International Union of Biochemistry & Molecular Biology Focused Meeting on Aminoacyl-tRNA Synthetases 2023

The 13th International Symposium on Aminoacyl-tRNA Synthetases

AARS2023 co-chairs
Ilka Heinemann & Patrick O'Donoghue
The University of Western Ontario

Table of Contents

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Welcome Words

Since its inception in 1990, AARS2023 is an international conference that brings together scientists from all over the world to advance the frontiers of research with respect to the essential functions of aminoacyl-tRNA synthetases (AARSs) in protein synthesis and translation fidelity and their relationship to disease caused by human pathogens or by genetic mutations in the human population. Our research community includes scientists, clinicians, and representatives from the pharmaceutical industry that will meet to reveal new knowledge regarding the origin of genetic coding, the structure and mechanism of the translational apparatus, the diverse roles of AARSs in protein synthesis, as well as non-translational functions and physiological regulation in human disease.

We welcome you to share in the exciting advances in our field at the 13th International AARS meeting, held at The Oakwood resort in Grand Bend, Ontario, Canada from June 4th to June 9th, 2023.

Sponsors

We are grateful to each of these sponsors for their generous and critically important support for the meeting. We are especially appreciative of the IUBMB and thankful to Charysse Austria of the IUBMB Secretariat and IUBMB president, Alexandra Newton, for their partnership and strong support for the meeting and AARS research.

Conference History

2025 – 14th International Symposium on AARSs, **Zagreb, Croatia**

2023 – 13th International Symposium on AARSs, **Grand Bend, Canada**
IUBMB Focused Meeting on AARSs

2019 – 12th International Symposium on AARSs, **Hangzhou, China**

2017 – 11th International Symposium on AARSs, **Clearwater, USA**
IUBMB Focused Meeting on AARSs

2015 – 10th International Symposium on AARSs, **Barcelona, Spain**

2013 – 9th International Symposium on AARSs, **Hakone, Japan**

2011 – 8th International Symposium on AARSs, **Salt Lake City, USA**

2008 – 7th International Symposium on AARS, **Veyrier du Lac, France**
International Conference on AARSs:
From basic mechanism to systems biology

2006 – 6th International Symposium on AARSs, **San Diego, USA**
2006 International Conference on AARSs:
From the Genetic Code to Human Disease & Medicine

2004 – 5th International Symposium on AARSs, **Barcelona, Spain**
2004 International Conference on AARSs:
Ancient Molecular for Future Biology and Medicine

2002 – 4th International Symposium on AARSs, **Asilomar, USA**
Asilomar Conference on AARSs in Biology, Medicine, & Evolution

1998 – 3rd International Symposium on AARSs, **Mittelwehr, France**
EMBO Workshop on Structure and Function of AARSs

1997 – 2nd International Symposium on AARSs, **Taos, USA**
Keystone Symposium on AARSs in Biology & Disease

1990 – 1st International Symposium on AARSs, **Autrans, France**
NATO Workshop on AARSs

Program

IUBMB Focused Meeting on Aminoacyl-tRNA Synthetases 2023

IUBMB Focused Meeting on Aminoacyl-tRNA Synthetases: AARS 2023 Program

Sunday, June 4, 2023

Registration	3:00 pm–8:30 pm
Welcome Cocktails	4:00 pm–6:00 pm
Dinner	6:30 pm–8:00 pm
Alexandra Newton, University of California San Diego *Opening remarks from the IUBMB president*	8:00 pm–8:10 pm
Patrick O'Donoghue & Ilka Heinemann, The University of Western Ontario *Opening remarks from the Organizers*	8:10 pm–8:20 pm
Keynote 1 Nahum Sonenberg, McGill University *The mRNA 5' cap-binding protein, eIF4E2 (4EHP) links with the AARS and miRNA apparatuses*	8:20 pm–9:00 pm

Monday, June 5, 2023

Session A: ***AARS Biochemistry***
Session Chair: Tamara Hendrickson, Wayne State University

Opening remarks from the Chair	8:55 am–9:00 am
Michael Ibba, Chapman University *Divergent changes in the synthetic and proofreading activities of aminoacyl-tRNA synthetases are required for a concerted response to oxidative stress*	9:00 am–9:20 am
Marie Sissler, Centre National de la Recherche Scientifique *Mitochondrial threonyl-tRNA synthetase (TARS2)-related disorders*	9:20 am–9:40 am
Thomas Carell, Ludwig Maximilian University *The prebiotic origin of the RNA nucleosides and translation*	9:40 am–10:00 am
Hubert Becker, University of Strasbourg *Saccharomyces organellar echoforms of cytosolic aminoacyl-tRNA synthetases: not simply mislocated*	10:00 am–10:20 am
Ita Gruic–Solvj, University of Zagreb *Mupirocin hyper-resistance secured by naturally altered class I signature motif*	10:20 am–10:40 am
Coffee Break	10:40 am–11:00 am

Magali Frugier, Centre National de la Recherche Scientifique 11:00 am–11:20 am
Two aminoacyl–tRNA synthetase complexes, membrane localization and unusual role in tRNA import: many questions remain about tRNA biology in Plasmodium

Natalia Mora, Radboud University 11:20 am–11:30 am
Loss of function mechanisms in CMT2D

Julian Ross, University of Vienna, Institute of Molecular Biotechnology 11:30 am–11:50 am
Functional characterization of a phenylalanyl-tRNA-synthetase-based selfish element in the nematode C. tropicalis

Lunch 11:50 am–1:15 pm

Session B: ***Synthetic Biology & Orthogonal Translation***
Session Chair: Jiqiang Ling, University of Maryland
Opening remarks from the Chair 1:15 pm–1:20 pm

Keynote 2
Dieter Söll, Yale University 1:20 pm–2:00 pm
Pyrrolysine and Selenocysteine - different designs to manifest the genetic code

Ren Nakazaki, The University of Tokyo 2:00 pm –2:20 pm
Translational regulation mediated by ligand-induced tRNA activation

Xiang-Lei Yang, Scripps Research 2:20 pm–2:40 pm
Probing the physiological role of nuclear tRNA synthetases in mammals

Jeffrey Tharp, Indiana University School of Medicine 2:40 pm–3:00 pm
Split aminoacyl–tRNA synthetases for proximity-induced stop codon suppression

Margaret A. Schmitt 3:00 pm–3:10 pm
Rapid evaluation of sense codon reassignment potential by the M. barkeri pyrrolysyl-tRNA/aaRS pair

Yane-Shih Wang, Academia Sinica 3:10 pm–3:30 pm
X-ray crystal structure of wild type G1PylRS reveals multiple binding modes and chiral degeneracy to accommodate pyrrolysine and noncanonical amino acids

Coffee Break 3:30 pm–3:50 pm

Wei Lu, University of Tokyo 3:50 pm–4:10 pm
Development of aminoacylation ribozymes capable of peptide elongation in one-pot in vitro translation

Natalie Krahn, Yale University 4:10 pm–4:30 pm
Without a recruitment domain, pyrrolysyl-tRNA synthetase requires a rigid tRNA identity structure for recognition

Session C: ***AARS genomics & tRNAomics***
Session Chair: Lluís Ribas de Pouplana, IRB Barcelona
Opening remarks from the Chair 4:35 am–4:40 pm

EMBO Young Investigator Lecture 4:40 pm–5:00 pm
Danny Nedialkova, Max Planck Institute of Biochemistry
Transfer RNA pools in human cells are controlled by selective gene expression

Todd Lowe, University of California, Santa Cruz 5:00 pm–5:20 pm
The complexity leap in tRNA evolution in vertebrates

Joe Chihade, Carleton College 5:20 pm–5:40 pm
Novel features of helminth aminoacyl-tRNA synthetases

Jordan Douglas, University of Auckland 5:40 pm–6:00 pm
Rethinking the taxonomy of AARS hierarchical modularity

Dinner 6:00 pm–7:30 pm

Tuesday, June 6, 2023

Session D1: ***AARSs in health and disease***
Session Chair: Haissi Cui, University of Toronto
Opening remarks from the Chair 8:55 am–9:00 am

Rob Burgess, The Jackson Laboratory 9:00 am–9:20 am
Rescue of tRNA synthetase-associated neuropathy by inhibiting GCN2 or supplementing tRNA expression

Karin Musier-Forsyth, The Ohio State University 9:20 am–9:40 am
Role of aminoacyl-tRNA synthetases in HIV-1 lifecycle

Sunghoon Kim, Yonsei University 9:40 am–10:00 am
An oncogenic variant of aminoacyl-tRNA synthetase-interacting multi-functional protein 2 (AIMP2) as a novel target to control KRAS-driven cancer

Albena Jordanova, VBI–University of Antwerpen 10:00 am–10:20 am
Unraveling the non-aminoacylation functions of tyrosyl-tRNA synthetase: insights from inherited peripheral neuropathies

Paul Fox, Cleveland Clinic 10:20 am–10:40 am
Defective m^6A methylation of EPRS1 mRNA in hypomyelinating leukodystrophy patients

Coffee break 10:40 am–11:00 am

AARS patient panel 11:00 am–12:00 pm
Victoria Mok Siu, The University of Western Ontario (panel chair)

Lunch 12:00 pm–1:25 pm

Session D2: ***AARSs in health and disease***
Session Chair: Michael Ibba, Chapman University
Opening remarks from the Chair 1:25 pm–1:30 pm

Victoria Mok Siu, The University of Western Ontario 1:30 pm–1:50 pm
Clinical strategies in the management of individuals with HARS (His-tRNA synthetase) syndrome

Marisa Mendes, Amsterdam UMC 1:50 pm–2:10 pm
Thermal characterization of human aminoacyl-tRNA synthetases in health and disease

Christina Nemeth Mertz, Kennedy Krieger Institute 2:10 pm–2:30 pm
The LBSL transcriptome: distinct and divergent gene expression profiles based on DARS2 mutation

Rachel Heilmann, The Rory Belle Foundation 2:30 pm–2:50 pm
Insights into NARS1-Associated Disease: A preliminary genotype–phenotype review by patient and research advocates

Justin Wang, The Scripps Research Institute 2:50 pm–3:10 pm
Seryl-tRNA synthetase inhibits breast cancer metastasis possibly through blocking Wnt signaling

Coffee 3:10 pm–3:25 pm

Session E: AARS & tRNA Therapeutics

Session Chair: John Nick Fisk, University of Colorado, Denver
Opening remarks from the Chair 3:25 pm–3:30 pm

Leslie Nangle, aTyr Pharma 3:30 pm–3:50 pm
Clinical proof-of-concept for a novel therapeutic based on histidyl-tRNA synthetase for treatment of interstitial lung diseases

Sarah Wilhelm, The University of Western Ontario 3:50 pm–4:10 pm
Histidine supplementation can escalate of rescue HARS deficiency in a Charcot-Marie-Tooth disease model

Edmund Grace, Oxford Drug Design 4:10 pm–4:30 pm
A novel class of Gram-negative antibacterial agents targeting leucyl-tRNA synthetase

Min-Xin Guan, Zhejiang University 4:30 pm–4:50 pm
Nuclear modifier mitochondrial tyrosyl-tRNA synthetase allele correction restored retinal ganglion cells–specific deficiencies in Leber's hereditary optic neuropathy

Ralph Mazitschek, Harvard Medical School 4:50 pm–5:10 pm
Development of novel ProRS inhibitors for malaria

Alice Hadchouel, Hôpital Universitaire Necker-Enfants Malades 5:10 pm–5:30pm
Methionine supplementation as a game–changer for severe pulmonary alveolar proteinosis related to MARS1 mutations

Guillaume Hoffmann, National Institute of Health and Medical Research (Inserm)
Institute for Advanced Biosciences (IAB) 5:30 pm–5:50 pm
Adenosine–dependent activation mechanism of prodrugs targeting an aminoacyl-tRNA synthetase

Trinayan Kashyap, hC Bioscience, Inc. 5:50 pm–6:10 pm
Anticodon engineered tRNA rescues expression of tumor suppressor proteins, resulting in growth inhibition of metastatic colorectal cancer models in vivo

Dinner 6:10 pm–7:30 pm

Keynote 3
Paul Schimmel, Scripps Research 7:30 pm–8:10 pm
Back to AARS beginnings and the layers of deep learning that followed

Poster Session & Social 8:10 pm–9:45 pm

Wednesday, June 7, 2023

Session F1: ***Structure, evolution, and cellular function of AARSs & tRNAs***

Session Chair: Yane-Shih Wang, Academia Sinica
Opening remarks from the Chair 8:55 am–9:00 am

Lluís Ribas de Pouplana, IRB Barcelona 9:00 am–9:20 am
Saturation of tRNA sequence space restricts genetic code growth

Aaron Voigt, University Clinic RWTH Aachen 9:20 am–9:40 am
PolyQure: Increasing translational error-rate by TRMT2A–inhibition, an avenue to cure polyQ diseases?

Rasangi Tennakoon, The University of Western Ontario 9:40 am–9:50 am
Inhibiting polyglutamine aggregation with mistranslating tRNAs

Elizabeth Kalotay, University of New South Wales 9:50 am–10:00 am
Novel pre-clinical HBSL models to enable proof-of-concept for AAV-mediated DARS1 gene therapy

Shigeyuki Yokoyama, RIKEN 10:00 am–10:20 am
Structure-based engineering of Methanomethylophilus alvus and ISO4-G1 PylRSs for genetic-code-expanded cell-free protein synthesis

Rylan Watkins, The Ohio State University 10:20 am–10:40 am
Trypanosoma brucei prolyl-tRNA synthetase and a trans–editing domain maintain proline codon translational fidelity

Coffee Break 10:40 am–11:00 am

Keynote 4 11:00 am–11:40 am
Zoya Ignatova, Universität Hamburg
Charcot-Marie-Tooth disease linked to mutations in aminoacyl-tRNA synthetases: tRNA-centered view of pathology (and cure)

Industry panel 11:40 am–12:40 pm
aTyr Pharma, hC Bioscience, Oxford Drug Design, BioCon

Lunch 12:40 pm–2:00 pm

Excursion 2:00 pm–5:00 pm

Pinery Provincial Park
Buses to Pinery: pickup at Oakwood – 2:00 pm
Buses back to Oakwood: pickup at Pinery – 5:00 pm

Dinner @ Hessenland 5:30 pm–8:00 pm
Buses to Hessenland for dinner: pickup at Oakwood – 5:45 pm
Buses back to Oakwood: pickup at Hessenland – 9:00 pm

Thursday, June 8, 2023

Session F2: ***Structure, evolution, and cellular function of AARSs & tRNAs***
Session Chair: Todd Lowe, University of California Santa Cruz
Opening remarks from the Chair 8:55 am–9:00 am

Umesh Varshney, Indian Institute of Science, Bangalore 9:00 am–9:20 am
IF3 interaction with initiator-tRNA elbow modulates translation initiation and growth fitness in Escherichia coli

Jiqiang Ling, University of Maryland, College Park 9:20 am–9:40 am
Coordination of aminoacylation and editing in proteotoxic stress

Yoav S. Arava, Technion – Israel Institute of Technology 9:40 am–10:00 am
tRNA-related modification and anticodon stem loop structure underlie translation regulation by yeast aaRS

Qi Chen, University of Utah, School of Medicine 10:00 am–10:20 am
Puzzles and solutions in resolving RNA modifications in tRNA-derived small RNAs

Parker Murphy, University of Maryland, College Park 10:20 am–10:30 am
Investigating the cellular impacts of translational fidelity mutations

Peter Rozik, The University of Western Ontario 10:30 am–10:40 am
Elucidating tRNA-dependent mistranslation rates in living cells

Coffee break 10:40 am–11:00 am

Ru-Juan Liu, ShanghaiTech University 11:00 am–11:20 am
Molecular basis of human Trmt13 in tRNA modification and transcriptional regulation

Bastien Muller, National Institute of Health and Medical Research (Inserm)
Institute for Advanced Biosciences (IAB) 11:20 am–11:40 am
Structural basis of the resistance mechanisms by an antibiotic targeting leucyl-tRNA synthetase of multi–drug resistant gram-negative bacteria

Tong Zhou, University of Nevada, Reno School of Medicine 11:40 pm–12:00 pm
Exploring the tRNA fragmentation principles across multiple species

Lunch 12:00 pm–1:25 pm

Session G: ***AARSs beyond translation***
Session Chair: Rebecca Alexander, Wake Forest University

Chairs opening remarks 1:25 pm–1:30 pm

Jie Chen, University of Illinois at Urbana–Champaign 1:30 pm–1:50 pm
Threnonyl-tRNA synthetase regulates signal transducer and activator of transcription 3

Luis Povoas, IRB Barcelona 1:50 pm–2:00 pm
Control of cell cycle progression by a (multifunctional) mitochondrial protein

Haissi Cui, University of Toronto 2:00 pm–2:20 pm
Arg-tRNA synthetase links inflammatory metabolism to RNA splicing and nuclear trafficking via SRRM2

Susan Martinis, University of Illinois at Urbana-Champaign 2:20 pm–2:40 pm
Novel function of LARS in maintenance of gastric carcinoma cell homeostasis

Keisuke Wakasugi, The University of Tokyo 2:40 pm–3:00 pm
Tryptophan depletion induces high-affinity tryptophan uptake mediated by tryptophanyl-tRNA synthetase into human cells

Mathew Sajish, University of South Carolina 3:00 pm–3:20 pm
Nuclear TyrRS stimulates topoisomerase 1-induced single strand DNA breaks to protect against tyrosine–induced neurotoxicity in primary cortical neurons

Coffee break 3:20 pm–3:40 pm

Myung Hee Kim, Korea Research Institute of Bioscience and Biotechnology 3:40 pm–4:00 pm
A gut-associated bacterial tRNA synthetase acting as an immune modulator

Debjit Khan, Lerner Research Institute, Cleveland Clinic Foundation 4:00 pm–4:20 pm
A viral pan-end RNA element and unconventional host aminoacyl–tRNA synthetase complex define a SARS-CoV-2 regulon

Mirim Jin, Gachon University 4:20 pm–4:40 pm
Tryptophan-dependent and -independent secretions of tryptophanyl- tRNA synthetase: Pathophysiological implications for innate immune responses

Jung Min Han, Yonsei University 4:40 pm–5:00 pm
Regulation of the metabolic fate of leucine by leucyl–tRNA synthetase 1

Dinner 5:00 pm–6:30 pm

Keynote 5
Faiza Fakhfakh, University of Sfax 6:30 pm–7:10 pm
Mutations in aARS genes revealed by next-generation sequencing in mitochondrial diseases: Clinical and molecular investigations

Keynote 6
Susan Ackermann, HHMI/University of California San Diego 7:10 pm–7:50 pm
tRNAs, ribosome stalling, and neuronal function

Closing remarks & Presentation prizes 7:50 pm–8:15 pm
Patrick O'Donoghue & Ilka Heinemann, The University of Western Ontario

Friday, June 9, 2023

To-go breakfast (front desk) 5:15 am–6:30 am

Breakfast 6:30 am–10:00 am

Bus departures to Toronto Person International Airport

Bus 1 – 6:00 am

Bus 2 – 9:00 am

Keynotes

The mRNA 5' cap-binding protein, eIF4E2 (4EHP) links with the AARS and miRNA apparatuses

Nahum Sonenberg

McGill University, Montréal, Canada
nahum.sonenberg@mcgill.ca

Eukaryotic mRNA translation is predominantly regulated at the initiation phase, which commences with the recognition of the m^7GpppN cap structure by the initiation factor complex 4F (eIF4F). The complex consists of eIF4E, the cap-binding subunit; eIF4A, and RNA helicase and eIF4G, a scaffolding protein, which bridges between the cap structure and the ribosome. The eIF4E Homolog Protein (4EHP, eIF4E2) is a cytoplasmic cap-binding protein that unlike eIF4E does not interact with eIF4G, and acts as a translation repressor in metazoans. eIF4E2 plays a crucial role in development in flies via interaction with morphogens such as Bicoid. eIF4E2 also mediates the translational repression of microRNAs (miRNAs) in metazoans via recruitment by the RISC (RNA-induced silencing complex). We discovered 2 proteins, 4E-T (eIF4E-transporter) and GIGYF2 (Grb10 interacting GYF protein-2), which bridge between eIF4E2, and the RISC. eIF4E2 suppresses IFN-β production by promoting miR-34a-induced translational silencing of *Ifnb1* mRNA. The SARS-CoV-2 encoded Non-Structural Protein 2 (NSP2), directly interacts with GIGYF2, and augments its binding to eIF4E2 and thereby enhancing translational repression of *Ifnb-1* mRNA. Depletion of eIF4E2 or GIGYF2 engendered enhanced IFN-ß expression accompanied by a significant reduction in SARS-CoV-2 replication.

In a collaborative study with the group of Jeong and Kim (Seoul, South Korea) we showed that eIF4E2 binds directly to threonyl-tRNA synthetase (TRS) and stimulate translation initiation by recruiting translation initiation factors.

We reported on another noncanonical function of a threonyl-tRNA synthetase, the mitochondrial TARS2, as a mediator of threonine-dependent mTORC1 (mechanistic target of rapamycin complex 1) activation. TARS2 interacts with Rag GTPase proteins, particularly RagC, to promote mTORC1 activity. Stimulation of mTORC1 activity in response to threonine is dependent on TARS2, but not cytoplasmic TARS.

References

Rom, E, et al (1998) Cloning and characterization of 4EHP, a novel mammalian eIF4E-related cap-binding protein. J. Biol. Chem. **273**, 13104-13109.

Zhang X, et al (2021) microRNA-induced translational control of antiviral immunity by the cap-binding protein 4EHP. Mol Cell. **81**,1187-1199.

Jeong SJ, et al (2019) A threonyl-tRNA synthetase-mediated translation initiation machinery. Nat Commun. **10**,1357-1372.

Kim SH, et al (2021) Mitochondrial Threonyl-tRNA Synthetase TARS2 Is Required for Threonine-Sensitive mTORC1 Activation. Mol Cell. **81**,398-407.

Pyrrolysine and Selenocysteine ─ different designs to manifest the genetic code

Dieter Söll

Yale University, New Haven, CT, USA
dieter.soll@yale.edu

This lecture will review native and synthetic biology routes for incorporation of these genetically encoded unusual amino acids into proteins.

References

Jiang HK, Ambrose NL, Chung CZ, Wang YS, Söll D, Tharp JM. Split aminoacyl-tRNA synthetases for proximity-induced stop codon suppression (2023) *Proc Natl Acad Sci USA* **120**:e2219758120.

Guo LT, Amikura K, Jiang HK, Mukai T, Fu X, Wang YS, O'Donoghue P, Söll D, Tharp JM (2022) Ancestral archaea expanded the genetic code with pyrrolysine. *J Biol Chem* **298**:102521.

Prabhakar A, Krahn N, Zhang J, Vargas-Rodriguez O, Krupkin M, Fu Z, Acosta-Reyes FJ, Ge X, Choi J, Crnković A, Ehrenberg M, Puglisi EV, Söll D, Puglisi J (2022) Uncovering translation roadblocks during the development of a synthetic tRNA. *Nucleic Acids Res* **50**:10201-10211.

Back to AARS Beginnings and the Layers of Deep Learning that Followed

Paul Schimmel

Department of Molecular Medicine, Scripps Research, La Jolla ,CA, USA
schimmel@scripps.edu

Aminoacyl tRNA synthetases arose early in evolution, ostensibly in conjunction with the establishment of the genetic code. They split into two classes of 10 enzymes each, with each class being further divided into subclasses. Early work suggested a rationale for the two classes. Recent x-ray structures of an 'orphan' tRNA synthetase have now provided support for early ideas on the origin of the two classes. From this origin, the aaRSs have further evolved through layer upon layer of deep learning, stimulated by strong selective pressures that engendered new functions with, among other faculties, deep roots in immunology and associated inflammatory responses. These new functions are now seen as essential and, when perturbed, result in diseases. These connections to new functions and disease are in the earliest stages of our understanding.

Charcot-Marie-Tooth Disease Linked to Mutations in Aminoacyl-tRNA Synthetases: tRNA-centered View of Pathology (and Cure)

Zoya Ignatova

Institute of Biochemistry and Molecular Biology, University of Hamburg, Germany
zoya.ignatova@uni-hamburg.de

Heterozygous mutations in six tRNA synthetases (aaRSs) are linked to Charcot-Marie-Tooth (CMT) peripheral neuropathy. I will discuss the underlying molecular mechanisms of different CMT forms, e.g. CMT2D pathology associated with mutations in glycyl-tRNA synthetase (GARS), a class II aaRS, and DI-CMTC pathology linked to tyrosyl-tRNA-synthetase (YARS), a class I aaRS. The sequestration of cognate tRNA by mutant CMT-aaRS variants follows a gradient along neuronal ends, which provides an explanation about the higher susceptibility of the distal motor neuronal ends to degeneration. Local administration of in vitro transcribed tRNA alleviates translation.

Mutations in aARS genes revealed by next-generation sequencing in mitochondrial diseases: Clinical and molecular investigations

Faiza Fakhfakh

Laboratory of molecular and functional genetics, Faculty of science of Sfax,
University of Sfax, Tunisia
faiza.fakhfakh02@gmail.com

Mitochondrial diseases are a clinically heterogeneous group of disorders characterized by mitochondrial dysfunction affecting particularly organs with high energy requirements such as the nervous system, skeletal and cardiac muscles, kidneys, liver, and endocrine system. Mitochondrial diseases can be caused by defects in mitochondrial DNA (mtDNA), but more frequently by mutations in nuclear DNA (nDNA) genes. A group of diseases due to defects of the mitochondrial aminoacyl-tRNA synthetases (mtARSs) has also been identified and encompassing variable symptoms as encephalopathy, myopathy, cardiomyopathy, anemia, tubulopathy and hearing loss. Currently, several human ARSs proteins have been identified and divided into three groups depending on the subcellular compartment where the aminoacylation occurs into the cytoplasm, the mitochondria or in both. We identified variants in different mtARS using Next Generation Sequencing (NGS) in patients from unrelated families with clinical features of mitochondrial disorders. Two homozygous variants were found in KARS (c.683C>T) and AARS2 (c.1150-4C>G), respectively in two patients, while two heterozygous variants in EARS2 (c.486-7C>G) and DARS2 (c.1456C>T) were concomitantly found in the third patient. Bio-informatic investigations predicted their pathogenicity and deleterious effects on pre-mRNA splicing and on protein stability. Our data illustrate the variability of clinical presentations associated to mutations in mt-aARS genes, rendering genotype phenotype correlations extremely complicated. They further contribute to extend the genetic diagnosis of mitochondrial diseases pointing to the crucial contribution of mt-aARS mutations in very severe pathologies.

tRNAs, Ribosome Stalling, and Neuronal Function

Susan L. Ackerman

Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA, USA

sackerman@health.ucsd.edu

Growing evidence indicates that neurons are particularly reliant on the spatial and temporal regulation of mRNA translation for their survival and function; and mutations in numerous components of the translational machinery have been linked to neurological disorders. Although the mammalian genome contains hundreds of nuclear-encoded transfer RNA (tRNA) genes, surprisingly no disease-linked mutations in these genes have been reported as of yet. We recently identified the first tissue-specific mammalian tRNA gene, *n-Tr20*. *n-Tr20* is one of 5 isodecoders in the nuclear-encoded $tRNA^{Arg}_{UCU}$ family and, in contrast to the other members of this family, is specifically expressed in the nervous system. Loss of *n-Tr20* in mice dramatically reduced the $tRNA^{Arg}_{UCU}$ pool in the nervous system, resulting in ribosome stalling on the cognate AGA codons, and activation of the integrated stress response (ISR). Here we present evidence from an allelic series of mutations in *n-Tr20* that loss of function of this tRNA can induce alterations in neuronal function. These changes are accompanied by widespread transcriptional reprogramming. Our work highlights the exquisite sensitivity of the nervous system to even subtle disruption of cellular homeostasis, and raises the possibility that the regulation of tRNA expression may play a critical role in complex neuronal processes.

Talks

Divergent changes in the synthetic and proofreading activities of aminoacyl-tRNA synthetases are required for a concerted response to oxidative stress

Michael Ibba*, Arundhati Kavoor, Paul Kelly & Rebecca Steiner

Affiliations:
Department of Microbiology, The Ohio State University, Columbus, Ohio, USA; Schmid College of Science and Technology, Chapman University, Orange, California, USA.

*Presenting author email address: ibba@chapman.edu

To maintain translation accuracy, aminoacyl-tRNA synthetases (aaRS) have evolved proofreading strategies that can hydrolyze a mis-activated aminoacyl adenylate in the active site or translocate a misacylated tRNA to a separate editing domain capable of hydrolysis. Recent studies showed that environmental challenges such as exposure to reactive oxygen species can also alter aaRS synthetic and proofreading functions, prompting us to investigate if oxidation might positively or negatively affect aaRS activity. Specifically, we investigated the impact of oxidative stress on the synthetic and editing activities of alanyl-tRNA synthetase (AlaRS) and phenylalanyl-tRNA synthetase (PheRS). We showed that AlaRS activity is resistant to oxidative stress, while PheRS proofreading activity actually increases in response to reactive oxygen species. Our findings illustrate one of the roles of aaRSs in the cellular responses that help to maintain the fidelity of the genetic code during growth under environmentally stressful conditions.

Mitochondrial threonyl-tRNA synthetase (TARS2)-related disorders

Andrea Accogli[1#], Sheng-Jia Lin[2#], Mariasavina Severino[3#], Sung-Hoon Kim[4#], ..., Nahum Sonenberg[4], Henry Houlden[5], **Marie Sissler[6^]*** , Gaurav K. Varshney[2^], Reza Maroofian[5^]

1. Division of Medical Genetics, McGill University Health Centre (MUHC) & Department of Human Genetics, McGill University, Montreal, QC, Canada; **2.** Genes & Human Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; **3.** Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy; **4.** Goodman Cancer Institute and Department of Biochemistry, McGill University, Montreal, QC, Canada; **5.** Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, United Kingdom; **6.** GMGM, UMR7156 - Institut de Physiologie et de Chimie Biologique, Strasbourg - France

[#],^These authors contributed equally

*Presenting author email address: **m.sissler@unistra.fr**

The human mitochondrial (mt) translation machinery is of dual genetic origin with RNA constituents encoded by the mitochondrial genome and protein constituents encoded by the nuclear genome, among which are the mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs). In the last decade, an increasing number of mutations affecting mt-aaRSs and leading to severe disorders has been reported [1,2]. Despite being ubiquitously expressed and apparently having a common role in a single cellular process, mt-aaRSs are impacted in various ways. Their mutations cause pleiotropic effects with an unexpected variety of phenotypic expressions, including mainly neurological disorders but also non-neurological symptoms [3]. After recalling some essential characteristics of these enzymes and the pleiotropy the related diseases, the recent data concerning mutations of *TARS2*, the nuclear gene coding for the mitochondrial threonyl-tRNA synthetase, will be presented. They are the first description where mutations likely lead to the pathology (characterized by intellectual disability and cerebellar atrophy among others) for distinct molecular/mechanistic reasons; some of the mutations impacting the canonical function of aminoacylation, others the non-canonical function of mt-ThrRS on the mTORC1 signaling pathway [4].

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The prebiotic origin of the RNA nucleosides and translation

Thomas Carell

Center for Integrative Protein Science at the Department of Chemistry, Ludwig Maximilians University, Munich; e-mail: thomas.carell@lmu.de; www.carellgroup.de

Keywords: Prebiotic chemistry, purine & pyrimidine bases, wet-and-dry-cycles, Origin of the ribosome

The widely accepted RNA world hypothesis suggests that life first emerged from RNA, which is able to (self)-replicate and evolve. Replication of RNA requires formation of the complementary pyrimidine-purine Watson-Crick base pairs A:U and G:C, which are a prerequisite for accurate genetic information transfer. Although prebiotic pathways to RNA building blocks have been reported, no pathway has been able to generate all four constituents of RNA simultaneously.[1, 2] We recently reported a prebiotically plausible new pathway (FaPy-pathway) that is able to generate purine nucleosides.[3] The chemistry is driven exclusively by fluctuations of physicochemical parameters such as pH, temperature and concentration. These conditions allow in addition the parallel formation of a variety of non-canonical purine nucleosides as living molecular fossil of an early abiotic world.[4] Many of the formed non-canonical RNA building blocks are today assumed to have been part of the genetic system of the last universal common ancestor (LUCA).[5] In order to find a prebiotically plausible scenario for the parallel formation of purine and pyrimidine bases to create the fundamental Watson-Crick base pairing system, I will report about new prebiotically plausible chemistry route to pyrimidines. Because the new chemistry is compatible with the purine procedures it allows to simulate the formation of all four RNA building blocks in the same geochemical environment.[6]

Next to the formation of nucleosides, the emergence of life also required amino acids and the process of translation, in which RNA information encodes the formation of proteins. We were able to show that certain RNAs have the property to self-decorate with amino acids and that these amino acids can react directly attached to RNA to peptides. This so far unknown property of RNA forces us to extend the RNA world to an RNA-peptide world theory.[7] We now see an RNA-peptide world at the beginning of the emergence of life.[8]

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Saccharomyces organellar echoforms of cytosolic aminoacyl-tRNA synthetases: not simply mislocated

Solène Zuttion[1], Marine Hemmerle[1], Johan-Owen De Craene[1], Bruno Senger[1], Roza Kucharczyk[2], Maya Schuldiner[3], Claudio De Virgilio[4], Sylvie Friant[1] and **Hubert Dominique Becker[1]**

(1) Génétique Moléculaire, Génomique, Microbiologie, UMR 7156, CNRS, Université de Strasbourg, 4 Allée Konrad Röntgen, 67084 Strasbourg Cedex, France. (2) Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland. (3) Weizmann Institute of Sciences, Rehovot, Israël. (4) Université de Fribourg, Fribourg, Switzerland.

We have shown that some of *Saccharomyces cerevisiae* cytosolic aminoacyl-tRNA synthetases (aaRS) can simultaneously be located in two to four different subcellular compartments. These different locations are not the result of a single nuclear gene being alternatively spliced or translated but instead the result of a differential distribution of the same protein echoforms[1] between various compartments. The nucleus and mitochondria are the compartments in which echoforms were often reported to be found in yeast[2] and we developed a yeast strain expressing a bi-genomic split-GFP[3] enabling specific visualization and purification of mitochondrial echoforms of any dual-localized cytosolic protein. To our surprise we recently found, using conventional subcellular fractionation methods, that a substantial proportion of the three components of the yeast multisynthetase AME complex are bound to the surface of the vacuole (yeast lysosome). To confirm this result engineered a yeast strain expressing a vacuolar-restricted split-CFP that allows visualization and purification of the vacuolar echoform. By probing the entire set of aaRS-encoding genes we discovered that in addition to the AME components, all cytosolic aaRSs we analyzed do possess a vacuolar echoform. To confirm that these vacuolar echoforms are not simply mislocated pool of cytosolic aaRSs we used our strain to compare the interactomes of both the cytosolic and vacuolar echoforms and found out that the vacuolar echoforms are not binding to components of the translation machinery but rather to nutrient sensing machineries. Finally, the preliminary results we have on the function of some of these vacuolar echoforms suggest that they might regulate TORC1 activity under nutritional needs.

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Mupirocin hyper-resistance secured by naturally altered class I signature motif

Ita Gruic-Sovulj

Department of Chemistry, Faculty of Science University of Zagreb, Horvatovac 102a, 10000 Zagreb, Croatia

*gruic@chem.pmf.hr

Mupirocin is a natural antibiotic produced by *Pseudomonas fluorescence*. It inhibits protein synthesis by competing with isoleucine and ATP for binding to the active site of isoleucyl-tRNA synthetase (IleRS). Two IleRS types exist in Bacteria; mupirocin-sensitive IleRS1 and the resistant type 2 enzymes, which exhibit mupirocin inhibitory constants (K_I) in a micromolar or even millimolar range (dubbed hyper-resistance). We found that hyper-resistance, remarkably, originates from the natural swap of the 1st and 3rd positions of the HXGH motif, a signature motif of class I AARSs to which IleRS belongs. Histidine at the 3rd motif position secures hyper-resistance by promoting a steric clash with mupirocin. Surprisingly, a number of IleRS2 enzymes comprise the swapped motif while keeping the housekeeping function. In sharp contrast, we could not identify any IleRS1 with an altered HXGH motif. X-ray structural analysis of *Bacillus megaterium* IleRS1 and IleRS2 enzymes (naturally comprising the HXGH motif) and their designed swapped motif mutants (GXHH-IleRS1 and GXHH-IleRS2) unravelled how the altered motif abolishes catalysis in IleRS1 but not in IleRS2. Specifically, in IleRS2, a unique conformational rearrangement of the α-helix containing the signature motif allows glycine to histidine functional exchange. This unique resistance mechanism evolved exclusively in IleRS2 presumably because IleRS1 was under stronger selection for fast aminoacylation (Zanki et al, Protein Sci. 2022).

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Loss of function mechanisms in CMT2D

Natalia Mora*, Erik Slot, Moushami Malik, Maria Menafra & Erik Storkebaum

Department of Molecular Neurobiology, Donders Institute for Brain, Cognition and Behaviour and Faculty of Science, Radboud University, Nijmegen, Netherlands.

* Natalia.moragarcia@donders.ru.nl

Several mutations in Glycyl-tRNA-synthetase (GlyRS) lead to Charcot-Marie-tooth type 2D (CMT2D); an axonal type with dominant inheritance. Recent studies in our lab have revealed that the netto addition of positive charges of some of these mutations lead to the sequestration of tRNA which is negatively charged[1]. This sequestration depletes the pool of free tRNA to be aminoacylated and results in ribosomal stalling. In this frame, the dominant inheritance of CMT2D is the result of the acquisition of a novel toxic function: tRNA sequestration. However, despite the consistency of the symptoms, not all CMT2D mutations result in the addition of positive charges, suggesting the existence of convergent mechanisms. In this work, we hypothesize a dominant loss of aminoacylation activity, which would lead to less aminoacylated-tRNA and cause ribosomal stalling, as the convergent mechanism. Using *Drosophila melanogaster,* we have evaluated the presence of CMT-like phenotypes in a loss of function paradigm (GlyRS-RNAi), the contribution of loss of function mechanisms in CMT existing models (E71G, G240R and G526R) that add positive charges, and we have created new models of disease-mutations that do not change the charge (S211F and H418R) or add negative charges (K510Q), to evaluate the contribution of dominant loss of function mechanisms to CMT2D.

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Two aminoacyl-tRNA synthetase complexes, membrane localization and unusual role in tRNA import: many questions remain about tRNA biology in *Plasmodium*

José Jaramillo Ponce, Martina Pitolli, Marta Cela, Delphine Kapps & **Magali Frugier***

*M.frugier@ibmc-cnrs.unistra.fr

The team identified the tRip protein encoded in the genome of *Plasmodium*, the parasite that causes malaria. We have established that (i) tRip binds to tRNAs, (ii) tRip is located on the surface of the parasite, with its tRNA binding domain exposed to the outside. (iii) tRip is expressed in both the vertebrate host and mosquito stages, (iv) While exogenous tRNAs enter living wild-type sporozoites, (v) the knockout parasite (tRip-KO) does not import tRNAs, its protein biosynthesis is significantly reduced, and its infectivity and growth are decreased in vertebrate blood (Bour et al., 2016).

We identified three aminoacyl-tRNA synthetases (aaRSs), namely glutamyl- (ERS), glutaminyl- (QRS) and methionyl- (MRS) tRNA synthetases, specifically co-immunoprecipitated with tRip. They all contained a GST-like domain involved in the assembly of the multi-synthetase complex (MSC). The four proteins form two exclusive heterotrimeric complexes: the Q complex (tRip:ERS:QRS) and the M complex (tRip:ERS:MRS) characterized by distinct biophysical properties (Jaramillo-Ponce et al., 2022) and different small-angle X-ray scattering analyses (Jaramillo-Ponce et al., 2023). Despite their structural discrepancies, the two *Plasmodium* MSCs allow for the joint presence of the C-terminal tRNA-binding domains of tRip outside of the parasite but raise the question of their potential implications in divergent functions. Indeed, tRip is the only known example to date of an AIMP with a decoupling between the aaRSs present in MSCs and its tRNA specificity (Cela et al., 2021), suggesting that imported tRNAs play a role beyond their aminoacylation by ERSs, MRSs or/and QRSs in *Plasmodium* homeostasis.

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Functional characterization of a phenylalanyl-tRNA synthetase-based selfish element in the nematode *C. tropicalis*

James Julian Ross[1,2]*, Polina Tikanova[1,2], Antonio Hohn[3], Andreas Hagmüller[1], Valeria Stefania[1], Manuel Hunold[1], Eyal Ben-David[4,5], Gang Dong[3], Alejandro Burga[1]

1) Institute of Molecular Biotechnology of the Austrian Academy of Sciences (IMBA), Vienna BioCenter (VBC), Vienna, Austria; 2) Vienna BioCenter PhD Program, Doctoral School of the University of Vienna and Medical University of Vienna; 3) Max Perutz Labs, Medical University of Vienna, Vienna BioCenter (VBC), Vienna, Austria; 4) Department of Biochemistry and Molecular Biology, Institute for Medical Research Israel-Canada, The Hebrew University School of Medicine, Jerusalem, Israel; 5) Illumina Artificial Intelligence Laboratory, Illumina Inc, San Diego, CA, USA

* julian.ross@imba.oeaw.ac.at

Toxin-antidote elements (TAs) are selfish genetic entities that spread in populations by selectively removing TA non-carrier progeny, who are unable to protect themselves from the effect of the parentally deposited toxin[1]. The underlying molecular mechanisms of eukaryotic TA function remain completely unresolved to date. We recently expanded the repertoire of known animal TAs in the nematode *Caenorhabditis tropicalis*[2]. Here, we focused on a single *C. tropicalis* element for mechanistic investigation. We identified the novel toxin and antidote genes, which we named *klmt-1* and *kss-1*, respectively. We found that KLMT-1, which possesses predicted intrinsically disordered termini, evolved from the phenylalanyl-tRNA-synthetase (PheRS) beta subunit via gene duplication. We therefore hypothesized that KLMT-1 kills worms by interfering with PheRS function. We quantified the activity of the*C. tropicalis* PheRS in *E. coli* and found that N-/C-terminally truncated KLMT-1 lacking the disordered termini strongly reduces the ability of the PheRS to charge its cognate $tRNA^{Phe}$, in contrast to full-length KLMT-1. Furthermore, we showed that this reduction of activity is accompanied or driven by a depletion of the intracellular pool of $tRNA^{Phe}$. The necessity for truncation may reflect an activation step required for full toxic function of KLMT-1, which could explain how KLMT-1 toxicity is spatiotemporally regulated. Currently ongoing characterization of PheRS structure and interactome as well as global tRNA profiling in the context of KLMT-1 activity will shed light on the details of toxin function. Our findings lay the foundations to formulate the first ever model for toxicity of an animal TA.

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Translational regulation mediated by ligand-induced tRNA activation

Ren Nakazaki*[1], Asuteka Nagao[1], Kensuke Ishiguro[1], Takeshi Yokoyama[2], Yoshikazu Tanaka[2] & Tsutomu Suzuki[1]

[1]Department of Chemistry and Biotechnology, Graduate School of Engineering, University of Tokyo, [2]Graduate School of Life Sciences, Tohoku University

*nakazaki-ren919@g.ecc.u-tokyo.ac.jp

Each tRNA species has 'tRNA identity' consisting of unique sequences, base pairs, modifications, and/or local structures that are recognized by each aaRS. Modulation of tRNA identity to control the aminoacylation would allow us to develop a novel regulatory system of protein synthesis available for synthetic biology and tRNA therapeutics.

In this study, we aim to develop an artificial tRNA (Art-tRNA) which can be activated by a specific ligand in the cell. To design an Art-tRNA, an aptamer motif for theophylline was fused to the V-arm of *Escherichia coli* amber suppressor $tRNA^{Tyr}$. The connection module between the aptamer motif and the tRNA was randomized and subjected to screen for Art-tRNAs which are strongly activated by theophylline to readthrough an amber codon of a reporter gene. The selected Art-tRNA was efficiently aminoacylated in a theophylline-dependent manner. To elucidate the mechanism of the theophylline-dependent activation, we analyzed cryo-EM structure of the Art-tRNA bound to the *E. coli* ribosome. Only in the presence of theophylline, we observed clear cryo-EM density of the connection module which exhibits a similar structure of V-arm of *E. coli* $tRNA^{Tyr}$, facilitating theophylline-dependent aminoacylation. We then introduced the Art-tRNA to human culture cells, and succeeded in regulating amber suppression of a reporter construct in a theophylline-dependent manner.

Probing the physiological role of nuclear tRNA synthetases in mammals

Xiang-Lei Yang

Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA USA

*Presenting author email address: xlyang@scripps.edu

Cytosolic aminoacyl-tRNA synthetases (aaRSs) have long been detected in the nucleus of eukaryotic cells, where translation generally does not occur. Recently, aaRSs were found to have diverse regulatory functions in the nucleus. However, due to the essentiality of the aaRS enzymes in protein synthesis, it is challenging to dissect the *in vivo* impact of nuclear aaRSs from that of their cytosolic enzymatic roles. Discovery of a nuclear localization signal (NLS) sequence in vertebrate seryl-tRNA synthetase (SerRS) enabled our lab to demonstrate for the first time that nuclear functions of aaRSs can be essential for organism development and survival (1). Disruption of the NLS does not affect the enzymatic function of SerRS but nevertheless causes abnormal vasculature in zebrafish, leading to their embryonic lethality (1, 2). In another study, we found that nuclear translocation of SerRS is stimulated by glucose (3), suggesting the potential involvement of nuclear SerRS in glucose sensing. To understand the physiological function of nuclear SerRS in mammals, our lab used CRISPR/Cas9 gene editing technology to disrupt the NLS and created nuclear SerRS deficient mice. Interestingly, under Western diet, the mutant mice develop glucose intolerance, insulin deficiency, and insulin resistance. Longitudinal study clarified that insulin deficiency in pancreatic beta-cells is a primary event. Importantly, glucose stimulation and Western diet strongly promote SerRS nuclear translocation in beta-cells. These and other observations suggest that nuclear translocation of SerRS serves as a glucose sensor that stimulates insulin production and secretion for beta-cells to adapt to the metabolic stress.

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Split Aminoacyl-tRNA Synthetases for Proximity-Induced Stop Codon Suppression

Han-Kai Jiang[1,3,4,5], Nicole L. Ambrose[1], Christina Z. Chung[1], Yane-Shih Wang[3,4,6], Dieter Söll[1,2], & **Jeffery M. Tharp**[7,*]

[1]Department of Molecular Biophysics and Biochemistry and [2]Department of Chemistry, Yale University, New Haven, CT 06511. [3]Institute of Biological Chemistry, [4]Chemical Biology and Molecular Biophysics Program, Taiwan International Graduate Program, Academia Sinica, Taipei 11529, Taiwan.[5]Department of Chemistry, National Tsing Hua University, Hsinchu 100044, Taiwan [6]Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan. [7]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202

*jemtharp@iu.edu

Synthetic biology tools for regulating gene expression in living cells have many useful applications in biotechnology and medicine. Most tools developed for this purpose regulate gene expression at the level of transcription, by controlling the rate at which DNA is transcribed to mRNA. In contrast, relatively few strategies have been developed for controlling gene expression at the level of translation. Here we describe the design and engineering of split orthogonal aminoacyl-tRNA synthetases (o-aaRSs) as novel tools for translational control of gene expression in bacteria and eukaryotes. We demonstrate that these split o-aaRSs can be used to regulate stop codon suppression, and thereby gene expression, in response to diverse input signals, including protein–protein interactions, and small molecule "molecular switches". We further show that the split o-aaRSs can function as genetically encoded AND gates where stop codon suppression is controlled by two distinct molecular inputs. Finally, we demonstrate that split o-aaRSs can be used as genetically encoded biosensors to detect diverse protein–protein interactions, including those involved in cancer, and those that mediate SARS-CoV-2 infection.

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Rapid Evaluation of Sense Codon Reassignment Potential by the *M. barkeri* Pyrrolysyl tRNA/aaRS Pair

Margaret A. Schmitt*, David G. Schwark, & John D. Fisk

Department of Chemistry, University of Colorado Denver, Denver CO 80204

*margaret.schmitt@ucdenver.edu

Increasing effort has been directed at employing the set of aaRS variants previously-evolved for amber suppression to incorporate noncanonical amino acids in response to *sense* codons. Predicting which sense codons are most amenable to reassignment and which orthogonal translation machinery is best suited to each codon is challenging. We previously evaluated reassignment of more than 30 sense codons by the native, tyrosine-incorporating *M. jannaschii* tRNA/aaRS pair using a gain of function fluorescence-based screen. We evolved a variant of the *Methanosarcina barkeri* pyrrolysyl aaRS that activates its cognate tRNA with tyrosine with an efficiency rivaling that of endogenous aaRSs and evaluated its ability to reassign sense codons in *E. coli*. Tyrosine represents an easy-to-evaluate surrogate for the nearly 200 noncanonical amino acids previously-incorporated into proteins biosynthetically. The ways in which the space of interactions that govern the fidelity of protein translation may be infiltrated and the extent to which an orthogonal tRNA/aaRS pair is better suited to reassignment of a subset of codons has not been systematically investigated. We present a comparison of reassignment efficiencies to tyrosine by the two orthogonal pairs most commonly used for genetic code expansion. Although the primary difference between the two pairs is expected to be a result of changes to the anticodon on the enzymatic efficiency of the aaRS, interactions between the two unique orthogonal tRNAs and the endogenous translation machinery may render certain codons more efficiently reassigned by one of the two pairs. The *in vivo* measurements provide a data set for understanding the factors affecting ncAA incorporation efficiencies of evolved variants.

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X-ray crystal structure of wild type *G1*PylRS reveals multiple binding modes and chiral degeneracy to accommodate pyrrolysine and noncanonical amino acids

Yane-Shih Wang*[1,2], Jo-Chu Tsou[1], & Dieter Söll[3]

[1] Institute of Biological Chemistry, Academia Sinica, Taipei 11529, Taiwan
[2] Institute of Biochemical Sciences, National Taiwan University, Taipei 10617, Taiwan
[3] Departments of Molecular Biophysics and Biochemistry, and of Chemistry, Yale University, New Haven, CT 06511-8902, USA

*E-mail: yaneshihwang@gate.sinica.edu.tw

To expand the genetic code, orthogonal pyrrolysyl-tRNA synthetase (PylRS)·tRNA^{Pyl} translation systems are exploited to incorporate non-canonical amino acids (ncAAs) at specified sites through amber codon (UAG) suppression. In this structural study, we examined the conformational changes within the N-terminally deleted, *methanogenic archaeon ISO4-G1* ΔPylRS (*G1*PylRS) arising from its binding to different cofactors and substrates. To characterize the ncAA substrates of *G1*PylRS, we measured their respective *sfGFP-UAG* expression levels and reconfirmed their homogeneous incorporation via ESI-MS experiments. We then determined *G1*PylRS' apo, AMP-bound, ATP-analog-bound forms, and its various structures in complex with either its cognate amino acid, pyrrolysine (Pyl) or ncAA substrates through X-ray crystallography. Notably, G1PylRS' crystallized structures with Pyl, lysine, histidine, phenylalanine, and D-phenylalanine analogues revealed the highly dynamic nature of its active site, exhibiting numerous distinct substrate accommodation modes. These new findings illustrated how three key residues, Y125, N165, and Y204, in G1PylRS' active site take part in coordinating counterbalance interactions toward its ncAA substrates to achieve its optimal positioning against ATP and tRNA^{Pyl}'s acceptor stem. We also identified chiral degeneracy in the binding pocket of *G1*PylRS that allows its binding to both enantiomers of 3-bromo-phenylalanine (BrPhe). Altogether, these results shed light on the underlying mechanisms of ncAA recognition utilized by *G1*PylRS, highlighting the flexibility and adaptability of native *G1*PylRS for accommodating Pyl and other structurally diverse ncAAs.

Development of aminoacylation ribozymes capable of peptide elongation in one-pot *in vitro* translation

Wei Lu*[1], Naohiro Terasaka[1], & Hiroaki Suga[1]

Affiliations: [1] Department of Chemistry, Graduate School of Science, The University of Tokyo
*Presenting author email address: wei_lu@chem.s.u-tokyo.ac.jp

Ribozymes catalyzing aminoacylation are considered as one of the most critical linkages between the hypothesized RNA world and the modern world. However, aminoacylation is now solely conducted by protein AARS. The lack of RNA catalysts in modern aminoacylation encouraged our discovery towards aminoacylation ribozymes that resemble the modern AARS functions.

Two decades ago, our group developed “flexizymes”, a series of aminoacylation ribozymes. Since the flexizymes only recognize 3’-RCCA of tRNA, tRNAs need to be pre-charged before added to *in vitro* translation. T-boxzyme is a tRNA-sensing aminoacylation ribozyme derived from T-box riboswitch. The T-boxzyme can specifically aminoacylate its cognate tRNA inside *in vitro* translation mixture and produce Biotin-Phenylalanine initiating peptides, without the need of pre-charging. However, the N-terminal biotinyl group of the substrate is involved in the aminoacyl-donor recognition of the T-boxzyme, its role is limited to aminoacylation of an initiator tRNA.

In this study, we aimed at developing ribozymes with dual recognition of cognate tRNA and N-terminal free substrates. These functions enable ribozymes to be used for peptide elongation in one-pot *in vitro* translation. We chose activated Para-azido-Phenylalanine as the substrate to recover catalytic sequences through bioorthogonal chemistry during *in vitro* selection. We also developed a new strategy to monitor tRNA aminoacylation status based on the idea of oxidative decomposition 3’-termini of non-aminoacylated tRNAs. A ribozyme newly enriched is capable of aminoacylation of its cognate tRNA within *in vitro* translation mixture and produce Para-azido-Phenylalanine containing peptides.

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Without a recruitment domain, pyrrolysyl-tRNA synthetase requires a rigid tRNA identity structure for recognition

Natalie Krahn[1]*, Jingji Zhang[2], Sergey Melnikov[3], Jeffery M. Tharp[1], Haben Gabir[4], Trushar R Patel[5], Alessandra Villa[6], Armaan Patel[1], Rebecca J Howard[7], Jörg Stetefeld[4,8], Joseph Puglisi[2], & Dieter Söll[1,9]

[1] Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA.[2] Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305, USA. [3] Biosciences Institute, Newcastle University, Newcastle upon Tyne, UK.[4] Department of Chemistry, University of Manitoba, Winnipeg, MB R3T 2N2, Canada. [5] Department of Chemistry and Biochemistry, Alberta RNA Research and Training Institute, University of Lethbridge, Lethbridge, AB T1K 2E1, Canada.[6] PDC-Center for High Performance Computing, KTH-Royal Institute of Technology, Stockholm, Sweden.[7] Department of Biochemistry and Biophysics, Science for Life Laboratory, Stockholm University, Solna, Sweden.[8] Department of Microbiology, University of Manitoba, Winnipeg, MB R3T 2N2, Canada. [9] Department of Chemistry, Yale University, New Haven, CT 06520, USA. *natalie.krahn@yale.edu

Pyrrolysyl-tRNA synthetases (PylRSs) are naturally encoded in some archaeal and bacterial genomes to acylate $tRNA^{Pyl}$ with pyrrolysine, the 22nd amino acid. Their large amino acid binding pocket and poor recognition of the tRNA anticodon have been instrumental in genetic code expansion. PylRS enzyme variants have been repurposed to aminoacylate their cognate $tRNA^{Pyl}$ with >200 non-canonical α-amino acids. The enzymes can be divided into three classes based on their genomic structure. Two of the classes contain both an N-terminal and C-terminal domain (both necessary for *in vivo* function), however the third class (*ΔpylSn*) lacks the N-terminal domain. Striking features of the *ΔpylSn* class is its high activity in *Escherichia coli* and its orthogonality with respect to $tRNA^{Pyl}$ from the other two classes of PylRS. In this study we explored the tRNA identity elements of a *ΔpylSn* $tRNA^{Pyl}$ from *Candidatus Methanomethylophilus alvus* which drives the orthogonality seen with its cognate PylRS (MaPylRS). Aminoacylation and translation assays identified unique structural elements important for MaPylRS recognition. High-resolution structure determination and molecular dynamic simulations led us to acknowledge that the tRNA elements we identified are not specifically bound by the enzyme. Rather, they form a rigid identity structure that facilitates aminoacylation by MaPylRS. This requirement is due to the absence of an N-terminal domain used to recruit the tRNA in solution.

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EMBO Young Investigator Lecture

Transfer RNA pools in human cells are controlled by selective gene expression

Lexi Gao[1,#], Andrew Behrens[1,#], Geraldine Rodschinka[1], Sergio Forcelloni[1], Sascha Wani[1], Katrin Strasser[1], & **Danny Nedialkova**[1,2,*]

1. Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
2. Department of Bioscience, School of Natural Sciences, Technical University of Munich, 85748 Garching, Germany

*nedialkova@biochem.mpg.de

Transfer RNAs are required for translating genetic information into protein sequence. The human genome contains hundreds of predicted tRNA genes, many of which in multiple copies. How their expression is regulated to control functional tRNA levels is unknown. Here, we combined quantitative tRNA profiling and ChIP-Seq to measure tRNA expression upon differentiation of human induced pluripotent stem cells (hiPSC) into neuronal and cardiac cells. We find that tRNA transcript pools vary substantially, while the abundance of tRNAs with distinct anticodons, which governs decoding rates, is more stable among cell types. Mechanistically, RNA Polymerase III (Pol III) samples a wide range of tRNA genes in hiPSC and becomes constrained to a housekeeping subset upon differentiation. This is mediated by diminished mTORC1 signaling, which activates the Pol III repressor MAF1. Our data rationalize how tRNA anticodon pools are buffered in different cellular contexts and reveal that mTORC1 activity drives selective tRNA expression.

The Complexity Leap in tRNA Evolution in Vertebrates

Todd Lowe*, Henry Moore, and Patricia Chan

Department of Biomolecular Engineering, Baskin School of Engineering, University of California, Santa Cruz, USA
*tmjlowe@ucsc.edu

While the dynamic regulatory roles of the various aminoacyl tRNA synthetases have been the subject of intense study for many decades, the differential regulation and continued evolution of tRNA functionality is only now coming into focus. In terms of raw tRNA gene counts per genome, eukaryotes usually have many times the number of tRNA loci relative to bacteria and archaea. The increased tRNA gene counts have often been attributed to the need for amplified tRNA production in larger eukaryotic cells. The eukaryotic model *Saccharomyces cerevisiae*, with only 55 unique gene sequences among the 275 different tRNA loci, is a prime example of tRNA isodecoder uniformity (i.e., all tRNAs with a particular anticodon have exactly the same mature transcript). In contrast, vertebrate genomes have at least 400-600 tRNA genes, but more than half are unique in sequence, hinting at much greater potential for diversified tRNA characteristics and functionality in higher eukaryotes.

Deep evolutionary study of tRNA genes, integrated with epigenetic and multi-tissue tRNA transcriptional data, has revealed several new levels of complexity in vertebrates: at the gene locus level (creating unique tissue or developmental transcriptional programs); and at the tRNA transcript level (producing unique tRNA isodecoder family profiles). I detail five major regulatory classes for human tRNA genes, plus the striking observation that vertebrates have evolved at least 140 different cross-species conserved isodecoders, each family with potentially altered tRNA modification patterns, processing efficiency, stability, derived small RNAs, and/or efficiency of decoding in translation.

Novel features of helminth aminoacyl-tRNA synthetases

Joseph Chihade*, Alison Block, David Wilson, Noah Mueller, Samuel Diaz de Leon, Molly Kamman, Sara Abraha, Seth Warner, Jevon Robinson, & Marie Sissler

•Department of Chemistry, Carleton College, Northfield, MN 55057, USA
•Institut de Physiologie et de Chimie Biologique, Université de Strasbourg, Strasbourg, France

*Presenting author email address
jchihade@carleton.edu

Helminth is a generic term for the parasitic worms that negatively impact health outcomes for roughly a quarter of the global population. Although more than 150 helminth genomes have now been sequenced, protein sequence predictions available in compiled genomes and public databases are often not completely correct. These errors, usually due to incorrectly predicted intron-exon boundaries, hinder identification of novel drug targets and the development of a more complete understanding of helminth biology. Our work has focused on identifying and correcting predictions for aminoacyl-tRNA synthetase genes. Using published genomic information available in the WormBase ParaSite[1] compilation from a set of twelve human-infecting helminths, we initially corrected exon boundary predictions using RNAseq data. The proposed protein sequences were then validated using multiple-sequence alignments of enzymes from helminths and other organisms, allowing us to identify helminth-specific features. We confirmed a high error rate in predictions of the studied genes. 60% of the sequences required at least one correction and an average of 4.3 corrections were made per gene.

Our analysis identified several unique features of helminth aminoacyl-tRNA synthetases, particularly among trematode (fluke) and platyhelminth (flatworm) proteins. Notable examples include unusual sequences of mitochondrially-targeted enzymes that lack known RNA-binding domains. In several cytosolic sequences, appended domains, known to mediate assembly of the multisynthetase complex in humans, are missing or replaced with unique domains in helminth sequences.

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Rethinking the taxonomy of AARS hierarchical modularity

Jordan Douglas*[1], Remco Bouckaert[2], Peter Wills[1], & Charlie Carter[3]

[1] Department of Physics, University of Auckland, New Zealand
[2] School of Computer Science, University of Auckland, New Zealand
[3] Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, USA
*jordan.douglas@auckland.ac.nz

Inferring the pre-LUCA evolution of genetic coding through Bayesian phylogenetic analysis is beset with ambiguities arising from the hierarchical and modular AARS architecture. To address this, we have compiled a taxonomically representative structural database of class I and II AARS to clarify the evolution of AARS modules and to map the early evolution of core functional modules that have been characterised experimentally. The database seamlessly integrates crystal structures, structural predictions from AlphaFold2, and curated amino acid and nucleotide sequence alignments with visualisation tools, allowing for comparison of modules at all scales. This tool was used to classify the AARS into 14 class I families and 21 class II families, based on the sequence, structure, and functional activity of their catalytic domains. Each family in the database, where possible, contains a taxonomically representative sample from all three domains of life, including also organelles and viruses. This database highlights the idiosyncrasies of the AARS and systematises the elusive evolutionary history of catalytic domain insertion modules. This can materially strengthen efforts to build phylogenetic trees with experimentally testable intermediates. In this talk I will demonstrate functionalities of this database, show how we have used it to produce an evolutionary model describing the accretion of insertion modules in the catalytic domains, and discuss the development of novel Bayesian phylogenetic methods (BEAST 2) applicable to the pre-LUCA evolution of genetic coding consistent with the concurrent expansion of the coding alphabet.

Rescue of tRNA synthetase-associated neuropathy by inhibiting GCN2 or supplementing tRNA expression

Robert W. Burgess[1]*, Abigail Tadenev[1], Robert Schneider[1], & Scott Q. Harper[2]

[1]The Jackson Laboratory, Bar Harbor, ME 04609 USA.[2]Center for Gene Therapy, Research Institute of Nationwide Children's Hospital, Columbus, OH, 43205 USA

*Robert.burgess@jax.org

Charcot-Marie-Tooth disease is a collection of inherited peripheral neuropathies associated with mutations in at least 100 different genes. The gene family with the most CMT-associations is the aminoacyl tRNA-synthetase family (aaRSs), with dominant mutations in at least five aaRS gene leading to CMT. We have shown in mouse models of *Gars1*/Charcot-Marie-Tooth type 2D that the integrated stress response (ISR) is selectively activated in motor and sensory neurons through the kinase GCN2 and inhibiting GCN2 greatly mitigates peripheral neuropathy (1). Importantly, we now show this strategy is still beneficial even when started after the onset of disease, and it appears to benefit a related mouse model, *Yars1*/diCMTC. We have also shown that transgenic overexpression of wild-type *GARS1* does not rescue the neuropathy in mice; however, transgenic overexpression of $tRNA^{Gly}_{GCC}$ was able to completely prevent disease (2, 3). Consistent with this, we find that AAV9-mediated delivery of $tRNA^{Gly}$ genes is also beneficial, and efficacy correlates with tRNA (anticodon) abundance and codon usage. The suppression of the ISR and delivery of $tRNA^{Gly}$ genes represent potential therapeutic strategies for CMT2D and related diseases.

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Role of aminoacyl-tRNA synthetases in HIV-1 lifecycle

Karin Musier-Forsyth*, William A. Cantara, Alice Duchon, Danni Jin, Chathuri Pathirage, Yingke Tang

Department of Chemistry and Biochemistry, Center for Retrovirus Research and Center for RNA Biology, Ohio State University, Columbus, OH 43210, USA

*musier-forsyth.1@osu.edu

Host factor tRNAs facilitate the replication of retroviruses such HIV-1, which uses human $tRNA^{Lys3}$ as the primer for reverse transcription. The assembly of HIV-1 Gag at the plasma membrane is also regulated by tRNA interactions with the matrix domain of Gag. Proteomic studies have identified the multi-aminoacyl-tRNA synthetase complex (MSC) as part of the HIV-1 Gag interactome. We recently mapped the primary interaction site to the linker domain of bi-functional human glutamyl-prolyl-tRNA synthetase (EPRS). While the precise function of the Gag-EPRS interaction remains uncertain, possible effects of the interaction will be discussed.

Another MSC member, lysyl-tRNA synthetase (LysRS), is phosphorylated at S207, released from the MSC and partially re-localized to the nucleus of HIV-1 infected cells. Proper targeting of $tRNA^{Lys3}$ to the primer binding site (PBS) of the HIV-1 genomic RNA (gRNA) is facilitated by LysRS binding to a PBS-adjacent tRNA-like element (TLE). The full-length gRNA has two fates—it serves as an mRNA for translation of viral polyproteins and as a genome that is packaged in two copies as a dimer. LysRS binds preferentially to the TLE of dimeric gRNA, suggesting that the synthetase selects for packaging-competent gRNA for Gag-chaperoned annealing of the tRNA to the PBS. We have also investigated the nuclear function of pS207-LysRS in the HIV-1 lifecycle. LysRS phosphorylation up-regulates HIV-1 transcription, as does direct transfection of Ap4A, a transcription factor activator that is synthesized by pS207-LysRS. We propose that nuclear pS207-LysRS generates Ap4A, leading to activation of HIV-1 transcription.

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An oncogenic variant of aminoacyl-tRNA synthetase-interacting multi-functional protein 2 (AIMP2) as a novel target to control KRAS-driven cancer

Sunghoon Kim

Yonsei University, College of Pharmacy and Severance Hospital, Institute for Artificial Intelligence and Biomedical Research

While AIMP2 works as a scaffold factor for the assembly of human multi-tRNA synthetase complex (MSC), it also serves as a potent tumor suppressor by controlling diverse cancer pathways depending on upstream signal and cell context. Decreased activity and expression of AIMP2 enhances the propensity of tumor formation. Expression of its alternative splicing variant, AIMP2-DX2, lacking exon 2 of AIMP2, is induced by tumorigenic insults and frequently observed in various cancer cells. AIMP2-DX2 augments tumorigenesis by compromising the tumor suppressive activities of AIMP2. AIMP2-DX2 specifically interacts with KRAS, preventing KRAS from ubiquitin-mediated degradation. Chemical inhibition of these two oncogenic factors effectively reduced the cellular level of KRAS and inhibited tumor growth driven by KRAS regardless of its mutation type. Thus, AIMP2-DX2 provides a unique route to control the cancers driven by KRAS mutations.

Unraveling the non-aminoacylation functions of tyrosyl-tRNA synthetase: insights from inherited peripheral neuropathies

Albena Jordanova

Center for Molecular Neurology, VIB, University of Antwerp, Antwerpen, Belgium; Department of Biomedical Sciences, University of Antwerp, Antwerpen, Belgium; Department of Medical Chemistry and Biochemistry, Medical University-Sofia, Sofia, Bulgaria.
Albena.jordanova@uantwerpen.vib.be

Dominant mutations in the gene (YARS1) encoding the cytoplasmic tyrosyl-tRNA synthetase (TyrRS) and six other tRNA-ligases cause Charcot-Marie-Tooth peripheral neuropathy (CMT). Loss of aminoacylation is not required for their pathogenicity, suggesting a gain-of-function disease mechanism. By an unbiased genetic screen in *Drosophila*, we link TyrRS dysfunction to actin cytoskeleton organization. Biochemical studies uncover yet unknown actin-bundling property of TyrRS to be enhanced by a CMT mutation, leading to actin disorganization in the *Drosophila* nervous system, human SH-SY5Y neuroblastoma cells, and patient-derived fibroblasts. Genetic modulation of F-actin organization improves hallmark electrophysiological and morphological features in neurons of flies expressing CMT-causing TyrRS mutations. Similar beneficial effects are observed in flies expressing a neuropathy-causing glycyl-tRNA synthetase. Our findings demonstrate that TyrRS is an evolutionary-conserved F-actin organizer which links the actin cytoskeleton to tRNA-synthetase-induced neurodegeneration.

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Defective m^{6}A methylation of *EPRS1* mRNA in hypomyelinating leukodystrophy patients

Debjit Khan,[1] Kommireddy Vasu,[1] Arnab China,[1] Iyappan Ramachandiran,[1] Krishnendu Khan,[1] Briana Long,[1] Gregory Costain,[2] Susan Blaser,[3] Amanda Carnevale,[2] Valentin Gogonea,[4] Ranjan Dutta,[5] Grace Yoon,[2,6] & **Paul L. Fox**[1]*

[1]Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA. [2]Division of Clinical and Metabolic Genetics, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. [3]Division of Neuroradiology, Department of Diagnostic Imaging, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada. [4]Department of Clinical Chemistry, Cleveland State University, Cleveland, Ohio, USA. [5]Department of Neuroscience, Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio, USA. [6]Division of Neurology, Department of Paediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

*Presenting author: foxp@ccf.org

Pathogenic variants in more than twenty-five genes cause hypomyelinating leukodystrophy (HLD), characterized by defective central nervous system myelination, and disturbed motor and cognitive function. Exome sequencing of two siblings with severe cognitive and motor impairment and progressive hypomyelination on brain magnetic resonance imaging, characteristic of childhood-onset HLD, revealed homozygosity for a novel missense variant in the ProRS domain of *EPRS1* (c.4444C>A; p.Pro1482Thr), which encodes glutamyl-prolyl tRNA synthetase. Analysis of patient lymphoblastoid cell lines revealed reduced level of EPRS1 protein, but unaltered specific GluRS and ProRS tRNA charging activity. Reduced EPRS1 protein in patient cells was not due to altered *EPRS1* mRNA level, but rather from dual defects in nuclear export and translation of variant *EPRS1* mRNA. Molecular analysis showed defective m^{6}A modification at two sites surrounding the c.4444C>A variant site was responsible for disturbed *EPRS1* mRNA processing and protein expression. Our findings reveal a new mechanism of disease causation, and suggest novel mRNA-targeted therapeutic approaches.

Clinical strategies in the management of individuals with HARS syndrome

Siu VM[1,2,5], Leat S [3], McCulloch D [3], Hutchings N [3], Parker C [4,5], Miller M[2], Rupar CA [2,5,6] .
[1]Division of Medical Genetics, Department of Pediatrics and Biochemistry, Western University, London, ON, Canada, [2]Children's Health Research Institute, London, ON, [3]School of Optometry, University of Waterloo, ON, Canada, [4]Audiology, [5]London Health Sciences Centre, London, ON, Canada, [6]Department of Pathology, Western University, London, ON, Canada

Homozygosity for the Y454S variant in HARS1 is associated with the autosomal recessive disorder originally described as Usher syndrome type 2W (OMIM#604504), and referred to as HARS syndrome in the Old Order Amish community in Ontario, Canada. Carrier rate is about 1 in 5 in this population, with 20 living affected children. The HARS1 protein has a non-canonical role in regulation of the inflammatory response. In-vitro studies have shown decreased incorporation of histidine during protein synthesis at higher temperatures which can be rescued by histidine.

Affected children have cystic placentas with massive perivillous fibrin deposition (MPFD), low birthweights, neonatal hypoglycemia, and normal cognition. Historically, viral illnesses in early childhood can trigger a life-threatening deterioration with fever, vomiting, acute respiratory distress syndrome, encephalopathy, visual and hearing loss, and visual hallucinations. Mortality and stillbirth rates have been high. Progressive hearing loss often leads to the need for cochlear implants while progressive retinopathy can result in blindness.

Febrile illnesses are currently managed with acetaminophen, nonsteroidal anti-inflammatory medications and cautious oral fluid replacement, avoiding intravenous fluids if possible. In October 2018, we initiated a formal clinical trial (Clinical Trial ID: NCT02924935) of oral l-histidine supplementation 50mg/kg BID in 14 children with HARS syndrome, ages 1 to 17, for up to 3 years. Twelve children had hearing loss (7 with cochlear implants) and 5 had vision loss at baseline. Histidine was well tolerated with no adverse effects, and compliance was reasonably good. Plasma histidine levels increased once supplementation started. Hearing remained stable in the children who did not have a cochlear implant at baseline. Vision showed no deterioration. Colour vision and appearance of the retina seemed improved in some cases. During minor illnesses, histidine dose was doubled. There were no serious episodes. By the end of the trial, 9 children had evidence of prior natural infection with COVID. We conclude that histidine supplementation in children with HARS syndrome is safe, inexpensive, and well tolerated, with potential for stabilization of hearing and vision. Given the high stillbirth risk and placental changes in affected pregnancies, as well as the high safety profile for histidine supplementation, we propose offering prenatal histidine supplementation once a pregnancy is confirmed, as well as acetyl-salicylic acid (ASA) to address the MPFD. Further follow-up of individuals with HARS syndrome on histidine supplementation will be needed to determine if the natural history of the disorder is significantly altered in the long term.

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Thermal characterization of human aminoacyl-tRNA synthetases in health and disease

Marisa I. Mendes*, Gajja S. Salomons & Desiree Smith

Laboratory Metabolic Diseases, Amsterdam UMC, Amsterdam, The Netherlands
*m.mendes@amsterdamumc.nl

Background: Mutations in each of the 37 human genes that encode the aminoacyl-tRNA synthetases (aaRSs) family have been associated with disease. A relationship between fever and clinical worsening of patients with mutations in several *ARS* genes has been observed, therefore, we investigated the effect of hyperthermia on aaRS activities in mainly non aaRS deficient cell lysates.

Methods: To investigate the effect of temperature on aminoacylation activity, we pre-incubated six control fibroblast lysates at 37, 42 and 46°C for 10 min. After incubation, the activities of cytosolic aaRSs were determined simultaneously by LC-MS/MS.

Results: Temperature increases resulted in statistically significant decrease in aminoacylation activity of AlaRS, LeuRS, MetRS, TyrRS and ValRS, whereas the activity of CysRS, GlyRS, LysRS, GluProRS and SerRS slightly increased with temperature. Furthermore, the LeuRS activity of patients with pathogenic variants in *LARS1* is even more sensitive to an increase in temperature than controls.

Conclusion & Discussion: We observed that the different aaRSs respond to the temperature challenge in a variable manner. The thermal sensitivity of aaRS variants should be further studied, particularly in patients with pathogenic variants in *AARS1*, *LARS1*, *MARS1*, *YARS1* and *VARS1*. This could have an important impact on patient treatment, especially during episodes of fever, which are common in early infancy.

The LBSL transcriptome: distinct and divergent gene expression profiles based on DARS2 mutation

Nemeth CL[1,2*], Guang S[1,2], Ying M[2,3], Fatemi A[1,2]

[1]Moser Center for Leukodystrophies, Kennedy Krieger Institute, Baltimore MD [2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore MD [3]Hugo W. Moser Research Institute at Kennedy Krieger Institute, Baltimore MD

*mertz@kennedykrieger.org

Leukoencephalopathy with brain stem and spinal cord involvement and lactate elevation (LBSL) is a rare neurological disorder caused by the mutations in *DARS2*, encoding the mitochondrial aspartyl-tRNA synthetase.[1] LBSL has a wide phenotypic spectrum, characterized by childhood- or juvenile-onset slowly progressive spasticity, cerebellar ataxia and dysfunction of the dorsal column.[2] The objective of this study was to understand the impact of *DARS2* mutations on cell processes through the evaluation of LBSL patient stem cell derived cerebral organoids. We generated human cerebral organoids from seven LBSL patients and three controls using an unguided protocol and performed SMARTseq2 high resolution single-cell RNA sequencing.[3] After quality control, a total of 809 cells were used for downstream analysis and uncovered two patient subgroups which presented distinct and divergent gene expression profiles. Patients with at least one missense mutation showed downregulated expression of mRNA catabolic processes and RNA splicing, which was exacerbated in neurons. Patients carrying two splice-site mutations showed upregulation of RNA splicing and translation. Inspired by dysregulation of splicing factors, we identified some genes, *LGMN*, *SLIRP*, *CAST* and *PDCD4*, which were spliced differently across LBSL patient groups. At the transcript level, we found decreased levels of full-length *DARS2* transcripts in LBSL patients, and within individual cells, transcripts skipping exon 3 coexist with normal transcripts in controls, but LBSL cells express solely abnormal transcripts. From these data, we show that dysregulated RNA splicing, protein translation and metabolism may underlie at least some of the pathophysiological mechanisms in LBSL.

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Insights into NARS1-Associated Disease: A Preliminary Genotype-Phenotype Review By Patient and Research Advocates

Rachel Heilmann[1,2]*, Irem Karagoz[3], Henry Houlden[3], Stephanie Efthymiou[3]

1. The Rory Belle Foundation, Colorado U.S.A 80220. 2. COMBINEDBrain. Brentwood, TN 37027, USA. 3. Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, Queen Square, London, UK (s.efthymiou@ucl.ac.uk)

*rachel@therorybellefoundation.org

Asparaginyl-tRNA synthetase1 (NARS1) is a highly conserved and ubiquitously expressed cytoplasmic tRNA synthetase necessary for protein translation. Mutations in the 20 class II cytoplasmic aminoacyl-tRNA synthetases (AARSs) result in neurological disorders, ranging from mild late-onset peripheral neuropathy to severe multi-systemic neurodevelopmental disorders. Due to the rarity of this disorder, there is no formal genotype-phenotype study for NARS1; however, the literature describes 33 individuals with clinical characteristics including: global developmental delay, delayed speech and motor function with or without microcephaly and/or seizures. Together, with a patient advocacy group, additional impacted-individuals have been discovered with genotype and phenotype information collected as part of the RARE-X platform. Preliminary data from 10 additional individuals with bi-allelic or de-novo variants in NARS1 show an overlapping phenotype. The majority of individuals presented with generalized tonic-clonic seizures within the first year of life. Early-onset epilepsy shared phenotypic features of epileptic encephalopathy and was characterized by a more severe disease course and poorer survival. The aim here is to describe and differentiate the collective symptoms, variants, and functional impact between de-novo and bi-allelic cases, as well as to discuss potential biomarkers and identify therapeutic options.

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Seryl-tRNA synthetase inhibits breast cancer metastasis possibly through blocking Wnt signaling

Justin Wang[1,+,*], Lei Jiang[1,+], Ze Liu[1], & Xiang-Lei Yang[1,#]

[1] Department of Molecular Medicine, Scripps Research Institute, La Jolla, CA, 92037, USA
[+] The authors wish it to be known that, in their opinion, the first 2 authors should be regarded as joint First Authors.

*Presenting author email address: jjwang@scripps.edu
[#] To whom correspondence should be addressed. Email: xlyang@scripps.edu

Protein synthesis is required for tumor growth, and aminoacyl-tRNA synthetases, as critical components of the translation machinery, are expected to play a positive role in supporting cancer progression. Interestingly, overexpression of seryl-tRNA synthetase (SerRS) has been shown to have the opposite effect in the context of breast cancer[1,2]. Through its nuclear translocation and its ability to regulate transcription of genes involved in angiogenesis and lipid biosynthesis, SerRS inhibits primary breast cancer growth. However, the effect of SerRS on metastasis was not studied, although metastasis is the dominant cause of mortality in breast cancer[3]. Through multiple mouse metastasis models using breast cancer cell lines overexpressing SerRS, we show that SerRS impedes not only the growth of primary tumors but also the establishment of metastases. By inducing SerRS overexpression after tumor establishment, we mimic a clinical treatment setting and further demonstrate the potential of SerRS as an anticancer therapeutic. RNA-seq analysis of tumor tissues from these experiments identified Wnt signaling as the top pathway regulated by SerRS. The Wnt signaling pathway has been widely implicated in tumorigenesis by affecting cancer stemness and metastasis[4]. Following up in vitro, we confirm that SerRS expression suppresses Wnt signaling in breast cancer cells and cell activities related to metastasis, such as cell migration and invasion. To our knowledge, this study provides the first observation that a tRNA synthetase can act as a tumor and metastasis suppressor.

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Clinical Proof-of-Concept for a Novel Therapeutic Based on Histidyl-tRNA Synthetase for Treatment of Interstitial Lung Diseases

Nangle LA[1], Adams RA[1], Xu Z[1], Siefker D[1], Guy L[1], Burman L[1], Burkhart C[1], Zhai L[2,3], Glendening L[1], Yu E[1], Rauch K[1], Klopp-Savino S[1], Wang A[1], Yang XL[4], Kinnersley N[5], Walker G[1], Carey L[1], Schimmel P[4], Shukla S[1]

[1]aTyr Pharma, San Diego, CA, USA, [2]Pangu Biopharma, Hong Kong, China, [3]IAS HKUST – Scripps R&D Laboratory, Institute for Advanced Study, Hong Kong, China, [4]The Scripps Laboratories for tRNA Synthetase Research, The Scripps Research Institute, La Jolla, CA, USA, [5]Octa Consulting Services Ltd, England, Email: lnangle@atyrpharma.com

Extracellular tRNA synthetase biology represents a novel set of potential physiological modulators and therapeutic targets. aTyr has developed a process to advance novel tRNA synthetase domains from a concept to clinical product candidate. This process leverages our early discovery work as well as current scientific understanding of tRNA synthetase evolution, protein structure, gene splicing and tissue-specific regulation to identify potentially active protein domains. The most advanced of these molecules, efzofitimod, is an immunomodulatory Fc fusion protein based on a naturally occurring splice variant of histidyl-tRNA synthetase (HARS) that is enriched in human lung. Extensive preclinical work on efzofitimod has demonstrated efficacy in the regulation of inflammatory responses through binding to the cell-surface receptor neuropilin-2 (NRP2) that is present on multiple immune cell types and is often upregulated upon insult or stimulation/maturation. Subsequent studies have demonstrated its activity in multiple animal models of disease, significantly reducing lung inflammation, immune cell infiltration, and fibrosis. Clinical development of efzofitimod is being pursued for the treatment of fibrotic lung diseases with high unmet medical need, including interstitial lung diseases (ILDs), a group of fibrotic lung disorders in which immune cells play a predominant role, . The mechanism of action of efzofitimod on immune cells, particularly myeloid cells, overlaps with the cellular pathology observed in pulmonary sarcoidosis, a major form of ILD, and recent data has demonstrated a connection between the target receptor for efzofitimod (NRP2) and this disease. The safety and preliminary efficacy of efzofitimod for the treatment of pulmonary sarcoidosis was recently demonstrated in a Phase 1b/2a randomized, placebo-controlled study in patients with symptomatic disease. In this study, efzofitimod was safe, well tolerated, and associated with dose-dependent reductions in steroid use and improvements in lung function and patient reported outcomes compared to placebo. Additionally, efzofitimod treatment resulted in control of pro-inflammatory biomarker secretion that was consistent with changes observed in preclinical animal models. The results of this study represent the first clinical proof-of-concept for a tRNA synthetase-based therapeutic, paving the way for development of a new class of drugs derived from this family.

Histidine Supplementation can escalate of rescue HARS deficiency in a Charcot-Marie-Tooth Disease model

Sarah Wilhelm* & Ilka Heinemann

Department of Biochemistry, The University of Western Ontario, London, Ontario Canada

*swilhel2@uwo.ca

Aminoacyl-tRNA synthetases (ARSs) are essential enzymes responsible for charging amino acids onto cognate tRNAs during protein synthesis. In histidyl-tRNA synthetase (HARS), autosomal dominant mutations V133F, V155G, Y330C, S356N in the HARS catalytic domain cause Charcot Marie Tooth Disease Type 2W (CMT2W), while RNA-binding domain mutation Y454S causes recessive Usher Syndrome Type IIIB (USH3B). In a yeast model, all human HARS variants complemented a genomic deletion of the yeast ortholog *hts1* at high expression levels. CMT2W associated mutations, but not Y454S, resulted in reduced growth. We show mistranslation of histidine to glutamine and threonine in V155G and S356N but not Y330C mutants in yeast, leading to accumulation of insoluble proteins, which was rescued by histidine. Mutants V133F and Y330C showed the most significant growth defect and decreased HARS abundance in cells. Here, histidine supplementation led to insoluble protein aggregation and further reduced viability, indicating histidine toxicity associated with these mutants. V133F proteins displayed reduced thermal stability *in vitro,* which was rescued by tRNA. Our data will inform future treatment options for HARS patients, where histidine supplementation may either have a toxic or compensating effect depending on the nature of the causative HARS variant.

A novel class of Gram-negative antibacterial agents targeting leucyl-tRNA synthetase

Grace Edmund*, Michael Charlton, Michael Dawson, & Paul W. Finn

Oxford Drug Design, OCFI, New Road, Oxford, OX1 1BY, U.K.

*grace.edmund@oxforddrugdesign.com

Using rational drug design, a novel class of antibacterial agents inhibiting the catalytic site of leucyl-tRNA synthetase (LeuRS) have been developed. Aminoacyl-tRNA synthetases play an essential role in protein synthesis, catalysing the transfer of amino acids to their cognate tRNA. These enzymes are conserved across bacteria and, at the same time, exhibit considerable evolutionary divergence with respect to the human enzymes. This makes them promising targets for the discovery of broad-spectrum antibiotics. To date, no catalytic site leucyl-tRNA synthetase inhibitor has been advanced to clinical investigation.

Enzymatic assays show potent inhibition of E. coli leucyl-tRNA synthetase and antibacterial activity against a range of Gram-negative bacteria, including drug-resistant strains, with no cytotoxicity against eukaryotic cells. We have validated the mechanism of action, and have determined co-crystal complexes in LeuRS with our compounds to aid optimization. Initial in vivo tests look promising with results in line with comparator compounds for our preferred clinical indication.

This novel class shows promise for the treatment of Gram-negative bacterial infections. Because of the novel mechanism of action, cross-resistance with existing classes of antibiotics should be limited.

Nuclear modifier mitochondrial tyrosyl-tRNA synthetase allele correction restored retinal ganglion cells-specific deficiencies in Leber's hereditary optic neuropathy

Jia-Rong Chen, Chao Chen and **Min-Xin Guan***

Institute of Genetics, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China. Correspondence to: Min-Xin Guan, Ph.D., Institute of Genetics, Zhejiang University School of Medicine, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, China; Tel: 86-571-88206916; Fax: 86-571-88982377; E-mail: gminxin88@zju.edu.cn

Leber's hereditary optic neuropathy (LHON) is a maternally transmitted eye disease due to the degeneration of retinal ganglion cells (RGC). Mitochondrial 11778G>A mutation is the most common LHON-associated mitochondrial DNA (mtDNA) mutation. Our recent studies demonstrated some LHON families manifested by synergic interaction between m.11778G>A mutation and YARS2 allele (c.572G>T, p.Gly191Val) encoding mitochondrial tyrosyl-tRNA synthetase. However, the RGC-specific effects of LHON-associated mtDNA mutations remains elusive and there is no highly effective therapy for LHON. Here, we generated patients-derived induced pluripotent stem cells (iPSCs) from fibroblasts derived from a Chinese LHON family (both m.11778G>A and c.572G>T mutations, only m.11778G>A mutation, and control subject). The c.572G>T mutation in iPSC lines from a syndromic individual was corrected by CRISPR/Cas9. Those iPSCs were differentiated into neural progenitor cells (NPCs) and subsequently induced RGC-like cells using a stepwise differentiation procedure. Those RGC-like cells derived from symptomatic individual harboring both m.11778G>A and c.572G>T mutations exhibited greater defects in neuronal differentiation, morphology including reduced area of soma, numbers of neurites, and shortened length of axons, electrophysiological properties than those in cells bearing only m.11778G>A mutation. Furthermore, these RGC-like cells revealed more drastic reductions in oxygen consumption rates, levels of mitochondrial ATP and increasing productions of reactive oxygen species than those in other cell models. These mitochondrial dysfunctions promoted the apoptotic process for RGC degenerations. Correction of YARS2 c.572G>T mutation rescued deficiencies of patient-derived RGC-like cells. These findings provide new insights into pathophysiology of LHON arising from RGC-specific mitochondrial dysfunctions and step toward therapeutic intervention for this disease.

Development of Novel ProRS Inhibitors for Malaria

Ralph Mazitschek

Center for Systems Biology, Massachusetts General Hospital, Boston, MA 02114, USA. Harvard T.H. Chan School of Public Health, Boston, MA 02115, USA. Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA

*Presenting author email address: ralph@broad.harvard.edu

The development of next-generation antimalarials that are efficacious against the human liver and asexual blood stages is recognized as one of the world's most pressing public health challenges. In recent years, aminoacyl-tRNA synthetases, including prolyl-tRNA synthetase, have emerged as attractive targets for malaria chemotherapy. We have developed a single-step biochemical assay for *Plasmodium* and human prolyl-tRNA synthetases that overcomes critical limitations of existing technologies and enables quantitative inhibitor profiling with high sensitivity and flexibility. Importantly, we have shown that this assay approach can be readily extended to other aaRS isoforms.

Supported by this assay platform and co-crystal structures of representative inhibitor-target complexes, we develop a set of high-affinity prolyl-tRNA synthetase inhibitors, including previously elusive aminoacyl-tRNA synthetase triple-site ligands that simultaneously engage all three substrate-binding pockets.

Several compounds exhibit potent dual-stage activity against *Plasmodium* parasites and display good cellular host selectivity. Our data inform the inhibitor requirements to overcome existing resistance mechanisms and establish a path for rational development of prolyl-tRNA synthetase-targeted antimalarial therapies.

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Methionine supplementation as a game-changer for severe pulmonary alveolar proteinosis related to *MARS1* mutations

Alice Hadchouel[1,2,3*], MD, PhD, David Drummond[1,2], MD, PhD, Clément Pontoizeau[2,4], MD, PhD, Laura Aoust[1,2], MD, Elsa Gachelin[5], MD, Caroline Perisson[6], MD, Clémentine Vigier[7], MD, Manuel Schiff[8,9], MD, PhD, Florence Lacaille[10], MD, Thierry Jo Molina[9,11], MD, PhD, Laureline Berteloot[9,12], MD, Sylvain Renolleau[2,13], MD, PhD, Chris Ottolenghi[2,4], MD, PhD, Jean-Marc Tréluyer[2,14] MD, PhD, Jacques de Blic[1,2], MD, PhD, and Christophe Delacourt[1,2] MD, PhD

[1]AP-HP, Hôpital Universitaire Necker-Enfants Malades, Service de Pneumologie Pédiatrique, Centre de Référence pour les Maladies Respiratoires Rares de l'Enfant, Paris, France [2]Université Paris Cité, Faculté de Médecine, Paris, France. [3]INSERM U1151, Institut Necker Enfants Malades, Paris, France. [4]AP-HP, Hôpital Universitaire Necker-Enfants Malades, UF de Métabolomique, Paris, France. [5]CHU Reunion site Félix Guyon, Service de Pédiatrie, Saint Denis, France. [6]CHU Reunion site Sud, Service de Pédiatrie, Saint Pierre, France. [7]CHU de Rennes, Service de Pédiatrie, Rennes, France. [8]AP-HP, Hôpital Necker-Enfants Malades, Service de Maladies Héréditaires du Métabolisme, Centre de Référence Maladies Héréditaires du Métabolisme, Paris, France. [9]Institut Imagine, Inserm UMRS 1163, Paris, France. [10]AP-HP, Hôpital Necker-Enfants Malades, Service de Gastroentérologie-Hépatologie-Nutrition Pédiatrique, Paris, France. [11]AP-HP, Hôpital Universitaire Necker-Enfants Malades, Service de Pathologie, Paris, France. [12]AP-HP, Hôpital Universitaire Necker-Enfants Malades, Service d'Imagerie Pédiatrique, Paris, France. [13]AP-HP, Hôpital Universitaire Necker-Enfants Malades, Service de Réanimation médico- chirurgicale pédiatrique, Paris, France. [14]Groupe Hospitalier APHP Centre Université de Paris Recherche Clinique et Pharmacologie Necker Cochin, Paris, France.

*alice.hadchouel-duverge@aphp.fr

Pulmonary alveolar proteinosis related to mutations in the methionine tRNA synthetase (*MARS1*) gene is a severe, early-onset disease that results in death before the age of 2 years in one-third of patients. It is associated with a liver disease, growth failure and systemic inflammation. As methionine supplementation in yeast models restored normal enzymatic activity of the synthetase, we studied the tolerance, safety and efficacy of daily oral methionine supplementation in patients with severe and early disease.

Four patients received methionine supplementation and were followed for respiratory, hepatic, growth, and inflammation-related outcomes. Their course was compared to those of historical controls.

Methionine supplementation was associated with respiratory improvement, clearance of the extracellular lipoproteinaceous material, and discontinuation of whole-lung lavage in all patients. The three patients who required oxygen or non-invasive ventilation could be weaned off within 60 days. Liver dysfunction, inflammation, and growth delay also improved or resolved.

Methionine supplementation was associated with important improvements in children with pulmonary alveolar proteinosis related to mutations in the *MARS1* gene. Since the clinical trial, 16 additional *MARS1* patients have been successfully treated. This study paves the way for similar strategies for other tRNA synthetase deficiencies.

Adenosine-Dependent Activation Mechanism of Prodrugs Targeting an Aminoacyl-tRNA Synthetase

Guillaume Hoffmann[a]*, Madalen Le Gorrec[a], Emeline Mestdach[b], Stephen Cusack[c], Loïc Salmon[b], Malene Ringkjøbing Jensen[d], and Andrés Palencia[a].

1. Institute for Advanced Biosciences (IAB), Structural Biology of Novel Targets in Human Diseases, INSERM U1209, CNRS UMR5309, Université Grenoble Alpes, 38000 Grenoble, France. 2. Centre de Résonance Magnétique Nucléaire à Très Hauts Champs, (CRMN), UMR 5082, CNRS, ENS Lyon, UCBL, Université de Lyon, 69100 Villeurbanne, France. 3. European Molecular Biology Laboratory, 38042 Grenoble, France. 4. Université Grenoble Alpes, CEA, CNRS, IBS, 38044 Grenoble, France.

*Presenting author email address: guillaume.hoffmann@inserm.fr

Prodrugs have little or no pharmacological activity and carry a promoiety group to improve druglike properties such as solubility, permeability, transport, and stability toward enzymatic and metabolic degradation [1]. They are converted to active drugs in the body by enzymes, metabolic reactions, or through human-controlled actions. However, prodrugs promoting their chemical bioconversion without any of these processes have not been reported before. In a recent article [2], we presented an enzyme-independent prodrug activation mechanism by boron-based compounds (benzoxaboroles) targeting leucyl-tRNA synthetase (LeuRS), including an antibiotic that recently has completed phase II clinical trials to cure tuberculosis [3]. We combined nuclear magnetic resonance spectroscopy and X- ray crystallography with isothermal titration calorimetry to show that these benzoxaboroles do not bind directly to their drug target LeuRS, instead they are prodrugs that activate their bioconversion by forming a highly specific and reversible LeuRS inhibition adduct with ATP, AMP, or the terminal adenosine of the $tRNA^{Leu}$ [4]. We demonstrated how the oxaborole group of the prodrugs cyclizes with the adenosine ribose at physiological concentrations to form the active molecule. This bioconversion mechanism explains the remarkably good druglike properties of benzoxaboroles [5] showing efficacy against radically different human pathogens and fully explains the mechanism of action of these compounds. Thus, this adenosine-dependent activation mechanism represents a novel concept in prodrug chemistry that can be applied to improve the solubility, permeability and metabolic stability of challenging drugs.

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Anticodon engineered tRNA rescues expression of tumor suppressor proteins, resulting in growth inhibition of metastatic colorectal cancer models in vivo

Trinayan Kashyap*, Alan Leggett, Lingyue Yan, Sagi Ravid, Conlin O'Neil, Monika Tasak, Ruan Zhang, Alysia Cox, Sarani Ghoshal, Ali Dahaj, Philip McGilvray, Chris Katanski, Leslie Williams, Gautam Goal, Dave Altreuter and Yosef Landesman

hC Bioscience, Inc., 22 Liberty Drive, 9J Boston, MA 02210, USA.

*Presenting author: trinayan.kashyap@hcbioscience.com

Background: Colorectal cancer (CRC) is the second leading cause of cancer related deaths worldwide. Liver metastases present in 75% metastatic CRC patients drive morbidity. Adenomatous polyposis coli (APC) tumor suppressor gene is frequently mutated in CRC and 30% of all mutations are nonsense mutations. Reintroduction of full-length APC protein into cancer cells reduces tumorigenicity. We used anticodon engineered tRNAs (ACE-tRNA) to enable readthrough of nonsense mutation in mRNA transcripts by inserting a cognate amino acid, restoring full length protein expression. The highly metastatic CRC LoVo cell line (KRAS mutated), carrying APC R1114X premature termination codon (PTC) was used as a model cell line. ACE-tRNAs rescued full-length APC protein, reactivated the APC pathway, and induced anti-cancer activity. Systemic delivery of ACE-tRNAs in NOD-SCID mice inhibited LoVo tumor growth in xenograft and orthotopic models for metastatic CRC.

Results: ACE-tRNAs restored full-length APC protein expression, activating downstream pathway. Rescue of APC protein resulted in G1 phase cell cycle arrest and dose dependent cytotoxicity of LoVo cells. In vivo, systemic delivery of ACE-tRNAs dosed at 1 or 2mg/kg QoD (3 times a week) resulted in 76% tumor growth inhibition after 3 weeks. Mice gained weight and low inflammatory cytokine levels indicated ACE-tRNAs treatment tolerability.

Conclusions: ACE-tRNAs rescued full length protein expression of the tumor suppressor protein APC in metastatic CRC model. ACE-tRNA treatment showed anti-tumor activity and was well tolerated in vivo. These data support the development of ACE-tRNA as a therapeutic agent for CRC with liver metastases, which has limited treatment options.

Saturation of tRNA sequence space restricts genetic code growth

Jorge Garcia Lema [1,2], Montserrat Fàbrega [1,2], Noelia Camacho [1], Luca Maggi[1], Modesto Orozco[1,3], Miquel Pons[1,2], & **Lluís Ribas de Pouplana** [1,4]

1 Institute for Research in Biomedicine (IRB Barcelona), Barcelona Institute of Science and Technology, 08028 Barcelona, Catalonia, Spain. 2 Molecular Biology Institute of Barcelona, Consejo Superior de Investigaciones Científicas, 08028 Barcelona, Catalonia, Spain. 3 Dept. of Chemistry, University of Barcelona, 08028 Barcelona, Catalonia, Spain 4 Catalan Institution for Research and Advanced Studies (ICREA), 08010 Barcelona, Catalonia, Spain.

Early in evolution, the incorporation of new amino acids to the Genetic Code reached a limit that has been maintained for billions of years. Why the canonical Genetic Code can only house twenty tRNA identities remains a fundamental question in the evolution of life. This riddle is nicely exemplified by $tRNA^{Gly}_{ACC}$ which, despite being a perfectly suitable substrate for glycyl-tRNA synthetase, does not exist.

We have proposed that an ACC anticodon sequence is incompatible with $tRNA^{Gly}$ because it destabilizes the folding of the anticodon loop of this tRNA, thus representing a clear example of forbidden tRNA identity.

To test this hypothesis we have characterized the structure of $tRNA^{Gly}_{ACC}$ in solution, and we have solved the crystal structure of this molecule. We find that $tRNA^{Gly}_{ACC}$ in solution forms a dimeric structure that is dependent on its anticodon sequence. The crystal structure of this molecule reveals a new type of tRNA dimeric complex that relies on two consecutive Hogsteen base pairs formed by bases of the anticodon loops of the two tRNAs that form the complex. Thus, we propose that $tRNA^{Gly}_{ACC}$ is absent in all known Genetic Codes because of its tendency to form unproductive adducts.

PolyQure: Increasing translational error-rate by TRMT2A-inhibition, an avenue to cure polyQ diseases?

Aaron Voigt[1,2]*, Sabine Hamm[1], Laura Wend[1], Jörg B. Schulz[1,2], Clevio Nobrega[3], & Dierk Niessing[4]

[1]Department of Neurology, University Hospital, RWTH Aachen University, Aachen, Germany; [2] JARA-BRAIN Institute Molecular Neuroscience and Neuroimaging, Forschungszentrum Jülich GmbH and RWTH Aachen University, Aachen, Germany; [3]Faculty of Medicine and Biomedical Sciences, University of Algarve, Portugal; [4]Helmholtz Zentrum München, Germany

*avoigt@ukaachen.de

Background: Genes linked to polyglutamine (polyQ) diseases share expanded CAG stretches within the coding region, translated into glutamine tracts in disease-linked proteins[1]. These polyQ tracts are eponymous and causative for disease[2]. No therapy is available to stop neuronal decline in polyQ patients.

Results: To identify genetic suppressors of polyQ-induced neurotoxicity, we performed an unbiased RNAi screen in *Drosophila*. We found that loss of the tRNA-methyl transferase 2 homolog A (TRMT2A) suppresses polyQ-induced toxicity and aggregation not only in flies[3], but also yeast and human cells[4]. Similarly, TRMT2A-deficient mice display reduced polyQ-aggregates and are protected from polyQ-dependent neuronal decline. On the other hand, loss of TRMT2A did not result in detrimental phenotypes in all analyzed organisms.

TRMT2A is a conserved protein that facilitates the methylation of tRNAs generating m5U at position 54 (m5U54)[5]. We show that loss of TRMT2A causes reduced m5U54 levels and these coincides with an increased error-rate in translation. Accordingly, polyQ stretches display frequent interruptions by non-glutamines. Interrupted polyQ stretches are known to be less toxic compared to their uninterrupted counterparts[6].

Conclusion: Specific inhibition of TRMT2A in asymptomatic stages of polyQ diseases will delay or even prevent disease onset. Our data on TRMT2A knockout mice strongly suggest that an increased error-rate upon TRMT2A inhibition is unlikely to cause any side effects. Thus, inhibition of TRMT2A represents a prime target for the rational design of therapies to cure polyQ diseases like HD.

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Inhibiting polyglutamine aggregation with mistranslating tRNAs

Rasangi Tennakoon[1,*] & Patrick O'Donoghue[1,2]

Departments of [1]Biochemistry and [2]Chemistry, The University of Western Ontario, London, Ontario, Canada.

*rtennako@uwo.ca

Naturally occurring human transfer RNA (tRNA) variants can cause mistranslation across the proteome through amino acid mis-incorporation[1]. Mistranslation resulting from human tRNA variants can modify protein aggregation associated with Huntington's disease (HD)[2]. Serine tRNA ($tRNA^{Ser}$) variants with anticodon mutations are especially likely to mistranslate because their cognate aminoacyl-tRNA synthetase does not recognize the anticodon[1]. HD is caused by expansion of the poly-glutamine (polyQ) repeat found in exon 1 of the huntingtin gene[3]. Introducing interrupting residues to the disease-associated polyQ tracts has been found to slow protein aggregation[4, 5]. We hypothesize that tRNA-dependent mistranslation of serine at glutamine codons in cellular models of HD will reduce polyQ aggregate formation. In live mouse neuroblastoma cells, levels of protein aggregation were visualized using a green fluorescent protein fused to huntingtin. We assessed two $tRNA^{Ser}$ variants that mis-read glutamine codons ($tRNA^{Ser}_{CUG}$ and $tRNA^{Ser}_{UUG}$) in cells expressing wild-type (23Q) or disease-causing (74Q) huntingtin proteins. We found that expression of $tRNA^{Ser}_{CUG}$ caused a reduction in production of both the 23Q and 74Q alleles, while $tRNA^{Ser}_{UUG}$ caused a significant and selective decrease in production of 74Q and did not affect production of 23Q protein. Both mistranslating tRNAs caused no significant changes in cytotoxicity. Our findings indicate that glutamine codon mistranslating tRNAs have the potential to modify huntingtin protein aggregate formation without increasing cytotoxicity.

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Novel pre-clinical HBSL models to enable proof-of-concept for AAV-mediated *DARS1* gene therapy

Elizabeth Kalotay*, Elena Venuti, Gary Housley, Matthias Klugmann & Dominik Fröhlich

CNS Gene Therapy group, Translational Neuroscience Facility, School of Biomedical Sciences, University of New South Wales.

*e.kalotay@unsw.edu.au

Hypomyelination with brainstem and spinal cord involvement and leg spasticity (HBSL) is a debilitating and currently untreatable childhood leukodystrophy caused by biallelic mutations of the *DARS1* gene, which encodes the cytoplasmic aspartyl-tRNA synthetase (AspRS). Clinically, HBSL leads to regression of motor skills and progressive lower limb spasticity, sometimes accompanied by additional neurological abnormalities including epilepsy and cognitive impairments. Being a monogenic disease, HBSL is an ideal candidate for gene replacement therapy, however, a prerequisite to testing this treatment strategy is the development of accurate mammalian disease models. Our group's initial attempts to model HBSL in mice demonstrated that complete loss of *Dars1* gene function is embryonically lethal, and that heterozygous knockout of the *Dars1* gene does not produce a disease phenotype. In contrast to human patients, homozygous murine carriers of HBSL-causing mutations do not exhibit neurological deficits. Expression of HBSL-causing variants *in trans* to a *Dars1*-null allele reproduces some HBSL phenotypes but does not recapitulate the full disease spectrum. Through conditional knockout of *Dars1* in neurons ($Dars1^{NeuroKO}$) and in oligodendrocytes ($Dars1^{OligoKO}$), we have created additional HBSL mouse models, which exhibit pronounced neurological dysfunction. Characterisation of these models will advance the understanding of HBSL pathophysiology, including the contribution of specific cell populations to the disease process. In addition, we have developed recombinant AAV vectors for *in vivo* delivery of an optimised human *DARS1* coding sequence, which has shown great efficacy in ameliorating neurological deficits observed in the pre-clinical HBSL models, providing the first proof-of-concept for a HBSL gene replacement therapy.

Highly Productive Cell-Free Genetic Code Expansion by Structure-Based Engineering of *Methanomethylophilus alvus* and ISO4-G1 Pyrrolysyl-tRNA Synthetases

Eiko Seki[1,2,3], Tatsuo Yanagisawa[3], Hiroaki Tanabe[1,2,3], Kensaku Sakamoto[2,3], **Shigeyuki Yokoyama[1,2,3]**
[1]RIKEN, Yokohama, [2]Tokyo Medical and Dental University, Tokyo, [3]Shinshu University School of Medicine, Matsumoto, Nagano, Japan.

Pairs of pyrrolysyl-tRNA synthetase (PylRS) and $tRNA^{Pyl}$ from *Methanosarcina mazei* and *Methanosarcina barkeri* are widely used for site-specific incorporations of non-canonical amino acids into proteins (genetic code expansion). We are engineering *Methanomethylophilus alvus* and ISO4-G1 PylRSs for genetic code expansion with bulky lysine analogues, on the basis of their crystal structures. We also use the cell-free protein synthesis method, as it is advantageous for production of human membrane proteins such as GPCRs. We achieved full productivity of cell-free protein synthesis for bulky non-canonical amino acids, including N^{ε}-((((*E*)-cyclooct-2-en-1-yl)oxy)carbonyl)-L-lysine (TCO*Lys), by using *M. alvus* PylRS with structure-based mutations in and around the amino acid binding pocket (first-layer and second-layer mutations, respectively). We attempted the site-specific incorporation of N^{ε}-(*p*-ethynylbenzyloxycarbonyl)-L-lysine (*p*EtZLys) into proteins on the basis of the ISO4-G1 PylRS structure, as it was much less efficient than that of TCO*Lys with *M. alvus* PylRS mutants. A couple of first-layer mutations of ISO4-G1 PylRS, with no additional second-layer mutations, increased the protein productivity with *p*EtZLys up to ca. 60% of that with TCO*Lys at high enzyme concentrations in the cell-free protein synthesis.

Trypanosoma brucei prolyl-tRNA synthetase and a *trans*-editing domain maintain proline codon translational fidelity

Rylan Watkins[1,3], Anna Vradi[1,3], Irina Shulgina[1,3], Juan Alfonzo[2,3], and Karin Musier-Forsyth[1,3]

[1]Department of Chemistry and Biochemistry, [2]Department of Microbiology, and [3]Center for RNA Biology, Ohio State University, Columbus, Ohio

Email: Watkins.819@osu.edu

Trypanosoma brucei (Tb) is a eukaryotic pathogen that causes Human African Trypanosomiasis—a fatal disease that lacks reliable therapeutics. Before infecting mammals, *Tb* resides in the midgut of the tsetse fly. Differentiation of *Tb* from the insect form to the mammalian bloodstream form leads to metabolic reprogramming. In a nutrient-rich mammalian host environment, *Tb* uses glucose for ATP production via glycolysis. In the insect form, glucose availability is low and proline is imported as a carbon source to make ATP, producing alanine as a major side product. Thus, $tRNA^{Pro}$ aminoacylation errors may be elevated during insect-stage catabolism, as prolyl-tRNA synthetases (ProRSs) across all domains of life mischarge alanine onto cognate $tRNA^{Pro}$. Here, we characterize recombinant *Tb* ProRS and multi-tRNA synthetase complex 3 protein (MCP3), a homolog of the known ProXp-ala *trans*-editing domain found in humans and some bacteria. Both *Tb* ProRS, which encodes an appended ProXp-ala domain, and MCP3 display robust Ala-$tRNA^{Pro}$ editing activity. We show that the unique N-terminal domain of MCP3 facilitates homodimerization and $tRNA^{Pro}$ binding. The deacylation activity of the free-standing *Tb* ProXp-ala domain is reduced ~17-fold relative to its activity in the context of full-length ProRS—preliminary assays suggest this is due to improved $tRNA^{Pro}$ binding. We propose that *Tb* evolved to encode functionally redundant Ala-$tRNA^{Pro}$ proofreading machinery to maintain translational fidelity at proline codons during peaks in the intracellular alanine pool and MCP3, which is not encoded in humans, may be a novel drug target. Studies are currently underway to test our hypothesis *in vivo.*

IF3 interaction with initiator-tRNA elbow modulates translation initiation and growth fitness in *Escherichia coli*

Jitendra Singh[1], Rishi Kumar Mishra[2], Shreya Ahana Ayyub[1], Tanweer Hussain[2], & **Umesh Varshney**[1]

[1]Departments of Microbiology and Cell Biology; and [2]Molecular Reproduction, Development and Genetics, Indian Institute of Science, Bangalore, 560012, India

*varshney@iisc.ac.in

Initiation factor 3 (IF3) regulates the fidelity of translation initiation in bacteria by avoiding the use of non-canonical start codons or non-initiator tRNAs and prevents premature docking of the 50S ribosomal subunit to the 30S preinitiation complex (PIC). The C-terminal domain (CTD) of IF3 can carry out most of the known functions of IF3 and sustain *Escherichia coli* growth. However, the roles of the N-terminal domain (NTD) have remained unclear. We hypothesize that the interaction between NTD and initiator $tRNA^{fMet}$ (i-tRNA) is essential to coordinate the movement of the two domains during initiation pathway to ensure fidelity of the process. Now, using atomistic molecular dynamics (MD) simulation run we show that R25A/Q33A/R66A mutations do not impact NTD structure but disrupt its interaction with i-tRNA. These NTD residues modulate the fidelity of translation initiation and are crucial for bacterial growth. Our observations also implicate the role of these interactions in the subunit anti-association activity of IF3 CTD. Overall, the study shows that the interactions between IF3 and i-tRNA are crucial for coupling the movements of NTD and CTD of IF3 during the initiation pathway and in imparting growth fitness to *E. coli*.

Coordination of aminoacylation and editing in proteotoxic stress

Jiqiang (Lanny) Ling* & Hong Zhang

Department of Cell Biology and Molecular Genetics, The University of Maryland, College Park, MD 20742, USA

*jling12@umd.edu

Approximately half of the aminoacyl-tRNA synthetases utilize an editing domain to hydrolyze misacylated tRNAs and maintain overall translational fidelity (1, 2). The evolutionally conserved editing function and the mild fitness changes caused by editing defects have remained a puzzling question. We have used yeast as a model organism to examine the physiological role of editing in alanyl- (AlaRS) and threonyl- (ThrRS) tRNA synthetases. We show that mutating a conserved editing-site cysteine residue in either AlaRS (3) or ThrRS (unpublished) leads to similar cellular responses, including decreased protein synthesis, activated integrated stress response, and increased heat sensitivity. We then performed evolution experiments of the AlaRS and ThrRS editing-defective mutants under heat-stress conditions. Unexpectedly, all suppressor mutations mapped to the genes encoding AlaRS and ThrRS without rescuing the editing defect. Follow-up experiments led us to propose a new unified model to explain the proteotoxicity caused by AlaRS and ThrRS editing defects.

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tRNA-related modification and anticodon stem loop structure underlie translation regulation by yeast aaRSs

Yoav S. Arava*

Faculty of Biology, Technion – Israel Institute of Technology, Haifa, 3200003, ISRAEL

*arava@technion.ac.il

tRNAs are recognized by cognate aminoacyl tRNA synthetases (aaRS) through various sequence and structure identity elements. Since aaRSs also emerge as important mRNA binding proteins *(1)*, we investigated whether tRNA-like elements underlie binding, and subsequent mRNA expression regulation. Comprehensive transcriptomic screen for mRNAs bound by *S. cerevisiae* cytosolic aaRSs, revealed enrichment for target mRNAs with features that resemble cognate tRNAs *(2)*. Further detailed analysis of ThrRS and MetRS provided mechanistic understanding regarding two tRNA-like elements: i) A tRNA anticodon-stem loop (ASL) mimic was found to be important for mRNA binding by ThrRS. Unbiased random mutagenesis of its top target mRNA (encoding the RPC10 transcription factor) revealed that almost any change to the ASL-mimic hampered binding. Importantly, ThrRS binding was found to affect RPC10 protein levels and consequently expression of $tRNA^{Thr}$ *(3)*. These data substantiate the role of ASL-mimics in aaRS-mediated regulation. ii) A pseudouridine modification within an mRNA target of MetRS *(YEF3)* was found to be critical for its binding. Deletion of the pseudouridine synthase which modifies $tRNA^{Met}$ (Pus6) led to a decrease in MetRS binding to both cognate tRNA and mRNA targets. Furthermore, translation of YEF3 mRNA increased upon pseudouridine removal *(5)*. This reveals that pseudouridines are common denominators between tRNA and mRNA regulation, and may coordinate global and gene-specific translation. Altogether, our findings reveal that key tRNA features are also present in mRNA and exert a regulatory role on translation.

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Puzzles and solutions in resolving RNA modifications in tRNA-derived small RNAs

Qi Chen[1,2,*]

[1]Molecular Medicine Program, Division of Urology, University of Utah School of Medicine, Salt Lake City, UT, USA. [2]Center for RNA Biology and Medicine, Division of Biomedical Sciences, University of California, Riverside, CA, USA.

*qi.chen@hsc.utah.edu

tRNA-derived small RNAs (tsRNAs or tDRs) are a type of widely existed small non-coding RNA with their mechanisms of action underexplored. tsRNAs are sophisticatedly modified, creating series of challenge to study their expression and biological function. The RNA modifications can lead to biases in the expression analysis of tsRNAs when performing high-throughput sequencing and Northern blot analyses; and synthetic tsRNAs without proper modifications may not fully mimic endogenous tsRNAs when analyzing their cellular functions. Our group strive to develop new analyzing tools such as PANDORA-seq, to sequencing modified tsRNAs that are previously undetectable; and to use newly developed mass spectrometry-based direct sequencing, such as MLC-seq, to de novo analyze the full scope of RNA modifications of individual tsRNAs, based on which we can synthesize engineered tsRNAs with added modifications, to mimic or enhance the function of endogenous tsRNAs. Our efforts may guide future understanding and designing of modified tsRNAs for precise gene regulation with a comprehensive tsRNA modification profile identified.

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Investigating the Cellular Impacts of Translational Fidelity Mutations

Parker Murphy*, Hong Zhang, & Jiqiang Ling

University of Maryland College Park, MD, USA.

*pmurphy1@umd.edu

Translational fidelity mutations have become a growing field due to their wide ranging impacts on the cell or whole organism. Particularly recent studies have shown translational fidelity mutations may play a role in cancer[1], and they have also been shown to influence the speed of ageing[2,3]. With this growing interest we have investigated some of the cellular impacts these mutations have. Initially the growth rates, protein synthesis rates, and response to heat shock was tested for our high and low fidelity mutations. We then moved to examining the predicted structure of the mutated proteins to understand the possible impacts on the ribosome they have. Neither high or low fidelity mutations impacted the individual protein structure, indicating there may be changes in protein-protein interactions in the ribosome. Next we examined polysome profiling to understand the possible effects on polysome formation as well as monosome formation, and found that the low fidelity mutations did not indicate changes when compared to wild type profiles. Finally we looked into the Ribosome Quality Control (RQC) Pathway utilising a GFP poly basic plasmid. We found that there were apparent aberrations in the activation or action of the RQC pathway in the mutant strains.

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Elucidating tRNA-dependent mistranslation rates in living cells

Peter Rozik[1,*], Patrick O' Donoghue[1,2].

Departments of [1]Biochemistry and [2]Chemistry, The University of Western Ontario, London, Ontario, Canada.

The 'frozen accident' hypothesis suggested that deviations from the genetic code would result in so much non-functional proteins that cells cannot survive[1]. In the information transfer from DNA to RNA to protein, translation of mRNA is the most error prone step[2]. Typically, error rates in protein synthesis are low and estimates suggest 1 in every 10,000 codons are mistranslated by the ribosome[3]. Recent studies show cells can survive significantly higher levels of mistranslation of 1-10% per codon[4,5,6]. There are limited approaches to measure the level of translation error in living cells. We engineered a novel mCherry reporter that is sensitive to serine mis-incorporation at proline (Ser151Pro)[7] or phenylalanine (Ser151Phe) codons. Ser mis-incorporation was validated by fluorescence restoration of the mutant mCherry and with mass spectrometry. We next fused the mCherry reporter to a normal GFP and quantified mistranslation in individual mammalian cells expressing a natural human mutant $tRNA^{Ser}$ G35A ($tRNA^{Ser}_{AAA}$), which is found in 2% of the population in the SER-AGA-2-3 gene. The mutant tRNA mis-incorporated Ser at Phe codons and restored fluorescence to the mCherry Ser151Phe reporter. We found that the mistranslation level ranges from 10-30% and is dependent on the tRNA gene in which the mutant was located and in the cell line in which the tRNA allele was expressed. The ability to accurately measure mistranslation rates is essential to investigate how cells tolerate different levels of mistranslation resulting from natural or engineered tRNA variants.

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Molecular basis of human Trmt13 in tRNA modification and transcriptional regulation

Ru-Juan Liu*, Di-Jun Du, Hao Li, Pei-Yu Tian & Hui-Min Zhao

School of Life Science and Technology, ShanghaiTech University, Shanghai 201210, China

*liurj@shanghaitech.edu.cn

Modification at the acceptor stem of tRNAs is rare, but 2'-*O*-methylation at the 4th position (Nm4) is conserved in eukaryotes. In yeast, a special RNA methyltransferase Trm13p, which has no sequence similarity with other known methyltransferases, was identified as the modifying enzyme for Nm4 of tRNA (Wilkinson *et al*, 2007). Recently, we identified Trmt13 as the human homolog of Trm13p that catalyzes Nm4 formation on tRNA^{Gly} and tRNA^{Pro} and regulates protein translation (Li *et al*, 2022). On the other hand, Trmt13 directly binds DNA as a transcriptional co-activator of key epithelial-mesenchymal transition factors, thereby promoting cell migration independent of tRNA-modification activity (Li *et al*, 2022). So far, the molecular basis of the Trm13/Trmt13 as a tRNA methyltransferase or a transcriptional co-activator remains elusive.

Here, we used Cryo-Electron Microscopy to determine a high-resolution structure of human Trmt13 in complex with SAM and tRNA substrates, providing insights into the molecular mechanisms of Trmt13 binding and catalyzing tRNA. Human Trmt13 contains a main methyltransferase domain, two zinc fingers that are involved in tRNA binding, and a coil-coiled domain that links them. Although Trmt13 has no sequence similarity to other known methyltransferases, its methyltransferase domain presents as a classical Rossman fold, as seen in other RNA methyltransferases. Combining with more biochemical assays, we revealed how Trmt13 binds with tRNA or DNA using the specific Zinc-finger domain. Our work provides a molecular basis to understand the dual function of Trmt13 in tRNA methylation and transcriptional regulation.

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Structural Basis of The Resistance Mechanisms By An Antibiotic Targeting Leucyl-tRNA Synthetase of Multi-Drug Resistant Gram-negative Bacteria

Bastien Muller[1], Guillaume Hoffmann[1], Franziska Fries[2], Lukas Junk[2], Jennifer Herrmann[2], Rolf Müller[2] and Andrés Palencia[1*]

[1]Institute for Advanced Biosciences (IAB), Structural Biology of Novel Drug Targets in Human Diseases, 38700 Grenoble, France

[2]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), 66123 Saarbrücken, Germany **Contact:** bastien.muller@univ-grenoble-alpes.fr; andrés.palencia@inserm.fr ; guillaume.hoffmann@inserm.fr

AN3365, a boron-based inhibitor targeting the editing site of leucyl-tRNA synthetase (LeuRS)[1,2], entered phase two clinical trials for the treatment of complicated bacterial infections, however the study was halted due to the emergence of antibiotic resistance in the trial with patients presenting complicated urinary tract infections [3]. Here, we unveil the structural basis of the resistance mechanisms by the five most prevalent *E. coli* LeuRS mutants resistant to AN3365 identified during the clinical trials, which mapped to the *E. coli* LeuRS editing site. The mutants were characterized by X-ray crystallography and by isothermal titration calorimetry binding experiments, which provided an explanation to the decreased drug susceptibility.

In addition, as the LeuRS mutants could have a compromised editing activity, growth kinetics tests were performed in presence of norvaline (main substrate of the LeuRS editing site) with several *E. coli* resistant strains. This showed a lower growth of the mutants compared with the wild-type strain that confirmed a higher norvaline sensitivity by the mutants.

In a second approach to minimize the drug resistance problem, we are functionally and structurally characterizing *E. coli* isoleucyl-tRNA synthetase (IleRS), a validated antibacterial target with a high structural similarity with the LeuRS editing site.

Our data provide insights to design new derivatives of AN3365 targeting *E. coli* LeuRS, by using a "structure guided-drug design" approach and, based on the structural similarity of the editing sites, to design a potential dual inhibitor of both LeuRS and IleRS, which would significantly reduce the emergence of resistance.

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Exploring the tRNA fragmentation principles across multiple species

Junchao Shi[1], Musheng Li[2], Bailong Zhang[1], Edward A. Vizcarra[1], Emma H. Wilson[1], Weifeng Gu[3], Xuemei Chen[4], Rachana Ramarao[5], Catherine Merrick[5], Thomas Kidd[6], Qi Chen[1,7], **Tong Zhou[2]***

[1]*Center for RNA Biology and Medicine, Division of Biomedical Sciences, University of California, Riverside, CA, USA.*
[2] *Department of Physiology and Cell Biology, University of Nevada, Reno School of Medicine, Reno, NV, USA*
[3]*Department of Molecular, Cell and Systems Biology, University of California, Riverside, Riverside, CA, USA*
[4]*Department of Botany and Plant Sciences, University of California, Riverside, Riverside, CA, USA*
[5]*Department of Pathology, University of Cambridge, Cambridge, UK*
[6] *Department of Biology, University of Nevada, Reno, Reno, NV, USA*
[7]*Molecular Medicine Program, Division of Urology, Department of Surgery, University of Utah School of Medicine, Salt Lake City, UT, USA*

*Presenting author email address: tongz@med.unr.edu

The fragmentation of tRNAs generates a class of small non-coding RNAs, tRNA-derived small RNAs (tsRNAs). tsRNAs have been discovered across three domains of life, and play regulatory roles in various biological processes [1]. We recently developed a new small RNA sequencing protocol, PANDORA-seq, which overcomes the potential biases caused by RNA modifications harbored in tsRNAs compared with traditional small RNA sequencing protocols, thus providing an opportunity to comprehensively analyze tsRNAs with unprecedented resolution [2]. Here, we applied PANDORA-seq to profile tsRNAs from multiple species, including human, mouse, fly, worm, arabidopsis, protozoa, yeast, and bacteria. We found that species taxa are the dominant factor shaping tRNA fragmentation patterns, which is followed by an organ/cell type specificity within each species. Computational analyses of tRNA sequences across species further reveal potential sequence features that shape the fragmentation patterns, in addition to the contributions of RNA modifications and cleavage enzymes. Our study brings new insights into the principles of tsRNA biogenesis and contributes to our knowledge on the expanding universe of small RNAs [3].

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Control of Cell Cycle Progression by a (Multifunctional) Mitochondrial Protein

Luís F.C. Póvoas [1]*; Lluís Ribas de Pouplana [1,2]

[1] Institute for Research in Biomedicine (IRB), C/Baldiri Reixac 12, 08028 Barcelona, Catalonia, Spain
[2] Catalan Institution for Research and Advanced Studies (ICREA), Passeig Lluís Companys 23, 08010 Barcelona, Catalonia, Spain
* Presenting author email address: luisfilipe.povoas@irbbarcelona.org

SLIMP, a novel Mitochondrial Seryl-tRNA synthetase – like protein, has been described as a multifunctional protein originated from a duplication of the mitochondrial seryl-tRNA synthetase (SerRS2), early in animal evolution. These two proteins form a heterodimeric structure, essential for their aminoacylation function in *Drosophila melanogaster* mitochondria [1, 2]. Moreover, SLIMP interacts with LON protease, being essential for the control of mitochondrial DNA copy number [3]. Others have shown that an *in vivo* co-depletion of SLIMP and E2F1, a major regulator of cell cycle, is able to rescue cell viability [4], however, the involvement of SLIMP in this pathway is still not well understood.

Here we describe a novel non-canonical function of SLIMP related to cell cycle progression. Upon *in vitro* knockdown of this protein, cells accumulate in G2 and show an increase of E2F mRNA targets, essential for G1-S transition. In addition, this transition is faster in SLIMP depleted cells, showing an involvement of this protein in cell cycle progression. Furthermore, a rescue experiment of SLIMP without the mitochondrial signal peptide (MSP) was able to reverse the G2 accumulation phenotype obtained upon knockdown of this protein. This indicates a potential extra mitochondrial function of SLIMP, where its cell cycle function is independent from its mitochondrial location.

To our knowledge, this is the first description of a mitochondrial protein involved in cell cycle progression from G1-S phase, providing new insight into understanding the importance of this organelle in cell cycle regulation.

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Threonyl-tRNA synthetase regulates signal transducer and activator of transcription 3

Chong Dai, Adriana Reyes-Ordoñez, Pallob Barai, and **Jie Chen***

Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA

*jiechen@illinois.edu

Like many aminoacyl-tRNA synthetases, threonyl-tRNA synthetase (ThrRS) has been found to play a role in regulating cellular processes beyond protein synthesis. We have reported that ThrRS directly regulates a JNK signaling pathway in a translation-independent manner during skeletal muscle differentiation. We now identify another non-canonical target of ThrRS as signal transducer and activator of transcription 3 (STAT3). ThrRS positively regulates STAT3 phosphorylation, nuclear translocation, and transcriptional activation. This regulation is independent of ThrRS function in protein synthesis, and it involves the kinase of STAT3, Jak. STAT3 is a proto-oncogene, and its aberrant activation is associated with the formation of several types of tumors, including non-small cell lung cancer (NSCLC). Interestingly, ThrRS is found in the human cancer databases to be frequently amplified in NSCLC. A potential link between elevated ThrRS levels and hyperactive STAT3 in NSCLC is currently explored.

Arg-tRNA synthetase links inflammatory metabolism to RNA splicing and nuclear trafficking via SRRM2

Haissi Cui[1*], Jolene K. Diedrich[1], Douglas C. Wu[2], Justin J. Lim[3,4], Ryan M. Nottingham[2], James J. Moresco[1,5], John R. Yates III[1], Benjamin J. Blencowe[3,4], Alan M. Lambowitz[2], Paul Schimmel[1]

[1]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA
[2]Institute for Cellular and Molecular Biology and Departments of Molecular Biosciences and Oncology, University of Texas at Austin, TX 78712, USA
[3]The Donnelly Centre, University of Toronto, Toronto, ON M5S 3E1, Canada
[4]Department of Molecular Genetics, University of Toronto, ON M5S 1A8, Canada
[5] Center for Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX 75390, USA

*haissi.cui@utoronto.ca

Cells respond to perturbations like inflammation by sensing changes in metabolite levels. Especially prominent is arginine, which has known connections to the inflammatory response. Aminoacyl-tRNA synthetases, enzymes that catalyze the first step of protein synthesis, can also mediate cell signaling. Here, we show that depletion of arginine during inflammation decreased levels of nuclear-localized arginyl-tRNA synthetase (ArgRS). Surprisingly, we found that nuclear ArgRS interacts and co-localizes with serine/arginine repetitive matrix protein 2 (SRRM2), a spliceosomal and nuclear speckle protein, and that decreased levels of nuclear ArgRS correlated with changes in condensate-like nuclear trafficking of SRRM2 and splice-site usage in certain genes. These splice-site usage changes cumulated in the synthesis of different protein isoforms that altered cellular metabolism and peptide presentation to immune cells. Our findings uncover a mechanism whereby a tRNA synthetase cognate to a key amino acid that is metabolically controlled during inflammation modulates the splicing machinery.

Novel Function of LARS in Maintenance of Gastric Carcinoma Cell Homeostasis

Susan A. Martinis*, Giyeong Kim, Allison Kim, & Lin-Feng Chen

Department of Biochemistry, University of Illinois Urbana-Champaign, Urbana, IL 61801

*martinis@illinois.edu

The aminoacyl-tRNA synthetases have become well known for essential non-canonical roles that are distinct from their critical house-keeping function in protein synthesis. Disruptions in these idiosyncratic activities can trigger shifts in homeostasis and cause disease. Bioinformatics showed that gastric cancer patients have up-regulated LARS mRNA, which inversely correlates with patient survival. Knockdown of LARS decreased viability, proliferation, and migration of carcinoma cells without altering global translation, suggesting a unique function that is independent of leucine aminoacylation. Analysis via RNA-seq, qRT-PCR and immunoblotting of key proteins, revealed that LARS regulates the p53 pathway, as well as other genes in the apoptosis pathway. Specifically, LARS knockdown increased p53 expression and tumor suppressor genes that include p21 and p27, triggering cell cycle arrest, and ultimately apoptosis. This study revealed a novel function of LARS in maintaining cancer cell homeostasis, indicating that LARS could be a potential therapeutic target in cancer.

Tryptophan depletion induces high-affinity tryptophan uptake mediated by tryptophanyl-tRNA synthetase into human cells

Takumi Yokosawa [1], & **Keisuke Wakasugi** [1, 2, *]

[1] Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2] Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

*Presenting author email address: wakasugi@bio.c.u-tokyo.ac.jp

The novel high-affinity tryptophan (Trp)-selective transport system is present at elevated levels in human interferon-γ (IFN-γ)-treated and indoleamine 2,3-dioxygenase 1 (IDO1)-expressing cells. High-affinity Trp uptake into cells causes immune suppression. We have previously shown that both IDO1 and tryptophanyl-tRNA synthetase (TrpRS), whose expression levels are increased by IFN-γ, have a crucial function in high-affinity Trp uptake into human cells. In the present study, we aimed to elucidate the relationship between TrpRS and IDO1 in high-affinity Trp uptake. We demonstrated that overexpression of IDO1 in HeLa cells drastically enhances high-affinity Trp uptake upon addition of purified TrpRS protein to uptake assay buffer. We also clarified that high-affinity Trp uptake by Trp-starved cells is significantly enhanced by the addition of TrpRS protein to the assay buffer. Taken together, we conclude that Trp deficiency is crucial for high-affinity Trp uptake mediated by extracellular TrpRS.

Nuclear TyrRS Stimulates Topoisomerase 1-induced Single Strand DNA Breaks to Protect against Tyrosine-induced Neurotoxicity in Primary Cortical Neurons

Sajish Mathew

College of Pharmacy, University of South Carolina, SC 29208

Email: mathew2@cop.sc.edu

The molecular basis of the development of Alzheimer's Disease (AD) still remains a scientific conundrum. Although amyloid beta (Aβ) plaques remained at the center of many therapeutic strategies to target AD, paradoxically, inhibition of Aβ peptide (Aβ40/42) generation using small molecule inhibitors caused cognitive impairments in AD patients. Moreover, AD patients exhibit low cerebrospinal fluid (CSF) Aβ42 levels, suggesting that endogenous Aβ42 has a central role in human cognition and memory formation. We recently showed that tyrosyl-tRNA synthetase (TyrRS) is decreased in AD brains and tyrosine depletes neuronal TyrRS[1]. Our unpublished data show that picomolar Aβ42 increases nuclear TyrRS protein levels and potentiates topoisomerase 1 (TOP1)-induced neuronal single strand DNA breaks (SSBs) in a protein synthesis-dependent manner. In contrast, Aβ40 depleted nuclear TyrRS and protected against camptothecin (CPT)-induced neurotoxicity in cortical cultures. Similarly, tyrosine that depletes nuclear TyrRS also activated neuronal DNA repair and protected against etoposide-induced neurotoxicity. Finally, *cis*-resveratrol (*cis*-RSV) that facilitates the nuclear localization of TyrRS also induced DNA damage in primary cortical neurons upon long time exposure (16 hr) whereas *trans*-RSV that depleted nuclear TyrRS failed to evoke DNA damage in presence of tyrosine. Together, our work suggests that nuclear TyrRS is a potent stimulator of neuronal activity-induced DNA damage and an age-associated increase in serum tyrosine levels may attenuate nuclear TyrRS-induced DNA damage and protein synthesis to affect neuronal activities associated with cognition, memory, and survival.

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A gut-associated bacterial tRNA synthetase acting as an immune modulator

Su-Man Kim[1,2], Shinhye Park[1,2], & **Myung Hee Kim**[1]*

[1]Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Korea
[2]These authors contributed equally

*mhk8n@kribb.re.kr

Co-evolution of the host and its gut microbiota has led to a highly mutualistic relationship in which the gut microbiota plays fundamental roles in maintaining host homeostasis. Growing evidence shows that perturbation of the gut microbiota (i.e., dysbiosis) has a marked effect on metabolic and immune processes. Failure to shape gut immune activity results in deregulated and excessive inflammatory responses that are linked to chronic inflammatory disorders such as inflammatory bowel diseases. Due to their multifaceted roles, aminoacyl-tRNA synthetases (ARSs) are considered as mediators that maintain homeostasis by tuning translation, as well as other biological roles[1-5]. So far, studies of the non-translational roles of ARSs have focused largely on eukaryotes. To date, little attention has been paid to the role of ARSs of commensals in regulating host homeostasis. We hypothesized that gut commensal ARSs may contribute to maintenance of host homeostasis via immune regulation. We found that a gut-associated bacterium secretes threonyl-tRNA synthetase (TARS) and that secreted TARS acts as an immune homeostatic mediator by activating the TLR2-CREB anti-inflammatory immune axis in macrophages. The immune axis led to efficient production of IL-10 and suppression of the central inflammatory mediator NF-κB. Intraperitoneal administration of the TARS restored IL-10-positive macrophage numbers, increased IL-10 levels in serum, and ameliorated the pathological effects in colitis mice. Thus, our results provide a new perspective of gut microbiota–host mutualism, in which commensal ARSs may have co-evolved as intrinsic immune mediators that shape holobiont homeostasis.

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A viral pan-end RNA element and unconventional host aminoacyl-tRNA synthetase complex define a SARS-CoV-2 regulon

Debjit Khan[1*], Fulvia Terenzi[1], GuanQun Liu[2], Prabar Kumar Ghosh[1], Arnab China[1], Iyappan Ramachandiran[1], Fengchun Ye[4], Kien Nguyen[4], Shruti Chakraborty[1], Jennifer Stefan[1], Krishnendu Khan[1], Kommireddy Vasu[1], Franklin Dong[1], Belinda Willard[3], Jonathan Karn[4], Michaela U. Gack[2], Paul L. Fox[1,5]

[1]Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195, USA. [2]Florida Research and Innovation Center, Cleveland Clinic Foundation, Port St. Lucie, FL 34987, USA. [3]Lerner Research Institute Proteomics and Metabolomics Core, Cleveland Clinic Foundation, Cleveland, OH 44195, USA. [4]Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA. [5]Lead contact

[*]Presenting author: khand@ccf.org

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, generates multiple protein-coding, subgenomic RNAs (sgRNAs) from a longer genomic RNA (gRNA)[1,2], all bearing identical termini with poorly understood roles in regulating viral gene expression. Insulin and interferon-gamma (IFN-γ), two host-derived, stress-related agents, and virus spike protein subunit 1 (S1), induce binding of glutamyl-prolyl-tRNA synthetase (EPRS1), within an unconventional, heterotetra-aminoacyl-tRNA synthetase complex, to the sgRNA 3'-end thereby enhancing sgRNA expression. The *s*arbecoviral *p*an-*e*nd *a*ctivating *R*NA (SPEAR) element also enhances viral -1 programmed ribosomal frameshifting, thereby expanding its functionality. EPRS1 knockout in adipocytes and knockdown in a lung cell line show loss of agonist-induced SPEAR-mediated translation induction, demonstrating importance of EPRS1 in sgRNA translation. SARS-CoV-2 replicon-derived sgRNAs as well as gRNA associate with EPRS1 upon agonist-induction, highlighting biological significance. In lung cell lines co-treated with spike and IFN-γ, lysyl- (K), arginyl- (R) and methionyl- (M) tRNA synthetases, all multi-tRNA synthetase complex (MSC) components[3], associate with EPRS1 as a unique extra-MSC *t*etra-*a*minoacyl tRNA-synthetase *s*arbecoviral *R*NA-*i*nteracting (TASRI) complex– to our knowledge, the largest known complex of aaRSs aside from the MSC itself, and localize to endoplasmic reticulum. Enhanced EPRS1/SPEAR element interaction in adipose tissue from fat-fed, obese mice underscores a potential in vivo significance. We report an RNA-based approach reduces SARS-CoV-2 titer, protein and RNA levels, and kinetics, suggesting a pan-sarbecoviral therapeutic modality. By co-opting noncanonical activities of a family of essential host proteins - the aminoacyl-tRNA synthetases, SARS-CoV-2 establishes a post-transcriptional regulon enhancing global viral RNA translation.

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Tryptophan-dependent and -independent secretions of tryptophanyl-tRNA synthetase: Pathophysiological implications for innate immune responses

Tram Thuy Thuy Nguyen[1], Sunghoon Kim[2] and **Mirim Jin**[1,3,4,*]

[1] Department of Health Sciences and Technology, GAIHST, Gachon University, [2]Medicinal Bioconvergence Research Center, College of Pharmacy & College of Medicine, Yonsei University, Incheon 21983, Korea, [3]Department of Microbiology, College of Medicine, [4]Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon 21999, Korea

*Presenting author email address: mirimj@gachon.ac.kr

While cytoplasmic tryptophanyl-tRNA synthetase (WARS1) ligates tryptophan (Trp) to its cognate tRNAs for protein synthesis, it also plays a role as an innate immune activator in extracellular space. However, its secretion mechanism remains elusive. Here, we report that in response to stimuli, WARS1 can be secreted via two distinct pathways: via Trp-dependent secretion of naked protein and via Trp-independent plasma-membrane-derived vesicles (PMVs). In the direct pathway, Trp binding to WARS1 induces a ‘‘closed’’ conformation, generating a hydrophobic surface and basic pocket. The Trp-bound WARS1 then binds stable phosphatidylinositol (4,5)-biphosphate and inner plasma membrane leaflet, passing across the membrane. In the PMV-mediated secretion, WARS1 recruits calpain 2, which is activated by calcium. WARS1 released from PMVs induces inflammatory responses *in vivo*. These results provide insights into the secretion mechanisms of WARS1 and improve our understanding of how WARS1 is involved in the control of local and systemic inflammation upon infection.

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Regulation of the metabolic fate of leucine by leucyl-tRNA synthetase 1

Jung Min Han

College of pharmacy, Yonsei University, South Korea

*jhan74@yonsei.ac.kr

Leucine is one of the three proteogenic branched-chain amino acids (BCAAs) and is among the nine essential amino acids for human, accounting for about 35% of the essential amino acids in muscle proteins. Leucyl-tRNA synthetase 1 (LARS1) mediates leucine incorporation into proteins through tRNA charging of leucine. Metabolically, BCAAs are rapidly oxidized into the TCA cycle, with the greatest quantity occurring in most tissues. Especially, pancreas supplies 20% of its TCA carbons from BCAAs. Therefore, it is very important to regulate whether leucine is used for protein synthesis in the cytosol or for the TCA cycle through catabolism in the mitochondria. In this study, we show that glucose availability affects the metabolic fate of leucine through intracellular leucine sensor LARS1. Glucose starvation results in O-GlcNAcylation of LARS1 on residue S1042. Subsequently, this modification promotes S720 phosphorylation of 'KMSKS' signature motif which is required for leucine-binding by the autophagy-activating kinase ULK1, resulting in locking LARS1 in a "sensing-off" state. As a result, global protein synthesis is inhibited and therefore leucine is redirected toward mitochondrial catabolism to support energy production and cell survival upon glucose starvation.

The lack of LARS1 O-GlcNAcylation constitutively activates protein synthesis and deregulates mitochondrial leucine catabolism under glucose starvation. This work demonstrates that LARS1 integrates leucine and glucose availability to regulate protein synthesis and metabolism.

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Posters

Poster 1:
Identification of Key Fibrotic Extracellular Targets for Alanyl- and Aspartyl-tRNA Synthetases

Ryan A. Adams[1]*, Ying-Ting Wang[1], Andrew Imfeld[1], Yeeting E. Chong[1], Kristina Hamel[1], Alison G. Barber[1], Wayne Liu[1], Cory Soto[1], Eileen Sun[1] and Leslie Nangle[1]

[1]aTyr Pharma, San Diego, CA, USA

*radams@atyrpharma.com

Throughout the course of evolution, aminoacyl-tRNA synthetases have acquired diverse functional domains capable of impacting an expansive range of biology. These signaling activities often result from the extracellular locality of these domains generated as either splice variants or proteolytic fragments from full-length synthetases. Efforts to characterize a splice variant of His-tRNA synthetase (HARS) have resulted in a therapeutic molecule, shown to interact with the extracellular receptor Neuropilin-2 (NRP2), which is currently engaged in a Phase 3 trial for Pulmonary Sarcoidosis, a form of inflammatory and fibrotic lung disease.

In an effort to discover novel functions from other members of the aminoacyl-tRNA synthetase family, we set out to identify extracellular interacting targets for domains from alanyl (AARS) and aspartyl (DARS) tRNA synthetases. To uncover these novel targets, we initially identified cell types that bound domains from AARS and DARS. Utilizing a ligand-based receptor-capture mass spectrometry technique on these bound cells, we identified Fibroblast Growth Factor Receptor 4 (FGFR4) as a receptor for a domain of AARS and Latent-Transforming Growth Factor Beta-binding Protein 1 (LTBP-1) as a binding partner for DARS. These interactions were confirmed through siRNA knockdown experiments, immunocytochemistry for colocalization and AlphaLISA for direct protein-protein interactions. On-going functional characterization of these interactions has revealed potential novel fibrotic mechanisms that underscore the therapeutic potential within the tRNA synthetase family of molecules.

Poster 2:
Secreted human cysteinyl-tRNA synthetase1 mediates immunogenic responses in ferroptosis induced by cysteine depletion

Hamin Ban[a,b,c,*], Uijoo Kim[a,b,c], Seongmin Cho[b,c], Hyeong Yun Kim[a,b,c], Sunghoon Kim[a,b,c,d,**]

[a] College of Pharmacy, Yonsei University, Incheon 21983, Korea. [b] Medicinal Bioconvergence Research Center, Incheon 21983, Korea. [c] Institute for Artificial Intelligence and Biomedical Research, Incheon 21983, Korea. [d] College of Pharmacy & College of Medicine, Interdisciplinary Biomedical Center, Gangnam Severance Hospital, Yonsei University, Incheon 21983, Korea. *hamin@target.re.kr **Correspondence

Ferroptosis is a type of programmed cell death, which caused by cysteine deprivation and induces immunogenic response through damage-associated molecular patterns (DAMPs). However, the mediator that senses the cysteine depletion and induces an immunogenic response has not been found. Previously, we reported that cysteinyl-tRNA synthetase1 (CARS1) is secreted from tumor cells and induces immune stimulation. As CARS1 uses cysteine as a catalytic substrate for charging cysteinyl-tRNAs, we investigated the role of CARS1 in ferroptosis conditions induced by cysteine deprivation. Interestingly, we found that the extracellular secretion of CARS1 from tumor cells was increased by cysteine deprivation or ferroptosis inducers and decreased by the addition of cysteine. We prepared the antibody specific to human CARS1 and found that the antibody treatment reduced immunogenic response of PMA-differentiated monocyte, THP-1. These results suggest that CARS1 secretes from tumor cells in ferroptosis condition and plays its unique signaling role in immunogenic cell death.

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Poster 3:

Enzymatic Characterization of Full-length Glutamyl-Prolyl-tRNA Synthetase (EPRS)

Morgan Bauer[1]*, Sabat Gonzalez-Serrano[2], David Wood[2], & Karin Musier-Forsyth[1]

1 Department of Chemistry and Biochemistry, Center for RNA Biology, Ohio State University, Columbus, OH,
USA.
*Bauer.715@buckeyemail.osu.edu

2 William G. Lowrie Department of Chemical and Biomolecular Engineering, Ohio State University, Columbus,
OH, USA

Glutamyl-prolyl-tRNA synthetase (EPRS) catalyzes the attachment of proline and glutamic acid onto their respective tRNAs. Out of all the aminoacyl-tRNA synthetases, EPRS is the only bifunctional synthetase, with two catalytic cores connected by a linker domain. EPRS resulted from a fusion event between stand-alone, glutamyl- and prolyl-tRNA synthetase (ERS and PRS, respectively) during evolution around the creation of metazoans, and remained encoded in all animals. It has been proposed by Fox and co-workers that this fused protein is metabolically advantageous for organisms by keeping the proline and glutamic acid pool at cellular homeostasis. Whether EPRS catalytic activity differs relative to the stand-alone synthetases is unknown. Previous work on EPRS has shown that it performs many non-canonical functions, such as translational repression of inflammatory mRNAs, increasing the intake of long-chain fatty acids and repressing viral replication. In addition, there are multiple reports of mutations in EPRS which are linked to patients with hypomyelinating leukodystrophy and general multi- organ diseases. Given its function in a variety of cellular processes and role in disease, studying the full-length protein is of great interest. We successfully purified full-length EPRS in good yield from mammalian cells and characterized its enzymatic activity by performing steady-state aminoacylation and ATP-exchange assays. Compared to ERS and PRS domains alone, the fused catalytic domains of EPRS are more efficient at aminoacylating tRNA. These preliminary findings reveal a new advantage of ERS and PRS fusion.

Poster 4:
Engineered tRNA therapeutics for progranulin PTCs in Frontotemporal Dementia

Aruun Beharry[1,*] & Patrick O'Donoghue[1,2]

Department of [1]Biochemistry and [2]Chemistry, The University of Western Ontario, London, Ontario, Canada.

*abeharr3@uwo.ca

Premature termination codons (PTCs) account for 11% of inherited human diseases including forms of Cystic Fibrosis, Frontotemporal Dementia (FTD), various cancers, and several others (1,2). For many of these diseases, treatments are often limited or unavailable. A novel therapeutic approach is the use of non-sense suppressor transfer RNAs (sup-tRNAs); synthetically altered tRNAs bearing a mutated anticodon corresponding to one of the three stop codons (3). In this work, sup-tRNAs are generated from natural human $tRNA^{Arg}$ variants and evaluated for their ability to read through an R493X PTC in the progranulin protein, a mutation that has been linked to the development of FTD (4). We aim to restore full-length protein production to ameliorate the pathogenic phenotype. The Arg $tRNA^{UCG}$ variants were amplified from human genomic DNA and single-base mutagenesis was employed to produce a $tRNA^{UCA}$ capable of decoding the aberrant UGA stop codon. The mutagenesis avoids discriminator bases A20 and C35, so aminoacylation by the cognate synthetase is unaffected (5). The sup-tRNA were ligated into a plasmid and co-transfected into HEK 293T with a synthetic readthrough reporter to determine readthrough efficiency by live cell fluorescence microscopy. Treatment with the sup-tRNA restored fluorescence to approximately 16% of wild-type levels. As a disease model, a plasmid carrying GFP-$progranulin^{R493X}$ fused protein will be transfected into N2a cells. The effectiveness of sup-tRNA readthrough will be evaluated by Western Blotting for full-length protein, live cell fluorescence for protein localization, and a neurite outgrowth assay to evaluate progranulin functionality as described in previous works (6).

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Poster 5:
EPRS1-related disorder: A genotype-phenotype correlation study reveals distinct neurological disease types and wide phenotypic variability

Alexandra Chapleau[1,2]*, Reza Maroofian[3], Morgan Bauer[4], Stephanie Efthymiou[3], Gaurav Varshney[5], Wolfgang Kohler[6], Hanna Mierzewska[7], Marcelo Kauffman[8,9], Davide Tonduti[10,11,12], Chloe Stutterd[13,14,15,16], Konstantinos Tsiakas[17], Farrah Rajabi[18,19,20], Vernet Machado Matheus[21], Nicole Wolf[22,23], Karin Musier-Forsyth[4], Henry Houlden[3], Genevieve Bernard[1,2,24,25,26]

[1]Child Health and Human Development Program, Research Institute of the McGill University Health Centre, Montreal, Canada [2]Department of Neurology and Neurosurgery, McGill University, Montreal, Canada [3]Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, University College London, London WC1N 3BG, UK [4]Department of Chemistry and Biochemistry, Center for RNA Biology, Ohio State University, Columbus, Ohio, USA. [5]Genes & Human Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK, USA [6]Department of Neurology, University Hospital Leipzig, Liebigstrasse 20, 04103 Leipzig, Germany. [7]Clinic of Pediatric Neurology, Institute of Mother and Child, Warsaw, Poland. [8]Consultorio y Laboratorio de Neurogenética, Centro Universitario de Neurología "José María Ramos Mejía" y División Neurología, Hospital JM Ramos Mejía, Facultad de Medicina, UBA, Buenos Aires, C1221ADC, Argentina. [9]School of Medicine, UBA, CONICET, Buenos Aires, C1121ABG, Argentina. [10]Child Neurology Unit, V. Buzzi Children's Hospital, Milan, Italy [11]Center for Diagnosis and Treatment of Leukodystrophies and Genetic Leukoencephalopathies (COALA), V. Buzzi Children's Hospital, Milan, Italy. [12]Department of Biomedical and Clinical Sciences, University of Milan, Milan, Italy. [13]Department of Clinical Genetics, Austin Health, Heidelberg, Australia 3084. [14]Victorian Clinical Genetics Services, Melbourne, Parkville, Victoria, Australia 3052. [15]Royal Children's Hospital Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia 3052. [16]Murdoch Children's Research Institute, Melbourne, Parkville, Victoria, Australia 3052. [17]Department of Pediatrics, University Medical Center Hamburg-Eppendorf, Martinistr. 52, 20246, Hamburg, Germany. [18]Division of Genetics and Genomics, Boston Children's Hospital, Boston, MA, USA. [19]The Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA. [20]Department of Pediatrics, Harvard Medical School, Boston, MA, USA. [21]Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brazil. [22]Department of Child Neurology, Amsterdam Leukodystrophy Center, Amsterdam UMC location Vrije Universiteit Amsterdam, Emma's Children's Hospital, Boelelaan 1117, Amsterdam, The Netherlands. [23]Amsterdam Neuroscience, Cellular & Molecular Mechanisms, Amsterdam, The Netherlands. [24]Department of Pediatrics, McGill University, Montreal, Canada [25]Department of Human Genetics, McGill University, Montreal, Canada [26]Division of Medical Genetics, Department of Specialized Medicine, McGill University Health Centre, Montreal, Canada

*alexandra.chapleau@mail.mcgill.ca

Aminoacyl tRNA synthetases (ARS) are a group of ubiquitously expressed enzymes essential for catalyzing the esterification reaction to load amino acids onto their cognate tRNAs. A growing number of neurological disorders have been associated with mutations in *ARSs*. Glutamyl-prolyl-tRNA synthetase, EPRS1, is a bifunctional ARS with two catalytic domains joined by a linker region. Biallelic pathogenic variants in *EPRS1* have been shown to cause an ultra-rare hypomyelinating leukodystrophy previously reported in 4 patients. Here, we present a cohort of 22 individuals from 20 families (19 previously unpublished patients) and expand the disease spectrum of EPRS1-related disorder. 26 new DNA sequence changes were detected, including 3 affecting splicing and 1 deletion. 10 individuals were found to display a novel disease phenotype. These patients do not have hypomyelination and display a milder clinical presentation characterized by developmental delay with or without ocular abnormalities, microcephaly and ataxia. In our cohort, neurological manifestations are universal, and visual loss (59.1%), short stature (31.8%), dental anomalies (31.8%) and sensorineural hearing loss (18.2%) were also commonly seen. Genotype-phenotype correlations revealed segregation of phenotypes to distinct protein domains, with most patients' mutations with hypomyelination clustering in the proline catalytic domain and of the mildly affected individuals' mutations concentrating within the glutamate catalytic core or the non-canonical appended domains. This study delineates the phenotypic landscape of *EPRS1*-related-disorder, broadening the disease spectrum.

Poster 6:
Evolution of the primordial genetic code by salvaging ancient enzymes

Jordan Douglas*[1], Remco Bouckaert[2], Peter Wills[1], & Charlie Carter[3]

[1] Department of Physics, University of Auckland, New Zealand
[2] School of Computer Science, University of Auckland, New Zealand
[3] Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, USA

*jordan.douglas@auckland.ac.nz

Due to their critical role in protein synthesis, understanding the evolutionary history of AARS can provide valuable insights into the earliest stages of life on Earth. In this study, we compiled a taxonomically representative dataset of AARS protein structure predictions and introduced a Bayesian phylogenetic method that leverages information from both sequence and structure. 33 AARS families collectively encode for the 22 proteinogenic amino acids found in the modern genetic codes. By modelling accretion of the insertion modules which characterise these families, we reconstructed the evolution of AARS, from their low-specificity protozymic forms that arose over 3 billion years ago to the diverse range of highly-specific extant enzymes that we see today. We observed a negative correlation between the structural complexity of an AARS's catalytic domain and the amino acid it activates, suggesting that the more structurally primitive enzymes tend to activate amino acids that were not synthesised until much later in the history of cellular metabolism. To explain this curiosity, we propose that the most probable evolutionary route for an amino acid type to establish its place in the primordial genetic code was by salvaging and reinventing older, less specific AARSs, rather than tweaking the more contemporary ones.

Poster 7:

Human TRUB1 is a highly conserved pseudouridine synthase responsible for the formation of Ψ55 in mitochondrial $tRNA^{Asn}$, $tRNA^{Gln}$, $tRNA^{Glu}$ and $tRNA^{Pro}$

Zidong Jia , Feilong Meng , Hui Chen , and **Min-Xin Guan***

Institute of Genetics, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China. Correspondence to: Min-Xin Guan, Ph.D., Institute of Genetics, Zhejiang University School of Medicine, 866 Yuhangtang Road, Hangzhou, Zhejiang 310058, China; Tel: 86-571-88206916; Fax: 86-571-88982377; E-mail: gminxin88@zju.edu.cn

Pseudouridine (Ψ) at position 55 in tRNAs plays an important role in their structure and function. This modification is catalyzed by TruB/Pus4/Cbf5 family of pseudouridine synthases in bacteria and yeast. However, the mechanism of TRUB family underlying the formation of Ψ55 in the mammalian tRNAs is largely unknown. In this report, the CMC/reverse transcription assays demonstrated the presence of Ψ55 in the human mitochondrial $tRNA^{Asn}$, $tRNA^{Gln}$, $tRNA^{Glu}$, $tRNA^{Pro}$, $tRNA^{Met}$, $tRNA^{Leu(UUR)}$ and $tRNA^{Ser(UCN)}$. TRUB1 knockout (KO) cell lines generated by CRISPR/Cas9 technology exhibited the loss of Ψ55 modification in mitochondrial $tRNA^{Asn}$, $tRNA^{Gln}$, $tRNA^{Glu}$, $tRNA^{Pro}$, but did not affect other 18 mitochondrial tRNAs. An in vitro assay revealed that recombinant TRUB1 protein can catalyze the efficient formation of Ψ55 in $tRNA^{Asn}$ and $tRNA^{Gln}$, but not in $tRNA^{Met}$ and $tRNA^{Arg}$. Notably, the overexpression of TRUB1 cDNA reversed the deficient Ψ55 modifications in these tRNAs in TRUB1KO HeLa cells. TRUB1 deficiency affected the base-pairing (18A/G-Ψ55), conformation and stability but not aminoacylation capacity of these tRNAs. Furthermore, TRUB1 deficiency impacted mitochondrial translation and biogenesis of oxidative phosphorylation system. Our findings demonstrated that human TRUB1 is a highly conserved mitochondrial pseudouridine synthase responsible for the Ψ55 modification in the mitochondrial $tRNA^{Asn}$, $tRNA^{Gln}$, $tRNA^{Glu}$ and $tRNA^{Pro}$.

Poster 8:
Circulating His-tRNA Synthetase is Reduced in Patients Harboring the Usher Syndrome Type 3B-linked mutation Y454S

Lauren Guy[1]*, Elizabeth Yu[1], Yeeting E. Chong[1], Zhiwen Xu[1], C. Anthony Rupar[2], Victoria M. Siu[2], Ryan A. Adams[1], Leslie Nangle[1]

[1]aTyr Pharma, San Diego, CA, USA
[2]The University of Western Ontario, London, Canada
*lguy@atyrpharma.com

His-tRNA synthetase (HARS) is found in human circulation and its activity modulates the trafficking of immune cells to sites of inflammation in pre-clinical mouse models. This suggests a role for HARS and its WHEP-domain in dampening the immune response and restoring homeostasis in the setting of chronic inflammation. The HARS WHEP-domain can be liberated from the full-length enzyme by both alternative splicing and proteolysis to signal extracellularly, which is exemplified by its association with anti-Jo-1+ disease wherein neutralization of extracellular HARS by high-titer antibodies leads to an aberrant tissue-immune response characterized by interstitial lung disease and myositis.

While there have been no human disease linkages for mutations occurring in the HARS WHEP-domain, a mutation outside the catalytic domain of HARS, Y454S, is associated with the development of Ushers Syndrome Type 3B (USH3B) in young children. Biochemical characterization of this mutation revealed minimal enzymatic effects; however, the mutant enzyme exhibited worsened thermal stability. We hypothesized that the inherent instability introduced by the Y454S mutation could result in decreased levels of extracellular, circulating HARS protein available for signaling. Utilizing plasma samples from a recent clinical trial involving children with the Y454S homozygous mutation, circulating HARS was assessed and found to exhibit strikingly lowered circulating HARS levels compared to age-matched healthy controls. These findings bolster previous protein stability results and may suggest a functional role for extracellular HARS that is diminished in individuals with Y454S mutations, perhaps providing rationale for some of the overlapping clinical features observed between anti-Jo-1+ disease and USH3B.

Poster 9:
Aminoacyl-tRNA synthetases are altered in hypoxic ovarian and hepatic cells

Jenica Kakadia[1*], **Mallory I. Frederick**[1*], Owen J. H. Hovey[1], Cristiana H. Iosef[1,2], Shawn S. C. Li[1], Victor K. Han[1,2], and Ilka U. Heinemann[1]

[1] Department of Biochemistry, University of Western Ontario, London, Ontario, Canada
[2]Children's Health Research Institute, London Health Sciences Centre, London, Ontario, Canada
*these authors contributed equally: jkakadia@uwo.ca and mfreder8@uwo.ca

Epithelial ovarian cancer (EOC) is the fifth most common cancer in women and has a high rate of recurrence due to a propensity for metastasis. During EOC metastasis, adherent cells from the primary tumour detach, form spheroids in the peritoneal fluid, and readhere at secondary sites. Indeed, liver metastases have been found in the majority of patients with advanced ovarian cancer. Hypoxia is present in ovarian cancer, and can affect the cellular response to cancer treatment; alleviating hypoxia may improve treatment efficacy. Aminoacyl-tRNA synthetases (ARSs) are pathologically significant in tumorigenesis; ARS inactivation promotes survival during hypoxia. Here, we utilized OVCAR8 EOC metastasis and hepatic-derived HepG2 cells to determine if hypoxic signaling in metastasis alters ARSs expression. Proteomic analysis revealed enrichment of hypoxic markers in spheroid and re-adherent OVCAR8 cells relative to adherent cells, and increased spheroid expression of multiple ARSs, pointing toward a relationship between hypoxia and ARS expression. To determine whether hepatic hypoxia affects ARSs expression, we treated HepG2 cells with 20% (normoxic) or 1% (hypoxic) O_2 air; through RNA sequencing, we found hypoxic HepG2 cells have significantly increased transcripts for DARS1 and GARS1-DT and decreased WARS1 and MARS2 compared to normoxia controls. Therefore, ovarian metastasis models exhibit increased hypoxia signaling concurrent with increased ARS expression; similarly, hypoxic signaling in hepatic cells also shows changes in ARS. This work highlights the relationship between ovarian cancer and hepatic metastases, hypoxia, and aminoacyl-tRNA synthetases and may be utilized for further work to understand mechanisms regulating EOC metastasis.

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Poster 10:
The involvement of tRNA in the pathophysiology of Antisynthetase syndrome

Sachiko Kanaji[1]*, Taisuke Kanaji[1], Ryan Shapiro[1] Megumi Shigematsu[2], Yohei Kirino[2], Paul Schimmel[1] & Xiang-Lei Yang[1]

1: Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA USA
2: Computational Medicine Center, Sidney Kimmel Medical College, Thomas Jefferson University, Philadelphia, PA USA

*Presenting author email address: skana@scripps.edu

Antisynthetase syndrome (ASSD) is an autoimmune disease characterized by myositis and interstitial lung disease (ILD).[1] A key feature of ASSD is the presence of autoantibodies against aminoacyl-tRNA synthetases (aaRSs).[2] There are eight aaRSs (out of 20 family members) reported as targets of the autoantibodies, among which the most common one is anti-Jo-1 directed against histidyl-tRNA synthetase (HARS). To date, the mechanism through which only selective members of the aaRS family is involved in ASSD and how the disease is caused or exacerbated by the presence of autoantibodies against aaRSs remain unknown. Importantly, tRNA, a key partner of aaRS, has not been studied in the context of ASSD.[3] Growing evidence suggests the involvement of endosomal Toll-like receptor TLR7 and type I interferon (IFN-I) signaling in sustaining and spreading inflammation in autoimmune diseases.[2] Because TLR7 can be activated by single-stranded RNAs (ssRNAs), including tRNA fragments[4], we hypothesized that aaRS-bound tRNAs or tRNA fragments play critical roles in the pathogenesis of ASSD. To this end, we generated a mouse model of ASSD and demonstrated a role of TLR7 activation in exacerbating myositis. Our in vitro study using human lung epithelial cells (A549) and mouse myoblasts (C2C12) showed release of HARS in the form of extracellular vesicles (EVs) and the potential co-secretion with 5'-half $tRNA^{HisGUG}$ fragments. This study unveils previously unrecognized involvement of tRNA in the pathogenesis of ASSD and suggests new targets for therapeutic development of the disease.

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Poster 11:
A severe disease-associated *TRIT1* mutation in combination with a polymorphism in its tRNA-modification substrate

Abdul Khalique*[1], Sandy Mattijssen[1], Gal Zaks Hoffer[2], Naama Orenstein[2, 3], Robert W. Taylor[4], Richard J. Maraia[1]

[1]National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA, [2]Raphael Recanati Genetics Institute, [3]Sackler Faculty of Medicine, Tel Aviv University, Israel, [4]Wellcome Centre for Mitochondrial Research, Newcastle upon Tyne Hospitals
*Email: abdul.khalique@nih.gov, abdul.biotech07@gmail.com

Gene mutations in tRNA modification enzymes (TME) cause several human diseases. Multiple cases of childhood mitochondrial (mt) -disease have been attributed to mutations in *TRIT1* (tRNA isopentenyltransferase-1), that puts isopentenyl-N6-adenine-37 (i^6A37) on both cytoplasmic (cy) -tRNAs and mt-tRNAs. Prior work from our lab identified the first *TRIT1-R323Q* mutation; It decreases tRNA-i^6A37 levels and leads to neurodevelopmental disease. Yet molecular mechanisms that relate tRNA-i^6A37 hypomodification and disease severity are unexplored.
We report a new case with severe unexplained infant liver disease that has not yet been linked to *TRIT1* deficiency, due to a homozygous mutation *-R323W*. We found severe tRNA-i^6A37 hypomodification associated with *TRIT1-R323W* in patient fibroblasts. Several assays indicate that the *-R323W* reduced i^6A37 modifying activity much more than the other, the *-R323Q* variant. The sequential increasing over-expression of *TRIT1-R323Q* in yeast can overcome its tRNA mediated suppression deficiency phenotype, but *-R323W* could not. The mt-DNA sequencing of control and patient fibroblast revealed a 5773T (A>T) polymorphism in mt-$tRNA^{Cys}$ a *TRIT1* substrate, only in the *TRIT1-R323W*. We then found the ratios of *TRIT1* substrate mt-tRNAs accumulation were differentially altered in patient and control fibroblast, correlated with a mt-DNA 5773 (A>T) SNP in mt-$tRNA^{Cys}$ sequence. The retroviral transduction of *TRIT1* and catalytic mutant *TRIT1-T32A* fully and partially rescue the mt-Tyr/Cys accumulation ratio respectively. These data suggest compound effects of *TRIT1* and associated mutations on mt-tRNA modification, accumulation and disease severity including liver failure not previously reported, and lead to a new dimension of future analyses.

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Poster 12:

A unique domain with immune stimulation activity embedded in the catalytic domain of human cysteinyl-tRNA synthetase 1 (CARS1): Its potential implications for immunotherapy.

Hyeong Yun Kim[a,b,c,*], Seongmin Cho[b,c], Sunghoon Kim[a,b,c,d,**]

[a] College of Pharmacy, Yonsei University, Incheon 21983, Korea. [b] Medicinal Bioconvergence Research Center, Incheon 21983, Korea. [c] Institute for Artificial Intelligence and Biomedical Research, Incheon 21983, Korea. [d] College of Pharmacy & College of Medicine, Interdisciplinary Biomedical Center, Gangnam Severance Hospital, Yonsei University, Incheon 21983, Korea. *rona1228@yonsei.ac.kr **Correspondence

Human cysteinyl-tRNA synthetase 1 (CARS1) was previously shown to be secreted to activate immune system via its specific interaction with TLR2/6. CARS1 contains two unique domains (UNE-C1 and UNE-C2) that are not present in other tRNA synthetases, implying their distinct roles. UNE-C1 is located within the catalytic domain while UNE-C2 is attached to the C-terminal end of CARS1. Interestingly, UNE-C1 is indispensable for the catalytic activity of CARS1 and also responsible for the TLR2/6-mediated immune stimulating activity. The isolated UNE-C1 is expected to form a stable four helix bundle structure and shows extremely high thermostability and solubility. With its unique immune-stimulating activity and physicochemical properties, we anticipated its potential as an immune booster for cancer vaccines and immune checkpoint inhibitors. Here we tested and validated its potential as a novel immune booster using HPV-induced cervical cancer models. These findings suggest aminoacyl-tRNA synthetases not only as essential enzymes for protein synthesis but also as regulators for immune-homeostasis.

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Poster 13:
Usage of a unique domain UNE-C1 in cysteinyl-tRNA synthetase 1 as an immune stimulator of mRNA vaccine

Uijoo Kim[1,2,3], Seongmin Cho[2,3], Hyeong Yun Kim[1,2,3], Ha Min Ban[1,2,3], Sunkyo Hwang[1, 2, 3], Sunghoon Kim[1,2,3]

[1] College of Pharmacy, Yonsei University, Incheon, Korea.
[2] Medicinal Bioconvergence Research Center, Korea
[3] Institute for Artificial Intelligence and Biomedical Research, Korea
*Email: kujoo7060@gmail.com

mRNA-based cancer immunotherapy has emerged as a promising platform for treating cancer. However, the requirement for immunostimulation poses significant challenges to achieving therapeutic efficacy. In this study, we investigated the use of mRNA encoding a conjugate of antigen and UNE-C1, a TLR2/6 agonist derived from cysteinyl-tRNA synthetase 1, which activates the innate immune system and induces anti-tumor cytotoxic T lymphocyte responses. To evaluate the clinical potential of this approach, we assessed the efficacy of an mRNA vaccine encoding a conjugate of four KRAS mutations (G12D, G12C, G12V, G13D) and UNE-C1. Our results demonstrate that the mRNA encoding the conjugate enhanced the activation of dendritic cells and CD8+ T cell response specific to G12D peptide, compared to mRNA encoding only the antigens. Furthermore, the mRNA encoding the conjugate showed preventative effects in a CT26 tumor model by significantly improving infiltration of CD8+ T cells into the tumor. These findings suggest that mRNA encoding UNE-C1 fused to antigens is a rational design strategy for increasing the effectiveness of mRNA vaccines for cancer immunotherapy.

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Poster 14:
Glutamyl-prolyl-tRNA synthetase 1 coordinates early endosomal anti-inflammatory AKT signaling

Eun-Young Lee*, Shinhye Park, & Myung Hee Kim

[1]Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Korea

*krupi00@kribb.re.kr

AKT signaling pathway plays critical roles in the resolution of inflammation. However, the underlying mechanisms of anti-inflammatory regulation and signal coordination remain unclear. Here, we report that anti-inflammatory AKT signaling is coordinated by glutamyl-prolyl-tRNA synthetase 1 (EPRS1). Upon inflammatory activation, AKT specifically phosphorylated Ser999 of EPRS1 in the cytoplasmic multi-tRNA synthetase complex, inducing release of EPRS1. The EPRS1 compartmentalized AKT to early endosomes via selective binding to the endosomal membrane lipid phosphatidylinositol 3-phosphate and assembled an AKT signaling complex specific for anti-inflammatory activity. These events promoted AKT activation-mediated GSK3β phosphorylation, which increased anti-inflammatory cytokine production. EPRS1-deficient macrophages could not assemble the early endosomal complex and consequently exacerbated inflammation, decreasing the survival of EPRS1-deficient mice undergoing septic shock and ulcerative colitis. Collectively, our findings show that the housekeeping protein EPRS1 acts as a mediator of inflammatory homeostasis by coordinating compartment-specific AKT signaling.

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Poster 15:
Disease modelling for mitochondrial aminoacyl-tRNA synthetases using iPSC derived motor neurons and transcriptomic approaches

Pooja Manjunath[1], **Tiina Rasila**[1] Nelli Jalkanen[1], Jouni Kvist[1], Sami Jalil[1], Henna Tyynismaa[1,3]

[1]Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Finland

pooja.manjunath@helsinki.fi

As nuclear encoding and ubiquitously expressed essential enzymes, mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) bind to mitochondrial t-RNAs and their cognate amino acids. Even so, each of the 19 mitochondrial aaRSs are linked to a pathogenic variant that causes a disorder that only affects a specific tissue or a specific syndrome. Unknown molecular processes underlie tissue-specific or tissue-dominant phenotypes. We aim to elucidate cell type specific disease mechanisms for infantile-onset cardiomyopathy caused by an alanyl-tRNA synthetase (AARS2) mutation and for a splicing mutation in seryl-tRNA synthetase (SARS2) underlying early-onset spastic paraplegia. We produced disease-specific induced pluripotent stem cells (iPSCs) using CRISPR-Cas9 editing. To elucidate cell type specific disease mechanism, we aim to compare the gene expression patterns. AARS2 and SARS2 mutant iPSCs differentiate into mature motor neurons and RNA sequencing show nearly identical pathway enrichment of these mutant cells. Excitatory synapse pathways are enriched in the mutant motor neurons, showing probability of increased neuronal activity. Although, in live cell image analysis we see no change in the neurite length and neurite branch points. In future we aim to decipher the mechanism of the neuronal function and activity.

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Poster 16:
Uncovering the Role of RARS1 in Hypomyelinating Leukodystrophies

Samuel Nyandwi*& Haissi Cui

Department of Chemistry, University of Toronto, Toronto, Ontario M5S 3H6, Canada

*Samuel.nyandwi@mail.utoronto.ca

Neurological diseases make up almost half of the known rare diseases, yet many remain undiagnosed or receive symptomatic treatment without addressing the underlying cause. White matter disorders known as hypomyelinating leukodystrophies (HLDs) fall within this category and their clinical manifestations include delayed motor development and seizures. During the last decade, evidence has emerged that variants in arginyl tRNA synthetase (RARS1) which mediates aminoacylation of arginyl-tRNA are causative of HLDs. Interestingly, a recurring N-terminally truncated mutant retains its catalytic activity which suggests a disruption in noncanonical function of the enzyme. The disease-causing mechanism of remains elusive and no model exists that recapitulates the symptoms observed in patients. Here, we propose a three-pronged approach to characterizing the effects of an N-terminal truncated RARS1 variant: N-terminally truncated RARS1 will be tested for its tRNA-charging activity using enzymatic assays and northern blotting. Current RNA-seq data points to neural development pathways being affected by RARS1 truncation. Using a CRISPR vector designed to truncate the RARS1 N-terminal, neural stem cells will be cultured in the presence of basic fibroblast growth factor to recapitulate neural development *in vitro* and assess differences in development. RARS1 truncation will also be characterized at the tissue-level by conducting immunohistochemistry experiments on mouse brains. Altogether, this study provides a comprehensive molecular and cellular portrait of RARS1-related HLDs, a first step towards the exploration of treatment options in a pre-clinical model.

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Poster 17:
GST-like domains within the parasite multi-tRNA synthetase complex

Do Won Oh[1,2] & **Myung Hee Kim**[1*]

[1] Microbiome Convergence Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 34141, Korea
[2] Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Korea

* mhk8n@kribb.re.kr

Aminoacyl-tRNA synthetases (ARSs) are essential enzymes generating active aminoacyl-tRNAs for protein synthesis. There has been a clear, strong tendency towards the addition of new sequences and domains to ARSs during evolution. The emergence of such new domains is consistent with the involvement of ARSs in a broad range of biological functions beyond translation. Intriguingly, higher eukaryotic systems form a macromolecular complex called multi-tRNA synthetase complex (MSC). *Toxoplasma gondii* is a unicellular protozoan parasite. In *T. gondii* (Tg), the MSC is composed of four ARSs ((methionyl-tRNA synthetase (MARS1), glutamyl-tRNA synthetase (EARS1), glutaminyl-tRNA synthetase (QARS1), and tyrosyl-tRNA synthetase (YARS1)) and a scaffold protein (Tg-p43), where YARS1 is the only component without a GST-like domain. In this study, we analyzed a complex structure of GST-like domains within the MSC by single particle analysis using cryo-electron microscopy. Based on the electron density map, we determined an arrangement of the domains within the MSC using structural models produced by AlphaFold. The structure reveals that the Tg GST-like domains interact with each other via the canonical binding interfaces shown in the structure of GST-like domains within the human MSC.

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Poster 18:
Comparative metabolite profiling of stem cell derived motor neurons in different mitochondrial aaRS diseases

Tiina Rasila[1*], Pooja Manjunath[1], Nelli Jalkanen[1], Jouni Kvist[1], Sami Jalil[1], Anni Nieminen[2], Christina L Nemeth[3], Henna Tyynismaa[1]

[1]Stem Cells and Metabolism Research Program, Faculty of Medicine, University of Helsinki, Finland
[2]Metabolomics Unit, Institute for Molecular Medicine Finland (FIMM), HiLife, University of Helsinki, Finland
[3]Moser Center for Leukodystrophies, Kennedy Krieger Institute, Baltimore, MD 21205, USA.

*tiina.rasila@helsinki.fi

Mitochondrial aminoacyl-tRNA synthetases (mt-aaRS) are essential enzymes for mitochondrial protein synthesis and cellular respiration. Pathogenic variants in the nuclear genes encoding for mt-aaRS result in a variety of human disease phenotypes, often affecting the nervous system. Mitochondrial metabolism is complex and cell type specific, which contribute to the differential outcomes of the mt-aaRS disease variants of different severity. To gain understanding of the shared and gene-specific molecular mechanisms of mt-aaRS diseases, we used CRISPR-Cas9 gene editing to generate isogenic pairs of induced pluripotent stem cells (iPSC) with and without pathogenic mt-aaRS mutations in genes AARS2, SARS2, and DARS2. The AARS2 variant is severe being lethal within the first year of life (1), whereas the SARS2 variant resulted in early-onset motor neuron disease (2). For DARS2, which is a disease gene for leukoencephalopathy with brainstem and spinal cord involvement and lactate elevation (LBSL) (3), three isogenic pairs with compound heterozygous mutations were studied. To compare the metabolic effects of each mt-aaRS defect, we performed targeted metabolite profiling with each iPSC pair, motor neuron progenitors and mature motor neurons differentiated from the iPSC. Our results suggest that although stem cell derived neurons are efficient in compensating for the mt-aaRS defects, the motor neurons rewire their metabolism in response to a partial bioenergetic failure. These cell models could hopefully be used in identification and testing of drugs that restore the metabolic imbalance.

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Poster 19:
Experimental evolution of cysteinyl-tRNA synthetase for resistance to selenite toxicity leads to *cysS* duplication

Noah M Reynolds* & Emma Garcia

School of Integrated Sciences, Sustainability, and Public Health
University of Illinois Springfield

*nmreynol@uis.edu

Selenocysteine, the 21st genetically encoded amino acid, is used across the three domains of life. Selenocysteine is inserted into proteins at UGA codons using a special mRNA recognition sequence, a dedicated $tRNA^{Sec}$, and a specific elongation factor. Selenocysteine is synthesized in a multistep process that occurs on $tRNA^{Sec}$. Initially, $tRNA^{Sec}$ is aminoacylated with serine, producing seryl-$tRNA^{Sec}$ as an intermediate product. In the final step, selenide and ATP are used to form selenophosphate, which then acts as a selenium donor to form selenocysteyl-$tRNA^{Sec}$. In cells, selenite is reduced to selenide, which can enter the tRNA-dependent selenocysteine biosynthetic pathway or infiltrate the cysteine biosynthetic pathway, producing free selenocysteine. Due to the inability of cysteinyl-tRNA synthetase (CysRS) to effectively discriminate between cysteine and selenocysteine, the accumulation of selenocysteine in cells can lead to the production of $tRNA^{Cys}$ charged with selenocysteine (Sec-$tRNA^{Cys}$) and result in the misincorporation of selenocysteine at cysteine codons, causing selenium toxicity. To increase resistance to selenite through CysRS and provide further insight into selenite resistance, we used *Escherichia coli* expressing wild-type CysRS from a plasmid in a serial transfer evolution experiment with increasing concentration of selenite. We observed an increase in resistance up to 12 mM of selenite. The lower sensitivity to selenite was accompanied by plasmid duplication and concatenation, leading to an increase copy number of the CysRS gene (*cysS*). Our findings suggest that multiple copies of *cysS* may be responsible for increased selenite resistance in our evolved line.

Poster 20:
Delivery of Protein Kinase B (AKT1) with programmed phosphorylation to mammalian cells

Tarana Siddika*, Richard N. Shao, Nileeka Balasuriya,
Ilka U. Heinemann, & Patrick O'Donoghue

Departments of [1]Biochemistry and [2]Chemistry, The University of Western Ontario, London, Ontario, Canada.

* tsiddika@uwo.ca

Protein kinase B (AKT1) is a serine/threonine kinase and central transducer of cell survival pathways. Typical approaches to study AKT1 biology in cells rely on growth factor or insulin stimulation that activates AKT1 via phosphorylation at two key regulatory sites (Thr308, Ser473), yet cell stimulation also activates many other kinases. To produce cells with specific AKT1 activity, we developed a novel system to deliver active AKT1 to human cells. We recently established genetic code expansion methods to produce AKT1 phospho-variants from *Escherichia coli* with phosphoseryl-tRNA synthetase and phosphoinositide-dependent kinase-1 (PDK1). We fused AKT1 with an N-terminal cell penetrating peptide tag derived from the human immunodeficiency virus trans-activator of transcription (TAT) protein. The TAT-tag did not alter AKT1 kinase activity and was necessary and sufficient to rapidly deliver AKT1 protein variants to mammalian cells. TAT-pAKT1^{T308} induced selective phosphorylation of the known AKT1 substrate GSK-3α, but not GSK-3β, while both TAT-pAKT1^{S473} and TAT-pAKT1^{T308S473} increased the phosphorylation of GSK-3α and -3β. Each TAT-AKT1 phospho-variant simulated downstream AKT signaling as shown by phosphorylation of ribosomal protein S6 at Ser240/244. The data demonstrate efficient delivery of AKT1 with programmed phosphorylation to human cells, thus establishing a cell-based model system to investigate cellular signaling that is dependent on AKT1 activity.

Poster 21:
Domain Acquisition by Class I Aminoacyl-tRNA Synthetase Urzymes Coordinated the Catalytic Functions of HVGH and KMSKS Motifs

Guo Qing Tang, Jessica J. Hobson, and Charles W. Carter, Jr

Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599-7260

National Institute of Environmental Health Sciences, Research Triangle, NC 27709; Department of Structural Biology National Institute of Environmental Health Sciences, F034 Bldg 101, Research Triangle Park, North Carolina

Class I aaRS signature sequences, HIGH and KMSKS, have been mutated numerous times in various Class I enzymes, in order to implicate them mechanistically. However, to our knowledge, no one has carried out the 2^2 factorial experiment in which catalytic side chains in both sequences have all been mutated to alanine separately, and together in order to assess the interaction free energy, $\Delta(\Delta G^{\ddagger})$, between the two signatures during catalysis. Combinatorial mutagenesis and thermodynamic cycle analyses of these catalytic signatures in full-length LeuRS and the 129-residue urzyme ancestral model generated from it (LeuAC) provide quantitative insight into the evolutionary gain of function induced by the anticodon-binding (ABD) and connecting peptide (CP) domains. The free energy coupling terms, $\Delta\Delta G^{\ddagger}_{HVGH*KMSKS}$, are small and unfavorable for LeuAC, but large and favorable for LeuRS. Thus, the ABD and CP domains induce strong cooperativity between the two signature sequences, which are uncoupled in the urzyme, much as we previously demonstrated for TrpRS and its urzyme. Quantitative consistency of the extended comparison of catalytic signature participation in transition state stabilization: (i) further substantiates the authenticity of LeuAC urzyme catalysis, (ii) implicates domain motion in catalysis by full-length LeuRS, and (iii) suggests that backbone elements of secondary structures are responsible for a major portion of the overall transition-state stabilization by the LeuAC urzyme. The implication that backbone elements of secondary structures achieve a major portion of the overall transition-state stabilization by LeuAC, rather than configurations of specific side chain residues in the active site, is also consistent with coevolution of the genetic code and metabolic pathways necessary to produce histidine and lysine sidechains, which are produced by complex multi-enzyme metabolic pathways and were likely unavailable when genetic coding first appeared.

Poster 22:
A Novel Approach (Reverse Molecular Evolution) toward Primordial Gene Discovery for Our Understanding Life Origin *in vivo*

Guo Qing Tiger Tang, and Charles W. Carter

Department of Biochemistry and Biophysics, School of Medicine, University of North Carolina at 120 Mason Farmer Rd, Chapel Hill, Chapel Hill, North Carolina, NC27599

Forces driving the diversification of primordial genes are of central interest in evolutionary biology. We previously identified a nested hierarchy of highly active excerpts from full-length aaRS of both classes, with diverse biochemical and bioinformatic validation[1-6]. Combinatorial mutagenesis of Class I HIGH and KMSKS signatures in *Pyrococcus horikoshii* leucyl-tRNA synthetase using dual antisense mutagenic primers for the AVGA and AMSAS double mutant recently revealed, among the expected full-length double mutants, three other subsets of recombinant plasmids with long deletions. Surprisingly, the first group of deletions correspond to the anticodon-binding domain and Connecting Peptides, CP, resulting in striking similarity to the LeuRS urzyme, LeuAC, derived by deconstructing the same gene[1]. A second subset of sequenced plasmids deleted, in addition, most of the second β-α-β crossover connection of the Rossmann fold. This novel deletion suggests a tidy solution to the nagging problem of designing a bidirectional gene encoding a Class I urzyme from one strand and a Class II urzyme from the opposite strand comparable to the bidirectional protozyme gene[2, 7]. We suggest that at the high PCR temperatures, the template DNA forms transient tertiary structures promoting excision of the deletions we observe. If correct, the consistent location of these deletions suggest something akin to reversal of the processes by which insertion elements resulted in acquisition of novel domains by ancestral enzymes. Further, it furnishes unexpected validation that our published deconstruction of Class I aaRS enzymes to produce protozymes and urzymes did produce legitimate "molecular fossils". Finally, we suggest that such deletions likely represent a widespread phenomenon and may provide experimental access to processes by which other enzymes grew from rudimentary catalytic peptides into their sophisticated contemporary forms.

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Poster 23:
Aminoacyl-tRNA synthetases in human health and disease

Alexandra K. Turvey[1]*, Gabriella A. Horvath[2,3], & André R.O. Cavalcanti[1]

[1]Department of Biology, Pomona College, Claremont, CA, United States
[2]Division of Biochemical Genetics, Department of Pediatrics, University of British Columbia, BC Children's Hospital, Vancouver, BC, Canada
[3]Adult Metabolic Diseases Clinic, Vancouver General Hospital, Vancouver, BC, Canada
*atab2020@mymail.pomona.edu

The Aminoacyl-tRNA Synthetases (aaRSs) are an evolutionarily ancient family of enzymes that catalyze the esterification reaction linking a transfer RNA (tRNA) with its cognate amino acid matching the anticodon triplet of the tRNA. Proper functioning of the aaRSs to create aminoacylated (or "charged") tRNAs is required for efficient and accurate protein synthesis. Beyond their basic canonical function in protein biosynthesis, aaRSs have a surprisingly diverse array of non-canonical functions that are actively being defined. The human genome contains 37 genes that encode unique aaRS proteins. To date, 56 human genetic diseases caused by damaging variants in aaRS genes have been described: 46 are autosomal recessive biallelic disorders and 10 are autosomal dominant monoallelic disorders. Our appreciation of human diseases caused by damaging genetic variants in the aaRSs has been greatly accelerated by the advent of next-generation sequencing, with 89% of these gene discoveries made since 2010. In addition to these genetic disorders of the aaRSs, anti-synthetase syndrome (ASSD) is a rare autoimmune inflammatory myopathy that involves the production of autoantibodies that disrupt aaRS proteins. Our poster provides an overview of the basic biology of aaRS proteins and describes the rapidly growing list of human diseases known to be caused by genetic variants or autoimmune targeting that affect both the canonical and non-canonical functions of these essential proteins.

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Poster 24:
Mutation in AsnRS causes neurodevelopmental delays in a novel disease mechanism

Ingrid Vallee* & Xiang-Lei Yang

Scripps Research

*ivallee@scripps.edu

Aminoacyl-tRNA synthetases (aaRS) play a central role in protein biosynthesis by catalyzing the aminoacylation of tRNA for subsequent use by the ribosome. A *de novo* monoallelic deletion-causing nonsense mutation (R534*) and a biallelic missense mutation (R545C) in asparaginyl-tRNA synthetase (AsnRS) were recently linked to severe neurodevelopmental delays with onset at birth and comparable disease phenotypes[1]. In contrast to R534*, which exhibits dominant toxicity, the R545C mutant is not toxic when heterozygous and mutation in both alleles are required for disease phenotype. As both R534*and R545C mutations are located within the catalytic domain of AsnRS, we speculate that the disease mechanism involves a loss of enzymatic function, at least in part. We hypothesized that, compared with the R545C mutation, R534* deletion may damage the enzymatic activity of AsnRS more severely and/or the deletion mutation may have a stronger ability to poison the WT enzyme through heterodimer formation and allosteric regulation. Interestingly, our preliminary data provide support to at least the first possibilities - the active site of AsnRS is more severely damaged by R534* than the R545C mutation. Importantly, overexpression of the R534* mutant, but not the R545C mutant, in HEK293T cells confers cellular toxicity, suggesting a unique dominant-negative mechanism for the deletion mutation. Apart from the loss of enzymatic function mechanism, we are also investigating potential toxic gain of functions associated with the AsnRS mutations, such as tRNA sequestration and abnormal protein interactions. Together, our study will provide comprehensive analyses of AsnRS mutations that cause severe disease to shed light on their pathological mechanisms.

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Poster 25:
Discriminator Base is a Critical Recognition Element for *Trypanosoma brucei* Ala-tRNA^{Pro} Editing Domains

Anna Vradi, Rylan Watkins, Irina Shulgina, Karin Musier-Forsyth

Center for RNA Biology, Department of Chemistry and Biochemistry,
The Ohio State University, Columbus, Ohio 43210

Email: Vradi.1@osu.edu

Prolyl-tRNA synthetases (ProRSs) mischarge Ala onto cognate tRNA^{Pro}. In bacteria, this error can be corrected by a *cis*-editing domain appended to the synthetase (INS) or by a homologous freestanding *trans*-editing domain, ProXp-ala. *Trypanosoma brucei (Tb)* ProRS encodes an appended ProXp-ala domain and a unique free-standing INS homolog—MCP3 (multi-tRNA synthetase complex 3 protein). We hypothesize that *Tb* may have two Ala-tRNA^{Pro} editing domains due to its unusual metabolism. In the procylic form, the main carbon source is proline, with alanine produced as a metabolic by-product. With high intracellular concentrations of alanine, robust editing mechanisms may be needed to avoid misincorporation of Ala at Pro codons. Free-standing *homo Sapiens (Hs)* ProXp-ala recognizes nucleotides in the tRNA acceptor stem for optimal function. In contrast, the ProRS-appended *E. coli* INS domain relies exclusively on ProRS anticodon recognition. Here, we probed the acceptor stem specificity of *Tb* Ala-tRNA^{Pro} editing domains. Ala-tRNA^{Pro} deacylation assays revealed that the activities of ProRS-appended *Tb* ProXp-ala, as well as free-standing ProXp-ala and MCP3, are significantly decreased upon mutation of the discriminator base (C73A), with more relaxed recognition of the first base pair (G1:C72). Surprisingly, MCP3 deacylated the triple mutant (G1C:C72G, C73A) Ala-tRNA^{Pro} better than the C73A single mutant, and displayed robust deacylation of G1:C72, A73-containing Ala-tRNA^{Ala} *in vitro*. Thus, the context of the discriminator base and the Ala moiety appear to be important determinants for this editing domain. As MCP3 is not encoded in the human genome, results of these studies may have implications for new therapeutic strategies.

Poster 26:
Evolving tRNA variants to read-through premature termination codons that cause ALS

Cian Ward[1]* & Patrick O'Donoghue[1,2]

Department of [1]Biochemistry and [2]Chemistry, The University of Western Ontario, London, Ontario, Canada.

*cward72@uwo.ca

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder with no known cures or treatments. One ALS phenotype is caused by the introduction of a UGA premature termination codon (PTC) at Arg495 of the fused in sarcoma (FUS) protein (1). Efforts to read-through disease-causative PTCs have had mixed results, as small molecule treatments are not codon specific and do not reliably deliver the wild-type amino acid to the PTC (2, 3). Recent success with using engineered transfer RNAs (tRNAs) to treat cystic fibrosis in mice has engendered further exploration into tRNA-related medicine (4, 5). We aim to use arginine tRNA variants that decode UGA stop codons and hypothesize that suppressor $tRNA^{Arg}$ will read-through the FUS R495X PTC with arginine, reducing its degenerative effects. 5'-UCA-3' anticodons were introduced in three different natural $tRNA^{Arg}$ alleles expressed in the human genome. Each variant was co-transfected into HEK293T cells with a fluorescent reporter to evaluate read-through efficiency through live-cell fluorescence microscopy. The introduction of a G36A mutation in each $tRNA^{Arg}$ variant does not adversely affect aminoacylation by its cognate synthetase as identity elements A20 and G35 are unchanged (6). $tRNA^{Arg}$ toxicity was measured using a commercial cytotoxicity kit. The highest-performing suppressor $tRNA^{Arg}$ achieved 15% rescue of wild-type fluorescence and had no effect on cell viability. The efficacy of tRNA treatment on FUS-R495X will be evaluated by fluorescence microscopy and semi-denaturing agarose gel electrophoresis as previously described in a neuroblastoma model of ALS (7).

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Poster 27:
MARS2 Deficiency: Where are the missing patients?

Bryn D. Webb*[,1], Patricia G. Wheeler[2], & Sander M. Houten[3]

[1]Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA
[2]Division of Genetics, Arnold Palmer Hospital, Orlando, FL, USA
[3]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA

*bdwebb@wisc.edu

Previously we identified by whole exome sequencing that recessive single nucleotide variants in mitochondrial methionyl aminoacyl-tRNA synthetase (*MARS2*) cause a mitochondrial disease characterized by developmental delay, poor growth, and sensorineural hearing loss (MIM #616430; Combined oxidative phosphorylation deficiency 25). The compound heterozygous pathogenic variants c.550C>T;p.Gln184* and c.424C>T;p.Arg142Trp [NM_138395.3] were identified in two siblings and led to: decreased MARS2 protein levels in patient lymphoblasts; decreased Complex I and IV enzyme activities in patient fibroblasts; and reduced protein levels of NDUFB8 and COXII, representing Complex I and IV respectively, in patient fibroblasts and lymphoblasts. Overexpression of wild-type MARS2 in patient fibroblasts increased NDUFB8 and COXII protein levels (1). At the 2017 AARS meeting we reported the establishment of our *Mars2* mouse model of disease and reported differentially expressed gene signatures for homozygous mutant mice in liver and gastrocnemius (2). Now we present clinical follow-up on the original two siblings, the only family identified to date in the literature, and query the conference attendees whether others have identified patients with *MARS2* mutations. We have also completed RNA-seq in patient lymphoblasts and have identified disease signatures.

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Poster 28:
Asymmetric inhibition of prolyl-tRNA synthetase 1 controls fibrosis with enhanced safety

Ina Yoon[1,2]*†, Sulhee Kim[3]†, Minjae Cho[4], Kyung Ah You[1], Jonghyeon Son[3], Caroline Lee[4], Ji Hun Suh[1,2], Da-Jeong Bae[4], Jong Min Kim[4], Sinae Oh[1], Songhwa Park[1], Seonha Park[3], Kyuhyeon Bang[3], Minjeong Seo[3], Joon Seok Park[4], Kwang Yeon Hwang[3]‡, Sunghoon Kim[1,2,5]‡

[1]Institute for Artificial Intelligence and Biomedical Research, Medicinal Bioconvergence Research Center, Yonsei University, Incheon 21983, Korea.[2]Yonsei Institute of Pharmaceutical Sciences, College of Pharmacy, Yonsei University, Incheon 21983, Korea.[3]Department of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 02841, Korea. [4]Drug Discovery Center, Daewoong Pharmaceutical Co., Ltd., Yongin 17028, Korea. [5]College of Medicine, Gangnam Severance Hospital, Yonsei University, Seoul 06273, Korea.
*Presenting author email address: yin1988@target.re.kr
†These authors contributed equally to this work.
‡Corresponding authors.

Idiopathic pulmonary fibrosis is a disease with high unmet treatment needs due to the lack of drugs with desirable efficacy and safety margin. Since proline is highly present in collagen, the active site of prolyl-tRNA synthetase 1 (PARS1) has been identified as a potential drug target for fibrotic diseases. However, inhibiting PARS1 catalysis raises concerns about adverse effects due to its potential effect on global translation. To address this, we developed a novel PARS1 catalytic inhibitor, DWN12088, which passed human clinical phase 1 studies and exhibits enhanced safety compared to the well-known PARS1 inhibitor, halofuginone. Structural and kinetic analyses revealed that DWN12088 binds to the catalytic site of each protomer of the PARS1 dimer in an asymmetric mode with different affinity. This reduces responsiveness at higher doses and expands the safety window. Moreover, cells expressing PARS1 mutants disrupting PARS1 homodimerization showed restored sensitivity to DWN12088, confirming the negative allosteric communication between PARS1 promoters for DWN12088 binding. Our work suggests that DWN12088, as an asymmetric catalytic inhibitor of PARS1, is a novel therapeutic agent against fibrosis with enhanced safety. In addition, our data demonstrate that the catalytic sites of PARS1 (and potentially those of other human aminoacyl-tRNA synthetases) can be explored as effective pharmacological targets.

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Poster 29:
The role of nuclear EPRS1 in Akt-mediated PARP1 activi

Isaac Zin[a,b], Arnab China[a], Shinjini Ganguly[c], Valentin Gogonea[a,b], & Paul L. Fox[a,b]

[a] Department of Cardiovascular and Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA
[b] Department of Chemistry, Cleveland State University, Cleveland, OH 44115, USA
[c] Translational Hematology and Oncology Research, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195

ZINI2@ccf.org

Glutamyl-prolyl-tRNA synthetase (EPRS1), the only bifunctional aminoacyl-tRNA-synthetase (aaRS), is a constituent of the multi-tRNA synthetase complex (MSC). The two catalytic domains (GluRS and ProRS) of EPRS1 are linked by a peptide containing three WHEP domains. We show that a nuclear localization sequence (NLS) within the linker region of EPRS1 is responsible for nuclear translocation following multiple stimuli in breast cancer cells. In cells with low levels of phosphatase and tensin homolog (PTEN), EPRS1 has higher levels in the nucleus than in PTEN-positive cells. EPRS1 localization in the nucleus requires activation of Akt, which PTEN inhibits. In addition, we show EPRS1 nuclear localization following heat shock or hydrogen peroxide treatment. Nuclear EPRS1 binds and enhances the activity of poly-(ADP)-ribose polymerase 1 (PARP1), which induces DNA damage repair by depositing one or more ADP-ribose moieties on repair proteins (i.e., by PARylation). We show here that nuclear localization of EPRS1 serves as a link between Akt and PARP1 activation to enhance DNA repair, and thus is a potential therapeutic target in breast cancer.

Poster 30:
Control of the Tor pathway by vacuolar echoforms of yeast cytosolic aminoacyl-tRNA synthetases

Solène Zuttion[1], Marine Hemmerle[1], Nicolas Fournier[1], Ludovic Enkler[1,2], Johan-Owen De Craene[1,3], Bruno Senger[1], Claudio De Virgilio[4], Sylvie Friant[1] and Hubert Dominique Becker[1]

(1) Génétique Moléculaire, Génomique, Microbiologie, UMR 7156, CNRS, Université de Strasbourg, 4 Allée Konrad Röntgen, 67084 Strasbourg Cedex, France. (2) Present address: Biozentrum, University of Basel, 4056 Basel, Switzerland. (3) Present address : EA 2106 Biomolécules et Biotechnologies Végétales, Université de Tours, 31, Avenue Monge, 37200 Tours, France. (4) Université de Fribourg, Fribourg, Switzerland.

Mainly known for their essential cytosolic role in translation, aminoacyl-tRNA synthetases have the capacity to relocate from the cytosol to various organelles to exert a wide range of non-canonical functions[1].

Subcellular fractionation experiments revealed that each component of *Saccharomyces cerevisiae* AME complex, composed of the assembly factor ARC1 binding to methionyl (MRS)- and glutamyl (ERS)-tRNA synthetases, could possibly relocate to the vacuole (yeast lysosome).

Storage compartment for amino acids (aa), yeast vacuoles express on their surface a highly conserved aa-sensing machinery: the target of rapamycin complex 1 (TORC1), that has been shown to be controlled by the vacuolar echoform of the leucyl-tRNA synthetase, depending on aa availability in the cell[2].

Considering that the AME complex could also regulate TORC1 activity, we sought to confirm the vacuolar localization for each component by engineering a dedicated yeast strain expressing a vacuolar Split-CFP. We fused the first ten beta strands also bearing an interactomic tag, to the vacuolar membrane protein VPH1. Co-expression of any protein fused to the last beta strand of Split-CFP, allows specific visualization of the vacuolar echoform of any dual-localized protein.

Using this vacuolar Split-CFP strain, we *i)* visualized vacuolar echoforms of the AME components, *ii)* established their interactomes in differential nutritional conditions and *iii)* analyzed whether these echoforms modulate TORC1 activity through monitoring of the phosphorylation state of downstream targets of TORC1.

Our overall purpose is to understand how proteins relocate to the vacuolar membrane and by which mechanism they relay aa sensing and regulate TORC1.

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List of Participants

IUBMB Focused Meeting on Aminoacyl-tRNA Synthetases 2023

Last Name	First Name	Organization/Affiliation	Email
Ackerman	Susan	University of California, San Diego	sackerman@ucsd.edu
Adams	Ryan	aTyr Pharma	radams@atyrpharma.com
Alexander	Rebecca	Wake Forest University	alexanr@wfu.edu
Arava	Yoav	Technion - Israel Institute of Technology	arava@technion.ac.il
Ban	Hamin	Yonsei University	hmban@target.re.kr
Barrientos	Maximiliano	Board member CMT Research Foundation & CMT patient	Barrientosmaximiliano@gmail.com
Bauer	Morgan	The Ohio State University	bauer.715@buckeyemail.osu.edu
Becker	Hubert	University of Strasbourg	h.becker@unistra.fr
Beharry	Aruun	University of Western Ontario	abeharr3@uwo.ca
Burgess	Robert	The Jackson Laboratory	robert.burgess@jax.org
Carell	Thomas	LMU Munich	thomas.carell@lmu.de
Chakraborty	Shruti	Cleveland Clinic Lerner Research Institute	chakras6@ccf.org
Chapleau	Alexandra	McGill University	alexandra.chapleau@mail.mcgill.ca
Chen	Jie	University of Illinois at Urbana-Champaign	jiechen@illinois.edu
Chen	Qi	University of Utah School of Medicine	qi.chen@hsc.utah.edu
Chen	Siyu	University of California, San Francisco	siyu.chen2@ucsf.edu
Chihade	Joe	Carleton College	jchihade@carleton.edu
Chung	Christina	Alltrna	cchung@alltrna.com
Cui	Haissi	University of Toronto	haissi.cui@utoronto.ca
Douglas	Jordan	University of Auckland	jordan.douglas@auckland.ac.nz
Edmund	Grace	Oxford Drug Design	grace.edmund@oxforddrugdesign.com
Fakhfakh	Faiza	University of Sfax	faiza.fakhfakh02@gmail.com
Finn	Paul	Oxford Drug Design	paul.finn@oxforddrugdesign.com
Fisk	John	University of Colorado Denver	john.fisk@ucdenver.edu
Fox	Paul	Cleveland Clinic	foxp@ccf.org
Frederick	Mallory	University of Western Ontario	mfreder8@uwo.ca

Frugier	Magali	CNRS-ARN	m.frugier@ibmc-cnrs.unistra.fr
Gopalakrishnan	Rajaraman	Alltrna	rgopalakrishnan@alltrna.com
Gruic Sovulj	Ita	University of Zagreb Croatia	gruic@chem.pmf.hr
Guan	Min-Xin	Zhejiang University	gminxin88@zju.edu.cn
Guy	Lauren	aTyr Pharma	kbrown@atyrpharma.com
Hadchouel	Alice	Necker Enfants Malades Hospital	alice.hadchouel-duverge@aphp.fr
Han	Jung Min	Yonsei University	jhan74@yonsei.ac.kr
Heilmann	Rachel	The Rory Belle Foundation	rachel@therorybellefoundation.org
Heinemann	Ilka	The University of Western Ontario	ilka.heinemann@uwo.ca
Hendrickson	Tamara	Wayne State University	Tamara.Hendrickson@wayne.edu
Hoffmann	Guillaume	IAB, Inserm	guillaume.hoffmann@inserm.fr
Ibba	Michael	Chapman University	ibba@chapman.edu
Ignatova	Zoya	University of Hamburg	zoya.ignatova@uni-hamburg.de
Jin	Mirim	Gachon University	mirimj@gachon.ac.kr
Jordanova	Albena	VIB, University of Antwerpen, Medical University-Sofia	albena.jordanova@uantwerpen.vib.be
Kakadia	Jenica	The University of Western Ontario	jkakadia@uwo.ca
Kalotay	Elizabeth	University of New South Wales, Sydney	e.kalotay@unsw.edu.au
Kanaji	Sachiko	The Scripps Research Institute	skana@scripps.edu
Kanaji	Taisuke	The Scripps Research Institute	tkana@scripps.edu
Katanski	Chris	hC Bioscience, Inc.	chris.katanski@hcbioscience.com
Khalique	Abdul	National Institute of Health (NIH)	abdul.khalique@nih.gov
Khan	Debjit	Cleveland Clinic Lerner Research Institute	khand@ccf.org
Kim	Uijoo	Yonsei University	kujoo7060@gmail.com
Kim	Myung Hee	Korea Research Institute of Bioscience and Biotechnology (KRIBB)	mhk8n@kribb.re.kr
Kim	Hyeongyun	Yonsei University	rona1228@yonsei.ac.kr
Kim	Sunghoon	Yonsei University	sunghoonkim@yonsei.ac.kr
Krahn	Natalie	Yale University	natalie.krahn@yale.edu

Lee	Eun-Young	Korea Institute of Bioscience and Biotechnology	krupi00@kribb.re.kr
Lefel	Romi	The University of Western Ontario	romilefel@icloud.com
Ling	Jiqiang	University of Maryland, College Park	jling12@umd.edu
Liu	Ru-Juan	ShanghaiTech University	liurj@shanghaitech.edu.cn
Lowe	Todd	University of California, Santa Cruz	tmjlowe@ucsc.edu
Lu	Wei	The University of Tokyo	wei_lu@chem.s.u-tokyo.ac.jp
Manjunath	Pooja	University of Helsinki	pooja.manjunath@helsinki.fi
Martinis	Susan	University of Illinois Urbana Champaign	martinis@illinois.edu
Mathew	Sajish	University of South Carolina	mathew2@cop.sc.edu
Mazitschek	Ralph	Harvard Medical School/Mass. General Hospital	ralph@broad.harvard.edu
McGilvray	Phil	hC Bioscience, Inc.	phil.mcgilvray@hcbioscience.com
Mendes	Marisa	Amsterdam UMC	m.mendes@amsterdamumc.nl
Mora Garcia	Natalia	Radboud University	Natalia.moragarcia@donders.ru.nl
Muller	Bastien	IAB, Inserm	bastien.muller@univ-grenoble-alpes.fr
Murphy	Parker	University of Maryland College Park	pmurphy1@umd.edu
Musier-Forsyth	Karin	The Ohio State University	musier-forsyth.1@osu.edu
Nakazaki	Ren	The University of Tokyo	nakazaki-ren919@g.ecc.u-tokyo.ac.jp
Nangle	Leslie	aTyr Pharma	kbrown@atyrpharma.com
Nedialkova	Danny	MPI of Biochemistry	nedialkova@biochem.mpg.de
Nemeth Mertz	Christina	Kennedy Krieger Institute	mertz@kennedykrieger.org
Nyandwi	Samuel	University of Toronto	samuel.nyandwi@mail.utoronto.ca
O'Donoghue	Patrick	The University of Western Ontario	podonog@uwo.ca
Oh	Do Won	Korea Research Institute of Bioscience & Biotechnology(KRIBB)	5do1@kribb.re.kr
Palencia	Andres	IAB, Inserm	andres.palencia@univ-grenoble-alpes.fr
Park	Shinhye	Korea Research Institute of Bioscience and Biotechnology	shinhye0@kribb.re.kr
Plater	Andrew	University of Dundee	aplater001@dundee.ac.uk
Povoas	Luis	IRB Barcelona	luisfilipe.povoas@irbbarcelona.org

Rasila	Tiina	University of Helsinki	tiina.rasila@helsinki.fi
Reynolds	Noah	University of Illinois Springfield	nmreynol@uis.edu
Ribas de Pouplana	Lluís	IRB Barcelona	lluis.ribas@irbbarcelona.org
Ross	Julian	Institute of Molecular Biotechnology (IMBA)	julian.ross@imba.oeaw.ac.at
Rozik	Peter	The University of Western Ontario	prozik@uwo.ca
Schimmel	Paul	The Scripps Research Institute	schimmel@scripps.edu
Schmitt	Margaret	University of Colorado Denver	margaret.schmitt@ucdenver.edu
Siddika	Tarana	The University of Western Ontario	tsiddika@uwo.ca
Sissler	Marie	Université de Strasbourg	m.sissler@unistra.fr
Siu	Victoria	The University of Western Ontario	vmsiu@uwo.ca
Söll	Dieter	Yale University	dieter.soll@yale.edu
Sonenberg	Nahum	McGill University	nahum.sonenberg@mcgill.ca
Tang	GuoQing	University of North Carolina	Guoqing_Tang@med.unc.edu
Tasak	Monika	hC Bioscience, Inc.	monika.tasak@hcbioscience.com
Tennakoon	Rasangi	The University of Western Ontario	rtennako@uwo.ca
Tharp	Jeffery	Indiana University School of Medicine	jemtharp@iu.edu
Turvey	Alexandra	Pomona College	atab2020@mymail.pomona.edu
Tyynismaa	Henna	University of Helsinki	henna.tyynismaa@helsinki.fi
Vallee	Ingrid	Scripps Research	ivallee@scripps.edu
Vargas-Rodriguez	Oscar	University of Connecticut Health Center	vargasrodriguez@uchc.edu
Varshney	Umesh	Indian Institute of Science, Bangalore	varshney@iisc.ac.in
Voigt	Aaron	University Clinic RWTH Aachen	avoigt@ukaachen.de
Vradi	Anna	The Ohio State University	vradi.1@buckeyemail.osu.edu
Wakasugi	Keisuke	The University of Tokyo	wakasugi@bio.c.u-tokyo.ac.jp
Wang	Dan	UMass Chan Medical School	dan.wang@umassmed.edu
Wang	Justin	The Scripps Research Institute	jjwang@scripps.edu
Wang	Yane-Shih	Academia Sinica	yaneshihwang@gate.sinica.edu.tw

Ward	Cian	The University of Western Ontario	cward72@uwo.ca
Watkins	Rylan	The Ohio State University	watkins.819@osu.edu
Webb	Bryn	University of Wisconsin, Madison	bdwebb@wisc.edu
Wilhelm	Sarah	The University of Western Ontario	swilhel2@uwo.ca
Yang	Xiang-Lei	The Scripps Research Institute	xlyang@scripps.edu
Yokoyama	Shigeyuki	RIKEN	yokoyama@riken.jp
Yoon	Ina	Medicinal Bioconvergence Research Center	yin1988@target.re.kr
Zhou	Tong	University of Nevada, Reno School of Medicine	tongz@med.unr.edu
Zin	Isaac	Cleveland Clinic Lerner Research Instiute	ZINI2@ccf.org
Zuttion	Solène	University of Strasbourg	solene.zuttion@etu.unistra.fr

Industry Panel Biosketches

Leslie Nangle
aTyr Pharma, Inc.
Vice President for Research lnangle@atyrpharma.com

Dr. Leslie Nangle has over 20 years of experience in research dedicated to extracellular tRNA synthetases. Throughout her tenure at aTyr, she has co-developed and implemented the company's discovery platform to generate an intellectual property estate of over 300 potential therapeutically relevant proteins and has overseen research leading to the discovery of a splice variant of histidyl-tRNA synthetase that has been shown to modulate the immune system, providing the basis of aTyr's lead program, ATYR1923. Prior to joining aTyr, Dr. Nangle received a Ph.D. in Macromolecular Cellular Structure and Chemistry from The Scripps Research Institute in La Jolla, CA, studying the emerging linkage between mutations in tRNA synthetases and inherited neuropathies under the mentorship of Dr. Paul Schimmel, who co- founded aTyr in 2005. Dr. Nangle received a bachelor of science in biology from the University of California at Santa Barbara. She is co-author or co-inventor of over 75 publications and issued or pending U.S. patents.

Paul Finn
Oxford Drug Design Limited
Chief Scientific Officer paul.finn@oxforddrugdesign.com

Paul completed his undergraduate biochemistry degree at St Peter's College, Oxford in 1981 and holds a PhD from Manchester University (1985) on the prediction of protein structure by theoretical methods. Moving to industry, Paul worked as a scientist in the computational chemistry group at SmithKline Beecham, and then Pfizer. In 1998 Paul switched to biotech, joining Prolifix, subsequently acquired by TopoTarget, which focused on oncology. As Director of R&D Paul was responsible for TopoTarget's extremely successful HDAC inhibitor programme which led to the discovery and development of belinostat, which was approved by the FDA for the treatment of Peripheral T-cell Lymphoma in 2014. In 2004 Paul moved to InhibOx, now Oxford Drug Design, a spin-out company from the Chemistry Department of the University of Oxford, where he is CSO. Oxford Drug Design is a biotechnology company advancing a pipeline of drug candidates for the treatment of drug-resistant bacterial infections and cancer.

Suchul Jang
hC Biosciences
Director, In Vivo Pharmacology suchul.jang@hcbioscience.com

Dr. Jang leads a team responsible for the in vivo translation of tRNA based therapeutics. Prior to joining hC Bioscience, Dr. Jang was Director of Preclinical & Biomarkers and head of In Vivo Pharmacology at Codiak BioSciences. He has organized and oversighted operations of the

preclinical group and collaborations within the company as well as external partners. In addition, he led and participated in multiple programs including exoSTING, exoIL-12, exoASO-STAT6, and exoVACC, testing efficacy, pharmacokinetic, pharmacodynamic properties as well as mechanism of actions of therapeutic candidates. His research career was focused on how to utilize exosomes for therapeutics and diagnostics. He first invented exosome-mimetic nanovesicles to deliver anti-cancer therapeutics during his Ph.D. and co-invented the first clinical candidate of Codiak Biosciences, exoSTING. During his research career, he has invented about 20 patents and published 36 peer-reviewed articles. Dr. Jang finished B.S. and Ph.D. degree at Pohang University of Science Technology in Republic of Korea and then did postdoctoral training at Dr. Jan Lötvall's group at University of Gothenburg, Sweden.

Sunghoon Kim
Underwood Distinguished Professor, Yonsei University, College of Pharmacy & Severance Hospital, Institute of Artificial Intelligence and Biomedical Research
sunghoonkim@yonsei.ac.kr

Director, Medicinal Bioconvergence Research Center (Biocon): a Target Factory
sungkim@target.re.kr

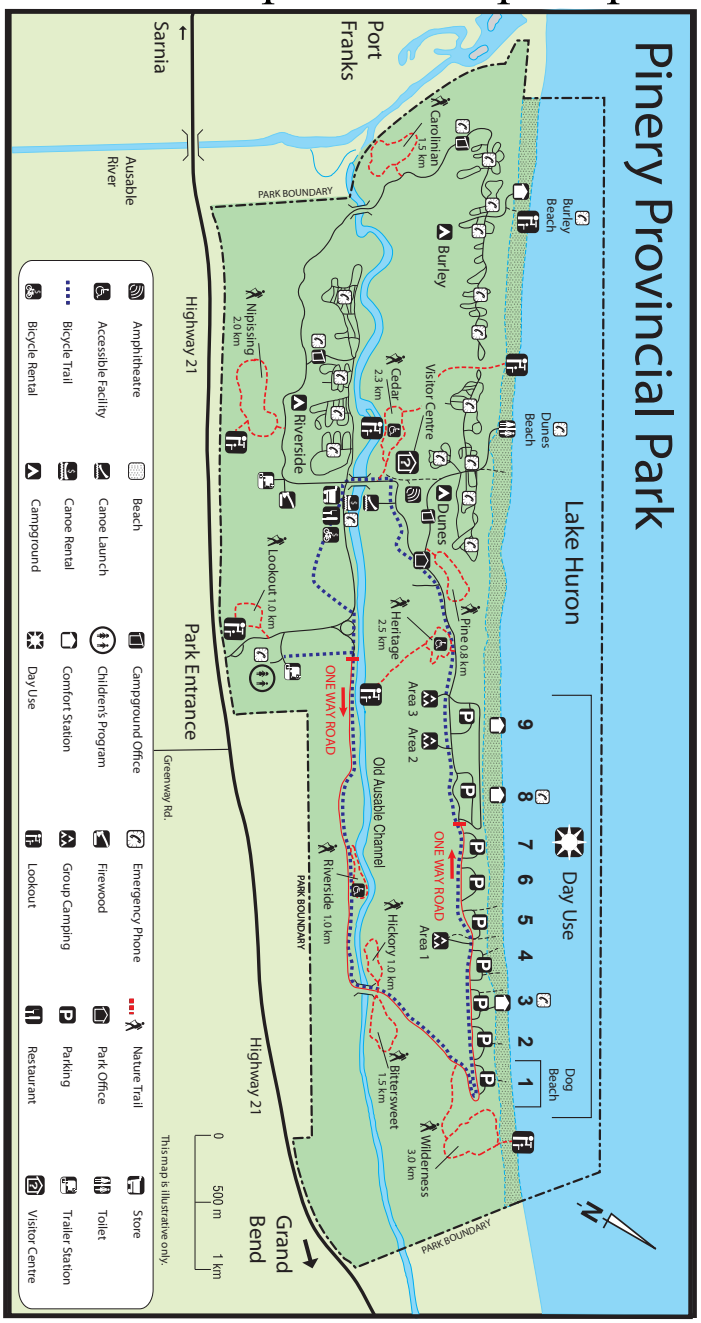
Sunghoon Kim received a PhD degree at Brown university and worked at MIT as a post-doc. Ever since he served as a professor at Seoul National University, Korea, he has unveiled novel biological activities of human aminoacyl-tRNA synthetases beyond their catalytic role in protein synthesis and published about 250 research articles with numerous patents. In 2010, he was assigned as a director of the global frontier project, Medicinal Bioconvergence Research Center (Biocon) that is supported by the Korea Ministry of Education, Science and Technology with a mission to establish an innovated target discovery platform for novel drug development (nicknamed by the Target Factory in Nat Biotech 2018). In 2021, he changed his academic affiliation to Yonsei University, the best private university in Korea, while continuing to lead the Biocon project. Through the unique target identification and validation platform of Biocon, Sunghoon has demonstrated unique druggability of human aminoacyl-tRNA synthetases that can be applied to various human refractory diseases (Nat Rev Drug Discov 2019) and generated numerous first-in-class drug candidates and leads that are under different developmental stages.

Resort Map

https://oakwoodresort.ca

Excursion

https://www.ontarioparks.com/park/pinery



Emergency Telephones: located at each comfort station in the campgrounds, Picnic Areas 3 & 8, Dunes and Burley Beach Access Areas, canoe rental dock and at the children's program chalet. **Public Telephones:** located at the park store and each campground office.

Visitor Centre: open daily, 10:00am - 5:00pm during the summer season. Open weekends year-round. **Park Store**: open daily, 8:00am - 10:00pm during July and August. **Dog Beach**: Picnic Area 1. **Dogs must be leashed at all times**, owners clean up after pets.

Firewood: available at the Main Gatehouse and the woodyard which is located between the park store and Riverside Campground Office. **Drinking Water**: treated water is available from all park taps. Visitors should not drink lake or river water. **Do not wash dishes at water taps.**

Trailer Sanitation: located adjacent to the Lookout Trail parking lot and the woodyard. **One Way Road**: Please note the day-use road between the traffic circle and Picnic Area 8 is a one way road; please obey signage.

IUBMB Information & Events

International Union of Biochemistry and Molecular Biology

The International Union of Biochemistry and Molecular Biology (IUBMB) – founded in 1955 – unites biochemists and molecular biologists in 75 countries and regions that belong to the Union as an Adhering Body or Associate Adhering Body represented by a biochemical society, a national research council or an academy of sciences. The IUBMB is devoted to promoting research and education in biochemistry and molecular biology throughout the world and gives particular attention to promoting opportunities for trainees and providing opportunities in areas where the biomolecular sciences are less well developed. The IUBMB sponsors a triennial International Congress of Biochemistry and Molecular Biology, co-sponsored by Regional Organizations of Biochemistry and Molecular Biology, in addition to multiple smaller annual Focused Meetings. The IUBMB also disseminates scientific knowledge through a host of publications: *Trends in Biochemical Sciences (TiBS); Biotechnology and Applied Biochemistry; IUBMB Life; Biochemistry and Molecular Biology Education; BioFactors; Molecular Aspects of Medicine; and Aspects of Molecular Medicine*. In addition to scientific meetings and publications, the IUBMB organizes or sponsors workshops, symposia and training sessions on biochemical and molecular biological education and provides free textbooks and journals to training institutions in developing nations. Furthermore, the IUBMB funds short-term fellowships for trainees and early/mid-career biochemists and molecular biologists to travel to other institutions to perform research not possible in their own laboratories. It also provides Travel Fellowships for trainees to attend meetings around the world. Established in 2022, the IUBMB Trainee Initiative, run by trainees and for trainees, engages biochemistry and molecular biology students around the world with activities related to career development, research, and education.

Uniting Biochemists and Molecular Biologists Around the World

IUBMB Focused Meeting on Aminoacyl-tRNA Synthetases 2023

IUBMB UPCOMING EVENTS

Check Out Our Upcoming Events for 2023 - 2024

https://iubmb.org/events/

2023

Sept 19-22

IUBMB Focused Meeting
Extremophilic Fungi (FUN- EX)
Ljubljana, Slovenia | https://www.fun-ex.si/

Oct 15-20

IUBMB Focused Meeting
Integrative Omics of Nuclear Functions
Munich, Germany | http://nuclearomics2023.org/index.html

Nov 23

IUBMB Educational Activities
Lifelong Learning for the Changing World in Biochemistry
Bangkok, Thailand

Nov 23-25

2nd FEBS-IUBMB-ENABLE Conference
THE EMERGING CHALLENGE: Environmental impacts on human health
Cologne, Germany | https://enablenetwork.eu/cologne-2023/

2024

Sept 21-22

IUBMB-FAOBMB-ComBio Young Scientist Program
SAVE THE DATE

June 4-8

IUBMB-FAOBMB-ComBio Congress
Biomolecular Horizons 2024: Discover, Create, Innovate
Melbourne, Australia | https://bmh2024.com/