

Do mammalian cells synthesize lipoic acid?

Identification of a mouse cDNA encoding a lipoic acid synthase located in mitochondria¹

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Abstract Lipoic acid is a coenzyme essential to the activity of enzymes such as pyruvate dehydrogenase, which play important roles in central metabolism. However, neither the enzymes responsible for biosynthesis nor the biosynthetic event of lipoic acid has been reported in mammalian cells. In this study, a mouse *mLIP1* cDNA for lipoic acid synthase has been identified. We have shown that the cDNA encodes a lipoic acid synthase by its ability to complement a mutant of *Escherichia coli* defective in lipoic acid synthase and that *mLIP1* is targeted into the mitochondria. These findings suggest that mammalian cells are able to synthesize lipoic acid in mitochondria. © 2001 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

Key words: Lipoic acid; Lipoic acid synthase; Mitochondrion; Pyruvate dehydrogenase; Thioctic acid

1. Introduction

Lipoic acid (6,8-thioctic acid) is a sulfur-containing cofactor required for the activity of enzyme complexes involved in central metabolism. Pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, branched-chain 2-oxo acid dehydrogenase complexes, and the glycine cleavage system are known as lipoic acid-requiring enzyme complexes [1–6]. In each protein complex, lipoic acid is covalently bound to one subunit of these complexes via an amide linkage to the ϵ -amino group of a specific Lys residue [7]. The lipoyl-lysine arm functions as

a carrier of reaction intermediates and interacts with the active sites of the components of the complexes [8,9]. Recently, lipoic acid has received attention for its role as a biological antioxidant. Extensive studies of lipoic acid and its reduced form, dihydrolipoic acid, have shown that both possess potent antioxidant activity [10,11]. Both forms protect membranes by interacting with ascorbic acid and glutathione, which may in turn recycle vitamin E. Furthermore, they can function as a redox regulator of transcription factors such as nuclear factor- κ B and activator protein-1 [12].

Despite the importance of lipoic acid as described above, the metabolism of lipoic acid in mammalian cells is poorly understood. It is certain that lipoic acid supplied by diet must be transported by the blood stream to tissues, and then incorporated into the cells. A major part of lipoic acid incorporated into the cells must be translocated into the mitochondria where lipoic acid-requiring enzyme complexes are located [1–6]. The translocated lipoic acid is activated to become lipoyl-AMP by lipoate-activating enzyme, and the lipoyl moiety is then transferred to E2 subunits of 2-oxo acid dehydrogenase complexes and H-protein of the glycine cleavage system by the action of lipoyltransferase [13,14]. In addition to the supply of lipoic acid through the diet, it might also be synthesized endogenously in mammalian cells [15,16]. However, there is no direct evidence that lipoic acid is synthesized in mammalian cells. Nothing is known regarding the enzymes responsible for biosynthesis of lipoic acid or the biosynthetic event in mammalian cells.

In the present study, we have identified and characterized a mouse cDNA, designated *mLIP1*, which encodes a lipoic acid synthase located in mitochondria. To our knowledge, this is the first identification of the cDNA for the enzyme involved in the biosynthesis of lipoic acid in mammalian cells. The present findings suggest that mammalian cells are capable of synthesizing lipoic acid in mitochondria.

2. Materials and methods

2.1. Materials

4-week-old mice (Jc1: ICR strain, male) were used for cDNA cloning. Chinese hamster ovary (CHO)-K1 cells were grown at 37°C under 5% (v/v) CO₂/air in F-12 nutrient mixture (Gibco BRL, Life Technology, Tokyo, Japan) supplemented with 10% (w/v) fetal bovine serum, 25 μ g/ml kanamycin, and 25 μ g/ml streptomycin.

2.2. cDNA cloning and analysis

Mouse EST clones encoding polypeptides homologous to lipoic acid synthase of *Arabidopsis thaliana* were obtained by screening of

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¹ The nucleotide sequence data of the *mLIP1* cDNA for lipoic acid synthase of the mouse were deposited in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession number AB057731.

Abbreviations: ACP, acyl carrier protein; CHO, Chinese hamster ovary; GFP, green fluorescent protein; ORF, open reading frame; PCR, polymerase chain reaction

the database of the National Center for Biotechnology Information using a BLAST search [17]. *mLIP1* cDNA including an open reading frame (ORF) was amplified by polymerase chain reaction (PCR). A primer, 5'-CAGACCTTCAGAAC-3', was used for the synthesis of cDNA using total RNAs isolated from the heart of a 4-week-old male mouse. A primer set, 5'-CCTTGACACTCCTGAGACCTAA-3' and 5'-TTGCACTCACAGGAATCCTGA-3', was used for the PCR. The amplified DNAs were subcloned into a pCR2.1 vector (Original TA Cloning kit, Invitrogen, Carlsbad, CA, USA), and their nucleotide sequences were determined.

DNA-sequencing reactions were performed using a sequencing kit (Thermo sequenase sequencing kit, Amersham, Buckinghamshire, UK) and sequences were determined by a DNA sequencer (ALF Red DNA sequencer, Pharmacia Biotech, Tokyo, Japan). Double-stranded DNAs were used as templates, and the sequence of each strand was determined. Nucleotide and deduced amino acid sequences were analyzed by suitable software (Genetyx-Mac, Software Development, Tokyo, Japan).

2.3. Southern and Northern hybridization analyses

Genomic DNA extracted from mouse testes and thymus was digested with the appropriate restriction enzymes, separated by electrophoresis on a 0.8% (w/v) agarose gel, and transferred to a nylon membrane (Hybond-N⁺, Amersham). The membrane was used for Southern hybridization analysis using a DNA-labeling and detection system (ECL kit, Amersham).

Northern hybridization analysis was performed with a blot (Multiple tissue Northern blot, Clontech, Palo Alto, CA, USA). The membrane was hybridized as described for the Southern hybridization analysis. Reverse transcription (RT)-PCR analysis of *mLIP1* was performed with an RT-PCR kit (Superscript One-Step System, Gibco BRL).

2.4. Functional complementation of the *Escherichia coli lipA* mutant

The regions of *mLIP1* cDNA encoding a precursor form and a putative mature form of mLIP1 were amplified by PCR with specific primers: a primer set 5'-CGCTGCAGCTCTACGCTGCTGGGAT-3' and 5'-CGCTGCAGCTTGAAGGGCTTGCTA-3', and another primer set 5'-CGCTGCAGCATTAAAGTTCTTTGCC-3' and 5'-CGCTGCAGCTTGAAGGGCTTGCTA-3'. The eight-nucleotide sequence 5'-CGCTGCAG-3' including a *Pst*I site was added to the 5'-end of each primer. PCR products were digested with *Pst*I and ligated into the *Pst*I site of an expression vector, pKK233-2 (Clontech), to give the in-frame desired product. The obtained plasmids were designated pmLIP1-Δ0 and pmLIP1-Δ26, where the number after the Δ represents the number of amino acid residues deleted from the N-terminus of the mLIP1 protein. These plasmids were used for the transformation of the *E. coli lipA* mutant KER176 [18]. The obtained transformants were used for complementation test as described previously [19]. The transformant of the *E. coli lipA* mutant with pKK233-2 was used as a negative control.

2.5. Intracellular localization analysis

The region of *mLIP1* cDNA encoding a putative presequence (a sequence from amino acids 1 to 59) presumably required for targeting into mitochondria was amplified by PCR with the following two primers: 5'-CGTATCTCGAGATGG-CTCTACGCTGCTGGGATA-3' and 5'-GCATAGTCGACCTCTGTCTGCAAGATCACCAG-3'. The 11-nucleotide sequences 5'-CGTATCTCGAG-3' and 5'-CGA-TAGTCGAC-3' including the *Xho*I and *Sal*I sites, respectively, were added to the 5'-end of each primer. The obtained PCR product was digested with *Xho*I and *Sal*I and ligated into the same sites of pEGFP-N1 (Clontech) to form the expression construct for the mLIP1 presequence-green fluorescent protein (GFP) (pPre-mLIP1::GFP). Using a transfection kit (CalPhosTM Mammalian Transfection kit, Clontech), the plasmid DNA (8 μg) was transfected into the CHO-K1 cells. The transfected CHO-K1 cells were incubated in the growth medium under 5% (v/v) CO₂/air at 37°C for 24 h. After incubation, the growth medium was changed to fresh medium supplemented with 500 μg/ml neomycin for selection and was incubated again under the same conditions. When the transfected cells became 60–90% confluent, they were seeded onto dishes at 37°C under 5% (v/v) CO₂/air. After 24 h, mitochondria were stained with Mito Tracker Red (CM-H₂Xros, Molecular Probes, Eugene, OR, USA), which stains mitochondria, and then cells were observed using a confocal Laser Scan Microscope (LSM410, Carl Zeiss, Jena, Germany).

3. Results

3.1. Identification of *mLIP1* cDNA for mouse lipoic acid synthase

A BLAST search [17] of the mouse EST database in the National Center for Biotechnology Information using the amino acid sequence of lipoic acid synthase of *Arabidopsis* (LIP1) resulted in identification of mouse EST clones encoding a polypeptide homologous to LIP1. cDNAs in these clones did not contain an ORF, and the nucleotide sequences of the cDNAs were partially overlapping. The overlapping sequences of each clone were combined, and a sequence including an ORF that encodes a polypeptide homologous to lipoic acid synthase of *Arabidopsis* was obtained. This sequence information was used for the design of primers needed for the amplification of *mLIP1* cDNA by PCR. The PCR products were subcloned into the vector pCR2.1 and sequenced. The total cDNA of *mLIP1* (1243 bp) was found to contain an ORF that encodes a polypeptide of 373 amino acids, which corresponds to a molecular mass of 41 878 Da.

The amino acid sequence of the mLIP1 protein was compared with those of lipoic acid synthases of *Arabidopsis*, *E. coli* and yeast, as shown in Fig. 1. The amino acid sequence identity between the mouse mLIP1 protein and the lipoic acid synthases of *Arabidopsis*, *E. coli* and *Saccharomyces cerevisiae* are 58%, 61%, and 39%, respectively. Several stretches of conserved residues that may be important to the function of this enzyme were found. Two conserved Cys motifs, C-E-E-A-X-C-P-N-X-X-E-C and C-T-R-X-C-X-F-C, were found at positions from 105 to 116 and 136 to 143 in the mouse mLIP1 protein, respectively. The second Cys motif is also conserved in biotin synthases of *Arabidopsis* [20,21] and microorganisms [22–24]. As it is known that lipoic acid synthase and biotin synthase are iron sulfur proteins and catalyze a similar reaction that inserts a sulfur atom into a hydrocarbon chain, the conserved Cys residues may play an important role in this process as a binding site for iron atoms. A comparison of the N-terminal region of the sequences clearly indicates that mLIP1 contains a 37-residue extension relative to the lipoic acid synthase of *E. coli*. The amino acid sequence of this region has some characteristics in common with the presequence for targeting into mitochondria [25,26]. In addition, the sequence R²⁶-A-L-S is similar to the (R-X-↓-X-S) motif, which may represent the potential cleavage site for a mitochondrial presequence [26]. If this sequence motif is the actual cleavage site, the mature form of the mLIP1 protein is a polypeptide of 347 amino acid residues with a molecular mass of 38 891 Da.

3.2. Complementation of the *E. coli lipA* mutant

To confirm that *mLIP1* cDNA encodes a lipoic acid synthase, it was expressed in an *E. coli lipA* mutant defective in lipoic acid synthase [18]. As shown in Fig. 2, when the *E. coli* mutant was transformed with pKK233-2 (control vector) and plated on lipoic acid-free medium, no growth of the transformant was observed, although the transformant could grow on a plate containing lipoic acid. In contrast, the transformant with the plasmid (pmLIP1-Δ0) carrying the *mLIP1* cDNA for a precursor form could grow slightly on a plate without lipoic acid and the transformant with the other plasmid (pmLIP1-Δ26) in which 26 amino acids were deleted at

Mouse	1		MALRCWDTARSLG
Arabi.	1		MHSRSALLYRFLR
Yeast	1		MMYRRSVGVLFVGRNTRWISSSTIRCGTSATRPIRSNALNTSDNASVVRVPGVNSTEVENA
▼			
Mouse	14		SRIFGRYAFTVRLSSLPDKKKEFLHNGPDLQDFVSGDLADKSTWDEYKGNLKRQKGERL
Arabi.	14		PASRCFSSSAVTPVTVTQSPKSLRALRARLAN-ESPSLTDIFHGDYTSVEVGKFK-KFL
Yeast	61		TSQLTGTSGKRRKGNRKRITFEFKDALNLGFSFAD-FVSGKASKMLDPLEKARQNT-EA
<i>E. coli</i>	1		MSKPIVMER-GVKYRDADKMALIPVKNVATEREALL
.			
Mouse	74		RLPPWLKTKIP-MGKNYNKLNKTLRNLSLHTVCEEARCPNIGECWGGGEYATATATIMLL
Arabi.	72		PKPKWMMKESIP-GGERYVQIKKLRDLKLHTVCEAEKCPNLGECWGGGTATATIMLL
Yeast	119		KKLPRWKVPIPK-GTNYHKLGDKVKEGLSTVCEEARCPNIGECWGGKDKSKATATIMLL
<i>E. coli</i>	36		RKPEWMMKLPADSTRIQGIKAAMRKNGLHSVCEEASCPNLAEC- ----FNHGATFMI
			. . . * . * . . * . . . * . . . * . . . * . . . * . . . * . . . * . . . * . . . *
Mouse	133		GDTCTRGCRFCVSKTARNPPLDANEPDNTAKAIAEWGLDYVVLTSVDRDDVADGGAHH
Arabi.	131		GDTCTRGCRFCNVKTSRTPPPDPNPNVVAEAIASWGVYVVLTSVDRDDLQDGGSGHF
Yeast	178		GDTCTRGCRFCVSKTNRTPSKPDPEPENTAEAIKRWGLGVVLTVDVDRDDLQVGDGANHL
<i>E. coli</i>	91		<u>GAICTRRCFCDVAHGR</u> -PVAPDANEPVKLAQTADMALRYVVITSVDRDDLQDGGGQHF
			* . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . *
Mouse	193		AKTVSCLKERNPKILVECLTPDFRQD-LRAVEKVALSGLDVYAHNVETVPELQKRVDRPR
Arabi.	191		AETVQRLKFLKPEMLIEALVPDFRQD-GGCVEKVSKEGLDLAHLNLETVEELQSFVRDHR
Yeast	238		AETVRKIKQKAPNTLVETLSGDFRQD-LKMVDIMAQCGLDVYAHNLETVELTPHVRDR
<i>E. coli</i>	150		ADCIITAIREKSPQIKIETLVPDFRQDRLDILTATPPDFVFNHNLENVPRISLQVVRPGA
			* * . . . * . * . * . * . * . * . * . * . * . * . * . * . * . * . *
Mouse	252		ANFDQSLRVLRHAVEVQP-DVSKTSSIMLGLGETDEQVYATLKAALRAADVCLTLGQYMQ
Arabi.	250		ANFKQSLDVLMAKEYAPAGTLTKTSVMLGCGETPDQVVKTEKVRAGVDVMTFGQYMR
Yeast	297		ATYRQSLSVLERAKATVP-SLITKTSIMLGLGETDEQITQTLKDLRNIQCDVVFQYMR
<i>E. coli</i>	210		DYNW-SLKLLERFKEAHP-ELPTKSGLMVGLGETNEEIIEVMRDLRRHGVTMLTLGQYLQ
			. . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . * . *
Mouse	311		PTKRHLKVEEYVTPKEK-FYWEKVGNELGFLYTASGPLVRSSYKAGEFFLKNLV-ARRKT
Arabi.	310		PSKRHMPVAEYVTPDAFERYR-LLGMEGFRYVAGPMVRSSYKAGEYIYKSMEADRVA
Yeast	356		PTKRHMKVVEYVTPKEKFDYWK-ERALEMGFLYCASGPLVRSSYKAGEAFIENVLK-KRNM
<i>E. coli</i>	268		PSRHPLVQRYVSPDFDEM-KAEALAMGFTHAACGPFVRRSSYHA-DLQAKGMEVK
			* . . * . * * . * . . . * . * . * . * . * . * . * . * . * . * . * . *
Mouse	369		KASKV
Arabi.	369		SPSTSP
Yeast	414		K

Fig. 1. Comparison of the amino acid sequences of lipotic acid synthases. The deduced amino acid sequence of lipotic acid synthase encoded by mouse *mLIP1* cDNA is compared with those of lipotic acid synthases of *Arabidopsis* (Arabi.) [19], *S. cerevisiae* (Yeast) [36], and *E. coli* [18]. The amino acid residues conserved in all sequences are indicated by asterisks, and those conserved in the three sequences are indicated by dots. Hyphens represent gaps to maximize sequence alignment. The conserved Cys motifs are underlined. The putative cleavage site for the mitochondrial transit peptide is indicated by an arrowhead.

the N-terminus could grow well on a plate without lipotic acid, nearly the same as on a plate containing lipotic acid. These findings clearly demonstrate that mouse *mLIP1* cDNA functionally complements the *E. coli lipA* mutant and that *mLIP1* cDNA encodes a lipotic acid synthase. The deletion of 26 residues, which probably gives a putative mature *mLIP1* protein, was found to enhance the growth of the mutant, suggesting that the deleted amino acid residues are not required for catalytic activity of the *mLIP1* protein, but presumably are required for targeting into mitochondria.

3.3. Organ-specific expression of *mLIP1*

Fig. 3A shows the result of a genomic Southern hybridization analysis of the *mLIP1* gene in the mouse. The 1.3-kb *EcoRI* fragment containing *mLIP1* cDNA was used as a probe. The genomic DNA was digested with *Bam*HI, *Eco*RI, or *Pst*I. A strong hybridizing band in *Pst*I, two bands in *Bam*HI, and four bands in *Eco*RI were detected. This result suggests that some genes homologous to *mLIP1* may be present in the genome. However, it should be noted that a single gene encoding a polypeptide homologous to *mLIP1* was only found by a BLAST search [17] in the database of ge-

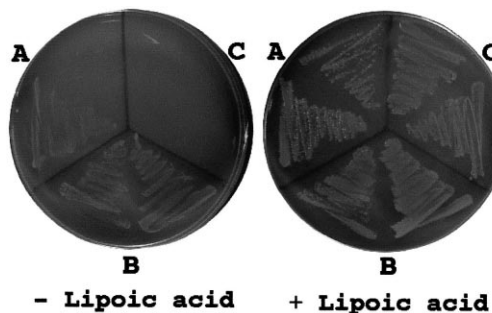


Fig. 2. Complementation of the *lipA* mutant of *E. coli* by expression of *mLIP1* cDNA. The *E. coli lipA* mutant (KER176), which is defective in lipotic acid synthase [18], was transformed with the plasmids pmLIP1- Δ 0 (A), pmLIP1- Δ 26 (B), and pKK233-2 (C, control). The number after Δ represents the number of amino residues deleted from the N-terminus of the *mLIP1* protein. The colonies of each transformant were streaked onto plates containing 50 μ g/ml ampicillin, 50 μ g/ml kanamycin, and with either 50 ng/ml lipotic acid or no lipotic acid, and the plates were incubated at 37°C for 3 days.

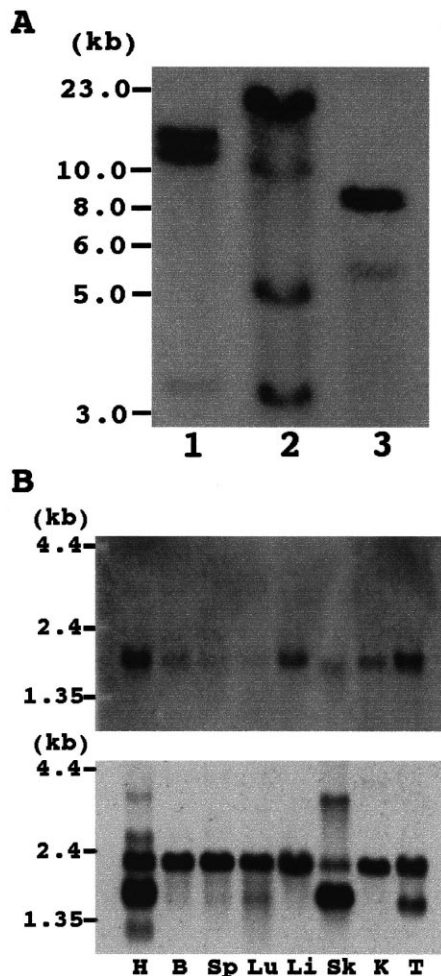


Fig. 3. Southern and Northern hybridization analyses of the mouse *mLIP1* gene. A: Southern hybridization analysis. Genomic DNA extracted from mouse testis was digested with *Bam*HI (lane 1), *Eco*RI (lane 2), or *Pst*I (lane 3). 15 μ g of DNA was applied to each lane. The 1.3-kb *Eco*RI fragment containing *mLIP1* cDNA was used as a probe. The positions of the DNA size markers (in kb) are indicated on the left. B: Northern hybridization analysis. The Northern blot membrane was hybridized with the probe corresponding to the *mLIP1* cDNA (upper panel). The blot was also hybridized with a cDNA for a mouse β -actin, which was used as a probe (lower panel). Approximately 2 μ g of poly(A)⁺ RNA was blotted to each lane in the membrane. H, heart; B, brain; Sp, spleen; Lu, lung; Li, liver; Sk, skeletal muscle; K, kidney; T, testis.

nomic sequence of human, suggesting that lipoic acid synthase is encoded by a single copy gene.

To investigate the organ-specific expression of the *mLIP1* gene, Northern hybridization analysis was carried out using a Northern blot containing approximately 2 μ g of poly(A)⁺ RNA each from the brain, heart, kidney, liver, lung, skeletal muscle, spleen and testis. The result (Fig. 3B) shows that *mLIP1* is primarily expressed in heart, testis, and liver tissues as an mRNA of 1.8 kb. The analysis of *mLIP1* expression by RT-PCR with specific primers also showed that *mLIP1* is primarily expressed in the tissues of those organs (data not shown).

3.4. Intracellular localization of lipoic acid synthase in CHO-K1 cells

As described above, mouse lipoic acid synthase contains a

putative mitochondria-targeting presequence at the N-terminus. This finding raises the possibility that lipoic acid synthase is located in the mitochondria. To investigate this possibility, we prepared a plasmid that encodes a GFP fused to the carboxyl-terminal of this presequence and transfected it into CHO-K1 cells. For a control, a plasmid (pEGFP-N1) encoding a GFP was also transfected into CHO-K1 cells. The transfected CHO-K1 cells were observed with a Laser Scan Microscope (Fig. 4). The red fluorescence (Fig. 4B,F) represents mitochondria, which were stained with Mito Tracker Red, and the green fluorescence (Fig. 4C,G) represents the sites where GFP is present. In the cells transfected with pPre-*mLIP1*::GFP, the green fluorescence from GFP was well overlapped with red fluorescence from mitochondria that were stained with Mito Tracker Red (Fig. 4D). By contrast, in the CHO-K1 cells transfected with pEGFP-N1, the fluorescence from GFP was observed in cytoplasm and nucleus (Fig. 4G). These results demonstrate that the presequence of *mLIP1* functions as a mitochondria-targeting signal.

4. Discussion

In the present study we cloned and characterized mouse *mLIP1* cDNA and demonstrated that it encodes a lipoic acid synthase located in mitochondria. In mammalian cells, all lipoic acid-requiring enzyme complexes and lipoyltransferase are located in mitochondria [1–6]. It is therefore reasonable to suppose that lipoic acid synthase is located in mitochondria. In *E. coli*, lipoic acid is synthesized from octanoic acid by the addition of two sulfur atoms to the octanoyl group bound to the acyl carrier protein (ACP). This reaction is catalyzed by lipoic acid synthase encoded by *lipA* as clearly demonstrated by Miller et al. [27]. In plants, we have previously shown that fatty acid synthesis takes place in mitochondria in addition to in plastids and it provides octanoyl-ACP, which can be used for the biosynthesis of lipoic acid by lipoic acid synthase [28]. We have also shown that a lipoic acid synthase and a lipoyltransferase of *Arabidopsis* homologous to those of *E. coli* are located in mitochondria [19,29]. These findings suggest that, in plants, lipoic acid is synthesized in mitochondria. The biosynthesis of lipoic acid in plant mitochondria is further supported by the finding of Gueguen et al. [30]. In the fungus *Neurospora crassa*, mitochondrial ACP has been identified [31] and shown to be involved in the biosynthesis of fatty acids in mitochondria [32]. Mitochondrial ACP has also been found in yeast [33] and bovine heart [34]. The disruption of the yeast gene for mitochondrial ACP markedly reduces lipoic acid content, resulting in a respiratory-deficient phenotype [33]. These findings, together with the identification of mouse *mLIP1* cDNA for lipoic acid synthase located in mitochondria as presented in this study, suggest that fatty acid synthesis in mitochondria takes place commonly in eukaryotic cells and that one of the most important roles of mitochondrial fatty acid synthesis is the biosynthesis of lipoic acid.

E. coli has two types of lipoic acid attachment enzymes, LplA and LipB, that use lipoic acid plus ATP or lipoyl-ACP as substrates, respectively [35]. An enzyme capable of transferring lipoic acid from lipoyl-AMP has been purified from bovine mitochondria [13]. Although this protein has strong homology with LplA (but only within the N-terminal half of the protein), the mammalian enzyme cannot make

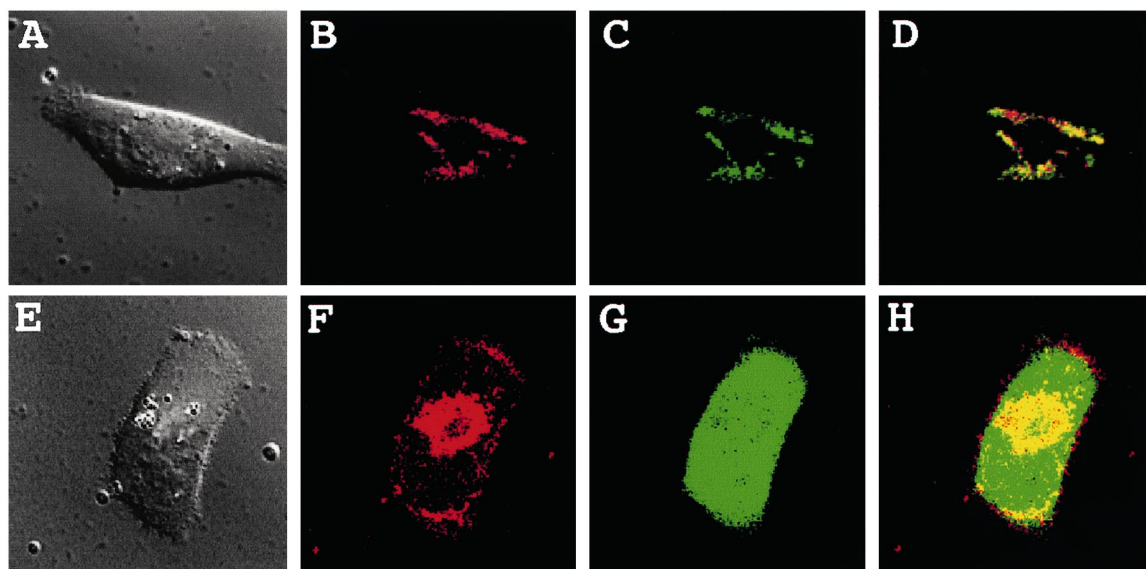


Fig. 4. Localization of GFP fused to the carboxyl-terminal region of the mLIP1 presequence. pPre-mLIP1::GFP encoding a GFP fused with presequence of mLIP1 was transfected to CHO-K1 cells (A–D). For a control, pEGFP-N1 vector was transfected to CHO-K1 cells (E–H). Confocal pictures (A and E), fluorescence images of GFP (B and F), and Mito Tracker Red (C and G) and merged images (D and H) are shown.

lipoyl-AMP from lipoic acid and ATP [13]. It therefore seems reasonable that mammals possess an LplA-like enzyme, since lipoic acid from intestinal bacteria and digestion of food can be salvaged for lipoylation of mitochondrial lipoate-dependent enzymes. However, in the present study, we found that *mLIP1* for lipoic acid synthase is primarily expressed in heart, liver, and testis of the mouse. This finding suggests that, in tissues of these organs, lipoic acid is endogenously synthesized in mitochondria and used for important purposes such as lipoylation of lipoic acid-requiring enzyme complexes. This utilization of endogenously synthesized lipoic acid is further supported by the evidence that several sequences encoding putative proteins having strong homology with a lipoyltransferase (LipB) of *E. coli*, which uses endogenously synthesized lipoyl-ACP as the substrate, are found in the EST databases of mouse and human. It can be assumed that lipoic acid is not sufficiently supplied by the diet to tissues of the heart, liver, and testis, where *mLIP1* is expressed, and that it therefore must be synthesized in mitochondria of those tissues.

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