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# Cholesterol in the rod outer segment: A complex role in a "simple" system

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ABSTRACT

The rod outer segment (ROS) of retinal photoreceptor cells consists of disk membranes surrounded by the plasma membrane. It is a relatively uncomplicated system in which to investigate cholesterol distribution and its functional consequences in biologically relevant membranes. The light sensitive protein, rhodopsin is the major protein in both membranes, but the lipid compositions are significantly different in the disk and plasma membranes. Cholesterol is high in the ROS plasma membrane. Disk membranes are synthesized at the base of the ROS and are also high in cholesterol. However, cholesterol is rapidly depleted as the disks are apically displaced. During this apical displacement the disk phospholipid fatty acyl chains become progressively more unsaturated, which creates an environment unfavorable to cholesterol. Membrane cholesterol has functional consequences. The high cholesterol found in the plasma membrane and in newly synthesized disks inhibits the activation of rhodopsin. As disks are apically displaced and cholesterol is depleted rhodopsin becomes more responsive to light. This effect of cholesterol on rhodopsin activation has been shown in both native and reconstituted membranes. The modulation of activity can be at least partially explained by the effect of cholesterol on bulk lipid properties. Cholesterol decreases the partial free volume of the hydrocarbon region of the bilayer and thereby inhibits rhodopsin conformational changes required for activation. However, cholesterol binds to rhodopsin and may directly affect the protein also. Furthermore, cholesterol stabilizes rhodopsin to thermal denaturation. The membrane must provide an environment that allows rhodopsin conformational changes required for activation while also stabilizing the protein to thermal denaturation. Cholesterol thus plays a complex role in modulating the activity and stability of rhodopsin, which have implications for other G-protein coupled receptors.



## 1. Introduction

Cholesterol has been implicated in the regulation of many G-protein coupled receptors (review (Burger et al., 2000; Oates and Watts, 2011)). These include the cholescytokinin (Gimpl et al., 1997), oxytocin (Gimpl et al., 1997, 1995, 2002; Klein et al., 1995) and serotonin (Pucadyil and Chattopadhyay, 2004) receptors. Additionally, other receptors such as the nicotinic acetylcholine (Narayanaswami and McNamee, 1993; Fernandez-Ballester et al., 1994) and transferrin (Nunez and Glass, 1982) receptors may also be modulated by cholesterol. The effect of cholesterol on rhodopsin, the visual photoreceptor and archtype for the family of G-protein receptors, has been investigated in model membranes as well as within its native membrane in the rod photoreceptor, providing complimentary insight into the interrelationship of the receptor and the lipid bilayer. The rod photoreceptor is a particularly useful system in which to study the synthesis, distribution and function of cholesterol within a single differentiated cell.

The rod photoreceptor cell in vertebrates is a terminally differentiated cell and is responsible for vision under conditions of low light. As diagramed in Fig. 1, it consists of an inner segment and an outer segment each of which has specialized functions. The biochemical machinery for metabolic processes, protein synthesis and the nuclei are located in the inner segment. The function of the outer segment is to capture photons of light and to initiate the visual signal transduction cascade. The mammalian visual transduction cascade culminates in hyperpolarization of the plasma membrane that triggers firing at the nerve synapse at the base of the inner segment. This nerve synapse interacts with an

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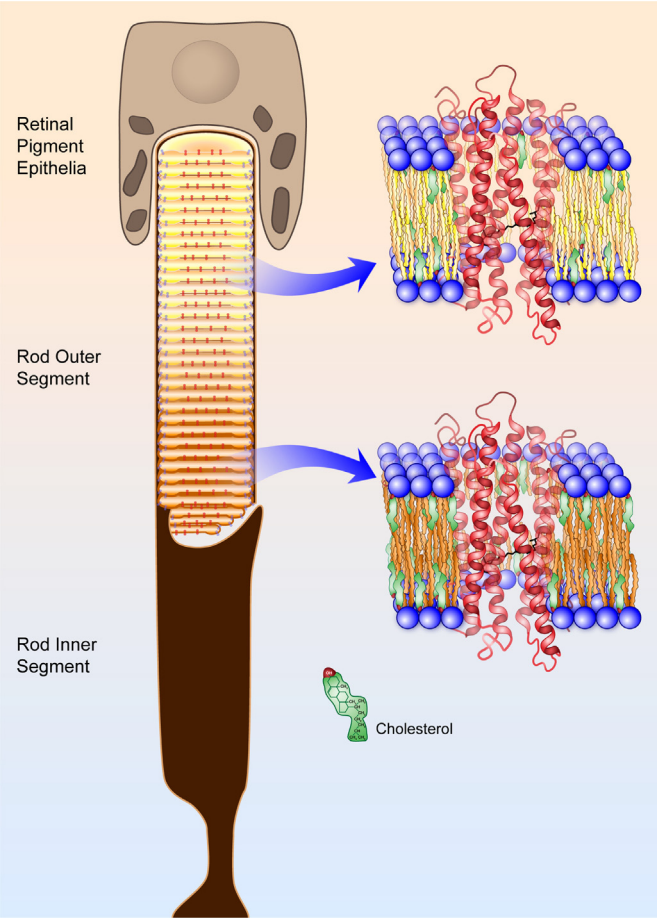


**Fig. 1.** Representation of the rod cell. Disk membranes, which are flattened vesicles, are stacked along the length of the rod outer segment. Disks are synthesized at the base of the outer segment and are apically displaced as additional disks are synthesized. The plasma membrane and newly synthesized disks are high in cholesterol while older apical disks are low in cholesterol. As membrane cholesterol decreases, the fatty acyl chain unsaturation increases. This is indicated by the dark to light gradient of orange to yellow, bottom to top. This change in the hydrocarbon unsaturation and cholesterol content alters the rhodopsin environment in the disk membrane in a manner that allows the protein to be more readily activated by light in the apical disks. Rhodopsin is depicted in red and the disk rim proteins depicted in blue. While the figure indicates the general location of these proteins, the proteins are more tightly packed than depicted here. Additionally, rhodopsin resides separately in both the upper and lower disk bilayers and does not bridge adjacent disk membranes. Rhodopsin in the plasma membrane is not depicted. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

array of retinal neural cells, which transmit the visual signal via the optic nerve to the brain visual cortex. The retinal pigment epithelia (RPE) surrounds the apical portion of the outer segment and phagocytoses disk packets which are shed at the apical tip. An immense amount of material is phagocytosed over a lifetime, disposing of photoreceptor cell waste while retaining useful content. It is essential that the rod cell and the pigment epithelia remain in constant contact for exchange of metabolites. In the absence of a functional RPE the rod cell degenerates and eventually dies (for review see Strauss, 2005; Kevany and Palczewski, 2010).

## 2. The rod outer segment plasma membrane and the disk membranes

Structurally the rod outer segment (ROS) is relatively simple. The plasma membrane and disk membranes constitute the two membranes of the outer segment. The disks are flattened membrane vesicles stacked along the length of the ROS and are surrounded by the plasma membrane. Despite the apparent simplicity of the ROS, it is exquisitely organized with a complex distribution of lipids and proteins. The plasma membrane and the disk membranes have different functions that are reflected in distinct lipid and protein compositions. The disk membrane is the site of light absorption and the initiation of visual transduction. The plasma membrane maintains the appropriate ion permeability under dark conditions and hyperpolarizes in response to the light-initiated transduction cascade. This ultimately results in a neural signal via the synaptic foot to the optic nerve.

### 2.1. Plasma membrane

A primary function of the plasma membrane is to maintain appropriate ion concentrations. Under dark conditions cGMP is bound to $Na^+$ channels, which stabilizes the open conformation of the channel. The $Na^+$ pumped out of the inner segment of the cell via the $Na^+K^+$ ATPase flows back into the outer segment via the $Na^+$ channels thus generating the dark current. As a consequence of visual transduction and the ensuing drop of cytoplasmic cGMP, the channels close and entry of $Na^+$ is blocked. This causes a hyperpolarization of the plasma membrane and triggers the inner segment synaptic response.

The protein and lipid compositions of the plasma membrane have been challenging to investigate because the plasma membrane constitutes less than ten percent of the total outer segment membrane (Boesze-Battaglia and Albert, 1992). However, certain characteristics are clear. The G-protein coupled receptor, rhodopsin is the major protein in the plasma membrane (40% of the total protein) (Molday and Molday, 1987; Molday, 1998) and is the only membrane protein identified to be located in both the plasma membrane and the disk membranes. The plasma membrane rhodopsin is identical to rhodopsin found in the disk with respect to glycosylation, light-stimulated phosphorylation, and primary structure (Hsu et al., 1993). However, as will be described later, high cholesterol inhibits rhodopsin initiation of visual transduction in the plasma membrane. Therefore rhodopsin function in the plasma membrane remains unclear tempting speculation of an alternate rhodopsin function. The $Na^+/Ca^{+2}$ exchanger (Reid et al., 1990) and the cGMP-gated channel (composed of α and β subunits (Cook et al., 1989)) are ion transport proteins responsible for maintaining normal ROS function and are found only in the plasma membrane. The GLUT-1 glucose transporter is also located in the ROS plasma membrane (Hsu and Molday, 1991). In addition to these membrane proteins, cytoskeletal proteins play an important role in ROS structure and function. These proteins form the unique structure of the photoreceptor transition zone (reviewed (Malicki (2012)) as well as the synaptic ribbon of rods and cones (reviewed in (Mercer and Thoreson (2011))

### 2.2. Disk membranes

Disk membranes have been proposed to originate at the base of the outer segment from evaginations of the plasma membrane (Steinberg et al., 1980), although this model has been questioned (Sung and Chuang, 2010). The Cryo-EM structures of the outer segment membranes support a model for disk morphogenesis in which basal disks are enveloped by the plasma membrane (Gilliam et al., 2012). However, it is agreed that new disks are synthesized at the base of the ROS and are displaced toward the apical tip over the course of several days (approximately 10 days in vertebrates (Young, 1971). At the apical tip old disks are shed and phagocytosed by the surrounding pigment epithelium. This mechanism continuously provides the ROS with new disk membranes (Bok, 1985;