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# Identification of the gene encoding the mitochondrial elongation factor G in mammals

Christopher Barker+, Antonios Makris, Christos Patriotis, Susan E.Bear and Philip N.Tsichlis*
Department of Medical Oncology, Fox Chase Cancer Center, Philadelphia, PA 19111, USA

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## ABSTRACT

**Protein synthesis in cytosolic and rough endoplasmic reticulum associated ribosomes is directed by factors, many of which have been well characterized. Although these factors have been the subject of intense study, most of the corresponding factors regulating protein synthesis in the mitochondrial ribosomes remain unknown. In this report we present the cloning and initial characterization of the gene encoding the rat mitochondrial elongation factor-G (rEF-$G_{mt}$). The rat gene encoding EF-$G_{mt}$ (r*Mef*-g) maps to rat chromosome 2 and it is expressed in all tissues with highest levels in liver, thymus and brain. Its DNA sequence predicts a 752 amino acid protein exhibiting 72% homology to the yeast *Saccharomyces cerevisiae* mitochondrial elongation factor-G (YMEF-G), 62% and 61% homology to the *Thermus thermophilus* and *E.coli* elongation factor-G (EF-G) respectively and 52% homology to the rat elongation factor-2 (EF-2). The deduced amino acid sequence of EF-G contains characteristic motifs shared by all GTP binding proteins. Therefore, similarly to other elongation factors, the enzymatic function of EF-$G_{mt}$ is predicted to depend on GTP binding and hydrolysis. EF-$G_{mt}$ differs from its cytoplasmic homolog, EF-2, in that it contains an aspartic acid residue at amino acid position 621 which corresponds to the EF-2 histidine residue at position 715. Since this histidine residue, following posttranslational modification into diphthamide, appears to be the sole cellular target of diphtheria toxin and *Pseudomonas aeruginosa* endotoxin A, we conclude that EF-$G_{mt}$ will not be inactivated by these toxins. The severe effects of these toxins on protein elongation in tissues expressing EF-$G_{mt}$ suggest that EF-$G_{mt}$ and EF-2 exhibit nonoverlapping functions. The cloning and characterization of the mammalian mitochondrial elongation factor G will permit us to address its role in the regulation of normal mitochondrial function and in disease states attributed to mitochondrial dysfunction.**

## INTRODUCTION

Elongation of the nascent protein chain during protein synthesis depends primarily on the sequential function of three factors: 1) Elongation factor-1$\alpha$ (EF-1$\alpha$) which binds aminoacyl tRNA and directs its codon dependent placement at the A site of the ribosome in a reaction regulated by EF-1$\alpha$ GTP binding and hydrolysis. 2) Elongation factor-1$\beta$ (EF-1$\beta$) which interacts with the released EF-1$\alpha$-GDP and promotes the exchange of GDP for GTP. This exchange is facilitated further by elongation factor 1$\gamma$ (EF-1$\gamma$). and 3) Elongation factor-2 (EF-2) which, following peptide bond formation, catalyzes the translocation of the peptidyl-tRNA from the A to the P site in the ribosome. The function of EF-2, similarly to the function of EF-1$\alpha$ and EF-1$\beta$, depends on GTP binding and hydrolysis. The procaryotic homologs of the eucaryotic factors EF-1$\alpha$, EF-1$\beta$ and EF-2 are called EF-Tu, EF-Ts and EF-G respectively (for review see 1–3).

Eucaryotic cells harbor two protein synthesis machineries: one of them is localized in the soluble and membrane bound cytoplasmic ribosomes. These ribosomes translate mRNAs transcribed in the nucleus and they synthesize proteins which are transported to the various cellular compartments through a complex network of processes that regulate intracellular protein trafficking (4, 5). The other machinery is localized in the mitochondria. These semi-autonomous organelles have their own genetic material which is transcribed and translated in ribosomes localized in the mitochondrial matrix (6–10). The factors regulating mitochondrial protein synthesis, however, are encoded by nuclear (non-mitochondrial) genes, they are synthesized in the cytoplasmic ribosomes, and they are transported to the mitochondria (11–15).

In this communication we report the cloning and initial characterization of the gene encoding the rat mitochondrial

* To whom correspondence should be addressed

+ Present address: Department of Cell Culture R & D, Centocor Inc., Malvern, PA 19355, USA

elongation factor G (rEF-$G_{mt}$). Our findings show that rEF-$G_{mt}$ is a highly conserved (10, 16–18) GTP binding protein (18–26) whose function is restricted to the mitochondrial ribosomes (27–30). Expression of rEF-$G_{mt}$ was detected in all tissues with the highest levels in liver, thymus and brain.

## MATERIALS AND METHODS

### Oligonucleotide probes and hybridization conditions

The gene encoding rEF-$G_{mt}$ was cloned by virtue of the fortuitous homology of one of its introns with a 43 base long oligonucleotide complementary to a conserved region of the Growth hormone receptor gene (*Ghr*) (31). The hybridization of this oligonucleotide probe to rat genomic DNA was carried out in the course of experiments designed to clone genes encoding growth hormone receptor related molecules (see Results section).

Three oligonucleotides corresponding to highly conserved regions of the growth hormone receptor gene (nucleotide position 264–308, 903–945, 4023–4053) (31) were synthesized. One of these oligonucleotides (PT36, nucleotide position 903–945) (5'TCCAGTTCCAAAGATTAAAGGAATCGATCCAGATCTCCTCAAG3') is complementary to a region of the *GHR* gene encoding a portion of the intracellular domain of the receptor. This region of the human *GHR* is identical in sequence to the rabbit *Ghr* at the nucleotide level and to the human prolactin receptor encoding gene at the amino acid level (31, 32). 100 ng of the oligonucleotide PT36 were labelled with $\gamma$-$^{32}$P ATP using T4 polynucleotide kinase (Boehringer Mannheim) according to the manufacturer's instructions. Labeled oligonucleotides were separated from unincorporated $\gamma$-$^{32}$P ATP by fractionation on a Bio-Gel P6 (Bio-Rad) column.

Long-Evans rat lung DNA (10 $\mu$g) were digested to completion with restriction endonucleases, electrophoresed in 0.7% agarose gels and blotted onto NYTRAN membranes (S&S) by standard methods (33–35). The filters were hybridized to $4\times10^5$ cpm/ml labeled PT36 in a solution containing 5×SSC (1×SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.0) and 0.5% SDS. Hybridizations were carried out at 37°C for 16–18 h. The filters were washed in 2×SSC 0.5% SDS, at 50° for at least 30 min.

### Construction and screening of partial genomic DNA libraries

Long-Evans rat thymus DNA (100 $\mu$g) was digested to completion with *Eco*RI and fractionated in a 0.7% agarose gel. Three fractions (8–10 kb, 6.5–8 kb and 4.5–6.5 kb) corresponding to the DNA bands detected in the initial screening experiments with the PT36 probe were purified by electroelution. The presence of the desired DNA fragment in each fraction was confirmed by analytical gel electrophoresis and Southern blotting. Partial genomic DNA libraries were prepared by ligating DNA from individual fractions to the *Eco*RI arms of the bacteriophage lambda vector λgtWES-λB'. The products of each ligation reaction were *in vitro* packaged with Gigapack Gold (Stratagene) and propagated in *Escherichia coli* LE392. The 8–10 kb library was screened with the oligonucleotide probe PT36 using the same hybridization conditions originally used to hybridize PT36 to genomic DNA blots. This screening yielded a clone, λ1D, which contained a 9.0 kb *Eco*RI insert.

To define the shortest DNA fragment, that was free of repetitive sequences and hybridized to PT36, we first isolated a 6.5 kb *Kpn*I-*Eco*RI subclone of λ1D (λ1D1/K1). After partial *Hae*III digestion of the clone λ1D1/K1, a 503 bp *Hae*III clone (p1DA1) lacking rat repetitive DNA sequences and hybridizing to the PT36 oligonucleotide probe was isolated.

### Construction and screening of cDNA libraries

Screening of a normal liver cDNA library (Stratagene) with the 503 bp probe p1DA1 under conditions of high stringency (50% formamide 5×SSC-0.1% SDS at 42°) yielded an 891 bp clone (pCRL11). Screening the same library with the cDNA clone pCRL11 yielded two additional larger clones, the 1.45 kb clone pCRL25 and the 1.2 kb clone pCRL27. Finally, screening a rat thymic lymphoma (6889) cDNA library, prepared from size-fractionated polyadenylated RNA (34) with the pCRL11 probe, yielded 26 additional independent cDNA clones (pAS1-pAS26) which ranged in insert size from 1.0 to 2.7 kb.

### Northern (RNA) blot analysis of polyadenylated cell RNA

Total cell RNA was isolated by the method of Chomczynski and Sacchi (36) following cell lysis with guanidinium isothiocyanate. Polyadenylated RNA was selected by affinity chromatography in oligo(dT)-cellulose. Polyadenylated RNA (5 $\mu$g) was electrophoresed in denaturing 1% agarose, 2.2 M formaldehyde gels, and after transfer onto nylon membranes it was hybridized to the pCRL11 probe at high stringency as previously described (33–35).

### Sequencing of cDNA clones and computer analysis

Bidirectional nested deletions of the clones pCRL11 and pCRL25 were generated using the exonuclease III/mung bean nuclease method (35). Selected subclones with overlapping deletions were sequenced. The sequencing reactions were carried out on alkali-denatured double stranded DNA templates by using the dideoxy chain termination method and employing Sequenase version 2.0 (U.S. Biochemicals) and [$\alpha$-$^{35}$S]dATP (NEN DuPont). The products of these reactions were analyzed on 6% polyacrylamide-8.3 M urea sequencing gels as described elsewhere (33–35). Since clones pAS1-pAS26 formed a nested set of clones, the sequencing of their ends allowed us to assemble nearly the entire sequence of EF-$G_{mt}$. Areas that failed to overlap or areas of ambiguity were resolved by sequencing multiple independent clones using specific oligonucleotide primers and the CircumVent sequencing system (New England Biolabs).

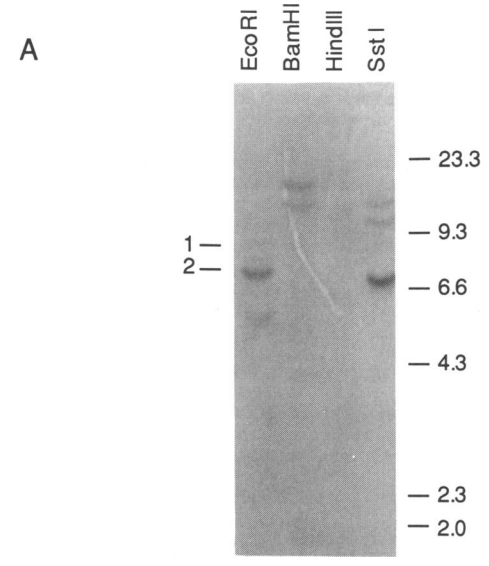
All computer analyses were performed using the Genetics Computer Group software package (37). The DNA sequence presented here has been submitted to GenBank.

## RESULTS

### Cloning of genomic DNA fragments containing regions with partial homology to oligonucleotides complementary to the gene encoding the growth hormone receptor (*Ghr*)

The gene encoding EF-$G_{mt}$ was cloned by virtue of the fortuitous hybridization of one of its introns to an oligoncucleotide probe (PT36), complementary to a conserved region of the gene encoding the growth hormone receptor.

In the course of our studies on Moloney murine leukemia virus (MoMuLV)-induced rat T cell lymphomas (33, 34, 38–40), we showed that *Mlvi*-2, a locus of common proviral integration identified in this laboratory (40), was genetically linked to the gene encoding the growth hormone receptor (*Ghr*) in humans, mice and rats (33). This suggested that *Mlvi*-2 may encode a




B

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1 CCCCGGAGGACTACACGAAGTTGGAGTTTTCCGATGAGACCTTTGGGGCCAATG
  ||||||||||||||||||||||||||||||||||||||||||||||||||||||
2 CCCCGGAGGACTACACGAAGTTGGAGTTTTCCGATGAGACCTTTGGGGCCAATGgtgtgcgccactcctgcgattccacc

2 ttgtctcagtatatgtgaaggtaactagtttactcccaatttacaaagttaaaaaggaagcagagacagatctc.taaat
                                | | || |||||  | |||||||| | ||  |||||||| | ||
3                               TCCAGTTCCAAAGAT.TAAAGGAATC.GATCCAGATCTCCTCAAG
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**Figure 1.** Cloning the r*Mef*-g gene. **A.** Southern blot analysis of restriction endonuclease digested DNA and hybridization to the oligonucleotide probe PT36 under conditions of reduced stringency (see text). The lines on the right show the migration pattern of *Hin*dIII digested bacteriophage lambda DNA size markers. The numbers define the size of the markers in kilobases (kb). The lines and numbers on the left show the two *Eco*RI DNA fragments which were cloned. A third band at approximately 5.5 kb has not been cloned. Band 2 was derived from the gene encoding the rat growth hormone receptor (*Ghr*) while band 1 was derived from the gene encoding the rEF-$G_{mt}$ (r*Mef*-g). **B.** Oligonucleotide PT36 hybridizes to an intron of the gene encoding EF-$G_{mt}$. Line 2 shows part of the nucleotide sequence of the genomic clone λ1D. This clone was obtained from a partial genomic DNA library constructed with DNA containing the larger of the two *Eco*RI bands shown in panel A. Screening was carried out using the oligonucleotide probe PT36. Capital and lower case letters depict exon and intron sequences respectively. Line 1 shows the nucleotide sequence of the portion of the pCRL11 cDNA clone displaying homology to the genomic clone λ1D. Line 3 shows the DNA sequence of oligonucleotide PT36. The PT36 sequence is written in capital letters and it is underlined and aligned with the λ1D derived intron sequences.

member of the *Ghr* family of receptors. Moreover, these data suggested that other members of this receptor family may cluster in the same chromosomal region and may be targeted by provirus integration in MoMuLV induced rat T cell lymphomas. To investigate these possibilities we synthesized three oligonucleotides complementary to selected regions (nucleotide position 264−308, 903−945, 4023−4053) of the *Ghr* RNA (31). The selection of these regions was based on their high degree of conservation between rabbits and humans. Hybridization of these oligonucleotide probes after end labelling with $\gamma$-$^{32}$P ATP to restriction endonuclease digested rat genomic DNA revealed several distinct bands. One of these oligonucleotide probes (PT36) extending from nucleotide position 903 to 945 in the human *GHR* cDNA (31), detected three DNA fragments (5.5, 7 and 9.0 kb) in *Eco*RI digested rat DNA (Fig. 1A). DNA fractions containing

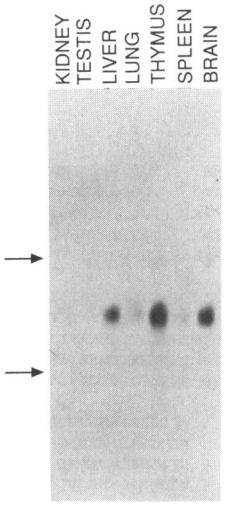


**Figure 2.** Expression of *Mef*-g in normal rat tissues. 5 μg polyadenylated RNA from normal rat tissues was electrophoresed in 1% agarose/2.2 M formaldehyde gels and it was hybridized to the rat EF-$G_{mt}$ probe pCRL11. All the lanes were loaded with approximately equal amounts of RNA, as determined by hybridization to a cDNA clone of the gene encoding the rat ribosomal protein 12 (rpL12) (data not shown).

these fragments were isolated by agarose gel electrophoresis and electroelution. The isolated DNA fractions were then used to generate partial genomic DNA libraries. Screening the 8−10 kb *Eco*RI library with a $\gamma^{32}$P labelled PT36 probe identified a 9.0 kb clone, λ1D. Restriction endonuclease digestion of the cloned DNA identified a 503 bp *Hae*III DNA fragment (p1DA1) which was free of repeats and hybridized to the PT36 probe. Sequence analysis of this DNA fragment revealed a region with 72% homology to the 43 bases long oligonucleotide PT36 (Fig. 1B). Using Southern blotting of genomic DNA from a panel of somatic cell hybrids, the cloned DNA fragment was mapped to rat chromosome 2 (data not shown), the same chromosome harboring the *Mlvi*-2 locus (33).

## The clone p1DA1 identifies a gene which is expressed in all normal rat tissues

The 503 bp *Hae*III subclone p1DA1 (Fig. 1B) was hybridized to a Northern blot of poly(A)$^+$ RNA isolated from a panel of normal rat tissues. This detected an approximately 3.0 kb mRNA transcript which was present in all tested samples (data not shown). The same genomic DNA probe was then used to screen a cDNA library constructed from poly(A) selected normal rat liver RNA. This yielded a clone, pCRL11, which, by sequence analysis, was shown to exhibit homology to several members of the family of the procaryotic elongation factor-G and the eucaryotic elongation factor-2. The pCRL11 clone was then used to probe a panel of poly(A)$^+$ RNAs isolated from normal rat tissues. The results (Fig. 2) revealed that the gene represented by this cDNA clone was expressed ubiquitously as an approximately 3.0 kb RNA transcript. The highest levels of expression were detected in liver, thymus and brain. To obtain full length cDNA clones we used the probe pCRL11 to screen the rat liver cDNA library and a cDNA library constructed from size selected oligo(dT) primed RNA isolated from the rat T cell lymphoma line 6889 (34). This yielded two liver cDNA clones

**Figure 3.** Comparison of the rat EF-$G_{mt}$ amino acid sequence (1) with the amino acid sequences of the yeast *Saccharomyces cerevisiae* YMEF-G (2), the *Thermus thermophilus* EF-G (3), the *E.coli* EF-G (4) and the rat EF-2 (5). Identical amino acids between the rat EF-$G_{mt}$ and any of the other elongation factors are shown in bold. The boxed amino acid sequences marked I, II and III define the characteristic consensus sequences shared by GTP binding proteins. Sequence I is alternatively called the A consensus or P loop and it is involved in phosphate binding (41, 42). Sequence III defines the nucleotide binding specificity (19). The underlined sequence is conserved among all GTP binding elongation factors and appears to be involved in the conformational change of these proteins following GTP hydrolysis (43). The D residue at amino acid position 621 corresponds to the H residue at amino acid position 715 of the mammalian EF-2 (18, 27–30). The sequence marked by the interrupted line defines the region of homology between the rat genomic DNA clone λ1D and the r*Mef-* cDNA (see Fig. 1).

(pCRL25 and pCRL27) and 26 additional 6889 cDNA clones (pAS1-pAS26) which ranged in size from 1.0 to 2.7 kb.

## The product of the gene identified by hybridization to clone p1DA1 exhibits sequence homology with the yeast *Saccharomyces cerevisiae* mitochondrial elongation factor G (YMEF-G), the procaryotic elongation factors G (EF-G) and its eucaryotic homolog, elongation factor-2 (EF-2)

The sequence of the gene identified by hybridization to clone p1DA1 was assembled as described in the Materials and Methods. Comparison of the assembled sequence with the sequence of the 503 bp p1DA1 genomic clone revealed a 54 bp region of identity representing the 3ó portion of an exon (Fig. 1B and Fig. 3). The genomic DNA sequence homologous to the PT36 oligonucleotide probe was mapped within the adjacent intron (Fig. 1B).

Translation of the nucleotide sequence of the full length cDNA revealed a 2256 nucleotide long (752 amino acids) open reading frame beginning with an in frame ATG codon (Fig. 3). Comparison of the deduced amino acid sequence with the GenBank protein sequence data base revealed that the cloned gene exhibits 72% homology with the yeast *Saccharomyces cerevisiae* mitochondrial elongation factor G (YMEF-G), 62 and 61% homology with the *Thermus thermophilus* and *E.coli* elongation factors G, respectively and 52% homology with the eucaryotic homolog of EF-G elongation factor-2 (EF-2) (Fig. 3 and Fig. 4). Based on these sequence similarities and using the PILEUP program of the Genetics Computer Group software package (37) we deduced the evolutionary relationship among these genes (Fig. 5) and we tentatively identified the cloned gene as the gene encoding the mammalian (rat) mitochondrial elongation factor G (rEF-$G_{mt}$). The predicted amino acid sequence of rEF-$G_{mt}$ suggests that it is a GTP binding protein (18–26). Thus, rEF-$G_{mt}$ contains a consensus GTP binding domain composed of three appropriately spaced consensus elements designated I, II and III (Fig. 3). Motif I, also known as the A consensus (41) or P-loop (42) is likely to form a flexible

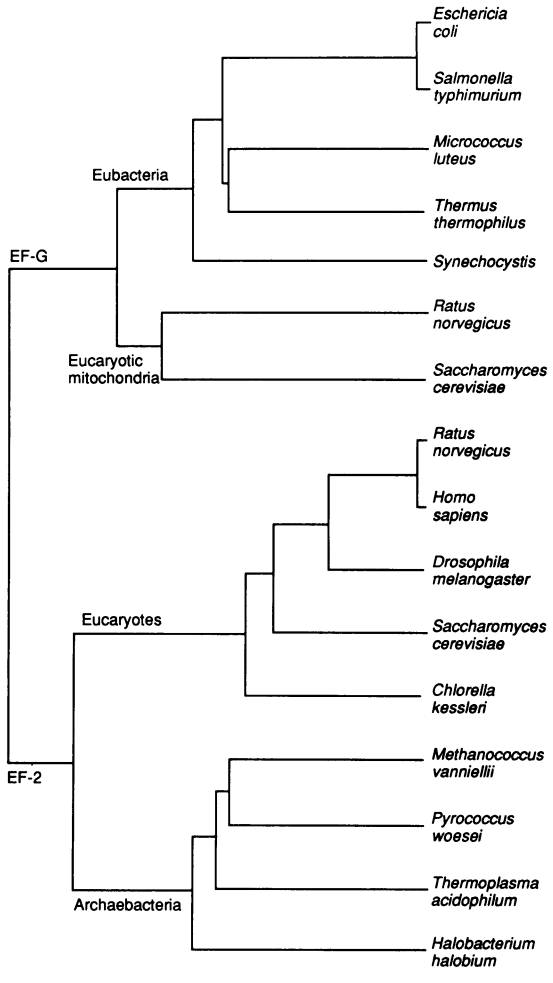


**Figure 4.** Evolutionary relationship between the rat EF-$G_{mt}$ and other known elongation actors. The depicted evolutionary tree was generated using the program PILEUP of the Genetics Computer Group software package (36).

loop between a beta strand and an alpha helix which may interact with one of the nucleotide phosphate groups. Consensus III appears to be responsible for base specificity. Thus, exchange of the aspartic acid with a tryptophan residue allows the binding of ITP as well as GTP (19). Finally, between consensus elements I and II there is a 13 amino acid region (underlined in Fig. 3) which has been conserved among all GTP binding elongation factors. In EF-1$\alpha$, this region has been shown to be involved in the conformational changes mediated by the hydrolysis of GTP to GDP (43).

Phosphorylation and dephosphorylation contribute to the physiological regulation of elongation factors. Thus, EF-2 can be phosphorylated by a $Ca^{++}$ calmodulin dependent protein kinase named EF-2 kinase (44, 45) and perhaps by additional kinases (46, 47). Moreover, EF-2 can be dephosphorylated by a type 2A phosphatase (48). Phosphorylation removes EF-2 from the active pool while dephosphorylation restores its activity (49, 50). The physiological significance of these changes in the phosphorylation of EF-2 is supported by the finding that the proportion of phosphorylated EF-2 changes in parallel with total protein synthesis during the cell cycle of human amniotic cells (1). Additionally, EF-2 phosphorylation is modulated during treatment of PC12 cells with nerve growth factor (NGF) (51), during treatment of epidermal cells with phorbol esters (52, 53), and during cAMP-dependent activation of protein synthesis in cell free reticulocyte lysates (54). To identify potential sites of phosphorylation in rEF-$G_{mt}$ its amino acid sequence was analyzed using the Motifs program of the Genetics Computer Group software package (36). This analysis revealed seven conserved putative protein kinase C (PKC) phosphorylation sites (55, 56) at amino acid positions 58, 64, 71, 389, 479, 518 and 523, two conserved Casein kinase II (CKII) phosphorylation sites (57) at position 62 and 76 and one conserved cAMP or cGMP dependent kinase phosphorylation site (58) at position 285.

## DISCUSSION

The factors involved in protein synthesis in the cytoplasmic and rough endoplasmic reticulum associated ribosomes have been the subject of intense study for at least two decades (for review, see 1–3). The characterization of the corresponding factors in the mitochondrial protein synthesis machinery (6), however, began only recently. Thus, the mitochondrial initiation factor 2 (IF-$2_{mt}$) (8), the mitochondrial elongation factors Tu (EF-$Tu_{mt}$) and Ts (EF-$Ts_{mt}$) (7) and the mitochondrial elongation factor G (EF-$G_{mt}$) (9), were only recently purified from bovine liver mitochondria. Moreover, the recent characterization of the yeast *Saccharomyces cerevisiae pet* mutants C155 and E252 led to the cloning of the genes encoding the yeast mitochondrial initiation factor 1$\alpha$ (IFM1) and the yeast mitochondrial elongation factor G (YMEF-G) (10).

In this report we present the cloning and characterization of the gene (r*Mef*-g) encoding the rat mitochondrial elongation factor G (rEF-$G_{mt}$). The identity of the protein encoded by r*Mef*-g was based on the following observations: a) The protein most closely related by sequence to the protein encoded by r*Mef*-g is the yeast mitochondrial elongation factor G (YMEF-G) (10). Moreover, rEF-$G_{mt}$ exhibits a closer evolutionary relationship with members of the family of the procaryotic elongation factor G than with members of the family of the eucaryotic elongation factor 2. b) The predicted molecular weight of the protein encoded by r*Mef*-g is approximately 80 kilodalton (kd) which is the same as the experimentally measured molecular weight of the bovine mitochondrial elongation factor G (9). c) Proteins encoded by nuclear genes and transported in the mitochondria harbor signal sequences which are usually localized at their N-terminal end and are rich in basic and hydroxylated amino acids while they are devoid of acidic amino acids and long hydrophobic stretches (11–15). The N-terminal portion of the predicted amino acid sequence of rEF-$G_{mt}$ fulfills these criteria. d) Finally, our preliminary data using antibodies raised against this protein indicate that it is localized, at least in part, in the mitochondrial fraction of heart muscle cells (A. Makris et al., unpublished).

The predicted amino acid sequence of the rEF-$G_{mt}$ indicates that, like other elongation factors, it is a GTP binding protein (18–26, 41–43). GTP hydrolysis by EF-$G_{mt}$, similarly to GTP hydrolysis by other elongation factors, is expected to be strictly conditional, that is, dependent on its association with ribosomal protein complexes which therefore function as GTPase activating proteins (GAPs) (25, 26). GTP and GDP bind weakly to EF-2 and EF-G (59, 60). Moreover, both EF-G and EF-2 are highly catalytic in the absence of auxiliary factors (61, 62). These data suggest that EF-2, EF-G, and perhaps EF-$G_{mt}$, may not be regulated by guanine nucleotide exchange factors (63). The GTP

binding domain of rEF-$G_{mt}$ is localized at the N-terminal portion of the protein. However, the homology between rEF-$G_{mt}$ and other elongation factors extends throughout the length of the molecule. These extended areas of homology may define other properties shared by these proteins. One such property is the binding to the ribosomes.

rEF-$G_{mt}$ cannot substitute for EF-2 since its function appears to be restricted in directing protein elongation in the mitochondrial protein synthesis machinery. This conclusion is based on the following observations. EF-2 contains a histidine residue at amino acid position 715 which undergoes posttranslational modification to diphthamide (18, 27–30). rEF-$G_{mt}$ contains an aspartic acid residue in the corresponding position of the molecule (amino acid 621). Bacterial toxins such as diphtheria toxin and *Pseudomonas aeruginosa* endotoxin A inactivate EF-2 by catalyzing the ADP-ribosylation of the diphthamide residue at position 715 (18, 27–30). Diphtheria toxin and pseudomonas endotoxin A resistant Chinese hamster ovary cells harbor mutations that inhibit the ADP ribosylation of EF-2 (30). Therefore, this modification appears to be necessary and sufficient for the expression of the toxic effects of diphtheria toxin and pseudomonas endotoxin A. The inability of the non ADP-ribosylatable EF-$G_{mt}$ to protect the cells from the effects of these toxins suggests that its function is restricted in the mitochondrial ribosomes.

Phosphorylation and dephosphorylation appear to contribute to the physiological regulation of elongation factors. Thus, the proportion of phosphorylated EF-2 changes in parallel with the total protein synthesis activity during the cell cycle in human amniotic cells (1). Moreover, EF-2 phosphorylation is modulated during treatment of PC12 cells with NGF (51), during treatment of epidermal cells with phorbol esters (52, 53) and during cAMP dependent activation of protein synthesis in cell free reticulocyte lysates (54). Phosphorylation of EF-2, catalyzed by a $Ca^{++}$/calmodulin dependent protein kinase (44, 45) and perhaps other additional kinases (46, 47), removes this factor from the active enzyme pool (45, 46). On the other hand, dephosphorylation of EF-2, catalyzed by a type-2A protein phosphatase (48), restores its activity (49, 50). Scanning the rEF-$G_{mt}$ sequence for potential consensus phosphorylation sites revealed several potential sites for PKC, CKII and cAMP/cGMP dependent kinase induced phosphorylation (55–58) which are conserved among members of the elongation factor G family. The significance of these potential phosphorylation sites is under investigation. Regarding the significance of the potential PKC sites, it should be pointed out that although PKC is not known to localize in the mitochondria, these sites may still be important targets for phosphorylation by related kinases (64) that may exhibit subcellular distributions different than PKC.

The evolutionary relationship between the members of the EF-G/EF-2 family of elongation factors supports the view that the primary kingdom of archaebacteria is evolutionarily closer to the primary kingdom of eucaryotes than to that of eubacteria (65). Moreover, it supports the view that mitochondria arose by invasion of aerobic or anaerobic photosynthetic bacteria into ancestral protokaryotic cells (66).

The cloning and characterization of the mammalian mitochondrial elongation factor G will permit us to address its role in the regulation of normal mitochondrial function and in disease states attributed to mitochondrial dysfunction (67).

## ACKNOWLEDGEMENTS

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