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Drosophila visual transduction

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Abstract

Visual transduction in the *Drosophila* compound eye functions through a pathway that couples rhodopsin to phospholipase C (PLC) and the opening of Transient Receptor Potential (TRP) channels. This cascade differs from phototransduction in mammalian rods and cones, but is remarkably similar to signaling in mammalian intrinsically photosensitive retinal ganglion cells (ipRGCs). This review focuses on recent advances in the fly visual system, including the discovery of a visual cycle and insights into the machinery involved in generating a light response in photoreceptor cells. These latest findings suggest that the mechanism of light detection in flies and mammals have important parallels. Thus, a better understanding of phototransduction in the fly has the potential to contribute to our understanding of light detection in mammalian ipRGCs.

Keywords

TRP channels; rhodopsin; phototransduction; phospholipase C

Introduction

Drosophila phototransduction has been scrutinized for more than 40 years, and provides a genetic paradigm for signaling cascades that employ phosphoinositides and TRP channels [1–4]. During the last decade it has become clear that there exists a new class of photoreceptor cells in mammals, referred to as ipRGCs [5], which function through a signaling cascade akin to fly visual transduction [6–9]. The ipRGCs contribute primarily to photoentrainment of circadian rhythm, pupillary constriction and sleep [10–15]. The current review focuses on recent advances in understanding the fly visual system. Such findings include the discovery of a visual cycle, insights into the mechanism activating the TRP channels, the demonstration of dynamic interactions with the INAD (Inactivation but No Afterpotential D) signaling complex, and plastic changes in rhodopsin expression. Furthemore, the finding that light-dependent shuttling of a signaling protein depends on a second type of cascade that is coupled to rhodopsin will be discussed.

Anatomy of compound eye

The *Drosophila* compound eye is comprised of ~750–800 reiterating hexagon-shaped ommatidia (Figure 1a), each of which contains 20 cells, including eight photoreceptors. Six photoreceptor cells (R1–6) extend the full depth of the retina, while the R7 and R8 cells are

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situated in the top (distal) and bottom (proximal) halves of the retina, respectively. Thus, only seven of photoreceptor cells lie in any plane of section (Figure 1b). Photoreception and signal transduction take place in the rhabdomeres, which are comprised of stacks of microvilli. ~50,000 are present in the larger R1–6 cells (Figure 1c and d). These ~1.2–1.5 μm long microvilli are only ~50 nm in diameter and contain an F-actin filament, but are devoid of internal organelles. The secondary retinal pigment cells (RPCs) are the main cells surrounding the photoreceptor cells (2° PC; Figure 1b). Tertiary RPCs and mechanosensory bristle cells occupy alternating vertices of the ommatidia (3° PC and bristle cell; Figure 1b).

In addition to the compound eye, adult flies have three smaller and simpler eyes (ocelli) located on the top of the head. Ocelli seem to be more important for detecting changes in light intensities during flight, than in image formation [16].

Fly phototransduction and its relationship to the cascades in rods and cones

Phototransduction cascades serve to amplify single photon responses, and to allow the cells to adapt to light with intensities that differ over many orders of magnitude. The initiation of these signaling pathways depend on light sensors that are comprised of a seven transmembrane-containing protein (opsin) linked to a chromophore (3-hydroxy 11-*cis*-retinal in *Drosophila*, and 11-*cis*-retinal in rods and cones). Light promotes isomerization of the chromophore to the all-*trans* configuration, thereby inducing a conformation change in the protein subunit. The photoactivated visual pigment stimulates GDP/GTP exchange in a heterotrimeric G-protein. In the fly eye, the G-protein (Gq) [17] activates the PLC encoded by *norpA* [18], which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2), leading to opening of the TRP channel and the related TRP-like (TRPL) cation channel in photoreceptor cells (Figure 2) [19–23]. NORPA also accelerates the intrinsic GTPase activity of the Gqα [24–26]. Thus, NORPA promotes activation and negative feedback regulation, and does so through distinct domains [24–26]. The rise in Ca^{2+} is counterbalanced by extrusion through the Na^{+}/Ca^{2+} exchanger, CalX [27].

Several of the key aspects of *Drosophila* phototransduction are distinct from the cascades in mammalian rods and cones. In flies, rhodopsin is bistable, i.e.—the chromophore usually stays bound to the opsin following exposure to light [28]. A second photon of light is necessary to convert the 3-OH-all-*trans* retinal back to the 3-OH-11-*cis*-retinal. If the flies are exposed to blue (480 nm) light, the major rhodopsin (Rh1) remains active in the dark. In rods and cones the light activated chromophore, all-*trans*-retinal, is released from the opsin and must be recycled through an enzymatic pathway [29, 30]. The heterotrimeric G-protein that is activated by the mammalian visual pigments (transducin; G_t) stimulates a phosphosphodiesterase, causing a decline in cGMP levels and subsequent closure of the cGMP-gated channels [31]. Thus, light induces opposite affects on the state of the cation channels in the rhabdomeres, and in the outer segments of rods and cones.

Mammals sensing light like flies

Direct light activation of the ipRGCs is mediated by the visual pigment melanopsin, which bears greater sequence homology to fly rhodopsin than to the mammalian rod and cone visual pigments [5, 8, 10]. Similar to the major *Drosophila* rhodopsin (Rh1), melanopsin is maximally activated by blue light, and appears to be a bistable photopigment [7, 32].

A variety of pharmacological, electrophysiological and expression studies in heterologous systems and in native cells indicate that melanopsin engages a Gq/PLC/TRPC signaling cascade [6, 7, 33, 34]. The requirement for PLC signaling has been solidified in a recent study demonstrating that loss of the mouse homolog of *Drosophila* NORPA (i.e. PLCβ4) dramatically reduces the light response of the M1-ipRGCs, which are the most light

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responsive ipRGCs [9]. Phototransduction in these cells depends on the TRP cation (TRPC) channels TRPC6 and TRPC7, since a double mutant eliminated photosensitivity [9]. Given that the *Drosophila* TRP and TRPL channels are the classical TRPC channels, these findings further underscore the common features of phototransduction in ipRGCs and fly photoreceptor cells.

The sphincter muscle cells in the iris of certain nocturnal and crepuscular (i.e. most active at dawn and dusk) mammals are also photosensitive and contribute to a local pupillary light response. It is proposed that the iris from nocturnal/crepuscular animals is directly photosensitive because their eyes are comprised mostly of rods, and under bright light the pupil may be constricted to such an extent that insufficient light reaches retinal photoreceptor cells to direct a pupillary response [9]. The phototransduction cascade in these photosensitive muscle cells is dependent on melanopsin and PLCβ4, as is the case for the ipRGCs [9]. However, their light response is not dependent on a TRPC channel, or on the TRP **V**anilloid (TRPV) channel, TRPV4, which functions in smooth muscle cells [9]. Nevertheless, the phototransduction cascade in the iris may be dependent on another TRP channel. If so, light signaling in these sphincter muscle cells would represent another *Drosophila*-like phototransduction cascade.

Drosophila rhodopsins and plasticity in the adult eye

Drosophila encodes seven opsin genes (Rh1–Rh7), six of which (Rh1–6) fully account for the photoresponses of the different classes of photoreceptor cells in the adult compound eyes and ocelli (Figure 3a). The major rhodopsin, Rh1, is expressed in the six outer photoreceptor cells (R1–6 cells), which respond to dim light (Figure 3a and b). The R7/R8 photoreceptor cells each express one of four different rhodopsins, and are involved in color vision, including the detection of ultraviolet light by the R7 cells (Figure 3a and b) [35]. A random set of ~30% of the ommatidia coordinately express Rh3 in the R7 cells and Rh5 in the R8 cells (pale ommatidia; Rh3/Rh5; Figure 3b). Most of the remaining ~70% of ommatidia express Rh4 and Rh6 in the R7/R8 photoreceptor cell pairs (yellow ommatidia; Rh4/Rh6). In addition, there are two minor classes of ommatidia located near the dorsal rim that deviate from this pattern.

The control of one opsin to one R7 or R8 neuron in the pale and yellow ommatidia begins with stochastic expression of the Spineless (Ss) transcription factor in 70% of R7 cells, and induction of Rh4 expression in these cells [36]. As a default, R7 cells that do not express Ss turn on Rh3 (Figure 3b). The Rh7 cells that express Rh3 then induce expression of Rh5 in the underlying R8 cells in pale ommatidia (Figure 3b). Expression of Rh6 in the R8 cells is the default if Rh3 is not expressed (Figure 3a).

The continued exclusion of Rh5 from R8 cells in yellow ommatidia occurs through a mechanism involving a negative feedback signal induced by Rh6, which inhibits transcription of *rh5* [37]. The signal requires activity of Rh6 and the Gqα, but not NORPA or the TRP and TRPL channels. In *rh6* mutant flies, expression of Rh5 gradually expands with age to include nearly all R8 cells, including those in yellow ommatidia (Figure 3c) [37]. If wild-type flies are maintained in the dark, a low level of Rh5 expression is turned on in some yellow R8 cells that also express Rh6 [37]. The mechanism that links Rh6 activity with transcriptional repression of *rh5* remains to be determined.

The finding that Rh6 prevents expression of Rh5 illustrates a role for a rhodopsin that is distinct from its classical function in vision. The major rhodopsin, Rh1, has two light-independent roles. These include a requirement for this rhodopsin for rhabdomere morphogenesis [38]. In addition, Rh1 initiates a thermosensory signaling cascade, which permits larvae to select their preferred temperature in the comfortable range [39]. Whether

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Drosophila rhodopsins have other light-independent or non-visual roles have not been reported, but are intriguing possibilities.

Bistable photopigments and an enzymatic visual cycle

The *Drosophila* visual pigments are bistable pigments, and therefore require absorption of one photon to activate rhodopsin, and a second photon to convert the light-activated metarhodopsin back to the non-active rhodopsin. Because the regeneration of the chromophore is normally a light-driven process, it has been assumed that *Drosophila* rhodopsins do not rely on an enzymatic cycle to regenerate the chromophore. However, recent findings demonstrate that flies use a visual cycle after all.

During light stimulation, a fraction of the rhodopsin pool is internalized and the opsin is degraded, thereby releasing the chromophore. The free 3-OH-all *trans* retinal is then recycled through an enzymatic pathway that includes the retinal dehydrogenases, PDH (Pigment Cell Dehydrogenase) [40] and RDHB (Retinal Dehydrogenase B) [41], and an isomerase that remains to be identified (Figure 4). These enzymatic steps take place in the RPCs that surround the photoreceptor cells. The essential role for the RPCs is reminiscent of the requirements for cells in the retinal pigment epithelium and Müller cells for regeneration of the chromophore used in rods and cones, respectively [29, 30].

In *Drosophila* the visual cycle allows the flies to maintain chromophore levels and a normal visual response under conditions in which dietary limitations prevent the flies from synthesizing new chromophore [40]. Melanopsin also appears to be a bistable pigment [7, 32], although it is not known if it depends on a visual cycle. If so, the Müller glia are candidate cells for participating in this process since they are situated near the ipRGCs and function in the visual cycle necessary for the cone pigments [15].

Mechanism of activation of TRP

TRP is the classical member of the TRP superfamily [19, 20, 42], and activation of this Ca^{2+}-permeable cation channel is strictly dependent on stimulation of the PLCβ encoded by *norpA* [18]. However, the mechanism linking PLC with the opening of TRP, and the related TRPL channels, remains controversial. Stimulation of PLC results in hydrolysis of PIP_2 and production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG; Figure 2). Although largely ignored, a single H^+ is also generated [43] (Figure 2). Thus, TRP and TRPL could be activated either by a reduction in inhibitory PIP_2, or an increase in H^+, IP_3, DAG, or by metabolites that are produced by these products, such as polyunsaturated fatty acids (PUFAs). IP_3 does not appear to contribute to channel gating since release of caged IP_3 does not mimic the light response, and mutation of the sole IP_3 receptor encoded in the fly genome does not impair activation [44–46].

There are currently two prevailing models. The first is that PUFAs activate the channels. This concept is supported by whole-cell and single-channel recordings of isolated ommatidia indicating that TRPL is activated by PUFAs [47, 48]. DAG lipases metabolize DAG, and mutation of a gene (*inaE*) encoding a putative DAG lipase (Figure 2), severely impairs the light response [49]. A second model, supported by a recent study, is that a decrease in inhibitory PIP_2, in combination with a local acidification, activates TRP and TRPL [43]. This work also provides *in vivo* evidence that light stimulation leads to PLC-dependent acidification. However, acidification with the protonophore 2,4-dinitrophenol is most effective at activating the channels, and this chemical is a mitochondrial uncoupler. Since metabolic stress can also activate the channels [50], it cannot be excluded that this is the basis for activation by 2,4-dinitrophenol. Another possibility is that full activation of the

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channel involves a decline in PIP_2 in combination with a rise in DAG [51], PUFA, H^+, and possibly other metabolites.

Light dependent movements of signaling proteins

Multiple signaling proteins in mammalian and fly photoreceptor cells undergo light dependent changes in spatial distribution [52]. In *Drosophila* photoreceptor cells, TRPL, Gq, and Arrestin1 and Arrestin2 (Arr1 and Arr2) shuttle in and out of the rhabdomeres dynamically [53–57]. These latter two proteins bind to rhodopsin, and contribute to termination of signaling by blocking the rhodopsin/Gq interaction.

The light-induced movements of some of these proteins are opposite in direction, and correlate with their roles during phototransduction. TRPL and Gq function in activation and are concentrated in the rhabdomeres of dark-adapted flies [56–59]. Upon light stimulation, these two proteins translocate to the cell bodies over the course of minutes. In contrast, Arr1 and Arr2 participate in termination of phototransduction, and shuttle from the cell bodies into the rhabdomeres in response to light [54, 55], and do so with a time-constant of under 10 seconds [60]. The opposite vector of these movements makes sense, since the light-dependent translocations participate in light adaptation [55, 56]. A decline in TRPL and Gq in the rhabdomeres, or an increase in Arr1 and Arr2, all serve to attenuate signaling.

Translocation of TRPL, Gq and Arr1/2 between the two major compartments of the photoreceptor cells depends on rhodopsin, which is expected since the trafficking is light dependent [59, 61, 62]. However, there are some surprising findings concerning the requirements for other phototransduction proteins. Two studies indicate that the phototransduction cascade comprised of PLC, TRP and TRPL are not required for movement of Gqα [57, 58]. Similarly, Arr2 can translocate into the rhabdomeres in the absence of any of classical proteins that function downstream of Rh1, such as PLC and TRP [61]. However, the kinetics of the movements is reduced at least 10-fold in the absence of these proteins due to a contribution of Ca^{2+} influx for the translocation [60]. Nevertheless, there is a Gq/PLC/TRP independent mechanism that contributes to light-induced movement of Arr2. This second pathway includes the small GTPase, Rac2, which associates either directly or with Rh1 *in vivo* [61]. Thus, in addition to the classical phototransduction cascade, there is a second Rac2-dependent cascade in *Drosophila* photoreceptor cells.

Several studies focusing on vertebrate rhodopsin indicate that a similar non-classical phototransduction pathway exists [63]. Mammalian Rac1, which is the homolog of fly Rac2, associates with mammalian rhodopsin and is activated by light. These findings raise the possibility that such as a cascade may also participate in Arr2 translocation in rod photoreceptor cells.

The effector proteins that couple with Rac2 in fly photoreceptor cells are not known. However, one candidate is phospholipase D (PLD), an enzyme that can be controlled by small G proteins [64]. G-protein coupled receptors of the rhodopsin family can activate PLD. Furthermore in bovine retinas the small GTPase RhoA has been reported to regulate PLD activity in a light-dependent manner [65], and the *Drosophila* genome encodes a PLD that appears to contribute to phototransduction [66].

The light-dependent movement of TRPL out of the rhabdomeres occurs in a two-step process. During the first stage, which occurs over the course of a few minutes, TRPL translocates to the apical region of the plasma membrane just outside the rhabdomeres (stalk membrane) [59]. The second stage proceeds for several hours and results in a redistribution of TRPL over the basolateral membrane of the photoreceptor cells. NORPA is required for the first stage, while the entire phototransduction cascade, including activation of the TRP

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channel participates in the second stage [59]. In addition, two small GTPases, Rab5 and RabX4 [67], as well as both the N- and C-terminal regions of TRPL appear to contribute to internalization of TRPL [68]. However, the proteins that bind to these domains and promote the translocation of TRPL are not known.

A controversial issue is whether the NINAC (Neither Inactivation Nor Afterpotential C) myosin III participates in dynamic movements of signaling proteins. Three independent studies have found that NINAC participates in the translocation of Gq, TRPL and Arr2 [58, 62, 69]. However, another study has challenged this conclusion, at least with respect to the contribution of NINAC to shuttling of Arr2 [53]. NINAC has been reported to promote the light-dependent translocation of TRPL from the rhabdomeres to the cell bodies [62]. In the case of Gqα (Gα49B) [58] and Arr2 [69], NINAC may function in movements from the cell bodies to the rhabdomeres, although this occurs under opposite light conditions—dark and light, respectively. The interaction between NINAC and Arr2 is indirect, and may be mediated by interactions with the same vesicles, since both proteins bind phosphoinositides [55, 69].

If NINAC participates in the movements of signaling proteins, the question arises as to whether this activity is mediated by the motor activity of this myosin III. Human myosin III is a plus ended motor [70]. However, NINAC motor activity has not been demonstrated, despite considerable effort (e.g. Porter, Sellers and Montell, unpublished observations). The plus end of F-actin orients towards the distal tips of the rhabdomeres [71], which is consistent with a function for NINAC in the translocation of Gq and Arr2 into the rhabdomeres. However, an activity as a plus-ended motor is inconsistent with a role in the shuttling of TRPL from the rhabdomeres to the cell bodies. Thus, NINAC may not retain motor activity. Rather, it is possible that the cell body and rhabdomere-specific isoforms of NINAC (i.e. p132 and p174, respectively) [72, 73] might function in dynamic spatial redistributions of signaling proteins by passive association, and therefore act as a sink in one compartment or the other. If so, this would support the recent contention that the shuttling of signaling proteins is driven by diffusion, rather than by an active motor [60].

The INAD signalplex and redox modulation of signaling

Many of the proteins that function in *Drosophila* phototransduction are grouped in the rhabdomeres into a large macromolecular assembly referred to as the signalplex [74–78]. The central scaffold in the signalplex is INAD, a protein consisting of five tandem ~90 amino protein interaction modules referred to as PDZ domains. The core complex includes INAD and three target proteins—TRP, PLC (NORPA) and a protein kinase C (PKC) encoded by the *inaC* gene [79]. Loss of INAD results in instability of these three proteins and disrupts their localization in the rhabdomeres [74, 80]. The stability and spatial distribution of INAD is reciprocally dependent on TRP [79, 81]. Thus, TRP is required both as a cation channel and as a molecular anchor. The concentration of INAD also declines if it not bound to the NINAC myosin III, or if there are mutations in a Membrane Occupation and Recognition Nexus (MORN)-domain containing protein, Retinophilin, which is required for stability of NINAC [82].

INAD also binds to itself and forms an homooligomer through a PDZ/PDZ interaction interface distinct from the surface groove that functions in target binding [77]. Thus, INAD may couple an extensive array of TRP channels with the other proteins required for phototransduction. Several other proteins that might bind to INAD include calmodulin, TRPL and a fraction of the rhodopsin (Rh1) pool [74, 77]. These signaling proteins are not dependent on this interaction for stability or localization, and their interactions with INAD might be dynamic. However, in contrast to the members of the core complex, there is not a consensus that these latter proteins bind INAD [83].

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Two studies demonstrate that interactions of at least some of the signaling proteins that bind to INAD are dynamic, and are regulated by light-dependent conformational changes in PDZ5 [84, 85]. PDZ domains are β-barrel structures that contain a surface groove into which target proteins form hydrogen bonds [86]. PDZ5 in INAD is unusual in that two cysteines juxtaposed on either side of the surface groove form disulfide bonds, but only upon light stimulation (Figure 5a) [84]. Once generated, this precludes binding of target proteins. Under dark conditions, the disulfide bond is reduced, thereby creating a typical binding pocket [84].

The dynamic oxidation and reduction of the disulfide bonds in PDZ5 depend on interactions with the adjacent PDZ4 domain [85]. In the dark, the two PDZ domains interact stably (Figure 5b), thereby promoting the reduced state by increasing the redox potential of the disulfide bond by 330 mV. Upon exposure to light, PDZ4/PDZ5 coupling is disrupted, resulting in disulfide formation. It is proposed that light dissociates PDZ4 from PDZ5 due to the acidification generated by hydrolysis of PIP_2 [85].

The light-induced oxidation of PDZ has physiological consequences, since it prevents target binding. One such INAD binding protein is TRP, which interacts with both PDZ3 and PDZ5 through distinct sites in the channel. TRP binds to PDZ5 through a classical C-terminal PDZ binding motif, and to PDZ3 via a site near the C-terminus [79, 85]. It is proposed that in the dark, when TRP is bound to both PDZ domains, TRP is maximally sensitive to activation [85]. Upon light stimulation, the TRP/PDZ5 interaction is disrupted, which is proposed to lead to full dissociation of the TRP/INAD interaction since TRP binding to PDZ3 is too weak to maintain the association between the two proteins (Figure 5b). As a consequence, the sensitivity of TRP to activation declines. Thus, production of H^+ by PIP_2 might contribute to both activation and negative feedback regulation.

Conclusions and future perspectives

The discovery that light-dependent changes in redox potential affects dynamic interactions of TRP with PDZ5 raises a number of questions. Is there a disulfide isomerase that promotes the conformational switch? A protein with this predicted activity is enriched in the fly eye [87]. Since the kinetics of the oxidation of PDZ5 appears to be too slow to account for the rapid kinetics of signaling, the mechanisms that work in concert with this reaction to promote the speed of phototransduction remain to be determined. If light causes full dissociation of TRP from INAD, how is TRP maintained in the rhabdomeres during light stimulation? This is an issue since TRP depends on its interaction with INAD for localization in the rhabdomeres [74], and TRP remains in the rhabdomeres during prolonged light stimulation [56]. Furthermore, mutation of the C-terminal PDZ5 binding site in TRP causes mislocalization of TRP [79]. Perhaps wild-type TRP remains bound to PDZ3 after dissociation from PDZ5, or the C-terminus of TRP binds to an alternative PDZ domain in INAD after its interaction with PDZ5 is occluded by the light-induced oxidation of PDZ5.

The nexus between PIP_2 hydrolysis and activation of TRP and TRPL remains controversial. While it is an intriguing concept that a decline in inhibitory PIP_2 combined with a rise in H^+ might be the mechanism [43], a recent study argues that PUFAs gate TRPL [88], as proposed previously [47]. While there are many reports describing the biophysical properties of TRPL in heterologous expression system [89–94], until now there have been only three groups that have reported functional expression of TRP *in vitro* [90, 95, 96]. Apparently, this is due to difficulties in obtaining surface expression of TRP in expression systems. However, this obstacle may be reduced by the recent demonstration that a protein called XPORT augments the transport of TRP from the endoplasmic reticulum to the plasma membrane in tissue culture cells [97].

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The recent insights concerning the machinery involved in the light response in *Drosophila* photoreceptor cells have potential implications for the ipRGCs, and for the light sensitive cells in the iris of nocturnal and crepuscular mammals [9]. Are mammalian TRPC6/TRPC7 activated by PUFAs or a combination of a decline in PIP_2 and a rise in H^+? The existence of a *Drosophila* visual cycle [40] despite the bistability of the fly visual pigments suggests the possibility that the melanopsin in ipRGCs also depends on an enzymatic pathway for regenerating the chromophore. The melanopsin and PLCβ-dependent light response in the iris sphincter muscle cells would appear to function through a TRP channel, although TRPC channels and TRPV4 have been excluded [9]. Nevertheless, the discovery of melanopsin-initiated signaling in mammalian eyes indicates that the mechanism of light detection in flies and mammals have a common and ancient origin.

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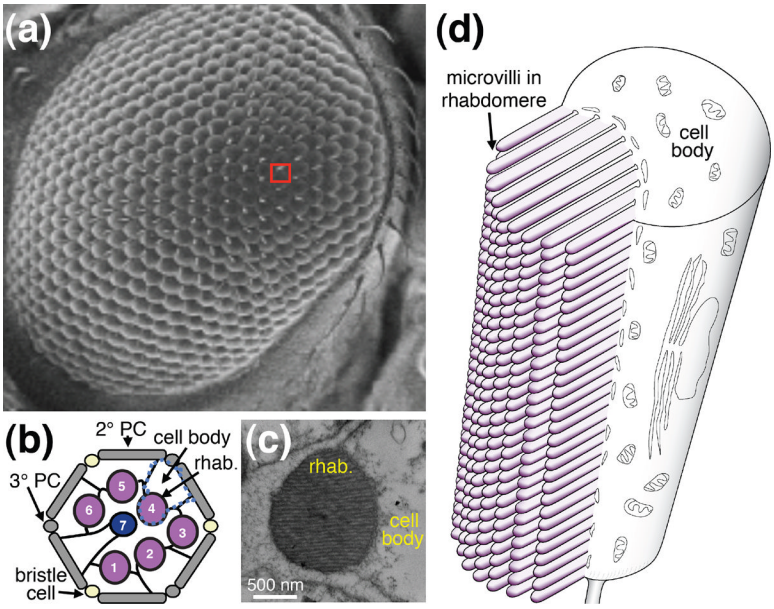

Figure 1.

Anatomy of *Drosophila* compound eye and photoreceptor cells. (a) Scanning electron microscope (EM) image of an adult compound eye. The eye contains ~750 – 800 ommatidia. The red box indicates one ommatidium. (b) Cartoon illustrating the various cell types in a cross-sectional view of an ommatidium (distal region). Seven photoreceptor cells, each containing a rhabdomere, are shown: bristle cell, mechanosensory bristle cell; cell body, photoreceptor cell body; rhab., rhabdomere; 2° PC, secondary retinal pigment cells; 3° PC, tertiary retinal pigment cells. The dashed blue line indicates a single photoreceptor cell. (c) Transmission EM cross-section through one photoreceptor cell. (d) Cartoon showing a longitudinal view of one photoreceptor cell. ~50,000 microvilli are present in the rhabdomeres of each R1-6 cell. The microvilli are not drawn to scale. Normally there are 30–35 (50 nm wide) microvilli per cross-section.

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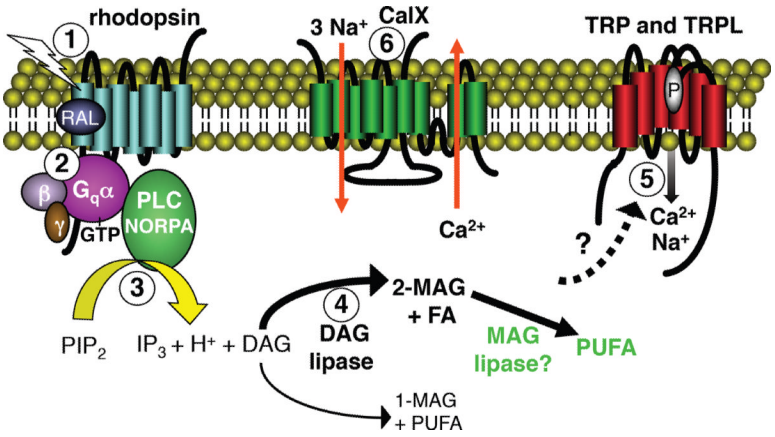

Figure 2.

Model of the *Drosophila* phototransduction cascade. Events in phototransduction: 1) Light-activates rhodopsin. 2) Coupling of light-activated rhodopsin with the heterotrimer Gq protein. The activated Gqα subunit associates with GTP. 3) Stimulation of PLC leads to hydrolysis of PIP_2 and production of IP_3, DAG and H^+. 4) A DAG lipase encoded by *inaE* hydrolyzes DAG to produce 2-MAG and FA. Minor products are 1-MAF and PUFA. The 2-MAG might be metabolized into PUFA by an unknown MAG lipase. 5) TRP and TRPL are activated following PLC stimulation, although the mechanism remains controversial. 6) Following activation of the channels, a Na^+/Ca^{2+} exchanger (CalX) extrudes Ca^{2+} out of the photoreceptor cell. Abbreviations: DAG, diacylglycerol; FA, saturated fatty acid; IP_3, inositol 1,4,5-trisphosphate; MAG, monoacylglycerol; PIP_2, phosphatidylinositol 4,5-bisphosphate;; P, pore loop indicated in TRP; PUFA, polyunsaturated fatty acid; RAL, the chromophore (3-OH-11-*cis*-retinal).

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(a)

rhodopsin	expression pattern	peak sensitivity rhodopsin (nm)	peak sensitivity metarhodopsin (nm)
Rh1	R1-R6	~486	~566
Rh2	ocelli	~418	~506
Rh3	R7 (~30%)	~331	~468
Rh4	R7 (~70%)	~355	~470
Rh5	R8 (~30%)	~442	~494
Rh6	R8 (~70%)	~515	~468
Rh7	?	?	?

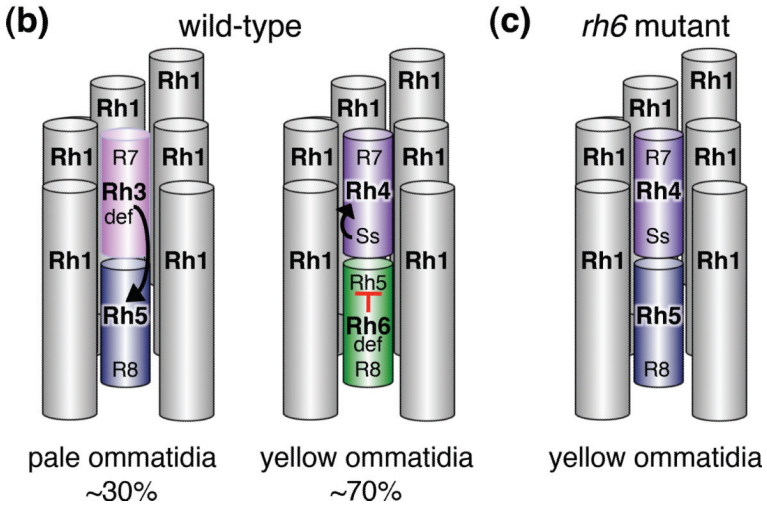

Figure 3.

Expression of *Drosophila* rhodopsins. (a) Five of the six characterized rhodopsins (Rh1 and Rh3 – Rh6) are expressed in photoreceptor cells in the compound eye. Rh2 is expressed in the ocelli. The peak light sensitivities of the non-activated (rhodopsin) and light activated pigments (metarhodopsin) are indicated. (b) Shown are the spatial distribution of Rh1 and Rh3 – Rh6 in the eight photoreceptor cells within an ommatidium (wild-type fly reared under a normal light/dark cycle). Rh1 is expressed in the R1-6 cells of all ommatidia. Rh3 – Rh6 are expressed in non-overlapping subsets of R7 and R8 cells as indicated. Yellow ommatidia normally express Rh4 and Rh6 in the R7 and R8 cells, respectively. Pale ommatidia express Rh3 and Rh5 in the R7 and R8 cells, respectively. Expression of Rh4 is induced by the Spineless (Ss) transcription factor [36]. If Ss is not expressed in a given Rh7 cell, then Rh3 is turned on as the default (def) state. Rh5 is induced in R8 cells that are below Rh3 expressed R7 cells. As a default, Rh6 is expressed in an R8 cell if it is not below an Rh7 cell that expresses Rh3. Expression of Rh6 in R8 cells inhibits expression of Rh5 in these cells. (c) Rh5 is turned on in nearly all yellow ommatidia of *rh6* mutant flies [37]. If wild-type flies are maintained constantly in the dark, some yellow ommatidia express low levels of Rh5 in addition to Rh6 [37].

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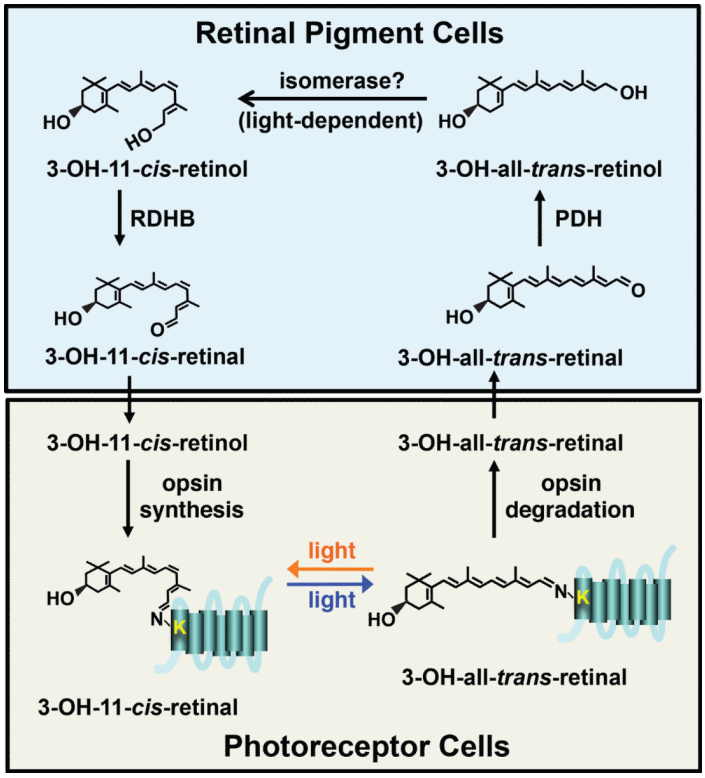

Figure 4.

Model of the visual cycle. Following light stimulation of rhodopsin, a proportion of the rhodopsin pool is internalized and degraded, thereby liberating the 3-OH-all-*trans*-retinal. The 3-OH-all-*trans*-retinal is then transported to primary and secondary retinal pigment cells where it is converted into 3-OH-11-*cis*-retinal through several enzymatic steps that depend on at least two retinal dehydrogenases, PDH (Pigment Cell Dehydrogenase) [40] and RDHB (Retinal Dehydrogenase B) [41], and a putative isomerase that has not been identified. The 3-OH-11-*cis*-retinal is then transported into the photoreceptor cells.

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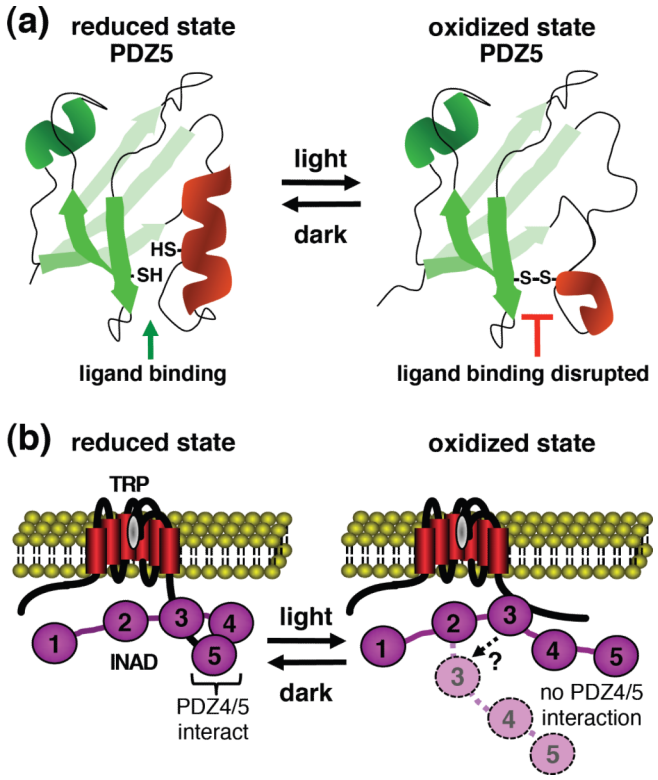

Figure 5.

Dynamic binding of TRP with INAD. (a) The two cysteines lining the surface groove in INAD PDZ5 are reduced in the dark [84, 98]. As a consequence, PDZ5 can bind to binding proteins, including the C-terminus of TRP [84]. Light results in oxidation of the two key cysteines in PDZ5, which precludes target binding. (b) In the dark, PDZ4 and PDZ5 interact, thereby promoting the reduced state in PDZ5 [85]. Under these conditions TRP binds to PDZ5 through the C-terminus, and to PDZ3 via a separate binding site near the C-terminus. Following light stimulation, the PDZ4/PDZ5 interaction is disrupted, leading to oxidation of PDZ5 [85]. This prevents binding of the TRP C-terminus to PDZ5. Since the affinity of the internal binding site in TRP to PDZ3 is weak, binding to PDZ3 may dissociate as a secondary consequence of the oxidation of PDZ5. However, the light-induced impairment of the TRP/PDZ3 interaction is speculative, and is therefore indicated by a question mark.