PDE6 Conformational Dynamics as a Molecular Driver of Light-Induced Photoreceptor Length Modulation

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1. Main Text

Sight is perhaps the most spectacular human sense. Light enters the eye and reaches the retina, where it triggers highly orchestrated biochemical reactions that are converted into electrical signals and ultimately processed into vision. At the heart of this cascade lies phototransduction, initiated in photoreceptor outer segments - highly ordered stacks of discs loaded with proteins critical for light detection. While the activation of the phototransduction cascade is well described, it has long been observed that light stimulation also evokes subtle, transient morphological changes in photoreceptors, specifically disc spacing alterations and outer segment length modulation [1]. However, the molecular driver behind these physical deformations remains elusive.

Recent structural data now suggest that phosphodiesterase 6 (PDE6), a core effector enzyme of phototransduction tethered to disc membranes, may directly contribute to these morphological changes. PDE6 undergoes activation-dependent conformational changes [2-4] and may serve as a dynamic mechanical linker between stacked discs [5]. These structural transitions, observed between distinct cryoEM states, modulate the effective length of the PDE6 molecule, potentially transmitting nanometer-scale forces to disc spacing and outer segment length.

To test this hypothesis, we combined the isolation of native rod PDE6 and rod outer segments (ROS) with multiple cryo-imaging modalities to directly investigate these structural alterations in their physiological context. While our cellular-resolution cryoET, cryoSXT, and cryoFIB-SEM experiments operate at a lower resolution compared to single-particle cryoEM, they provide valuable measurements of disc spacing under different conditions, with PDE6 situated in its native membrane environment. Understanding this coupling between molecular conformational changes and large-scale morphological responses may not only clarify fundamental phototransduction mechanics but also support functional imaging techniques such as optoretinography (ORG), and open avenues for therapeutic targeting in PDE6-related retinal diseases.

2. Methods and Results

- PDE6 isolation and purification from porcine eyes
- In vitro PDE6 catalytic activity assays
- ROS isolation from murine retinas
- CryoET, cryoSXT, cryoFIB-SEM, and TEM imaging of ROS

To elucidate the molecular basis of ORG signals, we employed spatiotemporal optical coherence tomography (STOC-T) and demonstrated that PDE6 conformational transitions propagate into nanometer-scale photoreceptor outer segment elongations, synchronized with light flicker stimuli [5]. Using sildenafil as a PDE6 inhibitor in rodent models, we confirmed that blocking PDE6 activity significantly attenuates these light-induced elongations, directly linking phototransduction to mechanical optical signals captured in ORG [5]. Additionally, we developed protocols to isolate rod outer segments (ROS) and apply cryoET, cryoSXT, cryoFIB-SEM, and TEM imaging to extract disc-spacing parameters (Fig. 1A–D), while in vitro assays with purified porcine PDE6 further characterized its catalytic activity (Fig. 1E). Mouse ROS were also imaged by cryoSXT, cryoFIB SEM, and TEM (Fig F-H).

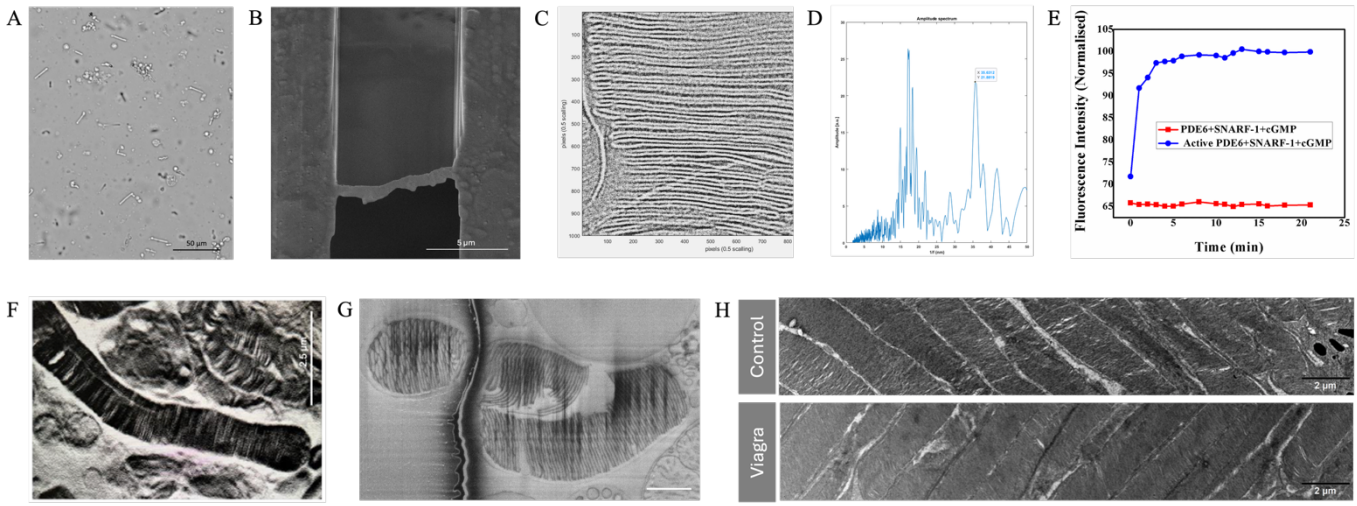

Fig. 1. Mouse ROS and isolation and characterization. A) Mouse ROS visualized under a light microscope. (B) FIB lamella preparation from ROS cryo grids. (C) CryoET ROS tomograms. (D) Distances between disc measurements. (E) PDE6 activity assay, measuring hydrolysis of cGMP. (F) CryoSXT. (G) CryoFIB SEM. (H) TEM.

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