UNIVERSITE BIOLOGIE BRETAGNE SANTE LOIRE

Année 20 /20

FICHE SUJET DE THESE

Sujet N° (à remplir par l'ED) :	FINANCEMENT : ☒ Demandé ☒ Acquis	Origine du financement : 50% HFSP CDA / 50% ARED (100% HFSP CDA si ARED non-acquis)
Titre de la thèse : **Understanding microtubule organization at spindle poles**		3 mots-clés : Spindle architecture, Microtubules, *Xenopus* egg extracts
Unité/équipe encadrante : **UMR CNRS 6290 IGDR, Equipe 'Tubulin and Interacting Proteins'**		
Directeur de thèse : **Romain GIBEAUX (Directeur) & Laurence DUCHESNE (Co-Directeur)**		N° de tél : 02 23 23 46 76 Mail : romain.gibeaux@univ-rennes1.fr

Contexte socioéconomique et scientifique (env. 10 lignes) :

Mitosis is key to the cell cycle, as it guarantees the accurate segregation of replicated chromosomes to daughter cells. This process relies on the spindle, a microtubule-based, dynamic, and bipolar structure. Spindle morphology varies greatly among species and cells to optimize its function. Size and architecture, in particular, are both essential for accurate chromosome segregation, cell division and cytokinesis. Defects in spindle morphology are thus detrimental to organism survival (fetal and birth defects) and health (sterility, cancer development and metastasis). However, despite decades of study and the investigation of hundreds of factors involved, it remains unclear how microtubule subpopulations organize into a complex spindle with well-established morphometrics, at both size and architectural levels. This project tackles this question by focusing on understanding the regulation of microtubule organization at spindle poles. Its goal is to elucidate the role of specific machineries in controlling the variability of spindle pole morphology and the possible contributions of microtubule dynamics and 3D organization in relation to molecular motor localization and activity.

Hypothèses et questions posées (env. 8 lignes) :

Two machineries play a role in organizing microtubules at spindle poles. The minus end-directed cytoplasmic dynein forms spindle poles by cross-linking and sliding microtubule minus ends together, an activity dependent on its interaction with its motility-activating complex dynactin and NuMA. Additionally, the microtubule nucleator TPX2 plays a role in modulating microtubule arrangement at the poles through the recruitment of the plus end-directed antiparallel-crosslinking motor Eg5. By characterizing microtubule 3D organization, localizing and quantifying the different motor activities in asters with unequalled precision, we aim to understand how each cytoplasm yields spindle poles with differing size and architecture. What are the mechanisms that control the variability of spindle pole morphology? What, in particular, is the relative contribution of the NuMA/Dynactin/Dynein and TPX2/Eg5 modules of opposite motilities to differential spindle pole size and architecture? What is the biological significance of the different architectures?

Grandes étapes de la thèse (env. 12 lignes) :

Using the egg extracts of *X. laevis* and *X. tropicalis*, two well-known models that recapitulate very different spindle sizes and architectures, we will reconstruct and compare microtubule asters. The first step of the project will be to combine cutting-edge fluorescence microscopy and electron tomography analyses to elucidate the dynamic and ultrastructural principles that control differential aster size and architecture. We will use live fluorescent imaging to quantify microtubule dynamics, deploy expansion microcopy to obtain a map of molecular motors' localization at high-resolution and finally employ large-scale electron tomography to resolve the organization of microtubules within the structures at the single-microtubule resolution. In addition, this first part of the project will allow us to extract quantitative parameters about microtubules and motors so far inaccessible that will be used in a second step to build physically realistic simulations. We will use Cytosim to perform 3D simulations that will accommodate the lateral interactions between microtubules and thus simulate their overall organization observed experimentally in the structures formed in egg extracts. Motor localizations obtained through our high-resolution maps will also be incorporated within these simulations. We will finally test the physical relevance as well as the necessity and sufficiency of the formulated hypotheses to explain morphometric variations.

Compétences scientifiques et techniques requises par le candidat (2 lignes) :

Knowledge in molecular and cell biology as well as proficiency in English language is required. Experience in microscopy (light and/or electron) as well as interest in bioinformatics/biophysics would be beneficial.

3 publications de l'équipe d'accueil relatives au domaine (5 dernières années) :

M. Kitaoka, R. Heald, R. Gibeaux. Spindle assembly in egg extracts of the Marsabit clawed frog, *Xenopus borealis*. Cytoskeleton; 2018, 75(6):244-257.

R. Gibeaux, R. Acker, M. Kitaoka, G. Georgiou, I. van Kruijsbergen, B. Ford, E. M. Marcotte, D. K. Nomura, T. Kwon, G. J. C. Veenstra, R. Heald. Paternal chromosome loss and metabolic crisis contribute to hybrid inviability in *Xenopus*. Nature; 2018, 553(7688):337-341.

A. Guesdon, F. Bazile, R.M. Buey, R. Mohan, S. Monier, R.R. García, M. Angevin, C. Heichette, R. Wieneke, R. Tampé, L. Duchesne, A. Akhmanova, M.O. Steinmetz, D. Chrétien. EB1 interacts with outwardly curved and straight regions of the microtubule lattice. Nature Cell Biol. ; 2016,18(10):1102-8.

Collaborations nationales et internationales :

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