Profile N° (à remplir par VAS) **FUNDING Planned** MEN-NF

Obtained

Sheet abstract of thesis 2017 **Disciplinary Fields** **Biologie fondamentale et Bio-informatique**

Thesis Title : (1-2 lines)
Mechanisms of GTP-hydrolysis by the tubulin molecule during microtubule assembly

3 keywords : (1 line) **ACRONYME** MT-GTP
Cytoskeleton / Microtubule / Cryo-electron tomography

Unit/Team of supervising : (1-2 lines)
UMR6290 Institute of Genetics and Development of Rennes / Team Tubulin and Interacting Proteins

Name of the scientific director and co-director : (1 line)

Chrétien Denis (director) and Duchesne Laurence (co-director)

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Socio-economic and scientific context : (10 lines)

Microtubules are constitutive fibers of the cytoskeleton composed of α,β tubulin-heterodimers. They are involved in many cellular processes such as cell division, motility and differentiation. Because of their importance in cell life, they are privileged targets for the development of new therapeutic drugs (e.g. as used in chemotherapy). During assembly, hydrolysis of the exchangeable GTP bound to β-tubulin is delayed with respect to microtubule assembly, giving rise to a protecting layer of GTP tubulin molecules at growing microtubule ends, the so-called 'GTP-cap'. However, direct evidence for such a mechanism at the molecular level has been lacking, due to the impossibility to visualize GTP tubulin molecules at microtubule ends. The recent discovery of a new class of proteins (EBs: End-Binding Proteins) that recognize and bind specifically to this GTP-cap gives us new tools to analyze its structure and its dynamics at growing microtubule ends.

Assumptions and questions (8 lines)

Recent results in the lab suggest that the GTP-cap region comprises the outer surface of outwardly curved and straight tubulin sheets, and extends into closed regions of the microtubule lattice (Guesdon et al., Nat. Cell Biol., 2016). According to our results, curve GTP-tubulin sheets gradually straighten and close into straight tubes during microtubule assembly. These structural changes would activate the GTPase activity of tubulin, giving rise to GDP-P_i intermediates in the straight regions of the microtubule tip structure followed by the progressive release of the inorganic phosphate. To investigate this model, we will correlate the conformational changes of tubulin to its nucleotide state (GTP/GDP-P_i/GDP) during microtubule assembly.

The main steps of the thesis and demarche (10-12 lines)

A significant part of the project will be devoted to the structural analysis of microtubules and of their assembly and disassembly products by cryo-electron tomography and 3D reconstructions. First, the architecture of the GTP-cap will be studied using EB-proteins conjugated to gold nanoparticles as probes of GTP-tubulin molecules present at the tip of growing microtubules. Second, the structure of microtubules ends assembled in the presence of GTP analogues will be analyzed by sub-tomogram averaging to correlate the conformational changes of tubulin to its nucleotide state (GTP/GDP-P_i/GDP). Disassembly experiments will be conducted to reveal the intrinsic conformation of tubulin in the presence of these analogues. Results will be compiled to derive a model that will describe tubulin conformational changes in relation with its GTPase cycle. This project is funded by the ANR for the period 2017-2020.

Methodological and technical approaches considered (4-6 lines)

Methodological approaches will include purification of tubulin and recombinant proteins, sample preparation for cryo-electron tomography, data acquisition (tilt series by electron microscopy), three-dimensional reconstructions and sub-tomogram averaging. Spinning disk microscopy will be used to follow microtubule dynamics.

Scientific and technical skills required by the candidate (2 lines)

Knowledge in biochemistry, molecular biology, structural biology and/or bio-informatics (image analysis). Knowledge of English language required.