof that several PRP genes act directly by encoding bona fide splicing factors was provided, in part, by the demonstration that cell extracts prepared from mutant cultures were temperature sensitive for splicing (31). Relative to extracts prepared from the parental strain, prp38 extracts are sensitive to heat inactivation (Fig. 2 and 3A); a 20-min incubation of the prp38 extract at 34°C reduced splicing to less than 30% of that achieved for wild-type extracts (Fig. 3A, lanes 7 to 9 and 10 to 12) or the untreated mutant extract (Fig. 2, lanes 1 and 2; Fig. 3A, lanes 4 to 6 and 10 to 12). Splicing activity was recovered, albeit incompletely, if the heat-inactivated mutant extract was complemented with an MN-treated wild-type extract (Fig. 2, lane 4) but not if the complementing activity was heat inactivated at 50°C (Fig. 2, lane 6) or was pretreated with NEM (Fig. 2, lane 9). Similar success in reconstituting the heat-inactivated prp38 extract was achieved by using a heat-inactivated prp2 or prp16 extract (data not shown). These data suggest that PRP38...
encodes a protein that directly contributes to spliceosome assembly or activation.

In contrast to the marked drop in splicing efficiency, spliceosome assembly appeared largely unaffected by preincubation of the prp38 extract at 34°C (Fig. 3). The time of appearance and relative amounts of the prespliceosome (complex III) and the complex I intermediate were comparable in the heat-treated and control samples. Although not well resolved on this gel, a complex comigrating with mature spliceosome (complex II [37]) was detected in heat-treated prp38 extracts (41a). We note, however, that the temperature-imposed splicing block was not absolute; limited splicing did occur in the heat-treated extracts. Furthermore, as the denatured (splicing-impaired) ts prp38 protein remained present during the assembly reaction, residual (or partial) PRP38 activity may have contributed to the formation of the observed complexes. Nevertheless, these results suggest an essential function for PRP38 late in spliceosome assembly or directly during splicing.

Isolation of PRP38. The wild-type PRP38 allele was isolated from a yeast DNA plasmid library (39) by in vivo complementation of the ts192 growth defect. Three different complementing plasmids were obtained, each containing a 5.0-kb Pvull fragment within a larger (15- to 20-kb) yeast DNA insert. This 5.0-kb fragment was subcloned from one isolate and found to complement the ts192 growth defect (Fig. 1A and 4).

To demonstrate that the cloned DNA contained the wild-type allele of PRP38 and not an extragenic suppressor, we mapped its chromosomal location relative to the ts192 lesion. The 5.0-kb Pvull fragment was subcloned into the URA3-containing integrating vector Ylp21 (16) and targeted to its resident chromosomal location in ts192 (ura3) cells (see Materials and Methods). Diploids were constructed by mating the resultant Ylp21/Pvull transformant with the splicing-competent strain MGD343 13D. In 10 dissected tetrads derived from this cross, the Ura+ phenotype segregated 2:2 and no temperature-sensitive spores were found. Thus, the integrated cloned DNA was tightly linked to the ts192 lesion and ostensibly contains wild-type PRP38 DNA.

The PRP38 gene was localized by additional complementation analysis to a DNA fragment containing an open reading frame of 726 bp, sufficient to encode a 242-amino-acid, 28-kDa protein (Fig. 5). The importance of this open reading frame was tested by site-directed mutagenesis. Two mutant alleles were created; in one, a base pair deletion was introduced at nucleotide position 846. This change creates a termination codon in the next position (amino acid Val172→STOP). In the second mutant allele, the LEU2 gene was inserted at a KpnI site within codon 66. Both mutant constructs failed to complement the ts prp38 lesion. Furthermore, when the LEU2 insertion derivative was used to replace the wild-type allele in a gene substitution experiment (see Materials and Methods), no viable Leu+ spore products were found in 15 dissected tetrads, thereby establishing PRP38 as an essential yeast gene.

PRP38 structure. The DNA sequence of PRP38 provides little clues as to its regulation or function. A putative TATA-box element is present 67 bp 5' to the presumed site of PRP38 translational initiation. The sequence context of this
FIG. 5. DNA and deduced protein sequences of *PRP38*. The putative TATA-box element and positions of the in vitro-generated single-base deletion and LEU2 insertion are underlined. The four cysteines and the acidic serine-rich regions of the protein are italicized.

initiating codon, ACTACAATGCGCT, is a suboptimal fit to the consensus sequence (A/T)A(A/C)AA(A/C)ATGTG(U/C) of well-expressed yeast genes (19). No intron consensus elements are found within *PRP38*. A comparison of the *PRP38* DNA sequence with the GenBank data base revealed that base pairs 1 to 524 were previously reported 3′ of *YMR26* (21), a nuclear-encoded mitochondrial ribosomal protein gene located on chromosome VII or XV.
Consistent with the NEM sensitivity of the PRP38-complementing activity, four cysteines are present in PRP38 (amino acids 87, 174, 184, and 209). At least one of these cysteines is dispensable for function, as mutant alleles encoding variants in which cysteine 174 is changed to either a histidine or a tyrosine fully complement a prp38 null mutation (20a). The most remarkable feature of PRP38 is its highly acidic, serine-rich C-terminal region; of the final 26 amino acids, 5 are aspartic acid, 6 are glutamic acid, and 11 are serine. This segment is responsible for the acidic character of PRP38; removing the terminal 26 amino acids increases the predicted pI from 5.0 to 8.1. The codon distribution of the C-terminal serines is typical of the protein as a whole and characteristic of poorly expressed genes; 8 of 11 are either AGT or AGC, very infrequent codons in yeast genes (48).

Inactivation of several PRP proteins alters the snRNA profile. The heat inactivation of PRP38 presumably perturbs its structure. Such a change might cause PRP38 or interacting components of the splicing apparatus to turn over at an accelerated rate. As a means of characterizing the splicing block imposed by prp38 and other prp mutations, we determined whether heat inactivation of their respective proteins altered the normal intracellular snRNA profile.

Beyond a slight decrease in the yield of U6 snRNA from the prp3 and prp4 strains and a somewhat lower yield of U2 snRNA from our wild-type strain and its prp4' and prp38 derivatives, no reproducible snRNA variation was observed at 23°C (Fig. 6A). In contrast, when prp38, prp3, prp4, prp6, prp19, and prp24 cultures were shifted to 37°C for 4 h, U6 snRNA levels dropped to 5 to 30% of the wild-type level (Fig. 6B; for unknown reasons, prp7 U6 snRNA levels were variable between experiments and in this example are exaggerated because of sample underloading). U6 snRNA reduction for this mutant set was observed in three separate experiments; similar observations for prp4 strains have been noted independently by Abovich and Rosbash (1a). U4 snRNA levels did not change even in strains in which U6 loss was almost complete (e.g., prp3 and prp4). The snRNA probe for prp26 and the intron-accumulating strains prp26 and prp27 (55) were also assayed and found to be similar to that of the wild type (data not shown). No other temperature-dependent changes in snRNA abundance were noted except for a decrease in the level of U5L snRNA in the prp8 strain.

The magnitude of U6 loss in the prp mutants did not correlate with the severity of the splicing defect. When the Northern (RNA) transfer filter used for snRNA analysis in Fig. 6B was probed with RPS5a, virtually no processed RPS5a mRNA was detectable in the prp2 and prp35 samples, indicating a severe splicing block (Fig. 6C), yet in these same cultures, U6 snRNA levels were comparable to that of the wild type. In contrast, the prp19 block to splicing was incomplete (i.e., comparatively low pre-mRNA/mRNA ratio [36]), yet U6 levels decreased markedly. Therefore, U6 snRNA levels are sensitive to particular RNA processing blocks and not simply responding to the loss of splicing competence.

**DISCUSSION**

The eukaryotic pre-mRNA splicing apparatus consists of five snRNAs and a complex, poorly defined array of proteins (17, 18). This study shows that a 28-kDa acidic protein, PRP38, is an essential splicing factor and, like PRP3, PRP4, PRP6, PRP19, and PRP24, is necessary for both intron excision and the maintenance of normal U6 snRNA abundance.

In vivo, loss of PRP38 function correlates with an increase in the pre-mRNA/mRNA ratio of intron-containing trans- 
scripts and a decrease in intracellular U6 snRNA. Time course studies indicate that U6 loss is a consequence, rather than a primary cause, of the initial prp38 splicing block. Decreased splicing efficiency, as evidenced by a 5- to 10-fold increase in the pre-mRNA/mRNA ratio, is evident 1 to 2.0 h after a temperature shift. In contrast, U6 snRNA abundance remains virtually unchanged during this period (58a).

While the loss of PRP38 activity might lower U6 levels through either decreased synthesis or accelerated decay, we favor the latter interpretation for three reasons. First, the measured rate of U6 snRNA turnover is slower than that observed with the prp38 strain. Blocking U6 synthesis by inactivating a temperature-sensitive polymerase III mutant results in only a minor decrease in U6 snRNA after 5 h (33).
Second, multiple means of U6 destabilization clearly exist, as intracellular U6 snRNA levels drop in response to inactivation of any one of at least five other PRP gene products (PRP3, PRP4, PRP6, PRP19, and PRP24). With the possible exception of the untested PRP19, each of these proteins functions by binding to a U6 snRNA-containing snRNP complex, that is, U6, U4/U6, or U4/U6/U5 (1, 3, 5, 35, 47, 59). Reported genetic or biochemical links between these products, e.g., suppression of a prp4 mutation by overexpression of PRP3 (25) or failure of heat-inactivated prp3 and prp4 extracts to complement one another (31), may relate to their joint contributions to U6 snRNA stability. Finally, it is clear from our in vitro studies that PRP38 functions, at least to support splicing, in the absence of appreciable transcription.

With extended incubation at the restrictive temperature, less U6 is recovered from prp38 mutant cultures, decreasing to 20 to 30% of the wild-type level 4 h after the temperature shift. We interpret these data to indicate that the initial effect of the prp38 lesion is to arrest splicing in a state that ultimately compromises the integrity of a U6-containing snRNP particle. The concordance of a rapid (<4 h) and extensive (>70%) reduction of U6 snRNA with strains bearing mutations in known U6 (or U4/U6)-associating proteins suggests a direct PRP38/U6 association. However, it is also possible that U6 snRNA is simply the most labile of the spliceosome-associated snRNAs and shows the greatest sensitivity to general spliceosomal or snRNP perturbation. Under different laboratory conditions (e.g., higher temperatures, longer periods of inactivation, and complete metabolic depletion), the removal of splicing factors other than those deemed sensitive in this study may result in U6 snRNA loss. Indeed, Brown and Beggs have observed U4 and U6 as well as U5 snRNA decreases in cells with diminished PRP8 activity (7a). While it is not yet clear why we do not see this prp8 effect on U4 or U6, their result is consistent with the general view that the removal or inactivation of an snRNP-specific polypeptide, that is PRP8/U5, can directly impact the stability of the associated snRNA and that U6 snRNA is sensitive to snRNP (presumably U4/U5/U6 tri-snRNP) perturbation.

In vitro, the prp38 block to splicing appears to occur late in the maturation of the splicing complex or during the splicing reaction itself; spliceosome assembly progresses efficiently to the formation of a structure similar to the complete spliceosome, yet splicing progresses poorly. While we have not analyzed the snRNA content of these complexes, spliceosomes stalled after the dissociation of U4 snRNA might present particularly vulnerable targets for U6 snRNA degradation. U4 release by itself appears insufficient to promote U6 degradation, however, as splicing-defective prp2 spliceosomes release U4 snRNA (60) and yet U6 is not essentially unstable in cells bearing this mutation.

The intracellular U6 snRNA concentration is normally at least twofold greater than that of U4 snRNA (11, 50, 59). This bias is expected to drive the formation of U4/U6 snRNA hybrids, and indeed, relatively little free U4 snRNA is apparent in yeast cell extracts (11, 50). Nevertheless, as noted for heat-treated prp3 and prp4 cultures, U4 snRNA levels remain constant even in the virtual absence of U6 snRNA. The U4 snRNP liberated after U6 snRNA degradation may be similar to the U4 snRNP released during normal spliceosome maturation. Regardless, it is clear that U4 snRNA stability is not obligatorily linked to the presence of U6 snRNA.

PRP38 terminates in a very acidic, serine-rich segment. The carboxy terminus is apparently needed for protein stability or function, as a frameshift mutation designed to generate a truncated, two-thirds peptide fails to complement the ts prp38 allele. Acidic regions are found in a variety of nuclear proteins (14), although the significance of such domains remains obscure. The yeast NSR1 protein (26) contains a serine-rich acidic domain similar to but more extensive than that found in PRP38 (Fig. 7). NSR1 is a 67-kDa nuclear protein, identified by virtue of its ability to bind nuclear localization sequences. Intriguingly, NSR1 also contains a pair of RNP consensus sequences, suggesting that it may function in the trafficking of RNPs. Whether mediated by the acidic serine-rich segment or not, nuclear localization and RNP association are characteristics likely to be shared between PRP38 and NSR1.

Recent studies on snRNP structure have revealed remarkable complexity in the protein composition of the various U6-containing complexes (4); also see references in references 17, 18, and 46). Protein-protein interactions appear to mediate complex assembly and disassembly. For example, the human U4/U6/U5 snRNP contains five proteins, including a 27-kDa peptide similar in size to PRP38, required for the assembly of the U4/U6/U5 snRNP but not stably bound to either the U4/U6 or U5 snRNP (4). It remains to be seen whether PRP38 also participates in U4/U6/U5 snRNP biogenesis or, like the acidic peptides of the ribosome (reviewed in reference 58), associates with assembled structures to promote or regulate RNP function.

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