Mammalian Mitochondrial Methionyl-tRNA Transformylase from Bovine Liver

PURIFICATION, CHARACTERIZATION, AND GENE STRUCTURE*

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The mammalian mitochondrial methionyl-tRNA transformylase (MTFmt) was partially purified 2,200-fold from bovine liver mitochondria using column chromatography. The polypeptide responsible for MTFmt activity was excised from a sodium dodecyl sulfate-polyacrylamide gel and the amino acid sequences of several peptides were determined. The cDNA encoding bovine MTFmt was obtained and its nucleotide sequence was determined. The deduced amino acid sequence of the mature form of MTFmt consists of 357 amino acid residues. This sequence is about 30% identical to the corresponding Escherichia coli and yeast mitochondrial MTFs. Kinetic parameters governing the formylation of various tRNAs were obtained. Bovine MTFmt formylates its homologous mitochondrial methionyl-tRNA and the E. coli initiator methionyl-tRNA (Met-tRNAMet) with essentially equal efficiency. The E. coli elongator methionyl-tRNA (Met-tRNAmMet) was also formylated although with somewhat less favorable kinetics. These results suggest that the substrate specificity of MTFmt is not as rigid as that of the E. coli MTF which clearly discriminates between the bacterial initiator and elongator Met-tRNAs. These observations are discussed in terms of the presence of a single tRNAMet gene in mammalian mitochondria.

During the initiation process of protein biosynthesis the initiator methionyl-tRNA is bound to the ribosomal P-site. In prokaryotes, this step is facilitated by initiation factor 2 (IF-2) while in the eukaryotic cytoplasm this step is mediated by eIF-2. In contrast, all other aminoacyl-tRNAs function as elongator tRNAs and enter the A-site of the ribosome in a complex with elongation factor Tu (EF-Tu) in prokaryotes or eEF-1 in the eukaryotic cell cytoplasm (1). In most organisms, the initiator tRNA has distinct features that ensure its selection during the initiation process and its exclusion from the steps of polypeptide chain elongation. In prokaryotes and eukaryotic organelles, such as mitochondria and chloroplasts, the methionine attached to the initiator tRNA undergoes formylation at its amino group through the action of the enzyme methionyl-tRNA transformylase (MTF) (2–4). In Escherichia coli, MTF discriminates strictly between the initiator tRNA and the tRNAs used for chain elongation by recognizing specific determinants in the initiator tRNA (5). Formylation of methionyl-tRNA is necessary for the interaction of the tRNA with IF-2. Formylation also eliminates any significant interaction with EF-Tu. In the yeasts and plants, the initiator tRNA is not formylated. However, Met-tRNAMet is excluded from chain elongation by the presence of a 2’-O-ribosyl phosphate modification at position 64 of the initiator tRNA (6).

The translational system in animal mitochondria is thought to be more closely related to that of prokaryotes than to that of the eukaryotic cell cytoplasm (7, 8). This idea is based on the use of Met-tRNA for initiation, on the antibiotic sensitivity of the ribosomes, and on the ability of the mammalian mitochondrial elongation factors to function on bacterial ribosomes. However, animal mitochondrial protein synthesis has a number of unusual features that distinguish it from other translational systems. In general, mitochondrial tRNAs are shorter than their prokaryotic or eukaryotic cytoplasmic counterparts (59–75 nucleotides in length). They display numerous primary structural differences from “normal” tRNAs. In some cases, they cannot be folded into the typical cloverleaf secondary structure and lack one or more of the invariant or semi-invariant residues found in other tRNAs (9). There are genes for 22 tRNAs in the mammalian mitochondrial genome (10). This number is sufficient to read the altered genetic code found in this organelle. There is a single tRNA for each amino acid except for leucine and serine for which two tRNAs are required. A single gene for tRNAMet is present and no tRNAs appear to be imported into mammalian mitochondria (11). It is unclear how a single tRNAMet species can play the dual roles of an initiator and an elongator tRNA. Translational initiation in mammalian mitochondria requires Met-tRNA for the IF-2-dependent binding to ribosomes. However, the unformylated form is required by EF-Tu for chain elongation (12, 13). Thus, the single tRNAMet gene must give rise to two species of tRNA (Met-tRNA and Met-tRNA). This process requires a mechanism to adjust the ratio of formylated to non-formylated Met-tRNAs to meet the needs of both initiation and elongation. As a first step toward the investigation of this process, we report here the purification, cloning, and characterization of bovine mitochondrial MTF (MTFmt).
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Experimental Procedures

Materials

Folinic acid and CHAPS were purchased from Sigma. [35S]Methionine (37 TBq/mmol) and [14C]methionine (1.85 GBq/mmol) were obtained from Amersham. DEAEE-Sepharose fast flow, Mono S (HR5/5), Hi Trap Blue, and Hi Trap Heparin columns were purchased from Pharmacia. An affinity column using an immobilized E. coli tRNA mixture was prepared as described (14).

Buffers

Buffer TG contains 20 mM Tris-HCl (pH 7.6), 1 mM MgCl₂, 0.1 mM EDTA, 6 mM β-mercaptoethanol, 10% glycerol, 0.1 mM phenylmethanesulfonyl fluoride. Buffer PG contains 20 mM potassium phosphate (pH 6.8), 10% glycerol, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfon fluoride, 0.5% CHAPS.

Analytical Methods

Protein concentrations were determined by the Bio-Rad protein assay kit using bovine serum albumin as a standard. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (15).

Preparation of Mitochondrial Methionyl-tRNA<sup>Met</sup> and E. coli Methionyl-tRNA<sup>Met</sup>

Mitochondrial Met-tRNA synthetase (MetRS<sub>mt</sub>) was partially purified from mitochondrial extracts by chromatography on DEAEE-Sepharose and ceramic hydroxyapatite (Bio-Rad). E. coli MetRS was partially purified from E. coli extracts by chromatography on DEAEE-Sepharose and ceramic hydroxyapatite (Bio-Rad). Mitochondrial tRNA<sup>Met</sup> was purified by a solid-phase hybridization method using DNA probes complementary to the 30 bases at the 3′-end of the tRNA (16). E. coli tRNA<sup>Met</sup> and tRNA<sup>Met</sup> were purified as described (17, 18). Further purification was carried out on a 6% native polyacrylamide gel if necessary.

Aminoacylation of mitochondrial tRNA<sup>Met</sup> was carried out in reaction mixtures (100 μl) containing 100 mM Tris-HCl (pH 8.5), 14 mM Mg(OAc)<sub>2</sub>, 20 mM KCl, 2 mM dithiothreitol, 4 mM ATP, 1 mM spermine, 20 μM [35S]methionine (200 GBq/mmol), 2–4 μM tRNA<sup>Met</sup>, and saturating amounts of partially purified MetRS<sub>mt</sub>. The tRNA was extracted using phenol equilibrated at pH 5.0 and the remaining ATP and methionine were removed on a Hi Trap desalting gel (Pharmacia). Aminoacylation of E. coli tRNA was carried out with [35S] or [14C]methionine as described (19) and the Met-tRNAs were purified as described above.

Purification of Bovine Liver Mitochondrial MTF

The bovine liver mitochondria were prepared as described (20). About 50 g of mitochondria were resuspended in 240 ml of Buffer TG containing 0.005 M KCl (TG.005), and disrupted by sonication using five 20-s bursts at 100 watts followed by 40-s cooling periods. The homogenate was subjected to centrifugation at 100,000 × g for 180 min. The supernatant fraction (S100) was either processed immediately or frozen and stored at −70 °C.

Step 1: Chromatography on DEAEE-Sepharose—CHAPS was added to a final concentration of 0.2% (w/v) to all the buffers indicated below. The S100 (5,700 mg) was applied to a 100-ml DEAEE-Sepharose fast flow column (17.5 × 2.7 cm) equilibrated with Buffer TG.005, and a flow rate of about 4 ml/min. The column was washed with Buffer TG.005 until the absorbance at 280 nm became less than 0.1 and the proteins bound to the column were eluted by a 1.0-liter linear gradient of 5–400 mM KCl in Buffer TG. Fractions (10 ml) were collected at a flow rate of about 20 ml/min. Fractions containing MTF<sub>mt</sub> activity were pooled and concentrated by ammonium sulfate precipitation (45–60% saturation). The pellet was then dissolved in Buffer PG and dialyzed against Buffer PG containing 0.15 M KCl (PG.15) for 6 h with two changes of buffer.

Step 2: Chromatography on Mono S—The sample (1,300 mg) was applied to a Mono S column (0.5 × 5 cm) equilibrated in Buffer PG.15 at a flow rate of 0.25 ml/min. The column was then washed with Buffer PG.15 and developed with a 10-ml linear gradient from 0.15 to 0.4 M KCl in Buffer PG. Fractions (0.25 ml) were collected at the flow rate of 0.25 ml/min. The fractions with MTF<sub>mt</sub> activity were pooled and dialyzed with Buffer PG until the concentration of KCl was less than 0.25 M. The sample was then frozen quickly and stored at −70 °C.

Step 3: Chromatography on Hi Trap Blue—The sample (1.6 mg) was applied to a flow rate of 0.25 ml/min to a Hi Trap Blue column (1 ml) equilibrated in Buffer PG containing 0.25 M KCl (PG.25). After washing with Buffer PG.25, bound proteins were eluted with a 10-ml linear gradient from 0.25 to 0.75 M KCl in Buffer PG. Fractions (0.25 ml) were collected at the flow rate of 0.25 ml/min. The fractions with MTF<sub>mt</sub> activity were pooled and dialyzed with Buffer PG to decrease the KCl concentration to less than 0.10 M. The sample was then frozen quickly and stored at −70 °C.

Step 4: Chromatography on a Column Carrying Immobilized tRNA—E. coli tRNA was immobilized on CNBr-activated Sepharose 4B (Pharmacia). The partially purified sample (0.24 mg) containing MTF<sub>mt</sub> activity was applied to the column (0.5 × 2.1 cm) which had been equilibrated in Buffer PG containing 0.1 M KCl (PG.10). The column was developed with a linear gradient (0.10 to 0.90 M KCl in Buffer PG). Fractions (0.1 ml) were collected at the flow rate of 0.1 ml/min. The fractions showing MTF<sub>mt</sub> activity were dialyzed with Buffer PG to reduce the concentration of KCl to less than 0.25 M and stored at −70 °C.

Step 5: Chromatography on Hi Trap Heparin—The sample (0.13 mg) was applied to a Hi Trap Heparin column (1 ml) equilibrated with Buffer PG.25. The column was washed with Buffer PG.25 and developed with a 10-ml linear gradient from 0.25 to 0.75 M KCl in Buffer PG. Fractions of 0.25 ml were collected at the flow rate of 0.25 ml/min. Fractions with MTF<sub>mt</sub> activity were pooled and dialyzed against buffer containing 20 mM Tris-HCl (pH 7.6), 10 mM KCl, 5 mM MgCl₂, 10% glycerol, 1 mM dithiothreitol, and 0.5% CHAPS. The sample was divided into small aliquots, fast-frozen, and stored at −70 °C.

Assays of Bovine MTF<sub>mt</sub> Activity

The assay of the formylating activity was carried out according to Ref. 21 with a slight modification as follows. Reaction mixtures (50 μl) contained 20 mM Tris-HCl (pH 7.6), 10 mM KCl, 5 mM MgCl₂, 0.1 mg/ml bovine serum albumin, 0.5% (w/v) CHAPS, 1 mM dithiothreitol, 1 μM E. coli [35S]Met-tRNA<sup>Met</sup>, 0.3 mM N5-formyltetrahydrofolate, and the indicated amounts of MTF<sub>mt</sub>.

Determination of the Amino Acid Sequence of MTF<sub>mt</sub>

The partially purified sample containing MTF<sub>mt</sub> was subjected to SDS-PAGE and blotted onto a siliconized glass-fiber membrane (22). The band believed to be MTF<sub>mt</sub> (based on the correlation between the intensity of this band and the activity of MTF<sub>mt</sub>) was excised. The amino-terminal sequence was obtained on an Applied Biosystems 477A/120A protein sequencer. The sequences of internal peptides were obtained according to Cleveland et al. (23) with modifications indicated in Ref. 24.

Screening of cDNA Libraries and DNA Sequencing

Approximately 1 × 10⁶ plaques from a bovine heart cDNA library (Uni-ZAP<sup>TM</sup> XR, Stratagene) were screened by hybridization with a putative human MTF<sub>mt</sub> cDNA probe labeled by random priming (25). Hybridized clones were washed out at 65°C with 6 × SSC buffer containing 20 mM NaH₂PO₄ and 0.4% (w/v) SDS (26). Positive plaques were isolated and the pBluescript SK(−) plasmid clones were excised in vivo

| Purification step | Protein Total units | Specific activity<sup>a</sup> | Total recovery Purification |
|-------------------|-------------------|----------------|---------------------------|
| Mitochondrial extract<sup>b</sup> (S-100) | 5,700 ×10⁶ | 1,100 | 0.19 | 100 | 1 | -fold |
| DEAEE-Sepharose | 1,300 | 420 | 0.32 | 38 | 1.7 |
| Mono S | 1.6 | 27 | 17 | 2.5 | 89 |
| Hi Trap Blue | 0.24 | 7.5 | 31 | 0.068 | 160 |
| Affinity column (tRNA<sub>E. coli</sub>—Sepharose) | 0.13 | 7.0 | 54 | 0.064 | 280 |
| Hi Trap Heparin | 0.010 | 4.2 | 420 | 0.038 | 2,200 |

<sup>a</sup> Specific activity; 1 unit of the enzyme is the capacity of formylating 1 pmol of E. coli Met-tRNA<sup>Met</sup> in 1 min at 30 °C.

<sup>b</sup> From 63 g (wet weight) of bovine mitoplast.
according to the manufacturer's instructions (Stratagene). Plasmid DNA was subjected to autosequencing using a HITACHI SQ-5500 sequencer (27).

RESULTS AND DISCUSSION

Purification of MTFmt—The initiation of protein synthesis in mitochondria requires the use of the formylated initiator tRNA (Met-tRNA). Hence, this organelle must possess a factor equivalent to the bacterial methionyl-tRNA transformylase. When extracts of bovine mitochondria were tested for a factor that could carry out the formylation of E. coli Met-tRNAfMet, a small amount of activity could be detected. The partial purification of this activity (MTFmt) was carried out by successive column chromatography as described under "Experimental Procedures" (Table I).

The purification scheme resulted in a 2,200-fold purification of MTFmt with an overall yield of about 0.04%. Throughout the purification scheme, the recovery of MTF mt was significantly improved by the addition of the detergent CHAPS to all of the buffers used. This observation suggests that there are hydrophobic patches on MTFmt that lead to the absorption of this factor on the matrices of various resins or that reduce its solubility resulting in substantial losses of activity. Analysis of the partially purified preparation of MTFmt on SDS-PAGE (Fig. 1B) showed the presence of three major polypeptide bands. The band with a molecular mass of about 40,000 daltons was tentatively identified as MTFmt. The intensity of this band correlated with the amount of MTFmt activity observed. In addition, the transformylase would be expected to be about this size. This polypeptide represented about 25% of the protein in the partially purified sample. About 2.5 μg of MTFmt were obtained from 2 kg of bovine liver.

Amino Acid Sequence Determination of Peptides Derived from MTFmt and cDNA Cloning—In order to obtain cDNA clones of MTFmt, partial peptide sequences were determined. MTFmt was first subjected to NH2-terminal Edman degradation. Second, for the determination of internal amino acid sequences, peptides resulting from digestion with endoproteinase V8 were purified by polyacrylamide gel electrophoresis and subjected to Edman degradation. Three peptide sequences were obtained (Table II).

These sequences were then used to search the data bases. The partial sequence of one human cDNA (GenBank number 108908) included the sequence EVVTVPSPSP found as an internal peptide in bovine MTFmt. The nucleotide sequence of this cDNA contained a 400-bp region which was homologous to E. coli MTF. This cDNA clone was, thus, predicted to encode a portion of human MTF mt. Probes prepared from this putative human MTF mt cDNA hybridized with the E. coli MTF gene (data not shown). A bovine heart Uni-ZAP™XR cDNA library was screened using the human cDNA clone as a probe. Five positive plaques were isolated among 1 × 106 plaques, and the
plasmids carrying the cDNA inserts of interest were excised in vivo. The largest clone characterized carried a 1355-bp insert (Fig. 2). Sequence analysis indicated that this clone contained the entire coding region for the mature form of MTFmt (1071 bp) and a portion of a putative mitochondrial import signal (45 bp). The 3'-untranslated region was 220 bp in length and contained a conventional polyadenylation signal (AAUAAA) 17 nucleotides upstream of the poly(A) tail (Fig. 2) (28).

Characterization of the Sequence of Bovine MTFmt—The mature form of MTF mt is 357 amino acids in length and has a molecular weight of 40,017. This value is consistent with the molecular weight of the band identified as MFTmt on SDS-PAGE. The amino acid sequence of MTF mt is about 30% identical to the corresponding prokaryotic factors (Fig. 3A) (29–33).

It is interesting to note that the sequence of bovine MTF mt is also only 28% identical to that of yeast MTF mt (34) (Table III). It should be noted that, while the NH2-terminal amino acid sequence obtained by peptide sequencing was ASPGWED, the cDNA sequence obtained gave the sequence ASPPWED when translated into the amino acid sequence. This apparent discrepancy could arise if there is more than one copy of MTF mt gene in the bovine nuclear genome with slightly different sequences.

The crystal structure of E. coli MTF has recently been determined (35). Analysis of this structure indicates that MTF contains two domains, NH2-terminal and COOH-terminal domains. NH2-terminal domain carries the tetrahydrofolate (THF)-binding site in which the THF binding motif (SLLP motif) is contained, as well as a Rossman fold (35). The longest stretch of conserved residues among the MTFs from various sources is located in the THF-binding site proposed for E. coli MTF (35) (Fig. 3). The COOH-terminal domain provides a positively charged surface oriented toward the active center of the enzyme and is, presumably, involved in positioning the Met-tRNA substrate for the formylation reaction. The THF binding motif is present as SCLP in bovine MTFmt, and the Phe at position 14, which is assumed to be responsible for the interaction of E. coli MTF with the 3'-end of the Met-tRNA (35), is also conserved in the bovine mitochondrial counterpart (Phe-29) (Fig. 3). The COOH-terminal domain of E. coli MTF
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### Table III

| Organism          | E. coli | T. thermophilus | H. influenzae | M. genitalium | B. subtilis | Yeast (mt) | Bovine (mt) |
|-------------------|---------|-----------------|---------------|---------------|-------------|------------|-------------|
| %                 | %       | %               | %             | %             | %           | %          | %           |
| E. coli           | 100     | 100             | 100           | 100           | 100         | 100        | 100         |
| T. thermophilus   | 44      | 100             | 100           | 100           | 100         | 100        | 100         |
| H. influenzae     | 65      | 40              | 40            | 29            | 29          | 29         | 29          |
| M. genitalium     | 26      | 23              | 23            | 29            | 29          | 29         | 29          |
| B. subtilis       | 43      | 39              | 40            | 100           | 100         | 100        | 100         |
| Yeast (mt)        | 29      | 29              | 29            | 29            | 29          | 29         | 29          |
| Bovine (mt)       | 29      | 28              | 28            | 28            | 28          | 28         | 28          |

### Table IV

| Met-tRNA          | $V_{max}$ ($\times 10^{-2}$ M/min) | $K_m$ ($\mu$M) | Relative $V_{max}/K_m$ |
|-------------------|----------------------------------|----------------|------------------------|
| E. coli (f)       | 2.4                              | 0.091          | 0.83                   |
| E. coli (mt)      | 0.28                             | 0.095          | 0.991                  |
| Bovine mitochondria| 0.8                             | 0.025          | 1                      |

*Relative $V_{max}/K_m$ is the ratio of $V_{max}/K_m$ of mitochondrial Met-tRNA to $V_{max}/K_m$ of each Met-tRNA.*

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Characterized by an oligonucleotide binding fold termed the OB fold. The OB fold is formed by two orthogonally sheets consisting of five antiparallel strands folded into a $\beta$-barrel surrounded by $\alpha$-helices (35). Secondary structure predictions using the Chou-Fasman or Robson methods (36, 37) have not been able to clearly identify a similar structure in MTF$_{mt}$. The Lys at position 207 in the linker region between the two domains of E. coli MTF is thought to be involved in the interaction with the 3′-end of the tRNA (19). This residue (Lys-214) has been conserved in the spacer region between the domains of MTF$_{mt}$ (Fig. 3).

**Characterization of Substrate Specificity of MTF$_{mt}$**—The initiation of translation in most prokaryotic organisms requires the formylation of the initiator Met-tRNA by MTF. In E. coli, the formyl group is a positive determinant for the specific formylation of the initiator Met-tRNA by MTF. In contrast to all other systems, animal mitochondria do not contain two distinct methionyl-tRNA species that are used exclusively for the initiation or elongation phases of protein synthesis. Mammalian mitochondria have a single tRNA$_{Met}$ gene which is encoded in the organelle genome (10). There is no evidence that cytoplasmic tRNAs are imported into animal mitochondria (11). Thus, the single tRNA$_{Met}$ gene must, in some unknown manner, give rise to both an initiator tRNA (fMet-tRNA) and an elongator tRNA (Met-tRNA) (35, 19).

The unique presence of a single Met-tRNA species in mammalian mitochondria made it of considerable interest to address the substrate specificity of MTF$_{mt}$. The kinetic parameters governing the formylation of three native tRNA molecules, bovine mitochondrial Met-tRNA, E. coli Met-tRNA$^{Met}$, and E. coli Met-tRNA$^{Met}$, were measured (Table IV). The results of these experiments indicated that MTF$_{mt}$ is clearly able to use E. coli Met-tRNA$^{Met}$ with a $V_{max}$ that is about 3-fold higher than that observed with the mitochondrial Met-tRNA$^{Met}$. The $K_m$ observed with the E. coli initiator tRNA is a little over 3-fold higher than with the mitochondrial tRNA$^{Met}$. The net result is that the relative $V_{max}/K_m$ for these two tRNAs are essentially the same. Surprisingly, MTF$_{mt}$ was also able to formylate the E. coli elongator Met-tRNA$^{Met}$. This tRNA is never a substrate for formylation by the homologous E. coli MTF (38). The $K_m$ value observed with Met-tRNA$^{Met}$ is essentially the same as that observed for E. coli Met-tRNA$^{Met}$, clearly does not discriminate between the bacterial initiator and elongator Met-tRNAs. This observation is compatible with the fact that there is a single Met-tRNA species in mammalian mitochondria.

**E. coli MTF** is known to have some affinity for many different tRNAs that is mediated through an interaction between the 3′-end of the acceptor stem of the tRNA and regions surrounding Lys-207 in MTF (19) (Fig. 4). However, only Met-tRNA$^{Met}$ is a substrate for the actual formylation process. It has been speculated that the formylation reaction is triggered by the melting of the acceptor stem of Met-tRNA$^{Met}$ which is facilitated in the initiator tRNA by the unstable acceptor stem found in this tRNA (19, 39). Several residues in MTF are thought to be involved in this melting process, such as basic residues in the loop I, and Lys-207 in E. coli MTF, see text. This topological difference might allow MTF$_{mt}$ to formylate Met-tRNA with the rigid acceptor stem.

### Table III

| Organism          | E. coli | T. thermophilus | H. influenzae | M. genitalium | B. subtilis | Yeast (mt) | Bovine (mt) |
|-------------------|---------|-----------------|---------------|---------------|-------------|------------|-------------|
| %                 | %       | %               | %             | %             | %           | %          | %           |
| E. coli           | 100     | 100             | 100           | 100           | 100         | 100        | 100         |
| T. thermophilus   | 44      | 100             | 100           | 100           | 100         | 100        | 100         |
| H. influenzae     | 65      | 40              | 40            | 29            | 29          | 29         | 29          |
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| Bovine mitochondria| 0.8                             | 0.025          | 1                      |

*Relative $V_{max}/K_m$ is the ratio of $V_{max}/K_m$ of mitochondrial Met-tRNA to $V_{max}/K_m$ of each Met-tRNA.*

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**Fig. 4**. Schematic drawing of tertiary structure of MTFs from prokaryotes (A) and bovine mitochondria (putative) (B). The region around the insertion sequence in the Rossman fold of MTF$_{mt}$ is shorter than that of prokaryotic MTFs. The important elements for tRNA recognition in MTF$_{mt}$ might be located closer compared with those of prokaryotic MTF (Phe-14, basic residues in the loop I, and Lys-207 in E. coli MTF; see text). This topological difference might allow MTF$_{mt}$ to formylate Met-tRNA with the rigid acceptor stem.
length in class I aminoacyl-tRNA synthetases (41), is shorter in the case of bovine MTF<sub>mt</sub> than in prokaryotic MTF (Figs. 3 and 4). The difference in this distance would be expected to place a number of residues in somewhat different positions in MTF<sub>mt</sub>. This difference might permit the mitochondrial factor to form the transition state with the elongator Met-tRNA easily. Hence it might be able to formylate the elongator Met-tRNA without the necessity for melting the acceptor stem. Further experiments will be designed to evaluate this idea.

How the single tRNA<sup>Met</sup> gene gives rise to both an initiator and an elongator tRNA<sup>Met</sup> species still remains unknown at this time. One possibility is that the formylation of Met-tRNA converts it from the elongator to the initiator tRNA since formylation increases its affinity for IF-2<sub>mt</sub> and diminishes its ability to form a complex with EF-Tu<sub>mt</sub>. The ratio of formylated tRNA<sup>Met</sup> to the non-formylated Met-tRNA might be regulated by a component that is present in the mitochondrial extracts but not in the cytosolic extracts. Further experiments are required to investigate this issue by using purified IF-2<sub>mt</sub> and EF-Tu<sub>mt</sub>. The ratio of formylated to the non-formylated Met-tRNA might be regulated by an unknown mechanism in vivo in conjunction with the initiation of mitochondrial protein synthesis. An investigation of this possibility must await the development of a better understanding of the initiation of mitochondrial protein synthesis.

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