



# 25<sup>th</sup> tRNA Conference 2014

21-25 September 2014 • Kyllini, Greece



University of Patras  
GREECE



*Co-chairs: D. Drinas & C. Stathopoulos*



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University of Patras  
GREECE



*Plato & Aristotle The School of Athens by Raphael (1509)*

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# ABSTRACTS

## **Conference Chairs**

**Prof. Denis Drinas**, University of Patras

**Prof. Constantinos Stathopoulos**, University of Patras

## **Local Organizing Committee**

George DINOS, University of Patras

Kriton KALANTIDIS, IMBB-FORTH & University of Crete

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Dimitrios KALPAXIS, University of Patras

Dimitris KLETSAS, NCSR "Demokritos" (President of HSBMB)

George SIMOS, University of Thessaly

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Tina HENKIN, Ohio State University, USA

Michael IBBA, Ohio State University, USA

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Tao PAN, University of Chicago, USA

Lluís RIBAS DE POUPLANA, IRB Barcelona, Spain

Manuel SANTOS, University of Aveiro, Portugal

Tsutomou SUZUKI, The University of Tokyo, Japan

Xiang-Lei YANG, The Scripps Research Institute, USA

Marat YUSUPOV, IGBMC- CNRS, Strasbourg, France

## W E L C O M E   M E S S A G E

**Dear Colleagues and Friends,**

We welcome you to Greece and the 25th tRNA Conference.

We would like to thank you for your participation, which has exceeded our expectations. Your contribution through excellent scientific work of the highest caliber reassures the continuation of the excellent research in the field and highlights interdisciplinary and innovative approaches to unravel novel pathways in biology. Most encouraging is the participation of new groups and young investigators who bring fresh and novel perspectives on tRNA biology.

Research on tRNA has always been interconnected with the evolution of the genetic code and translation. Today, more than ever, it is evident that tRNA research continues to reveal new and surprising aspects of cellular metabolism which will be presented in sessions devoted to synthetic biology, tRNA biogenesis, processing, editing, transfer and localization, the recognition of tRNA by aminoacyl-tRNA synthetases and their role in many diseases, the relationship between structure and function of translation factors, the regulation of translation at many levels, the role of many tRNA-related proteins or protein networks outside translation and finally, the regulatory role of tRNA itself and its fragments, post-transcriptionally.

We would like also to thank the members of the scientific committee, the session chairs, the speakers and poster presenters and all the academic, scientific and commercial entities that support this 25th anniversary tRNA Conference. Their encouragement and help towards a successful and memorable conference is gratefully acknowledged.

We welcome you to the place that gave birth to the great ideas of the modern world and we invite you to enjoy both the 25th tRNA Conference and your stay in Greece.

**Denis Drainas & Constantinos Stathopoulos**  
*25<sup>th</sup> tRNA Conference Co-chairs*  
*Department of Biochemistry*  
*School of Medicine*  
*University of Patras*

## Sponsors

The Organizing Committee gratefully acknowledges  
the contribution to the organization of the Conference of



The Sidney Altman Endowment

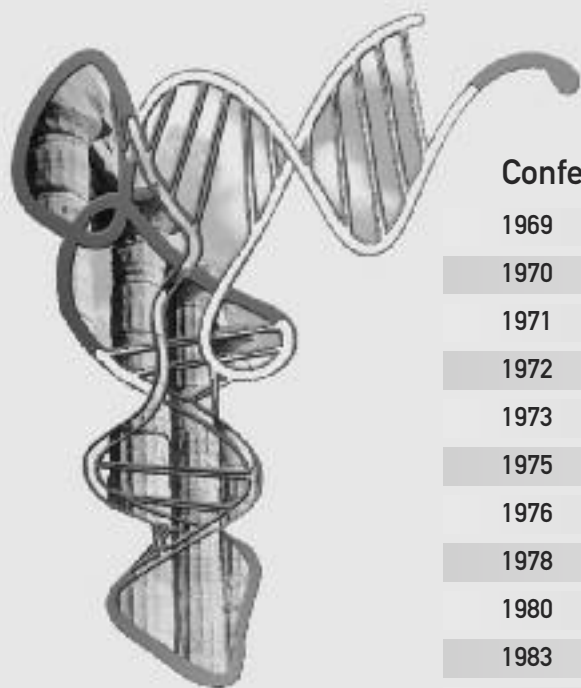


"ARISTEIA I"



**Lab Supplies  
Scientific**





## Conference History

1969	Cambridge, UK
1970	Riga, Soviet Union
1971	Göttingen, Germany
1972	Princeton, USA
1973	Göteborg, Sweden
1975	Nof Ginosar, Israel
1976	Sandbjerg, Denmark
1978	Cold Spring, Harbor, USA
1980	Strasbourg, France
1983	Hakone, Japan
1985	Banz, Germany
1987	Umea, Sweden
1989	Vancouver, Canada
1991	Rydzyzna, Poland
1993	Cap d'Agde, France
1995	Maddison, USA
1997	Chiba, Japan
2000	Cambridge, UK
2002	Shangai, China
2003	Banz, Germany
2005	Bangalore, India
2007	Uppsala, Sweden
2010	Aveiro, Portugal
2012	Olmue, Chile
<b>2014</b>	<b>Kyllini, Greece</b>



# Conference PROGRAM

Sunday, September 21<sup>st</sup>

From 15.00	<b>Arrivals and Registration</b> at Grecotel Olympia Oasis Conference Venue
<b>18.30-19.15</b>	<b>Welcome Cocktail</b>
<b>19.15-19.30</b>	<b>Welcome from the Organizers</b> <b>D. Drainas</b> (Greece), <b>C. Stathopoulos</b> (Greece), <b>D. Kletsas</b> (Greece)
<b>19.30-20.30</b>	<b>Session 1 - 50 years of tRNA research and 25 Conferences</b>
19.30-20.00	<b>Keynote Lecture 1: Paul Agris</b> (USA) Implications of RNA being modified: Basic and translational science
20.00-20.30	<b>Keynote Lecture 2: Paul Schimmel</b> (USA) The new biology of tRNAs and AARSs
20.30	<b>Dinner</b>

Monday, September 22<sup>nd</sup>

<b>09.00-11.00</b>	<b>Session 2 - tRNA: Genetic code evolution and Synthetic Biology</b> <i>Chairs:</i> <b>D. Söll</b> (USA), <b>S. Yokoyama</b> (Japan)
09.00-09.20	<b>O1 Shigeyuki Yokoyama</b> (Japan) Structural studies on specific aminoacyl-tRNA synthesis and genetic code expansion
09.20-09.40	<b>O2 Dieter Söll</b> (USA) Rewiring E. coli translation for synthesis of selenoproteins
09.40-10.00	<b>O3 Jesse Rinehart</b> (USA) Probing proteins and proteomes from organisms with expanded genetic codes
10.00-10.15	<b>O4 Naohiro Terasaka</b> (Japan) - <b>Sidney Altman Endowment Lecture</b> An orthogonal ribosome-tRNAs pair by the engineering of peptidyl-transferase center
10.15-10.30	<b>O5 Takahito Mukai</b> (Japan) A rare codon can be captured by a non-canonical amino acid
10.30-10.45	<b>O6 Joanne Ho</b> (USA) Sense codon recoding in Escherichia coli
10.45-11.00	<b>O7 Jeffrey R. Sampson</b> (USA) Synthetic biology: A measurement solutions provider's perspective
11.00-11.30	<b>Coffee break</b>
<b>11.30-13.30</b>	<b>Session 3 - tRNA: Biogenesis and Modification I</b> <i>Chairs:</i> <b>D. Engelke</b> (USA), <b>E. Phizicky</b> (USA)
11.30-11.50	<b>O8 David Engelke</b> (USA) Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing

# Conference PROGRAM

11.50-12.10	<b>O9 Eric Phizicky (USA)</b> Identification of the determinants of tRNA function and susceptibility to rapid tRNA decay by high throughput in vivo analysis
12.10-12.30	<b>O10 Walter Rossmannith (Austria)</b> RNase P: convergent evolution within a structurally diverse enzyme family
12.30-12.45	<b>O11 Stewart Shuman (USA)</b> Biology and chemistry of tRNA damage and its repair
12.45-13.00	<b>O12 Mario Mörl (Germany)</b> The identity of the discriminator base has an impact on CCA-addition
13.00-13.15	<b>O13 Aaron Cozen (USA)</b> Coupling demethylation to RNA sequencing provides a high-throughput view of tRNA processing and modification
13.15-13.30	<b>O14 Carine Tisné (France)</b> 1-methyladenosine in transfer RNA
13.30-15.00	<b>Conference Photo &amp; Lunch</b>
15.00-17.05	<b>Session 4 - tRNA: Biogenesis and Modification II</b> <i>Chairs: T. Suzuki (Japan), V. deCrecy-Lagard (USA)</i>
15.00-15.15	<b>O15 Tsutomu Suzuki (Japan)</b> Biogenesis and function of cyclic t6A and its derivatives
15.15-15.35	<b>O16 Valerie de Crecy-Lagard (USA)</b> Complex modification of tRNA, interplay with DNA metabolism?
15.35-15.55	<b>O17 Juan Alfonzo (USA)</b> A tRNA modification pathway in mitochondria unveils the origin of wybutosine in eukaryotes
15.55-16.10	<b>O18 Ya-Ming Hou (USA)</b> A divalent metal ion-dependent N1-methyl transfer to G37-tRNA
16.10-16.25	<b>O19 Kazuhito Tomizawa (Japan)</b> Cdk5rap1-mediated 2-methylthio modification of mitochondrial tRNAs controls precise mitochondrial protein translation and contributes to myopathy
16.25-16.40	<b>O20 Sebastian A. Leidel (Germany)</b> Wobble uridine hypomodification triggers protein misfolding by reducing decoding speed in vivo
16.40-16.55	<b>O21 Marc Graille (France)</b> Structure-function analysis of the Trm9-Trm112 complex, a methyltransferase involved in the mcm5U34 tRNA modification and in response to genotoxic stresses
16.55-17.05	<b>O22 Takaaki Taniguchi (Japan)</b> A Rossmann-fold superfamily enzyme, TmcAL, is a novel acetate ligase responsible for the formation of N4-acetylcytidine in tRNAMet in <i>Bacillus subtilis</i>
17.05-17.30	<b>Coffee break</b>



## Monday, September 22<sup>nd</sup>

17.30-19.30

### Session 5 - tRNA: Processing, Transport, Localization

*Chairs:* **L. Marechal-Drouard** (France), **A. Hopper** (USA)

17.30-17.50

#### **O23 Laurence Marechal-Drouard** (France)

Metazoan mitochondrial genome expression: the tRNA punctuation model revisited

17.50-18.10

#### **O24 Anita Hopper** (USA)

Asking the cells: genome-wide screen of yeast uncovers the mechanisms for tRNA intron turnover and targeting the SEN complex to mitochondria

18.10-18.30

#### **O25 Sidney Kushner** (USA)

Multiple pathways for processing of tRNA primary transcripts in *Escherichia coli*

18.30-18.45

#### **O26 Philippe Giegé** (France)

Exploring the mode of action, diversity and evolution of protein-only RNase P

18.45-19.00

#### **O27 Malgorzata Dobosz-Bartoszek** (USA)

The crystal structure of human selenocysteine tRNA-specific elongation factor, eEFSec

19.00-19.15

#### **O28 Jana Alexandrova** (France)

Elaborate uORF/IRES features control expression and localization of human moonlighting glycyl-tRNA synthetase

19.15-19.30

#### **O29 Mary Anne T. Rubio**

Editing and methylation at a single tRNA site by functionally interdependent activities: Keeping a mutagenic enzyme in check

19.30-20.30

### POSTER EXHIBITION

*(Open also after dinner until 23.00)*

20.30

### Dinner

## Tuesday, September 23<sup>rd</sup>

09.00-11.00



### Session 6 - IUBMB Symposium "The dynamic networks of the tRNA protein community" I

*Chairs:* **S. Kim** (Republic of Korea), **X-L. Yang** (USA)

09.00-09.30

**O30 Sunghoon Kim** (Republic of Korea)

Implications of tRNA synthetase network in cancer microenvironment

09.30-10.00

**O31 Xiang-Lei Yang** (USA)

Nuclear function of TyrRS under oxidative stress

10.00-10.20

**O32 Susan Ackerman** (USA)

Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration

10.20-10.40

**O33 Pavel Ivanov** (USA)

Angiogenin-mediated tRNA cleavage in neurodegeneration

10.40-11.00

**O34 Min Guo** (USA)

Structural basis for multiplexed inhibition of translational and non-translational functions on a human tRNA synthetase

11.00-11.30

**Coffee break**

11.30-13.30



### Session 7 - IUBMB Symposium "The dynamic networks of the tRNA protein community" II

*Chairs:* **H.D. Becker** (France), **P. L. Fox** (USA)

11.30-12.00

**O35 Hubert D. Becker** (France)

The yeast AME multisynthetase complex controls mitochondrial ATP production by coordinating expression and assembly of the F1FO ATP synthase

12.00-12.30

**O36 Paul L. Fox** (USA)

Glutamyl-prolyl tRNA synthetase and metabolism: New functions for an ancient enzyme

12.30-12.50

**O37 Ignacio Luque** (Spain)

Role of an alternative threonyl-tRNA synthetase in the adaptation to zinc deficiency in prokaryotes

12.50-13.10

**O38 Malcom R. Whitman** (USA)

EPBS inhibition signals to a novel matrix remodeling pathway in multiple cell types

13.10-13.30

**O39 Marc Mirande** (France)

Aminoacyl-tRNA synthetase complexes in evolution

13.30-15.00

**Lunch**

## Tuesday, September 23<sup>rd</sup>

15.00-17.00

### Session 8 - tRNA: Recognition and Aminoacylation I

*Chairs: K. Musier-Forsyth (USA), I. Gruić Sovulj (Croatia)*

15.00-15.20

#### **O40 Ita Gruić Sovulj (Croatia)**

Class I aaRS quality control mechanisms preserve canonical translation in *Escherichia coli*

15.20-15.40

#### **O41 Karin Musier-Forsyth (USA)**

Homologous trans-editing factors with broad substrate specificity prevent global mistranslation

15.40-16.00

#### **O42 Mark Saftro (Israel)**

Universal pathway for post-transfer editing reactions: insights from the crystal structure of TtPheRS with puromycin

16.00-16.15

#### **O43 Saumya Dasgupta (India)**

Functional evolution of bacterial GlxRS facilitated by isoacceptor plasticity of tRNA<sup>Gln</sup>

16.15-16.30

#### **O44 John S. Reader (USA)**

Key immunity determinants in agrobacterial LeuRS prevent self-poisoning of plant tumor biocontrol

16.30-16.45

#### **O45 Adam C. Mirando (USA)**

A bioengineered macrolide inhibitor that separates the translational and angiogenesis functions of threonyl-tRNA synthetase

16.45-17.00

#### **O46 Young Ho Jeon (Korea)**

Interaction of lysyl-tRNA synthetase and laminin receptor in the control of cell migration and cancer metastasis

17.00-17.30

### Coffee break

17.30-19.30

### Session 9 - tRNA: Recognition and Aminoacylation II

*Chairs: M. Ibba (USA), S. Martinis (USA)*

17.30-17.50

#### **O47 Michael Ibba (USA)**

Oxidation of cellular amino acid pools leads to cytotoxic mistranslation of the genetic code

17.50-18.10

#### **O48 Susan Martinis (USA)**

Quality control mechanisms of leucyl-tRNA synthetase

18.10-18.30

#### **O49 Henna Tynismaa (Finland)**

The importance of editing by mitochondrial alanyl-tRNA synthetase for mitochondrial protein quality control in mammals

18.30-18.45

#### **O50 Jana Ognjenović (USA)**

Structural basis for the neurological disorders caused by mutations in human cytosolic GlnRS

18.45-19.00

#### **O51 Yi Shi (USA)**

Seryl-tRNA synthetase counteracts c-Myc to develop functional vasculature

19.00-19.15	<b>O52 Michael Schwartz</b> (USA) Methionyl-tRNA synthetase alters its tRNA substrate specificity to facilitate adaptation to varying environments
19.15-19.30	<b>O53 Tamara Hendrickson</b> (USA) Roles of non-canonical proteins and enzymes in indirect tRNA aminoacylation in <i>Helicobacter pylori</i>
19.30-20.30	<b>POSTER EXHIBITION</b> (Open also after dinner until 23.00)
20.30	<b>Dinner</b>

## Wednesday, September 24<sup>th</sup>

<b>09.00-11.00</b>	<b>Session 10 - tRNA: Translation, Structure and Function</b> <i>Chairs: C. Gualerzi</i> (Italy), <i>O. Nureki</i> (Japan)
09.00-09.20	<b>O54 Osamu Nureki</b> (Japan) Structural insights into non-canonical aminoacylation
09.20-09.40	<b>O55 Claudio Gualerzi</b> (Italy) Structural and dynamic aspects of initiator tRNA interactions with the bacterial ribosome and other ribosomal ligands
09.40-10.00	<b>O56 Marina Rodnina</b> (Germany) Translational recoding as a kinetic event
10.00-10.15	<b>O57 Joachim Frank</b> (USA) Progression of tRNAs during elongation, visualized by cryo-EM of a single sample
10.15-10.30	<b>O58 Wolfgang Wintermeyer</b> (Germany) Single-molecule fluorescence reveals multiple chimeric tRNA states during EF-G-induced translocation on the ribosome
10.30-10.45	<b>O59 Daniel N. Wilson</b> (Germany) Structural insight into drug-dependent ribosome stalling
10.45-11.00	<b>O60 Axel Innis</b> (France) A proton wire to couple aminoacyl-tRNA accommodation and peptide bond formation on the ribosome
11.00-11.30	<b>Coffee break</b>

## Wednesday, September 24<sup>th</sup>

11.30-12.00	<b>The EMBO Keynote Lecture</b> <b>Marat Yusupov</b> (France) X-ray crystallography for ribosome function study
12.00-13.30	<b>Lunch</b>
13.30-22.00	<b>Conference Tour to Olympia Archaeological Museum and Site</b> <b>&amp; Dinner at Mercouri winery</b>

## Thursday, September 25<sup>th</sup>

09.00-11.00	<b>Session 11 - tRNA: Regulation, Genomics, Disease I</b> <i>Chairs: I. Tarassov</i> (France), <b>A. Ferré-D'Amaré</b> (USA)
09.00-09.20	<b>O61 Ivan Tarassov</b> (France) Modeling gene therapy of mitochondrial diseases by imported small RNA
09.20-09.40	<b>O62 Adrian Ferré-D'Amaré</b> (USA) - <b>Sidney Altman Endowment Lecture</b> Co-crystal structure of a tRNA-T box riboswitch complex: molecular basis for the control of gene expression by transfer RNA
09.40-10.00	<b>O63 Orna Dahan</b> (Israel) Exploring the dynamic and evolution of the tRNA pool through systematic deletion of tRNA genes
10.00-10.15	<b>O64 Umesh Varshney</b> (India) An extended Shine-Dalgarno sequence in mRNA functionally bypasses a vital defect in initiator tRNA
10.15-10.30	<b>O65 Jennifer Gebetsberger</b> (Switzerland) - <b>Sidney Altman Endowment Lecture</b> tRNA-derived fragments target the small ribosomal subunit to fine-tune translation
10.30-10.45	<b>O66 Jordi Gómez</b> (Spain) Messenger RNAs bearing tRNA features exemplified by a tRNA-mimic motif within interferon alfa 5 mRNA
10.45-11.00	<b>O67 Francesca Tuorto</b> (Germany) The tRNA methyltransferase Dnmt2 is required for accurate protein synthesis during haematopoiesis
11.00-11.30	<b>Coffee break</b>

<b>11.30-13.30</b>	<b>Session 12 - tRNA: Regulation, Genomics, Disease II</b> <i>Chairs: M. Frugier (France), L. Ribas de Pouplana (Spain)</i>
11.30-11.50	<b>O68 Magali Frugier (France)</b> Malaria sporozoites import exogenous tRNAs
11.50-12.10	<b>O69 Lluís Ribas de Pouplana (Spain)</b> Signal saturation limits the development of tRNA identities and the size of the genetic code
12.10-12.30	<b>O70 Philip Farabaugh (USA)</b> A model to explain frequent misreading of a subset of codons by individual tRNAs
12.30-12.45	<b>O71 Jingji Zhang (Sweden)</b> Accuracy of codon recognition by ternary complex on the ribosome
12.45-13.00	<b>O72 Hila Gingold (Israel)</b> A dual program for translation regulation in cellular proliferation and differentiation
13.00-13.15	<b>O73 Rafael R. Argüello (France)</b> Cracking the genomic code of codons: Using dendritic cells to explore beyond the genetic code
13.15-13.30	<b>O74 Salvador Meseguer (Spain)</b> Control of mitochondrial tRNA modification enzymes in MELAS cells by the ROS-regulated microRNA miR-9/9*
13.30-15.00	<b>Lunch</b>
<b>15.00-17.00</b>	<b>Session 13 - tRNA: Regulation, Genomics, Disease III</b> <i>Chairs: T. Pan (USA), E. Razin (Israel)</i>
15.00-15.20	<b>O75 Tao Pan (USA)</b> Discovering tRNA-protein interactome and its function in cellular communication between translation and other processes
15.20-15.40	<b>O76 Ehud Razin (Israel)</b> The role of the LysRS-Ap4A-Hint-1-MITF pathway in health in disease
15.40-16.00	<b>O77 Maria Hatzoglou (USA)</b> tRNAs as potential inhibitors of apoptosis during hyperosmotic stress
16.00-16.15	<b>O78 Sotiria Palioura (Greece)</b> Selenoprotein biosynthesis and human disease
16.15-16.30	<b>O79 Veerle Eggens (The Netherlands)</b> Mutation in RNA kinase CLP1 causes neurodegeneration
16.30-16.45	<b>O80 Elisa Vilardo (Austria)</b> Pathogenic mutations in SDR5C1 impair the tRNA maturation activities of human mitochondrial RNase P
16.45-17.00	<b>O81 Susan Robey-Bond (USA)</b> Molecular basis of histidyl-tRNA synthetase-associated Usher syndrome type 3B in cochlea-derived mouse cells and zebrafish
17.00-17.30	<b>Coffee break</b>



## Thursday, September 25<sup>th</sup>

17.30 - 18.30	<b>Session 14 - tRNA: Systems Biology</b> <i>Chairs: J. Pütz (France), D. H. Ardell (USA)</i>
17.30-17.45	<b>O82 David H. Ardell (USA)</b> Molecular evolution of tRNAs and tRNA CIFs in <i>Drosophila</i>
17.45-18.00	<b>O83 Tasos Gogakos (USA)</b> tRNAseq: An experimental and computational pipeline to characterize expression and processing of precursor and mature human tRNAs
18.00-18.15	<b>O84 Jonathan Morrand (USA)</b> A novel program for analysis of tRNA expression from RNASeq datasets
18.15-18.30	<b>O85 Patricia Chan (USA)</b> An improved tRNAscan-SE and genomic tRNA database: new capabilities and features to enhance tRNA research
18.30-19.15	<b>Session 15 - Closing Session</b> <i>Chairs: C. Stathopoulos (Greece), D. Drainas (Greece), G. Simos (Greece)</i>
18.30-19.00	<b>Keynote Lecture 3: Henri Grosjean (France)</b> The expanding world of (t)RNA modification and editing: 50 years of personal recollections
19.00-19.15	<b>Closing remarks and invitation to attend the 26th tRNA Conference</b>
21.00	<b>Gala Dinner</b>

- P1** Predicting the minimal translation apparatus: Lessons from the reductive evolution of *Mollicutes*  
H. Grosjean, M. Breton, P. Sirand-Pugnet, F. Tardy, F. Thiaucourt, C. Citti, A. Barré, S. Yoshizawa, D. Fourmy, V. de Crécy-Lagard, A. Blanchard
- P2** The identity elements for aminoacylation of metazoan mitochondrial tRNA<sup>Arg</sup> have been widely conserved throughout evolution  
Gabor L. Igloi, Anne-Katrin Leisinger
- P3** A comprehensive evolutionary analysis uncovers an unexpected complexity of tRNA modifications in yeast  
L. Peter Sarin, Fiona Alings, Hannes C. A. Drexler, Sebastian A. Leidel
- P4** Molecular characterization of *Naegleria gruberi* selenophosphate synthetase  
Natália K. Bellini, Ivan R. e Silva, Marco T. A. da Silva, Otavio H. Thiemann
- P5** A genetic code without the sulfur containing amino acids  
Kazuaki Amikura, Daisuke Kiga
- P6** Ancient genes: prediction, RNASeq detection, and ancestral tracing of tRNA in plantae  
Jonathan Morrand, Kirtan Joshi, Kirstyn Tan, Matt Spencer, Bill Spollen, Chris Bottoms, and William Folk
- P7** Intra-organism variation in tRNA structural type  
Aditi Banerjee, Hanchao Zhao, Susan A. Martinis, Margaret E. Saks
- P8** *In vivo* function of RtcA in tRNA processing  
Bart Appelhof, Veerle R.C. Eggens, Tessa van Dijk, Anneloor ten Asbroek, Marian A.J. Weterman, Joseph G. Gleeson, Frank Baas
- P9** Identification of a new folate-dependent rRNA methyltransferase that catalyses m<sup>5</sup>U1939 modification in 23S Rrna  
C. Lartigue, A. Lebaudy, A. Blanchard, B. El Yacoubi, S. Rose, S. Douthwaite, H. Grosjean
- P10** Biosynthesis of wyosine derivatives in tRNA<sup>Phe</sup> of Archaea: Role of a remarkable bifunctional tRNA<sup>Phe</sup>: m<sup>1</sup>G/imG2 methyltransferase  
J. Urbonavicius, R. Rutkiene, J. Napryte, D. Tauraitė, J. Stankeviciute, A. Aucynaite, R. Meskys, H. Grosjean
- P11** Structural characterisation of two homologous 2'-O-methyltransferases showing different specificities for their tRNA substrates  
Bart Van Laer, Jonathan Somme, Martine Roovers, Jan Steyaert, Louis Droogmans, Wim Versées
- P12** NMR conformational dynamics of La protein domains in interaction with pre-tRNA ligands from a lower eukaryote exhibiting identical structural organization with its human homolog  
Dionysios Vourtsis, Maria Apostolidi, Aikaterini Argyriou, Christos Chasapis, Parthena Konstantinidou, Denis Drainas, Detlef Bontrop, Constantinos Stathopoulos and Georgios A. Spyroulias
- P13** Peculiarities of queuosine biosynthesis in trypanosomes  
Zdeněk Paris, Alan Kessler, Mary Anne T. Rubio, Juan D. Alfonso
- P14** The differential activity of tRNA modifying enzyme: the role of anticodon stem loop sequence  
Bhavik Sawhney, N Saraswathi, Akash Ranjan
- P15** Pre-tRNA capping  
Takayuki Ohira and Tsutomu Suzuki
- P16** A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs  
Takeo Suzuki and Tsutomu Suzuki
- P17** How does the folate dependent tRNA (m<sup>5</sup>U54) methyltransferase (TrmFO) recognize substrate tRNA?  
Ryota Yamagami, Koki Yamashita, Hiroshi Nishimasu, Chie Tomikawa, Anna Ochi, Chikako Iwashita, Ryuichiro Ishitani, Osamu Nureki, and Hiroyuki Hori
- P18** Alteration of the solid-phase DNA probe method for large-scale tRNA purification  
Ai Kazayama, Ryota Yamagami, Takashi Yokogawa, and Hiroyuki Hori
- P19** CmoM, the novel methyltransferase responsible for the last step of uridine-5-oxyacetic acid methyl ester (mcm<sup>5</sup>U) biogenesis  
Yusuke Sakai, Kenryo Miyauchi, Satoshi Kimura and Tsutomu Suzuki
- P20** Topological knot tRNA methyltransferase (TrmH) discriminates substrate tRNA from non-substrate tRNA by a multistep recognition mechanism  
Anna Ochi, Koki Makabe, Ryota Yamagami, Akira Hirata, Reiko Sakaguchi, Ya-Ming Hou, Kazunori Watanabe, Osamu Nureki, Kunihiro Kuwajima, and Hiroyuki Hori
- P21** Proteinaceous vs. bacterial RNase P: a comparative mechanistic analysis  
Nadine Wäber, Dennis Walczyk, Sören Seidler, Roland K. Hartmann

# POSTERS

- P22** A rapid semiquantification of tRNA precursors in human cultured cell lines  
Tamara Fernández, Florencia Cabrera, Danilo Segovia, Mónica Marín
- P23** Unprecedented archaeal tRNA modifications found in *Thermoplasma acidophilum*  
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# TALKS







## Keynote Lecture 1

### Implications of RNA being modified: Basic and translational science

**Paul Agris**

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Long before the term non-coding RNA was coined, there was tRNA, now the most understood of all non-coding RNAs. tRNAs were found to contain nucleosides that had been enzymatically modified after transcription, which is also true for other non-coding RNAs. Inosine was the first modified nucleoside of note found in tRNA, identified and sited at the first position of the anticodon during sequencing of yeast tRNA<sup>Ala</sup> by Holley in 1965. This important discovery laid the foundation for the Wobble Hypothesis postulated by Crick in 1966. He proposed that inosine bound adenosine, as well as uridine and cytosine. Crick's hypothesis has been altered to include the wobble base pairing of other modified nucleosides. We have now shown that inosine's ability to decode A, U and C is regulated by another anticodon loop modification. The first half century of the tRNA Workshop/Conference, was also the first half century of tRNA modified nucleoside investigations. Initially, it was replete with the biochemical analyses of tRNA modifications by TLC, then by HPLC, and finally by LC-MS. Simultaneously, there was a hunt for the enzymes responsible for modified nucleoside synthesis, followed by their mechanisms of action, particularly those of the methyltransferases and pseudouridine synthases. An understanding of modified nucleoside contributions to the thermal stability and dynamics of the anticodon gave insights into the functions of these numerous and complex chemistries. Chief among them were the functional analyses of tRNA modification, and concurrently, structure-function relationships that contributed to tRNA's codon recognition, biases and wobbling. The early introduction of NMR and later x-ray crystallography has revealed that wobble codon binding is stabilized through uridine and cytosine modifications, facilitating tautomer formation. Today, a combination of ultra-highly sensitive techniques in modified nucleoside analysis and a variety of approaches to structure-function relationships have and can continue to relate tRNA modification to basic biology, expansion of the genetic code and to solutions to human health problems.



## Keynote Lecture 2

### The new biology of tRNAs and AARSs

**Paul Schimmel**

*The Scripps Laboratories for tRNA Synthetase Research, Scripps Florida Research Institute, Jupiter FL and The Scripps Research Institute, La Jolla, CA*

New biology has emerged from the recent realization that the functions of tRNAs and AARSs in translation per se also, surprisingly, link translation to cellular events such as genomic stability and auto-immunity. In another vein, the discovery of many alternative forms of AARSs in higher organisms has opened a frontier for exploration of a previously unrecognized deep layer of biology that is controlled by a network of non-translational activities of these natural, novel forms of AARSs. These novel forms, together with the recently discovered nuclear and extracellular non-translation activities of the native AARSs, suggest that investigations of these unanticipated functions of AARSs will be essential for uncovering new pathways that impact broadly on all areas of biology.



## Keynote Lecture 3

### The expanding world of (t)RNA modification, editing and repair: 50 years of personal recollections

Henri Grosjean

*Emeritus Scientist at the Center of Molecular Genetics of the CNRS in Gif-sur-Yvette / France*

The first evidence for the presence of modified nucleosides in RNA was obtained in 1951 (the fifth nucleoside, now designated Psi), while the first RNA modification enzyme was identified in 1962 (tRNA:m<sup>5</sup>U methyltransferase, now designated TrmA or Trm2). In 1986, a process of post-transcriptional insertion of non-genomically encoded uridylate residue within the coding region of mitochondria mRNA of kinetoplastid protozoa was discovered (RNA editing). Soon it was demonstrated that RNA editing also occurs by well-established RNA modification enzymes. Another remarkable discovery (1996) was that RNA guides some RNA modification enzymes within multiprotein complexes catalysing formation of 2'-O-ribose methylation and pseudouridine in certain RNAs in Eukarya and Archaea. Recently (2003), demethylases, analogous to those acting on DNA, were demonstrated to act also on RNA (RNA repair). To date, 114 structurally distinguishable naturally occurring modified nucleosides originating from different types of cellular RNAs and from many organisms of the three biological domains have been identified. This is not an upper limit and every year new modified nucleosides are still discovered, especially that new tools for their easier identification exist and RNA of different organisms are studied. A large number of enzymes catalysing the formation of these modified nucleosides or converting one canonical base into another at the posttranscriptional level have been identified and their mechanisms, structures and evolutionary relationship of a large number of them become well established. The effect of modifications on RNA structure and function was for a long time elusive but nowadays it is clear that they play major roles in controlling RNA quality and improve the performance of the matured RNAs by working more efficiently and accurately in various steps of cellular metabolism, including their regulation. Their effects can be subtle and not easy to demonstrate in vivo or in vitro. Despite the considerable progress made during these last five decades on our understanding of the biosynthesis and functions of modified nucleosides in RNAs, still much remains to be solved. I began to be interested to this problem in October 1964, when I started my PhD thesis at University of Brussels in Belgium. In this talk I shall present a brief history of this expanding World of RNA modification, editing and repair, using examples of my own research work.



# The EMBO Keynote Lecture

## X-ray crystallography for ribosome function study

**Marat Yusupov and Gulnara Yusupova**

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Ribosomes from bacteria consist of a large and a small subunit, which together compose the 2.5 megadalton (MDa) 70S ribosome. Their eukaryotic counterpart is the 80S ribosome (from 3.5 MDa in lower eukaryotes to 4.5 MDa in higher). Many ribosomal key components are conserved across the three kingdoms of life: bacteria, archaea, and eukarya and constitutes a common core undertaking the fundamental processes of protein biosynthesis.

The mechanism for decoding based on X-ray structures of bacterial 70S ribosome determined at 3.1-3.4 Å resolution and modeling cognate or near-cognate states of the decoding center has been investigated. We show that the 30S subunit undergoes an identical domain closure upon binding of either cognate or near-cognate tRNA. This conformational change of the 30S subunit forms a decoding center that constrains the mRNA in such a way that the first two nucleotides of the A codon are limited to form Watson-Crick base pairs. When a U•G or G•U mismatch, generally considered to form a Wobble base pair, is at the first or second codon-anticodon position, the decoding center forces this pair to adopt the geometry close to that of a canonical C•G pair. This by itself or together with distortions in the codon-anticodon mini-helix and the anticodon loop causes the near-cognate tRNA to dissociate from the ribosome. Our study provides structural insights into a universal principle of decoding on the ribosome.

The ribosome is a major target for small-molecule inhibitors. We used X-ray crystallography to determine 16 high-resolution structures of the full 80S ribosome from *Saccharomyces cerevisiae* in complexes with 12 eukaryote-specific and 4 broad-spectrum inhibitors. All inhibitors were found associated with messenger RNA and transfer RNA binding sites. The study defines common principles of targeting and resistance, provides insights into their mode of action and unveil the structural determinants responsible for species selectivity.



## Structural studies on specific aminoacyl-tRNA synthesis and genetic code expansion

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The genetic code assigns each of the twenty amino acids to its codon(s). The strict molecular recognition of both the amino acid and tRNA by aminoacyl-tRNA synthetases guarantees the genetic code. The amino acid specificity depends primarily on the interaction of the substrate amino acid with the binding pocket in the aminoacylation catalytic site on the aminoacylation domain. Several of the twenty aminoacyl-tRNA synthetases have an editing domain to correct the possible errors in the amino acid recognition, by hydrolyzing misformed non-cognate aminoacyl-tRNAs. We engineered the aminoacylation and/or editing domains of tyrosyl- and pyrrolysyl-tRNA synthetases, on the basis of their crystal structures, in order to aminoacylate tRNAs with unnatural amino acids. On the other hand, the tRNA specificities of aminoacyl-tRNA synthetases depend mostly on the interactions of aminoacyl-tRNA synthetases with small numbers of nucleotides (the identity determinants), such as the anticodon and the discriminator nucleotide adjacent to the invariant 3'-terminal CCA region, mostly via base-specific interactions. The identity determinants are 15–75 Å distant from the 3' terminus on the common L-shaped tertiary structures of tRNAs. Aminoacyl-tRNA synthetases exhibit about 100 times faster  $k_{\text{cat}}$  values for their cognate tRNAs than non-cognate tRNAs, with much smaller differences in the  $K_{\text{m}}$  values. In this study, we have elucidated novel structural bases for the dynamic mechanisms for tRNA selections at the  $k_{\text{cat}}$  level. By using tRNA variants with the anticodon complementary to UAG, we expanded the genetic code to assign UAG to unnatural amino acids. We knocked out the release factor 1 gene of *Escherichia coli*, and enabled the complete reassignment of UAG to unnatural amino acids.



## 02

## Rewiring *E. coli* translation for synthesis of selenoproteins

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At the time of its elucidation the genetic code was suggested to be universal in all organisms, and the result of a 'frozen accident' unable to evolve further (1). Today we know 22 natural amino acids (2): selenocysteine, the 21<sup>st</sup>, and pyrrolysine, the 22<sup>nd</sup>, are directly inserted into growing polypeptides during translation. The incorporation of selenocysteine directed by UGA requires the action of specific RNA and protein elements, a fact that has restricted engineering of selenoproteins. Based on the realization that protein plasticity is a feature of living cells (3), man-made expansion of the genetic code based on orthogonal translation systems (OTSs) is an active research field (4,5). However, the successful design of *in vivo* specific and highly active OTS systems is far from ideal (6). This will be illustrated at examples of co-translational insertion of selenocysteine (7-9).

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## Probing proteins and proteomes from organisms with expanded genetic codes

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Orthogonal translation systems (OTS) that direct nonstandard amino acid (NSAA) incorporation into recombinant proteins are increasing in their diversity and utility in biotechnology applications. Recently, *E. coli* strains that lack release factor one (RF1) have shown improvements in their ability to “reassign” the amber codon from stop to sense function. We have made significant contributions to this effort<sup>1,2,3</sup> and have focused on improving phosphoprotein synthesis *via* an OTS for phosphoserine. During these efforts we recognized the need for robust proteomics methodologies to characterize proteins in this new biological context where the absence of RF1 dramatically changes the natural properties of translation at so called “open” codons. We wondered what the extent of on and off target amino acid insertions would be in various *E. coli* strains with different engineered OTSs. We also identified the need for a general strategy to characterize translation products derived from TAG codons in recombinant proteins or in the host genome. To this end, we will present a versatile proteomics platform to probe proteins and proteomes from organisms with expanded genetic codes. Our workflow accurately identifies standard and non-standard amino acids inserted at reassigned TAG codons. We utilized our methods to demonstrate on and off target amino acid insertions in both recombinant proteins and across the *E. coli* proteome. We extended our method to quantify the fidelity of several phosphoserine OTSs engineered for enhanced performance. We have applied these improved OTSs to understand the role of protein phosphorylation in human signaling networks.

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## 04

## An orthogonal ribosome-tRNAs pair by the engineering of peptidyl transferase center

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The 3'-CCA sequence of tRNA is conserved among all organisms. In bacteria, the CCA end makes Watson-Crick base pairs with 23S rRNA in both A and P sites during translation. These base pairs are important for translational activity, and the compensatory mutations in these base pairs are tolerated during peptidyl transfer reaction. However, it is yet empirically unknown whether such mutations accommodates translation in its entirety.

In this study, we evaluated the translational activity of mutant ribosomes and CCA-mutated tRNAs pairs using a reconstituted in vitro translation system, and discovered a compensatory mutant ribosome-tRNA pair which works independently of the wild type ribosome-tRNA pair. We developed a simple method to prepare CCA-mutated aminoacyl-tRNAs by using the aminoacylation ribozyme, "flexizyme", because CCA-mutated tRNAs are poor substrates for aminoacyl-tRNA synthetases. Flexizyme recognizes tRNA by base pairing with the CCA end of tRNAs, and therefore flexizymes, which are engineered to be complementary to the CCA-mutated tRNAs, can readily aminoacylate CCA-mutated tRNAs. Ribosome mutants were prepared by MS2-tag affinity purification, which have point mutations corresponding to the Watson-Crick base pairs with the CCA-mutated tRNAs.

We discovered several mutant ribosome-tRNA pairs having translational activity and one of these pairs showed orthogonality with the wild type pair. In addition, we demonstrated that two different peptides were simultaneously translated from single mRNA by these wild type and mutant ribosome-tRNA pairs without mis-incorporation of undesired amino acids, which indicates two different genetic codes can work independently in one pot. This work thus identifies a new way to reprogram the genetic code.

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### Acknowledgements

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## 05

## A rare codon can be captured by a non-canonical amino acid

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Codon reassignment occurs when the original decoding factors of a particular codon are superseded by new ones while changing the meaning of the codon. Previously, we found that UAG stop codon reassignment in *E. coli* required only the prior elimination of 7 TAG codons and expression of a suppressor tRNA. Active suppressor tRNAs such as *supP* and *supE* sufficiently complemented the loss of the release factor 1. Several unnatural amino acids, including 3-iodotyrosine and Aloc-lysine, were also efficiently assigned to UAG. To generalize the assumption that the genetic code remains flexible and mutable even in a modern organism, we extended our rapid strategy for UAG to sense codon reassignment. The steps are: 1) Breaking the redundancy of degenerate codons for the codon-specific reassignment. 2) Eliminating the minimum numbers of the codon by synonymous substitution for partial recoding. 3) Overwriting the assignment of the codon by an analogue of the cognate amino acid not to significantly disturb the proteome. Among the sense codons and non-canonical amino acids, we chose AGG and L-homoarginine, as the AGG codon is the rarest in *E. coli* (about 1500 times in total, and only 38 times in the essential genes), and because L-homoarginine is thought to be a good or better alternative to arginine. We found that the slow growth of the *E. coli* Magic21(DE3) strain so constructed was dependent on the L-homoarginine (assigned to the AGG codon). The upcoming challenge will be to select/screen for more active translation by the orthogonal aaRS/tRNA/amino acid system.



## 06

Sense codon recoding in *Escherichia coli*

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Expansion of the genetic code is an active research topic in synthetic biology (1). Recoding of two stop codons (UAG, UGA) has been studied for three decades, but sense codon recoding is experimentally still poorly addressed. Natural recoding of leucine CUG codons to serine in *Candida* species has led to exciting results (2). However, to date, no successful *in vivo* recoding has been reported with synthetic systems. A recent attempt in *Mycoplasma capricolum* to recode the arginine CGG codon using the pyrrolysyl-tRNA synthetase: tRNA<sup>Pyl</sup> pair failed, probably since the endogenous arginyl-tRNA synthetase charged the exogenous tRNA<sup>Pyl</sup><sub>CCG</sub> with arginine. Most endogenous aminoacyl-tRNA synthetases recognize their tRNA substrates via their anticodon sequences (3); this phenomenon increases translation fidelity but complicates sense codon recoding. In order to reassign sense codons using orthogonal synthetase: tRNA pairs that do not crosstalk with the endogenous aminoacylation machinery, one needs to address the challenges posed by tRNA identity (3). We will report data from our current attempts to recode a number of sense codons in *Escherichia coli*.

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## 07

## Synthetic biology: A measurement solutions provider's perspective

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Synthetic Biology is an emerging discipline at the intersection of biology and engineering with the goal of designing and constructing biological devices, pathways, systems, and entire genomes for useful purposes. The advance of synthetic biology relies on several key enabling technologies provided at ever increasing throughput and lower cost. DNA sequencing, the synthesis of genes, pathways and genomes and precisely measuring gene behavior are essential tools in synthetic biology. It is also clear that RNA is playing an ever increasing role in advancing this field.

In this talk, I will provide a brief overview of Agilent's view of synthetic biology and why we, as a measurement solutions provider for life sciences and chemical analysis, are so excited about this emerging field. I will also touch on the central role that tRNA plays in genetic code reassignment and expansion which is major thrust of the synthetic biology field and a great research opportunity for the tRNA community.



## 08

## Diverse roles of the prion-like protein, Mod5, in tRNA-modification and RNA-silencing

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Mod5 is a highly conserved tRNA modifying enzyme residing primarily in the cytoplasm where it modifies a small subset of tRNAs by transferring a isopentenyl group from dimethylallyl pyrophosphate to A37 adjacent to the anticodon. A small population of Mod5 also resides with nuclear tRNA gene transcription complexes and nascent pre-tRNAs, and is required for silencing RNA polymerase II transcription near tRNA genes (1). Yeast Mod5 can misfold into heritable prion-like aggregates, allowing more of the dimethylallyl pyrophosphate substrate to be diverted to the essential prenylation pathways. The human homolog of Mod5, TRIT1, complements both the tRNA-modification and tRNA gene-mediated silencing functions in yeast and has similar cytoplasmic/nuclear localization in human cells. We are investigating the folding behavior of Mod5/TRIT1 in yeast and in human cells, addressing whether amyloid formation affects the nuclear or cytoplasmic functions of Mod5. Although it is known that tRNA modification in the cytoplasm is significantly reduced when Mod5 aggregates, our studies demonstrate that tRNA gene-mediated silencing in the nucleus is unaffected. Thus, either the nuclear Mod5 is aggregation-resistant or threshold level for the nuclear function is different.

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## Identification of the determinants of tRNA function and susceptibility to rapid tRNA decay by high throughput in vivo analysis

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Sequence variation in tRNA genes influences the structure, modification, and stability of tRNA, affects translation fidelity, impacts the activity of numerous isodecoders in metazoans, and leads to human diseases. To comprehensively define the effects of sequence variation on tRNA function, we developed a high throughput in vivo screen to quantify the activity of the nonsense suppressor *SUP4<sub>oc</sub>* of *Saccharomyces cerevisiae*. Using a highly sensitive GFP fluorescent reporter gene with an ochre mutation (Dean, K. M. and Grayhack, E. J. (2012) *RNA* **18**:2335-2344), fluorescence-activated cell sorting of a library of *SUP4<sub>oc</sub>* mutant yeast strains, and deep sequencing, we scored 25,491 variants for function. Unexpectedly, we find that *SUP4<sub>oc</sub>* tolerates numerous sequence variations without significant loss of function, accommodates slippage in several tertiary and secondary interactions, and exhibits genetic interactions that suggest an alternative functional tRNA conformation.

We have also applied this methodology to comprehensively define tRNA variants subject to rapid tRNA decay (RTD). Although RTD normally degrades tRNAs with exposed 5' ends, mutations that sensitize *SUP4<sub>oc</sub>* to RTD were found to be located throughout the sequence, including the anticodon stem. Thus, the integrity of the entire tRNA molecule is under surveillance by cellular quality control machinery.

This approach to assess activity by high throughput quantification in vivo is widely applicable to many problems in tRNA biology.



## 010

## RNase P: convergent evolution within a structurally diverse enzyme family

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The RNase P family is a diverse group of endonucleases responsible for the removal of 5' extensions from tRNA precursors. The diversity of enzyme forms finds its extremes in the eukaryal nucleus where RNA-based catalysis by complex ribonucleoproteins in some organisms contrasts with single-polypeptide enzymes in others. Such extraordinary contrast in composition and structural complexity raises the question of whether the different enzymes use similar or distinct enzymatic mechanisms, but also whether they are at all functionally equivalent. The complexity of the ribonucleoprotein was indeed proposed to broaden its functionality beyond tRNA processing. To explore functional overlap and differences between most divergent forms of RNase P *in vivo*, we replaced yeast nuclear RNase P, a 10-subunit ribonucleoprotein, with *Arabidopsis* PRORP3, a single monomeric protein. Surprisingly, the RNase P-swapped yeast strains were not only viable, but had essentially unchanged growth properties and fitness, indicating the full functional exchangeability of the dissimilar enzymes. The molecular analysis showed a minor disturbance in tRNA metabolism, but did not point to any RNase P substrates or functions beyond that. An in-depth analysis of substrate recognition and cleavage site selection by PRORP3 indicated a high degree of similarity to what is known about nuclear ribonucleoprotein enzymes. Together these data establish the RNase P family, combining structural diversity with functional and mechanistic uniformity, as an extreme case of convergent evolution. It moreover suggests that the apparently gratuitous complexity of some RNase P forms is the result of constructive neutral evolution rather than reflecting increased functional versatility.





## Biology and chemistry of tRNA damage and its repair

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tRNA anticodon breakage inflicted by bacterial and eukaryal “ribotoxins” (a phenomenon referred to as tRNA restriction) underlies a rudimentary innate immune system that distinguishes self from non-self species, defends cells against virus infection, and modulates protein synthesis in response to stress. *Pichia acaciae* toxin (PaT) arrests the growth of the non-self species *Saccharomyces cerevisiae* by incising the anticodon loop of tRNA<sup>Gln</sup> to form 2,3'-cyclic phosphate and 5'-OH ends at the break. I will present the atomic structure of PaT, the first of a eukaryal tRNA restriction enzyme.

RNA repair enzymes capable of sealing 2',3'-cyclic phosphate and 5'-OH ends are present in diverse taxa in all phylogenetic domains of life. Repair pathways anchored by “classic” ATP-dependent RNA ligases entail the joining of 3'-OH and 5'-PO<sub>4</sub> termini. To satisfy the ligase specificity for 3'-OH/5'-PO<sub>4</sub> ends, the original broken 2',3'-cyclic phosphate and 5'-OH ends must be “healed” before they can be sealed. Healing entails two discrete reactions: hydrolysis of the 2',3'-cyclic phosphate by a phosphoesterase enzyme to form a 3'-OH; and phosphorylation of the 5'-OH by a polynucleotide kinase enzyme to form a 5'-PO<sub>4</sub>.

We have elucidated a wholly different mechanism of RNA break repair whereby 3'-PO<sub>4</sub> and 5'-OH ends are spliced by a new flavor of RNA ligase, exemplified by *E. coli* RtcB. RtcB executes a four-step pathway of end joining that requires GTP as an energy source and Mn<sup>2+</sup> as a cofactor. RtcB first reacts with GTP to form a covalent RtcB-(histidinyl-N)-GMP intermediate. It then hydrolyzes the RNA 2',3'-cyclic phosphate end to a 3'-phosphate and transfers guanylate from His337 to the RNA 3'-phosphate to form an RNA-(3')pp(5')G intermediate. Finally, RtcB catalyzes the attack of an RNA 5'-OH on the -N(3')pp(5')G end to form the splice junction and liberate GMP.

RtcB is structurally *sui generis* and its chemical mechanism is unique. The wide distribution of RtcB proteins in bacteria, archaea and metazoa raises the prospect of an alternative enzymology based on covalently activated 3' ends.



012

## The identity of the discriminator base has an impact on CCA-addition

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CCA-adding enzymes synthesize and maintain the C-C-A sequence at the tRNA 3'-end, generating the attachment site for amino acids. While tRNAs are the most prominent substrates for this polymerase, CCA-additions on non-tRNA transcripts are described as well. To identify general features for substrate requirement, a pool of randomized transcripts was incubated with the human CCA-adding enzyme. Most of the RNAs accepted for CCA-addition carry an acceptor stem-like terminal structure, consistent with tRNA as the main substrate group for this enzyme. While these RNAs show no sequence conservation, the position upstream of the CCA-end was in most cases represented by an adenosine residue. In tRNA, this position is described as discriminator base, an important identity element for correct aminoacylation. Mutational analysis of the impact of the discriminator identity on CCA-addition revealed that purine bases (with a preference for adenosine) are strongly favoured over pyrimidines. Furthermore, depending on the tRNA context, a cytosine discriminator can cause a dramatic number of misincorporations during CCA-addition. The data correlate with a high frequency of adenosine residues at the discriminator position observed *in vivo*. Originally identified as a prominent identity element for aminoacylation, this position represents a likewise important element for efficient and accurate CCA-addition.



## Coupling demethylation to RNA sequencing provides a high-throughput view of tRNA processing and modification

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Transfer RNAs are among the most abundant RNAs in cells, but their abundant post-transcriptional modifications pose special challenges for high throughput sequencing-based profiling. We have developed an approach to facilitate sequencing of RNAs modified with 1-methyladenosine (m1A), which is among the most common tRNA modifications in many organisms, or the less common 3-methylcytosine (m3C), both of which cause problematic stops during the reverse transcription step of RNA-seq library preparation. Our method uses enzymatic pre-treatment to demethylate these modified bases, revealing a large class of modified tRNA fragments, precursors, and processing intermediates that are otherwise absent or grossly underrepresented in libraries prepared using common RNA-seq protocols. Treatment of samples from *Saccharomyces cerevisiae* and from several human cell lines shows that demethylation produces striking changes in tRNA fragment profiles. Furthermore, comparing treated versus untreated samples provides a high-throughput, statistically-supported readout of modification state for multiple tRNA species in parallel, enabling new investigations of modification dynamics over time, cell environment, and cell type. These comparative analyses support established patterns of modification for most tRNAs in *S. cerevisiae*, and provide new modification predictions for a large number of human tRNAs. Further analyses yielded a range of new observations: modified tRNA fragments from most tRNA isoforms greatly outnumber unmodified tRNA fragments; numerous human tRNAs appear to be modified at an early stage of processing, prior to removal of pre-tRNA trailer sequences; and there are clear differences in the tRNA fragment profiles of human cancer versus non-cancer cell lines that were not apparent without demethylation treatment. Thus, the rich landscape of tRNA processing and modification illuminated by this method sets the stage for understanding the dynamics of tRNA function and regulation at new levels of complexity.



## 014

## 1-methyladenosine in transfer RNA

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RNA modification is a post-transcriptional process by which certain nucleotides are altered after their initial incorporation into an RNA chain. Transfer RNA is the most heavily modified class of RNA molecules. These modifications expand the chemical and functional diversity of tRNA, and enhance its structural stability. We focussed our research on m<sup>1</sup>A tRNA modification. m<sup>1</sup>A nucleotide is found at position 9, 14, 22, 57 and 58 in tRNA. We solved the X-ray structures of the *T. thermophilus* m<sup>1</sup>A<sub>58</sub> (TrmI) and of the *B. subtilis* m<sup>1</sup>A<sub>22</sub> tRNA (TrmK) methyltransferases. We then conducted biophysical studies using mass spectrometry, NMR, site-directed mutagenesis and molecular docking to obtain data on the tRNA recognition mode specific to each methyltransferase and on the reaction mechanism. We showed that TrmI has to maintain its tetrameric organization in order to achieve its enzyme activity whereas TrmK modifies the tRNA as a monomer. TrmI presents two grooves that are large enough and electrostatically compatible to accommodate one tRNA per face of TrmI tetramer. TrmK presents two domains : the 'Rossman-fold' domain responsible for the methyltransferase activity and a domain that presents two helices that formed a coiled-coil, probably necessary to bind to tRNA. These two domains form a concave surface of positive electrostatic potentials favorable to the tRNA binding just below the catalytic pocket. Recent results regarding the specific recognition with tRNA substrates and the reaction mechanisms of these two enzymes will be presented.

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## Biogenesis and function of cyclic t<sup>6</sup>A and its derivatives

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N<sup>6</sup>-threonylcarbamoyladenosine (t<sup>6</sup>A) and its derivatives are universally conserved, essential modified nucleosides found at position 37 of tRNAs responsible for ANN codons in all three domains of life. t<sup>6</sup>A plays a crucial role in maintaining decoding accuracy during protein synthesis, and it is also required for aminoacylation of tRNA, tRNA binding to the A-site codon, efficient translocation, reading-frame maintenance, and prevention of leaky scanning of initiation codons and read-through of stop codons. We reported that t<sup>6</sup>A is a hydrolyzed artifact of cyclic t<sup>6</sup>A (ct<sup>6</sup>A), a bona fide modified base of *E. coli* tRNAs (Miyauchi et al., 2013). ct<sup>6</sup>A is an additional modification of t<sup>6</sup>A that enhances tRNA-decoding activity. ct<sup>6</sup>A is widely distributed among tRNAs from a certain group of bacteria, fungi, plants, and some protists, whereas t<sup>6</sup>A is present in tRNAs of mammals, archaea, and other group of bacteria. We identified an E1-like enzyme named tRNA threonylcarbamoyladenosine dehydratase A (TcdA) which catalyzes ATP-dependent dehydration of t<sup>6</sup>A to form ct<sup>6</sup>A. Detailed catalytic mechanism of t<sup>6</sup>A dehydration has been investigated by structural and biochemical approaches. In addition, we identified a cysteine desulfurase (CsdA) and a sulfur carrier protein (CsdE) to be required for efficient ct<sup>6</sup>A formation, indicating that sulfur relay system is involved in this reaction.

N<sup>6</sup>-methyl-N<sup>6</sup>-threonylcarbamoyladenosine (m<sup>6</sup>t<sup>6</sup>A) is a t<sup>6</sup>A derivative found in tRNAs from bacteria, *Drosophila*, plants, and mammals. In *E. coli*, we recently identified *trmO* responsible for the N<sup>6</sup>-methyl group of m<sup>6</sup>t<sup>6</sup>A in tRNA<sup>Thr</sup> specific for ACY codons. TrmO has a unique single-sheeted  $\beta$ -barrel structure and does not belong to any known classes of methyltransferases. Recombinant TrmO employs S-adenosyl-L-methionine (AdoMet) as a methyl donor to methylate t<sup>6</sup>A to form m<sup>6</sup>t<sup>6</sup>A in tRNA<sup>Thr</sup>. Therefore, TrmO represents a novel category of AdoMet-dependent methyltransferase (Class VIII). In a  $\Delta$ *trmO* strain, m<sup>6</sup>t<sup>6</sup>A was converted to ct<sup>6</sup>A, suggesting that t<sup>6</sup>A is a common precursor for both m<sup>6</sup>t<sup>6</sup>A and ct<sup>6</sup>A. N<sup>6</sup>-methylation of t<sup>6</sup>A enhanced the attenuation activity of the *thr* operon, suggesting that TrmO ensures efficient decoding of ACY. We also identified a human homolog, TRMO, indicating that m<sup>6</sup>t<sup>6</sup>A plays a general role in fine-tuning of decoding in organisms from bacteria to mammals.



## 016

**Complex modification of tRNA, interplay with DNA metabolism?****Valérie de Crécy-Lagard**<sup>1,2</sup><sup>1</sup>*Dpt of Microbiology and Cell Science, University of Florida, Gainesville, Florida, USA;*<sup>2</sup>*University of Florida Genetics Institute, Gainesville, Florida, USA**vcrcry@ufl.edu*

The synthesis of complex modifications of tRNAs requires multiple enzymes, precursors and cofactors. Our long-standing interest in both the queuosine (Q) modification found at position 34 of tRNAs decoding GUN codons and the threonylcarbamoyladenosine (t<sup>6</sup>A) modification found at position 37 of tRNAs decoding ANN codons led to the discovery of their synthesis genes by combining *in silico* data-mining and experimental validations. As more whole-genome sequences became available, we have used this knowledge to discover remaining Q salvage genes in bacteria and eukaryotes, with some clearly derived from DNA metabolism. Additionally, we discovered the unexpected presence of Q precursors in DNA. These 7-deazaguanosine derivatives in DNA could provide protection against physical denaturation and/or act as self-recognition markers. Finally, the identification of inactive paralogs of the t<sup>6</sup>A tRNA modification enzyme TsaC of the YciO subfamily, probably linked to DNA repair, raises the question of how RNA and DNA modification synthesis enzymes discriminate between the closely related DNA and RNA substrates.



## A tRNA modification pathway in mitochondria unveils the origin of wybutosine in eukaryotes

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Strategies for reading frame maintenance were likely prerequisites to establish the early genetic code and must have inevitably involved tRNA post-transcriptional modifications. Given their well-documented effects on translational fidelity, wyosine/wybutosine found in tRNA<sup>Phe</sup> of Archaea and Eukarya probably played a central role as one of the drivers of translational accuracy. In its most common form in eukaryotes, hydroxywybutosine is the product of five highly conserved enzymes that use a methylated guanosine (m<sup>1</sup>G<sub>37</sub>) as a precursor, but its function has been relegated to cytosolic translation. Here we present genetic, molecular and mass spectrometry data demonstrating the first example of a wyosine pathway in mitochondria, a situation thus far unique to the kinetoplastid lineage. Although two forms of wyosine/wybutosine-modified tRNA<sup>Phe</sup> exist in *Trypanosoma brucei* mitochondrion, the organellar pathway has features in common with that of Archaea. Based on molecular phylogeny arguments, we suggest that mitochondrial wyosine biosynthesis represents an ancestral pathway dating back to the last common ancestor with the Archaea. These findings are discussed in the context of the extensive RNA editing in the trypanosomatid mitochondrion, whereby editing, in generating potentially “slippery” U-rich sequences, provided the selective pressure to maintain mitochondrial wyosine.



018

## A divalent metal ion-dependent N<sup>1</sup>-methyl transfer to G37-tRNA

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The N<sup>1</sup>-methylation of G37 to synthesize m<sup>1</sup>G37 on the 3' side of the anticodon of tRNA is essential for the survivability of cells in all three domains of life. The m<sup>1</sup>G37 is a post-transcriptional modification that prevents frameshift errors during tRNA-mediated protein synthesis on the ribosome. Unlike mis-sense errors, frameshift errors interrupt the reading frame of protein synthesis, resulting in premature termination. While m<sup>1</sup>G37 is conserved in evolution, the bacterial enzyme TrmD that synthesizes this modification is not related to its eukaryotic or archaeal counterpart Trm5. We have shown that while TrmD and Trm5 both use S-adenosyl methionine (AdoMet) as the methyl donor and G37-tRNA as the acceptor, these two enzymes are fundamentally distinct in structure and in mechanism. While TrmD uses a deep topological knotted protein fold to bind the methyl donor, Trm5 uses an open dinucleotide fold. Recently, we have discovered that TrmD differs from Trm5 in one major mechanistic aspect, in that TrmD requires Mg<sup>2+</sup> to catalyze methyl transfer whereas Trm5 does not. The Mg<sup>2+</sup>-dependence of TrmD is highly unusual, because the great majority of AdoMet-dependent methyl transferases require no Mg<sup>2+</sup>, due to the facile cationic nature of the methyl sulfonium center of AdoMet. More importantly, we showed that TrmD uses Mg<sup>2+</sup> not to modulate substrate binding, but to activate the nucleophile for methyl transfer. Our new finding demonstrates how Mg<sup>2+</sup> contributes to the catalytic mechanism of AdoMet-dependent methyl transfer in one of the most crucial post-transcriptional modifications to tRNA.





## Cdk5rap1-mediated 2-methylthio modification of mitochondrial tRNAs controls precise mitochondrial protein translation and contributes to myopathy

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Transfer RNA (tRNA) contains a variety of posttranscriptional modifications in all species. Some of the modifications identified in bacteria have been inherited in mammalian mitochondria, but the physiological role of the modifications and modifying enzymes have remained largely unknown. Here, we report that four mitochondrial tRNAs (mt-tRNAs) contain 2-methylthio (ms<sup>2</sup>) modifications, which are catalyzed by Cdk5 regulatory subunit associated protein 1 (Cdk5rap1) in mammalian cells. The ms<sup>2</sup>-modified mt-tRNAs are essential for oxidative phosphorylation activity by regulating the accurate and efficient translation of mitochondrial proteins. Deficiency of ms<sup>2</sup>-modification markedly impairs mitochondrial quality and accelerates myopathy and heart failure under stress. Furthermore, we show that ms<sup>2</sup>-modifications of mt-tRNAs are highly sensitive to oxidative stress and associated with mitochondrial disease. These findings highlight the fundamental role of ms<sup>2</sup>-modifications of mt-tRNAs in the quality control of mitochondrial translation, as well as their importance in mitochondrial disease.

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## 020

## Wobble uridine hypomodification triggers protein misfolding by reducing decoding speed *in vivo*

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Chemical modifications of wobble uridine (U34) in tRNAs are found in all domains of life. They are dispensable for tRNA stability and aminoacylation in eukaryotes but are essential for cellular fitness. However, the molecular events linking U34 modification loss to cellular dysfunction remain unknown. Interestingly, phenotypes of yeast lacking U34 modifications are rescued by elevated levels of tK(UUU) and tQ(UUG), pointing to inefficient translation of the cognate codons as a phenotypic trigger. To test this hypothesis, we used ribosome profiling and compared ribosomal distributions along endogenous mRNAs in wild type and U34 modification-mutants of yeast and nematodes.

Importantly, in both organisms, U34 hypomodification led to increased A-site ribosome occupancy at certain codons, indicating slowed translation elongation. Surprisingly, while eleven tRNAs had unmodified U34, we detected slowdown only for tK(UUU) and tQ(UUG), implying that chemical groups at U34 in other tRNAs play little functional roles. Second, we examined ribosome occupancy under stress conditions that exacerbate the phenotypes of U34 modification-deficient yeast. Interestingly, A-site codon occupancies in mutant strains were similar under stress, suggesting that the stress sensitivity of those strains has another trigger. Indeed, we found that U34 hypomodification leads to disrupted protein homeostasis. Cells with hypomodified U34 accumulate protein aggregates, and the ability to induce and maintain high chaperone and proteasome levels is critical for cell survival. Importantly, overexpression of tK(UUU) and tQ(UUG) reduces aggregate formation and relieves proteotoxic stress, along with restoring codon translation speed.

Our findings establish a link between anticodon modification, codon translation rates and protein folding *in vivo*, suggesting that the phenotypes of U34 hypomodification mainly stem from the toxicity of misfolded proteins.



## Structure-function analysis of the Trm9-Trm112 complex, a methyltransferase involved in the mcm<sup>5</sup>U34 tRNA modification and in response to genotoxic stresses

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Trm112 is truly unique acting as an activating platform of four eukaryotic methyltransferases (MTases) involved in rRNA, tRNA and translation factor modifications, ideally placing it at the interface between ribosome synthesis and function.

The Mtq2-Trm112 complex methylates the translation termination factor eRF1 on the glutamine side chain of its universally conserved GGQ motif, which is directly involved in the release of newly synthesized proteins (1). The Bud23-Trm112 complex is implicated in ribosome biogenesis by methylating guanosine 1575 of 18S rRNA (2). Trm11-Trm112 forms 2-methylguanosine at position 10 on tRNAs, a modification assumed to stabilize tRNA structure. The Trm9-Trm112 complex participates in the modification of wobble uridine 34 of some tRNAs. It catalyses the methylation of the cm<sup>5</sup>U (5-carboxymethyl Uridine) into mcm<sup>5</sup>U (5-methoxycarbonylmethyl Uridine). This methylation enhances the decoding accuracy of specific codons highly represented in some key genes of DNA damage response. ALKBH8, the human orthologue of Trm9 is over-expressed in various types of cancer and its absence significantly suppresses invasion, angiogenesis, and growth of bladder cancers.

We will present the crystal structure as well as *in vitro* and *in vivo* functional studies of the Trm9-Trm112 complex. In addition, we will compare the known crystal structures of Trm112-MTase complexes, revealing the structural plasticity allowing Trm112 to interact with all its MTase partners, which share less than 20% sequence identity, through a very similar mode.

(1) Liger et al; 2011; *Nuc.AcidsRes*; 39(24); 6249-59

(2) Figaro et al; 2012; *Mol.Cell.Biol*; **32(12)**; 2254-67



## 022

## A Rossmann-fold superfamily enzyme, TmcAL, is a novel acetate ligase responsible for the formation of $N^4$ -acetylcytidine in tRNA<sup>Met</sup> in *Bacillus subtilis*

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$N^4$ -acetylcytidine (ac<sup>4</sup>C) is a widespread modified nucleoside found in tRNAs and rRNAs from all domains of life. In *Escherichia coli* and other bacteria, ac<sup>4</sup>C is present at the wobble position (34) of the elongator tRNA<sup>Met</sup>. ac<sup>4</sup>C34 is thought to prevent misreading of AUA codon. Our group previously reported that ac<sup>4</sup>C34 in *E. coli* tRNA<sup>Met</sup> is synthesized by TmcA which catalyzes the acetylation of tRNA using acetyl-CoA and ATP as substrates (Ikeuchi et al., 2008). TmcA is a GNAT family acetyltransferase conjugated with Walker-type ATPase domain. Although homologs of TmcA occur in many Eukarya and Archaea, bacterial TmcA appears to be limited to the  $\gamma$ -proteobacterial subphylum. In *Bacillus subtilis*, the wobble position of the elongator tRNA<sup>Met</sup> was reported to be unmodified. In addition, TmcA homolog is not encoded in *B. subtilis* genome. However, we clearly detected ac<sup>4</sup>C34 in the elongator tRNA<sup>Met</sup> isolated from *B. subtilis*, indicating the presence of novel enzyme responsible for ac<sup>4</sup>C34 in *B. subtilis*. Taking advantage of comparative genomics using *Mycoplasma* species, we narrowed down candidate genes responsible for the ac<sup>4</sup>C formation, and then succeeded in identifying a novel enzyme which belongs to Rossmann-fold superfamily with HIGH motif, in which class I aminoacyl-tRNA synthetases are also classified. Intriguingly, ac<sup>4</sup>C34 in tRNA<sup>Met</sup> was successfully reconstituted by the recombinant protein using acetate and ATP as substrates. Hence, we named this enzyme as tRNA<sup>Met</sup> cytidine acetate ligase (TmcAL) which catalyzes two consecutive reactions. TmcAL first synthesizes acetyladenylate (Ac-AMP) as a reaction intermediate, then the acetyl group of Ac-AMP is transferred to  $N^4$ -amino group of C34 in tRNA<sup>Met</sup> with releasing AMP. TmcAL is the first enzyme, which utilizes acetate as a substrate to modify RNA molecule, urging us to reconsider our common knowledge that acetyl-CoA is a sole substrate for acetylation. Based on this discovery, ac<sup>4</sup>C34 might be established by convergent evolution in bacterial system.



## Metazoan mitochondrial genome expression: the tRNA punctuation model revisited

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Reminiscent of its prokaryotic origin, mitochondria contain their own DNA (mtDNA). Metazoan mtDNA presents a highly compact gene organization and is transcribed as large polycistronic transcripts. The tRNA processing is then essential for the release and maturation of mt mRNAs and rRNAs, instituting the tRNA punctuation model. The existence of this punctuation model has become a dogma<sup>1</sup>.

With more than 2000 completely sequenced mt genomes, *in silico* observations suggesting that this punctuation model cannot always be applied are emerging. Here, using the isopod crustacean *Armadillidium vulgare* as a model organism, we now provide experimental evidence.

In this arthropod, only a partial set of tRNA genes was identified by computational means<sup>2,3</sup>. Interestingly, a constitutive heteroplasmic site forming a dual tRNA gene was found<sup>4</sup>. Based on these observations, we first reanalyzed the tRNA gene content and experimentally validated the tRNA candidates. Several essential mt tRNA genes are missing thus likely mt import of nucleus-encoded tRNAs compensate for this lack. Then we demonstrated that a tRNA expressed from a single mt gene is extensively repaired both at the 5' and 3' ends. Then, we showed that out of the 13 expressed tRNA genes, 9 overlap with other genes. Extreme cases were encountered such as a huge overlap between 2 tRNA genes transcribed in the same direction or a tRNA gene fully inserted into a protein-coding gene.

Altogether, the data obtained provide experimental evidence that contradicts the dogma describing tRNA processing as essential for the release and maturation of mt mRNAs and rRNAs and support the existence of new biochemical mechanisms to count for mtgene expression.

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024

## Asking the cells: genome-wide screen of yeast uncovers the mechanisms for tRNA intron turnover and targeting the SEN complex to mitochondria

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We conducted a systematic and unbiased screen of the yeast proteome for gene products involved in tRNA biology. We employed the yeast deletion and temperature-sensitive collections, that together provide mutant alleles of >90% of the annotated genes, to assess the impact of each gene upon tRNA biology. Genetic and biochemical analyses of some of the identified mutants have provided surprising insights for two steps in tRNA biology and cell biology. First, although tRNA splicing has been studied for years, the fate of released introns has been a mystery. Even though 600,000 such intron molecules are generated each generation, they are barely detectable, indicating a very efficient turnover mechanism. We discovered that deletion of *XRN1*, encoding the cytoplasmic 5' to 3' exonuclease, causes accumulation of free tRNA introns. Further studies revealed that tRNA turnover requires collaboration by the tRNA splicing ligase (healing by phosphorylation of the 5' terminus of the linear intron) and destruction by the cytoplasmic tRNA quality control 5' to 3' exonuclease, Xrn1, that has specificity for 5' phosphorylated RNAs. Second, previous studies by Yoshihisa *et al.* (2003) showed that pre-tRNA splicing catalyzed by the tRNA splicing endonuclease, SEN, in yeast occurs on the mitochondrial surface, but the mechanism to direct and assemble the SEN complex on mitochondria was unknown. We discovered that deletion of *TOM70*, encoding a mitochondrial outer membrane protein, causes accumulation of end-matured intron-containing tRNAs. The tRNA splicing defect occurs because Tom70 is required for proper localization, assembly, and function of the SEN complex subunits onto the mitochondrial surface. Numerous additional mutations that affect other aspects of tRNA biology were also identified. Thus, our genome-wide screen has led to discoveries of novel gene products that function in eukaryotic tRNA biology.

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## 025

**Multiple pathways for processing of tRNA primary transcripts in *Escherichia coli*****Sidney R. Kushner, Bijoy K. Mohanty and Ankit Agrawal***Department of Genetics and Microbiology, University of Georgia, Athens, GA, USA*

The *E. coli* genome contains 86 tRNA genes that are organized as either monocistronic transcripts or complex operons containing other tRNAs, messenger RNAs (mRNAs) or ribosomal RNAs (rRNA) genes. Every tRNA is transcribed as a precursor that contains an encode CCA determinant and requires subsequent processing at both ends to generate mature species that are charged by their cognate aminoacyl tRNA synthetases. In addition, tRNAs that are part of polycistronic transcripts require initial endonucleolytic cleavages to generate the pre-tRNAs that undergo further processing at their 5' and 3' termini. The generally accepted model for such processing proposes that endonucleolytic cleavages of polycistronic transcripts by RNase E generate pre-tRNAs. Subsequently, the ribozyme RNase P endonucleolytically removes the extra nucleotides at the 5' terminus, while the 3' terminus is processed exonucleolytically by a combination of RNase T, RNase PH, RNase BN/Z, RNase D, RNase II and PNPase. However, we have now shown that a significant portion of *E. coli* primary transcripts are initially processed by RNase P and not RNase E. Thus all seven leucine tRNAs (found in three polycistronic transcripts) are initially separated into pre-tRNAs exclusively by RNase P. Furthermore, RNase P processes the *valU* and *lysT* operons in the 3'→5' direction by first removing the Rho-independent transcription terminators. Furthermore, we demonstrate that polyadenylation of pre-tRNAs by poly(A) polymerase I exacerbates the conditional lethality associated with mutations in the protein subunit of RNase P, such that inactivation of PAP I leads to partially suppression of the lethality. Of even greater interest is our data demonstrating that short unprocessed 5' regions (1-5 nt) on pre-tRNAs do not interfere with aminoacylation if the 3' ends are fully processed. Finally, we will present data to show that the three proline tRNAs are processed in such a fashion that they do not require any 3'→5' processing by RNase T, RNase PH, RNase BN/Z, RNase D, RNase II or PNPase. This work was supported in part by research grants from the National Institutes of Health to S.R.K. (GM57220 and GM81554).



026

## Exploring the mode of action, diversity and evolution of protein-only RNase P

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The dogma of the universal conservation of RNase P as a ribonucleoprotein (RNP) has been challenged with the discovery of protein-only RNase P enzymes called PRORP (Holzmann, 2008; Gobert, 2010). We have shown that these enzymes are responsible for RNase P activity in both organelles and the nucleus in plants (Gutmann, 2012). Although plant PRORP act as single subunit enzymes, we provide evidence to show their interaction with a number of factors *in vivo*, thus showing the integration of PRORP functions among other gene expression processes.

Beyond plants, it becomes evident that PRORP proteins are prominent in many distantly related eukaryote lineages. We thus explore the diversity of PRORP function and mode of action in a number of representative eukaryote model species.

Finally, mechanistic data show that PRORP proteins have evolved a mode of tRNA recognition reminiscent from the one used by RNP RNase P (Gobert, 2013).

The diversity of PRORP and RNP enzymes is compared and should give clues to understand the evolutive history of RNase P.

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## The crystal structure of human selenocysteine tRNA-specific elongation factor, eEFSec

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Faithful translation of mRNA is critical for the structure and function of proteins and for the maintenance of vital cellular functions. Elaborate machinery composed of the aminoacyl-tRNA synthetases, ribosome, tRNAs, translational protein factors, and auxiliary *in-cis* and *in-trans* factors is responsible for gene translation in all organisms. Because of its importance for protein synthesis, the role of translational elongation factors has been a subject of intense scientific scrutiny during the last two decades. In a canonical pathway, the general elongation factor EF-Tu/EF1A delivers aminoacyl-tRNAs to the ribosome, and EF-G/EF2 promotes the ratcheting of the small ribosomal subunit and translocation of the deacylated tRNAs and mRNA. While the mechanism of the prokaryotic translation factors has largely been elucidated at atomic resolution, the corresponding information about the eukaryotic system, in general, and the human system, in particular, is scarce if not completely absent. Perhaps the least understood is the mechanism governing decoding and co-translational insertion of the 21<sup>st</sup> amino acid, selenocysteine. This fundamental process, which is distinct from the canonical pathway facilitating insertion of the 20 standard amino acids into nascent polypeptides, instructs synthesis of 25 human selenoproteins and selenoenzymes. The process is dependent on a specialized elongation factor eEFSec (SelB in prokaryotes), which evolved to bind only selenocysteinyl-tRNA<sup>Sec</sup> and to promote decoding of the selenocysteine UGA codon. However, the mechanism at the structural level by which eEFSec exerts its function(s) is not well understood. Herein, we present the crystal structure of human eEFSec, which provides a platform for explaining unique functional properties of this specialized elongation factor and represents, to our knowledge, the first structure of a component of the human translational machinery.

### Acknowledgements

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028

## Elaborate uORF/IRES features control expression and localization of human moonlighting glycyl-tRNA synthetase

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Human Glycyl-tRNA synthetase (GRS) is a housekeeping enzyme with a key role in protein synthesis, responsible for the charging of glycine on its cognate tRNAs. In metazoans, there is a unique gene, GARS, which encodes both cytoplasmic and mitochondrial forms of GRS by using alternative start codons. These enzymes are thus responsible for maintaining cytosolic and mitochondrial translations. However, outside translation GRS was also shown to participate in many other functions, amongst which its involvement in peripheral axonal degeneration (Charcot-Marie Tooth disease) is still not understood. We identified two mRNA isoforms both capable of sustaining translation of cytosolic and mitochondrial GRSs. Using immunolocalization assays, *in vitro* translation assays and bicistronic constructs, we provided experimental evidences that expression and localization of human GRS are tightly controlled. An intricate regulatory domain was found in the 5' UTR of one of the mRNA isoforms only and displays a functional Internal Ribosome Entry Site and a short Upstream Open Reading Frame. Together, both elements hinder the synthesis of the mitochondrial GRS and target the translation of the cytosolic enzyme to ER-bound ribosomes. We uncovered an original post-transcriptional regulatory mechanism that is conserved in mammals. This finding sheds light on the regulation of mRNA directed trafficking to the ER and reveals a complex picture of GRS translation and localization. In this context, we discuss how GRS expression could influence moonlighting activities of human GRS and its involvement in diseases.



## Editing and methylation at a single tRNA site by functionally interdependent activities: keeping a mutagenic enzyme in check

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In all cells tRNAs undergo extensive post-transcriptional modifications, which collectively play important roles in tRNA structure and function. RNA editing, a specialized form of modification, has a wide phylogenetic distribution and in tRNAs may either expand their decoding capacity or generate conserved structural features. We showed that tRNA<sup>Thr</sup><sub>AGU</sub> undergoes both adenosine to inosine (A to I) and cytosine to uridine (C to U) editing in the anticodon loop, while robust A to I editing was reconstituted in vitro, C to U editing activity was not. Yet in vivo the TbADAT2/3 deaminase was essential for both editing events. Adding to the puzzle was the finding that the C to U edited position is also methylated leading to formation of 3-methylcytosine (m<sup>3</sup>C) and 3-methyluridine (m<sup>3</sup>U), respectively. This finding raised the possibility that editing and methylation are interconnected events. Here we present the identification of tRNA methylase for position 32 in tRNA<sup>Thr</sup>. We show that reconstitution of methylase activity requires the presence of the TbADAT2/3 deaminase. These findings are discussed in the context of the intracellular localization of both enzymes and how the potentially mutagenic deaminase is kept in check while it traffics to the nucleus of *T. brucei*.

**O30**

## Implications of tRNA synthetase network in cancer microenvironment

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Aminoacyl-tRNA synthetases (ARSs) are pleotropic proteins that are not only involved in diverse signal pathways as signal mediators, but also in protein synthesis as a catalysis. Although most of ARSs can perform their catalytic roles by themselves, they form dynamic functional network through protein-protein interactions with each other and with other cellular factors. Some ARSs are even secreted out of the cells to mediate diverse cell-cell and cell-extracellular matrix communications. Their functional flexibility appears to be achieved by post-translational modifications, extra-domain acquisition, and alternative splicing. The functional significance of their network are also pathologically implicated in various human diseases such as cancer, neurodegenerative and immune-related diseases (1). For instance, lysyl-tRNA synthetase plays a critical roles in cancer microenvironment as signal mediator in plasma membrane of cancer cells (2) and also as secreted inflammatory signaling factor (3). On the contrary, glycyl-tRNA synthetase is secreted from macrophages upon challenges by cancer cells to suppress specific cancer cells (4). All of these activities involve specific post-translational modifications or proteolytic cleavage of the corresponding ARSs.

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## Nuclear function of TyrRS under oxidative stress

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The demand of global protein synthesis decreases under stress conditions, as the translation machinery is diverted to preferentially promote synthesis of stress-response gene. Here we report that oxidative stress stimulates tyrosyl-tRNA synthetase to translocate into the nucleus, where the tRNA synthetase performs dual functions through transcriptional regulation. Nuclear TyrRS upregulates the expression of DNA damage repair genes for protection against DNA damage, while simultaneously it downregulates the expression of protein synthesis genes to suppress global translation. The mechanism and the interaction network behind how TyrRS achieves this dual regulation will be presented.

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**032**

## Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration

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In higher eukaryotes, tRNAs with the same anticodon are encoded by multiple nuclear genes and little is known how mutations in these genes affect translation and cellular homeostasis. Similarly, the surveillance systems that respond to such defects in higher eukaryotes are not clear. Here, we discover that a loss-of-function mouse mutation in a member of the nuclear-encoded tRNA<sup>Arg</sup><sub>UCU</sub> family that is expressed specifically in the central nervous system (CNS), leads to low but detectable levels of ribosome stalling. In the absence of a newly identified binding partner of the ribosome recycling protein Pelota, ribosome stalling increases, leading to widespread neurodegeneration. Our results not only define a novel ribosome rescue factor, but also unmask the disease potential of mutations in nuclear-encoded tRNA genes.



## Angiogenin-mediated tRNA cleavage in neurodegeneration

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Angiogenin (ANG) is a stress-activated ribonuclease that promotes the survival of motor neurons. Ribonuclease inactivating point mutations are found in a subset of patients with amyotrophic lateral sclerosis, a fatal neurodegenerative disease with no cure. We recently showed that ANG cleaves tRNA within anticodon loops to produce 5'- and 3'-fragments known as tiRNAs (tRNA-derived, stress-induced RNAs). Selected 5'-tiRNAs (e.g., tiRNA<sup>Ala</sup> and tiRNA<sup>Cys</sup>) co-operate with the translational repressor YB-1 to displace eIF4F from m<sup>7</sup>G-capped mRNA, inhibit translation initiation and induce the assembly of stress granules (SGs). Here we show that translationally active tiRNAs assemble unique G-quadruplex structures that are required for translation inhibition. We show that tiRNA<sup>Ala</sup> binds the cold shock domain of YB-1 to activate these translational reprogramming events. We discovered that 5'-tiDNA<sup>Ala</sup> (the DNA equivalent of 5'-tiRNA<sup>Ala</sup>) is a stable tiRNA analogue that displaces eIF4F from capped mRNA, inhibits translation initiation, and induces the assembly of SGs. 5'-tiDNA<sup>Ala</sup> also assembles a G-quadruplex structure that allows it to spontaneously enter primary motor neurons and trigger a neuroprotective response in a YB-1-dependent manner. Our results explain why the ribonuclease activity of ANG is required for its cytoprotective properties and introduce 5'-tiDNA<sup>Ala</sup> as a lead compound for the development of a new class of neuroprotective drugs.

**O34**

## Structural basis for multiplexed inhibition of translational and nontranslational functions on a human tRNA synthetase

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The polyketide natural product borrelidin (BN) has multiple biological activities including antibacterial, antifungal, antimalarial, insecticidal and herbicidal, and recently anti-VEGF-induced angiogenesis activities, through selective inhibition of threonyl-tRNA synthetase (ThrRS). How BN simultaneously attenuates angiogenesis and suppresses a variety of infections in plants and animals is not known. Using x-ray crystal structure and functional analysis, binding of a single molecule of BN is shown here to simultaneously block four distinct sites on ThrRS. These include three used for redundant inhibition of its aminoacylation function in protein synthesis, and a fourth 'orthogonal' site that suppresses angiogenesis in vascularized metastasis. The results highlight a remarkably natural design to intervene across diverse diseases via a diverged family of orthologous enzyme targets. The commodious active site cavities for aminoacylation in other tRNA synthetases may also provide previously unappreciated opportunities for identifying powerful agent targeting translational and nontranslational activities of AARSs with therapeutic benefits.





## The yeast AME multisynthetase complex controls mitochondrial ATP production by coordinating expression and assembly of the F<sub>1</sub>F<sub>0</sub> ATP synthase

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In eukaryotic cells, oxidative phosphorylation involves multisubunit respiratory complexes (RC) of mixed genetic origin. Assembling these complexes requires an organelle-independent synchronizing system for the proper expression of nuclear and mitochondrial genes (1). Here we show that expression of the mitochondrial F<sub>1</sub>F<sub>0</sub> ATP synthase (complex V) depends on the cytosolic multisynthetase AME complex made of two aminoacyl-tRNA synthetases (aaRSs): glutamyl- (cERS) and methionyl-tRNA synthetase (cMRS) attached to the assembly protein Arc1 (2). When yeast cells switch from fermentation to respiration the Snf1/4 glucose-sensing pathway (3) inhibits expression of Arc1 thereby triggering disassembly of the AME complex and simultaneous release of both aaRSs. The free cMRS and cERS then relocate to the nucleus and mitochondria respectively. To decipher their organellar roles and verify whether the synchronicity of cERS and cMRS relocation is crucial for the adaptation to the respiratory metabolism, we engineered strains unable to release synchronously the two aaRSs. Four strains were constructed: the ARC strain that constitutively expresses Arc1, the nE and nM strains able to only release cERS and cMRS respectively and the nE/nM strain in which both aaRSs are constitutively released. Desynchronizing release of cERS and cMRS from Arc1 resulted in aberrant assembly of ATP synthase. In depth analysis of these strains reveals that nuclear cMRS regulates transcription of the Atp1 subunit of the F<sub>1</sub> catalytic head of ATP synthase, while mitochondrial cERS regulates translation of the critical Atp9 subunit of the F<sub>0</sub> rotor. By doing so, synchronously relocated cERS and cMRS enable coordinated assembly of the F<sub>1</sub> catalytic subunit of ATP synthase with the F<sub>0</sub> proton channel (4). Finally, we show that defects in Atp9 subunit expression in the desynchronized nM strain, can be suppressed by ectopic expression of *ATP1* suggesting that the F<sub>1</sub> domain of ATP synthase somehow regulates expression of the mitochondrial-encoded F<sub>0</sub> proton channel.

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## 036

## Glutamyl-prolyl tRNA synthetase and metabolism: New functions for an ancient enzyme

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Glutamyl-prolyl tRNA synthetase (EPRS) is the unique component of the GAIT (Interferon-Gamma Activated Inhibitor of Translation) complex that binds GAIT elements in the 3'UTR of inflammation-related mRNAs and inhibits their translation. Induced phosphorylation at Ser<sup>886</sup> and Ser<sup>999</sup> in the non-catalytic linker domain of human EPRS orchestrates its release from the parental multi-aminoacyl tRNA synthetase complex, assembly of the GAIT complex, mRNA binding, and translational silencing activity. Cyclin-dependent kinase 5 (Cdk5), in conjunction with regulatory protein Cdk5R1 (p35), induces the initial phosphorylation at Ser<sup>886</sup> of EPRS. We show that mammalian target of rapamycin complex (mTORC)1, in coordination with Cdk5, phosphorylates ribosomal protein S6 kinase-1 (S6K1), which in turn phosphorylates Ser<sup>999</sup> to generate translation silencing-competent EPRS, and establishing EPRS as a novel mTORC1-S6K1 axis target. Remarkably, S6K1 exhibits an unprecedented “kinase phospho-code” that determines target selectivity. mTORC1-dependent S6K1 phosphorylation induces phosphorylation of RPS6, eIF4B, and eEF2K, all components of the protein synthetic apparatus that stimulate translation globally. In contrast, dual S6K1 phosphorylation by mTORC1 and Cdk5 directs specific phosphorylation of EPRS that selectively inhibits translation of an ensemble of inflammation-related genes. To investigate the role of EPRS phosphorylation *in vivo*, we have generated a loss-of-function mouse strain with knock-in of Ser<sup>999</sup>-to-Ala in the EPRS gene. Adult homozygous mutant mice exhibit markedly reduced body weight and adipose tissue mass, approximately phenocopying S6K1-null and adipocyte-specific mTORC1-null mice, implicating EPRS as a critical mTORC1-S6K1 effector regulating metabolism and growth. In view of previous findings that LeuRS is an upstream nutrient sensor that regulates mTORC1 activation, and our new results establishing EPRS as a critical downstream mTORC1 effector, we propose these synthetases act as macromolecular “bookends” encasing this critical metabolic pathway.

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## Role of an alternative threonyl-tRNA synthetase in the adaptation to zinc deficiency in prokaryotes

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Threonyl-tRNA synthetases (ThrRS) are dimeric class II enzymes containing a zinc atom in the active site which directly interacts with the amino acid substrate. Some prokaryotic organisms contain duplicated *thrS* genes. The cyanobacterium *Anabaena* sp. PCC 7120 contains a housekeeping gene, *thrS1*, and an alternative one, *thrS2*, inducible under low zinc conditions and required for survival when zinc is limiting. Despite their high sequence similarity and their comparable affinity for zinc, ThrRS1 and ThrRS2 show a striking different behavior under low zinc *in vitro*, i.e. ThrRS1 dissociates and becomes inactive, whereas ThrRS2 also loses activity but remains stable as a dimer. Substitution of the three residues coordinating zinc in ThrRS1 rendered a mutant protein unable to dimerize, indicating that the zinc cofactor is required to maintain the dimeric structure of ThrRS1. In an effort to elucidate the structural basis for the dissociation of ThrRS1, we have deleted two short insertions of 3 and 6 amino acids in its catalytic domain, generating a stably dimeric (and active) ThrRS1 protein resistant to dissociation under low zinc. As zinc is not located in the dimerization surface, it seems plausible that dissociation of ThrRS1 occurs by the transmission at-a-distance of a conformational rearrangement provoked by cofactor loss. It is thus possible that the two insertions are involved in the propagation of the structural change from the zinc-binding pocket to the dimerization surface. These results provide a link between the structural and functional differences observed for ThrRS1 and ThrRS2. We hypothesize that the higher robustness and stability of ThrRS2 in low zinc are probably key evolutionary adaptations of this enzyme to zinc limitation.

### Acknowledgements

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038

## EPRS inhibition signals to a novel matrix remodeling pathway in multiple cell types

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The Whitman Lab recently identified glutamyl-prolyl-tRNA synthetase (EPRS) to be the molecular target of febrifugine and halofuginone (HF), and showed that EPRS-inhibition underlies the broad therapeutic activities of these natural products. Febrifugine is the bioactive constituent of Chang Shan, one of the 50 fundamental herbs of traditional Chinese medicine. Febrifugine and its derivative HF have been used to treat malaria, cancer, fibrosis and inflammatory disease, but the cellular target and mechanism of action have proved elusive. We previously showed that HF inhibits the differentiation of pro-inflammatory Th17 cells in vitro and in vivo, in part through activation of the cellular amino acid stress response (AAR) pathway. We now have found that HF-inhibition of differentiation of Th17 cells and HF-inhibition of inflammatory cytokine-driven extracellular matrix remodeling program in stromal cells can occur independent of GCN2, the kinase that activates the canonical AAR. Inhibition of EPRS by HF, therefore, leads to activation of the canonical AAR, and activation of a novel cellular pathway that dampens tissue response to inflammatory cytokines. We propose that HF inhibits a broad set of cytokine responses, in multiple tissue types, via a non-canonical pathway downstream of EPRS inhibition.



## Aminoacyl-tRNA synthetase complexes in evolution

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Aminoacyl-tRNA synthetases are interpreting the genetic code and are among the most ancient protein families. Along evolution, these proteins acquired new domains that are involved in protein:protein interactions, and in the assembly of supramolecular assemblies. In human, two types of stable complexes containing one or several aaRSs have been described. The VEGA (ValRS-EF1A-GEF-Assembly) associates valyl-tRNA synthetase (ValRS) with the four subunits of elongation factor 1 (EF1A, and the guanine nucleotide exchange factors EF1Ba, EF1Bb and EF1Bg). The N-terminal appended domain of eukaryote ValRS is required for its interaction with the EF1Bb subunit of the complex. The MARS (Multi-Aminoacyl-tRNA Synthetase Complex) contains the nine aminoacyl-tRNA synthetases ArgRS, AspRS, GlnRS, GluRS, IleRS, LeuRS, LysRS, MetRS, ProRS and the three nonsynthetase components p18, p38 and p43. Polypeptide extensions appended to eukaryotic enzymes are landmarks of their assembly within the MARS.

Analysis of the sequences of AARS throughout evolution of eukaryotes suggests that the VEGA only occurs in the deuterostome branch of bilaterians, as opposed to the MARS that also exists in the protostome branch. However, analysis of the domain organization of eukaryote aaRSs is not always sufficient to unambiguously establish whether a particular enzyme will be a member of the MARS or could be referred to as a 'free' enzyme.



## O40

**Class I aaRS quality control mechanisms preserve canonical translation in *Escherichia coli***

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Norvaline, a side product of the leucine biosynthetic pathway, accumulates in *Escherichia coli* under downshift of oxygen to the concentration capable to jeopardize accuracy of Ile-tRNA<sup>Ile</sup> and Leu-tRNA<sup>Leu</sup> synthesis. Incorporation of this non-canonical amino acid into the cell proteome is however prevented by rapid post-transfer editing within the IleRS and LeuRS CP1-editing domains. IleRS, unlike LeuRS, proofreads norvaline also at the level of tRNA-dependent pre-transfer editing. Intriguingly, non-proteinogenic norvaline, not isoleucine, sets up the requirement for LeuRS editing. This conclusion follows from a reassessment of the LeuRS discriminatory power using isoleucine that was additionally purified from traces of cognate leucine. Indeed, opposite to the prevailing opinion, we demonstrate that LeuRS discriminates against isoleucine with specificity of better than 10<sup>4</sup>-fold, and thus obviates need for post-transfer editing of Ile-tRNA<sup>Leu</sup>. Kinetic, structural and thermodynamic approaches establish that both very weak ground state binding and the decreased rate of the chemical step contribute to isoleucine specificity. Both features clearly distinguish isoleucine from norvaline which exhibits only 10<sup>2</sup>-fold specificity in the LeuRS synthetic reaction. The growth of an *E. coli* strain deprived of LeuRS post-transfer editing displays accordingly a high intolerance towards a surplus of norvaline, but not isoleucine, in the media. Likewise, editing-dependent cell viability correlates well with micro-aerobic but not normal growth conditions. It thus appears that class I aaRS editing plays an important role in the *E. coli* adaptive response to quickly changing oxygen environments by preserving canonical translation under these natural error-prone conditions.



## 041

**Homologous *trans*-editing factors with broad substrate specificity prevent global mistranslation****Karin Musier-Forsyth***Ohio State University*

Aminoacyl-tRNA synthetases (ARSs) establish the rules of the genetic code, whereby each amino acid (aa) is attached to a cognate tRNA. Errors in this process lead to mistranslation, which can be toxic to bacteria and mammalian cells. Recent studies suggest that the selective forces exerted by cell-specific requirements and environmental conditions potentially shape quality control mechanisms. Approximately half of the ARSs possess a proofreading function to hydrolyze mischarged aa-tRNAs. Interestingly, single-domain proteins homologous to ARS editing domains are encoded in many genomes but the physiological function of these putative *trans*-editing proteins is still largely unknown. Infiltration of the genetic code by non-protein aa's is likely to pose an even greater threat to cell viability than misincorporation of protein aa's into the proteome. While proofreading of genetically-encoded aa's by ARSs is well documented, much less is known about how the translation quality control machinery prevents misincorporation of non-protein aa's. A family of *trans*-editing factors collectively known as the "INS superfamily" is encoded in organisms from bacteria to humans. This family includes the *cis*-editing domain (INS) of bacterial ProRS, which clears Ala-tRNA<sup>Pro</sup>, the *trans*-editing factor YbaK, which clears Cys-tRNA<sup>Pro</sup>, and 4 additional ProXp's. The distinct substrate specificities of these *trans*-editing domains, which extend beyond aa-tRNA<sup>Pro</sup> and include non-protein aa's, will be discussed.



042

## Universal pathway for post-transfer editing reactions: Insights from the crystal structure of *TtPheRS* with puromycin

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At the amino acid binding and recognition step, Phenylalanyl-tRNA synthetase (PheRS) faces the challenge of discrimination between cognate phenylalanine and closely similar non-cognate tyrosine (1). Resampling of Tyr-tRNA<sup>Phe</sup> to PheRS increasing the number of correctly charged tRNA molecules have recently been revealed (2). Thus, the very same editing site of PheRS promotes hydrolysis of sterically different 2' and 3'-OH misacylated tRNA species, associated with cis- and trans-editing pathways. Here we present the crystal structure of the *T. thermophilus* PheRS (*TtPheRS*) at 2.6Å resolution, in complex with phenylalanine and aminonucleoside antibiotic puromycin mimicking the A76 of tRNA acylated with Tyr. Starting from the complex structure and using hybrid quantum mechanics/molecular mechanics (QM/MM) approach we investigate the pathways of editing mechanism catalyzed by *TtPheRS*. We showed that both 2' and 3' isomeric esters undergo mutual transformation via the cyclic intermediate orthoester (3) and the hydrolytic cleavage of the ester bond proceeds without regard to whether the 2' or the 3' isomer is approaching the editing site. The suggested pathway of hydrolytic reaction at the editing site of PheRS is of sufficient generality to warrant comparison with those of other class I and class II aaRSs.

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## Functional evolution of bacterial GluRS facilitated by isoacceptor plasticity of tRNA<sup>Gln</sup>

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Glutamyl tRNA-synthetase (GluRS) is non-discriminatory in most bacteria, capable of glutamylating both tRNA<sup>Glu</sup> and tRNA<sup>Gln</sup>. During the course of bacterial evolution, acquisition of glutamyl tRNA-synthetase (GlnRS) by horizontal gene transfer from eukaryotes, or the appearance of an additional GluRS (GluRS2), triggered the parent non-discriminatory GluRS to evolve into a discriminatory (against tRNA<sup>Gln</sup>) GluRS in some bacteria. For example, majority of proteobacteria, thought to have diverged more recently than most other bacteria, either contain a single copy of discriminatory GluRS or contain two copies of GluRS (GluRS1 and GluRS2), GluRS1 being discriminatory. Mere acquisition of GlnRS or GluRS2 in a bacterium is not sufficient for a non-discriminatory GluRS to evolve into a discriminatory GluRS. The newly acquired GlnRS (or GluRS2) and the parent non-discriminatory GluRS need to reoptimize their interactions with tRNA<sup>Gln</sup> in a concurrent fashion. The double optimization can be fatal for bacteria that contain only one tRNA<sup>Gln</sup> isoacceptor. From an analysis of whole genome bacteria sequences we show that the nature and number of extant tRNA<sup>Gln</sup> isoacceptors and the tRNA<sup>Gln</sup>-specificity of GluRS are strongly coupled in proteobacteria. The result not only highlights an undocumented aspect of GluRS evolution in bacteria, it also establishes an important role that tRNA isoacceptor plasticity plays in functional evolution of proteins, emphasizing that genome plasticity is an important requirement for robustness of functional proteins. This has important implications in design of synthetic life using a minimalist approach or incorporation of non-coded amino acids using redundant codons.



044

## Key immunity determinants in agrobacterial LeuRS prevent self-poisoning of plant tumor biocontrol

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*Agrobacterium radiobacter* strain K84 is a bacterial biocontrol that prevents the spread of tumors in a wide variety of commercially important plant species. The biocontrol secretes a pathogen-specific Trojan Horse antibiotic, agrocin 84, which targets the causative agent of the tumors pathogenic strains of *Agrobacterium tumefaciens*. After selective uptake by *A. tumefaciens*, agrocin 84 is processed into the toxin TM84 that closely resembles a stable form of leucyl-adenylate and potentially inhibits leucyl-tRNA synthetase (LeuRS). We recently showed that TM84 does indeed bind to the catalytic domain of LeuRS but uses a novel tRNA-dependent inhibition mechanism (1). In order to provide self-immunity to the antibiotic, *A. radiobacter* K84 expresses a second functional LeuRS, AgnB2, which is resistant to TM84. The mechanism by which AgnB2 LeuRS is resistant to TM84, yet is still capable of catalyzing the aminoacylation of tRNA<sup>Leu</sup>, is currently unknown. Here we identify key immunity determinants within AgnB2 whose cumulative action is responsible for imparting a substantial degree of TM84 resistance to the enzyme while still allowing AgnB2 to catalyze the aminoacylation reaction. We use a combination of steady state enzyme kinetics, thermodynamic and structural approaches to analyze wild-type and mutant forms of AgnB2 LeuRS to reveal the role these determinants play in destabilizing the TM84 ternary inhibition complex. Our results also show that it is possible to manipulate some of the corresponding determinants in the LeuRS from *A. tumefaciens* to convert a TM84 sensitive enzyme into a resistant form.

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## A bioengineered macrolide inhibitor that separates the translational and angiogenesis functions of threonyl-tRNA synthetase

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In addition to its canonical role in aminoacylation, threonyl-tRNA synthetase (TARS) possesses pro-angiogenic activity susceptible to the TARS-specific antibiotic borrelidin (BN). However, the therapeutic benefit of BN is offset by its strong toxicity to living cells. The removal of a single methylene group from the parent BN generates BC194, a modified compound with significantly reduced toxicity but comparable anti-angiogenic potential. Biochemical analyses reveal that the difference in toxicities is due to BN's stimulation of amino acid starvation at ten-fold lower concentrations than BC194. However, both compounds were found to inhibit *in vitro* and *in vivo* models of angiogenesis at sub-toxic concentrations, suggesting a similar mechanism that is distinct from the toxic responses. Crystal structures of TARS in complex with each compound indicate that the decreased contacts in the BC194 structure may render it more susceptible to competition with the canonical substrates and permit sufficient aminoacylation activity over a wider concentration of inhibitor. Conversely, both BN and BC194 induce identical conformational changes in TARS, providing a rationale for their comparable effects on angiogenesis. The mechanisms of TARS and BN-based compounds on angiogenesis were subsequently tested using zebrafish and cell-based models. These data revealed ectopic branching, non-functional vessels, and increased cell-cell contracts following BC194-treatment suggesting a role for TARS in the maturation and guidance of nascent vasculature. *In situ* hybridization and RT-qPCR data also demonstrate changes in VEGF and TARS expression in response to TARS inhibition, possibly indicating a receptor-mediated response for TARS signaling. However, the identity of this receptor and the exact mechanism of BN's anti-angiogenic effects remain to be determined.



## 046

## Interaction of lysyl-tRNA synthetase and laminin receptor in the control of cell migration and cancer metastasis

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Lysyl-tRNA synthetase (KRS) is the ARS ligating the lysine to tRNA<sup>Lys</sup>. Although human lysyl-tRNA synthetase (KRS), an enzyme for protein synthesis, is often highly expressed in various cancer cells, its pathophysiological implications have not been understood. KRS enhances the cell migration in membrane through the binding with 67kDa laminin receptor (67LR), dimerized form of p40/37LRP which is one of the ribosomal components. 67LR was well reported as a critical factor in cell migration and cancer metastasis. Here we present the structural study and the binding analyses of KRS with the selected inhibitors of KRS-laminin receptor interaction. Using NMR-based study, we identified that the anticodon-binding domain of KRS binds directly to the C-terminal region of 37LRP, and the previously found inhibitors BC-K-01 and BC-K-YH16899 interfere the KRS-37LRP binding. Not only laminin receptor, KRS has affinity to the laminin molecules suggesting the interaction of KRS-laminin receptor is stabilized in extracellular matrix. Restrained docking study with NMR-derived experimental data elucidates the binding mode of BC-K1 and BC-K-YH16899 into the pocket of KRS anticodon binding domain. These results suggest a mechanism of action to prevent cancer metastasis by modulating the interaction of KRS with MSC and laminin receptor in cellular membrane.

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## Oxidation of cellular amino acid pools leads to cytotoxic mistranslation of the genetic code

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Aminoacyl-tRNA synthetases use a variety of mechanisms to ensure fidelity of the genetic code and ultimately select the correct amino acids to be used in protein synthesis. The physiological necessity of these quality control mechanisms in different environments remains unclear, as the cost versus benefit of accurate protein synthesis is difficult to predict. We show that in *Escherichia coli*, a non-coded amino acid produced through oxidative damage is a significant threat to the accuracy of protein synthesis and must be cleared by phenylalanine-tRNA synthetase in order to prevent cellular toxicity caused by mis-synthesized proteins. These findings demonstrate how stress can lead to the accumulation of non-canonical amino acids that must be excluded from the proteome in order to maintain cellular viability.

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## Quality control mechanisms of leucyl-tRNA synthetases

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Leucyl-tRNA synthetases activate diverse sets of standard and non-standard amino acids.<sup>1</sup> CP1 domain-specific mutants induce mischarging of tRNA<sup>Leu</sup>.<sup>2</sup> These mischarged tRNAs can be cleared by the CP1 domain hydrolytic editing site to clear mistakes and guard against statistical mutations in the proteome that might be detrimental or even toxic to the organism. A new class of antimicrobial benzoxaborole compounds is a potent inhibitor of leucyl-tRNA synthetase (LeuRS) and relies on a mechanism of action that involves cross-linking tRNA<sup>Leu</sup> in the editing site.<sup>3</sup> Resistance mutations to the oxaborole include CP1-based active site mutations, but also some that are distal to the editing site. A combination of X-ray crystallography, molecular dynamics, biochemical experiments, and mutational analysis of one of these mutations uncovered a eukaryote-specific tyrosine “switch” with three states that are critical to tRNA-dependent post-transfer editing. The oxaborole’s mechanism of action capitalizes upon one of these editing active site states. Evolution of this tunable editing mechanism in eukaryotic and archaea LeuRSs is proposed to enable precise control of aminoacylation fidelity.

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## The importance of editing by mitochondrial alanyl-tRNA synthetase for mitochondrial protein quality control in mammals

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Mischarged tRNAs leading to intracellular accumulation of misfolded proteins can underlie pathologies such as neurodegeneration. The editing functions of aminoacyl-tRNA synthetases play an important role in maintaining translational fidelity by removing mischarged tRNAs. Consequently it has been shown in mice that editing-defective cytoplasmic alanyl-tRNA synthetase causes ataxia and cerebellar Purkinje cell degeneration because of protein misfolding in neurons (1).

Mitochondria are essential organelles with their own protein synthesis machinery. Protein quality control is important for mitochondrial function but the role of editing by aminoacyl-tRNA synthetases has not been clearly demonstrated in mammalian mitochondria. In fact, most of the nuclear-encoded mitochondrial aminoacyl-tRNA synthetases lack editing activity. Thus it has been suggested that mitochondria mainly operate protein quality control at a post-synthesis level to maintain the correct assembly of the oxidative phosphorylation complexes (2). The mitochondrial alanyl-tRNA synthetase (mtAlaRS, encoded by AARS2) is an exception, with a conserved editing domain. We have previously identified a pathogenic mutation in human AARS2 that affects an amino acid in the mtAlaRS editing domain and leads to an early-onset cardiomyopathy (3).

As an effort to create a mouse model for mitochondrial mistranslation, we have generated proofreading-deficient Aars2 knock-in mice. The heterozygous mice are viable and show no overt phenotype. The homozygous knock-in mice will clarify the importance of mischarged tRNA editing in mammalian mitochondria, and may enable studies addressing the consequences and responses of mistranslated mitochondrial proteins in different tissues.

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O50

## Structural basis for neurological disorders caused by mutations in human cytosolic GlnRS

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Aminoacyl-tRNA synthetases (aaRS), ubiquitously expressed enzymes, are essential for protein synthesis in all organisms. Each aaRS couples a specific amino acid to the cognate tRNA and thus ensures faithful translation of the genetic information. The importance of these enzymes for the integrity of the cellular proteome is further emphasized by the fact that the overall error rate of translation corresponds to the error rate displayed by aaRSs. It is, therefore, generally accepted that mutations affecting aaRS catalytic activities (i.e. aminoacylation and editing) would have detrimental effects on vital cellular functions. A number of biochemical and genetic studies have demonstrated that mutations in AlaRS, GlyRS, LysRS, and TyrRS are causatively linked to heritable peripheral neuropathy, Charcot-Marie-Tooth (reviewed in [1]), and that mutations in SepSecS, the terminal synthetic enzyme of the selenocysteine pathway, cause severe neurodegenerative disorders (reviewed in [2]). Most recently, two sets of compound heterozygous mutations in a gene encoding human cytosolic GlnRS (*QARS*) have been reported to cause progressive microcephaly, cerebral-cerebellar atrophy, and intractable seizures [3]. In one instance, the siblings harbored G54V and R403W mutations, whereas in the other, a patient carried Y57H and R515W mutations on different *QARS* alleles. To understand how mutations in GlnRS affect the enzyme's structure and function, and the downstream cellular processes, we have determined the crystal structure of the intact wild-type human cytosolic GlnRS. Also, we solved structures G54V and Y57H point mutants of GlnRS. The crystal structures reveal the fold of the N-terminal domain, which is involved in binding to tRNA and multi-synthetase complex. Further, using the three-dimensional structure, we speculate how each of the mutations may affect the GlnRS function.

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## Seryl-tRNA synthetase counteracts c-Myc to develop functional vasculature

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Seryl-tRNA synthetase (SerRS) plays an essential role in vascular development<sup>1-3</sup>. This role is independent of the aminoacylation function of SerRS. We have previously reported that a unique nucleus-directing domain, added at the invertebrate-to-vertebrate transition, confers a non-translational activity of SerRS in modulating VEGF expression<sup>4</sup>. Using in vitro, cell and animal experiments, we further discovered that SerRS controls VEGF expression by counteracting the activity of c-Myc and that vertebrate SerRS and c-Myc is a pair of 'Yin-Yang' transcriptional regulator for proper development of a functional vasculature<sup>5</sup>. Because c-Myc is a major transcription factor promoting expression of 15% of all genes including VEGF, the non-translational role of SerRS may go beyond vascular development. Current studies are focused on how the non-translational role of SerRS is regulated through post-translational modifications and our result will be discussed.

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052

## Methionyl-tRNA synthetase alters its tRNA substrate specificity to facilitate adaptation to varying environments

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Bacteria must subsist throughout the extensive variations of their natural habitats in order to survive and reproduce. Despite this crucial tenet of fitness, all organisms universally encode, synthesize, and utilize proteins that function optimally within subset of growth conditions. Although it may be deleterious to maintain differentially adapted protein variants in the genome to preserve optimal protein functionality during environmental changes, recent evidence has revealed that cells can circumvent their genetic confines by altering their existing proteins during conditions which may otherwise compromise protein activity. In *Escherichia coli*, we have characterized one mechanism by which proteins can be globally altered through the incorporation of nongenetically encoded methionine residues into specific amino acid positions. This process is accomplished through regulated misacylation of particular nonmethionyl-tRNAs with methionine by the methionyl-tRNA synthetase (MetRS). We identified distinct molecular states of the MetRS which facilitate the adaptable tRNA substrate specificity of the enzyme. Remarkably, two redundant succinyl-lysine modifications at the tRNA binding interface of the MetRS mediate its high aminoacylation fidelity, while the unmodified MetRS is capable of accepting specific nonmethionyl-tRNAs as substrates. This adaptive translational response does not occur under the standard conditions for laboratory cultivation, where proteomes are presumably best adapted, but it can be activated rapidly in response to certain growth conditions. We are testing the hypothesis that adaptive translation can simultaneously adjust the proteome of an organism for optimal function in varying environments. Our investigation aims to establish that deviation from the central dogma has evolved to facilitate adaptation to fluctuating growth conditions in natural habitats.



## Roles of non-canonical proteins and enzymes in indirect tRNA aminoacylation in *Helicobacter pylori*

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Most microorganisms and organelles rely on indirect aminoacylation to produce Asn-tRNA<sup>Asn</sup> and/or Gln-tRNA<sup>Gln</sup>. In these cases, tRNA<sup>Asn</sup> and tRNA<sup>Gln</sup> are misacylated to produce Asp-tRNA<sup>Asn</sup> and Glu-tRNA<sup>Gln</sup>. These intermediates are converted to Asn-tRNA<sup>Asn</sup> and/or Gln-tRNA<sup>Gln</sup> by an amidotransferase (GatCAB in bacteria). Asp-tRNA<sup>Asn</sup> and Glu-tRNA<sup>Gln</sup> are rarely used erroneously in protein translation. In at least some bacteria, efficient sequestration and transamidation of Asp-tRNA<sup>Asn</sup> is ensured by formation of a transamidosome complex.<sup>1</sup> We have recently shown that transamidosome assembly in *Helicobacter pylori* is tRNA-independent and requires a novel protein factor called Hp0100.<sup>2</sup> Since this report, our characterization of Hp0100 has revealed that this enzyme uses bifurcated hydrolysis of ATP to improve the transamidation efficiency of both Asp-tRNA<sup>Asn</sup> and Glu-tRNA<sup>Gln</sup> by GatCAB and experimental observations suggest that these ATPase activities are regulated by metal ions. These results will be discussed and put into context of Hp0100 function *in vivo*.

We first identified Hp0100 from a protein-protein interaction map of the *H. pylori* proteome.<sup>3</sup> This same map hints at other roles for Hp0100 and the possibility that the  $\alpha$ -proteobacteria rely on additional unknown proteins for tRNA aminoacylation and perhaps for the non-canonical use of different aminoacyl-tRNAs. In particular, we have discovered that Hp0495, an 86 amino acid protein, preferentially forms a 1:1 complex with elongation factor Tu and either tRNA<sup>Glu1</sup> or tRNA<sup>Gln</sup>. Possible roles for this small protein will be discussed.

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## Structural insights into non-canonical aminoacylation

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Aminoacyl-tRNA synthetases link the correct amino acid to the corresponding tRNAs in a canonical aminoacylation pathway. However, there exist noncanonical aminoacylation pathways devoted for protein biosynthesis or other biological activities. Glutaminyl-tRNA (Gln-tRNA<sup>Gln</sup>) in archaea or organelle is synthesized in a pretranslational amidation of misacylated Glu-tRNA<sup>Gln</sup> by the heterodimeric or heterotrimeric Glu-tRNA<sup>Gln</sup> amidotransferase, respectively. We reported the first crystal structure of archaeal GatDE amidotransferase complexed with tRNA<sup>Gln</sup>, which revealed the ammonium shuttling mechanism from GatD to GatE active sites. The GatE enzyme recognizes tRNA<sup>Gln</sup> by indirect readout based on shape complementarity of the D-loop. In yeast mitochondria, trimeric GatFAB amidotransferase converts Glu-tRNA<sup>Gln</sup> (generated by cytoplasmic mis-charging GluRS) to Gln-tRNA<sup>Gln</sup>. The GatF subunit is a fungi-specific ortholog of the GatC subunit found in all other known heterotrimeric amidotransferase (GatCAB). The N-terminal extension of GatF forms several additional hydrophobic and hydrophilic interactions with GatA, which works as a trans-acting scaffolding peptide for the GatA glutaminase active site.

Aminoacyl-phosphatidylglycerol synthases (aaPGSs) are membrane proteins that catalyze the biosynthesis of aminoacyl-phosphatidylglycerol (aa-PG) by transferring amino acids from aminoacyl-tRNAs (aa-tRNAs) to PG in bacteria, thus catalyzing a non-canonical aminoacylation pathway. Incorporation of amino acids into membrane PG reduce the net negative charge of the outer surface of the membrane, facilitating the cellular tolerance to cationic antimicrobial peptides and increasing antibiotic tolerance. Thus, aaPGSs have attracted strong medical interest as a novel therapeutic targets. Most aaPGSs have two domains. The N-terminal domain is a trans-membrane domain composed of 6 to 14  $\alpha$ -helices and works as a flippase. The C-terminal domain is a cytosolic domain and responsible for the aminoacylation of PG. Here, we present the crystal structure of the C-terminal domain of aaPGS (aaPGS CTD). The overall structure of aaPGS(CTD) is composed of an a/b mixed fold and belonged to the GCN5-related N-acetyltransferase (GNAT) family with tandem GNAT domains. Structural similarity with FemX, which catalyzes transfer of Ala from Ala-tRNA<sup>Ala</sup> to a peptidoglycan precursor, combined with biochemical data provided us the clues to understand the molecular mechanism of aaPGSs.

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## Structural and dynamic aspects of initiator tRNA interactions with the bacterial ribosome and other ribosomal ligands

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Bacterial initiator tRNA (fMet-tRNA) is structurally different from all other cellular tRNAs and its participation in protein synthesis relies on unique and specific interactions with the ribosome and other components of the translational apparatus. Several cis-acting and trans-acting factors may influence the interactions of fMet-tRNA and the ribosome thereby determining efficiency and accuracy of translation initiation and sometimes playing a key role in regulating gene expression. The nature, the dynamics and the functional consequences of these interactions, canonical or non-canonical, during the various steps of the translation initiation pathway will be described.

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**O56****Translational recoding as a kinetic event****M. V. Rodnina**

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The translation of the genetic code is normally very accurate. However, during the expression of certain genes the information is recoded to yield an alternative protein product. Recoding occurs in response to special signals in mRNA. Frameshifting or bypassing are best-known examples of such exceptions to the canonical co-linear information flow. To understand molecular mechanisms that govern -1 programmed ribosomal frameshifting and translational bypassing, we monitored the movement of the ribosome over the respective recoding sites, one codon at a time, by rapid kinetics. Recoding is explained in terms of kinetic bifurcation events leading to the commitment to the alternative coding.



## 057

## Progression of tRNAs during elongation, visualized by cryo-EM of a single sample

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Cryo-electron microscopy of ribosomes engaged in translation yields large heterogeneous projection data sets, numbering in the hundreds of thousands, from which subpopulations in individual states can be recovered by using classification (1). Thanks to the advent of direct electron detectors, the resolution of some of the resulting reconstructions is now reaching the atomic level. I will present three studies in which the relative movement of the subunits, their individual domains, and the progression of the tRNAs through the ribosome has been visualized in this manner: one for yeast (2), one for *T. brucei* (3), and one for *E. coli* (4).

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**058**

## Single-molecule fluorescence reveals multiple chimeric tRNA states during EF-G-induced translocation on the ribosome

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Coupled translocation of tRNA and mRNA through the ribosome entails involves large-scale structural rearrangements, including the stepwise movement of tRNAs relative to the ribosomal subunits. Recent structural work has visualized intermediates of tRNA translocation induced by elongation factor G (EF-G) with the two tRNAs trapped in chimeric states with respect to the 30S and 50S subunits. The functional role of the chimeric states is not known. Here we use a single-molecule FRET assay to characterize EF-G-promoted translocation intermediates. Using EF-G mutants and antibiotics that slow down or block translocation, we identify several chimeric states on the translocation pathway and show that these intermediates readily interconvert with the pre- and post-translocation states. EF-G engagement disallows backward transitions at an early stage of translocation and increases the dynamics of tRNA fluctuations between pre-translocation hybrid, chimeric, and post-translocation states. The results suggest how the engagement of EF-G alters the energetics of translocation towards a flat, low-energy landscape.





## Structural insight into drug-dependent ribosome stalling

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In bacteria, ribosome stalling during translation of Erm leader peptide occurs in the presence of the antibiotic erythromycin and leads to induction of expression of the downstream macrolide resistance Erm methyltransferases<sup>1</sup>. Here we present our recent cryo-electron microscopy (EM) structure of the erythromycin-dependent ErmBL-SRC<sup>2</sup>. The structure reveals that the antibiotic does not interact directly with ErmBL, but rather redirects the path of the peptide within the tunnel. Furthermore, we identify a key peptide-ribosome interaction that defines an important relay pathway from the ribosomal tunnel to the peptidyltransferase centre (PTC). The PTC of the ErmBL-SRC appears to adopt an uninduced state that prevents accommodation of Lys-tRNA at the A-site, thus providing a structural basis for understanding how the drug and the nascent peptide cooperate to inhibit peptide bond formation and induce translation arrest. In addition, we now have cryo-EM structure of the ErmCL-SRC at near-atomic resolution. In comparison to ErmBL, the ErmCL peptide adopts a distinct conformation within the ribosomal tunnel directly interacting with the drug. Surprisingly, ErmCL induces large scale conformational rearrangements within the PTC, thus illustrating how Erm peptides can inhibit translation using vastly distinct mechanisms of action. Such insights will be important for development of new improved macrolide antibiotics that circumvent induction of Erm mediated erythromycin resistance.

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**060**

## A proton wire to couple aminoacyl-tRNA accommodation and peptide bond formation on the ribosome

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During ribosome-catalyzed peptide bond formation, the  $\alpha$ -amino group of an aminoacyl-tRNA attacks the ester carbonyl carbon of a peptidyl-tRNA to yield a peptide that is lengthened by one amino acid. This process is further accompanied by the net transfer of a proton from the attacking amine to the 3'-oxygen leaving group of the P-site tRNA. While the ribosome has been shown to decrease the entropy of activation for the reaction, the lack of high-resolution structural data for the complete ribosomal active site with bound full-length substrates or products has made it difficult to assess what role the ribosome might play in coordinating proton movement during catalysis. Using different combinations of natural and non-hydrolyzable aminoacyl-tRNAs, we have determined the crystal structures of pre-attack and post-catalysis complexes of the *Thermus thermophilus* 70S ribosome at a resolution of  $\sim 2.6$  Å resolution. These structures reveal a previously unseen network of hydrogen bonds, or "proton wire", along which hydrogen transfer from the attacking amine could take place to ensure the concerted, rate-limiting formation of an intermediate. An alternate reaction model that differs substantially from the prevailing models in the field is presented.



## Modeling gene therapy of mitochondrial diseases by imported small RNA

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Mitochondria are essential organelles of eukaryotic cells, taking part in several essential cellular processes. They carry their own genome (mtDNA) assembled into nucleoid complexes. Mutations in mtDNA, often in tRNA genes, have been associated with a variety of human pathologies. In patients with mtDNA defects, it is common to find mutant and normal (wild type) mtDNA molecules in the same cell, a phenomenon described as heteroplasmy. Manifestation of biochemical and clinical defects occurs only when a threshold level of heteroplasmy (>60% of mutant genomes) has been reached. Since there is no effective treatment for these disorders, one attractive approach would be to either address into mitochondria RNAs which would functionally replace mutant mtDNA encoded ones, the strategy termed as “allotopic”, or to specifically target mutant mtDNA with mitochondrially imported aptamers to prevent it from replicating, thereby allowing propagation of wild type genomes, the strategy termed as “antigenomic”.

We first validated the allotopic approach for two pathogenic mtDNA mutations, associated with the syndromes MERRF (mutation in tRNA(Lys)) and MELAS (mutation in tRNA(Leu)) (Kolesnikova et al., *Hum. Mol. Genet* 2004; Karicheva et al., *Nucleic Acids Res*, 2011). The antigenomic strategy is confronted to 2 challenges: translocation of the anti-genomic molecules through the double mitochondrial membrane, and their access and specific binding to mutated region of mtDNA. Our study of the natural pathway of RNA import into mitochondria permitted to identify the import determinants in tRNA and 5S rRNA structures. Basing on these data, a set of small RNA molecules with significantly improved efficiency of mitochondrial import was constructed (Kolesnikova et al. *RNA*, 2010; Smirnov et al., *Genes and Dev.*, 2011). To create a vector system able to target therapeutic oligonucleotides into deficient human mitochondria, we inserted into these RNAs short sequences corresponding to the boundaries of a large deletion in mtDNA associated with a neuromuscular syndrome KSS. Recombinant RNAs, introduced into cultured human cells containing KSS deletion, were shown to be stable in the cytosol, partially imported into mitochondria, and induced a decrease of the mutant mtDNA proportion, thus validating the potential of our approach to rescue the deleterious mtDNA mutations (Comte et al., *Nucl. Acid Res.*, 2013).

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**062**

## Co-crystal structure of a tRNA-T box riboswitch complex: molecular basis for the control of gene expression by transfer RNA

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T-box riboswitches are widespread in Gram-positive bacteria, where they regulate the expression of genes encoding aminoacyl-tRNA synthetases and other proteins in response to the intracellular concentration of their cognate aminoacyl-tRNA. These regulatory mRNA elements function by binding to tRNA and concurrently decoding its anticodon and sensing its aminoacylation status (Grundy & Henkin, Cell 74:474, 1993). T-box riboswitches comprise two conserved domains. First, an ~100 nucleotide Stem I domain is necessary and sufficient for high affinity ( $K_d \sim 150$  nM), anti-codon dependent binding to tRNA. Second, an antiterminator domain senses the charge status of the tRNA acceptor end, and makes the transcription termination decision. Recently, we reported the first crystal structure of a T-box Stem I domain in complex with its cognate tRNA (Zhang & Ferré-D'Amaré, Nature 500:363, 2013). Our structure reveals that the Stem I domain not only binds to the anticodon, as predicted genetically, but in fact recognizes the overall architecture of tRNA by interacting extensively with its elbow region. Remarkably, the T-box employs the same structural motif (two interdigitated T-loops) to recognize the elbow of tRNA as RNase P and the E-site of the ribosome. We find that the T-box and tRNA undergo mutually induced fit (taking advantage, among other elements, of the intrinsic flexibility of tRNA and of its post-transcriptional modifications); this rearrangement may help orchestrate the genetic control decision. We have also investigated how the T-box determines the aminoacylation state of tRNA. We have developed a method to generate biophysical quantities of >95% pure aminoacylated tRNA. Using such preparations, we show that, in vitro, the glyQ T-box can selectively terminate transcription in response to glycylation of tRNA-gly, without a need for accessory factors, such as EF-Tu (Zhang & Ferré-D'Amaré, Mol. Cell, in press).

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## Exploring the dynamic and evolution of the tRNA pool through systematic deletion of tRNA genes

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Translation is regulated by many factors acting at the levels of initiation, elongation and termination. The efficiency of the elongation step is dependent, among other things, on the balance between levels of the different tRNA molecules (tRNA supply) and the representation of the various codons in the transcriptome (tRNA demand). In this work we aimed to dissect the specific contribution of each tRNA gene to the tRNA pool, and explore the mechanisms by which the translational machinery adapts to changes in the balance between tRNA supply and demand. Towards this challenge we have systematically deleted most tRNA genes in *S.cerevisiae* generating tRNA deletion library. We found extensive backup interactions between different tRNA genes that endowed yeast with robustness to genetic perturbation especially under optimal growth conditions, yet under more challenging conditions, additional phenotypes were observed. Interestingly, we detect differences in the essentially of various gene copies coding the same tRNA molecule, suggesting differential regulation between identical tRNA gene copies located at different genomic locations. Finally, using lab evolution experiments we have shown that mutants deleted for specific tRNA can adapt to such perturbation and restore the balance between tRNA supply and demand. This adaptation was based on strategic mutations that changed the anticodon of other tRNA genes to match that of the deleted one. We believe that our tRNA deletion library is a novel invaluable genetic tool that can provide insights into the dynamic nature and evolution of the tRNA pool.



064

## An extended Shine-Dalgarno sequence in mRNA functionally bypasses a vital defect in initiator tRNA

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Initiator tRNAs are special in their direct binding to the ribosomal P-site due to the hallmark occurrence of the three consecutive G-C base pairs (3GC pairs) in their anticodon stems. How the 3GC pairs function in this role, has remained unsolved. We show that mutations in either the mRNA or rRNA leading to extended interaction between the Shine-Dalgarno (SD) and anti-SD sequences compensate for the vital need of the 3GC pairs in tRNA<sup>fMet</sup> for its function in *Escherichia coli*. *In vivo*, the 3GC mutant tRNA<sup>fMet</sup> occurred less abundantly in 70S ribosomes but normally on 30S subunits. However, the extended SD:anti-SD interaction increased its occurrence in 70S ribosomes. We propose that the 3GC pairs play a critical role in tRNA<sup>fMet</sup> retention in ribosome during the conformational changes that mark the transition of 30S pre-initiation complex into elongation competent 70S complex. Furthermore, treating cells with kasugamycin, decreasing ribosome recycling factor (RRF) activity or increasing initiation factor 2 (IF2) levels enhanced initiation with the 3GC mutant tRNA<sup>fMet</sup>, suggesting that the 70S mode of initiation is less dependent on the 3GC pairs in tRNA<sup>fMet</sup>.



## O65

**tRNA-derived fragments target the small ribosomal subunit to fine-tune translation****Gebetsberger J.<sup>1</sup>, Polacek N.<sup>1\*</sup>**<sup>1</sup> *Departement for Chemistry and Biochemistry (DCB), University of Bern, Switzerland*\* *Correspondence: Norbert.polacek@dcb.unibe.ch*

Post-transcriptional cleavage of RNA molecules to generate smaller fragments is a widespread mechanism that enlarges the structural and functional complexity of cellular RNomes. In particular, fragments deriving from both precursor and mature tRNAs represent one of the rapidly growing classes of post-transcriptional RNA pieces. Importantly, these tRNA-derived fragments (tRFs) possess distinct expression patterns, abundance, cellular localizations, or biological roles compared with their parental tRNA molecules (1).

Here we present evidence that tRFs from the archaeon *Haloferax volcanii* directly bind to ribosomes. In a previous genomic screen for ribosome-associated small RNAs we have identified a 26 residue long fragment originating from the 5' part of valine tRNA (Val-tRF) to be by far the most abundant tRF in *H. volcanii* (2). The Val-tRF is processed in a stress-dependent manner and was found to primarily target the small ribosomal subunit *in vitro* and *in vivo*. Translational activity was markedly reduced in the presence of Val-tRF, while control RNA fragments of similar length did not show inhibition of protein biosynthesis. Crosslinking experiments and subsequent primer extension analyses revealed the Val-tRF interaction site to surround the mRNA path in the 30S subunit. In support of this, binding experiments demonstrated that Val-tRF does compete with mRNAs for ribosome binding. Therefore this tRF represents a ribosome-bound non-protein-coding RNA (ncRNA) capable of regulating gene expression in *H. volcanii* under environmental stress conditions probably by fine-tuning the rate of protein production (1).

(1) Gebetsberger J. and Polacek N. (2013), *RNA Biol.* 10:1798-1808

(2) Gebetsberger J. et. al. (2012), *Archaea*, Article ID 260909



## O66

## Messenger RNAs bearing tRNA features exemplified by a tRNA-mimic motif within interferon alfa 5 mRNA

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RNase P is a structure-dependent nuclease that specifically cleaves tRNA precursors (pre-tRNAs). The purpose of this work was to ascertain whether liver mRNA species share common structural features with hepatitis C viral (HCV) mRNA that allow them to support the RNase P cleavage reaction *in vitro* and, if so, whether any of the mRNA species identified could provide clues regarding the advantages to the viruses of incorporating tRNA-mimicking elements in their genomes. We observed that human liver mRNA competed with pre-tRNA cleavage in a human RNase P competition, and subsequently identified a set of sensitive mRNA species for which three cleavage sites were characterised. One of these sites was found in interferon-alfa mRNA within a large RNase T1-resistant fragment of approximately 59-nts. Structural probing revealed a pseudoknotted four-branched structure compatible with tRNA-like folding. This element mapped in a position that coincided with a cytoplasmic-accumulation-region (CAR) -a signal described during functional analysis- thereby positioning both of its consensus CAR-E sequences in symmetrical and equivalent positions in the secondary structure thereof. The pseudoknotted core structure, a similar CAR-E sequence and other similar features can be identified in the tRNA-like element of HCV. The shared ability of an antiviral host defence and a viral mRNA to mimic tRNA is discussed in biosemiotic terms.





## The tRNA methyltransferase Dnmt2 is required for accurate protein synthesis during haematopoiesis

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RNA modifications have long been considered to modulate RNA activity, but their function is poorly characterized. Dnmt2 is a unique enzyme that utilizes the catalytic mechanism of eukaryotic DNA methyltransferases to methylate several tRNAs at cytosine 38. Dnmt2 mutant mice, flies and plants were reported to be viable and fertile. Recently we demonstrated that cytosine-5 tRNA methylation is important for tRNA homeostasis, protein synthesis and cellular differentiation during mammalian development. To further characterize the function of cytosine-5 tRNA methylation, we now performed a more detailed analysis of the Dnmt2 mutant mouse phenotype using the mouse hematopoietic system as paradigm of stem cell renewal and differentiation. In Dnmt2-deficient mice, haematopoiesis was impaired and haematopoietic progenitors were reduced, as observed by *in vitro* colony formation assays and *in vivo* by serial transplantation of bone marrow cells. Further analysis also revealed an impaired stromal support, as demonstrated by mesenchymal stem cells (MSC) differentiation defects. Using RNA bisulfite sequencing, we show that Dnmt2 methylates C38 of tRNA Asp<sup>GTC</sup>, Gly<sup>GCC</sup>, and Val<sup>AAC</sup> in bone marrow as well in MSC and differentiated cells. Dnmt2-mediated C38 methylation modulates the steady state and fragmentation of tRNA substrates as demonstrated by Northern blotting and small RNA sequencing. Proteomic analysis of bone marrow from Dnmt2-deficient mice and dynamic SILAC analysis from primary bone marrow cells in combination with gene expression profiles revealed that the differences in protein expression are not due to altered mRNA levels but caused by codon bias related to the tRNA methylation function of Dnmt2. Our observations demonstrate that Dnmt2 plays an important role in hematopoiesis and define a function of C38 tRNA methylation in the accuracy of protein synthesis

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## Malaria sporozoites import exogenous tRNAs

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*Plasmodium falciparum* belongs to the phylum of *Apicomplexa*; it causes the most severe form of malaria in humans. It is responsible for the infection of 300 to 500 million people and leads to one million deaths every year (the Malaria Foundation International website). We used *Saccharomyces cerevisiae* ARC1p and *Homo sapiens* AIMP1 (p43) to look for proteins containing a tRNA binding module (tRBD) in *P. falciparum*. This allowed us to identify a protein, that we named tRip (for tRNA import protein), present exclusively in *Apicomplexa*. This protein is characterized by a unique modular organization. The N-terminal domain of tRip shows no significant homology with any known protein but does contain a predicted short internal transmembrane region. Besides, as expected, the C-terminal tRBD of tRip specifically binds tRNAs *in vitro*, by recognizing the elbow of the tRNA structure. Although tRNA trafficking has only been observed between the cytoplasm and different organelles of eukaryotic cells, here we show that tRip is expressed at the surface of the parasite and facilitates import of exogenous tRNAs into *Plasmodium* sporozoites (the infectious form of the parasite that accumulates in mosquito salivary glands and are injected into the vertebrate host). We propose a specific stage where *Plasmodium* can divert tRNA's from host cells and we will discuss the biological pertinence of this original host-pathogen interaction as well as the possible fate of imported tRNAs.



## Signal saturation limits the development of tRNA identities and the size of the genetic code

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The canonical genetic code contains sixty-four codons, but only codes for twenty amino acids. Francis Crick labeled the code as a frozen accident, and a vast body of literature has accumulated that attempts to explain how did the code emerge, evolve, and reach its extant complexity. However, the question of why does it contain twenty amino acids and not more has been rarely addressed and remains unanswered.

The importance of the question is not limited to the academic understanding of the origin of life. Many biotechnological applications, and the ultimate goal of systems biology, depend upon our ability to manipulate the genetic code and introduce new amino acids into artificial proteomes. It is unlikely that this can be done effectively if we don't understand where do the limits of the universal code lie.

We would like to propose that the limit of the universal genetic code was set by the saturation of identity elements in tRNA molecules. Using experimental data, structure modeling, and mathematical analysis we will defend the idea that the structural and functional constraints that operate on the seventy-six bases of tRNA have reached an operational limit that impedes the incorporation of new tRNA identities (and hence new amino acids) to the system.



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## A model to explain frequent misreading of a subset of codons by individual tRNAs

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The accuracy of the process of translation is essential to cellular health. Cells use a variety of mechanisms to insure optimal accuracy by blocking codon recognition by other than correct, cognate aminoacyl-tRNAs. tRNA discrimination is efficient but not completely so. As a result, incorrect amino acids can be incorporated by misreading by near-cognate tRNAs, which we define as those making no more than two legal base pairs with the codon. We have developed sensitive and quantitative in vivo assays to measure the frequency of all possible near-cognate decoding events by individual tRNAs. We find that the frequency of errors varies among codons and that they are extremely low for the majority of codons. The lowest frequencies of errors, by a tRNA<sup>Glu</sup>, were on the order of  $2 \times 10^{-6}$  per codon. For each tRNA tested errors at a few codons are much more frequent, up to several in 1000 codons. We are interested in the molecular reason for this variation in error frequency.

We had found that errors by tRNA<sup>Lys</sup> were highest for codons recognized by a low-abundance competing cognate tRNA, for example at the Arg codons AGA and AGG in *E. coli*. Further work has shown that poor availability of the cognate tRNA is not required for frequent errors although it tends to increase errors at error-prone codons. Analysis of errors by multiple tRNAs in *E. coli*, the yeast *Saccharomyces cerevisiae* and human cells (HEK 293T) suggests that the most frequent errors require a small set of mismatched base pairs. Post-transcriptional modification of tRNAs modulates error frequency but the same modification can have opposite effects on “strong” and “weak” codons. These data suggest a general model to explain frequent misreading at a small number of codons based on the ribosome’s inability to reject some near-cognate events.

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## Accuracy of codon recognition by ternary complex on the ribosome

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On mRNA programmed ribosome, the accuracy of A-site codon recognition between the cognate and non-cognate aminoacylated transfer RNAs (aa-tRNAs) could be separated by GTP hydrolysis into initial selection and proofreading step. In initial selection, aa-tRNAs together with elongation factor Tu (EF-Tu) and GTP consist of ternary complex. The cognate ternary complex associates high probability of codon recognition followed by the GTP hydrolysis, while the non-cognate case dissociates from the A-site codon with high probability. Previously, tRNA<sup>Lys</sup> displayed a linear trade-off between efficiency of cognate codon reading and the accuracy of codon selection<sup>1</sup>. In that paper, the *d*-values varied from 1 500 to 25 000. Now we find that the *d*-values varies tremendously from 200 to 80 000 as the set of six additional tRNAs, which are tRNA<sup>Phe</sup>, tRNA<sup>Glu</sup>, tRNA<sup>Asp</sup>, tRNA<sup>His</sup>, tRNA<sup>Cys</sup> and tRNA<sup>Tyr</sup>. After GTP hydrolysis, the proofreading step could enhance the accuracy by high probability of non-cognate aa-tRNAs rejection. Interestingly, with the low *d*-values are error hot spots. In the whole selection steps, our in vitro accuracy hot spots are identical to Farabaugh and collaborators' in living *E. coli* cells  $\beta$ -galactosidase assay accuracy data<sup>2</sup>. We can even further see the partition of the initial selection and proofreading with those hot spots. So our in vitro biochemistry accuracy data could calibrate to the Farabaugh's in vivo data. From this, we could even estimate error levels which have not obtained or remain in the background of  $\beta$ -galactosidase measurements.

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072

## A dual program for translation regulation in cellular proliferation and differentiation

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A central choice for metazoan cells is between proliferation and differentiation. Measuring tRNA pools in various cell-types, we found two distinct subsets, one that is induced in proliferating cells, and repressed otherwise, and another with the opposite signature. Correspondingly, we found that genes serving cell-autonomous functions and genes involved in multi-cellularity obey distinct codon-usage. Proliferation-induced and differentiation-induced tRNAs often carry anti-codons that correspond to the codons enriched among the cell-autonomous and the multi-cellularity genes, respectively. Since mRNAs of cell-autonomous genes are induced in proliferation and cancer in particular, the concomitant induction of their codon-enriched tRNAs suggests coordination between transcription and translation. Histone modifications indeed change similarly in the vicinity of cell-autonomous genes and their corresponding tRNAs, and in multi-cellularity genes and their tRNAs, suggesting the existence of transcriptional programs coordinating tRNA supply and demand. Hence, we describe the existence of two distinct translation programs that operate during proliferation and differentiation.



## Cracking the genomic code of codons: Using dendritic cells to explore beyond the genetic code

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During the last decade the advances in computational sciences and sequencing technologies allowed to identify most coding sequences from the genome of model organisms and also to identify the gene copy number for each anticodon containing tRNAs. Using proprietary softwares, we were able to identify that immune genes from *Homo sapiens*, *Mus musculus* and *Caenorhabditis elegans* use a particular common set of codons. The conservation during evolution of the same group of codons in genes with similar functions, suggests that codon usage is part of a previously not described gene expression regulatory program during stress and immunity.

Dendritic cells are cells are particular immune cells that change their expression pattern rapidly after activation and are key for correct functioning of the immune system. In order to investigate the importance of tRNA metabolism and the changes in translatability of synonymous codons in dendritic cells, we developed two strategies. First, we analyzed the regulation of most known genes involved in synthesis, splicing, modification, transport, aminoacylation and degradation of tRNAs in a genomic dataset of 96 microarrays performed in bone marrow derived Dendritic cells (bmDCs). Second, we generated recombinant reporter genes and transduced them on bmDCs in order to monitor the translatability of synonymous codons and the changes in speed of translation after pattern recognition receptors stimulation.

Our results indicate that DCs modulate their mRNA decoding abilities during PRR activation and this reprogramming might add a new layer of regulation for gene expression.



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## Control of mitochondrial tRNA modification enzymes in MELAS cells by the ROS-regulated microRNA miR-9/9\*

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The molecular mechanisms underlying mitochondrial (mt) human diseases associated with defects in mt-tRNA modification like MELAS still remain unresolved. MELAS syndrome is caused by mutations in mtDNA affecting mt-tRNA<sup>Leu(UUR)</sup>. Patient and cybrid cells exhibit elevated oxidative stress. Moreover, mutant mt-tRNAs<sup>Leu(UUR)</sup> lack the taurine-containing modification normally present at the wobble uridine of wild-type mt-tRNA<sup>Leu(UUR)</sup>, which is considered an etiology of MELAS. Wobble modifications are thought to contribute to translational accuracy and/or efficiency, so modification defects may lead to a defective translation of mtDNA-encoded proteins and, accordingly, to functional perturbations of the OXPHOS system. However, several studies in MELAS and similar diseases suggest that defects of mitochondrial protein synthesis cannot fully explain the mitochondrial dysfunction manifested. We tested the hypothesis that regulation of the mt-tRNA modification enzymes may participate in the pathogenic mechanisms underlying these diseases. In fact, we found a significant decrease in the steady-state levels of such enzymes in MELAS cybrids. Since ROS are increased in MELAS cells and these species have been hypothesized to cause a microRNA-mediated response in mtDNA disorders, we wondered whether under-expression of the tRNA modifying enzymes in MELAS could be a consequence of a microRNA-directed regulation. We found that the ROS-sensitive-microRNA, microRNA-9/9\*, is over-expressed through a ROS/NF-κB signaling pathway in cells carrying a MELAS mutation. We showed that miRNA-9/9\* is responsible of the post-transcriptional negative regulation of mitochondrial tRNA modification enzymes, thus contributing to mitochondrial dysfunction in MELAS. Our data demonstrate that the modification status of mt-tRNAs is dynamic and that cells respond to stress by modulating the expression of the mt-tRNA modifying enzymes. We conclude that microRNA-9/9\* is a crucial player in mitochondria-nucleus retrograde signaling as it regulates the expression of nuclear genes in response to changes in the functional state of mitochondria.





## Discovering tRNA-protein interactome and its function in cellular communication between translation and other processes

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Communications between cellular processes are essential in homeostasis and in stress response. Protein synthesis consumes a vast amount of materials and energy in the cell. Hence, coordination of protein synthesis activity with other cellular processes such as cell cycle control, histone modification, membrane trafficking and others should be crucial for cell physiology. We discovered recently that tRNAs may serve as a major class of communicators in the cell (1). We found that tRNAs bind to a wide range of human proteins such as the mitogen-activated protein kinase kinase (MEK), histone H3K9 methyltransferase, membrane trafficking protein SAR1a, farnesyl-transferase, glutathione synthetase, and phosphoenolpyruvate carboxykinase in cells; none was known previously to interact with nucleic acids. We hypothesize that tRNA binding regulates the activity of these proteins in response to the translation activity in the cell. When translation activity is high, only a small amount of tRNA is available, and these tRNA-protein interactions are present at low levels. When translation activity decreases, more tRNA becomes available to increase the level of such tRNA-protein interactions in order to up- or down-regulate the cellular processes these proteins participate in. We are testing this functional hypothesis of tRNA as communicators to further expand extra-translational functions of tRNA.

(1) Parisien, M., Wang, X-Y., et al.: *Discovering RNA-protein interactome using chemical context profiling of the RNA-protein interface*, *Cell Reports*, 3, 1703-13 (2013).



## 076

## The role of the LysRS-Ap<sub>4</sub>A-Hint-1-MITF pathway in health in disease

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Microphthalmia transcription factor (MITF) is a basic helix-loop-helix leucine zipper (bHLH-Zip) DNA-binding protein. This transcription factor has a crucial role in the physiological and pathological development of many distinct organs, including the eye, ear, immune system, bone, and skin.

During the last decade, by using cellular and structural biology approaches to investigate this pathway in gene regulation we elucidated the mechanistic and molecular bases of MITF-activation in several cell types. Upon physiological activation, lysyl-tRNA synthetase (LysRS) is phosphorylated and translocates into nucleus where it forms a complex with MITF. Phosphorylated LysRS also produces a critical signaling molecule, diadenosine tetraphosphate (Ap<sub>4</sub>A) that binds and releases the MITF-suppressor protein Hint-1 from MITF. This LysRS-MITF pathway results in transcriptional activation of MITF target genes (please see the following link: <http://www.youtube.com/watch?v=NGgMEz79NX4>). Using mass spectrometry, we found that Hint-1 was subjected to K21 acetylation and Y109 phosphorylation upon immunological activation of Mast cells. These modifications appear upon Hint-1 dissociation from MITF caused by Ap<sub>4</sub>A binding. Based on structural analyses, we predict that the binding interface of Hint-1-MITF is close to this Ap<sub>4</sub>A binding cleft. The identified residues are located in a proximity to the Ap<sub>4</sub>A binding region. We have also started to investigate mutant mice including Hint-1(-/-) and LoxP-Cre Ap<sub>4</sub>A hydrolase mice, which allow us to conditionally delete Ap<sub>4</sub>A hydrolase, and to explore the important role of this LysRS-Hint1-Ap<sub>4</sub>A-MITF pathway under physiological conditions.



## tiRNAs as potential inhibitors of apoptosis during hyperosmotic stress

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An imbalance between extracellular and intracellular fluid osmolarity can cause osmotic stress. Increased extracellular osmolarity is associated with inflammatory diseases. Cells adapt to changes in osmolarity with adaptive mechanisms. However, chronic exposure to hyperosmotic stress induces apoptosis. We have previously shown that hyperosmotic stress induces the cleavage of tRNAs via the increased function of the nuclease angiogenin, thus causing the accumulation of tRNA halves, known as tiRNAs. We investigated the function of the tiRNAs in stressed cells. Accumulation of tiRNAs was accompanied by increased survival in hyperosmotic-stressed mouse embryonic fibroblasts. Induction of apoptosis during hypertonic stress was dependent on Cytochrome *c* (Cyt *c*) release from mitochondria and subsequent apoptosome formation which was negatively influenced by the increased accumulation of tiRNAs in stressed cells. Released Cyt *c* from mitochondria formed a ribonucleoprotein particle (Cyt *c*-RNP), with twenty tiRNAs highly enriched. 5'- and 3'-tiRNAs were components of this complex. Using *in vitro* binding assays of cellular tiRNAs with recombinant Cyt *c*, we identified a subpopulation of tiRNAs that directly bind Cyt *c*, thus identifying potential tiRNA candidates as inhibitors of apoptosis *in vivo*. Our findings reveal a connection between angiogenin-generated tiRNAs and cell survival in response to hyperosmotic stress, and suggest a novel cellular complex involving Cyt *c* and tiRNAs that inhibits apoptosome formation.



078

## Selenoprotein biosynthesis and human disease

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The essential micronutrient selenium is found in proteins as selenocysteine (Sec), the only genetically encoded amino acid whose biosynthesis occurs on its cognate tRNA in humans. In the final step of selenocysteine formation, the essential enzyme SepSecS catalyzes the conversion of Sep-tRNA to Sec-tRNA. We demonstrate that mutations in SepSecS cause two autosomal recessive neurodegenerative disorders, progressive cerebellocerebral atrophy (PCCA) (1) and progressive encephalopathy with edema, hypsarrhythmia, and optic atrophy (PEHO-like) syndrome. Such mutations are predicted to disrupt the known human SepSecS:tRNA<sup>Sec</sup> structure and their effect was shown to be detrimental in functional *in vivo* assays. Finally, the human SepSecS protein is also known as soluble liver antigen/liver pancreas (SLA/LP) (2), which represents one of the antigens of autoimmune hepatitis. The human SepSecS:tRNA<sup>Sec</sup> structure suggests the predicted antigenic site to be at the binding interface of SepSecS to tRNA<sup>Sec</sup> (3) and provides a better understanding of the immunogenicity of SLA/LP in autoimmune hepatitis.

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## Mutation in RNA kinase *CLP1* causes neurodegeneration

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Cleavage and polyadenylation factor I subunit 1 (*Clp1*) is an important kinase in RNA metabolism. It is involved in processes as tRNA maturation and mRNA 3' end processing. Recently, we identified a homozygous p.R140H missense mutation in the *CLP1* gene in patients with atrophy of the cerebellum, pons and corpus callosum.

We show that *Clp1* knockout zebrafish do not survive beyond 4 days post fertilisation (dpf), have a reduced head size and show an S-curved body. At 2dpf, *Clp1* knockout fish show reduced expression of midbrain marker *otx2*, increased cell death in the brain and a disturbed organisation of motor neurons. The phenotype can be partly rescued by injecting human wild type, but not by mutant p.R140H *CLP1* mRNA. Morpholino knock down of p53 partially rescues the phenotype as well.

Furthermore, we show that the p.R140H mutation impairs the kinase function and nuclear localisation of the CLP1 protein, and that tRNA cleavage efficiency is reduced in patients.

We show that *Clp1* is an essential gene in both the central and peripheral nervous system. Similar to the human situation, *clp1* mutations cause neurodegeneration and motor neuron problems in zebrafish. The neurodegeneration is mediated by the p53 apoptosis pathway. Our data supports the hypothesis that amongst other RNA processing genes, *CLP1* plays a crucial role in neurodevelopment.

**O80**

## Pathogenic mutations in SDR5C1 impair the tRNA maturation activities of human mitochondrial RNase P

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SDR5C1 is a short-chain amino and fatty acid dehydrogenase/reductase, moonlighting as component of the human mitochondrial RNase P complex. RNase P is the enzyme responsible for the removal of extra nucleotides at the 5'-end of tRNAs, an early and crucial step in tRNA maturation. The human mitochondrial enzyme moreover catalyzes the N<sup>1</sup>-methylation of purines at position 9, a modification found in all mitochondrial tRNAs and assumed to stabilize their structure. Ten different missense mutations in SDR5C1 were reported to cause a human disease characterized by progressive neurodegeneration and cardiomyopathy, originally called 2-methylhydroxy-butyryl-CoA dehydrogenase (MHBD) deficiency (referring to SDR5C1's dehydrogenase function). However, the dehydrogenase activity of the different mutant forms of SDR5C1 does not correlate with the severity of the associated disease. Two out of the 10 known mutations of SDR5C1 were reported to cause a decrease in the protein steady state level, and the consequent accumulation of mitochondrial RNA precursors, while for others the total amount of protein seems to be unaffected, suggesting possible differences in the pathomechanisms associated with the different mutations.

We are investigating the effect of the different mutations of SDR5C1 on its role in tRNA maturation. We have expressed and purified SDR5C1 and its partner proteins, and tested the activity in vitro of the human mitochondrial RNase P complex reconstituted with mutant forms of SDR5C1 in comparison to the wild type. Here we show that pathogenic mutations in SDR5C1 impair the dehydrogenase, methyltransferase, and endonucleolytic activities of the human mitochondrial RNase P.



## Molecular basis of histidyl-tRNA synthetase-associated Usher syndrome type 3B in cochlea-derived mouse cells and zebrafish

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Usher syndrome Type 3B is a syndrome comprising deafness, blindness, and fever-induced hallucinations, recently discovered in Amish children. It is caused by a mutation in histidyl-tRNA synthetase (HARS), a class II aminoacyl-tRNA synthetase required for protein production and which has not previously been associated with Mendelian disease. In order to determine the mechanism by which the Usher-associated Y454S mutation has tissue-specific effects, we have designed a multiple level approach, studying HARS at the molecular, cellular and organismal levels. The molecular approach is described by Abbott et al. (this conference). Cells isolated from embryonic mouse ears, comprising either neural or mechanosensory cell phenotypes, were chosen to investigate cellular expression, localization and binding partners of HARS and Y454S HARS. Immunocytochemistry of cells transfected with FLAG-tagged HARS or Y454S HARS reveals that both wild type and mutant protein distribute throughout the cell with a punctate morphology, associate with cytoskeletal elements, are present in cellular extensions, and demonstrate greater concentration near parts of the cellular membrane. Co-localization studies of HARS with various cellular markers are underway. In parallel work, immunoprecipitation of transfected HARS or Y454S followed by quantitative proteomics analysis reveals that cellular yield of Y454S HARS is reduced two-fold compared to HARS, suggesting increased turnover rate of mutant enzyme. Proteins that co-immunoprecipitate with HARS or Y454S HARS include those previously reported (e.g. eEF family). Notably, some co-immunoprecipitated proteins preferentially bound either Y454S HARS or wild-type HARS. Proteins chosen for validation by reverse immunoprecipitation or by confocal microscopy include Rab7a, involved in Charcot-Marie-Tooth neuropathy. Finally, we are developing a model for Usher Type 3B in zebrafish by knocking down HARS with embryonic morpholino injections. These embryos exhibit disruption of the laminar development of the eye. Co-injections of morpholino with human HARS (for rescue) or Y454S HARS mRNA (to recapitulate the Usher phenotype) are underway.



## Molecular evolution of tRNAs and tRNA CIFs in *Drosophila*

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Patterns of substitution rates footprint functional constraints and reveal potentially adaptive evolutionary change in macromolecules. The publication of twelve *Drosophila* genomes in 2007 facilitated one of the first highly resolved molecular evolutionary analyses of a noncoding RNA family at single-site resolution, specifically for microRNAs. Yet, despite that tRNAs were the first RNAs to ever be sequenced or structurally solved, detailed molecular evolutionary analysis of tRNAs at single-site resolution has not yet been published. Orthology assignment remains challenging for short and repetitive genes. We exploited curated tRNA orthology sets in *Drosophila* to accurately measure evolutionary rates of individual sites and structural elements of tRNAs. We found that sites in the tRNA “variable pocket” show rapid rates of evolution across different species and classes of tRNAs. These sites form part of an ion-binding pocket, often bound to Mg<sup>2+</sup>, but also to other ions. We also integrated our molecular evolutionary analysis with the first detailed analysis of tRNA Class-Informative Features (CIFs) and their evolution in a eukaryotic genus, reporting here the first analyses of base-pair and base-triple CIFs. Generally, CIF-estimation appears to be superior and more refined for eukaryotes over prokaryotes because single genomes with larger gene complements may be analyzed individually. We have found that CIFs have turned over surprisingly rapidly in *Drosophila* with evidence of cross-talk or CIF coevolution across functional classes, including between initiators and elongators.





## tRNAseq: An experimental and computational pipeline to characterize expression and processing of precursor and mature human tRNAs

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Despite their importance in fundamental aspects of biology and disease, human tRNAs remain an understudied class of non-coding RNAs on a genome-wide scale. Currently, no experiment-based and transcriptome-wide annotation of precursor and mature tRNAs exists, mainly because secondary structure and modifications, together with tRNA gene multiplicity, complicate sequencing and mapping efforts.

To address these issues we developed tRNAseq, a novel method, based on partial alkaline hydrolysis to fragment tRNAs into 20-40 nt long pieces with less rigid secondary structures, followed by standard small RNA cDNA library preparation and sequencing. We also established a bioinformatic annotation pipeline for the analysis of the sequencing results. We show that only 45% of previously predicted tRNA genes show convincing evidence of expression in human cell lines. We identify also editing positions, as well as determine expression levels of tRNA precursors, including leader, trailer, and intron sequences.

Our methodology can also be used to assess abundance changes of tRNAs across tissues and cell lines. In a recent study we identified and quantified tRNA introns accumulating in the context of a novel human neurodevelopmental syndrome caused by mutations in CLP1, a member of the tRNA splicing complex. In summary, we present a human tRNA expression atlas, and an RNA-sequencing-based methodology for determining expression levels of mature and processing intermediates of tRNAs in human cells.



## 084

## A novel program for analysis of tRNA expression from RNASeq datasets

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Next-Generation Sequencing (NGS) mediated RNA sequencing (RNA-seq) is now the method of choice for accurate measurements of gene expression. However, algorithms developed for mRNA are not suitable for expression analyses of tRNA. We have developed a novel computer program that expands the use of the sequence alignment program Bowtie<sup>1</sup> to map RNA-seq reads to tRNA genes in any genome. The reads are divided into groups that can be customized according to the user's desired classifications. We have designated these groups to classify RNA-seq reads of plant species; Name, Family, Isotype, Splice, Globalso, and Unclassified. Name matched reads to a specific tRNA gene at a specific location in the genome that allowed comparisons of location-specific expression of the same genetic sequence. Family matched reads to a specific sequence, but not to a specific genomic location. This allowed comparison of a specific anticodon containing tRNA gene type against another. Isotype reads matched to multiple sequences, but the resulting tRNA all code for the same amino acid. Globalso reads matched to both tRNA genes in the nuclear DNA and tRNA genes in the mitochondria or chloroplast, separating them from genomic DNA-specific expression data. Unclassified reads matched several different isotype tRNA genes.

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## An improved tRNAscan-SE and genomic tRNA database: new capabilities and features to enhance tRNA research

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tRNAscan-SE [1] has been widely used for whole-genome tRNA gene prediction for nearly two decades. In that time, we have developed a more detailed understanding of tRNA gene features and inherent variability between genomes. tRNAscan-SE was initially designed to detect phylogenetic domain-specific tRNA genes using just one general tRNA model at a time. Due to this limitation, we have found numerous cases where it fails to detect or classify genes. We have now addressed this shortcoming by adding a more diverse set of specific tRNA models, and enabling the ability to automatically scan for multiple types of tRNAs simultaneously.

The first example of improved tRNA detection performance was shown in the human genome. Recently, over 700 segments of nuclear mitochondrial sequences (NumtS) were found scattered across the human nuclear genome [2]. To detect these mitochondrial tRNA genes, we integrated the latest version of Infernal [3] into tRNAscan-SE and added mt-tRNA specific covariance models [4]. We identified 465 likely mt-tRNAs in the nuclear chromosomes, 33 of which are located outside of the previously noted NumtS regions. Notably, these nuclear-encoded mt-tRNAs are nearly as numerous as the updated set of 576 cytosolic human tRNA genes.

To provide easy access to the more complete collections of human and all other species' tRNA gene sets, we are expanding the Genomic tRNA Database [5] to include classifications of newly detected nuclear mt-tRNAs, distinct from cytosolic tRNAs. We also have worked with model species consortia to establish a new tRNA naming convention that is more informative and stable between genome sequence updates. New tRNA gene names will be included in the Genomic tRNA Database along with legacy names. These and other new enhancements of tRNAscan-SE and the Genomic tRNA Database will provide researchers more accurate detection and more comprehensive annotation for over a million tRNA genes.

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# POSTERS





## P1

## Predicting the minimal translation apparatus: Lessons from the reductive evolution of *Mollicutes*

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The minimal set of proteins that could sustain ribosome biogenesis and translation has been evaluated in *Mollicutes*. These are a class of bacteria that have evolved from *Firmicutes* ancestor by massive genome reduction. With genomes less than one Mbp in size, most species retain the capacity to grow autonomously. Using the experimentally validated genes from *E. coli* and *B. subtilis* as input, genes encoding proteins of the core translation machinery were predicted in 39 *Mollicutes* species. The set of selected 260 input genes encodes proteins involved in ribosome biogenesis, tRNA maturation/aminoacylation, protein cofactors and RNA decay. A core set of 104 proteins is found in all species analysed. Genes encoding proteins involved in post-translational modifications of r-proteins, translation cofactors, post-transcriptional modifications of t+rRNAs, ribosome assembly and RNA degradation are the most frequently lost. Only genes coding for aa-tRNA synthetases, r-proteins and translation factors are conserved in a majority of genomes. Enzymes introducing nucleotide modifications in the anticodon loop of tRNA, in helix 44 of 16S rRNA and in helices 69 and 80 of 23S rRNA are maintained in all species. Reconstruction of genome evolution in *Mollicutes* revealed that, beside many gene losses, occasional gains by HGT also occurred, attesting that slightly different solutions for preserving a functional, albeit minimal, protein synthesizing machinery have emerged during reductive evolution of *Mollicutes*. Implications in guiding the reconstruction of a minimal cell by synthetic biology approaches will be discussed.



## P2

## The identity elements for aminoacylation of metazoan mitochondrial tRNA<sup>Arg</sup> have been widely conserved throughout evolution

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Eumetazoan mitochondrial tRNAs possess idiosyncratic structures that require the specific recognition by their cognate nuclear-encoded aminoacyl-tRNA synthetases. The AGA (arginine) codon of the standard genetic code has been reassigned to serine in eumetazoan organelles and is translated in some organisms by a mitochondrially encoded tRNA<sup>Ser</sup><sub>UCU</sub>. Translation of arginine codons is performed in mitochondria by the single mitochondrial tRNA<sup>Arg</sup><sub>UCG</sub> isoacceptor. We have performed in vitro aminoacylation using a recombinant insect mitochondrial arginyl-tRNA synthetase. Base substitution and loop transplantation studies have revealed that recognition is defined by the anticodon loop in which C35 and G36 play a dominant role that is modulated by the nature of the base combination at position 32:38.

With a knowledge of these identity elements, one may consider their evolutionary functional relationship to other metazoan species. We tested mitochondrial tRNA transcript arginylation of nine representative eumetazoan species from six classes. The human tRNA<sup>Arg</sup> stood out in its substantially greater activity compared to the cognate insect tRNA. The others of this clade were generally slightly lower in their activity.

The metazoan kingdom also includes the Porifera (sponges) and Placozoa phyla. In contrast to eumetazoans, most members of both these phyla translate AGA as arginine and encode both tRNA<sup>Arg</sup><sub>UCU/UCG</sub> isoacceptors. Placozoan tRNA<sup>Arg</sup><sub>UCG</sub> (*Trichoplax adhaerens*) is a good substrate for insect mitochondrial arginyl-tRNA synthetase. The tRNA<sup>Arg</sup><sub>UCG</sub> from the Poriferan *Iotrochota birotulata* proved to be recognized by the insect mitochondrial arginyl-tRNA synthetase but the tRNA<sup>Arg</sup><sub>UCU</sub> transcript, having the U36 negative determinant was inactive. Yeast arginyl-tRNA synthetase will not arginylate insect mitochondrial tRNA<sup>Arg</sup>. However, the Poriferan and Placozoan mitochondrial tRNA<sup>Arg</sup><sub>UCG</sub> and tRNA<sup>Arg</sup><sub>UCU</sub> (with canonical-type tRNA structures) are good substrates. Having screened the genome sequence of the sponge *Amphimedon queenslandica* for the presence of arginyl-tRNA synthetases, we propose that in these organisms an imported cytoplasmic arginyl-tRNA synthetase replaces the dedicated mitochondrial form.



## P3

## A comprehensive evolutionary analysis uncovers an unexpected complexity of tRNA modifications in yeast

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Yeasts inhabit a wide range of ecological niches, requiring them to cope with a multitude of environmental stress factors. Thus, different strains - even within the same species - have adapted their cellular metabolism to specific types of stress. To elucidate how tRNA modification systems have evolved to modulate cellular stress adaptation, we quantitatively characterize modified tRNA nucleosides in a collection of yeast isolates from various habitats using moderate temperature stress as an experimental regime. To this end, we combine (i) affinity gel electrophoresis and Northern blotting, (ii) thin layer chromatography, and (iii) mass spectrometry. Importantly, this allows us to establish a quantitative, high-resolution inventory of tRNA modification levels at physiologically relevant growth temperatures in 12 representative yeast strains.

Applying this experimental approach we find that nucleoside modification levels vary significantly between different strains, suggesting that tRNA pools are not functionally equivalent even within the same species. Second, we are able to cluster all strains according to their modification levels. Third, we observe a global remodelling of tRNA modifications in response to heat stress. Interestingly, most tRNA modifications are reduced at high temperatures. However, certain methylations, such as 2'-O-methyladenosine (Am) and 3-methylcytidine (m<sup>3</sup>C), become more prominent. Finally, by performing a fine mapping of 5-methoxycarbonylmethyl-2-thiouridine (mcm<sup>5</sup>s<sup>2</sup>U) levels, we find that individual tRNAs are differentially modified in different yeast isolates.

Importantly, our high-resolution analysis will generate the first comprehensive species-wide quantitative analysis of tRNA modification. However, our work already reveals an unexpectedly elaborate nucleoside modification pattern in wild yeast, highlighting the intricate complexity and interplay of cellular stress response networks.



## P4

## Molecular characterization of *Naegleria gruberi* selenophosphate synthetase

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The selenocysteine (Sec), the 21<sup>st</sup> amino acid, is presented in selenoproteins, whose functions in cell growth and proliferation and redox balance have been described. Sec biosynthesis require a complex biosynthesis pathway. Sec is incorporated at an in frame UGA codon and an mRNA secondary structure named Sec Insertion Sequence Element (SECIS). All potential genes involved in selenocysteine pathway were identified in *Naegleria gruberi* genome, an organism belonging to the Jakobs Elegans Heteroloboseans (JEH) group. Among the identified proteins is selenophosphate synthetase (SPS), the enzyme that catalyzes the formation of monoselenophosphate, a highly reactive selenium donor compound. In *N. gruberi*, SPS is present as a gene fusion between two domains. The C-terminal domain exhibit identity with other SPS described and it is essential to selenocysteine incorporation and the N-terminal domain possess similarity with unicellular algae methyltransferases and probably is involved in selenium cell detoxification. SPS full length gene (*sps.fl*) and the C-terminal domain (*sps.d*) were cloned into pET32a(+) vector and expressed in *Escherichia coli* BL21 (DE3) cells. The C-terminal domain was analyzed by gel electrophoresis in native, nondenaturing conditions, and by dynamic light scattering (DLS). A 100KDa protein, representing a dimer in solution (approximately 47KDa per monomer) is observed, consistent with SPS homologues of *Trypanosoma brucei* and *Escherichia coli*. Polyclonal anti-rabbit antibodies have been produced against *N. gruberi* SPS.d and SPS.fl for immunolocalization and the characterization of the gene products along the developmental stages of *N. gruberi*. Thus, these findings bring a new perspective to the study of the origin and evolution of the selenocysteine incorporation machinery, and in particular may bring alternatives roles to SPS protein by investigating its association with methyltransferase, besides contributing to the comparative understanding of the pathway between the three domains of life.





## P5

## A genetic code without the sulfur containing amino acids

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At earlier stages in the evolution of the universal genetic code, fewer than 20 amino acids were considered to be used. Although this notion is supported by a wide range of data, the actual existence and function of the genetic codes with a limited set of canonical amino acids have not been addressed experimentally. Recently, we constructed artificial genetic codes involving a reduced alphabet [1]. In one of the codes, a tRNA<sup>Ala</sup> variant with the Trp anticodon reassigns alanine to an unassigned UGG codon in the Escherichia coli S30 cell-free translation system lacking tryptophan. We confirmed that the efficiency and accuracy of protein synthesis by this Trp-lacking code were comparable to those by the universal genetic code, by an amino acid composition analysis, GFP fluorescence measurements and the crystal structure determination. The generality of our method for the simplification allowed us constructions of further simplified codes including a 16-amino-acid code [2]. Another simplified genetic code without both of Ser and Met was also constructed by introduction of engineered initiator tRNA. This method will provide not only new insights into primordial genetic codes, but also an essential protein engineering tool for the assessment of the early stages of protein evolution and for the improvement of pharmaceuticals.

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**P6****Ancient genes: prediction, RNASeq detection, and ancestral tracing of tRNA in plantae**

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*Brachypodium distachyon* and *Oryza sativa* are widely studied monocots whose genomes have been sequenced and well annotated. We have predicted their tRNA gene repertoire using well-established algorithms, and the data was paired with RNAseq expression data obtained from plant seedlings to assess expressed tRNAs. We have created a new computer program that maps RNA-seq reads and detects expression of individual genes within isoacceptor families (because of sequence polymorphisms).

The predicted genes of these two plants were compared with all available plant genomes to develop a phylogeny of tRNA genes in Plantae. We were able to obtain a hypothesized progenitor tRNA sequence for every version of tRNA present in each of these two plants. Most of the progenitor sequences for these closely related plants also appear to be the progenitor sequences for Angiosperms and, in some cases, for land plants in general.

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## Intra-organism variation in tRNA structural type

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In addition to donating amino acids to protein synthesis, aminoacyl-tRNA participates in the modification of peptidoglycan precursors and lipids, tagging proteins for recycling, and antibiotic synthesis. Inhibition of protein synthesis by a novel class of boron-containing oxaborole antibiotics also requires tRNA.<sup>1</sup> While it is well established that structural features can be integral to tRNA performance throughout the translation pathway, there are only a few instances where distinctive structures are known prerequisites for tRNA entry into a non-canonical biosynthetic pathway. Indeed, it remains unclear whether these cases are the exception or the rule. As a first step in addressing this problem, we used bioinformatics to identify unusual tRNA structures. Until now, it seemed that bacteria only encode a type II (long stem-loop) structural form of leucine, serine, and tyrosine tRNAs. Yet because tRNA<sup>Tyr</sup> is type I (short variable loop) in the archaea and eukarya, it is clear that type switching has occurred over the course of evolution. We now report the unexpected phenomenon of intra-organism type variation in which the chromosomes of some medically important organisms encode both type I and type II leucine tRNAs. This is particularly surprising given that these organisms encode a single form of the cognate synthetase. We are currently evaluating the role that this structural variation plays in protein synthesis and other tRNA-dependent biochemical transformations.

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## P8

***In vivo* function of RtcA in tRNA processing**

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The human genome encodes for 506 tRNA genes of which 6% contain an intron. The tRNA splicing endonuclease (TSEN) complex is responsible for removing introns out of pre-tRNAs. After splicing, the ligation of the two tRNA halves is facilitated by either CLP1 and a still unknown tRNA ligase or possibly by RtcA and RtcB.

In humans, mutations in genes encoding for the TSEN complex and in *CLP1* result in pontocerebellar hypoplasia (PCH), a heterogeneous group of neurological disorders with a prenatal onset. Knockdown of the *tSEN* genes or *clp1* in zebrafish results in abnormal brain development.

The exact *in vivo* function of RtcA remains elusive. The two tRNA halves resulting from intron removal by the TSEN complex are possibly ligated directly by RtcB. First the 5'-half tRNAs' 2',3'-cyclic phosphate can be cleaved to form a 3'-end phosphate or a 2'-end phosphates. The 3'-end phosphate is ligated to the hydroxyl group of the 3'-half tRNA. However the 2'-end phosphate cannot be ligated and is cyclized by RtcA, thereby rescuing aberrant RtcB product. To further assess the tRNA splicing and ligation pathway we have made a zebrafish knockdown model for *RtcA* and *RtcB*. Knockdown of *RtcA* in zebrafish results in defects in brain development, resembling PCH, whereas knockdown of *RtcB* gives no phenotype. Furthermore, we have identified sequence variants in the *RtcA* gene in several families with PCH.

*RtcA* is another PCH related gene involved in tRNA processing. This finding invigorates that proper tRNA processing is essential for cerebellar development and enables us to start revealing the underlying mechanism further.

**Acknowledgements**

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## Identification of a new folate-dependent rRNA methyltransferase that catalyses m<sup>5</sup>U1939 modification in 23S Rrna

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Efficient protein synthesis in all organisms requires the post-transcriptional methylation of specific rRNA and tRNA nucleotides. Enzymes that use S-adenosylmethionine as the methyl group donor almost invariably catalyse the t+rRNA methylation reactions. One noteworthy exception is seen in some bacteria, where the TrmFO enzyme adds the conserved tRNA methylation at m<sup>5</sup>U54 of the T-loop using N<sup>5</sup>, N<sup>10</sup>-methylenetetrahydrofolate as the one-carbon donor. The minimalist bacterium *Mycoplasma capricolum* possesses two homologs of TrmFO (COG1206), but surprisingly lacks the m<sup>5</sup>U54 tRNA modification. We created a single and dual deletion(s) of the *trmFO* genes using a novel synthetic biology approach (1). Subsequent analysis of the *M. capricolum* RNAs by mass spectrometry shows that the TrmFO homolog encoded by Mcap0476 specifically modifies m<sup>5</sup>U1939 in 23S rRNA. This conserved methylation is catalysed by AdoMet-dependent enzyme (RlmD or RlmCD, COG2265) in all other characterized bacteria, the corresponding gene being missing in the genome of *M. capricolum*. These findings suggest that during evolution some of the COG1206 enzymes have undergone a series of changes in target specificity, and that their functions have converged to find independent solutions to adding what are (presumably) important tRNA and/or rRNA modifications. The Mcap0476 methyltransferase (renamed RlmFO) represents the first folate-dependent enzyme seen to modify rRNA. The target nucleotide of the second TrmFO orthologous Mcap0613 is still under investigation.

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**P10**

## Biosynthesis of wyosine derivatives in tRNA<sup>Phe</sup> of Archaea: Role of a remarkable bifunctional tRNA<sup>Phe</sup>:m<sup>1</sup>G/imG2 methyltransferase

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The presence of tricyclic wyosine derivatives located 3'-adjacent to the anticodon is a hallmark of tRNA<sup>Phe</sup> in Eukarya and Archaea. In yeast, formation of wybutosine (yW) results from five enzymes acting in a strict sequential order. In Archaea, the intermediate compound imG-14 (4-demethylwyosine) is a target of three different enzymes leading to the formation of three distinct wyosine derivatives (yW-86, imG, and imG2). Based on our previous experimental data and comparative genomics analysis, we predicted the existence in several archaeal species of a peculiar Trm5-like methyltransferase (aTrm5a) displaying a dual-specificity. Combining a thin-layer chromatography and HPLC/MS analysis, we now demonstrate that the recombinant pfTrm5a of *Pyrococcus abyssi* catalyses in vitro both the N<sup>1</sup>-methylation of guanosine (producing m<sup>1</sup>G) and the C<sup>7</sup>-methylation of imG-14 (producing imG2). This last compound is the intermediate of the 7-methylwyosine (mimG) of the archaeal biosynthetic pathway. Based on the formation of mesomeric forms of imG-14, a rationale for such dual enzymatic activities is proposed. This bifunctional tRNA:m<sup>1</sup>G/imG2 methyltransferase, acting on two chemically distinct guanosine derivatives located at the same position of pre-tRNA<sup>Phe</sup>, is unique to a few Archaea and has no homologues in Eukarya. This aTrm5 enzyme belongs to the same family of enzymes (COG 2520) that catalyses the addition of amino-carboxypropyl group (acp) on C<sup>7</sup>-atom of imG-14 of the wye-base metabolism in yeast (Tyw2) and in certain Archaea (Taw2). This bifunctional m<sup>1</sup>G/imG2 methyltransferase, renamed as Taw22, probably played an important role in the emergence of the sequential multistep biosynthetic pathway of wyosine derivatives in Archaea and in Eukarya.



## P11

## Structural characterisation of two homologous 2'-O-methyltransferases showing different specificities for their tRNA substrates

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The 2'-O-methylation of the nucleoside at position 32 of tRNA is found in organisms belonging to all domains of life. Unrelated enzymes catalysing this modification in Bacteria (TrmJ) and Eukarya (Trm7) have been identified, but until now no information is available for the archaeal enzyme. We have identified the methyltransferase of the archaeon *Sulfolobus acidocaldarius* responsible for the 2'-O- methylation at position 32, which is a homologue of the bacterial TrmJ. Remarkably, both enzymes have different specificities for the nature of the nucleoside at position 32. While the four canonical nucleosides are substrate of the *E. coli* enzyme, the archaeal TrmJ can only methylate the ribose of a cytidine. Moreover, the two enzymes recognise their tRNA substrates in a different way. We present the first crystal structures of the catalytic domain of both the archaeal and bacterial TrmJ enzyme, which together with biochemical data, aided in unravelling the molecular determinants of the difference in specificity. Both TrmJ proteins are dimeric and consists of 2 autonomously folding domains in which the N-terminal domain adopts a SPOUT fold and the C-terminal domain is predominately helical in conformation. Interestingly, the difference in substrate specificity might be linked to a different conformation of the ligand SAM, which adopts an unusual conformation in *E. coli* TrmJ.



## P12

## NMR conformational dynamics of La protein domains in interaction with pre-tRNA ligands from a lower eukaryote exhibiting identical structural organization with its human homolog

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The La autoantigen, a key protein for proper folding of pre-tRNA transcripts, encompasses a characteristic La motif, an N-terminal RRM1 domain conserved in higher eukaryotes and a C-terminal RRM2 domain absent in lower eukaryotes. So far, the full-length La protein structure along with the role of RRM2 domain, remain elusive. Scattered structural data of the La and the RRM motifs from few eukaryotes (including human) provide only limited information on the possible roles of the La protein as a whole, in a more dynamic tRNA-dependent cellular network. Therefore, we initiated an extensive structural and functional characterization of a La "domain library" from the lower eukaryote *Dictostelium discoideum*, which interestingly contains both RRM domains unlike yeast. We present the NMR structure of La and RRM1 motifs and preliminary data from RRM2 domain. La and RRM1 motifs were found well-folded in "winged-helix" and classical RRM structures, respectively, as revealed by high resolution NMR spectroscopy. In addition, the distribution of secondary structure elements of RRM2 was  $\alpha\beta1\alpha1\beta2\beta3\alpha2\beta4\alpha3$ , similar to the NMR structure of human RRM2 domain. Finally, the RNA binding properties of the La motif were investigated and the interaction interface was identified through chemical shift perturbation of amide groups in  $^1\text{H}$ - $^{15}\text{N}$  HSQC spectra. Interestingly, both NMR analysis and biochemical experiments indicate that La motif alone can mediate interaction with pre-tRNAs, an observation which raises questions on the actual role of La motif in combination with the two RRM motifs, during tRNA biogenesis in this organism.

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## P13

## Peculiarities of queuosine biosynthesis in trypanosomes

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A general feature of tRNAs is a high number of nucleotide chemical modifications that are introduced post-transcriptionally. Queuosine (Q) is one of the most complex tRNA modifications found at the first position of the anticodon (wobble base) of several tRNAs. Despite its omnipresence in bacteria and eukaryotes, the function of Q in tRNA is not completely clear. In this study, we have used the protozoan parasite *Trypanosoma brucei* as a model for a comprehensive analysis of the tRNA guanine transglycosylase (TGT), the enzyme responsible for Q-tRNA formation in eukaryotes. Unlike its bacterial counterpart, in most eukaryotes TGT predominantly functions as a heterodimeric enzyme. In order to investigate the composition and function of the trypanosomal TGT, we used the sequence of the human TGT to search for potential homologues in the *T. brucei* genome. Analogous to humans, is the presence of two homologues of the TGT enzyme (TbTGT1 and TbTGT2) in *T. brucei*. However, we showed using RNAi knock-down strategy that only the most divergent TbTGT2 is responsible for Q-tRNA formation, while Q-tRNA levels in the RNAi cells of the conserved subunit TbTGT1 remained minimally affected. This striking observation suggests that in contrast to mammals, the trypanosomal TGT subunits don't form a complex. Furthermore, we propose that TbTGT1 subunit is involved in the recycling and salvaging of queuine from (QMP). These observations are discussed in the context of the possible roles of the differential intracellular localization of Q-tRNA and modification enzymes in respect to their role in cytosolic and mitochondrial translation.

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**P14****The differential activity of tRNA modifying enzyme: the role of anticodon stem loop sequence****Bhavik Sawhney<sup>1</sup>, N Saraswathi<sup>1</sup>, Akash Ranjan<sup>\*1</sup>**

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Post-transcriptional modifications of tRNA play a crucial role in its acquisition and stabilization of three dimensional structure. Modifications at and around wobble position is of immense functional importance because of their profound influence on translational fidelity and efficacy. The conversion of adenosine at wobble position to inosine, catalysed by tRNA adenosine deaminase, augments the decoding capacity of a tRNA molecule. This enzyme acts as a homodimer complex of TadA in prokaryotes, and hetero-dimer complex of ADAT2/3 in eukaryotic organisms. In the present study, conducted on *P. falciparum*, possible correlation between conversion of A to I at the wobble position in deciphering iso-acceptor codons was examined. Furthermore, the function of individual constituent subunits (ADAT2 and ADAT3) of this hetero-dimeric enzyme in selective binding to tRNA and in catalysis was delineated. In addition to identify the pertinent residues of anticodon stem loop which discriminates between the substrate vs non-substrate, it was also established that the degree to which a particular tRNA molecule undergoes modification is intrinsically linked with the residues at 3' end of wobble base. The rationale about ineptness of prokaryotic enzyme to act on eukaryotic tRNA molecules, while maintaining its absolute specificity towards a single tRNA molecule, i.e. Arg tRNA<sup>ACG</sup> was determined to be attributed by presence of pyrimidine at 3' end adjacent to adenosine in Arg tRNA<sup>ACG</sup>.

Additionally, we have also identified tRNA guanosine transglycosylase in *P. falciparum* that displays a greater phylogenetic resemblance with the prokaryotic enzyme. Computational analysis suggested that this enzyme had much higher binding affinity with quinazoline derivatives in comparison to human counterpart.

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## P15

## Pre-tRNA capping

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Efficient maturation of tRNAs is required for rapid cell growth. In eukaryotes, a primary tRNA transcribed by RNA polymerase III is subjected to consecutive processing events, including removal of 5' leader and 3' trailer sequences, addition of CCA sequence to the 3' terminus, and decoration with various post-transcriptional modifications. If tRNA has an intron, it should be removed by tRNA splicing. However, precise timing of tRNA processing associated with the order of tRNA modifications remains elusive. To analyze the modification status during tRNA maturation, tRNA precursors at various stages were isolated from *Saccharomyces cerevisiae*, and subjected to mass spectrometric analysis. Intriguingly, we detected 5' cap structure with methylated guanosines at the 5' terminus of the tRNA precursor bearing 5' leader sequence. The capped pre-tRNAs were significantly accumulated by the inhibition of RNase P activity. When the temperature-sensitive mutant of Ceg1p capping enzyme (*ceg1<sup>ts</sup>*) was cultured at non-permissive temperature, the steady-state level of the capped pre-tRNA was markedly reduced. In addition, the capped pre-tRNAs were accumulated in  $\Delta$ *MET22* strain in which 5' exonucleases are inhibited, indicating that the 5' cap structure plays a role in protecting pre-tRNA from 5' exonucleolytic degradation during maturation.

**P16****A complete landscape of post-transcriptional modifications in mammalian mitochondrial tRNAs****Takeo Suzuki and Tsutomu Suzuki\****Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo**Email: [ts@chembio.t.u-tokyo.ac.jp](mailto:ts@chembio.t.u-tokyo.ac.jp)*

In mammalian mitochondria, 22 species of tRNAs encoded in mitochondrial DNA play crucial roles in the translation of 13 essential subunits of the respiratory chain complexes involved in oxidative phosphorylation. Following transcription, mitochondrial tRNAs (mt tRNAs) are modified by nuclear-encoded tRNA-modifying enzymes. These modifications are required for the proper function of mt tRNAs, and the absence of these modifications can cause pathological consequences. To date, however, the information available about these modifications has been incomplete. To address this issue, we isolated all 22 species of mt tRNAs from bovine liver and comprehensively determined the post-transcriptional modifications in each tRNA by mass spectrometry. Here, we describe the primary structures with post-transcriptional modifications of 7 species of mt tRNAs which were previously uncharacterized, and provide revised information regarding base modifications in 5 other mt tRNAs. In the complete set of bovine mt tRNAs, we found 15 species of modified nucleosides at 118 positions (7.48% of total bases). This result provides insight into the molecular mechanisms underlying the decoding system in mammalian mitochondria and enables prediction of candidate tRNA-modifying enzymes responsible for each modification of mt tRNAs.



## P17

## How does the folate dependent tRNA (m<sup>5</sup>U54) methyltransferase (TrmFO) recognize substrate tRNA?

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Transfer RNA contains various modified nucleotides, which are introduced by site-specific tRNA modification enzymes. In particular, methylated nucleosides such as m<sup>5</sup>U, m<sup>1</sup>A, m<sup>7</sup>G, and m<sup>5</sup>C are most fundamental modifications, which are found in three domains of life. Folate dependent tRNA methyltransferase (TrmFO) converts uridine at position 54 in tRNA to 5-methyluridine using N<sup>5</sup>, N<sup>10</sup>-methylenetetrahydrofolate as a methyl group donor. In 2009, we determined the crystal structure of *Thermus thermophilus* TrmFO. Furthermore, alanine substitution analysis enabled us to predict the catalytic reaction and tetrahydrofolate binding mechanism<sup>(1)</sup>. However the substrate tRNA recognition mechanism was still unknown. To address this issue, we designed 42 tRNA mutant variants and measured their methyl group acceptance activity. The results indicated that TrmFO essentially recognizes the conserved U54U55C56 sequence and G53-C61 base pair in the T-arm. Furthermore, we found that A38 prevents the incorrect methylation of U32 in the anticodon-loop. Moreover, we found that the existence of m<sup>1</sup>A58 modification accelerates the TrmFO reaction. This result suggests that there is a synergistic effect of m<sup>5</sup>U54, m<sup>1</sup>A58 and s<sup>2</sup>U54 modifications on m<sup>5</sup>s<sup>2</sup>U54 formation. Based on these results, we proposed a docking model of TrmFO-T-arm complex<sup>(2)</sup>.

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**P18****Alteration of the solid-phase DNA probe method for large-scale tRNA purification****Ai Kazayama<sup>1</sup>, Ryota Yamagami<sup>1</sup>, Takashi Yokogawa<sup>2</sup>, and Hiroyuki Hori<sup>1</sup>**<sup>1</sup> Ehime University, Bunkyo 3, Matsuyama, Ehime 790-8577, Japan<sup>2</sup> Gifu University, Yanagido 1-1, Gifu, Gifu 501-1193, JapanHiroyuki Hori: [hori@eng.ehime-u.ac.jp](mailto:hori@eng.ehime-u.ac.jp)

To clarify how tRNA works on protein synthesis systems, it is necessary to purify specific tRNA species. For this purpose, the solid-phase DNA probe method has been already established. A specific tRNA is recovered from tRNA mixture via the principle of hybridization. In this method, DNA probe with 3'-biotin, which contains a complementary nucleotide sequence of target tRNA, is used. Although this method is convenient, there are some problems. In the case of tRNA purification from thermophiles, modified nucleosides have effects on the stability of tRNA-DNA duplex and recovery of tRNA is difficult. This problem was solved by using tetraalkylammonium salts [1]. Indeed, this method enabled us to purify tRNAs from thermophiles. However, because yield of purified tRNA was not so large, repeated purification steps were required. In the current study, we tried to improve the solid-phase DNA probe method for large-scale preparation of tRNA.

Initially, we used a pre-packed column in an incubator instead of Eppendorf tubes. Then, we optimized several conditions such as temperature, incubation time, flow rate, and order of the connected equipment. These alterations were succeeded to establish the large-scale tRNA purification method. This new method requires only a small number of manual operations and is simpler as compared to the previous method. Furthermore, the yield of purified tRNA was improved by approximately 15 times. Now we are trying to develop a new method for tRNA purification, in which the existence of modification(s) in tRNA is distinguished by solid-phase DNA probes.

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## P19

**CmoM, the novel methyltransferase responsible for the last step of uridine-5-oxyacetic acid methyl ester (mcmo<sup>5</sup>U) biogenesis****Yusuke Sakai, Kenryo Miyachi, Satoshi Kimura and Tsutomu Suzuki\****Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, Bunkyo-ku, Tokyo, Japan**\* E-mail: ts@chembio.t.u-tokyo.ac.jp*

Uridine-5-oxyacetic acid (cmo<sup>5</sup>U) is found at the wobble position of tRNAs for Leu, Val, Ser, Pro, Thr and Ala in gram negative bacteria. In tRNAs for Ser and Ala, cmo<sup>5</sup>U is further methylated to form uridine-5-oxyacetic acid methyl ester (mcmo<sup>5</sup>U). These modified uridines enable non-Watson-Crick base pairing with pyrimidines at the third position of codons so as to expand the decoding capacity. Therefore, the tRNAs with these wobble modifications can read any of four codons in the family boxes.

It is known that two enzymes CmoA and CmoB cooperatively synthesize cmo<sup>5</sup>U from 5-hydroxyuridine (ho<sup>5</sup>U) (Näsvall *et al.* 2004, Kim *et al.* 2013, Byrne *et al.* 2013). CmoA synthesizes S-adenosyl-S-carboxymethyl-L-homocysteine (SCM-SAH) from S-adenosylmethionine (SAM) and prephenate. CmoB employs SCM-SAH and catalyzes a carboxymethyltransfer reaction to convert ho<sup>5</sup>U to cmo<sup>5</sup>U at the wobble position of tRNA. However, the enzymes responsible for the hydroxylation at the initial reaction and the methyl ester formation of mcmo<sup>5</sup>U are still missing. We here report cmo<sup>5</sup>U methyltransferase (CmoM) responsible for converting cmo<sup>5</sup>U to mcmo<sup>5</sup>U in *Escherichia coli*. By mass spec analysis of wobble modifications in the individual tRNAs isolated from *E. coli*, we detected mcmo<sup>5</sup>U as a major wobble modification in tRNA<sup>Thr4</sup> and tRNA<sup>Pro3</sup>, in addition to tRNA<sup>Ser1</sup> and tRNA<sup>Ala1B</sup>. mcmo<sup>5</sup>U in these tRNAs was completely converted to cmo<sup>5</sup>U in  $\Delta$ cmoM strain. In addition, we successfully reconstituted mcmo<sup>5</sup>U from cmo<sup>5</sup>U on tRNA<sup>Ser1</sup> *in vitro* with recombinant CmoM in the presence of SAM.



## P20

## Topological knot tRNA methyltransferase (TrmH) discriminates substrate tRNA from non-substrate tRNA by a multistep recognition mechanism

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A conserved guanosine at position 18 (G18) in the D-loop of tRNAs is often modified to 2'-O-methylguanosine (Gm). Formation of Gm18 in eubacterial tRNA is catalyzed by tRNA (Gm18) methyltransferase (TrmH) [1]. Our previous crystal study revealed that *T. thermophilus* TrmH is a Class IV AdoMet-dependent methyltransferase, which maintains a topological knot (trefoil knot) structure in the catalytic domain [2]. Because TrmH enzymes have short stretches at the N- and C-termini instead of a clear RNA binding domain, these stretches are believed to be involved in tRNA recognition [3-5]. In the current study, we demonstrate that both N- and C-terminal regions really function in tRNA binding. However, *in vitro* and *in vivo* chimera protein studies demonstrated that the catalytic domain discriminates substrate tRNAs from non-substrate tRNAs. Thus, the N- and C-terminal regions do not function in the substrate tRNA discrimination process. Pre-steady state analysis of complex formation between mutant TrmH proteins and tRNA by stopped-flow fluorescence measurement revealed that the C-terminal region works in the initial binding process, in which non-substrate tRNA is not excluded, and that structural movement of the motif 2 region of the catalytic domain in an induced-fit process is involved in substrate tRNA discrimination [6, 7].

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## P21

## Proteinaceous vs. bacterial RNase P: a comparative mechanistic analysis

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Recently, a new type of RNase P was found in human mitochondria, land plants and kinetoplastida, which lacks an RNA subunit and consists of 1 to 3 polypeptides, thus termed proteinaceous RNase P (PRORP) [1-3]. For bacterial RNase P, single-site *Rp*- or *Sp*-phosphorothioate modification at the canonical cleavage site in precursor tRNA substrates in combination with metal ion rescue experiments indicated that the *pro-Rp* oxygen is coordinated by two magnesium ions in the transition state [4]. Additional active site constraints could be inferred from kinetic studies using 2'-ribose modifications at the -1 nucleotide, such as 2'-H, 2'-F, 2'-NH<sub>2</sub> or 2'-OCH<sub>3</sub> substitutions [5]. Based on the crystal structure of *Arabidopsis thaliana* PRORP1, a mechanistic model was proposed, involving three active site aspartates and two metal ions that simultaneously interact with the *pro-Sp* oxygen in the transition state [6].

Here, we studied the effects of the abovementioned modifications on the cleavage kinetics by PRORP. We could show that cleavage by PRORP is essentially unaffected by an *Rp*-phosphorothioate at the cleavage site, in sharp contrast to bacterial RNase P [7]. An *Sp*-phosphorothioate, although inhibitory, was not amenable to rescue by thiophilic metal ions. Thus, potential direct metal ion coordination to the *pro-Sp* oxygen remains elusive at present. Kinetic studies using 2'-ribose modifications are currently underway to shed more light on active site constraints in reactions catalyzed by PRORP enzymes.

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**P22****A rapid semiquantification of tRNA precursors in human cultured cell lines****Tamara Fernández, Florencia Cabrera, Danilo Segovia, Mónica Marín***Sección Bioquímica -Biología Molecular, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay. [marin@fcien.edu.uy](mailto:marin@fcien.edu.uy)*

Most amino acids are encoded by more than one codon. Synonymous codons are not used with identical frequency. Organisms exhibit a preferential usage of one codon over the others for encoding the same amino acid. Codon usage bias can be influenced within and among species by several factors such as expression level, GC content, recombination rates, RNA stability, splicing pattern, gene length and others. Additionally, a role for codon usage bias has been proposed related to translation kinetics and protein folding during in vivo biosynthesis. In particular, frequently used codons were associated to abundant tRNAs, highly expressed proteins, translation accuracy and protein folding. Therefore, the analysis of tRNA abundances in light of codon usage is especially important. However, the experimental measure of tRNA abundance has been a challenge difficult to overcome. For this purpose, some experimental approaches have been described: the separation of tRNAs by two-dimensional polyacrylamide gel electrophoresis; an approach based on microarrays with probes specific to individual tRNAs, and recently, relative quantitation of tRNAs using liquid chromatography mass spectrometry and signature digestion products. In this work, we assayed an experimental approach for a rapid evaluation of the expression level of proline-tRNAs precursors, in human cells. Proline can be encoded by 4 codons used with different frequency (CCC, 1.98; CCG, 0.69; CCU, 1.75; CCA, 1.69). In the human genome, Pro-tRNAs are encoded by 21 genes and 1 pseudogene. We assayed an experimental method based on qPCR and sequencing and discuss preliminary results obtained in the determination of precursors expression levels in two human derived cell lines.



## P23

## Unprecedented archaeal tRNA modifications found in *Thermoplasma acidophilum*

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*Thermoplasma acidophilum* is a thermo-acidophilic archaeon, and there is little knowledge concerning its tRNA modifications except for early studies [1-3]. In 1991, Edmonds *et al.* reported that a tRNA mixture from *T. acidophilum* contains *N*<sup>7</sup>-methylguanine (m<sup>7</sup>G) [3]. The m<sup>7</sup>G modification is commonly found at position 46 in cytoplasmic tRNAs from eubacteria and eukaryotes and found in anticodon-loop in tRNAs from organelle [4, 5]. Thus, the m<sup>7</sup>G modification in *T. acidophilum* tRNA is an exception in archaeal tRNA. However, for more than 20 years, the position of m<sup>7</sup>G modification in *T. acidophilum* tRNA has remained to be unidentified. In the current study, we therefore focused on the m<sup>7</sup>G modification in *T. acidophilum* tRNA.

We purified tRNA<sup>Leu</sup> (UAG) from *T. acidophilum* using a solid-phase DNA probe method and determined the RNA sequence after determining that this tRNA contains m<sup>7</sup>G *via* nucleoside analysis and m<sup>7</sup>G-specific aniline cleavage. RNA sequencing and liquid chromatography-mass spectrometry revealed that the m<sup>7</sup>G modification exists at a novel position 49 although this tRNA<sup>Leu</sup> (UAG) belongs to class II tRNA, which has a long variable region. Furthermore, we found several distinct modifications, which have not been found in archaeal class II tRNA, such as 4-thiouridine9 (s<sup>4</sup>U9), archaeosine13 (G<sup>+</sup>13), *N*<sup>2</sup>, *N*<sup>2</sup>-dimethylguanosine26 (m<sup>2</sup><sub>2</sub>G26), and 5-carbamoylmethyluridine34 (ncm<sup>5</sup>U34) [6].

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## P24

## Substrate recognition by proteinaceous RNase P

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RNase P is ubiquitously responsible for the 5' processing of tRNA primary transcripts. For more than two decades, the presence of a catalytic RNA subunit was thought to be the hallmark of this class of enzymes; such RNA-based RNases P are found in Bacteria, Archaea and Eukarya. However, the recent discovery of the protein-based human mitochondrial RNase P and related single-protein enzymes in plants and protists indicated that proteinaceous/protein-only RNase P (PRORP) enzymes are common as well, at least in Eukarya. The fundamental difference in molecular composition and the apparently independent evolutionary origin raise the question whether the different types of RNase P enzymes use similar or different mechanisms of substrate recognition and catalysis. However, while substrate recognition and catalysis by (bacterial) RNA-based RNase P are well characterized, such information is scarce for proteinaceous RNase P. Here we report the results of an in-depth analysis of substrate recognition and cleavage site selection by PRORP3 from *Arabidopsis thaliana*. Starting from a well-studied and conformationally stable class I tRNA, we systematically deleted or varied conserved structural elements and nucleotide identities. The kinetic parameters of cleavage of the substrate variants were determined under *single turnover* conditions. This enabled us to pinpoint specific structural determinants for substrate binding and positioning of the cleavage site, and allowed us to define minimal substrates for PRORP. Our findings indicate that substrate recognition by PRORP is mechanistically more similar to that of the RNA-based eukaryal nuclear enzymes than to recognition by bacterial RNase P.



## P25

## Control of pre-tRNA processing by environmental conditions in *S.cerevisiae*

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Multiple studies have shown that both tRNA transcription and tRNA modifications change under various growth conditions. In contrast, little is known how primary steps of tRNA maturation in the nucleus are affected by stress. In this work we studied the response of end-processing of intron-containing yeast tRNAs to heat shock and change from fermentable to nonfermentable carbon source.

By Northern analysis we show that upon shift to elevated temperature and/or glycerol-containing medium the pattern of tRNA precursors change in a tRNA-type and condition-specific manner. Under stress conditions additional forms of pre-tRNAUAIle and pre-tRNAUUULys were observed; they migrate slower than the forms previously designated as the primary transcripts. Similar aberrant precursors were detected in *rex1D* strain that is defective in exonucleolytic trimming of tRNA 3' ends. To verify that tRNA end processing in a wild type strain is affected by stress we employed tRNA circularization, RT, and subsequent DNA sequencing. The results suggest that the pre-tRNAUAIle 3' trailer is trimmed while 5' leader is still present even though it is generally thought that 5' processing precedes 3' processing. These results were confirmed by additional sequencing employing linker ligation to the 3' tRNA ends. Moreover, accumulation of tRNAUAIle precursor longer forms was detected in yeast grown under stress conditions, indicating lower efficiency of 3' processing. To exclude that observed phenotype is caused by transcription termination defects we sequenced pre-tRNAUAIle from *rex1D* strain. These results show that under stress most tRNAs had longer trailers. We are in process of deciphering the mechanism by which tRNA end processing is regulated by stress.

**P26****The threonylcarbamoyladenosine (t<sup>6</sup>A) tRNA modification is universally conserved but not universally essential**

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The threonylcarbamoyladenosine is a complex modification occurring at position 37 of tRNAs decoding ANN. The t<sup>6</sup>A biosynthesis pathways have now been identified. Two families, the TsaC/Sua5 and the TsaD/Kae1 are conserved across kingdoms and are at the core of the synthesis machinery, whereas the TsaB/E in bacteria and the KEOPS subunits in Eukaryotes are kingdom specific. The mitochondrial system is minimal and only requires the TsaD homolog Qri7 together with a TsaC/Sua5 homolog. However, the question of the essentiality of the genes and/or the modification has remained controversial. While in Yeast, t<sup>6</sup>A is essential in the mitochondria; it appears dispensable in the cytoplasm. Similarly, in some bacteria, such as *E. coli*, the modification/genes appear to be essential, but viable knockout mutants of t<sup>6</sup>A genes have been reported in other systems. We sought to understand this discrepancy and propose hypotheses explaining why some organisms cannot survive in the absence of t<sup>6</sup>A while others can. This is particularly relevant, as the t<sup>6</sup>A pathway genes had been proposed as an anti-bacterial target, even before the elucidation of the biosynthesis pathways.



## P27

**Psychrophilic tRNA nucleotidyltransferases: new insight into flexibility and fidelity****Felix G. M. Ernst, Joana Sammler, Heike Betat and Mario Mörl\****Institute for Biochemistry, University of Leipzig, Brüderstr. 34, 04103 Leipzig, Germany**Email: mario.moerl@uni-leipzig.de*

During evolution, organisms showed remarkable capabilities to adapt to cold habitats including oceans, polar and permafrost regions, representing the majority of the earth surface. In such psychrophilic organisms, cold adaptation of enzymatic activities is achieved by reducing optimal reaction temperatures via an increased structural flexibility of the corresponding proteins. One enzyme with certain flexible elements even in meso- or thermophilic organisms is tRNA nucleotidyltransferase, which is responsible for addition and maintenance of the CCA-terminus of tRNAs. Here, we investigate the molecular adaptation of this type of enzyme and the corresponding consequences in fidelity in several psychrophilic bacteria.

Sequence alignments indicate that even in the psychrophilic enzymes, the active site motifs A to E show a very high level of conservation. Hence, as the recombinant CCA-adding enzymes from two psychrophilic bacteria show a lowered optimal reaction temperature compared to mesophilic and thermophilic counterparts, cold adaptation obviously involved less conserved protein regions. Furthermore, the unfolding properties of these enzymes matched their activity temperature profiles. An initial analysis of the polymerization fidelity indicates that for one of the psychrophilic tRNA nucleotidyltransferases, the cold adaptation comes at the price of additionally incorporated nucleotides, leading to a considerable amount of heterogeneous tRNA 3'-ends. Whether an increased flexibility of the catalytic core or the tRNA binding region of this enzyme is responsible for this reduced fidelity is currently not known.

**P28**

## Biochemical characterization of Sua5, a universal protein present in the last universal common ancestor

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All living organisms, archaea, bacteria and eukaryotes, descend from a Last Universal Common Ancestor (LUCA). A number of laboratories are currently working on universal proteins (present in all living beings) in order to try to reconstruct the LUCA cell. We are studying two universal proteins which are involved, together with other accessory proteins, in the synthesis of a modified base, called t<sup>6</sup>A, present in all tRNAs that read codons ANN. This modification is essential for a correct reading of the genetic code and its lack is deleterious for cells.

One of these universal proteins exhibits two forms: short form called YrdC and long form called Sua5. Sua5 proteins are composed of a catalytic YrdC-like domain which is connected to a small globular domain of unknown function via a flexible linker. This domain is always associated to YrdC-like domain and has a discontinuous and puzzling phylogenetic distribution across the three domains of life. It is present in some archaea, yeast and several parasites such as *Plasmodium falciparum* (which causes malaria) but it is absent in humans. Our preliminary data show that in archaea, this particular non-catalytic domain of Sua5 is essential for its activity.

We have recently resolved the structure of Sua5 from the hyperthermophilic archaeon *Pyrococcus abyssi* including, for the first time, the linker. In this structure the linker is positioned in close proximity to the substrate binding site and we hypothesize that it might have a role of a gating loop. In order to test this hypothesis we have recently produced several mutants of Sua5 from *P. abyssi* and we are currently pursuing their biochemical characterization. The latest results issued from this work will be presented. We anticipate that this work will contribute significantly to the understanding of the catalytic mechanism of Sua5 proteins and will help evaluate the validity of these proteins as antiparasitic drug targets.





## P29

## Where have all the inosines gone? Conflicting evidence for A-to-I editing of the anticodon of eukaryotic tRNA<sup>Arg</sup><sub>ACG</sub>

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Codon-anticodon recognition between triplets of an mRNA and a specific tRNA is the key element in the translation of the genetic code. In general, the precision of this process is dominated by a strict Watson-Crick base-pairing scheme. However, the degeneracy of the genetic code led Crick to propose the Wobble Hypothesis, permitting a less restraining interaction with the third base of the codon and involving the participation of inosine for decoding C-ending codons.

The validity of this insight has subsequently been confirmed. However, in the case of the tRNA<sup>Arg</sup><sub>ACG</sub> isoacceptors only a single higher eukaryotic tRNA sequences (mouse) is available and a few exceptions from other sources (archaea, the mitochondria of single-cell organisms and metazoan mitochondria) are known to lack inosine at position 34 of tRNA<sup>Arg</sup><sub>ACG</sub>.

We have recently established that in higher plant cytoplasmic tRNA<sup>Arg</sup><sub>ACG</sub> no A-to-I deamination of the anticodon occurs [1] unless the gene is expressed in *E.coli*. A screening of the sequence of this tRNA from various sources by the accepted RNA-targeted reverse transcription/PCR technique has surprisingly revealed the absence of inosine in the anticodon of the cytoplasmic tRNA<sup>Arg</sup><sub>ACG</sub> in nematode, bovine, murine and coleopteran total tRNA preparations.

These results conflict with the conventionally sequenced isoacceptor from mouse leukaemia cells [2] and with the recent analysis of human tRNA using the inosine-specific endonuclease V [3]. We are currently unable to resolve this discrepancy.

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**P30****Pus3p function becomes essential for yeast viability when the Elongator complex subunit Elp1p is mutated****Emmanouil Venieris and George Simos\****Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece**\*simos@med.uth.gr*

*PUS3* encodes the enzyme that introduces pseudouridines ( $\Psi$ ) at position 38 or 39 in yeast tRNA (1). Disruption of the *PUS3* gene is not lethal but reduces cell growth rate. To elucidate *PUS3* function we sought genetically interacting genes by performing a synthetic lethality (sl) screen using a strain lacking Pus3p. This yielded a mutant sl strain that was unviable in the absence of Pus3p. Complementation with a yeast genomic library led to the identification of a chromosome fragment containing *ELP1*. Elp1p is part of the Elongator complex consisting of six proteins (Elp1-Elp6). This complex, originally thought to be involved in transcription, has been more recently suggested to mediate tRNA modification by forming 5-methoxycarbonylmethyl-2-thiouridine ( $\text{mcm}^5\text{s}^2\text{U}$ ) at the wobble position (2). To corroborate the screening results, the sl mutant was transformed with plasmids encoding all the subunits of the complex but only the plasmid containing *ELP1* complemented the sl mutation. As the phenotypes of *ELP1*-deficient cells are suppressed by over-expression of specific tRNAs that normally contain  $\text{mcm}^5\text{s}^2\text{U}$ , we also attempted to complement the sl mutant by over-expressing each of the 7 tRNA species that normally contain both  $\text{mcm}^5\text{s}^2\text{U}$  and  $\Psi 38$  or  $\Psi 39$ . However, none of the tRNAs suppressed the sl mutation. These data suggest that pseudouridine formation at position 38 or 39 is essential for the activity of tRNA with under-modified wobble position, probably by supporting proper anticodon loop structure.

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## P31

## Divergent amidinotransferases in the modification of archaeal tRNA

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*Key words: tRNA, modified nucleoside, archaeosine, amidinotransferase*

Archaeosine (G<sup>+</sup>) is a structurally complex modified nucleoside ubiquitous to the tRNA of Archaea, where it's located at position-15 in the D-loop, a site not modified in any tRNA outside of the Archaea. G<sup>+</sup> is characterized by an unusual 7-deazaguanosine core, a structure shared with the hypermodified nucleoside queuosine (Q) found at the wobble position of a subset of tRNA in Bacteria and Eukarya, but the two differ in substitution at the 7-position; G<sup>+</sup> possesses a formamidine group, while Q has an aminomethyl group appended to a cyclopentendiol moiety. Despite the phylogenetic segregation of G<sup>+</sup> and Q, the early steps of their biosynthesis are carried out by homologous enzymes. Notably, these steps occur outside the context of RNA and lead to the formation of the common intermediate 7-cyano-7-deazaguanosine (preQ<sub>0</sub>). In Bacteria preQ<sub>0</sub> is converted by the enzyme QueF to 7-aminomethyl-7-deazaguanine, which is subsequently inserted into the appropriate tRNA's by the enzyme tRNA-guanine transglycosylase (TGT), while in Archaea preQ<sub>0</sub> is inserted into the tRNA by an archaeal TGT. The enzyme(s) responsible for the conversion of the nitrile group of preQ<sub>0</sub>-modified tRNA to the formamidine functional group in archaeosine has, until recently, remained elusive.

We reported several years ago our discovery in Euryarchaeota of a novel amidinotransferase we named archaeosine synthase (ArcS) that catalyzes the last step in archaeosine biosynthesis, the conversion of preQ<sub>0</sub>-tRNA to G<sup>+</sup> modified tRNA. However, ArcS is not present in most Crenarchaeota. Presented here is our discovery of two discrete, non-homologous enzymes that catalyze the ArcS reaction in Crenarchaeota, and the characterization of the enzymatic activity of one of these (QueF-L), an enzyme homologous to the bacterial Q biosynthetic enzyme QueF. Remarkably, despite catalyzing very different reactions, the mechanisms employed by QueF and QueF-L share a number of common steps.



## P32

### Structural characterization of a novel eukaryotic family of proteinaceous RNase P

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The RNase P activity is ubiquitous and consists of the 5' maturation of pre-tRNAs. For a long time it has been thought that all RNase P were ribozymes. However, the characterization of the human mitochondrial RNase P revealed a novel kind of RNase P composed of proteins only, called PRORP for "Proteinaceous RNase P" [1]. Whereas in human mitochondria it is formed by a complex of three subunits, RNase P activity is held by single proteins in *Arabidopsis thaliana*, which possesses three PRORP homologs: PRORP1 located in mitochondria and chloroplasts, PRORP2 and PRORP3 in the nucleus. Each protein presents a pentatricopeptide repeat domain (PPR) and a metallonuclease domain [2].

Here we provide a first biophysical and functional characterization of PRORP enzymes [3]. Activity assays and footprinting experiments show that the anticodon domain of tRNA is dispensable, whereas individual residues in D and T $\psi$ C loops are essential for recognition. The affinity constant between a minimal substrate and a catalytic mutant of PRORP2 determined by microscale thermophoresis and isothermal titration calorimetry is in the 0.6-1  $\mu$ M range. Sequence and small-angle X-ray scattering analyses confirm that PRORP proteins are made of two main domains linked by a conserved zinc binding motif. This combination of approaches leads to a model of PRORP/tRNA complex suggesting that the recognition of pre-tRNA substrates by PRORPs is similar to that by ribonucleoprotein RNase P [4].

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## P33

## Residue 248 and RNase P RNA mediated cleavage

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The tRNA genes are transcribed as precursors and ribonuclease P, RNase P, is responsible for generating the 5' end of tRNAs in the cell. Bacterial RNase P consists of one protein and one RNA subunit (RPR) and *in vitro* tRNA precursors are correctly cleaved by the RPR without the protein. The RPR interacts with several regions in the substrate such as the T<sub>Ψ</sub>C-stem/loop (TSL) and the 3' end. Moreover, it has been suggested that residue -1 (the residue immediately 5' of the scissile bond) in the substrate interacts with A248, *Escherichia coli* numbering, referred to as "the N<sub>-1</sub>/A<sub>248</sub>-interaction". The way A248 interacts with residue -1 in the substrate is not clear but the possibility that A248 base pairs with residue -1 has been discussed in the literature.<sup>1-5</sup> To understand the nature of "the N<sub>-1</sub>/A<sub>248</sub>-interaction" and thereby get a deeper insight into RPR-mediated cleavage we have undertaken a systematic analysis using both tRNA precursors and model substrates carrying substitutions at position -1 in combination with *E. coli* RPR variants with A, C, G or U at position 248. We will provide data were we studied cleavage site recognition and rate of cleavage for the different substrate-RPR combinations and discuss the nature of "the N<sub>-1</sub>/A<sub>248</sub>-interaction" and possible role of A248.

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**P34****Antisense inhibition of bacterial RNase P RNAs by oligonucleotides identified via SELEX****Roland K. Hartmann<sup>1,\*</sup>, Dennis Walczyk<sup>1</sup>, Dagmar K. Willkomm<sup>2</sup>**<sup>1</sup>*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marburg, Germany*<sup>2</sup>*Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Schleswig-Holstein, Lübeck, Germany*\* [roland.hartmann@staff.uni-marburg.de](mailto:roland.hartmann@staff.uni-marburg.de)

Ribonuclease P (RNase P) is a ribonucleoprotein enzyme that processes precursor tRNAs to yield mature 5' tRNA ends in all domains of life. Bacterial RNase P is an attractive drug target because the enzyme is essential for viability but differs substantially in composition between Eukarya and Bacteria.

Here we explored antisense inhibition of bacterial type B RNase P (*Bacillus subtilis*), representing severe pathogens such as *B. anthracis* and *Staphylococcus aureus*, by a SELEX approach. This identified the L5.1 region as a target site, which forms a tertiary interaction with the L15.1 loop in natively folded type B RNase P RNA. Antisense or mutational disruption of this tertiary contact strongly impaired catalytic activity; a major contribution to this effect seems to be indirect through decreasing the accessibility of loop L15 toward interaction with tRNA CCA 3'-ends. We are currently testing RNA/LNA mixmer antisense oligonucleotides for their enhanced efficiency to invade the L5.1 stem-loop structure and disrupt the L5.1:L15.1 contact.



## P35

**Pus10 is involved in production of pseudouridine 54 of mammalian tRNAs****Manisha Deogharia, Archi Joardar and Ramesh Gupta\****Biochemistry & Molecular Biology, Southern Illinois University, Carbondale, IL 62901, USA**rgupta@siu.edu*

The classical TΨC sequence of tRNA reflects T (ribothymidine or 5-methyluridine) at position 54 in most Bacteria and Eukarya, and Ψ and C at positions 55 and 56, respectively, in nearly all tRNAs. TrmA and TruB homologs produce T54 and Ψ55, respectively, in Bacteria and Eukarya. However, archaeal tRNAs commonly have Ψ54 (or m<sup>1</sup>Ψ54) instead of T54, and Pus10 produces both Ψ54 and Ψ55 in these tRNAs. The *pus10* gene is present in nearly all Archaea and most higher eukaryotes, but not in Bacteria and yeast. This coincides with the presence of Ψ54 in archaeal tRNAs and certain tRNAs (for Gln, Asn, Trp, etc.) of animals, and its absence in the tRNAs of Bacteria and yeast. Certain tRNAs that function as primers for replication of retroviruses also contain Ψ54. The enzyme for tRNA Ψ54 synthase activity in eukaryotes has not yet been identified. We show here that HeLa cell extracts contain Ψ54 synthase activity that is specific for some tRNA transcripts but not for others. This activity is reduced in the extracts of Pus10-knock down cells with a concomitant increase in tRNA T54 methyltransferase activity, suggesting that both activities compete for modification of the U at the 54 position of the tRNA.



## P36

**Trit1 is a tRNA<sup>[Ser]Sec</sup>-isopentenyl-transferase**

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The biological effects of selenium have been attributed mainly to selenoproteins in which selenium is incorporated as the amino acid selenocysteine (Sec). Selenocysteine is encoded by a UGA codon. Recoding UGA as Sec requires a specific protein machinery, a mRNA stem loop in the 3'UTR of the selenoprotein genes, called Sec insertion sequence (SECIS), as well as a particular transfer RNA, tRNA<sup>[Ser]Sec</sup>.

Transfer RNA<sup>[Ser]Sec</sup> contains five posttranscriptional modifications:  $\psi_{55}$  (pseudouridine<sub>55</sub>), m<sup>1</sup>A<sub>58</sub> (1-methyladenosine<sub>58</sub>), i<sup>6</sup>A<sub>37</sub> (N<sup>6</sup>-isopentenyl-adenosine<sub>37</sub>), mcm<sup>5</sup>U<sub>34</sub> (5-methoxycarbonylmethyluridine<sub>34</sub>) and its further methylated form, mcm<sup>5</sup>Um<sub>34</sub> (5-methoxycarbonylmethyl-2'-O-methyluridine<sub>34</sub>). Although these modifications have been shown to be necessary for selenoprotein expression, however most of the enzymes responsible for tRNA<sup>[Ser]Sec</sup> modifications are unknown.

Isopentenyladenosine is found at position 37 following the anticodon in tRNAs decoding codons starting with uridine. This modification is thought to enhance translational accuracy. Isopentenylation is catalyzed by tRNA isopentenyl transferases, which add an isopentenyl group to the tRNA, using dimethylallyl pyrophosphate (DMAPP) as donor. Trit1 is an isopentenyl transferase present in mouse and human.

In this work, we demonstrate that murine Trit1 catalyzes the isopentenylation of tRNA<sup>[Ser]Sec</sup> at adenine 37. Recombinant Trit1 was able to transfer a isopentenyl group from [<sup>14</sup>C]DMAPP to tRNA<sup>[Ser]Sec</sup>. Trit1 mutants, D55G and T32A, which affect the active site of Trit1, showed a reduced activity. Mutant A37G tRNA<sup>[Ser]Sec</sup> was not isopentenylated in the presence of recombinant Trit1 protein as revealed by minor base analysis. Knockdown of Trit1 expression in mouse and human cell lines exhibited a significant decrease in selenoprotein expression under selenium deficient conditions, which it was compensated by the presence of selenium in the medium or when Trit1 was overexpressed. Recently, patients carrying a missense mutation in TRIT1 were identified. The patients suffer from mitochondrial disease suggesting that mitochondrial tRNAs also depend on TRIT1-mediated isopentenylation. We will present results on selenoprotein expression in samples of these patients.





## P37

**A kinetic isotope effect to probe the mechanism of pseudouridine synthases****Govardhan Reddy Veerareddygari and Eugene G. Mueller\***

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Earlier work with the substrate analog 5-fluorouridine in place of uridine in RNA suggested that the pseudouridine synthases may proceed through a mechanism involving the removal of the 2'-hydrogen to form a glycal intermediate.<sup>1</sup> To probe that possibility, the kinetic isotope effect was measured with [2'-<sup>2</sup>H]uridine in RNA, which is expected to slow the overall reaction if the proposed deprotonation occurs and is rate-limiting. The results and their mechanistic interpretation will be presented.

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**P38****Effect of osmotic shock on tRNA synthesis****Ewa Morawiec<sup>1</sup>, Olivier Lefebvre<sup>2</sup> and Magdalena Boguta<sup>1</sup>**

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Maf1 is required to repress transcription of tRNA genes and mediates regulation of various signaling pathways. Maf1 also indirectly affects tRNA processing. Activity of Maf1 is regulated by its phosphorylation and cellular localization. In yeast *Sacharomyces cerevisiae*, a change of growth conditions from fermentable carbon source to non-fermentable carbon sources or addition of rapamycin, induces the Maf1 dephosphorylation and its translocation to the nucleus to repress RNA polymerase III transcription.

We report a different mode of Maf1 regulation upon osmotic shock. In the presence of high salt concentration Maf1 is excluded from the nucleus in dephosphorylated form, independently on carbon source and independently on Msn5, only one known exportin of Maf1. Moreover, in Northern blot analysis of tRNAs of cells grown on glycerol and subjected to osmotic shock, we observed the disappearance of primary and 5'-end processed transcripts and in contrast, the accumulation of intron-containing end-processed transcript. This suggest, that tRNA transcription and processing is tightly regulated upon osmotic shock despite Maf1 is out of the nucleus. This regulation which depends on carbon source in media: on glycerol – tRNA transcription is switched off, on glucose – it is switched on, still exists even if osmotic shock applied.

The growth of yeast under conditions of osmotic stress is controlled by cell wall integrity pathway (CWI). Importantly Maf1 is excluded from the nucleus in CWI mutants.

In conclusions, these results suggest existence of another Pol III regulator, possibly linked to CWI pathway, which represses RNA polymerase III transcription independently on Maf1.



## P39

## Structural insights into elongator function

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The eukaryotic Elongator complex consists of six highly-conserved subunits and was initially described as a transcription elongation factor for RNA polymerase II. Although this large molecular machine (~900 kDa) has been associated with a broad range of different cellular activities (e.g. histone acetylation, exocytosis, and zygotic paternal genome de-methylation), there is accumulating evidence that its genuine cellular function is the specific modification of uridines at the wobble base position of tRNAs. Notably, the tRNA-modifying function could plausibly explain all of the above proposed roles of Elongator, through translational regulation of a multitude of target mRNAs. Deciphering Elongator's function has clinical importance as Elongator dysfunction leads to certain neurodegenerative diseases.

We will present high resolution structural information of individual Elongator subunits and insight into the architecture of fully assembled holoElongator. In particular, we will present structural insights into the Elp456 subcomplex, which forms a hetero-hexameric ring-like structure that unexpectedly resembles hexameric RecA-like ATPases. We will show that this hexameric assembly is essential for binding to tRNAs and analyze the regulatory role of ATP for this interaction. We will also provide insight into the stoichiometry of holoElongator, novel details of its subunit communication and recent intermediate resolution information of the fully-assembled holoElongator complex. In addition, we will show very recent data on two additional regulatory factors that are important for the proper activation of Elongator. Our results support a role of Elongator in translation regulation, explain the importance of each of the subunits for complex integrity, and suggest a model for the overall architecture of this large macromolecular machine.

**P40****Unravelling the trl1-like enzyme function in *Trypanosoma brucei***

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Transfer RNAs (tRNAs) play a central role in protein synthesis as translators of the genetic code connecting the information found in genes to that ultimately deposited into proteins. The biosynthesis of mature and functional tRNAs involves many processing steps, including 5' and 3' end trimming, incorporation of numerous chemical post-transcriptional modifications and addition of a CCA sequence at the 3' end. In some organisms, a subset of tRNAs also contain introns, which are cleaved by a tRNA splicing endonuclease. The generated exon halves are subsequently sealed by a splicing-specific tRNA ligase. A homolog of the yeast tRNA ligase was previously identified in trypanosomatids (1) and is presumably needed for joining the two tRNA exon halves generated by endonuclease cleavage of tRNA<sup>Tyr</sup>; the only intron-containing tRNA in these organisms. Here, we have preliminarily characterized the Trl-1 homolog of *T. brucei* (TbTrl1-like). Induction of *tbtrl1-like* gene silencing in a *T. brucei* RNAi strain leads to a severe growth defect and, unexpectedly, caused accumulation of intron-containing tRNA, as opposed to unligated exon halves, and almost a complete disappearance of mature tRNA. Furthermore, a pronounced cell-cycle arrest was observed. These results demonstrate the essentiality of the TbTrl1-like homolog for cell viability and its role tRNA<sup>Tyr</sup> processing. The restricted phylogenetic distribution to fungi and trypanosomatids for Trl1-like-splicing ligases shown in this work makes this family of enzymes promising therapeutic targets for such medically important organisms.

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## P41

## Novel insights into RNA structure-function relationships in RNase P from bacteria and organelles

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RNase P is the enzyme responsible for the 5' maturation of all pre-tRNAs in most organisms, and thus essential for cellular protein biosynthesis. This ribonucleoprotein enzyme is composed of one RNA and one to several proteins in bacteria, archaea and most unicellular eukaryotes. However, in the nuclei of higher plants and the energy generating organelles of all multicellular organisms the enzyme has a completely different composition.

Cyanobacteria are the evolutionary ancestors of chloroplasts and of the highly variable types of photosynthetic organelles found in diverse phylogenetic groups of algae. The primitive plastids from certain unicellular algae contain an essential RNase P RNA, with strikingly variable structures. These RNAs show only minor ribozyme activity *in vitro*. However, fully functional chimaeric holoenzymes can be reconstituted from these organellar RNAs and cyanobacterial protein subunits.

Although the RNase P holoenzymes of these plastids have a higher protein content than those of bacteria, they are immunologically related to cyanobacterial RNase P proteins. Novel results concerning the catalytic core and substrate binding sites of RNase P RNAs from plastids and cyanobacteria, as well as their evolutionary relationships will be presented.

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**P42****Structural basis of mitochondrial tRNA maturation****Sagar Sridhara<sup>1,2</sup>, Linda Reinhard<sup>1,2</sup>, B. Martin Hällberg<sup>1,2,3</sup>**<sup>1</sup> *Department of Cell & Molecular Biology (CMB), Karolinska Institutet, Stockholm, Sweden*<sup>2</sup> *Röntgen-Ångström Cluster, Karolinska Institutet outstation, Centre for Structural Systems Biology (CSSB), DESY campus, Hamburg, Germany*<sup>3</sup> *European Molecular Biology Laboratory, Hamburg Unit, 22603 Hamburg, Germany*

The mammalian mitochondrial tRNAs (mt-tRNAs) belong to a unique class of organellar tRNAs with marked deviations from the classical canonical tRNA cloverleaf structure and hence are often referred to as 'bizzare tRNAs'. These mt-tRNAs undergo extensive post-transcriptional processing, initiated by mt-RNase P, that processes the 5'-end, followed by mt-RNase Z, that processes the 3'-end to yield mature tRNAs. tRNA processing and modification are crucial steps to attain proper folding and render the intended function. The 5'-processing enzyme mt-RNase P is also unique in the sense that it is made up of protein sub-units only, lacking any RNA component unlike its bacterial, archaeal or human nuclear counterpart. Our long-term aim is to understand the molecular aspects of mt-RNase P substrate recognition and catalysis.



## P43

## Molecular basis for the differential interaction of plant mitochondrial VDAC proteins with tRNAs

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In plants, the voltage-dependent anion-selective channel (VDAC) is a major component of a pathway involved in tRNA translocation through the mitochondrial outer membrane (1). However, the way in which VDAC proteins interact with tRNAs is still unknown. *Solanum tuberosum* (potato) mitochondria contain two major VDAC proteins, VDAC34 and VDAC36, present in equal amounts in the outer mitochondrial membrane. These two proteins, composed of a N-terminal  $\alpha$ -helix and of 19  $\beta$ -strands forming a  $\beta$ -barrel structure, share 75% sequence identity. Interestingly, using both Northwestern and gel shift assays, we show that these two proteins interact differentially with nucleic acids: VDAC34 strongly interacts with tRNAs and other nucleic acids whereas VDAC36 only poorly does. In order to identify specific features and critical amino acids on VDAC34 required for tRNA binding, 23 VDAC protein mutants were constructed and analyzed by Northwestern. This allowed us to show that the  $\beta$ -barrel structure of VDAC34 and the first 50 amino acids that contain the  $\alpha$ -helix are essential for the tRNA binding. Altogether the data let us hypothesize that upon gene duplication, the two major *S. tuberosum* VDAC isoforms acquired specialized function and that VDAC34 evolved to develop a dedicated gate for tRNA import in plants.

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**P44****Role of Rsp5 ubiquitin ligase in tRNA biogenesis in yeast****Anna Domańska<sup>1</sup>, Anita Hopper<sup>2</sup>, Teresa Żołądek\*<sup>1</sup>**<sup>1</sup>*Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland*<sup>2</sup>*The Ohio State University, Columbus, Ohio 43210, USA*

Rsp5 is an ubiquitin protein ligase that is involved in tRNA biogenesis. Both, *rsp5-1* (mutation in catalytic Hect domain) and *rsp5-19* mutant cells (mutation in WW3 domain) accumulate initial pre-tRNA transcripts when grown in YPD medium and shifted for 4 hrs to an elevated temperature. Moreover, by FISH analysis tRNA accumulates in nuclei in both mutants after 2 and 4 hrs shift to repressive temperature. Since tRNA nuclear accumulation was detected earlier than the defect in processing of initial tRNA transcripts, we investigated whether retrograde traffic of tRNA from the cytoplasm to the nucleus contributed to the tRNA nuclear pools. Consistent with this notion upon treatment of cells with thiolutin (inhibitor of RNA transcription) accumulation of tRNA in the nucleus in *rsp5* mutants after shift to elevated temperature was observed. The results indicate that Rsp5 ubiquitin ligase may be involved in both, processing of initial tRNA transcripts and transport of mature tRNA between the nucleus and the cytoplasm *via* Msn5 and/or Mtr10 proteins. Further analysis showed that the level of importin Mtr10 is decreased whereas the level of exportin Msn5 is increased in *rsp5* mutants. We propose that under stress conditions when tRNA in the nucleus is too abundant, the level of proteins involved in tRNA transport is adapted to decrease the nuclear pool of tRNA.

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## P45

## Study of a unique tRNA snatcher and its putative function in *Plasmodium* development

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*Plasmodium*, the parasite responsible for malaria is mainly intracellular and has a complex life cycle within the mosquito vector and its vertebrate host.

The laboratory identified a surface protein, expressed all along the life cycle of *Plasmodium* parasites. Yet, we chose to work with the sporozoite stage of the parasite. Indeed, this stage has the particularity to be transiently in direct contact with the host cytosol (no parasitophorous vacuole). We demonstrated that this protein, called tRip for tRNA import protein, displays a tRNA binding domain outside of the parasite and mediates the import of exogenous tRNAs into its cytosol. Yet, we don't know the role of these tRNAs once inside the parasite.

My PhD project begun with the design and the construction of a *P. berghei* parasite Knock Out for tRip it is not an essential protein, however, its phenotype is significantly modified compared to the wild-type parasite. We investigated tRip oligomerization as well as the complex formed between tRip and tRNAs and compared the behavior of both the KO and the wt sporozoites *in vitro*.

(i) tRip as well as tRNA/tRip complexes were further characterized by analytical centrifugation, fluorometry, Dynamic light scattering, mass spectrometry...

(ii) We looked for putative changes in the tRNA structure after its interaction with tRip.

(iii) We compared the capacities of the KO and wt sporozoites to traverse host cells.

(iv) Since tRNAs are key molecules in translation, we looked for *de novo* protein synthesis in KO and wt sporozoites freshly isolated from mosquito salivary glands.

All together, these experiments should give us insightful information about the role that imported tRNAs could play in the sporozoites. This would bring light on the molecular mechanisms that are involved during the stage that precedes hepatocytes invasion, characterized by a rapid development and many morphological and physiological changes.



## P46

**The yeast Cex1p is not only involved in tRNA transport**

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In yeast *Saccharomyces cerevisiae*, aminoacylated-tRNAs are exported from the nucleus in a Los1p-dependant pathway and directly brought to ribosomes. Among the required factors, Cex1p was identified as a link between nucleoporins, exportins and the elongation factor eEF1a by the Dev Mangroo lab (Guelph University, Canada). Both biochemical and structural characterization of Cex1p gave clear insights into this channeling mechanism (Nozawa et al., 2013). The human counterpart of Cex1p, Scyl1, was first identified as an accessory protein in the Golgi-to-ER retrograde pathway. Although little is known about *CEX1* role in the latter pathway, the overall findings on mammalian Scyl1 suggest that, in addition to tRNA export, Cex1p might perform other tasks in yeast and more specifically in membrane trafficking. We analyzed the expression pattern of *CEX1* and looked for additional roles under different conditions. Using a combination of *in vivo* imaging and biochemical approaches, we show that *CEX1* is transcriptionally up-regulated in respiration and is implicated in intracellular membrane trafficking by acting at the level of late endosomes. We also bring up several evidences for Cex1p being important in trafficking when yeast cells divide.

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## Nuclear import pathway and interactome of *Saccharomyces cerevisiae* methionyl-tRNA synthetase

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In higher eukaryotes, nearly half of the aminoacyl-tRNA synthetases (aaRS) are embedded in a multisynthetase complex (MSC) together with non-enzymatic assembly proteins (Bandyopadhyay and Deutscher, 1971). Some of these MSC-participating aaRSs can be detached from the latter complex and exert additional, non-canonical functions (Hausmann and Ibba, 2008). In the model organism *Saccharomyces cerevisiae*, a small MSC has been identified nearly two decades ago (Simos *et al.*, 1996). This cytosolic MSC is composed of an anchoring assembly protein (Arc1p) and two aaRSs, methionyl-tRNA synthetase (cMRS) and glutamyl-tRNA synthetase (cERS). It is well known that, when given glucose as carbon source, yeast will first ferment to produce glycerol and ethanol. When glucose becomes low, an adaptation called diauxic shift occurs and yeast uses respiration to degrade glycerol and ethanol. During the diauxic shift, *ARC1* transcription is repressed and the complex dissociates to participate to the nucleus-mitochondria crosstalk. Indeed, in respiration the cERS relocates to the mitochondria and cMRS enters the nucleus. Here we present data concerning the nuclear import of cMRS. We show that cMRS possesses at least two different nuclear localization signals (NLSs) and identified, through proteomics, two different potential importins that could direct the import of cMRS *via* these NLSs. Finally, the identification of cMRS' nuclear partners confirms its involvement in regulating gene transcription upon switch from the fermentative to the respiratory metabolism.

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P48

## A variant of human glutamyl-prolyl tRNA synthetase involved in mitochondrial translation

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Aminoacyl-tRNA synthetases (aaRS) can associate into a multisynthetase complex (MSC) as exemplified with the yeast AME complex made of 2 aaRS (cMRS and cERS) and an anchoring protein, Arc1p or the human MSC made of 8 aaRS and 3 auxiliary proteins. Human Glutamyl-Prolyl tRNA synthetase (EPRS) is a bifunctional protein of the human MSC. Upon Interferon  $\gamma$  response, EPRS is released from human MSC assembles into the GAIT complex that operates a translational silencing of proinflammatory genes (Sampath et al., 2004). More recently, the group of Paul Fox published the discovery of a transcriptional variant of this enzyme (EPRS<sup>N1</sup>) that associates with the mRNAs targeted by the GAIT complex and thus prevents GAIT-mediated translational silencing (Yao et al. 2012). EPRS<sup>N1</sup> has similarities with the yeast cytoplasmic Glutamyl-tRNA synthetase (cERS) that we showed to be imported into mitochondria to misacylate mitochondrial tRNA<sup>Gln</sup> (mtRNA<sup>Q</sup>). Here we tested whether: *i*) EPRS<sup>N1</sup> can be imported into human mitochondria and increases organellar translation, *ii*) the human EPRS<sup>N1</sup> can functionally replace yeast cERS and bind to Arc1p and *iii*) EPRS<sup>N1</sup> can be imported into yeast mitochondria to misacylate yeast mtRNA<sup>Q</sup>.

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## P49

## Expression analysis of rodent-specific alternative splice variant of tryptophanyl-tRNA synthetase

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Tryptophanyl-tRNA synthetase (TrpRS) catalyzes the aminoacylation of tRNA<sup>Trp</sup>. In mouse embryonic stem (ES) cells, mRNA of a rodent-specific alternative splice variant of TrpRS (SV-TrpRS) has been identified<sup>1</sup>. Compared to mouse full-length TrpRS (FL-TrpRS), the SV-TrpRS has an additional heptapeptide (Cys-Phe-Cys-Phe-Asp-Thr) at the C-terminus. In the present study, we evaluated the expression of mouse TrpRS mRNA by real-time reverse transcription PCR. We found that the mRNA expression levels of FL- and SV-TrpRSs are high in mouse ES cells, embryo, spleen, lung, liver and uterus. The expression pattern of mouse TrpRS among tissues was similar to that of human TrpRS. And we elucidated that the relative expression of the SV-TrpRS compared to the FL-TrpRS is significantly less in the brain. Moreover, we demonstrated that interferon- $\gamma$  increases mRNA and protein expression of FL- and SV-TrpRSs in a mouse cell line. These results provide the first evidence for tissue-specific expression and splicing of mouse TrpRS.

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**P50**

## Characterization of the ProRS/tRNA<sup>Pro</sup>/YbaK ternary complex by analytical ultracentrifugation and native mass spectrometry

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Aminoacyl-tRNA synthetases (ARS) catalyze the attachment of cognate amino acids (aa's) onto tRNAs, which deliver aa's to the ribosome for use in protein synthesis. During the aminoacylation step, high fidelity is generally required to maintain the accurate flow of genetic information and cellular homeostasis. However, discrimination of structurally similar non-cognate aa's is challenging for many ARSs; thus, editing is required for quality control of aminoacylation. In many bacterial systems, a triple-sieve mechanism evolved to maintain tRNA<sup>Pro</sup> aminoacylation fidelity. While rejecting the majority of non-cognate aa's, prolyl-tRNA synthetase (ProRS) misactivates and mischarges Ala and Cys. Ala-tRNA<sup>Pro</sup> is specifically hydrolyzed by the insertion domain (INS) of ProRS *in cis*, while YbaK, a free-standing homolog of the INS domain, clears Cys-tRNA<sup>Pro</sup> *in trans*. YbaK does not appear to possess tRNA specificity, readily deacylating Cys-tRNA<sup>Cys</sup>. *In vitro* crosslinking data suggest YbaK interacts with ProRS in the presence of tRNA<sup>Pro</sup>, and *in vivo* evidence for ProRS-YbaK interaction has also been obtained using crosslinking strategies. We hypothesize that YbaK binds to ProRS to gain specificity for Cys-tRNA<sup>Pro</sup> and avoid deacylation of Cys-tRNA<sup>Cys</sup>. The ProRS/tRNA<sup>Pro</sup>/YbaK ternary complex has been difficult to characterize due to its apparently transient nature, and the stoichiometry of the complex is unknown. Here, analytical ultracentrifugation (AUC) and native mass spectrometry (MS) were used to investigate binary and ternary complex formation. AUC confirmed the binary interactions between ProRS/tRNA<sup>Pro</sup> and YbaK/tRNA<sup>Pro</sup>, however a binary ProRS/YbaK complex was not detected. In addition, AUC results are consistent with the existence of a ProRS/tRNA<sup>Pro</sup>/YbaK ternary complex. Furthermore, based on nanoESI ion-mobility MS data, we conclude that the stoichiometry of the complexes is as follows: ProRS:tRNA<sup>Pro</sup> (2:1), YbaK:tRNA<sup>Pro</sup> (1:1), and ProRS:tRNA<sup>Pro</sup>:YbaK (2:1:1). Taken together, our results support the hypothesis that YbaK specificity toward Cys-tRNA<sup>Pro</sup> is determined by the formation of a ternary complex with ProRS and tRNA<sup>Pro</sup>.



## *Plasmodium falciparum* tryptophanyl-tRNA synthetase and identification of potential inhibitors

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The human malaria pathogen, *Plasmodium falciparum*, replicates more than 10-fold every 48 hours and has a large requirement for rapid protein translation, so the loss of function of factors involved in protein biosynthesis could be detrimental to the parasites. Aminoacyl-tRNA synthetases (aaRSs) are enzymes that are key to the production of substrates for protein translation, an event that occurs in three cellular components of *Plasmodium*: the cytosol, the mitochondrion, and a remnant chloroplast called the apicoplast. *P. falciparum* expresses two forms of the *tryptophanyl-tRNA synthetase* (*TrpRS*) gene, and by analysing the subcellular localisation of the two isoforms we have shown that one localises to the apicoplast and the other to the cytosol. This enzyme is an attractive drug target in malaria parasites as several protein translation inhibitors have been developed as antimalarial drugs. To test for inhibition in malaria parasites, TrpRS inhibitors and analogues were identified and screened according to their pharmacokinetics properties. Assessment of dose-response curves reveal that several of these bacterial-type TrpRSs inhibitors effectively arrest parasite growth at low  $\mu\text{M}$  range. One, Indolmycin, kills with a delayed death effect characteristic of apicoplast inhibitors. We are now testing the effect of these compounds *in vitro* on the aminoacylation activity of the *Plasmodium* TrpRSs. We anticipate that the major differences between the host and the parasite TrpRSs will allow us develop parasite-specific inhibitors that may be used as starting points for drug development.

**P52**

## Quantitative analysis of the *Escherichia coli* proteome in the absence of LeuRS proofreading

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Leucyl-tRNA synthetase (LeuRS) efficiently activates norvaline, a non-canonical amino acid that accumulates in *Escherichia coli* during micro-aerobic growth. Remarkably, in spite of the prevailing opinion that LeuRS does not discriminate well against the non-cognate isoleucine, we have recently demonstrated that isoleucine does not pose a threat for LeuRS fidelity *in vitro* or *in vivo*. This points to norvaline as a prime candidate for proofreading activity. To establish an impact of translational quality control on maintaining a functional proteome and also to quantitate the level of mistranslation triggered by inactivation of LeuRS editing, the proteomes of the LeuRS deacylation-defective or wild-type *E. coli* strain grown under aerobic or micro-aerobic conditions were quantitated and compared using stable isotope labeling by amino acids in cell culture (SILAC). By this approach, we unambiguously demonstrate that LeuRS editing is indeed critical under micro-aerobic conditions to prevent non-canonical mistranslation. *E. coli* strain that lacks LeuRS post-transfer editing substitutes leucine with norvaline throughout the proteome, resulting in approximately 14% mistranslation level. In contrast, no significant isoleucine misincorporation is detected in the same strain even when non-physiologically high concentration of isoleucine is added to the growth media. Colony-forming unit assay reveals that norvaline misincorporation is followed by a drop in cell viability, confirming the importance of LeuRS editing. This study provides the first comprehensive quantitative analysis of mistranslation using SILAC methodology and high resolution mass spectrometry. It also characterizes the completely unknown scale and dynamics of the norvalylated proteome.





## P53

**tRNA charging pattern changes in different media and growth conditions**

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The concentration of aminoacyl -tRNA shapes the kinetics of translational elongation and influence protein production. *Bacillus licheniformis*, an important host for enzymes production, shows different tRNA aminoacylation patterns in different growth condition. We used tRNA microarray to analyze the charging level of each tRNA. Surprisingly, the charging of tRNA of many tRNAs is far below 80% even when cells grow in a balanced complex medium containing oligopeptides. The presence of free amino acids in the medium influences the charging pattern for specific tRNA. Additional supply of these amino acids may modulate the translation rate and increase the yield of expression in *Bacillus*.



P54

## Development of cysteine-specific tRNA and tRNA synthetase for site-specific protein fluorescence labeling in co-translational protein folding studies

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Protein fluorescent labeling is a challenge in protein folding studies for the need of ensuring labeling specificity. Recently, the importance of studying protein folding during translation has emerged, as many processes happen before the full length protein is synthesized. Due to the nature of ribosome-bound nascent chains (RNCs), fluorescent labeling must be coupled with translation during which tRNA acts as the carrier of fluorescent amino acid. In this work, we have developed a novel overexpressed suppressor tRNA<sup>Amber<sub>Cys</sub></sup> for the production of BODIPY FL-labeled RNCs. In order to simplify the purification procedures, *Bacillus subtilis* tRNA<sup>Amber<sub>Cys</sub></sup> has been selected for its distinctive sequence from any endogeneous *E. coli* RNA. In a single purification step, ample amounts of tRNA<sup>Amber<sub>Cys</sub></sup> have been obtained. As cysteinyl-tRNA synthetase was previously shown to aminoacylate tRNA<sup>Amber<sub>Cys</sub></sup> with lower efficiency, several point mutations were introduced into the C-terminus of cysteinyl-tRNA synthetase to compensate for the Amber mutation in the anticodon loop. Out of the cysteinyl-tRNA synthetase mutants, one has shown to improve aminoacylation efficiency towards tRNA<sup>Amber<sub>Cys</sub></sup>. In addition, overexpressed BODIPY FL-cysteinyl- tRNA<sup>Amber<sub>Cys</sub></sup> indicates improved stability of this tRNA compared to *in vitro* transcribed tRNA. Applying this tRNA, we have studied the dynamics of single-labeled RNCs by time-resolved anisotropy to reveal information about the protein folding on the ribosome. This tRNA tool may be beneficial on the site-specific labeling of full-length proteins as well as RNCs for different biophysical and biological research.



## P55

## New antibacterials with inhibitory activity on aminoacyl-tRNA synthetases (NABARSI)

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The major limitation of currently-used antibiotics is that in many cases they have been rendered ineffective by the emergence of bacterial resistance. This widespread resistance to antimicrobials is now a global threat and there is an urgent need for chemically-novel antibacterial agents. The NABARSI consortium, which consists of 5 partners, undertakes a cutting-edge drug discovery project to identify such agents.

The main goal is to find new chemical entities (NCEs) with antibacterial efficacy in an animal model of multi-drug resistant bacterial infection. The targets of these NCEs are the aminoacyl-tRNA synthetases (aaRS). aaRS are genetically and chemically-validated targets, with proof of principle provided by IleRS inhibitor mupirocin, which is a marketed drug for the treatment of topical methicillin-resistant *Staphylococcus aureus* (MRSA) infections.

Evolutionary divergence between prokaryotic and eukaryotic enzymes enables the development of aaRS inhibitors that selectively inhibit the bacterial enzymes over their human orthologues. However, across different bacterial pathogens individual aaRS enzymes are generally well conserved, making possible the development of broad-spectrum antibacterial agents. We take advantage of the similarities in catalytic sites of LeuRS, IleRS and ValRS to obtain NCEs with inhibitory activity on the three enzymes.

To speed up the discovery of pharmacological molecules, Omnia Molecular applies a completely new approach to the selection of anti-infective lead compounds. We take advantage of proprietary human cell lines where the activity of pathogenic aaRS can be monitored. These cell lines are used as a tool for the selection of specific and non-toxic aaRS inhibitors. Hit compounds are further characterized using our validated proprietary platform and those of our partners. Those lead compounds proven effective in animal models will provide selective NCEs with high antibacterial efficacy and low resistance potential.



## P56

## Formation of a macromolecular complex between *Escherichia coli* selenophosphate synthetase (SPS), selenocysteine synthase (SelA) and tRNA<sup>Sec</sup> (SelC)

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The biosynthesis pathway of selenocysteine (Sec or U) involves the interaction of Selenocysteine Synthase (SelA), tRNA (SelC or tRNA<sup>Sec</sup>), Selenophosphate Synthetase (SPS), a specific elongation factor (SelB) and a specific mRNA sequence known as SElenocysteine Insertion Sequence (SECIS). Since selenium compounds are highly toxic in cellular environment, selenium association with proteins throughout its metabolism is essential for cell survival and involves a specific and unique tRNA. In this study, we demonstrate the interaction of SPS with the SelA-tRNA<sup>Sec</sup> binary complex, resulting in a large ternary complex, unknown to this biosynthetic pathway, with stoichiometric ratios of 1SelA<sub>decamer</sub>:10tRNA<sup>Sec</sup>:5SPS<sub>dimer</sub>. In order to assemble the ternary complex, SPS and SelA undergo structural conformational changes. We show that SPS glycine-rich N-terminal region and SelA N-terminal domain are crucial for SelA-tRNA<sup>Sec</sup>-SPS interaction and selenoprotein biosynthesis, as revealed by functional complementation experiments. Together, our results present new insights into selenium metabolism, demonstrating for the first time the formation of the ternary catalytic SelA-tRNA<sup>Sec</sup>-SPS complex and the probable mechanism by which the toxic selenium intermediate is sequestered in the complex.

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## P57

## The dynamic tRNA<sup>His</sup> aminoacylation kinetics of human histidyl-tRNA synthetase and two human disease variants

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Human histidyl-tRNA synthetase (hHARS) is a homodimeric class II aminoacyl-tRNA synthetase that utilizes ATP to attach histidine accurately to histidyl-tRNA (tRNA<sup>His</sup>) during aminoacylation. Inhibition of this reaction can, in principle, cause ribosome stalling at histidine codons. We hypothesize that such events could be directly linked to human diseases, particularly those associated with neurodegeneration. Currently, two human diseases, Usher Syndrome IIIB and a peripheral neuropathy, have been linked genetically to single point mutations in the *HARS* gene. Usher Syndrome IIIB patients, whose *HARS* genes encode the Y454S substitution, lose sight and hearing during their second decade of life. An isolated patient whose *HARS* gene encodes a heterozygous R137Q mutation developed axonal peripheral neuropathy by the fifth decade of life. The Y454S substitution is localized at an interface between the anticodon-binding domain and the catalytic domain of the opposing subunit, while the R137Q mutation disrupts an important salt bridge at the dimer interface. Here, we describe the biochemical characterization of the Y454S and R137Q hHARS enzymes. Steady state kinetic analysis utilizing *in vitro* human tRNA<sup>His</sup> transcript and hHARS purified from HEK293 cells, showed that the catalytic turnover ( $K_{cat}$ ) of Y454S hHARS enzyme is greater for both histidine and tRNA<sup>His</sup> than WT hHARS enzyme. Single turnover kinetics demonstrated that the Y454S mutation does not significantly affect the rate of transfer of histidine from the adenylate to the tRNA<sup>His</sup>. By contrast, the R137Q substitution significantly compromised adenylation activity, but pre-steady state kinetics showed that the adenylation reaction was efficiently rescued by the addition of tRNA<sup>His</sup>. These results suggest that Usher Syndrome IIIB and HARS-linked peripheral neuropathy phenotypes are unlikely to be a consequence of a simple loss of aminoacylation function. The hHARS system also represents a notable example in which two different complex human diseases arise from distinct mutations in the same parent gene.

**P58**

## **Solution NMR study of the Interaction between Lysyl-tRNA Synthetase and Laminin Receptor**

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Lysyl-tRNA synthetase (KRS), which is an enzyme for aminoacylation of tRNA in protein synthesis, interacts with the laminin receptor (LR/RPSA) and enhances laminin-induced cell migration in cancer metastasis. Although the biological implication of the interaction with LR has been suggested, the structural aspect of the interaction remains elusive. Using NMR-based study, here we show that the anticodon-binding domain of KRS binds directly to the C-terminal region of 37LRP, and the previously found inhibitors BC-K-01 and BC-K-YH16899 interfere the KRS-37LRP binding. In addition, the anticodon-binding domain of KRS binds to laminin, observed by NMR and SPR studies. These results provide crucial insight into the structural characteristics of the KRS-LR interaction on cell surface.

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## P59

**Multi-dimensional interactions between tRNA synthetases and cofactor: Implications for translation and signaling****Yunje Cho**

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Aminoacyl-tRNA synthetases (ARSs) catalyze the attachment of amino acids to their cognate tRNAs, the essential step for the protein synthesis in all life. In higher eukaryotes, ARSs have additional motifs or domains to their catalytic domains and organized with auxiliary proteins to form a multisynthetase complex (MSC). MSC is composed of nine ARSs and three accessory proteins (AIMPs) and can be grouped into three sub-complexes. One complex is formed with RRS, QRS, and AIMP1/p43. Another complex is comprised of MRS, IRS, LRS, EPRS, DRS, and AIMP3. The MSC is believed to facilitate the protein synthesis through channeling mechanism, to enhance ARS activity by recruiting non-specific tRNA-binding domains, and to regulate the balance between translation and non-canonical functions. Here, I will describe the potential models of the MSC, and the interactions between components of the MSC through integrative approaches.



## P60

## The selective tRNA aminoacylation mechanism based on a single G•U pair

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Alanyl-tRNA synthetase (AlaRS) selectively ligates tRNA<sup>Ala</sup> with alanine in the process of the genetic code translation into proteins. AlaRS selects tRNA<sup>Ala</sup> by recognizing G3•U70 in the middle of the acceptor stem. Replacement of G3•U70 by the Watson-Crick base pair results in the loss of activity by affecting the  $k_{\text{cat}}$  level. Reversely, if G3•U70 is introduced into some tRNAs other than tRNA<sup>Ala</sup>, the tRNAs often become to be aminoacylated by AlaRS. Nevertheless, the mechanism by which tRNA<sup>Ala</sup> is selectively aminoacylated had been a longstanding mystery. The two crystal structures of AlaRS complexed with wild-type tRNA<sup>Ala</sup> having G3•U70 and a mutant tRNA<sup>Ala</sup> having A3•U70, and structure-based mutant analyses revealed that AlaRS strictly locates the base pair at the position 3•70 on the proper position, and then structural differences between G•U and Watson-Crick base pair (A3•U70) are transmitted toward the CCA terminus. The CCA of tRNA<sup>Ala</sup> with G3•U70 can be reach the aminoacylation active site, whereas that with the Watson-Crick base pair is located at the site far from the active site. Thus, AlaRS achieves the selective aminoacylation of tRNA dependent on G3•U70. Furthermore these findings explain why tRNA mutations at the site far from the active site affect  $k_{\text{cat}}$  not but their affinity.





## P61

**Aminotransferase activity of a novel aminoacyl-tRNA synthetase appended domain****Sandhya Bharti Sharma and Rebecca Wagner Alexander***Wake Forest University, Department of Chemistry, 1834 Wake Forest Road, Winston-Salem, NC 27109-7486*

Aminoacyl-tRNA synthetases (AARSs) are modular proteins, with separate polypeptide domains responsible for tRNA binding and catalysis; additional domains on some AARSs contribute oligomerization, localization, and editing functions. The opportunistic pathogen *Mycoplasma penetrans* expresses an unusually long version of methionyl-tRNA synthetase (MetRS) that contains an extra N-terminal domain with sequence homology to class V aminotransferases. We previously showed that the appended domain was not required for the canonical tRNA aminoacylation activity.<sup>1</sup> We anticipated that this domain carries out pyridoxal phosphate-dependent aminotransferase activity, possibly to modify Met-tRNA<sup>Met</sup> for enhanced pathogenicity.

We overexpressed and purified *M. penetrans* MetRS (MpMetRS) and used the amino-reactive aromatic dialdehyde *naphthalene-2,3-dicarboxaldehyde* followed by HPLC to analyze enzyme activity *in vitro*. Initial characterization confirms the proposed aminotransferase activity, with several amino acids and  $\alpha$ -keto acids serving as amino group donors and acceptors, respectively. Substitution of amino acids in the transferase and synthetase catalytic sites suggest that the activities may be interdependent. Ultimately we seek to identify the cellular substrates and implications of the MpMetRS aminotransferase activity.

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**P62****The charging level of different *E. coli* tRNA isoacceptors vary in different media****Irem Avcilar\*, Juan Varela, Alexander Leow, Zoya Ignatova***Institute of Biochemistry and Biology, University of Potsdam, Potsdam, Germany**\*iavcilar@uni-potsdam.de*

tRNAs are an important gateway to adapt translation to new environments. The concentration of the tRNA isoacceptors mostly correlates with doubling time in *E. coli*. In balanced growth medium, the majority of the tRNA isoacceptors are charged to almost 70-90%. However, few of them, e.g. His, Cys, Ser, Thr, Phe and Pro are charged to very low level, from 10% to 50%. In minimal medium, the overall aminoacylation levels are higher with an average of app. 50% for tRNAs with hydrophilic amino acids and an average of app. 80% for tRNAs with hydrophobic amino acids. Ribosomal profiling enables to find which codons account for ribosomal stalling. Ribosomal profiling reveals that tRNA isoacceptors with extremely low charging cause accumulation of ribosomal read over those codons (Ser-UCA, Ser-UCG, Ser-AGU) residing in the A-site.



## P63

**Nuclear localization, high-resolution structure, and potential stress-response function of human C-Ala****Litao Sun<sup>1</sup>, Youngzee Song<sup>1</sup>, Leslie Nangle<sup>2</sup>, Kyle Chiang<sup>2</sup>, Paul Schimmel<sup>1</sup>**

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Through proteomics based on mass spectrometry, a natural C-terminal fragment of human alanyl tRNA synthetase (AlaRS) was detected in cultured Jurkat T-cell lysates. Separate analysis established that alternative splicing or proteolysis generated C-Ala. Human C-Ala was secreted under stress conditions and could be re-internalized into human immune cells. While both monomeric and dimeric forms were present, the dimer specifically localized to the nucleus and associated with DNA. (Our earlier results (Guo et al (2009) Science 325: 744-747) showed that the orthologous *A. aeolicus* C-Ala formed a single-stranded nucleic acid binding motif.) A crystal structure at 2.0 Å resolution revealed how C-Ala may interact with DNA. Specifically, the dimer forms an inter-subunit positively charged groove into which double-stranded B-form DNA can be fit. CHIP analysis revealed several target genes associated with the stress response that bound C-Ala. Thus, a new protein, devoid of catalytic activity, is generated from AlaRS and appears to have a nuclear function related to the stress response.

**P64**

## Evolution of bacterial glutamyl- and glutaminyl-tRNA synthetase: New insights from whole genome analysis

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The aminoacylation machinery of bacterial tRNA<sub>Gln</sub> is strictly dependent on the convoluted evolutionary histories of the associated enzymatic partners glutamyl-tRNA synthetase (GluRS), amidotransferase (gatCAB) and glutaminyl-tRNA synthetase (GlnRS). After the divergence of eubacteria and eukarya, bacterial tRNA<sub>Gln</sub> used to be glutaminylated by the GluRS-gatCAB pair until GlnRS appeared by horizontal gene transfer (HGT) from eukaryotes or a second copy of GluRS (GluRS2), that only glutamylates tRNA<sub>Gln</sub>, appeared. The origin of GluRS2 is poorly understood in the above scenario derived from a limited sequence data. We have revisited the evolution of bacterial GluRS and GlnRS from a whole genome viewpoint. A large database of bacterial GluRS, GlnRS, tRNA<sub>Gln</sub> and the gatCAB, constructed from whole genomes by functionally annotating and classifying these enzymes according to their mutual presence and absence in the genome, was analyzed. Our analysis broadens the current understanding of bacterial GluRS evolution with some unique findings — i) Unlike non-functional GluRS2 (as in *Thermotoga maritima*), the functional GluRS2 (as in *Helicobacter pylori*) is found to be a chimera of mismatching catalytic and anticodon-binding domains, ii) the appearance of GlnRS and GluRS2 in bacterial genomes are not mutually exclusive indicating that their evolutionary histories are distinct, iii) GlnRS is more widespread in bacteria than is believed, iv) bacterial GlnRS appeared both by HGT from eukarya and intra-bacterial HGT, v) presence of GlnRS pseudogene (in *Sorangium cellulosum*) shows that many bacteria could not retain the newly acquired eukaryal GlnRS. The functional annotation of GluRS, without recourse to experiments, performed in this work, demonstrates the inherent and unique advantages of using whole genome over isolated sequence databases.<sup>1</sup>

<sup>1</sup>Dasgupta and Basu *BMC Evolutionary Biology* 2014, 14:26



## P65

## Molecular Basis for CC-addition by *Aquifex aeolicus* CC-adding enzyme

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3'-CCA of tRNA is synthesized by CCA-adding enzyme using CTP and ATP as substrates without using a nucleic acid template. In some eubacteria, such as *Aquifex aeolicus*, CC-adding and A-adding enzymes collaboratively synthesize the 3'-CCA of tRNA. Molecular mechanism of CCA-addition by the CC-adding and A-adding enzymes has remained obscure.

We present complex crystal structures of *A. aeolicus* CC-adding enzyme representing CC-addition onto the 3'-end of tRNA. Upon binding of acceptor/T helix of tRNA to the C-terminal body and tail domains of the enzyme, the 3'-end of tRNA is placed into the cleft between catalytic head and nucleobase-interacting neck domains. The base pair at the top of the acceptor stem of tRNA stacks with the beta-sheet in the head domain. As a result, 3'-end of tRNA is oriented into an enclosed active site in the head domain. CTP is selected by Watson-Crick like base-pairing between the cytosine of CTP and conserved Asp and Arg in the neck domain, and CMP incorporation proceeds in the spatially limited pocket. After first CMP incorporation, tRNA rotates and translocates backward by one nucleotide, and second CMP incorporation proceeds in the same mechanism using the same catalytic pocket as for the first CMP incorporation. After two CMP incorporations and pyrophosphate release, tRNA translocates backwards, and tRNA is dropped off from the enzyme, and the enzyme terminates RNA synthesis. tRNA ending 3'-CC is, then, loaded onto the A-adding enzyme, and a single AMP is incorporated on the 3'-end of tRNA, and the 3'-CCA synthesis of tRNA completes.

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## P66

## Translation using non-natural aminoacyl-tRNAs in cells and cell-sized liposomes

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Incorporation of non-natural amino acids into protein would expand protein's function. In this study, we tried to develop methods using *in vitro* prepared-aminoacyl-tRNA (aa-tRNA) to introduce non-natural amino acids into proteins in cells and cell-sized liposomes.

In mammalian cells, non-natural amino acids incorporation is generally achieved using engineered aminoacyl-tRNA synthetases (aaRSs). However, engineered aaRSs are not always available for non-natural amino acids, which can be attached to a tRNA by chemical acylation. The chemical acylation method enables us to attach various non-natural amino acids to tRNAs, although it can be used only *in vitro*. Thus we tried to introduce *in vitro* prepared-aa-tRNAs into mammalian cells for non-natural amino acid incorporation. Injection of the exogenous amber suppressor aa-tRNA into CHO cells resulted in that the amber codon was hardly translated by the aa-tRNA. In contrast, co-injection of the amber suppressor aa-tRNA and eEF1A-1B induced amber suppression. These data suggests that exogenous aa-tRNA cannot efficiently enter the mammalian cytoplasmic translation system by itself, and that the binding of eEF1A supports the efficient use of the exogenous aa-tRNA.

The integral membrane protein bacteriorhodopsin, containing a fluorescent amino acid at a specific position, was synthesized in the presence of liposomes using an *in vitro* translation system expanded with a four-base codon/anticodon pair. Cell-sized liposomes with the labeled protein inserted into the liposome membranes were generated after the translation reaction.<sup>1</sup> This study also demonstrated that this labeling method could be used to analyze the dynamic properties of membrane proteins *in situ* by fluorescence correlation spectroscopy.

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## P67

## Photoregulation of translation using a caged aminoacyl-tRNA

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Genetic code expansion has facilitated the site-specific incorporation of unnatural amino acids (uaas) into proteins in *Escherichia coli*, yeast, cultured animal cells and, most recently, *Caenorhabditis elegans* [1]. We previously developed a method for incorporation of uaas into proteins [2]. In this study, we designed a protected aa-tRNA with a photocleavable group (caged aa-tRNA). Caged aa-tRNA is expected as a new approach for temporal control of translation through photo-triggered production of aa-tRNA.

This approach is used in in vitro translation system. For this approach to be successful, however, the caged aa-tRNA must show the following properties. (1) The caged aa-tRNA is stable and not deaminoacylated for long enough time during the translation. (2) The caged aa-tRNA does not inhibit the translation system. (3) The caged amino acid in the caged aa-tRNA does not participate to the translation. (4) The photocleavable protecting group must be removed in much shorter irradiation time than the period for the translation. In addition, the irradiation does not influence the translation. (5) The free aa-tRNA after deprotection must go into ribosome according to a unique codon/anticodon pair that is independent from the standard codon/anticodon pairs.

We here developed the caged aa-tRNA which met these conditions. We demonstrated photo-triggered temporal control of translation using the caged aa-tRNA.

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**P68****Molecular mechanism of replication initiation of Q $\beta$  RNA by Q $\beta$  replicase****Daijiro Takeshita<sup>1</sup>, Kozo Tomita\*<sup>1</sup>**<sup>1</sup>*National Institute of Advanced Industrial Science and Technology (AIST), Biomedical Research Institute, Tsukuba, Ibaraki, Japan**correspondence e-mail: kozo-tomita@aist.go.jp*

Q $\beta$  virus has a single and positive strand genomic RNA. It infects *E. coli* and replicates the viral RNA by using Q $\beta$  replicase, a tetrameric protein complex of the viral RNA-dependent RNA polymerase ( $\beta$ -subunit), translational elongation factor (EF) -Tu, EF-Ts and ribosomal protein S1. The S1 is indispensable for the initiation of the negative strand RNA synthesis from the positive strand Q $\beta$  RNA. Although structures of core Q $\beta$  replicase consisting of the  $\beta$ -subunit, EF-Tu and EF-Ts is available, structures of holo Q $\beta$  replicase, containing S1, has not been reported yet. The molecular mechanism of S1 interaction with core Q $\beta$  replicase, and the involvement of S1 in replication initiation of Q $\beta$  RNA have remained obscure.

We present crystal structure of Q $\beta$  replicase, containing  $\beta$ -subunit, EF-Tu, EF-Ts and N-terminal half of S1 containing three OB-fold, capable of initiating the negative strand synthesis from the positive strand Q $\beta$  RNA. The structural and biochemical studies revealed that the N-terminal two OB-folds anchor the S1 onto the  $\beta$ -subunit, and the third mobile OB-fold interacts with the specific RNA fragment derived from the internal region of Q $\beta$  RNA. The specific interactions between the internal region of Q $\beta$  RNA and the third mobile OB-fold of S1, spatially anchored near the surface of the  $\beta$ -subunit of Q $\beta$  replicase, would recruit the 3'-end of Q $\beta$  RNA in the proximal to the active site of the  $\beta$ -subunit. Thus, S1 acts as a replication initiation factor for efficient and specific replication initiation of Q $\beta$  RNA, beyond its established function in translation initiation.

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## P69

## Chemical synthesis of hydrolysis-resistant 3'-peptidyl-tRNA mimics that bind to the ribosome

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The ribosomal elongation of the peptide chain takes place at the peptidyl transferase center (PTC) of the ribosome. As translation proceeds, the nascent peptide chain passes from the PTC through the exit tunnel and emerges from the ribosome at the tunnel opening. Hydrolysis-resistant peptide-RNA conjugates that mimic peptidylated tRNA termini are thus valuable compounds for functional and structural studies of the ribosome. However, they represent a remarkable synthetic challenge, in particular if they contain amino acids with complex side-chain functionalities.

Here, a novel approach that combines solid-phase synthesis and bioconjugation is demonstrated to obtain these conjugates with high efficiency and purity. The key step is native chemical ligation of cysteine-modified RNA fragments to highly soluble peptide thioesters.[1] To expand the sequence variety of peptidyl-tRNA mimics obtained by native chemical ligation, metal-free desulfurization of cysteine to alanine is presented.[2] The so-prepared peptidyl-tRNA mimics relate to resistance peptides that can render the ribosome resistant to macrolide antibiotics by a yet unknown ribosomal translation mechanism.[3] In addition, one of the mimics was enzymatically ligated to the chemically synthesized 5'-fragment of tRNA-Cys to obtain the corresponding full-length tRNA-peptide conjugate.[1] Furthermore the hydrolysis-resistant 3'-amide linkage between the peptide and the RNA moiety allows the formation of crystallisable complexes without deacylation. Using X-ray crystallography, structural studies of a short peptidyl-tRNA mimic bound to the large ribosomal subunit of *D.radiodurans* will be presented (unpublished data).

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## P70

## Use of tRNA-specific recognition to test miR-122-induced structural changes in the tRNA-like domain of the hepatitis C virus internal ribosome entry site

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Hepatitis C virus (HCV) genome comprises a 5' untranslated region, which functions as an internal ribosome entry site (IRES) including the AUG start. It was demonstrated that the host pre-tRNA processing enzyme, RNase P, can cleave the HCV RNA genome at a site in the IRES near the AUG initiator triplet. This reaction indicates the presence of a tRNA-like structure in the IRES. We and others have previously shown that the HCV (IRES) resides within the 1-570 genomic region in a locked conformation and switches to an open conformation triggered by the binding of two miR-122 molecules at its 5' flank.

In this study we have tested quantitatively the ability of miR-122 to interact with HCV RNA in either the closed or the opened form. Surprisingly, control truncated RNAs from which the miR-122-triggering sites in the 5' flank of the IRES were removed continued to actively bind miR-122. Using classic RNA probing methods two consecutive miR-122 binding sites at the 3' flank of the IRES were found to be responsible. The effects of binding on the 3' IRES flank of miR-122 on single and double stranded specific RNases (RNase A, T1 and V1) or chemicals (DMS) were limited. In contrast, the activity of factors that specifically recognize the tRNA-like region containing the AUG start codon used as probes was highly affected, whether prevented or activated. These factors included human and cyanobacterial RNase P and *E. coli* RNase Z. This suggested that binding of miR-122 at the 3' flank of the IRES, in the vicinity of the AUG start codon might affect binding of the 40S ribosomal subunit which was subsequently demonstrated. In conclusion, the use of specific tRNA-related factors provided evidences that miR-122 binds to HCV RNA at both flanks of HCV IRES and induces a global rearrangement of its structure.



## Simulating ribosome dynamics and tRNA translocation

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With advances in structure determination and continued growth in high-performance computing (HPC), molecular dynamics (MD) simulations can now be employed to study large-scale conformational rearrangements in molecular machines, such as the ribosome. In the cell, proteins are synthesized by the joint action of the ribosome and transfer RNA (tRNA) molecules, enabling messenger RNA (mRNA) to be translated into peptides. In the elongation cycle of translation, tRNA molecules and the associated mRNA move between binding sites, a process known as tRNA translocation. During translocation, tRNA-mRNA movement ( $\sim 20\text{-}50\text{ \AA}$ ) is coupled to large-scale collective rotations in the ribosomal subunits. In order to better understand the physical relationship between these rotations and tRNA displacements, we use MD simulations that employ a simplified description of the energetics, which elucidate the role of sterics, and molecular flexibility during tRNA translocation. For the ribosome, we construct forcefields for which each experimentally-derived configuration is a potential energy minimum. Using these models, we are able to simulate spontaneous tRNA translocation events and identify robust aspects of the dynamics. We find that detailed steric interactions are a dominant contributor to tRNA translocation dynamics. These results provide a framework for understanding the interplay between structure and dynamics, and suggest strategies to experimentally modulate the physical-chemical features that govern ribosome function.

*Keywords: large biomolecular machines, energy landscapes, translation.*

**P72**

## Processive sliding of ribosomes through the non-coding gap in gene 60 mRNA of bacteriophage T4

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Translational bypassing is an unusual mechanism employed by the ribosome to skip a non-coding region in the mRNA. While the best-characterized example of bypassing occurs in gene 60 mRNA of bacteriophage T4 (Wills et al 2008), recent evidence suggests that bypassing phenomena are much more common, e.g. there are 81 translational bypassing elements in mitochondria of the yeast *Magnusiomyces capitatus* (Lang et al 2013). We studied how the ribosome bypasses a 50-nt non-coding segment of gene 60 mRNA *in vitro*. To identify the minimal set of signals required for bypassing, we recapitulated efficient translational bypassing in a reconstituted translation system from *Escherichia coli*. We show that the signals that promote efficient and accurate bypassing are specified by the gene 60 mRNA sequence itself. Systematic analysis of the mRNA suggested unexpected contributions of mRNA sequences upstream and downstream of the non-coding gap region as well as of the nascent peptide. During bypassing ribosome glides forward on the mRNA track in a processive way. At any time during bypassing, >97% of ribosomes successfully move by one nucleotide towards the landing site, while <3% are lost due to drop-off. We propose a novel model of bypassing, where the mRNA elements 5' of the take-off site and 3' of the landing site, together with the nascent peptide sequences in the ribosome exit tunnel, coordinate the ribosome movement over the gap. Gliding may play a role not only for gp60 synthesis, but also during regular mRNA translation for reading frame selection during initiation or tRNA translocation during elongation.



## P73

## A non-canonical tRNA-protein complex involved in tRNA mitochondrial import

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Mitochondrial genomes encode far less molecules than they need to fulfill their biological roles and thus rely on cytosolic transcription and translation. In yeast, mitochondrial translation at non-permissive temperature that suppresses the function of the mitochondrial-encoded tRNA(lys)3 (tRK3), relies on import of cytosolic tRK1, and not tRK2. tRK1 import is a chaperone-mediated process involving the glycolytic enzyme enolase 2 (Eno2p), which shuttles tRK1 to the precursor of the ultimately imported mitochondrial lysyl-tRNA synthetase. This process depends on the refolding of tRK1 in a so-called F-form based on three hairpins, with only the D-stem and loop structurally conserved. The two other hairpins result from re-shuffling of anticodon, T-stem and 3' acceptor strands giving rise to two neo-loops. In the F-form of tRK1, the participation of the CUU anticodon is required for formation of the second hairpin. Moreover, the entire anticodon can be deleted without significant alteration of the import process. This indicates that subtle differences in sequence and/or RNA post-transcriptional modification patterns between tRK1 and tRK2 may account for the canonical L-shape to F-form refolding, and consequently, stringent import specificity of the former, perhaps helped by Eno2p. Small angle X-ray scattering (SAXS) experiments have been overtaken to characterize the shapes of the complex and its individual components. The SAXS shapes show potential extended bi-lobal structures, which could accommodate both tRK1 and Eno2p. A small bridge at the junction of the two lobes indicates that contact with Eno2p could be mediated by the F-loop of tRK1.

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**P74**

## Artificial codon box division to encode multiple non-proteinogenic amino acids besides 20 proteinogenic ones

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In living organisms, mRNA codons are decoded to typically 20 proteinogenic amino acids according to the genetic code. The genetic code reprogramming method enables multiple kinds of non-proteinogenic amino acids to be encoded in a reprogrammed genetic code. In this method, several kinds of proteinogenic amino acids and/or aminoacyl tRNA synthetases (ARSs) are omitted from a reconstituted cell-free translation system, and instead aminoacyl-tRNAs with non-proteinogenic amino acids are added to reassign the codons corresponding to the excluded proteinogenic amino acids to the non-proteinogenic ones. As a limitation of the methodology, it is difficult to encode more than 20 kinds of amino acids in the reprogrammed genetic code because proteinogenic amino acids are necessarily excluded. To overcome this limitation, we have developed a new method, which is referred to as artificial codon box division, to encode multiple non-proteinogenic amino acids without the need to exclude any proteinogenic ones. For example, the valine GU(U/C/A/G) codon box can be divided into two segments GU(U/C) codons and GUG codon which are assigned to *N*-methyl tyrosine (<sup>Me</sup>Tyr) and valine, respectively. <sup>Me</sup>Tyr-tRNA<sub>GAC</sub> is prepared by a tRNA aminoacylation ribozyme “flexizyme” and added into a reconstituted cell-free translation system. On the other hand, valine is charged to tRNA<sub>CAC</sub> by valyl tRNA synthetase *in situ*. Similarly, *N*-methyl serine (<sup>Me</sup>Ser) and *p*-iodo phenylalanine (<sup>iod</sup>Phe) are assigned to CG(U/C) codons and GG(U/C) codons, respectively.

Artificial codon box division was examined by translation of a peptide containing three non-proteinogenic amino acids. The MALDI-TOF mass spectrometry of the peptide product demonstrated that all NNC codons of the divided codon boxes were accurately assigned to each non-proteinogenic amino acid. In conclusion, artificial codon box division enables three kinds of non-proteinogenic amino acids to be encoded in the reprogrammed genetic code without the need to exclude any proteinogenic ones.



## P75

## Modulation of organismal growth by initiator tRNA modification

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The number of cells and their size determine organism dimensions. Nutrients and hormones influence growth; these adjust translation to sustain animal growth.

Tor kinase transduces the growth-promoting signals to set, regulating the translational machinery, the metabolic state of the cell. tRNAs stand at the core of this machinery and need a variety of posttranscriptional modifications to function. N6-threonylcarbamoyl-adenosine (t<sup>6</sup>A) occurs in tRNAs that recognize ANN codons to permit accurate translation. Recent reports have revealed that KEOPS (Kinase, putative Endopeptidase and Other Proteins of Small size) complex together with Sua5 are responsible for this modification in yeast. Mutants for KEOPS members display low t<sup>6</sup>A and slow-growth phenotypes.

Recent experiments have shown that increasing the amount of the initiator transfer RNA (tRNA<sub>i</sub><sup>met</sup>) stimulates cell proliferation in yeast and promotes oncogenic transformation in mammals. Furthermore, tRNA<sub>i</sub><sup>met</sup> locus duplication in *Drosophila* produces larger animal. These growth effects are induced even although growth-promoting signals are kept constant, suggesting that changes in tRNA<sub>i</sub><sup>met</sup> levels are sufficient to set new growth outlines.

Using the advantages of *Drosophila* genetics we have investigated how the mechanisms that transduce the environmental cues are adjusted by the growth potential of the cells, specifically by the level of t<sup>6</sup>A modification. Our results show that KEOPS activity is conserved in metazoans, t<sup>6</sup>A is not only required for efficient translation and in consequence necessary for cell growth, but the amount of this modification present in tRNAs determine the growth potential of the organism, thus tuning the biosynthetic challenge to support sustainable cell and animal growth.

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**P76****Decoding mechanism of CGN codon box in *Bacillus subtilis***

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According to the conventional Watson-Crick and wobble base pairing, tRNA<sup>Arg</sup>(ICG) has been predicted to decode three arginine codons, CGU, CGC and CGA codons, whereas CGG codon is decoded by tRNA<sup>Arg</sup>(CCG). The inosine (I) modification through the adenosine deamination at the first position of the anticodon (position 34) has been considered essential to decode the CGA codons in most eubacteria. However, we found that *Bacillus subtilis* yaaJ encoding tRNA adenosine deaminase is dispensable and the cell harboring tRNA<sup>Arg</sup>(ACG) without inosine modification exhibited no adverse effect on their growth. A reporter assay analysis clarified that the depletion of inosine formation caused no defect on the decoding of the CGN four-codon-box but rather increased the overall translational activity. On the other hand, the lack of I34 increased the frameshifting rate at the CGA codon, indicating that the entry of arginyl-tRNA<sup>Arg</sup>(ACG) to the A-site on the ribosome was slowed and unstable at the CGA codon. Thus, the inosine modification of *B. subtilis* tRNA<sup>Arg</sup> is not essential but contributes to the fidelity of decoding CGA codons probably by stabilizing the codon:anticodon interactions. We found that either tRNA<sup>Arg</sup>(ICG) or tRNA<sup>Arg</sup>(ACG) alone is sufficient for decoding the CGN codon box in *B. subtilis* cells. This indicates that the CGN codon box is decoded based on the super-wobbling by I34 and A34. Similar results were obtained for the other four-codon-boxes showing the relatively relaxed decoding strategy in *B. subtilis*.





## Rescue of fatal base-pair substitutions in the T stem by residues in distant tRNA domains

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There is no doubt that, on account of its structural flexibility, tRNA is able to access the variety of conformational states that are central to its performance before and after it arrives at the ribosome. However, the question remains as to whether tRNA's structural adaptability is dictated by the characteristics of localized sequence elements or is the result of functional coupling among residues in distant structural domains. To address this important, but difficult, problem we took advantage of tRNA<sup>Thr</sup><sub>UGU</sub> T-stem variants that we previously characterized<sup>1</sup>. Variants that are aminoacylated by threonyl-tRNA synthetase, and bind to EF-Tu with an affinity similar to that of wt tRNA<sup>Thr</sup><sub>UGU</sub>, but fail to confer viability to an *E. coli* tRNA<sup>Thr</sup><sub>UGU</sub> knockout strain, were subjected to error-prone PCR and selected using a genetic screen. In addition to recovering the anticipated reversions in the T stem, the screen identified mutations outside of the T stem – in the tRNA core, variable pocket, and anticodon stem loop – that individually restore function in the context of particular T-stem sequences. Together, these studies reveal unanticipated specific coupling between residues in the T stem and those in distant structural domains.

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P78

## Multiple site-specific installations of $N^{\epsilon}$ -monomethyl-L-lysine into histone proteins by cell-based and cell-free protein synthesis

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Lysine methylation is one of the important post-translational modifications of histones, and produces an  $N^{\epsilon}$ -mono-, di-, or trimethyllysine residue. Multiple and site-specific lysine methylations of histones are essential to define epigenetic statuses and control heterochromatin formation, DNA repair, and transcriptional regulation. A method was previously developed to build an analogue of  $N^{\epsilon}$ -monomethyllysine, with cysteine substituting for lysine. In this study, we developed a novel method to prepare histones bearing multiple  $N^{\epsilon}$ -monomethyllysine residues at specified positions. Release factor 1 (RF1)-knockout (RFzero) *Escherichia coli* cells or the cell-free system using the RFzero cell lysate was used for protein synthesis, since in RFzero cells, UAG is completely redefined as a sense codon for non-canonical amino acids. Therefore, during protein synthesis, a *tert*-butyloxycarbonyl-protected  $N^{\epsilon}$ -monomethyllysine analogue is ligated to *Methanosarcina mazei* pyrrolysine tRNA (tRNA<sup>Pyl</sup>) with *M. mazei* pyrrolysyl-tRNA synthetase mutants, and is translationally incorporated into one or more positions specified with the UAG codon. The protecting groups on the protein are then removed with trifluoroacetic acid, to generate  $N^{\epsilon}$ -monomethyllysine residues. We installed  $N^{\epsilon}$ -monomethyllysine residues at positions 4, 9, 27, 36, and/or 79 of human histone H3. Each of the  $N^{\epsilon}$ -monomethyllysine residues within the produced histone H3 was recognized by its specific antibody. Furthermore, the antibody recognized the authentic  $N^{\epsilon}$ -monomethyllysine residue at position 27 better than the  $N^{\epsilon}$ -monomethyllysine analogue built with cysteine. Mass spectrometric analyses also confirmed the lysine modifications on the produced histone H3. Thus, the present method enables the installation of authentic  $N^{\epsilon}$ -monomethyllysines at multiple positions within a protein for large-scale production.



## P79

## Elongation factors on tRNA docking models

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The present author has proposed the A-P and P-E tRNA docking modes at the pre- and posttranslocational states (1). These structures of tRNAs represent a dynamic behavior of the whole ribosome under a physiological condition and based on a universal rule on intersubunit complementarities of ribosomal RNAs and tRNAs (2). From this point of view, the recent result of the crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA (3) does not seem to fully explain the mechanism of EF-Tu in the selection of a correct tRNA. Accordingly, the effector region of EF-Tu around Val 42 and Ile 63, between which the atomic coordinates of 15 peptides are missing, and also the region near uridine 2653 of 23S ribosomal RNA of the crystal structure (3) were fitted to the uridine 2653 of 23S rRNA in our model as close as possible at the distance of 40 Å, and connected at the phosphate group of the 73rd nucleotide of the A-site tRNA. In this conformation, EF-Tu molecule makes contacts with the waist regions of both A- and P-site tRNAs, and allows a sterical interaction of peptidyl transfer between the two O3' position of 76th adenines of both A- and P-site tRNAs. Since the A-site tRNA bound to EF-Tu can be considered highly homologous to the structure of EF-G bound to P-site tRNA. In this structure, the domain IV of EF-G makes a contact with P-site tRNA, which is quite similar to the crystal structure of the ribosome with EF-G trapped in the posttranslocational state (4).

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## P80

## tRNA dependence of stringent response factor RelA inhibition by thiostrepton suggests two conformations of the ribosome-bound RelA

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The stringent response is a central bacterial regulatory pathway mediated by the alarmone nucleotide ppGpp<sup>1</sup>. In *E. coli*, stringent response factor RelA synthesizes ppGpp in response to increased level of deacylated tRNAs caused by the amino acid starvation. ppGpp exerts its regulatory role by modulating the activity of numerous enzymes<sup>1</sup>: RNA polymerase, translational GTPases, and by activating the ppGpp-synthetic activity of RelA itself<sup>2</sup>. Since the stringent response regulates bacterial virulence and antibiotic tolerance<sup>4</sup>, development of specific inhibitors is a promising strategy for the design of novel antibacterials.

Using an *in vitro* stringent response and translation system<sup>2</sup> we have tested the inhibition of *E. coli* stringent response factor RelA by antibiotics tetracycline, thiostrepton, chloramphenicol and recently developed RelA inhibitor Relacin<sup>3</sup>. Both Relacin and tetracycline inhibit RelA inefficiently and the inhibition is insensitive to the A-site tRNA while the inhibition of RelA by thiostrepton is strongly dependent on the presence of deacylated tRNA. We hypothesize that RelA enzyme acquires two different conformations in the presence and absence of tRNA leading to dramatically different sensitivity to thiostrepton.

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## P81

**tRNA-derived RNAs are the most abundant small RNA in chronic viral hepatitis**

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Small, non-coding RNAs control gene expression post-transcriptionally and play important roles in virus-host interactions. Hepatitis B and C viruses persistently infect hundreds of millions of people worldwide, and are associated with chronic liver inflammation, cirrhosis and cancer. We sequenced small RNAs (14-40 nts) in liver from subjects with chronic hepatitis B or C and associated cancer, as well as uninfected controls. Surprisingly, we found a high abundance of reads mapping to tRNAs. Specifically, we identified reads mapping to 348 of the 458 known unique tRNA sequences. Approximately 90% of these reads mapped to the region extending from the 5'-end of the tRNA to the anticodon triplet (these reads are ~32-35 nts and are referred to as tRNA-halves or tRHs). More than 60% of these tRHs originated from tRNA<sup>Gly</sup> and tRNA<sup>Val</sup>. In most infected livers, tRH levels exceeded that of miRNAs. In contrast, in the cancer tissue from the same subjects tRH abundance was reduced, concomitant with decreased expression of the tRNA-cleaving ribonuclease, angiogenin, and increased expression of angiogenin inhibitor, RNH1. Previous studies in cell culture have suggested that tRHs confer global reduction in protein translation. Using two different assays, we found that ectopic expression of the most common intrahepatic tRHs (5'-tRH<sup>Gly</sup> and 5'-tRH<sup>Val</sup>) does not repress 5'-cap-dependent translation in human hepatoma cells, and may alter hepatitis C internal ribosomal entry site (IRES)-initiated translation. In summary, our results show that tRHs are abundantly expressed in primary human tissue, increased in chronic viral infection, and decreased in associated cancer. These findings highlight the potential biological and clinical relevance of these small non-coding RNAs.



## P82

## Upregulated tRNA level alters insulin signaling in C2C12 myotubes

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Insulin signaling, through PI3K/Akt kinase, activates mTORC1 which directly phosphorylates and inhibits Maf1 thus increases tRNA transcription. Since palmitic acid disrupts insulin signaling in muscle cells and increases mTORC1 activity independently of PI3K/Akt pathway, we asked whether either altered Maf1 activity or tRNA cellular level contribute to palmitic acid induced insulin resistance. The tRNA level was upregulated in palmitic acid treated C2C12 myotubes as well as in white *Gastrocnemius* of Wistar rats fed for 16 weeks with high fat diet (HF). Overexpression of tRNA decreased insulin sensitivity of C2C12 myotubes treated with palmitic acid. Similarly, hyperactive Maf1 conveyed the same effect. Surprisingly, increased tRNA level was accompanied by dephosphorylation of Maf1 which suggests that Maf1 may be activated by the tRNA excess. Our results indicate that Maf1, apart from Pol III repressor function, modulates lipid metabolism therefore we investigated if unbiased tRNA level contributes partially to this effect. We found upregulated tRNA level to induce lipolysis and  $\beta$ -oxidation suggesting increased lipid utilization. Moreover in palmitic acid treated C2C12 myotubes we found upregulated tRNA level to induce ROS production. In conclusion we believe that disturbances in RNA Pol III transcription due to either Maf1 activity or increased tRNA cellular content and possibly non canonical tRNA functions may affect lipid metabolism thus alter insulin signaling.

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## Plant tRNA-derived RNA fragments: origin, biogenesis and function

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In the expanding repertoire of small noncoding RNAs, tRNA-derived RNA fragments (tRFs) have been identified in all domains of life. Accumulating evidence suggest that they are not just random degradation fragments but rather stable entities which may have major biological functions.

So far, only few data on plant tRFs are reported. Neither in-depth analysis of their identity was performed, nor their biogenesis and roles studied. Using high-throughput sequencing technology and in silico analyses, tRFs were retrieved from 30 *Arabidopsis* small RNA libraries, thanks to a new PlantRNA database we recently created. The RNA libraries were generated from various tissues, from plants submitted to biotic or abiotic stress and from immunoprecipitated argonaute fractions. Among the remarkable observations, it is to note that numerous tRFs originate from organellar tRNAs and with very specific cleavage sites. Examining the AGO1- or AGO4- associated tRFs also reveals bias in term of identity and size and shows the presence of several organellar tRFs in immunoprecipitates.

Having validated the major tRFs by northern blots, we then asked the question of their biogenesis. An *in vitro* cleavage assay was set up from *Arabidopsis* leaves. We provide evidence that plastidial or mitochondrial tRFs are generated outside the organelles by cytosolic endonucleases. While in mammals, short tRFs may be produced by Dicer, this is not the case in plants according to *Arabidopsis* mutant analysis. Rather, two *Arabidopsis* endonucleases were identified and shown to cleave both nuclear and organellar tRNAs. Their characterization is under way. All these observations strongly suggest that some tRFs play important regulatory functions within the cell. Beyond translation, our data open new perspectives for nucleus- and organelle-encoded tRNAs as major actors of gene expression.

**P84****mTOR regulates the degradation of initiator tRNA<sup>Met</sup> under heat stress****Kazunori Watanabe<sup>1,2</sup>, Kenichi Ijiri<sup>2</sup>, Takashi Ohtsuki<sup>1</sup>**<sup>1</sup> *Department of Biotechnology, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan*<sup>2</sup> *Radioisotope Center, The University of Tokyo, Tokyo, Japan**correspondence e-mail: k\_watanabe@okayama-u.ac.jp*

Stress induces various responses including translational arrest and tRNA degradation and accumulation in mammals. Previously, we showed that heat stress induces the nuclear accumulation of initiator tRNA<sup>Met</sup> (iMet) and the degradation of its through 5'-3' exoribonuclease Xrn1 and Xrn2, which are localized primarily to the cytoplasm and nucleus including the nucleolus, respectively (1, 2). In addition, we found that rapamycin inhibits the degradation of iMet under heat stress (2). Here, we report that the mammalian target of rapamycin (mTOR), a rapamycin binding protein, regulated the diffusion of Xrn2 from the nucleolus to the nucleoplasm, facilitating the degradation of iMet under heat stress. Moreover, Xrn2 protein synthesis was regulated by mTOR and heat stress induced the degradation of Xrn2 protein. Our results suggest a mechanism of translational arrest through mTOR-regulated iMet degradation in mammal cells.

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## P85

## Regulation of tRNA transcription by TFIIC in budding yeast

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Transfer RNAs (tRNAs) belong to the group of short non-coding (nc) RNAs that are transcribed by the RNA Polymerase III (Pol III) system. Other targets include the 5S ribosomal and U6 spliceosomal RNA. Transcription factors (TF) IIIA, IIIB and IIC are involved in Pol III transcription regulation, binding to different promoter sequences and the transcription of each ncRNA is regulated by a different combination of TF and promoters. In particular, to transcribe tRNAs TFIIC binds to the intragenic A and B boxes and it has been suggested to stay bound to these sites until displaced by TFIIB/Pol III when transcription is initiated.

Each of the six subunits of TFIIC is essential for cell survival, making it difficult to perform *in vivo* studies to analyze the function of each subunit separately. We have studied the interactions between TFIIC subunits using fluorescence-cross-correlation spectroscopy (FCCS). In addition, we have generated viable yeast strains where we can deplete each subunit independently via an inducible degron system and we can follow the depleted subunit via GFP fluorescence and microscopy over time. Using these strains and quantitative PCR, we analyze the effect on the levels of two different tRNA genes, U6 and 5S RNA in yeast cells, nine hours after inducing the degradation of each TFIIC subunit. Unexpectedly, our initial results show a clear increase in the tRNA levels, whereas no change is observed in the levels of the U6 and 5S RNAs. These results suggest that TFIIC acts to block and not enhance for Pol III transcription, an idea that agrees with previous hypothesis concerning the role of TFIIC in transcription. Whole transcriptome studies are now being established to verify these results.



## P86

## CLP1 as a novel player in linking tRNA splicing to neurodegenerative disorders

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Transfer RNAs (tRNAs) undergo a series of posttranscriptional processing and modification events to mature into functional tRNAs. Processing entails intron excision by the tRNA splicing endonuclease (TSEN), ligation of the resulting exon halves by a tRNA ligase and removal of 5' leader and 3' trailer sequences by RNase P and RNase Z, respectively. Unique to mammals is the association of the TSEN complex with CLP1, a polynucleotide kinase that phosphorylates the 5'-hydroxyl group of 3' tRNA exons *in vitro*. We recently investigated the *in vivo* role of CLP1 through the generation of a kinase-dead (*Clp1<sup>k/k</sup>*) mouse harboring a lysine to alanine substitution at amino acid position 127 (K127A) in CLP1. Surprisingly, these CLP1 mutant mice exhibited a progressive loss of lower motor neurons resulting in fatal deterioration of motor functions. In addition, *Clp1<sup>k/k</sup>* mice accumulated a novel class of tRNA fragments composed of a tri-phosphorylated 5' leader followed by a 5' exon, that sensitize cells to p53 activation in response to oxidative stress. These fragments are also generated in cells upon exposure to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). This new class of tRNA fragment adds to a long list of tRNA pieces that arise with stress conditions. Furthermore, we recently characterized homozygous mutations in CLP1 (R140H) that were identified in Turkish families, with patients experiencing severe motor-sensory defects, cerebellar neurodegeneration, cortical dysgenesis and microcephaly. Biochemical dissection revealed accumulation of linear introns, lack of interaction between CLP1 and TSEN subunits and a consequent impairment in pre-tRNA cleavage activity. Upon re-examination, microcephaly was also found in *Clp1<sup>k/k</sup>* mice. Our findings linking tRNA splicing defects with neurodegeneration suggest that tRNA processing, in particular tRNA splicing, is an Achilles heel for neuron function.



## Dissecting the regulatory role in translation and cell wall formation of *Staphylococcus aureus* *glyS* T-Box riboswitch

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In *Staphylococcus aureus*, the solely existing GlyRS provides the necessary Gly-tRNA<sup>Gly</sup> molecules as substrates for glycine incorporation during translation and cell wall stabilization through pentaglycine bridges via the FemXAB factors. We have previously identified 5 tRNA<sup>Gly</sup> isoacceptors exhibiting differential binding affinity to EFTu<sup>1</sup>. We show herein, that *glyS* transcription is controlled by a T-box riboswitch which interestingly contains the shortest intergenic sequence of all known T-boxes, so far. After predicting the secondary structure and cloning the *glyS* T-box we screened the two proteinogenic tRNA<sup>Gly</sup> isoacceptors (P1 and P2) and the three non proteinogenic (NP1, NP2 and NEW) for binding. We observed binding of P1 tRNA which is the only one with different anticodon that the other four molecules that matches the anticodon region of the specifier loop. Using various mutants and the riboswitch as a whole, we performed chemical probing and primer extension, as well as enzymatic probing which unravelled and verified the secondary structure of the *glyS* T-box riboswitch. In addition, *in vivo* experiments tested the ability of various T-box mutants to trigger antitermination in the presence of each tRNA<sup>Gly</sup> isoacceptor, using a modified *E. coli* plasmid-based antitermination system. Moreover, tRNA-directed antitermination *in vitro* assays with all five different tRNA isoacceptors validated the differential read-through of the *glyS* TBox terminator. Finally, the *in vivo* experiments were repeated using directly transformed *S. aureus* specific strains, detecting the endogenous GlyRS expression levels. In conclusion, the *S. aureus* *glyS* T-box represents the only known tRNA-dependent regulatory element that controls two essential, but unrelated pathways and could be considered as a novel antimicrobial target.

1. Giannouli S., Kyritsis A., Malissovass N., Becker H. D. and Stathopoulos C. (2009) On the role of an unusual tRNA<sup>Gly</sup> isoacceptor in *Staphylococcus aureus*. *Biochimie* 91: 344-351

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## Active methionyl-tRNA synthetase (MRS) controls cell cycle promoting triple-negative breast cancer proliferation

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Aminoacyl-tRNA synthetases (ARSs), charging amino acid to the cognate tRNA, mediate various signal pathways in response to growth, stress and apoptotic stimuli. Among them, methionyl-tRNA synthetase (MRS) is known to relocate from cytosol to nucleus under growth condition, however the meaning and function of MRS in the nucleus is not clearly understood.

We found that knockdown of MRS triggered growth retardation of triple negative breast cancer (TNBC) cells. G1/S transition of cell cycles was impaired by MRS knockdown based on the flow cytometry analysis suggesting that MRS may be involved in the cell cycle control. We, therefore investigated the relationship between MRS and cell cycle regulation-related proteins and observed that level of cyclin-dependent kinase 4 (CDK4) was critically affected by MRS expression level. MRS activity is related to the control of CDK4 stability, and inhibition of MRS activity significantly reduced the level of CDK4 resulting in cell growth arrest under growth condition. MRS knockdown-mediated cell growth retardation was recovered by overexpression of CDK4 suggesting that maintenance of CDK4 stability via interaction with MRS is critical for cell proliferation. This study suggests the novel function of MRS regulating cell cycle via increasing the stability of CDK4 and the possible crosstalk with translation for the control of cancer cell proliferation.



## Monitoring the expression dynamics of networks controlling tRNA expression, signaling and translation in lung cancer

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Lung cancer is a leading cause of death worldwide. Although many expression-based biomarkers have been reported to regulate the disease progress, there are limited reports on the expression level of genes related to tRNA transcription, maturation, aminoacylation, translation initiation, cleavage and signalling, in combination with tRNA expression and the identification of tRNA fragments. In the current study, we investigate the expression levels of more than 60 genes related to those events. Many of the proteins encoded by the genes under investigation appear interconnected in a putative protein network model proposed based on experimentally verified data. Finally, we provide a first landscape of the tRNAs, tRFs and miRNAs expression levels using NGS from cancer cell lines and biopsies.

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