The molecular basis of mechanisms underlying polarization vision

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The underlying mechanisms of polarization sensitivity (PS) have long remained elusive. For rhabdomeric photoreceptors, questions remain over the high levels of PS measured experimentally. In ciliary photoreceptors, and specifically cones, little direct evidence supports any type of mechanism. In order to promote a greater interest in these fundamental aspects of polarization vision, we examined a varied collection of studies linking membrane biochemistry, protein–protein interactions, molecular ordering and membrane phase behaviour. While initially these studies may seem unrelated to polarization vision, a common narrative emerges. A surprising amount of evidence exists demonstrating the importance of protein–protein interactions in both rhabdomeric and ciliary photoreceptors, indicating the possible long-range ordering of the opsin protein for increased PS. Moreover, we extend this direction by considering how such protein paracrystalline organization arises in all cell types from controlled membrane phase behaviour and propose a universal pathway for PS to occur in both rhabdomeric and cone photoreceptors.

Keywords: polarization sensitivity; polarization vision; protein interactions; molecular tethering; membrane composition; lipid rafts

1. INTRODUCTION

Polarization vision epitomizes sensory biology; it is a complex, integrated and plastic modality that detects, encodes and processes the polarization information contained within the visual light environment. It has been just over 60 years since Karl von Frisch discovered that bees (Apis mellifera) see and use polarized light [1], and we know now that sensitivity to linearly polarized light occurs widely throughout the animal kingdom (figure 1). Most recently we have seen how animals can even detect and discriminate the handedness of circularly polarized light [2]. However, while polarization vision has been recognized as an important sensory modality for many animals, the underlying mechanisms have yet to be understood.

Like the spectral component of light (e.g. colour), the polarization of light provides visual information that can be used by animals. The information gained from discriminating between different electric field vectors of light augments normal visual functionality, providing visual contrast enhancement for object detection and recognition [3], a navigational compass [4–6], an aid for orientating within the environment [7] and a separate channel for communication and display [8,9]. In many animals, polarization vision is truly a multi-level pathway, leading from molecular properties defining a cellular response through several layers of neuronal processing and interpretation in higher brain areas. Recent works have clearly demonstrated the processing of polarization information downstream of the photoreceptor layer in both invertebrates [10–12] and vertebrates [13–15], and suggested how that information can inform and direct behaviour [3,6,16,17]. However, the mechanisms that begin the sequence by creating a polarized light detector at the level of the photoreceptors have received surprisingly little attention.

The foundation of animal polarization sensitivity (PS) is in the visual pigment molecules, which are inherently dichroic because the light-absorbing chromophore, retinal, is itself a dichroic molecule. Based on the inherent visual pigment dichroism alone, rhabdomeric photoreceptors can theoretically achieve PS values of 2 [18,19]. Much higher PS values of 5–15 in a plethora of animals have been discovered (figure 1; [20–25]), and such large sensitivities are only attainable if molecular ordering and alignment of the visual pigment elevate the overall dichroism of the photoreceptor. Molecular-level considerations have been discussed previously [26], and these discussions still represent the current state of knowledge. While oligomerization, cytoskeletal tethering and protein–protein and protein–lipid interactions present possible mechanisms for achieving the hypothesized order and alignment at the molecular level, these interactions have been little investigated in photoreceptors. This has left the problem of the molecular mechanisms...
underlying polarization vision unsolved and even uninvestigated.

The aim of this paper is to bring together a diverse range of literature on photoreceptor membrane biochemistry and biophysics in order to place the spotlight on a neglected area of polarization vision. While this paper may not provide the answers to these questions, it will hopefully inspire new research directions.

![Evolutionary distribution of polarization vision in animal visual systems.](http://rstb.royalsocietypublishing.org/)
within the field to provide a more comprehensive understanding of the specific molecular mechanisms underlying the detection of polarized light in photoreceptor membranes.

2. PHOTORECEPTORS AS POLARIZATION DETECTORS: CURRENT KNOWLEDGE OF GEOMETRIC MECHANISMS

Polarization vision was first described in bees [1], and has since been widely investigated in arthropod compound eyes, vertebrate and cephalopod camera-type eyes and in several extracellular photoreceptors. For an extensive review of behavioural and electrophysiological demonstrations of polarization vision, the reader is referred to *Polarized Light in Animal Vision* [27], which provides a very complete review of the literature. Also, Waterman’s [28] classic review in the *Handbook of Sensory Physiology* is worth seeking out. What is important in the context of this paper is that underlying all such forms of polarization vision are photoreceptor detectors that respond differentially to different polarizations of light. However, it is clear throughout the literature, and as we will demonstrate below, that the mechanisms of detection have been primarily considered in terms of geometric arguments of photoreceptor structure.

(a) Rhabdomeric photoreceptors and microvillar geometry

Rhabdomeric photoreceptors are by far the best studied with respect to how geometric arrangements contribute to PS. In rhabdomeric photoreceptors, the dichroic visual pigments are found in microvilli, which extend in groups from one margin of the cell (figure 2a). De Vries et al. [29] first suggested that if (i) microvilli were assembled in a parallel arrangement and (ii) the collective visual pigment displayed a degree of linear dichroism in an aligned manner, we would be able to explain overall photoreceptor PS. This prediction turned out to be broadly true and the subsequent 50 years of microscopic data have demonstrated how arthropod (in particular insect and crustacean) and cephalopod photoreceptors are superbly equipped both structurally (e.g. microvillar geometric structure) and genetically (e.g. constant cell numbers in ommatidia) for polarization vision [30].

An individual microvillus displays dichroism for several reasons. Since the foundation of all PS lies in the dichroic chromophore of the visual pigment, one could hypothesize that a degree of preferential alignment of chromophores between multiple visual pigments is required for an overall dichroism of the membrane. However, the cylindrical nature of the microvillus creates dichroism simply owing to geometric arguments; even when the dipoles are ‘end-on’, most spectral absorbance experiments have provided the clearest evidence for one mechanism, owing to the unusual arrangement and the morphology of the cones in their retina. Fineran & Nicol [35] first demonstrated that the long, bi-lobed anchovy cones have the visual-pigment-containing membranes orientated axially, such that normally incident light illuminates the visual pigment transversely. As the chromophore lies closely parallel (approx. 17°) to the plane of the membrane [38–40] in all vertebrate photoreceptors that have been examined, the resulting transverse dichroism [37], coupled with the orthogonal arrangement of the long, bi-lobed cell types, could provide a dichroic detection system.

In more typical vertebrate photoreceptors, only one study [41] has provided direct experimental measurements of dichroism with the cell under physiological illumination. While light in the eye enters these cells ‘end-on’, most spectral absorbance experiments use ‘side-on’ microspectrophotometry. Roberts & Needham [41] used a laser tweezer system to control the photoreceptor cell orientation in three dimensions, and discovered that the mid-wavelength-sensitive members of double cones in goldfish (*Carassius auratus*) are axially dichroic and thus could display PS *in vivo*. In contrast, rods absorb all orientations of polarized light to the same degree when illuminated.
axially. This is consistent with earlier indirect evidence of axial dichroism in the cones of salmonids [42–44].

In the absence of a body of experimental evidence, several theoretical models have also been proposed to explain the mechanism underlying PS in vertebrates. Flamarique et al. [45] proposed that the curved limiting membrane between double cone inner segments could act as a directional dichroic reflector of incident light, thus transversely illuminating the outer segments of the corner ultraviolet-sensitive cones within a square cone mosaic. The resulting difference in the polarization of the transmitted light could then be analysed by the double cone outer segments. However, the theory used by Flamarique et al. [45] is based on macroscopic Fresnel equations that are not valid for sub-wavelength thicknesses of cell membranes. In a separate study, Cameron & Pugh [46] proposed that refractive index gradients in inner segments of twin cones could operate as anisotropic polarization waveguides. They supported this hypothesis with both optical modelling and an electrophysiological study discovering PS in the orthogonal array of twin cones in green sunfish [47]. However, Flamarique & Hawryshyn [48] could not verify the PS measurements in green sunfish, and Roberts & Needham [41] measured isotropic transmission through similar double cone inner segments.

(c) The limits of geometric considerations in the detection of polarized light

We described earlier how rhabdomeric photoreceptors are inherently polarization sensitive owing to the geometry of the microvilli and their arrangement in the cell. However, neither the cylindrical shape nor microvillar packing explain the levels of PS measured in many arthropod species. For example, Labhart [22] found PS values of 18 in honeybees (figure 1). It is worth noting that experimentally, PS is generally measured from electrophysiological recordings, however there is a direct equivalence to optical dichroic ratio measurements intrinsic to the photoreceptor cell (see eqns (14)–(18) in [43]) and not subject to any neural processing. Similarly, as described, the literature still lacks any broad base of experimental evidence for why typical vertebrate ciliary photoreceptors exhibit PS. Logically, PS can be increased through the ordering and alignment of the chromophores with the plane of the cell membranes in both rhabdomeric and ciliary cell types [26]. In the following sections, we discuss how protein–protein and protein–lipid interactions may underlie the dichroic alignment of visual pigments and how membrane composition lamellar ordered phases may bring about such interactions, thus explaining the high values of PS measured experimentally.

3. PHOTORECEPTORS AS POLARIZATION DETECTORS: VISUAL PIGMENT DIMERS AND higher order structural protein architecture contributing to polarization sensitivity

Snyder & Laughlin [26] first calculated how the alignment of the visual pigment chromophore within the microvilli membranes could increase the dichroic ratio to reach the measured levels of rhabdomeric PS. However, few studies have investigated the potential of protein–protein interactions and cytoskeletal coupling as mechanisms of visual pigment alignment. A number of potential molecular interaction mechanisms have been previously suggested to occur within photoreceptor membranes, including: (i) visual pigment dimerization and ordered protein–protein arrays [49, 50]; (ii) direct anchoring of visual pigment molecules to a relatively fixed axial microvillar cytoskeleton [51–53]; and (iii) extracellular tethering of visual pigment molecules across microvilli membranes [53]. Studies of these mechanisms are in their infancy, and
in many cases are still contentious. We will highlight the information suggesting that these ordering and anchoring mechanisms significantly contribute to the observed PS values in both rhabdomeric and ciliary photoreceptors.

(a) Visual pigment interactions in rhabdomeric photoreceptors
Cephalopods present some of the best-studied rhabdomeric photoreceptor systems in terms of microvillar structure within the retina and structure of the visual pigment. In fact, rhodopsin from the squid Todarodes pacificus is only the second opsin, and the single non-vertebrate opsin, to have been crystallized [50]. Early studies of cephalopod photoreceptor structure reveal that the microvilli contain a cytoplasmic core bundle of actin filaments with crossbridges to the membranes, as well as special membrane junctions linking adjoining microvilli [53]. Microvillar actin cores have also been characterized in Drosophila and crayfish rhabdams [54,55]. The observed actin cores and the core-to-membrane crossbridges fit with the idea from studies of Drosophila phototransduction of a ‘signalplex’—a macromolecular complex linking the visual pigment and associated phototransduction proteins with the cytoskeleton (figure 3; for review see [56]). Although these protein cross-microvillar and cytoskeletal interactions have been studied in very few invertebrate species, together they suggest a highly structured mechanism for increasing the ordering of chromophore alignment.

In conjunction with these studies, evolutionary studies of expressed opsin genes in stomatopod crustaceans have provided some tantalizing evidence that higher order protein interactions may be a common feature of rhabdomeric photoreceptors. In most arthropod visual systems, the photoreceptors that detect polarized light express visual pigments that are also expressed in photoreceptors devoted to the task of colour discrimination. However, in some stomatopod crustacean visual systems, these two tasks have been de-coupled, with the photoreceptors specialized for polarized light detection expressing different opsin genes than those optimized for colour discrimination [57]. This allows for the comparison of opsin gene sequences from photoreceptors devoted to different tasks, with the goal of elucidating specific mechanisms within the opsin protein that may contribute to PS. Preliminary studies indicate that the genes expressed in polarization-sensitive photoreceptors are evolutionarily distinct from those in colour-sensitive photoreceptors (figure 4). Furthermore, comparative evolutionary analyses have identified a set of amino acids that are diversifying among stomatopod opsins that are likely to interact with machinery inside the cell, either in the phototransduction system or with cytoskeletal elements similar to the Drosophila signalplex [58].

In addition to these hypothetical protein/cytoskeleton interactions, Murakami & Kouyama’s [50] crystallization study of squid rhodopsin suggested several protein–protein interactions among visual pigments. First, squid visual pigments are thought to form dimers in the membrane. Second, Murakami & Kouyama [50] also found a tight association across microvillar membranes between the amino-terminal polypeptides of neighbouring monomers, which is suggested to play a role in the hexagonal packing of the microvilli. In fact, these authors suggested that the across-membrane protein–protein interactions may be stronger than the dimer interactions within the membrane. This is one of the first indications that visual pigments could have protein–protein contacts across adjacent microvillar membranes. These proposed protein–protein interactions within and across membranes form a tetrameric structure in which four chromophores are oriented in a nearly parallel arrangement, and they may also play a role in the highly parallel ordering of microvilli needed for polarized light detection.

Functioning either individually or in combination, these visual pigment protein–protein and protein–cytoskeletal interactions observed in Drosophila and cephalopods, and suggested in stomatopods, may provide a rigid organization of visual pigment molecules that contributes to PS by ordering and aligning the chromophores parallel to the axis of the microvillus. However, these studies represent a very limited sampling of invertebrate diversity. Much more research on the interactions among visual pigments and cytoskeletal elements from animals containing rhabdomeric photoreceptors is needed.

Figure 3. Hypothetical arrangement of anchoring and tethering mechanisms within rhabdomeric receptors. Visual pigments are arranged in dimers, which are anchored to each other across microvillar membranes (represented by dashed lines), and tethered to cytoskeletal elements (actin core represented by double helix in the middle of the microvilli) by phototransduction molecules (represented by shaded forms).
linking across the extra- and inter-faces of the membranes. These studies show remarkable similarities with the work described above by Saibil [53] of the linkages between microvilli.

Further evidence of ordered arrays of rhodopsin has been obtained using recombinant membranes via saturation-transfer spin-label electron spin resonance [63]. The key findings of Ryba & Marsh [63], using different synthetic membranes, were that the level of oligomerization was correlated with a reduction of rotational diffusion and was principally dependent on the lipid membrane composition. They found that greater levels of protein aggregation take place with longer lipid chain lengths in these artificial membranes. Botelho et al. [64] concurred with this finding, providing direct fluorescence resonance energy transfer (FRET) evidence that the membrane lipid composition causes oligomerization of rhodopsin.

(c) Implications of visual pigment interactions and common themes in both rhabdomeric and ciliary photoreceptors

Long-range spatial organization in both rhabdomeric and ciliary photoreceptors provides an ideal mechanism for cooperatively ordering the visual pigment to produce the experimentally measured levels of dichroism seen in all cell types. In ciliary cells, such organization would seem to be incompatible with several studies [65,66] that have concluded that the visual pigment rotates in the membrane resulting in isotropic absorbance. Note, however, that both Brown's [65] and Cone's [66] studies were conducted on rods of *Rana pipiens*, which is not a known polarization-sensitive animal. In general, rods are known not to be polarization sensitive, and recent studies have shown that rotational diffusion may be inhibited in cone outer segment membranes [41]. Furthermore, a very recent study by Govardovskii et al. [67] has demonstrated that similar lateral diffusion measurements made at about the same time as these earlier studies [68] may have over-estimated levels of fluidity in ciliary membranes by an order of magnitude and that protein–protein interactions are physiologically relevant in vertebrate photoreceptors.

Therefore, in the remainder of this paper, we consider how the protein–protein interactions may occur in both rhabdomeric and ciliary cell types and how these may be linked by a common theme. As the current understanding is that both rhabdomeric and cone photoreceptors mediate polarization information, we ask the question: are there reasons to suspect that such protein–protein interactions and protein oligomerization as discussed above occurs with more prevalence in rhabdomeric cells and cones than in rods?

4. PHOTORECEPTORS AS POLARIZATION DETECTORS: THE COMPOSITION AND PHASE BEHAVIOUR OF CELL MEMBRANES CONTRIBUTING TO POLARIZATION SENSITIVITY

The local lipid environment within a membrane is crucial for correct protein function and therefore, in photoreceptors, visual function. The effects of the

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**Figure 4.** Evolutionary history of stomatopod crustacean opsins, illustrating a separation between opsins involved in colour vision from those expressed in photoreceptors specialized for the detection of polarized light (for more detail, see [57]). Phylogeny is derived from Porter et al. [58]. Stomatopod opsins form six groups, labelled A–F, and the sequence groups (triangles) have been shaded grey. The sequence groups coloured white represent opsin sequences from other crustaceans.

(b) Visual pigment interactions in ciliary photoreceptors

Over the last 20 years, numerous studies have detailed how oligomerization via receptor–receptor interaction is a universal aspect of G-protein coupled receptor (GPCR) biology [59]. While there is still no conclusive answer to whether a particular order of oligomerization is prevalent (from dimers to large complexes), oligomerization appears to be a pivotal component of the structure and function of GPCRs. Opsin proteins, as members of subfamily A and some of the best-studied GPCRs, also exhibit oligomerization. Two atomic force microscopy (AFM) studies of native rod outer segments (ROS) discs clearly show that rhodopsin dimerizes and forms long-range-ordered paracrystalline protein arrays of those dimers (figure 5) [49,60]. Further X-ray powder diffraction results confirmed the double row and axial repeat periodicities of the rhodopsin dimers to be 8.4 and 4.2 nm \(^{-1}\), respectively [49]. To exclude the possibility that the absorption of the disc membrane onto mica promotes the formation of the arrays, disc membranes were also absorbed onto carbon-coated electron microscopy (EM) grids. The power spectra from the EM images of these discs confirmed the unit size and array order seen in the AFM image.

Other levels of order in rod and cone outer segments have also been consistently revealed by EM. Coreless et al. [61,62] showed extensive areas of order within the membranes—not only in the membrane plane but also between bilayers, where the inter-discal space seemed expanded, with processes

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lipid composition on membrane structure have wide reaching implications. In tertiary model systems, particular lipid compositions create lipid tubes or discs (analogous to microvilli or outer segment structures) with prescribed spatial order in the plane of the membrane [69]. These lamellar-ordered (L\textsubscript{o}) phases [70] exhibit higher degrees of spatial order compared with the fluid lamellar (L\textsubscript{a}) and are currently an extremely exciting area of research.

(a) The effect of membrane lipid composition on visual pigment function in rhabdomeric photoreceptors

Lipid analysis of rhabdomeric membranes indicates a high concentration of cholesterol (Limulus [71]; squid [72]; insects [73]), which was originally believed to imply less-fluid homogeneous membranes and correspondingly slower visual pigment diffusion than in ciliary types [52]. Studies of arthropod and cephalopod photoreceptor fatty acid compositions also found large variations among species [74]. The effect of membrane lipid composition on visual pigment function is indicated by recent in vitro expression studies of honeybee opsins [75]. Historically, invertebrate visual pigments have been difficult to express in cultured cells, and most attempts at the functional expression of arthropod visual pigments have been unsuccessful. While it is possible to express invertebrate opsin genes and make protein in mammalian cell lines, once solubilized from the membrane, the reconstituted visual pigments are not normally functional. However, honeybee UV and blue-sensitive visual pigments have been successfully expressed in a mammalian cell line, solubilized and functionally characterized using spectrophotometry. To functionally characterize these visual pigments, the purified pigments were reconstituted in lipid vesicles, suggesting that lipid composition plays an important role in insect visual pigment function. It is also worthy of noting that, using these same techniques, Terakita et al. [75] were unable to functionally express the honeybee long-wavelength-sensitive opsin, suggesting that membrane lipid composition affects visual pigment spectral classes differently.

(b) The effect of membrane lipid composition on visual pigment function in ciliary photoreceptors

We currently understand that ROS discs and the surrounding outer cell membrane differ significantly in lipid composition. Work by Boesze-Battaglia et al. [76,77] has shown that the photoreceptor plasma membranes have considerably higher levels of cholesterol and a markedly different ratio of saturated to unsaturated fatty acids compared with the intercellular discs. In this regard, altering the lipid and cholesterol composition has been shown to...
increase photoreceptor PS beyond the limits set by light. A diverse set of potential mechanisms that may act in concert to achieve the highly ordered chromophore arrangement needed to produce the PS observed in some animals. The evidence suggests it is possible that a carefully controlled membrane composition, with a higher cholesterol content in rhodobacter and cone ciliary photoreceptors, may be responsible for driving the formation of liquid-ordered $L_n$ and $L_o$ micro-domains in the membranes. Opsin proteins then affiliate with the $L_o$ micro-domains enabling protein–protein and protein–cytoskeletal (in rhodobacter cases) interactions. This association allows rhodopsin to oligomerize and thus aligns the chromophores into a cellular-scale polarization detector, producing the higher levels of dichroism measured experimentally than predicted theoretically. This would be a common mechanism used by both rhodobacter and ciliary photoreceptors, a possibility up until now not considered.

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