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 : UMR 7592 Université Paris Diderot **Intitulé Equipe :** Regulation of microtubule nucleation **ED d'appartenance :** BioSPC **Responsable de l'Equipe :** Paul Conduit	**Responsable du Stage :** Isabelle Bécam **Contacts** Adresse : Insitut Jacques Monod 15 rue Hélène Brion 75013 PAris Email : isabelle.becam@ijm.fr Tel : 0157278095

Titre du projet : Characterisating how the mode of nucleation influences microtubules properties

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

Microtubules are typically nucleated and organised at **microtubule organising centres** (**MTOCs**), such as the centrosome, certain membrane-bound organelles, or specialised areas of the cell cortex. **γ-TuRCs** are recruited and activated at MTOCs and are considered the principal microtubule nucleator. Yet several studies, including ours, have shown that microtubules can still be nucleated and organised within cells in the absence of γ-TuRCs. The proteins most frequently implicated belong to the XMAP215 family that contain **T**umour **O**verexpressed **G**ene (TOG) domains, including ***Drosophila*** **Mini-spindles** (Msps). Intriguingly, we recently showed that microtubules nucleated from *Drosophila* centrosomes lacking γ-TuRCs were unusually resistant to cold-induced depolymerisation (Zhu et al., 2023). Our hypothesis is that the **mode of microtubule nucleation** (γ-TuRC-nucleated vs γ-TuRC-independent) influences the **subsequent properties of the microtubule**.

So far, we have examined the stability of γ-TuRC-nucleated and γ-TuRC-independent microtubules organised by centrosomes within larval brain cells. These centrosomes contain many proteins that could influence microtubule dynamics. To test whether microtubule stability is directly related to the mode of microtubule nucleation (γ-TuRC vs Msps), the student will use a powerful assay developed in the lab to reconstitute MTOCs in *Drosophila* eggs (Tovey et al., 2021). The student will generate MTOC-encoding mRNA in vitro and inject it into eggs, leading to the spontaneous MTOC formation. By modifying the sequence of the mRNA, these artificial MTOCs can be manipulated to recruit γ-TuRCs, Msps, or both γ-TuRCs and Msps, allowing the student to then analyse the properties of microtubules nucleated in different ways using a state-of-the-**art imaging system.**

The student will learn a range of skills, including **molecular cloning**, **molecular biology** techniques, **fly genetics** and manipulation, and **fluorescent microscopy** approaches. The results from the project will strengthen our first conclusion that the mode of microtubule nucleation influences the subsequent properties of the microtubule, a new and potentially important concept **in human health and disease.**

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

-Zhu Z *et al*. Multifaceted modes of γ-tubulin complex recruitment and microtubule nucleation at mitotic centrosomes
JCB 2023 Oct 2;222(10):e202212043.
- Tovey, C. A. *et al.* Autoinhibition of Cnn binding to γ-TuRCs prevents ectopic microtubule nucleation and cell division defects. *J Cell Biol* **220**, e202010020 (2021).