Imaging capabilities allow researchers to peer into protein transport systems

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A new study from University of Georgia cell biologists analyzes the transport system that builds cell organelles called cilia. Defective cilia are directly connected to a host of diseases and conditions, including inherited bone malformations, blindness, male infertility, kidney disease and obesity. Knowledge of how cilia are built and the ability to manipulate their structure can inform future medical treatments.

Led by Karl Lechtreck, assistant professor in the department of cellular biology, a team of researchers utilized Total Internal Reflection Fluorescence microscopy to analyze moving protein particles inside cilia of Chlamydomonas reinhardtii, a widely used unicellular model for the analysis of cilia. Results of the study were published in the online version of *Current Biology* on December 5.

The interdisciplinary team included researchers from the UGA Franklin College of Arts and Sciences and the College of Engineering, Dartmouth College and the University of Minnesota.

"Because cilia are very complex and their construction requires the transport of hundreds of different proteins, direct evidence at the molecular level requires a very sensitive imaging technique," said Lechtreck, who is a member of the Integrated Life Sciences Program.

In TIRF microscopy, laser light is reflected to generate an evanescent field that allows for the imaging of single proteins. "That field is very thin-30 to 300 nanometers in thickness-and flagella have a thickness of 200 nm, approximately 500 times thinner than a human hair. TIRF allows us to now precisely see what is going on inside the flagella of living cells," Lechtreck explained.

Lechtreck and his team used the technique to load the cilia-building transport mechanism with an actual protein and watch it throughout the process of delivery and assembly into cilia.

"In cell biology, it is very important to understand how a cell determines the size of its cilia, and our observations on intraflagellar transport and its cargoes suggest a much more sophisticated mechanism than previously assumed," he said. "The ability to directly watch how cilia are assembled and alter their composition during signaling is a major result for our field. Because defects in ciliary length and protein transport are linked to disease, our observations have direct biomedical implications."

More information: Kathryne N. Wren, Julie M. Craft, Douglas Tritschler, Alexandria Schauer, Deep K. Patel, Elizabeth F. Smith, Mary E. Porter, Peter Kner, Karl F. Lechtreck, A Differential Cargo-Loading Model of Ciliary Length Regulation by IFT, *Current Biology*, Available online 5 December 2013, ISSN 0960-9822, dx.doi.org/10.1016/j.cub.2013.10.044.

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