Depletion of *Saccharomyces cerevisiae* ribosomal protein L16 causes a decrease in 60S ribosomal subunits and formation of half-mer polyribosomes

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We constructed yeast strains containing deletion-insertion null alleles of the *RPL16A* or *RPL16B* genes encoding the 60S ribosomal subunit protein L16 to determine the role of L16 in the synthesis and function of ribosomes. Strains lacking a functional *RPL16A* gene grow as rapidly as wild type, whereas those containing a null allele of *RPL16B* grow more slowly than wild type. RNA analysis using *RPL16* probes revealed that both *RPL16* genes are transcribed and that *RPL16B* transcripts accumulate to twice the level of *RPL16A* transcripts. No evidence was obtained for the occurrence of dosage compensation at the level of *RPL16* mRNA accumulation in either mutant. Strains lacking both *RPL16* genes are apparently inviable, demonstrating that L16 is an essential yeast ribosomal protein. Introduction of an extra copy of either *RPL16* gene into *rpl16b* mutants restored wild-type growth rates, indicating that the two forms of the L16 protein are interchangeable.

**rp116** mutants are deficient in 60S ribosomal subunits relative to 40S subunits. 43S preinitiation complexes accumulate in half-met polyribosomes in the absence of sufficient 60S subunits. We postulate that the slow-growth phenotype of *rp116* mutants results from the perturbation of initiation of protein synthesis.

**Key Words:** Yeast ribosomal protein mutants; half-mer polyribosomes

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transcripts using S1 mapping. We also characterized the structure of the \textit{RPL16} genes and transcripts by S1 mapping, primer extension, and sequencing of the cDNA and genomic \textit{RPL16} clones (J.L. Woolford, R. Last, M. Rotenberg, and A. Levy, in prep.). The polypeptides predicted from the nucleotide sequences of \textit{RPL16A} and \textit{RPL16B} are nearly identical, differing only at their third amino acid residues, threonine and alanine, respectively (Leer et al. 1984; Teem et al. 1984; J.L. Woolford, R. Last, M. Rotenberg, and A. Levy, in prep.). In this report we describe the use of mutant \textit{S. cerevisiae} strains lacking functional copies of one or the other or both of the \textit{RPL16} genes to study the expression and function of ribosomal protein L16.

**Results**

**Disruption of the \textit{RPL16A} and \textit{RPL16B} genes**

Deletion–insertion mutations of \textit{RPL16A} or \textit{RPL16B} were constructed in diploids by the one-step gene replacement method of Rothstein (1983). The \textit{RPL16A} gene was disrupted by inserting the yeast \textit{URA3} gene.

![Figure 1](image-url)  
**Figure 1.** Replacement of the \textit{RPL16A} gene with an \textit{rpl16a :: URA3} disruption allele. (A) Construction of the \textit{rpl16a :: URA3} deletion–insertion null allele. A synthetic \textit{XhoI} site was inserted into a plasmid containing the 5' end of \textit{RPL16A} to create a 2-bp deletion plus \textit{XhoI} linker insertion within codon 30 of \textit{RPL16A}. The yeast \textit{URA3} gene was inserted into that \textit{XhoI} site to produce plasmid \textit{pI16aAURA}. A \textit{BamHI} fragment containing the \textit{rpl16a :: URA3} allele was isolated from \textit{pI16aAURA} and used to transform yeast diploid JW100 such that one wild-type allele of \textit{RPL16A} was replaced by the deletion–insertion allele. The sizes of genomic \textit{EcoRI} restriction fragments from the \textit{RPL16A} wild-type allele and predicted fragments from the null allele are shown. Restriction enzyme sites: [Bg] \textit{BglII}; [X] \textit{XhoI}; [R] \textit{EcoRI}; [B] \textit{BamHI}; [H] \textit{HaeIII}. (B) Gel transfer hybridization analysis of yeast DNA from strains transformed with the \textit{rpl16a :: URA3} construct. Total DNA was purified from a diploid transformant [lane 1], from four progeny spore colonies from each of two tetrads derived from the transformed diploid [lanes 2–9], and from the untransformed parental diploid JW100 [lane 10]. The DNA was digested with \textit{EcoRI}, subjected to electrophoresis on a 1% agarose gel, transferred to nitrocellulose, and probed with a \textsuperscript{32}P-labeled 1.2-kb \textit{BamHI–BglII} fragment containing \textit{RPL16A}. This probe hybridizes to both a 3.0-kb and a 4.4-kb \textit{EcoRI} fragment, each containing part of \textit{RPL16A} as well as to an 11.0-kb \textit{RPL16B EcoRI} fragment, as shown. The 4.4-kb fragment is converted to a 5.6-kb \textit{EcoRI} fragment in the disruption allele. The \textit{Ura} phenotype of each diploid strain and haploid spore clone is shown above the lanes.
between the endpoints of a deletion from nucleotide +87 to nucleotide +89, i.e., within codon 30 of the \textit{RPL16A} protein-coding region [Fig. 1A]. An \textit{rpl16b} :: \textit{LEU2} disruption was constructed by deleting the sequences from nucleotide +259 to +443 of \textit{RPL16B} (codons 87–148) and replacing them with the yeast \textit{LEU2} gene [Fig. 2A]. Linear DNA fragments containing each of these \textit{RPL16} deletion–insertion mutations were used to separately transform the diploid strain JW100, selecting for \textit{URA3} or \textit{LEU2}.

The Ura\textsuperscript{+} or Leu\textsuperscript{+} diploid transformants were sporulated, and tetrads were analyzed. Most tetrads yielded four viable spores in which all markers displayed the expected segregation patterns, including 2 : 2 segregation for either the \textit{URA3} or \textit{LEU2} disruption markers. Replacement of the wild-type \textit{RPL16} alleles with the \textit{rpl16a} :: \textit{URA3} or \textit{rpl16b} :: \textit{LEU2} alleles was confirmed by gel transfer hybridization experiments [Figs. 1B and 2B]. In each case, DNA from the untransformed diploid, transformed diploid, and four haploid spore clones from each of two tetrads was analyzed. Ura\textsuperscript{+} cosegregated with replacement of the 4.4-kb \textit{RPL16A} EcoRI fragment by a 5.6-kb EcoRI fragment predicted to contain \textit{rpl16a} :: \textit{URA3} [Fig. 1B]. Similarly, Leu\textsuperscript{+} cosegregated with the 8.0-kb HindIII fragment containing \textit{rpl16b} :: \textit{LEU2} (Fig. 2B). The transformed diploids were

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**Figure 2.** Replacement of the \textit{RPL16B} gene with an \textit{rpl16b} :: \textit{LEU2} disruption allele. (A) Construction of the \textit{rpl16b} :: \textit{LEU2} deletion–insertion null allele. A 185-bp deletion within the coding region of \textit{RPL16B} was constructed using BAL31 exonuclease. Synthetic \textit{XhoI} linkers were added to the deletion endpoints, and the yeast \textit{LEU2} gene was inserted into the \textit{XhoI} site to create plasmid \textit{pl16b\text{\textregistered}LEU}. A linear HindIII-BamHI fragment containing the \textit{rpl16b} :: \textit{LEU2} allele was transformed into yeast diploid JW100 such that one wild-type allele of \textit{RPL16B} was replaced with \textit{rpl16b} :: \textit{LEU2}. The sizes of genomic HindIII fragments from the \textit{RPL16B} wild-type and mutant alleles are shown. Restriction enzyme sites: (H) HindIII; (X) \textit{XhoI}; (B) BamHI; (K) KpnI. (B) Gel transfer hybridization analysis of yeast DNA from strains transformed with the \textit{rpl16b} :: \textit{LEU2} construct. Total DNA was purified from four progeny spore colonies from each of two tetrads derived from the transformed diploid (lanes 1–8), from the transformed diploid (lane 9), and from the untransformed parental diploid JW100 (lane 10). The DNA was digested with HindIII, subjected to electrophoresis on a 1% agarose gel, transferred to nitrocellulose, and probed with a \textit{32P}-labeled 3.0-kb BamHI–HindIII fragment containing \textit{RPL16B}. This fragment hybridizes to a 6.0-kb HindIII fragment containing \textit{RPL16B} and to an 11.0-kb \textit{RPL16A} HindIII fragment (visible only in the original autoradiogram). The 6.0-kb \textit{RPL16B} fragment is converted to an 8.0-kb HindIII fragment as a result of the deletion–insertion mutation. The Leu phenotype of each strain is shown above the lanes.
heterozygous for the expected \textit{RPL16} restriction fragments, whereas the untransformed diploids and Leu- Ura- haploids contained only wild-type \textit{RPL16} fragments. From these results, we conclude that neither \textit{Ura-} haploids contained only wild-type fragments, whereas the untransformed diploids and Leu- \textit{rpll6a :: URA3} \textit{rpll6b :: LEU2} \textit{haploid} spore clones grew with doubling times identical \textit{min}. The \textit{rpll6b :: LEU2} \textit{haploid} cells grew more slowly than \textit{rpll6a} or wild-type cells in defined synthetic medium as well. The same results were obtained when the \textit{rpll6a :: URA3} and \textit{rpll6b :: LEU2} \textit{deletion-insertion alleles were introduced independently by direct transformation into either of two different haploid strains, JL8 (Fig. 3) and SC252. The phenotype of strains disrupted for \textit{RPL16A} \textit{rpll6a :: URA3} and \textit{rpll6b :: TRP1} and for \textit{RPL16B} \textit{rpll6b :: LEU2} and \textit{rpll6b :: URA3} was independent of the disruption marker used.

\textit{L16 is an essential yeast ribosomal protein}

To determine whether \textit{L16} is an essential protein, we constructed a diploid strain (JW450) heterozygous for disruptions in both \textit{RPL16} loci \textit{rpll6a :: URA3/RPL16A rpll6b :: LEU2/RPL16B}. Following sporulation of JW450, asci were dissected and spore clones were analyzed. Gel transfer hybridization analysis verified that both the parental diploid and the haploid spore clone DNA contained the expected \textit{RPL16} alleles. The observed ratio of parental ditype : nonparental ditype : tetratype tetrads of 1 : 1 : 4 was consistent with that expected for random segregation of two unlinked genes. This agrees with preliminary mapping data that indicate \textit{RPL16A} is on either chromosome VII or XV and that \textit{RPL16B} is on chromosome XVI [M. Moritz and M. Rotenberg, unpubl.]. All \textit{rpll6a :: URA3} \textit{RPL16B} \textit{(Ura"-Leu+" haploid} spore clones grew as fast as wild-type \textit{(Ura"-Leu+")} spore clones, whereas the slow-growth phenotype cosegregated with \textit{RPL16A} \textit{rpll6b :: LEU2} \textit{(Ura"-Leu+")} cells. No \textit{Ura"-Leu+" \textit{rpll6a :: URA3} \textit{rpll6b :: LEU2)} \textit{spore} clones were recovered in cases where this genotype was predicted from the segregation pattern of the other \textit{RPL16} alleles in the same ascus. This latter result is explained most simply if \textit{L16} is an essential ribosomal protein in yeast. Essentially the same results were obtained upon sporulation of diploid MM1402, which is heterozygous for the \textit{rpll6a :: TRP1} and \textit{rpll6b :: LEU2} mutations. The lethality was a direct consequence of the absence of \textit{L16} function, because viable spore clones containing chromosomal disruptions of \textit{RPL16A} and \textit{RPL16B} were obtained when either \textit{RPL16A} or \textit{RPL16B} was provided on a complementing plasmid. The fact that null mutations in either \textit{RPL16} gene alone are not lethal indicates that both \textit{RPL16A} and \textit{RPL16B} are expressed and complement each other.

\textit{The \textit{RPL16B} gene is expressed at twice the level of \textit{RPL16A}}

The more deleterious effect of the \textit{rpll6b} mutations relative to the \textit{rpll6a} mutations might be due to higher expression of the \textit{RPL16B} gene in wild-type cells or to some specialized role performed by the \textit{L16b} protein for which the \textit{L16a} protein cannot substitute. We measured the relative steady-state levels of \textit{RPL16A} and \textit{RPL16B} mRNA in wild-type haploid strains and in those containing a disruption allele of \textit{RPL16A} or \textit{RPL16B}. A 0.3-\textit{kb} HindIII fragment from the protein-coding region of \textit{RPL16A} was used as a probe for gel transfer hybridization analysis of RNA extracted from these strains. This \textit{RPL16A} fragment is 99% homologous to the nucleotide sequence in the same region of \textit{RPL16B} [J.L. Woolford, R. Last, M. Rotenberg, and A. Levy, in prep.] and hybridizes with equal efficiency to either \textit{RPL16} mRNA. The amount of \textit{RPL16} mRNA was quantitated by densitometry of several exposures of the hybridization filters and, in each case, was normalized to the amount of mRNA homologous to plasmid pY11-10, which carries a gene encoding an abundant yeast transcript. Data obtained by comparing the levels of \textit{RPL16A} mRNA or \textit{RPL16B} mRNA to each other in the disruption strains [Fig. 4, lanes 2 and 3] or to total \textit{RPL16} mRNA in wild-type cells [Fig. 4] suggest that \textit{RPL16B} is expressed at approximately twice the level of \textit{RPL16A}. This interpretation is based on the assumption...
The abundance of $RPL16$ mRNA is decreased in haploids containing a null allele of $RPL16A$ or $RPL16B$. Strains JL8, JL8Δ16a and JL8Δ16b were grown to a density of $2 \times 10^7$ to $4 \times 10^7$ cells/ml in defined synthetic medium. Total yeast RNA was extracted, and about 15 μg samples of each were fractionated on a 1.2% agarose-formaldehyde gel. RNAs were blotted to nitrocellulose and hybridized with both a $^{32}$P-labeled 0.3-kb $RPL16A$ HinfI fragment and plasmid pYll-10. The $RPL16A$ fragment is from a portion of the $RPL16A$-coding region that is 99% identical in nucleotide sequence to the equivalent region of $RPL16B$ and hybridizes with equal efficiency to both species of $RPL16$ transcript. The 11-10 RNA signal (Woolford and Rosbash 1979) indicates the relative amounts of total RNA loaded in each lane. The amount of $RPL16$ mRNA relative to 11-10 RNA in each sample was determined by densitometry of several exposures of each hybridization filter. (Lane 1) JL8 (wild type); (lane 2) JL8Δ16a ($rpl16a :: URA3 RPL16B$); (lane 3) JL8Δ16b ($RPL16A rpl16b :: LEU2$). That there is no dosage compensation affecting either the rate of transcription or the stability of $RPL16$ mRNA in strains containing disruptions of $RPL16A$ or $RPL16B$. This assumption was proven to be correct, using hybridization probes specific for each $RPL16$ mRNA (see below).

No dosage compensation of $RPL16$ transcripts occurs in the rpl16 mutants

We constructed two $RPL16A$ and $RPL16B$ gene-specific oligonucleotide probes complementary to a highly divergent region of the respective 3’-transcribed non-coding regions. These probes differ in 15 out of 19 nucleotides and hybridized to only their complementary RNAs. No $RPL16$ mRNA was detected when the $RPL16A$ oligonucleotide probe was hybridized to blots of RNA from the $rpl16a$ mutant [Fig. 5, lane 2] or when the $RPL16B$-specific oligonucleotide was hybridized to RNA from the $rpl16b$ null mutant [Fig. 5, lane 6]. Furthermore, no transcripts differing in electrophoretic mobility from the $RPL16$ mRNA were detected in blots of RNA from wild-type cells or $rpl16$ null mutants using either the gene-specific probes (Fig. 5) or the $RPL16A$ HinfI fragment homologous to both $RPL16$ mRNAs (Fig. 4).

These probes were employed in a dot blot hybridization assay to measure the relative levels of $RPL16A$ and $RPL16B$ mRNAs in wild-type haploid cells. The $RPL16A$-specific probe hybridized to complementary denatured DNA with approximately twice the efficiency of the $RPL16B$-specific probe (Fig. 6), presumably because of its significantly greater G + C content. When the same DNA samples were then washed free of these probes and subsequently hybridized to the yeast LEU2 gene, the signal intensities for the two sets of DNA samples were much more similar (Fig. 6). This confirmed that the different relative signal intensities observed for the two $RPL16$ oligonucleotide probes were actually due to the differing hybridization efficiencies of these probes. When used to probe a dilution series of RNA extracted from wild-type strain JL8, the two $RPL16$ oligonucleotide probes yielded hybridization signals of approximately equal intensity (Fig. 6). Taking into ac-
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PROBE: RPL16A

Figure 6. Quantitation of RPL16A and RPL16B mRNA levels by dot blot hybridization. The indicated quantities of plasmids YEp13L16A and YEp13L16B containing the RPL16A and RPL16B genes, respectively, and the LEU2 gene, and total RNA samples prepared from yeast strain JL8 were denatured and transferred to nitrocellulose sheets. DNA standards were included for determination of the relative hybridization efficiencies of 32P-labeled probes. Oligonucleotides specific for RPL16A and RPL16B and a 32P-labeled 2.2-kb XhoI-SalI fragment containing the yeast LEU2 gene were hybridized to the filters.

count the differing hybridization efficiencies of the two probes, we conclude that there is approximately twice as much RPL16B mRNA as RPL16A mRNA in wild-type cells. This is consistent with our determination that there are one third and two thirds of wild-type amounts of RPL16 mRNA in rpl16a and rpl16b null mutants, respectively (Fig. 4). Therefore, the synthesis and stability of mRNA encoded by either RPL16 gene is apparently not affected by a null mutation in the other.

There is no functional distinction between the L16a and L16b proteins

We tested whether the difference in phenotype between rpl16a and rpl16b null mutants resulted from functional distinctions between the L16a and L16b proteins by asking whether an extra copy of RPL16A could suppress the growth deficiency produced by the rpl16b null mutation. RPL16A, cloned in the yeast integrating plasmid YIp5 (YIpL16A), was directed to integrate into RPL16A rpl16b :: LEU2 yeast at the ura3-52 locus by linearizing the plasmid within URA3. DNA gel blot hybridization analysis of uracil prototrophic transformants confirmed that a single copy was integrated at ura3-52 and that the RPL16A and rpl16b :: LEU2 alleles were also present [data not shown]. One extra copy of RPL16A fully restored a wild-type growth rate to the rpl16b :: LEU2 strain [Ab−, Fig. 3]. Unless RPL16A is overexpressed at the ura3-52 locus due to a position effect, a total of two normally expressed copies of RPL16A are fully capable of replacing the function of RPL16B. Not surprisingly, a wild-type growth rate was also restored to the rpl16b :: LEU2 strain by integration of one copy of RPL16B at ura3-52 [Ab−, Fig. 3]. The same results were obtained when extra copies of RPL16A or RPL16B were introduced on either the low copy centromere plasmid YCP50 or the high copy 2μm plasmid YEp24 [data not shown]. We conclude that either of the two L16 proteins can perform the essential functions in vivo and that the rpl16b :: LEU2 mutation is recessive, encoding no observably deleterious gene product.

rpl16b mutants have decreased pools of free 60S ribosomal subunits and form half-mer polyribosomes

We examined the effects of disruption of the RPL16 genes and the presumed reduction of L16 protein levels upon ribosome biogenesis and function by comparing the levels of free ribosomal subunits, 80S monosomes, and polyribosomes in wild-type cells and rpl16a and rpl16b null mutants. Extracts of these cells were fractionated by sucrose velocity gradient centrifugation. A typical absorbance profile for a wild-type strain is shown in Figure 7A. There is a 2 : 1 ratio of absorbance for the peak of free 60S ribosomal subunits compared to the peak of free 40S subunits. Because the mass ratio of the two subunit particles is about 2 : 1, the absorbance ratio indicates that free 60S and 40S subunits are equimolar in wild-type cells. These wild-type cells contain an excess of 80S monosomes compared to free subunits. Distinct polyribosome peaks containing two to nine ribosomes can be observed. In contrast, the rpl16b cells contain a fourfold excess of free 40S subunits relative to 60S subunits, fewer 80S monosomes relative to free subunits,
and fewer larger polyribosomes than wild-type cells do [Fig. 7B]. Most strikingly, there are discrete peaks sedimenting at positions in the gradient intermediate to those polyribosome peaks containing integral numbers of ribosomes [Fig. 7B, vertical arrows]. These intermediate peaks are reminiscent of the half-mer peaks observed in polyribosome profiles of yeast treated with low

concentrations (10 μg/ml) of cycloheximide. Under these conditions, the initiation step of yeast protein synthesis is inhibited [Lin et al. 1966; Baliga et al. 1969; Cooper and Bossinger 1976; Helser et al. 1981]. The rpl16a mutant that is depleted of one third of RPL16 mRNA has a polyribosome profile intermediate to that of wild-type and rpl16b cells [Fig. 7C], the latter of which are depleted of two thirds of RPL16 mRNA. There are roughly twice as many free 40S subunits as free 60S subunits in the rpl16a cells. Although the half-mer peaks are clearly visible in extracts of rpl16a cells, they are less pronounced than in rpl16b extracts. These biochemical defects in rpl16 mutants are direct results of the RPL16 disruption, because introduction of a single extra wild-type copy of either RPL16A or RPL16B restores the wild-type profile to rpl16 mutant cells [data not shown].

Helser et al. (1981) showed that yeast half-mers consist of polyribosomes containing mRNA associated with one or more 80S ribosomes and a 43S preinitiation complex (derived from the 40S subunit and associated translation initiation factors). We suggest that in rpl16 mutants deficient in L16 protein, 60S subunits fail to accumulate in quantities equimolar to 40S subunits. The excess 40S subunits may form 43S preinitiation complexes and attach to mRNAs to initiate protein synthesis but may then remain at the translation initiation site in the absence of a free 60S subunit with which to associate. In that case, the intermediate peaks containing putative half-mer polyribosomes would contain an excess of 40S subunits compared to 60S subunits. We have analyzed the ribosomal RNA present in each fraction of the polyribosome gradients from extracts of rpl16b cells to determine whether the putative half-mer fractions do indeed contain an excess of 40S subunits. The rRNA was quantitated by gel blot hybridization analysis using a DNA probe containing a portion of the yeast rDNA repeat that is complementary to 18S and 25S rRNA. As shown in Figure 8, fractions 8–11 contain predominantly 18S rRNA and fractions 13 and 14 contain mostly 25S rRNA, consistent with their containing free 40S and 60S subunits, respectively. Equimolar quantities of 18S and 25S rRNA can be seen in fractions 15 and 16 containing 80S monosomes (Fig. 8). Due to the difference in size of these rRNAs, the hybridization signal for 25S rRNA exceeds that for 18S rRNA when equimolar amounts are present and probed with comple-

Figure 7. Decrease in free 60S ribosomal subunit pool and accumulation of half-mer polyribosomes in rpl16 mutant strains. [A] MM1401 [wild type], (B) MM1405 [RPL16A rpl16b :: LEU2], (C) MM1404 [rpl16a :: TRP1 RPL16B] cells [200 ml] were grown in defined synthetic medium to 1 x 10^8 to 2 x 10^8 cells/ml. Cells were harvested, and 50 A_260 units of lysate from each culture were analyzed on 7–47% sucrose gradients centrifuged for 4 hr at 27,000 rpm. The positions of 40S and 60S ribosomal subunits, 80S monosomes, and polyribosomes containing 2–10 ribosomes are indicated above the absorbance profiles. The vertical arrows indicate intermediate-sized polyribosomes, or half-mers.
Formation of half-mer polysomes in rpl16 yeast

Figure 8. Decreased ratio of 25S rRNA to 18S rRNA in half-mer polyribosome fractions isolated from rpl16b mutants. (Top) Polyribosome profile of an rpl16b mutant. Yeast strain MM1405 [RPL16A rpl16b :: LEU2] was grown to 1 × 10⁸ to 2 × 10⁸ cells/ml and harvested, and a lysate was prepared and subjected to sedimentation on a 7–47% sucrose gradient. The 40S and 60S ribosomal subunits, 80S monosomes, and polyribosomes associated with two to seven ribosomes are labeled above the absorbance profile of the gradient. Half-mer polyribosomes are denoted by vertical arrows. (Bottom) RNA gel blot hybridization analysis of 18S and 25S rRNAs in fractions of the rpl16b mutant polyribosome gradient. Fractions of 1.2 ml each were collected from the gradient as indicated. Each fraction was concentrated by ethanol precipitation. RNA was isolated and subjected to electrophoresis, transferred to nitrocellulose, and hybridized with 32P-labeled DNA containing the yeast rRNA repeat unit to detect 18S and 25S rRNAs. Fractions from the polyribosome gradient are indicated above each gel lane. Differences in signal strengths among different fractions presumably reflect different recovery and loading of total RNA in each gel lane. Presumably there was no differential recovery of 18S vs. 25S rRNA.

mentary DNAs of equal specific activity. The ratio of 18S rRNA to 25S rRNA is clearly increased in fraction 17 [containing the putative '1 1/2-mer'], compared to fractions 15 and 16 (containing monosomes). These rRNA ratios were quantitated by densitometric scanning of several exposures of the autoradiograms of the RNA blots [data not shown]. The putative 1 1/2-mer peak contained an approximately 1.5-fold higher ratio of 18S rRNA to 25S rRNA than the fractions containing monosomes or disomes. Likewise, we predict that the 2 1/2-mer should have a 3:2 ratio of 40S to 60S subunits, unless multiple 40S subunits accumulate on these polysomes. We have been unable to resolve 2 1/2-mers from disomes or trisomes sufficiently well to test this hypothesis.

To demonstrate that the deficiency of 60S subunits in rpl16 strains is not a result of the slow growth of these strains, we examined the polyribosome profile of wild-type cells constrained by a poor carbon source to grow with a doubling time equivalent to that of the rpl16b strain. The polyribosome profiles of these slow-growing wild-type cells were identical to those obtained when wild-type cells were grown in rich medium [data not shown].

Discussion

We have constructed null mutations in the yeast RPL16A and RPL16B genes encoding ribosomal protein L16 to study the expression and function of this protein in ribosome biogenesis. Both copies of the RPL16 genes are expressed and encode proteins with indistinguishable functions. Disruption of RPL16B results in a significant increase in the doubling time of haploid strains, whereas inactivation of RPL16A has no measurable effect upon growth rate. This is consistent with our finding that the steady-state level of RPL16B mRNA is approximately twice that of RPL16A. We do not know whether the approximately twofold difference in abundance of RPL16A and RPL16B mRNAs results from differential rates of transcription, differences in mRNA stability, or both. However, it is not due to differential mRNA splicing ef-
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ficiencies, as the two RPL16 genes are not interrupted by intervening sequences (J.L. Woolford, R.L. Last, M.O. Rotenberg, and A. Levy, in prep.). Although the arrangement of the tripartite consensus promoter motifs common to yeast ribosomal protein genes (Rotenberg and Woolford 1986; Larkin et al. 1987) differs between the two RPL16 genes (see Fig. 7; Rotenberg and Woolford 1986), we have not been able to correlate these specific arrangements with levels of transcription of each gene. It seems unlikely that the RPL16A and RPL16B mRNA differ significantly in their translational efficiency, given our observation that cells containing two copies of RPL16A and the rpl16b null allele grow at the same rate as cells containing one copy of RPL16B and an rpl16a null allele.

The growth rates and molecular phenotypes of the rpl16 mutants suggest that there is no regulatory response to a decrease in RPL16 gene dosage manifest at the level of increased expression of the remaining RPL16 gene. There is no dosage compensation of either RPL16A or RPL16B mRNA abundance in cells containing a null allele of the other gene. Because rpl16b mutants containing one-third the wild-type level of RPL16B mRNA grow more slowly than wild-type cells, we infer that the stability or translational efficiency of RPL16 mRNA is not enhanced significantly in the rpl16 mutant cells.

The failure to recover haploid spore clones containing null mutations in both RPL16A and RPL16B indicates that L16 is essential for spore germination. Recent experiments using a strain conditional for synthesis of L16 (J.R. Thompson, J.C. Larkin, M.O. Rotenberg, and J.L. Woolford, in prep.). This is in marked contrast to the observation, that the absence of any one of at least 16 different ribosomal proteins from Escherichia coli is not a lethal event (reviewed in Dabbs 1985).

Why are there two functional copies of most yeast ribosomal protein genes? Although L16a and L16b differ by one amino acid, either protein is sufficient for wild-type growth when supplied in adequate amounts. Cells containing two thirds of wild-type levels of L16 protein (provided by one RPL16B gene or two RPL16A genes) grow at or near wild-type rates. Cells that contain one third of wild-type levels do not; therefore, there must be a minimum amount of L16 required that is somewhere between one third and two thirds of that which normally accumulates in wild-type cells. We cannot rule out the possibility that one form of L16 serves a specific function under certain physiological conditions not yet tested. However, the existence of functionally distinct subclasses of ribosomes derived from specific combinations of ribosomal proteins contributed by one or the other of each of the duplicate protein genes seems unlikely. Nevertheless, there is at least one precedent in Plasmodium for stage-specific ribosomes containing structurally distinct rRNAs (Gunderson et al. 1987).

We have shown that disruption of either RPL16 gene leads to excess 40S ribosomal subunits relative to 60S subunits. Ribosomal protein L16 must be necessary for the assembly of stable 60S subunits. In the absence of sufficient L16 protein with which to assemble, other 60S components may be synthesized but are not assembled and are then degraded. Under these conditions, 40S particles do accumulate and are apparently functional, as judged by binding to mRNA. Such turnover of excess molecules produced during ribosome biogenesis also occurs under several other circumstances. When the synthesis of ribosomal proteins is blocked, rRNA is transcribed but is not processed and is instead degraded (Shulman and Warner 1978; Stoyanova and Hadjiolov 1979). When rRNA synthesis or processing is blocked, ribosomal proteins are synthesized but degraded (Craig and Perry 1971; Gorenstein and Warner 1977; Tsurugi and Ogata 1977, Warner 1977; Jacobs et al. 1985; Pierandrei-Amaldi et al. 1985). Several yeast ribosomal proteins have been shown to be degraded when synthesized in excess of other ribosomal components (Abovich et al. 1985; Warner et al. 1985; El Baradi et al. 1986; Y.F. Tsay, J.R. Thompson, J.C. Larkin, M.O. Rotenberg, and J.L. Woolford, in prep.). We have also found that depletion of a 40S ribosomal subunit protein, rp59, results in a decrease in the pool of 40S subunits and accumulation of extra 60S subunits (A.G. Paulovich, J.C. Larkin, J.G. Anthony, E.R. Reynolds, and J.L. Woolford, in prep.). Similar results were obtained for rp51, another 40S subunit protein (Abovich et al. 1985). Accumulation of the pools of either 40S or 60S ribosomal subunits is apparently uncoupled.

Initiation of protein synthesis is slowed in rpl16 mutants as a consequence of the deficit of functional 60S ribosomal subunits relative to 40S subunits. Half-mer polysomes accumulate in both rpl16a and rpl16b mutants, but to a greater extent in the latter. The average number of ribosomes associated with mRNAs is less in the rpl16b mutants than in wild-type strains. Presumably, this difference in the polysome size distribution is a result of the decreased rate of initiation of protein synthesis in the rpl16b mutants and results in the slower rate of growth of rpl16b strains than wild-type strains. The polysome size distribution and growth rate of rpl16a mutants are not distinguishable from those of wild-type cells, even though RPL16 mRNA and 60S subunits are depleted by one-third and half-mer polysomes accumulate in rpl16a cells. This suggests that wild-type yeast contains an excess of both RPL16 mRNA and functional 60S subunits under the growth conditions tested.

Materials and methods

Nomenclature

The duplicated yeast genes encoding the 60S ribosomal subunit protein L16 are designated RPL16A and RPL16B, based on standard nomenclature of yeast ribosomal proteins (Warner 1982). We previously denoted this protein RP39 and its genes RP39A and RP39B (Woolford et al. 1979; Teem et al. 1984; Rotenberg and Woolford 1986).
Plasmids, strains and media

Plasmid pY11-40HB contains a 3.0-kb yeast genomic fragment that includes the intact RPL16B gene, cloned between the HindIII and BamHI sites of plasmid pBR322. pBR1078B is a derivative of pBR322 that contains the 1.2-kb HaeIII fragment extending from 1.0 kb upstream of RPL16A to nucleotide +148 within the coding region of RPL16A. BamHI linkers were added to this HaeIII fragment, which was then inserted into the BamHI site of pBR322 [Rotenberg and Woolford 1986]. Yeast strains used in this study are listed in Table 1. Growth of S. cerevisiae was at 30°C in YESD [1% yeast extract, 2% peptone, 2% dextrose], YEPG (1% yeast extract, 2% peptone, 3% glycerol), or defined synthetic medium with 2% dextrose as a carbon source [Jones and Lam 1973; Mackay and Manney 1974]. Yeast strains were constructed by standard genetic manipulations [Mortimer and Hawthorne 1969]. Matings between cells of the same mating-type (strains JLSΔ16a and JLSΔ16b) were obtained by selection, as described previously [MacKay and Manney 1974; Strathern et al. 1981].

Materials and enzymes

Two oligonucleotides (19-mers), 5’ CACTGACTATACCGA-GACC 3’ and 5’ ATTTCTTCACAAACTAATT 3’, complementary to sequences within the 3’-noncoding regions of the RPL16A and RPL16B transcripts, respectively, were obtained from the University of Pittsburgh DNA Synthesis Facility. T4 DNA ligase and [α-32P]dCTP were obtained from New England Nuclear Corp. [γ-32P]dCTP was purchased from ICN Biomedicals, Inc. Restriction endonucleases, nuclease BAL31, and calf alkaline phosphatase were purchased from Boehringer-Mannheim Biochemicals. BamHI- and XhoI-linker peptides were obtained from New England Biolabs. T4 polynucleotide kinase was a generous gift of Dr. William McClure, Carnegie Mellon University. E. coli DNA polymerase I holoenzyme and Klenow fragment were kindly provided by Dr. William E. Brown, Carnegie Mellon University. DEAE–nitrocellulose paper (NA45) was purchased from Schleicher & Schuell.

DNA and RNA isolation and manipulation

Plasmid DNA, DNA restriction fragments, yeast genomic DNA, and yeast RNA were all purified by methods described previously [Rotenberg and Woolford 1986]. Restriction endonuclease digestions, BAL31 digestions, conversion of cohesive ends to blunt ends, and DNA ligations were also all performed as described [Woolford et al. 1979; Last et al. 1984; Rotenberg and Woolford 1986].

Isolation and construction of plasmid DNAs

A yeast genomic Sau3A library, cloned into plasmid YEp24 in E. coli [Carlson and Botstein 1982] was screened for the presence of plasmids carrying RPL16A by the colony hybridization procedure of Gergen et al. [1979]. Seven plasmids were identified in which the cloned yeast DNAs contained overlapping restriction patterns that matched those previously identified for the RPL16A genomic region [Woolford et al. 1979]. Therefore, only one plasmid, YEp24Δ16A-7, was selected for further analysis. RPL16A occurs on a 2.3-kb BamHI fragment within the 10-kb genomic insert of this plasmid.

The RPL16A deletion–insertion plasmid p16aΔURA was constructed as follows. An XhoI linker insertion derivative of plasmid pBR1078B, pBR1078B+87, was identified in which the XhoI linker was inserted between the endpoints of a deletion from nucleotides +87 to +89 within codon 30 of RPL16A [Rotenberg and Woolford 1986]. XhoI linkers were added to the filled-in ends of the 1.1-kb HindIII URA3 fragment of plasmid YEp24. This URA3 fragment was inserted into the XhoI site of plasmid pBR1078B+87 such that URA3 is transcribed toward the 3’ end of RPL16A. The RPL16A deletion–insertion mutation pl16aΔ was derived from plasmid pBR1078B+87. This plasmid was digested with XhoI, and the sticky ends of the XhoI sites were filled in. The 1.45-kb TRP1 EcoRI fragment was purified from plasmid pTC3 [Shaw and Olson 1984], and the sticky ends were filled in. The TRP1 fragment was ligated into the flush-ended XhoI site of pBR1078B+87 to create plasmid p16aΔTRP, such that TRP1 is transcribed toward the 5’ end of RPL16A.

An RPL16B deletion–insertion allele was constructed by first linearizing plasmid pY11-40HB at the unique KpnI site near the 3’ end of RPL16B. The linearized DNA was then treated with nuclease BAL31, as described by Maniatis et al. [1982], and synthetic XhoI linkers were attached to the resulting deletion endpoints. The 2.2-kb SalI–XhoI fragment of plasmid YEp13 [Broach et al. 1979] containing LEU2 was converted to an XhoI fragment by filling in and linker ligation and was inserted into the XhoI site of one of the BAL31 deletion derivatives of pY11-40HB. One resulting plasmid, p16bΔLEU, was chosen for use in further studies. In this construct, the 185bp of RPL16B between nucleotide +259 and +443 [codons 87–148] are deleted, and LEU2 is inserted such that it is transcribed in the direction opposite that of RPL16B. A second RPL16B deletion–insertion allele was constructed by excising the LEU2 fragment from pl16bΔLEU and inserting in its place the 1.1-kb HindIII fragment encoding URA3. Prior to ligation, the XhoI ends of the opened plasmid and the HindIII ends of the URA3 fragment were rendered flush through treatment with DNA polymerase I holoenzyme. In the resulting plasmid, pl6bΔURA, both Hinc

### Table 1. Yeast strains used in this study

| Strain | Genotype |
|--------|----------|
| JW100  | MATα/MATα ade1+/+ gal4/+ GAL80f/+ leu2-3,112/leu2-3,112 ura3-52/ura3-52 |
| IL8    | MATα cry1 leu2-3,112 trp1 ura3-52 |
| IL8Δ16a| MATα cry1 leu2-3,112 trp1 ura3-52 rpl16a::URA3 |
| IL8Δ16b| MATα cry1 leu2-3,112 trp1 ura3-52 rpl16b::LEU2 |
| SC252  | MATα ade1 leu2-3,112 ura3-52 |
| JW450  | MATα cry1/cry1 leu2-3,112/leu2-3,112 trp1/trp1 ura3-52/ura3-52 rpl16a::URA3/rpl16b::LEU2 |
| MM1401 | MATα ade1-101/+ can1/+ his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ101/trp1-Δ101 ura3-52/ura3-52 rpl16a::TRP1/rpl16b::LEU2 |
| MM1402 | MATα ade1-101/+ can1/+ his3-Δ200/his3-Δ200 leu2-Δ1/leu2-Δ1 lys2-801/lys2-801 trp1-Δ101/trp1-Δ101 ura3-52/ura3-52 |
| MM1403 | MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ101 ura3-52 |
| MM1404 | MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ101 ura3-52 rpl16a::TRP1 |
| MM1405 | MATα ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ101 ura3-52 rpl16b::LEU2 |
dIII sites are regenerated. URA3 is oriented in pl16bURA such that its transcription is toward the 5' end of RPL16B. Plasmids YEp24L16A-5, YEp13L16A, and YCp50L16A were constructed by inserting the 2.3-kb RPL16A BamHI fragment of plasmid YEp24L16A-7 into the unique BamHI sites of vectors YEp24 (Botstein et al. 1979), YEp13, and YCp50 (Kuo and Campbell 1983), respectively. Plasmids YEp24L16B, YEp13L16B, and YCp50L16B were similarly constructed by inserting the 3.0-kb RPL16B HindIII—BamHI fragment of plasmid pY11-40HB into the BamHI sites of vectors YEp24, YEp13, and YCp50. The RPL16B HindIII—BamHI fragment was converted to a BamHI fragment by linker addition prior to ligation. Integrating plasmids YIpL16A and YIpL16B were produced by excising the 2.2-kb EcoRI fragment carrying the 2 μm origin of DNA replication from plasmids YEp24L16A-5 and YEp24L16B, respectively.

**Yeast transformation**

Yeast was transformed by the lithium acetate method of Ito et al. [1983], using 2–5 μg of episomal DNAs. Integration of plasmids YIpL16A and YIpL16B was directed to the ura3-52 locus of strain JL8All6b by the method of Orr-Weaver et al. [1983], using 2–5 μg of episomal DNAs. Integration of plasmids YEp13L16A and YIpL16B was linearized at the unique SmaI site within URA3 prior to transformation. One-step gene replacements were performed by the method of Rothstein [1983], using 5 μg of linear DNA isolated from plasmids p16adURA, p16aATRP, p16bALEU, or p16bAURA (e.g., see Figs. 1 and 2). The stability of all integrative transformants was assessed as described by Last et al. [1984].

**Preparation of DNA and RNA dot blot samples**

Samples (10 μg) of plasmids YEp13L16A and YEp13L16B were digested to completion with BamHI and extracted once with phenol : CHCl3 (1 : 1) and twice with CHCl3 : isomyl alcohol (24 : 1). The DNAs were precipitated with ethanol at −80°C, collected by centrifugation, washed twice with 70% ethanol, dried, and suspended in TE to a final concentration of 50 ng/μl. Multiple aliquots of these samples were diluted with sterile water to a volume of 8.3 μl each, heated to 100°C for 10 min, and quick-chilled in an ice-water bath. An equal volume of each reaction mixture to chromatography through a 0.9-ml Bio-Gel P2 spin column.

**Fractionation and characterization of polyribosomes**

Selected yeast strains were grown to a density of 1.0–1.5 × 108 cells/ml in 200 ml of YEPD or YEPG medium at 30°C. Polyribosomes were extracted and analyzed on 7–47% sucrose velocity gradients essentially as described by Baim et al. [1985] using an ISCO density gradient fractionator, model 640. The presence and amounts of rRNA molecules in fractions collected from the polyribosome gradients were determined as follows. Two volumes of ice-cold ethanol were added to each fraction, RNA was precipitated at −20°C overnight and pelleted by centrifugation for 5 min, then air-dried at room temperature, and subjected to autoradiography at −70°C, as described previously. If filters were reprobed, they were first soaked in boiling water for 10 min to remove old probes.

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