**Drosophila** photoreceptors and signaling mechanisms

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Fly eyes have been a useful biological system in which fundamental principles of sensory signaling have been elucidated. The physiological optics of the fly compound eye, which was discovered in the *Musca, Calliphora* and *Drosophila* flies, has been widely exploited in pioneering genetic and developmental studies. The detailed photochemical cycle of bistable photopigments has been elucidated in *Drosophila* using the genetic approach. Studies of *Drosophila* phototransduction using the genetic approach have led to the discovery of novel proteins crucial to many biological processes. A notable example is the discovery of the inactivation no afterpotential D scaffold protein, which binds the light-activated channel, its activator the phospholipase C and it regulator protein kinase C. An additional protein discovered in the *Drosophila* eye is the light-activated channel transient receptor potential (TRP), the founding member of the diverse and widely spread TRP channel superfamily. The fly eye has thus played a major role in the molecular identification of processes and proteins with prime importance.

**Keywords:** optics of compound eyes, bistable pigments, phosphorylated arrestin, G-protein, phospholipase C, TRP channels, phosphoinositide cycle, INAD scaffold protein

**INTRODUCTION**

Vision of invertebrate species has been one of the first senses to be thoroughly studied, and many fundamental principles relevant to all senses have been first discovered in invertebrate eyes. A notable example is the discovery of lateral inhibition in the compound eye of the *Limulus* by the Nobel Prize Laurie, Haldan Keffer Hartline (Ratliff, 1990). Surprisingly, invertebrate phototransduction, the process by which light quanta are translated into electrical signal is still not entirely understood in terms of its underlying molecular mechanism. The pioneering experiments, which exploited the size of giant photoreceptor cells in some invertebrate species like the *Limulus* (reviewed in Dorlochter and Stieve, 1997), were followed by studies on *Drosophila melanogaster*, exploiting its great molecular genetics power (reviewed in Minke and Hardie, 2000; Montell, 1989; Pak, 1995; Ranganathan et al., 1995). In the present review, we focus on processes and molecules that have been discovered in invertebrate eyes in general and in the *Drosophila* eye in particular, which shed light on crucial functions of other cells and tissues. These landmark discoveries include: (i) Structural and optical properties of *Diptera* compound eyes. (ii) Bistable photopigments in which both the rhodopsin (R) and metarhodopsin (M) states of the photopigment are dark stable and photoconvertible. (iii) The photochemical cycle in which phosphorylated arrestin (ARR) and ARR translocation play a major role. (iv) Light-induced translocation of Gα and the excess of Gβ over Gα, which prevents spontaneous activation of the Gq protein in the dark. (v) The dual role of light-activated phospholipase Cβ (PLCβ) in vivo as a G-protein-mediated activator and negative regulator of phototransduction via its action as a GTPase activating protein (GAP). (vi) Unitary signaling events (e.g. single photon responses, quantum bumps). (vii) The light-activated channels, TRP and TRPL, the founding members of the TRP superfamily channel proteins. (viii) Light-induced translocation of the TRPL channel. (ix) The inactivation no afterpotential D (INAD) multimolecular signaling complex, which binds the TRP channel, its activator, the PLC and its regulator, eye-specific protein kinase C (ePKC).

**STRUCTURAL AND OPTICAL PROPERTIES OF THE DIPTERA COMPOUND EYE**

**GENERAL ANATOMY**

Two distinct types of eyes have evolved through evolution; the lens eye (or camera eye) typically encountered in vertebrates, and the compound eye typically encountered in arthropods. Many insects encompass both types of eyes. While, the compound eye is the primary image forming organ, the ocelli lens eye is small and primitive (Kirschfeld and Franceschini, 1968, 1969). The compound eyes are composed of many repeat and well-organized units termed ommatidia (*Figure 1B*) embedded in a sphere (*Figure 1A*). The number of ommatidia in insects vary from just a handful in the primitive *Arachaeognatha* (jumping bristletails) and *Thysanura* (silverfish or bristletails) to several hundred up to thousands in *Diptera* (which includes the house fly *Musca* and the fruit fly *Drosophila*). In *Drosophila*, the ommatidium consists of about 20 cells, in which 8 (6–21 in different insect species) are the photoreceptor cells (RZ, *Figure 1A*). Each ommatidium contains a dioptic apparatus composed of transparent chitinous cuticle, which forms the cornea (C, *Figure 1A*) and an extracellular fluid-filled cavity, called the pseudocone (PC, *Figure 1A*). The floor of the cavity is formed by four
Semper cells (SZ, Figure 1A) and the walls by primary pigment cells (PZ, Figure 1A, red), which together circle the pseudocone, shielding the photoreceptor from stray light coming from adjacent ommatidia. The photoreceptor cells are highly polarized epithelial cells, with a specialized compartment known as the rhabdomere (Rh, Figure 1A), consisting of a stack of ∼30,000–50,000 microvilli each ∼2 µm long and ∼60 nm in diameter. The transduction machinery is located in these tightly dense structures, while the nucleus and cellular organelles (N, Figure 1C), such as submicrovillar cisternae (SMC, Figure 1C) reside in the cell body. Pioneering studies conducted by Franceschini and Kirschfeld in Diptera (mainly in Musca) have elucidated the remarkable optics of the compound eye. In their studies, they showed that the highly ordered rhabdomeres form light guides (Kirschfeld and Snyder, 1976) that have been widely exploited experimentally (Figure 3). For example, the screening for retinal degeneration mutants of Drosophila has used the optical phenomenon designated deep pseudopupil (dpp), by Franceschini and Kirschfeld (1971), that is associated with their light guide property (see Figure 3). The dpp, which disappears in retinal degeneration mutant flies such as in R defective mutants, has been used as an efficient tool for a fast screen of large populations of putative mutant flies. Other examples are spectral measurement of the compound eye such as the eye shine, resulting from tapetal reflection, transmittance spectra of photopigments and fluorescent measurements of M.

OPEN AND CLOSED RHABDOM

Two kinds of rhabdomere architecture exist: closed rhabdom, in which all rhabdomeres are fused at the center of the ommatidium (Figures 2A,C) and open rhabdom, in which the rhabdomeres are separated (Figures 2A,B), forming a polygon pattern depending on the number of photoreceptors (hexagonal in Drosophila). Each ommatidium is connected by axons to the ganglionic layers providing a single or several image elements of space, depending on the rhabdomere architecture (Figure 1A). In open rhabdomere, the repeated elements are arranged in a specific geometrical pattern and spacing, ensuring visual connectivity between adjacent ommatidia. Accordingly, the angles between the individual rhabdomeres in one ommatidium are identical to those between adjacent ommatidia. As a result, each of the seven rhabdomeres in one ommatidium portrays the same field of view as a rhabdomere in a neighboring ommatidium (Figures 2D,E; Kirschfeld, 1967).

In addition, all six rhabdomeres that share a common field of view send their axons to the same place in the first ganglionic layer – the lamina (La, Figures 1A and 2E). The central rhabdomeres send their axons to the second ganglionic layer – the medulla (Me, Figure 1A). In Drosophila, the seven rhabdomeres of each ommatidium are separated from each other and function as independent light guides (Figure 1D) forming open rhabdomere architecture (Figures 2A,B). In contrast, bees, beetles and various mosquitoes have a closed rhabdom architecture, in which rhabdomeres within each ommatidium are fused to each other, thus sharing the same visual axis (Figures 2A,C). Recently, the power of Drosophila genetics was exploited to elucidate the molecular factors participating in the transition between open and closed rhabdom architecture by screening, isolating and characterizing Drosophila genes involved in this process. The study identified two genes, spacemaker (spam) and prominin (prom) which when mutated cause the collapse of the intra-rhabdomere space (IRS; Figure 2B) resulting in the conversion of an open rhabdom system into a closed rhabdom architecture. Further analysis showed that SPAM is a secreted protein...
expressed in the IRS, which acts together with PROM, which is an evolutionary conserved transmembrane (TM) protein often associated with microvilli. Secretion of SPAM into the IRS forces the separation of the stalk membrane, pushing the rhabdomere apart, and the recruitment of SPAM to the microvilli surface by the binding to PROM prevents inter-rhabdomere adhesion. Furthermore, targeted expression of spam to photoreceptors of a closed system markedly reorganizes the architecture of the compound eyes to resemble an open system (Zelhof et al., 2006).

The unusual stiffness of SPAM has been exploited in mechanoreceptors of Drosophila. Accordingly, a recent study has demonstrated the involvement of SPAM in maintaining cell shape and tone, crucial for integrity of the mechanosensory neurons. The authors argued that for poikilothermic organisms, like insects, changes in temperature may impact the function of mechanoreceptor neurons. SPAM role was found as protective of mechanosensory organs from massive cellular deformation caused by heat-induced osmotic imbalance, by forming an extracellular shield that guards mechanosensory neurons from environmental insult (Cook et al., 2008).

FUNCTIONAL RETINAL ORGANIZATION
Drosophila ommatidia consist of eight photoreceptors that can be divided into two functional groups according to their position, functional involvement, spectral specificity and axonal projection. The R1–R6 cells (marked 1–6 in Figure 1B) represent the major class of photoreceptors in the retina and are involved in image formation and motion detection. These cells have peripherally located rhabdomeres extending from the basal to the apical side of the retina. They express a single opsin called Rh1, which when
combined with 11-cis 3-hydroxy retinal, forms a blue-absorbing R and orange-absorbing M. The R1–R6 cells (Figure 1B) project their axons to the first optic lobe, the lamina (La, Figure 1A green). The second group consists of two cells in the center of each ommatidium termed, R7 (marked 7 in Figure 1B) and R8 (located below R7) each spanning only half of the retina in length. The central cells R7 and R8 are involved in color vision and detection of polarized light and project their axons to the second optic lobe, the medulla (Me, Figure 1A, pink; Wernet et al., 2006).

Color vision requires comparison between the electrical signals of photoreceptors that are sensitive to different ranges of wavelengths of light. In *Drosophila*, this is achieved by the inner photoreceptors (R7 and R8) that contain different Rs. The R7 rhabdomere is located distally in the retina and expresses one of two opsins, Rh3 or Rh4, characterized by a UV-absorbing R and blue-absorbing M. The R8 rhabdomere is located proximally in the retina, beneath the R7 rhabdomere (not shown) and expresses one of three opsins, Rh3, Rh5 or Rh6, characterized by a UV-, blue- or green-absorbing R, respectively. On the basis of opsin expression in the R7 and R8 cells, three ommatidial subtypes can be distinguished. The R7 and R8 cells in ommatidia, residing in the dorsal rim area of the eye, which functions as a polarized light detector, both express Rh3 opsin. The “pale” ommatidia subtype express Rh3 in R7 cells and Rh5 in R8 cells and constitute ∼30% of the total ommatidia, while the “yellow” ommatidia subtype express Rh4 in R7 cells and Rh6 in R8 cells and constitute ∼70% of the total ommatidia. Two types of comparisons, required for color vision, can thus occur in the fly: between the R7 (UV sensitive) and R8 (blue or green sensitive) photoreceptor cells within one ommatidium or between different ommatidia that contain spectrally distinct inner photoreceptors (Wernet et al., 2006).

The intriguing repeated structure of fly compound eye has been a major scientific preparation for research of various aspects of cell differentiation and development. For example, in *Drosophila*, the hexagonal chiral orientation of the six rhabdomeres in the ommatidia is identical at the upper hemisphere of the compound eye and is reverted by 180° at the equator (Figure 1D). This phenomenon is generally referred to as the planar cell polarity (PCP) of a tissue, a mechanism that is reverted by 180° hexagonal chiral orientation of the six rhabdomeres in the ommatidia that functions as a polarized light detector, both express Rh3 opsin.

**THE PHOSPHOINOSITIDE CASCADE OF VISION**

The signaling proteins of the phototransduction cascade are tightly assembled in the microvillar structure and linked to the actin cytoskeleton (F-actin) via two proteins: Dmoesin, which binds the TRP and TRPL channels, at the base of the microvilli to F-actin (Chorna-Ornan et al., 2005), and no inactivation no afterpotential C (NINAC), that associates INAD to F-actin (Li et al., 1998). The only protein that diffuses through the phototransduction cascade is Gα (Figure 4).

Upon absorption of a photon, R is converted into the active state of the photopigment, M (Figure 4). This leads to the activation of heterotrimeric G-protein (DG₃) by promoting the guanosine diphosphate (GDP) to guanosine triphosphate (GTP) exchange. In turn, this leads to activation of PLCβ, which hydrolyzes the minor phospholipid, phosphatidylinositol 4,5-bisphosphate (PIP₂) into the soluble inositol 1,4,5-trisphosphate (InsP₃) and the membrane-bound diacylglycerol (DAG). Subsequently, two classes of light-sensitive channels, TRP that is highly permeable to Ca²⁺ and TRPL that is a non-selective cation channel, open by a still unknown mechanism. PLC also promotes hydrolysis of the bound GTP, resulting in Gα₃ binding to GDP and this ensures the termination of Gα₃ activity. The TRP and TRPL channel openings lead to elevation of calcium ions extruded by the Na⁺/Ca²⁺ exchanger CALX. Elevation of DAG and Ca²⁺ promote ePKC activity, which regulates channel activity. PLC, ePKC and the TRP ion channel form a supramolecular complex with the scaffolding protein INAD (for reviews on the phototransduction cascade see Hardie and Raghu, 2001; Minke and Cook, 2002; Montell, 1989).

**UNITARY EVENTS**

Dim light stimulation induces discrete voltage (or current) fluctuations in most invertebrate species, which are called quantum bumps (Yeandle and Spiegler, 1973; see Figure 5A). Each bump is assumed to be evoked by the absorption of a single photon. The
Discrete nature of the unitary events of the photoreceptor cells is not due to the quantized nature of light. This has been demonstrated by the application of a non-quantized stimulus such as GTPγS, which elicit quantum bump-like events (Fein and Corson, 1981). The bumps vary in latency, time course and amplitude for identical stimulation and are the consequence of synchronized activation of many light-sensitive channels. The number of channels, which are activated to produce a bump vary greatly in different species: few tens in *Drosophila* and up to several thousands in *Limulus* ventral photoreceptors (Nasi et al., 2000). Bump generation is a stochastic process described by Poisson statistics where each effective absorbed photon elicits only one bump (Yeandle and Spiegler, 1973). However, the photoconversion leads to the activation of heterotrimeric G-protein (Gqα) by promoting the GDP to GTP exchange. In turn, this leads to activation of phospholipase Cβ (PLCβ), which hydrolyzes PIP2 into the soluble InsP3 and the membrane-bound DAG. Subsequently, two classes of light-sensitive channels, the TRP and TRPL open by a still unknown mechanism. PLC also promotes hydrolysis of the bound GTP, resulting in Gqα bound to GDP and this ensures the termination of Gqα activity. The TRP and TRPL channel openings lead to elevation of cellular Ca2+. Elevation of DAG and Ca2+ promote eye-specific protein kinases C activity, which regulates channel activity. PLC, PKC and the TRP ion channel form a supramolecular complex with the scaffolding protein INAD.

**FIGURE 4** | Schematic representation of the molecular components of the signal transduction cascade of *Drosophila*. Upon absorption of a photon, rhodopsin (R) is converted into metarhodopsin (M). This photoconversion leads to the activation of heterotrimeric G-protein (Gqα) by promoting the GDP to GTP exchange. In turn, this leads to activation of phospholipase Cβ (PLCβ), which hydrolyzes PIP2 into the soluble InsP3 and the membrane-bound DAG. Subsequently, two classes of light-sensitive channels, the TRP and TRPL open by a still unknown mechanism. PLC also promotes hydrolysis of the bound GTP, resulting in Gqα bound to GDP and this ensures the termination of Gqα activity. The TRP and TRPL channel openings lead to elevation of cellular Ca2+. Elevation of DAG and Ca2+ promote eye-specific protein kinases C activity, which regulates channel activity. PLC, PKC and the TRP ion channel form a supramolecular complex with the scaffolding protein INAD.

**FIGURE 5** | Slow response termination in *arr2* and *ninaC* null mutants. (A–C) Upper panels: Whole-cell voltage clamp recordings of quantum bumps in response to brief (1 ms) dim flashes of light with intensity sufficient to activate only a single rhodopsin molecule upon photon absorption in wild-type (WT), *arr2* and *ninaC* null *Drosophila* mutant flies. In WT, only a single bump is induced by a single flash and some flashes do not elicit any bump (middle trace). In contrary, a single flash in *arr2* and *ninaC* mutant flies elicits a train of bumps. (A–C) Lower panels: Whole-cell voltage clamp recordings of normalized macroscopic responses of WT and the corresponding mutants in response to 500-ms light pulses. A slow termination of macroscopic response is observed in *arr2* and *ninaC* mutant flies relative to WT.
in at least two Drosophila mutants (ninaC and arr, see Figure 5), absorption of a single photon elicits a train of bumps which do not overlap but are separated by intervals (Figures 5B,C). This train of bumps is thought to be caused by a failed R inactivation process and a refractory period of the microvilli (Scott et al., 1997).

A detailed study in Limulus photoreceptors has indicated that the latency of the bump is not correlated with the bump waveform, thus strongly suggesting that the triggering mechanism of the bump arises from different molecular processes than those determining the bump waveform (Dorlochter and Stieve, 1997). These findings are partly explained by models in which the amplification process is preceded by a series of non-amplifying latency producing steps. To produce realistic bumps by such a model means that no step in the transduction cascade could have a life time greater than the duration of a bump generating mechanism which includes the latency, bump duration and bump refractory period. The single photon-single bump relationship requires that each step in the cascade must have not only an efficient “turn-on” mechanism, but also an equally effective “turn-off” mechanism (see below). The functional advantage of such a transduction mechanism is obvious; it produces a sensitive photon counter, very well suited for both the sensitivity and the temporal resolution required by the visual system.

A recent study has presented a quantitative model explaining how bumps emerge from stochastic non-linear dynamics of the signaling cascade. Three essential “modules” govern the production of bumps in this model: (i) an “activation module” downstream of PLC but upstream of the channels, (ii) a “bump-generation module” including channels and Ca^{2+}-mediated positive feedback and (iii) a Ca^{2+}-dependent “negative-feedback module”. The model shows that the cascade acts as an “integrate and fire” device conjectured formerly by Henderson et al. (2000) much like the generation of spikes. The model explains both the reliability of bump formation and low background noise in the dark and is able to capture mutant bump behavior and explains the dependence on external calcium, which controls feedback regulation (Pumir et al., 2008).

THE PHOTOCHEMICAL CYCLE: THE “TURN-ON” AND “TURN-OFF” OF THE PHOTOPIGMENT BISTABLE PIGMENTS

The G-protein-coupled receptor (GPCR), R, is composed of a 7-TM protein, opsin and the chromophore, 11-cis 3 hydroxy retinal (in Diptera; Vogt and Kirschfeld, 1984). Isomerization of the chromophore by photon absorption induces conformational change in the opsin, which is photoconverted into the dark stable physiologically active photoproduct, M. The action spectrum of this reaction depends on the R type (see above) and spans a wavelength range between UV and green lights. To ensure high sensitivity, high temporal resolution and low dark noise of the photoreponse, the active M has to be quickly inactivated and recycled (Figure 6). The latter requirement is achieved, in invertebrates, by two means: the

![FIGURE 6](The photochemical cycle: the “turn-on” and “turn-off” of the photopigment. Upon photoconversion of rhodopsin (R) to metarhodopsin (M), by illuminating with blue light (wavy blue arrow), M is phosphorylated at multiple sites by rhodopsin kinase and the fly ARR2 binds to phosphorylated M. ARR2 is then phosphorylated by Ca^{2+} calmodulin-dependent kinase (CaMKII). Photoconversion of phosphorylated M (M_{pp}) back to phosphorylated R (R_{pp}) is achieved by illuminating with orange light (wavy red arrow). Upon photoregeneration of M_{pp} to R_{pp}, phosphorylated ARR2 is released and the phosphorylated rhodopsin (R_{pp}) is exposed to phosphatase activity by rhodopsin phosphatase (encoded by the rdgC gene). Unphosphorylated ARR2 also binds to myosin III (ninaC) in a Ca^{2+}-calmodulin (Ca-CaM)-dependent manner (modified from Liu et al., 2008; Selinger et al., 1993).)
absorption of an additional photon by the dark stable M, which photoconverts M back to R (Hillman et al., 1972, 1983), or by a multistep photochemical cycle (Figure 6). The action spectrum of M to R conversion in the R1–R6 cells of Drosophila is in the orange range. The red screening pigment of the Drosophila eye prevents massive conversion of R to M, by formation of a red filter, which is preferential for M to R conversion. Genetic removal of the red screening pigment and application of blue light (which is preferentially absorbed by the R state) enables a large net photoconversion of R to its dark stable photoprodut M with a minimal conversion of M back to R (Figure 6). A large net photoconversion of R to M, prevents transduction termination at the photopigment level when light is turned off (Minke et al., 1975a). This is because the net photoconversion of R to M exceeds the amount of ARR (see below) and thereby its ability to inactivate M, resulting in a large amount of dark stable M, which does not undergo inactivation and thus remains physiologically active in the dark (Byk et al., 1993; Dolph et al., 1993). This brings the capacity of the phototransduction process to its upper limit and results in a phenomenon called prolonged depolarizing afterpotential (PDA; Hillman et al., 1972, 1983). Illumination with red light photoconverts M back to R and terminates the PDA after the light is turned off. The PDA protocol has been used efficiently to screen for phototransduction defective Drosophila mutants (Pak, 1995) and has been widely exploited in studies of Drosophila phototransduction.

THE ROLE OF ARRESTIN IN PHOTON ACTIVATION

The AR family of proteins plays a key role in regulating the activity of GPCRs (Violin and Lefkowitz, 2007). In Drosophila, two homologues of vertebrate ARR exist, which participate through binding, in M inactivation. Both ARRs undergo light-dependent phosphorylation by Ca" calmodulin-dependent kinase II (CaMKII) originally discovered by Matsumoto (Kahn and Matsumoto, 1997). This phosphorylation is unique to the invertebrate visual ARRs and crucial for ARR dissociation from M (Alloway and Dolph, 1999; Kiselev et al., 2000; Yamada et al., 1990).

The study, which clarified the regulatory role of ARR2, used in vitro assays of ARR2 and M, in Drosophila and Musca eyes. Upon photoconversion of R to M, by illumination with blue light (wavy blue arrow, Figure 6), the fly ARR2 is found predominantly in the membrane fraction, while photoconversion of phosphorylated M (Mpp) back to phosphorylated R (Rpp), by illumination with orange light (wavy red arrow), result in the detection of ARR2 in the supernatant fraction (cytosol). ARR1 on the other hand, always remains membrane bound. The in vitro studies indicated that the functional role of ARR2 binding to M is to terminate its activity (Byk et al., 1993). The isolation of Drosophila mutant fly arrestin2 (arr2), enabled demonstrating the physiological effect, in vivo, of ARR2 on the light response (Dolph et al., 1993). Accordingly, these flies showed a slow response termination at the macroscopic level (Figure 5B). Further investigations have shown that single photon absorption in these flies results in a train of quantum bumps while in wild-type flies it elicits a single bump (Figure 5B). The train of bumps is a manifestation of the M’s incapability to inactivate, and explains the slow response termination seen at the macroscopic level (Scott et al., 1997). Moreover, under the assumption that each bump is produced in a single microvillus, the train of bumps separated by intervals suggests a possible inactivation process of the microvilli (Hardie and Raghu, 2001).

The binding of ARR2 also protects the Mpp from phosphatase activity (Figure 6). Only upon photoregeneration of Mpp to Rpp, is ARR2 released and the Rpp is exposed to phosphatase activity by rhodopsin phosphatase, encoded by the rdgC gene (Steele et al., 1992). These combined actions are crucial for preventing reinitiating of phototransduction in the dark, as the dissociation of ARR2 is coupled to conversion of Mpp to Rpp, thereby directing the protein phosphatase only towards the inactive Rpp (Byk et al., 1993). Subsequent studies have revealed that both CaMKII-dependent phosphorylation of ARR2 at Ser366 and photoconversion of Mpp are required to release phosphorylated ARR2. They furthermore showed that the phosphorylation of ARR2 is required for its dissociation from Mpp upon photoconversion and that ARR2 phosphorylation prevents endocytotic internalization of the ARR2-Mpp complex by a clathrin-mediated mechanism (Alloway and Dolph, 1999; Alloway et al., 2000; Kiselev et al., 2000).

Upon illumination, ARR2 translocates from the cell body to the rhabdomere, thereby elevating its concentration in the signaling compartment (Byk et al., 1993). This process enables the ARR2-dependent inactivation of M, operating in massive photoconversion of R to M in bright daylight, thus preventing response saturation and ensures sufficient time resolution of the light response. A further study has shown that ARR2 translocation requires a phosphoinoside-mediated interaction with myosin III (NINAC; Lee and Montell, 2004). Interestingly, the electrophysiological phenotype of the ninaC mutant is similar to that of arr2 mutant (Figures 5B,C) and may be the consequence of reduced ARR2 concentration in the rhabdomere caused by the ninaC mutation. A recent study suggests that under low Ca" conditions, ARR2 binding to M is slowed down by its sequestration to NINAC. Accordingly, in physiological conditions, light-induced Ca" influx acting via CaM (Ca-CaM), rapidly releases ARR2 from NINAC and allows its binding to M and consequently, M inactivation (Lee et al., 2008; Figure 6).

LIGHT-ACTIVATED G Protein: The Roles of Gα and Gβ

It has been well established in photoreceptors of several invertebrate species that photoexcited R activates a heterotrimeric G-protein (Fein, 1986). The first experiments, conducted on fly photoreceptors, showed that when pharmacological agents, known to activate G-proteins, are applied to Musca photoreceptors in the dark, they mimic the light-dependent activation of the photoreceptor cells (Minke and Stephenson, 1985). Later studies using genetic screens isolated two genes encoding visual specific G-protein subunits. These genes, dsg (Lee et al., 1990) and gβec (Dolph et al., 1994), encode a Gα and Gβ subunit, respectively. The isolated eye-specific DG α shows ~75% identity to mouse Gα, which is known to activate PLC (Lee et al., 1990). The most direct demonstration that DG α participates in the phototransduction cascade came from studies of mutants defective in Gα which showed highly reduced sensitivity to light. In the isolated GagI’ mutant, DG α protein levels are reduced to ~1%, while Gβ, PLC and R protein levels are virtually normal. The GagI’ mutant exhibits a ~1000-fold reduced sensitivity to light and slow response termination (Scott et al., 1995), strongly suggesting that there is no parallel pathway mediated by the G-protein, as proposed for the
Limonus eye (Dorlochter and Stieve, 1997). Manipulations of the DGα protein levels by the inducible heat-shock promoter made it possible to show a strong correlation between the sensitivity to light and DGα protein levels, further establishing its major role in Drosophila phototransduction (Scott et al., 1995).

The Drosophila fly has an eye-specific Gβ (Gβ2) which shares 50% amino acid identity with other Gβ homologue proteins. Two defective Gβ (GBβ1 and GBβ2) mutants with highly reduced Gβ levels were isolated and showed a greatly (~100-fold) decreased sensitivity to light and slow response termination (Dolph et al., 1994). Studies conducted on these mutants revealed that Gα is dependent on Gβγ for both membrane attachment and targeting to the rhabdomere, suggesting that the decreased light sensitivity of these mutants may result from the mislocalization of the Gα subunit (Elia et al., 2005). Attachment of Gα to Gβγ prevents spontaneous GDP-GTP exchange and anchors Gα to the plasma membrane. Therefore, in Gβ mutants Gα concentration is highly reduced in the rhabdomere (Figures 7A,B). Analysis of the stoichiometry between the Gα and Gβ subunits revealed a twofold excess of Gβ over Gα. Genetic elimination of the Gβ excess leads to spontaneous activation of the visual cascade in the dark, demonstrating that Gβ excess is essential for the suppression of dark electrical activity produced by spontaneous GDP-GTP exchange of Gα. Reestablishing the excess of Gβ over Gα by a double heterozygote mutant fly, suppresses the dark electrical activity (Elia et al., 2005; Figures 7C bottom trace and D). These studies show a dual role for Gβ: retention of Gα in the signaling membrane and prevention of spontaneous activation of Gα in the dark.

Heterotrimeric G-proteins relay signals between membrane-bound receptors and downstream effectors. Little is known, however, about the regulation of Gα subunit localization within the natural endogenous environment of a specialized signaling cell. Studies using Drosophila flies showed that prolonged lights cause massive and reversible translocation of Gα to the cytosol (Kosloff et al., 2003), in similar manner to light-induced translocation of the vertebrate Gα transducin (Arshavsky, 2003; Trojan et al., 2008). A long exposure to light followed by minutes of darkness resulted in reduction in the efficiency with which each absorbed photon elicited single photon responses, while the size and shape of each single photon response did not change. To dissect the physiological significance of Gα translocation by light, a series of Drosophila mutants were used. Genetic dissection showed a pivotal role for light-induced translocation of Gα from the signaling membrane and the cytosol. Biochemical studies revealed that the sensitivity to light depends on the membrane Gα concentration, which can be modulated either by light or by mutations that impair its membrane targeting. Thus, long-term adaptation is mediated by the movement of Gα from the signaling membrane to the cytosol, thereby reducing the probability of each photon to elicit a bump (Frechter et al., 2007).

DUAL ROLE FOR LIGHT-ACTIVATED PLC

PLC ROLE IN LIGHT EXCITATION

Evidence for a light-dependent Gα-mediated PLC activity in fly photoreceptors came from combined biochemical and electrophysiological experiments. These experiments, conducted in membrane preparations and intact Musca and Drosophila eyes, showed illumination and Gα-dependent accumulation of InsP3 and InsP2, derived from PIP2 hydrolysis by PLC (Devary et al., 1987; Figure 4).

The key evidence for the participation of PLC in visual excitation of the fly was achieved by the isolation and analysis of Drosophila PLC gene, designated no receptor potential A (norpA). The norpA gene encodes a β-class PLC, predominately expressed in the rhabdomeres. Mutant flies in the norpA gene show a drastically reduced receptor potential. Transgenic Drosophila, carrying the norpA gene on a null norpA background, rescued the transformant flies from all the physiological, biochemical and morphological defects, which are associated with the norpA mutants (Bloomquist et al., 1988). The norpA mutant thus provides essential evidence for the critical role of inositol-lipid signaling in phototransduction, by showing that no excitation takes place in the absence of functional PLC (Bloomquist et al., 1988; Minke and Selinger, 1992). However, the events required for light excitation downstream of PLC activation remain unresolved.

PLC ROLE IN RESPONSE TERMINATION

In general, the cytoplasmic GTP concentration in cells is much higher than GDP, making the inactivation process of Gα by hydrolysis of Gα-GTP to Gα-GDP unfavorable. In order to accelerate the GTPase reaction and terminate Gα activity, a specific GAP exists (Mukhopadhyay and Ross, 1999). In vitro studies of mammalian PLC-β1 reconstituted into phospholipid vesicles with recombinant M1 muscarinic receptor and Gq/11 (Berstein et al., 1992) have shown that, upon receptor stimulation, the addition of PLC-β1 increases the rate at which Gα hydrolyses GTSP by three orders of magnitude, suggesting its action as GAP. A reduction in the levels of PLC in mutant flies affects the amplitude and activation kinetics of the light response (Pearn et al., 1996), but also mysteriously slows response termination (compare Figure 8A to Figure 8B, lower panels). Biochemical and physiological studies conducted in Drosophila have revealed the requirement for PLC in the induction of GAP activity in vivo. Using several Drosophila norpA mutant flies, a high correlation between PLC protein level, GAP activity and response termination was observed (Cook et al., 2000). The virtually complete dependence of GAP activity on PLC provides an efficient mechanism for ensuring the one photon, one bump relationship (Yeandle and Spiegler, 1973), which is critical for the fidelity of phototransduction in dim light. The apparent inability to hydrolyze GTP without PLC ensures that every activated G-protein eventually encounters a PLC molecule and thereby produces a response by the downstream mechanisms. The instantaneous inactivation of the G-protein by its target, the PLC, guarantees that every G-protein produces no more than one bump (Cook et al., 2000). This apparently complete dependence of GTPase activity on its activator PLC, in flies, differs from the partial dependence of GAP factors in vertebrate phototransduction (Chen et al., 2000). Vertebrate phototransduction depends on specific GAPs (Arshavsky and Pugh-EN, 1998; Makino et al., 1999). Accordingly, genetic elimination of regulators of G-protein signaling (RGS) proteins reduces and slows down GAP activity and leads to slow response termination to light (Chen et al., 2000).

The dual action of PLC as an activator and a negative regulator nicely accounts for all features of the PLC-deficient mutants. A
striking demonstration of the poor temporal resolution of mutants with reduced PLC levels relative to wild-type flies is shown in Figure 8C, which compares the ability of wild type and the PLC-deficient mutant norpA\textsuperscript{76} to discriminate between intense lights of different durations (flash, red arrow, pulse, blue line). In contrast to the wild-type fly, where there is a pronounced difference between the responses to a flash compared with a long stimulus, no such difference is observed in the norpA mutants, where the two responses overlap (Figure 8C). This result indicates that the PLC-deficient mutants cannot discriminate between long and short light stimuli. When PLC levels in the signaling membranes are low relative to the amount of the active G-protein, light induces production of G\textsubscript{q}\textalpha-GTP at a higher rate than it is inactivated by PLC. The G\textsubscript{q}\textalpha-GTP that has accumulated during illumination continues to produce bumps in the dark until all active G\textsubscript{q}\textalpha-GTP molecules are hydrolyzed via GAP activity of the scarce PLC (Figure 8B, lower panel, inset). Hence, the flies’ temporal resolution is reduced and become virtually blind at low levels of PLC (Cook et al., 2000).
THE PHOSPHOINOSITIDE (PI) CYCLE
In the phototransduction cascade of Drosophila, light triggers the activation of PLCβ. This catalyzes hydrolysis of the membrane phospholipid PIP2 into water soluble InsP3 and membrane-bound DAG (Berridge, 1993). The continuous functionality of the photoreceptors during illumination is maintained by rapid regeneration of PIP2 in a cyclic enzymatic pathway (the PI pathway, Figure 9). Moreover, the PI pathway has emerged to be most important for activation of the TRP and TRPL channels (Hardie, 2003; Raghu and Hardie, 2009).

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**Figure 8** | Slow response termination composed of bumps characterizes norpA mutants. (A, B) Upper panels: Whole-cell voltage clamp recordings of quantum bumps in response to continuous dim light in wild-type and the weak allele of norpA, norpAP57 mutant flies. (A, B) Lower panels: Whole-cell voltage clamp recordings of normalized macroscopic responses of wild-type and the corresponding mutants in response to 200-ms light pulses. In contrast to the fast response termination of wild-type, slow termination of the light response of norpAP57 mutant flies is revealed.

This slow response termination can be resolved into continuous production of bumps in the dark at a later time (inset, at higher magnification).

**Figure 9** | The phosphoinositide cycle. In the phototransduction cascade, light triggers the activation of phospholipase Cβ (PLCβ). This catalyzes hydrolysis of the membrane phospholipid PIP2 into water soluble InsP3 and membrane-bound DAG (Berridge, 1993). DAG is transported by endocytosis to the endoplasmic reticulum and inactivated by phosphorylation converting it into phosphatidic acid (PA) via DAG kinase (DGK) and to CDP-DAG via CDP-DAG syntase. Subsequently, CDP-DAG is converted into phosphatidylcholine (PC) by phospholipase D (PLD). DAG is also hydrolyzed by DAG lipase into polyunsaturated fatty acids (PUFA).
The phospholipid branch of the PI cycle, following PLC activation, begins by DAG transport through endocytosis to the endoplasmic reticulum (SMC) and subsequently, inactivation by phosphorylation and conversion into phosphatidic acid (PA), via DAG kinase (DGK), encoded by the retinal degeneration A (rdgA) gene (Masai et al., 1993, 1997). Then, CDP-DAG syntase encoded by the cds gene (Wu et al., 1995) produces DAG-CDP from PA. Both RDGA and CDS are located in the SMC (Figure 1C). Subsequently, DAG-CDP is converted into phosphatidylchinositol (PI), which is transferred back to the microvillar membrane, by the PI transfer protein (PITP), encoded by the rdgB gene (Vihitelic et al., 1991) located in the SMC. PIP and PIP2 are produced at the microvillar membrane by PI kinase and PIP kinase, respectively. PA can be reconverted back to DAG by lipid phosphate phosphohydrolase, LPP, also designated phosphatidic acid phosphatase, PAP, encoded by the laza gene (Garcia-Murillas et al., 2006; Kwon and Montell, 2006) or produced from phosphatidylcholine (PC) by phospholipase D, PLD, encoded by the Pld gene (LaLonde et al., 2005). DAG is also hydrolyzed by DAG lipase encoded by the inaE gene (Leung et al., 2008) predominantly localized outside the rhabdomeres, into polyunsaturated fatty acid (PUFA, Figure 9).

Mutations in most proteins of the PI pathway result in retinal degeneration. For example, rdgA mutant flies show light-independent retinal degeneration, thought to occur due to a sustained Ca2+ influx through the light-activated TRP and TRPL channels, making the PI pathway crucial for understanding phototransduction and TRP channels activation. Although it is possible to partially rescue the degeneration phenotypes by reducing the level of TRP (Raghu et al., 2000b), it is still unclear whether this mutation promotes channel opening directly or through an indirect change in the photoreceptor, leading to channel opening.

THE LIGHT-ACTIVATED CHANNELS, TRP AND TRPL, THE FOUNDING MEMBERS OF THE TRP SUPERFAMILY

THE trp MUTANT AND THE DISCOVERY OF THE TRP CHANNEL

A spontaneously occurring Drosophila mutant, showing a decline in the receptor potential to baseline during prolonged illumination (Cossens and Manning, 1969), was designated transient receptor potential (trp) by Minke et al. (1975b) (Figure 10B, right). Minke and Selinger suggested in a review article, that the trp gene encodes a Ca2+ channel/transporter, mainly because application of the Ca2+ channel blocker La3+ to wild-type photoreceptors mimicked the trp phenotype (Minke and Selinger, 1991). The cloning of the trp locus by Montell and Rubin (1989) revealed a novel membrane protein. The available sequence of the trp gene led, several years later, to the discovery of mammalian TRPs and the TRP superfamily (Wes et al., 1995; Zhu et al., 1995). However, the significance of the trp sequence, as a gene encoding a putative channel protein, was only first appreciated after a trp homolog, the trp-like (trpl) gene was cloned. This was done by a screen for calmodulin-binding proteins which identified a TM protein. A comparison of its TM domain to that of voltage gated Ca2+ channels and the TRP protein led to the conclusion that this protein is a putative channel protein with high identity to TRP (Phillips et al., 1992). The first direct physiological evidence for the notion that TRP is the major light-activated channel came from a comparative patch clamp study of isolated ommatidia of wild type and the trp mutant (Hardie and Minke, 1992). The use of Ca2+ indicator dyes and Ca2+-selective microelectrodes, directly demonstrated that the TRP channel is the major route for Ca2+ entry into the photoreceptor cell (Peretz et al., 1994a,b). The final evidence showing that TRP and TRPL are the light-activated channels came from the isolation of a null mutant of the trpl gene and the construction of the double mutant, trpl;trp, which is blind (Niemeyer et al., 1996). A third TRP homolog channel designated TRPγ has been cloned and sequenced (Xu et al., 2000). Heterologous expression in HEK293 cells has revealed a functional channel (Jors et al., 2006; Xu et al., 2000). However, in Drosophila photoreceptors this channel cannot generate any light-activated conductance in isolation as revealed in the trpl;trp double null mutant and therefore its role in phototransduction, if any, is not clear.

BIOPHYSICAL PROPERTIES OF THE TRP AND TRPL CHANNELS

The Drosophila light-sensitive channels, TRP and TRPL, can be studied separately by utilizing the trp<sup>α6</sup> and trpl<sup>α4</sup> null mutants, respectively (Scott et al., 1997, Figure 10). The channels are permeable to a variety of monovalent and divalent ions including Na+, K+, Ca2+ and Mg2+ and even to large organic cations such as TRIS and TEA (Ranganathan et al., 1991). The reversal potential of the light-induced current (LIC) shows a marked dependence on extracellular Ca2+ indicating a high permeability for this ion. Permeability ratio measurement for a variety of divalent and monovalent ions, determined under bi-ionic conditions, confirmed a high Ca2+ permeability of 57:1 = Ca<sup>2+</sup>:Cs<sup>+</sup> in the trpl mutant and 4.3:1 = Ca<sup>2+</sup>:Cs<sup>+</sup> for the trp mutant (Reuss et al., 1997). The large Ca2+ permeability of TRP is reflected in its positive reversal potential (E<sub>rev</sub>; Figures 10C,D).

The TRP and TRPL channels show voltage-dependent conductance during illumination. An early study revealed that the light response can be blocked by physiological concentrations of Mg2+ ions (Hardie and Mojet, 1995). The block mainly influenced the TRP channel and affected its voltage dependence. Later, detailed analyses described the voltage dependence of heterologously expressed TRPL channels in S2 cells and of the native TRPL channels, using the Drosophila trp null mutant. These studies indicated that the voltage dependence of the TRPL channel is not an intrinsic property, as is thought for some other members of the TRP family, but arises from divalent cations open channel block that can be removed by depolarization. The open channel block by divalent cations is thought to play a role in improving the signal to noise ratio of the response to intense light and may function in light adaptation and response termination (Parnas et al., 2007).

A comparison with voltage-gated K+ channels and cyclic nucleotide gated (CNG) channels, postulates that both TRP and TRPL are assembled as tetrameric channels, thus raising the question whether they assemble as homomultimers or as heteromultimers. Since null trp and trpl mutants both respond to light, each can clearly function without the other. However, heterologous co-expression studies and co-immunoprecipitation, led to the suggestion that the TRP and TRPL channels can assemble into heteromultimers (Xu et al., 1997). Detailed
FIGURE 10 | The electrophysiological properties of WT, trp and trpl mutants.
(A) Whole-cell voltage clamp recordings of quantum bumps in response to continuous dim light in wild-type, trp<sup>302</sup> and trp<sup>P343</sup> null mutant flies. Highly reduced amplitude of trp<sup>P343</sup> bumps is observed.
(B) Whole-cell voltage clamp recordings in response to a 3-s light pulse of WT and the corresponding mutants. The transient response of the trp<sup>P343</sup> mutant is observed. (C) A family of light-induced currents to 20-ms light pulse at voltage steps of 3 mV measured around E<sub>rev</sub>. (D) Histogram plotting the mean E<sub>rev</sub> of WT and the various mutants, error bars are SEM. E<sub>rev</sub> of wild-type is between the positive E<sub>rev</sub> of trp<sup>302</sup>, which expresses only TRP and the E<sub>rev</sub> of trp<sup>P343</sup> mutant, which expresses only TRPL.
Reuss et al., 1997). Second, the localization of Acharya et al., Hardie and Minke, 2004) further imply that TRPL assembles as homomers.

**LIGHT-REGULATED SUBCELULAR TRANSLLOCATION OF DROSOPHILA TRPL CHANNELS**

In neurons the expression pattern of ion channels determines the physiological properties of the cell. Besides regulation at the level of gene expression that determines which channels are present in a given neuron, trafficking of ion channels into and out of the plasma membrane is an important mechanism for manipulating the number of channels at a specific cellular site (for reviews see Lai and Jan, 2006; Sheng and Lee, 2001). In Drosophila photoreceptors activation of the phototransduction cascade and the influx of Ca\(^{2+}\) through the TRP channels initiate the translocation of the TRP but not the TRPL channels from the signaling compartment, the rhabdomere, to the cell body (Bahnner et al., 2002; Meyer et al., 2006). The TRPL translocation process occurs in two stages, a fast translocation (5 min) to the neighboring stalk membrane and a slow translocation (over 6 h) to the basolateral membrane (Cronin et al., 2006). Thus, the TRPL translocation timescale conforms to day night cycle and act in light adaptation (Bahnner et al., 2002). While, Ca\(^{2+}\) influx has been shown to be necessary for TRPL translocation the molecular mechanism and structural determinants of the TRPL involved in translocation, are still unknown. Signal dependent translocation of mammalian TRP channels was found to be a widespread phenomenon (Bezzerides et al., 2004; Kanzaki et al., 1999; Zhang et al., 2005). Nevertheless, many of these researches are conducted on TRP channels expressed in tissue culture cells. This makes the Drosophila photoreceptors a unique system in which TRPL channels translocation can be studied in vivo.

**ACTIVATION MECHANISMS OF TRP AND TRPL CHANNELS**

It has been well established that hydrolysis of PIP\(_2\) by PLC, encoded by the norpA gene, activates the light-sensitive channels TRP and TRPL in Drosophila photoreceptors. However, the mechanism by which PLC activity results in channels opening is still under debate. Several hypotheses have been presented through the years. (i) The InsP\(_3\) hypothesis, suggested that the elevation of InsP\(_3\), following PIP\(_2\) hydrolysis, activates the InsP\(_3\) receptor (InsP\(_3\) receptor) resulting in Ca\(^{2+}\) store depletion and activation of the channels in a store-operated manner (Hardie and Minke, 1993). This mechanism of activation has also been suggested for a number of mammalian TRPC channels (Putney, 2007; Yuan et al., 2007). In addition, direct activation of the channels as in the Limulus ventral photoreceptors (Payne et al., 1986) using caged Ca\(^{2+}\) or InsP\(_3\) to elevate Ca\(^{2+}\) did not activate the channels (Hardie, 1995; Hardie and Raghu, 1998). Rather, direct application of Ca\(^{2+}\) in excised inside-out patches inhibits expressed TRPL channels in S2 cells by an open channel block mechanism (Parnas et al., 2007), suggesting an inhibition rather than activation effect of Ca\(^{2+}\). Furthermore, genetic elimination of the only InsP\(_3\)R in Drosophila had no effect on the light response (Acharya et al., 1997; Raghu et al., 2000a). Therefore, the InsP\(_3\) hypothesis was abundant. It therefore became evident that the alternative branch of PLC, DAG production should be investigated. The most familiar action of DAG is to activate the classical protein kinase C (PKC) synergistically with Ca\(^{2+}\). However, mutations in the ePKC, encoded by the inaC gene lead to defects in response termination with no apparent effects on activation (Hardie et al., 1993; compare Figure 11A to Figure 11B). (ii) The PUFA or DAG hypothesis argues that the elevation of DAG and consequently of PUFA acting as second messengers results in channel opening. This hypothesis emerged from a detailed pharmacological study which tested the effect of various fatty acids (including PUFAs) on TRP and TRPL channels activation in vivo and TRPL expressed in Drosophila S2 cells (Chyb et al., 1999). In addition, a detailed analysis of the rdgA mutant encoding DAG kinase has established the importance of the DAG branch in channel activation. This mutant shows light-independent retinal degeneration and constitutive activity of the light-activated channels, while a partial rescue of the degeneration is achieved by eliminating the TRP channel in the double mutant rdgA;trpP343 (Raghu et al., 2000b). Furthermore, it has been shown that the double mutant norpA\(^{P344}\), rdgA partially rescues the light response in the almost null norpA\(^{P29}\) mutant. This finding further supports the hypothesis that DAG or its surrogate PFUA are involved in channel activation (Hardie et al., 2003). Several lines of evidence challenge this hypothesis: first, application of DAG to intact ommatidia does not activate the channels (unpublished data), while application of DAG analogs 1-oleoyl-2-acetyl-sn-glycerol (OAG) at low concentration (2 μM) in inside-out patches excised from the microvilli of dissociated ommatidia result in activation of the TRP and TRPL channels in kinetics slower by three orders of magnitude (~60 s after application) compared to the light stimuli (Delgado and Baccigalupo, 2009). Second, the localization of RDGA in the SMC, a relatively distant cellular compartment from the transduction machinery (Masai et al., 1997) makes it unlikely that DAG could act as a second messenger without considerably slowing response termination kinetics, which does not fit to the fast termination of the response to light. Further establishment of this hypothesis requires identification of a functional-binding domain for DAG or PUFA on the TRP and TRPL channel and further elucidating the complex enzymatic machinery of PUFA production by DAG lipases. Recently, the inaE gene was identified as encoding a homologue of mammalian sn-1 type DAG lipase and was shown to be expressed predominantly in the cell body of Drosophila photoreceptors (Figure 9). Mutant flies, expressing low levels of the inaE gene product, have an abnormal light response, while the activation of the light-sensitive channels was not prevented (Leung et al., 2008). The discovery of the inaE gene is a first step in an endeavor to elucidate lipids regulation of the channels (see review, Raghu and Hardie, 2009). Thus, the participation of DAG or PUFAs in TRP and TRPL activation in vivo needs further exploration. (iii) The PIP\(_3\), depletion and DAG accumulation hypothesis argues that PIP\(_3\), acts as a negative modulator, while DAG or its surrogates acts as positive modulators of the TRPL channel. Schilling and colleagues demonstrated
in Sf9 cells expressing TRPL that application of DAG or PUFA activates the channels, while application of PIP$_2$ in inside-out patches inhibit their activity (Estacion et al., 2001). However, Hardie et al. (2001) showed that the trpP343 mutant phenotype (in which the light response decays to baseline) is a result of PIP$_2$ depletion which is not compatible with a PIP$_2$ inhibitory action. In addition, a PIP$_2$-binding domain has not been functionally identified in the TRP or TRPL channels. Together, the above arguments put the PIP$_2$ depletion and DAG accumulation hypothesis in question. (iv) A recently new hypothesis was formulated, suggesting that plasma membrane lipid–channel interactions controls channel gating. Accordingly, disruption of this interaction by membrane lipid modification through PLC activation causes the opening of the channels (Parnas et al., 2009). It is important to realize that PLC activation, which converts PIP$_2$, into DAG, devoid of the hydrophilic head-group, is known to cause major changes in lipid packing and lipid–channel interactions (Janmey and Kinnunen, 2006). It is therefore possible that neither PIP$_2$ hydrolysis nor DAG production affect the TRP and TRPL channel as second messengers, but rather act as modifiers of membrane lipid–channel interactions. This may in turn act as a possible mechanism of channel activation (Parnas et al., 2009).

This hypothesis evades the two main problems of the DAG or PUFA hypothesis: the need of RDGA at closed proximity to the channels and a channel-binding domain for DAG and/or PUFA. This hypothesis suffers from insufficient direct demonstration both in cell expression systems and in vivo.

**ORGANIZATION IN A SUPRAMOLECULAR SIGNALING COMPLEX VIA THE SCAFFOLD PROTEIN INAD**

An important step towards understanding Drosophila phototransduction has been achieved by the finding that some of the key elements of the phototransduction cascade are incorporated into supramolecular signaling complexes via a scaffold protein, INAD (Figure 4). The INAD protein was discovered using a PDA screen which isolated a defective Drosophila mutant (inaD). The first discovered inaD mutant, the inaD$^{P215}$, was isolated by Pak (1995) and was subsequently cloned and sequenced by Shieh and Niemeyer (1995). Later studies in Calliphora have shown that INAD binds not only TRP but also PLC (NORPA) and ePKC (INAC) (Huber et al., 1996). The interaction of INAD with TRP, NORPA and INAC was later confirmed in Drosophila (Tsunoda et al., 1997). It was further found that inaD is a scaffold protein, which consists

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**FIGURE 11** The inaC$^{P209}$ and inaD$^{P215}$ mutants reveal slow response termination of the macroscopic response to light and of the single bumps. (A–C) Upper panels: Whole-cell voltage clamp quantum bump responses to continues dim light in wild-type, inaC$^{P209}$ and inaD$^{P215}$ mutant flies. A slow termination of the bumps is observed in inaC$^{P209}$ and inaD$^{P215}$ mutant flies. (A–C) Lower panels: Whole-cell voltage clamp recordings of normalized responses to a 500-ms light pulse of the above mutants. A slow termination of macroscopic response is observed in inaC$^{P209}$ null mutant and in the inaD$^{P215}$ mutant in which the binding of INAD to TRP is disrupted (Chevesich et al., 1997; Shieh and Zhu, 1996).
of five ~90 amino acid (aa) protein interaction motifs called PDZ (PSD95, DLG, ZO1) domains. These domains are recognized as protein modules which bind to a diversity of signaling, cell adhesion and cytoskeletal proteins (Dimitratos et al., 1999; Schillace and Scott, 1999) by specific binding to target sequences typically, though not always, in the final three residues of the C-terminal. The PDZ domains of INAD bind to the signaling molecules as follows: PDZ1 and PDZ5 bind PLC (Shieh et al., 1997; van Huizen et al., 1998), PDZ2 or PDZ4 bind ePKC (Adamski et al., 1998) and PDZ3 binds TRP (Chevesich et al., 1997; Shieh and Zhu, 1996). This binding pattern is still under debate due to several contradictory reports. Contrary to TRP, TRPL appears not to be a member of the complex, since unlike INAC, NORPA and TRP it remains strictly localized to the microvilli of the inaD null mutant (Tsunoda et al., 1997). Several studies have suggested that, in addition to PLC, PKC and TRP, other signaling molecules such as CaM, R, TRPL and NINAC bind to the INAD signaling complex. Such binding, however, must be dynamic. Biochemical studies conducted in Calliphora have revealed that both INAD and TRP are targets for phosphorylation by the nearby ePKC (Huber et al., 1998). Accordingly, the association of TRP into transduction complexes may be related to increasing speed and efficiency of transduction events as reflected by the immediate vicinity of TRP to its upstream activator, PLC, and its possible regulator, ePKC (Huber et al., 1998). Indeed, genetic elimination of INAC affected the shape of the quantum bump of the inaC null mutant, by inducing slow termination of the bump, composed of damped oscillating current noise of an unclear underlying mechanism (Hardie et al., 1993; Henderson et al., 2000; Figure 11B). Interestingly, a similar phenotype was observed in the inaD7515 mutant, whereby the INAD complex and TRP channel are dissociated (Henderson et al., 2000; Figure 11C), also with a still unclear underlying mechanism.

TRP plays a major role in localizing the entire INAD multimolecular complex. Association between TRP and INAD is essential for correct localization of the complex in the rhabdomeres, as found in other signaling systems (Arnold and Clapham, 1999). This conclusion was derived from the use of Drosophila mutants in which the signaling proteins, which constitute the INAD complex, were removed genetically, and also by deletions of the specific binding domains, which bind TRP to INAD. These experiments showed that INAD is correctly localized to the rhabdomeres in inaC mutants (where ePKC is missing) and in norpA mutants (where PLC is missing), but severely mislocalized in null trp mutants (Li and Montell, 2000; Tsunoda et al., 2001), thus indicating that TRP but not PLC or PKC is essential for localization of the signaling complex to the rhabdomere. To demonstrate that a specific interaction of INAD with TRP is required for rhabdomeric localization of the complex, the binding site at the C-terminal of TRP was removed or three conserved residues in PDZ3, which are expected to disrupt the interaction between PDZ domains and their targets were modified. As predicted, both TRP and INAD were mislocalized in these mutants. The study of the above mutants was also used to show that TRP and INAD do not depend on each other for targeting to the rhabdomeres. Thus, INAD–TRP interaction is not required for targeting but for anchoring of the signaling complex (Li and Montell, 2000; Tsunoda et al., 2001). Additional experiments on TRP and INAD further showed that INAD has other functions in addition to anchoring the signaling complex. One important function is to preassemble the proteins of the signaling complex. Another important function, at least in the case of PLC, is to prevent degradation of the unbound signaling protein.

A recent study by Ranganathan and colleagues has suggested that the binding of signaling proteins to INAD may be a dynamic process that allows an additional level of phototransduction regulation. Their study showed two crystal structural states of isolated INAD PDZ5 domain, differing mainly by the formation of a disulfide bond. This conformational change has light-dependent dynamics that was demonstrated by the use of transgenic Drosophila flies expressing INAD with a point mutation disrupting the formation of the disulfide bond. They proposed a model in which, ePKC phosphorylation at a still unknown site promotes the light-dependent conformational change of PDZ5, distorting its ligand-binding groove to PLC and thus regulating phototransduction (Mishra et al., 2007).

CONCLUDING REMARKS

The study of fly photoreceptors has opened new avenues in biological research, mainly through the exploitation of the power of Drosophila molecular genetics. Processes and proteins that were discovered in Drosophila have been found to be highly conserved through evolution and thus paved the way for the discovery of important proteins and mechanisms in development and cell signaling in mammals. A striking example is the discovery of the TRP channel protein in the Drosophila photoreceptors, which led to the discovery of the widespread TRP superfamily, which plays crucial roles in sensory signaling of insects and mammals. The activation and regulation of Drosophila TRPs by the inositol-lipid signaling pathway and the major role of PLC in the activation of these channels has wide implications for understanding the activation and regulations of mammalian TRPs. Even today Drosophila photoreceptors are one of the few systems in which TRP channels are studied in vivo. Another novel molecule that was discovered in Drosophila photoreceptors is the INAD scaffold protein which forms a supramolecular signaling complex. This protein has introduced new concepts in cell signaling dynamics which are still under investigations. An additional advantage of using the fly for research on cellular signaling is that frequently the fly system is less evolutionary evolved relative to mammals, making it simpler to study, while maintaining its core function. It is therefore anticipated that research using the Drosophila sensory and motor systems will continue to identify new proteins and mechanisms of high biological importance.

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REFERENCES

Acharya, J. K., Jalink, K., Hardy, R. W., Hartenstein, V., and Zakeri, C. S. (1997). InsP3 receptor is essential for growth and differentiation but not for vision in Drosophila. Neuron 18, 881–887.

Adamski, F. M., Zhu, M. Y., Bahiraei, F., and Shieh, B. H. (1998). Interaction of eye protein kinase C and INAD in Drosophila. Localization of binding domains and electrophysiological characterization of a loss of association in transgenic flies. J. Biol. Chem. 273, 17173–17179.

Alloway, P. G., and Dolph, P. J. (1999). A role for the light-dependent phosphorylation of visual arrestin. Proc. Natl. Acad. Sci. U. S. A. 96, 6072–6077.

Alloway, P. G., Howard, L., and Dolph, P. J. (2000). The formation of stable rhodopsin-arrestin complexes induces apoptosis and photoreceptor cell degeneration. Neuron 28, 129–138.

Arnold, D. B., and Clapham, D. E. (1999). Molecular determinants for subcellular localization of PSD-95 with an interacting k+ channel. Neuron 23, 149–157.

Arshavsky, V. Y. (2003). Protein translocation in photoreceptor light adaptation: a common theme in vertebrate and invertebrate vision. Sci. STKE 2003, E43.

Arshavsky, V. Y., and Pugh-EN, J. (1998). Lifetime regulation of G protein-effector complex: emerging importance of RGS proteins. Neuron 20, 11–14.

Bahner, M., Frechter, S., Da, S. N., Minke, B., Paulsen, R., and Huber, A. (2002). Light-regulated subcellular translocation of Drosophila TRPL channels induces long-term adaptation and modifies the light-induced current. Neuron 34, 83–93.

Berridge, M. J. (1993). Inositol trisphosphate and calcium signalling. Nature 361, 315–325.

Berstein, G., Blank, J. L., Hardy, R. W., Hartenstein, V., and Zakeri, C. S. (1997). An eye-specific Gβ subunit essential for termination of the phototransduction cascade. Nature 379, 589–591.

Berridge, M. J. (2000). Slowed recovery of rod photoreceptor in mice lacking the GTPase activating protein RGS9-1. Nature 403, 557–560.

Chavezis, J., Kreuz, A. J., and Montell, C. (1997). Requirement for the PDZ domain protein, INAD, for localization of the TRP store-operated channel to a signaling complex. Neuron 18, 95–105.

Choma-Ornan, I., Tzafyfat, V., Ankri-Eilahoo, G., Joel-Almogar, T., Meyer, N. E., Huber, A., Payre, F., and Minke, B. (2005). Light-regulated interaction of Dmoesin with TRP and TRPL channels is required for maintenance of photoreceptors. J. Cell Biol. 171, 143–152.

Chyb, S., Raghu, P., and Hardie, R. C. (1999). Polysaturated fatty acids activate Drosophila light-sensitive channels TRP and TRPL. Nature 397, 255–259.

Cook, B., Bar, Y. M., Cohen-Bein, A. H., Goldstein, R. E., Paroush, Z., Selinger, Z., and Minke, B. (2000). Phospholipase C and termination of G-protein-mediated signalling in vivo. Nat. Cell Biol. 2, 296–301.

Cook, B., Hardy, R. W., McConnaughey, W. B., and Zaker, C. S. (2008). Preserving cell shape under environmental stress. Nature 452, 361–364.

Cosens, D. J., and Manning, A. (1969). Abnormal electroretinogram from a Drosophila mutant. Nature 224, 285–287.

Cronin, M. A., Lieu, M. H., and Tsunoda, S. (2002). Two stages of light-dependent phosphorylation of the light-regulated subcellular channel TRPL in Drosophila photoreceptors. Neuron 36, 634–637.

Fein, A. (1986). Blockade of visual excitation and adaptation in Limulus photoreceptor by GDP-β-S. Science 232, 1543–1545.

Fein, A., and Corson, D. W. (1981). Excitation of Limulus photoreceptors by vanadate and by a hydrolysis-resistant analog of guanosine triphosphate. Science 212, 555–557.

Fischer, A., and Horstmann, G. (1971). Fine structure of the eye of the meal moth, Ephesia kuehniella Zeller (Lepidoptera, Pyralidae). Z. Zellforsch. Mikrosk. Anat. 116, 275–304.

Franceschini, N., and Kirschfeld, K. (1971). In vivo optical study of photoreceptor elements in the compound eye of Drosophila. Kybernetik 6, 1–13.

Frechter, S., Elia, N., Tzarfaty, V., Selinger, Z., and Minke, B. (2007). Translocation of Gβγ mediates long-term adaptation in Drosophila photoreceptors. J. Neurosci. 27, 5571–5583.

García-Murillas, I., Pettitt, T., Macdonald, E., Okkenhaug, H., Georgiev, P., Trivedi, D., Hassan, B., Wakeham, M., and Raghu, P. (2006). Lazo-5 encodes a lipid phosphate phosphatase that regulates phosphatidylinositol turnover during Drosophila phototransduction. Neuron 49, 533–546.

Hardie, R. C. (1995). Photolysis of caged Ca2+ facilitates inactivation but does not directly excite light-sensitive channels in Drosophila photoreceptors. J. Neurosci. 15, 889–902.

Hardie, R. C. (2003). Regulation of TRP channels via lipid second messengers. Annu. Rev. Physiol. 65, 735–759.

Hardie, R. C., Martín, F., Chyb, S., and Raghu, P. (2003). Rescue of light responses in the Drosophila “null” phospholipase C mutant, norpA−/−, by the dicyclerglycerol kinase mutant, rdk−, and by metabolic inhibition. J. Biol. Chem. 278, 18851–18858.

Hardie, R. C., and Minke, B. (1992). The trp gene is essential for a light-activated Ca2+ channel in Drosophila photoreceptors. Neuron 8, 643–651.

Hardie, R. C., and Minke, B. (1993). Novel Ca2+ channels underlying transduction in Drosophila photoreceptors: implications for phosphoinositide-mediated Ca2+ mobilization. Trends Neurosci. 16, 371–376.

Hardie, R. C., and Mojet, M. H. (1995). Magnesium-dependent block of the light-activated and trp-dependent conductance in Drosophila photoreceptors. J. Neurosci. 74, 2590–2599.

Hardie, R. C., Peretz, A., Suss-Toby, E., Rom-Glas, A., Bishop, S. A., Selinger, Z., and Minke, B. (1993). Protein kinase C is required for light adaptation in Drosophila photoreceptors. Nature 363, 634–637.

Hardie, R. C., and Raghu, P. (1998). Activation of heterologously expressed Drosophila TRP channels: Ca2+ is not required and InsP3 is not sufficient. Cell Calcium 24, 153–163.

Hardie, R. C., and Raghu, P. (2001). Visual transduction in Drosophila. Nature 413, 186–193.

Hardie, R. C., Raghu, P., Moore, S., Juusola, M., Baines, R. A., and Sweeney, S. T. (2001). Calcium influx via TRP channels is required to maintain PIP levels in Drosophila photoreceptors. Neuron 30, 149–159.

Henderson, S. R., Reuss, H., and Hardie, R. C. (2000). Single photon responses in Drosophila photoreceptors and their regulation by Ca2+. J. Physiol. 524, 129–138.

Hillman, P., Hochstein, S., and Minke, B. (1972). A visual pigment with two physiologically active stable states. Science 175, 1486–1488.

Hillman, P., Hochstein, S., and Minke, B. (1983). Transduction in invertebrate photoreceptors: role of pigment bistability. Physiol. Rev. 63, 668–772.

Huber, A., Sander, P., Bahner, M., and Paulsen, B. (1998). The TRP Ca2+ channel assembled in a signaling complex by the PDZ domain protein INAD is phosphorylated through the interaction with protein kinase C (εPKC). FEBS Lett. 425, 317–322.

Huber, A., Sander, P., Gobert, A., Bahner, M., Hermann, R., and Paulsen, R. (1996). The transient receptor potential protein (TRP), a putative store-operated Ca2+ channel essential for phosphoinositide-mediated phototransduction, forms a signaling pathway.
complex with NorpA, InaC and InaD. *EMBO* J. 15, 7036–7045.

Janny, P.A., and Kinnunen, P.K. (2006). Biophysical properties of lipids and dynamic membranes. *Trends Cell Biol*. 16, 723–728.

Jors, S., Kazanski, V., Foik, A., Kratwurst, D., and Harteneck, C. (2006). Receptor-induced activation of Drosophila TRP by polysaturated fatty acids. *J. Biol. Chem.* 281, 29693–29702.

Kahn, E. S., and Matsumoto, H. (1997). *Lai, H. C., and Jan, L. Y. (2006). The distribution and targeting of neuronal voltage-gated ion channels. *Nat. Rev. Neurosci.* 7, 548–562.*

LaLonde, M. M., Janssens, H., Rosenbaum, E., Choi, S. Y., Gergen, J. P., Colley, N. J., Stark, W. S., and Lehman, M. A. (2005). Regulation of phototransduction responsiveness and retinal degeneration by a phosphodiester D-generated signaling lipid. *J. Cell Biol.* 169, 471–479.

Lee, S. J., and Montell, C. (2004). Light-dependent translocation of visual arrestin regulated by the NINAC myosin III. *Neurosci. 45*, 95–103.

Lee, Y. J., Dobbs, M. B., Verardi, M. L., and Hyde, D. R. (1990). dgg: a Drosophila gene encoding a visual system-specific G molecule. *Neuron* 5, 889–898.

Leung, H. T., Tseng-Crank, J., Kim, E., Mahapatta, C., Shino, S., Zhou, Y., An, L., Doerge, R. W., and Pak, W. L. (2008). DAG lipase activity is necessary for TRP channel regulation in Drosophila photoreceptors. *Neuron* 58, 884–896.

Li, H. S., and Montell, C. (2000). TRP and the PDZ protein, INAD, form the core complex required for retention of the signalplex in Drosophila photoreceptor cells. *J. Cell Biol.* 150, 1411–1422.

Li, H. S., Porter, J. A., and Montell, C. (1998). Requirement for the NINAC kinase/myosin for stable termination of the visual cascade. *J. Neurosci.* 18, 9601–9606.

Liu, C. H., Satoh, A. K., Postma, M., Huang, I., Ready, D. F., and Hardie, R. C. (2008). Ca2⁺-dependent metathoraxi inactivation mediated by calmodulin and NINAC myosin III. *Neuroscience 59*, 778–789.

Lo, M. V., and Pak, W. L. (1981). Light-induced pigment granule migration in the retinal cells of *Drosophila mela*- nogaster. *Comp. Gen. Physiol.* 77, 155–175.

Makino, E. R., Handy, J. W., Li, T., and Arashvay, V. Y. (1999). The GTPase activating factor for transducin in rod photoreceptors is the complex between RGS9 and type 3 G protein βsubunit. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1947–1952.

Masai, I., Okazaki, A., Hosoya, T., and Hoti, Y. (1993). Drosophila retinal degeneration A gene encodes an eye-specific diacylglycerol kinase with cysteine-rich zinc-finger motifs and ankyrin repeats. *Proc. Natl. Acad. Sci. U. S. A.* 90, 11157–1116.

Masai, I., Suzuki, E., Yoon, C. S., Kohyama, A., and Hotta, Y. (1997). Immunolocalization of Drosophila eye-specific diacylglycerol kinase, rdgA, which is essential for the maintenance of the photoreceptor. *J. Neurobiol.* 32, 695–706.

Meyer, N. E., Joel-Almagor, T., Frechter, S., Minke, B., and Huber, A. (2006). Subcellular translocation of the cGPTagged TRP channel in Drosophila photoreceptors requires activation of the phototransduction cascade. *J. Cell Sci.* 119, 2592–2603.

Minke, B., and Cook, B. (2002). TRP channel proteins and signal transduction. *Physiol. Rev.* 82, 429–472.

Minke, B., and Hardie, R. C. (2000). Genetic dissection of Drosophila phototransduction. In *Molecular Mechanisms in Visual Transduction*, D. G. Stavenga, D. J. N. van der Hoeve, and E. Pugh, eds (North Holland, Elsevier), pp. 449–525.

Minke, B., and Selinger, Z. (1991). Inositol lipid pathway in fly photoreceptors: excitation, calcium mobilization and retinal degeneration. In Progress in Retinal Research, N. A. Osborne and G. J. Chader, eds (Oxford, Pergamon Press), pp. 99–124.

Minke, B., and Selinger, Z. (1992). The inositol lipid pathway is necessary for light excitation in fly photoreceptors. Sensory Transduction, D. Corey and S. D. Roper, eds (New York, The Rockefeller University Press), pp. 202–217.

Minke, B., and Selinger, Z. (1996). The roles of trp and calcium in regulating photoreceptor function in *Drosophila*. *Curr. Opin. Neurobiol.* 6, 459–466.

Minke, B., and Stephenson, R. S. (1985). The characteristics of chemically induced noise in *Musca* photoreceptors. *J. Comp. Physiol. 156*, 339–356.

Minke, B., Wu, C.-F., and Pak, W. L. (1973a). Isolation of light-induced response of the central retinal cells from the electoretinogram of *Drosophila*. *J. Comp. Physiol.* 98, 345–355.

Minke, B., Wu, C.-F., and Pak, W. L. (1973b). Induction of photoreceptor voltage noise in the dark in Drosophila mutant. *Nature* 258, 84–87.

Mishra, P., Socolich, M., Wall, M. A., Graves, J., Wang, Z., and Ranganathan, B. (2007). Dynamic Scaffolding in a G Protein-Coupled Signaling System. *Cell 131*, 80–92.

Montell, C. (1989). Molecular genetics of *Drosophila* vision. *Bioessays* 11, 43–48.

Montell, C., and Rubin, G. M. (1989). Molecular characterization of the *Drosophila* trp locus: a putative integral membrane protein required for phototransduction. *Neuron* 2, 1313–1323.

Mukhopadhyay, S., and Ross, E. M. (1999). Rapid GTP binding and hydrolysis by G protein-gated receptor and GTPase-activating proteins. *Proc. Natl. Acad. Sci. U. S. A.* 96, 9539–9544.

Nasi, E., del Pilar Gomez, M., and Payne, R. (2000). Phototransduction mechanisms in microvillar and cili- ary photoreceptors of invertebrates. In *Molecular Mechanisms in Visual Transduction*, D. G. Stavenga, D. J. N. van der Hoeve, and E. Pugh, eds (North Holland, Elsevier), pp. 389–448.

Niemeier, B. A., Suzuki, E., Scott, K., Jalink, K., and Zuker, C. S. (1996). The Drosophila light-activated con- ductance is composed of the two channels TRP and TRPL. *Cell 85*, 651–659.

Pak, W. L. (1995). *Drosophila* in vision research. The Friedenwald Lecture. *Invest. Ophthalmol. Vis. Sci.* 36, 2340–2357.

Parnas, M., Katz, B., Lev, S., Tzarfaty, V., Dadon, D., Gordon-Shaag, A., Metzner, H., Yaka, R., and Minke, B. (2009). Membrane lipid modifications remove divalent open channel block from TRP-like and NMDA channels. *J. Neurosci.* 29, 2371–2383.

Parnas, M., Ares, R., and Minke, B. (2007). Open channel block by Ca2⁺ underlies the voltage dependence of Drosophila TRP channel. *J. Gen. Physiol*. 129, 17–28.

Payne, R., Corson, D. W., and Fein, A. (1986). Pressure injection of calcium both excites and adapts *Limulus* ventral photoreceptors. *J. Gen. Physiol*. 88, 107–126.

Pearn, M. T., Randall, L. I., Shortridge, R. D., Burg, M. G., and Pak, W. L. (1996). Molecular, biochemical, and electrophysiological characterization of *Drosophila* norpA mutants. *J. Biol. Chem.* 271, 4937–4945.

Peretz, A., Sandler, C., Kirschfeld, K., Hardie, R. C., and Minke, B. (1994a). Genetic dissection of light-induced Ca2⁺ influx into *Drosophila* photoreceptors. *J. Gen. Physiol*. 104, 1027–1077.

Peretz, A., Suss-Toby, E., Rom-Glas, A., Arnon, A., Payne, R., and Minke, B. (1994b). The light response of *Drosophila* photoreceptors is accom- panied by an increase in cellular calcium: effects of specific mutations. *Neuron* 12, 1257–1267.

Phillips, A. M., Bull, A., and Kelly, L. E. (1992). Identification of a *Drosophila* gene encoding a calmodulin-binding protein with homology to the trp phototransduction gene. *Neuron* 8, 631–642.

Pumir, A., Graves, J., Ranganathan, R., and Shraiman, B. I. (2008). Systems analysis of the single photon response in invertebrate photoreceptors. *Proc. Natl. Acad. Sci. U. S. A.* 105, 10354–10359.

Putney, J. W. Jr (2007). Inositol lipids and TRPC channel activation. *Biochem. Soc. Symp.* 74, 37–45.
Raghu, P., Colley, N. J., Wébel, R., James, T., Hasan, G., Danin, M., Selinger, Z., and Hardie, R. C. (2000a). Normal phototransduction in Drosophila photoreceptors lacking an InsP3 receptor gene. Mol. Cell Neurosci. 15, 429–445.

Raghu, P., and Hardie, R. C. (2009). Regulation of Drosophila TRPC channels by lipid messengers. Cell Calcium. [Epub ahead of print].

Raghu, P., Usher, K., Jonas, S., Chyb, S., Polyansky, A., and Hardie, R. C. (2000b). Constitutive activity of the light-sensitive channels TRP and TRPL in the Drosophila diacylglycerol kinase mutant, rdd4. Neuron 26, 169–179.

Ranganathan, R., Harris, G. L., Stevens, C. F., and Zuker, C. S. (1995). A Drosophila mutant defective in extracellular calcium-dependent photoreceptor deactivation and rapid desensitization. Nature 354, 230–232.

Ranganathan, R., Malicki, D. M., and Zuker, C. S. (1995). Signal transduction in Drosophila photoreceptors. Annu. Rev. Neurosci. 18, 283–317.

Radliff, E. (1990). Haldan Keffe Hartline: December 22, 1903–March 18, 1983. Biogr. Mem. Natl. Acad. Sci. 59, 197–213.

Reuss, H., Mojet, M. H., Chyb, S., and Hardie, R. C. (1997). In vivo analysis of the Drosophila light-sensitive channels, TRP and TRPL. Neuron 19, 1249–1259.

Sato, A. K., Li, B. X., Xia, H., and Ready, D. F. (2008). Calcium-activated myosin V closes the Drosophila pupil. Curr. Biol. 18, 931–935.

Schillace, R. V., and Scott, J. D. (1999). Organization of kinases, phosphatases, and receptor signaling complexes. J. Clin. Invest. 103, 761–765.

Scott, K., Becker, A., Sun, Y., Hardy, R., and Zuker, C. (1995). G protein function in vivo: genetic dissection of its role in photoreceptor cell physiology. Neuron 15, 919–927.

Scott, K., Sun, Y., Beckingham, K., and Zuker, C. S. (1997). Calmodulin regulation of Drosophila light-activated channels and receptor function mediates termination of the light response in vivo. Cell 91, 373–383.

Selinger, Z., Doza, Y. N., and Minke, B. (1993). Mechanisms and genetics of photoreceptors desensitization in Drosophila flies. Biochim. Biophys. Acta 1179, 283–299.

Sheng, M., and Lee, S. H. (2001). AMP receptor trafficking and the control of synaptic transmission. Cell 105, 825–828.

Shieh, B. H., and Niemeyer, B. (1995). A novel protein encoded by the InaD gene regulates recovery of visual transduction in Drosophila. Neuron 14, 201–210.

Shieh, B. H., and Zhu, M. Y. (1996). Regulation of the TRP Ca2+ channel by INAD in Drosophila photoreceptors. Neuron 16, 991–998.

Shieh, B. H., Zhu, M. Y., Lee, J. K., Kelly, I. M., and Bahraiini, F. (1997). Association of INAD with NOSPA is essential for controlled activation and deactivation of Drosophila phototransduction in vivo. Proc. Natl. Acad. Sci. U. S. A. 94, 12682–12687.

Steel, F. R., Washburn, T., Rieger, R., and O’Toole, J. E. (1992). Drosophila retinal degeneration C (rdgC) encodes a novel serine/threonine protein phosphatase. Cell 69, 669–676.

Tajon, P., Krauss, N., Choe, H. W., Gless, A., Pulvermüller, A., and Wolfrum, U. (2008). Centrin in retinal photoreceptor cell regulators in the connecting cilium. Prog. Retin. Eye Res. 27, 237–259.

Tsunoda, S., Sierralta, J., Sun, Y., Bodner, R., Suzuki, E., Becker, A., Socolich, M., and Zuker, C. S. (1997). A multivalent PDZ-domain protein assembles signalling complexes in a G-protein-coupled cascade. Nature 388, 243–249.

Tsunoda, S., Sun, Y., Suzuki, E., and Zuker, C. (2001). Independent anchoring and assembly mechanisms of INAD signaling complexes in Drosophila photoreceptors. J. Neurosci. 21, 150–158.

van Huizen, R., Miller, K., Chen, D. M., Li, Y., Lai, Z.-C., Raab, R. W., Stark, W. S., Shortridge, R. D., and Li, M. (1998). Two distantly positioned PDZ domains mediate multivalent INAD-phospholipase C interactions essential for G protein-coupled signaling. EMBO J. 17, 2285–2297.

Vihitelc, T. S., Hyde, D. R., and O’Toole, J. E. (1991). Isolation and characterization of the Drosophila retinal degeneration B (rdgB) gene. Genetics 127, 761–768.

Violin, J. D., and Leffkowitz, R. J. (2007). Beta-arrestin-biased ligands at seven-transmembrane receptors. Trends Pharmacol. Sci. 28, 416–422.

Vogt, K., and Kirschfeld, K. (1984). Chemical identity of the chromophores of fly visual pigment. Naturwissenschaften 71, 211–213.

Wang, Y., and Nathans, J. (2007). Tissue/planar cell polarity in vertebrates: new insights and new questions. Development 134, 647–658.

Wernet, M. F., Mazzoni, E. O., Celik, A., Duncan, D. M., Duncan, L., and Desplan, C. (2006). Stochastic spineless expression creates the retinal mosaic for colour vision. Nature 440, 174–180.

Wes, P. D., Chevesich, J., Jeromin, A., Rosenberg, C., Stetten, G., and Montell, C. (1995). TRPC1, a human homolog of a Drosophila store-operated channel. Proc. Natl. Acad. Sci. U. S. A. 92, 9652–9656.

Wu, L., Niemeyer, B., Colley, N., Socolich, M., and Zuker, C. S. (1995). Regulation of PLC-mediated signaling in vivo by CDP-diacylglycerol synthase. Nature 373, 216–222.

Xu, X. Z., Chien, F., Butler, A., Salkoff, L., and Montell, C. (2000). TRPγ2, a Drosophila TRP-related subunit, forms a regulated cation channel with TRPβ1. Neuron 26, 647–657.

Xu, X. Z. S., Li, H. S., Guggino, W. B., and Montell, C. (1997). Coassembly of TRP and TRPL produces a distinct store-operated conductance. Cell 89, 1155–1164.

Yamada, T., Takeuchi, Y., Komori, N., Kobayashi, H., Sakai, Y., Hotta, Y., and Matsumoto, H. (1990). A 49-kilodalton phosphoprotein in the Drosophila photoreceptor is an arrestin homolog. Science 248, 483–486.

Yeandle, S., and Spiegler, J. B. (1973). Light-evoked and spontaneous discrete waves in the ventral nerve photoreceptor of Limulus. J. Gen. Physiol. 61, 552–571.

Yuan, J. P., Zeng, W., Huang, G. N., Worley, P. F., and Mualem, S. (2007). STIM1 heteromultimerizes TRPC channels to determine their function as store-operated channels. Nat. Cell Biol. 9, 636–645.

Zelhof, A. C., Hardy, R. W., Becker, A., and Zuker, C. S. (2006). Transforming the architecture of compound eyes. Nature 443, 696–699.

Zhang, X., Huang, J., and McNaughton, P. A. (2005). NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J. 24, 4211–4223.

Zhu, X., Chu, P. B., Peyton, M., and Birnbaumer, L. (1995). Molecular cloning of a widely expressed human homologue for the Drosophila trp gene. FEBS Lett. 373, 193–198.

Zuidervaart, H., Stavenga, D. G., Stark, W. S., and Bernard, G. D. (1979). Pupillary responses revealing receptors characteristics in wild-type and mutant Drosophila. Abstr. Soc. Neurosci. 5, 814.

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