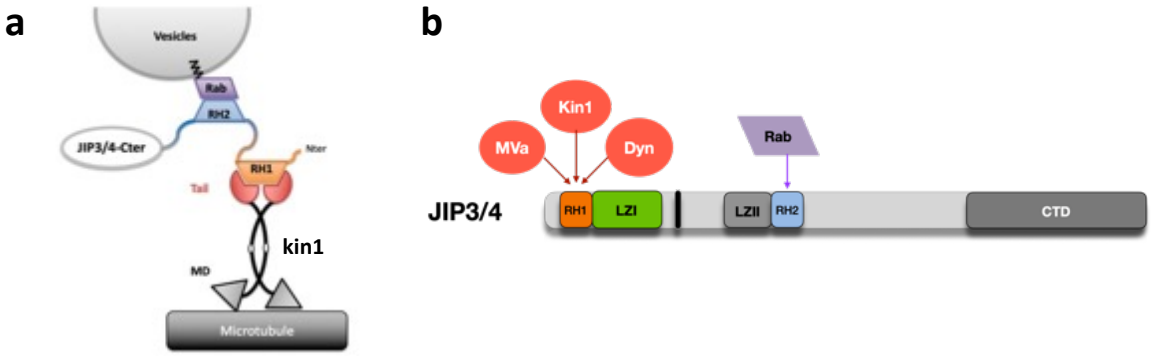
Proposition de stage M2 2021-2022

Regulation of a regulating hub: structural characterization of the auto-inhibited form of JIP3/4 motor adaptors.

JIP3 and JIP4 (JIP3/4) are cargo adaptors for cytoskeletal motors. But they are more than passive adaptors able to link cargoes to motors, JIP3/4 are regulating hubs able to activate, recruit and coordinate three different cytoskeletal motors. They enable bi-directional transport on Microtubule track (MT) by recruiting kinesin-1 (kin1) (Figure 1a) and dynein/dynactin (dyn) complex and may allow switching of cargo between MT and actin tracks by recruiting Myosin V. JIP3/4 can be recruited by Rab GTPases, which are crucial regulators of the intracellular trafficking. Finally, they are also scaffolding proteins that mediates JNK signaling. Then, JIP3/4 play a role in signaling, trafficking and in the coordination of intracellular cargo transport. Defects in these processes could lead to neurological disorders or cancer for example.

JIP3 and JIP4, which are two close homologues, are large dimeric proteins containing a RH1 and RH2 domains, both flanked by coiled-coil regions, namely Leucine Zipper I (LZI) and II (LZII) (Figure1b). The RH1-LZI and LZII-RH2 tandems, located on the N-terminal half of JIP3/4, are separated by an intrinsically disordered region. The RH1 domain of JIP3/4 is the binding site for kin1 and dyn motors (Figure1b). The RH2 domain is the binding site for Rab36 and the phosorylated form of Rab10 (Figure1b). One recent exciting hypothesis focus on the ability of JIP3/4 to be autoinhibited through intramolecular interactions between the RH1 and RH2 domains. Because such intramolecular interactions involved RH1 and RH2 domains, Rab GTPases and cytoskeletal motors recruitment should be impacted. We would like to study these regulation mechanisms as they are essential for understanding intracellular transport and will provide clues for new opportunities to control and manipulate transport.



We propose a Master 2 internship with the purpose to continue a thesis project on JIP3/4 regulation. The objectives of this Master 2 internship will be to characterize the interaction between the RH1 and RH2 domains of JIP3/4 using separate isolated fragments. To do so, the student will design the RH1 and RH2 domain fragments using molecular biology, will express them using a bacterial system, purify proteins using Akta system and characterize their structural integrity using biophysical approaches (CD, MALS, MS, DLS, nanoDSC, AUC). Finally, the student will characterize the interaction using biophysical approaches in solution (ITC, MST and BLI) and perform crystallization assays on the RH1:RH2 complex in order to determine its 3D structure.

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https://www.i2bc.paris-saclay.fr/structural-biochemistry-of-microtubules-kinesins-and-their-cargos/

Références

Vilela F, et al. Structural characterization of the RH1-LZI tandem of JIP3/4 highlights RH1 domains as a cytoskeletal motor-binding motif. *Scientific Reports 9* : 16036 (2019). https://www.nature.com/articles/s41598-019-52537-3.pdf