

SOUSSION D'UN SUJET DE THÈSE – ECOLE DOCTORALE VIE ET SANTÉ

UNITÉ : UMR7156

EQUIPE

Intitulé de l'équipe : Traffic intracellulaire d'ARN et maladies mitochondriales

Responsable de l'équipe: Nina Entelis

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COMPOSITION DE L'ÉQUIPE

Nombre de chercheurs : 4

Nombre de ITA : 1

Nombre de doctorants : 2

Nombre de post-docs : 1

Autres : 1 (stagiaire)

CONCERNANT LA THÈSE

Nom du Directeur de thèse : Benoît Masquida

Mail du Directeur de thèse : b.masquida@unistra.fr

Téléphone : +33 3 68 85 14 81

Thèse en co-encadrement ~~oui~~? non ?

Thèse en co-direction ~~oui~~? non ?

Thèse en co-tutelle *oui* ? ~~non~~?

Nombre de thèses en cours pour le Directeur de thèse : 0

En cas de co-direction, co-encadrement ou co-tutelle :

Nom du co-directeur/encadrant : Prof. Dr. Mark Helm

Université du co-directeur/encadrant : Mainz University

Nombre de thèses en cours pour le co-directeur/encadrant : ?

THÈSE(S) EN COURS POUR LE DIRECTEUR DE THÈSE

Nombre de thèses en cours : 0

Pour chaque doctorant en cours :

Nom :

Prénom :

Début de la thèse :

Fin prévisionnelle :

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DOCTEURS ISSUS DE L'ÉQUIPE (durant les 5 dernières années)

Nombre de docteurs issus de l'équipe (max 3) :

Nom du docteur : Baleva
Prénom du docteur : Mariia
Date de soutenance : Décembre 2016
Situation actuelle : Postdoc

Nom du docteur : Meyer
Prénom du docteur : Mélanie
Date de soutenance : Septembre 2013
Situation actuelle : Postdoc

Nom du docteur : Beckert
Prénom du docteur : Bertrand
Date de soutenance : Octobre 2010
Situation actuelle : postdoc

idem pour chaque docteurs issus de l'équipe

CONCERNANT LE SUJET PROPOSÉ

Titre : (max 190 caractères, espaces compris)

Search and characterization of molecular partners involved in RNA mitochondrial import in yeast and human

Projet : (max 3990 caractères espaces compris, pas de caractères spéciaux)

Transfer RNAs (tRNAs) are imported into mitochondria. Many organisms have mitochondrial genomic DNA lacking tRNA genes or only partial set. The mitochondrial translation necessary for the synthesis of key oxidative phosphorylation proteins thus requires the import of cytosolic tRNAs. However, organisms encoding a complete mitochondrial set of tRNA maintain an import mechanism, the biological consequences of which are still not understood.

The import of tRNA into yeast mitochondria has been shown experimentally in our laboratory. We study tRK1, tRNALysCUU. Its import would restore an effective mitochondrial translation when mitochondrial tRNA (tRK3) is impaired at non-permissive temperature. The import of tRK1 seems to overcome this defect because its anticodon CUU is active without modification.

TRK1-derived RNAs artificially expressed in human cells in culture are also imported into mitochondria. We use this property to import RNAs in the mitochondria for therapeutic purposes. Our strategy consists in inserting into the imported RNA a sequence hybridizing to a region of the mitochondrial mutated "sick" genomic DNA. In the case of efficient hybridization, inhibition of replication of mutant DNA copies should have a curative effect.

As part of the LabEx MitoCross, our main objective is to better understand the mechanisms of mitochondrial tRNA import. We want to acquire the necessary knowledge to control the

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mechanisms of RNA import in mitochondria. In addition to the fundamental aspect of our research, these discoveries would allow us to improve therapeutic approaches.

Only few molecular partners are identified in the mitochondrial import mechanisms. In the case of tRK1, two steps follow. TRK1 is first recognized by the glycolytic enzyme enolase, which facilitates its conformational change so as to mask it from cytosolic pathways. Once in the vicinity of the outer mitochondrial membrane, the tRNA is handed to the mitochondrial lysyl-tRNA synthetase precursor (preMSK1p) which then crosses the membranes up to the matrix, where it is expected to participate in mitochondrial translation.

Our recent experiments (Thesis in 2016) show that the picture is more complex. Our work shows that other factors as enolase are required to carry tRK1 to the mitochondrial surface. In accordance with our results, the literature describes numerous functions for enolase. Enolase is involved in many finely regulated processes, which influence each other, thus adding to the complexity of our model system. Moreover, our affinity assays based on the use of RNA tags allowed the isolation of peptides corresponding to enzymes of tRNA modifications using tRK1 as bait and enolase-enriched yeast extracts as prey. The project is presented in this context.

Our work confirms that enolase participates in mitochondrial import and establishes that enolase does not bind tRK1 without the support of protein helpers. We now wish to identify the proteins involved in the interaction between enolase and tRK1. The structure of a stable complex would be solved by combining mass spectrometry, crystallography, XFEL, and SAXS. We also want to investigate the role of preMSK1p. Evaluating the role of chemical modifications of tRNAs in relation to the import process using a genetic approach conducted in yeast is also our goal. We will study the impact of stress on changes in the profiles of tRNAs purified from mitochondria by high-throughput sequencing (CLIP-seq) methods. This part of the project will be carried out in the laboratory of Professor Mark Helm (University of Mainz, Germany). Mark Helm is a expert in analytical chemistry and RNA bio-conjugate chemistry. His expertise in organic chemistry will also allow us to improve the methods of affinity purification by using click chemistry to link the molecules destined to covalently interact to stabilize the complexes of interest.

Compétences souhaitées : *(max 590 caractères, espaces et sauts de lignes compris)*

The candidate should be very motivated, interested in experimental lab work as well as bioinformatics. The candidate will show good human skills to work in team as well as demonstrate autonomy when necessary. Moreover the candidate will have to develop experimental approaches and as such should be imaginative and capable of going beyond the knowledge.

Expertises qui seront acquises au cours de la formation : *(max 590 caractères, espaces et sauts de lignes compris)*

Along the PhD, the candidate will become an expert in overexpression and purification of proteins and RNAs. The biochemical and biophysical (crystallization, crystallography) methods allowing characterization of these molecules will be mastered as well as the associated computer scientific and other necessary skills. Yeast genetics and human cell culture skills will also be acquired.

Mot clé (servira pour la consultation des sujets) : *(max 40 caractères, espaces compris)*

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RNA mitochondrial import, RNP

PUBLICATIONS MAJEURES DE L'ÉQUIPE RELATIVES AU SUJET AU COURS DES 3 DERNIÈRES ANNÉES. SI NOUVEAU SUJET SANS PUBLICATION, MERCI D'INDIQUER 3 PUBLICATIONS RÉCENTES DU DIRECTEUR DE THESE.

1) (*max 590 caractères, espaces et sauts de lignes compris*)

Baleva, M.; Gowher, A.; Kamenski, P.; Tarassov, I.; Entelis, N.; Masquida, B.* A moonlighting human protein is involved in mitochondrial import of trna. *International Journal of Molecular Sciences* **2015**, *16*, 9354-9367

2) (*max 590 caractères, espaces et sauts de lignes compris*)

Baleva, M.; Meyer, M.; Kamenski, P.; Tarassov, I.; Entelis, N.; Masquida, B.* Factors beyond enolase 2 and mitochondrial lysine trna synthetase precursor are required for trna import in yeast mitochondria. *Soumis*.

3) (*max 590 caractères, espaces et sauts de lignes compris*)

Meyer, M.; Nielsen, H.; Olieric, V.; Roblin, P.; Johansen, S.D.; Westhof, E.; Masquida, B. Speciation of a group i intron into a lariat capping ribozyme. *Proceedings of the National Academy of Sciences of the United States of America* **2014**, *111*, 7659-7664.

CONTRATS DE L'ÉQUIPE (*max 140 caractères, espaces et sauts de lignes compris*)

LabEx MitoCross (2012-2020, Directeur Scientifique Ivan Tarassov)

IDEX Contrat Doctoral (2017-2020, Benoît Masquida)

FRM Contrat de Recherche (2016-2019, Bruno Sargeuil, Université Paris Descartes)

LIA Mitocure (2014-2020, Echanges Strasbourg-Moscou-Novossibirsk, Ivan Tarassov)

Partie à rédiger en anglais :

TOPIC

Title : (*max 190 caractères, espaces compris*)

Project : (*max 3990 caractères espaces compris, pas de caractères spéciaux*)

Wished skills : (*max 590 caractères, espaces et sauts de lignes compris*)

Expertises which will be acquired during the training : (*max 590 caractères, espaces et sauts de lignes compris*)

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Key word : *(max 40 caractères, espaces compris)*

Commentaires éventuels pour l'Ecole Doctorale *(max 590 caractères, espaces et sauts de lignes compris)*