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Adaptations in rod outer segment disc membranes in response to environmental lighting conditions

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ABSTRACT

The light-sensing rod photoreceptor cell exhibits several adaptations in response to the lighting environment. While adaptations to short-term changes in lighting conditions have been examined in depth, adaptations to long-term changes in lighting conditions are less understood. Atomic force microscopy was used to characterize the structure of rod outer segment disc membranes, the site of photon absorption by the pigment rhodopsin, to better understand how photoreceptor cells respond to long-term lighting changes. Structural properties of the disc membrane changed in response to housing mice in constant dark or light conditions and these adaptive changes required output from the phototransduction cascade initiated by rhodopsin. Among these were changes in the packing density of rhodopsin in the membrane, which was independent of rhodopsin synthesis and specifically affected scotopic visual function as assessed by electroretinography. Studies here support the concept of photostasis, which maintains optimal photoreceptor cell function with implications in retinal degenerations.

1. Introduction

Two types of photoreceptor cells are present in the vertebrate retina that initiate vision upon photon capture via phototransduction. Rod photoreceptor cells are responsible for scotopic vision whereas cone photoreceptor cells are responsible for photopic vision. Rod photoreceptor cells are the most abundant photoreceptor cell type in most mammalian retina. These cells are exquisitely sensitive, capable of generating a response to a single photon of light [1]. Photoreceptor cells are highly compartmentalized neurons comprised of an inner segment, where biosynthesis of proteins occurs, and an outer segment, where phototransduction occurs to initiate vision (Fig. 1A). The rod outer segment (ROS) exhibits a highly-organized membrane structure comprised of membranous discs stacked one on top of another that are encased by a plasma membrane [2,3]. ROS discs consist of a double lamellar membrane connected by a rim region (Fig. 1B and C).

The ROS is a dynamic compartment. Up to 500–2000 discs are stacked in a single ROS, depending on the species [4]. New discs are continually formed at the base of the ROS whereas old discs at the distal tip of the ROS are phagocytized each day by retinal pigment epithelial cells [5–7]. Rhodopsin is the light receptor in rod photoreceptor cells and is the predominant protein species in ROS discs. The light receptor is synthesized in the rod inner segment and transported to the base of the ROS, where it is incorporated into the membrane of newly synthesized ROS discs [8–12]. Rhodopsin initiates the first steps of vision via phototransduction by absorbing incoming photons and, upon photoactivation, binding and activating the heterotrimeric G protein transducin [13].

Photoreceptor cells must adapt to the lighting environment for optimal function and survival of the organism. Short-term adaptations of rod photoreceptor cells occurring in the seconds to minutes range, typically referred to as light or dark adaptation, have been studied in much molecular detail (reviewed in [14–21]). The molecular details of more long-term adaptations of rod photoreceptor cells to the lighting environment are less clear. The concept of photostasis was introduced to describe long-term adaptations to the lighting environment requiring days to weeks to develop [22]. Photostasis has been proposed to occur in rod photoreceptor cells to maintain an optimal constant photon absorption capacity in the retina and to saturate rod photoreceptors cells under photopic conditions [23,24]. Photostasis is a phenomenon also thought to occur in invertebrate photoreceptor cells, plants, green algae, and cyanobacteria [25–28], where adaptations occur in the cells and proteins that capture and detect photons.

Primary changes thought to be associated with photostasis in

Abbreviations: AFM, atomic force microscopy; ERG, electroretinography; ROS, rod outer segment(s); TEM, transmission electron microscopy

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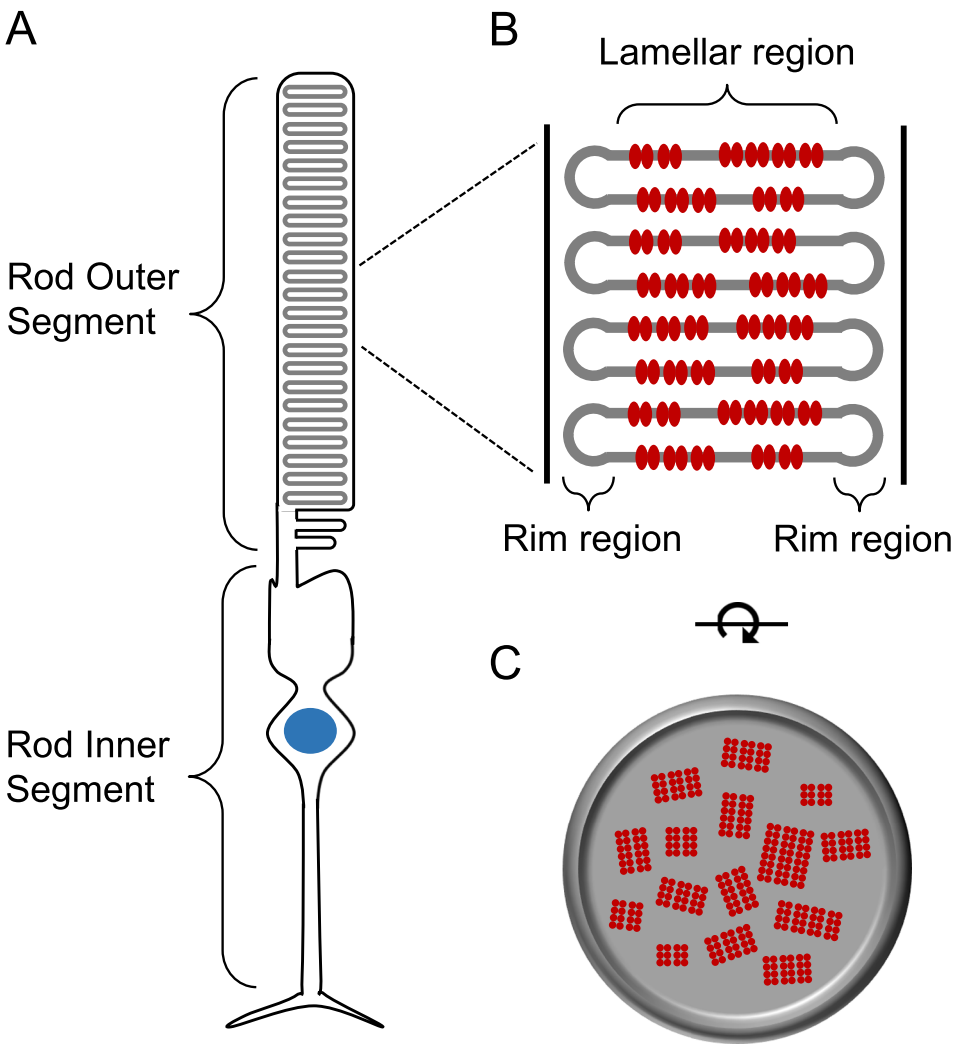

Fig. 1. Cartoon of a rod photoreceptor cell and rod outer segment discs. A, Side view of a rod photoreceptor cell. Rhodopsin is synthesized in the rod inner segment and then transported and incorporated into the membrane of discs in the rod outer segment. B, Zoomed in view of discs. Discs are stacked one on top of another and encased by a plasma membrane. Rhodopsin (red ellipses) is present within the lamellar region and excluded from the rim region of discs. C, Top view of a disc. Rhodopsin is organized into nanodomains within disc membranes forming oligomeric complexes.

vertebrate rod photoreceptor cells include changes in the length of the ROS and the expression level of rhodopsin [22,23,29–32]. The concept of photostasis has been recently questioned based on the absence of significant changes observed in the length of the ROS in mice housed in different lighting regimes when improved statistics were utilized [33]. Furthermore, changes in ROS length and rhodopsin expression levels is minimal in pigmented mice compared to albino animals [34,35], which raises the question of whether or not photostasis is a general adaptive mechanism in vertebrate rod photoreceptor cells and whether or not long-term changes occur in rod photoreceptor cells in response to changes in the lighting environment.

Are there adaptive changes in rod photoreceptor cells in response to long-term changes in environmental lighting? The incorporation of ectopically expressed rhodopsin into ROS disc membranes appears to be regulated by the lighting environment [36,37]. Although it is unclear whether or not endogenously expressed rhodopsin exhibits similar behavior, these studies raise the possibility that adaptations to the lighting environment may occur at the level of ROS discs, the site of photon absorption by the light receptor rhodopsin. Previous studies have been unable to explicitly characterize ROS discs with sufficient resolution to understand the effects of long-term changes in lighting environment at the molecular level.

Atomic force microscopy (AFM) is a nanoscale imaging tool useful for visualizing the structures of biological membranes and membrane proteins under the physiological conditions of a biological membrane and buffer conditions [38]. AFM is suited to examine the structure of ROS discs and the packing of rhodopsin within disc membranes. AFM has revealed that rhodopsin oligomerizes in ROS disc membranes forming nanodomains [39–42] (Fig. 1C). This membrane organization of rhodopsin has also been observed by cryo-electron tomography of vitrified ROS and may be required for signaling efficiency and single photon response [2,43–46]. In the current study, AFM was utilized to directly investigate the effects of prolonged housing in dark or light conditions on the structure of disc membranes and rhodopsin packing in the ROS of pigmented mice.

2. Materials and methods

2.1. Mice and housing conditions

Wild-type mice used in the current study were C57Bl/6J mice (The Jackson Laboratory, Bar Harbor, ME). *Gnat*$^{-/-}$ mice were generated as reported previously [47], and were backcrossed for 10 generations with C57Bl/6J mice. All mice were housed in cage racks except for those housed in constant light conditions. Mice housed in constant light were stored on top of a cart to fully expose mice to room lighting. Mice housed in cyclic lighting conditions were on a 12 h dark/12 h light cycle and were investigated at 6 weeks of age. Mice housed in constant dark or constant light conditions were born and reared in cyclic lighting conditions. An exception were mice reared in constant darkness, which were born and reared in constant dark conditions until they were used for experiments at 6 weeks of age. Mice housed for 10 days in constant darkness or 10 days in constant light were transferred from cyclic lighting conditions to the respective lighting conditions after 4.5 weeks