Homology of Yeast Mitochondrial Leucyl-tRNA Synthetase and Isoleucyl- and Methionyl-tRNA Synthetases of Escherichia coli*

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Alexander Tzagoloff, Anna Akai, Maryellen Kurkulos, and Barbara Repetto
From the Department of Biological Sciences, Columbia University, New York, New York 10027

Respiratory deficient mutants of Saccharomyces cerevisiae previously assigned to complementation group G59 are pleiotropically deficient in respiratory chain components and in mitochondrial ATPase. This phenotype has been shown to be a consequence of mutations in a nuclear gene coding for mitochondrial leucyl-tRNA synthetase. The structural gene (MSL1) coding for the mitochondrial enzyme has been cloned by transformation of two different G59 mutants with genomic libraries of wild type yeast nuclear DNA. The cloned gene has been sequenced and shown to code for a protein of 894 residues with a molecular weight of 101,836. The amino-terminal sequence (30–40 residues) has a large percentage of basic and hydroxylated residues suggestive of a mitochondrial import signal.

The cloned MSL1 gene was used to construct a strain in which 1 kb of the coding sequence was deleted and substituted with the yeast LEU2 gene. Mitochondrial extracts obtained from the mutant carrying the disrupted MSL1::LEU2 allele did not catalyze acylation of mitochondrial leucyl-tRNA even though other tRNAs were normally charged. These results confirmed the correct identification of MSL1 as the structural gene for mitochondrial leucyl-tRNA synthetase. Mutations in MSL1 affect the ability of yeast to grow on nonfermentable substrates but are not lethal indicating that the cytoplasmic leucyl-tRNA synthetase is encoded by a different gene.

The primary sequence of yeast mitochondrial leucyl-tRNA synthetase has been compared to other bacterial and eukaryotic synthetases. Significant homology has been found between the yeast enzyme and the methionyl- and isoleucyl-tRNA synthetases of Escherichia coli. The most striking primary sequence homology occurs in the amino-terminal regions of the three proteins encompassing some 150 residues. Several smaller domains in the more internal regions of the polypeptide chains, however, also exhibit homology. These observations have been interpreted to indicate that the three synthetases may represent a related subset of enzymes originating from a common ancestral gene.

Eukaryotic cells have compartmentally segregated sets of aminoacyl-tRNA synthetases to accomodate the cytoplasmic and organelar protein synthetic machineries. In Saccharo-

yces cerevisiae five of the mitochondrial synthetases have been ascertained to be encoded by nuclear genes distinct from those coding for the homologous cytoplasmic enzymes (1–4). Recent studies, however, indicate that not all mitochondrial aminoacyl-tRNA synthetases are separately encoded (5). The mitochondrial and cytoplasmic histidyl-tRNA synthetases have been shown to be products of a single gene (5). Targeting of the two proteins to their appropriate subcellular compartments is achieved by differential transcription of the gene such that only one of the mRNAs contains the sequence coding for the mitochondrial amino-terminal import signal. The shorter transcript lacking this sequence acts as the mRNA for the cytoplasmic enzyme (5).

In the present study we have cloned the yeast nuclear gene for the mitochondrial leucyl-tRNA synthetase. In situ disruption of the chromosomal copy of the gene induces a respiratory deficient phenotype but is not lethal to the cell, indicating the existence of a separate gene for the cytoplasmic synthetase. The primary structure of the mitochondrial leucyl-tRNA synthetase predicted by the gene sequence has been compared to the sequences of the isoleucyl- and methionyl-tRNA synthetases (6, 7) of Escherichia coli. Although the three proteins have undergone significant changes in their primary structures, they exhibit a number of homologous domains suggestive of a common evolutionary ancestry. The recently reported sequences of the valyl-tRNA synthetases of yeast (8) and E. coli (9) suggest that this synthetase is also a member of the subgroup (8). These results imply that earlier proteins may have been composed of a set of amino acids that included only one of the above four amino acids.

**MATERIALS AND METHODS**

**Yeast Strains and Growth Media**—The genotypes of the yeast strains used in this study are described in Table I. The leucyl-tRNA synthetase mutants were identified among a collection of respiratory defective pet1 mutants (12). Yeast were grown on the following media: YPD (2% glucose, 2% peptone, 1% yeast extract), YEPG (3% glycerol, 2% ethanol, 2% peptone, 1% yeast extract), WO (2% glucose, 0.67% yeast nitrogen base without w/o amino acids (Difco), YPGal (2% galactose, 2% peptone, 1% yeast extract). Solid media contained 2% agar.

**Cloning of the MSL1 Gene**—The MSL1 gene was cloned by transformation of two different leucyl-tRNA synthetase mutants with yeast genomic libraries constructed by ligation of 5–15-kb partial Sau3A fragments of nuclear DNA to the BamHI site of the shuttle vectors YEp13 (13) or YEp24 (14). Transformant clones complemented for the respiratory deficiency and for the auxotrophic markers were selected on minimal glycerol medium as described previously (15). The clone C151/T1 was obtained by transformation of C151/L1 (leu2, msl1–1) with the YEp13 library. This clone yielded plasmid pJ5/T1. The second clone C169/T2 was obtained by transformation

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J03495 and J03496.
of C169/U9 (ura3,msl1-2) with a YEp24 library kindly provided by Dr. Marian Carlson, Department of Human Genetics, Columbia University. C169/T2 contained the recombinant plasmid pG59/T2 whose nuclear DNA insert overlapped partially with that of pG59/T1.

Procedures Involving Manipulation of DNA—Standard protocols were used for transformation of E. coli, preparation of plasmid DNA, restriction endonuclease analysis of DNA, and purification of and ligation of DNA fragments (16). All DNA sequences were obtained by the chemical derivatization procedure of Maxam and Gilbert (17) using 5'-end-labeled single-stranded DNA fragments.

Aminoacylation of Mitochondrial tRNAs—A crude RNA synthetase fraction was prepared from wild type and mutant mitochondria by extraction with cholate. Mitochondria suspended in 10 mM Tris-HCl, pH 7.5 at a protein concentration of 10 mg/ml were treated with 1/20 volume of 20% potassium acetate, and separated by reverse phase chromatography on RPC-5 (19). The RPC-5 column was developed with 200 ml of a linear gradient of NaCl from 0.38 to 0.75 M. Fractions of 2 ml were collected and treated with trichloroacetic acid in the presence of 0.5 mg of carrier RNA. The precipitates were harvested by filtration over Whatman GF/A glass filters and counted in a scintillation counter.

RESULTS

Properties of Leucyl-tRNA Synthetase Mutants—The pet complementation group G59 consists of 19-independent respiratory deficient mutants. The strains lack cytochromes a, a', b, and oligomycin-sensitive ATPase. The pleiotropic deficiency of the respiratory chain carriers and of ATPase can be accounted for by the inability of the mutants to carry out mitochondrial protein synthesis as assayed by in vivo incorporation of radioactive methionine in the presence of cycloheximide (data not shown). Mutants in this group produce cytoplasmic petites at a high frequency (50-85% of the vegetative progeny in a stationary phase culture containing of a and a' mutants) indicating that the nuclear mutations have a secondary effect on the stability of mitochondrial DNA. Furthermore, in situ disruption of the nuclear gene defined by this complementation group (see below) was found to cause a quantitative loss of wild type mitochondrial DNA. Similar properties have previously been noted in pet strains with lesions in mitochondrial ribosomal proteins, elongation factor, and aminoacyl-tRNA synthetases (20).

The above phenotype suggested that the primary lesion of G59 mutants is in a component necessary for mitochondrial protein synthesis. This was confirmed by aminocacylation assays of mitochondrial tRNAs. The results of such assays indicate that the mutant mitochondria are unable to acylate the mitochondrial leucyl-tRNA (Fig. 1). Since yeast mitochondria do not recognize the CUN family as leucine codons, they have only one leucyl-tRNA for the UUA and UUG codons. As shown in Fig. 1, this tRNA is charged by extracts of wild type mitochondria. No charging of the leucyl tRNA was observed by a similar extract obtained from the msll mutant C151/L1.

C151/T1 is a transformant harboring a multicopy plasmid with the wild type MSL1 gene coding for the mitochondrial leucyl-tRNA synthetase (see below). This plasmid complements the mutation of C151/L1 and renders it respiratory competent. The fact that more efficient charging of mitochondrial leucyl tRNA was seen with mitochondrial extracts obtained from the transformant provided additional evidence that the mutation of C151/L1 is in the structural gene for the mitochondrial leucyl-tRNA synthetase.

Cloning of the MSL1 Gene—The nuclear gene coding for mitochondrial leucyl-tRNA synthetase was selected from two

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TABLE 1

Genotypes and sources of Saccharomyces cerevisiae strains

<table>
<thead>
<tr>
<th>Strain Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>D273-10B/A1</td>
<td>s, a', met6</td>
</tr>
<tr>
<td>W303-1A</td>
<td>s, a', ade2-1, his3-11, 15, leu2-3, 112, ura3-1, trpl-1, can1-100</td>
</tr>
<tr>
<td>W303-1B</td>
<td>a, a', ade2-1, his3-11, 15, leu2-3, 112, ura3-1, trpl-1, can1-100</td>
</tr>
<tr>
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<td>a, a', ade1</td>
</tr>
<tr>
<td>C151</td>
<td>a, a', met6, ms1-1</td>
</tr>
<tr>
<td>B151</td>
<td>a, a', ade1, ms11-1</td>
</tr>
<tr>
<td>C151/L1</td>
<td>a, a', ade2-3, 112, ms11-1</td>
</tr>
<tr>
<td>C169</td>
<td>a, a', ade1, ms11-2</td>
</tr>
<tr>
<td>C169/U9</td>
<td>a, a', ura3-1, ms11-2</td>
</tr>
<tr>
<td>W303VMSL1-1</td>
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</tr>
<tr>
<td>W303VMSL1-2</td>
<td>a, a', ade2-1, his3-11, 15, leu2-3, 112, ura3-1, trpl-1, can1-100, MSL1::LEU2</td>
</tr>
</tbody>
</table>

*Department of Human Genetics, Columbia University, New York, NY.

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Fig. 1. Acylation of mitochondrial leucyl- and tryptophanyl-tRNAs by mitochondrial extracts of wild type, mutant, and transformed yeast. Mitochondria were prepared from the msl1 mutant C151/L1, from the transformant C151/T1 harboring the MSL1 gene on the autonomously replicating plasmid pG59/T1, and from the respiratory competent haploid strain D273-10B. The mitochondria were used to prepare a crude aminocyl-tRNA extract as described under "Materials and Methods." The extracts were used to acylate total mitochondrial tRNAs with a mixture of [3H]leucine and [3H]tryptophan. The acylated mixture was separated on an RPC-5 column eluted with a gradient of sodium chloride from 0.36-0.75 M (18). The elution positions of the acylated tRNAs are indicated in the figure.
different genomic libraries. A library constructed with the shuttle vector YEp13 (13) was used to transform C151/L1. Transformants complemented for both the leucine auxotrophy and respiratory deficiency were selected on minimal glycerol medium. One such strain contained a recombinant plasmid (pG59/T1) with a nuclear DNA insert of 11 kb (Fig. 2). The second plasmid studied, designated pG59/T2, was obtained by transformation of C159/U9 with another library constructed with the shuttle vector YEp24 (14). This plasmid had a smaller insert of 7.5 kb. A comparison of the restriction maps of pG59/T1 and pG59/T2 indicated that the two plasmids had a common region of approximately 6 kb (Fig. 2).

To further localize the region of DNA responsible for complementation of the msl1 mutations, different segments of the nuclear DNA insert of pG59/T1 were transferred to the shuttle vector YEp351 (21). The new constructs were tested for their ability to complement C151/L1. The results of these transformations, summarized in Fig. 2, indicate that the smallest plasmid (pG59/ST1) capable of complementing the mutant had a 4.7-kb insert spanning a region from the left-most side of the insert in pG59/T1 to the proximal XbaI site. The inability of the smaller plasmids (pG59/ST2-4) to complement C151/L1 indicated that the gene crosses the internal XbaI and the two BamHI sites.

Sequence of the MSL1 Gene—A region spanning approximately 5 kb of the insert in pG59/ST1 was sequenced by the strategy depicted in Fig. 3. All the sequences were obtained from single-stranded restriction fragments labeled at their 5′ ends and derivatized by the method of Maxam and Gilbert (17). All the restriction sites used for 5′-end-labeling were crossed from neighboring sites and most of the sequence was confirmed from the complementary strands.

Analysis of the sequence revealed the presence of an open reading frame starting at an ATG codon at nucleotide +1 and ending at an ochre termination codon at nucleotide +2,583 (Fig. 4). This reading frame includes the two BamHI and the XhoI sites that were found to be in the region necessary for complementation. The protein encoded by the reading frame is 894 residues long and has a molecular weight of 101,936. This value is consistent with the size estimates of bacterial (22, 23) and yeast cytoplasmic (24) leucyl-tRNA synthetases both of which have been reported to have subunit sizes of 105 and 120 kDa, respectively. The amino-terminal sequence of the protein encoded by the reading frame has a preponderance of basis (Arg, Lys) and hydroxylated (Thr, Ser) amino acids, a common feature of mitochondrial presequences (25). Assuming that the first 30–40 residues comprise an import signal, the size of the mature protein would be some 3–4 kDa smaller.

Disruption of the MSL1 Gene—The identification of the reading frame in pG59/ST1 as the structural gene for mitochondrial leucyl-tRNA synthetase was corroborated by in situ disruption of this gene in wild type strains of yeast. The construct used for the one-step gene replacement (26) is shown in Fig. 5. The 2.7-kb SstI-XhoI fragment of pG59/ST1 was ligated to the SstI and SaI sites of YEp352 (21). This construct was digested with BamHI to remove a 1-kb sequence internal to the reading frame. The deleted sequence was replaced with a 3-kb BglII fragment containing the yeast LEU2 gene. The resultant plasmid served as the source of a 4.7-kb SstI-XhoI fragment containing the disrupted gene. The 4.7-kb linear fragment was used to transform the isogenic haploid strains of S. cerevisiae W303-1A and W303-1B. Following transformation leucine prototrophic clones were selected on minimal glucose medium supplemented with all the auxotrophic requirements of W303 except for leucine. Among a large number of Leu+ Glyceraldehyde-3-phosphate dehydrogenase transformants obtained most were not complemented for the respiratory defect when mated to tester strains with either the msl1– or msl1– mutations indicating linkage of MSL1::LEU2 to these alleles.

The results of the genetic crosses suggested that the Glyceraldehyde-3-phosphate dehydrogenase transformants had acquired the disrupted allele MSL1::LEU2. This was confirmed by Southern blot analysis of the genomic DNA in two independent Glyceraldehyde-3-phosphate dehydrogenase transformants (W303-VMSL1-1 and W303-VMSL1-2). Nuclear DNA purified from the respiratory competent parental strain W303-1A and from each of the two Glyceraldehyde-3-phosphate dehydrogenase transformants were digested with a combination of XhoI and SstI. Following separation on agarose and transfer to nitrocellulose, the blot was hybridized to the 2.7-kb SstI-XhoI fragment of wild type yeast nuclear DNA. As expected the probe detects the homologous

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**Fig. 2.** Restriction maps of pG59/T1, pG59/T2, and derivative subclones. A partial restriction map of the nuclear DNA insert in pG59/T1 is shown in the lower part of the figure. The second plasmid pG59/T2 obtained from another transformation had a smaller insert but included a sequence of approximately 1.5 kb that is absent in pG59/T1. The bar in the upper part of the figure denote the regions subcloned in YEp351 and YEp352 (21). Complementation of the msl1−1 or msl1−2 mutations is indicated by the plus signs and lack of complementation by the minus signs. The locations of the recognition sites for SstI (S), BamHI (B), XhoI (O), and XbaI (X) are shown for pG59/T1. The insert of this plasmid starts just short of the SstI site that is present in the insert of pG59/T2.

**Fig. 3.** Restriction sites used to determine the DNA sequence of the MSL1 gene. The region of pG59/ST1 sequenced is shown in the figure. The arrows indicate the restriction sites used for 5′-end-labeling and the directions and approximate lengths of sequences obtained. The location of the reading frame identified as the MSL1 gene is depicted by the open bar in the upper part of the figure. The symbols used for the various restriction sites are: PstI (O), BamHI (B), XhoI (O), and XbaI (X). The region of pG59/T2 obtained from another transformation had a smaller insert of 7.5 kb. A comparison of the restriction maps of pG59/T1 and pG59/T2 indicated that the two plasmids had a common region of approximately 6 kb (Fig. 2).

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Yeast Mitochondrial Leucyl-tRNA Synthetase
Yeast Mitochondrial Leucyl-tRNA Synthetase

**FIG. 4.** Nucleotide sequence of MSL1. The sequence starts from the leftmost side of the insert in pG59/T1 (see Fig. 2). The BamHI site at −185 has been created as a result of ligation of the insert to the BamHI site of YEpl3 and does not exist in the genomic DNA. Only the sequence of the sense strand is shown. The reading frame proposed to code for the mitochondrial leucyl-tRNA synthetase has been translated into the amino acid sequence. The EcoRI, XhoI, and PstI sites are marked for reference.

2.7-kb fragment in the wild type strain W303-1A (Fig. 5). Both transformants, however, had two new fragments of approximately 3.2 and 1.5 kb. Based on the restriction map of the BglII fragment used for the disruption, the larger 3.2-kb fragment contains 0.5 kb of the reading frame plus almost the entire BglII insert. The smaller 1.5-kb fragment should correspond to 5' flanking sequence of MSL1 starting at the SstI site, the amino-terminal-coding sequence of MSL1 up to the first BamHI site and approximately 200 bp of the BglII fragment. The agreement between the observed and expected results of the Southern analysis together with the results of the genetic crosses constitute strong evidence for the substitution of the wild type by the disrupted gene in both of the transformants examined.

To study the effect of the disruption of MSL1 on the aminoacylating activity of mitochondria, synthetase extracts prepared from the wild type parent W303-1A and from W303VMSL1, were compared for their ability to charge mitochondrial leucyl tRNA. The extracts were also tested for acylation of methionyl, cysteinyl, valyl, and isoleucyl tRNAs. The aminoacylating enzymes of *E. coli* responsible for charging of these tRNAs have been reported to have molecular weights similar to the protein encoded by MSL1 (27). As shown in Fig. 6, of all the tRNAs assayed only the leucyl tRNA failed to be charged by the mitochondrial extract of W303VMSL1. These data provide further supporting evidence for the identity of MSL1 as the structural gene for yeast mitochondrial leucyl-tRNA synthetase.

**Homology of Leucyl-, Isoleucyl-, and Methionyl-tRNA Synthetases**—A search for homology of the yeast mitochondrial leucyl-tRNA synthetase to other proteins in the Claverie library (28) by the DFASTP program (29) revealed the highest scores to be with the *E. coli* isoleucyl- and methionyl-tRNA synthetases. The homology of the three synthetases, shown in Fig. 7, is based on alignments of synthetase pairs using the MFALGO program (30). Positions with identical or with chemically similar residues (Lys, Arg; Gln, Asp; Ala, Gly; Phe, Tyr; Met, Ile, Leu, Val; Gln, Asn, Thr, Ser) are indicated. Even though deletions and insertions were necessary to achieve an optimal alignment, the three proteins share a number of consecutive regions of sufficiently high primary sequence similarity to suggest they may be evolutionarily related. The longest and most homologous region extends over 150 residues near the amino-terminal ends (residues 56-
were identical to those described by Myers et al. (20). The migration of known size standards is shown in the left-hand margin of the figure. The lower part of the figure shows the restriction maps of the wild type MSL1 and disrupted MSL1::LEU2 genes. The MSL1 coding region is denoted by the solid bars. The 3-kb BgII fragment with the LEU2 gene is depicted by the dashed line. The locations of the SstI (S), BamHI (B), XhoI (O) sites, and the BamHI-BgII (B/G) junctions. The smaller fragment detected in the mutant DNAs has a size consistent with the size of the SstI-XhoI fragment. The larger radioactive band has a size expected for the XhoI fragment containing most of the BgII fragment used for the disruption.

218 of the leucyl-tRNA sequence). In this region 21–24% of the residues are identical (33–39% when conservative substitutions are included) between any two sequences. More significantly there are 13% identical amino acids shared among the three sequences. This region has been shown to form an adenine mononucleotide-binding fold in the methionyl-tRNA synthetase (31, 32). The conserved high sequence (residues 63–66) in the leucyl- and isoleucyl-tRNA synthetase has also been shown to be part of the nucleotide-binding fold of the Bacillus stearothermophilus tyrosyl-tRNA synthetase (33) and the identical sequence has been reported to be present in the glutaminyl-tRNA synthetase of E. coli (6). This sequence occurs in the loop intervening between the first β-strand and the connecting α-helix of the mononucleotide-binding fold in the methionyl- and tyrosyl-tRNA synthetases (31, 33). Both histidines in this sequence have been proposed to interact or be closely apposed to the adenylated substrate in the tyrosyl-tRNA synthetase (33).

The second homologous region (residues 444–459 in the leucyl-tRNA synthetase) is comprised of a short sequence of 16 amino acids with five identities. This small domain is also found in the valyl-tRNA synthetase (8, 9) but not in any of the other published synthetase sequences. Another region of homology is included between residues 607 and 670 of the leucyl-tRNA synthetase. In this segment there are 15% identities between the three sequences. The similarities are higher when individual pairs are compared to one another. For example, the isoleucyl- and methionyl-tRNA synthetase have 28% identical residues (42% when residues with similar chemical properties are included in the calculation), while the other two pairs are 20% identical. It is of interest that this region of the methionyl-tRNA synthetase encompasses a conserved lysine (Lys<sub>59</sub>) within the sequence KMSKS that has been implicated to interact with the 3′ ACC of the tRNA (34). The KMSKS sequence is also present in the leucyl-, isoleucyl-, and valyl-tRNA synthetases (8, 9). Houtondji et al. (35) have noted the occurrence of similar sequence motifs in other synthetases as well.

The last homology worthy of mention is seen in the carboxyl-terminal regions of the proteins (residues 678–743 of leucyl-tRNA synthetase). Although identical residues shared between the three proteins is only 10% and the sequences are more difficult to align, the overall character of the sequences suggests they may also represent an evolutionarily conserved domain.

**DISCUSSION**

The protein synthetic system of yeast mitochondria utilizes a set of aminoacyl-tRNA synthetases that are compartmentally separated from those functioning in cytoplasmic protein synthesis. As part of our efforts to clarify the relationship of
Fig. 7. Primary sequence homology of the yeast mitochondrial leucyl-tRNA synthetase to the isoleucyl- and methionyl-tRNA synthetases of E. coli. The sequence of the leucyl-tRNA synthetase (LEU) is derived from the sequence of the MSLe gene. The isoleucyl- (ISI) and methionyl-tRNA synthetase (MET) sequences were taken from Refs. 6 and 7, respectively. Positions with identical or chemically similar amino acids are boxed. Identical residues are shaded.
the mitochondrial and cytoplasmic aminoacyl-tRNA synthetases of yeast we have screened respiratory deficient pet strains of S. cerevisiae for defects in charging of mitochondrial tRNAs. These studies have enabled us to identify mutants with lesions in several mitochondrial aminoacyl-tRNA synthetases. The mutants have been useful in cloning the genes and deriving the primary sequences of their encoded products (1-4).

In the present paper we report the properties of mutant strains assigned to complementation group G59. A representative mutant from this complementation group has been found to be impaired in acylation of the mitochondrial leucyl-tRNA. A nuclear gene capable of complementing two-independent mutants has been cloned by transformation. Several lines of evidence confirm that this gene codes for the mitochondrial leucyl-tRNA synthetase. 1) Transformation of a mutant with the gene on a multicopy plasmid restores its ability to charge the leucyl-tRNA. Furthermore, a mitochondrial extract from the transformant exhibits a higher level of acylating activity than a comparable extract obtained from a wild type strain of yeast. 2) Partial deletion of the gene in wild type yeast induces a phenotype similar to that observed in the original mutant used to clone the gene. 3) The gene codes for a protein with a molecular weight compatible with previous estimates of bacterial and yeast cytoplasmic leucyl-tRNA synthetases (24, 25).

Based on these results the gene has been named msl1 (mitochondrial synthetase leucine) in accord with the previous convention (1-4). Since msl1 mutants, including a strain with a partially deleted copy of MSL1, are respiratory deficient but are capable of growing on fermentable substrate such as glucose or galactose, yeast must have another gene for the cytoplasmic leucyl-tRNA synthetase.

Aminoacyl-tRNA synthetases constitute an old family of enzymes whose evolution is poorly understood at present. Although the primary structures of a substantial number of bacterial synthetases are known, searches for sequence homologies have failed to detect any clear evidence for common evolutionary ancestry. The sequence homologies that have been reported are confined to small regions some of which have been shown to participate in binding of various substrates (6, 32-35). The sequence of the yeast mitochondrial leucyl-tRNA synthetase derived from the MSL1 gene has been compared to all the known sequences of bacterial and eukaryotic synthetases. The computer searches have revealed at least three and possibly four blocks of significant homology to the E. coli isoleucyl- and methionyl-tRNA synthetases. The most extensive homology occurs in the amino-terminal part of the three proteins spanning a region of approximately 150 residues. This region of the E. coli methionyl-tRNA synthetase has been shown to form an adenine mononucleotide binding fold that is believed to interact with ATP and the adenylated amino acid (31, 32, 34). The three proteins also have several more internal domains of high primary sequence homology. One of these has recently been proposed to interact with the acceptor stem of the tRNA substrate (34). Finally, a reasonably good alignment of the three sequences can be made in the carboxyl-terminal region where approximately 10% of coincident positions are occupied by identical amino acids. Similar but not identical alignments of the sequences have been made by Heck et al. (9). In addition, these authors find the valyl-tRNA synthetase of E. coli is another member of this subfamily of synthetases sharing the same common sequence elements (9). Jordana et al. (8) have recently cloned and characterized the cytoplasmic valyl-tRNA synthetase of S. cerevisiae. Based on primary sequence homology of the yeast valyl- and the E. coli isoleucyl-tRNA synthetases, these authors have concluded that the two synthetases may have originated from a common ancestral gene (8). The present studies suggest that the leucyl-, isoleucyl-, valyl-, and methionyl-tRNA synthetases form a group that may have evolved from a common precursor gene.

Note Added in Proof—We have recently noted that except for a few nucleotides, the sequence of the MSL1 gene is identical to the sequence of the NAM2 gene whose product has been implicated in splicing of the yeast apocytochrome b pre-mRNA (Labouesse, H., Herbert, C. J., Dujardin, G., and Slonimski, P. P. (1987) EMBO J. 6, 713-721).