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Published in:

DOI:
10.1007/BF00986960

Citation for published version (APA):

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Protein synthesis in mitochondria

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Received 18 December 1993; accepted 21 February 1994

Key words: mitochondria, protein synthesis, yeast

Introduction

Mitochondria and chloroplasts are unique among eukaryotic organelles in possessing their own genome together with the machinery necessary to express the information contained within it. The mitochondrial genetic system is required for the synthesis of a limited number (in the range of 7 to 13, dependent on the organism) of protein subunits of enzyme complexes of the inner membrane that are involved in respiration and oxidative phosphorylation [1–3]. Additional single genes encode components of the mitochondrial expression system itself, e.g. ribosomal RNAs, transfer RNAs [4, 5], proteins involved in the processing of mitochondrial transcripts [6] or ribosomal proteins [7]. The total number of mitochondrial translation products varies from 8 in certain yeasts [6], 13 in mammals [8] to about 20 in plants [2].

The mitochondrial expression system most extensively studied to date is that of the yeast Saccharomyces cerevisiae. This organism is highly suitable for genetic analyses of mitochondrial gene expression, since mutations in, or even a complete deletion of its mitochondrial genome lead to a respiratory–deficient phenotype, but do not affect its viability on fermentable carbon sources (for recent reviews covering many aspects of yeast mitochondrial biogenesis see [1, 9, 10]). Moreover, mutational analysis of the mitochondrial translation apparatus is greatly facilitated by the fact that all components identified so far are encoded by single–copy genes. In this review we will focus mainly on components of the yeast mitochondrial translation machinery identified so far. Some mutations recently identified in human mtDNA that affect components of the mitochondrial translational apparatus are also briefly discussed.

General features of the mitochondrial translation system

Mitochondrial translation systems more closely resemble their prokaryotic than their eukaryotic cytoplasmic counterparts in a number of respects. First, the spectrum of antibiotics inhibiting mitochondrial translation parallels that found in prokaryotes [11]. Second, components of the mitochondrial translation machinery that have homologs in other translation systems are consistently more similar to bacterial than to the corresponding eukaryotic cytoplasmic counterparts. Besides these similarities, the study of mitochondrial translation systems has disclosed a number of unusual features.

1. Mitochondrial genetic systems of various species use some codon assignments at variance with the ‘universal’ genetic code and with each other (see [12–14] for reviews).

2. Mitochondria use a restricted number of tRNAs that is far below the minimum number necessary to translate all the codons of the genetic code according to the wobble hypothesis, a feature that has its origins in an expansion of the normal codon recognition pat-
ter. For example, in *S. cerevisiae* mitochondria, single tRNAs are able to decode all four triplets in a four-codon family due to the presence of an unmodified uridine in the first anticodon position. Two-codon families ending in a purine are recognized by single tRNAs containing 5-[[(carboxymethyl)amino]methyl]uridine (pcmmnm5U) in the wobble position [15]. Organisms other than yeast probably employ a similar mechanism to prevent misreading of pyrimidine at the third codon position [15].

3. Despite their high similarity to prokaryotic ribosomes, mitoribosomes display several unusual structural features, as will be outlined in the next paragraph.

4. Translation in yeast mitochondria depends on the action of a number of messenger-specific translational activators. Properties and possible functions of these factors are discussed in subsequent sections of this review.

The mitochondrial ribosome

Mitochondrial ribosomes of various species differ markedly from each other [7, 13, 16]. A typical 5S rRNA has been found only in plant mitochondrial large ribosomal subunits; the other ribosomal RNAs show an extreme variability in length (the small rRNA varies from about 600 nucleotides in flagellates, 950 in mammals to 2000 nucleotides in plants; [106]). Mitoribosomes of a variety of organisms possess smaller sedimentation coefficients as compared to those of cytoplasmic ribosomes of the same organism, a feature that is explicable in terms of a higher protein to RNA ratio found in mitoribosomes [7]. For example, mammalian mitoribosomes have a protein content that is almost three times higher than that of *Escherichia coli* ribosomes [13, 17]. Generally speaking, mitoribosomes contain more and also different ribosomal proteins compared to prokaryotic and eukaryotic cytoplasmic ribosomes [7]. Of the 23 yeast mitoribosomal proteins whose primary sequence has been elucidated to date, 10 contain stretches of amino acids that display similarity to known *E. coli* ribosomal proteins ([7, 18] and references therein). It has been speculated that the yeast mitoribosome consists of a core structure of homologous components, responsible for carrying out central steps of protein synthesis common to both eubacteria and mitochondria, and an additional set of proteins with a more specialized function in mitochondrial protein synthesis [19]. In this respect it is worth noting that none of the three mitoribosomal proteins (PET123, MRP1, MRP17) found to functionally inter-

act with the unique mRNA–specific translational activator PET122 (see below) displays homology to other known ribosomal proteins [20–22].

Several of the yeast mitoribosomal proteins that display sequence similarity to eubacterial ribosomal proteins are considerably larger than their prokaryotic homologs due to amino- and/or carboxy-terminal extensions lacking sequence similarity. Recent analysis of the various sequence domains of MrpS28, the yeast mitochondrial homolog of *E. coli* S15, demonstrates that an amino–terminal extension of 117 amino acids and the S15–like domain are both essential for respiratory growth [23]. Surprisingly, both regions can functionally complement each other in trans, suggesting that each fragment facilitates the incorporation of the other into 37S ribosomal subunits [23]. The S15–like domain of MrpS28 partially complements the cold–sensitive phenotype of an *E. coli* mutant producing reduced levels of S15 [24]. The MrpS28–specific domain is inactive by itself. However, in cis it enhances the activity of the S15–like domain [24]. On the one hand these data underline the similarity between the yeast mitochondrial and *E. coli* ribosome. On the other, they also imply that functions present in an *E. coli* ribosomal protein can be split in two separate domains in a larger mitochondrial homolog. Whether this organization applies to more of the extended mitoribosomal proteins remains to be seen.

General components of mitochondrial translation systems

Due mainly to the ease with which yeast mutants affected in mitochondrial biogenesis can be isolated and complemented, most of the genes encoding components of the mitochondrial translational apparatus have been identified in the yeast *S. cerevisiae*. Interestingly, three yeast genes involved in post–transcriptional modification of mitochondrial tRNAs, *TRM1, TRM2* and *MOD5*, are also responsible for the modification of cytosolic tRNAs. Both *TRM1* and *MOD5* encode proteins with a dual location in the cell. The coding sequences contain two in–frame initiation codons whose use results in the production of two proteins that differ only at their amino–terminus, the longer product being more efficiently imported into mitochondria [25–27]. A dual location has also been reported for the valyl– and histidyl–tRNA synthetases, two of the ten *S. cerevisiae* mitochondrial tRNA synthetases identified so far (reviewed in [28]). Surprisingly, the yeast mitochondrial leucyl– as well as the Neurospor-
ra crassa mitochondrial tyrosyl-tRNA synthetase (and more recently also the homologous Podospora anserina mitochondrial tyrosyl-tRNA synthetase, [29]) have been implicated in a second process, the splicing of one or more group I introns [30, 31]. The RNA binding properties of these proteins are presumably exploited to stabilize a catalytically–competent conformation of unspliced precursor RNAs [32].

Genes encoding yeast mitochondrial homologs of prokaryotic initiation factor IF–2, elongation factors EF–Tu and EF–G, as well as termination factor RF–1 have been identified [33–35]. Recently, also a first mammalian gene encoding a general mitochondrial translation factor, the rat mitochondrial elongation factor G, has been cloned and sequenced [36]. All these factors are more similar to their prokaryotic than their eukaryotic cytoplasmic counterparts (although a cytoplasmic termination factor has yet to be identified). Of the encoded proteins, only mEF–Tu and mEF–G have been studied biochemically. The properties of these and several other purified mammalian mitochondrial translation factors are discussed in later sections dealing with the various phases of translation.

Recently a new yeast gene that seems to play a role in mitochondrial translation has been identified. The gene, MSS1, was cloned by complementation of an unusual yeast mutation that causes respiratory–deficiency only when combined with a mutation conferring resistance to the aminoglycoside antibiotic paromomycin (par R454, located in the gene for the mitochondrial 15S rRNA in a region implicated in translational decoding; [37]). Inactivation of the MSS1 gene gives essentially the same phenotype as the original mutation, thus confirming its identity with the mutant locus. Analysis of mitochondrial translation products of various mss1 mutants containing par R454 revealed, depending on both mutant allele and genetic background of the strain used, only a diminution of cytochrome c oxidase subunit 1 or severely reduced amounts of all major mitochondrial translation products. The latter effect suggests that MSS1 may play a general role in mitochondrial translation. Mitochondrial revertants of three mss1 mutants have been isolated and turn out to be second–site mutations in the gene encoding 15S rRNA. Taken together, these data indicate that MSS1 functionally interacts with the small ribosomal subunit. The protein encoded by MSS1 contains a sequence motif characteristic for a number of GTPases and displays a high sequence similarity to the products of so–called ‘50K’ genes in E. coli, Bacillus subtilis and Pseudomonas putida [38]. The 50K gene of E. coli is known to be essential [38], but its function has not yet been established (however, according to release 25 of the SwissProt database, the 50K genes may play a role in ‘oxidation of thiophene and furan’).

The yeast nuclear PET112 gene was originally identified by a mutation (pet112–1) that specifically blocks accumulation of coxII at a post–transcriptional level [39]. Cloning and sequence analysis of the PET112 gene revealed that it could encode a 62 kDa protein that does not resemble any proteins found in the databases [40]. In contrast to the pet112–1 mutation itself, disruption of the PET112 gene destabilizes the mitochondrial genome [40], a hallmark for a strict lesion in the general mitochondrial expression machinery [41]. Taken together these phenotypes suggest that PET112 could play an important, general role in mitochondrial translation.

Earlier reports have implicated the yeast nuclear NAM1 gene in mitochondrial translation [42]. More recently, however, it has become clear that nam1 mutants exhibit defects in RNA processing and/or stability of ATP6 and intron–containing transcripts of COX1 and COB genes [43]. It has been speculated that the previously described translational defects are caused by a shortage of tRNA G1u, a tRNA co–transcribed with the COB gene [43].

Phases of mitochondrial translation

Initiation

Mitochondrial mRNAs display a number of unusual characteristics. In mammals they are uncapped, contain a poly(A) tail that immediately follows or even forms part of the stop codon, but contain no or very few 5' untranslated nucleotides. The small subunit of animal mitoribosomes has the ability to bind these mRNAs tightly in a sequence–independent manner [44, 45] and this ability has led to the suggestion that the first step in initiation in animal mitochondria may in fact be the binding of the small subunit to the message [45]. A first mammalian initiation factor has been purified from bovine liver [46, 47]. This factor, mitochondrial initiation factor 2 (mIF–2), promotes binding of fMet–tRNA to bovine mitochondrial and E. coli ribosomes. Similar to bacterial, but in contrast to eukaryotic cytoplasmic initiation systems, binding of the initiator tRNA.mIF–2.GTP complex to the small ribosomal subunit requires the presence of mRNA [46, 47].
Yeast mRNAs are also uncapped, but unlike their mammalian counterparts, they lack a poly(A) tail and contain untranslated leader and trailer sequences that vary in size from about 50 up to several hundred nucleotides. Little is known about start site selection in these mRNAs. ‘Shine–Dalgarno’ type interactions between the 3′ end of 15S rRNA and specific sites in these leaders have been proposed, but seem unlikely to account for start-site selection [48]. In E. coli, Shine–Dalgarno elements act thanks to their fixed distance from the start codon, whereas in yeast mitochondria this distance is highly variable. Moreover, a chimeric mRNA lacking the putative Shine–Dalgarno box was faithfully translated in vivo [49]. On the other hand, the leader sequences of these mRNAs may be difficult for a ribosome to scan, since they often contain false upstream initiation codons, short open reading frames and stable secondary structures formed by short G+C rich clusters. Other mechanisms, such as entry at an internal landing site may therefore be used to guide the ribosome to the initiation site. How this site is recognized remains unclear, although sequence/structure elements additional to the initiator AUG must be involved, as shown by an elegant study in which the technique of biolistic transformation was used to replace the AUG start codon of the yeast mitochondrial COX3 gene by AUA. The coxIII protein specified by the mutant mRNA comigrated with the wild-type protein during SDS gel electrophoresis, suggesting that the ribosomes were able to recognize the proper initiation codon, albeit less efficiently, despite the fact that the COX3 mRNA contains 98 upstream AUA codons [50]. The generality of the phenotypic consequences of AUG to AUA initiation codon mutations has recently been demonstrated by the analysis of a mutant in which the COX2 initiation codon has been changed to AUA [51]. The 54 nt-long leader of the COX2 mRNA contains three upstream AUA codons. Nevertheless, the mutated initiation codon is still used for the synthesis of a reduced amount of normal coxII. These results suggest that COX2 and COX3 translational start sites are specified both by AUG and other local features of the mRNA [51]. Further work is required to uncover the sequence and/or structural determinants that govern recognition of translational initiation sites by ribosomes. These determinants may be recognized by one or more of the messenger-specific translational activators, as will be discussed below.

**Elongation**

A tightly associated complex consisting of mEF–Tu and mEF–Ts has been isolated from bovine liver mitochondria [52]. mEF–Ts facilitates guanine nucleotide exchange with E. coli EF–Tu and mEF–Tu is active on E. coli ribosomes. A detailed analysis of the complex reveals several remarkable features. First, the complex is not readily dissociated by GDP or GTP [52]. Second, guanine nucleotide binding to the complex requires the presence of aminoacyl–tRNA [53]. Third, the mEF–Tu.Ts complex promotes the binding of one round of phenylalanyl–tRNA to ribosomes in the absence of GTP. Recycling of the factors requires the presence of GTP [53]. These results indicate that there are distinct differences in the intermediates that can be observed in the elongation cycles found in mammalian mitochondria and in the prokaryotic and eukaryotic cytoplasmic systems. Based on these data a speculative model has been proposed in which mEF–Tu.Ts first interacts with the ribosome (step 1), where it promotes the binding of aminoacyl–tRNA (step 2). Upon subsequent GTP binding (step 3), EF–Tu catalyzes GTP hydrolysis, after which GDP and the mEF–Tu.Ts complex are separately or simultaneously released from the ribosome (step 4 [53]). Although the accuracy of this model still has to be verified, it clearly illustrates some of the unique properties of mammalian mitochondrial elongation factors. Puzzling results were obtained during the study of yeast mitochondrial elongation factors. S. cerevisiae mitochondrial EF–Tu has been isolated and is active together with E. coli ribosomes [54]. Just like bovine mEF–Tu, the protein has a low affinity for guanine nucleotides. However, there are no indications that the yeast mEF–Tu is complexed to EF–Ts. In fact, all efforts to detect EF–Ts activity in yeast mitochondrial extracts using E. coli ribosomes and EF–Tu have so far failed [54]. These results could mean that a yeast mitochondrial equivalent to E. coli EF–Ts does not exist. However, in view of the results obtained with the bovine mEF–Ts, it may just be that some unusual properties have prevented its detection up till now.

Mitochondrial EF–G (mEF–G) has been purified from yeast [55] and bovine liver [56]. Both factors are active on E. coli ribosomes but not on cytoplasmic ribosomes. The size of the purified proteins (80 kDa) corresponds well with that predicted from the cloned yeast [34] and rat [36] mEF–G genes. Unlike other translases however, both yeast and mammalian mEF–G are resistant to fusidic acid, a steroidal
antibiotic that inhibits translocation by stabilizing the ribosome–translocase.GDP complex [56].

**Termination**

A protein factor with polypeptide chain release activity has been purified from rat liver mitochondria [57]. This factor resembles prokaryotic RF–1 in recognizing the codons UAA and UAG, but not UGA; a factor of the bacterial RF–2 type, recognizing UAA and UGA, could not be detected [57]. Both observations are in line with the fact that in these, as in most mitochondria, UGA specifies tryptophan instead of a translational stop [12].

We have recently cloned and characterized the yeast MRF1 gene encoding a mitochondrial release factor (mRF–1 [35]). Since UGA codes for tryptophan also in yeast mitochondria, one release factor of the RF–1 type should suffice to direct translational termination. In this respect it may also be significant that mRF–1 is more similar to E. coli RF–1 than RF–2 [66]. Several mutants suppressing mitochondrial nonsense mutations have been isolated in yeast [58–62]. For two of these mutants, the component affected by the second-site suppressor mutation has been identified. One suppressor mutation changes a single nucleotide in the highly conserved ‘530 loop’ of 15S mitoribosomal RNA [63]. The second mutation affects the mitochondrial homolog, NAM9, of E. coli ribosomal protein S4 [62]. The effect of both nonsense suppressor mutations could be eliminated by overproduction of mRF–1 (antisuppression [35, 64]). A similar antisuppressive effect has previously been exploited for the cloning of the gene coding for E. coli RF–1 [65].

We have described two mrf1 mutants that display specific defects in mitochondrial translational termination [35]. In a recent study we have reported the cloning, sequencing, as well as an analysis of residual activities of both mutant mrf1 alleles [64]. Each allele specifies a different single amino acid substitution located one amino acid apart in a domain which, based on a comparative analysis of different release factor sequences, has been implicated in ribosome binding [66]. The amino acid changes do not affect the level or cellular localization of the mutant proteins, since equal amounts of wild type and mutant mRF–1 were detected in the mitochondrial compartment. Overproduction of the mutant release factors in wild type and mrf1 mutant yeast strains produces phenotypes consistent with a reduced affinity of the mutant release factors for the ribosome, indicating that the mutated release factor domain could have a primary function in ribosome binding [64].

**Modulation of mitochondrial translation**

**mRNA–specific translational activators**

One of the most peculiar features of the yeast mitochondrial genetic system is that translation of at least five mRNAs requires the action of one or more nuclear encoded gene–specific translational activator proteins (Table 1). Mutants deficient in one of these proteins have a respiratory–deficient phenotype due to the absence of one specific mitochondrial translation product, even though the corresponding mRNA is present in relatively normal amounts (see [1, 9] for reviews).

Three nuclear genes, PET494, PET54 and PET122, are specifically required for COX3 mRNA translation [49, 69, 75, 76]. Both mitochondrial and nuclear mutations capable of suppressing the respiratory–deficiency caused by inactivation of individual PET genes have been isolated. In the mitochondrial suppressors, leader sequences derived from another mitochondrial gene replace part of the COX3 leader (reviewed in [1]), implying that the factors act by interacting with the 5' untranslated leader of COX3 mRNA. An exact substitution of the COX2 for the COX3 5' untranslated leader places the expression of the coxII protein under the control of the COX3 mRNA–specific translational activator PET494 [68]. Additional strong evidence for an interaction is provided by the observation that a translational defect resulting from a partially deleted
COX3 leader is efficiently suppressed by a second-site mutation in the PET122 gene [77]. Moreover, the same leader mutation is slightly alleviated by over-expression of PET494, an effect which in turn is slightly increased upon co-overproduction of PET122 [77]. Alongside its role in translation, PET54 is also required for the excision of the aI5b intron in the COX1 gene [78]. Mutational analysis suggests that the two activities are located in different domains of the protein [79].

A second important feature of the translational activators involved in COX3 expression was uncovered by the study of four nuclear suppressors that partially compensate for the loss of PET122 carboxy–terminal amino acids. Three of these suppressors have turned out to be mutations in genes encoding proteins of the small mitoribosomal subunit [20–22, 80], thus strongly suggesting that PET122 activates translation of the COX3 mRNA via an interaction with this ribosomal subunit. The fourth nuclear gene isolated in this screen, PET127, differs from the previous three in that its function is not absolutely required for mitochondrial gene expression [21]. Inactivation of the PET127 gene leads to a partial heat–sensitive respiratory deficiency and has very little effect on mitochondrial translation as visualized by specific labeling of mitochondrial translation products. Inactivation of PET127 suppresses the effects of the carboxy–terminal truncation of PET122 [21]. Whether this protein plays a direct role in mitochondrial gene expression remains as yet unclear.

A third important feature of the three translational activators involved in COX3 expression was uncovered by studying their mitochondrial localization [81]. About half of PET54 detected in wild type mitochondria was found to be peripherally bound to the inner membrane whereas the remainder was present in a soluble form. PET122 and PET494, which both have to be overproduced in order to be detectable, appear to be tightly membrane–bound proteins. Although the possibility that they get stuck in the membrane as a result of their overproduction has not been formally excluded, the results indicate that these three factors may be naturally associated with the mitochondrial inner membrane (Fig. 1). PET494, PET54 and PET122 probably work together to carry out the same function. Using a GAL4–based two–hybrid system physical interactions have been detected between PET54 and PET122, and between PET54 and PET494 [82]. Genetic analysis of pet54 mutants revealed functional interactions between PET54 and both PET122 and the COX3 mRNA 5′ leader. Taken together, these data suggest that a complex containing PET54, PET122 and PET494 mediates the interaction of the COX3 mRNA with mitochondrial ribosomes at the surface of the inner membrane [82].

A similar picture arises for CBS1 and CBS2 (CBP7), two proteins required for proper translation of the COB mRNA [83]. The respiratory deficiencies due to mutations in the genes CBS1 and CBS2 are suppressed by mitochondrial mutations replacing the COB leader by the untranslated leader of the ATP9 gene [84]. Antibodies against CBS2 detect a spot on a two–dimensional separation of small mitoribosomal subunit proteins [85], and localization studies reveal association of the two proteins with mitochondrial membranes, which is tight in the case of CBS1 and loose for CBS2 [85]. The analysis of CBS1 and CBS2 has been complicated by the complex RNA maturation pathway of COB mRNA. Depending on the yeast strain, a varying number of introns can be present in COB pre-mRNA. Processing of most of these introns depends on the translation products of intronic reading frames, the so–called RNA maturases [32]. Synthesis of these maturases requires translation of the upstream exons starting at the genuine COB initiation codon ([85] and references therein). Unexpectedly, CBS1 and CBS2 appear to be dispensable for the expression of the maturase involved in the processing of intron b14 [85, 86]. This finding suggests that CBS1 and CBS2 may play other roles in translation than start site selection, at least as far as the synthesis of an RNA maturase is concerned (see below).

The translational activator PET111 is specifically required for the proper translation of COX2 mRNA [87]. The respiratory deficiency resulting from a mutation in PET111 is suppressed by an exact substitution of the COX2 for the COX3 5′ untranslated leader, thus demonstrating that PET111 also acts via sequences upstream of the initiation codon [68]. A translational defect resulting from a point mutation in the COX2 leader can be efficiently suppressed by a second–site mutation in the PET111 gene [88]. Interestingly, the same PET111 missense mutation partially suppressed the leaky respiratory–deficient growth phenotype of a mutant in which the COX2 initiation codon has been changed to AUA [51]. Moreover, both the leader and the start site mutation are slightly alleviated by over–production of PET111 [51, 88]. Like PET122 and PET494, overproduced PET111 has been detected in mitochondria as a tightly bound inner membrane protein [68]. No functional link between PET111 and the mitochondrial ribosome has been demonstrated so far.
At least three distinguishable functions can be proposed for the mRNA-specific activators on the basis of the features discussed above. All three functions suppose an interaction between (a complex containing) these factors and the 5'-leaders of the respective target mRNAs, a feature which has unfortunately not been demonstrated as yet. As a first function, the mRNA-specific activators may bring mRNAs and small ribosomal subunits to the mitochondrial inner membrane. From this it follows that translational initiation could occur at the inner membrane, thus allowing the encoded proteins to be synthesized near their site of assembly into multisubunit respiratory enzymes. Support for this idea comes from the observation that a mutation in CBS2 can be partially suppressed by overproduction of ABC1, a protein implicated in the correct folding or assembly of apocytochrome b [89].

Alternatively, several mRNA-specific activators may play a role in translational start site selection. As outlined above, an as yet unidentified mechanism must act together with the AUG start codon in the specification of correct translational start sites on COX2 and COX3 mRNAs. Two observations can be interpreted as evidence that some of the mRNA-specific activators participate in translational initiation. First, a mutation of the COX2 initiation codon could be suppressed by a missense mutation in PET111 [88]. Second, the leaky respiratory—deficient growth caused by this mutation was sensitive to alterations in PET111 gene dosage [51]. In parallel, the effect of a similar mutation of the COX3 initiation codon was found to be sensitive to alterations in PET122 and PET494 gene dosage [50]. These findings suggest a link between the recognition of a mutated initiation codon and a specific factor, but at present, alternative explanations, involving indirect interactions between factor and mutated mRNA cannot be ruled out. As outlined above, a role in translational start site selection seems less likely for CBS1 and CBS2, since these factors appear non—essential for RNA maturase synthesis. Definitive proof for a function of the mRNA-specific activators in translational initiation will require suitable in vitro assays using active yeast mitochondrial ribosomes. In this context, it is interesting to note that all attempts to develop an mRNA-dependent in vitro translation system using yeast mitochondrial ribosomes have met with a singular lack of success. However, the recent finding that in vitro translation of yeast mitochondrial mRNAs stalls...
at the level of start codon recognition [90] suggests that the absence of mRNA–specific activators may be responsible for the lack of translational initiation in vitro.

A third possible function of the mRNA–specific activators involves the modulation of translation of specific mRNAs. As reviewed by Costanzo and Fox [1], the expression of yeast mitochondrial proteins is at least partially regulated at the translational level. Several of the mRNA–specific activators have also been shown to be expressed in a regulated fashion [1]. Together this opens the possibility that the activator proteins form part of a regulatory network that tailors the level of mitochondrial biogenesis in response to intra- and extracellular conditions.

At present four other genes (MSS51, CBP6, AEP1 and ATP13) have been proposed to be involved in translation of specific mitochondrial mRNAs (see Table 1 and references therein). The general phenotype associated with mutations in any one of these genes includes absence of a specific mitochondrial translation product, while the corresponding mature mRNA is present in approximately normal amounts. As with the COX3–specific activators discussed above, the primary function of these genes remains obscure.

**Yeast mitochondrial NAD\(^{+}\)-dependent isocitrate dehydrogenase binds to leaders of mitochondrial mRNAs**

An additional protein that specifically interacts with leader sequences of all major mRNAs has been identified by a biochemical approach [91, 92]. This 40 kDa protein (p40) is nuclear encoded, exclusively present in mitochondria and highly abundant [93]. On SDS–polyacrylamide gels p40 consists of two closely migrating bands of Mr 39 and 40 kDa. Recent sequence analysis of p40–derived fragments identified the protein as the Krebs cycle enzyme NAD\(^{+}\)-dependent isocitrate dehydrogenase (Idh [94]). The two subunits of this enzyme, encoded by the nuclear genes IDH1 and IDH2, indeed have apparent molecular weights of 39 and 40 kDa [95, 96]. Disruptions in either IDH1 or IDH2 eliminate both enzyme and RNA–binding activities, confirming identity of p40 and Idh and demonstrating that both activities are dependent on the simultaneous presence of both subunits.

Another protein that combines functions in both translation and metabolism, is the iron–responsive element binding protein (IRBP), which also doubles as cytoplasmic aconitase. The RNA–binding activity of this protein is modulated in response to iron levels in the cell by changes in an Fe–S cluster and is reciprocally related to its enzymic activity as cytosolic aconitase [97]. For isocitrate dehydrogenase, however, it is as yet unclear why two so disparate activities are combined in a single protein. It has previously been speculated that p40 may act as a translational repressor [92]. Such a role would account for the finding that chimeric mRNAs apparently lacking p40 binding sites are still translatable and goes some way towards explaining why p40 disrupted strains maintain the ability to grow (albeit slowly) on certain non-fermentable substrates [94]. It is therefore attractive to speculate that the combination of RNA–binding and dehydrogenase activities forms part of a regulatory circuit that links the rate of mitochondrial biogenesis to the need for bioenergetic function. Synthesis of Idh is subject to carbon catabolite repression [98] and it will be of great interest to analyze whether the two functions of p40 are regulated in a different manner. A further analysis of the RNA–binding activity of p40 would be greatly facilitated by a separation of enzymic and RNA–binding activities. Ongoing mutational analysis should reveal whether such a separation is achievable or not.

**Human disease and defects in mitochondrial translation**

Over the past five years, interest in mitochondrial gene expression has increased sharply following the discovery that a number of human neuromuscular diseases result from mutations in the mitochondrial genome (for review see [19]). That such mutations occur should not come as a surprise to anyone, since due to shortcomings of their replication and repair systems, mammalian mtDNAs display a sequence evolution rate that is some 10–20 times higher than that of nuclear DNA. That most of these mutations are, in addition, deleterious is also not unexpected: many cell types are vitally dependent for their survival on mitochondrial energy production and the genomes encode essential components of the energy transduction machinery. Mammalian mtDNAs are extremely compactly organized, with no or few non–coding bases interspersed between genes [99]. Transcription of these DNAs produces polycistronic RNAs in which nearly all m- and rRNAs are separated by one or more tRNAs. Processing of these transcripts occurs via removal of the tRNAs, a process in which the tRNAs themselves are
thought to play an important role by guiding endonucleases to the correct cleavage site [100].

Several of the mutations characterized so far lie in genes for mitochondrial tRNAs and predictably lead to defects in mitochondrial translation [101–103, see also 104]. For example, the MERFF (myoclonic epilepsy and ragged–red fibers) syndrome is caused by a mutation in the TYC loop of tRNA<sup>Leu(UUR)</sup>, and MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and strokelike episodes) is associated with a mutation in the DHU loop of tRNA<sup>Leu(UUR)</sup>. In contrast, a different mechanism has been proposed for a mutation thought to underlie a mitochondrial myopathy characterized by a severe deficiency in NADH dehydrogenase (complex I) in skeletal muscle. This mutation is an A-G transition in the aminoacyl stem of this same tRNA<sup>Leu(UUR)</sup> [105], whose gene is located between that for the 16S large rRNA and subunit 1 of NADH dehydrogenase (ND1). Analysis of mitochondrial transcripts in muscle cells revealed a marked accumulation of a precursor RNA containing tRNA<sup>Leu(UUR)</sup>, together with transcripts from genes located immediately upstream and downstream [105]. Interestingly, important qualitative differences in transcript patterns are observed when comparing muscle cells and skin fibroblasts (the latter cells do not express a biochemical defect, despite containing the mutation). In muscle, processing appears to occur first at the 5'-end of the tRNA, generating 16S rRNA plus a tRNA-ND1 intermediate. In fibroblasts, however, processing occurs at the 3'-end of the tRNA, generating a 16S rRNA-tRNA intermediate. These observations suggest that the mutation causes a structural alteration in the tRNA that leads to a tissue-specific primary defect in the processing of the polycistronic transcript. It is this defect, rather than a defect in translation per se, that gives rise to the biochemical abnormalities, thus illustrating the importance of careful analysis when mutations in components of the mitochondrial translation machinery are concerned.

**Concluding remarks**

**Why study mitochondrial protein synthesis?**

One of the most striking features of mitochondrial protein synthetic systems is the great inter-species variability of their RNA and protein components. It has been speculated that this variability is directly attributable to the fact that mitochondrial translation systems produce only a very limited number of proteins. As previously outlined by Benne and Sloof [13], this idea implies that many of the special features of mitochondrial translation systems may not be applicable to non-mitochondrial translation systems because they are only tolerable in a context that makes so few demands on them. Whether or not this idea turns out to be true, it should not deter anyone from studying mitochondrial translation. The characterization of these systems and of analyzing how they have adapted to the specific needs of the organelle that contains them has taught us much about the basic principles of genetic decoding and protein synthesis.

**Saccharomyces cerevisiae as a model for the study of mitochondrial protein synthesis**

The yeast *Saccharomyces cerevisiae* is highly suitable for the study of mitochondrial protein synthesis. First, this facultative anaerobe does not depend for its survival on the mitochondrial genome, thus allowing a mutational approach to the study of components which are absolutely essential for cell viability in other systems. Second, the development of a biolistic procedure to transform yeast mitochondria has opened up possibilities for site-directed mutagenesis of mtDNA. This means that both the nuclear- and mitochondrially-encoded components of the mitochondrial translational apparatus can be mutated and subsequently studied *in vivo*. The fact that all components identified so far are encoded by single-copy genes means that the mutated components can easily be studied in a homozygous system. Notwithstanding these attractive features, it must be admitted that there are a couple of disadvantages, which further research should attempt to eliminate in the near future. The first of these is the lack of a fully active mitochondrially-derived *in vitro* translation system (see below). A second is the instability of the mitochondrial genome which results from the introduction of mutations that severely hamper mitochondrial gene expression. A better understanding of this phenomenon could help to further increase the usefulness of yeast as a tool to study mitochondrial biogenesis.

**mRNA-specific translational activators and the unique features of translation initiation**

Mitochondrial translational initiation is likely to involve new principles of mRNA recognition and selection. Unfortunately, this notion is still largely based on the unusual features of the mitochondrial 5'-untranslated mRNA leaders, since little is known of the initiation process itself. Prime candidates for proteins that could function in translational initiation are...
the mRNA-specific translational activators. Definitive proof for a role of these proteins in translational initiation will require the development of suitable in vitro assays using active yeast mitochondrial ribosomes. As outlined in this review, attempts to develop an in vitro system capable of translating yeast mitochondrial mRNAs have been unsuccessful due to a failure to obtain correct translational initiation [90]. A better understanding of both the specific features of translational initiation and the function of the mRNA-specific translational activators awaits a biochemical study of purified activator proteins. Such an approach could not only be helpful to establish whether the activators physically interact with mitochondrial mRNA leaders and mitochondrial ribosomes or not, but could also help to elucidate the eventual roles of these proteins in various steps of mitochondrial protein synthesis.

Acknowledgements

We are grateful to Dr. Tom Fox for communicating results prior to publication. We thank Drs. Monique Bolotin-Fukuhara, Bertrand Daignan-Fornier, Peter Dekker, Sandra Elzinga, Martijn Rep, Hans van der Spek and Ruth Zelikson for their critical evaluation of the manuscript. Research carried out in our laboratory was supported by the Netherlands Foundation for Chemical Research (SON) and the Netherlands Organization for Scientific Research (NWO). H.J. Pel is a recipient of a long term EMBO Fellowship (ALTF 130-1992).

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194