Yeast ribosomal protein L1 binds to 5 S rRNA and can be released from 60 S ribosomal subunits as an intact ribonucleoprotein particle. To identify residues important for binding of Saccharomyces cerevisiae rpl1 to 5 S rRNA and assembly into functional ribosomes, we have isolated mutant alleles of the yeast RPL1 gene by site-directed and random mutagenesis. The rpl1 mutants were assayed for association of rpl1 with 5 S rRNA in vitro and assembly of rpl1 into functional 60 S ribosomal subunits. Consistent with previous data implicating the importance of the carboxyl-terminal 47 amino acids of rpl1 for binding to 5 S rRNA in vitro, we find that deletion of the carboxyl-terminal 8, 25, or 44 amino acids of rpl1 confers lethality in vivo. Missense mutations elsewhere in rpl1 also affect its function, indicating that multiple regions of rpl1 are important for its association with 5 S rRNA and assembly into ribosomes.

A useful approach to study ribosome biogenesis is to focus on a smaller, more compact particle within the ribosome and understand its assembly and function. This provides an opportunity to dissect the complex interactions that occur within the ribosome. One such particle that is a fundamental constituent of both prokaryotic and eukaryotic large ribosomal subunits is the complex between 5 S rRNA and ribosomal protein(s) that bind to it (Blobel, 1971; Chen-Schneisser et al., 1977; Smith et al., 1978; Nazar et al., 1979).

In the yeast Saccharomyces cerevisiae, this 5 S ribonucleoprotein complex (5 S RNP) consists of ribosomal protein L1 (rpl1 or L1, also known as L1a and YL3) and 5 S rRNA (Mazelis et al., 1973; Nazar et al., 1979). Similarly, in Xenopus, rat, chicken, and rice, the 5 S RNP complexes are comprised of rpl5, the homolog of yeast rpl1, and 5 S rRNA (Chan et al., 1987; Wormington, 1989; Kamochi et al., 1992; Kim and Wu, 1993). These eukaryotic 5 S rRNA-binding ribosomal proteins are greater than 80% similar in sequence (Tang and Nazar, 1991). The 5 S RNP complexes present in ribosomes of the archaebacterium Halobacterium cutirubrum contain two proteins, HL13 and HL19 (Smith et al., 1978), whereas those in Escherichia coli contain three proteins, EL5, EL18, and EL25 (Chen-Schneisser and Garrett, 1977). These bacterial proteins share fewer sequence similarities with the eukaryotic 5 S rRNA-binding ribosomal proteins.

5 S RRNA is the only rRNA known to form a stable RNP prior to its assembly into ribosomal subunits. In mammalian cells and Xenopus oocytes, 5 S rRNA interacts with rpl5 before assembling into ribosomes (Steitz et al., 1988; Guddat et al., 1990; Allison et al., 1991, 1993). Similarly, binding of 5 S rRNA to the three ribosomal proteins EL5, EL18, and EL25 is an obligatory step for its assembly into E. coli ribosomes in vitro (Yu and Wittmann, 1973).

Chemical and enzymatic analysis of the 5 S RNP complexes, and binding studies with mutant 5 S rRNA molecules have provided information about 5 S rRNA structure and about sequences within 5 S rRNA that interact with ribosomal proteins (Nazar, 1979; Garret et al., 1981; Christiansen and Garrett, 1986; Yeh et al., 1988; Yeh and Lee, 1988; Guddat et al., 1990; Allison et al., 1993). In contrast, very little is known about sequences in the ribosomal proteins that interact with 5 S rRNA. Chemical modification of lysines or arginines in yeast rpl1 abolishes its ability to form a stable complex with 5 S rRNA in vitro, suggesting that the basic residues of rpl1 are important for interaction with 5 S rRNA (Vioque et al., 1987). Additional studies point to the importance of the rpl1 carboxyl terminus in binding to 5 S rRNA. A CNBr-deaved peptide fragment containing the carboxyl-terminal 47 amino acids of rpl1 forms a stable complex with yeast 5 S rRNA in vitro (Nazar et al., 1979; Yaguchi et al., 1984). However, this binding is nonspecific; this fragment could also bind 5 S rRNA and tRNAs in vitro (Nazar et al., 1979). This 47-amino acid carboxyl-terminal peptide fragment is rich in basic amino acids, containing nine lysine and three arginine residues. Mutations in rpl1 that contained single methionine substitutions of either Lys-276, Lys-279, or Lys-289 did not affect its ability to bind to 5 S rRNA in vitro; however, multiple substitutions of Lys-289 in combination with other basic residues, particularly Arg-282 and Arg-285, result in the formation of a nonfunctional rpl1 protein that is deficient in 5 S rRNA binding in vitro (Yeh and Lee, 1995b). Because these residues are predicted to be located on the same side of an α-helix, it was proposed that 5 S rRNA may interact with multiple contact sites located on one side of such a helical structure in the carboxyl terminus of rpl1 (Yeh and Lee, 1995b).

In this paper, we describe further experiments to functionally dissect rpl1 and identify sequences that are important for its binding to 5 S rRNA or assembly into ribosomes. Several lethal and temperature-sensitive (Ts- ) alleles of the RPL1 gene
encoding rpl1 were constructed by both site-directed and random mutagenesis. To study how the mutations affect rpl1 function, we assayed the ability of the rpl1 mutant proteins to assemble into ribosomes in vivo or to bind to 5 S RNA in vivo and in vitro. Sequences in both the amino and the carboxyl terminus of rpl1 are required for its interaction with 5 S rRNA. An intact carboxyl terminus of rpl1 is necessary for its stable assembly into functional ribosomes.

**EXPERIMENTAL PROCEDURES**

**Strains, Plasmids, and Media**

Strains used in this study are described in Table I. JWB2671, the parent plasmid used for mutagenesis of the RPL1 gene, was constructed as follows: the 1.3-kilobase XbaI fragment containing an HA epitope-tagged allele of RPL1 (RPL1-HA) (Deshmukh et al., 1993) was cloned into the XbaI-PstI site of pRS315 (Sikorski and Hieter, 1989). Yeast strain J WY3733 (rpl1-1::TRP1 + pRS316-RPL1-HA) was used to assay the rpl1-HA mutant alleles. J WY3749, 3750, 3751, 3752, and 3753 were isolated by selecting for Ura+ growth (Deshmukh et al., 1993). JWY3749, JWY3750, JWY3751, and JWY3752 were transformed with pGAL1-RPL1. JWY3753, JWY3754, JWY3755, JWY3756, JWY3757, JWY3758, JWY3759, JWY3760, JWY3761, JWY3762, JWY3763, JWY3764, and JWY3767 were transformed with pGAL1-RPL1-5 S. For all of the rpl1-HA alleles, the nucleotide sequence of the entire RPL1 open reading frame was determined to identify the site of mutation.

**Site-directed mutagenesis**

The rpl1-HA gene was mutagenized in vitro as described below. Nucleotides and amino acids are numbered from the ATG translation initiation codon of RPL1. For all of the rpl1-HA alleles, the nucleotide sequence of the entire RPL1 open reading frame was determined to identify the site of mutation.

**Mutagenesis of RPL1-HA**

The RPL1-HA gene was mutagenized in vitro as described below. Nucleotides and amino acids are numbered from the ATG translation initiation codon of RPL1. For all of the rpl1-HA alleles, the nucleotide sequence of the entire RPL1 open reading frame was determined to identify the site of mutation.

**SITE-DIRECTED MUTAGENESIS**

The specific oligonucleotides used and the nucleotides changed are as follows: rpl1-HA-1 (5'-CCAGGTTTCTGACGAGTTGA-3' + Ala-760 > Thr), rpl1-HA-2 (5'-GCTTACGCTACTGGTACCTTGCAAAAGTTGG-3' + Cys-886 > Arg) (MD6: 5'-GCTTACGCTACTGGTACCTTGCAAAAGTTGG-3'), rpl1-HA-3 (5'-GCTTACGCTACTGGTACCTTGCAAAAGTTGG-3' + insertion of TAGA after nucleotide 867), rpl1-HA-4 (5'-GCTTACGCTACTGGTACCTTGCAAAAGTTGG-3', 9 nucleotides).
with the yeast 5 S-1 L RNP. The rabbit was immunized with 70 μg and subsequently, at four week intervals, with 301, 182, and 182 μg of the 5 S-1 RNPs, respectively; injection was intramuscular, and the immune was administered along with incomplete Freund's adjuvant. High titer anti-L1-NRP antibodies were detected in the serum after the third injection. A 1:30,000 dilution of these antibodies was used for immunoblot analysis with the enzyme chemiluminescence kit (Amersham Corp.).

Immunoprecipitation Analysis

Cell lysates for immunoprecipitation were prepared from logarithmic cultures of yeast cells by glass bead lysis in lysis buffer (10 mM KCl, 20 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 25 mM EDTA). The lysates were spun twice before using the supernatant for immunoprecipitation. The HA-tagged plasmid was immunoprecipitated essentially as described before (Deshmukh et al., 1993) with the modification that immunoprecipitation was carried out in lysis buffer and the immunoprecipitation pellet was washed twice in 1 ml of wash buffer (150 mM NaCl, 50 mM Tris-HCl, pH 7.5, 0.1% Nonidet P-40) and once in 1 ml of final wash buffer (50 mM NaCl, 50 mM Tris-HCl, pH 7.5). Proteins were eluted from half of the immunoprecipitation pellet in 1 X Laemmli buffer, boiled for 5 min, and separated by electrophoresis on SDS-10% polyacrylamide gels. After transfer of the proteins from the gel to a nitrocellulose membrane, the immunoblots were probed with anti-L1 RNP polyclonal antibodies. After transfer of the proteins from the gel to a nitrocellulose membrane, the immunoblots were probed with anti-L1 RNP polyclonal antibodies. Immunoblots were developed with the enzyme chemiluminescence kit (Amersham) according to the manufacturer's instructions. The amount of 5 S rRNA coimmunoprecipitated with rpl1-HA protein was assayed from the other half of the immunoprecipitation pellet as described before (Deshmukh et al., 1993). The signal was quantitated with an LKB Ultrascan XL densitometer.

Formation of 5 S rRNA-rpL1 Complex in Vitro

JWB2624-derived plasmids containing the appropriate mutation (rpl1-HA-1, rpl1-HA-2, rpl1-HA-3, or rpl1-HA-4) were digested with Sac I, filled in with Klenow fragment, and religated with T4 DNA ligase, to remove a 0.3-kilobase fragment from the 5'-untranslated region. Removal of this fragment was necessary to allow efficient transfection of the RPL1-HA gene. To determine RNA-protein complex formation, the derived plasmid was incubated in a coupled transcription-translation system from rabbit reticulocytes (Promega Corp.) in the presence of purified, exogenous yeast 5 S rRNA as described in Yeh and Lee (1995a). For a standard 25-μl reaction, 1 μg of 5 S rRNA and 12.5 μCi of [3H]leucine were included. After 2 h, one-fifth of the reaction mixture was analyzed on a SDS-containing polyacrylamide gel. The amount of radiolabeled protein or RNP complex was quantified by fluorography followed by image analysis (Bioimage 110).

To compare and normalize the extent of RNP formation with different mutant proteins, the amount of RNP formed in each case was divided by the amount of protein L1 synthesized.

RESULTS

Isolation of rpl1-HA Mutant Alleles—To map domains within rpl1 that interact with 5 S rRNA and are important for its assembly into ribosomes, we sought to isolate mutant alleles of RPL1-HA. The fully functional epitope-tagged RPL1-HA allele was used for mutagenesis, to enable detection and immunoprecipitation of mutant rpl1-HA proteins with high titer monoclonal anti-HA antibodies (Deshmukh et al., 1993). The RPL1-HA gene was mutagenized in vitro using site-directed mutagenesis (Kunkel et al., 1987), forced nucleotide misincorporation (Liao and Wise, 1990), and PCR mutagenesis (Muhlrad et al., 1992). The mutagenized RPL1-HA alleles were assayed by plasmid shuffling to determine whether the yeast strains carrying them were viable, lethal, or Ts' in vivo (Boeke et al., 1987; Moritz et al., 1991).

We targeted mutagenesis initially to the 3' end of RPL1-HA, because a fragment corresponding to the carboxyl-terminal 47 amino acids of rpl1 was previously shown to be capable of binding to 5 S rRNA in vitro (Yaguchi et al., 1984). We constructed four 3' terminal deletion mutant alleles, rpl1-HA-6, rpl1-HA-7, rpl1-HA-8, and rpl1-HA-9, by introducing a stop codon (Fig. 1). The rpl1-HA-6, rpl1-HA-7, and rpl1-HA-8 alleles, which result in deletion of the carboxyl-terminal 44, 25, and 8 amino acids of rpl1-HA, respectively, confer lethality in vivo (Fig. 1). However, the rpl1-HA-9 allele, which contains a deletion of the carboxyl-terminal 2 amino acids of rpl1-HA, does not result in lethality. These results are consistent with the previous in vitro 5 S rRNA binding data (Yaguchi et al., 1984) and indicate that the carboxyl-terminal 44 amino acids are functionally important in vivo as well as in vitro. An internal deletion of six amino acids, rpl1-HA-10 (∆103–108), which removes a domain (L1IARR), that is conserved among the euukaryotic 5 S rRNA-binding ribosomal proteins also causes lethality in vivo (Fig. 1).

Since the carboxyl-terminal deletions were lethal in vivo, we sought to identify functionally important residues in that region. Random mutations in the 3' end of RPL1-HA were generated by PCR mutagenesis (Ma et al., 1987; Zhou et al., 1991; Muhlrad et al., 1992). One Ts' allele, rpl1-HA-5, was isolated; this allele contains a lysine to glutamic acid change at codon 289 (Fig. 1).

To identify additional rpl1-HA Ts' alleles, we mutagenized...
the RPL1-HA gene by forced nucleotide misincorporation (Liao and Wise, 1990). Four rpl1-HA Ts alleles (rpl1-HA-1, rpl1-HA-2, rpl1-HA-3, and rpl1-HA-4) were isolated, all of which contain a single mutation (Fig. 1). The growth of the rpl1-HA Ts mutants was compared by spotting the cultures on plates incubated at different temperatures. The rpl1-HA-4 (G91R) and rpl1-HA-3 (V53G) alleles grew slower than the rpl1-HA-1 (K27E), rpl1-HA-2 (T28A), and rpl1-HA-5 (K289E) alleles at 37°C (Fig. 2). The rpl1-HA-3 (V53G) allele was also cold-sensitive for growth at 13°C (Fig. 2).

Accumulation of 60 S ribosomal subunits is diminished in the rpl1-HA Ts mutants—The assembly or stability of ribosomal subunits is often diminished when a protein component of that subunit is missing or inactivated (Nam and Fried, 1986; Sachs and Davis, 1989; Moritz et al., 1991; Deshmukh et al., 1993). We examined whether the amount or composition of ribosomal subunits is affected in the rpl1-HA Ts mutants. Extracts from these rpl1-HA Ts strains, grown at 23°C and shifted to the nonpermissive temperature of 37°C for 2 h, were fractionated by sucrose velocity gradient centrifugation to resolve 40 and 60 S ribosomal subunits, 80 S monoribosomes, and polyribosomes (Fig. 3). This assay has proven to be a sensitive method for detection of changes in ribosomal subunit ratios as a result of subunit assembly defects in vivo (Rotenberg et al., 1988; Moritz et al., 1990, 1991; Ripmaster et al., 1992, 1993; Deshmukh et al., 1993; Goyer et al., 1993). Each of the five rpl1-HA Ts mutants accumulated fewer 60 S ribosomal subunits compared with the wild-type control cells (JWY3739) at the nonpermissive temperature. This decrease in 60 S ribosomal subunits is likely to be responsible for the decrease in 80 S monoribosomes and the resulting increase in free 40 S ribosomal subunits. Additional peaks sedimenting faster than the 80 S monoribosome and polyribosome peaks (Fig. 3, arrows) represent half-mer polyribosomes that contain mRNAs associated with an integral number of ribosomes plus a stalled 48 S preinitiation complex. These peaks have been observed previously in cells containing diminished amounts of 60 S ribosomal subunits (Rotenberg et al., 1988; Moritz et al., 1990, 1991; Ripmaster et al., 1992, 1993; Deshmukh et al., 1993; Goyer et al., 1993).

In certain rpl1-HA mutant strains, 60 S ribosome subunit assembly was disrupted even in the presence of wild-type RPL1-HA protein. Yeast cells that express wild-type RPL1 from the Pgal1-RPL1 plasmid and mutant rpl1-HA alleles from another plasmid were grown in galactose-containing medium, allowing expression of wild-type RPL1, under conditions that are nonpermissive for the rpl1-HA mutant alleles: 37°C for the Ts rpl1-HA alleles and 30°C for the unconditionally lethal rpl1-HA alleles. The polyribosome profiles of cells expressing both rpl1-HA-1, rpl1-HA-2, rpl1-HA-4, and the wild-type RPL1 exhibited a reduction in the amounts of 60 S ribosomal subunits compared with the profile of wild-type cells (Fig. 4, compare A and D, E, or F). These dominant mutant phenotypes indicate that the rpl1-HA-1, rpl1-HA-2, and rpl1-HA-4 mutant proteins interfere with the assembly of wild-type 60 S ribosomal subunits and 80 S monoribosomes are labeled. Peaks representing two to six polyribosomes are also labeled for the wild-type profile. Vertical arrows indicate presumptive half-mer polyribosomes.

**Fig. 2.** Temperature-sensitive and cold-sensitive alleles of RPL1. Equal numbers of cells from wild-type and mutant rpl1 cultures were spotted at low density onto three YEPD plates and incubated at 30, 37, and 13°C. The plates grown at 30 and 37°C were photographed after 2 days; the plate grown at 13°C was photographed after 5 days. Yeast strains are: JWY3739 (RPL1-HA), JWY3750 (rpl1-HA-1), JWY3751 (rpl1-HA-2), JWY3749 (rpl1-HA-3), JWY3752 (rpl1-HA-4), and JWY3761 (rpl1-HA-5).

**Fig. 3.** The temperature-sensitive rpl1-HA mutants contain diminished amounts of 60 S ribosomal subunits and accumulate half-mer polyribosomes at 37°C. Polyribosome profiles are shown for JWY3739 (RPL1-HA), JWY3750 (rpl1-HA-1), JWY3751 (rpl1-HA-2), JWY3749 (rpl1-HA-3), JWY3752 (rpl1-HA-4), and JWY3761 (rpl1-HA-5) cells grown in rich medium at 23°C and shifted to 37°C for 2 h. Free ribosomal subunits and polyribosomes in cell extracts were separated on 7–47% sucrose gradients. Peaks representing 40 and 60 S ribosomal subunits and 80 S monoribosomes are labeled. Peaks representing two to six polyribosomes are also labeled for the wild-type profile. Vertical arrows indicate presumptive half-mer polyribosomes.
ribosomal subunits, most likely by competing with the wild-type rpl1. Differences in the size of the 80 S monoribosome peak in these mutants are extract rather than strain specific (data not shown). The 3'9-terminal deletion allele rpl1-HA-6 and the internal deletion allele rpl1-HA-10 do not interfere with the assembly of wild-type 60 S ribosomal subunits (Fig. 4, compare A with B and C). The other rpl1-HA mutant proteins were not tested.

Using these yeast strains, that express both the wild-type and mutant rpl1, we also examined the polysome phenotype of cells expressing only the nonconditionally lethal rpl1-HA alleles. We found that these rpl1-HA lethal mutants contain fewer 60 S ribosomal subunits and accumulate half-mer polyribosomes compared with the wild-type polysome profile, a phenotype that was similar to that obtained for the rpl1-HA Ts" mutants (data not shown, Fig. 5, C and D).

An Intact Carboxyl Terminus of rpl1-HA Is Necessary for Its Stable Assembly into Functional Ribosomes—Among the possible explanations for defective function of the mutant L1-HA proteins are that the mutant rpl1-HA proteins may fold into an inappropriate conformation and are degraded before they can assemble, or these mutant proteins could be specifically defective for assembly into ribosomes and thus are degraded. Alternatively, these mutant proteins may be unable to enter the nucleus or they could be defective for binding to 5 S rRNA or both. To distinguish among these possibilities, we first asked whether the mutant rpl1-HA proteins were stable and whether they could assemble into ribosomes.

To enable us to assay the mutant rpl1-HA proteins at their nonpermissive conditions in the absence of newly synthesized wild-type rpl1, we constructed yeast strains containing the rpl1-D1::TRP1 null allele, a plasmid that expresses wild-type RPL1 from the repressible GAL1 promoter (PGAL1-RPL1), and a second plasmid bearing one of the mutant rpl1-HA alleles. Because the mutant alleles are the only HA-epitope-tagged alleles in these cells, anti-HA antibodies can specifically detect and immunoprecipitate the mutant rpl1-HA proteins. We grew these cells in galactose-containing medium and shifted for 2 h to glucose-containing medium. Cell extracts were separated on a 7-47% sucrose gradient, and fractions across the gradient were collected. rpL16 and HA-tagged mutant rpL1 were detected in these fractions by trichloroacetic acid precipitation of total protein and immunoblot analysis with anti-rpL16 and anti-HA-epitope antibodies. Peaks are labeled as in Fig. 3.
rRNA or assembly into ribosomes. All the mutant rpl1-HA proteins except rpl1-HA-6 (Δ254–297) were detected in these extracts by immunoblot analysis (see Fig. 6), indicating that, with the exception of rpl1-HA-6, these mutant proteins are relatively stable.

To assay whether the mutant rpl1-HA proteins could assemble into ribosomes, yeast cells were grown as described above, and cell extracts were fractionated to resolve ribosomes and ribosomal subunits. Fractions across these gradients were examined by immunoblot analysis with anti-HA epitope monoclonal antibodies, to determine whether the mutant rpl1-HA protein was present in peaks corresponding to the 60 S ribosomal subunits, 80 S monoribosomes, or polyribosomes. The distribution of rpl16, another 60 S ribosomal subunit protein, serves as an internal control. We found that all five Ts- rpl1-HA proteins could assemble into ribosomes; these proteins were detected in fractions containing 60 S ribosomal subunits, 80 S monoribosomes, and polyribosomes (Fig. 5 and data not shown). This result is consistent with the fact that rpl1 is a 60 S ribosomal subunit protein and indicates that the Ts- rpl1-HA proteins are able to assemble into stable ribosomal subunits and polyribosomes.

The rpl1-HA-7 (Δ273–297) mutant protein was detected in fractions corresponding to the 60 S ribosomal subunit peak and to a lesser extent in the peak corresponding to the 80 S monoribosomes. However, no rpl1-HA-7 was detected in the peaks corresponding to polyribosomes (Fig. 5D). This result indicates that although the rpl1-HA-7 mutant protein is able to assemble into 60 S ribosomal subunits and to some extent in the 80 S monoribosomes, ribosomes containing rpl1-HA-7 are apparently unstable or nonfunctional. The rpl1-HA-10 (Δ103–108) mutant protein could also assemble into ribosomes; this protein was detected in fractions corresponding to the 60 S ribosomal subunits, 80 S monoribosomes, and polyribosomes (Fig. 5C). However, because the signal was weak, the rpl1-HA-10 protein may not be assembling efficiently or may be partially unstable.

Regions in Addition to the Carboxyl Terminus of rpl1-HA Are Necessary for Binding to 5 S rRNA—Yeast ribosomal protein L1 binds 5 S rRNA both in vivo (Nazar et al., 1979; Deshmukh et al., 1993) and in vitro (Yaguchi et al., 1984; Vioque et al., 1987; Yeh and Lee, 1988). We have begun to identify amino acid residues in rpl1-HA that are important for binding 5 S rRNA by determining whether the rpl1-HA mutant proteins could bind 5 S rRNA in vivo and in vitro.

For the in vivo analysis, the mutant proteins were immunoprecipitated with anti-HA-epitope monoclonal antibodies from yeast cell extracts and the amount of 5 S rRNA coimmunoprecipitated with the protein was assayed (Fig. 6 and Table II). To compensate for differences in stability of different mutant rpl1-HA proteins and for differences in the recognition of the various 5 S-rpl1-HA mutant complexes by the anti-HA antibodies, the amount of 5 S rRNA coimmunoprecipitated with a particular rpl1-HA mutant protein was divided by the amount of that protein precipitated. The amount of 5 S rRNA coimmunoprecipitated with the wild-type rpl1-HA protein was calculated as the control; the numbers obtained for various rpl1-HA alleles are expressed as percent of wild-type. The rpl1-HA-7 mutant protein that contains a deletion of the carboxyl-terminus 25 amino acids could bind 5 S rRNA to 52% of the wild-type levels in vivo; a smaller deletion of 8 amino acids at the carboxyl terminus (rpl1-HA-8) decreases binding to 65% of wild-type levels. These results indicate that, although the carboxyl terminus of rpl1-HA is necessary for binding, it is not the only 5 S rRNA binding domain in rpl1-HA. rpl1-HA-10 lacking amino acids 103–108 binds 5 S rRNA to only 7% of the wild-type levels. 5 S rRNA binding of the rpl1-HA-6 mutant protein could not be determined, because this protein was not detected in the extracts and hence was inferred to be unstable in cells (Fig. 6).

Among the five rpl1-HA Ts- mutant proteins, rpl1-HA-3 (V53G) is most defective in binding 5 S rRNA in vivo; binding to 5 S rRNA is reduced to only 17% of the wild-type levels. Binding of the rpl1-HA-1 (K27E) mutant protein to 5 S rRNA

![Fig. 6. Binding of the mutant rpl1-HA proteins to 5 S rRNA in vivo.](http://www.jbc.org/)

Yeast strains JWY3742 (RPL1-HA), JWY3755 (rpl1-HA-1), JWY3756 (rpl1-HA-2), JWY3754 (rpl1-HA-3), JWY3757 (rpl1-HA-4), JWY3764 (rpl1-HA-5), JWY3743 (rpl1-HA-6), JWY3762 (rpl1-HA-7), JWY3763 (rpl1-HA-8), and JWY3753 (rpl1-HA-10) were grown in galactose-containing medium and shifted for 2 h to glucose-containing medium. Cell extracts were treated with anti-HA-epitope antibodies to immunoprecipitate the mutant rpl1-HA proteins. L1-HA IP, the immunoprecipitated rpl1-HA proteins were detected by immunoblot analysis with anti-L1 RNP antibodies. 5 S rRNA co-IP, commmunoprecipitation of 5 S rRNA with the mutant rpl1-HA proteins. 5 S rRNA was detected by hybridization with oligonucleotide S5-2. These data were quantitated by densitometry (average of two experiments) and are represented in Table II. To normalize for differences in stability of different mutant rpl1-HA proteins, the amount of 5 S rRNA commmunoprecipitated with each mutant rpl1-HA protein was divided by the amount of that mutant protein precipitated.

| Mutant allele | Dominant polyribosome phenotype | Assembly into ribosomes | Binding to 5 S rRNA in vivo | Binding to 5 S rRNA in vitro |
|---------------|---------------------------------|-------------------------|-----------------------------|-----------------------------|
| rpl1-HA-1 (Lys-27 → Glu) | Yes | Yes | 65 ± 5 | 40 ± 5 |
| rpl1-HA-2 (Thr-28 → Ala) | Yes | Yes | 76 ± 8 | 50 ± 6 |
| rpl1-HA-3 (Val-53 → Gly) | ND* | Yes | 17 ± 6 | 0 |
| rpl1-HA-4 (Gly-91 → Arg) | Yes | Yes | 83 ± 9 | 50 ± 5 |
| rpl1-HA-5 (Lys-289 → Glu) | ND | Yes | 85 ± 2 | ND |
| rpl1-HA-6 (Δ254–297) | No | Unstable | ND | ND |
| rpl1-HA-7 (Δ273–297) | ND | 60 and 80 S only | 52 ± 2 | ND |
| rpl1-HA-8 (Δ290–297) | ND | 80 S only | 65 ± 2 | ND |
| rpl1-HA-10 (Δ103–108) | No | Yes (inefficiently) | 7 ± 1 | 0 |

* ND = not determined.
is 65% of wild-type levels; that for the rpl1-HA-2 (T28A) mutant protein is 76% of wild-type levels. 5 S rRNA binding is only marginally reduced in the rpl1-HA-4 (G91R) and rpl1-HA-5 (K289E) mutants; these proteins bind 82 and 85% of wild-type amounts, respectively, in vivo. These results indicate that valine 53 and amino acids 103–108 of rpl1-HA are very important in binding 5 S rRNA, either directly by interacting with 5 S rRNA or indirectly by maintaining the structure of rpl1-HA.

To extend our in vivo analysis and to assay 5 S rRNA binding by a different criterion, we assayed a subset of the rpl1-HA mutant proteins for their ability to bind 5 S rRNA in vitro. An in vitro system for the study of rpl1 and 5 S rRNA binding has been described recently (Yeh and Lee, 1995a). One advantage of the in vitro analysis is that it enables us to assay 5 S rRNA binding of the mutant rpl1-HA proteins independently of other factors, such as differences in stability, ribosome assembly, or competition with wild-type rpl1, which could affect 5 S rRNA binding in vivo. These rpl1-HA mutant proteins were expressed in a coupled transcription-translation system from rabbit reticulocytes in the presence of exogenous yeast 5 S rRNA and assayed for 5 S RNP formation on an 8% nondenaturing polyacrylamide gel (Fig. 7). The 5 S RNP formation for each mutant was normalized to the amount of that mutant protein synthesized and expressed as a percent of the binding observed for the wild-type rpl1-HA protein.

Consistent with the marginal 5 S rRNA binding of the rpl1-HA-3 and rpl1-HA-10 mutant proteins in vivo, these two mutant proteins did not form a detectable 5 S RNP complex in vitro as well (Fig. 7, data not shown). The percent RNA binding calculated for the rpl1-HA-1, rpl1-HA-2, and rpl1-HA-4 mutant proteins in vitro was 40, 60, and 50% of wild-type, respectively (Table II).

**DISCUSSION**

Temperature-sensitive and lethal mutations in ribosomal protein L1 were isolated by in vitro mutagenesis of rpl1-HA. Four carboxyl-terminal truncations and one internal deletion of rpl1-HA were constructed, and five conditional lethal alleles of rpl1-HA were isolated; one of the Ts alleles, rpl1-HA-3 (V53A), was also cold-sensitive. As has been suggested before, it is not uncommon to find cold-sensitive mutations in RNA binding proteins (Zavanelli et al., 1994). All five yeast rpl1 Ts alleles isolated contained mutations in amino acid residues that are highly conserved among all the known eukaryotic 5 S rRNA binding ribosomal proteins (Fig. 8). Since eukaryotic 5 S rRNAs have similar tertiary structure, it is likely that the mechanism of interaction between 5 S rRNA and these ribosomal proteins is also conserved in evolution. One prediction of this hypothesis would be that rpl5 proteins from different species containing similar mutations would also be defective for function.

The rpl1-HA mutant proteins could be defective for function, because the mutations cause structural changes in the protein. We have used the secondary structure prediction program described by Rost and Chris (1993, 1994) to determine whether mutations of rpl1-HA are likely to affect its secondary structure. None of the rpl1-HA point mutations were predicted to disrupt its major structural elements (data not shown). Previous studies have indicated that rpl1 is phosphorylated in vivo; up to two residues are thought to be phosphorylated per mol of rpl1 (Zinker and Warner, 1976; Campos et al., 1990). Although the significance of this phosphorylation is not yet known, it is possible that the rpl1-HA-2 (T28A) mutant protein and the rpl1-HA-6 and rpl1-HA-7 proteins that contain the carboxyl-terminal deletion are defective because they alter potential phosphorylation sites and thus affect rpl1 function.

Analysis of the rpl1-HA Mutants—The decrease in 60 S ribosomal subunits in the rpl1-HA Ts and nonconditionally lethal mutants is diagnostic of a defect in 60 S ribosomal subunit assembly; this polyribosome phenotype is similar to that of the rpl1-D1 conditional null mutant (Deshmukh et al., 1993) and of strains expressing the conditional mutant alleles of another eukaryotic 60 S ribosomal subunit protein gene, RPL16A (Moritz et al., 1990, 1991). The mutant rpl1-HA proteins either assemble less efficiently into 60 S ribosomal subunits or the 60 S ribosomal subunits containing the mutant
rpl1-HA proteins are unstable in vivo or both.

None of the rpl1-HA Ts− mutants appear to accumulate any precursor or aberrantly formed ribosomal subunit particles detectable by sucrose gradient analysis. This observation contrasts with results obtained for some bacterial ribosomal mutants, which accumulate aberrant ribosomal subunit particles (Nashimoto and Nomura, 1970; Marvaldi et al., 1979; Pichon et al., 1979). It appears that in eukaryotes, any aberrantly formed ribosome particles are degraded rapidly in vivo. Thus far, only one yeast ribosome assembly mutant that accumulates aberrantly formed ribosomal subunit particles has been described (Bayliss and Ingraham, 1974). This streptomycin-sensitive yeast mutant accumulates a 28 S RNP containing the 18 S rRNA. However, this RNP is not a precursor to the 40 S ribosomal subunits; the molecular nature of this mutation is not known (Bayliss and Ingraham, 1974).

Assembly of Mutant rpl1-HA into Ribosomes—We found that all of the rpl1-HA Ts− mutant proteins were at least partially capable of assembly into ribosomes, as they were detected in fractions corresponding to the 60 S ribosomal subunits and 80 S monoribosomes and polyribosomes (Fig. 5 and data not shown). Nevertheless, these mutant proteins are defective for function. One possible explanation for this result is that mutant 80 S monoribosomes are able to assemble onto mRNAs that are being actively translated by the wild-type ribosomes previously formed in the cells, but cannot subsequently translocate. In addition, these mutant proteins could be partially defective for 60 S subunit assembly as these strains also contain half-mere polyribosomes. Alternatively, the assembly defect may be combined with a decrease in the overall rate of translation, thus resulting in the growth defect.

The rpl1-HA-7 mutant protein, which lacks the carboxy-terminal 24 amino acids, is present in fractions corresponding to the 60 S ribosomal subunits. However, very little mutant protein is present in the fractions corresponding to the 80 S monoribosome and none is detected in the polyribosome fractions (Fig. 5D). Thus, an intact carboxy terminus of rpl1-HA is required for the formation of stable 80 S monoribosomes. Our results cannot distinguish whether these sequences are important for the formation of 80 S monoribosomes or for maintaining their stability. Other assays such as pulse-chase analysis of the mutant rpl1-HA protein would be able to distinguish whether the mutant proteins are degraded because they cannot assemble into ribosomes, or whether they assemble at normal rates, and subsequently, the ribosomes containing them are degraded.

5 S rRNA-binding Domain in rpl1-HA—Previous results indicated that a peptide fragment corresponding to the carboxy-terminal 47 amino acids of rpl1 can bind 5 S rRNA in vitro (Nazar et al., 1979; Yaguchi et al., 1984). Our results are consistent with the importance of an intact rpl1 carboxyl terminus for binding to 5 S rRNA (Table II). However, the rpl1-HA-7 mutant protein, which lacks the carboxyl-terminal 25 amino acids, still binds 5 S rRNA at 52% of wild-type levels in vivo (Fig. 6 and Table II). This result suggests that sequences in addition to the carboxyl-terminal 25 amino acids of rpl1 also interact with 5 S rRNA. Two previous observations are consistent with this hypothesis. First, fluorescence studies have indicated that the environment around two tryptophan residues in rpl1, located at positions 95 and 183, is influenced by association with the 5 S rRNA (Yeh et al., 1992). Second, an amino-terminal fragment of rpl1 containing the first 207 amino acids was shown to interact with 5 S rRNA. However, this complex was relatively insoluble and was not characterized further (Nazar et al., 1979). These results are also consistent with our observation that a point mutation that changes the valine residue at position 53 to glycine (rpl-HA-3) produces a Ts− mutant protein that is greatly defective in binding to 5 S rRNA at its nonpermissive temperature in vivo (17% of wild-type) and incapable of forming a 5 S RNP in vitro (Fig. 7 and Table I). Also, the rpl1-HA-10 mutant protein, which has an internal deletion of 6 amino acids (103–108), binds 5 S rRNA to only 7% of wild-type levels in vivo and does not bind 5 S rRNA in vitro (data not shown, Table I). However, such an internal deletion might cause structural changes in the protein that may indirectly affect its interaction with 5 S rRNA. Thus, regions in addition to the carboxyl terminus of rpl1-HA are required for its interaction with 5 S rRNA in vivo.

Several RRM-binding proteins have discrete domains, such as the RRM sequences, RGG box, or arginine motifs, which are necessary and sufficient to interact with RNA (Mattaj, 1993). Our results indicate that yeast rpl1 does not interact with 5 S rRNA via any discreet domains; both amino- and carboxyl-terminal sequences are required for efficient interaction. These results are consistent with studies from other RNA-binding ribosomal proteins. Mutagenesis of the E. coli S8 protein identified 39 different mutations that cause defects in binding to 16 S rRNA. However, these mutations are not localized to any specific regions within the protein (Wower et al., 1992). A deletion of either the amino-terminal 14 amino acids or the carboxyl-terminal 6 amino acids of E. coli S20 reduces its binding affinity for 16 S rRNA in vitro, indicating that the RRNA-binding domain in S20 is complex (Donly and Mackie, 1988). Structural elements necessary and sufficient for yeast rpS25 to bind to domain III of 25 S rRNA in vitro are contained between amino acids 62 and 126 (Rutgers et al., 1991; Koo et al., 1994).

One model for the biogenesis of the yeast L1-5 S RNP is that rpl1 interacts with 5 S rRNA first and subsequently the L1-5 S RNP assembles into 60 S ribosomal subunits. This is known to occur in mammalian cells and Xenopus oocytes where rpl5, a homolog of yeast rpl1, forms a L5-5 S RNP prior to its assembly into 60 S ribosomal subunits (Steitz et al., 1988; Guddat et al., 1990; Allison et al., 1991, 1993). Is binding of yeast rpl1 to 5 S rRNA a prerequisite for the assembly of rpl1 into 60 S ribosomal subunits? Perhaps a conformational change that occurs upon binding of rpl1 to 5 S rRNA is necessary for the L1-5 S RNP to subsequently assemble into 60 S ribosomal subunits; formation of the yeast L1-5 S RNP is known to result in a conformational change of the RNA (Yeh et al., 1988). This hypothesis would predict that mutant rpl1 proteins that are unable to interact with 5 S rRNA would also be unable to assemble into ribosomes. Alternatively if different sequences in rpl1 mediate its interactions with 5 S rRNA and assembly into ribosomes, some fraction of rpl1 that is not associated with 5 S rRNA could also independently assemble into ribosomes. Our results cannot distinguish between these two possibilities. The rpl1-HA-10 mutant protein that is greatly defective in binding to 5 S rRNA (7% of wild type) also appears to be defective in assembling into ribosomes (Fig. 5C and 6). However, the rpl1-HA-3 mutant protein that is defective in binding to 5 S rRNA (17% of wild type) does not appear to have an obvious assembly defect (Fig. 5B and 6). One caveat of this interpretation is that our assembly assay lacks the quantitation necessary for comparison of 5 S rRNA binding capability with ribosome assembly of these mutant proteins. Nevertheless, it would be useful to determine whether the rpl1-HA-3 mutant protein that is assembled into ribosomes is associated with 5 S rRNA. Isolation of rpl1 mutants that are completely defective for binding to 5 S rRNA in vivo may ultimately be necessary to determine whether prior binding of rpl1 to 5 S rRNA is a requirement for the assembly of rpl1 into 60 S ribosomal subunits.
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Mohanish Deshmukh, Jeremy Stark, Lee-Chuan C. Yeh, John C. Lee and John L. Woolford, Jr.

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