P1.1

The protein-only RNase P PRORP1 interacts with the nuclease MNU2 in Arabidopsis mitochondria

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The essential endonuclease activity that removes 5' leader sequences from transfer RNA precursors is called RNase P. While ribonucleoprotein RNase P enzymes containing a ribozyme are found in all domains of life, another type of RNase P called "PRORP", for "PROtein-only RNase P", only composed of protein occurs in a wide variety of eukaryotes, in organelles and the nucleus. Although PRORP proteins function as single subunit enzymes in vitro, we find that PRORP1 occurs in protein complexes and is present in polysome fractions in Arabidopsis mitochondria. The analysis of immuno- precipitated protein complexes identifies proteins involved in mitochondrial gene expression processes. In particular, direct interaction is established between PRORP1 and MNU2 another mitochondrial nuclease involved in RNA 5' processing. A specific domain of MNU2 and a conserved signature of PRORP1 are found to be directly accountable for this protein interaction. Altogether, results reveal the existence of an RNA 5' maturation complex in Arabidopsis mitochondria and suggest that PRORP proteins might cooperate with other gene expression regulators for RNA maturation in vivo.

P1.2

CytoRP, a cytosolic RNase P to target TLS-RNA phytoviruses

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In plants, PRORP enzymes are responsible for RNase P activity that involves the removal of the 5' extremity of tRNA precursors. We showed previously that in addition to the maturation of tRNA precursors, PRORP enzymes cleave tRNA-like structures (TLS) present on mitochondrial mRNA. Many plant viruses (phytoviruses) are RNA viruses containing TLS structures. These RNA elements are involved in virus genome replication. The best characterized TLS is found in the Turnip Yellow Mosaic Virus (TYMV) genome. TLS based viruses are found in numerous clades of viruses. Their impact on crop production is major with considerable crop yield decreases worldwide.

Our project is based on the modification of PRORP enzymes in order to be able to target TLS RNA viruses to obtain plant resistance to virus infections. For this, we produced a PRORP variant called "CytoRP", localized in the cytosol, where virus replication takes place. CytoRP is able to cleave the TYMV TLS in vitro. We have produced transgenic plants expressing CytoRP and a cisgenic plant where the nuclear localization signal of PRORP2 was deleted using CrispR/Cas9 technology. We show that the nuclear localization of these mutants is lost and CytoRP accumulates in the cytosol. The ability of these CytoRP plants to hinder TYMV infection in vivo is currently investigated.

P1.3

Structural probing of staphylococcal-specific glyS T-box domains and modulation of transcription by protein synthesis inhibitors

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Staphylococcus aureus glyS T-box contains the species-specific intervening sequence stem Sa as part of the antiterminator/terminator stem, which possibly confers selectivity for five different $tRNA^{Gly}$ isoacceptor ligands. In addition, stem Sa was found to accommodate binding of protein synthesis inhibitors which induce antitermination of glyS transcription. Structural probing and in vitro analysis of a series of mutants containing swaps of stem I or apical loop between O. iheyensis and S. aureus and stem Sa deletions revealed diverse effects in the presence of tigecycline and linezolid. Deletion of Stem Sa reduced the in vitro transcription independently of the presence or absence of antibiotics, suggesting that stem Sa is important for T-box-mediated transcription. Moreover, $tRNA^{Gly}$ mutations in crucial nucleotides of the “elbow” showed that stem I becomes insensitive of discriminating the isoacceptors and this phenotype is reversed when stem Sa is deleted. Taken together, our data support our previous observations on the role of stem Sa in the equilibrium of transcription termination/antitermination conformations, when all tRNA isoacceptors compete for T-box riboswitch binding.

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P1.4

Effects of Lupus antigen (La) expression in transcription and translation components in lung cancer

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La protein is the major antigen in lupus and Sjogren's syndrome, involved in folding of tRNAs and safeguarding miRNA pathway from tRNA fragments during selection of small non-coding RNAs for silencing events. Although aberrant expression of La has been linked with malignancy, its exact role in various cancer types remains uncharacterized. In the present study, we screened the NSCLC cell line A549 and various NSCLC biopsy samples to verify upregulation of La. Expression of La in A549 cells under various stress conditions, showed constant localization in the nucleus and nucleolus, in contrast to what has been reported for HeLa cells. Interestingly, expression of La both at transcriptional and translational level is increased under glucose deprivation but decreased under oxidative stress. Expression of La leads to upregulation of genes involved in cell cycle, tRNA maturation and transport and miRNA biogenesis and upregulation of specific miRNAs and tRFs species. Finally, FACS analysis showed that La expression does not lead to cell cycle arrest. Instead, wound healing and CFSE assays showed that La expression accelerates cell motility, suggesting possible involvement in metastasis.

P1.5

Nuclear regulation of mitochondrial tRNA production

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The size and organization of the animal mitochondrial genome has been reduced and compacted significantly since its endosymbiosis from an α-proteobacterial ancestor. This compaction has necessitated the evolution of unique mechanisms to facilitate rapid changes in gene expression in response to the changing energy demands of the cell. The mitochondrial transcriptome encodes proteins that are subunits of the respiratory chain, responsible for most of the energy production required by the cell. Consequently the coordinated regulation of the mitochondrial transcriptome by the nucleus is of particular importance for the maintenance of cell health and energy metabolism. We have been investigating the unusual features of mitochondrial tRNAs and the RNA-binding proteins that control their production, maturation, translation and stabilization to understand the regulation of mitochondrial gene expression and its contribution to health and disease. I will highlight the devastating consequences of dysregulated mitochondrial gene expression and protein synthesis in a new model of disease caused by genetic disruption of a tRNA-binding protein. Mouse models of disease have enabled us to understand the in vivo role of fundamental processes that regulate mitochondrial tRNA metabolism and the pathogenesis of diseases caused by impaired protein synthesis. This work illustrates that balanced mitochondrial tRNA production is required for the regulation of nuclear gene expression, as well as translation in multiple cellular compartments.

P1.6

Characterization of protein-only RNase P in complex with tRNA.

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RNase P activity is the essential cleavage that removes 5'-leader sequences from transfer RNA precursors. “PRORP” (PROteinaceous RNase P) is a category of protein-only RNase P. Before the characterization of PRORP, RNase P enzymes were thought to occur universally as ribonucleoproteins (RNP). The characterization of PRORP revealed an enzyme with two main domains, an N-terminal domain containing five PPR RNA binding motifs and a C- terminal NYN domain holding catalytic activity (1, 2).
We used a combination of biochemical and biophysical approaches to characterize the PRORP / tRNA precursor complex. Analytical ultracentifugation, microscale thermophoresis and activity assays show that the sole PPR motifs PPR2 and PPR3 are involved in PRORP/ tRNA precursor interaction. These results were combined with small angle X ray scaterring data of PRORP alone and with tRNA to build a model of the PRORP2 pre-tRNA complex (3). In this model, PPR2 and 3 interact, in accordance with the “PPR code”, repectively with C56 and G18 spacialy localized at the corner of the tRNA. This shows that PRORP target recognition process is in accordance with PPR proteins canonical mode of action (4). Altogether, our analysis reveals an interesting case of convergent evolution. It suggests that PRORP has evolved an RNA recognition process similar to that of RNP RNase P.
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P1.7

Substrate recognition by the novel single-subunit protein-only RNase P HARP

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RNase P is the essential tRNA 5'-end maturation endonuclease. Recently, a new form of RNA-free RNase P was discovered in the hyperthermophilic bacterium Aquifex aeolicus, termed AaRP (Aquifex aeolicus RNase P) [1]. Homologs of Aquifex RNase P (HARP) were identified in many Archaea and some Bacteria, of which all Archaea and most Bacteria also encode an RNA-based RNase P. We have begun to study substrate recognition by Aq_880 in comparison with HARPs from Methanothermobacter thermautotrophicus (archaeon) and Thermodesulfatator indicus (bacterium) using a series of substrate variants already used in the study of substrate recognition by A. thaliana PRORP3 [2]: precursor-$tRNA^{Gly}$ derivatives with (i) varying 5'-leader length, (ii) with 3'-CCA deletion and 3'-extensions, (iii) with other strategic deletions, such as those of anticodon and D arm and (iv) length variation of acceptor/T stems. This will provide a detailed and representative picture of substrate recognition by HARPs in comparison to PRORPs and RNA-based RNase P enzymes.

[1] A.I. Nickel et al. (2017) Proc. Natl. Acad. Sci. USA 114: 11121 [2] N. Brillante et al. (2016) Nucleic Acids Res. 44: 2323

P1.8

Ablation of RNase Z^{5} via CRISPR-Cas9 results in viable cells with altered phenotype

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Ribonuclease Z (RNase Z) is responsible for the 3' maturation of precursor tRNAs and tRF-1 production. In human, both activities are served predominantly by RNase Z^{L} (ELAC2), while the role of RNase Z^{5} (ELAC1) which is localized in the cytoplasm, has been overlooked. Overexpression of both genes in A549 and HEK-293T cells revealed differential responses on the expression of AGO genes, p21, p27, p53 and CCN genes and DANCR, GAS5, HULC, HOTAIR, MALAT1 lncRNAs, indicating a possible role of both enzymes in lncRNA biogenesis and turnover. In addition, ELAC1 was upregulated together with ANG under various stress conditions, indicating a possible concerted action in stress inducible tiRNA metabolism. To gain insights on the biological role of RNase Z^{5} we used CRISPR-Cas9 to successfully knockout the sole copy of ELAC1 encoded in HEK-293T cells. Ablation of ELAC1 did not affect cell viability and ELAC2 expression but upregulated ANG. Finally, it led to increased G1 phase, similar apoptotic behavior, reduced proliferation and motility, vulnerability to serum deprivation, higher resistance to glucose deprivation, increased mitochondrial mass per cell and overall altered morphology.

P1.9

Role of CCA adding enzyme in the mitochondrion of Trypanosoma brucei

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T brucei which is a single cell eukaryote presents a unique situation where all the tRNA genes have been thrown out of the mitochondrial genome and are imported from the nuclear encoded cytosolic pool of mature tRNAs. Hence, the tRNA processing enzymes are not needed in its mitochondria. Yet, recent result from our lab shows that CCA adding enzyme that adds CCA tail to the tRNAs in all eukaryotes is present in the mitochondria of trypanosomes. We found that CCA addition activity is also present in this mitochondrion along with the cytosol. And this activity is abolished by the knockdown of the single gene encoding for CCA adding enzyme in trypanosomes. Additionally, the knockdown shows shorter tRNA accumulation in mitochondria. Though it is not clear whether the CCA-less tRNAs are imported into the mitochondria because of the knockdown or lack of enzyme leads to accumulation of CCA-less tRNA during internal tRNA turnover of the organelle. Through dissecting its dual localisation mechanism we aim to create a mitochondrial knockout of this enzyme and ascertain its dispensability for the mitochondria and/or involvement in any other function apart from its canonical role in T brucei.

P1.10

Novel protein-only RNase Ps in prokarya -comparison of AaRP and archaeal HARPs

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RNase P is the essential tRNA 5'-end maturation endonuclease. Recently, a new form of RNA-free RNase P was discovered in the hyperthermophilic bacterium Aquifex aeolicus,termed AaRP (Aquifex aeolicusRNase P) [1]. In contrast to the known protein-only RNases P from Eukarya (PRORPs), AaRP is only a small protein (~23 kDa).We were able to detect homologs, termedHARPs (Homologs of Aquifex RNase P), in some bacteria and many archaea. In a few of these bacteria and in all of those archaea, the HARP coexists with the classical RNase P consisting of a catalytic RNA and the corresponding protein subunit(s). We have begun to characterize the HARP proteins of selected archaeal species that are amenable to genetic studies. Our studies include in vitro experiments with recombinant HARPs (cleavage of various model substrates to analyze substrate recognition, kinetics, crystallization, biophysical analyses etc.) as well as in vivostudies (complementation analyses on solid and in liquid media, physiological characterization of HARP knockout strains).

[1] Nickel A. I. et al., PNAS,2017 Oct 17;114: 11121

P2.1

Characterization of RNA post-transcriptional modifications in *Staphylococcus aureus* during infection and antibiotic stresses

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S. aureus is a Gram-positive major human pathogen involved in a wide range of human infectious diseases. The treatment of *S. aureus* infection is very challenging due to the emergence of multiple antibiotic resistant isolates. The high diversity of clinical symptoms caused by S. aureus, as well as its drug resistance mechanisms depend on the precise expression of numerous virulence factors and stress response pathways, which are tightly regulated at several levels (transcription, translation, mRNA decay).
During the last two decades, it has become evident that small regulatory RNAs (sRNAs) play a major role in these adaptive responses, mainly targeting mRNA translation. Besides, tRNAs and codon bias are impacting translation elongation and protein folding during stress responses and adaptation. In these RNA mediated regulatory mechanisms, RNA post- transcriptional modifications which can be modulated in response to adaptive processes could add an additional layer of control. Altering the chemical and physical properties of nucleotides, they affect RNA base-pairing formation, protein recognition, RNA structure and stability, mRNA translation and the wobble-base tRNA decoding properties. Their impact in pathogenic bacteria during growth adaptation and infection has just started to be appreciated. Using mass spectrometry and RNA-Seq methods, we are characterizing the epitranscriptome of *S. aureus* in various conditions encountered during infection and by mutagenesis analysis the machineries involved in their synthesis and regulations. The methods used and some preliminary data showing differences in tRNAs modifications in different stresses will be discussed.

P2.2

Time-resolved NMR monitoring of RNA maturation in cellular extracts revealed complex circuits of modifications in tRNAs

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Decades of exploration in the field of RNA modification have revealed more than 90 different ribonucleoside structures incorporated as post-transcriptional modifications in tRNAs, which not only display the largest variety of post-transcriptional decoration among RNA molecules, but also the highest density of modification per RNA transcript. For instance in yeast, 13 modifications are typically found per tRNA molecule, which corresponds to ~12-20% of its nucleotides bearing a chemical modification. Many of these modifications are introduced at positions in the neighbourhood of others, enabling potential interplay in their incorporation. Although these circuits of modifications have been investigated and identified in some cases of rather strong dependency, their study remains difficult since monitoring the maturation of tRNA in real-time at a single nucleotide level is technically challenging. Here, using an original methodology, we show that nuclear magnetic resonance spectroscopy (NMR) is a powerful tool to monitor tRNA maturation events in a non-disruptive and continuous fashion. Time-resolved NMR measurements of different modification events in complex environments such as yeast cell extracts revealed a defined chronology in the incorporation of tRNA modifications. Furthermore, a detailed analysis of snapshots measured along the tRNA maturation route under various conditions revealed a complex circuitry of modifications where most modifications are seen to have positive and/or negative impact on the incorporation of other ones. We believe that the NMR-based methodology presented here can be adapted to investigate many aspects of tRNA maturation and RNA modifications in general.

P2.3

Loss of tRNA modification i^6A_{37} leads to mitochondriopathy and increased +1 frame-shifting in mice

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In mammals, cytoplasmic tRNAs carrying N^6-isopentenyl adenosine (i^6A) are $tRNA^{SEr(UCN)}$ and $tRNA^{Ser(UGA)}$Mitochondrial isopentenylated tRNAs, Tyr, Trp, Phe, and Ser(UCN), are further thiomethylated to ms^2i^6A (2-methylthio-N^6-isopentenyl adenosine). While i^6A is among the tRNA modifications longest known, its significance in animals remained unclear. We have conditionally inactivated Trit1 (tRNA:dimethylallyl-isopentenyltransferase 1) in mouse hepatocytes and neurons accordingly abrogating tRNA isopentenylation in targeted cells. Ribosomal profiling of nuclear transcripts in liver reveals increased +1 frame-shifting. A-site occupancy of codons requiring wobble base pairs is changed in mitochondria. Expression of mitochondrial electron transport chain subunits is impaired and the mitochondrial integrated stress response pathway is mounted. Neuron-specific Trit1 knockout mice exhibit microcephaly and seizures and upregulate markers of the unfolded protein response pathway. Inactivation of tRNA isopentenylation is more severe than inactivation of tRNA thiomethylation underlining the fundamental role of i^6A in mammalian biology.

P2.4

Queuosine and 5-methylcytosine tRNA modifications: Impact on cellular processes and *in vivo* consequences

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To date, many conserved tRNA modifications have been catalogued. Recent studies have provided novel insights into how these modifications and the responsible enzymes influence various biological processes. However, very little is known about their contribution to cell fate decisions. To comprehensively understand the influences of these modifications on various cellular processes, we aim to functionally characterize Q and m5C38 modifications, which are found in the anticodon loop. We recently generated Qtrt1, Qtrtd1, Qtrt1-Dnmt2, and Qtrtd1-Dnmt2 single and double knockout mESCs using CRISPR-Cas9 technology. Preliminary experiments have been performed to study the functional relevance of Q and m5C38 modifications and to reveal the effect of these modifications on proliferation, protein translation, and protein homeostasis. Firstly, the influence of micronutrient Q on mammalian Dnmt2 activity has been analyzed in both mouse and human cell lines using bisulfite conversion based high- throughput sequencing. Secondly, dynamic regulation of protein synthesis is currently being analyzed by SuNSET and polysome profiling. Furthermore, using ribosome footprinting and computational data analysis, the impact of Q deficiency on protein translation fidelity at single-codon resolution will be determined. Lastly, to provide insights into the consequences of specific modifications in vivo, Qtrt1 and Qtrtd1 knockout mice are being generated and will be crossed with Dnmt2 knockout mice to further analyze the biological functions of these modifications in vivo. Taken together, this project will have significant implications for connecting tRNA modifications with key cellular processes and, in the long term, with human diseases.

P2.5

tRNA modifying enzymes: study of expression changes during an epithelial-mesenchymal - like transition in a breast cancer cell model, by ribosome profiling

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Multiple species and subspecies of isoacceptor and isodecoder tRNAs constitute the tRNA population of a particular cell in a defined state. The diversity of the tRNA population results from the differential expression of tRNA genes and their diverse state of post-transcriptional modification. Modification of tRNA bases involves a large set of enzymes exhibiting high specificity (target tRNA, specific base and precise location in the structure). Given the emerging evidence that links tRNA population with cell fate, our aim is to understand if particular cells states have a defined distribution of its tRNA variants, adapted to the ongoing cellular program and to metabolic conditions. As an approach to better understand adaptive changes in tRNA populations, we studied the variations in the expression of the enzymes involved in tRNA modifications at the transcriptional and translational levels, during an epithelial-mesenchymal - like transition in a breast cancer cell model (MCF7 derived). Enzymes expression levels were obtained by ribosome profiling. The results are discussed considering the state of the main metabolic pathways that provide substrates for tRNA modification enzymes.

P2.6

Dissecting the role of the tRNA modifiers ADAT3 & WDR4 in the wellness/illness of the developing cerebral cortex

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Development and functioning of the cerebral cortex depends on the coordinated production, migration and differentiation of excitatory and inhibitory neurons. Disruption of these processes can cause socially-devastating neurodevelopmental disorders such epilepsy or severe learning and intellectual disabilities. Mutations in genes encoding tRNA modification enzymes have been increasingly linked to human neurological disorders. We aim at understanding the role in cerebral corticogenesis of ADAT3 and WDR4, two tRNA modifiers, which need to form active heterodimers with ADAT2 and METTL1, respectively, to modify tRNAs. Mutations in both genes have been linked to neurodevelopmental disorders. In mice embryos we have shown that ADAT3 and WDR4 as well as their partners show a dynamic expression during cortical development. By using in utero electroporation of miRNAs in mouse embryos during cortical development we have shown that depletion of both ADAT3 and WDR4 delays radial migration of cortical neurons. Furthermore, we have demonstrated that although mutant ADAT3 and WDR4 still form complex with their partners, the stability and folding of the mutant complexes are severely impaired.

P2.7

Queuine links translational control in eukaryotes to a micronutrient from bacteria

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In eukaryotes, the wobble position of tRNA with a GUN anticodon is modified to the 7- deaza-guanosine derivative queuosine (Q34), but the original source of Q is bacterial, since Q is synthesized by eubacteria and salvaged by eukaryotes for incorporation into tRNA. Q34 modification stimulates Dnmt2/Pmt1-dependent C38 methylation (m^5C38) in the $tRNA^{Asp}$ anticodon loop in Schizosaccharomyces pombe. Here, we show by ribosome profiling in S. pombe that Q modification enhances the translational speed of the C-ending codons for aspartate (GAC) and histidine (CAC) and reduces that of U- ending codons for asparagine (AAU) and tyrosine (UAU), thus equilibrating the genome-wide translation of synonymous Q codons. Furthermore, Q prevents translation errors by suppressing second-position misreading of the glycine codon GGC, but not of wobble misreading. The absence of Q causes reduced translation of mRNAs involved in mitochondrial functions, and accordingly, lack of Q modification causes a mitochondrial defect in S. pombe. We also show that Q-dependent stimulation of Dnmt2 is conserved in mice. Our findings reveal a direct mechanism for the regulation of translational speed and fidelity in eukaryotes by a nutrient originating from bacteria.

P2.8

Codon-specific effects of tRNA anticodon loop modifications on translational misreading errors in the yeast Saccharomyces cerevisiae

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Post-transcriptional modification of tRNAs have roles in stabilizing the three dimensional structure of the tRNA and supporting codon-anticodon recognition during aminoacyl-tRNA recruitment. Through these roles they stabilize the tRNA
to degradation and improve decoding. Much of the information about their roles in decoding have derived from studies of cognate decoding but arguably equally important is their roles in modulating erroneous, near-cognate decoding. We have used in vivo reporter systems to determine the frequencies of misreading events by individual tRNAs. The systems exploit essential amino acids in the reporter proteins E. coli 🚲galactosidase and Photinus pyralis (firefly) luciferase. We have used these systems to test the role of tRNA modifications on near-cognate decoding by $tRNA^{Glu}{}_{UUC}$ and $tRNA^{Lys}{}_{UUU}$ in the bacterium E. coli and the yeast S. cerevisiae. We find different roles for x^5m^2 s U_{34} in the two species. In E. coli, $mnm^5s^2U_{34}$ either increases or decreases misreading regardless of the codon recognized while in S. cerevisiae the effect of $mcm^5s^2U_{34}$ the same modification on a particular tRNA can either increase or decrease misreading depending on the codon being decoded. In E.coli, therefore, the modification affects a general property of the tRNA while in S. cerevisiae the modification modulates the codon-anticodon interaction context specifically. We also found that t^6A_{37} modification in yeast has distinct effects on misreading depending on the position of the base mismatch. It strongly decreases misreading involving first position U_1-U_{36} mismatches but increase misreading involving second or wobble position mismatches. This contrasts with its role in cognate decoding in which it stabilizes the weak A_1-U_{36} pair; whereas t^6A stabilizes cognate decoding by a cross-strand stacking interaction with the first codon nucleotide, its unkown interaction with the extremely weak U_1-U_{36} interferes with its formation as part of recruitment of the near-cognate tRNA. These results suggest that prediction of the function of tRNA modifications during misreading cannot be predicted by their role in cognate decoding and that even closely related modifications may have drastically different functions in modulating misreading.

P2.9

Structural and biochemical analysis of the dual-specificity Trm10 enzyme from *Thermococcus kodakaraensis* prompts reconsideration of its catalytic mechanism

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N1 methylation of purines at position 9 of tRNA is catalyzed by the SPOUT methyltransferase (MTase) Trm10. While certain Trm10 orthologues are specific for either G or A, others show a dual specificity. We report the structure and biochemical analysis of the dual specificity enzyme from *T. kodakaraensis* ($_{TK}$Trm10). We solved the crystal structure of a construct of this enzyme, consisting of the N-terminal domain and the catalytic SPOUT domain. Crystal structures of the SPOUT domain, either in the apo form or bound to SAM or SAH reveal conformational plasticity of two active site loops upon substrate binding. Kinetic analysis shows that $_{TK}$Trm10 has a high affinity for its tRNA substrates. Mutation of either of two active site residues (D206, D245) results in only modest effects on the methylation reaction, with a small shift toward a preference for m^1G over m^1A formation. Only a double D206A/D245A mutation severely impairs activity. These results are in line with the recent finding that the single active-site D was dispensable for activity in the G-specific yeast Trm10, and suggest that also dual specificity Trm10 use a mechanism without residues acting as general base catalysts.

P2.10

Unexpected effect on selenoprotein expression by lack of i^6A modification in $tRNA^{[Ser]Sec}$

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Selenoproteins contain the amino acid selenocysteine (Sec) encoded by UGA. Recoding of UGA as Sec requires a 3' stem loop in selenoprotein mRNAs, protein factors, and $tRNA^{[Ser]Sec}$(UGA). This tRNA is isopentenylated at adenosine 37 (i^6A37) by the enzyme Trit1. Several lines of evidence suggested that i^6A37 in $tRNA^{[Ser]Sec}$ is essential for efficient recoding of UGA: 1) Lovastatin treatment reduced selenoprotein expression in cultured cells. 2) Mice expressing $tRNA^{[Ser]Sec}$ carrying A37G do not express all selenoproteins. 3) Knockdown of TRIT1 in HepG2 cells showed decreased selenoprotein expression under low selenium conditions. In order to address the role of isopentenylated $tRNA^{[Ser]Sec}$, we have generated Trit1-knockout mice and analysed selenoprotein translation by western-blot and ribosome profiling. There was no general downregulation of selenoprotein expression in Trit1-knockout mice. Ribosome profiling revealed unchanged UGA recoding efficiency. Selenoprotein expression in patient fibroblasts carrying an inactivating TRIT1 mutation was also not affected. We speculate that a role of i^6A37 modification of $tRNA^{[Ser]Sec}$ may only be revealed under conditions of selenium deficiency.

P2.11

Cytosine-5 tRNA methylation links protein synthesis to cell metabolism

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Chemical modifications in tRNA are often critical for normal development as they adapt protein synthesis to a dynamically changing microenvironment. However, the precise cellular mechanisms linking the extrinsic stimulus to the intrinsic RNA modification pathway remain unclear. Here, we identified the cytosine-5 RNA methyltransferase NSUN2 as a sensor for external stress stimuli. Exposure to oxidative stress efficiently repressed NSUN2 causing a reduction of methylation at specific tRNA sites. Using metabolic profiling, we showed that loss of tRNA methylation captured cells in a distinct catabolic state. Mechanistically, loss of NSUN2 altered the biogenesis of tRNA-derived non-coding fragments (tRFs) in response to stress leading to impaired regulation of protein synthesis. The intracellular accumulation of specific tRFs was sufficient to dynamically repress global protein synthesis. Finally, NSUN2-driven RNA methylation was functionally required to adapt cell cycle progression to the early stress response. In summary, we revealed that changes in tRNA methylation profiles were sufficient to specify cellular metabolic states and efficiently adapt protein synthesis rates to cell stress.

P2.12

Reprogramming tRNA modifications to combat cancer

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Transfer RNAs (tRNAs) are richly decorated with various post-transcriptional modifications that influence the rate and accuracy of translation. Although perturbations in tRNA modification unbalance translation, their exact role as mediators in tumour formation and proliferation is only starting to emerge. Hence, we aim to (i) identify tRNA modifications with significantly altered abundance and (ii) investigate tRNA modification-mediated translational changes in various cancers. We hypothesize that cancer cells can be rescued by pinpointing tRNA modifications that maintain tumorigenic growth and correct their modification levels to that of healthy, non-oncogenic cells. To this end, we use mass spectrometry and ribosome profiling to quantitatively determine tRNA modification levels and monitor translational changes in healthy vs. cancerous cells. The follow-up correlation of RNA modification and translation changes will create a detailed characterization of tRNA-mediated translational responses and identify key regulatory modifications on specific tRNA species. This study will highlight the regulatory function of tRNA modifications and expand our understanding of cancer formation.

P2.13

A hypertension-associated mitochondrial DNA mutation introduces an m1G37 modification into tRNAMet, altering its structure and function

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Defective nucleotide modifications of mitochondrial tRNAs have been associated with several human diseases, but their pathophysiology remains poorly understood. In this report, we investigated the pathogenic molecular mechanism underlying a hypertension-associated 4435A>G mutation in mitochondrial tRNAMet. The m.4435A>G mutation affected a highly conserved adenosine at position 37, 3' adjacent to the tRNA's anticodon, which is important for the fidelity of codon recognition and stabilization. We hypothesized that the m.4435A>G mutation introduced an m1G37 modification of tRNAMet, altering its structure and function. Primer extension and methylation activity assays indeed confirmed that the m.4435A>G mutation created a tRNA methyltransferase 5 (TRMT5)–catalyzed m1G37 modification of tRNAMet. We found that this mutation altered the tRNAMet structure, indicated by an increased melting temperature and electrophoretic mobility of the mutated tRNA compared with the wild-type molecule. We demonstrated that cybrid cell lines carrying the m.4435A>G mutation exhibited significantly decreased efficiency in aminoacylation and steady-state levels of tRNAMet, as compared with those of control cybrids. The aberrant tRNAMet metabolism resulted in variable decreases in mitochondrial DNA (mtDNA)-encoded polypeptides in the mutant cybrids. Furthermore, we found that the m.4435A>G mutation caused respiratory deficiency, markedly diminished mitochondrial ATP levels and membrane potential, and increased the production of reactive oxygen species in mutant cybrids. These results demonstrated that an aberrant m1G37 modification of mitochondrial tRNAMet affected the structure and function of its tRNA and consequently altered mitochondrial function. Our findings provide critical insights into the pathophysiology of maternally inherited hypertension, which is manifested by the deficient tRNA nucleotide modification.

P2.14

Investigating Geobacillus kaustophilus TilS dynamics using molecular dynamic simulations

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Nucleotide modifications contribute to the stability, structure, and function of tRNA molecules. Some modifications are essential for translational fidelity and their loss scan affect cell viability. One such modification proposed to be essential in prokaryotes is the lysidine installed at the $tRNA^{Ile2}$ wobble position by tRNA isoleucine lysidine synthetase (TilS). Addition of lysine at the C2 position is essential for decoding the rare isoleucine AUA codon in prokaryotes. Because TilS is thought to be essential for specific and accurate protein synthesis, it is useful to investigate the dynamics and mechanism of the enzyme to gain a better understanding of its ability to recognize and bind its cognate substrate, including both the anticodon in the enzyme's active site and the acceptor end at a distance ~80 Å away. Through the use of molecular dynamics, the flexibility and mobility of TilS can be monitored. We performed simulations using the 3.65 Å Geobacillus kaustiphilus $TilS:tRNA^{Ile2}$ crystal structure, including protein-only and tRNA-bound systems, to better understand the changes in flexibility upon complex formation.

P2.15

Role of tRNA binding protein Kti12 for function of the Elongator complex

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Certain tRNA species contain Elongator complex dependent modifications at anticodon wobble uridines (U_{34}). These are targets for zymocin, a fungal ribotoxin produced by Kluyveromyces lactis. Zymocin cleaves in the anticodon of modified tRNAs and thereby results in cell death. Mutations in the Elongator complex result in loss of U_{34} modification and resistance towards zymocin. Here, we demonstrate that similar U_{34} modification defects are observable if KTI12, a gene coding for an Elongator partner protein, is inactivated or mutated. Interestingly, the U_{34} modifications depend on an NTP binding P-loop domain in Kti12, which is conserved with a tRNA kinase (PSTK) and whose mutation abolishes interaction with Elongator in yeast. This strengthens our view that physical contact between Kti12 and Elongator is required for anticodon modification. Indeed, LC-MS/MS measurements performed on tRNAs extracted from P-loop mutants verify loss of U_{34} modification. In addition, we show that Kti12, like PSTK, is able to bind to tRNAs in a fashion that appears to be P-loop independent in vitro.

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P2.16

Nuclear tRNA export in *Trypanosoma brucei*

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Protein and RNA transport across the nuclear envelope occurs through the nuclear pore complex (NPC) and requires proteins of the karyopherin family (exportins). Xpo-t in vertebrates and Los1 and Msn5 in yeast specifically export tRNAs from the nucleus. T. brucei is a single-cell parasite, that, in contrast with other eukaryotes, lack most of the transcriptional control; the bulk of gene expression regulation occurring post-transcriptionally. Nuclear tRNA export might provide an additional level of regulation of gene expression during the complex life cycle of these parasites. However, only a limited set of the export factors, conserved in other organisms, can be easily identified in the T. brucei genome. Our results indicate that similar to other eukaryotes, TbXpo-t is not essential for growth of trypanosomes. Moreover, RNAi silencing of TbXpo-t did not result either in disruption of the tRNA translocation, or in intron-tRNA accumulation in the nucleus, both phenotypes described for yeast mutants. These observations are discussed in the context of the possible roles of the mRNA export pathway in the translocation of mature tRNAs through the NPC and its role on tRNA processing.

P2.17

Long and branched polyamines are required for maintenance of the ribosome, tRNA^{His}, and tRNA^{Tyr} in Thermus thermophilus cells at high temperatures

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Thermus thermophilus is an extremely thermophilic eubacterium that produces various polyamines. Aminopropylagmatine ureohydrolase (SpeB) and SAM decarboxylase-like protein 1 (SpeD1) are involved in the biosynthesis of polyamines. The speB and speD1 gene-deleted strains (ΔpeB and ΔpeD1, respectively) cannot synthesize long and branched polyamines. Although neither strain grew at high temperatures (>75oC), both strains survived at 80oC when they were cultured at 70oC until the mid-log phase and then shifted to 80oC. We prepared the ΔpeB and ΔpeD1 cells using this method. Microscopic analysis showed that both strains can survive for 10 h at 80oC. Although the modification levels of 2’-O-methylguanosine at position 18, 7-methylguanosine at position 46, 5-methyluridine at position 54 and 1-methyladenosine at position 58 in tRNA from both strains were normal, amounts of tRNA^{Tyr}, tRNA^{His}, rRNAs, and 70S ribosomes were decreased. Furthermore, in vivo protein synthesis in both strains was completely lost 10 h after the temperature shift. Thus, long and branched polyamines are required for at least the maintenance of 70S ribosome and some tRNA species at high temperatures.

P2.18

Specificity in the Biosynthesis of the Universal tRNA Nucleoside Threonylcarbamoyl Adenosine (t^6A)

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N6-threonylcarbamoyl adenosine (t^6A) is a universal nucleoside modification found at position 37 of tRNAs decoding ANN codons. In Bacteria the proteins TsaB, TsaC (or C2), TsaD, and TsaE, comprise the biosynthetic apparatus responsible for t^6A formation. TsaC catalyzes the formation of the intermediate threonylcarbamoyl adenosinemonophosphate (TC-AMP) from ATP, threonine, and CO_2, and TsaD catalyzes the transfer of the threonylcarbamoyl moiety from TC-AMP to A_{37} of substrate tRNAs. Several related modified nucleosides, including hydroxynorvalyl carbamoyl adenosine (hn^6A), have been identified, but nothing is known about their biosynthesis. To better understand structural constraints on t^6A formation, and to determine if related modified nucleosides are formed via parallel biosynthetic pathways or the t^6A pathway, we investigated the specificity of the t^6A systems from *E. coli* and *T. maritima*. Our data demonstrate that TsaC/C2 exhibit relaxed substrate specificity, producing a variety of TC-AMP analogs that can differ in both the identity of the amino acid and nucleotide component, while TsaD displays high specificity in *E. coli* and relaxed specificity in *T. maritima*.

P2.19

Leber's hereditary optic neuropathy is potentially associated with a novel m.5587T>C mutation in Chinese pedigrees

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Mitochondrial (mt)DNA mutations have been revealed to be associated with Leber's hereditary optic neuropathy (LHON). Our previous investigation has shown the mutational incidence and spectrum of the ND1, ND4 and ND6 gene in a large cohort of 1281 Chinese subjects with LHON. Resultant variants were evaluated for evolutionary conservation, allelic frequencies, and structural and functional consequences. However, the mutational spectrum and incidence in the tRNA gene are poorly understood in the LHON patients. Furthermore, it is anticipated that additional mutations can be found in the tRNA gene. In this investigation, a total of 4 (3 men and 1 female) out of 14 matrilineal relatives in two Chinese families exhibited visual impairment with variable severity and age of onset. The average age of onset of visual loss was 20.5 years old. Molecular analysis of the complete mitochondrial genome in these pedigrees demonstrated that the three primary mutations associated with LHON were not detected; however, the homoplasmic m.5587T>C mutation was identified, which was localized at the end of the mitochondrially encoded transfer (t)RNA alanine gene and may alter the tertiary structure of this tRNA. Subsequently, this structural alteration may result in tRNA metabolism failure. In addition, distinct sets of mtDNA polymorphisms belonging to haplogroup F1 were detected in both families tested. The findings of the present study suggested that the m.5587T>C mutation may be involved in the pathogenesis of visual impairment. In addition, the mtDNA variant m.15024G>A(p.C93H) in the mitochondrially encoded cytochrome B gene was detected in both families, which exhibited evolutionary conservation, indicating it may serve a potential modifying role in the development of visual impairment associated with m.5587T>C mutation in these families. Furthermore, other modifying factors, including nuclear modifier genes, and environmental and personal factors may also contribute to the development of LHON in subjects carrying this mutation. Our findings may provide valuable information for pathophysiology, management, and genetic counseling of LHON.

P2.20

Mitochondrial tRNA(Glu) 14693A>G mutation is a potentially risk factor in the phenotypic manifestation of Leber's hereditary optic neuropathy in a cohort of Chinese families

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Mitochondrial DNA (mtDNA) mutations have been associated with Leber's hereditary optic neuropathy (LHON). In this study, A cohort of 1741 Han Chinese probands and 485 control subjects underwent sequence analysis of mitochondrial (mt)DNA. Resultant variants were evaluated for evolutionary conservation, allelic frequencies, and structural and functional consequences. we investigated the pathophysiology of a LHON susceptibility allele (m.14693A>G) in the mitochondrial tRNA gene. The m.14693A>G mutation affected a highly conserved at position 54 of the TΨC loop of tRNA(Glu). The destabilization of base-pairing (54A-U58) caused by the m.14693A>G mutation perturbed the conformation and stability of tRNA(Glu). The incidence of m.14693A>G mutation was 1.8%, either isolation in 15 pedigrees or together with the m.11778G>A in 12 pedigrees, and m.14484T>C mutations in 3 pedigrees, and m.3460G>A mutations in one pedigree in these probands with LHON. The occurrence of m.14693A>G mutation in these genetically unrelated pedigrees affected by LHON and differing considerably in their mtDNA haplotypes (M7, N9, D4, D5, Y) strongly indicated that this mutation is involved in the pathogenesis of this disorder. Furthermore, the average penetrance of LHON among 12 Han Chinese pedigrees carrying m.14693A>G and m.11778G>A mutations, and 3 pedigrees bearing both m.14693A>G and m.14484T>C mutations, and one pedigree bearing both m.14693A>G and m.3460G>A mutations were significantly higher than those carrying only m.14693A>G, m.11778G>A, m.14484T>C or m.3460G>A mutation ($p<0.0001$). In particular,the average penetrances of optic neuropathy of 26 families carrying only m.3394T>C mutation were 12.27%, while these among 21 pedigrees harboring both m.3394T>C and m.11778G>A or m.14484T>C mutations were 39.47% , 46.28% and 42.85%, respectively. Therfore, we hypothesized that the biochemical consequences caused by the m.14693A>G mutation may deteriorate the mitochondrial dysfunction associated with primary mutation, thereby increasing the penetrance and risk of LHON. Our findings provide new insights into the pathophysiology of LHON arising from the synergy between mitochondrial ND1 and ND4 mutations.

P2.21

Post-transcriptional modification and cellular energy levels control degradosome-dependent tRNA clearance

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The factors and mechanisms that mediate and control tRNA stability in bacteria are not well understood. Here, we studied the roles of post-transcriptional modification in bacterial tRNAs (tRNA modification) in tRNA stability. We focused on 4-thiouridine (s^4U), a modified nucleoside found in bacterial and archaeal tRNAs. Comprehensive tRNA quantification revealed that the abundance of a subset of tRNA species decreased in a s^4U deficient (ΔthiI) strain of Vibrio cholerae in a stationary phase specific fashion. Multiple mechanisms, including rapid degradation of hypomodified tRNAs, account for the reduced level of tRNAs in the absence of thiI. We identified additional tRNA modifications that promote tRNA stability and bacterial growth. Genetic analysis of suppressor mutants as well as biochemical analyses revealed that degradation of hypomodified tRNAs is mediated by the RNA degradosome. Finally, we found that low cellular NTP levels trigger the decay of hypomodified tRNAs. Together, these observations support the existence of a cellular energy responsive bacterial tRNA quality control mechanism in which hypomodification sensitizes tRNAs to RNA degradosome mediated decay.

P2.22

NSUN2 introduces 5-methylcytidines in mammalian mitochondrial tRNAs

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Human mitochondrial (mt-) tRNAs contain 16 species of modified nucleosides at 135 positions. These modifications play a critical role in precise decoding of genetic information and stability of tRNA structure. However, biogenesis and functional roles of these modifications are not fully understood. We mapped eigjht 5-methylcytidine (m^5C) at positions 48-50 in the extra loop of six mt-tRNA species. The cytidine methyltransferase NSUN2 introduces m^5C at the same positions of cytoplasmic tRNAs. Lack of m^5C reduces tRNA stability and accumulates stress-induced tRNA fragments. Loss-of-function mutations in NSUN2 gene cause autosomal-recessive intellectual disability and Dubowitz syndrome. Super-resolution microscopy using structured illumination microscopy (SIM) revealed that NSUN2 partially localizes in mitochondria, though it is predominantly in nucleus. Then, we conducted mass spec analysis of mt-tRNAs isolated from Nsun2 null mouse liver as well as from NSUN2 knockout HEK293T cells, and found complete loss of m^5C in any of mt-tRNAs, demonstrating that NSUN2 is also responsible for m^5C formation in mt-tRNAs. We are trying to identify mitochondria-related phenotype of NSUN2 KO cells.

P2.23

Combined tRNA modification defects activate multiple transcriptional starvation responses

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Simultaneous removal of critical tRNA anticodon loop modifications induces synthetic negative phenotypes in yeast. We characterized proteomic and transcriptomic changes in mutant combinations previously shown to affect either $tRNA^{Lys}UUU$ or $tRNA^{Gln}UUG$. While translational inefficiencies of mRNAs highly enriched for either of the cognate codon could be demonstrated, most significant changes occurred at the transcriptional level. We observed a transcriptional upregulation of different starvation responses, including general amino acid control, carbohydrate catabolite and nitrogen catabolite repressed pathways independent of whether $tRNA^{Lys}UUU$ or $tRNA^{Gln}UUG$ were affected. In addition, all combined modification mutants inadequately induced autophagy under nutrient replete conditions, suggesting that the modification status of different tRNAs is crucial for sensing nutrient availability. Since transcriptional starvation responses were suppressed by elevated levels of the hypomodified tRNAs, these are likely a consequence of translational inefficiency rather than loss of the modifications itself.

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P2.24

Structural insights into regulatory mechanisms of Elongator complex

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The cm^5 modification, which is provided by the Elongator complex at the wobble U_{34} position of certain tRNA species, is a major mechanism of translational control.[1] The structure of the Elongator complex was recently solved using electron microscopy,[2] however, regulatory mechanisms, which enable cm^5 modification, remain elusive. Here we provide structural insights into the function of Kti12, a transiently associated regulatory factor of Elongator. We demonstrate that Kti12 is an tRNA-induced ATPase and solved the crystal structure of Kti12's ATPase domain bound to a transition state mimic of ATP hydrolysis. Additionally, we mapped the interaction site of the Kti12 protein on the Elp1 protein, a "docking hub" of the Elongator complex. Understanding the biogenesis of cm^5 modification may pave the way for novel treatment strategies of Elongator associated pathophysiologies, like familial dysautonomia, intellectual disability, and rolandic epilepsy.[3]

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P2.25

Queuosine: The role of an essential tRNA modification in parasitic protist Trypanosoma brucei.

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Queuosine (Q), is a modified base, found at the wobble position of the anticodon of several tRNAs. Despite its omni- presence in all domains of life, its function is not clear. In this study, we used protozoan parasite T. brucei as a model for comprehensive analysis of tRNA guanine transglycosylase (TGT), the enzyme catalyzing the Q-modification. Unlike its bacterial counterpart, in most eukaryotes TGT predominantly functions as a heterodimer. We identified two TGT subunits in T. brucei, TbTGT1 and TbTGT2. Both subunits are necessary for the catalytic activity. Interestingly, unlike higher eukaryotes, TbTGT heteromer is localized to the nucleus. In order to examine the physiological role of Q, we generated a knock-out of TbTGT2 and performed in vivo characterization, to simulate natural parasite infection in its mammalian host. Upon infection of mice, the mutant parasites showed significantly delayed parasitemia, and conse- quently, prolonged survival of mice, as compared to WT parasites. Our data suggests that Q plays an important role during survival of the parasites inside the mammalian host and may be at the heart of virulence.

P2.26

Mitochondrial tRNAs epitranscriptome in plants

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Transfer RNAs are universally conserved molecules essentially known for their implication in translation, and more and more non- canonical functions are currently described. They are also the most post-transcriptionnally modified RNAs, with an average of 17% of modified residues. However, while these modifications are of great importance to understand tRNAs structure, stability and functions, data regarding them in plants are still very scarce. In order to resolve this lack of knowledge, we want to get a complete mapping of the plant tRNAs epitranscriptome. A focus is made on mitochondrial tRNAs, with the model plants *Arabidopsis thaliana* and *Solanum tuberosum*. As first experiment, total *S. tuberosum* mitochondrial tRNAs have been digested into mononucleosides and analysed by HPLC-MS. In parallel, some specific mitochondrial tRNAs from A. thaliana have been purified with a complementary oligonucleotide and 1) digested into mononucleosides in order to know what modifications are carried by these specific tRNAs. 2) partially digested in order to map these modifications on the tRNAs sequences. These preliminary results are a first step before getting a complete mapping plant mitochondrial tRNAs modifications.

P2.27

tRNA Modification Mapping using Mass Spectrometry

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tRNAs are the key for mRNA translation by connecting the amino acids to specific codon sequences. Importantly, they carry chemically modified nucleosides. Hypomodification can disturb protein homeostasis, which is a hallmark of several human diseases. However, the role of tRNA modifications in such pathologies remains largely enigmatic and it will be crucial to quantify tRNA modifications in the sequence context to change this. Currently, using sequence-specific RNAses followed by LC-MS/MS is the only applicable method. Two challenges of this method exist: 1) RNAses are toxic to the expression host, 2) due to the short length of the digestion products of commercially available RNAses, sequence coverage is low. Hence, it is critical to combine RNAses of different specificities. We express three base-specific RNAses in the cytoplasm of E. coli, which combined will improve the sequence coverage by 20%. We currently develop a LC-MS/MS method and a tailored software for data analysis. This will allow us to quantify chemical RNA modifications and to localize them within tRNA sequences, enabling us to shed light on their biological role during stress and in the etiology of human diseases.

P2.28

tRNA methyltransferase TrmX promote breast cancer metastasis through transcriptional regulation

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tRNA undergoes numerous chemical modifications which may affect its structure and function. Aberrant tRNA modifications were found in numerous cancers, while the mechanism remains largely unclear. In the current study, we found out that the gene TrmX is extremely high-expressed in some tumor tissues versus adjacent from breast cancer patients. The tRNA methyltransferase enzymatic activities of TrmX were confirmed by in vitro and in vivo analyses, and the RNA substrate and RNA recognition mechanism of TrmX were identified. The corresponding tRNA modification is up regulated as the expression of TrmX in tumor tissues. Results from loss- or gain-of function assays in cell line and in mouse show that TrmX promote cell migration and tumor metastasis. Interestingly, TrmX is located in both the nucleus and cytoplasm. Subsequently, we found out that TrmX regulates the expression of oncogenes through directly binding to DNA and further modulate gene transcription in nucleus. Chip-seq was performed to identify the down-stream target genes of TrmX, and the result is highly consistent with the global transcriptome analyses. Collectively, our results show that TrmX is a human tRNA modification enzyme that regulates breast cancer metastasis through transcriptional regulation.

P2.29

A totally unexpected mechanism of the bacterial t^{6}A tRNA-modification

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The universal N^6-threonylcarbamoyladenosine (t^{6}A) modification at position A37 of ANN-decoding tRNAs is essential for translational fidelity. In bacteria the TsaC enzyme first synthesizes an L- threonylcarbamoyladenylate (TC-AMP) intermediate. In cooperation with TsaB and TsaE, TsaD then transfers the L-threonylcarbamoyl-moiety from TC-AMP onto tRNA. We will present the crystal structure of the TsaB-TsaE- TsaD (TsaBDE) complex of *Thermotoga maritima* in presence of a non-hydrolysable AMPCPP. TsaE is positioned at the entrance of the active site pocket of TsaD, contacting both the TsaB and TsaD subunits and prohibiting simultaneous tRNA binding. AMPCPP occupies the ATP binding site of TsaE and is sandwiched between TsaE and TsaD. Unexpectedly, the binding of TsaE partially denatures the active site of TsaD causing loss of its essential metal binding sites. TsaE interferes in a pre- or post-catalytic step and its binding to TsaBD is regulated by ATP hydrolysis. This novel binding mode and activation mechanism of TsaE offers good opportunities for antimicrobial drug development.

P2.30

Acid/Base Residues in 4-Thiouridine Synthetase

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4-Thiouridine at position 8 in tRNA serves as a photosensor in bacteria by cross-linking to cytidine-13 upon exposure to UV light, which triggers controlled growth arrest. Conserved acid/base residues in 4-thiouridine synthetae (also called ThiI) were mutated to test for their possible participation in tRNA binding or catalysis. The effect of substitution at those positions in the E. coli enzyme was examined by in vivo screening and in vitro 4-thiouridine generation assays. The results will be presented and interpreted in light of a homology model based on the crystal structure of the shorter 4-thiouridine synthetase from T. maritima.

P2.31

Characterization of fitness-enhancing Burkholderia cenocepacia tRNA^{Ile2} variants

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Bacterial tRNA ^{Ile2} is initially transcribed with a CAU anticodon that is modified to LAU by the enzyme tRNA isoleucine lysidine synthetase (TilS). Installation of lysidine at the wobble position restricts tRNA ^{Ile2} pairing at the ribosome to the rare AUA isoleucine codon. Single nucleotide polymorphisms (SNPs) in the Burkholderia cenocepacia TilS and tRNA ^{Ile2} genes were identified following forward evolution experiments in a nutrient-limited environment. Selected strains exhibited fitness increases of up to 25%. We have shown that the enzyme variants display significant functional loss, and we are now characterizing the tRNA ^{Ile2} variants U15C and U63G as substrates for BcTilS. The efficacy of tRNA ^{Ile2} variants as substrates of TilS is quantified in vitro using radiolabeled lysine and a filter binding assay. In vivo lysidine levels are identified by LC/MS of cellular tRNA. The TilS:tRNA binding affinity is evaluated by an electrophoretic mobility gel shift assay.

P2.32

Comparison of non-essential *Burkholderia cenocepacia* TilS to its vital *Escherichia coli* homolog

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tRNA isoleucine lysidine synthetase (TilS) is proposed to be an essential prokaryotic gene product, as demonstrated using temperature sensitive TilS (TilS^{TS}) variants expressed in *Escherichia coli*. During growth at TilS^{TS}permissive temperatures, cells replicate in a normal fashion compared to cells harboring the WT TilS; while at elevated temperatures that inactivate TilTS, AUA dependent translational deficiencies lead to cell death. We have previously shown that mutations causing severe functional loss in *Burkholderia cenocepacia* TilS (BcTilS) are associated with a gain-of-fitness phenotype under amino acid and trace metal starvation conditions. Here we identify key differences in substrate recognition between *E. coli* TilS (EcTilS) and BcTilS and present a comparative kinetic analysis of these enzymes. EcTilS enzymatic efficiency is substantially higher than that of its *B. cenocepacia* homolog. Furthermore we show that BcTilS uses a unique set recognition elements within its tRNA^{Ile2} substrate compared to those previously reported from EcTilS. We also demonstrate that the loss-of-function substitutions identified in *B. cenocepacia* have vastly different effects on enzymatic function when transplanted into the *E. coli* enzyme.

P2.33

Functional analysis of pre-tRNA capping in budding yeast

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In eukaryote, 5' methylguanosine cap structure is installed co-transcriptionally on RNA pol II transcripts, and play pivotal roles in RNA stability, export and translation. Previously, we happened to find that tRNA precursors (pre-tRNAs) bearing 5' leader sequences had methylguanosine cap structures in Saccharomyces cerevisiae, nervertheless tRNA genes are transcribed by RNA pol III. We named this phenomenon "pre-tRNA capping" (Ohira and Suzuki, 2016). Genetic study revealed that 5' capping stabilizes pre-tRNAs by protecting from 5' exonucleolytic degradation. However, the biological significance of pre-tRNA capping remains to be investigated. To identify pre-tRNA species with 5' capping, pre-tRNAs accumulated in RNase P-repressed cells were immunoprecipitated with anti-methylguanosine antibody and subjected to deep sequencing, elucidating a subset of pre-tRNAs 5' capped efficiently. In addition, we found that eIF-4E interacts with 5' capped pre-tRNAs in the cell, indicating that 5' capped pre-tRNAs have a functional role in sequestering eIF-4E to modulate translation initiation.

P2.34

Structural and functional study of two tRNA thiolation [4Fe-4S] cluster dependent enzymes

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Post-transcriptional modifications of transfer RNA (tRNA) are essential for translational fidelity. Sulfur is present in several nucleosides within tRNAs. The thiolation of the universally conserved methyl-uridine at position 54, catalyzed by the enzyme called TtuA, stabilizes tRNAs from thermophilic bacteria and hyperthermophilic archaea and is required for growth at high temperature. Moreover, the thiolation of uridine 34 in the anticodon loop of tRNAs, which is required for normal growth and stress resistance in yeast, is carried out by two completely different systems: the well-studied MnmA protein (present in bacteria and in the eukaryotic mitochondrion) and the Nsc6/NcsA/Ctu1 proteins in all other organisms, including the eukaryotic cytoplasm.

Spectroscopic and enzymatic studies demonstrate that TtuA and NcsA catalyze the simple non-redox substitution of the C2-uridine carbonyl oxygen by sulfur using a [4Fe-4S] cluster and thus are representatives of a new enzymes superfamily. A series of crystal structures of TtuA and NcsA show that: (i) the [4Fe-4S] cluster is ligated by three cysteines only that are fully conserved, allowing the fourth unique iron to bind an exogenous sulfide, which likely acts as the sulfurating agent; (ii) the ATP-binding site is adjacent to the cluster [1]. A new mechanism for tRNA sulfuration is proposed, in which the unique iron of the catalytic cluster functions as a sulfur carrier, opening new perspectives regarding functions of iron-sulfur proteins in biology.

Interestingly, TtuA and NcsA show the highest structural similarity with lysidine synthetase (TilS), an N- type ATP pyrophosphatase that targets position 34 in tRNA, whose structure has been determined in complex with either ATP or the tRNA substrate. Mdels of the TtuA/tRNA and NcsA/tRNA complexes suggest that a flipped adenylated uridine intermediate is formed during catalysis by the tRNA thiolases and that two tRNA molecules are likely bound on each side of one TtuA/NcsA dimer, with the two zinc finger domains belonging to different polypeptide chains being used to clamp the loop containing the target uridine on opposite sides.

[1]. Nonredox thiolation in tRNA occurring via sulfur activation by a [4Fe-4S] cluster. Arragain S, Bimai O, Legrand P, Caillat S, Ravanat JL, Touati N, Binet L, Atta M, Fontecave M, Golinelli-Pimpaneau B. Proc Natl Acad Sci U S A. 2017, 114(28):7355-7360

P2.35

Nuclear-cytoplasmic tRNA dynamics in required for tRNA maturation in *Trypanosoma brucei*

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A lingering question in retrograde tRNA transport is whether it is relegated to S. cerevisiae and multicellular eukaryotes or alternatively, is a pathway with deeper evolutionary roots. In the early branching eukaryote T. brucei, tRNA splicing, like in yeast, occurs in the cytoplasm. In this report, we used a combination of cell fractionation and molecular approaches that show the presence of significant amounts of spliced tRNATyr in the nucleus of T. brucei. Notably, the modification enzyme tRNA-guanine transglycosylase (TGT) localizes to the nucleus and, as shown here, is not able to add queuosine (Q) to an intron-containing tRNA. We suggest that retrograde transport is partly the result of the differential intracellular localization of the splicing machinery (cytoplasmic) and a modification enzyme, TGT (nuclear). In addition, RNAi silencing of a novel tRNA exporter led to accumulation of mature spliced tRNATyr supporting the existence of the tRNA retrograde pathway. Moreover, the level of Q modified tRNA is influenced by the efficiency of the tRNA nuclear export. These findings expand the evolutionary distribution of retrograde transport to include early diverging eukaryotes, while highlighting its importance for Q biosynthesis.

P2.36

THG1L catalyses the addition of the aminoacylation determinant G_{-1} on human mitochondrial tRNAHis

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Almost all $tRNA^{His}$ molecules analysed to date deviate from the canonical structure of tRNAs by the presence of an additional 5’ guanine residue (G_{-1}). The source of this additional nucleotide has been well characterised in *Saccharomyces cerevisiae*, where it has been shown to be post-transcriptionally added by Thg1 in the cytosol, and genomically encoded and retained after cleavage in mitochondria. Here, we demonstrate that human mitochondria differ from yeast through the utilisation of the ‘cytosolic pathway’ of G_{-1} inclusion, namely, post-transcriptional addition by Thg1-like 1 (THG1L). We further show that the presence of G_{-1} is critically required for the aminoacylation of mitochondrial $tRNA^{His}$ and therefore mitochondrial translation as a whole.

P2.37

CCA-addition in the cold: characterization of the enzyme from *Planococcus halocryophilus*

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Enzymes from psychrophilic organisms usually achieve catalysis at low temperatures by a reduction of secondary structure elements to increase their flexibility. For CCA-adding enzymes, there is a discrepancy between the need of a tightly controlled flexibility during polymerization and an increased flexibility as a strategy for cold adaptation. Using biochemical and structural analyses, we investigated the properties of the CCA-adding enzyme (48 kDa) from *Planococcus halocryophilus*, a bacterium from the Arctic permafrost which grows at -15 °C. This enzyme is strongly cold-adapted and catalyzes CCA-addition down to 0°C *in vitro*.
Crystal structures of the apo enzyme and of the complex with CTP were determined at high resolution. The nucleotide binding pocket is located in the groove of the neck domain, next to the body domain displaying 10% less alpha-helices than that of *Geobacillus stearothermophilus* enzyme.
Detailed mutational analysis revealed that residues in a flexible element between head andneck domain – responsible to modulate domain movements during CCA addition – contribute to cold- adaptation. Furthermore, deep sequencing of the tRNA pool of *P. halocryophilus* indicates that the increased flexibility of the enzyme has an impact on the polymerization fidelity. Thus, CCA-addition in the cold is achieved at the expense of an increased error rate during CCA synthesis.

P2.38

Regulation of Elongator's U34 modification function by tRNA binding protein Kti12

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Zymocin is an anticodon nuclease that attacks tRNA species which carry Elongator dependent wobble uridine (U34) modifications. Hence, Elongator mutants help yeast cells to survive zymocin. Here, we show that deletion of KTI12, a gene coding for an Elongator interactor, causes zymocin resistance, too. In addition, Kti12 contributes to tRNA nonsense and missense suppression. Both depend on an NTP binding P-loop domain in Kti12 that is conserved with a tRNA kinase (PSTK), and when mutated, abolishes binding to the Elongator complex. This reinforces our view that Kti12 association to Elongator promotes U34 modification. In support of this notion are data showing that Elongator binding P-loop mutants have U34 modification defects. Intriguingly, like PSTK, Kti12 is able to bind tRNAs and NTPs. Together with NTP dependent effects on the capacity of Kti12 to bind Elongator, our data imply a potential cofactor requirement for the regulation of Elongator's U34 modification function by Kti12.

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P2.39

Hierarchical modification of tRNA by TrmA, TruB, and TrmB

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About 10% of nucleosides within tRNA are post-transcriptionally modified. TrmA and TruB are the only two enzymes that modify every tRNA introducing 5-methyluridine (m^5U) 54 and pseudouridine (Ψ) 55, respectively. TrmB generates 7- methylguanosine (m^7G) 46 in many tRNAs. Despite the abundance of tRNA modifications, it remains unclear how the tRNA modifications by many enzymes are concerted. TrmA, TruB and TrmB can each act on unmodified tRNA; however, in vivo they cannot interact simultaneously with nascent, unmodified tRNA due to steric hindrance. Thus, we hypothesize that these enzymes are likely to modify tRNA in a preferential, hierarchical fashion rather than a stochastic order. To test this, we used *in vitro* transcribed, single-modified tRNAs in binding and modification assays. Thereby, we show that TruB prefers to bind and act upon unmodified tRNA, whereas TrmA binds tRNA containing Ψ55 most strongly. Both TrmA and TruB prefer to bind and modify tRNA lacking m^7G46. Our results suggest that TrmA and TruB act early during tRNA maturation, whereas TrmB is likely to act later. This research lays the foundation to uncover how tRNAs are so highly modified in the cell.

P2.40

Characterization of decoding of the CGN codon family in *C. merolae* organelles

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As organelle genomes of various organisms do not code for the complete set of tRNA genes, their translation often requires import of nuclear-encoded tRNAs from cytoplasm. On the other hand, tRNA import does not necessarily explain all cases of incomplete tRNA sets, and no import of cytosolic tRNA has been suggested in chloroplasts or in human mitochondria. As for the CGN codon family of arginine, most eubacteria and organelles contain two species of anticodons; $tRNA^{Arg}$(ICG) of which the wobble position has a deaminated form of adenosine (I, inosine) recognizes CGU, CGC, and CGA codons, whereas $tRNA^{Arg}$ (CCG) recognizes the CGG codon. Organelle genomes of some plants, including P. patens, encode for both $tRNA^{Arg}$ (A/ICG) and $tRNA^{Arg}$ (CCG), while most of others, including A. thaliana and C. merolae, encode only for $tRNA^{Arg}$ (A/ICG), and the decoding mechanim of the latter is not clarified. To see how the plant organelles with $tRNA^{Arg}$ (A/ICG) decode the CGN codons, we analysed the inosine modification at the wobble position of $tRNA^{Arg}$ (A/ICG) and the tRNA import from cytoplasm in C. merolae, a unicellular red alga.

P2.41

Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease

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Modified uridine containing taurine, 5-taurinomethyluridine (τm^5U), is found at the wobble position of mitochondrial (mt-)tRNAs. We previously reported that τm^5U is absent in mt-tRNAs with pathogenic point mutations associated with mitochondrial diseases. However, biogenesis and physiological role of τm^5U remain elusive.
Here, we successfully reconstituted τm^5U on mt-tRNA in vitro catalyzed by MTO1 and GTPBP3 compelx in the presence of 5,10-methylene-tetrahydrofolate and taurine as substrates. GTPBP3-knockout cells exhibited respiratory impairment and reduced mitochondrial translation. Very little τm^5U was detected in patient's cells with the GTPBP3 mutation, showing that the lack of τm^5U results in pathological consequences. Taurine depletion downreguled τm^5U frequency in cultured cells and animal tissues (cat liver and flatfish muscle), suggesting that mitochondrial protein synthesis is dynamically regulated by sensing intracellular metabolic status. Moreover, we happened to detect 5- carboxymethylaminomethyluridine ($cmnm^5U$), in which taurine moiety of τm^5U is replaced with glycine, in mt-tRNAs under taurine-depleted conditions. This is the first instance of chemical switching of RNA modification under physiological conditions in human mitochondria.

P2.42

Pseudouridines in tRNA play a role in maintaining genomic mutation frequency in Pseuduomonas species

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tRNAs are the most abundantly modified nucleic acids in the cell. tRNA molecules have numerous interaction partners that are crucial for their functionality and this may be the reason for the complex set of modifications. Still, the function of many modifications is not clear. Modifications in the anticodon loop can facilitate anticodon-codon recognition and help to maintain translation fidelity. We are studying the role of pseudouridines (Ψ) at tRNA positions 32 or 38-40 in Escherichia coli MG1655, Pseudomonas aeruginosa PAO1 and P. putida PaW85.

In our previous studies we identified the tRNA modification enzyme TruA as a factor in mutation frequency, as its disruption significantly increases mutation rate in P. putida. TruA catalyzes the isomerization of uridine to Ψ in positions 38-40. On the other side of the anticodon, position 32 is pseudouridylated by the enzyme RluA. Here we show that the lack of either of these modification enzymes causes a 3-5-fold rise in mutation frequency in P. putida, whereas in P. aeruginosa the effect on mutation frequency is less relevant. This effect is specifically caused by the lack of pseudouridylation activity, as mutating the catalytic aspartic acid of either enzyme caused the same phenotype in P. putida as deletion of truA. Contrary to the effect on mutations the truA and rluA-deficient strains display different non- mutation related phenotypes in P. putida, P. aeruginosa and E. coli (e. g. growth rate, stress tolerance). These intriguing results indicate that tRNA modifications can affect DNA mutations via yet unknown mechanism.

P2.43

The role of tRNA modification on proteome homeostasis

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Protein synthesis is central to life and is being intensively studied at various levels. The exception is mRNA translation fidelity whose study has been hampered by technical difficulties in detecting amino acid misincorporations in proteins. We are investigating the role of RNA modification by RNA modifying enzymes (RNAmods) in protein synthesis efficiency and accuracy. Thus, we have set up a yeast genetic screen to identify tRNAmods that play a role on protein solubility and proteome homeostasis and identified a subgroup of yeast tRNAmods that play roles in protein synthesis fidelity and folding, preventing protein aggregation. The data indicate that some tRNAmods influence tRNA stability and decoding efficiency while others have direct impact on mRNA decoding fidelity. We identified the aggregated proteins in tRNAmods KOs and found that genes encoding insoluble proteins were enriched in codons decoded by the hypomodified tRNAs. Amino acid misincorporations detected at these codon sites showed that tRNA U_{34} modifications play an important role in the control translational accuracy.

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P2.44

The crystal structure of the enterohemorrhagic *E. coli* AtaT-AtaR toxin-antitoxin complex

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Bacterial toxin-antitoxin (TA) systems control key cellular processes to facilitate cell survival during stress conditions by causing cell dormancy and persistence. In the case of type II TA systems, toxins are bound and inactivated by antitoxins under normal growth conditions, whereas stress conditions induce specific degradation of antitoxins and lead toxin activation. AtaT (toxin) -AtaR (antitoxin) is a type II TA system found in enterohemorrhagic *E. coli* O157:H7. Recent study showed that AtaT is a GNAT family enzyme and a dimeric form of AtaT transfers acetyl group from acetyl-CoA to the amine group of methionyl aminoacyl moiety of initiator $tRNA^{fMet}$, thereby AtaT inhibits the initiation of protein synthesis. Here we present the crystal structure of E. coli O157:H7 AtaT-AtaR complex in the presence or absence of acetyl-CoA. AtaT-AtaR is a heterohexameric complex ($AtaT_2$-$AtaR_4$), where two dimeric AtaRs bridge two monomeric AtaTs and prevent AtaTs from forming an active homodimer. Since AtaT alone forms an active homodimer, it is likely that AtaT dimer is dissociated, when it is complexed with dimeric AtaRs or that AtaT is prevented from its dimerization in the presence of AtaR.

P2.45

Direct nutritional control of protein translation by queuosine

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Global protein translation as well as translation at the codon level can be regulated by tRNA modifications. In eukaryotes, levels of tRNA queuosinylation reflect the bioavailability of the precursor queuine, which is salvaged from the diet and gut microbiota. We have shown that nutritionally determined Q-tRNA levels promote Dnmt2 mediated methylation of tRNA- Asp and control translational speed of Q-decoded codons as well as at near-cognate codons. Deregulation of translation upon queuine depletion result in unfolded proteins that trigger endoplasmic reticulum stress and activation of the unfolded protein response, both in cultured human cell lines and in germ-free mice fed with a queuosine-deficient diet. Taken together, our findings comprehensively resolve the role of this anticodon tRNA modification in the context of native protein translation and describe a novel mechanism that links nutritionally determined modification levels to effective polypeptide synthesis and cellular homeostasis.

P2.46

A totally unexpected mechanism of the bacterial t^{6}A tRNA-modification

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The universal N^6-threonylcarbamoyladenosine (t^{6}A) modification at position A37 of ANN-decoding tRNAs is essential for translational fidelity. In bacteria the TsaC enzyme first synthesizes an L- threonylcarbamoyladenylate (TC-AMP) intermediate. In cooperation with TsaB and TsaE, TsaD then transfers the L-threonylcarbamoyl-moiety from TC-AMP onto tRNA. We will present the crystal structure of the TsaB- TsaE-TsaD (TsaBDE) complex of Thermotoga maritima in presence of a non-hydrolysable AMPCPP. TsaE is positioned at the entrance of the active site pocket of TsaD, contacting both the TsaB and TsaD subunits and prohibiting simultaneous tRNA binding. AMPCPP occupies the ATP binding site of TsaE and is sandwiched between TsaE and TsaD. Unexpectedly, the binding of TsaE partially denatures the active site of TsaD causing loss of its essential metal binding sites. TsaE interferes in a pre- or post-catalytic step and its binding to TsaBD is regulated by ATP hydrolysis. This novel binding mode and activation mechanism of TsaE offers good opportunities for antimicrobial drug development.

P2.47

Characterization of the human TRM10 family of tRNA methyltransferases

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To date, more than 100 RNA modifications have been described, mostly in tRNAs. However, the synthesis pathways of many modifications, and their impact on the RNA molecule remain poorly understood. TRM10 is a widely conserved family of methyltransferases found in Archaea and Eukaryotes, responsible for the methylation of nitrogen 1 of purine-9 of tRNAs. The human genome encodes three forms of TRM10 methyltransferase; however, the specific role of the different forms remains unclear. The methyl group introduced interferes with canonical base pairing, and causes errors during reverse transcription. The modification can thus be identified as a hyper-error signature in NGS data. Here we used NGS to identify the full repertoire of tRNAs methylated at position-9 in a human cell line. Using CRISPR/Cas we have generated cell lines deleted for the members of the TRM10 family to investigate their role in tRNA modification. We found that the different members of the family have evolved distinct substrate specificity. We aim to elucidate the function of the expanded TRM10 family in vertebrates, their role in tRNA modification, and the importance of R9 methylation for translation.

P2.48

The crystal structure of Nanoarchaeum equitans tRNA (uracil-54, C5)-methyltransferase–RNA– cofactor ternary complex provides insights into substrate selectivity and enzymatic function

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The 5-methyluridine (m^5U, also termed rT for ribothymidine) is invariably found at position 54 in almost all tRNAs from Bacteria and Eukarya but rarely in Archaea. Ribothymidine is additionally present in rRNAs of all Bacteria and certain Archaea. We have recently shown that no m^5U is found in rRNAs from the archaeal N. equitans. However, its genome displays one gene possibly coding for a S-adenosyl-L-methionine-dependent RNA (uracil, C5)-methyltransferase (MTase). Here, we demonstrate that this enzyme (renamed $_{Neq}$TrmU54) catalyzes in vitro the formation of m^5U54 in tRNA. The crystal structure of $_{Neq}$TrmU54 in complex with its RNA substrate and cofactor at 2.3 Å resolution reveals strong similarity to its closest homolog $_{Pab}$TrmU54 from P. abyssi. The protein is organized into three domains like E. coli RlmD, which catalyzes the same reaction at position 1939 of 23S rRNA. Interestingly, the presence of two additional flexible loops in the central domain of $_{Neq}$TrmU54, involved in RNA binding, may explain the difference in substrate selectivity of both enzymes. The structure of the $_{Neq}$TrmU54–RNA–cofactor ternary complex also confirms the proposed catalytic mechanism of RNA m^5U MTases.

P2.49

Deletion of Trmu in Zebrafish revealed the essential role of tRNA modification in mitochondrial biogenesis and hearing function

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Trmu is a highly conserved tRNA modifying enzyme responsible for the biosynthesis of τm^5s^2U at the wobble position of $tRNA^{Gln}$, $tRNA^{Glu}$ and $tRNA^{Lys}$. Our previous investigations showed that TRMU mutation modulated the phenotypic manifestation of deafness-associated mitochondrial 12S rRNA mutation. However, the pathophysiology of TRMU deficiency remains poorly understood. Using the trmu knock-out zebrafish generated by CRISPR/Cas9 system, we demonstrated the abolished 2-thiouridine modification of U34 of mitochondrial $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$ in the trmu knock-out zebrafish. The elimination of this post-transcriptional modification mediated mitochondrial tRNA metabolisms, causing the global decreases in the levels of mitochondrial tRNAs. The aberrant mitochondrial tRNA metabolisms led to the impairment of mitochondrial translation, respiratory deficiencies and reductions of mitochondrial ATP production. These mitochondria dysfunctions caused the defects in hearing organs. Strikingly, $trmu^{-/-}$ mutant zebrafish displayed the abnormal startle response and swimming behaviors, significant decreases in the sizes of saccular otolith and numbers of hair cells in the auditory and vestibular organs. Furthermore, $trmu^{-/-}$ mutant zebrafish exhibited the significant reductions in the hair bundle densities in utricle, saccule and lagena. Therefore, our findings may provide new insights into the pathophysiology of deafness, which was manifested by the deficient modifications at wobble position of mitochondrial tRNAs.

P2.50

Gtpbp3 deficiency caused mitochondrial tRNA mobility shift and leads to hypertrophic cardiomyopathy in zebrafish

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Wobble uridine modificaitons of some mt-tRNAs are carried out by a set of nuclear-encoded mitochondrial genes GTPBP3, MTO1 and TRMU. It was reported mutations in GTPBP3 are associated with hypertrophic cardiomyopathy (HCM). However, the function of GTPBP3 in myocardium and cardiac development is not fully understood. Here, we generated a zebrafish gtpbp3 mutant line using the CRISPR/Cas9 system. Compared to control, gtpbp3 mutant exhibited delayed embryonic heart development, impaired heart function and developed HCM in adult. For the first time, we used native gel electrophoresis to show mobility shift in related mt-tRNAs, indicating tRNA structure change due to lack of modification and providing more direct evidence for Gtpbp3 mutation to cause a translation defect. Interestingly, we also found a huge increase in the aminoacylation level of mt tRNAs, and we deduced this as a result of the delayed translation process. This imbalance between free mt tRNAs and aminoacylated mt tRNAs might act together with translation defect to break mitochondrial homeostasis and lead to dysfunctional mitochondria.

P3.1

Shortening of tRNAs as a mechanism of translational silencing during stress

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Regulation of gene expression is essential for cell survival, especially under adverse growth conditions. The pathogenic protozoan parasite Trypanosoma brucei largely lacks the ability to regulate transcription of protein coding genes and all protein-coding genes are transcribed polycistronically. Therefore they rely on posttranscriptional means to regulate gene expression. We are particularly interested in understanding how translation is regulated, focusing on the role of tRNA in this process. One of our discoveries was the shortening of the bulk of the tRNAs during nutritional stress at the expense of the conserved 3'-CCA tail, which renders tRNAs unsuitable substrates for translation. Trimmed tRNAs can be repaired by the CCA adding enzyme and are therefore recycled for translation once normal growth conditions are restored. Despite the strong decrease in polysomes triggered by starvation the tRNAs lacking the CCA-tail are enriched in the polysome fraction, suggesting a function for these tRNA species on the ribosomes in translation control. We are at the moment working on the identification of the nuclease involved in CCA-tail shortening.

P3.2

Systematic detection of amino acid substitutions in proteome reveals a mechanistic basis of ribosome errors.

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Errors in translation can result either from the charging of a tRNA with a non-cognate amino acid or from the ribosome failing to discriminate against imperfect codon-anticodon complexes. Currently, our understanding of the causes of amino acid mis-incorporations is hindered by the lack of methods to observe errors at the full proteome. In this project, we have developed a pipeline aiming to systematically detect errors in entire proteomes using mass spectrometry data. We used this pipeline in order to characterize the spectrum of amino acid substitutions in both *E. coli* and *S. cerevisiae*. We found that most substitutions occur due to codon-anticodon mispairing rather than charging errors. We further showed that errors patterns seem to be similar in bacteria and yeast. By analyzing the set of substations obtained, we found that translation errors tend to occur at positions that are less evolutionarily conserved, minimally affect protein stability and are fastly translated. Finally, we explored how environmental perturbations affect translation error pattern by treating E. coli cells with a drug known to affect ribosomal proofreading as well as starving them to several amino acids.

P3.3

Oxidative stress response and adaptation by tRNAs mismethionylation and methionine misincorporation in the yeast *S. cerevisiae*

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The oxidative stress results in an excess of electrons carried by oxidants such as Reactive Oxygen Species. Misincorporation of methionine (Met) has been proposed to constitute an important antioxidant defense mechanism. It has been shown by tRNA microarrays that methionylation of non-cognate tRNAs by the methionyl-tRNA synthetase (MetRS) increased under oxidative stress and that some tRNAs are more likely mischarged by Met than others. To confirm these results and go further, we developed a bifluorescent reporter to compare misincoporation of Met for all codons in the yeast S. cerevisiae. Results show a preference for Met incorporation at all Leu codons in the absence of any oxidative stress (fermentation growth condition) in a wild type strain. After treatment with H_2O_2, misincorporation of Met at these codons increased substantially. Using an arc1Δ strain that mimics the diauxic shift and mitochondrial oxidative phosphorylation activity, the level of Met misincorporation at Leu codons increases and various new other codons trigger Met misincorporation. All these data suggest a global methionine mischarging during oxidative stress involving mismethionylation of unexpected tRNAs.

P3.4

Microfluidic high-throughput screening of codon-anticodon pairs accepted by the ribosome reveals the critical role of base modifications in tRNAs

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In order to determine the codon-anticodon pairing combinations efficiently accepted by the eukaryotic ribosome in the absence of modification in the anticodon loop and bias from competition between tRNAs or protein amino acid sequence, we took advantage of the IRES from the intergenic region (IGR) of the Cricket Paralysis Virus. It contains an essential pseudo knot PKI that structurally and functionally mimics a codon-anticodon helix. With a reporter containing the IGR upstream of the GFP coding sequence, we screened the entire set of 4,096 possible combinations using high-throughput screenings combining *in vitro* coupled transcription/translation and droplet-based microfluidics. Only 97 combinations are efficiently accepted and accommodated for translocation and further elongation: 38 combinations involve cognate recognition and 59 involve near-cognate recognition. A/U-rich codon-anticodon combinations did not promote GFP translation and the majority of missing combinations have a U at the first anticodon position. These data comfort the contribution of nucleotide modifications to the stability of triplet formation. Most of the near-cognate combinations (51) contain a G at the first position of the anticodon (numbered 34 of tRNA). In short, on the basis of the accepted combinations, we show that (1) in the absence of competition between tRNAs from the tRNA pool, as well as between tRNAs and release factors, the stability of the codon/anticodon triplet is a sufficient and major factor for productive translation; (2) in the absence of tRNA modifications in the anticodon loop, tRNA binding to the ribosomal decoding centre is either not productive (in A/U rich codon/anticodon triplets) or lead to extensive miscoding, especially with G34-containing tRNAs.

P3.5

Intron removal from tRNA genes in *S. cerevisiae*

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In yeast, total 59 tRNA genes generate intron-containing pre-tRNAs subjecting tRNA splicing on the mitochondrial surface. For decades mechanistic processes of tRNA splicing have been studied, whereas the biological roles of tRNA introns remain to be elucidated. We eliminated yeast tRNA introns from different isodecoder tRNA genes to evaluate their impact on cellular mechanisms mainly focused on protein synthesis. Here, we report that all intronless mutants maintained tRNA aminoacylation status derived from intronless tRNA genes and had little effect on global translation monitoring by polysome profiles. We also examined the nucleolar morphology by immunofluorescence for nucleolar marker Nop1 due to some mutants possessing cold sensitivity and 5.8S rRNA reduction. However, all mutants displayed typical round nucleolar shape, suggesting intron removal from certain tRNA genes could affect rRNA biogenesis but that may be independent of general nucleolus functions affecting its morphology. Together, tRNA introns in yeast genes could have minor roles on protein synthesis under non-stressed conditions, while they may have advantages for other cellular processes in different conditions.

P3.6

LeuRS-Urzyme Minihelix Aminoacylation

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The origin of codon-dependent translation remains one of science's quintessential mysteries. Aminoacyl-tRNA synthetases (aaRSs) likely played a central role in these events, as they enforce the rules of the genetic code by activating amino acids and attaching them to the corresponding tRNAs. Our long-term goal is to recapitulate as much of translation as possible with plausible ancestral components. We hypothesize that extant aaRSs and tRNAs are likely elaborations on much simpler prebiotic translational apparatuses. To test this hypothesis, we are reconstituting and testing the aminoacylation activity of an "ancestral" LeuRS, LeuRS- Urzyme, on a set of designed "ancestral" tRNA minihelices. Previously, we demonstrated aminoacylation activity for two other "ancestral" aaRSs, the TrpRS- and HisRS-Urzyme, on full length tRNAs, but have yet to demonstrate this activity for the LeuRS- Urzyme. Moreover, we have not tested whether any Urzyme aminoacylates minihelices. As Urzymes can interact only with the 3'CCA end, we expect that the LeuRS-Urzyme will acylate minihelices. Cognate Urzyme-minihelix pairs will provide a new paradigm for studies of the origin of codon-dependent translation.

P3.7

Physiological consequences of a decreased $tRNA^{Gly}$ activity under oxidative stress in *Escherichia coli*

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Escherichia coli is a model organism used to analyze the bacterial response to oxidative stress. There is plenty knowledge about transcriptional regulation of the response, but little is known about the role of the translation machinery in the adaptation to this condition. We performed a screening for changes in tRNA levels and found an inactivation of $tRNA^{Gly}$ and the consequent decrease of glycine aminoacylation under oxidative stress. These changes probably explain an altered efficiency of translation of glycine codons we have observed in this condition. Interestingly, this translational effect depends on the availability of that amino acid in the culture media. Modifying the *in vivo* concentration of $tRNA^{Gly}$ altered the response to oxidative stress suggesting the existence of a mechanism whereby bacteria modulate the translational machinery as a response to environmental alterations and the metabolic state of the cells in order to regulate its response to environmental changes.

P3.8

On the function of cryptic tRNA arrays in prokaryotes

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The number of tRNA genes varies dramatically, from less than 100 to more than 10^4, among the species of the tree of life (1). However, in Bacteria the number of tRNA genes is relatively low and consistent, with an average of 40-50 genes encoding non-redundant isoacceptor tRNAs per genome (2). In the vast majority of bacterial phyla individual tRNA genes are found scattered in the genome or occasionally forming short clusters of two to three genes. However, in addition to this complement of tRNA genes, some species sprinkled in the bacterial radiation contain one or two long tRNA gene arrays probably acquired by horizontal gene transfer (HGT) from a donor of the Firmicutes phylum, where such gene arrays are common (2-4). The function of these arrays in species outside of the Firmicutes phylum is intriguing and has been little investigated. Previous work showed that in addition to a set of 48 genes scattered in the chromosome encoding the housekeeping tRNA complement, the cyanobacteriun Anabaena sp PCC 7120 contains a tandem array of 23 genes (hereafter trn operon) in a plasmid. The tRNAs encoded in this array can be processed and charged but their level in the cell is extremely low (orders of magnitude below the level of the housekeeping tRNAs). Deletion of the array did not impair cell growth in diverse conditions (5), which suggests that this array, probably acquired by HGT, constitutes a non-functional relic in Anabaena (5).

To gain insights into the possible function of tRNA gene arrays in bacteria outside of the Firmicutes we analyzed the expression of the Anabaena trn operon in a wide variety of conditions and found that it is induced up to 10^3-fold in diverse stress conditions. Furthermore, most of the tRNAs encoded in this array are charged under induction conditions and some are found in the polysomal fraction. This indicates that these tRNAs participate in protein synthesis in stress conditions and suggests that they may play a role in preserving cell viability under stress. Interestingly, some observations indicate that a few of the tRNAs encoded in the trn operon are misacylated (i.e. charged with a non-canonical amino acid). Further consequences of the expression of the trn operon are still under investigation.

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P3.9

DNA damage-induced cell death relies on SLFN11-dependent cleavage of distinct type II tRNAs

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DNA-damaging agents (DDAs) represent the largest group of cancer drugs, but primary or secondary resistance severely limits their effectiveness. Reports of two large-scale screening efforts revealed that human Schlafen 11 (SLFN11) – a protein we previously found to inhibit translation of HIV encoded proteins due to atypical codon-usage in the viral RNA – sensitizes cancer cells to DDAs. We now show here that SLFN11 also inhibits translation of ATR upon DNA damage and thereby causes sensitivity to DDAs without disrupting early events of the DNA damage response. Type II tRNAs, which have a long variable stem of 13-19 bases instead of a short variable loop (4-5 bases), are cleaved by a SLFN11-dependent mechanism in response to DDAs. Type II tRNAs accounts for all serine and leucine codons, and we demonstrate that genes making frequent use of TTA (Leu) codon like ATR are subject to selective translational suppression by SLFN11. Gapmer-mediated down-regulation of tRNA-Leu-TTA resulted in ablated ATR protein expression and re-sensitization of SLFN11-deficient cells to DDAs. Our study uncovered a novel mechanism of codon-specific translational inhibition by SLFN11-dependent tRNA cleavage in the DNA damage response, and suggest that SLFN11-deficient cancer cells can be re- sensitized to DDA therapy by targeting ATR or tRNA-Leu-TAA.

P3.10

Cell cycle protein cdc13 and growth of *Schizosaccharomyces pombe* are affected upon overexpression of tRNA.

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Levels and chemical modifications of tRNA can affect their functions, including translation rate. The purpose of this work was to determine whether increasing the tRNA levels affects the expression and folding of proteins and/or the cell growth in *S. pombe*. We induced oxidative stress on S. pombe cells cultured in liquid medium by H_2O_2 and analyzed the tRNA levels. Under these conditions $tRNA^{Gly}_{UCC}$ and $tRNA^{Arg}_{UCU}$ increased their levels while $tRNA^{Thr}_{UGU}$ did not change. We studied the effect of the overexpression of these tRNAs in the expression and solubility of cdc13, a cyclin required for the G_2/M transition. Cdc13 mRNA possesses rare codons recognized by these tRNAs. Overexpression of tRNA genes in *S. pombe* increased aggregation of cdc13, but did not affect the mRNA and protein levels. Cells harboring increased tRNA levels showed a reduced colony size. Syncronized cells showed an elongated phenotype revealing a defect in cell division. In conclusion, overexpression of these tRNAs affected the solubility of cdc13 and the cell cycle of *S. pombe*. Whether aggregation of cdc13 and/or other cyclins is responsible for the phenotypes is under investigation.

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P3.11

Investigating biases in tRNA sequencing libraries

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Recent developments in RNA sequencing techniques have broadened our understanding of many small RNAs, such as microRNAs (miRNAs), small nucleolar RNAs (snoRNAs) and piwi-interacting RNAs (piRNAs). However, the application of small RNA sequencing technologies to better understand tRNA biology remains challenging. This is mainly due to abundant post-transcriptional modifications in tRNAs that inhibit typical approaches to library preparation, including cDNA synthesis and adapter ligation. Multiple techniques that rely upon high-throughput sequencing have been developed to reduce these problems and to ascertain tRNA expression in a quantitative manner. However, relatively little validation of the sequencing data has been conducted so far, and no comparison between these methods has been reported.
We have investigated the validity of several methods to prepare cDNA libraries of mature tRNAs from human cells. In particular, we have assessed the efficiency of reverse transcription and adapter ligation for individual demethylated tRNAs and investigated sources of sequence bias in tRNA-derived sequencing libraries. Based on these experiments, we offer recommendations for preparing cDNA libraries for tRNAs, and small RNAs in general, which could assist a more reliable interpretation of results from high-throughput sequencing experiments.

P3.12

Different effects of tRNA pseudouridines on translational fidelity in *E. coli* and Pseudomonas species

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Modified nucleosides play several roles in modulating tRNA structure and functions. Modifications in the anticodon loop direct codon recognition and facilitate codon-anticodon recognition. Modifications around the anticodon are important for optimal translational fidelity. Importance of pseudouridines outside anticodon is still enigmatic. Translational accuracy has been studied mostly in *E. coli*. Moreover, molecular mechanisms of aatRNA selection and control of error frequency have been elucidated using *in vitro* translation derived of *E. coli* system. We are interested to elucidate the role of pseudouridines in translational fidelity in diverse bacteria. Here we study translational fidelity of wt and pseudouridine deficient strains using three bacterial species: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Ps. putida*.

Pseudouridines are conserved around the anticodon stem at the positions 32, 38, 39, and 40 of many tRNA species. These psi residues are made by enzymes RluA (pos 32) and TruA (38-40). Physiological role of these pseudouridines is not known. We have devised a broad range plasmid derived dual luciferase mRNA translation fidelity assay system. The E. coli MG1655, Pseudomonas aeruginosa PAO1, and Ps. putida PaW85 strains lacking either TruA, RluA or both were tested for frameshift frequency at -1 (-1FS) and +1 (+1FS) sites and readthrough at UGA stop codon. Deletion of RluA does not have effect on the error frequency in any species. In contrast, deletion of TruA leads to increased -1FS at specific context in E. coli and Ps putida but not in Ps aeruginosa. Similar effect was found with UAG reathrough. Frequency of +1FS is hardly affected by absence of psi residues. The results point to the species specific effect of the conserved tRNA modification to translational fidelity. When wild-type bacteria are compared, frameshift is more frequent in Pseudomonas species as compared to E. coli. The difference between *E. coli* and Pseudomonas can be partially due to the E. coli derived programmed frameshift signal used. On the other hand, UGA readthrough occurs with the similar frequency in all three bacteria species. The results open new aspects of mRNA context specific effects on the translation and new physiological role of pseudouridine in tRNA.

P3.13

Investigating the role of MetRS on persister formation.

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Persisters cells enter a dormant state in which they become highly tolerant to antibiotics. After antibiotic treatment, persisters are able to reactivate and resume infection, leading to relapses and chronic disease. The mechanism of persister formation remains poorly understood. Recent studies identified Escherichia coli metG mutants (encoding methionyl-tRNA synthetase) with increased persister formation rates, establishing a link between aminoacyl-tRNA synthetases and persister formation. Characterization of these metG mutants revealed striking differences in the K_M of the substrates as well as variation in k_{cat}. Because of the prominent role aaRSs play in translation, we also explored effects of the metG mutants on cellular protein synthesis and ATP homeostasis in the presence of both cognate and non-cognate amino acids. Intriguingly, the MetRS mutants are sensitive to the non-cognate homocysteine, and we also have evidence that $tRNA^{Met}$ is mischarged with this amino acid. Under some stress conditions a certain degree of mistranslation may be beneficial as a means to expand phenotypic heterogeneity, suggesting a novel link between MetRS mistranslation and persister formation.

P3.14

The dual role of the 2'-OH group of A76 $tRNA^{Tyr}$ in the discrimination of D-Tyrosine during the first steps of translation

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Tyrosyl-tRNA-synthetase (TyrRS) is exceptional in its biochemical context: it weakly distinguishes enantiomers of Tyr and demonstrates dual aminoacylation on 2'- and 3'-OH groups of A76 $tRNA^{Tyr}$ To correct the mistakes in recognition of Tyr isomers, an additional enzyme, D-aminoacyl-tRNA-deacylase (DTD), exists. However, the catalytic role of OH groups of the $tRNA^{Tyr}$ A76 in the catalysis by TyrRS and DTD remained unknown. To address this issue, [^{32}P]-labelled $tRNA^{Tyr}$ substrates (A76, 2'd, 3'd) have been tested in vitro in aminoacylation and deacylation assays with Thermus thermophilus TyrRS and DTD. The aminoacylation activity of L-Tyr is similar on both 2' and 3'-OH groups of $tRNA^{Tyr}$. D-Tyr attaches to the 2'-OH group of A76 2800-fold faster comparing to the 3'-OH. Thus, TyrRS demonstrates significant preference toward 2'-OH in charging with D-Tyr. DTD catalyses the hydrolysis of D-Tyr-$tRNA^{Tyr}$ specifically from the 3'-OH group while the 2'-OH assists in this hydrolysis. It is worth to mention that the transacylation, occurring from the primary site (2'-OH) to the neighbor OH-group (3'-OH) is dramatically essential for further hydrolysis, similar to IleRS.

P3.15

Implications of discriminator based operational code for chirality based proofreading system DTD

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D-aminoacyl-tRNA deacylase (DTD) removes D-amino acids mischarged on tRNAs and therefore are implicated in enforcing homochirality in proteins. DTD's mechanistic design principle is based only on L-amino acid rejection and hence it acts on achiral glycine attached to tRNA. The activity on glycyl-tRNA is advantageous as DTD clears glycine mischarged on tRNA(Ala). We have identified ATD (Animalia-specific tRNA deacylase) as a new variant of DTD which rectifies a unique tRNA mis-selection problem of eukaryotic AlaRS. The structural basis of ATD function and its substrate specificity will be discussed in detail. Our recent studies have led us to identify key elements in tRNA which influence DTD's enzymatic activity. The study has revealed the role of discriminator base in ensuring faithful glycine delivery for protein biosynthesis. It throws light on the fundamental and underappreciated problem of discriminator base bias in all three branches of life and the evolutionary selection pressures that act on it.

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P3.16

KRAS^{G12C} mutation differentially rewires protein synthesis *via* c-MYC modulated translation initiation machinery recruitment in NSCLC

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KRAS mutants affect translation initiation by activating the MAPK and PI3K/AKT/mTOR signaling pathways, which functionally converge with components of the translational machinery, altering protein synthesis rates of several oncoproteins, including c-MYC. Concomitantly, c-MYC is recruited to control transcription by all RNA polymerases thus rewiring protein synthesis *via* modulation of ribosome biogenesis, tRNA transcription and formation of eIF4F complex. To decipher the effect of KRAS^{G12C} on translation initiation in NSCLC, we constructed TetON-inducible KRAS^{G12C} stable A549 and CL1-5 cell lines using TALENs. We observed that eIF4F formation is differentially regulated in a c-MYC-depictive manner. The 43S PIC formation along with the tRNAi^{Met} levels indicated variations in global protein synthesis rates. Several mitochondrial tRFs were found modulated and corelated with a possible translocation of KRAS^{G12C} towards mitochondrial membrane. In conclusion, we report a divergent response among two epithelial-derived NSCLC cell lines upon induction of the same KRAS mutant, which affects the rewiring of protein synthesis, tRNA fragmentation and possibly mitochondrial integrity.

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P3.17

tRNA structures in the ribosome

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The tRNA L-shape structure rationally demonstrates its functions, base-pairing with mRNA and transferring the cognate amino acid to the peptidyl transferase center. The 3D structure is organized mainly by interactions between D and TΘ loops together with participation of the variable region. Recently, structural dynamics of tRNA is explored, but there is little structural information about a class II tRNA. Here, we have determined the crystal structures of the 70S ribosome from *T. thermophilus* HB8 complexed with *E. coli* $tRNA^{Ser}$ cognate for AGY codons, SerV (93 nt). In obtained structures, the electron density for tRNA in the P-site was unambiguously visible and the structure of all nucleotides including a long variable region (21 nt) was determined confidently. The variable region forms eight basepairs starting from A45-U47p, which interacts with an insertion residue of D loop, G20b. The tip residue in the loop region (V-loop), A47f interacts with the edge of the A-site finger, helix 38 in the 23S rRNA (H38). In addition, the edge of H38, A887 and A47g in V-loop are close to ribosomal protein S13. Meanwhile, variable regions of E-site and A-site tRNAs exhibit poor electron density, suggesting that there is no significant interaction with any ribosomal components. Structural changes in the vicinity of V-loop during translocation will be discussed.

P3.18

tRNA-dependent mechanism of the errors editing in translation quality control

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Fidelity of translation is controlled by several mechanisms including the proofreading mechanisms by aminoacyl- tRNA-synthetases and trans-editing factors. We have studied molecular mechanisms of editing by two different editing enzymes, leucyl-tRNA synthetase (LeuRS) and D-aminoacyl-tRNA-deacylase (DTD) from Thermus thermophiles, using a number of approaches, including molecular modeling, quantum-mechanical calculations, site-directed mutagenesis enzyme and modification of tRNA. Our intensive alanine scanning mutagenesis of LeuRS and DTD editing sites has failed to identify catalytic residues for hydrolysis within the active site. On the other hands, modification of $tRNA^{Tyr}$ at the 2'-OH of A76 and $tRNA^{Leu}$ at the 3'-OH of A76 by replacing them each with a proton, revealed an essential function for these groups in hydrolysis. On the basis of obtained experimental results and our QM calculations we suggest tRNA-dependent mechanism of post-transfer editing by LeuRS and DTD. The most important element of that mechanism is the formation of intramolecular hydrogen bond between the 2'-OH or 3'-OH of A76 and carbonyl group of the substrate, which strongly facilitate the hydrolysis reaction.

P3.19

Mechanism of EF-P(Arg32) rhamnosylation revealed by the crystal structure of glycosyltransferase EarP·translation factor EF-P complex

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Translation elongation factor P (EF-P), a ubiquitous protein over the entire range of bacterial species, rescues ribosomal stalling at consecutive prolines in proteins. In *Escherichia coli* and *Salmonella enterica*, the 🚲lysyl modification of Lys34 in EF-P is important for the EF-P activity. The 🚲lysylation pathway is conserved among less than 30% of bacteria. Recent reports found that the *Shewanella oneidensis*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis* EF-P proteins, containing an Arg residue at position 32 (corresponding to Lys34 in E. coli EF-P), are modified with the sugar rhamnose by the glycosyltransferase EarP. In addition to the 🚲lysylation of EF-P(Lys34), the rhamnosylation of EF-P(Arg32) is important for the EF-P activity, cell growth, pathogenicity, and antibiotic resistance.

EarP specifically transfers the rhamnosyl moiety from dTDP-🚲L-rhamnose to Arg32 of EF-P. To elucidate the detailed reaction mechanisms of EF-P(Arg32) rhamnosylation, we solved the crystal structures of the N. meningitidis EarP apo form, and the EarP/dTDP-rhamnose and EarP/EF-P complexes. The EarP structure contains two tandem Rossmann-fold domains, thus classifying EarP in glycosyltransferase superfamily B. EarP forms numerous interactions that specifically recognize the conserved residues of EF-P, and binds the entire 🚲sheet structure of EF-P domain I. Thereby, Arg32 of EF-P is properly positioned at the EarP active site, and causes a structural change in a conserved dTDP-🚲 L-rhamnose-binding loop of EarP. Furthermore, Asp20 of EarP was identified as the general base residue in the S_N2 reaction. We propose that the Arg32 binding and the accompanying structural change of EarP convert the rhamnose-ring conformation, thereby facilitating the rhamnosylation of EF-P(Arg32). Understanding the EarP structure and its unique reaction mechanism will enable the structure-based design of drug candidates against a specific group of pathogenic bacteria, including *N. meningitidis*, *Neisseria gonorrhoeae*, and *Bordetella pertussis*.

P4.1

Examining the relation between tRNA expression and cellular proliferation state

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We have previously shown that proliferating and differentiated mammalian cells express distinct sets of tRNA molecules, that are compatible with the codon usage of either pro-proliferation genes or pro-differentiation genes, respectively (Gingold et al., Cell 2014). This study aimed to assess the essentiality of the coordinated changes in the tRNA pool and the proliferative state of the cell. We used CRISPR-iCas9 technique to target and manipulate the pro-proliferation and pro- differentiation tRNA levels in HeLa cell line using sgRNA library we designed, in which each sgRNA is targeting one tRNA isoacceptor family. To explore the effect of tRNA knock-out on HeLa cells, we activated iCas9 in a heterogeneous cell population, in which each cell carries one of the sgRNA types, and sequenced the sgRNAs. The results indicate that reduction in the pro-proliferation tRNA levels reduced the growth rate of the cells, while pro-differentiation tRNA knock- outs showed little effect on cellular growth. We further explored the impact of editing on the tRNA gene sequence and expression by targeting single tRNA isoacceptor family, following activation of iCas9. We then sequenced the targeted tRNAs, both in the DNA and RNA level. Our results suggest that HeLa cells do not tolerate Indel mutation in pro-proliferation tRNA genes, as indicated by the rapid elimination of such cells from the population. In contrast, cells carrying Indel mutation in the pro-differentiation tRNA genes didn’t show any growth defect and continued to propagate. These results indicate an active role of pro-proliferation tRNAs in the cellular proliferation state.

P4.2

An improved workflow for quantitative tRNA transcriptomics.

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Accurate, global tRNA quantification is necessary for understanding how tRNA pools impact cell physiology. High-throughput tRNA transcriptomics has been impeded experimentally and computationally by tRNA modifications and structure, as well as sequence similarity between multi-copy isoacceptor families. We present improvements to transcriptome-wide tRNA abundance measurements in both of these regards. We compared recent approaches to overcoming reverse transcription roadblocks to find a striking variation in their ability to mitigate tRNA sequencing bias. By using a highly thermostable reverse transcriptase under conditions that favor misincorporation at modified positions, we achieve a uniform tRNA coverage without biases towards particular tRNA species. Accounting for misincorporations at modified residues in our computational analysis drastically increases read alignment sensitivity and specificity, further improving tRNA quantification. We have used our optimized workflow for quantitative tRNA transcriptomics to define how modification defects impact translationally available tRNA pools.

P4.3

New tyrosine identity set in Plasmodium falciparum apicoplast

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Aminoacylation is a highly specific process that relies on the presence of identity elements, usually conserved throughout evolution. However, the tyrosylation system escapes this rule: $tRNA^{Tyr}$ and tyrosyl-tRNA synthetases (TyrRS) have developed features that prevent cross-tyrosylation between different phylae. On the tRNA side, this barrier consists in a unique element: the 1-72 base pair (G-C in prokaryotes, C-G in eukaryotes/archaea). Surprisingly, in a relict plastid (apicoplast) in the human malaria parasite P. falciparum, $tRNA^{Tyr}$ share an uncommon A1-U72 pair. Given the importance of the aminoacylation process and the apicoplast for the parasite survival, we aimed to decipher the identity rules that govern apicoplastic tyrosylation.
We produced an "harmonized version" of the apicoplastic TyrRS (apiTyrRS); and the $tRNA^{Tyr}$ sequences were in vitro transcribed, both WT and mutated at strategic positions. Kinetic tyrosylation parameters were determined and compared to the WT molecule. Results are in favor of an unusual recognition pattern between the apicoplastic $tRNA^{Tyr}$ by its cognate TyrRS. These peculiar properties make apiTyrRS a promising candidate for antimalarial drug development.

P4.4

tRNAs with Multiple Introns Are Widely Spread in Fungi

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A portion of precursor transfer RNAs contain introns that must be removed during maturation. Most tRNA introns are located one nucleotide downstream of the anticodon, although numerous tRNAs introns in archaea have been found in other, seemingly random “noncanonical” positions. In eukaryotes, noncanonical tRNA introns were believed to be rare, given they have been identified only in two algae. Using tRNAscan-SE and comparative analyses, we show that these noncanonical introns are spread across at least nine species in Pezizomycotina of Fungi, with Penicillium rubens having the most (20 out of 217) among the studied genomes. The introns are located in different positions within the genes, similar to those previously found in archaea and algae. Unlike well-studied fungi such as Saccharomyces cerevisiae, many species in Pezizomycotina also have a majority of tRNAs that contain introns. Interestingly, most of the new noncanonical introns were found in tRNA genes that also have a canonical intron. Although the acquistion mechanism of these introns is still unknown, our findings provide a new perspective on eukaryotic tRNA gene evolution.

P4.5

Looking at the end: Sequencing tRNA 3'-termini provides new information about maturation and integrity

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Anticodon and 3'-terminus are crucial parts for canonical tRNA function. The essential mature 3'-CCA-tail is created by ribonucleases during 3'-trailer processing and maintained by the CCA-adding enzyme. Furthermore, the 3-terminus can be elongated by poly(A) polymerase or the CCA-adding enzyme to generate a tag for degradation. We employed a new sequencing strategy by preparing libraries from the 3'-terminal part of E. coli tRNAs only. By using this short hypo- modified segment we avoid sequencing biases from secondary structure or modifications. This allowed us to not only quantify mature tRNA, but also precursors of different lengths, shortened and elongated tRNA. Our results revealed that 3'-trailer processing efficiency varies greatly among different tRNA species. In addition, in contrast to stationary growth phase, in the exponential phase a significant amount of tRNAs has shortened CCA-tails and is translation-incompetent. Integrity varied largely among different tRNA species and seemed to depend on the identity of discriminator base. We hypothesize, that the CCA-tail of certain tRNAs is damaged during 3'-trailer processing and needs to be repaired by the CCA-adding enzyme.

P4.6

Murine aminoacyl-tRNA synthetases: current knowledge representation

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Aminoacyl-tRNA synthetases are well studied as part of research efforts to understand the mechanisms of translation. Data about murine aminoacyl-tRNA synthetases come from the peer-reviewed literature or from inferences from comparative context of orthologs or phylogenetics. Comprehensive integration of these data is essential for data analysis
The Mouse Genome Informatics (MGI, http://www.informatics.jax.org,) is the international database resource for the laboratory mouse, providing integrated genetic, genomic, and biological data to facilitate the study of human health and disease. MGI uses the Gene Ontology (GO, http://www.geneontology.org) for functional annotation of mouse genes. GO defines concepts used to describe gene product functioning, location, and participation in biological processes, as well as relationships between these concepts. Because a single eukaryotic gene can encode multiple protein isoforms, MGI uses Protein Ontology (PRO (http://proconsortium.org/pro) which supplies unique identifiers to specific proteoforms. These forms are organized in an ontological framework that explicitly describes how these entities relate to each other. These resources provides an overview of the genomic and proteomic data associated with usage of alternate promoters or polyadenylation sites, alternative splicing of the primary transcript to generate different mRNAs, selection of alternative start sites during translation of an mRNA, and/or single or multiple post-translational processing events including proteolytic cleavage as well as amino acid modifications. An overview of genomic and proteomic data associated with murine aminoacyl-tRNA synthetases is outlined here, using Gars as an example.
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P4.7

Insights into Genome Recoding from the Mechanism of a Classic Quadruplet-Codon Reader

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While quadruplet codons are attractive for incorporating non-canonical amino acids to expand the genetic code, little is known of how they are translated. Here we provide mechanistic insight into translation of quadruplet codons by a classic quadruplet-reading tRNA, isolated as SufB2 with GGG-G as the expanded anticodon. In contrast to the conventional perception, we show that SufB2 is a triplet reader of the proline CCC codon at the ribosomal A-site but that it transforms to a quadruplet-reader of CCC-C during translocation to the P-site. This transformation, however, is suppressed upon inhibition of quadruplet pairing or upon post- transcriptional modifications that occur at purine 37 on the 3'-side of the anticodon. This study establishes a framework for how to improve quadruplet reading in new strategies of genome expansion and highlights the potential of tRNA translocation across the ribosome as a regulator of quadruplet reading.

P4.8

CoLoC-seq, a new genome-wide approach to profile organellar RNA importomes

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Mitochondria are essential organelles of eukaryotic cells involved in several key processes, including cellular respiration, apoptosis and iron-sulfur clusters formation. Despite the presence of their own small genome, mitochondrial functions critically depend on the import of proteins and noncoding RNAs (tRNAs, 5S rRNA, miRNAs...) encoded by the nucleus. However, detection of partially imported RNAs is often confounded by the persistence of cytosolic contaminant RNAs even after most thorough purification of organelles. To distinguish between RNAs genuinely present inside mitochondria and mere contaminant, our laboratory developed a conceptually novel methodology, Controlled Level of Contamination coupled with sequencing (CoLoC-seq). Using CoLoC-seq procedure we comprehensively landscaped the RNA importome of human HEK293T cells, confirmed the mitochondrial import status of some nuclear-encoded RNAs and identified a few novel imported RNAs. Our analyses also clarified the unresolved import status of RNAse MRP and RNAse P RNAs, suggested to be involved in mitochondrial DNA replication and tRNA processing.

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P4.9

Proglambile misincoporation of amino acids increases probability of protein evolution

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Considering that high fidelity in the present translation is achieved by evolved machinery such as editing in aaRS, early evolutionary stages of translation had low fidelity polymerization system. To explore properties of proteins evolved in early life with low fidelity, we created genetic codes with adjustable low fidelity by engineering of cell-free translation. Fidelity in our code was decreased by the addition of $tRNA^{Ser}$ variants with anticodon replacements corresponding to Thr codons. By a dose-dependent manner of tRNA variants addition, GFP lost its fluorescence despite no change in amount of protein production. We created other misincorporations easily, because of SerRS and AlaRS accept anticodon replacements in their tRNA.
Our engineered code is expected to produce a more smooth protein fitness landscape on DNA sequence space, because not only protein with the encoded amino acid sequence, but protein variants with point mutations are produced from one DNA sequence. Thermostability analysis of GFP variants showed that the smoothing effect of our low fidelity code diminished local optimum points and thus expanded the number of evolutionary pathways to the global optimum.

P4.10

Integrative tRNAs, tRFs and miRNAs profiling in lung cancer biopsies

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Several cancer types exhibit tRNAs overexpression, tRFs production and altered miRNA levels that drive cell proliferation. In the present study we analyzed the differential expression of tRNAs, tRFs, and miRNAs in lung cancer biopsies with similar classification in comparison to healthy tissue samples from the same patients. Analysis of miRNAs revealed among others, upregulation of miR-210 and downregulation of miR-126. A modified protocol for NGS was used to discriminate the tRNAs and tRFs pools and the data were analyzed using tREFL tool. The differentially expressed tRNAs and tRFs were validated *via* RT-qPCR analysis. Interestingly, tRF-5003 is significantly upregulated whereas tRF-3021 is downregulated in tumours. Filtering of data using CLASH tool predicted possible targets for both tRFs *via* AGO-mediated loading and silencing. Effective transfection of A549 cell line using siRNAs corresponding to both tRFs showed that tRF-3021 effectively downregulates *ARIH1*, whereas tRF-5003 effectively downregulates *PAQR4* thus, verifying the targets prediction. These are among the few examples of tumor-derived tRFs that affect regulation of essential genes controlling tumor progression.

P4.11

Analysis of tRNA Gene Copy Number Variation and Codon Usage Patterns in 460 Fungal Species For Predictive Modeling of Highly Expressed Genes

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Numerous studies show tRNA abundance, proxied by tRNA gene copy number, as a major contributor of codon usage bias which has been shown to correlate with gene expression level (Ikemura 1982, Plotkin et al 2011). We have characterized the copy number variation of tRNA genes, obtained from tRNAscan-SE (Lowe et al 2016), and the codon usage patterns in over 6 million protein coding sequences, across several hundreds of fungal genomes that were sequenced at the Joint Genome Institute for projects like the *1000 Fungal Genomes Project*, making it the most extensive and comprehensive study of its kind within the Kingdom Fungi. We have analyzed how codon usage correlates with tRNA gene content between species. We have identified both highly conserved and rare tRNA. Finally, armed with the gene expression data of each genome, we propose to develop a predictive machine learning based model to identify highly expressed genes based on features of codon usage and tRNA gene content.

P4.12

Characterization of the Arabidopsis tRNAome in Pattern Triggered Immunity

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Previous findings have indicated that plants rapidly accumulate high levels of both charged and uncharged forms of specific tRNAs in response to pathogen recognition by cell surface localized Pattern Recognition Receptors. In addition, we have identified a pattern of differential codon usage in pathogen induced transcripts. Recently, novel approaches applied in yeast and mammalian systems have successfully overcome earlier problems associated with Next-gen sequencing of highly structured tRNAs. These advances presented the opportunity to adapt methods in order to perform the first ever high resolution analysis of a plant "tRNAome" as a means of further exploring the function of differential accumulation of specific tRNAs in plant immune responses. We are examining the abundance and charging of tRNA during the induced immune response in Arabidopsis through a combination of biochemical and next-generation sequencing techniques. This is being done under conditions identical to genome-wide transcriptional and translational studies from our laboratory, allowing for comparative analysis of output from multiple genetic mechanisms. Preliminary results suggest that changes in codon optimality resulting from coordinated modification of tRNA supply (isoacceptor abundance) and demand (codon usage), may facilitate rapid global changes in gene expression. This work is anticipated to provide a comprehensive analysis of the dynamics and function of tRNA in rapid plant immune responses.

P5.1

A tRNA half modulates translation as stress response in *Trypanosoma brucei*

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The pathogenic parasite Trypanosoma brucei lacks extensive transcriptional control of protein coding genes and thus is crucially dependent on post-transcriptional mechanisms to modulate gene expression. Despite this, how parasites regulate translation has been so far unexplored. Analyses of ribosome-associated small ncRNAs allowed the identification of several life stage dependent and stress-induced tRNA-halves in T. brucei. One of the most abundant ones, the $tRNA^{ThrAGU}$ 3’ half is predominantly present during nutritional stress. Interestingly, this half associates with ribosomes and polysomes in vivo and stimulates protein synthesis during nutritional stress recovery in vivo and in vitro. This stimulatory effect is conserved in other organisms, such as mammals and archaea. Depletion or blocking of the endogenous tRNA half showed a relaxation of the translation stimulation in vitro and in vivo. Additionally, the $tRNA^{Thr}$ half preferentially interacts with stressed ribosomes in ribosome binding studies. Our results suggest a novel biological function of a tRNA fragment, namely global stimulation of translation during the recovery from nutritional stress.

P5.2

A functional genomics approach to investigate Arabidopsis nuclear RNase P interactions

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Maturation of the 5' end of tRNA-precursors is catalysed by Ribonuclease P (RNase P) in all domains of life. RNase P, which can be a ribonucleoproteins or Protein-only RNase P (PRORP) enzymes have been shown to play a variey of additional roles, including the cleavage of tRNA-like structures (TLS) in mRNA, in both prokaryotes and eukaryotes. Using functional genomics, we aim to unravel Arabidopsis PRORP2 functional interactions at both the protein and RNA levels. Transgenic lines were generated with a tagged version of PRORP2 to identify by co-immunoprecipitation both its protein partners and RNA targets at the transcriptome wide level. Additionaly, the mRNA of the RNA polymerase III transcriptional repressor MAF1 was identified as a putative target for a regulation mediated by PRORP2. In vitro assays showed that MAF1 mRNA precursor is a PRORP2 substrate. Further work, using i.e. 5' RACE and virus induced gene silencing, is currently ongoing to investigate the effects and biological significance of this cleavage in vivo.

P5.3

$tRNA^{Gln}$ derived fragments mediate pancreatic β-cells dysfunction and apoptosis in TRMT10A diabetes

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Loss-of-function mutations in TRMT10A, a tRNA methyltransferase, cause early onset diabetes and microcephaly. Here we investigated the molecular mechanisms underlying β-cell demise in TRMT10A deficiency. iPSCs from controls and TRMT10A patients were successfully differentiated into β-like cells. TRMT10A expression was silenced in EndoC-βH1 cells. In patient-derived β-like cells and TRMT10A-depleted EndoC-βH1 cells m_1G^9 was reduced in a subset of cytosolic tRNAs, including $tRNA^{Gln}$. Hypomethylation of $tRNA^{Gln}$ resulted in fragmentation with accumulation of 5'-$tRNA^{Gln}$ fragments in patient-derived cells. Transfection of TRMT10A-competent EndoC-βH1 cells with synthetic $tRNA^{Gln}$ fragments induced apoptosis. Conversely, transfection of antisense oligonucleotides targeting $tRNA^{Gln}$ fragments inhibit β- cell apoptosis induced by TRMT10A-deficiency. TRMT10A silencing led to mitochondrial dysfunction, increased oxidative stress and activation of the intrinsic pathway of apoptosis. These findings provide unequivocal evidence for the importance of tRNA modifications in human pancreatic β-cells and identify tRNA hypomethylation and fragmentation as a novel mechanism of β-cell demise in diabetes.

P5.4

Effects of the tRNA^{Pro} half on translation in mammalian cells

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Recent data revealed tRNA-derived RNA as new class of ncRNAs that are able of regulating gene expression post- transcriptionally. The tRNA^{Pro} 5' half was detected in several mammalian cell lines. Northern blot analysis revealed the presence of the tRNA^{Pro} 5' half in the polysomal fractions. The ribosome association was confirmed via filter binding assays in vitro. Addition of synthetic tRNA^{Pro} half in in vitro translation reaction showed unique effects on translation in several species including yeast, CHO, HEK, rabbit reticulocytes, and HeLa cells. Addition of the synthetic tRNA^{Pro} half inhibits global translation and causes upregulation of a specific translational product consisting of both RNA and amino acids. Transfection of the synthetic tRNA^{Pro} half into HeLa cells lead to formation of the same product in vivo. The migration of the product in acidic gels, insensitivity to copper sulfate and to 3' polyadenylation, and association with 80S monosomes suggest that the accumulated product is peptidyl-tRNA. We speculate that binding of the tRNA^{Pro} 5' half to the ribosome leads to ribosome stalling, consequent translation inhibition and formation of peptidyl tRNA.

P5.5

Theoretical and experimental investigation of tRNA-protein interaction in Cytochrome C and tRNA nucleotidyltransferase models

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The RNA-protein communication is fundamental for many biological processes. RNA and proteins are critical in every biological system so the in-depth investigation of their interaction is vital to broaden our knowledge about those processes. Two pathways can be followed to study such arrangements. Experimental investigation of RNA-protein complexes with techniques like electrophoretic mobility shift assay (EMSA) or microscale thermophoresis (MST). The experimentally collected data however, still calls to be refined in order to reach a high resolution, which may be achieved following the theoretical path - computational prediction of the patterns governing the formation of the RNA-protein complex. Among RNAs, the tRNA deserves particular attention. It holds a highly conserved structure, serves as a core element in protein biosynthesis – translation and partake in apoptosis. Therefore, the cytochrome C and tRNA nucleotidyltransferase (TRNT) served as protein models for investigation of tRNA-protein complexes. A set of synthetic tRNA mimics was introduced to each of the protein models for the experimental analysis and were subjected to the computational complex analysis.

P5.6

Angiogenin modulates the levels of tRNA fragments in cardiac microvascular endothelial cells

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The diabetes-induced hyperglycemic environment is an important contributor to microvascular endothelial dysfunction and the progression of heart failure with preserved ejection fraction (HFpEF). HFpEF is a disease without treatment options, and a better understanding of the underlying molecular mechanisms is urgently needed. Non-coding RNAs are important regulators of gene expression in the cardiovascular system, of which transfer RNA fragments (tRFs) comprise a novel group with great potential. Although the function and biogenesis of tRFs remains largely unknown, specific ribonucleases have been associated with tRF generation, including Angiogenin (ANG).

To identify cardiac differentially expressed tRFs in HFpEF and investigate whether tRF levels are affected by knock-down of the ribonuclease enzyme Angiogenin (ANG) in Human cardiac Microvascular Endothelial Cells (HMVECs). Methods and Results Cardiac small RNA-seq reads from 20 weeks-old lean ZSF1 control rats and obese ZSF1 HFpEF rats were mapped to the R. norvegicus tRNA database using an in house-designed protocol, and processed tRNAs were detected. HMVECs were exposed to basal and high glucose to induce endothelial dysfunction and siRNA-mediated ANG knock-down. tRF levels were determined via Northern blot in these samples. In the obese ZSF1 left ventricle, there was a significant induction of ~30nt tRFs (tRNA-halves) compared to the lean ZSF1 LV. 5’tRNA-half G98* was upregulated both in the obese LV and in high-glucose treated HMVECs and confirmed via Northern blot. ANG knock-down in HMVECs resulted in a profound alteration of the profile of tRFs in multiple tRNA anticodon families.

Glucotoxic stimulus affects the processing of specific tRNAs in the LV of obese ZSF1 rats and in glucose-challenged cardiac HMVECs. ANG knock-down altered the profiles of tRFs deriving from multiple tRNAs, suggestive of an important role of ANG in differentially regulating the cleavage of specific tRNA templates. Our current investigation focuses on the effect of ANG knock-down and the functional role of 5’tRNA-half G98* in microvascular endothelial dysfunction.

*arbitrary gene name; unpublished data

P5.7

Tetra-molecular 5'tiRNAs inhibit 48S ribosome scanning through direct inhibition of eIF4F

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In response to stressful environments, cells react by generating small non-coding RNAs that we have termed tRNA- derived stress-induced RNAs (tiRNA). These small RNAs function to modulate translation in an effort to conserve and redirect cellular resources with the aim of promoting survival. We show that two tiRNAs, derived from the 5' halves of $tRNA^{Ala}$ and $tRNA^{Cys}$ are potent inhibitors of translation ($5'tiRNA^{Ala/Cys}$). These tiRNAs are unique in that they contain a conserved cis element called a terminal oligoguanine (TOG) motif, which we show is required for activity. We further show that this motif folds into parallel RNA G-quadruplex (G4) and that this structure, rather than solely TOG sequence, is required for bioactivity. By preventing the formation of G4 through ionic equilibration or through use of modified nucleic acids, we can prevent the activity of TOG tiRNAs. Mechanistically, we show that these tetrameric tiRNAs directly interact with and inactivates the heterotrimeric eIF4F complex composed of eIF4E, eIF4G and eIF4A. Consistent with eIF4F inactivation, these tiRNAs inhibit 48S ribosome scanning to block translation initiation.

P5.8

Characterization of aminoacyl-tRNA transferases related to bacterial MprF in Aspergillus fumigatus

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Aspergillus fumigatus is the most common mold pathogen in immunocompromised patients. The mortality, despite advances in diagnosis and treatment remains unacceptably high at 50- 80% of patient. Numerous virulence determinants have been characterized in A. fumigates but other remain to be identified in order to decrease complications and mortality. We identified in fungi, and especially in A. fumigatus, two MprF-like (Multiple Peptides Resistance Factor) proteins with a DUF2156 domain. In bacteria, MprF proteins are aminoacyl-tRNA transferases that catalyse the transfer of lysine from Lys-tRNA^{Lys} onto membrane lipids. Aminoacylation of lipids modifies the overall charge of the membrane, which increases the resistance of bacteria to cationic antimicrobial peptides. Characterization of the two MprF-like proteins in A. fumigatus shows that both proteins are involved in the synthesis of novel aminoacylated lipids. Our preliminary results suggest that fungal DUF2156 proteins influence central cellular processes such as virulence or sporulation in A. fumigatus.

P5.9

Transfer RNA-derived fragments target and regulate ribosome-associated aminoacyl-transfer RNA synthetases

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Recently, a number of ribosome-associated non-coding RNAs (rancRNAs) have been discovered in all domains of life. Our recent studies in Saccharomyces cerevisiae have shown that processing of tRNA molecules is widespread in a number of growth conditions. Moreover, we discovered that tRNA fragments bind to the ribosomes reveal inhibitory activity on in vitro translation. In this present study, we functionally characterized the molecular activity of S. cerevisiae transfer RNA (tRNA)-derived fragments (tRFs) during protein biosynthesis. Our results indicate ribosome-associated tRFs derived from both 5' (ranc-5'-tRFs) and 3'-part of tRNAs (ranc-3'-tRFs) have regulatory roles during translation. We demonstrated five 3'-tRFs and one 5'-tRF associate with a small ribosomal subunit and aminoacyl-tRNA synthetases (aa-RSs) in yeast. Furthermore, we discovered that four yeast aa-RSs interact directly with yeast ribosomes. tRFs interactions with ribosome-associated aa-RSs correlate with impaired efficiency of tRNA aminoacylation.

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P5.10

Alanine tRNAs translate environment into behavior in *Caenorhabditis elegans*

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The *Caenorhabditis elegans* worms produce and keep imprints of the attractive chemosensory cues to which they are exposed early in life. Imprinting enhances transiently or permanently chemoattraction to early olfactory cues.

We identified $tRNA^{Ala}$ (UGC) as the unique olfactory imprinting molecule. Feeding naive unexposed worms on $tRNA^{Ala}$ (UGC) from odor-exposed transiently or stably enhance odor-specific responses, suggesting worms produce odor-specific forms of $tRNA^{Ala}$ (UGC).

Different mutations in the tRNA modifying Elongator complex sub-units 1 or 3 genes either abolish chemo-attraction, or stably suppress attraction to the odor worms have been exposed to. Feeding Elongator mutants on $tRNA^{Ala}$ (UGC) produced by wild-type unexposed worms however rescues both behavioral phenotypes.

We conclude that $tRNA^{Ala}$ (UGC), through the Elongator complex activity, can translate the early olfactory environment and stably reprogram the C. elegans chemoattractive behavior.

P5.11

Characterization of tRNA^{Ile} (UAU)-like small RNAs in *Lactobacillus plantarum*

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In most eubacteria, a single tRNA^{Ile} (CAU) (where C34 is modified to lysidine) is found for decoding the rare AUA isoleucine codon instead of a tRNA^{Ile} (UAU), except for a few cases such as Mycoplasma mobile. TilS enzyme that catalyzes lysidine34 formation is present in all eubacteria lacking the tRNA^{Ile} (UAU) without any exception. However, Lactobacillus plantarum has genes not only for tRNA^{Ile} (CAU) and TilS, but also for tRNA^{Ile} (UAU)-like small RNAs. If the tRNA^{Ile} (UAU)-like RNAs are involved in decoding, it may cause some ambiguity in the genetic code since the tRNA can decode AUG methionine codon as well as AUA isoleucine codon. In order to elucidate the functional role of the tRNA^{Ile} (UAU)-like RNA in L. plantarum, we characterized the small RNA. The tRNA^{Ile} (UAU)-like RNA was not charged with isoleucine by isoleucyl-tRNA synthetase. Also, enzymatic probing results obtained on native tRNA^{Ile} (UAU)-like RNA suggest that the RNA adopts a structure less stable than the one found canonical tRNAs. The results suggest the tRNA^{Ile} (UAU)-like RNA does not function as a canonical isoleucine tRNA.

P5.12

Global identification of tRNA cleavage fragments in budding yeast

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Since S. cerevisiae lacks RNAi machinery, the pool of small RNAs in this model eukaryote is limited in its complexity. Small (18-70nt) RNA sequencing revealed a population of RNAs predominantly comprised of tRNA halves and rRNA fragments (Drinnenberg et al. 2008; Thompson et al. 2008). We took advantage of this limited repertoire of small RNAs to examine tRNA cleavage and processing in S. cerevisiae cells that lack 5′→3′ RNA decay. The tRNA ligase Trl1 is required for tRNA splicing and essential for growth but can be genetically bypassed by expression of "pre-spliced tRNAs." The 5′-kinase domain of Trl1 phosphorylates the 5′ end of 5′-OH RNA substrates, making them competent for ligation or 5′→3′ decay by Xrn1. We reasoned that bypassed trl1Δ cells would accumulate RNA products of endonuclease cleavage and the 5′-ends of these fragments would be resistant to Xrn1-mediated decay. We isolated small RNAs from trl1Δ and wild-type cells and end-repaired these molecules to enable detection of endonuclease- cleaved RNAs with 5′-OH and 2′,3′-cyclic phosphate ends. As expected, cells lacking TRL1 accumulate tRNA splicing intermediates from SEN cleavage, including 5′- and 3′-exons of intron-containing tRNAs, as well as liberated tRNA introns. In addition, we identify other signatures of endonuclease cleavage of tRNAs, including 5′- and 3′- tRNA half molecules consistent with Rny1 cleavage and a handful of smaller tRNA-derived RNA fragments (tRFs). Together, these data provide a comprehensive picture of tRNA processing in S. cerevisiae and reveal previously uncharacterized sites of endonuclease cleavage.

P6.1

A tissue-specific exon skipping event separates the catalytic and alternative functions of human leucyl-tRNA synthetase

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The aminoacyl-tRNA synthetases (AARS) set the genetic code by attaching amino acids to their cognate tRNAs. Throughout their lengthy evolutionary history, the AARS have also developed many functions beyond aminoacylation. For example, human cytoplasmic leucyl-tRNA synthetase (LARS) has been adapted as an intracellular leucine sensor for the mammalian target of the rapamycin complex 1 (mTORC1) master regulatory pathway. Here we describe an alternatively-spliced transcript variant of human LARS which lacks an exon coding for key enzymatic residues in the aminoacylation active site. Expression of this LARS splice variant (LSV1) is elevated specifically in leukocytes, where it comprises greater than 20% of LARS gene transcripts. In human cell culture, LSV1 overexpression results in a stable protein product. This LSV1 protein also increases leucine- dependent mTORC1 activity with a potency comparable to that of full length LARS. These data indicate that LSV1 may have evolved to decouple the canonical aminoacylation and alternative leucine-sensing functions of human LARS, and that this distinction is important in leukocyte biology.

P6.2

Aminoacyl-tRNA Synthetases: Investigations of Specificity for Application in ProxiMAX / Synthetic Biology

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'ProxiMAX' randomisation technology (ColibraTM) is a defined saturation mutagenesis process that delivers precision control of identity and relative ratio of amino acids (AAs) at specified locations within a protein library. This non-degenerate process allows encoding of all 20 or any desired subset of AAs with ease, without constraints imposed by the genetic code. Using a maximum of 20 codons in saturated positions means many codons remain available to encode additional, unnatural amino acids (UAAs). Incorporation of UAAs is particularly relevant in expanding engineered protein repertoires. Ultimately, we aim to combine ProxiMAX with an in vitro transcription/translation system to design and express synthetic protein libraries containing multiple UAAs simultaneously. We have employed ProxiMAX randomisation to encode 18 natural AAs in various, putative AA-binding locations of an E. coli alanyl-tRNA synthetase. Our poster describes the synthesis of two variant libraries, each encoding >10^7 novel proteins, their composition, quality and our progress towards screening and deconvolution of the libraries to discover novel enzymes, with an initial focus on specificity for D-AA.

P6.3

Contribution to the characterization of the human mitochondrial arginyl-tRNA synthetase: What we know and what is new

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Human mitochondrial aminoacyl-tRNA synthetases (mt-aaRSs) are key enzymes of the mitochondrial translation. Mutations in their nuclear genes are correlated with pathologies with a broad spectrum of clinical phenotypes. Despite being ubiquitously expressed, mutations on mt-aaRSs show a convergence of injuries on the central nervous system. As remarkable examples, mutations in mt-AspRS are correlated with a "mild" neuro-degenerative leukodystrophy, while mutations in the mt-ArgRS are correlated with a severe neuro-developmental pontocerebellar hypoplasia. Previous investigations performed in the laboratory contributed to the characterization of the mt-AspRS.

In this study, we focused on the determination of cellular properties of the mt-ArgRS and its functional characterization. We further investigated the impact of a series of disease-associated mutations on two main properties of mt ArgRS: the *in cellulo* sub-mitochondrial localization and the *in vitro* aminoacylation activity of this enzyme. Combined with previous works, the present results expand the knowledge of the mt-aaRSs, shedding new light on the link between mt-aaRSs-mutations and disease.

P6.4

IleRS editing primarily targets norvaline whose misincorporation is more toxic than of valine

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Translational errors may induce loss of protein stability and function. They are partly prevented by the editing activity of aminoacyl-tRNA synthetases (aaRSs). Norvaline (Nva) and valine are two isoleucine surrogates with identical mass: Val is a proteinogenic beta-branched amino acid, while the linear Nva sporadically accumulates in cells, but is not normally incorporated to proteins. We showed that Nva and Val are equally good non- cognate substrates of the deacylation-defective IleRS, both in vitro and in vivo. Surprisingly, however, IleRS distinguishes between Nva and Val at the CP1 editing domain that hydrolyses Nva-tRNA^{Ile} faster than Val- tRNA^{Ile}. We further demonstrated that incorporation of Nva into proteome is more deleterious, although Nva is mistranslated with similar frequency to Val and largely at the same Ile positions. Comparison of Nva-tRNA editing by IleRS, LeuRS and ValRS suggests Nva editing was present in their common ancestor, prior to its duplication and divergence to three specialized enzymes. This raises an intriguing hypothesis that elimination of Nva, which may had been present in primordial proteins, likely drove the acquisition of the CP1 editing domain.

P6.5

Intracellular leucine sensing mechanism coordinated by leucyl-tRNA synthetase

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Leucyl-tRNA synthetase (LRS), an enzyme involved in protein synthesis, serves as an intracellular leucine sensor for mTORC1 signaling pathway, which is a central effector for protein synthesis, metabolism, autophagy, and cell growth. The Rag GTPase cycle mediates leucine signaling to mTORC1. In this presentation, we would like to introduce our current understanding of intracellular leucine sensing mechanism controlled by leucyl-tRNA synthetase. Here we show the dynamics of Rag GTPase cycle during leucine signaling and that LRS serves as an initiating “ON” switch via GTP hydrolysis of RagD that drives the entire Rag GTPase cycle in the RagGTPase-mTORC1 axis. The LRS-RagD axis showed a positive correlation with mTORC1 activity in cancer cells and tumor tissues. In addition, we clarify the role of another leucine binding protein, Sestrin2, in the RagGTPase-mTORC1 axis. This study suggests that the GTPase cycle of RagD/RagB coordinated by LRS and Sestrin2 is critical for controlling mTORC1 activation, and thus will extend the current view of amino acid sensing by mTORC1 and will be invaluable for the development of novel approaches to combat mTORC1-related human diseases.

P6.6

Membrane aminoacyl-tRNA synthetases and signaling pathways

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Aminoacyl-tRNA synthetases (aaRS) are ubiquitous enzymes, which conventional role is to form aminoacyl-tRNA. Despite their well-known role in protein synthesis, studies have highlighted their implication in many other cellular processes including apoptosis and autophagy. In the yeast Saccharomyces cerevesiae the methionyl-tRNA synthetase (MRS) and the glutamyl-tRNA synthetase (ERS) are found in a complex together with the protein Arc1. Inside this complex the protein Arc1 acts as a cytosolic anchor and an aminoacylation cofactor for the two aaRS.

Even if Arc1 was shown to be essentially cytosolic, studies have revealed that it also interacts with lipids such as phosphoinositides (PIPs). Using membranes coated with PIPs, subcellular fractionation experiments and a new fluorescent tool developed by our lab, we confirmed the interaction of Arc1 with PIPs present in the vacuolar membrane. Moreover, these preliminary results indicate that the vacuolar Arc1 may trigger anchoring of two aaRS at the vacuolar membrane too. The physiological conditions that provoke this vacuolar relocation of Arc1 and the role of vacuolar Arc1 and the of two membrane-bound aaRSs will be discussed.

P6.7

Functional analysis of plant double-length tyrosyl-tRNA synthetase from Arabidopsis thaliana.

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Despite large numbers of non-canonical functions of aminoacyl-tRNA synthetases (aaRSs) being discovered in mammals, those in plants and fungi are largely unexplored. During our attempt to identify the function of plant aaRSs, we noticed a presence of a double-length tyrosyl-tRNA synthetase (TyrRS) gene in the model plant Arabidopsis thaliana. Such a TyrRS was found only in limited plants and were more broadly found among trypanosomatids. However, unlike those from trypanosomatids, both the N-terminal half full lenth (designated TyrRS-1N) and the C-terminal half full length (TyrRS-2C) contained the class I conserved motifs and were seemed active. Functional analysis using yeast complementary assay showed that TyrRS-1N itself was inactive while TyrRS-2C itself was active. Trypanosomatids double-length TyrRS was known to form an intramolecular pseudo-dimer accomodating one molecule of tRNA across the pseudo-dimer interface. In order to analyze the similarity and discrepancy of A. thaliana TyrRS function compared to trypanosomatids, mutations at active site residues were introduced into TyrRS-1N and TyrRS-2C portions of the full length. Intriguingly, mutations in already inactive TyrRS-1N portion resulted in inactivation of the full length TyrRS. Therefore, these mutated residues were suspected to somehow participate in a pseudo-dimer formation. By comparing the sequence of TyrRS-1N with TyrRS-2C and other TyrRSs, we noticed several residues that were highly conserved among TyrRSs were missing in TyrRS-1N. Although an attempt to restore TyrRS activity in TyrRS-1N by mutating back these residues have failed, the catalytic domain of TyrRS-1N seemed originally inactive while the anticodon binding domain was speculated to be active. Therefore, the tRNA substrate most likely binds to the catalytic domain of TyrRS-2C and the anticodon binding domain of TyrRS-1N across the pseudo-dimer interface.

P6.8

Asparginyl tRNA synthetase (NRS) is a novel regulator in hippo signaling

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An aminoacyl tRNA synthetase (ARS) is a key enzyme that plays an important role in protein translation. Recent studies suggest that the non-canonical function of ARSs is involved in various pathological mechanisms including cancer, autoimmune disorders, and neurological disorders. However, the molecular mechanisms of ARSs in cancer are largely unknown. Here, we established Drosophila carcinoma model by overexpressing of yorkie (yki), a well-known oncogenic protein in Hippo signaling pathway. Under genome-wide RNAi screening of all Drosophila ARSs, we identified asparginyl tRNA synthetase (NRS) as the one of cancer modifier gene. Yki-induced tumor phenotype was significantly rescued by knockdown of NRS in the adult eye. Furthermore, knockdown of NRS strongly suppressed the mitotic index (phosphor-H3) of yki-induced tumor in the larval eye disc. We also confirmed that increased phosphorylation activity of Akt and S6k caused by yki overexpression were also reduced by NRS knockdown. Interestingly, NRS inhibitor also suppressed tumor phenotype. Thus, we hypothesize that NRS is a novel regulator in hippo signaling. Based on genetic and biochemical analysis, we showed that NRS modulates hippo singlaing through interacting with Salvador, a binding substrate of hippo kinase. Taken together, these results provide a possibility for developing an anti-cancer drug using NRS as a novel target.

P6.9

Abiogenic evolution of aminoacyl-tRNA synthetase

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The origin of life on earth demands a prerequisite for abiogenic evolution of RNA which is believed to be highly destructive on the earth covered by boiling ocean. I have proposed a mechanism of evolution of RNA as a left-handed single-stranded lattice with a trend of condensation and dehydration of organic compounds under a condition of high pressure and high temperature in the mantle of the earth. All organic compounds and energy-rich polyphosphates were synthesized from a large amount of methane hydrate, inorganic phosphate and oxygen radicals that were produced from ferrous oxides by disintegration of uranium (1). The six fold symmetrical structure of the lattice allowed to stack a lot of energy-rich compounds such as adenosine triphosphate and phospho-lipids which were required for formation of cell membranere at the later stage (1). More importantly, the cavity formed inside the lattice allowed polymerization of small L-amino acids such as glycine, alanine, proline and serine that were required to protect RNAs from hydrolysis (1). The 6.5 fold symmetrical lattice allowed a little more larger L-amino acids to incorporate the protective proteins and formation of α–helices, but insertion of larger amino acids such as arginine, methionine, phenylalanine and tryptophan were rather difficult (1). However, in the next stage of evolution of the lattice formation of left-handed single stranded RNA, the 8 fold symmetric lattice seems to incorporate arginines and also the dimeric relationship between the two neighbor strands suggests a possibility of aminoacyl-tRNA synthetase to evolve because some of the aminoacyl–tRNA synthetases were found to be in dimeric form. Extension of protecting L-amino acid polymer at the dimeric shape strongly suggests evolution of aminoacyl-tRNA synthetase. Moreover, formation of small tRNA seems possible under the function of aminoacyl-adenylate in the process of evolution of the synthetases in its dimeric form. The aminoacyl group of the tRNA at the dimeric form, which behaves as A site tRNA, can be transferred to the neighbouring tRNA, which can be supposed to be P site tRNA. However, existence of E site tRNA cannot be confirmed in this stage of evolution.

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P6.10

Regulation of translational fidelity in Salmonella Typhimurium

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Translation is an essential cellular process that involves intricate quality control mechanisms to ensure fidelity. Aminoacyl tRNA synthetases function during translation to ligate tRNA to the cognate amino acid. Phenylalanyl tRNA synthetase (PheRS) has a proofreading mechanism to prevent misaminoacylation of non-cognate amino acids to tRNA. Oxidative stress has been shown to alter quality control of threonyl tRNA synthetase. In addition, treating E. coli with hydrogen peroxide (H_2O_2) alters the amino acid pool by oxidizing phenylalanine into tyrosine isomers, increasing availability of non-cognate amino acids. Due to increased non-cognate availability, PheRS must maintain accuracy during oxidative stress; however, how PheRS changes enzymatically and structurally has yet to be elucidated. Upon treatment of PheRS with H_2O_2, we observed differential oxidation patterns between subunits, paired with secondary structural changes. Interestingly, H_2O_2 did not impact the cognate amino acid aminoacylation activity, but lead to increased proofreading activity. These results suggest a possible mechanism for the cell to protect itself from amino acid pool changes during oxidative stress.

P6.11

Lysyl-tRNA synthetase producing second messenger Ap_4A to trigger polymerization of tumor suppressor HINT1 for allergy response

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Lysyl-tRNA synthetase (LysRS) is the essential enzyme that acylates $tRNA^{Lys}$ with amino acid lysine, and also the major generator of second messenger diadenosine tetraphosphates (Ap_4A). Ap_4A is a universal pleiotropic signaling molecule needed for various cell regulation pathways. During allergic mast cell activation, LysRS is phosphorylated on Ser207 and boost its activity to condense ATP with Lys-AMP to form Ap_4A. Accordingly, the cellular concentration of Ap_4A increases up to 3-10 fold in 15 min and drops back to basal level in 60 min. Ap_4A binds the tumor suppressor HINT1 directly, and disrupts the interaction between HINT1 and transcription factor MITF, thus releasing MITF for the targeted genes transcription essential for immune response. Using crystal structures, emission microscopy analyses, and multiple biochemical assays, we found that, instead of inducing allosteric conformational change to propagate signals like previous reported second messengers, Ap_4A triggers HINT1 to form polymer both in vitro and in mast cell in concentration-dependent manner. In addition, the polymerization of HINT1 block the interacting surface for MITF and release MITF transcriptional accordingly. These findings reveal a novel regulating mechanism of LysRS on gene transcription in allergy response, through the tethering manner performed by its noncanonical product - second messenger Ap_4A.

P7.1

Functional and structural study of *Plasmodium* tRNA import machinery

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Malaria continues to be a global burden for health, with 445,000 deaths and 216 million cases in 2016 (1). Our results bring an unprecedented observation: mosquitoes salivary gland sporozoites import exogenous host tRNAs. This tRNA trafficking is made possible by a unique surface protein, named tRip (transfer RNA import protein). The protein tRip mediates tRNA entrance in the parasite, and this mechanism is crucial for infection by a presently unknown mechanism (2). So far, we established that (i) *in vitro* tRip binds tRNAs with high affinity and recognizes the elbow of the tRNA molecule through its C-terminal domain. (ii) *In vivo* immunolocalization experiments found tRip in both the liver and the blood stages of *Plasmodium* in the mammalian host, as well as in the mosquito vector. (iii) tRip is located at the surface of the parasite, its tRNA binding domain being exposed to the outside. (iv) *In vitro*, exogenous tRNAs enter rapidly live sporozoites. Protein biosynthesis is reduced in a knock-out parasite, deleted for the TRIP gene (tRip-KO) and its infectivity is diminished at the blood stage as compared to the wild-type parasite. Moreover, the gene-encoding tRip is specific for *Apicomplexa* parasites, such as *Plasmodium*, *Toxoplasma*, and *Cryptosporidium*.
How are tRNAs imported into *Plasmodium*? What is their role in the infectious process of the parasite? We provide a functional and structural description of this parasite-specific import process by investigating recognition between tRip and host tRNAs, comparative proteomics between wild type and tRip-KO parasites, identification of tRip partners and structure of tRip, tRip/tRNA complex and the complex formed between tRip and its *Plasmodium* protein partners.

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P7.2

Contribution of the tRNAIle 4317A>G mutation to the phenotypic manifestation of the deafness-associated mitochondrial 12S rRNA 1555A>G mutation

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The 1555A>G mutation in mitochondrial 12S rRNA has been associated with aminoglycoside-induced and nonsyndromic deafness in many individuals worldwide. Mitochondrial genetic modifiers are proposed to influence the phenotypic expression of m.1555A>G mutation. Here, we report that a deafness-susceptibility allele (m.4317A>G) in the tRNAIle gene modulates the phenotype expression of m.1555A>G mutation. Strikingly, a large Han Chinese pedigree carrying both m.4317A>G and m.1555A>G mutations exhibited much higher penetrance of deafness than those carrying only m.1555A>G mutation. The m.4317A>G mutation affected a highly conserved adenine at position 59 in the T-loop of tRNAIle. We therefore hypothesized that the m.4317A>G mutation alters both structure and function of tRNAIle. Using lymphoblastoid cell lines derived from members of Chinese families (three carrying both m.1555A>G and m.4317A>G mutations, three harboring only m.1555A>G mutation, and three controls lacking these mutations), we found that the cell lines bearing both m.4317A>G and m.1555A>G mutations exhibited more severe mitochondrial dysfunctions than those carrying only m.1555A>G mutation. We also found that the m.4317A>G mutation perturbed the conformation, stability, and aminoacylation efficiency of tRNAIle. These m.4317A>G mutation-induced alterations in tRNAIle structure and function aggravated the defective mitochondrial translation and respiratory phenotypes associated with m.1555A>G mutation. Furthermore, mutant cell lines bearing both m.4317A>G and m.1555A>G mutations exhibited greater reductions in the mitochondrial ATP levels and membrane potentials and increasing production of reactive oxygen species than those carrying only m.1555A>G mutation. Our findings provide new insights into the pathophysiology of maternally inherited deafness arising from the synergy between mitochondrial 12S rRNA and tRNA mutations.

P7.3

Mutations in H. sapiens tRNase Z^L(ELAC2) associated with hypertrophic cardiomyopathy impair mitochondrial tRNA 3'-end processing

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Dysfunction of mitochondrial gene expression, caused by mutations in either the mitochondrial or nuclear genomes, is associated with a diverse group of human disorders characterized by impaired mitochondrial respiration. An increasing number of mutations have been identified in nuclear genes involved in mitochondrial RNA metabolism. Within this group are pathogenic mutations which have been identified in the genes encoding enzymes involved in the precursor transcript processing, including ELAC2 coding for the mitochondrial tRNase Z^L. Here, we report the identification of sixteen novel ELAC2 variants in individuals presenting with mitochondrial respiratory chain deficiency, hypertrophic cardiomyopathy and lactic acidosis. First, we provide further evidence for the pathogenicity of the three previously reported variants (p.Phe154Leu, p.Leu423Phe and p.Thr520Ile) by studying the tRNase Z activity in an in vitro system. Next, we applied this recombinant system to investigate all novel missense variants, confirming the pathogenic role of these new ELAC2 mutations. The residues in which HCM-associated substitutions were found were effectively modelled in solved tRNase Z structures, providing insight into enzyme structure and function. Finally, we show that primary fibroblasts from the individuals with novel ELAC2 variants have elevated levels of unprocessed mitochondrial RNA precursors. Our study thus broadly confirms a correlation of genotype/phenotype for variants in ELAC2 and suggests the value of screening for mutations in the ELAC2 gene in severe infantile-onset of hypertrophic cardiomyopathy and mitochondrial respiratory chain dysfunction.

P7.4

Fine-tuning of protein synthesis by tRNA 2'-O-methylation is required for learning and memory

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FTSJ1 is a tRNA modification enzyme for 2'-O-methylation of cytosolic tRNAs at positions 32 and 34. Pathological mutations in FTSJ1 gene have been linked to the nonsyndromic X-linked mental retardation, but the precise mechanism has been unclear. We have generated Ftsj1 knockout (KO) mice and investigated the pathophysiological roles. Deficiency of Ftsj1 resulted in hypomodification of a subset of tRNAs, leading to the translational stalling in the ribosome. Consequently, the overall protein synthesis was significantly reduced in Ftsj1 KO brain. The aberrant protein synthesis led to the electrophysiological and morphological abnormalities in both cortical and hippocampal neurons. Importantly, the spatial memory and fear memory were impaired in Ftsj1 KO mice, which mirrors the symptoms of human patients. Taken together, these results demonstrate that Ftsj1-medicated tRNA modification is essential for the fine-tuning of protein synthesis, and dysregulation of this process can cause the development of neurological disease.

P7.5

RNA modification plays a role in resistance to chemotherapeutics

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Ovarian cancer in women is among cancers with low five-year survival rate. So far, the treatment is based on oxidative alterations of the growth and division of cancer cells with chemotherapeutic drugs, like cisplatin and cisplatin-related compounds. However, development of cisplatin resistance is currently a major challenge leading to recurrence of the disease. The underlying mechanisms of cisplatin resistance are elusive and elucidating them will foster the development of new approaches to overcome resistance. We compared ovarian cell lines which span the spectrum from cisplatin-susceptible to cisplatin-resistant. Using tRNA-microarray-based technology, we observed alterations in charging of some tRNA isoacceptor families following cisplatin treatment. We complemented this data with Ribo-Seq (or ribosome profiling) and RNA-Seq and elucidated deregulations in amino- acid metabolism. Guided by our observation from the deep sequencing analysis on disbalance in the SAM synthesis in the cisplatin-resistant cells, we determined the RNA methylation pattern using m^6A-specific antibodies. Interestingly, following cisplatin treatment we observed a strong persistence on the m^6A-modification pattern in the cisplatin-resistant cells contrasting the loss of m^6A modifications in the cisplatin-sensitive cells. Our results suggest that metabolic activities linked to m^6A modification of RNAs play a role in cisplatin-resistance.

P7.6

Modulation of translation efficiency: a new player in dendritic cell function

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Dendritic cells (DC) are immune cells with the capacity to initiate immune responses though the presentation of antigens to naïve T cells. DCs change their gene expression pattern rapidly after activation by microbes and are key immune regulators. The control of protein synthesis during DC activation is a major determinant of proper function; tRNAs are fundamental for this process as they translate mRNA templates into corresponding proteins. However, stress conditions can alter their pool and modifications dynamics, which on its own can skew protein synthesis accuracy and efficiency. Currently we are studying the mechanisms that allow DCs to coordinate their gene expression program both at the transcriptional and translational levels upon activation. Using transcriptomic analysis and tRNAs expression, upon microbe sensing, we have observed a connection between variations in the tRNA species and immune function. Indeed protein synthesis regulation and tRNA abundance regulation are vital for DC activation and T cell priming. Therefore we believe regulation of protein synthesis quality can affect DC fitness and immune function.

P7.7

Overcoming a "molecular ruler" mechanism: the unusual heterotrimeric tRNA splicing endonuclease of *Trypanosoma brucei*

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Introns interrupt tRNA sequences in all major lines of descent (Bacteria, Archaea and Eukarya), rendering them nonfunctional for protein synthesis. Intron removal is therefore, essential. In all known eukaryotes, intron cleavage, the first step in the tRNA splicing pathway, is catalyzed by a conserved heterotetrameric tRNA splicing endonuclease (Sen) composed of four subunits: Sen54, 34, 15 and 2. Bioinformatics analysis using previously published eukaryotic Sen sequences led us to the identification of only one homolog of the tRNA splicing endonuclease in Trypanosoma brucei (Sen34), suggesting that, either the other subunits are missing or, as a whole, the enzyme is highly divergent in these organisms. In this work, we present evidence for a divergent and unique enzyme composed of three subunits: homologs of Sen34, 15 and 2. By performing tandem affinity chromatography followed by mass spectrometry analysis we purified and identified T. brucei Sen (TbSen) subunits from a T. brucei S100 fraction. Gel filtration chromatography and in vitro activity assays revealed that the active enzyme had a size within the range of 58 to 72 kDa, consistent with that of a heterotrimer. Furthermore, immunofluorescence localization assays showed that the enzyme was cytoplasmic, in stark contrast to the nuclear localization of Sen in most eukaryotes. The results presented here demonstrate that TbSen greatly diverges from previously described eukaryotic enzymes in both structure and localization. Interestingly, in most eukaryotes, Sen54 serves as a "molecular ruler" that carefully measures the distance between the splice sites and the backbone of the folded tRNA, aiding in substrate identification and catalytic site positioning. Our finding of a heterotrimeric endonuclease then obviates the need for a Sen54 subunit and may remove the substrate recognition restriction set forth by the "molecular ruler" mechanism. These observations have direct implications for both the evolution of the enzyme in trypanosomes and its potential for targeting of additional substrates while not just being limited to tRNAs.

P7.8

Identification of tRNA modifying enzymes as novel proteostasis players in human cells

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Accumulation of protein aggregates, impaired protein synthesis and proteotoxic stress are hallmarks of protein conformational diseases. Previously, we have shown that tRNA mistranslation induces proteotoxic stress and transcriptome deregulation in zebrafish. Since tRNA modifying enzymes catalyze tRNA modifications, which are essential for both tRNA stability and efficient codon-anticodon recognition, we hypothesize that deregulation of these molecules also affect translation fidelity, resulting in protein aggregation and UPR activation in human cells.
High content screenings using HeLa cells expressing a protein aggregation fluorescent sensor were performed and particular tRNA modifying enzymes that catalyse modifications at position 34 were identified. Besides affecting proteome stability, absence of these enzymes decreased both proteasome activity and protein synthesis rate and induced stress response pathways. We are now performing codon usage analysis and identifying which proteins are more prone to aggregate.
Together this data will contribute to elucidate the role of tRNA modifying enzymes in proteostasis and in disease onset.

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P7.9

A homoplasmic mitochondrial tRNA^{Pro} mutation causing exercise intolerance with muscle swelling

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Mitochondrial DNA mutations are at the origin of incurable human diseases (1). If the vast majority of mtDNA mutations are hetroplasmic (simultaneous presence of wild-type and mutant mtDNA), a very few number of homoplasmic pathogenic mutations of mtDNA were also described (2). We describe a new homoplasmic mtDNA mutation found in 5 independent families characterized by exercise intolerance and muscle swelling, m.15992A>T. It is localized in the mt-tRNA^{Pro}gene creating an U34>A change in the anticodon wobble position. A34 had never been described in mt- tRNAs and is predicted to induce problems of decoding or aminoacylation. Patients' fibroblasts and cybrids were characterized by low activity of mitochondrial complexes I and III. Mitochondrial translation was only moderately decreased in fibroblasts. The level of mt-tRNA^{Pro} was not changed, meaning that the mutation does not affect the tRNA stability. On the other hand, we found that the relative amount of other mitochondrial tRNAs were altered in human cells bearing the mutation.

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P7.10

Biochemical and structural studies of the plasmodial protein tRip, free or in the presence of various ligands

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Malaria is a disease caused by Plasmodium parasites transmitted to humans by infected mosquitoes. These parasites develop into two hosts: the mosquito Anopheles and a vertebrate organism. In the genome of Plasmodium, we identified a surface protein containing a tRNA binding domain. This protein was named tRip (tRNA import protein) in reference to its capacity to import exogenous transfer RNA (tRNA) by an active process.
In the absence of tRip, the parasite has a reduced protein biosynthesis and develops slowly in the blood of the vertebrate host. These observations suggest that host tRNAs affect the protein synthesis and/or the regulation of gene expression in the parasite. However, the mechanism by which this happens is still unclear. Beside its ability to form a complex in the presence of tRNA, tRip interacts also specifically with plasmodial cytosolic aminoacyl-tRNA synthetases. We are aiming to reconstitute a full complex in vitro in order to study its function.
In addition to crystallization attempts, different biochemical (PAGE, HPLC) and biophysical (MS, DLS, CD, AUC, SAXS, ...) techniques are used in order to characterize the size, shape and oligomerization state of tRip in the different complexes.

P7.11

Physiological alterations in HEK293 cells caused by tRNA misexpression

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Alterations in protein synthesis components, namely tRNAs, aminoacyl-tRNA synthetases or tRNA modifying enzymes, increase the level of protein synthesis errors (PSE) and are related with diseases, from cancer to neurodegeneration. Still, the cause-effect mechanisms remain to be elucidated. In order to understand the physiological effects of PSE in human cells, we modified the anticodon of a human $tRNA^{Ser}$, to incorporate serine at various non-cognate sites. Stable HEK293 cell lines expressing these tRNAs were analyzed at different cell passages. tRNAs misexpression led to the accumulation of misfolded proteins and protein aggregates, but activation of the ubiquitin-proteasome system and the unfolded protein response (UPR) maintained cell viability. Deregulated genes encoding plasma membrane and extracellular proteins suggest that levels of mistranslation that have minor or no visible effects on cell viability impact cell physiology through deregulation of ion homeostasis and cell adhesion.

P7.12

A deafness-associated tRNA mutation alters the m1G37 modification in tRNAIle and mitochondrial dysfunction

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Several mitochondrial tRNA mutations have been associated with maternally inherited deafness. However, the pathophysiology of these tRNA mutations remains poorly understood. In present study, we investigated the pathogenic mechanism underlying the deafness-associated mitochondrial tRNAIle 4295A > G mutation, which is localized at a highly conserved nucleotide (A37) for the fidelity of codon recognition and stabilization. Primer extension and methylation activity assays indeed confirmed that the m.4295 A>G mutation created a tRNA methyltransferase 5 (TRMT5)-catalyzed m1G37 modification of tRNAIle. Using cybrid cell lines, we demonstrated the significant decreases in the efficiency of aminoacylation and steady-state level of mt-tRNAIle in mutant cybrids, compared with control cybrids. A failure in metabolism of mt-tRNAIle caused the variable reductions in mitochondrial functions in mutant cybrids. Our findings may provide new insights into the pathophysiology of maternally transmitted deafness that was manifested by altered nucleotide modification of mitochondrial tRNA.

P7.13

A futile tRNA cycle drives energy expenditure and reprograms metabolism in Maf1$^{-/-}$ mice.

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As a master regulator of transcription by RNA polymerase (Pol) III, Maf1 responds to changes in nutrients and cellular stress conditions to balance the production of tRNAs, 5S rRNA and other small non-coding RNAs with protein synthetic need, cell growth and maintenance. So why are Maf1$^{-/-}$ mice lean and why do they resist weight gain on normal and high fat diets (1)? One proposal is that these phenotypes result from increased energy expenditure due to a futile cycle of tRNA synthesis and degradation that is enhanced in Maf1$^{-/-}$ mice. In agreement with this view, Pol III ChIP-seq, tRNA expression and metabolomic data support the function of Maf1 as a chronic repressor of Pol III transcription. Metabolite profiling points to increased activity in the TCA cycle, the pentose phosphate pathway and the urea cycle and confirms key predictions of the futile tRNA cycle hypothesis by identifying changes in pathways of nucleotide synthesis and turnover. Thus, constitutively high levels of Pol III transcription in Maf1$^{-/-}$ mice reprogram central metabolic pathways and waste metabolic energy through a futile tRNA cycle.

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ADDITIONAL POSTERS

P2.51

The critical role of queuosine modifications in the human oxidative stress response

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Cells respond to environmental changes and xenobiotic exposures by altering their phenotypes through signal transduction, transcriptional regulation, and protein secondary modifications. Using a unique computational and analytical platform, we recently described a novel tRNA-based mechanism of translational control of the cell stress response in bacteria and yeast. In this model, stressors and toxicants induce the reprogramming of dozens of modified ribonucleosides in tRNA that modulate levels of protein production by promoting the selective translation of codon- biased mRNAs representing families of stress-response genes. Here, we use LC-MS/MS analysis and multivariate statistics to move this discovery into human cells and show that oxidative stress induces specific changes in queuosine modifications that in turn regulate protein levels by way of selective translation of codon-biased stress response transcripts. Our findings have the potential to transform our understanding of human cell and exposure biology to reveal new mechanism of toxicant-induced pathology with direct relevance to many human diseases and to be exploited for diagnostic and therapeutic purposes.

P2.52

Nano LC-MS using capillary columns enables accurate quantification of modified ribonucleosides at low femtomol levels

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tRNA modifications are crucial modulators of translation and gatekeepers of proteome integrity. Despite their biological importance, technical challenges have limited their detection and accurate quantification. We established a sensitive capillary nanoflow liquid chromatography mass spectrometry (nLC-MS) workflow for quantitative high-resolution analyses of modified nucleosides. We evaluated different stationary phases for reversed-phase separation: two porous graphitic carbon (PGC) and an end-capped C18 material. When used in a nLC-MS setup, PGC and C18 capillaries provided excellent signal-to-noise ratios spanning up to six orders of magnitude, allowing the analysis of individual nucleosides down to femtomol levels. The matrices differ in their separation capability and nucleoside sensitivity, nicely complementing one another. Absolute or relative quantification was attained by defining the linear detection range through synthetic nucleosides or by normalizing signal intensities to a stable isotope labelled spike-in.

We show that capillary columns in a nLC-MS setup are a powerful and sensitive tool to quantitatively analyze modified ribonucleosides in complex biological samples.

P2.53

A pipeline for total tRNA composition analysis broken down to isoacceptors and differential modification content

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The composition of a cell's tRNA population crucially affects its various functions, including in particular protein biosynthesis but also immunostimulation and fragmentation into tRNA fragments (tFRs). The tRNA population of a cell is a highly diverse mixture, whose composition is traditionally analyzed in preparations of so-called total tRNA. These are thought to contain some forty-odd major species in a canonical system, i.e. in bacteria, archeae, or in eukaryotic cytosol. However, a closer look reveals higher diversity: in higher eukaroytes, tRNA genes number in the hundreds, many containing minor sequence deviations. More importantly, extensive post-transcriptional modification of tRNAs, which was long thought to be static and of uniform stoichiometry, has turned out to be dynamically involved in cellular responses to outside stimuli. Hence, a detailed understanding of functional aspects of total tRNA including translation but also non-canonical effects such as immunostimulation, requires quantification of different tRNA sequences as well as of their modification status.
Here we present an integrated approach allowing fast quantification of individual tRNA sequences by microscale thermophoresis (MST). Using complementary cDNA carrying a fluorophore and a biotin residue, the content of a given tRNA in total tRNA can be quantified in less than one hour, allowing a near-comprehensive analysis of the forty-odd conventional components of total tRNA inside three days. Such quantification also constitutes an optimization step for subsequent affinity based tRNA isolation using the same biotinylated cDNA. Thus "sequence-purified" tRNAs can then be assessed for their modification content, allowing the definition of a so-called modivariants, i.e. tRNA sequences that differ only in their modification status. Combination with RNA-seq based methods can pinpoint observed changes to defined positions inside the tRNA sequences. This combination of MST, LC-MS and RNA-seq based analytics allows tracing changes in the total tRNA landscape in response to various outside stimuli liable to change gene expression patterns.

P2.54

Human mitochondrial tRNA processing by ribonuclease P

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Protein coding and rRNA genes are separated (or 'punctuated') by tRNA encoding sequences in the human mitochondrial DNA. Therefore, mitochondrial tRNA ((mt)tRNA) maturation is essential for the processing of other key mitochondrial RNAs. While (mt)tRNA sequences comprise only a small portion of the mitochondrial DNA sequence (~10%), over 50% of the disease-causing mutations in DNA are located within (mt)tRNA coding sequences. Here, we investigate how patient mutations found in precursor (mt)tRNAs impair (mt)pre-tRNA recognition by the three-protein complex responsible for tRNA 5' end maturation, mitochondrial RNase P (mtRNase P). We find that only a subset of mutations influence (mt)pre- tRNA binding, and that all of the mutations we investigated impact the rate of (mt)pre-tRNA processing by mtRNase P. Our findings may be partially explained by the altered UV melting profiles of the mutant (mt)pre-tRNAs which are likely to adopt alternatively folded structures. Our results demonstrate that disease-linked (mt)DNA mutations can impair early steps of mitochondrial tRNA maturation.

P4.13

Application of improved sequencing methods reveals a dominant isoacceptor in angiosperm tRNA profiles

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The development of improved tRNA sequencing methods has now opened the door to exploring the tRNA profiles of multiple organisms. Here, I present tRNA-seq data from four angiosperm species (flowering plants), using a combination of AlkB treatment to remove inhibitory base modifications (Cozen et al., 2015 and Zheng et al., 2015) and YAMAT-seq (Y-shaped Adapter-ligated MAture TRNA sequencing; Shigematsu et al. 2017). Mature tRNAs were sequenced from total cellular RNA from four diverse angiosperms: *Arabidopsis thaliana*, *Solanum tuberosum*, *Medicago truncatula, and Oryza sativa*. Utilization of adapters complementary to CCA tails resulted in libraries composed almost entirely of mature tRNAs (up to 99%), demonstrating the utility of the method to generate libraries highly enriched in tRNA sequences. The resulting reads were dominated by a single tRNA family, as tRNAs from the cytosolic proline isoacceptor family made up the majority of sequenced reads in all angiosperm species (up to 78%), followed by the plastid trnG(GCC). Droplet digital PCR (ddPCR) analysis confirmed the exceptionally high expression level of trnP. Why a single tRNA would be in such high relative abundance is yet to be determined, but the advancement of tRNA profiling methods will lead to a better understanding of the multiple roles tRNAs play in diverse biological systems.