Architecture of rod sensory cilium disrupted by mutation

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Using a new technique called cryo-electron tomography, two research teams at Baylor College of Medicine have created a three-dimensional map that gives a better understanding of how the architecture of the rod sensory cilium (part of one type of photoreceptor in the eye) is changed by genetic mutation and how that affects its ability to transport proteins as part of the light-sensing process.

Almost all mammalian cells have cilia. Some are motile and some are not. They play a central role in cellular operations, and when they are defective because of genetic mutations, people can go blind, have cognitive defects, develop kidney disease, grow too many fingers or toes or become obese. Such mutations cause cilia defects known in the aggregate as ciliopathies.

"The major significance of this report lies in our being able to, for the first time, look in three dimensions at the structural alterations in ciliopathies," said Dr. Theodore G. Wensel, chair of biochemistry and molecular biology at BCM and corresponding author of the report that appears in the journal *Cell*. The report is spotlighted on the issue's cover.

In collaboration with the National Center for Macromolecular Imaging, led by Dr. Wah Chiu, professor of biochemistry and molecular biology at BCM, Wensel and his colleagues established such three dimensional images for cilia in three examples of mice known to have cilopathies.

These mice have genetic mutations that lead to defects in the structure of

the rod outer segment. The rod outer segment is part of the photoreceptor in the retina called a rod. The rod outer segment contains photosensitive disk membranes that carry rhodopsin, the biological pigment in photoreceptor cells of the retina responsible for the first events that result in the perception of light.

Using cryo-electron tomography, the scientists compared the structures of the rod outer segment in the mutant mice to those in normal mice.

"This is one of the few places in the world where you could do this," said Wensel. The Center, run by Chiu, has powerful cryo-electron microscopes that make tomography possible. To achieve the three-dimensional reconstruction, Dr. Juan T. Chang in Chiu's Center froze the photoreceptors purified by then-graduate student Jared Gilliam in a special way that made it possible to perform electron microscopy. During the microscopy session, the frozen samples were carefully tilted allowing the researchers to take many two-dimensional images that were used in the computer reconstruction of the three-dimensional map.

The light-sensing outer segments of photoreceptors in the retina are connected to the machinery responsible for protein production in the inner segment by a thin cylindrical bundle of microtubules known as the connecting cilium.

"There is a huge flux of material from the inner segment to the outer segment of the photoreceptor," said Wensel. "When there is a defect, then the animal or patient goes blind."

The three-dimensional structure showed that there are vesicles (small sacs) tethered to membrane filaments.

"It looks as though these vesicles that are tethered contain material that will fuse to the plasma membrane and go up the membrane to the outer

segment," said Wensel.

In studies of a mouse model of a disease called Bardet Biedl syndrome, developed by the laboratory of Dr. James Lupski professor of molecular and human genetics at BCM, Wensel and first author Gilliam saw something that was almost shocking – a huge accumulation of these vesicles. The Bardet Biedl genes contain the code for a BBsome that forms a membrane coat that makes transport possible through the connecting cilium to the outer coat.

"We would now surmise that the BBsome coat is required for fusion of the plasma membrane or transport up to the outer segment," said Wensel. "It gives us a whole new model for how this works. We need to do more now to nail it down."

"It suggests that aberrant trafficking of proteins is responsible for photoreceptor degeneration," said Gilliam, who is now a postdoctoral associate at The University of Texas Health Science Center at Houston.

Provided by Baylor College of Medicine