New Roles for the Snp1 and Exo84 Proteins in Yeast Pre-mRNA Splicing*

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The mammalian 70K protein, a component of the U1 small nuclear ribonucleoprotein involved in pre-mRNA splicing, interacts with a number of proteins important for regulating constitutive and alternative splicing. Similar proteins that interact with the yeast homolog of the 70K protein, Snp1p, have yet to be identified. We used the two-hybrid system to find four U1-Snp1 associating (Usa) proteins. Two of these proteins physically associate with Snp1p as assayed by communoprecipitation. One is Prp8p, a known, essential splicesomal component. This interaction suggests some novel functions for Snp1p and the U1 small nuclear ribonucleoprotein late in splicesomal development. The other, Exo84p, is a conserved subunit of the exocyst, an eight-protein complex functioning in secretion. We show here that Exo84p is also involved in pre-mRNA splicing. A temperature-sensitive exo84 mutation caused increased ratios of pre-mRNA to mRNA for the Rpl30 and actin transcripts in cells incubated at the non-permissive temperature. The mutation also led to a defect in splicing and presplicesomal formation in vitro; an indication that Exo84p has a direct role in splicing. The results elucidate a surprising link between splicing and secretion.

The U1 snRNP1 has an early, hierarchic role in pre-mRNA splicing in the yeast Saccharomyces cerevisiae (1–3). It must be bound to pre-mRNA for subsequent stable association of the other four splicesomal snRNPs with the pre-mRNA. Once U1 snRNP is bound, U2 snRNP binds, and the presplicesome is formed. The tri-snRNP complex, U4/U6/U5, then binds to form the spliceosome. The spliceosome next undergoes a number of coordinated rearrangements (4). The duplexes between the U4 and U6 snRNAs and between U1 snRNA and the 5′-splice site (SS) of the pre-mRNA are disrupted, whereas new pairings between U2 and U6 snRNAs, U6 snRNA and the 5′-SS, and U5 snRNA and the pre-mRNA exons 1 and 2 are formed. These rearrangements lead to the formation of the active catalytic site of the spliceosome. Splicing of the pre-mRNA then ensues by two transesterification reactions.

The yeast U1 snRNP recognizes both the 5′-SS and the branchpoint region of the pre-mRNA (3, 5). From 5 to 7 nucleotides of the 5′ end of the U1 snRNA base pair with the 5′-SS of the pre-mRNA to form the short U1/5′-SS duplex (3). Additionally, eight proteins of the U1 snRNP, including the C and Snp1p proteins, contact the 5′-SS region and exon 1 and may stabilize the U1/5′-SS duplex (6). Proteins bound to the pre-mRNAs branchpoint region associate with at least one protein component of the yeast U1 snRNP, Prp40p, to form a bridge to the 5′-SS (7). Similar protein-protein interactions suggest such a protein bridge in metazoans as well (8).

During splicesome formation, the 5′-SS switches its pairing from the U1 to the U6 snRNA (4). It is not understood when and how the U1 snRNP is displaced from the 5′-SS. It was first suggested that the U1 snRNP physically dissociates from the developing splicesome before the tri-snRNP binds, but it is now thought that the displacement occurs when the tri-snRNP enters the spliceosome or shortly thereafter (9–11). One of the DEAD box helicases, Prp28p, may eliminate the U1/5′-SS pairing by unwinding the duplex, by promoting the pairing of U6 with the 5′-SS to displace U1, or by altering C protein binding to destabilize the U1/5′-SS duplex (11, 12).

In metazoans, the U1 snRNP also functions in regulating both constitutive and alternative splicing. An element of the U1 snRNP important in this function is the 70K protein that interacts with a number of factors. The 70K protein binds to stem-loop 1 of the U1 snRNA via an RNA recognition motif in its central domain. Its N-terminal domain interacts with the U1 C protein to help stabilize the U1/5′-SS duplex (13). Its C-terminal domain associates with the SR protein, ASF/SF2 (14), and additional SR proteins (8, 15, 16). The SR proteins, so-called because they contain serine-arginine dipeptide repeats, enhance or repress splicing by recruiting or inhibiting other splicing factors, especially the U1 and U2 snRNPs (17, 18). Some SR proteins along with 70K form the protein bridge between the 5′-SS and the branchpoint region (8). Several interactions between the SR proteins and 70K are regulated by phosphorylation (14, 19), and several steps in the splicing pathway, including splicesome assembly, depend on the phospho-
rylated state of these proteins (20). Both the SR (17, 21) and 70K (22) proteins are targets of specific kinases.

We considered that Snplp (23), the yeast homolog of the 70K protein, might also physically associate with proteins that regulate splicing in yeast. Like the 70K protein with which it shares 30% identity, Snplp has three domains (23, 24), with the central domain binding via an RNA recognition motif to the U1 snRNA (25). Snplp is not required for viability in all yeast strains (23, 24), but it is required for efficient splicing (24), a phenotype suggestive of a regulatory role. Unlike metazoans that have large numbers of SR proteins, yeast may have only a few such proteins.

In this study we used the yeast two-hybrid system (26) to screen yeast sequences for those encoding U1 Snplp associating (Usa) proteins. We found four Usa proteins. One of these is Prp8p, a known, integral component of the U5 snRNP (27). Another, Usa3p, has also been identified as Exo84p, a component of the evolutionarily conserved exocyst complex of the secretory pathway (28). Our characterization of a Ts exo84 mutant indicates that Exo84p in vivo affects the expression of some genes with introns and in vitro has a direct role in splicing. Both interactions, Snplp-Prp8p and Snplp-Exo84p, may be involved in splicing regulation. The Snplp-Prp8p interaction may be important in coordinating the 5' SS switch

EXPERIMENTAL PROCEDURES

The following enzymes, chemicals, and antibodies were obtained from commercial suppliers as follows: restriction enzymes, Deep Vent DNA Polymerase, T7 RNA polymerase, and T4 DNA ligase were from New England Biolabs; avian myeloblastosis virus reverse transcriptase was from Life Sciences Research, Inc.; actinomycin D and 3-AT were from Sigma; RNasin RNase inhibitor, RRQ RNase-free DNase, and rabbit reticulocyte lysate were from Promega; zymolase was from Seikagaku Corp.; Sequenase version 2.0 kit was from U. S. Biochemical Corp.; DC protein assay kit was from Bio-Rad; anti-FLAG M2 immobilized antibody was from Eastman Kodak Co.; [35S]methionine (1000 Ci/mmol), [32P]UTP (3000 Ci/mmol), anti-mouse and anti-rabbit IgG antibodies conjugated with peroxidase, chemiluminescent detectors were from Amersham Pharmacia Biotech; and anti-HA monoclonal antibody 12CA5, originally from Babco, Inc., was a gift from Amersham Pharmacia Biotech; and anti-HA monoclonal antibody

Oligodeoxynucleotides

The oligos were synthesized by Genosys, Inc., or the University of New Mexico Center for Genetics in Medicine. Their sequences are available upon request.

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EXO84

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ingly, Exo84p has two copies of the consensus target site for an

SR protein kinase.

Pseudo Plasmids—The 1.4-kb NcoI-HindIII fragment from pJ215 was ligated into the blunt StyI sites of plasmid pBlD thereby replacing the 500-bp SNP1 fragment to generate plasmid pPS4 (snplp-HIII). For constructing pCite9-SNP1, BamHI and EcoRI sites were added to the 5' and 3' ends, respectively, of the SNP1 ORF by PCR using oligos oSR37 and oSR38 and template plasmid pRS3D. The amplified fragment was cut with BamHI and EcoRI restriction endonucleases and then ligated into pGBT9 immediately downstream of the GAL4 DNA binding domain codons 1–147. The SNP1 BamHI-EcoRI insert was subcloned from pGBT9 into pGAD424 (pGAD-SNP1) and pMA424 (pMA424-SNP1) downstream of the GAL4 activation domain.

In vitro expression of FLAG-tagged Snplp, unique BglII and HindIII sites at the 5' and 3' ends of the SNP1 ORF were introduced with oligos oSR66 and oSR67 and template pRS61 and template pRS61/H11033.

The resulting DNA fragment was cut with BglII and HindIII and then subcloned into the corresponding sites of pGAD424 to create pJ.D1. Hybridized oligos oSR68 and oSR69 encoding the FLAG-tag were ligated into the XhoI and BamHI sites of pJ1D1 to create plasmid pJ171. The BglII-BamHI SNP1 fragments from pJ1D1 and pJ271 were subcloned into the BamHI and BglII sites of pCite2a to generate plasmids pSJR174 and pSR175, respectively.

EXO84 Plasmids—The 5.4-kbp SacI-SalI EXO84 genomic DNA frag-

ment from phage λ was subcloned into plasmids pKSII1 + pUC119 to create pCM28 and pCM29, respectively. The Eco47III–SalI fragment containing EXO84 and 610 and 80 bp of its 5'- and 3'-flanking sequences, respectively, was subcloned from pCM29 into pRS316 and pRS313 to create pSAl3 (CEN-URA3-EXO84/Eco47III–SalI) and pSA20 (CEN-HIS3-EXO84/Eco47III–SalI). Because of the small amount of sequence from the EXO84 stop codon to the SalI site, we subcloned additional 3'-flanking DNA from a wild type yeast strain. For this purpose we constructed the plasmid pSA26 (HIS3-BglII-exo84) containing ~600 bp of the N terminal of EXO84 with a BglIII site just 5' to EXO84 codon 1. Plasmid pSA26 was target-integrated into genomic locus at the EXO84 site in strain SACL 6686-3A (MATa leu2-3,112 trp1-289 his3-200 ura3-52 ale3-1). The amplified fragment was cut with BglII and HindIII and then subcloned into the corresponding sites of pGAD424 to create pJ.D1. Hybridized oligos oSR68 and oSR69 encoding the FLAG-tag were ligated into the XhoI and BamHI sites of pJ1D1 to create plasmid pJ171. The BglII-BamHI SNP1 fragments from pJ1D1 and pJ271 were subcloned into the BamHI and BglII sites of pCite2a to generate plasmids pSJR174 and pSR175, respectively.

For constructing the exo84 null mutation, the Pet-S1a1 LEU2 fragment from pJ272 was inserted into the Pet1 and blunt EcoRI sites of pCM28 to form pCM32 (exo484:LEU2). For constructing deletion mutations in EXO84 in vitro, a unique Ncol site was introduced just 3' of the translational stop codon by mutagenesis of pSA35 with oligo oSA11 to create pSA45. A unique MluI site just 5' to the translational start codon was introduced by mutagenesis of pCM29 with oligo oSA10 to create pSA44. DNA fragments containing the 5' and 3' mutations were used to replace the corresponding fragments in pCM29 to create pSA46. To create the exo84-2 mutation, codons 636 and 637 were changed from AGA-CTC to AGC-CCT in pSA46 with oligo oSA15 to create a unique StuI site in pSA50. The Xcm1-Ncol fragment from pSA50 was subcloned into pSA45 to form pSA55. A stop codon immediately after codon 636 was then introduced by ligating hybridized oligos oSA19 and oSA20 into pSA53 DNA cut with StuI and Ncol to create plasmid pSA55. Plasmid pSA57 (CEN-HIS3-exo84-2 (Eco47III–Ncol)) was created by subcloning the Xcm1-Ncol fragment from pSA53 was subcloned into pCJ1 and blunt SalI sites of pSA20 to create pSA36 (CEN-URA3-EXO84 (Eco47III–Ncol)).
fragment of pksEXO84-HA was subcloned into the corresponding sites of pSA57 generating pEXO84-HA2. For expression of pEXO84 in *in vitro*, additional restriction enzyme sites were introduced by ligating hybridized oligos oSA5 and oSA6 into the PstI and BglII sites of pSR165 to create pSA23. The NspI-SalI EXO84 fragment was subcloned into pSA23 to create pSA24 (CEN::LEU2::BglII-EXO84); this encodes EXO84 lacking the first 14 codons. The BglII-SalI fragment containing the EXO84 ORF was subcloned from pSA24 in two steps into the corresponding sites of pCite2a to create pSA40; this construction added on the first 32 codons of the pCite2a (from NcoI to BglII sites) plus one codon (ATC) 5' to codon 15 of EXO84. PRPS and Other Plasmids—The 2.9-kbp, EcoRV-EcoRI, PrpF fragment from pJD13 was subcloned into corresponding sites of pCite2a to create pSR190. Hybridized oligos oSR95 and oSR96 encoding two copies of the HA tag were ligated into the NcoI and XhoI sites of pSR190 to generate pSR207. For generating the leu2d595 allele, the blunted ends of the 3.9-kbp BglII-BamHI HisG-URA3-HISG fragment from pNK1009 were ligated into the EcoRV and Blunted Clal sites of pJ293 to generate plasmids pFS95 and pFS96.

**Yeast and Bacterial Strains**

*Strains Obtained from Others—* Yeast strains HFC7 (MATa, ura3-52, his3-200, ade2-101, lys2-801, trpl-1001, leu2-3, 112, gal4-542, gal80-538, LYS2::GAL1-HIS3, URA::GAL1-7CYC1-lacZ) and SFY526 (MATa, ura3-52, his3-200, ade2-101, lys2-801, trpl-1001, leu2-3, 112, gal4-542, gal80-38, URA::GAL1-lacZ) were obtained from CLONTECH and S. Fields, respectively. Bacterial strains DH10B (F−, mcrA Δ[mrr-hs- drom]::kanMX1, galK::kanMX4, hisG464, lacZ, lacY, lacX, araD139, araA1, araG1, araB1, araC1, araD139, araI1, araJ1, araK1, araL1, araM1, araN1, araS1, araT1, leu2-1, trp1-1, his3-11, his4-101, rpsL1002, metH1002, rfaC1, supE44, thi-1, thy-1, recA4, orfAmAm1, orfBmAm1) were diluted 1:2000 in antibody buffer (5% rabbit anti-Gal4p antibody was diluted 1:2000 in antibody buffer (5% rabbit anti-Gal4p antibody was diluted 1:2000 in antibody buffer). Proteins from WCE and extract fractions were analyzed as just described except that 0.5 µg of single-strand salmon sperm DNA (average molecular mass of 300 nucleotides) and 1–2 µg of library DNA were added to 400 µl of electrophoretic competent cells.

Transformants were selected by growth on medium lacking leucine, tryptophan, and histidine and supplemented with 1 µg/mL sorbitol and either 0, 5, or 20 mM 3-AT. The prototrophs obtained were further screened, if necessary, by replica plating onto synthetic medium containing 5 or 20 mM 3-AT. His' prototrophs which grew in the presence of 5 or 20 mM 3-AT were selected onto medium supplemented with 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal) to detect β-galactosidase activity. The His' lacZ' candidate transformants were then grown in liquid selective media at 30°C to midlog phase and assayed quantitatively for β-galactosidase activity by the β-galactosidase assay described in the Methods (46). Units of activity were calculated as units = (A420 × 1000/min) × 7.050/1000 µl.

The pGAD library plasmids from these 24 yeast candidates were recovered from total yeast DNA by electroporation into *E. coli* BA1 cells. Transformed bacterial cells were first selected on LB medium with 100 µg/ml ampicillin and then replica-plated onto M9 medium lacking leucine to detect LEU2-marked pGAD-library plasmids. The LEU2-marked plasmids were then amplified in *E. coli* DH10B cells and sequenced with Primer 1 (CLONTECH). Dependent protein sequences were compared using BLAST, FASTA, and TFAST algorithms (47–49). The recovered plasmids were then electroporated into strain TS952-2 (strain SFY526 with the bait plasmid pMA424-BD-SNP1) and tested again for transcription activation via quantitative β-galactosidase assay. The combination of the strain (SFY526) and vector (pMA424) often gives higher β-galactosidase activities than the strain (H7C) and vector (pGBT9) used in the initial screen (50). In *vitro* Transcription, Translation, and Coimmunoprecipitation

The following plasmids were cut with restriction endonucleases to produce linear templates for *in vitro* transcription: pSR174 and pSR175 with SpI1; pSA40 with SacI; and pSR190 and pSR207 with XhoI. The templates were transcribed with T7 RNA polymerase and then treated with RNase Q1. The RNAs were purified from the reactions by phenol and CHCl3/isoamyl alcohol extractions and by ethanol precipitation after which they were dissolved in water and stored at −70°C. RNAs were translated in *in vitro* translation reactions with wheat germ and micrococcal nuclelease-treated rabbit reticulocyte lysate according toPromega’s conditions (1992 bulletin TM232). After the reactions were incubated for 30–60 min at 37°C, they were treated with RNase A for 30 min. In *vitro* translation products were separated by SDS-PAGE (43) and analyzed by fluorography and autoradiography. In *vitro* translations for coimmunoprecipitation experiments were conducted supplemented with protease inhibitors (0.1 mg/ml chymostatin, 2 mg/ml aprotinin, 1 mg/ml pepstatin A, 2 mg/ml trans-epoxysucillyl-l-leucylamido-(4-guanidino) butane, and 0.5 mg/ml leupeptin), and incubated at 30°C for 30 min. The average was then washed 4 times with NET-2. Proteins were released from the agarose by heating at 95°C for 5 min in 50 µl Tris-Cl, pH 8, 200 µM β-mercaptoethanol, 2% SDS, 0.1% bromphenol blue, and 10% glycerol, separated by SDS-PAGE, and visualized as described above.

**Western Analysis**

For the detection of GALA BD-SNP1 fusion in TS385-1, exponentially growing cells were harvested and resuspended in 250 µL sodium phosphate, pH 7.0, 10 µM EDTA supplemented with 0.1 mg/ml chymostatin, 2 mg/ml aprotinin, 1 mg/ml pepstatin A, 2 mg/ml trans-epoxysuccinyl-l-leucylamido-(4-guanidino) butane, and 0.5 mg/ml leupeptin (Buffer A). Cells lysates were prepared by grinding with glass beads (29). Samples containing 50–150 µg of protein were fractionated by SDS-PAGE and then transferred onto Immobilon-P membranes (Millipore) in Towbin buffer containing 0.1% SDS and no methanol (43). Primary antibody anti-Gal4p antibody was diluted 1:2000 in antibody buffer (5% nonfat milk, 10 µM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.01% Tween 20). The secondary antibody, sheep-anti-rabbit IgG conjugated with horseradish peroxidase, was diluted 1:5000 in antibody buffer. Proteins were visualized by chemiluminescence.

Proteins from WCE and extract fractions were analyzed as just described except that the primary antibodies (anti-HA 12CA5 or 16B12 mAbs, and anti-FLAG M2 mAb) were diluted 1:3000 and 1:1500, and the secondary antibody, sheep-anti-mouse IgG conjugated with horse-radish peroxidase, was diluted 1:10,000.

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**H7C cells** were transformed with the pGBT9-Snp1p to obtain the strain TS385-1. The pGAD library was then introduced into TS385-1 by electroporation as described (32) except that 0.5 µg of single-strand salmon sperm DNA (average molecular mass of 300 nucleotides) and 1–2 µg of library DNA were added to 400 µl of electrophoretic competent cells.

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RNA Analyses

Total RNA from yeast cells was extracted as described previously (51). Oligos were radiolabeled at their 5′ ends with polynucleotide kinase as described (43) except that PEG8000 was added to 3%. Primer extension reactions for ScR1 and either U5 or RpL30 RNAs used 25 μg of total RNA per sample and oligos oSR139 and either oSR90 or oSR126 as described (52), except that the reactions were supplemented with 50 ng/ml actinomycin D. Reactions for target RNAs used oligos oSR180 and oSR139 and the conditions of Rymond et al. (53). The extension products were fractionated by electrophoresis in 6% polyacrylamide gels in 8 M urea, 89 mM Tris borate, and 2 mM EDTA, pH 8.3 (43), and measured with a Molecular Dynamics Storm PhosphorImager. The peak areas of the bands were determined with Molecular Dynamics ImageQuant software. The 0.5-h samples were not included in our analyses due to technical difficulties with some samples. The data were analyzed by the Anderson-Darling test for normal distribution and by both the Bartlett and Levene tests for variance homogeneity in Minitab (Mac version 8). Differences in the means were analyzed with SAS Institute Statistical software (version 8.1) using the General Linear Model ANOVA procedure with repeated measures and least squares means. The Greenhouse-Geisser adjustment for covariance homogeneity and circularity was used for multivariate ANOVA (54, 55). In one case (RpL30 mRNA) where the variances were not homogeneous (p < 0.01), the non-parametric Mood’s median test (in Minitab) was also used to compare the 4-h levels in the mutant and wild type strains.

In Vitro Splicing Reactions

The yeast wild type, radiolabeled actin promoter RNA for splicing assays was synthesized in vitro as described (56). WCEs were prepared in liquid nitrogen by the method described previously (56). The fractions 40P3 and 40W were made from yeast strains Tsr1210, Tsr1210, and Tsr1280, as described previously (57). For testing heat sensitivity and extension reactions for ScR1 and either U5 or RpL30 RNAs used 25 μg/H11032.

RESULTS

Prp8p and Exo84p Are Identified as Proteins Interacting with Snplp—To use the two-hybrid system to identify proteins that interact with Snplp, we constructed a fusion between the Gal4p DNA binding domain and the entire Snplp as bait. The resulting fusion protein, BD-Snp1p, was expressed and fully functional in yeast cells (see “Experimental Procedures”). The plasmid pGBT9-BD-SNP1 encoding this fusion was then put into a yeast strain with the HIS3 and lacZ reporter genes for the initial selection and screen with the two-hybrid system. A library of yeast genomic DNA fragments fused to the sequence encoding the Gal4p activation domain (pGADf-library) was constructed and the entire Snp1p as bait. The yeast wild type, radiolabeled actin precursor RNA for splicing

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We obtained coimmunoprecipitations for both combinations of proteins. Snp1p coprecipitated when it was incubated with HA-tagged Prp8p and anti-HA antibody (Fig. 2, lane 12). Similarly, Exo84p coprecipitated when it was incubated together with FLAG-tagged Snp1p and anti-FLAG antibody (lane 19). As controls, the HA-tagged Prp8p alone precipitated with anti-HA antibody (lanes 11), but little or none of it precipitated in the absence of antibody (lane 9) or the HA-tag (data not shown). Little or no Snp1p precipitated in the absence of HA-tagged Prp8p (lane 13) or antibody (lane 10). Similarly, little or no Exo84p precipitated in the absence of FLAG-tagged Snp1p (lanes 16) or anti-FLAG antibody (lane 17). Snp1p did not precipitate with anti-FLAG antibody unless it had the FLAG tag (data not shown). Both coprecipitations (Snp1p with Prp8p and Exo84p with Snp1p) required the proteins to be incubated together at 30 °C for at least 15 min (data not shown). Neither coprecipitation depended on RNA binding as the individual translations were treated with RNase A before they were mixed together. Furthermore, the coprecipitations likely do not depend on most other splicing factors because reticulocyte lysates do not complement several yeast prp5 mutant extracts in vitro (70, 71), an indication that the lysate lacks several proteins that could functionally interact with the yeast spliceosome. We conclude that Snp1p does physically associate with Prp8p or Exo84p in the absence of RNA and most other yeast splicing factors.

A Ts exo84 Mutation Causes Increased Percentages of Pre-mRNA for Some Transcripts at the Non-permissive Temperature—Often proteins that physically associate with one another function in the same biological process. To determine whether Exo84p functions in splicing, we first engineered some deletion mutations in vitro using convenient restriction endonuclease sites to delete N- and C-terminal portions. The mutations were then introduced into yeast cells by the plasmid shuffling method to test their effects in vivo (29). One of these, exo84:2, is recessive and results in Ts mitotic growth; mutant cells in the asynchronous population either did not divide or divided only one to a few times after being shifted to 37 °C (Fig. 3). The mutant grew more slowly than the wild type at lower temperatures. Another possibility, however, is that normally the Chs1p-Snp1p interaction would not occur in the cell because the Chs1p-Snp1p interaction would not occur in the cell because the Chs1p-Snp1p interaction is physiologically relevant to any ORF in the data bases to date. Its only remarkable feature as noted in the Prosite data base (68) is a ubiquitin-like domain encoded by amino acids 259–318.

The fourth protein, Usa4/Chs1p, synthesizes chitin for closing the daughter cell wall after cytokinesis (69). Perhaps the interaction between Chs1p and Snp1p is physiologically relevant. Another possibility, however, is that normally the Chs1p-Snp1p interaction would not occur in the cell because the region of Chs1p with which Snp1p interacts is not exposed or the two proteins do not colocalize.

Snplp Physically Associates with Prp8p and Exo84p—To investigate further the interactions of Snp1p with Prp8p and Exo84p, we tested if the proteins physically associate by an immunological assay. The entire SNP1 and EXO84 coding regions were subcloned for expression in vitro. The FLAG epitope tag was also added to the C-terminal end of Snp1p. Because the large size of Prp8p precludes efficient expression in vitro, a DNA fragment encoding a 115-kDa fragment of Prp8p (Prp8f) containing the Snp1p interaction region was used. Prp8f was also engineered with the HA tag at its C-terminal end. Radiolabeled forms of Snp1p, Exo84p, and Prp8f were obtained by in vitro translation in the presence of [35S]methionine (Fig. 2).

Although the predominant form of Exo84p in the translation was 24 kDa smaller than the predicted molecular mass of 85.5 kDa, the 61-kDa fragment still contained the Snp1p-interacting region and was probably derived from the full-length protein by cleavage near the N terminus (data not shown). The individual translations were treated with RNase A and then incubated together at 30 °C for 30 min after which they were subjected to immunoprecipitation with either anti-FLAG or anti-HA monoclonal antibody.

We obtained coimmunoprecipitations for both combinations of
temperatures (Fig. 3) and also showed cold-sensitive growth arrest at 16 °C (data not shown). The exo84-2 mutation substitutes arginine 636 with proline and introduces a stop codon immediately thereafter such that the mutant protein also lacks the last 117 amino acids of its C-terminal end. The other deletion mutations and their phenotypic effects will be described elsewhere.

We next analyzed the transcripts of the intron-containing RPL30 and ACT genes and the intron-less SNR5 and SCR1 genes in the wild type and exo84-2 mutant strains. The yeast strains were grown to midlog phase at the permissive temperature (26 °C) and then shifted to the non-permissive temperature (37 °C). RNAs were extracted from the yeast cells incubated at 26 and 37 °C and analyzed by primer extension assays. We found that the levels of Rpl30 and actin mRNAs decreased in the exo84-2 mutant after the shift to 37 °C (Fig. 4, A, B, and D); the levels of Rpl30 and actin mRNAs went from 100% at 0 h to 36 and 40%, respectively, at 4 h. The levels of both the Rpl30 and actin mRNAs in the mutant were statistically significantly less than the wild type levels during incubation at 37 °C (p < 0.01 and p < 0.05 respectively). Although there were some differences in Rpl30 mRNA levels among the wild type isolates during the temperature shift (one isolate (Fig. 4D)) showed less of a heat shock response than the other two (Fig. 4A)), the levels of Rpl30 mRNA went from 100% at 0 h to 28% at 4 h. The levels of actin mRNA went from 73% at 0 h to 49% at 4 h. These results suggest that the defect caused by the exo84-2 mutation is due to the decreased levels of total Rpl30 and actin mRNA.

The exo84-2 Mutation Inhibits Splicing Activity in Vitro—If the exo84-2 mutation directly affects splicing, it should cause a splicing defect in vitro. To test this, we utilized the Ts phenotype of the exo84-2 mutation. Previously it has been shown that a Ts mutation in a splicing factor frequently confers heat or cold sensitivity for splicing activity in vitro (58, 74). We therefore made active splicing whole cell extracts (WCEs) from the isogenic wild type and exo84 mutant cells grown at the permissive temperature and then tested these extracts for heat sensitivity in vitro at different temperatures. The mutant WCE was inactivated for splicing by short heat treatments at 41 °C that did not inactivate wild type WCE (Fig. 5A). When radiolabeled actin pre-mRNA was added to the inactivated WCE, no splicing intermediates or products were formed. Addition of magnesium to exo84-2 WCE shortened the incubation period required for inactivation, but ATP had no effect (data not shown). These results suggest that the defect caused by the short heat treatments of the exo84-2 mutant WCE is due to the Ts exo84-2 mutation and occurs before the first splicing reaction.

That the heat sensitivity for the mutant extract is due to the exo84-2 mutation was assayed in two ways. First, we determined if an inactivated exo84-2 mutant WCE would complement inactivated WCEs with some Ts prp mutations in known splicing factors. If a distinct component is inactivated in each
extract, then different extracts should complement one another. If the inactivated components are the same or do not readily exchange, then the extracts will fail to complement. WCE from exo84-2, prp2-1, prp5-3, and prp9-1 mutants were individually heated and then mixed pairwise and assayed for splicing activity at 23 °C (Fig. 6B). Each heated extract had little or no splicing activity but complemented each of the other extracts. This result indicates that the heat sensitivity of each extract is due to the inactivation of specific, different, exchangeable components in each extract.

Second, we tested if splicing activity of the inactivated exo84-2 mutant WCE could be restored by complementation with two fractions (40P3 and 40W) made from wild type WCE. Each extract fraction, 40P3 and 40W, has been shown previously to be enriched for a subset of splicing factors but inactive for splicing (57). Splicing activity can be restored when the two fractions are mixed together. To detect Exo84p in WCE and extract fractions, we created an HA-tagged form of Exo84p in vitro and substituted it for the wild type protein in vivo. We found that the fraction 40P3, which contains about 10% of the total protein in the original WCE, was enriched for HA-tagged Exo84p about 5-fold relative to WCE (Fig. 7A). Moreover, the
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FIG. 5. Levels of Rpl30 and actin pre-mRNAs in wild type and exo84-2 mutant strains at 26 and 37 °C. The levels of pre-mRNAs and mRNAs in cells removed immediately before or at 1–4 h during incubation at 37 °C were assayed by primer extension (Fig. 4, A and B) and measured with a Molecular Dynamics PhosphorImager. Three each of wild type and exo84-2 mutant yeast isolates were assayed. The percent pre-mRNA equals (100 × units pre-mRNA/units pre-mRNA + mRNA). The probabilities of the means in the mutant and wild type strains at 37 °C being equal are indicated by asterisks.

40P3 fraction with either HA-tagged Exo84p (not shown) or untagged Exo84p complemented the heat-inactivated exo84-2 mutant WCE for splicing (Fig. 7B). The other fraction, 40W, was deficient in Exo84p (Fig. 7A) and did not restore splicing activity to inactivated exo84-2 WCE (Fig. 7B). Additionally, we made the 40P3 extract fraction from the exo84-2 mutant and tested its heat sensitivity and complementing activity in vitro. When exo84-2 mutant WCE and mutant 40P3 fraction were heated together, splicing activity was lost. When mutant WCE and wild type 40P3 were heated together, however, splicing activity was retained (data not shown). These results indicate that mutant Exo84p in the 40P3 fraction is also heat-sensitive in vitro and that this sensitivity is recessive in vitro as it is in vivo. The collective results from all these in vitro splicing assays are consistent with the exo84-2 mutation having a direct effect on splicing.

The exo84-2 Mutation Inhibits an Early Step in Spliceosome Development—The results of the in vitro splicing assays indicate that the mutation inhibits splicing before the first catalytic splicing reaction, or even earlier, during assembly of the spliceosome onto the pre-mRNA. We therefore assayed spliceosome formation in isogenic exo84-2 mutant and wild type WCEs at 23 °C before and after heat inactivation at 41 °C for 7 min in vitro. The spliceosome assembly assay uses radiolabeled actin pre-mRNA as substrate and polyacrylamide gel electrophoresis under nondenaturing conditions to separate the complexes formed on the substrate (9). These complexes normally migrate as three bands, δ, β, and α, with the β and α bands migrating closely to one another. We found that the untreated mutant and both the untreated and heat-treated wild type extracts formed all five spliceosomal complexes that contribute to these three bands (Fig. 8). In contrast, the inactivated mutant extract formed the ATP-independent δ complex but little or none of the other complexes. Thus the exo84-2 mutation blocks pre-spliceosome formation.

The interaction of Exo84p with Snp1p, a component of the U1 snRNP, suggests that Exo84p associates with the U1 snRNP. We assayed for such an association by in vitro immunoprecipitation assays using WCE with HA-tagged Exo84p, and immobilized anti-HA antibody. Any spliceosomal snRNAs coprecipitating with HA-tagged Exo84p were detected by primer extension using radiolabeled oligos specific for each spliceosomal snRNA. We were unable to detect an association of Exo84p with any of the five snRNAs, even under low salt stringency (50 mM NaCl) (data not shown). Similarly, we could not detect an association of Exo84p with radiolabeled pre-mRNA added to the extract (data not shown). Therefore, either Exo84p does not associate with a spliceosomal snRNP or pre-mRNA or the assay failed to detect the association.

DISCUSSION

In this study we found two proteins to associate physically with Snp1p, a component of the yeast spliceosomal U1 snRNP. One of the proteins, Prp8p, is a known spliceosomal component essential for splicing. The other, Exo84p, has recently been found by others (28) to be essential and required for the secretory pathway. We have discovered that Exo84p is also involved in pre-mRNA splicing. An exo84-2 mutation engineered in vitro was found to be Ts for mitotic growth in vivo and to alter expression of two genes containing introns. The exo84-2 mutation, like the prp5-3 mutation in a known splicing factor, increased the levels of actin and RPL30 pre-mRNA relative to the spliced mRNA at the non-permissive temperature. In vitro assays indicated that Exo84p plays a direct role in splicing in that inactivation of the protein in vitro resulted in a loss of splicing activity. When the Ts exo84-2 mutant extract was heat-treated in vitro, it became defective for splicing and pre-spliceosome formation. These results suggest that Exo84p could be a splicing factor, a factor that modifies a splicing factor, or both.

The Interaction between Prp8p and Snp1p Suggests New Roles for Snp1p—Although it has been conjectured earlier that the U1 snRNP leaves the spliceosome before the tri-snRNP binds, our finding that Snp1p interacts with Prp8p strongly suggests that the U1 snRNP is acting at additional, later steps in the pre-mRNA splicing pathway. Prp8p is a very large protein component of the U5 snRNP that is required for several steps in the pre-mRNA splicing pathway (75, 76). It is necessary for the U4/U6.U5 tri-snRNP to form and bind to the prespliceosome. It is essential for both the first and second transactinidation reactions of splicing during which it can be cross-linked to both the 5'- and 3'-SS. It is highly conserved, sharing 65 and 61% identity with the human and worm Prp8 proteins throughout nearly its entire length of 2400 amino acids (60, 77). Yet, Prp8p appears to be relatively featureless, others have noted only predicted amphipathic helices (amino acids 643–699 and 1626–1651) (78) and a non-conserved, proline-rich region at the N terminus (60).

The 28 amino acids (1166–1193) of Prp8p that associate with Snp1p in the two-hybrid assay (Fig. 1) delineate a very small, charged region in the middle of Prp8p. Remarkably, substitution mutations in some of these amino acids (1191–1194) also suppress the cold-sensitive U4cs1 mutation in the U4 snRNA (78). U4cs1 extends the base pairing of the U4 and U6 snRNAs in the U4/U6 snRNP. This extension occludes the ACAGA box of U6 snRNA (the region which normally pairs with the 5'-SS during catalysis) and enhances the stability of the U4/U6 duplex. Because of these and other genetic interactions, Kuhn and Brow (10) suggested that Prp8p may also coordinate unwinding of the U4/U6 snRNAs with unwinding of the U1/5'-SS duplex during spliceosome activation to catalytic competency. The Snp1p-Prp8p interaction could also be involved in both unwinding the U4/U6 snRNAs and destabilizing the U1/5'-SS duplex and in coordinating these two events. The 70K protein, the human homolog of Snp1p, interacts with the U1 C protein which stabilizes the U1/5'-SS duplex (13). Both the yeast U1 Snplp and C proteins contact the 5'-SS (6). Recently, the yeast C protein has also been found to counteract Prp28p, a helicase that disrupts U1/5'-SS stability (12). Therefore, it seems likely the yeast Snp1p and C proteins also interact and that this interaction would help stabilize the U1/5'-SS duplex. Furthermore, the Snplp-Prp8p interaction might antagonize the Snp1p-C interaction and help to destabilize the duplex. This function of the Snp1p-Prp8p interaction is consistent with observations that about the time the tri-snRNP complex containing Prp8p becomes part of the spliceosome, the U1 snRNP
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Fig. 6. The exo84-2 mutant splicing extract is heat-sensitive in vitro and complements other inactivated mutant extracts. A, the isogenic exo84-2 mutant and wild type WCE were incubated at 41°C for the indicated times (min) after which they were assayed for splicing activity at 23°C for 10 min with radiolabeled actin pre-mRNA as substrate. The radiolabeled RNAs in the reactions were extracted, fractionated by denaturing PAGE, and visualized by autoradiography as shown here. The symbols from top to bottom represent splicing intermediate lariat-exon 2, lariat product, pre-mRNA, spliced mRNA, and intermediate exon 1. B, active WCE from mutants exo84-2, prp2-1, prp5-3, and prp9-1 (84, 2, 5, and 9; lanes 1–4) were heat-inactivated in vitro (lanes 5–8). The inactivated extracts were then mixed pairwise in various combinations (lanes 9–14). Splicing assays were initiated by the addition of splicing buffer components, ATP, and radiolabeled pre-mRNA and incubated for 15 min at 23°C. The RNAs were analyzed as in A.

Fig. 7. The splicing defect caused by the exo84-2 mutation can be complemented in vitro with a fraction enriched for wild type Exo84p. A, Western blot analysis of HA-tagged Exo84p in WCE and extract fractions 40P3 and 40W. The fractions 40P3 (P3) and 40W (W), and WCE (CE) were made from a yeast strain with HA-tagged Exo84p (+HA tag). Aliquots of these extracts as well as WCE from a wild type strain (−HA tag) were fractionated by SDS-PAGE, electrophoretically transferred to a membrane, and probed with anti-HA monoclonal and anti-Bcy1 polyclonal antibodies. Ha-tagged Exo84p and the control, Bcy1p (105) which is not a spliceosomal factor, were visualized by chemiluminescence as shown here. The following amounts of extracts were loaded onto the gel: 47 µg of CE, lane 1; 14 µg of P3, lane 2; 28 µg of P3, lane 3; 64 µg of P3, lane 4; 22 µg of W, lane 5; and 40 µg of CE (−HA tag), lane 6. B, in vitro complementation of heat-inactivated exo84-2 mutant WCE with a fraction enriched for wild type Exo84p. Active exo84-2 WCE (lane 1) was heat-inactivated for 7 min at 41°C. The inactivated WCE was then combined with buffer D only (lane 2), wild type 40P3 (0.02 µg, lane 3; 1.7 µg, lane 4; 4.2 µg, lane 5; and 16.7 µg, lane 6) or wild type 40W (5 µg, lane 7; 25 µg, lane 8; and 50 µg, lane 9) and assayed for splicing activity using radiolabeled actin pre-mRNA as described in Fig. 6A. As controls, the active mutant extract was assayed with added 40P3 (16.7 µg, lane 10) or 40W (50 µg, lane 11). The fractions 40P3 (16.7 µg, lane 12) and 40W (100 µg, lane 13) individually had no splicing activity.

becomes less stably associated with the spliceosome (9, 11). Snp1p may also physically contact Prp44p (Brr2/Rss1/Sih22/Snu246p) (79). Prp44p is now thought to be the most likely DEAD box helicase to unwind the paired U4/U6 snRNAs (80).

It is also possible that the Snp1p-Prp8p interaction functions in docking the U4/U5/U6 tri-snRNP onto the pre-spliceosome and, more specifically, in guiding Prp8p to the 5′-SS region. Consistent with this possibility, the U1 and U5 snRNPs are both present in at least one spliceosomal complex during spliceosome development (9, 11). Moreover, U1/U5 snRNP complexes have been observed in both yeast (9) and human (81) splicing extracts, and the human U1 and U5 snRNAs can be cross-linked. Finally, there is the possibility to consider that Snp1p has a function involving Prp8p that is independent of the U1 snRNP. For example, the 70K protein shuttles between the nucleus and cytoplasm without U1 snRNA (82).

Role of Exo84p in Pre-mRNA Splicing—We have shown here that exo84-2 mutant extracts are heat-sensitive for splicing in vitro. Several lines of evidence support our conclusion that this sensitivity is due to inactivation of the exo84-2 mutant protein in vitro, thereby indicating that Exo84p has a direct role in splicing. The inactivated exo84-2 extract complemented inactivated splicing extracts with defects in other splicing factors. Moreover, the inactivated exo84-2 extract was complemented by the extract fraction 40P3 that was enriched for wild type Exo84p, and the 40P3 fraction made from the exo84-2 mutant was heat-sensitive in vitro. The complementation patterns of the inactivated exo84-2 mutant extract with different mutant
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Exo84p physically interacts with the spliceosome or at least the U1 snRNP. It therefore seems more likely that the inability of the purified GST-Exo84 fusion protein to complement the inactivated extract is due to (a) the altered activity of fusion or (b) the absence of a required, Exo84p-associating factor that is not readily exchangeable in the inactivated exo84-2 extract. Such a factor would probably not be Prp2p, Prp5p, or Prp9p because the corresponding inactivated mutant extract readily complemented the inactivated exo84-2 extract in vitro. In conclusion, the in vitro and in vivo results collectively indicate that the heat sensitivity of the mutant exo84-2 extract is due to a loss of function of mutant exo84-2p. The simplest explanation for the in vitro complementation pattern is that normally wild type Exo84p has a direct role in splicing.

Inactivation of mutant exo84-2p prevents prespliceosome formation in vitro (Fig. 8). This defect is compatible with the physical interaction of wild type Exo84p with Snp1p. Yeast prespliceosome formation requires an intact U1 snRNP bound correctly to the pre-mRNA in the δ or commitment complex (3, 5). Furthermore, factors bound to the branchpoint region of pre-mRNA (83), as well as the activities of Prp5p (45, 84) and at least seven other proteins including Prp9p, are important for U2 snRNP binding and prespliceosome formation (4, 85). Therefore, inactivation of Exo84p may affect the δ complex, the U2 snRNP, or some other factor in the extract that prevents the U2 snRNP from stably associating with the complex. However, we were unable to detect a physical association of Exo84p with any of the spliceosomal snRNPs. The interactions of Exo84p with these factors may be transient or unstable and therefore hard to detect. We must also consider that Exo84p could modify or be a cofactor for modifying a spliceosomal factor.

The in vivo assays showed 1) that the levels of spliced mRNAs decreased and 2) that the percentage of transcript as unspliced pre-mRNA increased for the Rpl30 and actin transcripts at 37 °C in the exo84-2 mutant. The percent pre-mRNAs for the RPL30 and actin transcripts were 3.8- and 3-fold, respectively, that at 26 °C in the mutant, as well as 2- and 4-fold, respectively, the percent pre-mRNA in the wild type strain at 37 °C. These in vivo effects are relatively mild compared with the in vitro effects on splicing. As our in vitro data indicate a direct role for Exo84p in splicing, why did we not see a larger accumulation of pre-mRNA in the exo84-2 mutant cells in vivo? Like mutations in some other splicing factors (86), the exo84-2 mutation is more severe in vitro than in vivo. The severity of a Ts mutation in vivo can depend on the genetic background of a strain (58). Furthermore, several RNA processing pathways can influence the turnover of RNAs in vivo and could lower the percent pre-mRNA (87–90). Conversely, the exo84-2 mutation could also affect other intron-dependent processes in addition to splicing that modulate RNA levels in vivo. We would not have detected these processes in our in vitro splicing assays.

The decreased levels of Rpl30 and actin RNAs seen in vivo could also be part of a stress response provoked by interrupting the secretory pathway (91). Several Ts sec mutations conferring defects in different steps in the secretory pathway also lead to repression of transcription of the ribosomal RNA and protein genes but not other genes (92, 93). To our knowledge, no mutations in the exocyst subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Eox70, and Exo84 proteins (28)) have been tested for this phenomenon. However, the effects of the exo84-2 mutation on actin RNA levels suggest that there could be some differences in the response invoked by this mutation compared with the sec mutations that have been examined. We observed in this study that both the splicing and total level of actin RNAs decreased as a result of the exo84-2 mutation. Warner and colleagues (92) have found that actin mRNA levels are unaf-

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*Fig. 8. The inactivated exo84-2 mutant extract is defective in prespliceosome formation.* Active and heat-treated exo84-2 and wild type WCE were assayed for spliceosome assembly activity as shown here. Half of each wild type and mutant extract was heated at 41 °C for 7 min. Splicing buffer components, ATP, and radiolabeled actin pre-mRNA were then added to the untreated and treated extracts and incubated at 23 °C. At the times indicated, samples were removed, quenched on ice, and then separated by native PAGE. Splicing-specific complexes containing radiolabeled RNA were visualized by autoradiography as shown here. The sequence of complex formation on the pre-mRNA is δ → β, (the prespliceosome) → α → α1 → β2 (the active spliceosome) (9); however, the individual complexes within the α and β bands are not distinguishable in assays using radiolabeled pre-mRNA as substrate. The δ complex contains U1 snRNP. The β1 complex (the prespliceosome) contains both U1 and U2 snRNPs.

and wild type extracts and extract fractions are consistent with the recessive nature of the exo84-2 mutation in vivo and a loss of Exo84p function. However, we were not able to restore activity to the mutant extract by adding back a GST-Exo84 fusion protein isolated from yeast cells and therefore cannot exclude the possibility that the mutation indirectly causes a Ts defect in splicing.

We think the possibility that the Ts defect is indirect is unlikely for two reasons. First, the heat sensitivity of the mutant 40P3 fraction argues against the idea that the temperature sensitivity in vivo and in vitro is due to the indirect effect of a decreased concentration of an essential splicing factor. Second, the number and types of interactions detected among Exo84p and some other splicing factors reveals a functionally congruent pattern. Interactions between Exo84p and Snp1p (this study), Exo84p and Prp40p, another U1 snRNP-specific protein, Prp8p and Prp40p (7), and Snp1p and Prp8p (this study) have been detected. Exo84p itself may also interact with the U5 snRNP as a two-hybrid study identified three clones interacting with Prp8p that encode amino acids 615–753 of Exo84p (78). Interestingly, most of these Exo84p amino acids are deleted in the exo84-2 mutation. When the interactions between the U1 and U5 snRNPs as discussed above are considered as well, the pattern of interactions suggests that

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*S. Ruby, unpublished data.
3 P. Siliciano, personal communication.
fected in the non-exocyt sec mutants. Furthermore, these sec mutations act earlier in the pathway than when the exocyt functions. It will thus be interesting to see whether the exo84-2 mutation also causes decreased levels of non-intron-containing, ribosomal protein gene RNAs.

A Dual Role for Exo84p in Secretion and Pre-mRNA Splicing?—Most of the secretory pathway in yeast is dedicated to the synthesis of cellular membranes and the secretion of proteins for the cell wall (96). As part of the exocyt complex, Exo84p tethers secretory vesicles onto the plasma membrane (28). These vesicles contain the proteins and lipids necessary for membrane expansion, as well secreted proteins such as invertase. Perhaps Exo84p has two distinct functions in the cell, one for secretion and one for pre-mRNA splicing. Alternatively, the activity of Exo84p in secretion may be somehow functionally linked to pre-mRNA splicing. The effect, if any, of the exo84-2 mutation on secretion will thus be interesting to test.

There have already been some genetic hints of interactions between pre-mRNA splicing and the secretory pathway in S. cerevisiae. A mutation in LUC7, which encodes another U1-snRNP-specific protein essential for splicing (72), also leads to enhanced export of secreted proteins (97). A mutation in SLT11, which is essential for splicing in vivo and in vitro, also causes aberrant cell wall synthesis or structure (98). In the case of the slt11 mutation, however, the effect on splicing may be indirect; slt11 reduces splicing of Sar1 pre-mRNA that encodes a protein essential for endoplasmic reticulum-to-Golgi trafficking (99). Finally, several proteins involved in vesicular transport in addition to Exo84p have been found to interact with at least 10 splicing factors in two-hybrid screens (79, 100). Although some of these two-hybrid interactions may not be physiologically relevant, the number of interactions between components of the secretory pathway and spliceosomal proteins suggest that there could be an extensive network linking splicing and secretion.

Exo84p could have a regulatory role for splicing by linking the functional state of the secretory pathway to pre-mRNA splicing. For example, in yeast cells the stress of accumulated, unfolded proteins in the endoplasmic reticulum induces Hac1 RNA splicing, although this splicing involves non-spliceosomal factors (94). In mammalian pituitary cells, depolarization leads to changes in the alternative splicing of some ion channel factors (94). In mammalian pituitary cells, depolarization leads to changes in RNA splicing, although this splicing involves non-spliceosomal components of the secretory pathway and spliceosomal proteins (79, 100). We thank D. A. Brow and M. A. Oasley for comments on the manuscript; T. Chang, A. Kuhn, D. Brow, and P. Siliciano for communicating results prior to publication; and the University of New Mexico Center for Genetics in Medicine for oligo synthesis and some DNA sequencing.

When and Where Do Exo84p and Snplp Interact in the Yeast Cell?—Finding that Exo84p interacts with Snplp and that it is required for splicing in vitro raises the questions of when and where Exo84p and Snplp interact in the yeast cell. A number of scenarios can be considered at this point. Because pre-mRNA splicing occurs in the nucleus, the simplest hypothesis is that Exo84p interacts there with the spliceosomal components. However, Exo84p, as part of the exocyst complex, has been shown to localize to regions of plasma membrane expansion and cell wall synthesis (28). Nonetheless, there is a significant pool of free Exo84p that does not cosediment with the exocyst complex. We have noted that Exo84p has possible sequences for nuclear localization and for nuclear export, and thus it may shuttle between the nucleus and cytoplasm. Npl3p, another protein that may interact with the U1 snRNP (101), also shuttles between the nucleus and cytoplasm (102). Interestingly, nuclear import of Npl3p is regulated in part by the cytoplasmic Sky1 kinase, a conserved SR protein kinase (64). Sky1p phosphorylates a single serine within a consensus target site on Npl3p. Exo84p has two putative copies of this site.

Another possibility is that Exo84p interacts with splicing factors while they are in the cytoplasm. In mammalian cells, the snRNPs are assembled in the cytoplasm, and the mature snRNPs are then transported to the nucleus (103). Additionally, some snRNP factors, like the 70K protein, may shuttle between the nucleus and cytoplasm independently of their snRNP partners (82). Finally, Snplp, or even the U1 snRNP, could also relocate to the cytoplasm as part of a stress response. Some yeast Ts sec mutations that interrupt secretion and induce repression of transcription of ribosomal protein genes also cause relocation of nucleolar and nucleoplasmic proteins, such as Npl3p and some U1 snRNP proteins, to the cytoplasm (104). By whatever mode Snplp and Exo84p come together, their interaction links RNA processing to the secretory pathway. The link may be simply that Exo84p has two independent functions in the cell. Alternatively, Exo84p and Snplp may be components of a larger regulatory network modulating the two processes. In either case, this link may provide one of only a few examples discovered in yeast of the regulation of splicing in response to a physiological stimulus.
New Roles for the Snp1 and Exo84 Proteins in Yeast Pre-mRNA Splicing
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