The Membrane-bound GTPase Guf1 Promotes Mitochondrial Protein Synthesis under Suboptimal Conditions*‡§

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Recently, the bacterial elongation factor LepA was identified as critical for the accuracy of in vitro translation reactions. Extremely well conserved homologues of LepA are present throughout bacteria and eukaryotes, but the physiological relevance of these proteins is unclear. Here we show that the yeast counterpart of LepA, Guf1, is located in the mitochondrial matrix and tightly associated with the inner membrane. It binds to mitochondrial ribosomes in a GTP-dependent manner. Mutants lacking Guf1 show cold- and heat-sensitive growth defects on non-fermentable carbon sources that are especially pronounced under nutrient-limiting conditions. The cold sensitivity is explained by diminished rates of protein synthesis at low temperatures. At elevated temperatures, Guf1-deficient mutants exhibit defects in the assembly of cytochrome oxidase, suggesting that the polypeptides produced are not functional. Moreover, Guf1 mutants exhibit synthetic growth defects with mutations of the protein insertase Oxa1. These observations show a critical role for Guf1 in vivo. The observed defects in Guf1-deficient mitochondria are consistent with a function of Guf1 as a fidelity factor of mitochondrial protein synthesis.

Cells of animals and fungi contain two translation machineries, one in the cytosol and one in the mitochondrial matrix. Whereas the cytosolic machinery synthesizes thousands of different polypeptides, the mitochondrial one produces only a handful of translation products. In most animal cells, including humans, 13 different proteins are synthesized on mitochondrial ribosomes, whereas the yeast *Saccharomyces cerevisiae* only eight proteins are mitochondrially encoded (1). These translation products constitute the membrane-embedded cores of the respiratory chain complexes and the F$_{1}$F$_{0}$-ATPase and hence are essential for respiratory activity of eukaryotic cells. Despite this extreme discrepancy in their number of products, both machineries are similarly complex. The number of proteins that constitute the mitochondrial ribosome even exceeds the number of the cytosolic 80 S ribosome (2, 3). Many factors were identified over the last decades that assist or coordinate mitochondrial protein synthesis. Little is known about the specific molecular function of most of these components. In contrast to other translation machineries, that of mitochondria is intimately coupled to the inner membrane, which might explain why a membrane-free in vitro translation system with mitochondrial ribosomes could not be established thus far (4–8). The lack of such in vitro systems is one of the reasons why the molecular roles of most of these factors are still elusive.

The translation machinery of mitochondria resembles that of eubacteria in a number of respects. The mitochondrial ribosome consists of two RNA subunits and ~70–80 different proteins. The rRNAs are related to the bacterial counterparts 16 S and 23 S, and more than half of the protein components of the mitochondrial ribosome have homologues in bacteria (9, 10). Elongation of translation is energetically driven by three GTPases, Mef1, Mef2, and Tuf1 (11–14). Tuf1 is the homologue of EF-Tu, which delivers the aminoacyl-tRNA to the A site of the ribosome. Mef1 and Mef2 are related to the bacterial elongation factor $G$ (EF-$G$), which transfers peptidyl-tRNA from the A to the P site of the ribosome. In *S. cerevisiae* mitochondria, a homologue of the nucleotide-exchange factor EF-Ts is missing. Besides EF-$G$ and EF-Tu, bacteria contain a third highly conserved GTPase, named LepA, that is proposed to be involved in translational elongation (15–18). In contrast to the other elongation factors, LepA is not essential and the deletion of LepA causes no obvious growth defects (15, 19). The function of LepA remained unclear for a long time. In a recent study it was shown that in vitro LepA stimulates the back-translocation of the ribosome along the mRNA and thereby antagonizes the function of EF-$G$ (15). Addition of increasing amounts of LepA reduced the error rate of in vitro protein synthesis assays, suggesting that LepA plays a role as a fidelity factor of the translation reaction. In *Escherichia coli* the gene of LepA is located on the same operon as that of leader peptidase. This, and the fact that LepA was reported to be membrane-associated in bacteria, suggested a role of LepA in the synthesis of membrane proteins (17). Experimental evidence for such a function in membrane protein biogenesis does, however, not exist.

LepA contains close homologues in eukaryotes, where they are named Guf1 for GTPase of unknown function 1 (20). Although these proteins were identified several years ago, their physiological role stayed elusive. Our results show that LepA/
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Guf1 plays a critical function in vivo. We present evidence that Guf1 is a mitochondrial protein that, like LepA, interacts with translating ribosomes. Mutants lacking Guf1 show reduced translation rates only at low temperature. However, Guf1 becomes important under nutrient-limiting conditions. In mitochondria of starving Guf1 deletion mutants, the rate of protein synthesis is not decreased, but only very low steady state levels of cytochrome oxidase are found. This points to a role, direct or indirect, of Guf1 in the assembly or stability of mitochondria of starving Guf1 deletion mutants, the rate of translation rates only at low temperature. However, Guf1 shows reduced translation rates only at low temperature. However, Guf1 becomes important under nutrient-limiting conditions.

EXPERIMENTAL PROCEDURES

Yeast Strains and Growth Medium—The wild type strain W303-1A (21) was used for all biochemical experiments. For growth experiments shown in Fig. 5, strains isogenic to the wild type BY4742 (22) were used. For generation of the Guf1-HA mutant, a sequence encoding three hemagglutinin (HA) epitopes and a TRP1 marker gene was amplified from the pYM22 plasmid (23) and inserted after the chromosomal GUF1 gene, resulting in the expression of the entire Guf1 protein carrying three HA epitopes at its C terminus. Yeast cultures were grown at 15, 30, or 37 °C in 1% yeast extract, 2% peptone (YP) medium supplemented with 2, 0.5, or 0.1% glucose, glycerol, or galactose.

Ribosomal Isolation—500 μg of isolated mitochondria were lysed in 1% Triton X-100, 50 mM KCl, 20 mM HEPES, pH 7.4, 1 mM phenylmethylsulfonyl fluoride, and protease inhibitor mix (Calbiochem) in the presence of 1 mM GTP, 5 mM MgCl2, 1 mM GDP and 5 mM MgCl2, or 5 mM EDTA for 15 min at 4 °C. After a clarifying spin for 5 min at 15,000 × g at 4 °C, the extracts were layered onto continuous 4 ml of 10–30% sucrose and centrifuged for 80 min at 485,000 × g in a Beckman SW60 Ti rotor at 4 °C. Fractions were collected, and proteins were precipitated with 12% trichloroacetic acid.

RESULTS

Guf1 Is the Eukaryotic Homologue of LepA—The LepA protein of E. coli was recently identified as a highly conserved GTPase involved in translational elongation (15, 28). Homologues of LepA are conserved throughout bacteria and eukaryotes (where they are referred to as Guf1) but absent from archaea (28, 29). The LepA/Guf1 family members of bacteria, plants, fungi, and animals form a separate sub-branch on the phylogenetic tree of GTPases that is distinct from the sub-branches of the elongation factors EF-G and EF-Tu (Fig. 1A). This suggests a common origin of pro- and eukaryotic LepA/Guf1 proteins after which these factors were maintained throughout the evolution of eukaryotes. This evolutionary conservation is further supported by the high degree of structural identity of Guf1 proteins with LepA. In bacteria, LepA is one of the proteins with the highest degree of sequence identity (66–93%), and only EF-Tu is even more conserved (28). The E. coli protein was reported to form five structural domains (15) that are all present in a consistent order in the Guf1 homologues of plants, fungi, and animals (Fig. 1B). Each of these domains shows a very high degree of identity to those of the LepA protein of E. coli, in the range of 35 to 65% (Fig. 1B). Their outstanding conservation throughout evolution suggests that LepA/Guf1 homologues exhibit a critical conserved function in pro- and eukaryotes.

Guf1 Is Located in the Mitochondrial Matrix—While prokaryotic LepA and eukaryotic Guf1 proteins are highly conserved, the eukaryotic proteins consistently show N-terminal extensions that are absent in LepA proteins (Fig. 1B, Pre). These sequences show the characteristics of mitochondrial targeting signals. In a genome-wide screen a fusion protein of Guf1 and green fluorescent protein was localized to mitochondria (30). To confirm the mitochondrial location of Guf1, a yeast strain was generated that expresses Guf1 with a C-terminal HA epitope tag under control of the endogenous promoter. Upon subfractionation of cells, this Guf1-HA protein was specifically recovered with mitochondria (Fig. 2A). Guf1-HA was resistant against protease in mitochondria and in mitoplasts in which the...
cells and incubated under conditions that promote active protein synthesis. Mitochondrial translation was monitored by the incorporation of $[^{35}S]$methionine into newly synthesized polypeptides. After 10 min mitochondria were reisolated, split into three samples, and further incubated in the presence of (i) GTP and ATP, (ii) GDP, and (iii) the non-hydrolyzable GTP analogue GTPγS. Subsequently, the samples were lysed and ribosomes were isolated by centrifugation through high sucrose cushions (Fig. 3A). In the ribosomal pellet fraction (Fig. 3A, $P$), proteins of the large (Mrpl36) and small (Mrps51) ribosomal subunits were recovered as well as nascent chains in the autoradiogram. Soluble proteins (Fig. 3A, Hep1) and completed translation products were found in the supernatant ($S$). In the presence of ATP and GTP, about half of the Guf1-HA was found to be associated with ribosomes (Fig. 3A, lanes 1–3). In the presence of GDP, Guf1 was exclusively recovered with the post-ribosomal supernatant (Fig. 3A, lanes 4–6) whereas GTPγS stabilized Guf1 on the ribosomes (lanes 7–9).

The ribosomal association of Guf1 was further supported by velocity centrifugation of mitochondrial ribosomes on linear sucrose gradients (Fig. 3B). In the presence of GTPγS, but not in the presence of GDP or EDTA, a fraction of Guf1 comigrated with proteins of the large and small subunits of ribosomes. The much longer migration distance of the ribosomes in these gradients led obviously to some dissociation of Guf1 even in the presence of GTPγS. In summary, we conclude that Guf1 binds to mitochondrial ribosomes in a GTP-dependent manner, consistent with a direct role of Guf1 in mitochondrial protein synthesis.

**Guf1 Promotes Mitochondrial Protein Synthesis in Low Temperature**—To assess a function of Guf1 in mitochondrial protein synthesis, we purified mitochondria from wild type and Δguf1 cells. Mitochondria were incubated in translation reactions in the presence of $[^{35}S]$methionine at 15, 30, and 37 °C for 5, 10, or 20 min. To a fourth sample, cold methionine was added after 20 min of labeling and mitochondria were further incubated for 20 min to monitor the stability of translation products. Mitochondria were reisolated and subjected to SDS-PAGE; synthesized proteins were visualized by autoradiography (Fig. 4). The mitochondrial genome encodes eight major proteins (31), which all were synthesized both in wild type and Δguf1 mitochondria. The overall pattern of translation products was comparable between both strains, suggesting that the synthesis of none of the mitochondrionally encoded proteins strictly depended on Guf1 under the conditions of the experiment. However, at 15 °C, the translation efficiency in Δguf1 mitochondria was significantly lower than in wild type. This deficiency apparently affected the synthesis of all mitochondrially encoded proteins. In contrast, at 30 and 37 °C the translation efficiencies were not diminished and for Var1, the only soluble mitochondrial translation product, the synthesis was even increased in the absence of Guf1 (Fig. 4, right panels). This suggests that Guf1 is critical to promote protein synthesis at low temperature but at normal or elevated temperatures Guf1 is largely dispensable for the translation reaction per se.

**Deletion of Guf1 Leads to Reduced Growth at Suboptimal Temperature and under Starvation Conditions**—In an initial study it was reported that the deletion of GLF1 does not cause
FIGURE 3. Guf1 binds to mitochondrial ribosomes in a GTP-dependent manner. A, translation products were radiolabeled in isolated mitochondria for 10 min at 25 °C. The reaction was split into three aliquots to which the following reagents were added: 2 mM ATP, 1 mM GTP (lanes 1–3); 1 mM GDP (lanes 4–6); or 1 mM GTPγS (lanes 7–9). After a second incubation for 10 min at 25 °C, mitochondria were reisolated and lysed in 1% Triton X-100, 50 mM KCl, 5 mM MgCl2, 20 mM Tris/HCl, pH 7.5, keeping the corresponding nucleotide conditions as above. 10% of the samples were directly applied to the gel (lanes 1–3). The radioactive signals of the synthesized products were analyzed on a phospho-imager (Fuji BAS-5000), quantified using AIDA quantification software package (Raytest), and are shown on the right. Solid lanes show the proteins synthesized in wild type mitochondria and dashed lines those of Δguf1 mitochondria. The y-axis depicts relative intensities for which values are represented in relation to the strongest signals. The x-axis represents the times of translation as used in the experiment.

B, mitochondria were labeled with [35S]methionine under translation-promoting conditions. Aliquots were taken after 5, 10, and 20 min as indicated. To one sample, an excess of cold methionine was added and the reaction was further incubated for 20 min (Postinc., postincubation). Mitochondria were reisolated from the samples. The synthesized proteins were visualized by SDS-PAGE and autoradiography (left panels). The radioactive signals of the synthesized products were analyzed on a phospho-imager (Fuji BAS-5000), quantified using AIDA quantification software package (Raytest), and are shown on the right. Solid lanes show the proteins synthesized in wild type mitochondria and dashed lines those of Δguf1 mitochondria. The y-axis depicts relative intensities for which values are represented in relation to the strongest signals. The x-axis represents the times of translation as used in the experiment.

FIGURE 4. Guf1-deficient mitochondria show reduced protein synthesis at 15 °C. Mitochondria from wild type (wt) and Δguf1 cells were incubated at 15 °C (top), 30 °C (middle), and 37 °C (bottom) in the presence of [35S]methionine under translation-promoting conditions. Aliquots were taken after 5, 10, and 20 min as indicated. To one sample, an excess of cold methionine was added and the reaction was further incubated for 20 min (Postinc., postincubation). Mitochondria were reisolated from the samples. The synthesized proteins were visualized by SDS-PAGE and autoradiography (left panels). The radioactive signals of the synthesized products were analyzed on a phospho-imager (Fuji BAS-5000), quantified using AIDA quantification software package (Raytest), and are shown on the right. Solid lanes show the proteins synthesized in wild type mitochondria and dashed lines those of Δguf1 mitochondria. The y-axis depicts relative intensities for which values are represented in relation to the strongest signals. The x-axis represents the times of translation as used in the experiment.

any detectable growth defect (20). This is in agreement with our observation that at 30 °C, i.e. the standard growth temperature for S. cerevisiae, Δguf1 strains grew almost like wild type on fermentable (not shown) and non-fermentable (Fig. 5A) carbon sources. However, at high temperatures (40 °C) the doubling time of the Δguf1 strain on non-fermentable carbon source was significantly longer than for wild type cells (Fig. 5A). Similarly, at low temperatures (15 °C) the growth rate of the Δguf1 strain was reduced.

To determine the growth characteristics in more detail, a variety of different media and conditions was tested, like the addition of different salts, antibiotics, or pH values (data not shown). Most of the conditions were tolerated by the Δguf1 strain. However, when the amount of non-fermentable carbon sources was reduced to growth-limiting conditions Δguf1 cells were much more strongly affected than the wild type already at 30 °C (Fig. 5B). This effect was most prominent in liquid medium but also detectable on plates (Fig. 5C). Although no growth differences were observed on glucose-containing medium, in medium containing non-fermentable carbon...
**Guf1 Improves the Functionality of Translation Products at High Temperature**—The observed relevance of Guf1 at low and high temperature inspired us to assess the expression of Guf1 at different temperatures. Guf1-HA cells were grown at 15 °C, 30 °C, and 37 °C, and the amounts of Guf1-HA were tested by Western blotting (Fig. 6A). For control, the membrane was probed with antibodies against the ribosomal protein Mrp136 and the heat-inducible matrix protein Hep1. We observed that Guf1-HA was up-regulated at 15 °C and even more at 37 °C, supporting the importance of Guf1 at non-optimal temperatures.

To identify the molecular reason for the observed defects of Δguf1 cells, we measured the enzyme activities of the bc1 complex and of cytochrome oxidase (both containing mitochondrially encoded subunits) and of malate dehydrogenase (containing exclusively nuclear encoded subunits) (Fig. 6B). Although the activity of the cytochrome bc1 complex and of malate dehydrogenase was not altered in Δguf1 mitochondria, the activity of cytochrome oxidase was reduced to ~10%, explaining the impaired growth phenotype of these cultures on non-fermentable carbon sources.

The reduced levels of cytochrome oxidase in Δguf1 mitochondria might be caused by a defective synthesis or a diminished insertion of nascent chains into the inner membrane. The Oxa1 complex plays a critical role for the biogenesis of mitochondrial translation products, especially for the insertion and assembly of subunits of the cytochrome oxidase (Fig. 6C). Subunit 2 of cytochrome oxidase, Cox2, is the mitochondrial translation product that is most sensitive to defects in Oxa1 (32). To test whether Guf1 is critical for Oxa1-dependent membrane insertion of Cox2, we radiolabeled translation products in isolated mitoplasts and treated the mitoplasts with protease to digest membrane-embedded proteins (Fig. 6D). Both in wild type and in Δguf1 mitochondria, most translation products were degraded upon addition of protease. Exceptions were Var1, which is the only matrix protein encoded in mitochondria, and subunits 8 and 9 of the ATPase, which both are deeply embedded in the membrane. Mitoplasting was controlled by Western blotting using the inner membrane protein Tim50 and the matrix protein mHsp70. Because we did not observe any differences between wild type and Δguf1 mitochondria, we conclude that defects in the membrane integration of subunits of cytochrome oxidase do not account for the low amounts of cytochrome oxidase in Δguf1 mutants. Similarly, cross-linking experiments revealed an efficient interaction of Oxa1 with nascent chains in Δguf1 mitochondria (supplemental Fig. S2).

The mitochondrial translation machinery is intimately coupled to the insertion apparatus in the inner membrane, mainly due to a physical contact of the C terminus of Oxa1 to ribosomes (5–7) (Fig. 6C). Mutants in which only the C-terminal 71

**FIGURE 5. Δguf1 mutants show reduced growth at suboptimal conditions.** A, wild type and Δguf1 cells of the BY4742 background were grown in liquid YP medium containing 2% glycerol at the temperatures indicated. The minimal doubling time was calculated for each sample. Shown are means and standard variations of three independent measurements. B, YP media containing 2, 0.5, and 0.1% glycerol were inoculated with overnight cultures of wild type and Δguf1 cells and incubated under agitation at 30 °C, and optical densities were measured at 600 nm after the times indicated. Note that the scaling of the y-axes is different in the three panels. C, wild type and Δguf1 cells were grown at 30 °C on YP medium with 2% galactose to mid-log phase and diluted to an optical density of 0.5. 10-fold serial dilutions of the cultures were spotted on YP plates containing the carbon sources indicated. The plates were incubated at 30 and 37 °C for 2 and 3 days, respectively.
residues of Oxa1 are deleted show a diminished ribosome binding of Oxa1 but are still able to grow on non-fermentable carbon sources (5). To test for a genetic interaction of Guf1 and Oxa1, we constructed strains in which Guf1 and the C terminus of Oxa1 were individually or simultaneously deleted. As shown in Fig. 6E, we observed a synthetic growth defect of the double mutant that was most pronounced under nutrient-limiting conditions. These observations suggest that the combination of the defects of Oxa1ΔC and Δguf1 reduces the amounts of functional cytochrome oxidase they need for respiratory growth too much to allow respiratory growth of yeast cells.

It was shown previously that at elevated temperatures translation products tend to form insoluble membrane-associated aggregates (24, 33). To test the accumulation of such aggregates we radiolabeled mitochondrial translation products in wild type and Δguf1 mitochondria that were isolated from starvation cultures. The labeling reaction was carried out at 15, 30, or 37 °C before the mitochondria were lysed with detergent and fractionated into pellet and soluble fractions (Fig. 6F). Especially subunit 2 of cytochrome oxidase, Cox2, was found in the insoluble pellet fractions. Hardly any soluble Cox2 protein was found in Δguf1 mitochondria after translation at 30 or 37 °C (Fig. 6F, arrowheads). This indicates that the absence of Guf1 leads to an increased aggregation at least of this translation product. In addition, we observed the accumulation of a small translation product in Δguf1 mitochondria at 30 and 37 °C that aggregated very efficiently (Fig. 6F, asterisk). This product presumably represents the recently identified aberrant splice variant of the COX1 gene, mp15, which is characteristic for mutants defective in assembly of cytochrome oxidase (34). Both the aggregation of Cox2 and the accumulation of the mp15 product point to a diminished quality of the proteins synthesized in Δguf1 mitochondria at elevated temperatures.

**DISCUSSION**

In contrast to the cytosolic translation reaction, the processes underlying mitochondrial protein synthesis are still poorly understood. Here we describe the so far uncharacterized GTPase Guf1 that, in sequence, is related to the classical translation factors EF-Tu and EF-G (Tufl, Mefl, and Mef2 in mitochondria). *In vitro* studies on the bacterial translation reaction, the processes underlying mitochondrial protein synthesis are still poorly understood. Here we describe the so far uncharacterized GTPase Guf1 that, in sequence, is related to the classical translation factors EF-Tu and EF-G (Tufl, Mefl, and Mef2 in mitochondria). *In vitro* studies on the bacterial
homologue of Guf1, LepA, indicated a role of this protein in the fidelity of the translation reaction. LepA directly interacts with the bacterial ribosomes in a GTP-dependent manner and reduces the probability of translation errors in in vitro translation assays (15, 35). Comparable in vitro assays to monitor mitochondrial protein synthesis could not be established thus far, presumably due to the intimate association of the mitochondrial translation machinery with the inner membrane (5–8). This restricts the biochemical analysis of the molecular role of Guf1 to studies in vivo and in isolated organelles. Guf1 is structurally very similar to E. coli LepA; both show an identity over the entire sequence of 47.9%. This is an exceptionally high degree of sequence conservation between a bacterial and a mitochondrial orthologue, given the ~1.5 billion years of separate evolution, suggesting a highly conserved function of LepA and Guf1. We report here that Guf1, like LepA, is able to bind to ribosomes in a GTP-dependent manner. When mitochondria were incubated under conditions favoring protein synthesis, we found approximately half of the mitochondrial Guf1 bound to ribosomes. The addition of GTP-γS locked also the residual Guf1 in a tightly ribosome-bound state, whereas Guf1 was completely released into the supernatant by addition of GDP. Thus, under translation conditions, a significant fraction of Guf1 is associated with ribosomes. The binding of Guf1 to the mitochondrial translation machinery in a dynamic, GTP-dependent fashion is consistent with a LepA-like function as a fidelity factor for mitochondrial translation.

However, the physiological relevance of both LepA and Guf1 remained unclear, because initial studies reported no detectable growth defects in E. coli or yeast deletion mutants under normal laboratory cultivation conditions (19, 20). We also observed no growth defects on standard growth conditions, but the growth of guf1 deletion strains on non-fermentable carbon sources at low or high temperatures was significantly retarded. This growth defect was especially striking under starvation conditions, indicating that Guf1 becomes critical when nutrients are limiting. Similarly, studies by Kurland and co-workers (36) showed that high translational proofreading activities are not necessarily beneficial but, rather, can considerably reduce growth rates of bacterial cultures under certain conditions.

Guf1 was found to be tightly associated with the inner membrane of mitochondria. Similarly, LepA was found to be membrane-associated in E. coli (17). In contrast, EF-G and EF-Tu are soluble factors in both bacteria and mitochondria. The presence of the LepA gene on the same operon as that of leader peptides in E. coli led to the speculation that LepA plays a role in the biogenesis of membrane-inserted or -secreted proteins (16, 18). Experimental evidence for a specific role of LepA in the synthesis of membrane proteins was, however, lacking so far. Here, we show that Guf1 is critical for the production of functional membrane proteins. The observed growth defect is presumably caused by strongly reduced levels of cytochrome oxidase in the absence of Guf1. The molecular reason for the low cytochrome oxidase levels is not entirely clear. Both the amount of synthesized proteins and their membrane integration were not affected. Our observations suggest rather that the produced translation products fail to form functional and stable cytochrome oxidase complexes. This is reminiscent of experiments with bacterial in vitro translation reactions that showed that LepA had only a moderate effect on the amount of synthesized green fluorescent protein but the lack of LepA significantly reduced the fraction of produced proteins that were functional (15). Thus, our observations are compatible with the proposed role of LepA/Guf1 as a fidelity factor for translational elongation (15, 35).

If LepA/Guf1 GTPases perform such an important function as controlling the quality of the translation reaction, why are Δguf1 mutants then not more severely affected? It was suggested that mitochondrial translation products are produced in excess over their nuclear encoded assembly partners (37). Under normal conditions a large fraction of the initially synthesized proteins are degraded (38). Although the degradation of newly synthesized polypeptides seems not economical, the selection of functional translation products is presumably important to prevent the generation of potentially harmful variants of respiratory chain complexes. How this selection system specifically recognizes aberrant translation products is not known, but it seems likely that this selection is functionally and kinetically coupled to the complicated assembly process of mitochondrial translation products. This system of selection and degradation of aberrant proteins may prevent the incorporation of malfunctioning and potentially dangerous subunits into respiratory chain complexes. When, however, during starvation conditions cells are forced to use their energy supplies efficiently, an increased error rate of the translation process might become intolerable, explaining the pronounced growth defect of Δguf1 cells on nutrient-limiting conditions.

Recent studies on mammalian model systems suggest that the error rate of mitochondrial gene expression has a strong impact on health and directly contributes to the physiological consequences of the aging process of animals (39). It will be exciting in the future to assess the relevance of Guf1 for the accuracy of the translation process in mammalian mitochondria and its potential relevance for the aging process of animals and humans.

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