

Deciphering the Biochemistry, Structure, and Cellular Impact of a Mitochondrial tRNA-Modifying Complex

Host laboratory: RNAmoBio team, Institut de Biologie Paris-Seine (IBPS), Sorbonne Université

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Project Summary

Mitochondria are central to cellular life, providing ATP through oxidative phosphorylation. Disruption of mitochondrial translation leads to pathological disorders. The GTPBP3/MTO1 complex is responsible for installing a post-transcriptional modification ($\tau\text{m}^5\text{U34}$) in mitochondrial tRNAs, ensuring decoding fidelity in mitochondrial ribosomes. The enzymatic reaction requires a complex set of cofactors and substrates including GTP, FAD, NADH, an amino acid (taurine or glycine) and folate derivatives. Clinical mutations in this complex cause the mitochondrial syndrome COXPD23 associated with cardiomyopathies and encephalopathies, but the enzymatic mechanism, structural organization, and cellular consequences of these mutations remain poorly understood.

This project combines enzymology, structural biology, and cell biology to unravel how this unique pathway functions and how its failure leads to disease. In addition to the human complex, we will use the bacterial homologues MnmE/MnmG as a more simple-to-handle model.

Objectives of the Master 2

A. *Enzymology: Dissecting the catalytic mechanism*

(i) Expression and purification of recombinant GTPBP3 and MTO1 (wild-type and pathogenic variants) and their bacterial homologs MnmE/MnmG. (ii) Enzymatic assays to monitor *in vitro*: GTP hydrolysis kinetics, formaldehyde (CH_2O) production from folate derivatives, taurinomethylation of tRNA. (iii) Use of advanced techniques (HPLC, fluorometric detection, isotope tracing, stopped-flow kinetics) to define cofactor usage (GTP, FAD, NADH) and the sequence of catalytic events.

B. *Structural Biology: Visualizing the GTPBP3/MTO1 complex*

(i) Biophysical characterization to characterize complex assembly (SEC-MALS, DLS, Biacore). (ii) Cryo-EM or X-ray crystallography to resolve high-resolution structures of the complex and visualize substrate/cofactor-binding sites. (iii) Structural comparison of complexes to understand disease mutations

C. *Cellular Studies: Linking molecular defects to disease phenotypes*

(i) Quantification of $\tau\text{m}^5\text{U34}$ levels in mitochondrial tRNAs by LC-MS/MS. (ii) Measurement of mitochondrial translation efficiency (ribosome profiling, polysome analysis). (iii) Tracking the complex *in vivo* by microscopy.

Lab Environment

The student will join the RNAmoBio team at Sorbonne Université, a multidisciplinary group with expertise in enzymology, structural biology, RNA biology, and cellular models. The lab fosters an inclusive and collaborative environment and is equipped with advanced biochemical instrumentation (FPLC, stopped-flow, optical spectroscopies) and has access to several platforms (LC/MS, Cryo-EM, confocal microscopy...).

Student Profile

We are looking for a motivated Master 2 student with strong interest in Biochemistry. Prior experience in protein purification, structural biology, or mammalian cell culture is appreciated but not required.

Perspectives

This project offers a complete training pipeline: from enzymatic dissection to 3D structures to human cell models. The student will gain expertise on multiple disciplines while contributing to a project with direct biomedical relevance. This experience provides an excellent foundation for a future PhD in biochemistry and cellular biology.