STRUCTURAL BIOLOGY OF CILIA

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Our group investigates the cellular mechanisms that maintain the distinct composition of cilia and immunological synapses. In particular, we are interested in the trafficking of lipid-modified signalling proteins by a group of proteins called GDI-like solubilising factors. In our lab, we address our research questions using a combination of structural, biochemical and cellular biology approaches.

Primary cilia, found on almost all human cell types, are involved in the regulation of several signalling pathways and are reported to be lost in several cancer tissues. Lymphocytes are one of the few cell types that do not form cilia. However, they do form a structure called the immunological synapse, which shares similarities with cilia, at the interface between professional and non-professional antigen-presenting cells, including tumour cells.

GDP dissociation inhibitor (GDI)-like solubilising factors (GSFs) are a family of proteins, including PDE6D, UNC119a and UNC119b, which solubilise lipid-modified proteins and share structural homology with the Rho GDP dissociation inhibitors, a class of proteins known to bind prenylated Rho proteins. PDE6D binds to and is involved in the trafficking of prenylated proteins, whereas UNC119a and UNC119b are specific for myristoylated proteins (Wright *et al.* Genes Dev 2011; 25: 2347–60; Zhang *et al.* Nat Neurosci 2011; 14: 874–80; Zhang *et al.* Vision Res 2012; 75: 19–25).

Arl2 and Arl3 are small G-proteins that belong to the Arf (ADP ribosylation factor)-like small G-protein subfamily. They have a 52% sequence identity and share several interactors. Amongst Arl2 and Arl3 interactors are PDE6D, UNC119a and UNC119b, where the interactions are guanosine triphosphate (GTP) dependent and do not involve lipid moieties. Arl2 and Arl3 function as allosteric release factors for lipidated proteins bound to PDE6D and UNC119a/b in a GTP-dependent manner (Ismail *et al.* Nat Chem Biol; 2011; 7: 942–9; Ismail *et al. EMBO J*; 2012: 31: 4085–94). Furthermore, it has been reported that the ciliary protein Arl13b can act as a specific guanine nucleotide exchange factor (GEF) for Arl3 (Gotthardt *et al.* eLife 2015; 4: pii: e11859.

Last year we put forward a sorting model for prenylated protein INPP5E delivery to the cilia. The model depends on the affinity of cargo for PDE6D, the presence of an active Arl3 found exclusively in cilia, and the specific release of ciliary cargo by active Arl3.

Using INPP5E and Rheb as examples for ciliary and non-ciliary proteins, respectively, we show that lipidated cargoes are solubilised by binding to GSFs in the cytosol. If a cargo binds to GSFs with a low binding affinity, the complex will be disrupted by active Arl2GTP in the cell body. In the case of ciliary proteins, which bind to GSFs with strong binding affinities, the soluble complex can diffuse into the cilia, where it is released by Arl3, which is in turn activated by the ciliary protein Arl13b. The released cargo is then retained in cilia by associating with the ciliary membrane.

We are currently investigating this trafficking mechanism and its role in the formation of the immunological synapse between lymphocytes and cancer cells. Furthermore, using small molecules we are trying to manipulate the positioning of signalling proteins in the cilia and immunological synapse to alter their signalling output.

Publications listed on page 98

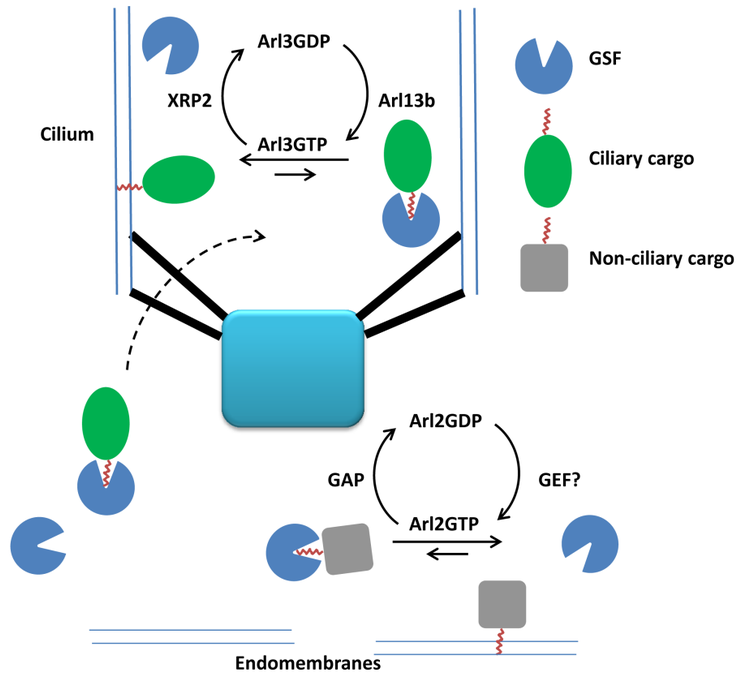

Figure 1
Sorting and shuttling of prenylated/myristoylated ciliary cargo into the cilium
The GSF (blue; e.g. Unc119b) binds to the lipid-modified tail of the ciliary cargo (green). The complex diffuses into the cilium, where Arl3, maintained in a GTP-bound state by Arl13b, binds the GSF, forcing a conformational shift that releases the ciliary cargo to the ciliary membrane. Binding of Arl3 to its GAP, XRP2, results in its inactivation. Non-ciliary cargo (grey) is solubilised by the GSF in the same manner before being released to endomembranes by Arl2GTP. The GEF required to activate Arl2, in its GTP-bound state, is not known.