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Dynamic Quantitative Imaging of Primary Cilium Assembly

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The primary cilium is a specialized organelle that projects from the surface of many cell types. Unlike its motile counterpart it does not actively move but acts to transduce extracellular stimuli into intracellular signals and provides a specialized subcellular compartment [1]. The cilium is built and maintained by the transport of proteins, membrane and other biomolecules into and out of this compartment. The two cellular pathways primarily responsible for cilium assembly and maintenance are: 1) Intraflagellar transport (IFT) and 2) membrane trafficking. We have developed methods to monitor and measure the flux of material into and out of primary cilia through these two pathways. By establishing these methodologies and measuring baseline activities, we can probe defects that underlie defects found in human ciliopathy syndromes.

IFT was originally identified in the green algae *Chlamydomonas* and has been discovered throughout the evolutionary tree [2]. The IFT machinery is widely conserved and acts to establish, maintain and disassemble cilia and flagella. Here we will describe current methods for directly observing the IFT process in mammalian primary cilia. Through the generation of fluorescently tagged fusion proteins that incorporate into endogenous IFT complexes, we can observe and measure the IFT process in living cells. High numerical aperture timelapse microscopy permits direct observation, and kymographic analysis reveals quantitative details of IFT motility. Moreover, mutations like those found to cause embryonic lethality [3] or perturbations by small molecules [4] can result in quantitative changes in IFT velocities modulating cilium signaling and length. Identifying defective IFT processes will help connect molecular defects with organismal disease pathophysiology.

Equally important in cilium physiology is the membrane trafficked from the intracellular membrane compartments that contribute to defining the cilium envelope [5]. The dynamics of membrane trafficking remain poorly defined however a wide variety of gene mutations result in inappropriate or defective protein trafficking to the cilium membrane. Observations using high numerical aperture imaging and a combination of photobleaching and photoactivation techniques permit the measurement of membrane protein residence time and the kinetics of entry and exit. A number of these gene mutations are being tested for their effect on membrane trafficking and resulting defects in cilium signaling and length regulation.

Detailed imaging of cilium dynamics will bridge molecular defects to mechanisms regulation cellular function and ultimately provide insights into the pathophysiologies that underlie human disease.

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