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Mpp10p, a U3 Small Nucleolar Ribonucleoprotein Component Required for Pre-18S rRNA Processing in Yeast

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We have isolated and characterized Mpp10p, a novel protein component of the U3 small nucleolar ribonucleoprotein (snoRNP) from the yeast *Saccharomyces cerevisiae*. The MPP10 protein was first identified in human cells by its reactivity with an antibody that recognizes specific sites of mitotic phosphorylation. To study the functional role of MPP10 in pre-rRNA processing, we identified the yeast protein by performing a GenBank search. The yeast Mpp10p homolog is 30% identical to the human protein over its length. Antibodies to the purified yeast protein recognize a 110-kDa polypeptide in yeast extracts and immunoprecipitate the U3 snoRNA, indicating that Mpp10p is a specific protein component of the U3 snoRNP in yeast. As a first step in the genetic analysis of Mpp10p function, diploid *S. cerevisiae* cells were transformed with a null allele. Sporulation and tetrad analysis indicate that *MPP10* is an essential gene. A strain was constructed where Mpp10p is expressed from a galactose-inducible, glucose- repressible promoter. After depletion of Mpp10p by growth in glucose, cell growth is arrested and levels of 18S and its 20S precursor are reduced or absent while the 23S and 35S precursors accumulate. This pattern of accumulation of rRNA precursors suggests that Mpp10p is required for cleavage at sites A0, A1, and A2. Pulse-chase analysis of newly synthesized pre-rRNAs in Mpp10p-depleted yeast confirms that little mature 18S rRNA formed. These results reveal a novel protein essential for ribosome biogenesis and further elucidate the composition of the U3 snoRNP.

In all eukaryotes, rRNA is transcribed as a single long transcript and processed by cleavages, nucleotide modification, and exonucleolytic degradation to generate the mature rRNAs. In the yeast *Saccharomyces cerevisiae*, these reactions result in the production of the mature 18S, 5.8S, and 25S rRNAs, which are assembled with the 5S ribonucleoprotein (RNP) and ribosomal proteins to form mature ribosomes. These events take place in the cell nucleolus. A number of small nucleolar ribonucleoproteins (snoRNPs) are required for many of these processing steps (25, 27, 40, 45).

Because it was readily identified in both vertebrate and yeast cells, the U3 snoRNP has been studied in a number of different organisms. Functional studies on the role of the U3 snoRNP in pre-rRNA processing have been carried out with cell extracts from mouse cells and *Xenopus* oocytes and in vivo in *Xenopus laevis* oocytes and in *S. cerevisiae* (4, 6, 18, 22, 30, 34). Collectively, the results from these experiments point to an obligate role for the U3 snoRNP in the cleavages in the 5′ external transcribed spacer (ETS) and in internal transcribed spacer 1 (ITS1) that generate the 18S rRNA (A0, A1, and A2 in Fig. 6).

The 5′-most U3-dependent cleavage site (A0) in yeast also requires the presence of the RNase III homolog, the Rnt1 protein (10). In fact, in vitro yeast RNase III will cleave at this site in the absence of any other factors. This suggests that RNase III is catalytic for this processing step and that the requirement for the U3 snoRNP may be to assist in the correct folding and orientation of the pre-rRNA. Therefore, the U3 snoRNP may function as a pre-rRNA chaperone.

The role that the U3 snoRNA itself plays in pre-rRNA processing has been studied in detail in yeast. The U3 snoRNA is essential and is required for processing at three sites that generate the 18S rRNA (18). The U3 snoRNA can be cross-linked to the 5′ ETS (6), and base pairing is required for its function (4, 5). Surprisingly, these sites of interaction of the U3 snoRNA with the nascent transcript are distant from the cleavage sites, further suggesting that the U3 snoRNP is acting as a chaperone. Recent experiments suggest that an additional interaction occurs in a sequence of the pre-18S rRNA that can form a pseudoknot (17). This suggests that the U3 snoRNP may play multiple roles, perhaps sequentially, in presenting or folding precursors to the 18S rRNA.

The RNA component of the U3 snoRNP is about 200 to 300 nucleotides in length and is found in a wide variety of species from humans to trypanosomes. The U3 snoRNA is bound to several proteins, including the mammalian autoantigen fibrillarin. The fibrillarin protein also binds to a number of other snoRNAs and is not unique to the U3 snoRNP (14, 20, 35, 41, 42). Little is known about the other protein components of the U3 snoRNP and whether they are specific for it. Only one has been identified so far: Sof1p (56 kDa). It was isolated as a suppressor of a temperature-sensitive fibrillarin protein and is essential for viability and for pre-rRNA processing in yeast (19).

We have identified, cloned, and studied another yeast U3 snoRNP component, which we call Mpp10p. This protein was discovered by homology to the human MPP10 protein (47). Screening of a HeLa cell expression library with an antibody that recognizes proteins that are phosphorylated during mitosis yielded the 120-kDa MPP10 protein (26, 47). Antibodies to this protein immunoprecipitate the U3 snoRNA, indicating that it is a component of the human U3 snoRNP (47a).

We were interested in whether MPP10 was required for pre-rRNA processing, and we sought to approach this problem by exploiting the genetics and biochemical tools available in yeast. We report here that the yeast Mpp10p homolog is 30% identical to the human protein and runs at 110 kDa in sodium dodecyl sulfate-polyacrylamide gel electro-

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phoresis (SDS-PAGE). Antibodies to it immunoprecipitate the U3 snoRNA, indicating that in yeast, as in humans, Mpp10p is a U3 snoRNP component. By targeted disruption of the *MPP10* gene, we show that it is essential for viability. Analysis of rRNA processing indicates that Mpp10p is required for processing at the three U3-dependent sites in the 5′ ETS and ITS1. In the absence of Mpp10p, 18S rRNA is not produced. Collectively, these results suggest that Mpp10p is a protein component of the U3 snoRNP that is required for pre-18S rRNA processing.

MATERIALS AND METHODS

Microbiological medium. *S. cerevisiae* cells were grown in the following media, where specified: YPD (1% yeast extract, 2% peptone, 2% glucose, YPG (1% yeast extract, 2% peptone, 2% galactose), SD (0.17% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose), SG (same as SD but with galactose instead of glucose), and His dropout medium (SD and a dropout supplement lacking histidine [Clontech]). The SD and SG media were supplemented with the following nutrients: adenine (40 μg/ml), histidine (20 μg/ml), leucine (60 μg/ml), lysine (30 μg/ml), and uracil (20 μg/ml).

Yeast strains. YPH260 (*MAT***a**/α *ura3-52/ura3-52 lys2-801^{amber}/lys2-801^{amber} ade2-101^{ochre}/ade2-101^{ochre} his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1*) was derived by mating YPH258 (*MAT***a** *ura3-52 lys2-801^{amber} ade2-101^{ochre} his3-Δ200 leu2-*Δ) and YPH259 (*MAT*α *ura3-52 lys2-801^{amber} ade2-101^{ochre} his3-Δ200 leu2-*Δ) (36).

Computer analysis. The amino acid sequence of the human MPP10 protein was used to search the *S. cerevisiae* database with the BLAST algorithm. Sequence alignments of the human, mouse, and yeast homologs were performed with Megalign in the DNASTAR program.

Cloning of the mouse and yeast homologs. The mouse homolog of the human *MPP10* cDNA (accession no. X98494) was obtained by screening a mouse myeloid leukemia cDNA library (LTR6) cloned in λZap (Stratagene) with a random-primed human *MPP10* probe. This library was obtained from Hui Zhang, Yale University. An *Nco*I fragment of the human *MPP10* gene was random primed in the presence of [^{32}P]dCTP as specified by the manufacturer (Amersham). Hybridization was carried out in 10% formamide at 52°C, and washes were performed at 52°C by standard methods (33). Automated DNA sequencing of the insert was carried out on an Applied Biosystems 373 Stretch sequencer by primer walking of both strands.

To make a probe to clone the yeast *MPP10*, two oligonucleotides were designed complementary to *MPP10* sequences. The sequences are 5′TTCTCACAA TCTCTCTCGATGGTAGCACCCT (ympp10.1) and 5′CCAATTGTTCTATC TGTTTTCTAATCTCTATTTGT (ympp10.2). They were used as primers for PCR with total yeast genomic DNA as a template. The approximately 600-nucleotide (nt) PCR product was cloned into pGEM-3Zf (Promega Biotech). A random-primed *Eco*RI-*Pst*I fragment containing *MPP10* sequences was used to probe a λ library of the yeast genome. A library that is available as an array on a filter was screened (ATCC 77284 from *S. cerevisiae* AB972). Hybridization was carried out in 50% formamide at 42°C. The cosmid corresponding to our positive clone was obtained from the American Type Culture Collection (ATCC). An *Eco*RV fragment containing the entire *MPP10* gene was subcloned into the *Sma*I site of pGEM-3Zf. The clone was confirmed by restriction digestion and by automated DNA sequencing on an Applied Biosystems 373 Stretch sequencer.

Expression and purification of the yeast Mpp10 protein in *E. coli*. The yeast *MPP10* gene was cloned into the pET-28a expression vector (Novagen) for expression in *Escherichia coli*. This places 6 histidines in frame with the MPP10 protein-coding sequences to facilitate purification. Two oligonucleotides were designed for PCR of yeast genomic DNA. The 5′ oligonucleotide includes the initiation codon and an upstream *Bam*HI site (5′CCGCGGATCCATGTCA GAACTCTTTGGAGTATTGAAATC; ympp10.3), and the 3′ oligonucleotide includes the stop codon and a downstream *Not*I site (5′GCATAGTTTAGCG GCCGCTGTTCAAAGTTTTATATTTGTGCTATC; ympp10.4). Following PCR, the DNA fragment was digested with *Bam*HI and *Not*I and cloned into the *Bam*HI and *Not*I sites of pET28a. This plasmid was transformed into *E. coli* BL21 (DE3) for expression. Induction of expression and analysis of solubility were carried out as specified by the manufacturer, except that induction was carried out overnight. Expression was evaluated by analysis of *E. coli* lysates on SDS–10% polyacrylamide gels.

Since Mpp10p was found primarily in inclusion bodies, purification was carried out under denaturing conditions by metal chelation affinity chromotography. We used a procedure that includes solid-state renaturation (16). Protein concentrations were quantitated by the method of Bradford, as specified by the manufacturer (Bio-Rad).

Antibodies and Western blots. The anti-fibrillarin antibody, 17C12, was obtained from Konstantin Konstantinov and Michael Pollard, Scripps Research Institute, and studied in yeast (2). The anti-trimethylguanosine (TMG) cap antibody was obtained from Oncogene Science. Antibodies to the purified Mpp10p were obtained by injection into rabbits, carried out at the Division of Animal Care at Yale University. Antibody production was monitored by Western blotting (Enhanced Chemiluminescence; Amersham) of yeast lysates (1).

Immunoprecipitations and RNA analysis. For immunoprecipitations analyzed by Northern blotting, antibodies were bound to 2.5 mg of protein A-Sepharose CL-4B (Pharmacia) as follows. Anti-Mpp10 (50 μl of rabbit serum), anti-fibrillarin (100 μl of culture supernatant), and preimmune rabbit serum (100 μl) were incubated with yeast cell lysates (see below) for 16 h at 4°C with rotation. A mock immunoprecipitation with no added antibody (PAS CL-4B alone) was also done. The same amounts of antibody were used for immunoprecipitations analyzed by 3′-end labelling, except that 10 μl of rabbit anti-Mpp10 and 10 μl of anti-TMG cap antibody were used. For the anti-TMG immunoprecipitation, 10 μl of a secondary antibody, rabbit anti-mouse antibody, was also used. The bound antibody was washed three times with NET-2 buffer (20 mM Tris-Cl [pH 7.5], 150 mM NaCl, 0.05% Nonidet P-40). All immunoprecipitations and washing of the immunoprecipitates were performed in NET-2 buffer.

For preparation of yeast cell lysates, exponentially growing cells were harvested at an optical density at 600 nm (OD_{600}) of 1 to 2, washed, and resuspended in NET-2 buffer. Protease inhibitors (2 μg of aprotinin per ml, 1 μg of leupeptin per ml, 1 μg of pepstatin A per ml) were included in the lysates used for Northern blots. The cells were lysed by vigorous vortexing (five times for 45 s) with 0.45- 0.5-mm-diameter glass beads. The lysate was cleared by centrifugation for 10 min at 13,000 × *g*. Immunoprecipitation was performed for 1 h at 4°C with lysate prepared from the equivalent of 5 OD_{600} units of cells. RNA was recovered by extraction with phenol-chloroform-isoamyl alcohol (25:24:1) and ethanol precipitated. For Northern blotting, the recovered RNA was separated on 8% denaturing polyacrylamide gels and analyzed by Northern blotting with antisense RNA probes (see below). For 3′-end- labelling, the recovered RNA was labelled with [^{32}P]pCp and T4 RNA ligase (11) and separated on 8% denaturing polyacrylamide gels.

Northern blotting with Zeta-Probe membrane (Bio-Rad Laboratories) was performed by the method of Kass et al. (22). The blots were probed with full-length antisense snoRNAs complementary to the indicated RNAs. The sequences of the U3, snR190, snR10, and U14 snoRNAs were amplified by PCR with primers which insert *Eco*RI and *Stu*I sites outside the 5′ and 3′ ends of the RNA, respectively, and cloned into pGEM-3Zf. Antisense RNAs were transcribed with SP6 RNA polymerase by the method of Melton et al. (29) with 1 μg of DNA template.

Disruption of the *MPP10* gene. Disruption of one allele of the gene encoding Mpp10p was carried out by homologous recombination via a PCR-based approach (3). Two oligonucleotides that have 50 nt complementary to the 5′- and 3′-flanking sequences of *MPP10* and 20 nt complementary to *HIS3* were designed. The sequences of the oligonucleotides are 5′TGAAGAAACAGTGTTT TGTTATGAATAAATGATGTGTGCACGAAAGACCCAAGCCTTGGCCC TCCTCTAG (ympp10.5) and 5′ATGAAGAAATAATTTTTTACCTAAATA CAAACTAAGTCTTCTTCAGGCGTCGTTCAGAATGACACG (ympp10.6). PCR was performed on a plasmid containing a clone of *HIS3* (pAD11) to generate a fragment of DNA that contains *MPP10*-flanking sequences and the *HIS3* gene. pAD11 (*ADE2 HIS3 CEN6 ARSH4*), obtained from R. Padmanabha and M. Snyder, Yale University, contains the *ADE2* gene cloned into pRS313 (36). By lithium acetate transformation (13), this fragment was transformed into the diploid strain, YPH260. Selection on histidine dropout medium allowed us to select colonies that have incorporated the DNA fragment. Verification that homologous recombination occurred at the *MPP10* locus was performed by Southern blot analysis on yeast genomic DNA (1). His$^+$ transformants heterozygous at the *MPP10* locus were sporulated, and tetrads were dissected. In each of the nine tetrads analyzed, only two spores were viable.

Cloning of *MPP10* under an inducible promoter and restoration of viability. A *Bam*HI-*Not*I fragment of the *MPP10* gene from the pET-28a *E. coli* expression plasmid was cloned into the *Bam*HI-*Not*I sites of the yeast expression plasmid pGAD3. pGAD3 was obtained from R. Padmanabha and M. Snyder and is a centromere-based plasmid with the *GALI-10* promoters and *URA3* and *ADE2* markers (described as plasmid p269 in reference 38). *pGAL1::MPP10* designates this plasmid (*GAL10/GAL1::MPP10 ADE2 URA3 CEN6 ARSH4*).

The *pGAL1::MPP10* plasmid was transformed into the heterozygous disruption strain, and colonies were selected on uracil dropout medium. Diploids were sporulated, and tetrads were dissected on plates containing galactose. The strains from the resulting spores were streaked onto 5-fluoroorotic acid (5-FOA) to verify growth dependence on the presence of the *pGAL1::MPP10* plasmid. We will refer to this strain as *mpp10::HIS3 pGAL1::MPP10.*

Analysis of pre-rRNA processing. For Northern analysis, the *mpp10::HIS3 pGAL1::MPP10* cells were grown in YPG medium to an OD_{600} of 1.0 and diluted into fresh YPD medium. At 0, 24, and 48 h after transfer to glucose-containing medium, total RNA was isolated and compared to total RNA isolated from the haploid strain YPH258 grown in YPD medium. Total RNA was isolated by the hot-phenol technique (1), 4.5 μg of total RNA was resolved on a 1% formaldehyde–agarose gel, and the RNA was transferred to Zeta-probe membrane.

The oligonucleotides used to probe for the rRNAs and their precursors were described by Berges et al. (8) and are depicted as bars in Fig. 5B. A 100-ng portion of each was labeled with 150 μCi of γ-ATP (5,000 Ci/mmol) and purified with the Qiaquick nucleotide removal kit (Qiagen). The blots were hybridized to 10^6 cpm of 5′-end-labeled oligonucleotide(s) per ml in 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7])–0.1% SDS at 37°C. The blots were washed twice in 5× SSPE–0.1% SDS at 37°C for 15 min each and

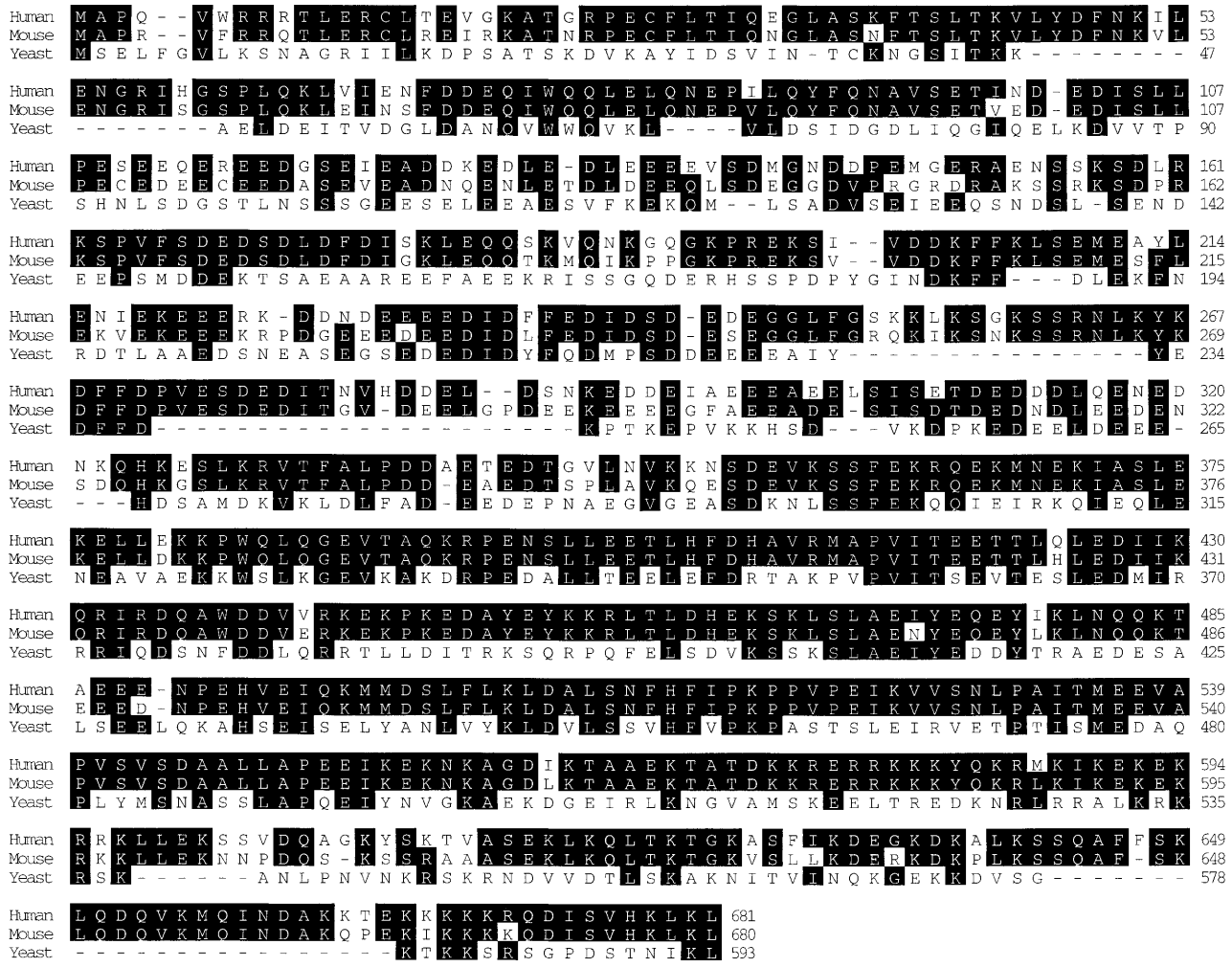

FIG. 1. Amino acid sequence of the MPP10 proteins from humans, mice, and the yeast *S. cerevisiae*. The white-on-black residues indicate sequence identity.

once in 1× SSPE–0.1% SDS at 37°C for 5 min. They were then exposed to X-ray film.

For pulse-chase analysis, YPH258 or *mpp10::HIS3 pGAL1::MPP10* cells were grown in SG (with supplements but without methionine) medium to an OD_{600} of 1.0. The cells were then washed in SD (with supplements but without methionine) medium and diluted into fresh SD medium. They were grown for 2 days, and then 30 ml was pelleted and resuspended in 3 ml of SD medium. The cells were pulsed with 60 μCi of [*methyl*-^{3}H]methionine per ml (75 Ci/mmol) at room temperature for 2.5 min. The cells were then chased with 500 μg of cold methionine per ml. Samples were taken at 0, 3, and 12 min after the chase, pelleted, washed, repelleted, and quick-frozen on dry ice-ethanol. Total RNA was isolated from cells by the hot-phenol technique (1). For each sample, 20,000 cpm of labeled RNA was resolved on a 1% formaldehyde-agarose gel. After transfer of labeled RNAs to Zeta-Probe membrane, the membrane was sprayed with EN^{3}HANCE (New England Nuclear) and then exposed to X-ray film.

RESULTS

Cloning of the mouse and yeast homologs of the human *MPP10* gene. To learn which sequences of the MPP10 protein play a functionally important role, we compared the human and mouse amino acid sequences (Fig. 1). We obtained a mouse cDNA by screening a mouse myeloid leukemia cDNA library with the human *MPP10* sequence. The mouse MPP10 protein is 680 amino acids long and has an overall identity of 83% (similarity of 92%) to the human protein. Interestingly, the extent of homology is not constant across the entire length of the protein. The two sequences are most similar over the last 300 amino acids (91% identity from amino acid 350 of the mouse sequence to the end), whereas the amino-terminal half of the protein is much less highly conserved. This sequence conservation suggests an important functional role for the carboxy-terminal half of the molecule. Also conserved are the predicted mitotic phosphorylation sites recognized by the MPM2 antibody (9) by which the human clone was originally detected (26).

A BLAST search of the translated GenBank database with the human MPP10 protein sequence yielded the *S. cerevisiae* homolog (Fig. 1). This was initially designated an open reading frame of unknown function on chromosome X (YJR002W, YJR83.5, J1411). The yeast Mpp10p is a protein of 593 amino acids, with an isoelectric point of 4.5 and a predicted molecular mass of 67 kDa. The human and yeast Mpp10p sequences are 30% identical and 50% similar over their length.

Although the mammalian and yeast proteins are only 30% identical, computer analysis indicates that they may share other important features. A search for motifs in the Mpp10p conserved among the three species yielded several predicted casein kinase II sites of protein phosphorylation, pointing to their potential functional significance. We have identified six sites that are conserved (SSGE, TSAE, SDDE, SSFE, SLAE,

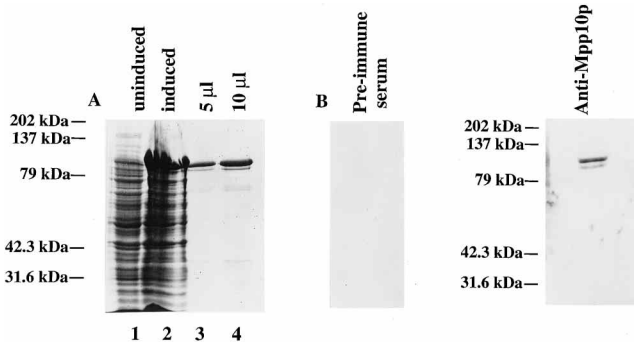

FIG. 2. Production of anti-yeast Mpp10p antibodies in rabbits. (A) Expression and purification of the yeast Mpp10 protein in *E. coli*. Lanes 1 and 2 contain lysates from *E. coli* before and after induction of expression of Mpp10p with isopropyl-β-D-thiogalactopyranoside (IPTG). Lanes 3 and 4 contain highly enriched Mpp10 protein resulting from metal chelation affinity chromatography. (B) Western blot of yeast lysates with either preimmune or anti-Mpp10p rabbit serum. The sera were used at a 1:20,000 dilution. The lower band is an Mpp10p breakdown product that appears in some preparations of yeast lysates.

and SMED). Furthermore, the predicted structure and charge distribution of the mammalian and yeast proteins is also very similar. All three are predicted to be largely alpha-helical and to have a similar distribution of acidic and basic amino acids. No known RNA binding motifs are present in any of the MPP10 proteins identified so far.

By using internal primers for the yeast *MPP10* gene, a 600-nt fragment was generated by PCR on yeast genomic DNA. This probe was radioactively labeled and used to probe a filter displaying a λ library of a robotic array of the yeast genome available from the ATCC. Two positives were obtained (data not shown). Subsequent computer analysis with the database available at the ATCC indicated that these two clones were overlapping fragments of chromosome X near the centromere, as predicted. Again using the ATCC database, we identified a cosmid clone that was likely to contain the *MPP10* gene. This cosmid was obtained from the ATCC, and an *Eco*RV fragment containing the entire MPP10 sequence was subcloned into the *Sma*I site of pGEM-3Zf.

Mpp10p expression in *E. coli* and production of antibodies. To investigate whether Mpp10p is a component of an RNP in yeast, we expressed it in *E. coli*, purified the fusion protein, and produced antibodies to it in rabbits. Primers were made to the 5′ (beginning at the AUG) and 3′ ends (including the stop codon) of the *MPP10* sequence. An in-frame *Bam*HI site was included at the 5′ end of the 5′ primer, and a *Not*I site was included at the 3′ end of the 3′ primer. PCR was performed on yeast genomic DNA, and a single band was obtained (data not shown). Following digestion by *Bam*HI and *Not*I, the fragment was cloned into the *Bam*HI and *Not*I sites of the pET-28a vector. This construct permits the expression of Mpp10p as a fusion protein with an amino-terminal six-histidine tag. SDS-PAGE analysis of the proteins produced in the induced and uninduced cells indicated the presence of high levels of expression of an approximately 110-kDa protein in the induced cells (Fig. 2A, lanes 1 and 2). The protein was purified by metal chelation affinity chromatography under denaturing conditions and obtained in soluble form following solid-state renaturation (lanes 3 and 4). A yield of at least 2 mg was obtained from 100 ml of induced *E. coli.*

The purified Mpp10p was injected into rabbits for the production of antibodies. The presence of antibodies was detected by Western blotting of yeast lysates with the rabbit antiserum. When preimmune rabbit serum was used, no cross-reactivity with yeast proteins was observed (Fig. 2B). However, when serum from an immune rabbit was used, a single band of approximately 110 kDa was observed in the yeast lysate (Fig. 2B), indicating that the antibodies recognize Mpp10p. The same results were obtained with the rabbit anti-Mpp10p serum at dilutions between 1:1,000 and 1:20,000, indicating a high titer.

Both the Mpp10p purified from *E. coli* and the Mpp10p in yeast lysates run at about 110-kDa in SDS-PAGE, which is considerably larger than the predicted molecular weight of 67 kDa. This aberrant migration on SDS gels has been noted previously for nucleolar proteins and is thought to result from either alternating positive and negative charges or heavily charged regions (7, 28).

Mpp10p is a U3 snoRNP component. The anti-Mpp10p antibody was used to investigate whether the Mpp10p protein is a component of the U3 snoRNP in yeast. Immunoprecipitations were performed on yeast extract with the rabbit anti-Mpp10p serum. RNA was extracted from the pellets and supernatants and analyzed by gel electrophoresis and Northern blots. For comparison, immunoprecipitations with anti-fibrillarin antibody were also performed. Antisense RNA probes for U3, U14, and snR190 were used. The results are shown in Fig. 3A. The anti-Mpp10p antibodies immunoprecipitate the U3 snRNA but not U14 or snR190 (lanes 3 and 7). In contrast, the anti-fibrillarin antibodies immunoprecipitate U3, U14, and snR190 (lane 4). Serum from the rabbit prior to injection (preimmune) does not immunoprecipitate U3 or any other of the snoRNAs. These results indicate that in yeast, as in humans, Mpp10p is likely to be a protein component specific to the U3 snoRNP.

Similar results were obtained following direct labeling of the snoRNAs after immunoprecipitation (Fig. 3B). U3 and many other snoRNAs are apparent in the anti-fibrillarin and anti-TMG immunoprecipitations (lanes 3 and 4). Only a few of these snoRNAs can be identified by size alone and are indicated. In the anti-MPP10p immunoprecipitation, the U3 snoRNA is greatly enriched over the other Box C/D snoRNAs (lane 5). Other RNAs, including the 5S and 5.8S rRNAs, are also present in this lane, but at levels comparable to that found when preimmune rabbit serum or beads alone are used (lanes 1 and 2). These results support our Northern blot results, again suggesting that Mpp10p is a component of the U3 snoRNP.

***MPP10* is an essential gene.** The *MPP10* gene was disrupted by transformation of a PCR fragment into the diploid yeast, YPH260. The PCR fragment was generated with primers designed to place the *HIS3* gene in the context of *MPP10* 5′- and 3′-flanking sequences, as depicted in Fig. 4A. This fragment replaces the entire coding sequence of *MPP10*. The diploid yeast cells were transformed with this PCR product and plated on histidine dropout medium to select for the presence of the *HIS3* marker. Genomic DNA was isolated from three of the resulting strains and the parent strain, and the presence of the disrupted *MPP10* allele was confirmed by Southern blot analysis. A 1.8-kb *Pst*I fragment which includes the gene and 3′-flanking sequences was used as a probe (Fig. 4A). *Eco*RV digestion of the intact *MPP10* followed by blotting with an *MPP10* probe yields a fragment of 2.9 kb, while the disrupted gene yields a fragment of 2.1 kb (Fig. 4B). These findings provide evidence that one allele of *MPP10* is disrupted in all three diploid strains (strains 5, 7, and 9). One heterozygous disruption strain was sporulated and tetrads were dissected to assess whether *MPP10* is essential for growth in yeast. Figure 4C shows that growth segregates 2:0 among the four spores, and the *HIS3* gene was not found in the surviving spores, indicating that *MPP10* is essential.

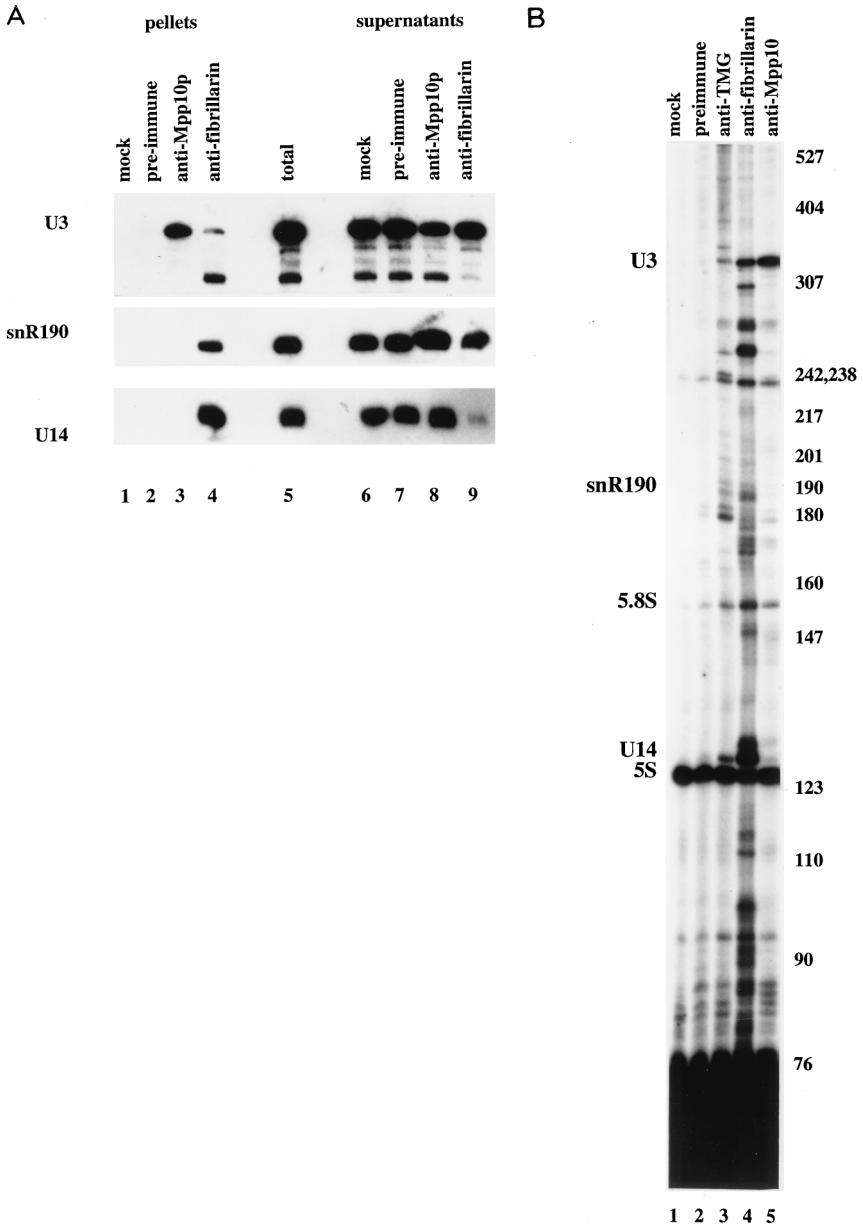

FIG. 3. Mpp10p is a U3 snoRNP component. (A) Immunoprecipitations were performed with either protein A-Sepharose CL 4B beads alone (lanes 1 and 6), preimmune (lanes 2 and 7) or anti-Mpp10p rabbit serum (lanes 3 and 8), or anti-fibrillarin antibody (17C12) (lanes 4 and 9). RNA was isolated from the pellets and supernatants from each immunoprecipitation and analyzed by electrophoresis on 8% denaturing polyacrylamide gels followed by Northern blotting. Antisense RNA probes were used to detect the yeast snoRNAs U3, snR190, and U14. The lower band in the U3 hybridization is a stable U3 snoRNA breakdown product. (B) Immunoprecipitations were performed with protein A-Sepharose CL 4B beads alone (lane 1), preimmune rabbit serum (lane 2), anti-TMG antibody (lane 3), anti-fibrillarin antibody (17C12) (lane 4), and anti-Mpp10 antibody (lane 5). The RNAs were directly labelled with [^{32}P]pCp and T4 RNA ligase and analyzed on an 8% denaturing polyacrylamide gel. The sizes of the labelled pBR322-Msp1 markers are indicated.

Expression of *MPP10* from a conditional promoter. To demonstrate that cell viability is dependent on an intact copy of *MPP10*, we restored growth to a strain carrying *mpp10::HIS3* by expression of Mpp10p from a plasmid. We cloned the *MPP10* gene into a plasmid with a galactose-inducible promoter and a *URA3* gene and have called it *pGAL1::MPP10*. When the heterozygous disruption strain bearing the *pGAL1::MPP10* plasmid was sporulated and tetrads were dissected on medium containing galactose, three or four spores grew from each tetrad (data not shown). These spores were tested for their dependence on the presence of the plasmid by streaking onto 5-FOA-containing medium. Some of the spores were inviable on 5-FOA, indicating that their growth was dependent on the presence of the *pGAL1::MPP10* plasmid. Therefore, growth can be restored to a strain with a disrupted *MPP10* gene by supplying Mpp10p.

Depletion of Mpp10p affects pre-18S rRNA processing. We hypothesized that the critical role played by Mpp10p in cell viability was in pre-rRNA processing. We tested this by depletion of Mpp10p by using the haploid strain with a disrupted chromosomal copy of *MPP10* (*mpp10::HIS3*) that maintains the *pGAL1::MPP10* plasmid. Mpp10p is expressed when this

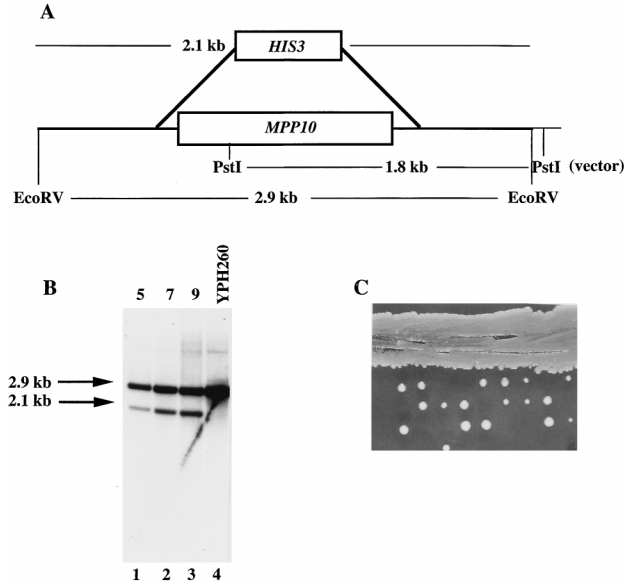

FIG. 4. *MPP10* is an essential gene in yeast. (A) Scheme for disruption of *MPP10*. Disruption was accomplished with a PCR product containing the *HIS3* gene and short 5′ and 3′ sequences identical to *MPP10* flanking sequences. (B) Disruption of *MPP10*. DNA was isolated either from three yeast strains (strains 5, 7, and 9) transformed with the PCR product in panel A or from the parent diploid strain, YPH260. Following digestion with *Eco*RV, the DNA was analyzed by agarose gel electrophoresis and Southern blotting for the presence of the disrupted *MPP10*. A random-primed *Pst*I fragment was used as a probe. The intact *MPP10* yields a fragment of 2.9 kb, while the disrupted *MPP10* yields a fragment of 2.1 kb. (C) *MPP10* is essential for viability. One heterozygous disruption strain from panel B was sporulated, and tetrads were dissected. The results from nine dissections are indicated.

strain is grown in galactose but not when it is grown in glucose. We assessed growth of the *mpp10::HIS3 pGAL1::MPP10* strain after transfer to glucose compared to growth of the otherwise isogenic *MPP10* strain (YPH258) in glucose and observed that growth continues for several hours after the switch to glucose but then slows and by 48 h is markedly impaired (Fig. 5A). At both 24 and 48 h after growth in glucose, little Mpp10p is detectable by Western blot analysis (data not shown). The Mpp10p that is still present is most probably due to incomplete repression of the *GAL* promoter in glucose (19, 23).

To investigate whether depletion of Mpp10p affects pre-rRNA processing, we extracted total RNA from the *mpp10::HIS3 pGAL1::MPP10* strain at 0, 24, and 48 h after growth in glucose and compared it to RNA from the strain that is isogenic except at the *MPP10* locus (YPH258). The presence of the mature 18S and 25S rRNAs and their precursors was assessed by Northern blot analysis with the specific oligonucleotides depicted in Fig. 5B. When oligonucleotides a and g were used to identify the mature 18S and 25S rRNAs, it is clear that 18S rRNA levels were reduced after depletion of Mpp10p whereas the 25S rRNA levels remained constant (lanes 1 to 4). The difference in intensities of the 18S and 25S signals is due to the oligonucleotides used and has been observed by others (8). When oligonucleotide b was used to detect precursors to the 18S rRNA, normal levels of the 20S precursor were present before the switch to glucose but absent after growth in glucose (lanes 5 to 8). In addition, the levels of 23S, a precursor that is normally present at low levels during normal pre-rRNA processing were increased, as were the levels of the 35S precursor (Fig. 6A shows the processing pathways, and Fig. 6B shows the Mpp10p-depleted processing pathway). The 23S precursor results from lack of cleavage at sites A0, A1, and A2 (15). Hybridization with an oligonucleotide that recognizes the 27SA2 precursor to 25S (oligonucleotide c) indicates that this precursor is absent after growth in glucose, again resulting from lack of cleavage at site A2 (lanes 9 to 12). An increase in the level of the 35S precursor after growth in glucose could also be seen after hybridization with this oligonucleotide. However, 25S synthesis proceeds in the Mpp10p depleted cells because the 27SB precursor is present (oligonucleotide e) from cleavage at site A3 or directly at B1 (lanes 13 to 16). This suggests that depletion of Mpp10p interferes with processing of pre-18S precursors and production of the mature 18S rRNA. Similar results were observed upon depletion of the U3 snoRNA (4–6, 17, 18).

The low 18S rRNA levels and absence of the 20S precursor seen in Fig. 5B could result from either decreased synthesis or increased lability of these RNAs. We used pulse-chase labeling of the rRNAs and their precursors to verify that the decrease we observed is due to decreased synthesis. We used [*methyl*-^{3}H]methionine to label the methyl groups of the newly synthesized rRNA in the isogenic strain (YPH258) and in the *mpp10::HIS3 pGAL1::MPP10* strain after 48 h in glucose. The results, shown in Fig. 7, demonstrate that the defect is in pre-18S rRNA processing. Little or no 18S rRNA was produced in the *mpp10::HIS3 pGAL1::MPP10* strain when grown in glucose (lanes 1 to 3), whereas even at time zero, 18S rRNA was detectable in appreciable amounts in the *MPP10* strain (lanes 4 to 6). The small amounts of mature 18S rRNA synthesized are due to the persistence of low levels of Mpp10p produced from an incompletely repressed *GAL* promoter (19, 23). The 35S precursor was also labeled at time zero in the Mpp10p-depleted strain (lane 1). In contrast, 25S rRNA was present at all time points in both strains.

Since depletion of the U3 snoRNA itself interferes with pre-18S rRNA processing (4–6, 17, 18), a defect in processing of 18S precursors following depletion of Mpp10p could occur because the levels of the U3 snoRNA are affected. To test this, RNA was isolated from the *mpp10::HIS3 pGAL1::MPP10* strain before and 24 and 48 h after the switch to glucose and analyzed for the presence of the U3 (snR17A), snR10, and U14 snoRNAs. As shown in Fig. 8, no change was seen in the levels of U3 compared to the other snoRNAs during this period (lanes 1 to 3). Therefore, accumulation of the U3 snoRNA was not affected by depletion of Mpp10p.

DISCUSSION

We have identified, cloned, and characterized the functional role of Mpp10p, a protein component of the yeast U3 snoRNP. The yeast homolog of the human Mpp10p was identified by database searching and is 30% identical to the mammalian MPP10 proteins. *MPP10* was cloned and expressed as a fusion protein in *E. coli*, purified, and injected into rabbits for production of antibodies. The antibodies recognize a protein of approximately 110 kDa on Western blots and selectively immunoprecipitate the U3 snoRNA, indicating that Mpp10p is a U3 snoRNP-specific component. Disruption of the *MPP10* gene followed by sporulation and tetrad analysis indicates that *MPP10* is essential. Depletion of Mpp10p with a conditional promoter indicates that, like the U3 snoRNA, it is required for processing of 18S rRNA precursors at sites A0, A1, and A2. Since the U3 snoRNA is stable after Mpp10p depletion, the observed effect on pre-rRNA processing is not due to an indirect effect of Mpp10p depletion on accumulation of the U3 snoRNA.

We also cloned the mouse MPP10 cDNA by screening a

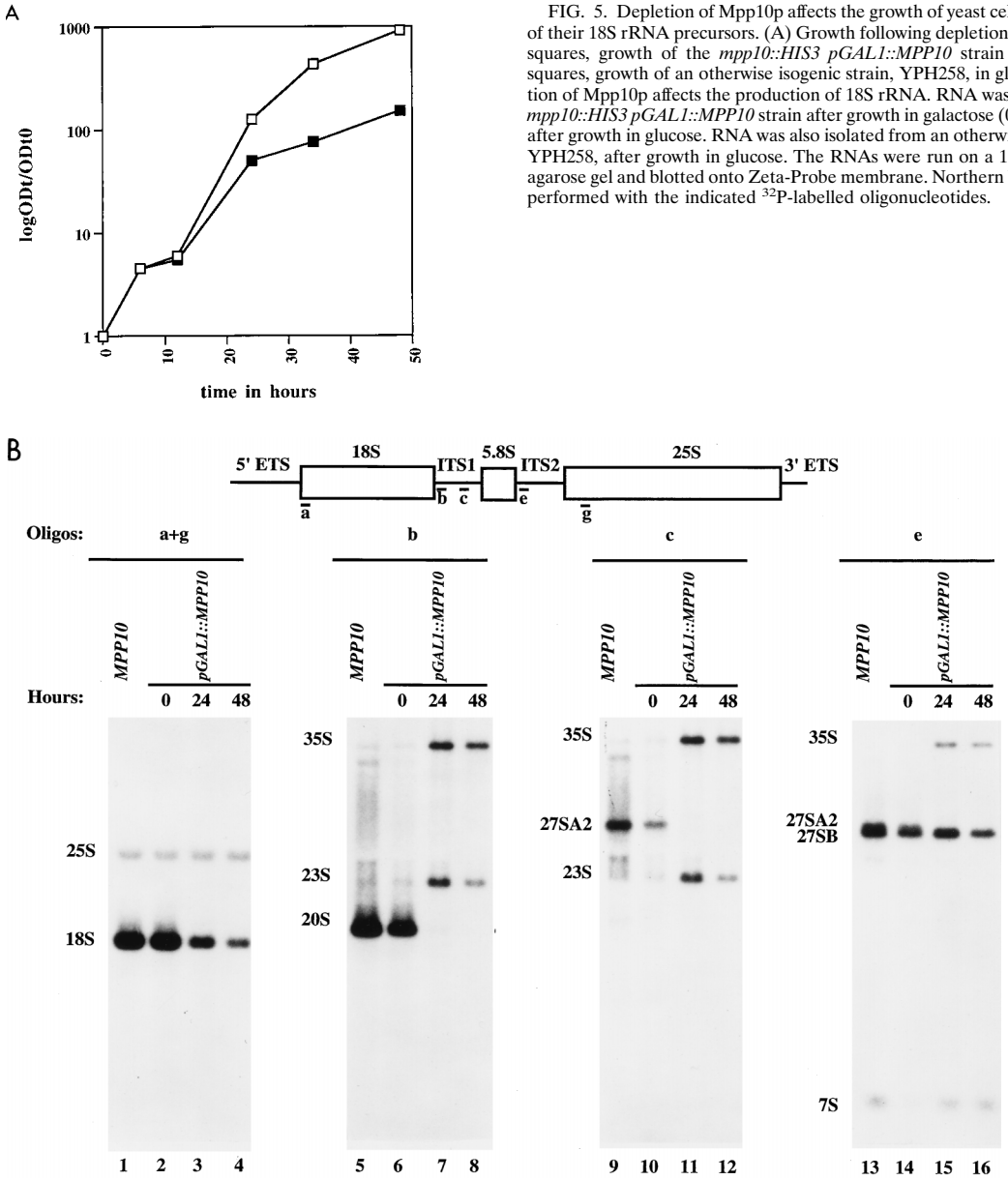

FIG. 5. Depletion of Mpp10p affects the growth of yeast cells and maturation of their 18S rRNA precursors. (A) Growth following depletion of Mpp10p. Solid squares, growth of the *mpp10::HIS3 pGAL1::MPP10* strain in glucose; open squares, growth of an otherwise isogenic strain, YPH258, in glucose. (B) Depletion of Mpp10p affects the production of 18S rRNA. RNA was isolated from the *mpp10::HIS3 pGAL1::MPP10* strain after growth in galactose (0 h) or 24 and 48 h after growth in glucose. RNA was also isolated from an otherwise isogenic strain, YPH258, after growth in glucose. The RNAs were run on a 1% formaldehyde–agarose gel and blotted onto Zeta-Probe membrane. Northern blot analyses were performed with the indicated ^{32}P-labelled oligonucleotides.

mouse myeloid leukemia cDNA library. The mouse MPP10 protein has an 83% overall identity to the human protein but exhibits greater identity at the carboxy terminus. The mouse cDNA can now be used to overexpress and purify the protein for production of antibodies in rabbits. This will facilitate study of the role of MPP10 in vitro in mouse cell extracts (21, 22).

The 30% identity between the human and yeast MPP10 proteins is not extraordinarily low for a human-yeast protein pair. Other human-yeast pairs of RNPs or proteins involved in RNA processing with 30% or less identity include the La protein (48), the AAUAAA binding subunit of the cleavage and polyadenylation specificity factor (CFT1) (39), the U1 snRNP 70,000-molecular-weight (70K) protein (37), and the splicing factor SAP 145 (Cus1) (46).

The predicted structure and charge distribution of the human and yeast MPP10 proteins are very similar. Of note are the several predicted casein kinase II phosphorylation sites that are conserved among species. It is unknown whether they are phosphorylated in vivo and whether phosphorylation at these residues is essential for the function of the protein. The human protein was identified by an antibody, MPM2, that recognizes certain sites of mitotic phosphorylation (26, 47). The function of mitotic phosphorylation in the human protein is not yet understood. The predicted MPM2 epitopes are conserved between mouse and human, but not yeast.

In spite of sequence and structural similarities among the MPP10 proteins, neither the human nor the mouse *MPP10* genes can complement the disrupted *mpp10::HIS3* gene (data not shown). We tested the vertebrate genes on both centromere-based and multicopy plasmids by using a highly expressing yeast promoter (31). In both cases, protein is produced but the yeast cells are inviable. Therefore, the mammalian MPP10 proteins cannot substitute for the yeast proteins.

The identification of Mpp10p in *S. cerevisiae* by homology to

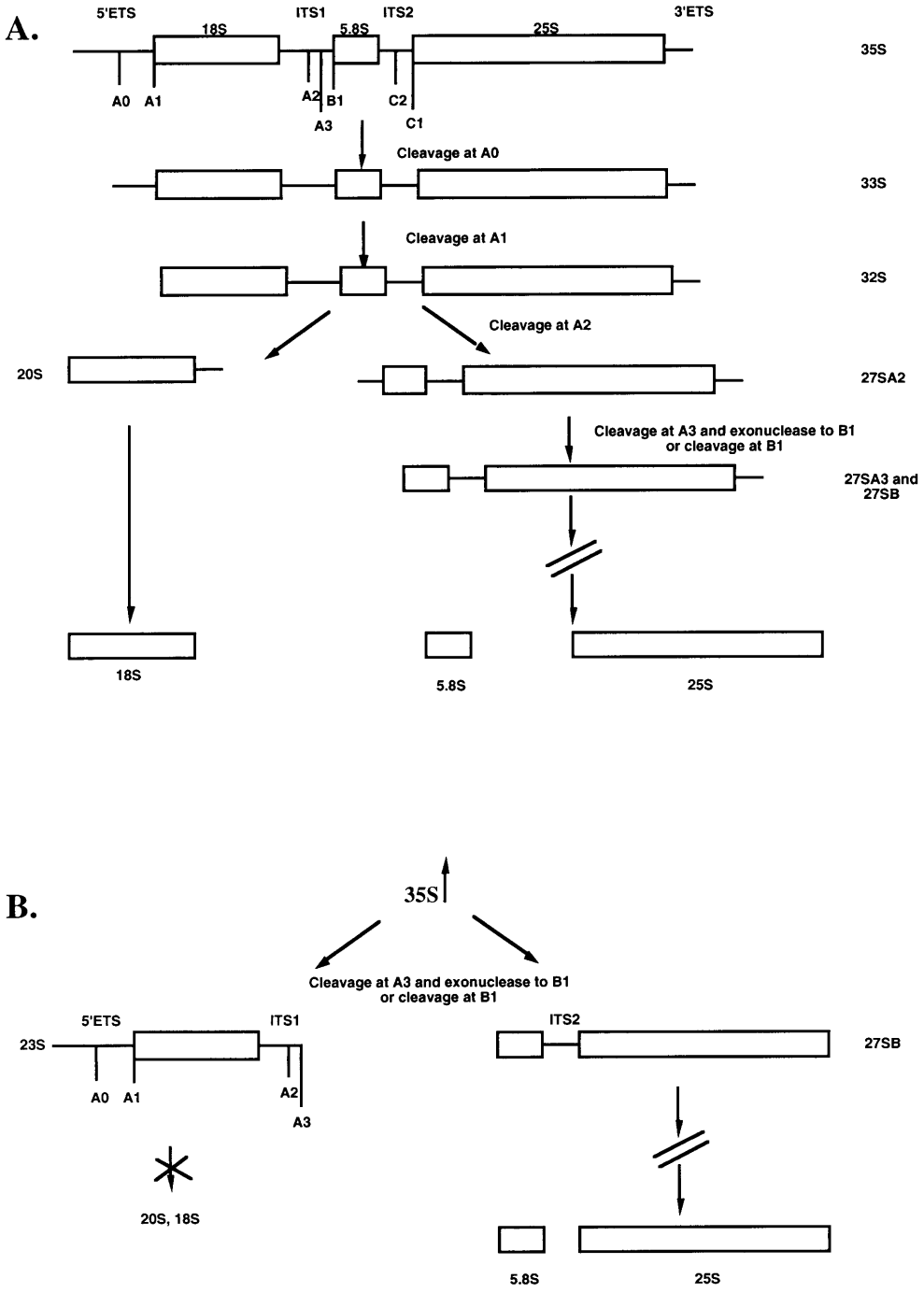

FIG. 6. Processing of pre-rRNA in *S. cerevisiae* and in Mpp10p-depleted yeast. The processing pathway is derived from reference 44 and includes only the relevant precursors and mature rRNAs. The 35S precursor, which contains the sequences destined to be the mature 18S, 5.8S, and 25S rRNAs is processed at A0 to yield the 33S precursor, at A1 to yield the 32S precursor, and at A2 to yield the 20S and 27SA2 precursors. The 20S precursor is processed to the mature small-subunit rRNA, 18S. The 27SA3 precursor is derived from cleavage at A3. The 27SB precursor arises from exonucleolytic degradation of 27SA3 to B1 or by direct cleavage at B1. The 27SB precursor is processed in a number of steps to generate the mature large-subunit rRNAs, 5.8S and 28S. (B) Processing pathways in Mpp10p-depleted yeast. Levels of the 35S precursor are increased. Accumulation of a 23S processing product that results from cleavage at A3 is apparent, indicating a lack of cleavage at A0, A1, and A2. Few or no 20S or 18S rRNAs are produced. Cleavage at A3 yields 27SA3 rRNA, which is not detectable by Northern blot analysis (23), and is processed to the 27SB pre-rRNA. The 27SB rRNA can also be generated by direct cleavage at B1. The 5.8S and 25S rRNAs are generated from this precursor.

the human protein is a direct result of the yeast genome sequencing project. The amino acid sequence of the human MPP10 protein was used in a database search to identify the yeast sequence. It is unlikely that experimental methods would have succeeded, since the human cDNA does not cross-hybridize with yeast DNA, even at low stringency (data not shown).

The yeast U3 snoRNP has a mobility of 10S to 12S in glycerol gradients (12). This predicts that there are roughly 10

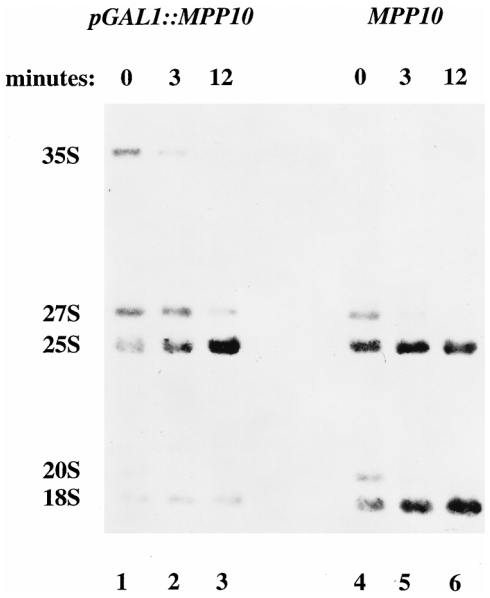

FIG. 7. Growth defect following Mpp10p depletion is due to a defect in processing of pre-18S rRNA precursors. The *mpp10::HIS3 pGAL1::MPP10* strain was grown in glucose for 48 h, labeled with [*methyl*-^{3}H]methionine, and chased with cold methionine for the indicated times. For comparison, similar labeling of the YPH258 strain grown in glucose was carried out. RNA was isolated, run on a 1% formaldehyde gel, and blotted to Zeta-Probe membrane. The membrane was sprayed with En^{3}Hance and exposed for autoradiography for 2 weeks.

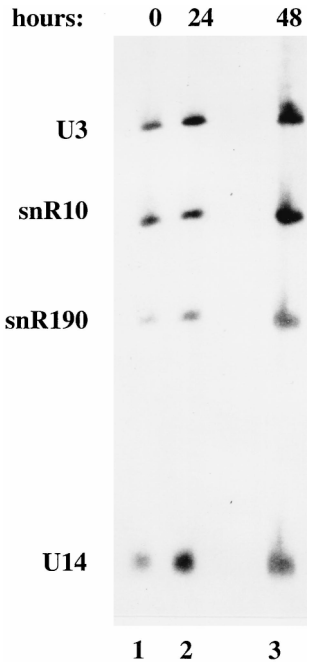

FIG. 8. Levels of the U3 snoRNA are not affected by Mpp10p depletion. RNA was isolated from the *mpp10::HIS3 pGAL1::MPP10* strain after growth in galactose (0 h) or 24 and 48 h after growth in glucose. The RNA was run on an 8% denaturing polyacrylamide gel, blotted to Zeta-Probe membrane, and probed with antisense RNA probes to the U3, snR10, snR190, and U14 snoRNAs.

proteins complexed with the U3 snoRNA in yeast. Only two other protein components of the yeast U3 snoRNP have been identified. These are the fibrillarin (Nop1) and Sof1 proteins (14, 19, 20, 35, 41, 42). The 34-kDa fibrillarin protein is a component of many other snoRNPs, including the snoRNPs involved in ribose methylation (2). Because of the salt-sensitive association of fibrillarin with the U3 snoRNA, it is thought that fibrillarin is not a core component of the U3 snoRNP (24, 32, 35, 43).

Sof1p, isolated in a screen for suppressors of a temperature-sensitive fibrillarin, is a 56-kDa protein with similarities to PRP4 and Gβ domains (19). Like Mpp10p, immunoprecipitation of an epitope-tagged Sof1p reveals association with the U3 snoRNA, with little or no association with other snoRNAs. Since anti-Mpp10p antibodies immunoprecipitate the U3 snoRNA, it is possible that Mpp10p and Sof1p coexist in the same RNP. However, since neither protein has a recognizable RNA binding motif, it is not clear that either of them binds the RNA directly. Both the *SOF1* and *MPP10* genes are essential, and both proteins are required for pre-18S rRNA processing at sites A0, A1, and A2 in the pre-rRNA. Mpp10p is therefore likely to be the second U3 snoRNP-specific component to be identified in yeast and the first described in both yeast and humans.

The RNase III homolog of yeast, Rnt1p, is required for cleavage at the 5′-most U3 cleavage site, A0 (Fig. 6), in vivo and cleaves at this site in the absence of any other factors in vitro (10). However, cleavage at site A0 in vivo requires base pairing of the "hinge" region of the U3 snoRNA with the pre-rRNA (5). Therefore, the requirement for the U3 snoRNP at this cleavage site is likely to be in folding or presentation of the pre-rRNA to RNase III, perhaps as a chaperone. RNase III is not involved in cleavage at site A1, and this cleavage reaction appears to be endonucleolytic (44). Mutations in the conserved 5′ sequences that include the Box A sequence in the U3 snoRNA have been isolated that affect cleavage at A1/A2, but not A0 (17). It will be interesting to investigate whether mutations that separate the two functions of the U3 snoRNP, the requirement for cleavage at A0 and at A1/A2, can be made in Mpp10p.

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D. A. Dunbar and S. Wormsley contributed equally to this work.

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REFERENCES

1. **Ausubel, F., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1995. Short protocols in molecular biology, 3rd ed. John Wiley & Sons, Inc., New York, N.Y.
2. **Balakin, A. G., L. Smith, and M. J. Fournier.** 1996. The RNA world of the nucleolus: two major families of small RNAs defined by different box elements with related functions. Cell **86:**823–834.
3. **Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin.** 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:**3329–3330.
4. **Beltrame, M., Y. Henry, and D. Tollervey.** 1994. Mutational analysis of an essential binding site for the U3 snoRNA in the 5′ external transcribed spacer of yeast pre-rRNA. Nucleic Acids Res. **22:**4057–4065.

5. **Beltrame, M., and D. Tollervey.** 1995. Base pairing between U3 and the pre-ribosomal RNA is required for 18S rRNA synthesis. EMBO J. **14:**4350–4356.
6. **Beltrame, M., and D. Tollervey.** 1992. Identification and functional analysis of two U3 binding sites on yeast pre-ribosomal RNA. EMBO J. **11:**1531–1542.
7. **Benton, B. M., J. H. Zang, and J. Thorner.** 1994. A novel FK506- and rapamycin-binding protein (FPR3 gene product) in the yeast Saccharomyces cerevisiae is a proline rotamase localized to the nucleolus. J. Cell Biol. **127:**623–639.
8. **Berges, T., E. Petfalski, D. Tollervey, and E. C. Hurt.** 1994. Synthetic lethality with fibrillarin identifies NOP77p, a nucleolar protein required for pre-rRNA processing and modification. EMBO J. **13:**3136–48.
9. **Davis, F. M., T. Y. Tsao, S. K. Fowler, and P. N. Rao.** 1983. Monoclonal antibodies to mitotic cells. Proc. Natl. Acad. Sci. USA **80:**2926–2930.
10. **Elela, S. A., H. Igel, and M. Ares, Jr.** 1996. RNase III cleaves eukaryotic preribosomal RNA at a U3 snoRNP-dependent site. Cell **85:**115–124.
11. **England, T. E., A. G. Bruce, and O. C. Uhlenbeck.** 1980. Specific labeling of 3′ termini of RNA with T4 RNA ligase. Methods Enzymol. **65:**65–74.
12. **Fabrizio, P., S. Esser, B. Kastner, and R. Luhrmann.** 1994. Isolation of S. cerevisiae snRNPs: comparison of U1 and U4/U6.U5 to their human counterparts. Science **264:**261–265.
13. **Gietz, R. D., R. H. Schiestl, A. R. Willems, and R. A. Woods.** 1995. Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:**355–360.
14. **Henriquez, R. G., G. Blobel, and J. P. Aris.** 1990. Isolation and sequencing of *NOP1*. A yeast gene encoding a nucleolar protein homologous to a human autoimmune antigen. J. Biol. Chem. **265:**2209–2215.
15. **Henry, Y., H. Wood, J. P. Morrissey, E. Petfalski, S. Kearsey, and D. Tollervey.** 1994. The 5′ end of yeast 5.8S rRNA is generated by exonucleases from an upstream cleavage site. EMBO J. **13:**2452–2463.
16. **Holzinger, A., K. S. Phillips, and T. E. Weaver.** 1996. Single-step purification/solubilization of recombinant proteins: application to surfactant protein B. BioTechniques **20:**804–807.
17. **Hughes, J. M.** 1996. Functional base-pairing interaction between highly conserved elements of U3 small nucleolar RNA and the small ribosomal subunit RNA. J. Mol. Biol. **259:**645–654.
18. **Hughes, J. M. X., and M. Ares, Jr.** 1991. Depletion of U3 small nucleolar RNA inhibits cleavage in the 5′ external transcribed spacer of yeast pre-ribosomal RNA and prevents formation of 18S ribosomal RNA. EMBO J. **10:**4231–4239.
19. **Jansen, R., D. Tollervey, and E. C. Hurt.** 1993. A U3 snoRNP protein with homology to splicing factor PRP4 and G beta domains is required for ribosomal RNA processing. EMBO J. **12:**2549–2558.
20. **Jansen, R. P., E. C. Hurt, H. Kern, H. Lehtonen, M. Carmo-Fonseca, B. Lapeyre, and D. Tollervey.** 1991. Evolutionary conservation of the human nucleolar protein fibrillarin and its functional expression in yeast. J. Cell Biol. **113:**715–729.
21. **Kass, S., and B. Sollner-Webb.** 1990. The first pre-rRNA-processing event occurs in a large complex: analysis by gel retardation, sedimentation and UV cross-linking. Mol. Cell. Biol. **10:**4920–4931.
22. **Kass, S., K. Tyc, J. A. Steitz, and B. Sollner-Webb.** 1990. The U3 small nucleolar ribonucleoprotein functions in the first step of pre-ribosomal RNA processing. Cell **60:**897–908.
23. **Lafontaine, D., J. Vandenhaute, and D. Tollervey.** 1995. The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. Genes Dev. **9:**2470–2481.
24. **Lubben, B., C. Marshallsay, N. Rottman, and R. Luhrmann.** 1993. Isolation of U3 snoRNP form CHO cells: a novel 55 kDa protein binds to the central part of U3 snoRNA. Nucleic Acids Res. **21:**5377–5385.
25. **Maden, T.** 1996. Click here for methylation. Nature **383:**675–735.
26. **Matsumoto-Taniura, N., F. Pirollet, R. Monroe, L. Gerace, and J. M. Westendorf.** 1996. Identification of novel M phase phosphoproteins by expression cloning. Mol. Biol. Cell **7:**1455–69.
27. **Maxwell, E. S., and M. J. Fournier.** 1995. The small nucleolar RNAs. Annu. Rev. Biochem. **64:**897–934.
28. **Meier, U. T.** 1996. Comparison of the rat nucleolar protein Nopp140 with its yeast homolog SRP40. J. Biol. Chem. **271:**19376–19384.
29. **Melton, D. A., P. A. Krieg, M. R. Rebagliati, T. Maniatis, K. Zinn, and M. R. Green.** 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Res. **12:**7035–7056.
30. **Mougey, E. B., L. K. Pape, and B. Sollner-Webb.** 1993. A U3 small nuclear ribonucleoprotein-requiring processing event in the 5′ external transcribed spacer of *Xenopus* precursor rRNA. Mol. Cell. Biol. **13:**5990–5998.
31. **Mumberg, D., R. Muller, and M. Funk.** 1995. Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. Gene **156:**119–122.
32. **Parker, K. A., and J. A. Steitz.** 1987. Structural analyses of the human U3 ribonucleoprotein particle reveal a conserved sequence available for base-pairing with pre-rRNA. Mol. Cell. Biol. **7:**2899–2913.
33. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
34. **Savino, R., and S. A. Gerbi.** 1990. In vivo disruption of Xenopus U3 snRNA affects ribosomal RNA processing. EMBO J. **9:**2299–2308.
35. **Schimmang, T., D. Tollervey, H. Kern, R. Frank, and E. C. Hurt.** 1989. A yeast nucleolar protein related to mammalian fibrillarin is associated with small nucleolar RNA and is essential for viability. EMBO J. **8:**4015–4024.
36. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
37. **Smith, V., and B. G. Barrell.** 1991. Cloning of a yeast U1 snRNP 70K protein homologue: functional conservation of an RNA-binding domain between humans and yeast. EMBO J. **10:**2627–2534.
38. **Sobel, S. G., and M. Synder.** 1995. A highly divergent gamma-tubulin gene is essential for cell growth and proper microtubule organization in Saccharomyces cerevisiae. J. Cell Biol. **131:**1775–1788.
39. **Stumbf, G., and H. Domdey.** 1996. Dependence of yeast pre-mRNA 3′-end processing on CFT1: a sequence homolog of the mammalian AAUAAA binding factor. Science **274:**1517–1520.
40. **Tollervey, D.** 1996. Small nucleolar RNAs guide ribosomal RNA methylation. Science **273:**1056–1057.
41. **Tollervey, D., H. Lehtonen, M. Carmo-Fonseca, and E. C. Hurt.** 1991. The small nucleolar RNP protein NOP1 (fibrillarin) is required for pre-rRNA processing in yeast. EMBO J. **10:**573–583.
42. **Tollervey, D., H. Lehtonen, R. Jansen, H. Kern, and E. C. Hurt.** 1993. Temperature-sensitive mutations demonstrate roles for yeast fibrillarin in pre-rRNA processing, pre-rRNA methylation, and ribosome assembly. Cell **72:**443–457.
43. **Tyc, K., and J. A. Steitz.** 1989. U3, U8, and U13 comprise a new class of mammalian snRNPs localized to the cell nucleolus. EMBO J. **8:**3113–3119.
44. **Venema, J., Y. Henry, and D. Tollervey.** 1995. Two distinct recognition signals define the site of endonucleolytic cleavage at the 5′-end of yeast 18S rRNA. EMBO J. **14:**4883–4892.
45. **Venema, J., and D. Tollervey.** 1995. Processing of pre-ribosomal RNA in *Saccharomyces cerevisiae*. Yeast **11:**1629–1650.
46. **Wells, S. E., M. Neville, M. Haynes, J. Wang, H. Igel, and M. Ares, Jr.** 1996. *CUS1*, a suppressor of cold-sensitive U2 snRNA mutations, is a novel yeast splicing factor homologous to human SAP145. Genes Dev. **10:**220–232.
47. **Westendorf, J. M., P. N. Rao, and L. Gerace.** 1994. Cloning of cDNAs for M-phase phosphoproteins recognized by the MPM2 monoclonal antibody and determination of the phosphorylated epitope. Proc. Natl. Acad. Sci. USA **91:**714–718.

47a. **Westendorf, J. M., K. N. Konstantinov, S. Wormsley, M. D. Shu, N. Matsumoto-Taniura, F. Pirollet, F. G. Klier, L. Gerace, and S. J. Baserga.** M phase phosphoprotein 10 (MPP10) is a U3 snoRNP-specific nucleolar protein. Submitted for publication.

48. **Yoo, C. J., and S. L. Wolin.** 1994. La proteins from *Drosophila melanogaster* and *Saccharomyces cerevisiae*: a yeast homolog of the La autoantigen is dispensable for growth. Mol. Cell. Biol. **14:**5412–5424.