Primary cilia assembly, disassembly, and cell proliferation

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Project description:

Primary (non-motile) cilia are hair-like extensions present on almost all animal cells that act as antenna for extracellular signals and are fundamental to proper metazoan development and ongoing health. Cilia extend from the mother centriole which precludes it use information of a mitotic spindle. Therefore, extension of cilia and entry into mitosis are, in most cases, mutually exclusive. Defects in cilia are linked to many inherited human diseases and more recent data has identified a key role for ciliary signalling in wound healing including resolving bone fractures.

Our recent work has focussed on the ciliary microtubule motor, dynein-2. We were first to define the composition of any metazoan dynein-2 motor complex, and have used genome engineering to demonstrate the function of key subunits in cilia assembly and function. Our work has defined the centriolar subdistal appendage protein CEP170 as the key site for assembly of the ciliary transport machinery. CEP170 is constantly expressed throughout the cell cycle and is phosphorylated during mitosis by Polo-like kinase 1 (Plk1) and Tankbinding kinase (TBK1). The impact of CEP170 phosphorylation on cilia function is unknown.

Here we propose a project based on our combined expertise in primary cilia biology and mitosis, with training and implementation of cutting-edge bioimaging methods, including super-resolution light microscopy and 3D electron microscopy, along with in vitro reconstitution experiments and phosphoproteomics. These technologies provide fantastic training opportunities for the student as well as ample opportunity to shape and define the scope of the project as they wish.

Our hypothesis is that CEP170 defines the location for the assembly of the ciliary machinery and that phosphorylation impacts that process. We also have evidence that CEP170 plays an important role in ciliary disassembly – a prerequisite for cell cycle entry. We propose that the CEP170-dynein-2 axis provides a critical point of integration of centriolar function and the decision between ciliary function and cell cycle entry. Genome editing, selective kinase inhibition, and recombinant protein expression provide opportunities to perturb the system. We can link these outcomes to key features of cell biology in the context of wound healing including proliferation, migration, and signal transduction. While amenable to work in diverse 2D and 3D cell culture models, in vitro work will be led by purification of centrosomes from mammalian cells for which established protocols exist.