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# Expression and purification of the mitochondrial serine protease LACTB as an N-terminal GST fusion protein in *Escherichia coli*

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**Abstract**

LACTB is a mammalian mitochondrial protein sharing sequence similarity to the β-lactamase/penicillin-binding protein family of serine proteases that are involved in bacterial cell wall metabolism. The physiological role of LACTB is unclear. In this study we have subcloned the cDNA of mouse LACTB (mLACTB) and produced recombinant mLACTB protein in *Escherichia coli*. When mLACTB was expressed as an N-terminal GST fusion protein (GST-mLACTB), full-length GST-mLACTB protein was recovered by glutathione–agarose affinity chromatography as determined by MALDI-TOF mass spectrometry and immunoblotting. Expression of mLACTB as a C-terminal GST fusion protein or with either an N- or C-terminal $His_6$-tag resulted in proteolytic degradation of the protein and we were not able to detect full-length mLACTB. Analysis of GST-mLACTB by Fourier transform infrared spectrometry revealed the presence of α-helices, β-sheets and turns, consistent with a well-defined secondary structure. These results show that mLACTB can be expressed as a GST fusion protein in *E. coli* and suggest that GST-mLACTB was properly folded.
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*Keywords:* LACTB; Serine protease; β-Lactamase; D-Transpeptidase; Penicillin-binding protein; Mitochondria

LACTB is a 60 kDa mammalian protein of unknown function sharing significant sequence similarity to β-lactamases and penicillin binding proteins (PBPs)[2] occurring in bacteria [1]. β-Lactamases and PBPs are involved in the synthesis of the peptidoglycan layer in bacteria. Mouse LACTB is 551 amino acids long and comprises of a predicted mitochondrial import sequence, a short putative transmembrane segment, and a β-lactamase homology domain containing the serine protease motif, –SXXK– [2]. Since peptidoglycan is not synthesized by eukaryotes the role of LACTB in mammalian cells is intriguing.

LACTB has been detected in mammalian heart and liver mitochondria [3,4]. Furthermore, LACTB is associated with the 39S subunit of the mammalian mitochondrial ribosome [5]. Interestingly, LACTB and five other additional proteins of the 39S subunit of the mammalian mitochondrial ribosome lack homologs in the bacterial ribosome and yeast mitochondrial ribosome [5]. Therefore, these uniquely mammalian mitochondrial ribosomal proteins may have other additional functions unrelated to mitochondrial protein synthesis [6]. The ability of

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[2] *Abbreviations used:* PBP, penicillin-binding protein; mLACTB, mouse LACTB; MALDI-TOF, matrix-assisted laser-induced ionisation/desorption and time-of-flight mass spectrometry; FTIR, fourier transform infrared spectrometry.

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mitochondrial ribosomal proteins to possess dual functionality has been demonstrated by MRP-S29/DAP3, and MRP-S30/p52 when they where implicated in the regulation of mitochondrial apoptotic events [7–11]. Because the ancestor of LACTB was a periplasmic serine protease, this suggests that LACTB also has functions outside the mitochondrial ribosome.

In this study, we report the subcloning of full-length mLACTB cDNA into GST-and His-tagged expression vectors and subsequent expression of mLACTB protein in *Escherichia coli*. Significant quantities of full-length protein was obtained when mLACTB was expressed as an N-terminal GST fusion protein. An analysis of the secondary structure of GST-mLACTB by FTIR suggested that the protein was correctly folded. These findings show that the N-terminal GST fragment protects LACTB from proteolytic processing and suggests that LACTB can undergo autoproteolysis.

## Materials and methods

### *PCR and cloning*

Mouse LACTB cDNA (clone BC046293: pCMV-SPORT6.1-mLACTB) sampled from mammary tumor tissue was purchased (I.M.A.G.E., CA, USA). The mLACTB insert was amplified using Phusion DNA-polymerase (Finnzymes, Finland), using the forward primer 5′-CACC ATGTACCG GCTCCTGTCAAG-3′ and the reverse primers 5′-TTAGTC AGCTCTGTCTTTATCAAATTC-3′ or 5′-GTCAGCTC TGTCTTTATCAAATTCC-3′. The purified PCR products were cloned into linearized pENTR/SD/D-TOPO vector according to the manufacturer's instructions (Invitrogen, USA). Competent *E. coli* strain Top-10 (Invitrogen) was transformed and colonies were picked for plasmid purification using the NucleoSpin Plasmid kit (Macherey–Nagel, Germany). Using the LR clonase mixture (Invitrogen) the mLACTB insert was transferred from the pENTR vector into four different expression vectors: pDEST15 yielding an N-terminal GST fusion product; pDEST17 yielding an N-terminal $His_6$-tagged product; pDEST24 yielding a C-terminal GST-fusion product; and pET-DEST42 yielding a C-terminal $His_6$-tagged product. All mLACTB plasmid constructs were confirmed by sequencing both strands using the Pharmacia ALFexpress DNA sequencing system (Amersham Biosciences, Buckinghamshire, UK).

### *LACTB expression in E. coli*

The expression vectors encoding mLACTB were transformed into *E. coli* cells strain BL21 (DE3). The cells were grown in 100 ml Luria–Bertani (LB) medium with 150 μg $ml^{-1}$ ampicillin for 16 h at 37 °C. This pre-culture was used to inoculate 1 L LB medium along with 64 mM Pi and 150 μg $ml^{-1}$ ampicillin, and the culture was allowed to grow at 37 °C until the optical density had reached 1.2 at $\lambda = 600$ nm. Expression of recombinant protein was induced by adding 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 2.5 h at 16 °C. Cells were harvested by centrifugation at 5000*g* for 20 min at 4 °C. The pellet was suspended in 60 ml phosphate-buffered saline (PBS) containing 150 mM NaCl and 10 mM Pi, pH 7.4, and centrifuged at 5000*g* for 20 min at 4 °C. The resulting pellet was stored at −70 °C and then subsequently used for protein analysis or purification.

### *Gel electrophoresis and immunoblotting*

Proteins were dissolved in SDS sample buffer containing 62.6 mM Tris–HCl, 2% SDS, 10% glycerol, and 5% 2-mercaptoethanol. The samples were heated at 97 °C for 10 min and separated on 10% SDS–PAGE gels. Proteins were visualized using Coomassie brilliant blue R-250 staining. For immunoblotting, proteins were electrotransferred onto PVDF membranes and incubated with primary antibodies diluted 1:1000 in Tris-buffered saline (TBS) containing 150 mM NaCl, 20 mM Tris–HCl, pH 7.6, and 5% non-fat milk powder for 12 h at 6 °C. The PVDF membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies diluted 1:10,000 in TBS supplemented with 0.1% Tween and 5% non-fat milk powder. Immunoreactive bands were visualized on Hyperfilm ECL (Amersham Biosciences) and developed using the ECL chemiluminescence detection kit (Amersham Biosciences) according to manufacturer's instructions. Coomassie-stained SDS–PAGE gels and developed film sheet were digitized in 8 bit grayscale at a resolution of 300 dpi using a Canon flatbed scanner. The resulting images were transferred directly into a Photoshop document and aligned without any further image processing.

### *Antibodies*

Two peptides with sequences corresponding to amino acid residues 279–294 (CRSAKPGKKKNDFEQG) and 506–520 (ELDSEAVNNKVPPRG) of mLACTB were synthesized. Five milligram of the peptides was coupled to hemocyanin and injected into a rabbit. After 12 weeks, serum was collected and affinity purified using the same peptides coupled to a TOYOPEARL AF-Amino-650M affinity resin (Tosoh Bioscience, Japan). Rabbit anti-$His_6$ antibody was purchased (Roche Diagnostics, Finland).

### *Protein purification*

Bacterial cells were suspended in 10 ml PBS supplemented with 0.1 mM EDTA, 10 mM dithiothreitol (DTT), 1% *N*-laurylsarcosine, 100 μg $ml^{-1}$ phenylmethylsulfonylfluoride (PMSF), 1 mg $ml^{-1}$ lysozyme, 250 U $ml^{-1}$ benzonase and Roche Protease inhibitor mixture (1:30), and incubated on ice for 30 min. The suspension was repeatedly sonicated for 5 min (20 s of sonication followed by 20 s of cooling) on ice using a tip sonicator (Branson, NY, USA). The suspension was centrifuged at 10,000*g* for 30 min at

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