Master Biologie Moléculaire et Cellulaire 'BMC',
Université Paris Cité - *UFR Sciences du Vivant*

Parcours : **Biologie et Développement Cellulaires 'BDC'**
https://master2bdc.ijm.fr/
Fiche de Projet de Stage de M2, 2024-2025

Unité INSERM ou CNRS ou Université : **CNRS** **Intitulé Equipe :** **Microtubule Regulation in Multi-cellular Animals** **ED d'appartenance :** **BioSPC** **Responsable de l'Equipe :** **Paul Conduit**	**Responsable du Stage : Paul Conduit** **Contacts** Adresse : Institut Jacques Monod, CNRS - Université de Paris, 15 rue Hélène Brion, 75013 Paris Email : paul.conduit@ijm.fr Tel : 01 57 27 80 95

Titre du projet : Identifying the long lost microtubule nucleation protein in Drosophila

Résumé du Projet de Stage (en 300 mots maximum, mots clés en gras)

Microtubules are nucleated by multi-protein gamma-tubulin ring complexes (γ-TuRCs). These essential cell components are potential targets for cancer therapy and have been genetically linked to neurodevelopmental disorders. Nevertheless, we still have much to learn about their assembly, recruitment and activation. For many years, γ-TuRCs within the same species were thought to have the same protein composition. We showed, however, that Drosophila γ-TuRCs vary in composition, with the small but essential γ-TuRC protein, Mozart1 (Mzt1), being expressed only within sperm cells. This was striking because Mzt1 homologues are essential for γ-TuRC assembly and mitosis in yeast, plants and human cells. So is there an uncharacterised Mzt1-like protein in other Drosophila cells? Recently, we found that γ-TuRCs purified from Drosophila embryo extracts contain an unknown protein at a similar size to Mzt1 (~8-10KDa). Mass spectrometry fails to identify this protein but a genome-wide Alphafold-based structural similarity search identifies an uncharacterised gene with a similar predicted structure to Mzt1. We hypothesise that this gene represents the long-lost Mzt1-like protein that will be essential for γ-TuRC assembly and recruitment in most Drosophila cells.

In this M2 project, the student will begin to characterise this gene by examining CRISPR-generated endogenously-tagged fly lines. We are currently in the process of tagging the gene with GFP and these flies will be ready to be analysed at the start of the M2 internship. The student will examine the localisation of the gene product within different cell types (embryos, brains, sperm), using both super-resolution live imaging and immunofluorescence. They will perform immunoprecipitation assays and sucrose gradient centrifugation to test whether the protein is associated with γ-TuRCs. In addition, they will perform molecular cloning to generate mutants (via CRISPR) that can then be analysed during a PhD project.

By the end of the project, the student will have gained experience in fly handling and genetic crosses, molecular biology, tissue dissection, and confocal imaging. The project is the perfect start to a PhD project and can be continued by the student should they wish to stay on for a PhD.

See lab website here: https://www.ijm.fr/research-topics/conduit-lab-va/?lang=en

Publications de l'équipe relatives au projet de stage (max 5)

- Tovey CA, Tubman CE, Hamrud E, Zhu Z, Dyas AE, Butterfield AN, Fyfe A, Johnson E, Conduit PT*. (2018). γ-TuRC heterogeneity revealed by analysis of Mozart1. Current Biology 28, 2314-2323.
- Tovey CA and Conduit PT*. (2018). Microtubule nucleation by γ-tubulin complexes and beyond. Essays in Biochemistry. DOI: 10.1042/EBC20180028
- Mukherjee A and Conduit PT*. (2019). γ-TuRCs (quick guide). Current Biology.