Initiation of Protein Synthesis in *Saccharomyces cerevisiae* Mitochondria without Formylation of the Initiator tRNA

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Protein synthesis in eukaryotic organelles such as mitochondria and chloroplasts is widely believed to require a formylated initiator methionyl tRNA (fMet-tRNA{sup fMet}) for initiation. Here we show that initiation of protein synthesis in yeast mitochondria can occur without formylation of the initiator methionyl-tRNA (Met-tRNA{sup fMet}). The formylation reaction is catalyzed by methionyl-tRNA formyltransferase (MTF) located in mitochondria and uses N{sup 10}-formyltetrahydrofolate (10-formyl-THF) as the formyl donor. We have studied yeast mutants carrying chromosomal disruptions of the genes encoding the mitochondrial C{sub 1}-tetrahydrofolate (C{sub 1}-THF) synthase (MIS1), necessary for synthesis of 10-formyl-THF, and the methionyl-tRNA formyltransferase (open reading frame YBL013W; designated FMT1). A direct analysis of mitochondrial tRNAs using gel electrophoresis systems that can separate fMet-tRNA{sup fMet}, Met-tRNA{sup fMet}, and tRNA{sup fMet} shows that there is no formylation in vivo of the mitochondrial initiator Met-tRNA in these strains. In contrast, the initiator Met-tRNA is formylated in the respective "wild-type" parental strains. In spite of the absence of fMet-tRNA{sup fMet}, the mutant strains exhibited normal mitochondrial protein synthesis and function, as evidenced by normal growth on nonfermentable carbon sources in rich media and normal frequencies of generation of petite colonies. The only growth phenotype observed was a longer lag time during growth on nonfermentable carbon sources in minimal media for the mis1 deletion strain but not for the fmi1 deletion strain.

Protein synthesis is initiated with methionine or formylmethionine in all organisms studied to date (23, 33). Of the two species of methionine tRNAs found in all organisms, the initiator is used for initiation of protein synthesis whereas the elongator is used for insertion of methionine into internal peptide linkages. In eubacteria such as *Escherichia coli*, following aminoacylation of the initiator methionine tRNA (tRNA{sup fMet}), the methionyl-tRNA (Met-tRNA{sup fMet}) is formylated in the respective "wild-type" parental strains. In contrast, the initiator Met-tRNA is formylated in eukaryotic organelles such as mitochondria and chloroplasts treated with puromycin (5, 6, 14, 16, 28, 35). These results, along with the identification of formylmethionine at the N terminus of several mitochondrially synthesized polypeptides with three enzyme activities: 10-formyl-THF synthase, 5,10-methylene-THF cyclohydrolase, and NADP-dependent 5,10-methylene-THF dehydrogenase. *S. cerevisiae* also expresses a monofunctional NAD-dependent 5,10-methylene-THF dehydrogenase in the cytoplasm, encoded by the *MIS1* gene. The formylation group donor in the formylation reaction is 10-formyl-THF (11). *S. cerevisiae* contains two C{sub 1}-THF synthase enzymes, as in *E. coli* and *Neurospora crassa*, and these enzymes are trifunctional polypeptides with three enzyme activities: 10-formyl-THF synthase, 5,10-methylene-THF cyclohydrolase, and NADP-dependent 5,10-methylene-THF dehydrogenase. *S. cerevisiae* also expresses a monofunctional NAD-dependent 5,10-methylene-THF dehydrogenase in the cytoplasm, encoded by the *MTD1* gene (57). The *ADE3* and *MTD1* gene products are responsible for mitochondrial one-carbon interconversions, whereas the *MIS1* gene product is responsible for mitochondrial one-carbon interconversions (3, 58).

Shannon and Rabinowitz (41) showed that disruption of the *MIS1* gene had no dramatic effects on the growth of *S. cerevisiae*, suggesting that the *MIS1* gene is dispensable in yeast. Also, disruption of the nuclear gene encoding the putative
mitochondrial MTF had no effect on viability (43), suggesting that the FMT1 gene is also dispensable in S. cerevisiae, although the growth conditions tested were not specified. These findings are rather surprising. If formylated Met-tRNA\(^{\text{fMet}}\) is required for initiation of mitochondrial protein synthesis, loss of the enzyme that produces the formyl donor or loss of the enzyme that synthesizes fMet-tRNA\(^{\text{fMet}}\) would be expected to affect protein synthesis and, thereby, mitochondrial function. Mitochondrial protein synthesis is required for respiratory function in mitochondria, and mutation of genes encoding mitochondrial translation components invariably leads to a respiratory-deficient (petite) phenotype (49). Thus, the lack of a dramatic effect on cell growth or respiratory function upon disruption of the genes coding for these two enzymes would suggest that protein synthesis can be initiated in S. cerevisiae mitochondria without formylation of the initiator tRNA. There are, however, several other possible explanations that need to be ruled out: (i) transport of the cytoplasmically made 10-formyl-TIF into mitochondria, (ii) alternate forms of MTF which do not use 10-formyl-TIF as a formyl donor (analogous to the formate-dependent glycaminide ribonucleotide transformylase [56]), or (iii) alternate genes for mitochondrial MTF with no homology to MTFs identified thus far.

A knowledge of the state of the initiator tRNA in mitochondria, whether it is in the form of fMet-tRNA or Met-tRNA\(^{\text{fMet}}\) (52), would allow one to distinguish among the above possibilities. This paper reports on a direct analysis of the state of the initiator tRNA in S. cerevisiae mitochondria in strains carrying the MIS1 and FMT1 gene disruptions. We show that there is no formylation of the initiator Met-tRNA in strains carrying these gene disruptions. Also, these strains grow at nearly wild-type rates in rich medium and on nonfermentable carbon sources requiring full mitochondrial function. There are also no changes in the frequencies of generation of petite colonies, indicating that MIS1 and FMT1 gene disruptions have no effect on mitochondrial protein synthesis. Thus, formylation of the initiator Met-tRNA is not essential for mitochondrial protein synthesis and for mitochondrial function in S. cerevisiae.

### MATERIALS AND METHODS

#### Strains, media, and plasmids

The S. cerevisiae strains used in this work are summarized in Table 1. Strains 1001, 1049, and 1052 were obtained from B. Purnelle (Université Catholique de Louvain, Louvain-la-Neuve, Belgium).

#### Strains Genotype Comments

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1001</td>
<td>α ade2 trp1 leu2 his3 ura3</td>
<td>Wild-type FMT1</td>
</tr>
<tr>
<td>1049</td>
<td>α ade2 trp1 leu2 his3 ura3 trn1::URA3</td>
<td>fmt1 disruptant</td>
</tr>
<tr>
<td>1052</td>
<td>α ade2 trp1 leu2 his3 ura3 FMT1</td>
<td>fmt1 disruptant</td>
</tr>
<tr>
<td>DAY4</td>
<td>a ser1 ura3 trp1 leu2 his4</td>
<td>Wild-type MIS1</td>
</tr>
<tr>
<td>DAY4mis1</td>
<td>a ser1 ura3 trp1 leu2 his4 Δmis1</td>
<td>mis1 disruptant</td>
</tr>
<tr>
<td>WHY2</td>
<td>a ura3 trp1 leu2 Δmis1::URA3</td>
<td>fmt1 fnd1 double disruptant</td>
</tr>
</tbody>
</table>

Electrophoresis of tRNAs on acid-urea polyacrylamide gels and Northern blot analysis. The various forms of tRNAs were separated and detected as described previously (52), except that 0.16 A\(_{260}\) unit of mitochondrial RNA was applied to the 6.5% polyacrylamide gel. The mitochondrial tRNAs migrate slower on the gel than corresponding Escherichia coli tRNAs. Therefore, tRNA standards in an 11-cm segment of gel, starting with the xylene cyanol dye and going toward the bromphenol blue dye, were transferred by electroblopting to Nytran Plus membrane (Schleicher & Schuell, Keene, N.H.) in 1× TAE (50× TAE is 242 g of Tris base, 57.1 g of glacial acetic acid, and 100 mM EDTA in 1 liter; pH 8.0) at 40 V for 2 h. The tRNAs in the membrane were detected by hybridization with sequence-specific oligonucleotide probes. Prehybridization and hybridization were carried out for 4 and 16 h, respectively, in 4× SET (20× SET contains 87 g of NaCl, 1.4 g of Tris base, and 0.25 g of EDTA in 1 liter) in the presence of 1 ng of salmon sperm DNA per ml, 1% sodium dodecyl sulfate, and 10× Denhardt's solution. The oligonucleotide 5′TAGCAATAATACGATTG3′, which is complementary to nucleotides 56 to 73 of the S. cerevisiae mitochondrial RNA\(^{\text{DM}6}\), was used to detect the mitochondrial RNA\(^{\text{DM}6}\).

#### Deacylation of aminoacyl-tRNAs

Aminoacyl-tRNA was decayed by 0.1 M Tris-HCl (pH 9.4) at 37°C for 1 h. Alternatively, the aminoacyl-tRNA was incubated with 10 mM CuSO\(_4\) in 0.1 M Tris-HCl (pH 8.0) at room temperature for 15 min. Copper sulfate treatment hydrolyzes aminoacyl-tRNAs but not formylaminoacyl-tRNAs (38).

#### Rates of chemical deacylation of Tyr-tRNA\(^{\text{DM6}}\) and Met-tRNA\(^{\text{DM6}}\)

Total E. coli tRNA (0.5 A\(_{260}\) unit) was aminoacylated at 37°C for 30 min in 20 mM imidazole (pH 7.5)–150 mM NaCl–10 mM MgCl\(_2\)–0.1 mM EDTA–10 μg of bovine serum albumin per ml–2 mM ATP, with either methionine (100 μM) plus a saturating amount of purified E. coli Met-tRNA synthetase or tyrosine (25 μM) plus a saturating amount of purified E. coli Tyr-tRNA synthetase. The tRNAs were quantitatively aminoacylated under these conditions. The aminoacyl-tRNAs were isolated by phenol-chloroform extraction followed by ethanol precipitation (52). For measurement of rates of deacylation, the aminoacyl-tRNAs were incubated with 0.1 M Tris-HCl (pH 9.4) at 37°C and aliquots were taken out at various times of the incubation. The samples were thawed and loaded onto a 6.5% acid-urea gel for separation of tRNA and aminoacyl-tRNA (12). The tRNAs in the gel were transferred to Nytran Plus membrane and detected by hybridization with oligonucleotides complementary to the nucleotides 10 to 25 of E. coli tRNA\(^{\text{DM6}}\) or aminoacyl-tRNA\(^{\text{DM6}}\). The amount of radioactivity in the tRNA bands was determined by quantification using a Molecular Dynamics PhosphorImager.
RESULTS

*S. cerevisiae* strains carrying disruptions in the *MIS1* and *FMT1* genes. The *MIS1* gene was disrupted as described in Materials and Methods in the haploid strain DAY4 to yield DAY4Δmis1. Haploid yeast strain 1052 harboring a disruption of the putative MTF ORF (*FMT1*; YBL013W) was obtained from B. Purnelle. The genotypes of these mutants and the parent strains are summarized in Table 1. The disruptions were verified by PCR analysis of genomic DNA isolated from each strain. Disruption of *MIS1* gave the expected 400-bp difference when genomic DNA from DAY4 and DAY4Δmis1 was amplified with primers 1 and 2 (Fig. 1, compare lanes 2 and 3). Disruption of *FMT1* gave the expected 1,100-bp difference when genomic DNA from 1001 (wild type) and 1052 (*fmt1* disrupted, lane 5). The *MIS1* locus was amplified with primers 1 plus 2; the *FMT1* locus was amplified with primers 3 plus 4. Lane 1 contains size standards. The numbers on the left indicate the sizes in kilobase pairs of the standard.

Lack of formylation in vivo of the mitochondrial initiator Met-tRNA leads to an essentially quantitative accumulation of the tRNA as Met-tRNA^{Met} (24, 53). The ester linkage between the methionine and the tRNA is known to be more labile than that between formylmethionine and the tRNA. Therefore, one possible explanation for the above result is that methionine is cleaved off the Met-tRNA^{Met} during the prolonged workup (6 h or longer) necessary for the isolation of yeast mitochondria prior to isolation of the mitochondrial RNA. To test this possibility, we probed another blot of the mitochondrial RNA preparation with an oligonucleotide complementary to nucleotides 56 to 73 of the yeast mitochondrial tRNAfMet into three forms: uncharged tRNAfMet, Met-tRNAfMet, and fMet-tRNAfMet. A labeled oligonucleotide complementary to nucleotides 56 to 73 of the tRNAfMet was used to probe a Northern blot of the gel. Figure 2 shows a typical Northern blot analysis of total yeast mitochondrial tRNA from the wild-type strain (lanes 2, 4, 6, and 8), the *fmt1*-disrupted strain (lanes 3 and 5), and the *mis1*-disrupted strain (lanes 7 and 9). Lanes 1 and 10 contain deacylated tRNAfMet as markers. Uncharged tRNAfMet (bottom band) was present in all strains, whereas formylated Met-tRNAfMet (middle band) was detectable only in the wild-type strains (1001 and DAY4). The mutant strains contained instead small amounts of charged but unformylated Met-tRNAfMet (top band, lanes 3 and 7). Treatment of tRNA with copper sulfate prior to electrophoresis resulted in the disappearance of the aminoacyl-tRNA band but not the formylated aminoacyl-tRNA band, confirming the identity of the upper bands seen in the mutant strains as unformylated Met-tRNAfMet (compare lane 3 to lane 5 and lane 7 to lane 9). These data show that there is no formylation of Met-tRNAfMet in mitochondria from either the *fmt1*-disrupted or *mis1*-disrupted strains and provide further support for the identification of ORF YBL013W as the yeast methionyl-tRNA formyltransferase gene.

A somewhat surprising result is the limited amount of aminoacylated mitochondrial Met-tRNAfMet (Fig. 2, lanes 3 and 7) found in strains carrying the *fmt1* or the *mis1* disruptions. This is unlike the situation in *E. coli*, where a block in formylation of the initiator tRNA^{Met} leads to an essentially quantitative accumulation of the tRNA as Met-tRNA^{Met} (24, 53). The ester linkage between the methionine and the tRNA is known to be more labile than that between formylmethionine and tRNA (38). Therefore, one possible explanation for the above result is that methionine is cleaved off the Met-tRNA^{Met} during the prolonged workup (6 h or longer) necessary for the isolation of yeast mitochondria prior to isolation of the mitochondrial RNA. To test this possibility, we probed another blot of the mitochondrial RNA preparation with an oligonucleotide complementary to yeast mitochondrial tRNAfMet. The results (Fig. 3) show that while this tRNA is present mostly in the...
a substantially amount of uncharged tRNA{sup}Tyr{sub}.

The difference between the extent of accumulation of Met-tRNA{sup}Met (Fig. 2) and Tyr-tRNA{sup}Tyr (Fig. 3) in the mitochondria of strains carrying the fmt1 and mis1 disruptions is probably due to the different stabilities of ester linkage between methionine and tRNA{sup}Met versus tyrosine and tRNA{sup}Tyr. To investigate this possibility, we prepared E. coli Tyr-tRNA{sup}Tyr and Met-tRNA{sup}Met and monitored the rates of base-catalyzed deacylation of these aminoacyl-tRNAs in 0.1 M Tris-HCl (pH 9.4) at 37°C (for details, see Materials and Methods). Radioactivity in the tRNA and aminoacyl-tRNA bands was quantified using a PhosphorImager. A plot of the percentage of residual aminoacyl-tRNA versus time (Fig. 4) showed that the ester linkage between tyrosine and tRNA{sup}Tyr is more stable (half-life of 10 min) than the ester linkage between methionine and tRNA{sup}Met (half-life of 15 min).

Growth rate of strains carrying the MIS1 and FMT1 gene disruptions. The complete absence of Met-tRNA{sup}Met in strains carrying the disruptions led us to investigate more closely whether these strains had any growth defects. A classic diagnostic test of mitochondrial function in yeast is to grow cells on nonfermentable carbon sources such as lactate or glycerol plus ethanol. Cells with defective mitochondria will grow poorly or not at all under these conditions (59).

The growth rates of the wild type and the single and double disruptants were determined in both rich and minimal media using 3% glycerol plus 2% ethanol as the nonfermentable carbon source. The mutant strains grew at nearly the same rates as the corresponding wild-type strains in rich medium (YPEG) (Table 2). When the strains were grown in a synthetic minimal medium on the glycerol-ethanol carbon source (YPEG), the mis1-disrupted strain (DAY4{sup}mis1) had a significantly longer lag time, although after about 80 h it achieved a growth rate approaching that of its wild-type parent, DAY4 (9.1- and 6.4-h doubling times, respectively [Fig. 5 and Table 2]). The fmt1-disrupted strain (strain 1052) grew similarly to its wild-type parent (strain 1001) (8.3- and 6.4-h doubling times, respectively). To confirm that the longer lag observed for DAY4{sup}mis1 on YMEG was due to loss of mitochondrial C1-THF synthetase, a plasmid carrying the wild-type MIS1 gene (YEpKS17) was introduced into the mutant strain. This plasmid also carries the URA3 gene, complementing the ura3-52 mutation in DAY4{sup}mis1. As a control, DAY4{sup}mis1 was transformed with another URA3 plasmid (pVT101U) that lacks the MIS1 gene. The plasmid-borne MIS1 gene completely rescued the long lag of DAY4{sup}mis1, whereas pVT101U had no effect on lag time (data not shown). One explanation for the lag observed in DAY4{sup}mis1 is that the mutation does limit growth on nonfermentable carbon sources and the eventual attain-

![FIG. 3. RNA blot hybridization of mitochondrial tRNA{sup}Tyr from wild-type (lane 1) and fmt1-disrupted (lane 2) strains of yeast. Other details are as in the legend to Fig. 2. Lane 3 contains the deacylated wild-type control. The blot was probed with an oligonucleotide complementary to mitochondrial tRNA{sup}Tyr.](http://jb.asm.org/)
ment of a normal growth rate is due to the appearance of cells harboring a second mutation that suppresses the growth defect of the \textit{mis1} disruption. This possibility was tested by harvesting cells from the DAY4\text{\textbar}mis1 culture at the end of the experiment in Fig. 5, and repeating the growth curve determination with cells grown in fresh YMEG. The same lag was observed (data not shown), ruling out the selection of a revertant or a second-site mutation. Thus, it seems likely that the pronounced lag time in growth seen with the \textit{mis1}-disrupted strain is more a reflection of a nutritional limitation than an effect on initiation of protein synthesis.

The \textit{mis1} \textit{fmt1} double-disruption strain (WHY2) also grew at nearly wild-type rates on glycerol plus ethanol on both rich (5-h doubling time) and minimal (8-h doubling time) media. Interestingly, on YMEG, WHY2 did not exhibit the long lag phase observed for DAY4\text{\textbar}mis1 and reached stationary phase at nearly wild-type rates on glycerol plus ethanol on both rich and minimal media containing glucose, indicating that cytoplasmic 10-formyl-THF does not enter the mitochondria to any significant extent. Similarly, disruption of the \textit{FMT1} gene in the \textit{mis1}-disruptant indicates that cytoplasmic 10-formyl-THF does not enter the mitochondria to any significant extent. Similarly, disruption of the \textit{FMT1} gene resulted in a total lack of formylation of the \textit{Met}-tRNA\textsuperscript{Met} in vivo. Besides providing strong support to the assumption that the \textit{S. cerevisiae} YBL013W ORF codes for the mitochondrial methionyl-tRNA formyltransferase, this result also rules out the possibility of the existence of any redundant and/or alternate forms of MTF for formylation of the initiator \textit{Met}-tRNA\textsuperscript{Met}.

The \textit{fmt} gene encoding methionyl-tRNA formyltransferase has also been disrupted in eubacteria such as \textit{E. coli} and \textit{Pseudomonas aeruginosa}. In \textit{E. coli}, this mutation causes a severe growth defect but the cells remain viable (18). In \textit{P. aeruginosa}, the disruption causes a less severe but still significant effect on the rate of cell growth, a 3-fold increase in doubling time for \textit{P. aeruginosa} compared to a 10-fold increase for \textit{E. coli} (31). In contrast, the growth rate of the \textit{fmt1}-disrupted yeast strain is essentially the same as that of the parental wild-type strain in both rich and minimal media containing glycerol and ethanol as the nonfermentable carbon sources. Furthermore, there was essentially no difference between the parental and \textit{fmt1}-disrupted strain in the frequency of formation of \textit{petite} colonies. These results suggest that the overall rates of protein synthesis in yeast mitochondria are not very different when initiated with \textit{fMet}-tRNA\textsuperscript{Met} versus \textit{Met}-tRNA\textsuperscript{Met}.

How is mitochondrial protein synthesis initiated in yeast lacking \textit{fMet}-tRNA\textsuperscript{Met}\textsuperscript{Met}? Genetic studies with eubacteria may provide some clues. Strains of \textit{Streptococcus faecalis} and mutant strains of \textit{E. coli} are known that can grow in media free of folic acid and its coenzymes, initiating protein synthesis with unformylated \textit{Met}-tRNA\textsuperscript{Met} (4, 36). These strains contain a tRNA\textsuperscript{Met} that is lacking in one of the base modifications, with uridine instead of ribothymidine (T) found in loop IV (also called the T loop) of all tRNAs (10). The absence of T in tRNAs from \textit{S. faecalis} grown in folate-free medium occurs because the source of the methyl group for the enzymatic methylation of U to T in tRNA is 5,10-methylene-THF in \textit{S. faecalis}, \textit{Bacillus subtilis}, and presumably other gram-positive eubacteria (45). In contrast, in \textit{E. coli}, as in many other organisms studied to date, the methyl group donor for this reaction is S-adenosylmethionine. The mutant \textit{E. coli} strain that can grow in the absence of folate is also partially lacking in T, but this is due to reduced activity of the tRNA uracil 5-methylase in the mutant strain (4). Preliminary results indicated that this mutant strain also overproduces initiation factor IF2 by about

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Strain & \% of petite derivatives in: & Tetrazolium assay \\
& 0.1% glucose assay & \\
\hline
1001 & 1 & 3.5 \\
1052 & 0.5 & 1.4 \\
DAY4 & 2.5 & 4.5 \\
DAY4\text{\textbar}mis1 & 4.3 & 4.1 \\
\hline
\end{tabular}
\caption{Frequency of \textit{petite} derivatives in various yeast strains}
\end{table}
three- to fourfold. The absence of T in tRNA\textsuperscript{Met} from two different organisms, S. faecalis and E. coli, that grow without requiring formylation of the initiator Met-tRNA\textsuperscript{Met} suggests that replacement of T with U in the tRNA\textsuperscript{Met} somehow enables the initiator Met-tRNA\textsuperscript{Met} to initiate protein synthesis without formylation.

It is unlikely that a similar “undermodification” of U to T in yeast mitochondrial initiator tRNA is responsible for initiation with Met-tRNA\textsuperscript{Met}. While N. crassa mitochondrial initiator tRNA normally contains U in place of T in loop IV (20), the S. cerevisiae mitochondrial initiator tRNA is known to contain T (45). S. cerevisiae has a single gene (TRM2) for tRNA uracil-5 methylase, which encodes both the cytoplasmic and mitochondrial forms of the enzyme (21). However, this enzyme is known to use S-adenosylmethionine for methylation of U to T (21). Therefore, both the mitochondrial misl- and fmitl-disrupted strains of S. cerevisiae would be expected to contain a full complement of the base modifications in their tRNAs, including the T in loop IV.

It is possible that disruption of MSL1 or FM1 genes results in overproduction of the yeast mitochondrial IF2 (IF\textsubscript{2mt}) and that this compensates for the lack of formylation of the initiator Met-tRNA\textsuperscript{Met} (4). This would mean that the yeast IF\textsubscript{2mt} is capable of interacting with unformylated Met-tRNA\textsuperscript{Met} in vivo. Yeast IF\textsubscript{2mt} has not been identified biochemically; however, the IFM1 gene (5) encodes a protein with significant homology to the human IF\textsubscript{2mt} (27) and disruption of the IFM1 gene causes a defect in mitochondrial protein synthesis, resulting in the petite phenotype (51), suggesting that the IFM1 gene product is important for mitochondrial protein synthesis. In contrast to yeast IF\textsubscript{2mt}, bovine IF\textsubscript{2mt} has been purified and shown to promote the binding of fMet-tRNA to mitochondrial ribosomes in a GTP- and AUG-dependent manner (26). Bovine IF\textsubscript{2mt} is reported to be inactive with unformylated Met-tRNA\textsuperscript{Met} in vitro (26).

Protein synthesis is initiated with formylmethionine in the mitochondria of a wide range of organisms from fungi to mammals. The finding that protein synthesis in S. cerevisiae can be initiated with methionine using an unformylated Met-tRNA\textsuperscript{Met} and without any significant effect on the overall growth rate in nonfermentable media requiring mitochondrial protein synthesis suggests that at least in S. cerevisiae, the role of formylation of the initiator Met-tRNA\textsuperscript{Met} is quite subtle. Given the strong conservation of initiator Met-tRNA formylation in mitochondria from such a wide range of organisms, it is likely that formylation of the mitochondrial initiator Met-tRNA provides at least an incremental advantage to the cell. The retention of initiator tRNA formylation in mitochondria of S. cerevisiae would thus be an example of what has been called “the ruthless delicacy of the selection” (48), which ensures the strict conservation, across a wide phylogenetic spectrum, of a feature that provides even the slightest advantage to the organism.

Finally, our finding that S. cerevisiae can grow quite well without formylation of the mitochondrial initiator Met-tRNA raises the question whether other eukaryotic cells will behave similarly. The yeast S. cerevisiae is, in many respects, an exception among eukaryotes in terms of mitochondrial function. First, S. cerevisiae, unlike most other eukaryotes, is a facultative anaerobe and can grow without mitochondrial function. Second, S. cerevisiae mitochondrial DNA encodes fewer species of mitochondrial membrane proteins than in other eukaryotes (15). For example, it does not encode any of the components of the multisubunit enzyme NADH-ubiquinone oxidoreductase (NADH dehydrogenase [ND]) whereas N. crassa and animal cell mitochondrial DNAs encode at least six or seven of the ND subunits (2, 7). In bovine heart mitochondria, besides cytochrome oxidase subunits I and II and the mitochondrially made subunits of ATPase (8, 46, 55), all of the mitochondrially made ND subunits are thought to retain the formylmethionine residue at the N terminus (J. E. Walker, personal communication). Therefore, formylation of the initiator Met-tRNA could well be important in beef heart mitochondria, although the retention of formylmethionine could also be simply due to the lack of a peptide deformylase activity in mitochondria. It would clearly be interesting to study whether formylation of the mitochondrial initiator Met-tRNA is more important in the mitochondria of Neurospora and animal cells than in S. cerevisiae.

Acknowledgments

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42. Skala, J., L. van Dyck, B. Purnelle, and A. Goffeau. 1992. The sequence of the 8 kb segment on the left arm of chromosome II from \textit{Saccharomyces cerevisiae} identifies five new open reading frames of unknown functions, two tRNA genes and two transposable elements. Yeast 8:777–785.


