
Identification and study of partners of C9orf72-SMCR8-WDR41 complex associated with amyotrophic lateral sclerosis

Sujet proposé par

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SPPIN

Membrane dynamics

M2 Research project title

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Keywords

ALS, C9orf72, cellular biology, proteomics, lysosomes

Description of the project

Mutations in the C9orf72 gene, causing both a gain and a loss of function, lead to the most common genetic forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (Balendra and Isaacs, 2018). C9orf72 protein forms a trimeric complex with SMCR8 and WDR41 proteins (CSW) that is recruited to the lysosomal surface upon amino acid starvation (Amick et al., 2020). There, CSW complex was proposed to have a GTPase activating protein activity for several monomeric G proteins (Su et al., 2020). However, the functions of the complex as well as its partners in vivo are still unknown. Here we propose to study the molecular mechanisms by which the CSW complex regulates cell processes during amino acid starvation, by identification of the partners of the complex in cell lines and in neurons using proximity biotinylation assays, and by investigating the functional interaction between the CSW complex and its partners. The proximity biotinylation relies on the fusion of an engineered biotin ligase to a target protein (Roux et al., 2012). Then, we induce biotinylation of interaction partners (in a radius of approximately 10 nm) by addition of biotin in the cell culture media. We then purify biotinylated proteins on streptavidin-conjugated beads and identify them by mass spectrometry and western-blot. In comparison with methods such as co-immunoprecipitation, this technique allows detection of even weak and transient interactions occurring for instance during the course of activation of the CSW complex. We already constructed plasmids expressing C9orf72 and SMCR8 fused to the biotin ligase (called UltraID; Kubitz et al., 2022) and validated their ability to induce biotinylation of proteins after transient expression. We are now in the process of obtaining cell lines stably expressing these constructs and setting up the method of purification of the biotinylated proteins. We wish to host an M2 student for the 2024-2025 academic year to continue this project. This internship will focus on the identification of partners of the CSW complex after mass spectrometry (performed in collaboration with a proteomics platform of the university), on the validation of the interaction of the most interesting partners with the complex and on the study of the roles of these partners in CSW signaling.

Methods and techniques

During the internship, the M2 student will learn and perform cell biology, imaging, and biochemistry techniques. He/she will perform cell culture (cell lines and possibly neurons), transfection, cell treatment, immunofluorescence as well as imaging by fluorescent and confocal microscopy. In addition, he/she will use biochemical techniques such as co-immunoprecipitation and western-blotting). Finally, the student will perform data analysis, including analysis of proteomics data with the assistance of the platform performing mass spectroscopy.

References (at least 3)

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