Description for the General Public

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Our cells use information encoded in their genomes (DNA) to produce proteins, including all active enzymes. These molecular machines are responsible for carrying out most fundamental cellular functions, like cell division, production of energy, sensing of the environment and reaction to external stimuli. During a process called "transcription" regions that are the blueprints for specific proteins (genes) are activated and give rise to messenger ribonucleic acid (mRNA) molecules. These mRNA molecules contain a copy of the sequence information stored in the DNA. Next, ribosomes and transfer RNA (tRNA) molecules convert the encoded information from mRNAs into correctly assembled chains of linked amino acids in a process called "translation". Transcription and translation are tightly controlled to assure the correct production of the right enzymes at the right time in the right cellular context. Mutated or incorrectly folded proteins, inappropriate expression levels as well as the production of a protein in the wrong cellular context can lead to cellular dysfunction and in the worst case promote the development of severe diseases, like cancer or epilepsy.

Evolutionary highly conserved machines coordinate pivotal modifications on tRNAs to ensure their correct function in properly decoding genetic information in our cells. One particular modification is the addition of a sulfur atom – termed *thiolation* – to wobble uridine bases located in anticodons of certain tRNAs. This thiolation reaction is important for the correct placement and function of tRNAs in the ribosome. Studies in yeast and worms have shown that the loss of tRNA thiolation can lead to ribosome stalling, which in turn causes proteins to aggregate during translation. Although tRNA thiolation has not been studied in detail thus far, we know that the human CTU1 and CTU2 proteins form a catalytic complex that is responsible for thiolating tRNAs. The recent description of patients carrying pathogenic *CTU2* mutations highlights the clinical necessity to study the human thiolation cascade in greater detail. The mechanisms behind the diagnosed disease, called DREAM-PL syndrome, remain unknown. The ultimate goal of the project is to elucidate the human tRNA thiolation reaction and understand the pathological consequences of its malfunction. Our main goals are (i) to establish model systems to study the loss of the tRNA modification in human cell lines as well as in a DREAM-PL mouse model and (ii) to determine the atomic structure of the human CTU1/CTU2 complex by cryo-EM to understand the mechanistic role of the detected mutations.

We plan to combine our expert knowledge of research teams in Krakow and Vienna to complement our individual strengths in cell biology, mouse genetics, tRNA biology and structural biology. We will perform pulldown proteomics of misfolded proteins and transcriptomics analyses in model systems using the state-of-the-art facilities at the Center for Molecular Medicine (CeMM) of the Austrian Academy of Sciences. The findings will be validated in primary cells derived from DREAM-PL patients. Furthermore, we have already edited mouse embryonic stem cells to carry a pathogenic *CTU2* variant reported in a DREAM-PL patient. Using these cells, we will establish a mouse model to study tRNA hypo-thiolation at the organismal level and in different tissues. The mice will also be analyzed for developmental, behavioral, cellular and molecular phenotypes. In parallel we will use various *in vitro* approaches in Krakow to reconstitute the thiolation reaction in a test tube and gain structural insights on the human tRNA thiolation cascade by cryogenic electron microscopy. We seek to combine the results from our *in vivo* and *in vitro* approaches to obtain a holistic molecular picture of the DREAM-PL syndrome.

Epitranscriptomics, the field of (t)RNA modifications, has become a heavily researched field in recent years, due to the direct link between the lack of certain modifications and the onset of neurodegeneration and cancer. However, much of our current knowledge on tRNA modification (e.g. thiolation) remains confined to studies in simple (mostly unicellular) model organisms. We now have the unique tools and opportunities to expand our current knowledge to human cells and patients. Carrying out this project as an international collaboration will allow us to create a comprehensive picture of the physiological role of tRNA thiolation and the pathological consequences when impaired. These studies on the cellular and molecular levels are required for developing future diagnostic and therapeutic approaches.

This project will substantiate our understanding on the interplay of tRNA thiolation, cellular homeostasis and its contribution to severe human pathologies. As neurons and certain types of cancer cells depend on tRNA thiolation for survival, this project will provide groundwork for targeting the tRNA modification machinery as a potential clinical treatment strategy in rare diseases, neurodegeneration, and cancer.