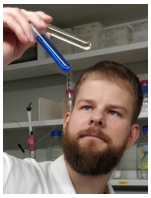

Molecular basis of ciliopathy diseases

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Group: 4 postdocs, 1 PhD student, 1 lab manager, 1 staff scientist, 1 Master student

Background: The **cilium** organelle projects from most cells in our body and serves important functions in motility and sensory reception (Fig. 1). Motile cilia power the movement of sperm cells towards the oocyte, clear our airways of pathogens and propel the cerebrospinal fluid. Immotile primary cilia are responsible for vision and important developmental signalling such as the sonic hedgehog pathway. The **Intraflagellar Transport** (IFT) machinery is responsible for the trafficking of protein factors into and out of the cilium organelle. Mutations in ciliary factors result in human diseases known as ciliopathies with diverse pathologies such as blindness, obesity, respiratory insufficiency and sterility.

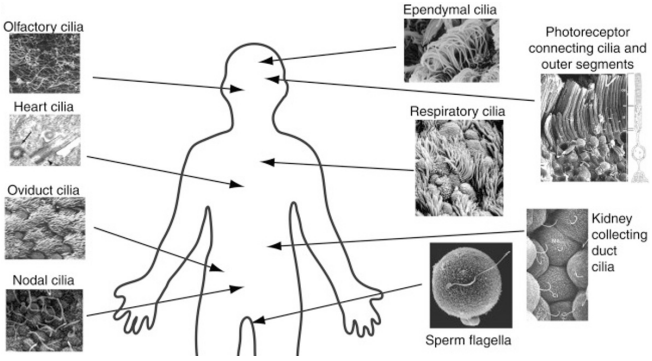

Fig. 1: Examples of cilia on different human cells and tissues

Project 1: Molecular mechanisms of mutations causing Joubert syndrome

Arl13B is a ciliary membrane-associated protein that localizes along the cilium (Fig. 2A). Arl13B is required for the proper ciliary localization of INPP5E, a phosphatase that converts PI(4,5)P_2 into PI4P to establish the correct composition of the ciliary membrane. Mutations in INPP5E cause Joubert syndrome, a genetic neurodevelopmental disorder with malformation of the mid and hindbrain, which results in developmental delay and intellectual disabilities. Pathologies can also include breathing problems, renal defects and polydactyly. Interestingly, mutations in Arl13B causing Joubert syndrome fail to import INPP5E into cilia (see Fig. 2B-C).

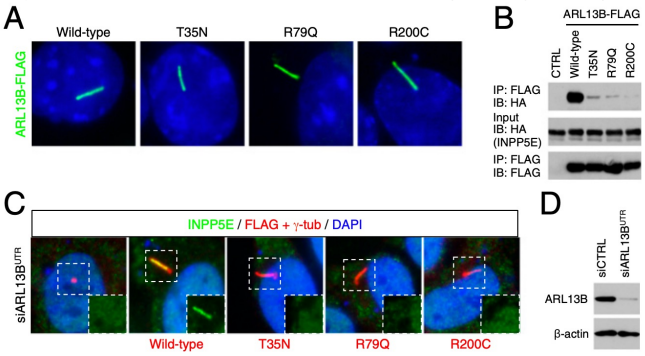

Fig. 2: Arl13B disease mutants fail to import INPP5E into cilia

- Structural modelling of the Arl13B-INPP5E complex using machine learning algorithms such as Alphafold
- Cloning, transformation, and expression of disease mutants of Arl13B and INPP5E
- Purifications of proteins and protein complexes for biochemical and structural investigations
- Biochemical investigation of the Arl13B-INPP5E complex: *Do ciliopathy causing mutations disrupt complex formation?*
- Thermal unfolding experiments. *Do disease mutations cause protein instability?*

Project 2: Molecular investigations of Bardet-Biedl syndrome proteins

Mutations in the Bardet-Biedl syndrome (BBS) gene family are associated with a range of pathologies that manifest in childhood and include kidney disease (50% of cases), polydactyly, intellectual disability and retinal dystrophy. BBS proteins associate into the BBSome complex and function together with the IFT machinery in ciliary export of proteins including signalling factors. Mutations in genes encoding BBS and IFT proteins cause BBS. BBS17 is likely the link between IFT and BBSome complexes (Fig. 3) and knock out of BBS17 disrupts hedgehog signalling by ciliary accumulation of the membrane protein Smoothened. In this project, you will investigate BBS17 as a molecular bridge required for ciliary export of signalling factors and examine the effect of BBS mutants on protein stability and complex formation.

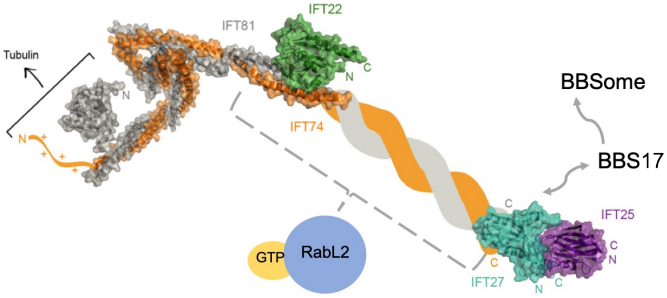

Fig. 3: Schematic of the IFT complex and factors in ciliary export

- Purifications of BBS17 and IFT complexes through IMAC, ion exchange, and SEC
- Assess BBS17 complex formation with IFT proteins through pull-downs, SEC and Alphafold
- Determine the influence of BBS17 on the GTPase activity of the small GTPases IFT27 and RabL2 using activity assays
- *Do ciliopathy mutations in IFT27 or BBS proteins influence protein stability and/or protein interactions?* This will be determined by biophysical investigations of proteins with disease mutations

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