SPATIAL SEGREGATION OF SIGNALLING

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At equilibrium, entropy (disorder) reaches maximum, since disorder is more probable than order. Life exists away from equilibrium and our cells have developed mechanisms to counteract disorder. Spatial and temporal segregation/organisation of proteins of opposite functions is crucial for controlling cell signalling output, e.g. separating phosphatases and kinases or the small GTPases regulators, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Our group is interested in studying self-organised cellular compartments that participate in cell signalling without being separated from the rest of the cell by membranes, such as the cilium and the immunological synapse. We combine structural biology, biochemistry and cell biology to investigate the atomic basis of cellular mechanisms that maintain and regulate the distinct composition of the cilia and immunological synapses in space and time. We take advantage of the similarities between cilia and immunological synapses and cross-test information that we generate from both cellular structures. We aim to develop cancer therapeutics by manipulating the spatial organisation of these protein networks and hence the output of their signalling compartments.

Mutations in the small GTPase ARL3 cause Joubert syndrome

The cilium is a hair-like protrusion on almost every cell in our body and functions as a signalling antenna. Concentrating signalling proteins and receptors inside the cilium is key to its function, and dysfunctional cilia result in many developmental diseases, collectively called ciliopathies. Previously, together with other groups, we have managed to identify and characterise the machinery that transports and concentrates those signalling proteins in the cilium (Ismail et al., Nat Chem Biol. 2011; 7: 942, Ismail *et al.*, EMBO J. 2012; 31: 4085, Watzlich *et al.*, EMBO Rep. 2013; 14: 465, Fansa *et al.*, Nat Commun. 2016; 7: 11360).

In collaboration with John Sayer (Newcastle University), we have reported *ARL3* missense variants as likely cause of Joubert syndrome (JBTS), making *ARL3* a novel JBTS gene. We show *in vitro* and in cells from patients that missense variants of *ARL3* arginine at position 149 disrupt the known interaction between ARL3 and ARL13B. This prevents the activation of ARL3 and in turn the correct release of intra-ciliary cargos. We propose ARL3 as a hub within the network of ciliopathy-associated genes, whereby perturbation of ARL3 results in the mislocalisation of multiple ciliary proteins, including INPP5E and NPHP3.

T cells repurpose ciliary machinery to traffic and concentrate LCK at the immunological synapse

Upon the engagement of a T cell receptor (TCR) with an antigen-presenting cell, LCK phosphorylates the immunoreceptor tyrosine-based activation motifs (ITAMs), initiating TCR signalling. Autophosphorylation of LCK tyrosine 394 (Y394) is critical for its kinase activity in cells. However, *in vitro*, phosphorylated Y394 results in only a two-fold increase of its catalytic activity, suggesting an additional layer of regulation. In this project we show that phosphorylation of LCK Y394 does not only alter the catalytic rate but also regulates its interaction with the ciliary UNC119A and thus LCK trafficking. UNC119A interacts with the unique domain of LCK with a high affinity, compared to other SRC family kinases. We show that the release at the target membrane is under the control of the ciliary ARL3/ARL13B. The UNC119A N terminus acts as a 'regulatory arm' by binding the LCK kinase domain, an interaction inhibited by LCK Y394 phosphorylation, thus together with the ARL3/ARL13B machinery ensuring immune synapse focusing of active LCK. We finally propose in this study that the ciliary machinery has been repurposed by T cells to generate and maintain polarized segregation of signals at the immune synapse.

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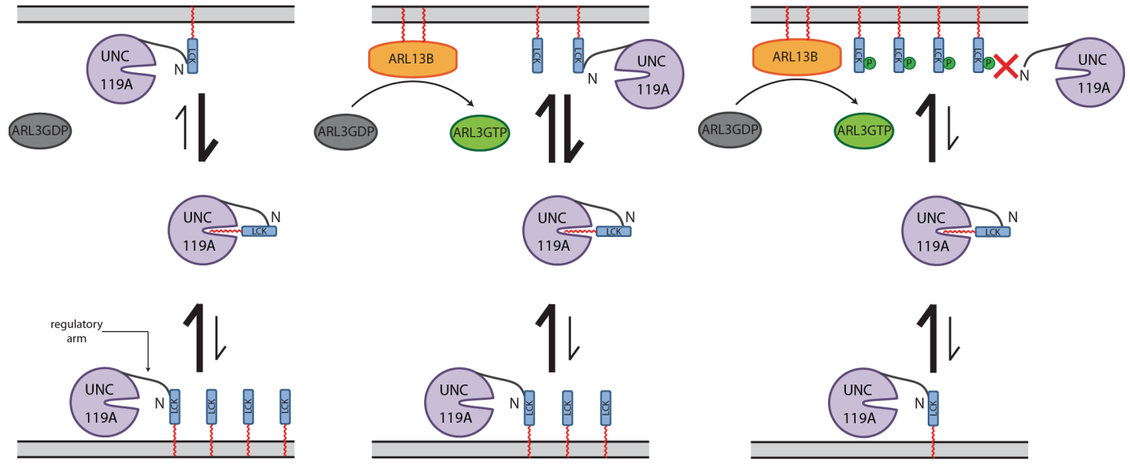

Figure 1
A working model of the interplay between ciliary ARL3 and LCK trafficking and phosphorylation
Myristoylated LCK at the endomembranes is extracted by UNC119A through a regulatory arm–kinase domain interaction as well as hydrophobic pocket-myristoyl group binding. Solubilised LCK can then be transported in the cytosol, but, in the absence of active ARL3GTP at the plasma membrane (left), very little release of LCK occurs at the cell surface. Localisation of guanine exchange factors (GEFs) such as ARL13B at the plasma membrane drives activation of ARL3. LCK released by ARL3GTP can then anchor to the plasma membrane, where it can be extracted again by UNC119A (middle). Phosphorylation of LCK due to its activation blocks the interaction between the kinase domain and UNC119A (right), thereby trapping LCK at the plasma membrane.