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Elevated Levels of a U4/U6.U5 snRNP-Associated Protein, Spp381p, Rescue a Mutant Defective in Spliceosome Maturation

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Received 13 May 1998/Returned for modification 2 July 1998/Accepted 22 September 1998

U4 snRNA release from the spliceosome occurs through an essential but ill-defined Prp38p-dependent step. Here we report the results of a dosage suppressor screen to identify genes that contribute to PRP38 function. Elevated expression of a previously uncharacterized gene, SPP381, efficiently suppresses the growth and splicing defects of a temperature-sensitive (Ts) mutant prp38-1. This suppression is specific in that enhanced SPP381 expression does not alter the abundance of intronless RNA transcripts or suppress the Ts phenotypes of other prp mutants. Since SPP381 does not suppress a prp38::LEU2 null allele, it is clear that Spp381p assists Prp38p in splicing but does not substitute for it. Yeast SPP381 disruptants are severely growth impaired and accumulate unspliced pre-mRNA. Immune precipitation studies show that, like Prp38p, Spp381p is present in the U4/U6/U5 tri-snRNP particle. Two-hybrid analyses support the view that the carboxyl half of Spp381p directly interacts with the Prp38p protein. A putative PEST proteolysis domain within Spp381p is dispensable for the Spp381p–Prp38p interaction and for prp38-1 suppression but contributes to Spp381p function in splicing. Curiously, in vitro, Spp381p may not be needed for the chemistry of pre-mRNA splicing. Based on the in vivo and in vitro results presented here, we propose that two small acidic proteins without obvious RNA binding domains, Spp381p and Prp38p, act in concert to promote U4/U5.U6 tri-snRNP function in the spliceosome cycle.

Pre-mRNA splicing occurs through a pair of transesterification reactions catalyzed by a complex enzyme, the spliceosome (reviewed in references 19, 23, and 26). Each round of intron removal progresses through an evolutionarily conserved cycle of subunit addition, catalysis, and subunit release from the surface of the splicing substrate. The great specificity of splicing is derived largely from the precise interaction of requisite intron consensus sequences with the dynamic spliceosomal apparatus that functions indirectly in this process, perhaps by facilitating substrate presentation or through the recruitment or activation of a helicase activity.

In this study, we used the conditional lethal mutant prp38-1 to screen for spliceosomal factors that interact with Prp38p. Elevated expression of the single gene SPP381 (for the suppressor of prp38-1) was found to suppress the temperature-sensitive growth and splicing defects of the prp38-1 mutant. The genetic and biochemical data presented suggest that the SPP381-mediated prp38-1 suppression occurs through direct contact between the two gene products. Like Prp38p, Spp381p is a small, acidic protein component of the U4/U6.U5 tri-snRNP particle. Curiously, Spp381p contains a PEST proteolysis motif that appears important for pre-mRNA splicing. This report identifies an unusual new component of the splicing apparatus that functions in concert with the Prp38p spliceosome maturation factor to support cellular pre-mRNA processing.

MATERIALS AND METHODS

Yeast strains. Yeast strains used were MGD353-46D (MATa leu2-3,112 trp1-289 ura3-52 his clyr), MGD353-13D (MATa leu2-3,112 trp1-289 ura3-52 arg4 ade2), MGD407 (MATa leu2-3,112 trp1-289 ura3-52; diploid of MGD353-
46 X MGD353-13D), ts192 (MATa prp38-1 leu2-3,112 trp1-289 ura3-52 his3-1102 his3-1101 hsl2-100 hsl2-901 his3-901 ura3-52 gal1-342 gal80-388, lys2-1 GAL1-H35 URA3-GAL1-2ac, URA3-his4), SUL1 (MATa spp381 HA prp38-1 leu2-3,112 trp1-289 ura3-52 his3-1102 his3-1101 hsl2-100 hsl2-901 his3-901 ura3-52 gal1-342 gal80-388, lys2-1 GAL1-H35 URA3-GAL1-2ac, URA3-his4), SUL2 (MATa prp38-1 leu2-3,112 trp1-289 ura3-52 his3-1102 his3-1101 hsl2-100 hsl2-901 his3-901 ura3-52 gal1-342 gal80-388, lys2-1 GAL1-H35 URA3-GAL1-2ac, URA3-his4), SUL3 (MATa ura3-52 leu2-3,112 spp381 LEU2 his3-1102, KBY2 (MATa prp38-1 LEU2 trp1-289 ura3-52 his3-1102 prVPc2 (TRP1 PRP38-1)), SYL2 (MATa leu2-3,112 trp1-289 ura3-52 asp384 LEU2 prVPc2 (PRP38-1)), and JX6Y (MATa prp38-1 LEU2 trp1-289 ura3-52 leu2-3,112 ade2 YCPc2 (TRP1 PRP38-1)).

Identification of suppressor plasmids. Standard protocols were used for yeast culture maintenance, plasmid isolation, and transformation (15). The Yepl3-based yeast genomic library was obtained from the American Type Culture Collection (ATCC stock no. 37323). Approximately 1 X 108 yeast transformants were prepared by the ground cell pellet method of Umen and Guthrie (41) to prepare yeast extract-derived plasmids of defined function. The internal strain SUL1 grown in YP-galactose was added to 2 liters of YP-glucose and grown at 30°C for 17.5 h prior to harvest. Immune precipitations were carried out essentially as described previously (21). Twenty microliters of yeast extract (at approximately 20 mg of protein per ml of extract) was mixed with 40 ml of a 50% slurry of protein A+G agarose (Oncogene, Inc.) bound with the HA.11 antibody (Babco) or with the mAb63 control antibody as described previously (25). To this was added 3 ml of 100 mM dithiothreitol, 50 U of RNasin (Promega), and HNT (20 mM HEPS [pH 7.9], 100 mM NaCl, 1.25 mM MgCl2, 0.05% Triton X-100) to 150 ml. The tubes were incubated with constant rotation at room temperature for 30 min. The unbound extract was removed by centrifugation at 4,000 X g for 1 min. The beads were then washed five times with 300 ml of HNT. Precipitations at 50 mM NaCl were carried out at this salt level in both the binding and the wash steps. All other samples were bound at 100 mM NaCl, and the salt was adjusted between 100 and 400 mM in the wash buffers. snRNA binding capacities of the antibodies were calculated using the detection buffer (100 mM Tris-HCl [pH 7.5], 12.5 mM EDTA, 150 mM NaCl, 1% sodium dodecyl sulfate, 2 mg of protease K/ml) for 10 min at 37°C. The samples were phenol extracted and precipitated with ethanol prior to Northern blot analysis with the previously described snRNA probe (5). Glycerol gradients (10 to 35%) were run and assayed for snRNA content by immune precipitation as described previously (25). Approximately 150 ml (20 mg of protein content/ml) of total extract protein was resolved and separated into 22 fractions of 0.5 ml each. One-third of each fraction was assayed directly for the HA content or precipitated with the HA.11 antibody and then scored for snRNA.

Western blotting was performed on 60 mg of total extract protein resolved on a 10 or 12% discontinuous polyacrylamide gel prepared with a 4% stacking gel. Proteins were electroblotted into a nitrocellulose membrane (Millipore) in a mini-V 8-10 apparatus (Bio-Rad/BRL) with transfer buffer (24.5 mM Tris-HCl/192 mM glycine) adjusted to 10% (vol/vol) with methanol. Immune detection was carried out with the anti-HA antibody HA.11 (Babco) diluted 1:1,500 in 100 mM sodium phosphate, 1% nonfat dry milk. The secondary antibody was a goat anti-mouse immunoglobulin G (heavy and light chains)-alkaline phosphatase conjugate (Bio-Rad/BRL) developed with the 5-bromo-4-chloro-3-indolylphosphate (BCIP)-nitroblue tetrazolium chloride (NBT) chromogenic substrate mixture as recommended by the supplier (Bio-Rad/BRL).

RESULTS

Selection of prp38-1 dosage suppressors. A yeast genomic library based on the high-copy-number plasmid Yepl3 was used to select plasmids that reduced the temperature-sensitive growth defect caused by the prp38-1 mutation in yeast strain ts192 (5). From approximately 20,000 yeast transformants, 11 that showed plasmid-dependent colony formation at 37°C were identified. DNA restriction site analysis and hybridization studies on the recovered plasmids revealed that three distinct types of genomic inserts were recovered. Plasmids with type-A inserts (e.g., Yepl3-2) or type-B inserts (e.g., Yepl3-7) relieved the prp38-1 growth defect with high efficiency, while plasmids with type-C inserts (e.g., Yepl3-5) suppressed it much more weakly (Fig. 1). The wild-type allele of PRP38 is present in both of the identified type-A plasmids. DNA sequence analysis showed that the type-C insert DNA was not present in the published yeast genome database or in any other publicly held databases. Plasmids of categories A and C were not studied further.

Each of the seven type-B plasmids contained identical or overlapping regions of the right arm of yeast chromosome II. Two genes of defined function, RB5 and RB7, were present in these library segments. RB5 encodes the 27-kDa subunit common to the three nuclear RNA polymerases (44). RB7 encodes an activity required for riboflavin biosynthesis (7). Besides these genes, two uncharacterized ORFs, YBR151w...
and YBR152w, were present. Of these, YBR152w had the capacity to code for a small acidic protein (Fig. 2). Embedded within its amino terminus are two serine-rich elements similar to a sequence found in the carboxyl terminus of the Prp38p protein (5). The second of the YBR152w-encoded serine-rich elements contains an exceptionally strong match to the PEST protein degradation signal (amino acids 56 through 95). Prp38p does not contain the PEST motif. YBR152w was subcloned free of the adjacent genes and assayed for suppression in the high-copy-number shuttle vector YEplac112 (13). The YEplac112-7A subclone efficiently suppressed the ts192 temperature-sensitive growth defect, confirming that YBR152w is gene restricted, as evidenced by efficient suppression on plasmid YEplac112-7A, or an efficient suppressor of prp38-1 (YEplac112-7A).

Prp38p, the latter possibility was ruled out with the demonstration that SPP381 on plasmid YEplac112-7A did not suppress a prp38::LEU2 null allele (reference 35) (data not shown). Thus, enhanced expression of SPP381 supports, but does not supplant, PRP38 activity.

Efficient pre-mRNA splicing is restored by enhanced SPP381 expression. A Northern blot of cellular RNA was used to score for the impact of plasmid-borne SPP381 expression on pre-mRNA splicing efficiency (Fig. 3). The PRP51A pre-mRNA–to–mRNA ratio of the wild-type parental strain was compared with that of the untransformed prp38-1 mutant and the prp38-1 mutant transformed with various suppressor plasmid constructs. RNA was extracted from cultures grown continuously at 23°C and from cultures shifted to the restrictive temperature of 37°C for 2.5 h. When assayed at the permissive temperature, all cultures showed abundant amounts of spliced PRP51A mRNA and little pre-mRNA (Fig. 3, lanes 1 to 7). In contrast, the pre-mRNA/mRNA ratio increased greatly with the temperature shift in the untransformed mutant (Fig. 3, lane 13) and in mutants transformed with the weak suppressor (lane 9) or a randomly chosen library plasmid (lane 12). Plasmid-based expression of the wild-type PRP38 gene (Fig. 3, lane 8) or enhanced expression of SPP381 (lanes 10 and 11) decreased the pre-mRNA/mRNA ratio in the mutant strain to near-wild-type levels (lane 14). By this measure, enhanced SPP381 expression reverses the pre-mRNA processing defect caused by the prp38-1 mutation.

SPP381 is an important but not an essential gene for normal yeast growth. The SPP381 gene was disrupted by replacement of approximately 90% of its coding sequence with the LEU2 selectable marker. When diploid yeast cells heterozygous for this mutation were sporulated, equal numbers of colonies of two distinct sizes were observed in the meiotic offspring. PCR and Southern blot analyses showed that colonies visible after 2 days of incubation on the tetrad dissection plate all possessed the uninterrupted SPP381 allele. Yeast cells that formed visible colonies only after 6 to 7 days of incubation all possessed the ssp381::LEU2 disruption. In liquid medium, the generation time of the ssp381::LEU2 disruptant cultures was approxi-
4 and data not shown). The limited this mutant, since a disruptant in which 17, 23, and 37°C compared with that of the sensitive, as colony formation appeared equivalently poor at versus 1.75 h). This mutant was not obviously heat or cold mately seven times that of the wild-type siblings (10 to 11 580 LYBARGER ET AL. MOL. CELL. BIOL. (53x418) and references therein), splicing was inhibited 10 to 11°C (Fig. 5). Yeast that expressed slightly better than the mutant bearing the null allele alone 4 and data not shown). Given the approximate sevenfold de-crease in the growth rate observed for the mutation (Fig. 5C) (unpublished data). Thus, the proposed PEST sequence contributes to Spp381p biological activity in vivo. Spp381p contributes to cellular pre-mRNA splicing. A di-
rect contribution of Spp381p to pre-mRNA splicing might un-de the genetic interaction between prp38-1 and SPP381. To address this, RNA samples isolated from a wild-type strain and from the spp381::LEU2 disruptant strain were analyzed by Northern blotting (Fig. 5). Indicative of decreased splicing efficiency, yeast with the prp38-1 mutation (Fig. 5A, lanes 1 and 2). Primer extension carried out with an RP51A exon II primer showed that most of this intron-bearing RNA was pre-mRNA rather than lariat intermediate, indicating that splicing was inhibited before 5' splice site cleavage. As a control for the specificity, a spp381::LEU2 strain transformed with the GAL1::SPP381::HA fusion gene was assayed in parallel. The spp381::LEU2 splicing defect was specifically reversed by GAL1::SPP381::HA under conditions that induced transcription of this fusion gene (i.e., growth on galactose). Similar to what has been reported for other splicing factors (see references 21 and 25 and references therein), splicing was inhibited 10 to 15 h after transcriptional repression of GAL1::SPP381::HA (Fig. 5A, lanes 3 and 4, and data not shown). While results with the CYH2 gene were somewhat less pronounced, transcripts of the CYH2 gene also showed decreased levels of mRNA and in-creased levels of pre-mRNA in the spp381::LEU2 mutant background (Fig. 5B). The more modest CYH2 defect suggests that not all introns are equally dependent upon Spp381p protein for excision. We note also that, compared with RP51A, the splicing of CYH2 precursors was somewhat less sensitive to Prp38-1p temperature inactivation (Fig. 5A and B, lanes 7 and 8). No reproducible changes with the intronless ADE3 mRNA, rRNA, or trimethylguanosine-capped snRNAs were associated with the spp381::LEU2 mutation (Fig. 5C) (unpublished data). Thus, the growth impediment of the spp381::LEU2 mutant can be accounted for by decreased pre-mRNA splicing efficiency in the absence of Spp381p. Unlike the prp38-1 mutation, the spp381::LEU2 splicing defect was not suppressed by overex-pres-sion of PRP38 on a high-copy-number plasmid or as a GAL1::PRP38 fusion gene. Together, these data provide strong evidence that PRP38 and SPP381 make important and independent contributions to cellular pre-mRNA splicing. Yeast that expressed GAL1::PEST-HA spliced RP51A pre-mRNA poorly (Fig. 5A and B, lanes 6) consistent with its weak complementation of the spp381::LEU2 mutation. Curiously, however, the high-copy-number ΔPEST construct continued to
5A, lane 5) compared with the wild-type strain (Fig. 5A, lanes 1 and 2). The RNA was recov-ered from cultures grown continuously at the permissive temperature for prp38-1 (23°C) or after 2.5 h at the restrictive temperature (37°C). The positions of pre-mRNA (P) and spliced mRNA (M) are indicated by arrowheads. (A) Hy-

FIG. 5. Contribution of SPP381 to the efficiency of pre-mRNA splicing in vivo. RNA was isolated from wild-type yeast (lanes 1 and 2) and the spp381::LEU2 disruptant before (lane 5) and after transformation with GAL1::SPP381::HA (lanes 3 and 4) or with GAL1::spp381::PEST-HA (lane 6). Galactose (gal) or glucose (glu) was used to activate or repress the GAL1 fusion constructs as indicated. Lanes 7 to 12. RNA from the untransformed prp38-1 mutant (lanes 7 and 8) and the same strain after transformation with the high-copy-number (i.e., YEp112) plasmid bearing SPP381 (lanes 9 and 10) or its ΔPEST (YEpplac112-based) derivative (lanes 11 and 12). The RNA was recov-ered from cultures grown continuously at the permissive temperature for prp38-1 (23°C) or after 2.5 h at the restrictive temperature (37°C). The positions of pre-mRNA (P) and spliced mRNA (M) are indicated by arrowheads. (A) Hy-

FIG. 4. Comparison of growth in SPP381 and spp381::LEU2 mutant yeast cells. Yeast cultures were grown to saturation in nonselective broth with 2% galactose. Each strain was adjusted to a culture density at 600 nm of 0.150. The presence of equivalent cell numbers in each culture was confirmed microscopi-cally. Serial 10-fold dilutions (positions 1 to 4) were spotted in 5-µl volumes on galactone-containing agar medium and incubated for 4 days at 30°C. The strains used were the wild-type parent (SPP381), the untransformed spp381::LEU2 mutant, and the spp381::LEU2 mutant transformed with the GAL1::SPP381::HA fusion gene or its ΔPEST-HA derivative.

SPP381
spp381::LEU2
GAL1::SPP381::HA
GAL1::spp381 ΔPEST-HA

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SPP381
spp381::LEU2
GAL1::SPP381::HA
GAL1::spp381 ΔPEST-HA

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RNAs (Total) were resolved in parallel (lanes 1 and 12 to 14). For comparison of relative snRNAs, nonprecipitated extract was hybridized with probes specific for the spliceosomal snRNAs (indicated by arrowheads). Immune pellets in lanes 3 to 8 were washed with buffer containing 100 mM NaCl. The immune pellets were fractionated on a linear 10 to 35% glycerol gradient. (A) Even-numbered fractions were assayed by Northern blotting for the presence of U5 (the long and short forms, U5L and U5S, respectively), U4, and U6 snRNA. (B) Spp381HAp was immune precipitated from the fractions presented in panel A with the anti-HA antibody HA.11 and was assayed for coassembled snRNAs by Western blotting with the same antibody.

FIG. 6. Coprecipitation of snRNA with HA-tagged proteins. Extracts of untagged yeast (lanes 11 and 14) and HA-tagged Spp381HAp (lanes 1 to 8), Prp38HAp (lanes 9 and 12), and Prp39HAp (lanes 10 and 13) were immune precipitated with the HA-specific antibody HA.11 (lanes 3 to 10) or the irrelevant antibody mAb63 (lane 2). The immune pellets were fractionated on a denaturing 5% polyacrylamide gel and transferred to a membrane, and the blot was hybridized with probes specific for the spliceosomal snRNAs (indicated by arrowheads). Immune pellets in lanes 3 to 8 were washed with buffer containing the indicated levels of NaCl; all other pellets were washed with buffer adjusted to at least 200 mM NaCl. Thus, Spp381p retained an aspect of its biological activity even in the absence of the PEST sequence. In addition, the splicing deficiency of prp38-1 mutant (Fig. 5A and B, lanes 7 to 12). Thus, Spp381p was likewise associated with snRNPs. In all extracts tested, the total (i.e., unfraccionated) snRNA levels were equivalent (Fig. 6, lanes 1 and 12 to 14). Extracts prepared from the GAL1::SPP381HA and control strains were incubated with the anti-HA antibody HA.11 or with the irrelevant control antibody mAb63. A Northern blot of the immune precipitate was assayed for the presence of snRNAs. Spp381p was present in the U4/U6.U5 tri-snRNP particle. This prediction was tested by glycerol gradient fractionation of the Spp381HAp splicing extract (Fig. 7). As described previously (6, 45), this fractionation technique resolves the free U6 snRNP particles (Fig. 7A, fractions 6 to 10) and free U5 snRNP particles (Fig. 7A, fractions 12 and 14) from the U4/U6.U5 tri-snRNP particles (fractions 16 and 18). The U4, U5, and U6 snRNAs coprecipitated efficiently with the HA.11 antibody only from the tri-snRNP fractions (Fig. 7B, fractions 16 and 18), even though both U6 and U5 are abundantly present elsewhere in the gradient. The cofractionation and coprecipitation results provide clear evidence for Spp381HAp association with the U4/U6.U5 tri-snRNP particle.

Prp38p and Spp381p interact in vivo. The dosage suppression and snRNA precipitation studies showed that Spp381p and Prp38p interact genetically and associate with the same biochemical complex. A two-hybrid analysis was next performed to investigate the possibility that these two proteins associate directly. Simultaneous expression of Spp381p and Prp38p as Gal4 fusion products led to strong transactivation of the lacZ and HIS3 reporter genes in the host strain (Table 1).
The Spp381p carboxyl terminus, since Spp381p(146–291) and Spp381p(1–291) did not interact with Prp38p(1–121) or Prp38p(122–242), suggesting that the interacting domain may comprise (or span) both halves of Prp38p. Supporting evidence for a critical N-terminal interaction was provided by the observation that either of two Ts mutations in this region, G66D (prp38-1), greatly reduced the level of transactivation with Spp381p(1–121) or Prp38p(122–242), indicating the necessity of two Ts mutations in this region, G66D (prp38-1), greatly reduced the level of transactivation with Spp381p(1–121) or Prp38p(122–242), suggesting that the interacting domain may comprise (or span) both halves of Prp38p. Supporting evidence for a critical N-terminal interaction was provided by the observation that either of two Ts mutations in this region, G66D (prp38-1) and C87Y (prp38-2), greatly reduced the level of transactivation with Spp381p(1–291). Overall, the two-hybrid results support an interaction between the carboxyl terminus of Spp381p and one or more regions of Prp38p.

The Spp381HAp fusion protein has a predicted molecular size of approximately 35 kDa. On polyclarimide gels, however, Spp381HAp migrates as a 51-kDa protein (Fig. 8, lanes 1 and 2). This anomalous migration might be caused by the highly acidic amino terminus of this protein, which also contains numerous possible sites for phosphorylation. Consistent with this, removal of the 4.6-kDa PEST sequence deletion caused an apparent 13-kDa shift to produce a protein with an apparent mass of 38 kDa (Fig. 8, lanes 4 and 5; predicted mass, 30.3 kDa). Equivalent amounts of the Spp381HAp and ΔPEST-HAp derivatives accumulated in cells when expressed from the GALI promoter, indicating that under these conditions the PEST sequence contributes little to stability. Curiously, given the importance of Spp381p to in vivo splicing, extracts prepared from the glucose-depleted GALI::SPP381HA culture (Fig. 8, lane 3) were found to splice RPS14 pre-mRNA through both chemical steps in vitro (data not shown). Thus, it appears that either Spp381p is not required in vitro, or it contributes to an activity not assayed under typical in vitro splicing conditions (see Discussion).

DISCUSSION

Prp38p was recently shown to be necessary for dissociation of the U4/U6 intermolecular helices (45), an essential maturation step that occurs prior to pre-mRNA 5′ splice site cleavage. In this study, we used the genetic approach of dosage suppression to identify a novel protein, Spp381p, that contributes to Prp38p function in splicing. The genetic and biochemical evidence indicates that Spp381 and Prp38p define a novel class of interacting acidic proteins which promote U4/U6.U5 snRNP activity in the spliceosome cycle. Multiple models for dosage suppression have been described based on kinetic or thermodynamic contributions of the overexpressed gene product to the process under study (for instance, see references 20 and 32). The fact that extra copies of SPP381 do not suppress a prp38::LEU2 null allele shows convincingly that increased Spp381p levels do not bypass the need for Prp38p in splicing. In principle, the elevated abundance of Spp381p might increase the stability or residual activity of the temperature-sensitive prp38-1 gene product. The latter suggestion appears more likely, since under a variety of conditions, we find no evidence for increased Prp38-1p abundance with enhanced Spp381p expression (unpublished data). While this result and the positive two-hybrid data are consistent with direct contact between Prp38p and Spp381p, we cannot rule out the possibility that a third component mediates this interaction. However, in the absence of data supporting a such a third factor, we favor the view that the genetic suppression occurs due to an increased frequency of Spp381p interaction with a functionally impaired Prp38-1p protein. An Spp381p–Prp38-1p interaction may promote a favorable structural change within Prp38-1p to facilitate the binding of Prp38-1p to the U4/U6.U5 tri-snRNP, promote Prp38-1 interaction with other splicing components, or in some other manner enhance the activity of the essential Prp38p protein.

Since Prp38p appears to be exclusively a U4/U6.U5 tri-snRNP protein (45), it is likely within this particle that Spp381p normally contacts Prp38p. Previously, two characterized proteins similar to Spp381p in size were reported in the...
yeast tri-snRNP (11). Glycerol gradient fractionation of yeast snRNP complexes presented here demonstrates the presence of Spp381HAp in the U4/U6.U5 tri-snRNP particle and the absence of antibody-accessible Spp381HAp in the free U5 or free U6 snRNP complexes. It remains possible, however, that Spp381HAp binds to the low-abundance U4/U6 di-snRNP precursor (19, 23, 26) or is present in an antibody-inaccessible form in other snRNP complexes. Both the two-hybrid results presented here and the presence of Spp381HAp in an immune pellet prepared with an anti-Prp38p antibody (32) support the view that Spp381p and Prp38p are ubiquitous (as opposed to alternative) components of the U4/U6.U5 tri-snRNP particle. Prp38p and Spp381p are clearly “weakly associated” snRNP proteins, similar in salt sensitivity to the phylogenetically conserved SF3a proteins of the 17S U2 snRNP (reference 3; see also references in reference 19), U1C (39), U1-Prp42p (25), and Prp38p tri-snRNP protein (45). No clear counterpart to Spp381p is known in mammals, although at least one small, highly charged phosphoprotein is a component of the mammalian U4/U6.U5 tri-snRNP particle (12).

What is the role of Spp381p in splicing? In vivo, enhanced SPP381 expression relieves the block to pre-mRNA S′ splice site cleavage imposed by the loss of Prp38p function. This observation suggests that Spp381p contributes to snRNP rearrangement events that lead to the catalytic activation of the spliceosome. Consistent with this, deletion of the SPP381 gene severely impairs yeast growth and inhibits step 1 in splicing. Surprisingly, we have not detected in vitro splicing defects in extracts prepared from glucose-repressed GAL1::SPP381HA cultures. U4 snRNA is released from the spliceosome, and both chemical steps in splicing occur unimpeded. Since SPP381 affects the efficiency (but not the absolute occurrence) of splicing in vivo, the lack of an obvious in vitro defect may indicate that Spp381p function is not rate limiting for the comparatively slow in vitro reaction. Alternatively, Spp381p function may be important in vitro but its function may not be obvious under standard assay conditions. Ampic precedent exists for such behavior by splicing factors. For instance, extracts deficient in Prp22p, Prp24p, or Prp43p can correctly process pre-mRNA through both chemical steps in splicing but are defective in postsplicing steps of mRNA release (Prp22p [10], U4/U6 snRNA recycling (Prp24p [30, 42]), and intron release (Prp43p [1]). The spliceosomal precursor pool in the Spp381HAp-depleted culture is an unknown and may increase by the release of endogenous spliceosomes during the 8-h extract preparation protocol. If Spp381p acts as a recycling or dissociation factor, its importance might not be obvious until the spliceosomal precursors become saturated with, or depleted of, the exogenously added pre-mRNA, as shown for Prp24p (30).

It is well established that the removal of a PEST sequence can greatly increase the half-life of an unstable protein (e.g., c-Fos [40]) and that the transfer of a PEST sequence to a naturally stable protein can greatly enhance its turnover (e.g., dihydrofolate reductase [22]; see references 31 and 33 for additional examples of PEST-mediated destabilization). Proteins with functionally defined PEST elements often have PEST values in the range of 4 to 16 (e.g., yeast Gcn4, mammalian Fos, ornithine decarboxylase, Aspergillus NIMA). The 40-amino-acid Spp381p element has a PEST sequence value of +29.8 and ranks higher than all of the 99 PEST elements presented in two recent reviews (2, 31). The presence of a PEST sequence raises the interesting possibility that proteolysis of Spp381p triggers a particular event of the spliceosome cycle. Many PEST-mediated proteolysis events are regulated (31). Perhaps the most obvious role for proteolysis would be to promote spliceosome disassembly following pre-mRNA splicing. A spliceosome-dependent turnover would explain the similar intracellular levels of Spp381HAp and its ΔPEST-HA derivative when these proteins are overexpressed (beyond the needs of splicing) by the GAL1 promoter. Intriguingly, while uncommon in spliceosomal proteins, the DEX/H-box proteins Prp22p, Prp28p, Brr2p, Prp43p, and Prp16p all possess good fits to the PEST consensus (PEST scores of 14.5, 13.4, 11.3, 7.3, and 5.7, respectively). Although highly speculative, it is conceivable that PEST sequence-mediated modification (i.e., phosphorylation or ubiquitination) subsequent to function rather than dissociation accounts for the “transient” association of members of this group with the spliceosome (see reference 38). Experiments are under way to define the function of Spp381p in splicing and to test the possibility that proteolysis contributes to the spliceosome cycle.

ACKNOWLEDGMENTS

We thank Frances McFarland, Martha Peterson, John Woolford, and our lab colleagues Seyung Chung, Mitch McLean, and Liz Otte for their helpful comments on the manuscript.

This work was supported by an HHMI summer support fellowship to V.B. and by National Institutes of Health grant GM42476 (to B.C.R.).

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