Generation of Affinity Reagents for the Study of Photoreceptor Neurons

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Background: Photoreceptor cells are highly polarized neurons with distinct compartments essential for visual function. Their outer segments contain specialized ciliary membranes, called disks, that detect photons. Actin contributes to disk morphogenesis, while rhodopsin, a transmembrane photopigment, is a major component of disks involved in phototransduction. Conventional fluorescence microscopy (200-300 nm resolution) is suboptimal for visualizing these subcellular structures (5–100 nm). The goals of this project are to enhance fluorescence microscopy resolution and refine protein labeling strategies to visualize actin and rhodopsin dynamics during disk formation. By generating recombinant affinity reagents (nanobodies and class-switched IgG), we aim to probe actin filaments in photoreceptor outer segments.

Methods: The DNA sequences encoding actin nanobody and its derivative were PCR-amplified and incorporated into the PET22B vector. Genetically modified nanobodies were expressed in Bl21 *E. Coli* for high-yield production, efficient purification, and detection via fluorescent tags or secondary IgG antibodies. In addition, a recombinant monoclonal antibody against rhodopsin was produced by introducing two expression vectors, coding 1D4 IgG heavy and light chains, to ExpiCHO cells. Western blotting was employed to measure the quality and quantity of these nanobodies and antibodies. These affinity reagents were used for immunofluorescence microscopy of mouse retinal sections with the Nikon AX R NSPARC super resolution confocal microscope.

Results: We successfully produced a recombinant rabbit monoclonal antibody against rhodopsin (1D4 mAb) in ExpiCHO cells. Based on immunoblotting analysis, we estimate approximately 1 mg of antibody is present in 35 ml of culture. The antibody labeled mouse rod outer segment structures, indicating it is specific to rhodopsin. We are currently attempting to express actin nanobodies in E. coli cells for fluorescence imaging.

Expected Impact: The successful staining of actin and rhodopsin in the outer segment of photoreceptors help reveal their spatial relationship during disk morphogenesis, highlighting a potential interplay between cytoskeletal elements and membrane proteins. This discovery offers new insight into how photoreceptor architecture is built and maintained, offering a foundation for understanding mechanisms linked to photoreceptor dysfunction and degeneration in diseases including retinitis pigmentosa and other inherited retinal dystrophies.