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Review

The role of cholesterol in rod outer segment membranes

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Abstract

The photoreceptor rod outer segment (ROS) provides a unique system in which to investigate the role of cholesterol, an essential membrane constituent of most animal cells. The ROS is responsible for the initial events of vision at low light levels. It consists of a stack of disk membranes surrounded by the plasma membrane. Light capture occurs in the outer segment disk membranes that contain the photopigment, rhodopsin. These membranes originate from evaginations of the plasma membrane at the base of the outer segment. The new disks separate from the plasma membrane and progressively move up the length of the ROS over the course of several days. Thus the role of cholesterol can be evlauated in two distinct membranes. Furthermore, because the disk membranes vary in age it can also be investigated in a membrane as a function of the membrane age. The plasma membrane is enriched in cholesterol and in saturated fatty acids species relative to the disk membrane. The newly formed disk membranes have 6-fold more cholesterol than disks at the apical tip of the ROS. The partitioning of cholesterol out of disk membranes as they age and are apically displaced is consistent with the high PE content of disk membranes relative to the plasma membrane. The cholesterol composition of membranes has profound consequences on the major protein, rhodopsin. Biophysical studies in both model membranes and in native membranes have demonstrated that cholesterol can modulate the activity of rhodopsin by altering the membrane hydrocarbon environment. These studies suggest that mature disk membranes initiate the visual signal cascade more effectively than the newly synthesized, high cholesterol basal disks. Although rhodopsin is also the major protein of the plasma membrane, the high membrane cholesterol content inhibits rhodopsin participation in the visual transduction cascade. In addition to its effect on the hydrocarbon region, cholesterol may interact directly with rhodopsin. While high cholesterol inhibits rhodopsin activation, it also stabilizes

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the protein to denaturation. Therefore the disk membrane must perform a balancing act providing sufficient cholestrol to confer stability but without making the membrane too restrictive to receptor activation. Within a given disk membrane, it is likely that cholesterol exhibits an asymmetric distribution between the inner and outer bilayer leaflets. Furthermore, there is some evidence of cholesterol microdomains in the disk membranes. The availability of the disk protein, rom-1 may be sensitive to membrane cholesterol. The effects exerted by cholesterol on rhodopsin function have far-reaching implications for the study of G-protein coupled receptors as a whole. These studies show that the function of a membrane receptor can be modulated by modification of the lipid bilayer, particularly cholesterol. This provides a powerful means of fine-tuning the activity of a membrane protein without resorting to turnover of the protein or protein modification.

Keywords: Cholesterol; Rhodopsin; Rod outer segment; GPCR

Contents

1. Introduction

Cholesterol is an essential membrane constituent of most animal cells. This sterol has been shown to be involved in a wide variety of critical cell functions, including modulation of enzyme function, permeability, fusion and receptor function. Because of its importance to normal membrane function, the mechanism of cholesterol action in biological membranes has been the focus of intensive investigation for decades. Not surprisingly, it is now clear that cholesterol exerts complex, multifaceted effects on cellular membranes. The critical importance of cholesterol to normal functioning of cell membranes may lay in its ability to both alter fundamental properties of the phospholipid bilayer and to interact directly with specific membrane proteins. This duel role of cholesterol was described in a review by Yeagle in 1991 [1]. In this review, the complex effects of cholesterol were used to explain the correlation between optimal protein function and the

normal cholesterol content of cells. One notable example is for the kidney Na^+/K^+ ATPase, which exhibits maximal activity when it is in a membrane containing the physiological levels of cholesterol. Studies since that time on the oxytocin and cholescytokinin receptors have further supported this duel role of cholesterol in membrane function [2]. That work indicated that the cholescyskinin receptor is modulated through the bulk lipid properties while the oxytocin receptor is modulated both through the bulk lipid and by direct cholesterol interactions. This was also further supported by modeling studies of cholesterol interaction with the cholescytokinin and oxytocin receptors [3]. Recently, direct cholesterol protein binding has been implicated in the mechanism for controlling membrane cholesterol composition [4].

The effects of cholesterol have been extensively investigated in many simple phospholipid bilayer systems. In these systems, it was demonstrated that cholesterol alters the properties of the bulk bilayer phase by interacting with the phospholipids. These lipid bilayer studies demonstrated that cholesterol increases the bilayer thickness [5] and decreases the membrane permeability both to small uncharged polar molecular species and to ions. This has been the subject of several reviews [6–10]. The effect of cholesterol on ion leakage is particularly intriguing. In animal cells the plasma membrane maintains an essential electrochemical gradient across the membrane through the pumping action of Na^+/K^+ ATPases. Therefore leakage of ions across the membrane entails a cost of metabolic energy in the form of ATP. Na^+ leakage across a bilayer that contains both phospholipids and proteins can be at least partially overcome if cholesterol is present in the membrane [11]. It was proposed that the importance of cholesterol in the plasma membrane may be to reduce the dissipation of the Na^+ gradient and thus conserve metabolic energy [9].

Many years ago, it was also observed that cholesterol broadens the gel to liquid crystal phase transition temperature of phospholipid bilayers as reviewed by Yeagle [7,12]. Cholesterol does this by disordering the packing of the phospholipid hydrocarbon acyl chains below the transition, but increasing the ordered packing of these hydrocarbons above the transition. This observation led to the hypothesis that cholesterol modulates the lipid gel to liquid crystalline phase transition in biological membranes. Since that time a role of cholesterol has been proposed to be that of a primary modulator of the ''fluidity'' of the hydrocarbon region of the bilayer. While ''fluidity is a poor term to describe membrane dynamics, in this context ''fluidity'' generally refers to the motional freedom and packing of the hydrocarbon side chains. In this context, cholesterol was acknowledged to be important in modulating the dynamics of the hydrocarbon region of the bilayer. As described later in this review, cholesterol is able to modulate membrane protein function through its impact on lipid packing.

No other sterol can completely substitute for cholesterol in mammalian cell membranes. That is, generally mammalian cells are not viable when totally depleted of cholesterol. This is likely because some cholesterol effects are due to unique structural features of cholesterol. However, other sterols may approximate certain of the cholesterol membrane effects and substitute for cholesterol in that particular function. Thus if the cell membrane cholesterol is depleted, but not eliminated, addition of another sterol can substitute for the bulk membrane cholesterol and produce a similar effect on membrane function as original level of cholesterol alone [13]. This is also consistent with a multifunctional role for cholesterol.

Rod photoreceptor cells provide a unique system in which to study the synthesis, distribution and function of cholesterol within a single differentiated cell. The synthesis of cholesterol, the cellular distribution of cholesterol and the effect of membrane cholesterol on various functional

parameters have been investigated in this cell using a range of techniques. Therefore, this system provides an ideal example of cellular membranes in which the in vivo role of cholesterol can be correlated with biophysical studies, in particular, the effect of cholesterol on rhodopsin, the photoreceptor and archetype for the family of G-protein receptors. This is particularly relevant because cholesterol has been implicated in the regulation of other G-protein receptors (review [14]). These include the nicotinic acetylcholine [15,16], cholescytokinin [2], oxytocin [17,18,2,19] serotonin [20] and transferrin [21] receptors. Therefore, an examination of the role of cholesterol on rhodopsin is likely to provide insight with respect to other G-protein receptors.

2. Rod outer segment structure and function

It is important to first consider the structure of the rod cell and its location in the retina. Rod photoreceptor cells are responsible for vision under conditions of low light. The rod cell is a terminally differentiated cell. As illustrated in Fig. 1, the cell consists of an inner segment and an outer segment. At the base of the inner segment the synapse of the rod cell interacts with a complex array of retinal neural cells. These cells ultimately are responsible for transmitting the visual signal to the brain visual cortex via the optic nerve. The inner segment contains nuclei and other organelles required for protein synthesis and the majority of the biochemical machinery for normal metabolic processes. The outer segment is composed of a stack of flattened membrane vesicles, the disks that are surrounded by the plasma membrane. Light capture occurs in the outer segment disk membranes that contain the photopigment, rhodopsin. The outer segment disk membranes carry out the initial events of the visual signal transduction cascade. The apical region of the outer segment is surrounded by the pigment epithelia. The rod cell and the pigment epithelia are in constant communication with an exchange of metabolites between the tissues. In the absence of the pigment epithelia the rod cell degenerates.

The disk membranes and the plasma membrane are distinct membranes with independent functions that share a common origin. The disk membranes originate from evaginations of the plasma membrane at the base of the outer segment. Although the rod cell does not divide, the outer segment is constantly undergoing renewal (Fig. 2) as new disks are formed at the base and are progressively displaced toward the apical tip. Old disks at the apical tip of the rod are shed and then phagocytosed by the overlying pigmented epithelium. Thus the outer segment is in a constant state of degradation and renewal [22,23]. In vertebrates the transit of disks from the base to the tip of the outer segment requires approximately 10 days [24]. Therefore, the age of disk membranes range from newly synthesized at the base to approximately 10 days old at the apical tip of an individual rod outer segment.

The unique morphology of the rod outer segment is complemented by a highly organized, complex distribution of lipids and proteins between the plasma membrane and disk membranes. Rhodopsin is the only membrane protein within the ROS that has been identified in both the plasma membrane and in the disk membranes. This prototypical G-protein receptor [25] is the dominant protein by mass in both the plasma membrane (60% total protein) [26] and in disk membranes, (85% total protein) [27,28]. ROS plasma membrane rhodopsin appears to be identical to the rhodopsin in disk membranes in regards to glycosylation, light-stimulated phosphorylation, and primary structure [29]. In contrast, the ion transport proteins responsible for maintaining normal

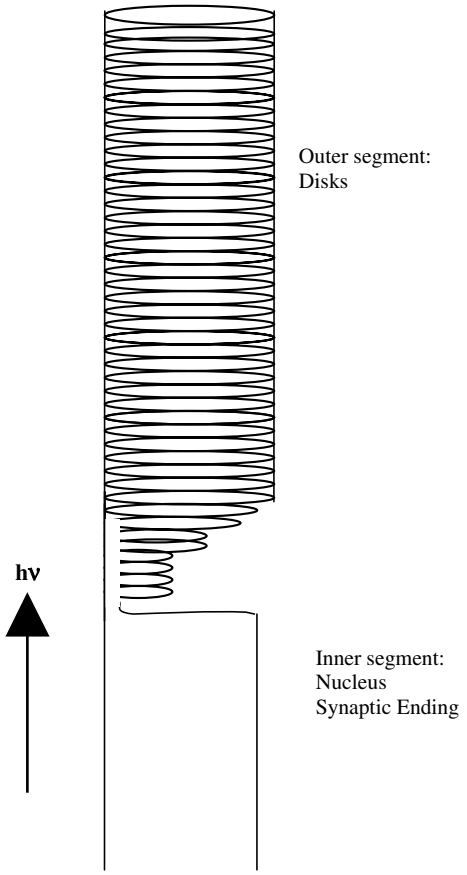

Fig. 1. The rod cell outer segment is responsible for initiating visual signal transduction. It consists of a stack of disk membranes, which are flattened membrane vesicles. The disks are surrounded by the plasma membrane. Light impinges upon the outer segment as indicated by the arrow. Activation of the transduction cascade on the disk membranes culminates in hyperpolarization of the plasma membrane. This is transmitted to the cell synapse.

retinal function, i.e., Na^+–Ca^{2+} exchanger [30] and the cGMP-gated channel, α and β subunits [31] are found exclusively in the plasma membrane. A minor component of the plasma membrane is the GLUT-1 glucose transporter [32]. Protein localization and distribution along the axial length of the plasma membrane is not homogeneous. A series of microscopy studies revealed a heterogeneous distribution of ricin-binding protein along the length of the plasma membrane [33–35]. Heavier labeling of basal disks with ricin communis agglutinin 120 was subsequently observed [36]. Since those studies, two ricin-binding proteins have been identified, the Na^+–Ca^{2+} exchanger and a 103 kDa protein with an as yet undetermined function [30].

Certain lipids exhibit a preferential localization to the plasma membrane of the ROS compared to the disk membranes. For example, the plasma membrane is enriched in cholesterol and in saturated fatty acids species relative to the disk membrane. The phosphatidylcholine (PC) to phosphatidylethanolamine (PE) ratio is also higher in the plasma membrane than in the disk membranes [37]. Additionally, squalene, a precursor of cholesterol is higher in the plasma

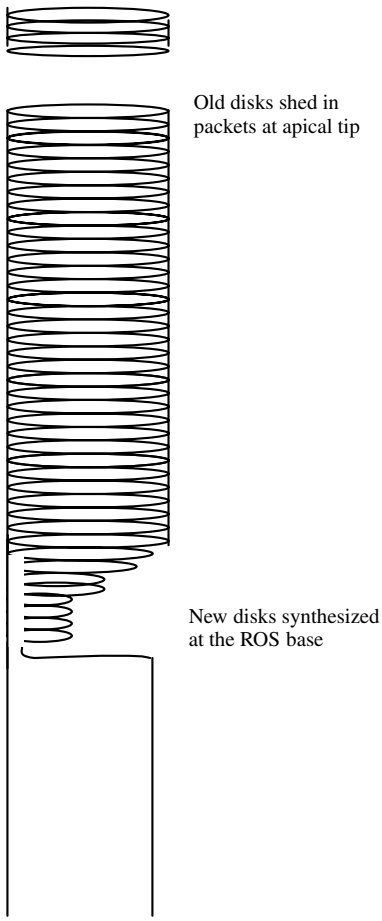

Fig. 2. New disks are synthesized at the base of the outer segment. New disks form from evaginations of the plasma membrane. New disks are apically displaced until they reach the tip of the outer segement. At the apical tip a packet of disks forms that is then phagocytosed by the pigement epithelium. This process of renewal maintains the outer segment at a constant length.

membrane than in the majority of mature disk membranes [38]. Although the role of squalene in the ROS plasma membrane is not known, it is interesting to note that Haines [9] has proposed a role for squalene in reducing H^+ leakage in some prokaryotic plasma membranes.

It is generally agreed that the integral membrane proteins of the disks do not undergo exchange or turnover during the lifetime of the disk. It has been shown that the rhodopsin originally inserted into the newly formed disk membrane remains with the disk as it transits the length of the outer segment [39,40]. In contrast, the lipid components of the disk bilayer undergo exchange between membranes and/or metabolic turnover [41,42]. During the disk transit from the ROS base to the apical tip the lipid composition can be altered. This dynamic state of the lipid environment allows the potential for modulation of function without alteration of the protein composition. The changes in lipid composition exhibited by disk membranes as they are apically displaced in the ROS are complex. As described below, dramatic change occurs in cholesterol composition.

The newly formed disk membranes have 6-fold more cholesterol than disks at the apical tip of the ROS [43,44]. Changes in the phospholipids are more subtle than that observed for cholesterol. There is little change in the overall phospholipid headgroup species as the disks are apically displaced. However, changes in fatty acid composition within a phospholipid headgroup class have been detected [45]. These changes in the lipids of the disk membranes as they are apically displaced demonstrate that the lipid bilayer is remodeled as the disk moves apically. This remodeling of the disk membrane may function directly in signal transduction. For example, studies summarized later in this review indicate that rhodopsin activation is sensitive to the lipid composition. Additionally, alterations in disk membrane composition could change the membrane susceptibility to fusion. This may be important for eventual shedding and phagocytosis by the pigment epithelium.

Prior to light exposure the Na^+ channels in the rod cell plasma membrane are maintained in the open configuration by bound cGMP. This allows Na^+ that is pumped out of the inner segment of the cell via the Na^+/K^+ ATPase to flow back into the outer segment through these protein channels. This flow of Na^+ out of the inner segment and into the outer segment is termed the dark current. The entry of Na^+ is blocked after light exposure because the signal transduction cascade culminates in a reduction of cGMP and closure of the channels. The resulting hyperpolarization of the plasma membrane triggers the synaptic response. Cholesterol may be important in reducing Na^+ leakage through the plasma membranes in both the inner and outer segments.

The disk membrane is the site of the initial events of visual signal transduction. Rhodopsin, a G-protein receptor, is responsible for initiating the conversion of the extracellular light signal into a cellular response. This is accomplished by a conformational change in the protein from the inactive, R state to the activated R* state. The rhodopsin chromophore, 11-*cis* retinal is bound via a Schiff's base linkage to a lysine residue in the seventh transmembrane helix. When light strikes the disk membrane 11-*cis* retinal absorbs a photon and isomerizes to the *all-trans* retinal form. After the retinal isomerizes, rhodopsin undergoes a series of conformational changes. These can be observed as a series of spectrally defined intermediates which culminates in the release of *all-trans* retinal from the apo-protein, opsin. The transition of the intermediate, metarhodopsin I to metarhodopsin II is associated with the initiation of the cGMP cascade [46]. Metarhodopsin II is thus the activated form of rhodopsin, R*. This activated form binds the heterotrimeric G-protein, transducin which in turn triggers GDP/GTP exchange and dissociation of the transducin into α and $\beta\gamma$ subunits. The α subunit then activates a phosphodiesterase. This cascade culminates in the phosphodiesterase mediated hydrolysis of cGMP. Reduction in cGMP leads to closure of the plasma membrane Na^+ channels and results in the hyperpolarization of the plasma membrane. Thus the disk membranes and the plasma membrane have distinct functions in visual signal transduction.

3. Cholesterol in rod outer segment membranes

3.1. Cholesterol biosynthesis

Cholesterol is by far the major sterol in the retina. It can be synthesized in the retina. However, the rate of cholesterol synthesis is low [47–50]. Furthermore, the rate of cholesterol turnover in photoreceptors is also very slow. It has been hypothesized that cholesterol can be obtained from

systemic sources and that it can be recycled within the retina [51]. This is consistent with the recycling of a major fatty acid, docosahexaenoic acid (DHA) in the retina [52–54]. In this recycling process lipids from phagocytosed apical disks are returned from the pigment epithelium to the rod cell and then reused in the synthesis of new disks. This recycling of the lipids is supplemented by lipids circulating in the blood. Thus the rod cell is able to use both lipids originating from the liver and lipids recycled from the pigment epithelium.

3.2. Cholesterol distribution among ROS membranes

Cholesterol distribution in the retina was initially investigated by freeze-fracture electron microscopy. In these studies fillipin was used to detect cholesterol in the membranes. The early freeze-fracture studies of mouse, frog and bovine photoreceptors indicated the presence of cholesterol-enriched, fillipin-induced pits. These regions were confined to particle free patches within the plasma membrane and in newly formed disk membranes. Based upon these freeze fracture data it was suggested that cholesterol was higher at the base of the ROS than at the apical tip [55,56].

The heterogeneous distribution of cholesterol among the ROS disk membranes detected by the above studies was demonstrated using biochemical techniques. The detergent, digitonin partitions preferentially into cholesterol containing membranes. This provided a means to separate membranes with different amounts of cholesterol. Bovine disk membranes with high levels of cholesterol were separated from those with low levels of cholesterol on a density gradient by exploiting the change in membrane buoyant density induced in the presence of subsolubilizing levels of digitonin. Analysis of the disk fractions isolated from the density gradient showed that cholesterol is not uniformly distributed among bovine ROS disk membranes [43]. Additional studies on rats demonstrated that rat ROS exhibit a similar cholesterol distribution among the disk membranes to that observed for bovine ROS disks [57]. In both these studies, the cholesterol to phospholipid mole ratio decreased from approximately 0.30 in basal disks to 0.05 in apical disks.

Although the above studies established a heterogeneous cholesterol distribution, the spatial arrangement within the ROS required additional investigation. As basal disks form, newly synthesized opsin is incorporated. Because this protein remains with disk as it progresses to the apical tip of the ROS, a labeled protein can serve as a marker of disk displacement. To determine if cholesterol within the outer segment as a function the spatial displacement of disks, cultured bovine retinas were incubated with ^{3}H leucine under conditions such that the label was incorporated into proteins, including opsin. The relatively short the culture times insured that only newly synthesized disks incorporated the labeled protein. Upon separation of the isolated disks into sub-populations containing high and low cholesterol it was observed that the labeled protein was associated with the high cholesterol disks [44]. These studies established that newly synthesized disk membranes contained high levels of cholesterol while older disks exhibited substantially lower cholesterol levels.

The plasma membranes of most cells are high in cholesterol content. While the average cholesterol content of the total ROS membranes is approximately 10 mol%, this largely reflects the cholesterol content of the disks, which constitute approximately 90% of the total ROS membranes. Therefore the plasma membrane of ROS was isolated and both the cholesterol content and the phospholipid composition investigated [37,58]. The ROS plasma membrane was found to contain approximately 30 mol% cholesterol. This is consistent with other plasma membranes and

established that the cholesterol composition in the plasma membrane is similar to that of newly formed disks, but distinctly higher than the older, apical disks. Squalene was also found in the plasma membrane at the surprisingly high concentration of 2 mol% [38]. This is especially intriguing in that this squalene does not continue on the pathway of cholesterol synthesis [59].

It is well known that cholesterol can readily exchange between membrane bilayers. Therefore the mechanism that maintains different cholesterol composition in adjacent disk membranes and the plasma membrane must be considered. The extent of cholesterol exchange is sensitive to the phospholipid headgroup and fatty acyl composition of the membranes [60]. Cholesterol favors a saturated fatty acyl environment to an unsaturated one. The phospholipid environment favorable to cholesterol follows the order of sphingomylin > phosphatidylserine > phosphatidylcholine > phosphatidylethanolamine [60,61]. This observation suggested that the heterogeneous distribution of cholesterol among cellular membranes could be explained in part by differential partitioning of cholesterol between membrane bilayers of different phospholipid composition. Therefore the phospholipid headgroup composition of disk membranes and the plasma membrane isolated from the same retina preparation were determined. Consistent with earlier studies [47], the disk membranes exhibited approximately equal amounts of PE and PC (42% and 45%, respectively). However, the plasma membrane was much lower in PE (10%) and much higher in PC (65%). PS was also lower in the disks (14%) than in the plasma membrane (24%) [37]. The fatty acyl chains of the phospholipids likewise exhibited a remarkable difference especially with respect to the enrichment of 22:6 (DHA) in the disk membranes relative to the plasma membrane [62]. Thus these findings support a role for the phospholipid composition in determining the cholesterol distribution.

The ability of cholesterol to partition between disk membranes and another bilayer was investigated to determine if the disk membrane could be readily enriched or depleted in cholesterol. Cholesterol was allowed to equilibrate between disk membranes and phospholipid vesicles of different composition [63]. The extent of cholesterol partitioning was sensitive to the phospholipid composition of the acceptor vesicles. Consistent with model studies, disk membrane cholesterol much more readily partitioned into PC membranes than membranes containing PE. Thus the partitioning of cholesterol out of disk membranes as they age and are apically displaced is consistent with the high PE content of disk membranes relative to the plasma membrane. Furthermore, it is also consistent with the high DHA content found in disks. Therefore, it is likely that the cholesterol distribution is influenced by the phospholipid content of the plasma membrane and the disk membranes.

The above studies support the preference of cholesterol for the plasma membrane relative to the "average" disk. Since disks are apically displaced over several days modification of lipid composition other than simply a decrease in cholesterol could occur. Alterations in disk phospholipids were correlated with the disk cholesterol composition and hence with the spatial displacement of the disks. Changes were primarily observed in the hydrophobic core of the bilayer, that is, in the fatty acids. The composition of the phospholipid headgroup classes did not change significantly with disks displacement either in rats [57] or in bovine [37]. While most of the fatty acids showed little or no change with age/spatial location, some pronounced changes were observed with specific fatty acids within particular phospholipid headgroup classes. The most remarkable change occurred in the relative distribution of fatty acids within the PC headgroup class. The 16:0 fatty acyl chain in PC dramatically decreased with disk age while the 22:6, (DHA) fatty acyl chain

increased with disk age. Thus the fatty acid side chains become progressively more unsaturated as they are displaced from base to tip of the ROS, approximately doubling the amount of DHA in PC [45]. This increase in unsaturation as the disks are apically displaced further promotes the depletion of cholesterol during spatial displacement. Phosphatidylinositol (PI) accounts for less than 2% of the total disk phospholipids. However, the importance of this phospholipid may not be its impact on the bulk bilayer properties. The content of arachidonic acid (20:4) increases within the PI class as the disks are displaced. This is particularly intriguing in that this fatty acid could be important in disk phagocytosis via its role in prostaglandin synthesis. While the PE class exhibits some fatty acid changes, they are small. The PS class exhibits no significant changes in fatty acid composition [45].

From these data, a picture of the ROS cholesterol distribution emerges (Fig. 3). The plasma membrane has the highest levels of cholesterol (almost 40 mol%). Newly synthesized disks are also

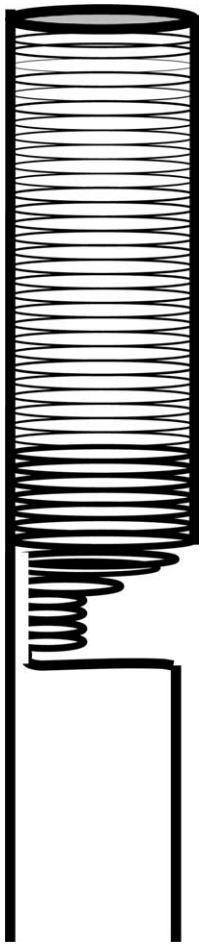

Fig. 3. The plasma membrane and newly synthesized disks are high in cholesterol. Older apical disks are low in cholesterol. This creates a gradation of membrane cholesterol from the basal to apical disks which is represented by the thickness of the line in this diagram.

high in cholesterol content, similar to the plasma membrane from which they form. The cholesterol is rapidly lost from the disks as they are apically displaced. This is driven at least partly by the high PE content of the disk membranes as well as the unsaturation of the fatty acyl side chains. The apical disks retain only 5 mol% or less membrane cholesterol [43,44].

The distribution of cholesterol in the ROS disks can be viewed as part of the complex, dynamic process of disk renewal in which lipid composition plays an important, but not well-understood role. Disks form from the plasma membrane. However, important membrane associated events occur much earlier. The lipid composition of rhodopsin containing membranes is important before disk formation. Prior to disk morphogenesis newly synthesized rhodopsin is transported by post-Golgi vesicles to the pericilliary ridge complex where it is incorporated into the membrane for disk formation. The post-Golgi vesicles that transport rhodopsin have a unique phospholipid composition, containing phosphatidylcholine and phosphatidylethanolamine with docosahexaenoyl (DHA) acyl chains [64]. Modification of phospholipid biosynthesis affected fusion and budding of the transport membranes differently [65] Furthermore, the organization of cholesterol within the disk membranes may also be determined prior to the formation of a new disk [66].

4. Cholesterol modulates rhodopsin function

The cholesterol composition of membranes has profound consequences on the protein functions of these membranes. The nascent disk membranes form at the base of the ROS. The new disk must separate from the plasma membrane. The disks must then be competent to carry out visual transduction. Finally disks must undergo fusion with the plasma membrane to form packets that are then phagocytosed by the pigment epithelium. At each of these junctures the composition of the membrane must be appropriate for function. An aberrant membrane lipid composition could be manifest in defects of visual signal transduction or in the dynamic renewal of the outer segment. It is likely the disk membrane lipid composition optimizes the ability of rhodopsin to undergo conformational changes required for visual signal transduction. A number of studies have focused on the effects of lipid composition on the properties of rhodopsin. These studies have been carried out both in reconstituted rhodopsin–phospholipid bilayers of well-defined composition as well as in native disk membranes.

4.1. Cholesterol and rhodopsin function in reconstituted rhodopsin–phospholipid bilayers

After light exposure rhodopsin undergoes a series of conformational changes. These conformations have well-defined spectral properties. An equilibrium is rapidly established between the conformational intermediates metarhodopsin I and metarhodopsin II. The activated state, R* corresponds to metarhodopsin II. Therefore, a shift in the metarhodopsin I $\Longleftrightarrow$ metarhodopsin II equilibrium alters the amount of R* available for signal transduction. Litman and colleagues investigated the influence of lipid dynamics on rhodopsin activation in well-defined rhodopsin–lipid reconstituted bilayers. Their studies demonstrated that systematic changes in the cholesterol composition of reconstituted phosphatidylcholine-rhodopsin vesicles has profound changes on the formation of R*. These changes correspond to changes in the dynamics of the bilayer hydrocarbon region [67–71]. These studies indicate that cholesterol can exert an effect on rhodopsin

function by reducing the partial free volume in the hydrocarbon core of the bilayer. This is because the transition of metarhodopsin I to metarhodopsin II involves an expansion of the protein in the plane of the bilayer, which requires the recruitment of partial free volume from the surrounding lipid bilayer. At high levels of cholesterol this volume is essentially occupied by cholesterol and is unavailable to rhodopsin. The transition is then inhibited. Their investigations using these well defined lipid model systems have shown that the formation of the activated state, R* (metarhodopsin II) is exquisitely sensitive to the membrane lipid composition.

It should be noted that cholesterol is not alone in its ability to alter the metarhodopsin I to metarhodopsin II equilibrium. The phospholipid hydrocarbon chain composition in reconstituted bilayers has been shown by Mitchell and Litman to regulate the metarhodopsin I $\Longleftrightarrow$ metarhodopsin II equilibrium. In particular, docosahexaenoic acid (DHA) strongly promotes the formation of metarhodopsin II [72]. This fatty acid is conserved in the retina, accounting for over 50% of the phospholipid hydrocarbon chains in the disk membranes. Not only does this highly unsaturated lipid modulate rhodopsin function in the membrane, but deprivation of this essential fatty acid degrades visual acuity as measured by electroretinograms [73–75]. Thus the affects seen on the molecular level have consequences on retinal physiology. The effects of DHA are not limited to the visual system. DHA deficit leads to reduced olfactory discrimination [76]. This suggests that the lipids are responsible for modulating the dynamic environment of the membrane, which in turn can affect the properties of the receptor protein. The role of the unsaturated acyl chains in the sn-2 position of phospholipids is distinct from that of cholesterol in modulating the metarhodopsinI/metarhodopsinII equilibrium [72]. It is clear that docosahexaenoic acid plays an important role in normal rod function, but the mechanism is not fully understood [74].

4.2. Cholesterol and rhodopsin function in ROS membranes

Model reconstituted membranes are invaluable systems for investigating the effects of the lipid bilayer on rhodopsin. These well-defined systems provide a means to study the effects of the lipid environment on protein function in a systematic manner. However, it is also essential to correlate these model studies with the behavior of rhodopsin in native membranes. Ultimately it is important to link biophysical effects of cholesterol on rhodopsin properties in well-defined systems with the effects of cholesterol on biological function in native disk membranes. To this end, rhodopsin properties have been compared in the low cholesterol environment found in average disk membranes to the high cholesterol environment found in the ROS plasma membrane. Additionally, signal transduction events subsequent to light activation were examined as a function of disk membrane cholesterol content.

Rhodopsin is the major protein found in disk membranes. It is also a major protein of the ROS plasma membrane. In the plasma membrane, it constitutes approximately 40% of the total membrane protein [77]. However, the plasma membrane lipid composition is distinctly different from that of the disks membranes [37,58]. The ROS plasma membrane thus provided another interesting native membrane in which to investigate rhodopsin function. The question arose as to whether plasma membrane rhodopsin could activate the visual signal transduction cascade. This cascade culminates in the activation of a phosphodiesterase and hydrolysis of cGMP. Therefore the extent of cGMP hydrolysis, which can be readily measured by a change in pH [78] can be used to monitor the activation of rhodopsin. A comparison of rhodopsin activation in the disks and in the

plasma membranes, normalized for rhodopsin content, indicated that the plasma membrane rhodopsin activated the cascade very poorly. There was little or no light-stimulated cGMP hydrolysis under the same conditions under which disk membrane rhodopsin fully activated the cascade. This differential activation is largely due to the high cholesterol content of the plasma membrane. When plasma membrane cholesterol was enzymatically oxidized to cholestenone the ability of rhodopsin in the plasma membrane to initiate the transduction cascade approximated that of disk membranes [58]. This is consistent with cholesterol modulation of R* in the plasma membrane. The high plasma membrane cholesterol content inhibits rhodopsin participation in the visual transduction cascade.

With this in mind it is interesting to speculate on the role of cholesterol in cone photoreceptors. Cone cells are approximately two orders of magnitude less sensitive to light than rod cells. In cone cells the disks are continuous invaginations of the plasma membrane and are not independent as are rod disks. It is reasonable to speculate that in these membranes cholesterol is high, typical of the plasma membrane. At this level cholesterol may thus play a role in decreasing the light sensitivity of cones by inhibiting the activity of the membrane receptor.

Since cholesterol inhibits rhodopsin activation in the native ROS plasma membrane and in model membrane systems, it was reasonable to predict that newly synthesized disks with their high cholesterol content may not activate the visual transduction cascade as efficiently as the older, low cholesterol disks. This hypothesis was tested by examining disks in which the membrane cholesterol compositions were adjusted to specific, intermediate levels by exploiting the ability of cholesterol to exchange between membranes. Cholesterol readily partitions between disks and phospholipid vesicles [63]. Cholesterol also partitions between disk membranes and β-methyl cyclodextran (MCD) [79]. This ability of cholesterol to partition into a low cholesterol membrane and out of a high cholesterol membrane provided a means with which to prepare native disks with systematically defined levels of cholesterol. The ability of rhodopsin to activate PDE in disk membranes as a function of cholesterol levels was examined by monitoring the hydrolysis of cGMP. PDE activation by disks with cholesterol levels that corresponded to levels found in basal disks was compared to activation by disks with average and low levels of cholesterol. These experiments indicated that disk membranes with low membrane cholesterol activated the cascade much more readily than those with high membrane cholesterol [80]. These data are consistent with the hypothesis that cholesterol renders the basal disks less efficient in activating the transduction cascade.

The differences in the response to light activation between basal and apical disks were further investigated in native disk membranes within the intact ROS. This was possible because disk membranes can be separated based on their membrane cholesterol content even after light exposure. Thus disks were exposed to light while still stacked within the ROS. Light activated rhodopsin, R* is the form which binds transducin. If cholesterol levels in the disk membrane modulate the formation of R*, R* should form more readily in the low membrane cholesterol environment of the apical disks. Thus apical disks would be expected to bind transducin more readily than high cholesterol basal disks. This was found to be the case. Upon exposure to light levels that bleached less than half of the rhodopsin, the transducin binding favored the apical disks [81]. Thus it is likely that the decrease in disk membrane cholesterol results in an environment that favors R* formation and the ability of transducin to bind to disk membranes increases as the disks age and are apically displaced. The change from the relatively rigid membrane environment of rhodopsin in

the basal disks to a less restricted membrane environment involves both loss of cholesterol and increased unsaturation of the hydrocarbon chains. This is qualitatively diagramed in Fig. 4.

The studies described above suggest that membrane cholesterol reduces the conformational freedom required for rhodopsin activation by making the bilayer environment conformationly rigid both in native disks and in reconstituted membranes. A rigid membrane environment may also inhibit the conformational changes associated with denaturation. For example, a rigid membrane environment could stabilize the native state if denaturation involves an increase in protein volume. Investigations of the effect of cholesterol on thermally induced bleaching of rhodopsin are consistent with this hypothesis.

The thermal denaturation of rhodopsin causes an irreversible loss of the retinal chromophore. This is in contrast to light induced bleaching where the protein does not denature and retains its

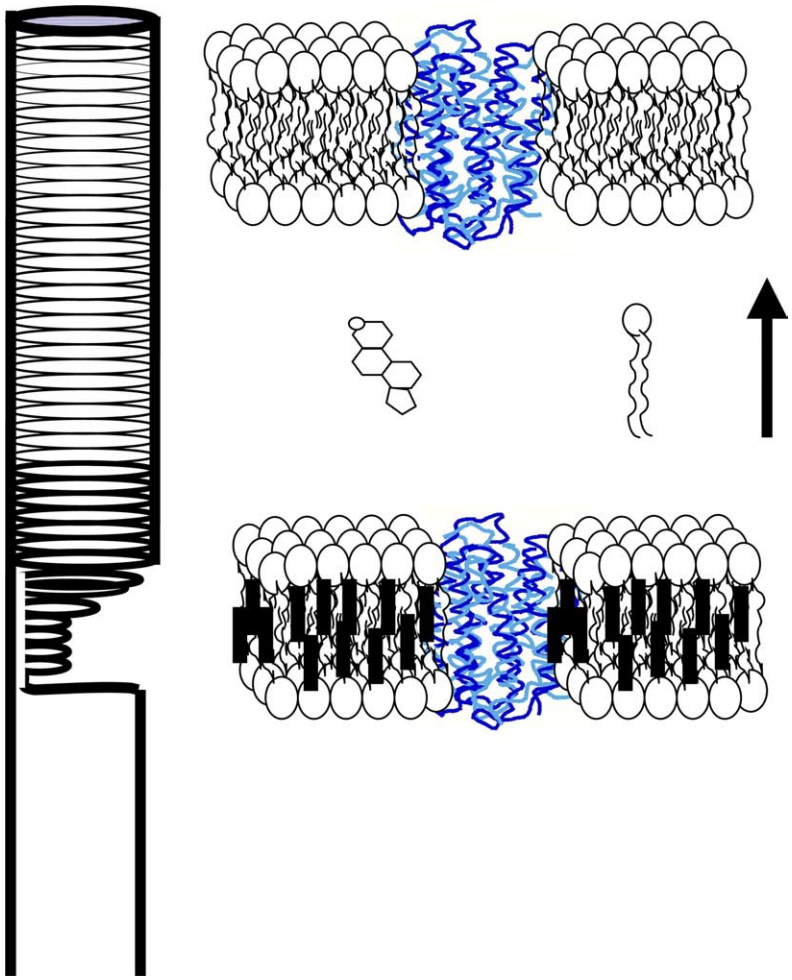

Fig. 4. The disk membranes undergo remodeling as they are apically displaced. Membrane cholesterol decreases and fattyacyl chain unsaturation increases. This alters the rhodopsin environment in a manner that allows the protein to be more easily activated by light.

ability to bind the retinal chromophore. To investigate the effect of membrane cholesterol on thermally induced bleaching, disk membranes with different levels of cholesterol were exposed to elevated temperature for fixed time periods. The loss of the characteristic rhodopsin absorption peak at 500 nm was used to evaluate the extent of rhodopsin thermal denaturation. The characteristic rhodopsin absorption was retained longest in membranes with the highest cholesterol levels [80].

The thermal bleaching experiments as detected through the retinal, can only evaluate the effect of cholesterol on the unbleached pigment, rhodopsin, not the bleached form, opsin. It must be noted that in contrast to thermal bleaching the opsin generated when rhodopsin is exposed to light is part of the normal light cycle and is not a denatured protein. If 11-*cis* retinal is available, rhodopsin will be regenerated. Therefore the stability of both opsin and rhodopsin is of interest. Differential scanning calorimetry (DSC) has been used to determine the temperatures at which opsin and rhodopsin undergo irreversible denaturation. These studies have shown that rhodopsin with a transition temperature of approximately 71 °C is considerably more stable than opsin, which exhibits a transition of approximately 55 °C [80,82]. This technique was further used to investigate the effect of cholesterol on the temperature of thermal denaturation for both rhodopsin and opsin. In agreement with the thermal bleaching studies these experiments also showed that the temperature of thermal denaturation increased in response to increased cholesterol content of the disks [80].

The full nature of cholesterol influence on rhodopsin however, is more complex than the above experimental evidence implies. The temperature of rhodopsin thermal denaturation as determined by DSC is scan rate dependent. That is, it is sensitive to the rate of heating and is therefore a kinetic process [83]. (For this reason, the transition temperatures for rhodopsin and opsin provided above are given as approximate values.) The effect of cholesterol composition on the activation energy of rhodopsin denaturation, a kinetic parameter, was further investigated by DSC at different scanning rates. Cholesterol content in disk membranes was altered. The cholesterol adjustment was achieved by using incubation with phosphatidylcholine vesicles or incubation with β-methyl cyclodextran. The activation energy for denaturation for rhodopsin was found to increase with increasing disk membrane cholesterol until the membrane was approximately 14 mol% cholesterol. After this point the activation energy decreased (Katragadda, unpublished data).

These experiments suggest that rhodopsin structure is very stable in the rigid environment imposed by cholesterol. However, this environment inhibits the conformational changes required for activation by light. This is because both the activation of rhodopsin and its thermal denaturation involve an expansion of the protein in the membrane. The condensing effect of cholesterol on the membrane creates an environment that inhibits both of these processes. The disk membrane must perform a balancing act. The protein must be in an environment that supports the stable, native state while allowing conformational flexibility. Since rhodopsin does not turnover in the disk membrane it must be stable during the lifetime of the disk (approximately 10 days) while maintaining sufficient flexibility to engage in the conformational changes essential for activation and binding transducin.

5. Organization of cholesterol in disk membranes

For many years the Singer–Nicholson fluid mosaic model [84] has dominated the image of biological membranes. In the simplest case, this model only requires the that the lipids form a bilayer

and that the integral membrane proteins are embedded in the bilayer. It imposes few restrictions on the protein movement. It allows the proteins and the lipids to be randomly distributed in the plane of the bilayer. It also allows the lipids to be randomly distributed between the bilayer leaflets. However, this model does not adequately deal with sophisticated complexity of true biological membranes. Disk membranes as well as many other membranes exhibit an asymmetric distribution of lipids between the inner and outer bilayer leaflets. Typically, for plasma membranes PC is high in the extracellular monolayer while PE and PS are high in the intracellular monolayer. In the case of disk membrane lipids PE is preferentially, although not exclusively located on the extradiskal surface and PC is preferentially located on the intradiskal surface [85,86]. This is consistent with the disk origin as an evagination of the plasma membrane. The intracellular surface of the plasma membrane becomes the outer surface of the nascent disk. Furthermore, it is likely that cholesterol composition of each of the bilayer leaflets is different. An asymmetric distribution of cholesterol was inferred from the thermodynamic behavior of extracted disk lipids [87].

In addition to bilayer asymmetry, proteins and lipids can be organized in the plane of the bilayer into microdomains. It has become widely accepted that regions of biological membranes exist which are highly specialized and which are enriched in specific proteins and lipids [88–102]. Microdomain organization may be driven by lipid–protein and/or by lipid/lipid interactions. Membrane lipids have been shown to play an active role in the organization of specialized microdomain regions [103]. In disk membranes rhodopsin must also be considered to play a role in organizing lipid microdomains. Rhodopsin interacts directly with approximately 30 phospholipids that form an annulus around the protein. These annular lipids are in exchange with the remaining bulk bilayer lipids [104]. However, further studies of the disk membrane showed that approximately 15 of these phospholipids are in slow exchange with the bulk of the phospholipid [105]. The ability of the lipids and rhodopsin to organize into complex lateral domains has also been investigated by Litman and colleagues. Their studies showed that there is a cholesterol-dependent recruitment of di22:6-PC by rhodopsin into lateral domains of the disk bilayer [106]. The cluster of rhodopsin and the lipids that formed this domain exceeded two adjacent lipid layers. This implies interactions between rhodopsin and the lipid environment that extends beyond direct protein–lipid interactions.

Direct interactions between rhodopsin and cholesterol have been shown by fluorescent energy transfer studies [107]. These studies suggested that cholesterol was associated with rhodopsin near a tryptophane residue. This was recently supported by electron crystallography [108]. Cholesterol was found to improve the crystallization of rhodopsin. Cholesterol was proposed as the likely candidate for density observed between helix 4 of one rhodopsin and helices 7 and 8 of the adjacent rhodopsin molecule. The case for direct cholesterol rhodopsin interactions is also supported by modeling studies using other G protein coupled receptors [3].

It is likely that the disk membrane lipid composition optimizes the ability of rhodopsin to undergo conformational changes required for function. Therefore microdomains of specific lipids are certainly relevant to visual transduction. Lipids that undergo slow exchange between the protein and the bulk bilayer may directly influence the light response of rhodopsin. For example, in the disk membrane it has been shown that the phospholipids exhibit a reorganization in response to light [109]. The effect of cholesterol on rhodopsin activity can be largely explained by its ability to alter bulk membrane properties. However, cholesterol has been shown to effect other membrane proteins both by altering the phospholipid properties and by direct interactions. Therefore the effect of direct cholesterol–rhodopsin interactions must also be considered.

Microdomains of cellular membranes that are enriched in both sphingomylin and cholesterol have been identified in several types of cells. These microdomains, known as rafts, are thought to represent a region of more highly ordered lipids than found in the rest of the membrane. Rafts have been linked to protein trafficking and to specific cell signaling events. These rafts are difficult to visualize in the cell membrane. In those cases in which visualization was achieved, clustering or cross-linking was involved. This has made investigation of the membrane microdomains exceedingly difficult. It was observed that certain components of the membrane are resistant to Triton X-100 at 4 °C. There is now a rapidly growing body of literature that describes the use of detergent resistant membranes (DRMs) to obtain information regarding the nature of the interactions that occur in the in vivo microdomains, rafts [88,89,110,111]. It has been proposed that DRMs, which are isolated at low temperature, reflect the composition of the rafts. However, because the lipids may undergo phase transitions that favor the ordered phase, DRMs, which are isolated at low temperature may overestimate the raft population and have an altered composition. The composition of DRMs may simply reflect the differential solubility of the proteins in the complex mix of detergent and lipids. Lowering the temperature can induce the lipid phase separation and this is reflected in the solubility of the lipids. Additionally, model membrane studies have suggested that DRMs may be the result of the partial solubilization process itself rather than pre-existing membrane microdomains [112]. Some of these concerns have been recently reviewed [113]. For these reasons, the extrapolation of DRMs to biologically relevant rafts, while intriguing, is speculative.

With these cautions in mind, the concept of rafts in the rod outer segment disks remains extremely interesting to consider. As described above, in addition to the asymmetrical cholesterol distribution across the disk bilayer, cholesterol may be clustered into micro-domains in the plane of the disk bilayer. This suggests the possibility of cholesterol-rich microdomains. There is some evidence for cholesterol microdomains in the disk membranes. The fillipin-pits observed by Andrews and Cohen [55] were attributed to a temperature induced lateral phase separation in these membranes. These freeze-fracture observations appear consistent with the formation of cholesterol-enriched membrane microdomains. That being the case these fillipin-pits or rafts are localized to the plasma membrane surface and are seen most often in the newly forming disks of mice. They are only observed in the frogs in these same locations when the frogs are reared at 37 °C. Since the ability of fillipin to partition uniformly throughout the entire length of the ROS maybe questionable, the rafts may not be limited in vivo to those areas detected by these freeze-fracture studies. However, ROS membranes have a non-uniform cholesterol composition [43,45] in which cholesterol is high in the membranes of basal disks and low in the membranes of apical disks [44]. Thus the biochemical analysis is consistent with the fillipin labeling studies.

DRMs have been isolated from rod outer segments [114,115]. Boesze-Battaglia et al. [116] as well as Elliott and Ghalanyini [117] have reported that caveolin is present in the rod outer segment membranes and that the caveolin was associated with DRMs. Furthermore, Boesze-Battaglia has shown that DRMs isolated from disk membranes using Triton X-100 are high in cholesterol (0.24 cholesterol/phospholipid mole ratio) and in sphingomylin (0.11 sphingomylin/phospholipid mole ratio) when compared to the total disk membrane. Moreover these DRMs were shown to contain in addition to caveolin, the tetraspanin protein rom-1. The presence of rom-1 in DRMs is sensitive to membrane cholesterol present in the original disk membrane. When the disk membranes were treated with β-methylcyclodextran to deplete cholesterol, the resultant DRM contained slightly lower levels of the dimeric form of rom-1. Cholesterol depletion of disk membranes also resulted in the

collapse of the large caveolin complex to a monomeric caveolin in the DRM. These observations suggest that cholesterol can influence the association of some membrane proteins. Assuming that Andrews and Cohen [55] were looking at membrane raft domains in photoreceptor cells, the raft-like species isolated by detergent are likely analogous to those observed microscopically. The interpretation of data derived from DRMs isolated from disk membranes remains challenging.

Regardless of whether or not cholesterol resides primarily in organized membrane rafts, its concentration in the disk membranes decreases as the disks are apically displaced from the base of the ROS. This loss of cholesterol either from membrane micro-domains or from the bulk lipid may play a role in the regulation of photoreceptor renewal processes. Rod cells are post-mitotic cells that utilize an elaborate light- or circadian rhythm-dependent renewal process to maintain a constant length and normal physiological function [118,119]. The processes of disk morphogenesis at the ROS base and shedding of old disks at the ROS apical tip coordinate to maintain a constant average length of the rod cell. These processes, are fundamental to normal visual function and share the common characteristic that each occurs through tightly regulated fusion of two biological membranes. Fusion occurs during disk morphogenesis as the newly formed disk is pinched off the plasma membrane. Fusion is again important for the formation of disk packets, which are then shed from the outer segment at the apical tip. The distribution of cholesterol along the length of the ROS may be important in regulating fusion events.

The formation of disk packets occurs when the rim region of an individual disk contacts and fuses with the opposing adjacent region of the plasma membrane [120]. The rim of a disk membrane is structurally and functionally unique; in particular, it contains tetraspanin protein complexes consisting of peripherin/rds and rom-1 homo and hetero-oligomers [121–124]. Fusion required during photoreceptor renewal has been studied using a reconstituted cell free assay system. These studies have provided in vitro evidence that the product of the *RDS* gene, peripherin/rds (a.k.a. peripherin-2) functions as a photoreceptor specific membrane fusion protein [125,126] and for review [66].

The spatial and temporal pattern of ROS fusion requires that peripherin /rds is fusion-active at distinct regions of the ROS: base and tip. Concomitantly, fusion at positions along the length of the OS must be suppressed. Unregulated fusion would have disastrous consequences to ROS structure and is predicted to result in increased membrane debris and the accumulation of lipofuscin, a hallmark of degenerative pathologies [127]. Thus, it has been hypothesized that fusion must be highly regulated. Early studies showed that the cholesterol content of the disk membranes did not alter in vitro membrane fusion [128]. However, subsequent studies [129] showed that the non-glycosylated homolog of peripherin/rds, rom-1, acts as an accessory protein that enhances peripherin/rds mediated fusion in vitro. As described above, rom-1 is found in DRMs and is therefore membrane raft associated [116]. Thus, it is tempting to speculate that as the disk membrane cholesterol content decreases during apical displacement the rom-1 associated with the membrane raft is released. Once released, it could interact with peripherin/rds to promote membrane fusion.

6. Retinal disease and cholesterol

Cholesterol clearly plays a vital and complex role in normal rod cell function. It influences visual transduction by modulating rhodopsin activation and may also be implicated in the dynamic

renewal of the outer segment membranes through a role in disk shedding. It is therefore anticipated that an alteration in the ROS cholesterol could result in visual defects and outer segment degenerative diseases. Although animal models in which to investigate the effect of altered cholesterol are rare, two models are currently available to investigate the link between cholesterol and vision disease.

Royal College of Surgeons (RCS) rats carry a recessive mutation that results in the degeneration of the retinal photoreceptor cells and ultimately in blindness. Characteristic of this strain is the inability of the pigment epithelium to phagocytose the outer segment tip. Freeze-fracture studies were exploited to link ROS cholesterol distribution as detected by fillipin binding with this retinal dystrophy. As described earlier, electron microscopy studies of ROS confirmed that the distribution of cholesterol is higher in the base than at the tip for normal rats. However, in the RCS dystrophic rats the cholesterol is homogeneously distributed from the base to the tip of the outer segment. This result was further confirmed by biochemical analysis of the layer of outer segment debris that accumulates in the interphotoreceptor space. Biochemical analysis revealed relative increases in cholesterol in rod outer segment disk and in debris membranes isolated from dystrophic retinas as compared to membranes isolated from normal retinas [56].

ROS disk membranes from RCS rats were isolated based on their age/spatial location as described earlier for normal rats and for bovine. The cholesterol distribution showed significant differences in cholesterol distribution when compared to normal rats. Unlike normal rats, the RCS rats showed little difference in cholesterol distribution among the disk membranes. However, the average molar ratio of cholesterol to phospholipid in the disk membranes of the RCS rats was approximately 0.14 while this ratio was approximately 0.11 in normal rats. Thus the overall level of cholesterol was marginally higher in RCS disks than in normal rat ROS disks. In contrast, the ROS plasma membrane of RCS rats had a lower cholesterol to phospholipid molar ratio (0.20) than was found in normal rats (0.40) [57].

The analysis of normal ROS disks and plasma membrane suggested that the cholesterol distribution is dependent on the phospholipid composition of the membranes. That is, the relative amounts of PC and PE in the disk and plasma membranes may play a role in determining the membrane cholesterol level. Analysis of the phospholipid composition of normal and RCS rat outer segment membranes showed that the composition of RCS disks and plasma membrane differed from normal animals. In RCS rats, the ratio of PE/PC was 0.6 in disks and 0.2 in the plasma membrane. In the normal rats, the PE/PC ratio was 0.9 in the disks and 0.4 in the plasma membrane. As described earlier, cholesterol tends to partition out of membranes with high PE/PC ratios. It was thus likely that the aberrant cholesterol distribution in RCS rats is the result of an abnormal phospholipid composition found in the RCS ROS [57].

These studies on the RCS rats are particularly interesting because the primary defect has been shown to reside in the inability of the pigment epithelia to appropriately phagocytose the disk packets shed from the ROS [130–132]. The aberrant cholesterol distribution may be a secondary effect of the mutation. However, it demonstrates the interrelationship between the pigment epithelium and the ROS membranes.

Smith–Lemli–Optiz (SLO) syndrome is a disease that is often fatal in humans and is characterized by high levels of 7-dehydrocholesterol (the precursor of cholesterol) and low levels of cholesterol [133,134]. In humans the defect can be due to an array of mutations [135–137] and the consequences of this syndrome are manifest in many tissues [138–140]. With respect to vision a spectrum of defects have been reported [141–151].

A potential animal model with which to investigate the visual effects of this disease became available when it was shown that the final step in cholesterol synthesis could be inhibited in rats using AY9944 [51]. This results in rats that exhibit high tissue levels of 7-dehydrocholesterol and low levels of cholesterol. After one month of treatment the rats exhibited physical manifestations of the defect. However, the retinas appeared normal [51]. In these animals the cholesterol was not completely depleted of cholesterol in the tissues. It is well known that some membranes can accommodate the limited substitution of other sterols for cholesterol and maintain functional integrity. However, extensive substitution often produces a functionally incompetent membrane [152]. This is consistent with the existence of more than one cholesterol environment. It was hypothesized that after one month of treatment a critical amount of cholesterol remained to maintain normal retina function. After three months of inhibition of cholesterol synthesis the rats exhibited serious retinal defects. The electroretinagrams of the treated rats indicated that normal function in both photoreceptor cells and neural cells was compromised. In addition, histological examination of the retina as well as changes in the cellular structure of retinal cells revealed changes that were consistent with retinal degeneration [153]. These changes could be due directly to the lack of membrane cholesterol or to the presence of 7-dehydrocholesterol and its cytotoxic oxysterol derivatives. This animal model system may provide valuable insight with respect to the in vivo effect of cholesterol.

7. Summary

The photoreceptor rod outer segment (ROS) provides a unique system in which to investigate the role of cholesterol in biological membranes. A dynamic image of cholesterol distribution in the ROS has emerged. This image depicts cholesterol in the ROS plasma membrane at the relatively high concentration typically found in plasma membranes (30 mol%). Disk membranes newly formed from the plasma membrane at the base of the ROS are also high in cholesterol. However, membrane cholesterol is rapidly depleted as the disks progress from the base of the ROS to the apical tip. This depletion of disk membrane cholesterol can have important effects on the major ROS specific protein, rhodopsin. Biophysical studies in both model membranes and in native membranes have demonstrated that cholesterol can modulate the activity of rhodopsin by altering the membrane hydrocarbon environment. These studies suggest that mature disk membranes initiate the visual signal cascade more effectively than the newly synthesized basal disks. Furthermore, rhodopsin has a marginal ability to be activated in the plasma membrane where cholesterol is high.

The effects exerted by cholesterol on rhodopsin function have far-reaching implications for the study of G-protein coupled receptors as a whole. These studies show that the function of a membrane receptor can be modulated by modification of the lipid bilayer, particularly cholesterol. This provides a powerful means of fine-tuning the activity of a membrane protein without resorting to turnover of the protein or protein modification.

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