THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 273, No. 24, Issue of June 12, pp. 15085–15090, 1998

Printed in U.S.A.

Mammalian Mitochondrial Methionyl-tRNA Transformylase from Bovine Liver

PURIFICATION, CHARACTERIZATION, AND GENE STRUCTURE*

(Received for publication, December 22, 1997, and in revised form, March 9, 1998)

Nono Takeuchi‡, Makoto Kawakami§, Akira Omori¶, Takuya Ueda‡, Linda L. Spremulli‖, and Kimitsuna Watanabe‡**

From the ‡Department of Chemistry and Biotechnology, School of Engineering, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan, the §Department of Molecular Biology, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan, the ¶Mitsubishi-Kasei Institute of Life Sciences, 11 Minamiooya, Machida-shi, Tokyo 194, Japan, and the ‖Department of Chemistry, University of North Carolina, Chapel Hill, North Carolina 27599-3290

The mammalian mitochondrial methionyl-tRNA transformylase (MTF_{mt}) was partially purified 2,200-fold from bovine liver mitochondria using column chromatography. The polypeptide responsible for MTF_{mt} activity was excised from a sodium dodecyl sulfate-polyacrylamide gel and the amino acid sequences of several peptides were determined. The cDNA encoding bovine MTF_{mt} was obtained and its nucleotide sequence was determined. The deduced amino acid sequence of the mature form of MTF_{mt} 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 MTF_{mt} formylates its homologous mitochondrial methionyl-tRNA and the *E. coli* initiator methionyl-tRNA (Met-$tRNA^{fMet}$) with essentially equal efficiency. The *E. coli* elongator methionyl-tRNA (Met-$tRNA^{mMet}$) was also formylated although with somewhat less favorable kinetics. These results suggest that the substrate specificity of MTF_{mt} 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 $tRNA^{Met}$ gene in mammalian mitochondria.

During the initiation 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)[1] 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-$tRNA^{iMet}$ 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 fMet-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 $tRNA^{Met}$ is present and no tRNAs appear to be imported into mammalian mitochondria (11). It is unclear how a single $tRNA^{Met}$ species can play the dual roles of an initiator and an elongator tRNA. Translational initiation in mammal mitochondria requires fMet-tRNA for the $IF\text{-}2_{mt}$-dependent binding to ribosomes. However, the unformylated form is required by $EF\text{-}Tu_{mt}$ for chain elongation (12, 13). Thus, the single $tRNA^{Met}$ gene must give rise to two species of tRNA (fMet-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 (MTF_{mt}).

* This work is supported by a Grant-in-Aid for Scientific Research on Priority Area from Ministry of Education, Science, Sports and Culture of Japan. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB004316.

** To whom correspondence should be addressed. Fax: 81-3-5800-6950; E-mail: kw@kwl.t.u-tokyo.ac.jp.

[1] The abbreviations used are: IF-2, initiation factor-2; PAGE, polyacrylamide gel electrophoresis; MTF, methionyl-tRNA transformylase; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; bp, base pair; THF, tetrahydrofuran.

EXPERIMENTAL PROCEDURES

Materials

Folinic acid and CHAPS were purchased from Sigma. [^{35}S]Methionine (37 TBq/mmol) and [^{14}C]methionine (1.85 GBq/mmol) were obtained from Amersham. DEAE-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 $MgCl_2$, 0.1 mM EDTA, 6 mM β-mercaptoethanol, 10% glycerol, 0.1 mM phenymethylsulfonyl fluoride. Buffer PG contains 20 mM potassium phosphate (pH 6.8), 10% glycerol, 1 mM dithiothreitol, 0.1 mM phenymethylsulfonyl 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^{Met} and E. coli Methionyl-tRNAs^{Met}

Mitochondrial Met-tRNA synthetase ($MetRS_{mt}$) was partially purified from mitochondrial extracts by chromatography on DEAE-Sepharose and ceramic hydroxyapatite (Bio-Rad). *E. coli* MetRS was partially purified from *E. coli* extracts by chromatography on DEAE-Sepharose and ceramic hydroxyapatite (Bio-Rad). Mitochondrial $tRNA^{Met}$ 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* $tRNA^{fMet}$ and $tRNA^{mMet}$ were purified as described (17, 18). Further purification was carried out on a 6% native polyacrylamide gel if necessary.

Aminoacylation of mitochondrial $tRNA^{Met}$ was carried out in reaction mixtures (100 μl) containing 100 mM Tris-HCl (pH 8.5), 14 mM $Mg(OAc)_2$, 20 mM KCl, 2 mM dithiothreitol, 4 mM ATP, 1 mM spermine, 20 μM [^{35}S]methionine (200 GBq/mmol), 2–4 μM $tRNA^{Met}$, and saturating amounts of partially purified $MetRS_{mt}$. 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 [^{35}S] or [^{14}C]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 60 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 quickly and stored at −70 °C.

Step 1: Chromatography on DEAE-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 DEAE-Sepharose fast flow column (17.5 × 2.7 cm) equilibrated with Buffer TG.005, at a flow rate of about 2.0 ml/min. The column was washed by 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 2.0 ml/min. Fractions containing MTF_{mt} 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_{mt} activity were pooled and diluted with Buffer PG until the concentration of KCl was less than 0.25 M. The sample was immediately frozen and stored at −70 °C.

Step 3: Chromatography on Hi Trap Blue—The sample (1.6 mg) was applied at 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_{mt} activity were pooled and diluted 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_{mt} 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 0.60 M KCl in Buffer PG). Fractions (0.1 ml) were collected at the flow rate of 0.1 ml/min. The fractions showing MTF_{mt} activity were diluted 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_{mt} activity were pooled and dialyzed against buffer containing 20 mM Tris-HCl (pH 7.6), 10 mM KCl, 5 mM $MgCl_2$, 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_{mt} Activity

The assay of the formylation 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 $MgCl_2$, 0.1 mg/ml bovine serum albumin, 0.5% (w/v) CHAPS, 1 mM dithiothreitol, 1 μM *E. coli* [^{14}C]Met-$tRNA^{fMet}$, 0.3 mM N^{10}-formyltetrahydrofolate, and the indicated amounts of MTF_{mt}.

Determination of the Amino Acid Sequence of MTF_{mt}

The partially purified sample containing MTF_{mt} was subjected to SDS-PAGE and blotted onto a siliconized glass-fiber membrane (22). The band believed to be MTF_{mt} (based on the correlation between the intensity of this band and the activity of MTF_{mt}) 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 × 10^6 plaques from a bovine heart cDNA library (Uni-ZAP^{TM} XR, Stratagene) were screened by hybridization with a putative human MTF_{mt} cDNA probe labeled by random priming (25). Hybridizations were carried out at 65 °C with 6 × SSC buffer containing 20 mM NaH_2PO_4 and 0.4% (w/v) SDS (26). Positive plaques were isolated and the pBluescript SK(−) plasmid clones were excised *in vivo*

TABLE I
Purification of mitochondrial MTFmt from bovine liver

Purification step	Protein	Total units	Specific activity[a]	Total recovery	Purification
	×10^3 mg	*×10^3*	*unit/mg*	*%*	*-fold*
Mitochondrial extract[b] (S-100)	5,700	1,100	0.19	100	1
DEAE-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 ($tRNA^{mix}_{E.\ coli}$-Sepharose)	0.13	7.0	54	0.064	280
Hi Trap Heparin	0.010	4.2	420	0.038	2,200

[a] Specific activity; 1 unit of the enzyme is the capacity of formylating 1 pmol of *E. coli* Met-$tRNA^{fMet}$ in 1 min at 30 °C.
[b] 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 MTF$_{mt}$—The initiation of protein synthesis in mitochondria requires the use of the formylated initiator tRNA (fMet-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-tRNA^{fMet}, a small amount of activity could be detected. The partial purification of this activity (MTF$_{mt}$) 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 MTF$_{mt}$ 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 MTF$_{mt}$ 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 MTF$_{mt}$ on SDS-PAGE (Fig. 1*B*) showed the presence of three major polypeptide bands. The band with a molecular mass of about 40,000 daltons was tentatively identified as MTF$_{mt}$. The intensity of this band correlated with the amount of MTF$_{mt}$ 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 MTF$_{mt}$ were obtained from 2 kg of bovine liver.

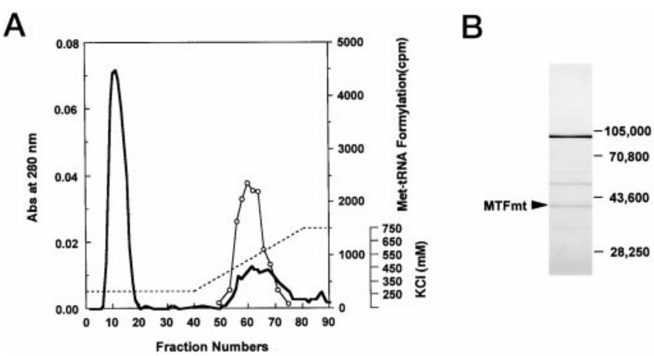

FIG. 1. **Purification of bovine MTF$_{mt}$.** *A*, elution profile of MTF$_{mt}$ on Hi Trap Heparin. The column was developed as described under "Experimental Procedures." Samples (10 μl) were assayed for MTF$_{mt}$ activity (*open circles*). Absorbance at 280 nm (*solid line*) was monitored using the scale of 0 to 0.1. The column was developed with the linear salt gradient (*dashed line*). *B*, SDS-PAGE analysis of MTF$_{mt}$. The sample (2 μg) purified from Hi Trap Heparin was analyzed on SDS-PAGE analysis. The molecular weight markers were phosphorylase *b* (105,000), bovine serum albumin (70,800), ovalbumin (43,600), and carbonic anhydrase (28,250). The *arrow* indicates the band that was excised for sequence analysis.

Amino Acid Sequence Determination of Peptides Derived from MTF$_{mt}$ and cDNA Cloning—In order to obtain cDNA clones of MTF$_{mt}$, partial peptide sequences were determined. MTF$_{mt}$ was first subjected to NH_2-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 MTF$_{mt}$. 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^{TM}XR cDNA library was screened using the human cDNA clone as a probe. Five positive plaques were isolated among 1×10^6 plaques, and the

TABLE II
Sequences of peptide fragments derived from bovine liver MTFmt

Peptide	Position
1 = NH_2-ASPPWED	1–7
2 = EVVTVPSPSP	53–62
3 = ETVPVPPKST	162–171

```
                                                                    ggc acg agg ccg aac cca cgc tgg cga gcg ctg gcc agg ctg agc
                                                                     G   T   R   P   N   P   R   W   R   A   L   A   R   L   S

    gcg tcc cct ggc tgg gag gac ggc cag ggc gct cga gtc cgc gag aag ccg cct tgg agg gtg ctc ttt ttc ggc aat gac cag ttc gcc cgc gaa acg ctg cgg
  1  A   S   P   G   W   E   D   G   Q   G   A   R   V   R   E   K   P   P   W   R   V   L   F   F   G   N   D   Q   F   A   R   E   T   L   R   35

    gcg ctg cac gcc gcc agg gaa aac aaa gag gaa gag tta att gag aag ttg gag gtg gtc aca gtg ccc tcc cca tca cca aaa gga ctg cca gtg aag caa tat
 36  A   L   H   A   A   R   E   N   K   E   E   E   L   I   E   K   L   E   V   V   T   V   P   S   P   S   P   K   G   L   P   V   K   Q   Y   70

    gct gtc cag tct cag ctt cca gtg tat gag tgg cca gat gtg gga tct gga gaa tac gat gtt ggc gtg gta gca tca ttt ggc cga ctt ttg agt gag gct ttt
 71  A   V   Q   S   Q   L   P   V   Y   E   W   P   D   V   G   S   G   E   Y   D   V   G   V   V   A   S   F   G   R   L   L   S   E   A   F  105

    att ctt aaa ttt ccc tac ggc atc ttg aat gtc cat ccc agt tgc ctt ccg agg tgg cgt ggt cca gcc cct ata atc cac acc atc ctg cat gga gac aca att
106  I   L   K   F   P   Y   G   I   L   N   V   H   P   S   C   L   P   R   W   R   G   P   A   P   I   I   H   T   I   L   H   G   D   T   I  140

    gct gga gta acg att atg caa att aaa ccc aga agg ttt gat gta ggt cca atc ctc aaa cag gag acg gtt cct gtg cca ccc aag agc acc tca aag gaa ttg
141  A   G   V   T   I   M   Q   I   K   P   R   R   F   D   V   G   P   I   L   K   Q   E   T   V   P   V   P   P   K   S   T   S   K   E   L  175

    gaa gca gtg ctg tca aga ctg ggt gcc aac atg ctt att tca gtt ttg aaa aat ttg cct gaa agt ttg aat aat gga aga cag cag cca gcc gag ggg gtg aca
176  E   A   V   L   S   R   L   G   A   N   M   L   I   S   V   L   K   N   L   P   E   S   L   N   N   G   R   Q   Q   P   A   E   G   V   T  210

    cac gcc cct aag att tct gct gct acc agc tgt ata aaa tgg gag gaa caa act tca gag cag ata ttc aga ctt tat cgt gct gtt gga aat ata att ccg ttg
211  H   A   P   K   I   S   A   A   T   S   C   I   K   W   E   E   Q   T   S   E   Q   I   F   R   L   Y   R   A   V   G   N   I   I   P   L  245

    cag aca ctc tgg atg gat aat acc att aaa ctt ctg gat ttg gta gaa gtt gat aat tca atc ctc agt gat tca aaa tta aca gga caa gct gtt att cca gga
246  Q   T   L   W   M   D   N   T   I   K   L   L   D   L   V   E   V   D   N   S   I   L   S   D   S   K   L   T   G   Q   A   V   I   P   G  280

    tca gta ata tac cac aaa cag tca caa ata ctg ctg gtt tgt tgc aag gat gac tgg att ggt gtt cga tca gtg atg ctc aag aaa aca cta aca gct act gac
281  S   V   I   Y   H   K   Q   S   Q   I   L   L   V   C   C   K   D   D   W   I   G   V   R   S   V   M   L   K   K   T   L   T   A   T   D  315

    ttc tac aat gga tat ttg cac ccc tgg tac cag aaa aat tcc caa gct caa cca agc caa tgc aga ttt cag act ctc aga ctt cca cca aag aag aag cag aaa
316  F   Y   N   G   Y   L   H   P   W   Y   Q   K   N   S   Q   A   Q   P   S   Q   C   R   F   Q   T   L   R   L   P   P   K   K   K   Q   K  350

    aaa aaa att gtt gct atg caa tag tgc acc aag tac ttt gga aga aga tgg aca aga acc tac tat ata ctt gta att tgt tag aag cct tat tta caa gga att
351  K   K   I   V   A   M   Q   *                                                                                                           357

    atc ttg act ttc aaa gaa ttt gat gcc gtt gaa agg gaa gat tat tgt caa aaa gat gaa tta cct cac ttt gca ttt tcc gtc taa cag tta aaa ata gct ttt
    tct gtt tga ata aaa aga gaa gtt tta aat t(a)n
```

FIG. 2. **Nucleotide and deduced amino acid sequences of bovine liver MTF$_{mt}$.** The amino acid numbered +1 corresponds to the first residue of the mature form of MTF$_{mt}$. The putative polyadenylation signals are *underlined*, and $(a)_n$ denotes the poly(a) tail.

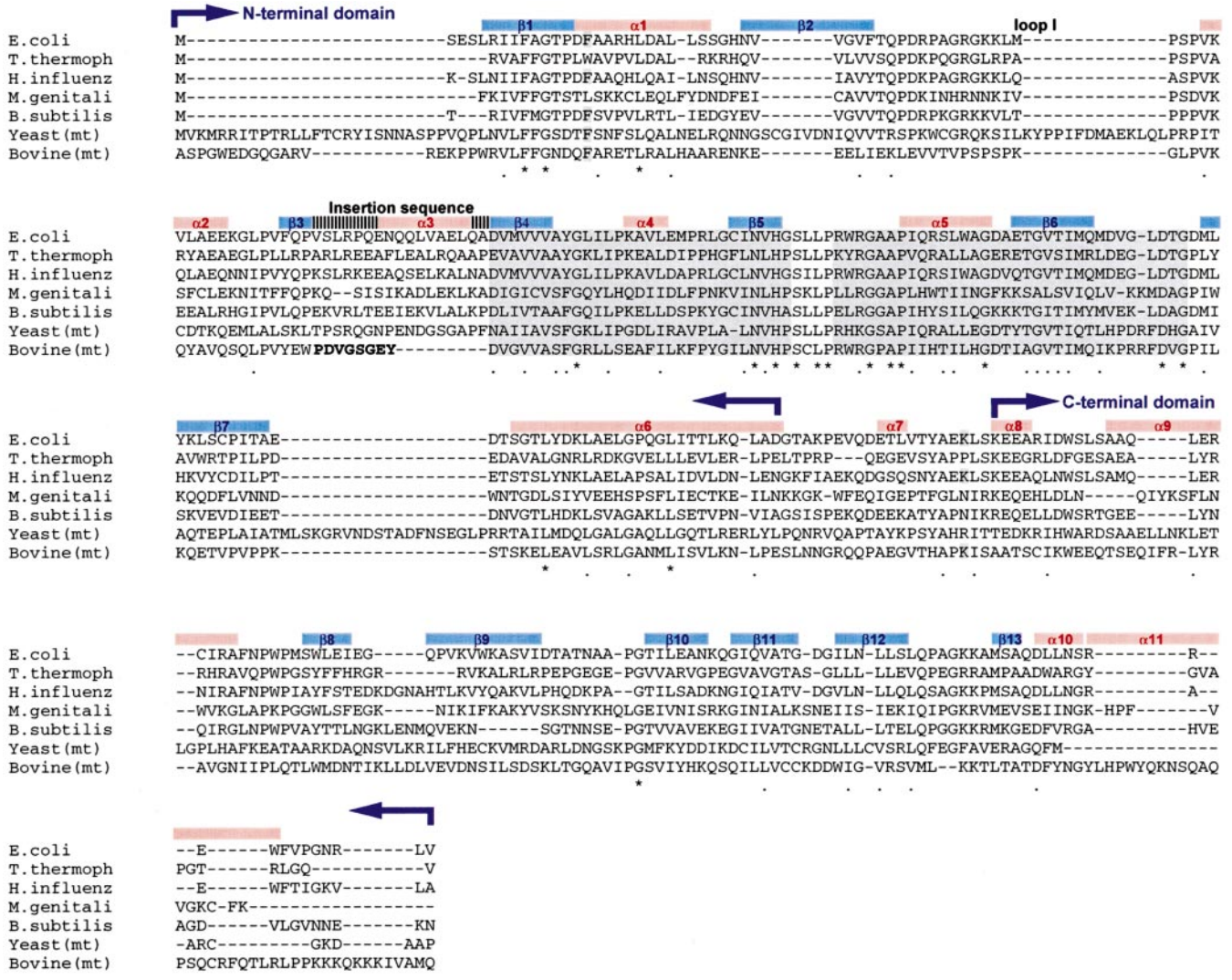

FIG. 3. **Multiple sequence alignment of MTF polypeptides from various sources.** The sequences are those of the enzymes from *E. coli*, *Thermus thermophilus* (*T. thermoph*), *Hemophilus influenzae* (*H. influenz*), *Mycoplasma genitalium* (*M. genitali*), *Bacillus subtilis* (*B. subtilis*), *Saccharomyces cerevisiae* mitochondria (*Yeast (mt)*), and bovine liver mitochondria (*Bovine(mt)*). Multiple sequence alignment of MTF polypeptides was performed with the CLUSTAL V program. The positions strictly conserved in the seven compared sequences are marked with an *asterisk below* the sequence. Positions with conservative replacements are designated by a *dot*. The THF-binding site containing the THF binding motif (SLLP motif) and the residues thought to interact with the 3′-end of $tRNA^{Met}$ in the case of *E. coli* MTF are conserved (*gray box*) (19, 35). The secondary structure of *E. coli* MTF is designated with *red* (α-helix) and *blue* (β-strand) *lines* above the sequence (35). The region that is speculated to correlate with the unique substrate specificity of bovine MTF_{mt} is shown with *bold letters*. This region corresponds to the insertion sequence in the Rossman fold which is usually variable in length in the class I aminoacyl-tRNA synthetases (41).

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 MTF_{mt} (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 MTF_{mt}—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 MFT_{mt} on SDS-PAGE. The amino acid sequence of MTF_{mt} is about 30% identical to the corresponding prokaryotic factors (Fig. 3*A*) (29–33). It is interesting to note that the sequence of bovine MFT_{mt} is also only 28% identical to that of yeast MTF_{mt} (34) (Table III).

It should be noted that, while the NH_2-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, NH_2-terminal and COOH-terminal domains. NH_2-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 MTF_{mt}, 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 is

TABLE III
Identity of the known MTF amino acid sequences from various organisms

Sequence comparisons were done with the Maximum Matching program in GENETYX version 7.0. The designations used are as follows: *E. coli, Thermus thermophilus (T. thermophilus), Haemophilus influenzae (H. influenzae), Mycoplasma genitalium (M. genitalium), Bacillus subtilis (B. subtilis), Saccharomyces cerevisiae* mitochondria (Yeast (mt)), and bovine liver mitochondria (Bovine (mt)).

	E. coli	*T. thermophilus*	*H. influenzae*	*M. genitalium*	*B. subtilis*	Yeast (mt)	Bovine (mt)
	%	%	%	%	%	%	%
E. coli	100						
T. thermophilus	44	100					
H. influenzae	65	40	100				
M. genitalium	26	23	29	100			
B. subtilis	43	39	40	32	100		
Yeast (mt)	29	29	29	24	27	100	
Bovine (mt)	29	28	29	24	28	28	100

characterized by an oligonucleotide binding fold termed the OB fold. The OB fold is formed by two orthogonal sheets consisting of five antiparallel strands folded into a β-barrel surrounded by α-helices (35). Secondary structure predictions using the Chou-Fasman or Robson methods (36, 37) have not able been 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 interaction of IF-2 with the initiator tRNA. It also serves as a negative determinant that nearly eliminates the binding of EF-Tu to the initiator tRNA (5). Bacterial MTF will not formylate the Met-$tRNA^{mMet}$ species used for chain elongation. The strict substrate specificity of *E. coli* MTF is essential to ensure the accuracy and the efficiency of the initiation process. 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-$tRNA^{fMet}$, and *E. coli* Met-$tRNA^{mMet}$, were measured (Table IV). The results of these experiments indicated that MTF_{mt} is clearly able to use *E. coli* Met-$tRNA^{fMet}$ 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-$tRNA^{mMet}$. This tRNA is never a substrate for formylation by the homologous *E. coli* MTF (38). The K_m value observed with Met-$tRNA^{mMet}$ is essentially the same as that observed for *E. coli* Met-$tRNA^{fMet}$ while the V_{max} is about 3-fold lower than that observed with the mitochondrial Met-tRNA. MTF_{mt} 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.

TABLE IV
Kinetic parameters in formylation of various Met-tRNAs

Met-tRNA	V_{max} ($\times 10^{-2}$ μM/min)	K_m	Relative V_{max}/K_m [a]
		μM	
E. coli (f)	2.4	0.091	0.83
E. coli (m)	0.28	0.095	0.091
Bovine mitochondria	0.8	0.025	1

[a] Relative V_{max}/K_m is the ratio of Vmax/Km of mitochondorial Met-tRNA to V_{max}/K_m of each Met-tRNA.

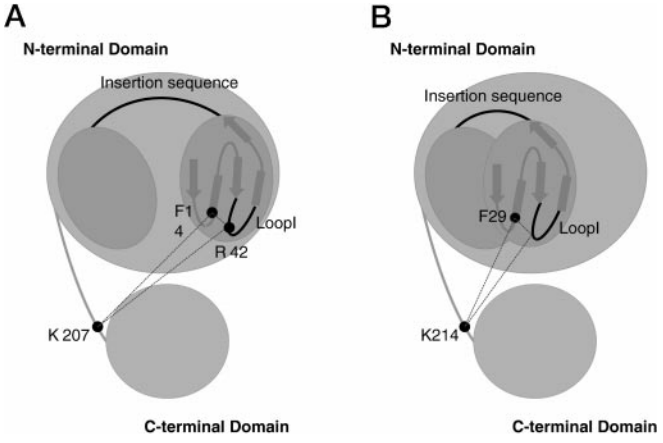

FIG. 4. **Schematic drawing of tertiary structure of MTFs from prokaryotes (*A*) and bovine mitochondria (putative) (*B*).** The region around the insertion sequence in the Rossman fold of MTF_{mt} is shorter than that of prokaryotic MTFs. The important elements for tRNA recognition in MTF_{mt} might 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.

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-$tRNA^{fMet}$ 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-$tRNA^{fMet}$ 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 Phe-14 (35, 40) (Fig. 4). Phe-14 corresponds to Phe-29 in MTF_{mt}, but Arg-42 in the loop I could not be identified clearly in MTF_{mt}. A comparison of the sequences of prokaryotic MTF and yeast MTF_{mt} with the sequence of bovine MTF_{mt} does not provide a clear rationale for the structural basis for the Met-tRNA specificities of these enzymes. However, it should be noted that the region termed the "insertion sequence" in the Rossman fold, which is usually variable in

length in class I aminoacyl-tRNA synthetases (41), is shorter in the case of bovine MTF_{mt} 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_{mt}. 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 $tRNA^{Met}$ gene gives rise to both an initiator and an elongator $tRNA^{Met}$ 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 $IF\text{-}2_{mt}$ and diminishes its ability to form a complex with $EF\text{-}Tu_{mt}$. The ratio of formylated to the non-formylated Met-tRNA might be regulated by a competition between MTF_{mt} and $EF\text{-}Tu_{mt}$ for Met-RNA. The purification and characterization of MTF_{mt} as described here will allow us to investigate this issue by using purified $IF\text{-}2_{mt}$ and $EF\text{-}Tu_{mt}$. Alternatively, the activity of MTF_{mt} 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.

Acknowledgments—We are indebted to our previous co-workers, Dr. T. Yokogawa (Gifu University), Dr. C. Takemoto (University of Tokyo), T. Koike (National Institute of Genetics), and Dr. L. Benkowski (University of North Carolina), for their experimental supports and critical discussions. We thank S. Yoshida, Mitsubishikasei Institute of Life Science, for technical assistance in peptide sequence determination.

REFERENCES

1. Kozak, M. (1983) *Microbiol. Rev.* **47,** 1–45
2. Marcker, K., and Sanger, F. (1964) *J. Mol. Biol.* **8,** 835–840
3. Mahler, H. (1973) *Crit. Rev. Biochem.* **1,** 625–637
4. Schwartz, J. H., Meyer, R., Eisenstadt, J. M., and Brawerman, G. (1967) *J. Mol. Biol.* **25,** 571–574
5. RajBhandary, U. L. (1994) *J. Bacteriol.* **176,** 547–552
6. Åström, S. U., and Byström, A. S. (1994) *Cell* **79,** 535–546
7. Galper, J. B., and Darnell, J. E. (1971) *J. Mol. Biol.* **57,** 363–367
8. Kumazawa, Y., Schwartzbach, C. J., Liao, H. X., Mizumoto, K., Kaziro, Y., Miura, K., Watanabe, K., and Spremulli, L. L. (1991) *Biochim. Biophys. Acta* **1090,** 167–172
9. Dirheimer, G., Keith, G., Dumas, P., and Westhof, E. (1995) *tRNA* (Süll, D., RajBhandary, U. L., eds) pp. 93–126, ASM Press, Washington, D. C.
10. Anderson, S., de Bruijin, M. H., Coulson, A. R., Eperon, I. C., Sanger, F., and Young, I. G. (1982) *J. Mol. Biol.* **156,** 683–717
11. Roe, B. A., Wong, J. F. H., and Chen, E. Y. (1981) *Proceedings of the Third Cleaveland Symposium on Macromolecules* (Walton, A. G., ed) pp. 167–176, Elsevier, Amsterdam
12. Liao, H. X., and Spremulli, L. L. (1990) *J. Biol. Chem.* **265,** 13618–13622
13. Schwartzbach, C. J., and Spremulli, L. L. (1989) *J. Biol. Chem.* **264,** 19125–19131
14. Cuatrecasas, P. (1970) *J. Biol. Chem.* **245,** 3059–3065
15. Laemmli, U. K. (1970) *Nature* **227,** 680–685
16. Wakita, K., Watanabe, Y., Yokogawa, T., Kumazawa, Y., Nakamura, S., Ueda, T., Watanabe, K., and Nishikawa, K. (1994) *Nucleic Acids Res.* **22,** 347–353
17. Nishimura, S. (1971) *Procedures in Nucleic Acid Research* (Cantoni, G. L., Davies, D. R., eds) Vol. 2, Harper & Row, New York
18. Yoshida, M., Takeishi, K., and Ukita, T. (1971) *Biochim. Biophys. Acta* **228,** 153–166
19. Gite, S., and RajBhandary, U. L. (1997) *J. Biol. Chem.* **272,** 5305–5312
20. Schwartzbach, C. J., Farwell, M., Liao, H. X., and Spremulli, L. L. (1996) *Methods Enzymol.* **264,** 248–261
21. Blanquet, S., Dessen, P., and Kahn, D. (1984) *Methods Enzymol.* **106,** 141–153
22. Eckerskron, C., Mewes, W., Goretzki, H., and Lottspeich, F. (1988) *Eur. J. Biochem.* **176,** 509–519
23. Cleveland, D. W., Fisher, S. G., Kirschner, M. W., and Laemmli, U. K. (1977) *J. Biol. Chem.* **252,** 1102–1106
24. Omori, A., and Yoshida, S. (1994) *J. Protein Chem.* **13,** 471
25. Gottesman, M. M. (1987) *Methods Enzymol.* **151,** 363–364
26. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
27. Biggin, M. D., Gibson, T. J., and Hong, G. F. (1983) *Proc. Natl. Acad. Sci. U. S. A.* **80,** 3963–3965
28. Wickens, M., and Stephenson, P. (1984) *Science* **226,** 1045–1051
29. Guillon, J. M., Meinnel, T., Mechulam, Y., Lazennec, C., Blanquet, S. and Fayat, S. (1992) *J. Mol. Biol.* **244,** 359–367
30. Meinnel, T., and Blanquet, S. (1994) *J. Bacteriol.* **176,** 7387–7390
31. Fleischmann, R. D., *et al*. (1995) *Science* **269,** 496–512
32. Fraser, C. M. *et al*. (1997) *J. Mol. Biol.* **266,** 939–949
33. Kunst, F., *et al.* (1997) *Nature* **390,** 249–256
34. Skala, J., Van Dyck, L., Purnelle, B., and Goffeau, A. (1992) *Yeast* **8,** 777–785
35. Schmitt, E., Blanquet, S., and Mechulam, Y. (1996) *EMBO J.* **15,** 4749–4758
36. Chou, P. Y., and Fasman, G. D. (1978) *Adv. Enzymol.* 45–148
37. Garnier, J., Osguthorpe, D. J., and Robson, B. (1978) *J. Mol. Biol.* **120,** 97–120
38. Dickerman, H. W., Steers, E., Jr., Redfield, B. G., and Weissbach, H. (1967) *J. Biol. Chem.* **242,** 1522–1525
39. Wallis, N. G., Dardel, F., and Blanquet, S. (1995) *Biochemistry* **34,** 7668–7677
40. Ramesh, V., Gite, S., Li, Y., and RajBhandary, U. L. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13524–13529
41. Arnez, J. G., and Moras, D. (1994) *RNA-Protein Interactions* (Nagai, K., and Mattaj, I. W., eds) pp. 52–81, Oxford University Press, Oxford