The yeast nuclear gene RML2, identified through genomic sequencing of Saccharomyces cerevisiae chromosome V, was shown to encode a mitochondrial homologue of the bacterial ribosomal protein L2. Immunoblot analysis showed that the mature Rml2p is a 37-kDa polypeptide component of the mitochondrial 54 S large ribosomal subunit. Null mutants of RML2 are respiratory-deficient and convert to [$rho^+$] or [$rho^-$] cytoplasmic petites, indicating that Rml2p is essential for mitochondrial translation. RML2 is regulated transcriptionally in response to carbon source and the accumulation of Rml2p is dependent on the presence of the 21 S large rRNA. Site-directed mutagenesis showed that a highly conserved 7-amino acid sequence (Val336 to Arg342) of Rml2p is essential for function. Substitution of Gln for His-343, the most highly conserved histidine in the L2 protein family, caused cold-sensitive respiratory growth but did not affect the assembly of 54 S ribosomal subunits. Mitochondrial protein synthesis was normal in the His343 to Gln (H343Q) mutant grown at the permissive temperature (30 °C) and was severely impaired after growth at the nonpermissive temperature (18 °C). His343 corresponds to His229 in Escherichia coli L2, which has been implicated in a direct involvement in peptidyl transferase activity. The conditional phenotype of the H343Q mutant indicates that His343 is not essential for peptidyl transferase activity in yeast mitochondria.

Members of the L2 family of ribosomal proteins are highly conserved and are found in eubacteria, archaeabacteria and in the cytoplasm and organelles of eukaryotes (1, 2). A large body of evidence from studies of bacterial ribosomes indicates that L2 is an important constituent of the peptidyl transferase center of the large ribosomal subunit. tRNA cross-linking data place L2 in the peptidyl transferase center (3, 4), and L2 is essential for in vitro reconstitution of peptidyl transferase activity (5, 6). Chemical modification of the histidine residues in the Escherichia coli and Bacillus stearothermophilus L2 proteins affected the assembly of the 50 S subunit and strongly inhibited peptidyl transferase activity (7, 8), and the imidazole functional group of histidine has been proposed to participate in peptidyl transferase through general acid-base catalysis analogous to the catalytic mechanism of serine proteases (9–11). Of the nine histidine residues in the E. coli L2 protein, His229 is the most highly conserved, occurring in the over 35 known L2 proteins from the eubacterial, archaeabacterial, and eukaryotic kingdoms. The lone exception is the possible substitution of glutamine at the position corresponding to His229 in the predicted L2 protein from Mycoplasma capricolum (12). It should be noted, however, that histidine is predicted at that position in Mycoplasma genitalium (13).

L2 is a primary RNA-binding protein in bacteria (14), and its binding site on 23 S rRNA has been characterized in detail by chemical and ribonuclease footprinting. The L2-binding site lies in domain IV of the 23 S rRNA, mainly on helix 66 (nucleotides 1792–1827) (15, 16), and there are several lines of evidence suggesting that domain IV is part of the peptidyl transferase region (17, 18). L2 has also been cross-linked by 2-iminothiolane to nucleotides 1819–1820 of the 23 S rRNA (19). The functional importance of the L2-binding region in the rRNA is suggested by the observation that a point mutation of U1696 to A in the yeast mitochondrial rRNA, which corresponds to U1796 of E. coli 23 S rRNA, caused cold-sensitive growth on nonfermentable carbon sources and reduced amounts of assembled large ribosomal subunits (20). There are no reports of E. coli mutants lacking L2.

Romero et al. (21) used in vitro mutagenesis to modify the extremely well conserved region between Gly221 and His331 of the E. coli L2 protein. The L2 variants included a single substitution of His229 to Gln, a 7-amino acid deletion ΔThr222 to Asp228, and the 2 amino acid deletions ΔGly231 to Thr222 and ΔAsp228 to His229. When these mutant proteins were overexpressed from a plasmid in the background of the normal chromosomal L2 gene, the cells could not grow at 37 °C, and sucrose gradient centrifugation of ribosomal particles from cells grown at 30 °C showed that all of the mutants accumulated abnormal 40 S particles in addition to the normal 50 S subunit. The 40 S particle isolated from the 7-aminoleucine deletion mutant contained the mutant L2 protein, completely lacked L16 and had reduced amounts of L28, L33, and L34. In in vitro assays, this particle did not associate with 30 S subunits and was inactive in poly-Phe synthesis. It was proposed that the region of L2 from Gly231 to His331 is required for the assembly of L16 into the 50 S subunit.

Recently, the His229 to Gln (H229Q) variant was used to replace wild-type L2 in the reconstitution of E. coli 50 S large subunit particles (22). The 50 S subunits reconstituted with H229Q-L2 appeared identical to subunits reconstituted with wild-type L2 with respect to overall protein composition, the interaction of L2 with 23 S rRNA and the ability to combine with 30 S subunits to form 70 S ribosomes. Significantly, however, the 50 S subunits containing H229Q-L2 were inactive in peptidyl transferase activity. These results support the possibility that His229 is an essential part of the peptidyl transferase catalytic center.

In this paper, we confirm that a yeast nuclear gene for an L2-like protein, designated RML2, encodes a component of the mitochondrial 54 S large ribosomal subunit. We also show by gene disruption analysis that Rml2p is an essential component of this function.
of the mitochondrial ribosome in vivo. Site-directed mutagenesis confirmed the functional importance of the conserved region corresponding to Thr222 to Asp228 in E. coli L2. We show, however, that the most highly conserved histidine in the L2 protein family is not required for peptidyl transferase activity in yeast mitochondria.

EXPERIMENTAL PROCEDURES

Plasmid Construction—Plasmids used in this study were constructed as follows. The 2.26-kb RML2 gene fragment amplified by PCR was cloned into pRS314 (23), a yeast centromere plasmid with the TRP1 gene. The 9.5-kb fragment encoding the COOH-terminal 378 amino acids of Rml2p was created by PCR-based oligonucleotide-directed mutagenesis. The same mutant DNA fragment corresponding to Thr222 to Asp228 in E. coli L2 was cloned into pRS314 (23), a yeast centromere plasmid with the TRP1 gene. The 9.5-kb fragment encoding the COOH-terminal 378 amino acids of Rml2p was created by PCR-based oligonucleotide-directed mutagenesis. The same mutant DNA fragment containing the LEU2 gene from the YEp24 plasmid was subcloned into the BglII site of pCP401. To express the Rml2 fusion protein in E. coli, the 1.1-kb fragment encoding the COOH-terminal 378 amino acids of Rml2p was cloned into pET-23a (Novagen), resulting in pCP405. pCP406 was generated by oligonucleotide-directed mutagenesis of pCP401 to change the codon for histidine at position 343 to a glutamine codon. pCP407 was created by PCR-based oligonucleotide-directed mutagenesis of pCP401 to change the codon for histidine at position 343 to an asparagine codon. The resultant plasmid pCP404 was transformed into the T76-2D strain with the 5.0-kb RML2 gene fragment amplified by PCR from genomic DNA of strain 22-2D. The amplified fragment was sequenced using oligonucleotides L2-5 and L2-3 to confirm the functional importance of the conserved histidine in the L2 protein family is not required for peptidyl transferase activity in yeast mitochondria.

Saccharomyces cerevisiae Strains—The yeast strains used in this study are listed in Table I. CPY401 was generated by transformation of the 22-2D strain with the 2.9-kb SalI-NheI fragment of pCP402 using the simplified lithium acetate transformation procedure described by Elble (24). CPY402 was obtained by transforming 22-2D with pCP404. The 1.0-kb fragment was used to express the Rml2 polypeptide in E. coli, and sporulation of CPY404 produced the haploid strain CPY407-SC. The same mutant DNA fragment was also cloned into the multicopy plasmids pJS92 (TRP1) and YEPT4 (URA3).

Synthetic Oligonucleotides Used in This Study—Oligonucleotides L2-5 (TGGTGCGACAATTTAGCATCAGT) and L2-3 (GCTAGCTACGATCAAGAGGAC) were used as primers for PCR amplification of the RML2 gene from the genomic DNA from 22-2D. The amplified 2.26-kb SalI-NheI fragment contained the 1182-bp RML2 I fragment of pCP401, which contains most of the RML2 open reading frame, with the 1.57-kb SalI-BamHI fragment of YEPT4 that contains URA3. pCP403 was constructed by inserting the 2.7-kb BamHI-BglII fragment of YEPT4 containing the LEU2 gene into the BglII site of pCP401. To express the Rml2 fusion protein in E. coli, the 1.1-kb fragment encoding the COOH-terminal 378 amino acids of Rml2p was cloned into pET-23a (Novagen), resulting in pCP405. pCP406 was generated by oligonucleotide-directed mutagenesis of pCP401 to change the codon for histidine at position 343 to a glutamine codon. pCP407 was created by PCR-based oligonucleotide-directed mutagenesis of pCP401 to change the codon for histidine at position 343 to an asparagine codon. The resultant plasmid pCP404 was transformed into the E. coli strain, BL21(DE3), pLyS8 (27) for protein expression. The overproduced Rml2 polypeptide was purified from inclusion bodies using HisTag® metal chelation chromatography (Novagen) according to the protocol provided by the manufacturer. The purified protein was used to immunize mice and hyperimmune ascites fluid was collected as described previously (28).

Analytical Methods—Total yeast genomic DNA and total RNA were isolated as described previously (29). RNA was fractionated by electrophoresis in a 1.1% agarose-formaldehyde gels as described (30). Southern and Northern blot analyses were performed using GeneScreen Plus membranes (DuPont) according to the manufacturer's instructions. The hybridization probes were [32P]-labeled as described (31). Previously described procedures were used for immunoblots analysis of ribosomal proteins in yeast subcellular fractions and in fractions from sucrose density gradients (29). Mitochondrial ribosomal subunits were analyzed by sucrose gradient centrifugation of mitochondrial lysates as described previously (32). Yeast mitochondrial translation were labeled in vivo with [35S]methionine as described previously (32) with modifications. Wild-type strain 22-2D and rml2-H343Q strain cells were harvested in Smax phase of the cell cycle as described (33). Cells were harvested and labeled with [35S]methionine for 1 h at 30 °C or 2 h at 18 °C. Radiolabeled mitochondrial proteins were resolved by electrophoresis at 4 °C in an 11% polyacrylamide gel containing SDS and visualized by exposing the dried gel to x-ray film.

RESULTS

Identification of the RML2 Gene—Sequencing of yeast chromosone V revealed an open reading frame for an L2-like protein (Swiss-Prot accession number P92611). This open reading frame was derived from the 36772-bp YCSYGP2 sequence of S. cerevisiae chromosome V (GenBank accession number L10830) and started at nucleotide 28420 and ended at nucleotide 30680 of the reverse complement sequence of YSCYGFP2. The gene encoding the potential yeast mitochondrial homologue of L2 was designated RML2. It encodes a 393-amino acid protein with a calculated pI of 11.50 and Mr of 43,755. This gene was amplified by PCR from genomic DNA of strains 22-2D and 22-2D using the L2-5 and L2-3 oligonucleotides. The amplified DNA fragment was cloned into the pRS314 vector. DNA sequencing of the PCR-generated RML2 gene confirmed that its sequence was identical to the GenBank YSCYGFP2 sequence. The codon usage of the RML2 gene was analyzed using the CODON PREFERENCE program of the GCG sequence analysis software package and its codon bias is similar to yeast proteins expressed to low levels, including several mitochondrial ribosomal proteins.
RML2 encodes a member of the L2 protein family that is most closely related to eubacterial L2 proteins. The predicted Rml2 protein has approximately 48% amino acid identity with the eubacterial L2 proteins from E. coli and B. stearothermophilus, and about 42% identity with chloroplast L2 proteins from tobacco and maize. The percentage of identical amino acids between Rml2p and archaebacterial and eukaryotic L2 proteins is much lower: 37 and 32% for the archaebacterial L2 protein from Methanococcus vannielii, respectively; and 30% for cytoplasmic L2 from rat and tobacco.

The sequence conservation of the Rml2 protein is very high compared with other yeast mitochondrial ribosomal proteins that are members of conserved protein families, such as the mitochondrial L27 and L16 homologues, Mrp7p (33) and Rnl16p (34), respectively.

Alignments with eubacterial L2 proteins show that Rml2p has a long NH2-terminal extension, part of which could be the mitochondrial targeting presequence. The sequence between the first 44 amino acids of the predicted protein contains five conserved histidine residues in the L2 protein family presequence. Histidine is found at this position in over 35 known L2 proteins. The lone exception is the presence of glutamine at this position in the L2 protein from Methanococcus vannielii, respectively; and 30% for cytoplasmic L2 from rat and tobacco.

Alignment experiments showed that the antibodies to Rml2p reacted specifically with a 37-kDa protein that was enriched in the mitochondrial fraction (data not shown). Furthermore, Rml2p cosedimented specifically with the 54 S large subunit in sucrose gradient centrifugation (data not shown). The predicted Rml2 polypeptide is 6 kDa larger than the size estimated from the electrophoretic mobility of the polypeptide detected by immunoblot analysis. This size discrepancy suggests that Rml2p is processed from a precursor with a relatively long mitochondrial targeting presequence. Although the amino terminus of the mature Rml2p has not been determined, it is noteworthy that the first 44 amino acids of the predicted protein contain five conserved histidine residues in the L2 protein family.

**Fig. 1.** Alignment of the most highly conserved region of representative L2 proteins. Representative L2 sequences were selected from eubacteria, archaebacteria, and organelar and cytoplasmic ribosomes in eukaryotes. Amino acid identities are shaded. The most highly conserved histidine residue of the L2 protein family is marked by an arrow. The sequences shown are: Yeast Mt, mitochondrial L2 protein from S. cerevisiae (GenBank accession number P32611); E. coli, L2 protein from E. coli (GenBank accession number P02387); Acant Mt, mitochondrial L2 protein from Acanthamoeba castellanii (GenBank accession number P46783); Yeast Cyt, cytoplasmic L2 protein from S. cerevisiae (GenBank accession number P05796); Pea Chl, chloroplast L2 protein from Pisum sativum (GenBank accession number P31169); Methan L2, protein from M. vannielii (GenBank accession number P21479).

| Yeast strain | Genotype | Source |
|--------------|----------|--------|
| 22–2D, [rho+] | MATa ura3–52 trp1 leu2–3,112 cyh2 can1 | G. R. Fink (Whitehead Institute, Cambridge, MA) |
| Cop161-U7, [rho+], [F11, rho-] and [rho+] derivatives | MATa ade2–101 lys2–801 ura3–52 | R. Butow (University of Texas Health Sciences Center at Dallas) |
| MH2, [rho+] | MATa ade2–101 ade2–101 his4–519 his4–519 leu2–117 leu2–117 trp1–101 trp1–101 ura3–52 ura3–52 gal2 gal2 | M. Fitzgerald-Hayes (University of Massachusetts, Amherst) |
| CPY401, [rho+] | MATa ade2–101 ade2–101 his4–519 his4–519 leu2–117 leu2–117 trp1–101 trp1–101 ura3–52 ura3–52 gal2 gal2 | This study |
| CPY402, [rho+] | MATa ade2–101 his4–519 leu2–117 trp1–101 ura3–52–trp1–101 ura3–52–gal2–112–cyh2–112–rml22–URA3/RML2 | This study |
| CPY403 | MATa ura3–52 trp1 leu2–3,112 cyh2 can1, pCP401 (CEN, TRP1, RML2) | This study |
| CPY404 | MATa ade2–101 ade2–101 his4–519 leu2–117 trp1–101 ura3–52 ura3–52 gal2 gal2 + rml22–URA3/RML2 cyh2–112–can1–112–cyh2–112–rml22–URA3/RML2 | This study |
| CPY405, [rho+] | MATa ade2–101 ade2–101 his4–519 leu2–117 trp1–101 ura3–52 ura3–52 gal2 gal2 + rml22–URA3/RML2 cyh2–112–can1–112–cyh2–112–rml22–URA3/RML2 | This study |
| CPY406 | MATa ura3–52 trp1 leu2–3,112 cyh2 can1, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY407, [rho+] | MATa ade2–101 ade2–101 his4–519 leu2–117 trp1–101 ura3–52 ura3–52 gal2 gal2 + rml22–URA3/RML2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY407–8C | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY411-U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY411–U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY415–U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY415–U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY417–U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
| CPY417–U | MATa leu2 ura3–52 trp1–101 rml2–LEU2, pCP404 (2 μm, TRP1, RML2) | This study |
positively charged residues and 11 residues with hydroxyl side chains, which are characteristics of cleavable mitochondrial targeting sequences (35). These results support the conclusion that RML2 encodes a protein component of the large subunit of the yeast mitochondrial ribosome.

**RML2 Encodes an Essential Yeast Mitochondrial Homologue of Bacterial Ribosomal Protein L2**—The rml2Δ::URA3 gene disruption allele was constructed to test whether Rml2p is an essential component of the mitochondrial ribosome. The rml2Δ::URA3 allele was generated by replacing the 1.0-kb SpeI-BglII fragment of pCP401, encoding the NH2-terminal 317 amino acids of Rml2p, with the URA3 gene. A linear DNA fragment containing the rml2Δ::URA3 allele was used to transform a wild-type diploid Ura+ strain MH2 (see Table I) to obtain the Ura+ strain CPY401 (36). CPY401 was sporulated and spores from 20 tetrads showed 2:2 segregation of Ura+ and Pet−, indicating that the replacement of RML2 in the chromosome with the gene disruption allele caused respiratory deficiency. The integration of the rml2Δ::URA3 disrupted gene at the RML2 chromosomal locus and the absence of Rml2p in the rml2Δ::URA3 mutants were confirmed by inspection of PCR-amplified DNA and immunoblot analysis of mitochondrial proteins from the spores of a representative tetrad (data not shown). To determine whether the disruption of RML2 caused conversion to [rho−] or [rho+] cytoplasmic petites, 16 representative rml2Δ::URA3 spores were crossed to RML2 [rho+] tester strains, either 22-2D MATa RML2 [rho+] or COP161-17 MATa RML2 [rho−], and the resulting diploids were checked for respiratory growth. All of the resulting diploids were respiratory deficient, indicating that the rml2Δ::URA3 spores had converted to [rho−] or [rho+] and were therefore unable to restore mitochondrial function in the rml2Δ::URA3/RML2 hetero-zygotes. This quantitative conversion to [rho−] or [rho+] indicates that RML2 encodes an essential mitochondrial homologue of ribosomal protein L2.

**Regulation of RML2**—The results of the Northern and Western blot analyses shown in Fig. 2 indicate that the steady-state levels of RML2 mRNA and Rml2p are regulated in response to carbon source, i.e., gene expression is derepressed in cells growing on nonfermentable carbon sources and repressed by growth on glucose. In addition, [rho+] cells lacking 21 S rRNA contained normal levels of the RML2 transcript but did not accumulate Rml2p. Since Rml2p accumulates at normal levels in [rho−] cells that lack the mitochondrial lon protease, unassembled Rml2p is apparently subject to rapid degradation.

**Mutagenesis of RML2**—Bacterial L2 has been implicated in several important ribosomal functions. Through the analysis of mutations generated by *in vitro* mutagenesis of the *E. coli* gene for L2, a region of the protein between Gly221 and His231 was found to be required for the *in vivo* assembly of L16 into large subunit particles (21). To determine whether the comparable region of Rml2p is involved in the assembly of the mitochondrial 54 S subunit, two targeted mutations were created in RML2. The first mutation, rml2Δ-H343-Q, caused the substitution of Gln for His343, which corresponds to His231 in *E. coli* L2 and is the most highly conserved histidine residue of the L2 family that has been implicated in peptidyl transferase activity (see Introduction). The second mutation, rml2Δ-D7, deleted the coding sequence for Val336 to Asp342. Romero et al. (21) found that overexpression of the corresponding variant of the *E. coli* L2 protein in the background of *wild-type* L2 caused a dominant negative phenotype; the cells could not grow at 37 °C and, at 30 °C, there was accumulation of abnormal 40 S ribosomal particles lacking L16.

![Regulation of RML2 and Rml2p in response to carbon source and mitochondrial genotype. The isogenic [rho−], [F11, rho−], and [rho+] derivatives of strain Cop161-U7 were grown in rich media with one of the following carbon sources: 5% glucose (Glu), 2% galactose (Gal), or 2% ethanol (GE). A, Northern blots. Total RNA was isolated from cells grown to 1.5 OD600.25 μg of total RNA was loaded in each lane and the blots were probed with the 32P-labeled 225-bp AccI-BglII fragment from pCP401, which contains the RML2 coding sequence. The blots were also probed with the yeast ACT1 probe (actin) as a control for equal loading of RNA. Only the relevant sections of the autoradiographs are shown. B, Immunoblots. Total cellular proteins were isolated from cells grown to 1.5 OD600.120 μg of total cellular protein was loaded in each lane and separated by SDS-polyacrylamide gel electrophoresis (12.5% acrylamide). The proteins were electroblotted onto a nitrocellulose filter and the blot was reacted with the mouse hyperimmunaseptic fluid against the Rml2 protein. The immune complexes were decorated with 125I-goat anti-mouse IgGs. Only the relevant sections of the autoradiographs are shown.](image-url)
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Fig. 3. Conditional respiratory deficiency of the rml2-H343-Q mutant and immunoblot analysis of representative ribosomal proteins in wild-type and mutant mitochondria. A, growth of RML2 and rml2-H343-Q cells on YPGE plates at 30, 37, and 18 °C. In the upper panel, YPGE plates were spotted with equal aliquots of wild-type and mutant cells and incubated at 30 °C (3 days), 37 °C (2 days), or 18 °C (5 days). The lower panel shows the size of the colonies formed by outgrowth of individual RML2 and rml2-H343-Q cells on YPGE plates at 18 °C. B, immunoblot of total mitochondrial proteins from RML2 and rml2-H343-Q cells grown at 18 °C in YPGal. Mitochondrial proteins (75 μg/lane) were reacted with antibodies to the following proteins from the yeast mitochondrial large ribosomal subunit: Mpr7p (33), Rml2p, Mrp20p (32), YmL9p (37) (C. Pan, unpublished data), and Rml16p (34).

COX1, and COX2, was impaired to a greater extent than the incorporation into the smaller polypeptides such as ATP6.

The 7-amino acid deletion allele rml2-Δ7 was expressed from either a centromere plasmid or a multicopy episomal plasmid, and, in each case, the mutants were incapable of respiratory growth at 18, 30, or 37 °C and converted to [rho−] or [rho+]. Thus, deletion of the Val336 to Asp342 (VAMNKCD) sequence functionally inactivates Rml2p.

DISCUSSION

In this paper we have confirmed that an open reading frame revealed in the sequence of yeast chromosome V encodes the yeast mitochondrial homologue of ribosomal protein L2 and have named the gene RML2 (Ribosomal Mitochondrial Large, following nomenclature suggested by B. Baum). Gene disruption analysis of RML2 showed that Rml2p is essential in vivo.

There are no known mutants of E. coli that lack L2, but in vitro mutagenesis has been used to generate L2 mutants that display trans-dominant phenotypes when the mutant proteins are overexpressed in the background of wild-type L2 (21). Two mutations have been particularly well characterized; a deletion of removing 7 amino acids from Thr222 to Asp228 and a substitution of Gln for His229. These mutations target the most highly conserved sequence among all L2 proteins. Since fragments of the B. stearothermophilus L2 protein containing amino acids 60–206 or 58–201 bind specifically to the 23 S rRNA, the 222–231 region is not part of the L2 RNA-binding domain (38). Cells overexpressing either of these mutant proteins could not grow at 37 °C and cells grown at 30 °C accumulated abnormal 40 S ribosomal particles in addition to normal 50 S subunits. The 40 S particles isolated from the 7-amino acid deletion mutant contained the mutant L2 protein, but had reduced amounts of L28, L33, and L34, and completely lacked wild-type L2 and L16. These particles also did not associate with 30 S subunits and were inactive in poly-Phe synthesis. It appears therefore that the Gly222 to His231 region of E. coli L2 is required for the in vivo assembly of L16 into the 50 S subunit.

The functional properties of the His229 to Gln mutant of E. coli L2 were further examined in in vitro reconstitution experiments by Cooperman et al. (22). Compared with subunits reconstituted with wild-type L2, the 50 S subunits reconstituted with mutant protein appeared normal with respect to overall protein composition and were able to combine with 30 S subunits to form 70 S ribosomes. Significantly, however, the 50 S subunits containing H229Q-L2 were completely inactive in peptidyl transferase activity. This result is consistent with an essential role for His229 in the peptidyl transferase catalytic center, perhaps as a catalytic residue in a mechanism involving general acid-base catalysis, similar to the proteolysis mechanism of serine proteases (9–11).

We created deletion and substitution mutations in RML2 that mimic those studies in the E. coli L2 gene. The rml2-Δ7 deletion removes 7 amino acids Val336 to Asp342 and the rml2-H343-Q substitution changes the most highly conserved histidine residue in Rml2p. Since yeast cells grow on a fermentable carbon source in the absence of mitochondrial protein synthesis, the phenotypes of the yeast mitochondrial L2 mutants could be examined in cells expressing only the mutant protein. Based on the reported results for the mutant L2 proteins in E. coli, we expected both the rml2-H343-Q and the rml2-Δ7 mutants to have strong Pet− respiratory deficient phenotypes. While this was the case for the rml2-Δ7 mutant, the rml2-H343-Q mutant had only a conditional respiratory growth phenotype.

Since respiratory growth and mitochondrial protein synthesis were normal in the rml2-H343-Q mutant grown at 30 °C, His343 is not essential for the formation of a functional peptidyl transferase center in yeast mitochondria. This result is significant because of the earlier proposal that the imidazole functional group of a histidine residue in L2 might participate directly in the catalysis of the peptidyl transfer reaction (22). Since His343 of Rml2p corresponds to the most highly conserved histidine in the L2 protein family, it is the best candidate to be an essential catalytic residue. It is surprising, therefore, that the His343 to Gln substitution is associated with only a conditional respiratory deficient phenotype, indicating that His343 is not essential in the yeast mitochondrial ribosome. Although Gln could conceivably replace His343 as a structural element in Rml2p, the Gln side chain amide cannot substitute for the His imidazole group in a catalytic mechanism involving general acid-base catalysis (22). Thus, it appears unlikely that His343 is directly involved in the chemistry of peptide bond formation.

E. coli cells expressing the His229 to Gln mutant L2 accumulate abnormal 40 S large subunit particles that lack L16 and contain reduced amounts of L28, L33, and L34 (21). In contrast to this, the yeast rml2-H343-Q mutant had no apparent defect in ribosome assembly, even when grown at the nonpermissive temperature (18 °C). The 54 S large subunit particle in the

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3 B. Baum, personal communication.
\textit{rml2-H343-Q} mutant contained the yeast mitochondrial homologue of bacterial L16, as well as the mitochondrial homologues of L27, L23, and L5 proteins. In the absence of a marked effect of the \textit{rml2-H343-Q} mutation on ribosome assembly at either the permissive (30 °C) or nonpermissive (18 °C) temperature, we conclude that the mutation either has a very subtle effect on the assembly of ribosomes at 18 °C or renders the ribosome cold-sensitive for function.

The \textit{rml2-H343-Q} mutant cells grown at the restrictive temperature had 40% of normal mitochondrial protein synthesis as measured by specific radioactivity of total mitochondrial protein isolated from cells labeled with [35S]methionine in the presence of cycloheximide. Inspection of the profile of radiolabeled mitochondrial translation products in the mutant grown at 18 °C indicates a preferential inhibition of the synthesis and accumulation of the larger polypeptides, such as VAR1, COX1, and COX2, and a less pronounced effect on the labeling of the smaller polypeptides (Fig. 5). The specific effect on longer polypeptides suggests that the mutant ribosomes suffer a defect in the elongation process rather than impaired translational initiation. Although more detailed studies will be required to pinpoint the functional impairment in the mutant ribosomes, the present results clearly show that the most highly conserved histidine in the L2 protein family is not an
essential catalytic residue in the peptidyl transferase center of yeast mitochondrial ribosomes.

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