Segregating phototransduction from morphogenesis in photoreceptor outer segments

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Outer segments are specialized compartments of photoreceptor cells which function in the detection of light and its conversion into electrical signals in a process known as phototransduction. In rod cells, the outer segment consists of a stack of hundreds of closed disks surrounded by a separate plasma membrane. Each disk is made up of two flattened membranes circumscribed by a hairpin rim region with one or more incisures. Outer segments undergo a continual renewal process in which packets of aged disks are removed at the distal end by phagocytosis while new discs are formed at the base or proximal end.

Phototransduction is initiated when a photon of light converts 11-cis retinal to its all-trans isomer within the G-protein coupled receptor rhodopsin densely packed in disk membranes. The ensuing protein conformational change leads to a cascade of reactions which culminate in phosphodiesterase (PDE) catalyzed hydrolysis of cGMP, closure of the cGMP-gated channels in the plasma membrane to the influx of Na\textsuperscript{+} and Ca\textsuperscript{2+}, and hyperpolarization of the rod cell. Return to the dark state occurs through inactivation of the phototransduction cascade, activation of guanylate cyclases (GCs) by the Ca sensor proteins GCAPs, and reopening of the channel in response to rising cGMP levels.

Previous studies have shown that disk and plasma membranes have distinct protein compositions.\textsuperscript{1} Disk membranes can be further separated into the lamellae containing high concentrations of rhodopsin oriented for maximum photon capture and the rim region containing the peripherin/rd complex important for membrane curvature, ABC transporter ABCA4 crucial for the clearance of retinoids, and GCs (Figure 1). In contrast the plasma membrane contains the cGMP-gated channel and the Na/Ca, K, exchanger (NCKX1) which control intracellular Ca levels, and moderate concentrations of rhodopsin. The cGMP-gated channel composed of 3 CNGA1 and 1 CNGB1 subunit associates with several proteins including NCKX1 in the plasma membrane,\textsuperscript{2} peripherin/rds complex in disc rims,\textsuperscript{3} and cytoskeletal proteins,\textsuperscript{4} but how the channel and cGMP signalling proteins are organized at a submembrane level has not been well-defined.

Nemet et al.\textsuperscript{5,6} have provided new insight into the compartmentalization of the rod outer segment plasma membrane and the arrangement of cGMP signalling proteins with implications in both phototransduction and disc morphogenesis. Using fluorescence imaging of immunolabeled endogenous proteins and over-expressed fluorescent-fusion proteins, they have shown that the plasma membrane of Xenopus laevis is organized into two submembrane domains, the ‘phototransduction domain’ and the ‘disk morphogenic domain’ (Figure 1). The cGMP-gated channel is trafficked directly to the phototransduction domain where it is organized in vertical striations possibly representing the immobile fraction of the channel. This organized array is localized in the plasma membrane across from the multiple disk incisures where GCs, GCAPs and PDE which control cGMP levels are concentrated.\textsuperscript{5,7} Since mammal rods typically have only 1-4 incisures, these structures may not be required for localizing the cGMP-signalling proteins across from the channel. Instead this may be accomplished in part through interactions of the channel with peripherin/rds or cytoskeletal proteins such as Band 4.1. The proximity of the channel-NCKX1 complex to the key cGMP and Ca signalling and regulatory proteins PDE, GCs and GCAPs in the disk rims can facilitate the rapid response of the rod cells to changes in cGMP and Ca during photoexcitation and recovery. Deletion studies further indicate that the GARP component of CNGB1 subunit is essential for trafficking of the channel to the rod outer segment. Interestingly, GARP is not present in the related cone cGMP-gated channels.

Disk morphogenesis occurs at the base of the outer segment (Figure 1). Two mechanisms have been proposed. In the evagination model, the ciliary plasma membrane protrudes to create disk-like structures which subsequently form closed disks,\textsuperscript{7} while in the vesicular model, vesicles translocated from the inner segment or produced at the base of the outer segment fuse to form nascent discs within the outer segment.\textsuperscript{8} Nemet et al.\textsuperscript{5,6} have shown that the plasma membrane within the disk morphogenetic domain is densely packed with rhodopsin, but devoid of the cGMP-gated channel. These studies indicate that the channels along with a relatively low amount of rhodopsin are directly trafficked to the phototransduction domain distal to the disk morphogenic domain. A diffusional barrier of unknown composition segregates these two domains. These and related studies employing overgrown nascent discs\textsuperscript{6} lend support to the actin-mediated evagination model and provide a framework for identifying the molecular mechanisms and components responsible for selective protein trafficking and disk morphogenesis in this unique light-sensing structure.
Figure 1. Diagram of the rod outer segment showing the domains which function in phototransduction ‘phototransduction domain’ and the disk morphogenesis ‘mophogenic domain’, The location of various proteins involved in phototransduction and outer segment structure and morphogenesis are shown. In the phototransduction domain the cGMP-gated channel in association with the sodium-calcium-potassium exchanger NCKX1 interact with peripherin/rds complex at the rim region of disks via the GARP domain of the CNGB1 channel subunit. Guanylate cyclases (GCs) in association with guanylate cyclase activating proteins (GCAPs) and phosphodiesterase (PDE) reside in the disk rim opposite the channel. In the morphogenic domain nascent disks densely packed with rhodopsin, but devoid of the channel are formed through actin-mediated evagination of the plasma membrane. The cytoskeletal protein Band 4.1 interacts with a fraction of the channels possibly during protein trafficking along the microtubules.

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