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RNP1, a new ribonucleoprotein gene of the yeast *Saccharomyces cerevisiae*

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ABSTRACT

A previously unidentified ribonucleoprotein (RNP) gene of yeast has been cloned and sequenced. The gene, named *RNP1*, was found adjacent to a previously sequenced gene encoding the second gene for ribosomal protein L4. *RNP1* contains two RNA Recognition Motifs (RRM), [alternatively known as RNA binding Domains (RBD)], but unlike most RNP genes does not contain any auxiliary simple sequence domains. The first RRM (RRM1) most resembles RRM domains found in the hnRNP A/B class of RNP proteins. The second RRM (RRM2) most resembles a RRM so far seen only in the single RRM of the yeast *SSB1* gene. Two null mutants of *RNP1* that were created, a frameshift disruption and a complete deletion of the gene, were viable, demonstrating that the gene is not essential for cell growth. Two double null mutants of yeast RNP genes that were created (Δ*RNP1*/Δ*SSB1* and Δ*SSB1*/Δ*NPL3*) were also viable. A fragment identical in size to the RRM1 domain could be amplified by PCR from the DNA of fungi, plants, and animals, using primers matching the ends of this domain, indicating that the structure of RRM1 is conserved. Another potential open reading frame on the same cloned fragment of DNA encodes a gene product whose structure resembles that of a seven-transmembrane-segment membrane receptor protein.

INTRODUCTION

The number of ribonucleoprotein (RNP) genes has expanded greatly in recent years, comprised at latest count of more than 100 genes in all types of eukaryotes, with a few in prokaryotes as well (1, 2). One family of RNP genes is defined by the RNA Recognition Motif (RRM) (3), or RNA Binding Domain (RBD) (4), an 80 to 100 amino acid residue domain, often present in multiple (2–4) copies. In the middle of each RRM is the RNP-1 octamer; approximately thirty amino acid residues N-terminal to the RNP-1 octamer is the RNP-2 hexamer sequence element (5, 6). Specific amino acid residues in RNP-1 and RNP-2 are not tightly conserved, but the character of residues (basic, aliphatic, aromatic, etc) is conserved. Other single amino acid residues between the RNP-1 and RNP-2 elements, and C-terminal to RNP-1, are also conserved (3).

Perhaps ten RRM-containing RNP genes have now been identified in yeast, most often serendipitously while searching for another gene. The first RNP gene identified in yeast was *PAB1*, encoding the Poly(A) Binding Protein (PABP) (7, 8). HnRNP proteins in human cells were identified by UV cross-linking of bound protein to RNA (5, 6), but the similar approach in yeast cells found PABP, not an hnRNP protein (7). *SSB1*, a nucleolar RNP (9, 10), is a protein purified on a single-stranded DNA cellulose column that was first thought to be an single-stranded binding (ssb) protein involved in DNA replication (11, 12). *SNP1*, the U1 snRNP 70K protein, was found in a project undertaking the full sequence of chromosome IX (13). *RNA15* is a gene that affects poly(A) tail length and mRNA stability (14). *PRP24*, found in a screen for mutants that accumulate unspliced pre-mRNA, is likely a U6 snRNP specific protein (15). Mutations in *PRT1* (allelic to *CDC63*) give rise to defects in initiation of protein translation and in cell cycle regulation. *NSR1* was identified as the gene for a protein that bound specifically to a synthetic peptide containing nuclear localization signal (17). Its location in the nucleolus seems antithetical to its assigned function in nuclear protein localization. *NSR1* mutants are deficient in pre-rRNA processing and in ribosome biogenesis (18). The *NPL3* gene also participates in transport of nuclear proteins to the nucleus (19). It's unclear why two nuclear protein localization gene products have RNA binding domains. The yeast poly(A) polymerase gene (*PAP1*) has a poorly conserved RRM, similar to its mammalian homolog (20). The *PUB1* gene, encoding a cytoplasmic RNP protein, was isolated independently in two labs by PCR amplification with degenerate primers to the RNP-1 and RNP-2 sequence elements (21–23). YCL11c is a previously unidentified open reading frame uncovered in the yeast Chromosome III sequencing project (24). It has high similarity to the poly(A)-binding proteins, but bears even greater similarity to a class of chloroplast RNPs discovered in tobacco and maize (25–27).

Most RNP genes of yeast have poor matches to the RNP-1 and RNP-2 consensus sequences, and so may be distant relatives of the canonical RNP proteins. It is therefore difficult to classify them with known RNP genes of higher eukaryotes, with the exception of *PAB1*. All yeast RNP genes identified to date are single copy genes. Remarkably, most of them are non-essential genes. Only *PAB1*, *PRT1*, and *RNA15* have been shown by disruption to be essential genes; *PRP24* is presumably essential

since *ts* alleles of it exist. *SSB1*, *NSR1*, *NPL3*, *SNP1* and *PUB1* (21–23) are not essential. The YCL11c URF has not yet been tested. Though full deletion of *PAB1* is lethal, a large deletion comprising three RRMs and the RNP-1 element of the fourth RRM is viable (28).

Several RNP proteins have been shown to occupy critical positions in cell physiology. The mammalian C1/C2 hnRNP proteins are a necessary part of the spliceosome, for in their absence mRNA splicing does not occur (29, 30). HnRNP A1 is instrumental in 5′ splice site selection (31, 32). Deletion studies of *PAB1* of yeast have shown that it functions in mRNA stability and in translation initiation by the ribosome (33). Beyond these few examples the physiological role of most known RNP proteins is as yet uncharacterized.

Reported here is the isolation of a new RNP gene of yeast. The initial aim of the study was to isolate yeast ssb proteins that would be RNP proteins. Since the overwhelming majority of eukaryote ssb's characterized to date have proven to be RNPs (26, 34–36), this approach seemed to have a good chance of success. The ssb so purified, migrating at 30 kDa, turned out to be *RPL4B*, a gene for ribosomal protein L4 (37). However, just 3′ to this gene was another open reading frame that encoded a novel RRM-containing RNP gene. Disruption or deletion of this gene, named *RNP1*, does not give rise to a lethal phenotype, so it, like other RNP genes of yeast, is not essential. The first RRM of this gene is highly conserved, as it can be identified by PCR amplification in a wide spectrum of eukaryotes. *RNP1* was mapped to the left arm of chromosome XII. A third open reading frame, on the same clone downstream of *RNP1*, has a seven-transmembrane-segment structure reminiscent of membrane receptor proteins that receive the signal for G-protein mediated signal transduction.

MATERIALS AND METHODS

Cloning and sequencing

A 4.4 kb *BamHI* fragment that was selected previously (37) was subcloned into pBS SK+, forming the plasmid p30B4.4. The *BamHI* insert was sequenced by the dideoxy method using the forward and reverse primers supplied by the manufacturer (US Biochemical) in the DNA sequencing kit. Nested deletions were created to sequence internal regions of the fragment (38). Regions of gaps not covered by any of the nested deletions were sequenced using synthetic DNA primers derived from DNA sequence already determined. Both strands of DNA were completely sequenced. All DNA manipulations and DNA cloning followed standard methodology (39). DNA sequence analyses were done with the Sequence Analysis Software Package of the University of Wisconsin Genetics Computer Group (UWGCG).

Southern and Northern blot-hybridization analysis

Small preparations of yeast genomic DNA were done as described (40). The probe was a PCR-amplified fragment of the *RNP1* gene using primers corresponding to the ends of the sequenced gene. The 5′ primer was TCTAGATGCTAATAGAGGAGATAGAG, corresponding to the first seven amino acids of the *RNP1* sequence plus an *XbaI* site (underlined) added at the 5′ end; the 3′ primer was GGATCCTATCTTAACCAACAATACTC, corresponding to six codons near the 3′ end and an added *BamHI* site (underlined). The PCR-amplified probe was purified in low-melting-point agarose and radioactively labeled by the random primer method (41). Southern blots onto nylon membranes and subsequent hybridization were done as described by the manufacturer (S&S).

Preparation of total RNA of yeast was done as previously described (42). The RNA was resolved on formaldehyde agarose gels before blotting onto a nylon membrane. Hybridization was according to directions supplied by the manufacturer (S&S). The probe was the PCR-amplified fragment of the *RNP1* gene described earlier.

Disruptions of the *RNP1* gene

To create a frameshift disruption, the *RNP1* gene was first subcloned apart from the other two ORFs on the cloned *BamHI* fragment. The two primers described earlier matching the 5′ and 3′ ends of *RNP1* were used to PCR amplify the coding region of the gene. The fragment so created was blunt ended with T4 DNA polymerase, then cloned into the HindII site in pBS+. Into a unique PflMI site in this clone (pRNP14) was inserted the URA3 gene, excised from YEP24 by ClaI/SmaI digestion. The gene so created (RNPΔ1::URA3) was excised from the plasmid using the unique restriction sites at each end of the original PCR primers (XbaI/BamHI), and this DNA was used to transform the yeast diploid SS328/330, homozygous for the ura3-52 mutation, by the one-step integration method (43). Sporulation, dissection, and analysis of colony genotype used standard yeast methods (44).

To create a complete deletion of *RNP1*, a 1.7 kb *SalI/StuI* fragment of the plasmid p30B4.4(ΔS) was replaced with a 2.0 kb *SalI/NruI* URA3-containing fragment taken from the plasmid YEP24. p30B4.4(ΔS) was created from p30B4.4 by excision of a *HindIII/XhoI* fragment to remove an interfering *SalI* site in the multiple cloning sequence of that plasmid. The RNP1::URA3 gene so created was excised from p30B4.4(ΔS) by *PvuII/BglII* digestion and transformed into the diploid strain SS328/330.

Subcloning *RNP1* into a episomal plasmid

The *RNP1* gene, from a SpeI site 272 bp upstream of the Met codon to a FspI site 148 bp downstream of the termination codon, was subcloned into the multiple cloning site of pBS+. From this plasmid the *RNP1* gene, on a 1172 bp SpeI/XhoI fragment, was subcloned into YEp24 cut with NheI and SalI, replacing the plasmid DNA between those sites with *RNP1*.

Double disruptions

To create the Δ*RNP1*×Δ*SSB1* double disruption, strain MCY76D (a, *his3Δ200, ade2-101, ura3-52, tyr1*$^-$, *RNPΔ1::URA3*), was mated to the strain MWCY003 containing the HIS3 gene inserted into a deletion of the *SSB1* gene (α, *his3Δ200, ade2-100, ura3-52, SSB1Δ::HIS3*). To create the Δ*SSB1*×Δ*NPL3* double disruption, strain MWCY003α was crossed to strain MBY101 (a, *his3Δ200, leu2-3,112, ade2, trp1, NPL3::URA3*). In both instances, diploids that were His$^+$, Ura$^+$ were selected, then sporulated, dissected, and analyzed by standard procedures (44).

PCR amplification

PCR was done in 100 μl volumes containing 10 mM Tris–HCl (pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 200 μM of each of the four deoxynucleoside triphosphates, 1 nmol of the two primers, and 2.5 U of *Taq* polymerase. For the first five cycles, the reaction was incubated for 1 min each at 95, 48, and 74°C; the annealing temperature was raised to 55°C for the remaining 25 cycles (45). After amplification, aliquots of each reaction was analyzed on a 4% composite agarose gel.

RESULTS

Cloning of the *RNP1* gene

The sequence determined here was on a clone obtained in a previous study (37). A single-stranded binding protein (ssb) of yeast was purified to homogeneity. From partial amino acid sequence of this protein degenerate oligonucleotides were synthesized and used to select a clone containing the gene from a yeast genomic library. The 4.4 kb BamHI fragment so selected (Fig. 1) was completely sequenced on both strands.

The DNA sequence showed three open reading frames (ORF), all transcribed in the same orientation. ORF1 was the gene for the 30 kDa ssb, and was identified as the second copy of the duplicated gene coding for ribosomal protein L4, designated *RPL4B* (37). ORF2 showed the sequence criteria typical of RRM-containing RNP proteins. ORF3 showed no resemblance to any known gene. A map of the clone showing the positions of the three ORF's is in Fig. 1, and the sequence of the full clone containing ORF2 and ORF3 is not shown but is available in the sequence databases (GenBank accession number M88604).

Sequence of the *RNP1* gene

The ORF2 sequence extends for 747 nucleotides, coding for a protein of 28,828 kDa from 249 amino acid residues. It is extremely basic, with a high concentration of lysine (17.3%), and an estimated pI of 10.7. Comparison of ORF2 to the protein sequence database showed significant homologies to many RNP proteins, so it was renamed *RNP1*. A profile comparing *RNP1* to known yeast RNP genes is shown in Fig. 2. *RNP1* has two RRMs. Comparisons to known RNP genes from many organisms, using available programs in the UWGCG package such as FASTA, BestFit, GAP, and PILEUP, revealed that the first RRM bears its best resemblance to hnRNPs of the A/B class, especially to the second RRM of that class of proteins (Fig. 3A). Similar comparisons with the second RRM of *RNP1* showed

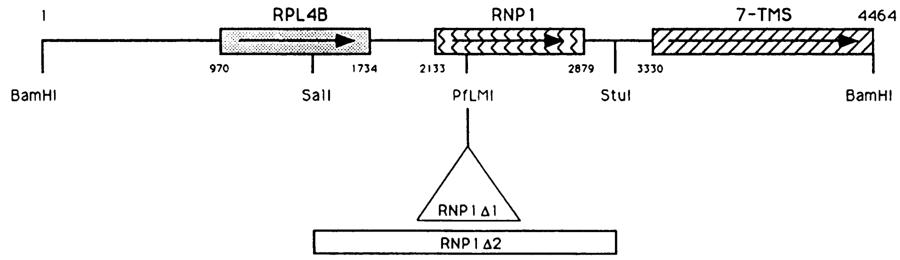

Figure 1. Map of the *RNP1* gene locus. The cloned 4.4 kb *BamHI* restriction fragment bearing *RNP1* is shown. Selected restriction sites are indicated. Boxes indicate open reading frames, arrows inside the boxes indicate direction of transcription. The sequences disrupted or deleted in the creation of the two null mutants (RNP1Δ1 and RNP1Δ2) are indicated.

		RNP-2		RNP-1	
RNP1#1	T	LYVGNL	PKNCRKQD--LRDLFEPNYGKITINMLKKKPLKKPL	KRFAFIEF	QEGVNLKKVKEKM-NG-KIFMNEKLVIENILTK
RNP1#2	T	LYVKNI	PMKSTNEDL-AKI-FGVDPKNINFVRRELVDL-RTN	KVFFSDEF	HTGEAFIKFDNLG-TGDSIQKKCRE-FKG--RK
U1-70K	T	IFIGRL	PYDL--DEIELQKYFVK-FGEIEKIRIVKDKITQKS	KGYAFIVF	KDPISSKMAFKEIGVHRGIQIKDRICIVDIERGRTVK
RNA15	V	VYLGSI	PYDQT-EEQILDL-CSN-VGPVINLKMMFDPQTGRS	KGYAFIEF	RDLESSASAVRNL-NGYQL-GSRFLKCGYSSN
NSR1#1	T	IFVGRL	SWSID-DEW-LKKEFEH-IGGVIGARVIYERGTDRS	RGYGYVDF	ENKSYAEKAIQEM-QGKEIDGRP-INCDMSTSK
NSR1#2	T	LFLGNL	SFNAD-RDA-IFELFAK-HGEVVSVRIPTHPETEQP	KGFGYVQF	SNMEDAKKALDAL-QGEYIDNRP-VRLDFSSSPR
PABP#1	S	LYVGDL	EPSVS-EAH-LYDIFSP-IGSVSSIRVCRDAITKTS	LGYAYVNF	NDHEAGRKAIEQL-NYTPIKGRL-CRIMWSQRD
PABP#2	N	IFIKNL	HPDID-NKA-LYDTFSV-FGDILSSKIATD-ENGKS	KGFGFVHF	EEEGAAKFAIDAL-NGMLLNGQE-IYVAPHLSR
PABP#3	N	LYVKNI	NSETT-DEQ-FQELFAK-FGPIVSASLEKD-ADGKL	KGFGFVNY	EKHEDAVKAVEAL-NDSELNGEK-LYVGRAQKK
PABP#4	N	LFVKNL	DDSVD-DEK-LEEEFAP-YGTITSAKVNRT-ENGKS	KGFGFVCF	STPEEATKAITEK-NQQIVAGKP-LYVAIAQRK
PRT1	A	PVIPSA	KVPVL-KKA-LTSLFSK-AGKVVNMEFPIDEATGKT	KGFLFVEC	GSMNDAKKIIKSF-HGKRLDLKHRLFL-YTMKD
PRP24#1	T	VLVKNL	PKSYN-QNK-VYKYFKH-CGPIIHVDVADSL--KKN	FRFARIEF	ARYDGALAAITK--THKVVGQNE-I-IVSHLTECT
PRP24#2	T	LWMTNF	PPSYT-QRN-IRDLLQD-INVVALSIRLPSLRFNTS	RRFAYIDV	TSKEDARYCVEKL-NGLKIEGYT-L-VTKVSNPLEK
PRP24#3	E	IMIRNL	STELL-DENLLRESFE-GFGSIEKINIPAGQKEHSFN	NCCAFMVF	ENKDSAERALQMN-RSL-L-GNREISVSLADKK
SSB1	T	LYINNV	PFKATKEEV-AE-FFGTDADSISLPMRKMRDQHTGRIFTSDSAN	RGMAFVTF	SGENVDIEAKAEEFKGK-VFGDRELTVDVAVIR
NPL3	R	LFVRPF	PLDV--QESELNEIFGP-FGPMKEVKIL--------	NGFAFVEF	EEAESAAKAIEEV-HGKSFANQP-LQVVYS--KL
PAP1	V	QVLKIL	------QE--LAQRFV--YE-VSKKKNMSDGMARDAGG	KIFTYGSY	RLGVHGPGSDIDTLVVVPKHVTRED-FFTVF---
PUB1#1	V	LYVGNL	DKAIT-EDI-LKQYFQV-GGPIANIKIMIDKNNKN-	VNYAFVEY	HQSHDANIALQTL-NGKQIENNI-VKINWAFQS
PUB1#2	N	LFVGDL	NVNVD-DET-LRNAFKD-FPSYLSGHVMWDMQTGSS	RGYGFVSF	TSQDDAQNAMDSM-QGQDLNGRP-LRINWAKRD
YCL11c#1	S	IFVRNL	TFDCTPED--LKELFGTVGEVVEADII---TSKGHH	RGHGTVEF	TKNESVQDAISKF-DGALFMDRK-LMVRQDNPP
YCL11c#2	E	VFIINL	PYSMNWQS--LKDMFKECGHVLRAD-VELDFN-GFS	RGFGSVIY	PTEDEMIRAIDTF-NGMEVEGRV-LEVREGRFNK
Consensus		LFVGNL	DE L F FG I I K	KGFGFV F	A N I G L V A K
		IYIKG	ED Y V V R	R YA I Y	Q V I I R

Figure 2. Comparison of RNP genes of yeast. The RRMs of yeast *RNP1* are lined up against other available RRMs of yeast. The well-conserved RNP-1 octamer and RNP-2 hexamer sequence elements are set apart. The consensus indicated at the bottom is taken from published compilations of RRM sequences (1−3). In the consensus the one or two most conserved amino acids in each position is listed. In the yeast sequences the amino acid residues corresponding in position to the consensus are boldfaced. U1-70K: the 70 kDa protein of the U1 snRNP (13); *RNA15*: product of an mRNA processing gene (14); *NSR1*: nuclear localization signal receptor; PABP: poly(A) binding protein (7, 8); *PRT1*: a gene in initiation of protein translation (16); PRP24: pre-mRNA processing gene (15); *SSB1*: single-stranded binding protein 1 (9); *NPL3*: nuclear protein localization gene 3 (19); *PAP1*: poly(A) polymerase (20); *PUB1*: poly(U) binding mRNP protein (21−23); YCL11c: an ORF of undetermined function uncovered in the chromosome III sequencing project (24).

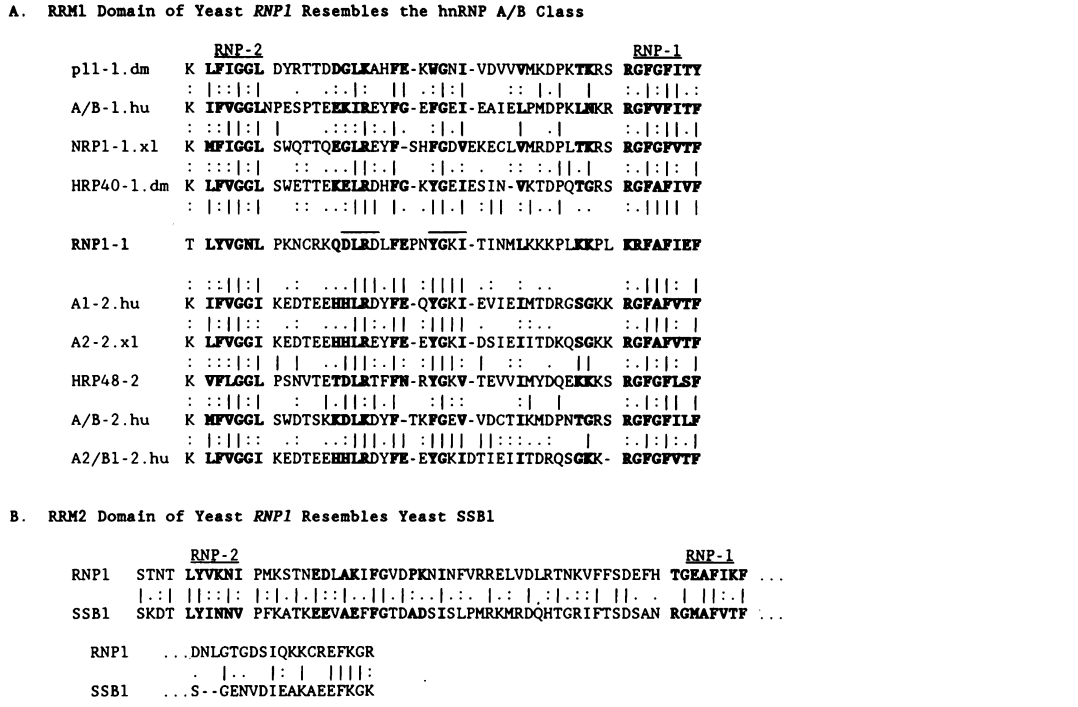

Figure 3. Sequence similarities of yeast *RNP1* to RNP proteins. **A.** Alignment of the first RRM of *RNP1* to RRMs of several hnRNP genes of the A/B class. The RNP-1 octamer and RNP-2 hexamer sequence elements are set aside from the rest of the sequence and are boldfaced. Other amino acids conserved in a published consensus sequence derived for RRMs (1–3) are also boldfaced. The first RRM of yeast *RNP1* is in the center, with RRM2 domains of hnRNP A/B proteins aligned below it and RRM1 domains of hnRNP A/B proteins aligned above it. A solid line indicates identity to a residue in *RNP1*, a double dot indicates a conservative substition, a single dot indicates less conservative similarity. Two sequence blocks conserved in hnRNP A/B RRM2 sequences are indicated by lines above the yeast *RNP1* sequence. **B.** Alignment of the second RRM of *RNP1* to the single RRM of yeast *SSB1*. The conventions followed are as in part A.

remarkable similarity (almost 40% identity) to yeast *SSB1* (Fig. 3B). These two RRMs of *SSB1* and *RNP1* have no remarkable similarity to any other known RRM besides themselves. RRM1 and RRM2 of *RNP1* have little resemblance to each other. There are no other remarkable sequence motifs in *RNP1*. In particular, there is no RGG box, no G-rich C-terminus, no P-rich or Q-rich areas, no GAR domains, no RS domains, and no zinc fingers. By examination of its sequence *RNP1* cannot be classified unequivocally as any one type of RNP gene.

Gene copy and expression of *RNP1*

Southern-blot hybridization analysis, with a PCR-amplified fragment of the full coding region of *RNP1* as a probe, showed that the gene was single copy in yeast (Fig. 4A). Though this gene is adjacent to *RPL4B*, a duplicated gene, it itself is not duplicated. Duplicated genes in yeast are rare, and no RNP gene found previously in yeast has been double copy. Hybridization with the full-length probe at low-stringency conditions, or hybridization with a probe comprised solely of the RRM1 domain of *RNP1* at high or low stringency conditions, revealed no other bands on the Southern blots (data not shown). There are no apparent homologs related to *RNP1* in the yeast genome.

Northern-blot analysis of total yeast RNA with the same probe shows an mRNA of about 0.9 kb (Fig. 4B), in line with expectation given the length (747 nt) of the open reading frame. The mRNA of this gene is about 100-fold less abundant than that of the adjacent *RPL4B* gene (data not shown). L4, like all ribosomal protein genes, is highly expressed.

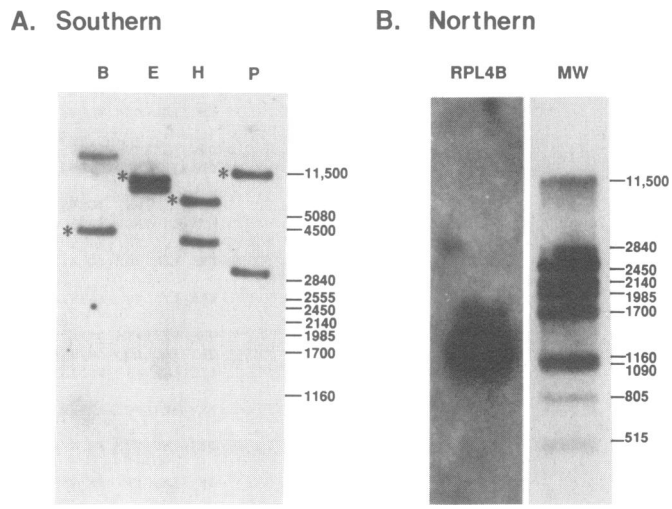

Figure 4. Southern and Northern blot-hybridization analysis of the RNP1 gene. **A.** Southern blot-hybridization. Total yeast genomic DNA digested with *BamHI* (B), *EcoRI* (E), *HindIII* (H), and *PstI* (P). The probe was a PCR-amplified fragment of the RNP1 coding region. Positions of molecular weight markers (a *PstI* digestion of phage lambda DNA), run in an adjacent lane, are indicated in base pairs. **B.** Northern blot-hybridization. Total yeast RNA separated on a formaldehyde-agarose gel and probed with a PCR-amplified fragment of the RNP1 coding region. On an adjacent lane are shown the molecular weight markers, indicated in nucleotides. These markers are lambda DNA *PstI* fragments that were run on the gel and blotted, then probed with ^{32}P-labeled total lambda DNA.

Table 1. Dissections of null mutants of *RNP1*

	*RNP1*Δ1	RNP1Δ2
A. Dissections		
# tetrads dissected:	47	62
# tetrads that grew 4:0	35	55
# 4:0 tetrads that segregated 2:2 for the URA3$^+$ marker	33	54

B. Growth Rate Determinations

Segregant	Phenotype	T_d	Phenotype	T_d
A	Ura$^-$: Rnp1$^+$	2.24 h	Ura$^+$: Rnp1$^-$	2.44 h
B	Ura$^+$: Rnp1$^-$	2.28 h	Ura$^-$: Rnp1$^+$	1.95 h
C	Ura$^+$: Rnp1$^-$	2.19 h	Ura$^-$: Rnp1$^+$	2.05 h
D	Ura$^-$: Rnp1$^+$	2.26 h	Ura$^+$: Rnp1$^-$	2.45 h

RNP1Δ1 is the frameshift disruption of the *RNP1* gene; RNP1Δ2 is the complete deletion of *RNP1*. T_d is the the doubling time determined on complete media (YPD). In each instance growth rate determinations were on segregants derived from a single tetrad.

Disruption of *RNP1* shows that it is non-essential

Two disruptions of *RNP1* were created to see if it is essential gene of yeast. In the first the URA3 gene was inserted just after the first RNP-1 hexamer sequence element, creating a frameshift of the open reading frame. Sequencing of the construct showed that the URA3 insert had been placed in reverse orientation, and that the reading frame of *RNP1* ended just four codons into the insert (data not shown). The diploid strain containing this disruption was sporulated and dissected. Most tetrads dissected produced four live spores, demonstrating that the RNP gene was not essential for growth (Table 1). All of the 4 colony tetrads gave a Ura$^+$/Ura$^-$ ratio of 2:2, as expected, showing that the disrupted (URA3-containing) *RNP1* gene grew. Southern blot-hybridization analysis done on one tetrad confirmed that the insertion had occurred (data not shown).

Since the coding region of the gene was still present, it could be argued that sufficient residual protein synthesis existed to give rise to a viable phenotype. To check against this possibility a complete deletion of *RNP1* was also constructed and tested. The deletion extended from a SalI site 5′ of the coding region (also removing about 1/3 of the adjacent RPL4B gene) to a StuI site well 3′ of the coding region (Fig. 1). Again, most dissected tetrads produced four viable segregants, all with a Ura$^+$/Ura$^-$ ratio of 2:2 (Table 1), showing that the deletion of *RNP1* did not restrict viability. Southern blot analysis confirmed that the *RNP1* locus had been deleted (data not shown). For both null constructs a battery of tests were applied to detect a phenotype. There was no substantial differences between the Ura$^+$ and Ura$^-$ colonies in: colony size or growth rate when grown on rich, complete synthetic, galactose, or glycerol media; mating efficiency; growth on high osmolarity media (1.8 M Sorbitol); germination of spores; heat sensitivity or cold sensitivity. The *RNP1*-deleted strain (RNP1Δ2) did display 25% slower growth than the non-disrupted control (Table 1), but since this construct also deleted part of the C-terminus of the RPL4B gene, and since disruption of either one of the two RPL4 genes leads to a slight growth retardation (46, 47), the interpretation is made that the slower growth is not due to loss of *RNP1*. *RNP1* is not essential for germination and growth of yeast cells.

Table 2. Genotypes of spores generated from double disruptions of yeast RNP genes

Phenotype : Genotype	Number
MCY76D×MWCY003 (ΔRNP1×ΔSSB1)	
Ura$^-$ His$^-$: RNP1$^+$ SSB1$^+$	44
Ura$^+$ His$^-$: RNP1$^-$ SSB1$^+$	30
Ura$^-$ His$^+$: RNP1$^+$ SSB1$^-$	31
Ura$^+$ His$^+$: RNP1$^-$ SSB1$^-$	47
ΔNPL3×ΔSSB1 (MBY101a×MWCY003α)	
Ura$^-$ His$^-$: NPL3$^+$ SSB1$^+$	30
Ura$^+$ His$^-$: NPL3$^-$ SSB1$^+$	11
Ura$^-$ His$^+$: NPL3$^+$ SSB1$^-$	23
Ura$^+$ His$^+$: NPL3$^-$ SSB1$^-$	21

For each cross the numbers of each genotype generated from the cross is indicated. In both instances, many viable spores were detected that contained disruptions in two RNP genes.

Double disruptions of yeast RNP genes

Other RNP genes of yeast are also non-essential. The fact that many single-copy RNP genes in yeast are non-essential implies that there is redundancy of function among them. Two double disruptions of yeast RNP genes were created in the hope of creating a strain with a lethal phenotype. Both the RNP1::URA3/SSB1::HIS3 and the SSB1::HIS3/NPL3::URA3 double disruptions were created. Diploids were selected, sporulated, and dissected. In both instances viable His$^+$, Ura$^+$ spores were generated, so the double disruptions were not lethal (Table 2). No reduction in growth rate was detected in the His$^+$, Ura$^+$ segregants on either complete or minimal media (data not shown), nor did they display either a heat sensitive (*ts*) or cold sensitive (*cs*) phenotype. Southern blot-hybridization analysis with probes for the *RNP1*, *SSB1*, and *NPL3* genes showed that both

disruptions were present in the His^+, Ura^+ spores (data not shown).

Overexpression of the *RNP1* gene

Sometimes overexpression of a gene in yeast has deleterious effects on growth (48). To test this the *RNP1* gene was subcloned into into the yeast high copy number episomal plasmid YEP24 (see Material and Methods). The plasmid (pYRNP24) so constructed was transformed into SS330. The growth rate of this strain is identical to the growth rate of the parent strain containing YEP24, even though Northern blot analysis showed that the RNP1 RNA was overexpressed about 50 fold (data not shown).

The first RRM of *RNP1* is conserved

A pair of PCR primers with exact matches to the RNP-1 octamer and RNP-2 hexamer sequence elements in the first RRM of *RNP1* amplified a DNA fragment of 153 bp in *Saccharomyces cerevisiae*. Surprisingly, with the same set of primers a PCR product of identical size is detected in DNA from a wide range of organisms, in fungi, plants, insects, and vertebrates (Fig. 5). No 153 bp PCR product was seen if one or the other of the primers was omitted from the reaction, if the DNA sample was omitted, or if DNA from several prokaryotic species was used (data not shown). It seems that the structure of the RRM1 of *RNP1* is conserved.

Mapping the *RNP1* gene

The *RNP1* gene locus was mapped to the left arm of chromosome 12. First, a probe consisting of only the RRM1 region of *RNP1* was hybridized to a blot containing an ordered lambda bank of *S.cerevisiae* genomic DNA available from ATCC. The only clone to which the probe hybridized was ATCC #70275, located on a previously unmapped region of chromosome 12L (data not shown). In this clone was a 7.0 kb EcoRI/HindIII fragment. Such large R/H fragments are rare in the collection of lambda clones; less than 1% of R/H fragments are that large or larger (49). Southern blot analysis showed that the *RNP1* probe hybridized to a 7.0 kb R/H fragment, confirming the original assignment (data not shown). Finally, as additional confirmation, *RNP1* hybridized to chromosome 12 on a 'ChromoBlot' (from CloneTech, Inc.) (data not shown). The region to which *RNP1* maps is approximately 43 kb from the *KNS1* locus.

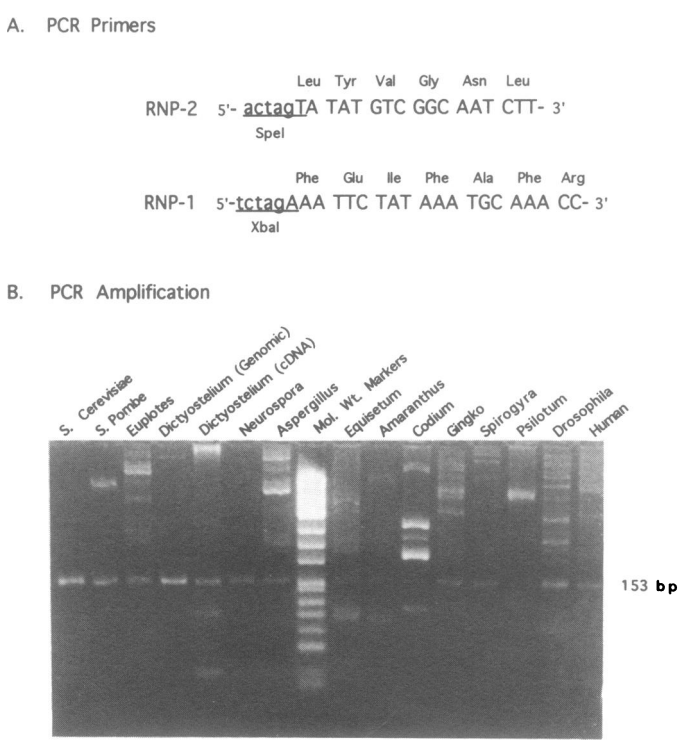

Figure 5. PCR Amplification of the first RRM of yeast *RNP1*. **A.** Oligonucleotides used as primers for the PCR reactions, derived from the RNP-1 and RNP-2 sequence elements of the *RNP1* gene. **B.** Results of the PCR amplification. Over each lane the source of the DNA that was amplified is indicated with the name of the genus. The expected product in the lane with *S.cerevisiae* DNA migrates at 153 bp A product of the same size is seen in every other lane.

The ORF3 gene resembles a seven-transmembrane-segment receptor protein

The amino acid sequence of ORF3 shows no marked similarities to any protein sequence in the SwissProt and PIR databases. A hydrophobicity profile, using the algorithm of Kyte and Doolittle, shows seven segments with hydrophobicity score greater than 1.36 (Fig. 6), implying that each such segment is capable of insertion through the membrane. This pattern of seven potential transmembrane segments is characteristic of membrane receptors that interact with G proteins. A large number of such proteins have been found; all share structural but not necessarily sequence similarity (50). Two such 7-TMS proteins previously discovered in yeast, the products of the *STE2* and *STE3* genes, which code for the α and a pheromone receptors, respectively, share no discernable sequence similarity with each other (51–53). The ORF3 gene shown here has no sequence similarity with either, and so represents a new potential 7-TMS protein of yeast. The C-terminal region, the cytoplasmic segment in 7-TMS proteins (50), is missing from the sequence reported here, as the cloned fragment ends at a *BamHI* site after 378 amino acid residues, before a termination codon is encountered.

DISCUSSION

One yeast RNP gene has been isolated (designated *RNP1*), adjacent to a gene for a single-stranded binding (ssb) protein that turned out to be a gene for ribosomal protein L4 (*RPL4B*). The *RNP1* gene has many criteria characteristic of a canonical RNP. It has two RNA Recognition Motifs (RRMs). Comparisons to known RNP genes from many organisms reveal that the first RRM bears its greatest resemblance to hnRNPs of the A/B class, especially to the second RRM of that class of proteins. Similar comparisons with the second RRM of *RNP1* showed a remarkable similarity (almost 40% identity) to yeast *SSB1*. RRM1 and RRM2 of *RNP1* have little resemblance to each other.

A lineup between the RRM1 of the *RNP1* gene and RRM2s of hnRNP A/B genes reveals two short conserved sequence elements in between the RNP-1 and RNP-2 sequence elements (Fig. 3A): a DLRD motif and a YGKI motif. Both these motifs are present in RRM1 domains of hnRNP A/B genes, though less well-conserved, but they are not often present in RRMs outside of the hnRNP A/B class. These two small motifs are part of a proposed consensus sequence for the second RRM of this class of RNP genes (54). The position 9 residues C-terminal to the RNP-1 sequence element is perhaps the most conserved residue in the RRM family (1, 54). This position is almost always an ala residue, except in the second RRM2 of hnRNP A/B genes, where it is an ile or val residue In this position in RRM1 of *RNP1*

there is a val residue. Taken together, these observations lend credence to the idea that *RNP1* might be an hnRNP gene of yeast.

The three-dimensional structures that have been determined for the first RRM of the two RRMs of the U1A snRNP protein (55, 56) and for the single RRM of the hnRNP C protein (57, 58) have in common a $\beta\alpha\beta\beta\alpha\beta$ structure, where the four β strands form a β sheet in contact with RNA, while the two α helices are packed behind the β sheet. The RNP-1 sequence element on β-strand 3 and the RNP-2 sequence elements on β-strand 1 are next to each other in the center of the antiparallel β sheet. In this structure, the DLRD motif falls in the middle of α-helix 1 while the YGKI motif is placed at the end of α-helix 1 extending into the short loop connecting α-helix 1 to β-strand 2. It is likely that residues in and adjacent to the α helices do not contact RNA (6, 58), and instead probably participate in protein−protein interactions. Multiple RRM-containing proteins combine to form complex hnRNP complexes (6). The conservation of the DLRD and YGKI motifs in yeast *RNP1* and vertebrate hnRNP A/B genes possibly indicates a possible conserved protein−protein interaction important to the function of these RNP proteins.

The second RRM of yeast *RNP1* has a structure altogether different from the first RRM. In RRM2 there seems to be two alternate forms of the RNP-1 octamer sequence element, both degenerate compared to the canonical RNP-1 sequence. Usually the RNP-1 and RNP-2 elements are 30 residues apart (1, 4, 5). If this pattern is followed, marking off 30 residues from the well-conserved RNP-2 element in RRM2, than the RNP-1 element is KVFFSDEF (Fig. 2). On the other hand, computer alignment suggests that RRM2 is most closely homologous to the single RRM of the yeast *SSB1* gene. In this assignment (Fig. 3B) the RNP-1 element is moved 9 residues to the C-terminus to become TGEAFIKF. If this alignment is accepted, then *SSB1* and the second RRM of *RNP1* have an lengthy loop preceding the RNP-1 element that is rarely seen in the large family of RNP proteins. This loop connects α-helix 1 and β-strand 3, and is the most variable region in length and residue type among RNPs (1, 2, 6). Which of the two possible RNP-1 octamer elements actually makes contact with RNA, if either actually does, will require further investigation.

Almost all RNP genes contain auxiliary, simple-sequence domains besides their RRMs (5, 6). The *RNP1* gene in this work represents a rare instance where there seems to be no auxiliary domain. In particular, there is no RGG box (59), no gly-rich C-terminus found in vertebrate and insect hnRNP A/B genes (60, 61), no pro-rich or gln-rich runs (19, 21), no GAR (gly−arg-rich) domains found in all nucleolar RNPs that presumably participate in pre-rRNA processing (62), no RS (arg−ser) domains found in many pre-mRNA splicing factors (63, 64), and no zinc fingers (65). Since auxiliary domains are missing it is not possible to classify *RNP1* unequivocally as any one type of RNP gene. It represents a peculiar hybrid not yet seen in the RNP superfamily of protein sequences, composed of an hnRNP-like RRM in tandem with a SSB1-like RRM that is unique to yeast.

Since RRM1 and RRM2 of *RNP1* are so different, it is conceivable that they can simultaneously bind to more than one RNA, or to two different RNA segments in the same RNA molecule. The RNA substrate(s) of rnp1p will have to be ascertained before this possibility can be tested. Alternatively, when there are multiple RRMs all of them could be required for RNA binding. In the only experiments testing this possibility to date, with the U1-A and U2-B″ snRNP proteins, both of which have two RRMs, only the first RRM is required for binding to the cognate RNA; the second RRM in both instances is dispensable (55, 66, 67). Perhaps the second RRM of *RNP1*, since it bears minimal resemblance to the RNP consensus sequence, is also dispensable for RNA binding.

The original aim of this line of investigation was to find new RNPs of yeast by isolating and identifying uncharacterized ssb proteins of yeast. The ssb protein so found was a ribosomal protein, not an RNP protein, but *RNP1* was adjacent to it. It's hard to know what to make of the proximity of an RNP gene to a ribosomal ssb gene. It could be that their position next to each other was merely fortuitous. Or, the two genes have related roles, and so their proximity is a means to keep their expression

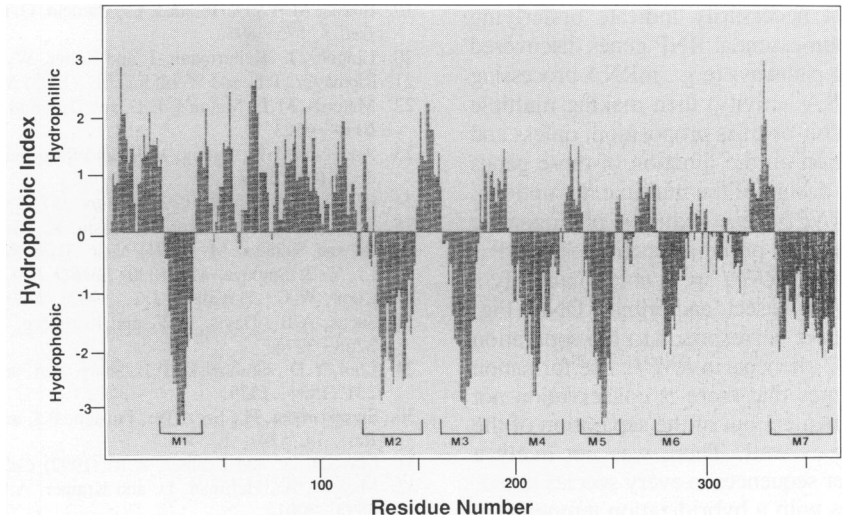

Figure 6. Hydrophobicity profile of the potential ORF3 gene product. Hydrophobic/hydrophilic values are computed from the algorithm of Kyte and Doolittle, with a window size of eight. Hydrophobicity above 1.36 indicates a potential membrane-spanning domain. The locations of the predicted seven transmembrane segments (M1−M7) of ORF3 are marked.

in tandem. Maybe *RNP1* is a nucleolar RNP with a role in ribosome assembly, or maybe the RPL4 gene product has a role in RNA metabolism beyond its role as a ribosomal protein. In regards to this speculation, it has been noted that the tertiary structure of snRNP U1A protein is similar to the tertiary structure of several ribosomal proteins (56, 68), although no sequence similarity between these ribosomal proteins and any RNP proteins is apparent. Structural studies on ribosomal protein L4 have not been done, so its not known if L4 is a member of this group of ribosomal proteins. Both RNP proteins and ribosomal proteins may have in common an ancestral RNA binding structural motif, and if so, the propinquity of the *RPL4B* and *RNP1* genes may not be so surprising. The propinquity of an RNP protein gene to a ribosomal protein gene may not be without precedent. *NAB2*, a putative RNP gene that does not have an RRM but does have a RGG box and seven zinc fingers, is adjacent to the gene for ribosomal protein S4 (65).

Both removal (in the *RNP1* disrupted and deleted strains) and overproduction of RNP1 (in strain containing the pRNP24 plasmid) had no obvious deleterious effects on cell growth. The yeast cell can apparently tolerate large variations in the amount of the *RNP1* gene product. Many yeast RNP genes are also not essential, making it hard to initiate further genetic analyses of RNP function in this genetically tractable organism. In cultured mammalian cells deletion of the hnRNP A1 locus does not reduce cell viability or cell growth (69). It seems surprising that hnRNP A1, despite its crucial importance to 5′ splice site selection in mammals (31, 32), is non-essential. If these findings are general, and if *RNP1* of yeast is an hnRNP A/B homolog, then its non-essentiality would not be unanticipated.

In yeast there may be other RNP proteins that can functionally substitute for *RNP1*. RNP genes in yeast form a moderately large family of related genes (Fig. 2), many of whose members are non-essential genes. Perhaps disruption of two of the functionally redundant RNP genes would give rise to a 'synthetic lethal' phenotype. With this hope in mind two double disruptions of RNP genes of yeast, *ΔRNP1/ΔSSB1* and *ΔSSB1/ΔNPL3*, were created in this work. Neither double disruption had any obvious effects on viability (Table 2). This approach to analysis of gene function has obvious limitations, in that structural homology indicated by sequence similarity does not necessarily indicate underlying functional homology. If the non-essential RNP genes discovered in yeast act in non-overlapping pathways (e.g., mRNA processing vs rRNA processing vs snRNA activity) then making multiple disruptions of RNP genes is a hit-or-miss proposition, unless and until further critical information on the function of these genes is available that could aid the design of the multiple disruptions.

The first RRM of the *RNP1* gene exhibits phylogenetic conservation. Unique sequence PCR primers matching the RNP-1 and RNP-2 sequence elements of *RNP1* were able to amplify a 153 bp product from fungal, plant, insect, and primate DNA (Fig. 5). The size of the PCR product corresponds to the separation between the RNP-1 and RNP-2 elements in *RNP1*. The formation of these PCR products indicates that there is conservation not only of the sequences of the primers but of the separation of the RNP-1 and RNP-2 elements as well. There was not likely a perfect match to the two primer sequences in every species tested. The PCR conditions (5 cycles with a hybridization temperature of 45°C followed by 25 cycles at the standard 55°C) were chosen to allow some mismatching (45). Two or three mismatches per primer were probably allowable. Nonetheless, the fact that there had to be moderate matches to two oligonucleotide primers, as well as the conservation of the size of the amplified product, indicates a substantial degree of conservation of RRM1. *RNP1* may not be an essential gene, but the strong conservation of its first RRM implies that it is an important gene.

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