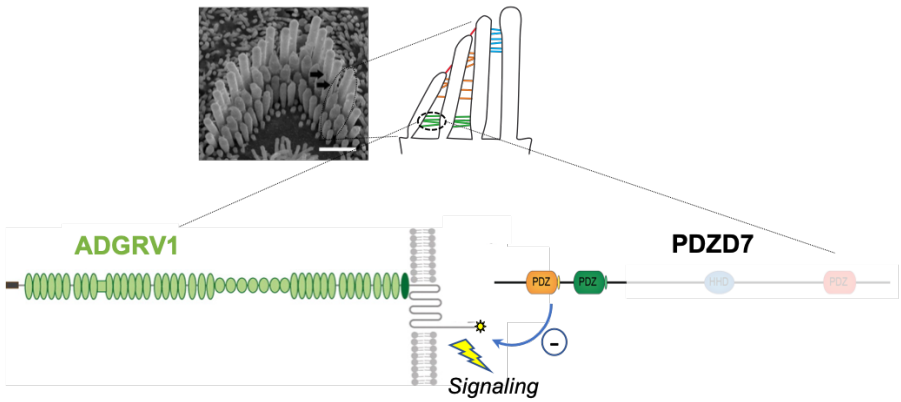
Molecular mechanism controlling the ADGRV1 activity, a GPCR protein involved in deafness and blindness.

1. Description

Hearing relies on the transduction of sound-evoked vibrations into electric signals, occurring in the stereocilia bundle of hair cells. The bundle architecture is pivotal to transduction and involves a network of scaffolding proteins with hitherto uncharacterized features. The study of Usher genes that are linked to deafness has provided insights into the cell biological mechanisms that control hair cell development and their function as mechanosensors.

The human Usher 2 syndrome is the most common genetic cause of combined deafness and progressive blindness. Associated to this disease, two transmembrane proteins, usherin and the G protein-coupled (GPCR) ADGRV1, and the cytoplasmic proteins whirlin and PDZD7, form a dynamic quaternary complex. Usher 2 proteins are transiently located at the base of the stereocilia during hair bundle development, contributing to the formation of the ankle links that connected stereocilia. The long extracellular domains of ADGRV1 and usherin build the core of the ankle link. Their cytoplasmic domains would then be connected to the PDZ domains of whirlin and PDZD7 that in turn associate to actin-binding proteins. In addition to the scaffolding role of the USH2 complex, the interaction between USH2 partners can induce or regulate intracellular signaling as proposed for the ADGRV1 G protein-coupled receptor, and then influence sensory cilia formation/organization/function and sensory cell physiology. The underlying molecular mechanisms of these scaffolding and signaling functions of USH2 complex are still unknown.

PDZD7 can regulate the GPCR activity of ADGRV1 in a PDZ-dependent manner



In this Master2 project, we propose to elucidate the network of interaction between ADGRV1 and PDZD7. The N-terminal part of PDZD7 directly binds to the cytoplasmic domain of ADGRV1 and this interaction regulates the GPCR activity of the membrane protein. This N-terminal region of PDZD7 encodes for two adjacent domains that can cooperate as a supramodule for the binding of partners. Two missense mutations leading to deafness were identified in the these PDZ domains of PDZD7 that interact with ADGRV1. We hypothesize that these mutations most likely affect the folding and/or the binding properties of PDZD7 and consequently alter the USH2 complex and the regulation of ADGRV.

2. Technics/methodology

The aim of the M2 project is to analyze the structure of the N-terminal region of PDZD7 using NMR spectroscopy, X-ray diffraction and SAXS experiments and its complex with the cytoplasmic region of ADGRV1. The impact of USH2 mutations on the stability, folding and binding, properties of PDZD7 will then be evaluated. In order to validate the significance of these protein interactions, we will perform validations in a cellular context, either in transient transfection experiments using HEK293/HeLa cells, or in whole-mount preparations of the mouse organ of Corti, the sensorial organ of hearing. We will analyze the cellular sub-localization of the selected partners by classical immunohistochemical analysis using UV microscopy.

3. Expected results

We will document the interaction between the N-terminal region of PDZD7 that encode for two folded PDZ domains involved in protein-protein interaction. The affinities of different constructs of PDZD7 for the cytoplasmic domain of ADGRV1 will be measured. In addition, we plan to solve the structures of PDZD7 PDZ domains in complex with the C-terminal region of ADGRV1 combining x-rays diffraction and NMR. These results will be compared to those obtained with mutated PDZD7 constructs to evaluate if these USH2 mutations impact the folding, the affinity for ADGRV1, and/or the stability of PDZD7. In parallel, these ADGRV1-PDZD7 interactions will be also documented in cell and in the organ of hearing extracted from mouse.

4. References

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