A protein containing conserved RNA-recognition motifs is associated with ribosomal subunits in *Saccharomyces cerevisiae*

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ABSTRACT

Using PCR cloning techniques, we have isolated a *Saccharomyces cerevisiae* gene encoding a protein that contains two highly conserved RNA-recognition motifs. This gene, designated *RNP1*, encodes an acidic protein that is similar in sequence to a variety of previously isolated RNA binding proteins, including nucleolin, poly (A) binding protein, and small nuclear ribonucleoproteins. The *RNP1* gene maps to the left arm of chromosome XIV centromere distal to *SUF10*. Haploid yeast containing a null allele of *RNP1* are viable, indicating that *RNP1* is dispensible for mitotic growth. However genomic Southern blot analysis indicated that several other loci in the *S.cerevisiae* genome appear to contain sequences similar to those in the *RNP1* gene. The majority of the Rnp1 protein is cytoplasmic. Extra copies of *RNP1* cause a decrease in levels of 80S monoribosomes. A fraction of Rnp1 protein cosediments on sucrose gradients with 40S and 60S ribosomal subunits and 80S monosomes, but not with polyribosomes.

INTRODUCTION

In eukaryotic cells, ribosomes are assembled in the nucleolus, a specialized organelle that is the site of ribosomal RNA (rRNA) synthesis. *Saccharomyces cerevisiae* ribosomal RNA is transcribed as a 35S precursor that is processed to mature 25S and 18S rRNAs as the ribosomal proteins assemble onto them to form ribosomal subunits. The mechanisms of rRNA processing and the role that non-ribosomal nucleolar proteins play in these events are of interest to our laboratory. We sought gene products that interact with RNA and are components of the yeast nucleolus by identifying genes encoding conserved sequence elements found in proteins known either to bind RNA, or reside in the nucleolus, or both. In particular, we were interested in identifying a *S.cerevisiae* homolog of nucleolin.

Many proteins that bind RNA or are involved in RNA metabolism contain a conserved 80–90 amino acid sequence

known as the RNA recognition motif (RRM) (1). The RRM consists of a highly conserved octamer or RNP-1 amino acid sequence and a less well-conserved hexamer or RNP-2 amino acid sequence, flanked by less conserved sequences. RRM sequences were originally identified in poly (A) binding protein (PABP) from *S.cerevisiae* (2), and have been found in numerous other RNA-binding proteins including the abundant nucleolar phosphoprotein nucleolin, as well as many types of hnRNP and snRNP proteins (reviewed in 3,4).

Over the past several years, genes encoding several yeast nucleolar proteins have been identified. Comparison of the inferred amino acid sequences of these yeast nucleolar proteins to each other and to the amino acid sequences of nucleolar proteins from higher eukaryotes, identified a conserved domain rich in arginine, glycine and phenylalanine residues, designated the GAR domain (5). Two yeast nucleolar proteins containing GAR domains, Nop1p and Ssb1p, are found associated with small nucleolar RNAs (snoRNAs), and also contain sequences resembling RRM (6,7). Gar1p, a yeast nucleolar protein containing an extensive GAR domain but no RRM, is also found associated with snoRNAs (5).

We have used the polymerase chain reaction (PCR) to identify a new gene from *S.cerevisiae* that encodes a protein containing RRM as well as a short GAR domain. This gene, designated *RNP1*, is dispensible for mitotic growth, however this gene may be functionally redundant in that several loci appear to have sequence similarity to *RNP1*. Overexpression of *RNP1* results in decreased numbers of 80S monoribosomes. A portion of the Rnp1 protein cosediments with ribosomal subunits and 80S monoribosomes.

MATERIALS AND METHODS

Strains and media

Saccharomyces cerevisiae strain JWY749 (*MATa/MATα ura3-52/ura3-52 trp1-Δ101/trp1-Δ101 lys2-801/lys2-801 leu2-1/leu2-1 his3-Δ200/his3-Δ200*) was the parent diploid in which the *mp1::HIS3* disruption was constructed. Genomic DNA

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prepared from *Saccharomyces cerevisiae* strain SC252 (*MAT α ade1 leu2-3,112 ura3-52*) was used for PCR amplification of the *RNP1* gene. Standard methods were employed for genetic analysis of yeast (8,9). Yeast strains were grown in either YEPD or defined synthetic media supplemented with 2% dextrose as a carbon source (8). Yeast were transformed by the lithium acetate method (10).

Materials

Restriction endonucleases and T4 DNA ligase were obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). *E. coli* DNA polymerase holoenzyme was a gift of Dr William E. Brown, Carnegie Mellon University. [α - ^{32} P]dATP and [α - ^{35} S]dATP were obtained from New England Nuclear (Boston, MA).

PCR amplification of yeast genomic DNA

PCR reactions were carried out using 500 μ g of yeast genomic DNA and the oligonucleotides shown in Fig. 1, in 50 μ l volumes using standard 1 $\times$ reaction buffer. Oligonucleotides were obtained from Operon Technologies (Alameda, CA). *AmpliTa*q DNA polymerase was obtained from Perkin Elmer Cetus (Norwalk, CT). Samples were amplified for 20 cycles, denaturing at 95°C for 1 min, annealing at 37°C for 1 min, and polymerizing at 72°C for 2 min with a 15 sec extension on the polymerization step at each cycle.

DNA manipulations and Southern analysis

Construction and analysis of recombinant DNAs, and electrophoresis, blotting, and hybridization of DNA were performed as described in Sambrook *et al.* (11). Nytran membrane (Schleicher and Schuell, Keene, NH) was used for DNA blots. Subclones designed to be used in both *E. coli* and yeast were cloned into the pRS series of vectors (12). High-copy yeast vector YEp352 was used for overexpression of cloned sequences in yeast (13). The library of overlapping yeast genomic fragments cloned into bacteriophage λ and cosmids was obtained from L. Riles and M. Olson and was screened as described in Bender and Pringle (14).

DNA sequence determination and analysis

To determine the nucleotide sequence of *RNP1* by the dideoxy chain termination method (15), a 5.0-kb *Hind*III fragment containing the *RNP1* gene was cloned into pKS⁺, and a series of deletions was created using an exonucleaseIII/mung bean nuclease kit from Stratagene (LaJolla, CA). Synthetic oligonucleotides synthesized by Operon Technologies (Alameda, CA) were used as primers to complete sequencing when necessary. Sequence analysis and data base searches for sequence similarities were performed using computer programs developed by the Genetics Computer Group's Sequence Analysis Software Package at the University of Wisconsin Biotechnology Center (16).

Fractionation and characterization of polyribosomes

Yeast strains were grown to a density of 4.0–6.0 $\times$ 10⁷ cells/ml at 30°C in 200 ml YEPD or defined synthetic media. Extracts containing ribosomal material were prepared and analyzed on 35 ml 7–47% sucrose gradients using a Model 640 ISCO density gradient fractionator as described by Baim *et al.* (17).

Epitope addition, cell fractionation, and immunoblot analysis

Oligonucleotide 5'-TCTGAAAATAACGAAGAATACCCAT-ACGACGTCCCAGACTACGCTCAACATCAACAACAA-CAA-3' encoding the nine amino acid influenza HA epitope plus *RNP1* flanking sequence was inserted into *RNP1* by site-directed mutagenesis (18). Yeast cells were spheroplasted and fractionated as described in Mirzayan *et al.* (19). Proteins from total cell extract, cytoplasmic and nuclear fractions, or TCA-precipitated from polyribosome gradient fractions were subjected to PAGE, electrophoretically transferred to nitrocellulose, and assayed by immunoblot analysis using the Protoblot Immunoscreening System (Promega, Madison, WI). Monoclonal antibody 12CA5, generated against the influenza HA epitope (20), was a generous gift of Dr Peter Kolodziej (U.C.S.F.). Antibodies against yeast Nop1p were a kind gift of Dr Robert Hamatake (N.I.E.H.S.).

RESULTS

Identification of a gene with conserved RNA binding motifs

We used PCR to determine whether the yeast genome contains sequences encoding proteins with RRM and GAR domains, with particular interest in identifying a homolog of nucleolin. Many RNA-binding proteins, including nucleolin contain multiple consecutive RRMs. Because the RNP-1 octamer is more conserved than the RNP-2 hexamer in RRMs, we used two oligonucleotides encoding RNP-1 motifs to screen for sequences

Figure 1. Oligonucleotides used for PCR cloning of the *RNP1* gene. The BOX3/RNP-1 and BOX4/RNP-1 oligonucleotides were designed to hybridize to DNA coding for the core octamer sequences conserved in many RNA binding proteins, including Xenopus nucleolin. The GAR oligonucleotide was designed to hybridize to DNA encoding a glycine-arginine rich motif that is conserved in many nucleolar proteins.

encoding more than one consecutive RRM. Oligonucleotides were synthesized based on the amino acid sequences of the RNP-1 octamer motifs contained within BOX3 and BOX4 of *Xenopus* nucleolin (21) (Fig. 1). An expected amplification product of 270 base pairs (bp) would represent the sequence encoding a portion of two consecutive RNA-binding motifs. A fragment of the predicted 270-bp size was amplified from total *S.cerevisiae* genomic DNA, cloned into plasmid pKS+, sequenced, and found to be 33.3% identical to RRM sequences from Chinese hamster ovary cell nucleolin (22) and 30.1% identical to RRM sequences from yeast polyadenylate binding protein (2). Alignment of the amino acid sequence encoded within the 270-bp fragment with the sequence of several RNA binding proteins revealed conservation of all of the amino acid residues defined as the ribonucleoprotein consensus sequence (RNP-CS) or RNA-recognition motif (RRM) found in many RNA-binding proteins (1, 4). To determine whether the amplified DNA originated from a gene expressed in yeast, the 270-bp fragment was radioactively labeled and hybridized to a blot of poly (A) RNA. An mRNA of approximately 1.6-kb was detected (data not shown). Because it encodes a sequence with similarity to RNA binding proteins and apparently is expressed in yeast, the 270-bp fragment was

then used as a probe to identify the entire gene from which it was derived.

Isolation of the *RNP1* gene

The 270-bp PCR amplified fragment was radioactively labeled and used as a hybridization probe to screen a library of yeast genomic DNA cloned in YCp50 (23). Five plasmids were identified that contained identical inserts of genomic DNA as determined by restriction enzyme mapping (data not shown). The 270-bp fragment could be amplified from these genomic clones by PCR. An additional oligonucleotide, GAR1, was synthesized based on the amino acid sequence surrounding the conserved glycine, arginine motif in *Xenopus* nucleolin and in the yeast nucleolar proteins Gar1p (5), Nop1p (6), and Ssb1p (7) (Fig. 1). A 450-bp fragment could be amplified from the cloned DNAs using the GAR1 and BOX3 oligonucleotides. We designated this gene to which the BOX3, BOX4 and GAR1 oligonucleotides hybridized *RNP1*.

The *RNP1* gene product contains two RNA-binding motifs

A restriction map of the cloned genomic DNA fragment containing *RNP1* and the complete sequence of the open reading frame of *RNP1* is shown in Fig. 2 (accession #L01797). The *RNP1* ORF is 1287 nucleotides in length, consistent with the size of the 1.6-kb mRNA to which *RNP1* DNA hybridizes. The 5' end of the *RNP1* transcript was mapped to nucleotide -73 by primer extension analysis (data not shown). Thus the Met codon indicated at +1 is most likely used as the codon for initiation of translation of *RNP1*. The Rnp1 protein (Rnp1p) is predicted to contain two consecutive RRM. The BOX3/RNP-1 and BOX4/RNP-1 sequences responsible for the original PCR amplification are located at nucleotides +343 to +366 and +607 to +630, respectively (Fig. 2). The alignment of the RNP consensus sequences RNP-1 and RNP-2 found in the RNA recognition motifs of Rnp1p with those from other known RNA binding proteins is shown in Fig. 3A. The entire ORF is 36.1% identical in sequence to that of human poly (A) binding protein (24), and 27.5% identical to that of CHO nucleolin (22). Sequences complementary to the GAR1 oligonucleotide lie between nucleotides +778 to +792 in *RNP1*, and are assumed to have been responsible for producing the observed 450-bp amplification product with the GAR1 and BOX3 oligonucleotides. Rnp1p contains an arginine-rich region similar to those found

Figure 2. Restriction map and nucleotide sequence of *RNP1*, and predicted amino acid sequence of Rnp1p. **A.** The cloned 5.0-kb *HindIII* restriction fragment bearing *RNP1* is shown. The *HindIII* restriction site in brackets was created by site-directed mutagenesis. **B.** Nucleotides are numbered above the lines. Amino acids are numbered to the left of each line. The sequences to which the BOX3, BOX4 and GAR1 oligonucleotides hybridized are underlined.

A.		B.	
PROTEIN	DOMAIN	PROTEIN	GAR DOMAIN
Nucleolin (<i>Xenopus</i>)	1	LFIGNL	RKFGYVDF
	2	LAWKNI	RGIAIYEF
	3	LVLISNL	RGFATIEF
	4	LFVRLG	RKGFVDF
PABP	1	LYVGDL	LGAIYVNF
	2	IFIKNI	RKGFVDF
	3	LYVRLG	RKGFVDF
	4	LFVRLG	RKGFVDF
Sex-Lethal	1	LIWVNL	RTGYSGFY
U1-70K	-	LFVAVR	RGVATIEY
RNP CONSENSUS		LFVGNL	RKGFVDF
<i>RNP1</i>		1	LYVGNL
		2	LFVGNL

Figure 3. Alignment of the conserved RNP-1 and RNP-2 motifs and the GAR domain found in Rnp1p with those from other RNA binding proteins and nucleolar proteins. **A.** Conserved RNP-1 and RNP-2 sequences within the 90 amino acid RRM defined by Bandziulis *et al.* (1) from several RNA binding proteins are aligned with the analogous domains in Rnp1p. **B.** GAR domains from *Xenopus* nucleolin, Nop1p and Ssb1p are aligned with that from Rnp1p.

Figure 4. *RNP1* is on chromosome XIV and cross-hybridizes with other loci in the yeast genome. **A.** A DNA fragment derived from within the *RNP1* coding region was used as a hybridization probe to a blot of yeast chromosomes separated by CHEF. **B.** A blot of *EcoRI* digested genomic DNA from *RNP1* and *rnp1::HIS3* containing strains. Lane 1: Wild-type diploid JWY749, Lane 2: Diploid strain heterozygous for *RNP1* and *rnp1::HIS3*. Lanes 3 and 4: Haploid spore clones containing the *rnp1::HIS3* allele. Lanes 5 and 6: Haploid spore clones containing the wild-type *RNP1* allele. The arrows and the bracket in panel B point to genomic *EcoRI* fragments that cross-hybridize with *RNP1* DNA. Stringent hybridization conditions were employed.

Figure 5. The majority of Rnp1p is cytoplasmic. Extract from a strain containing an *RNP1::HA* allele was separated into cytoplasmic and nuclear fractions. Equal microgram amounts of protein from each fraction were subjected to PAGE, blotted and probed with **A.** antibodies against Nop1p, a known yeast nucleolar protein serving as a control for the fractionation procedure or **B.** anti-HA antibodies to detect HA-tagged Rnp1p.

in *Xenopus* nucleolin, and yeast Nop1p and Ssb1p (Fig.3B). A proline-rich region extending from amino acid residue 293 to amino acid residue 374 of Rnp1p is similar to many such regions in snRNP proteins B and B' (25) and the U1 70K snRNP protein (26). The asparagine-rich region found between amino acids 243 and 289 and the glutamine rich-regions near the amino- and carboxy-termini of Rnp1p are similar to those observed in Rna14p, a yeast protein important for maintenance of poly (A) tail length and messenger RNA stability (27). Such homopolymeric regions are thought to be important for protein-protein interactions. Although sequence similarity does not indicate that *RNP1* encodes the homolog of nucleolin, it does represent a new member of the RRM family of proteins in yeast.

There are several sequences related to *RNP1* in the yeast genome

To map the location of *RNP1* within the yeast genome, *RNP1* DNA was hybridized first to a blot of electrophoretically separated yeast chromosomes and then to a set of bacteriophage λ and cosmid clones, containing overlapping fragments of the yeast genome. Stringent conditions for hybridization and washing were employed. The *RNP1* probe hybridized strongly to chromosome XIV and to several λ clones indicating that *RNP1* maps to the left arm of chromosome XIV, centromere distal to *SUF10*. Additional weak hybridization to chromosomes IX, XIII, XV, and V of *S.cerevisiae* was observed indicating that at least 4 other loci contain sequences related to *RNP1* DNA (Fig.4A). Similarly, *RNP1* DNA hybridized strongly to a 6.0-kb *EcoRI* fragment containing *RNP1* and weakly to several other fragments in a genomic Southern blot (data not shown and Fig.4B). We cannot rule out the possibility that these other loci cross-hybridize simply on the basis that they also contain sequences encoding RNA-binding motifs. However a restriction fragment containing the yeast *PAB1* gene which encodes four RRM, hybridized only to the *PAB1* locus in the yeast genome using the same stringency conditions (data not shown).

The *RNP1* gene is dispensable for mitotic growth

To determine whether *RNP1* is essential for mitotic growth of yeast, a deletion-disruption allele was constructed by replacing the coding sequence from nucleotide +238 to +658, which includes sequences encoding both RRM, with the 1.7-kb *BamHI* fragment containing *HIS3*. The *rnp1::HIS3* construct was transformed into diploid strain JWY749 homozygous for *his3-Δ200*, selecting for histidine prototrophy. Genomic Southern blot analysis confirmed that one of the two wild-type *RNP1* alleles was replaced by the *rnp1::HIS3* allele (Fig.4B). Upon sporulation and dissection, 66 tetrads were recovered, all of which contained two viable His⁺ and two viable His⁻ spores, indicating that *RNP1* is dispensable for mitotic growth. No effect on mitotic doubling rate, temperature sensitivity, or osmotic sensitivity could be detected in the His⁺ spores containing the *rnp1::HIS3* null allele.

We tested whether deletion of *RNP1* affected any of several processes in RNA metabolism known to involve RRM-containing proteins. Pre-mRNA processing and poly (A) tail length were normal in the *rnp1::HIS3* strain (data not shown). Levels of 40S and 60S ribosomal subunits and monoribosomes were identical in the His⁺ and His⁻ spore clones, indicating no apparent effects on ribosome assembly or translation (data not shown). A second more complete deletion-disruption allele was constructed by replacing the DNA between the *PstI* site at nucleotide -125, 5' of the *RNP1* ORF, and the *SphI* site at +875 (codon 292) with a DNA fragment containing *TRP1*. Sporulation and dissection of a diploid heterozygous for *RNP1* and *rnp1::TRP1* yielded four viable spores in each tetrad, confirming the dispensability of the *RNP1* gene for mitotic growth.

The Rnp1 protein is cytoplasmic and cofractionates with 40S ribosomal subunits and 80S monosomes

To determine the intracellular location of the Rnp1 protein, a sequence encoding an influenza hemmagglutinin epitope of nine amino acids was inserted between the ninth and tenth codons of *RNP1* by site-directed mutagenesis. The 4.2-kb *PstI-HindIII* fragment containing this HA-tagged allele of *RNP1* was cloned

Figure 6. Extra copies of *RNP1* cause a reduction of 80S monoribosomes. Cells were grown to a density of $4-6 \times 10^7$ cells/ml at 30°C. Extracts prepared from the indicated strains were loaded onto 7–47% sucrose gradients, subjected to centrifugation and fractionated. Peaks representing the 40S and 60S ribosomal subunits and the 80S monoribosomes are labeled.

into pRS315, a centromere-based plasmid containing *LEU2*. The *RNP1::HA* construct was transformed into a *mp1::HIS3* disrupted strain selecting for *Leu*⁺ colonies. Immunoblot analysis of yeast nuclear and cytoplasmic fractions isolated from a strain carrying this *RNP1::HA* allele indicated that *RNP1* encodes a protein of 61 kD, the majority of which was found in the cytoplasmic fraction (Fig. 5). When whole-cell extract containing HA-tagged Rnp1p was prepared and subjected directly to PAGE without freezing or fractionating, only the 61 kD species was observed (data not shown). When cell extract preparations were subjected to storage at –20°C or further fractionated, a 42 kD protein is also detected. We assume this faster migrating species is a truncated form of Rnp1p.

The molecular weight of Rnp1p predicted from the sequence of *RNP1* is 47 kD. This discrepancy between predicted and observed MW is not unusual; many RRM-containing proteins have slower electrophoretic mobilities in SDS PAGE gels than predicted, for example the U1 70K protein (26) and PABP (28). To confirm that the *RNP1* coding region does not extend beyond the sequenced 1.3-kb ORF, a *HindIII* restriction enzyme site was engineered 50 nucleotides 3' of the inferred stop codon of the HA-tagged *RNP1* allele. The 61 kD protein was detected by immunoblot analysis in a strain transformed with a plasmid bearing this smaller 1.8-kb *PstI-HindIII* fragment, confirming that the aberrant mobility of Rnp1p is due to its amino acid content.

Because the majority of Rnp1p is found in the cytoplasm, we tested whether Rnp1p is involved in ribosome function by assaying whether extra copies of *RNP1* have an effect on levels of ribosomal subunits, 80S monoribosomes, or polyribosomes. Extracts were prepared from *mp1::HIS3* haploid cells that contained either the high-copy vector YEp352 bearing *RNP1* or YEp352. Analysis of ribosomal material from these extracts indicated that extra copies of *RNP1* cause a reduction of 80S monoribosomes (Fig. 6). Polyribosome profiles of a strain

Figure 7. Rnp1p co-fractionates with 40S ribosomal subunits and 80S monosomes. Whole-cell extract was loaded onto a 7–47% sucrose gradient, subjected to centrifugation and fractionated. Proteins TCA-precipitated from fractions were subjected to PAGE, blotted to nitrocellulose and probed with anti-HA antibodies to detect tagged Rnp1p.

bearing YEp352 containing the HA-tagged allele of *RNP1* are identical to those of a strain containing the wild-type allele in YEp352, suggesting that the nine amino acid insertion is not perturbing the function of Rnp1p (data not shown). To determine whether Rnp1p is associated with ribosomal subunits, 80S monosomes, or polyribosomes, an extract from a strain containing the *RNP1::HA* allele on the centromere-based plasmid pRS315 was subjected to sucrose gradient analysis. Proteins from each fraction of the gradient were TCA-precipitated, subjected to electrophoresis on SDS PAGE gels, electrophoretically transferred to nitrocellulose, and subjected to immunoblot analysis using antibodies directed against the HA epitope. Rnp1p co-fractionated with free 40S and 60S ribosomal subunits and 80S monosomes; only a small amount of Rnp1p was detected in polyribosome fractions (Fig. 7A). A portion of Rnp1p was detected at the top of the gradient, where ribonucleoprotein particles (RNPs) have been detected (29). This analysis suggests that Rnp1p may interact with 40S and 60S ribosomal subunits and 80S monosomes, and may be present in a cytoplasmic ribonucleoprotein particle (RNP).

DISCUSSION

We have identified a new *S. cerevisiae* gene *RNP1*, using PCR amplification with oligonucleotides designed to hybridize to sequences that encode a conserved octamer found in nucleolin and many other RNA binding proteins. The *RNP1* gene product contains two RRMs, a small glycine-arginine rich region referred to as a GAR domain, and glutamine-rich, proline-rich and asparagine-rich motifs similar to those found in proteins involved in RNA binding and metabolism. Disruption of the *RNP1* gene produces no obvious phenotype that we have been able to detect.

To define the role that Rnp1p might be playing in yeast, we tagged the coding sequence with an HA epitope. Biochemical fractionation and immunoblot analysis were used to determine that the majority of Rnp1p is found in the cytoplasm. Efforts to localize Rnp1p by immunofluorescence microscopy using antibodies directed against the HA epitope were unsuccessful. Although the majority of Rnp1p was detected in the cytoplasmic fractions, there is some Rnp1p associated with the nuclear fraction, indicating that Rnp1p may reside in both compartments.

Rnp1p may shuttle between the nucleus and the cytoplasm, similar to nucleolin (30) and hnRNP protein A1 (31). The GAR domain in Rnp1p is similar to those found in many nucleolar proteins cloned from yeast and higher eukaryotes. We are currently generating polyclonal antibodies to Rnp1p to determine whether this protein resides in the yeast nucleolus or is able to shuttle between the nucleus and the cytoplasm.

The presence of extra copies of *RNP1* results in decreased levels of 80S monoribosomes. A portion of HA-tagged Rnp1p co-fractionates with ribosomal subunits and 80S monosomes, while very little protein is associated with polyribosomes. A fraction of Rnp1p sediments at the top of the gradient, indicating that Rnp1p may be part of a cytoplasmic RNP. Perhaps Rnp1p associates with cytoplasmic mRNA prior to interaction with the ribosome. In support of these observations, the *RNP1* gene product appears to be an abundant poly (A) RNA binding protein of *S. cerevisiae* (32, 33).

Because haploid strains bearing either the *rnp1::HIS3* null allele or *rnp1::TRP1* null allele are viable, we considered the possibility that other gene products with redundant functions may be able to compensate for the lack of Rnp1p. To test this hypothesis, we used a plasmid dependence assay (34) to screen for mutations in other genes that would result in synthetic lethality when in combination with a disruption of the *RNP1* gene. We were unable to recover such mutations after screening 140,000 mutagenized colonies. The inability to recover synthetic lethal mutations could indicate that the function of Rnp1p can be compensated by more than one other yeast gene product. This possibility is consistent with the observation that at least four other loci in the genome of *S. cerevisiae* cross-hybridize with *RNP1* DNA. Yeast contain multiple genes encoding cyclins (35) and heat shock proteins (36). In some combinations, two or more of the genes must be disrupted before a phenotype is detectable. The systematic identification and disruption of genes related to *RNP1* may be required to determine *RNP1* function.

PCR amplification of sequences encoding conserved elements of RNA-binding proteins was used successfully to identify at least 20 different RRM-containing genes from *Drosophila* (37). Based on these studies, the number of RRM-containing genes in *Drosophila* was estimated to be approximately 250. Our inability to amplify more than one sequence from *S. cerevisiae* is rather curious in that at least five genes containing RRM sequences have been identified in yeast. The BOX3/RNP-1 and BOX4/RNP-1 oligonucleotides that we employed are 24-fold and 32-fold degenerate respectively, while the RNP-1 and RNP-2 oligonucleotides used by Kim and Baker (37) are 64-fold to 1024-fold degenerate. Perhaps more degenerate oligonucleotides need to be employed to allow for all possible combinations of amino acid sequences found in yeast. Another possibility is that the RRM sequences in *S. cerevisiae* are more variable than in higher eukaryotes, making it difficult to design oligonucleotides that would hybridize to more than a few different sequences. Inspection of the DNA sequences encoding the six RNP-I octamer motifs contained in yeast *PAB1* and *NSR1* supports this hypothesis. An oligonucleotide designed to hybridize perfectly to all the DNA sequences would have to be 2.2×10^5 -fold degenerate. Future efforts to identify yeast RNA-binding protein genes by PCR should incorporate several degenerate oligonucleotides (200-fold to 1000-fold) in various combinations in order to provide the variety necessary to hybridize to all possible DNA sequences coding for RRMs.

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