Analysis of Protein Dynamics in Motile Cilia of Mouse Tracheal Epithelial Cells Using High-Resolution Live Cell Imaging

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Defects in motile cilia and flagella commonly result in the pediatric syndrome primary ciliary dyskinesia (PCD), an autosomal recessive disorder affecting 1 in 16,000 live births[1]. Affected individuals typically suffer from chronic rhinosinusitis, chronic otitis media, male infertility, and laterality defects, with some patients and models exhibiting female infertility and hydrocephalus. Motile cilia play a critical role in fluid flow, while the structurally related sperm flagella are required for sperm motility.

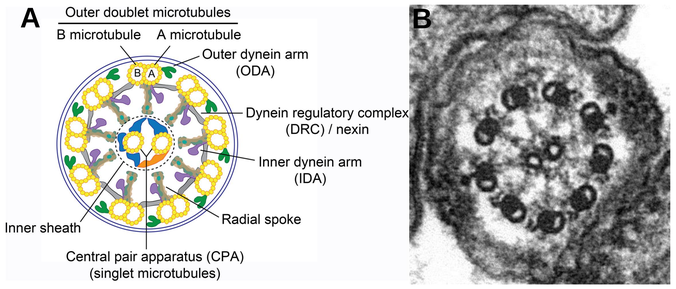

Figure 1. The ciliary axoneme. (A) Schematic diagram showing the ciliary core, or axoneme, which has a 9+2 microtubule structure comprised of nine outer microtubule doublets and a central pair apparatus. From Lee and Ostrowski, 2020[2]. (B) Electron micrograph of a mouse sperm flagellar axoneme. From Lee et al., 2008[3].

We previously showed that mice lacking central pair apparatus (CPA) proteins SPEF2, CFAP221, and CFAP54 each have a PCD phenotype resulting from a defect in ciliary motility[3,4,5], but the mechanisms regulating mammalian motile cilia are not fully understood. Here, we are using a novel application of high-resolution, live cell imaging to analyze ciliary protein dynamics in ciliated mouse tracheal epithelial cells (mTECs) cultured from PCD mice with a CPA defect.

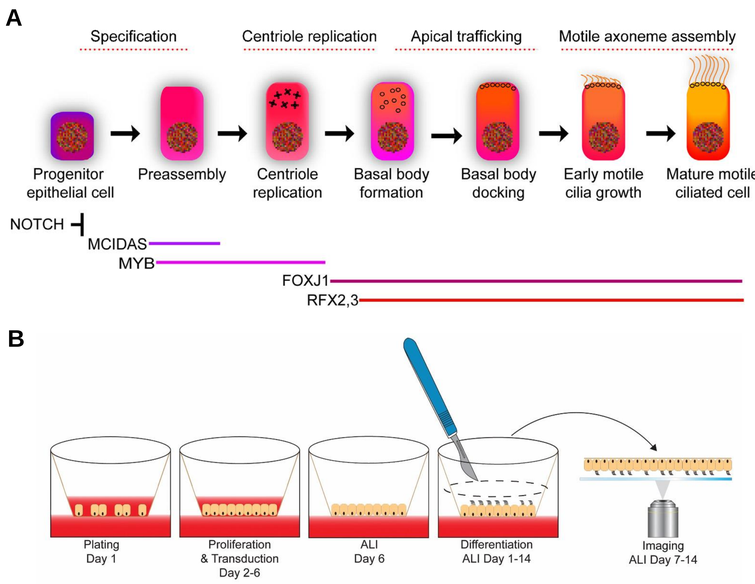

Figure 2. Motile ciliated cell differentiation. (A) Inhibition of the Notch signaling pathway initiates specification and differentiation of motile ciliated cells. From Horani et al., 2016[6]. (B) Culturing of mTECs involves isolation from a mouse trachea, growth and proliferation on a permeable membrane insert, and differentiation at an air-liquid interface (ALI) to form ciliated cells. The membrane is cut out and placed cells-down for live imaging at day 7-14 post-ALI.

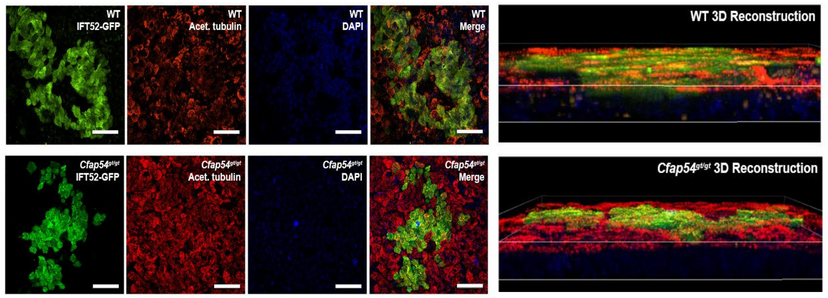

Figure 3. Confocal immunofluorescence analysis of exogenous IFT52-GFP expression in mTEC cilia. Analysis of wild type (top) and *Cfap54*$^{gt/gt}$ (bottom) mTECs expressing a C-terminal GFP-tagged IFT52, a component of the intraflagellar transport machinery required for ciliogenesis, following lentiviral transduction. Cells are stained with a GFP antibody (green), an antibody to ciliary marker acetylated tubulin (red), and nuclear marker DAPI (blue), showing that IFT52-GFP co-localizes with acetylated tubulin. Scale bar = 50μm (WT), 20μm (*Cfap54*$^{gt/gt}$). The reconstructed 3D side view of a confocal Z stack (right column) shows ciliary localization of IFT52-GFP on the surface of the cells.

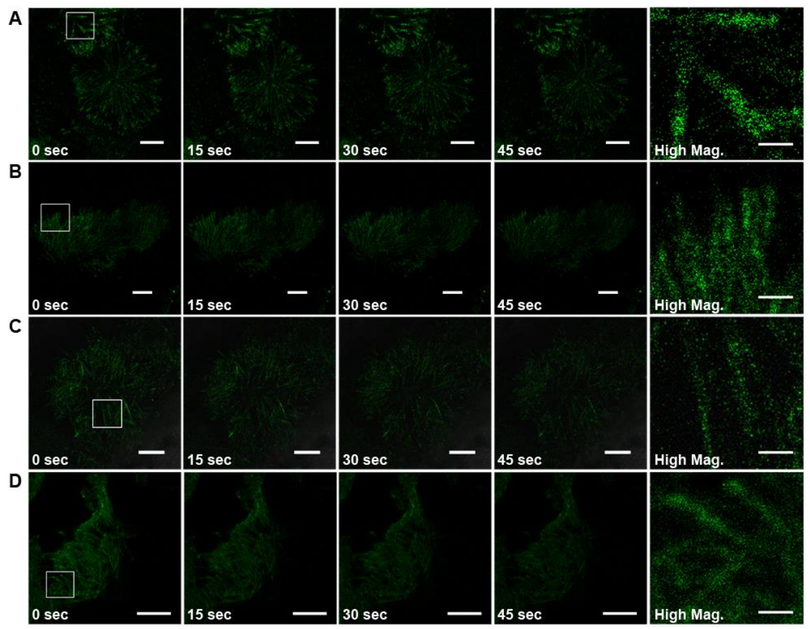

Figure 4. Live imaging of exogenous IFT52-GFP in mTEC cilia. Time-lapse photos from high-resolution movies show IFT52-GFP dynamics in WT (A,B) and *Cfap54*$^{gt/gt}$ (C,D) mTEC cilia from 0 to 45 seconds. High magnification images (High Mag) were digitally magnified from the 0-second images using Adobe Photoshop and show IFT52-GFP protein particles within ciliary structures. Boxes indicate digitally magnified fields. The cilia are immobilized for imaging by treatment with 100mM LiCl prior to imaging, and the membrane insert is transferred face-down onto a FluoroDish to flatten the cilia. The cells are imaged with an inverted Nikon A1R confocal microscope with 2% laser power, a Galvano scanner, 0.02-0.06 μm/px pixel sixe, and ~0.5 - 1 frame/second frame rate. Scale bar = 5μm, 1 μm (High Mag).

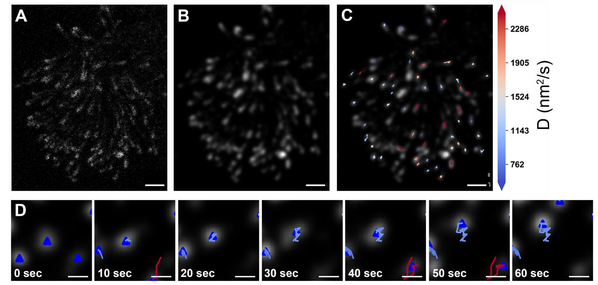

Figure 5. Single particle tracking analysis of time-lapse movies. Median filtering and Gaussian blurring were applied to the raw imaging files (A) during pre-processing to improve the signal-to-noise ratio (B). (C) The trajectory of each particle was traced at high magnification from the pixel at the center of the particle. D: diffusion coefficient. (D) Time-lapse images showing tracing of individual IFT52-GFP particles over time. The triangle represents the particle center, and the overlaid trajectory indicates movement of the particle center from time 0. Scale bars = 2 μm (A-C), 0.5 μm (D).

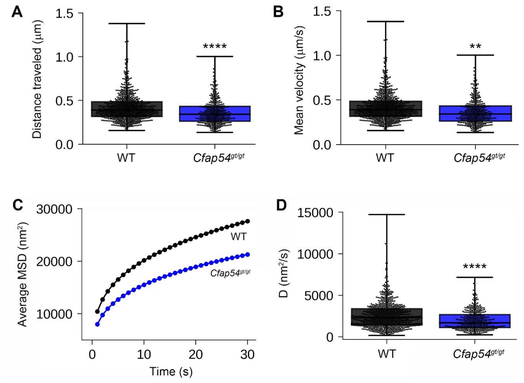

Figure 6. Loss of *Cfap54* impairs movement of IFT52-GFP in mTEC cilia. (A) Distance traveled by particles in WT and *Cfap54*$^{gt/gt}$ cilia. The mean distance is 0.422 mm in WT cilia and 0.366 mm in *Cfap54*$^{gt/gt}$ cilia. $p = 4.1 \times 10^{-10}$. (B) Velocity of particles in WT and *Cfap54*$^{gt/gt}$ cilia. The mean velocity is 0.0435 mm/s in WT cilia and 0.0425 mm/s in *Cfap54*$^{gt/gt}$ cilia. $p = 8.3 \times 10^{-3}$. (C) Average mean square distance (MSD) curve, applied commonly as a diffusion model, showing a more constrained mobility in *Cfap54*$^{gt/gt}$ mTEC cilia than WT. $p = 1.7 \times 10^{-5}$ for the comparison of average WT and *Cfap54*$^{gt/gt}$ MSD. (D) Diffusion coefficient, D, for IFT52-GFP in WT and *Cfap54*$^{gt/gt}$ cilia. Mean D value is 2605.5 for WT cilia and 1999.3 for *Cfap54*$^{gt/gt}$ cilia. $p = 3.3 \times 10^{-12}$.

Single particle tracking analysis of high-resolution, live cell imaging of mTECs enables assessment of protein dynamics in mammalian motile cilia and demonstrates that loss of the CPA protein CFAP54 results in impaired movement of exogenous IFT52-GFP. It is possible that the CPA defect compromises the integrity of the cilium, although further studies are required to uncover the molecular mechanism underlying the effect. These methods establish a platform for analysis of ciliary protein dynamics in PCD models, which will enable a better understanding of the mechanisms underlying mammalian cilia and PCD pathogenesis.

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ABSTRACT:

Dysfunction of motile cilia results in defects in fluid clearance and the pediatric syndrome primary ciliary dyskinesia (PCD). Motile cilia possess a 9+2 microtubule structure with nine outer microtubule doublets surrounding a central pair apparatus. While the importance of the motile cilia in human health is clear, the ability to study and understand protein dynamics in mammalian motile cilia has been limited, largely based on availability of useful tools and feasible strategies. In this study, we demonstrate a novel application of high-resolution, live confocal imaging to analyze ciliary protein trafficking and dynamics in ciliated mouse tracheal epithelial cells (mTECs). The mTECs are cultured at an air-liquid interface to enable optimal ciliogenesis, and GFP-tagged ciliary proteins are over-expressed using a lentiviral system. We have performed live single molecule imaging of GFP-tagged intraflagellar transport protein 52 (IFT52-GFP) in mTECs from wild type mice and a PCD mouse model lacking central pair apparatus protein CFAP54. Quantitative analysis by single molecule tracking identified the trajectory and velocity of IFT52-GFP and showed that movement is impaired in mice lacking CFAP54. This study establishes a powerful platform for analysis of protein dynamics to elucidate the cellular mechanisms underlying mammalian motile cilia and PCD pathogenesis.