

**The highly conserved LepA is a ribosomal
elongation factor that back-translocates the
ribosome and is essential for viability at high ionic
strength**

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Summary

The ribosomal elongation cycle describes a series of reactions prolonging the nascent polypeptide chain by one amino acid and driven by two universal elongation factors termed EF-Tu and EF-G in bacteria. EF-Tu brings aminoacyl-tRNA (aa-tRNA) to the ribosome in an aa-tRNA•EF-Tu•GTP ternary complex form, delivers the cognate aa-tRNA at the decoding center of the ribosomal A-site and – after EF-Tu dependent GTP cleavage - leaves the ribosome in the EF-Tu•GDP form. After aa-tRNA has been accommodated completely at the A site (the aminoacyl residue is now present at the peptidyltransferase center), the dipeptide bond will be catalyzed to form, namely the peptide will be transferred to the aminoacyl-tRNA so that the peptide is prolonged by one amino acid residue. After that, EF-G•GTP enters, triggers the translocation of the two tRNAs from A- and P-site to the P- and E- sites respectively, the GTP is hydrolyzed and EF-G•GDP leaves ribosome. Now the A-site is free and a new cycle starts.

In this work an extremely conserved protein LepA, a protein belonging to the G-class and presenting in all bacteria and mitochondria, is identified and characterized as a third elongation factor required for accurate and efficient protein synthesis. LepA is essential for cell viability at high ionic strength, where – without LepA - translocation is occasionally impaired thus provoking translational misreading or probably even a translational halt. LepA•GTP recognizes this unfavorable situation of the ribosome and restores the translational fidelity, in that it back-translocates the stuck ribosome thus giving EF-G a second chance to translocate the tRNAs correctly. However, LepA does not counteract decoding errors induced by antibiotics such as aminoglycosides and edeine. The novel function of LepA as a back-translocator also has implications for the fundamental process of translocation.

LepA has an important application potential, since only after addition a small amount of LepA (less than 0.3 molecules per ribosome) to coupled transcription-translation systems 100% active proteins can be synthesized *in vitro*. This application has been patented.

Zusammenfassung

Der ribosomale Elongationszyklus beschreibt eine Reihe von Reaktionen, welche die werdende Polypeptidkette, angetrieben durch zwei Proteinfaktoren EF-Tu und EF-G, um eine Aminosäure verlängern (in Archaea und Eukarya heißen die entsprechenden Faktoren EF1 und EF3). EF-Tu bringt Aminoacyl-tRNA (aa-tRNA) als ternären Komplex in der Form aa-tRNA•EF-Tu•GTP zum Dekodierungszentrum der ribosomalen A-Stelle. Die kognate aa-tRNA verbleibt dort, während nach EF-Tu abhängiger GTP Hydrolyse EF-Tu•GDP das Ribosom verläßt. Nachdem die kognate aa-tRNA vollständig in die A-Stelle akkommodiert, wird die Dipeptidbindung vom Peptidyltransferase Zentrum katalysiert. Dabei wird das Peptid auf die aa-tRNA übertragen, so dass das Peptid um einen Aminosäurerest verlängert wird. Schließlich dockt EF-G in seiner GTP-Form an das Ribosomen, katalysiert die Translokation der beiden tRNAs von der A- zur P-Stelle bzw. von der P- zur E-Stelle. Danach hydrolysiert EF-G das GTP und EF-G•GDP verlässt das Ribosom. Die A-Stelle ist nun frei und ein neuer Zyklus kann beginnen.

In dieser Arbeit wird das extrem konservierte Protein LepA, das in allen Bakterien und Mitochondrien vorhanden ist und zur Klasse der G-Proteine gehört, als dritter Verlängerungsfaktor identifiziert und charakterisiert. LepA ist für eine fehlerfreie und effiziente Proteinsynthese erforderlich und für die Lebensfähigkeit von Zellen unter hohen Ionenstärken wesentlich. Ohne LepA ist die bakterielle Translokation gelegentlich unvollständig, was zu Aminosäuren-Fehleinbau und wahrscheinlich auch zur Blockierung des Ribosoms führen kann. LepA•GTP erkennt diese Situation und löst eine Rück-Translokation aus, so dass EF-G eine zweite Chance für eine korrekte Translokation erhält.

LepA korrigiert nicht Dekodierungsfehler, die durch Antibiotika wie Aminoglykoside und Edein verursacht werden. Vielmehr verhindert es Fehleinbau als Folge von Translokationsdefekten. Die neuartige Funktion von LepA als Rück-Translokator lässt somit auch neue Schlüsse für den Translokationsprozess selbst zu.

LepA hat ein bedeutendes Anwendungspotential für *in vitro* Proteinsynthese. Nur nach Zugabe kleiner Mengen von LepA (unter 0.3 Moleküle per Ribosom) ist es möglich, im gekoppelten Transkriptions-Translations-System 100% aktive Proteine zu synthetisieren. Dieser LepA Effekt wurde von uns patentiert.

Table of contents

Table of contents	v
1.1 Ribosome: protein biosynthesis machinery.....	10
1.1.1 <i>Initiation</i>	13
1.1.2 <i>Elongation</i>	14
1.1.2.1 General description.....	14
1.1.2.2 Models for the elongation cycle: tRNA translocation motif.....	15
1.1.3 <i>Termination</i>	16
1.1.4 <i>Recycling</i>	16
1.2 Translational errors and two tRNAs on the ribosome.....	17
2.1 Chemicals and enzymes-suppliers.....	19
2.2 Buffers.....	24
2.2.1 <i>Buffers and Electrophoresis solutions</i>	24
2.2.2 <i>Buffers for microbiological and molecular methods</i>	27
2.2.3 <i>Buffers for the functional studies and ribosome preparation</i>	29
2.3 Analytical methods.....	32
2.3.1 <i>Determination of ribosome and nucleic acid concentrations</i>	32
2.3.2 <i>Conversion factors for the quantification of DNA and RNA</i>	32
2.3.3 <i>Radioactivity measurements</i>	33
2.3.4 <i>Cold TCA precipitation for the quantitative determination of aminoacylated tRNA</i>	34
2.3.5 <i>Polyuridin dependent Polyphenylalanin synthesis</i>	34
2.3.6 <i>Agarose gel electrophoresis of DNA and RNA</i>	34
2.3.7 <i>Specific activity determination of labelled [³²P]-tRNA</i>	35
2.3.8 <i>Western blot of LepA distribution in S30 fraction and membrane fraction</i>	36
2.3.9 <i>Western blot of EF-P distribution associated with ribosomes in S30 fraction</i>	37
2.3.10 <i>Chemical probing and primer extension assay</i>	37
2.3.11 <i>Toe-print assay</i>	38
2.3.12 <i>GTPase assay</i>	39
2.4 Working with DNA.....	40
2.4.1 <i>Preparation of E. coli competent cells for electroporation</i>	40
2.4.2 <i>Cloning strategies</i>	40
2.4.3 <i>Restriction with EcoRI and BamHI</i>	40
2.4.4 <i>Digestion with alkaline phosphatase</i>	41
2.4.5 <i>Synthesis of dsDNA and ligation to a linearized plasmid</i>	41
2.4.6 <i>Annealing and DNA filling reaction</i>	42
2.4.7 <i>Ligation to linearized plasmid</i>	42
2.4.8 <i>Transformation</i>	42
2.4.9 <i>Phenol/Chloroform extraction</i>	43
2.4.10 <i>Nucleic acid precipitation by ethanol or isopropanol</i>	43
2.4.11 <i>Plasmid isolation (miniprep)</i>	43
2.4.12 <i>Plasmid preparation (maxi prep)</i>	44
2.5 Working with RNA.....	45
2.5.1 <i>Transcription</i>	45
2.5.1.1 <i>Run-off transcription with T7 polymerase</i>	45
2.5.1.2 <i>PAGE purification of in vitro mRNA transcript</i>	46
2.5.1.3 <i>Separation at the single nucleotide level (sequencing gel)</i>	47
2.5.1.4 <i>Gel filtration for the separation of RNA preparations from low molecular weight contaminants</i>	48

2.5.1.5 List of messengers (mRNAs) used in this study	48
2.5.2 <i>tRNAs</i>	49
2.5.2.1 Analytical tRNA aminoacylation	49
2.5.2.2 Analytical enzymatic de-aminoacylation of aminoacyl-tRNA	49
2.5.2.3. Preparative tRNA aminoacylation and subsequent acetylation	50
2.5.2.4 Preparative de-aminoacylation of aminoacyl-tRNA remaining in the N-acetylaminoacyl-tRNA fraction	51
2.5.2.5 Reversed-Phase HPLC purification of aminoacyl-tRNA and acetylaminoacyl-tRNA	52
2.5.2.6 Preparation of N-formyl-methionyl-tRNA ^f (<i>E. coli</i>)	54
2.5.2.6.1 Preparation of the formyl donor	54
2.5.2.6.2 Synthesis and purification of fMet-tRNA ^f	54
2.5.2.7 Labelling of deacylated tRNA with γ -[³² P]-ATP	55
2.5.2.7.1 Dephosphorylation of tRNA with alkaline phosphates	55
2.5.2.7.2 [⁵]Phosphorylation with γ -[³² P]-ATP	56
2.6 Preparative Methods	56
2.6.1 <i>Large-scale cultures of Escherichia coli</i>	56
2.6.2 <i>Isolation of 70S ribosomes from Escherichia coli</i>	57
2.6.3 <i>Preparative isolation of 30S and 50S subunits</i>	58
2.6.4 <i>Preparation of re-associated 70S</i>	59
2.6.5 <i>Quality and functionality determination of the ribosomes preparation</i>	60
2.6.5.1 Analytical sucrose gradient centrifugation	61
2.6.5.2 Integrity of rRNA: 1D tube gel analysis	62
2.7 In vitro systems	63
2.7.1 <i>Estimation of the functional competence of ribosome preparations</i>	63
2.7.1.1 Poly(U)-dependent poly(Phe) synthesis	63
2.7.1.2 Determination of the AcPhe-tRNA ^{Phe} binding	63
2.7.2. <i>Watanabe assay: site specific binding of tRNA to ribosomes, translocation and puromycin reaction</i>	64
2.7.2.1 First step: P site binding or P _i complex formation	65
2.7.2.2 Second step: A site binding and PRE complex formation	66
2.7.2.3 Third step: Translocation reaction	66
2.7.2.4 Fourth step: puromycin reaction	66
2.7.3 <i>Di-peptide formation</i>	67
2.7.4 <i>RNase assay</i>	69
2.7.5 <i>RTS system</i>	69
2.7.5.1. RTS 100 High Yield <i>E. coli</i> Kit	69
2.7.5.2 RTS 500 High Yield <i>E. coli</i> Kit	71
2.8 Computational analysis	73
2.8.1 <i>Secondary structure prediction of synthetic RNA and estimation of its ΔG° of formation</i>	73
2.8.2 <i>Protein sequence analysis</i>	73
2.8.3 <i>Protein Modeling</i>	74
3.1 Conservation and domain structure of LepA	75
3.2 In vivo analyses of LepA	79
3.2.1 <i>Effects of LepA over-expression</i>	79
3.2.1.1 Construction of LepA over-expression vector and LepA purification	79
3.2.1.2 LepA over-expression inhibits cell growth	79

3.2.2 <i>Effects of lepA knock-out</i>	80
3.2.2.1 Wild type and <i>lepA</i> knock-out strain in LB medium.....	80
3.2.2.2 Wild type and <i>lepA</i> knock-out strain in LB medium +K ⁺	81
3.2.2.3 Wild type and <i>lepA</i> knock-out strain in LB medium plus Mg ²⁺	82
3.2.3 <i>LepA distribution in the cell (membrane versus cytoplasmic fraction)</i>	83
3.2.3.1 LepA distribution under normal condition (LB medium)	83
3.2.3.2 LepA distribution under high ionic condition	84
3.3 In vitro analyses of LepA	85
3.3.1 <i>LepA binding assay</i>	85
3.3.1.1 Saturating binding of LepA with re-associated 70S.....	85
3.3.1.2 Competitive binding of LepA and EF-G to re-associated 70S.....	87
3.3.1.3 Complex preparation for Cryo-EM	88
3.3.1.4 Cryo-EM reconstruction of LepA•POST complex	88
3.3.2 <i>GTPase assay of LepA</i>	89
3.3.2.1 GTPase activity of LepA with re-associated 70S.....	89
3.3.2.2 GTPase activity with ribosome complexes in functional states.....	90
3.3.3 <i>LepA's effect on puromycin activity of Pi or POST complex</i>	91
3.3.4 <i>Dipeptide formation analysis in the presence of LepA</i>	93
3.3.5 <i>LepA is a "back-translocator"?</i>	95
3.3.5.1 Footprint assay monitoring tRNA protection pattern.....	95
3.3.5.2 Toeprint assay monitoring mRNA movement	97
3.4 Effects of LepA on accuracy of protein in vitro synthesis	98
3.4.1 <i>LepA optimizing protein synthesis</i>	98
3.4.1.1 RTS system: GFP and luciferase synthesis	98
3.4.1.2 Promega system: GFP synthesis.....	100
3.4.2 <i>LepA's function in the presence of antibiotics</i>	100
3.4.2.1 Aminoglycosides	100
3.4.2.2 Edeine	102
3.4.3 <i>LepA's function in the presence of Mg²⁺</i>	102
3.5 Appendix	103
3.5.1 <i>Extended phylogenetic LepA</i>	103
3.5.2 <i>Studies with the Elongation Factor EF-P</i>	104
3.5.2.1 EF-P <i>in vivo</i> distribution.....	104
3.5.2.2 Preparation of EF-P ribosome complex for Cryo-EM	106
3.5.3 <i>Studies concerning the L7/L12 stalk</i>	107
3.5.3.1 Binding of EF-G to <i>E. coli</i> 70S minus L7/L12	107
3.5.3.2 Puromycin activity monitoring the EF-G dependent translocation of the PRE complex in the presence and absence of L7/L12	108
3.5.4 <i>Effect of the Shine-Dalgarno sequence on the first dipeptide formation</i>	109
4.1 Highly conserved bacterial factor LepA	111
4.2 LepA is strongly related to the cytomembrane.....	112
4.3 Structural mimicry of elongation factor	114
4.4 First back-translocator	115
4.5 LepA is an essential factor at high ionic condition	117
4.6 An application of LepA in protein in vitro synthesis system	118
REFERENCES.....	120

0 Abbreviations

AA	Acrylamide
aa-tRNA	aminoacyl-tRNA
AcPhe-tRNA ^{Phe}	N-Acetyl-Phe-tRNA ^{Phe}
Å	Angstrom
Asp	Aspartic acid
ATP	Adenosine tri-phosphate
BAA	Bis-acrylamide
BCIP	5-Bromo-4-Chloro-3-indolyl phosphate
BPB	Bromophenol blue
dsDNA	double strand DNA
EF-G	Elongation factor G
EF-Ts	Elongation factor thermo stable
EF-Tu	Elongation factor thermo unstable
eRF1	Eukaryote release factor 1
GDP	Guanine di-phosphate
Gly	Glycine
GTP	Guanine tri-phosphate
H _t M _u N _v SH _w Spd _x Spm _y	H Hepes t mM
	M MgAc ₂ u mM
	N NH ₄ Ac v mM
	SH β-Mercapto-ethanol w mM
	Spd Spermidine x mM
	Spm Spermine y mM
HPLC	High Performance Liquid Chromatography
IF	Initiation factor
kb	kilo bases
kJ	kilo Joules
kV	kilo Volts
Leu	Leucine
mA	milli Ampere

μCi	micro Curie
MDa	mega Dalton
MgAc_2	magnesium acetate
MQ	milli Q water
M.W.	molecular weight
N-AcPhe-tRNA ^{Phe}	N-Acetyl-Phe-tRNA ^{Phe}
NaAc	sodium acetate
NBT	nitroblue tetrazolium
NH_4Ac	ammonium acetate
nt	nucleotide(s)
NTP	Nucleoside tri-phosphate
Ω	Ohm
PEP	Phosphoenol pyruvate
PK	Pyruvate kinase
Phe	Phenylalanine
Poly(U)	Long poly-uridine mRNA
PP_i	Inorganic Pyrophosphate
PTF	Peptidyltransferase centre
RF	Release factor
rpm	revolutions per minute
rRNA	ribosomal RNA
RRF	Ribosome recycling factor
SD	Shine Dalgarno sequence
TFA	Trifluoroacetic acid
30S	small ribosomal subunit
V	Volts
v/v	volume/volume
w/v	water/volume