Phototransduction and retinal degeneration in *Drosophila*

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Abstract *Drosophila* visual transduction is the fastest known G-protein-coupled signaling cascade and has therefore served as a genetically tractable animal model for characterizing rapid responses to sensory stimulation. Mutations in over 30 genes have been identified, which affect activation, adaptation, or termination of the photoreponse. Based on analyses of these genes, a model for phototransduction has emerged, which involves phosphoinositide signaling and culminates with opening of the TRP and TRPL cation channels. Many of the proteins that function in phototransduction are coupled to the PDZ containing scaffold protein INAD and form a supramolecular signaling complex, the signalplex. Arrestin, TRPL, and $G\alpha_q$ undergo dynamic light-dependent trafficking, and these movements function in long-term adaptation. Other proteins play important roles either in the formation or maturation of rhodopsin, or in regeneration of phosphatidylinositol 4,5-bisphosphate (PIP₂), which is required for the photoreponse. Mutation of nearly any gene that functions in the photoreponse results in retinal degeneration. The underlying bases of photoreceptor cell death are diverse and involve mechanisms such as excessive endocytosis of rhodopsin due to stable rhodopsin/arrestin complexes and abnormally low or high levels of Ca²⁺. *Drosophila* visual transduction appears to have particular relevance to the cascade in the

intrinsically photosensitive retinal ganglion cells in mammals, as the photoreponse in these latter cells appears to operate through a remarkably similar mechanism.

Keywords *Drosophila* · Phototransduction · Retinal degeneration

Introduction

Overview and relationship of *Drosophila* to mammalian phototransduction

Drosophila vision is the first sensory modality to be subjected to detailed genetic analyses in any animal [1]. The appeal of this system stems in part from the observation that it functions through the fastest known G-protein-coupled signaling cascade as it is maximally activated in as little as 20 milliseconds [2]. As such it provides a model for characterizing the mechanisms underlying rapid responses to sensory stimulation.

Drosophila phototransduction offers the opportunity to combine classical and modern genetic approaches to identify genes and proteins that function in phototransduction using electrophysiological, cell biological, and biochemical approaches. This latter advantage, the ease of performing biochemical assays, is a consequence of the architecture of the fly's compound eye each of which is comprised of a reiteration of ~800 simple eyes. Consequently, all the photoreceptor cell proteins are expressed in many cells, greatly reducing a problem inherent in many signaling systems, the low abundance of the essential proteins, and signaling molecules. Not least among the tools that have driven the progress of *Drosophila* phototransduction are electroretinogram recordings (ERGs), an

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electrophysiological assay that is so simple that it can be used to perform large-scale genetic screens as pioneered by Pak and colleagues [3]. Once mutations are isolated, the bases of the defects can be assessed in greater detail by applying additional electrophysiological approaches, including intracellular recordings and patch clamp analyses [2, 4].

During the 40 years that have elapsed since the initial studies on *Drosophila* visual transduction, many of the mysteries underlying the cascade have been unraveled. These include the demonstration that the cascade operates through activation of a phospholipase C β (PLC) [5] and opening of the TRP and TRPL cation channels [6–9] (Fig. 1). These insights led to a puzzle, and later to an exciting surprise, concerning the parallels between *Drosophila* and mammalian phototransduction. While the cascades in vertebrate rods and cones, and fly photoreceptor cells are both initiated by activation of related rhodopsins and engagement of heterotrimeric G-proteins, there are notable differences. In contrast to fly photoreceptor cells, light stimulation of the rhodopsins in rods and cones leads to an increase in the activity of a cyclic guanosine monophosphate (cGMP) phosphodiesterase, a subsequent drop in cGMP levels, and closure of the cGMP-gated channels [10]. Thus, the effects of light stimulation on the channels are opposite in fly photoreceptor cells compared to rods and cones.

Therefore, what is the relationship between vertebrate and invertebrate phototransduction? Did they evolve separately? During the last few years, it has become clear that there is a small subset of retinal ganglion cells that are intrinsically photosensitive (ipRGCs), and which function in photoentrainment of circadian rhythm and in light-induced pupillary constriction [11]. Moreover, it appears that the phototransduction cascade in the ipRGCs bears striking similarities to that in fly photoreceptors. The cascade in the ipRGCs is initiated through a receptor, melanopsin, which has greater

sequence and biophysical similarities to the *Drosophila* rhodopsins [12–18] than the light receptors in rods and cones. Furthermore, pharmacological and electrophysiological studies suggest that the ipRGC cascade functions through a PLC and opening of channels that display features reminiscent of TRP channels [19–21]. Although, most of the specific signaling proteins in the ipRGCs remain to be identified, it appears that the *Drosophila* and mammalian ipRGC phototransduction cascades share common origins.

The *Drosophila* compound eye

The *Drosophila* compound eye is comprised of ~800 hexagonal units, ommatidia (Fig. 2a), each of which contains 20 cells including eight photoreceptor cells. Six of the photoreceptor cells (R1–6) extend the full depth of the retina (~85 μ m), while the remaining two (R7 and R8) occupy the distal and proximal regions of the ommatidia, respectively (Fig. 2c and d). The main cells surrounding the photoreceptor cells are secondary pigment cells. The vertices of the ommatidia alternate between tertiary pigment cells and mechanosensory bristle cells (Fig. 2d).

Each photoreceptor cell contains a microvillar structure, referred to as the rhabdomere, which is the functional equivalent of the rod and cone outer segment (Fig. 2e and f). The ~50,000 and 17,000 microvilli in the R1–6 and R7/8 cells, respectively, provide a massive plasma membrane surface to pack in a high concentration of rhodopsin and is also the site for most of the proteins that function in phototransduction. Due to a curvature of the eye (Fig. 2b), the diameter of the R1–6 cell rhabdomeres is nearly 2 μ m near the distal region and narrows to just over 1 μ m at the proximal end. The R7/8 rhabdomeral diameters are even smaller and are just slightly more than half that of the larger R1–6 cells.

Fig. 1 Model of the *Drosophila* phototransduction cascade. See the text for details

Fig. 2 Structure of the *Drosophila* compound eye. **a** Scanning EM of a compound eye. **b** Section through adult fly head: *br*, brain; *fb*, fat bodies; *la*, lamina; *lo*, lobula; *lp*, lobula plate; *me*, medulla; *re*, retina. **c** Single ommatidium at an angle 90° from the surface of the compound eye: *co*, cornea; *primary PC*, primary pigment cell; *psC*, pseudocone; *secondary PC*, secondary pigment cell; *R1–6*, photoreceptor cells 1–6; *R7*, photoreceptor cell 7; *R8*, photoreceptor cell 8. **d** Cross-sections through the distal and proximal regions of the ommatidia. Seven photoreceptor cells are present in any given plane of section. The *ovals* represent the rhabdomeres. The R cell bodies are numbered. A secondary pigment cell, a tertiary pigment cell and a mechanosensory bristle cell are indicated. **e** Longitudinal view through a photoreceptor cell. The rhabdomere, axon, and nucleus (*N*) are indicated. **f** Cross-sectional view through a photoreceptor cell. The approximate average dimensions of an R1–6 cell microvillus are indicated

The dimensions of each microvillus are quite small—they are only 50 nm wide and the space within the rhabdomeral cell bodies is limited further by the presence of an actin filament extending into the extra-rhabdomeral cell bodies (Fig. 2f). A single myosin III appears to span the small distance between the actin filament and the rhabdomeral membrane [22, 23]. The extremely small size of a microvillus has important implications concerning the mechanism of signal amplifica-

tion. According to one model, the extent of amplification by a photon of light is defined by the number of signaling molecules in a single microvillus [24–26].

Genetic approaches to *Drosophila* phototransduction

Since the compound eye is not required for viability, it is feasible to screen for mutations affecting the visual response

in adult flies. Some of the early genetic approaches relied on behavioral assays such as phototaxis or changes in body orientation in response to moving visual cues (the optomotor response; reviewed in [27]). Mutations affecting phototransduction have also been isolated by examining flies for evidence of retinal degeneration [28–30].

The most sensitive and productive screen for genes required for phototransduction relied on a simple electrophysiological test, the ERG recording [3]. ERGs are extracellular recordings, which measure the summed responses of all the retinal cells to light, and are typically performed with white light by placing a recording electrode on the surface of the compound eye. There are two primary features of the ERG, the maintained component (or light coincident receptor potential; LCRP) that results from the activity in the retina, and the on- and off-transients (Fig. 3a), which occur due to activity postsynaptic to the photoreceptor cells in the lamina (Fig. 2b). Mutations that affect the LCRP occur due to defects either in activation, adaptation, or termination of the photoresponse (Fig. 3b–e) and are considered in the current review.

A variation of the standard ERG paradigm has led to the identification of many mutations affecting the major rhodopsin, Rh1. Exposure of flies to blue (480 nm) light locks Rh1 in an activated metarhodopsin state. Therefore, upon termination of the light stimulus, there is a prolonged depolarization afterpotential (PDA; Fig. 3f). Termination of the PDA is achieved by orange (580) or white light, which triggers the conversion of the metarhodopsin back to the nonactivated rhodopsin (Fig. 3f). Mutations that reduce the PDA (Fig. 3g) affect either the synthesis, maturation, transport, or activity of the opsin or the chromophore.

The forward genetic screens for mutations affecting the photoresponse have led to the identification of many genes required for phototransduction (Table 1). However, the screens have not been carried to saturation. Therefore, as a complementary approach, a growing number of candidate genes, based on studies of mammalian phototransduction, have been disrupted using reverse genetic approaches. An alternative unbiased strategy to identify candidate phototransduction genes takes advantage of the observation that nearly all of the genes known to function in the visual response are expressed primarily in the eye. Using DNA microarrays, nearly 100 previously uncharacterized “eye-enriched” genes were identified (eye-enrichment from 2- to 500-fold) [31], two of which have been mutated and shown to function in the visual response [31–33].

Characterization of the >30 genes, which were identified using either forward or reverse genetic approaches (Table 1), has led to a formulation of a model for *Drosophila* phototransduction, as well as insights into a variety of highly related questions. These include the biosynthesis of the opsin and chromophore, the mechanisms and function

of light-dependent movements of signaling proteins, and the organization of a supramolecular complex comprising many of the proteins functioning in phototransduction. Of particular importance are the many advances concerning the mechanisms underlying the retinal degeneration that results from mutations in genes required for the visual response. Despite the differences between the cascades in *Drosophila* photoreceptor cells and rods and cones, there appear to be important parallels between the mechanisms underlying *Drosophila* retinal degeneration and human retinal dystrophies.

The light receptors: rhodopsins

The rhodopsins in both *Drosophila* and vertebrate photoreceptor cells are related molecules comprised of two types of subunits. These include a seven transmembrane protein, referred to as the opsin and a chromophore, which is covalently linked to a lysine residue in the seventh transmembrane domain through a Schiff base linkage. In vertebrates the chromophore is 11-*cis* retinal, while in *Drosophila* it is the highly related derivative, 11-*cis* 3-hydroxyretinal [34–36]. Light absorption results in isomerization of the 11-*cis* retinal to form all-*trans* retinal, which initiates a conformational change in the opsin. Metarhodopsin, the active form of rhodopsin, in turn activates heterotrimeric G-proteins [37–39]. In rods and cones light stimulation leads to dissociation of the all-*trans* retinal from the opsin through breakage of the Schiff base linkage. The rhodopsin is subsequently regenerated through recombination of the 11-*cis* retinal and opsin. In *Drosophila* photoreceptor cells the major Rh1 metarhodopsin is stable since the chromophore does not dissociate. Rather, regeneration of rhodopsin requires exposure to a second photon of white or orange light (Fig. 4). Consequently, a PDA is produced upon termination of a blue light stimulus (Fig. 3f). A similar two photon visual cycle may also exist in the ipRGCs in mammals [11].

Diversity of *Drosophila* rhodopsins

At least six rhodopsins are expressed in *Drosophila*, each of which displays a distinct spectral sensitivity and expression pattern (Table 2). The predominant *Drosophila* rhodopsin, Rh1, encoded by the *ninaE* (*neither inactivation nor afterpotential E*) gene is the only rhodopsin expressed in the R1–R6 photoreceptor cells [40, 41]. The minor opsins expressed in the central R7 and R8 photoreceptor cells are coordinately expressed in nonoverlapping subsets of cells. Rh3 and Rh4 are ultraviolet-absorbing visual pigments expressed in ~30 and 70% of the R7 cells respectively in a seemingly random pattern of distribution [42–45]. As

Fig. 3 ERG recordings from wild-type and representative mutants. **a–e** ERGs performed on wild-type and the indicated mutants using orange light. Amplitude (mV) and time-scale (s) markers are included. The event markers below the ERGs indicate the initiation and cessation of the light stimuli. The on- and off-transients and the maintained component (*mc*) are indicated. **f, g** The indicated strains of flies were exposed to multiple pulses of orange (*O*, 580 nm) and blue (*B*, 480 nm) light. The prolonged depolarization afterpotential (*PDA*) is indicated

with the R7 cells, the same relative proportions of R8 cells, 30 and 70%, express the blue (Rh5) and green (Rh6) absorbing pigments, respectively [46–48].

Every Rh3 expressing R7 cell is situated in the same ommatidium as the Rh5 expressing R8, while expression of Rh4 and Rh6 are coupled in the remaining ommatidia [46, 47, 49]. The different sets of Rh3/Rh5 and Rh4/Rh6 expressing R7/R8 cells appear to correspond to the previously described pairs of 7y/8y and 7p/8p photoreceptor cells of the housefly, so-named based on their yellow or pale appearance in transmitted light [50]. The coordinate expression of the two pairs of R7 and R8 rhodopsins may contribute to spectral tuning due to the presence of a screening pigment in the Rh4 expressing R7 cells, which

filters the light before it stimulates Rh6 in the R8 cells [51, 52]. In contrast to most ommatidia, the dorsal margin of the compound eye contains ommatidia that express Rh3 in both the R7 and R8 cells [45]. A violet-absorbing rhodopsin, Rh2, is expressed exclusively in the photoreceptor cells of the three ocelli, the simpler eyes localized on the vertex of the fly head [53–56].

Maturation of rhodopsin

Rh1 is synthesized in the endoplasmic reticulum (ER) and transported to the rhabdomere via the secretory pathway. The translocation of Rh1 from the ER to the rhabdomere depends on a protein related to peptidyl-prolyl *cis-trans*

Table 1 Genes required for a normal visual response

Gene	Identification method	Molecular function	ERG phenotype
<i>arr1</i>	Western blot screen	Minor arrestin	Enhances <i>arr2</i> phenotype
<i>arr2</i>	Western blot screen	Major arrestin	Slow termination
<i>calx</i>	ERG screen	Na ⁺ /Ca ²⁺ exchanger	Inactivation of light response
<i>cam</i>	Lethality/ERG	Ca ²⁺ regulatory protein; calmodulin	Slow termination
<i>cds</i>	<i>lacZ</i> reporter screen	CDP-DAG synthase	Low light sensitivity
<i>cnx</i>	Retinal degeneration screen	Rhodopsin chaperone; calnexin	No PDA
<i>dcamta</i>	ERG screen	CaM binding transcription factor	Slow termination
<i>dGα_q</i>	Western blot screen	α subunit of G protein	Low light sensitivity
<i>Gβ_e</i>	Western blot screen	β subunit of G protein	Low light sensitivity
<i>Gγ_e</i>	Transgenic flies: dominant negative isoform	γ subunit of G protein	Low light sensitivity
<i>GPRK1</i>	Transgenic flies: dominant negative isoform	Rhodopsin kinase	Larger amplitude
<i>inaC</i>	ERG screen	Protein kinase C	Slow termination
<i>inaD</i>	ERG screen	Scaffold protein; 5 PDZ domains	Small response and slow kinetics
<i>inaF</i>	ERG screen	Putative regulator of TRP	Transient receptor potential
<i>laza</i>	P-element	PA phosphatase	Small response/faster termination
<i>ninaA</i>	ERG screen	Cyclophilin	No PDA
<i>ninaB</i>	ERG screen	β, β'-carotene-15,15'-monooxygenase	No PDA
<i>ninaC</i>	ERG screen	Protein kinase/myosin III	Slow termination
<i>ninaD</i>	ERG screen	Class B scavenger receptor	No PDA
<i>ninaE</i>	ERG screen	Major rhodopsin	No PDA
<i>ninaG</i>	ERG screen	Oxidoreductase	No PDA
<i>norpA</i>	Phototaxis screen	Phospholipase C	No response
<i>pinta</i>	ERG screen	Retinoid binding	No PDA
<i>dpis</i>	Homolous recombination	PI synthase	Inactivation during light response
<i>pld</i>	Homolous recombination	Phospholipase D	Low light sensitivity
<i>rbo</i>	Paralysis/ERG	Possible lipase in PIP ₂ pathway	Transient response
<i>rdgA</i>	Retinal degeneration screen	DAG kinase	Constitutively active TRP channels
<i>rdgB</i>	Retinal degeneration screen	PI transfer protein	Inactivation after light response
<i>rdgC</i>	Retinal degeneration screen	Rhodopsin phosphatase	Slow termination
<i>santa maria</i>	ERG screen	Class B scavenger receptor	No PDA
<i>sun</i>	P-element insertion	Tetraspanin	–
<i>trp</i>	ERG	Cation channel	Transient receptor potential
<i>trpl</i>	Western blot screen	Cation channel	No response in combination with <i>trp</i>

Listed are the methods used to identify the mutant (or to generate transgenic flies expressing a dominant negative isoform), the proposed molecular functions of the corresponding proteins, and the ERG phenotypes due to loss-of-function mutations.

–Functions in degradation of Rh1

isomerases, cyclophilins, encoded by the *ninaA* locus [57–60]. NINAA is localized predominately in the ER and colocalizes with Rh1 in secretory vesicles. Moreover, NINAA forms a stable and highly specific complex with Rh1, and gene dosage studies demonstrate a quantitative requirement for NINAA for Rh1 transport [60, 61]. These findings suggest that NINAA functions as a molecular chaperon for Rh1 rather than as a catalytic enzyme that promotes folding of rhodopsin. Nevertheless, two *ninaA* alleles display temperature-sensitive peptidyl–prolyl *cis-trans* isomerase activity, suggesting that NINAA may also function catalytically to process Rh1 through the secretory pathway [62].

The translocation of rhodopsin also requires at least three Rab-GTPases, Rab1, Rab6, and Rab11, which mediate fusion of vesicles through interactions with effector molecules on the

target membranes. Rab1 is required for rhodopsin transport between the ER and Golgi complex [63], Rab6 functions in the transport through the ER–Golgi complex [64], and Rab11 mediates post-Golgi translocation of rhodopsin [65]. The function of Rab6 may be rhodopsin-specific, whereas Rab1 and Rab11 operate more generally in the trafficking of membrane proteins in photoreceptor cells [63–65].

Reminiscent of vertebrate rhodopsins, Rh1 undergoes N-linked glycosylation during biosynthesis [66]. The glycosylation takes place at single site (asparagine 20) and is absent from the mature form of Rh1 [67–69]. Mutation of asparagine 20 decreases the level of mature Rh1 and results in retention of this protein in the secretory pathway. In contrast to many proteins that require N-linked glycosylation for interactions with chaperons, N-linked glycosylation of Rh1 does not affect binding to its chaperon, NINAA [69].

Fig. 4 Rhodopsin cycle. Rhodopsin is converted into metarhodopsin by light. The receptor is subsequently phosphorylated through action of the GPRK1 and binds arrestin. The conversion of the light-activated metarhodopsin to the rhodopsin requires exposure to a second photon of light. The release of arrestin from the receptor requires $\text{Ca}^{2+}/\text{CaM}$ dependent phosphorylation of Arr2 by CaMKII. The dephosphorylation of the receptor is mediated by RDGC, which is a $\text{Ca}^{2+}/\text{CaM}$ -dependent protein phosphatase

Another ER transmembrane molecular chaperon, calnexin, is required in photoreceptor cells specifically for rhodopsin maturation [70]. Given that other calnexins interact with monoglycosylated glycans present in folding intermediates of glycoproteins, it is plausible that the *Drosophila* calnexin facilitates the transport of Rh1 through the secretory pathway by binding to the N-linked oligosaccharide attached to asparagine 20 [70].

Genes involved in chromophore biosynthesis: *nina*, *pinta* and *santa maria*

Since rhodopsin is comprised of an opsin and a chromophore, disruption of chromophore biosynthesis prevents

production of rhodopsin. In *Drosophila*, defects in the generation of the chromophore cause profound reductions in opsin levels [71], while this is not the case in vertebrate rods and cones [72]. It has been suggested that the chromophore accelerates opsin maturation by facilitating deglycosylation and transport of the opsin [73, 74].

In *Drosophila*, dietary β -carotene is the major substrate for production of vitamin A (all-*trans* retinol), and the vitamin A is subsequently converted into the chromophore (11-*cis* 3-hydroxyretinal; Fig. 5). Vitamin A is not required for viability in fruitflies but appears to function exclusively in the synthesis of the visual pigments [73]. As such, mutations affecting vitamin A production can be identified by screening for flies exhibiting a reduced PDA.

Three genes have been characterized that function in the conversion of β -carotene to vitamin A. Each function outside of the retina and the corresponding mutant phenotypes are rescued by supplementation of the food with vitamin A. The *ninaB* gene, which encodes a β , β' -carotene-15,15'-monooxygenase (BCO), catalyzes the centric cleavage of carotenoids to all-*trans* retinal (Fig. 5) [71, 75, 76]. Two other genes, *ninaD* [71, 77] and *santa maria* (scavenger receptor acting in neural tissue and majority of rhodopsin is absent) [78] encode proteins related to mammalian class B scavenger receptors. As such, NINAD and SANTA MARIA may mediate the transport of β -carotene into cells [77–79].

Table 2 Expression patterns and absorption maxima of the *Drosophila* rhodopsins

Rhodopsin	Expression pattern	Peak sensitivity of rhodopsin (nm)	Peak sensitivity of 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

Fig. 5 Model for the conversion of dietary β -carotene to the chromophore. The NINAD scavenger receptor is required for absorption of β -carotene into the midgut, which is subsequently taken up into extra-retinal neurons and glia in the head via the SANTA MARIA scavenger receptor and cleaved by the NINAB BCO. Vitamin A (all-*trans* retinol) is subsequently transported to the retinal pigment cells where it is converted to the chromophore through a process dependent on the PINTA retinoid-binding protein and the NINAG oxidoreductase. The chromophore is transported to the photoreceptor cells where it binds to the opsin resulting in the generation of rhodopsin

The question arises as to the identity of the scavenger receptor that may function in concert with NINAB. Although NINAD has been suggested to play such a role [80], the different expression patterns of *ninaB* and *ninaD* make this hypothesis unlikely [78]. Rather, *santa maria* and *ninaB* are expressed and function in the same extraretinal glia and neuronal cells in fly heads. Therefore, SANTA MARIA appears to mediate uptake of carotenoids from circulation into both neuronal and glia cells, thereby providing the substrates for processing of carotenoids to

retinal by NINAB [78]. NINAD appears to function in the uptake of carotenoids primarily in the midgut [78].

The subsequent conversion of vitamin A to the chromophore takes place in the retina and two genes that function in this transformation have been identified. The *pinta* (*PDA is not apparent*) locus [81] encodes a retinoid binding protein, which binds preferentially to all-*trans*-retinol, and is required in retinal pigment cells for production of the chromophore (Fig. 5). Since in mammals the transformation of dietary vitamin A to 11-*cis*-retinal occurs in the retinal

pigment epithelium (RPE) [72], it appears that *Drosophila* retinal pigment cells are the closest functional equivalent to the RPE. The *ninaG* gene encodes an oxidoreductase, which is proposed to act in the conversion of (3R)-3hydroxyretinol to the 3S enantiomer in the compound eye [82, 83]. However, it is not known whether *ninaG* functions in the retinal pigment cells or in photoreceptor cells. The conversion of all-*trans* retinal to 11-*cis* retinal also takes place in retinal tissues and does so in a light- rather than an enzyme-dependent manner [84, 85].

The combination of genetic and biochemical analyses permits a pathway to be proposed, which involves multiple cell types, in the conversion of dietary carotenoids to the rhodopsin chromophore (Fig. 5). The pathway begins with the uptake of dietary carotenoids into the midgut, through a process that involves the scavenger receptor NINAD. Instead of being metabolized in the midgut, β -carotene is delivered to neurons and glia through circulation and taken up into these cells via the SANTA MARIA scavenger receptor. The NINAB BCO subsequently functions in the same neurons and glia as SANTA MARIA in the centric cleavage of β -carotene to retinal [78]. The all-*trans*-retinol is then transferred to the retinal pigment cells, where it is converted into the chromophore, through a process involving the PINTA retinoid binding protein and the NINAG oxidoreductase [81, 82]. This proposed pathway is not yet complete as many factors are still unknown. For example, in the mammalian RPE there are several alternative reactions through which all-*trans* retinol is ultimately converted into the chromophore [86]. However, the proteins involved in the corresponding metabolic events have not been identified in *Drosophila*, including the relevant retinal dehydrogenases. Candidate dehydrogenases that may function in retinal pigment cells are three eye-enriched short-chain dehydrogenases, which were identified in a microarray analysis for genes preferentially expressed in the retina [31].

The rhodopsin cycle in *Drosophila*

Light activation of rhodopsin results in a *cis* to *trans* isomerization of the chromophore followed by phosphorylation of the receptor and binding of arrestin (Fig. 4). As is the case with the vertebrate rhodopsins, light-activated Rh1 is phosphorylated in a cluster of serine–threonine residues located in the C-terminal tail [87–89]. In *Drosophila*, phosphorylation of rhodopsin is catalyzed by a protein kinase (G-protein-coupled kinase 1; GPRK1), which bears greater amino acid sequence identities to mammalian β -adrenergic receptor kinases than to mammalian rhodopsin kinase [89]. Furthermore, the role of Rh1 phosphorylation by GPRK1 is more akin to the regulation of hormonally stimulated G-protein receptors by β -adrenergic receptor kinases than phosphorylation of mammalian rhodopsins by

rhodopsin kinase. Phosphorylation of Rh1 modulates the amplitude of the visual response because an increase or decrease in GPRK1 activity results in smaller or larger responses, respectively [89].

The termination of rhodopsin activity depends on binding of arrestin, which causes displacement of the $G\alpha_q$ subunit. It is proposed that the rate of arrestin binding determines the kinetics of rhodopsin inactivation in vivo [90]. In *Drosophila*, there are two arrestins expressed in photoreceptor cells, arrestin1 and arrestin2 (Arr 1 and Arr 2) [91–93]. Arr2, the major isoform, comprises ~85% of the total arrestin, whereas the remaining 15% is Arr1 [94]. Elimination of Arr2 significantly decreases the deactivation rate of the photo-response, whereas mutation of *arr1* has little effect [95]. Nevertheless, Arr1 may also participate in deactivation since termination is more severe when both *arr1* and *arr2* are mutated [95]. Unlike the case with the vertebrate rhodopsins, the binding of Arr2 to Rh1 is not dependent on phosphorylation of Rh1 [96–98]. However, phosphorylation of the C terminus of rhodopsin contributes to Arr1 binding and this interaction promotes endocytosis of Rh1, which is proposed to play a role in scavenging spontaneously activated, phosphorylated metarhodopsin [99].

The blue-light-induced PDA requires a molar excess of the Rh1 metarhodopsin over the available arrestin [95]. Normally, the concentration of Rh1 is fivefold higher than arrestin [94, 100]. Mutations that reduce the Rh1 levels to <20% of normal levels result in a molar excess of arrestin, thereby preventing a PDA. A PDA can be restored in flies expressing low levels of Rh1 if the Arr2 levels are also reduced [95]. If the Rh1 concentration is normal, but only the arrestin levels are reduced, a PDA is generated with less light intensity than in wild-type [95]. The minor rhodopsins are also bistable pigments. Misexpression of a minor opsin in the R1–6 photoreceptor cells, in the absence of Rh1, results in the production of a PDA according to the spectral characteristics of the minor rhodopsin [54, 56, 101]. For example, introduction of Rh2 in the R1–R6 cells results in a PDA that is produced at ~420 nm and terminated at ~520 nm, respectively.

After exposure of the metarhodopsin to a second photon of light, the regeneration of rhodopsin requires the release of Arr2 and dephosphorylation of rhodopsin (Fig. 4). Both of these steps are regulated by Ca^{2+} /calmodulin (CaM). Arr2 is phosphorylated by Ca^{2+} /CaM-dependent kinase II and this modification is required for dissociation of the rhodopsin/Arr2 interaction [87, 102–105].

Once the arrestin is released, rhodopsin is dephosphorylated by a serine/threonine protein phosphatase encoded by the *retinal degeneration C* (*rdgC*) locus [30, 96, 104, 106]. Maximal activity of RDGC requires direct interaction with Ca^{2+} /CaM, which relieves inhibition of the phosphatase activity by an autoinhibitory domain [107]. Mutation of

rdgC causes hyperphosphorylation of rhodopsin and consequently leads to a notable decrease in the rate of photoresponse deactivation. Normal deactivation kinetics is restored in the *rdgC* mutant flies by replacing wild-type Rh1, with the truncated version, Rh1 Δ^{356} which is missing the C-terminal phosphorylation sites [96]. It is proposed that the C terminus of rhodopsin functions as an autoinhibitory domain and deletion of the Rh1 C terminus may abrogate the requirement for Rh1 phosphorylation for maximal activity [96]. However, an alternative possibility is that hyperphosphorylation of rhodopsin in the *rdgC* mutant stabilizes the metarhodopsin state.

Based on the work described above, a model for the rhodopsin cycle can be formulated (Fig. 4). Absorption of the first photon converts rhodopsin into metarhodopsin, which is subsequently phosphorylated by GPRK1. Phosphorylated metarhodopsin is thermally stable and binds to arrestin, which promotes deactivation. After absorption of the second photon, the metarhodopsin is converted back to inactive rhodopsin, the arrestin dissociates, and the rhodopsin is dephosphorylated by RDGC.

G_q and PLC

In contrast to the phototransduction cascade in rods and cones, the pathway in *Drosophila* photoreceptor cells functions through phosphoinositol signaling. A similar cascade may be utilized in ipRGCs, although definitive evidence is lacking [11].

G_q protein in phototransduction

The effector for the light-activated rhodopsin is the heterotrimeric G-protein, G_q and it is encoded by *Gα_q* [108–110], *Gβ_e* [111, 112], and *Gγ_e* [113, 114], all of which are eye-enriched. After exchange of the guanosine diphosphate (GDP) bound to the Gα_q for GTP, the Gα_q and Gβγ subunits dissociate and the activated Gα_q-GTP binds to the PLC encoded by *norpA* (*no receptor potential A*) [5, 115]. The Gα_q is critical for the photoresponse since the *Gα_q* mutant (*dG_q¹*) shows a 1,000-fold loss in light sensitivity [110]. However, *dG_q¹* is not a null allele and a small residual light response remains.

The Gβγ subunit also affects activation of the PLC, but this appears to be indirect. A strong allele of *Gβ_e* mutant, *Gβ_e¹*, displays a ~100-fold reduction in light sensitivity [111], and a mutation in the farnesylation site of *Gγ_e* also results in a reduction in light sensitivity [114]. Moreover, light-simulated PLC activity is deficient in the *Gβ_e¹* mutant [116]. The contribution of the Gβγ complex to activation seems to be indirect through interaction with Gα_q, since unlike the Gα_q subunit, Gβγ does not physically interact

with NORPA [115]. The Gβγ subunit is also essential for targeting of Gα_q to the membrane [117, 118], and a twofold excess of Gβ_e over Gα_q in wild-type flies prevents spontaneous activity of photoreceptor cells, presumably by suppressing rhodopsin-independent activation of the Gα_q [117]. Since low spontaneous activity is required for high light sensitivity, a decrease in the concentration Gβγ subunit appears to reduce light sensitivity by altering the localization of the Gα_q subunit and increasing spontaneous activity of the Gα_q.

The Gβγ subunit is also required for termination of phototransduction, as the rate of deactivation of photoresponse is decreased in *Gβ_e¹* flies [111]. Other Gβγ subunits have been shown to be necessary for phosphorylation of GPCRs by recruiting GPCR kinases to the plasma membrane [119]. Since the *Drosophila* rhodopsin kinase, GPRK1, interacts with Gβ_e [89], it is possible that Gβ_e accelerates deactivation of rhodopsin by promoting phosphorylation of rhodopsin.

NORPA exhibits dual phospholipase C and GAP activities during phototransduction

The NORPA PLC, which is activated by the Gα_q-GTP, catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) to generate inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG; Fig. 6a) [5, 91]. The critical role for NORPA for activation is illustrated by the observation that strong alleles of *norpA*, such as *norpA^{P24}*, abolish the photoresponse (Fig. 3b) [5]. Weak alleles result in decreases in light sensitivity and activation rates, which are linearly dependent on the levels of PLC activity [120, 121]. Decreases in NORPA levels also result in slower termination of the photoresponse. This latter phenotype reflects a second role for NORPA as a GTPase activating protein (GAP) for the Gα_q [122], similar to that originally demonstrated for a mammalian PLCβ [123]. Thus, NORPA functions both in the activation of phototransduction and as a negative regulator of phototransduction.

At least two different forms of PLCs are encoded by *norpA*, only one of which (subtype I) is primarily expressed in the retina [5, 124, 125]. As is typical of other phospholipase Cβ proteins, both NORPA isoforms have a membrane binding PH domain, a Ca²⁺ interacting EF-hand, a divided phospholipase catalytic region consisting of X and Y core domains, and a putative Gα_q binding C2 domain (Fig. 6b). In addition, NORPA has a site that binds the INAD scaffold protein.

Consistent with the domain organization of NORPA, the PLC activity is regulated by Ca²⁺, both positively and negatively. Elevations in Ca²⁺ levels in the low range (10 nM to 1 μM) augment PLC activity, while higher concentrations of Ca²⁺ (>10 μM) suppress activity [91, 116]. Using a PIP₂ sensor to monitor NORPA activity in vivo, the

Fig. 6 The PIP₂ regeneration cycle and the domain organization of NORPA and TRP. **a** PIP₂ regeneration cycle. PIP₂ is hydrolyzed to IP₃ and DAG through action of NORPA. The DAG is converted into PIP₂ through a multistep process that involves the concerted actions of the RDGA DAG kinase (DGK), the CDP-diacylglycerol synthase (CDS), the PI synthase (PIS), and two PI kinases that have not been identified definitively. However, a candidate PIP kinase is encoded by the *skittles* (*sktI*) locus [32, 240]. A PI transfer protein (RDGB) is proposed to promote the transfer of PIs from the subrhabdomeral cisternae to the rhabdomeres. PIP₂ is converted into PIP₃ by phosphoinositide 3-kinase (PI3K), and back to PIP₂ through the activity of the PTEN phosphatase. A second pathway for production of DAG involves the PLD catalyzed

cleavage of phosphatidylcholine (*PC*) to generate phosphatidic acid (*PA*), and a PA phosphatase (*Laza*) promoted conversion of *PA* to DAG. **b** Domain organization of NORPA (PLC). Indicated are a possible membrane binding PH domain, an EF-hand, a split phospholipase catalytic region (X and Y core domains), a putative Gα_q binding C2 domain, which may bind Gα_q, and an INAD binding site. The *small rectangle* adjacent to the C-terminal end of the PH domain represents a region containing 14 amino acid differences between NORPA subtypes I and II. **c** Domain organization of TRP. The *six vertical rectangles* indicate the transmembrane segments. Four ankyrin repeats are situated near the N-terminus. The TRP domain is conserved among all TRPC proteins and some other members of the TRP superfamily [241]

PLC activity is inhibited by Ca^{2+} levels $\geq 50 \mu\text{M}$ [126, 127]. This rapid inhibition of NORPA PLC activity by local Ca^{2+} transients is proposed to prevent PIP_2 depletion during light stimulation [126, 127].

A positive regulator of NORPA activity has been proposed to be Rolling Blackout (RBO), which is distantly related to DAG lipases [128]. Temperature sensitive alleles of *rbo* result in loss of the photoresponse, if the flies are maintained at the restrictive temperature. An indication that PLC activity is lost in the *rbo* mutant is that PIP_2 levels actually rise and the DAG levels decline in response to light, which is the opposite of what should occur if PLCs were active. Whether RBO is a DAG lipase and how it functions in the regulation of NORPA or in the PIP_2 regeneration pathway, described in the following section, remains unresolved.

Regeneration of PIP_2 and dual pathways for production of DAG

Since hydrolysis of PIP_2 is catalyzed by NORPA, the PIP_2 must be rapidly replenished to maintain a sustained light response (Fig. 6a). A PI transfer protein and several of the enzymes that function in the PIP_2 regeneration cycle have been characterized. In those cases in which the proteins have been spatially localized, they are found in the subrhabdomeral cisternae (SRC) situated at the base of the rhabdomeres, or in other vesicles in the extra-rhabdomeral cell bodies [129–131].

Mutations in the first three enzymes that function in the conversion of DAG to PIP_2 have been identified. These include the DAG kinase (RDGA), cytidine diphosphate (CDP)-DAG synthase (CDS) and the PI synthase (PIS), which catalyzes the production of phosphatidic acid (PA), CDP-DAG, and PI, respectively (Fig. 6a) [28, 29, 131–133]. In addition, a PI transfer protein (RDGB) may function in the transfer of PIs from the SRC to the rhabdomeres [29, 134, 135]. If the levels of CDS or PIS are reduced but not eliminated, a light response can still be obtained if the flies are dark-adapting [131, 132]. However, the flies are not able to sustain a light response. Conversely, strong *rdgA* alleles display uncontrolled activity in the photoreceptor cells, even in the dark [136]. The implications of these findings are discussed below in the context of the mechanism of activation of the TRP and TRPL channels.

In addition to hydrolysis of PIP_2 by NORPA, there is a second pathway for production of DAG in fly photoreceptor cells. This alternate mechanism involves the concerted action of a phospholipase D (PLD), which cleaves phosphatidylcholine (PC) to generate PA, and a PA phosphatase, referred to as Lazaro (Laza), which subsequently converts the PA to DAG (Fig. 6a) [32, 33, 137]. As with the enzymes involved in regeneration of PIP_2 , the PLD and Laza are spatially localized to vesicles in the cell bodies [32, 137]. Mutations

that disrupt the *pld* or *laza* genes diminish the light response indicating that the DAG produced from this second pathway is required for a maximum light response [33, 137]. In addition, the rate of termination of the photoresponse is faster in the *laza* mutant flies than in wild-type flies [33], suggesting that DAG production under the control of Laza slows down response termination.

The TRPC cation channels

Activation of NORPA leads to opening of at least two TRPC channels, TRP and TRPL, which are ~50% identical over the N-terminal 700 residues. The TRP channel (Fig. 6c) is the founding member of the TRP superfamily of cation channels [6, 138], and mutation of *trp* results in a transient response to light (Fig. 3c) and a ~10-fold decrease in Ca^{2+} influx [8, 139]. Disruption of *trpl* alone results in a decreased response to long light stimulation, but the phenotype is much more subtle than the transient response that is characteristic of *trp* flies [140]. In addition, the *trpl* mutant displays a defect in adapting to dim background illumination [140]. Upon elimination of both *trp* and *trpl*, the flies are unresponsive to light [9, 141].

The subunit composition of the phototransduction channels may consist of TRP homotetramers and TRP/TRPL heteromultimers, although this issue remains controversial. TRP would appear to be present primarily as a homomultimer since it is ~10-fold more abundant than TRPL [142]. Analyses of TRP in the absence of TRPL (analyzed in the *trpl* mutant) indicates that the TRP-dependent current is relatively Ca^{2+} selective ($P_{\text{Ca}}:P_{\text{Na}}=100:1$), with a conductance of 4 pS [141]. The TRPL-dependent current (analyzed in the *trp* mutant) is nonselective ($P_{\text{Ca}}:P_{\text{Na}}=4.1$) and has a higher conductance than TRP (35 pS) [141]. Evidence that TRP and TRPL interact is that they coimmunoprecipitate from fly head extracts and interact in vitro [142]. In addition, TRPL can increase the concentration and activity of TRP when it is not bound to the scaffold protein, INAD [140]. TRPL is constitutively active in vitro; however, TRP/TRPL heteromultimers are regulated channels [142]. However, it has been argued that TRP and TRPL function as two independent homomultimers since the wild-type conductance can be accounted for by the summation of the conductances in the *trp* and *trpl* mutants [141]. An additional possibility is that the channels consist of TRP and TRPL homomultimers and TRP/TRPL heteromultimers. A third TRPC channel, TRP γ , can potentially interact with TRPL [143]. However, it is unclear whether TRP γ contributes to the light response, as no loss of function allele of *trp γ* has been described.

An important issue is the mechanism through which TRP and TRPL are activated. Since NORPA is required for activation of the channels, it would appear that gating

requires production of either IP₃, DAG, or a decrease in inhibitory PIP₂. A variety of studies indicate that IP₃ is not the critical second messenger. Release of caged IP₃ does not mimic the light response [144], and mutation of the only IP₃-receptor encoded in the *Drosophila* genome has no impact on phototransduction [145, 146]. Furthermore, IP₃ does not activate TRPL in an in vitro expression system [147]. A reduction in PIP₂ levels also does not appear to be sufficient for activation of the TRP channels in photoreceptor cells since a mutation in the only *Drosophila* PI synthase, which is required for regeneration of PIP₂, leads to a loss of the photoresponse rather than constitutive activation of the channels [131].

It appears that DAG, or a DAG metabolite, is critical for activation of the TRPC channels. In support of this conclusion, introduction of either DAG or PUFAs, such as linolenic acid, results in activation of the channels in photoreceptor cells or in vitro [148]. The channels are constitutively active in the *rdgA* mutant, which is missing the DAG kinase that metabolizes DAG to PA [136]. Conversely, in a mutant background (*laza*) that removes the second PLD/PAP pathway for production of DAG (Fig. 6a) [32, 33], the light response is smaller [33]. The faster termination of the photoresponse in the *laza* mutant also suggests that Laza-dependent production of DAG contributes to TRPC channel activation. Taken together, these results suggest that DAG is the essential second messenger necessary for activation of TRP and TRPL.

A protein that appears to be required for TRP activity is INAF since the absence of INAF results in an ERG phenotype very similar to that observed in *trp* mutant flies [149]. Moreover, the photoresponse is nearly eliminated in *inaF*; *trpl* double mutant flies. The concentration of TRP is reduced to ~15% of wild-type levels in the *inaF* mutant, and this effect appears to be specific as the levels of other proteins analyzed are unaffected. The *inaF* phenotype is not due simply to the lower concentration of TRP, since expression of TRP at 10% of the normal levels is sufficient to elicit a wild-type ERG [150]. INAF has no clear homology to other proteins or predicted transmembrane domains, and the mechanism through which it regulates TRP function remains to be elucidated. Nevertheless, INAF is not a chaperone required for rhabdomere localization since TRP displays a typical rhabdomere localization pattern in *inaF* mutant photoreceptor cells [149].

Extrusion of Ca²⁺ via the CalX Na⁺/Ca²⁺ exchanger

Within 20 ms of light stimulation, the Ca²⁺ concentration in the rhabdomeral microvilli can rise from a resting Ca²⁺ level of ~160 nM, to reach peak values higher than 200 μM, before declining to a steady-state of ~20 μM [151–153]. To

accomplish the dynamic changes in Ca²⁺ levels during the photoresponse, a rapid Ca²⁺ extrusion mechanism is required to counter the TRP-dependent influx of Ca²⁺. A Ca²⁺ efflux mechanism is also necessary to reset the normal resting Ca²⁺ levels after light stimulation. In *Drosophila* photoreceptor cells, a Na⁺/Ca²⁺ exchanger, CalX (Fig. 1) [154–156], colocalizes with TRP in the rhabdomeres and is the critical protein that mediates extrusion of Ca²⁺ [157]. As is typical of other Na⁺/Ca²⁺ exchangers, CalX extrudes one Ca²⁺ ion in exchange for entry of three Na⁺ ions [154–156].

Analyses of the *calx* mutant, as well as flies overexpressing *calx*, show that Ca²⁺ extrusion through the Na⁺/Ca²⁺ exchanger is required for many events during phototransduction [157] and support other studies indicating that Ca²⁺ both facilitates and inhibits different aspects of the photoresponse [25]. Loss of *calx* results in an inability to sustain a photoresponse of normal amplitude (Fig. 3d), hyperadaptation, possibly due to Ca²⁺-mediated inactivation of the TRP channels, and decreased amplification [157].

Signal amplification requires the G_q and NORPA

A phenomenon in both vertebrate and invertebrate phototransduction is that there is considerable signal amplification. The extent of amplification can be assessed by assaying the size of the quantum bumps (typically ~10 pA), which is defined by the number of TRPC channels opened by a photon of light. In flies, ~25 channels appear to be activated by a single photon [25]. Since each *Drosophila* microvillus may contain ~25 channels, it has been proposed that the extent of amplification of the cascade may be dictated by the size of the microvillus [24, 25]. Indeed, it has been reported that many TRP channels are gated in concert during the light response [158]. An important regulator of amplification is Ca²⁺, as the size of the quantum bumps is smaller in the *calx* mutant and larger in flies overexpressing *calx* [157].

A question concerns the steps in the cascade at which amplification takes place. According to one group, amplification occurs primarily downstream of PLC since mutations that reduce either the Gα_q or PLC concentration have little effect on the size of the quantum bumps [159]. However, a subsequent study showed that the quantum bump sizes were reduced in both *Gα_q* and *norpa* hypomorphs [26]. The reduced amplitude can be restored in either mutant by inhibiting the metabolism of DAG through mutation of the DAG kinase encoded by *rdgA* [26]. Since the DAG kinase activity is adenosine triphosphate (ATP)-dependent [26] it is suggested that the earlier results, which conclude that amplification is subsequent to the activity of NORPA [159], is probably due to omission of ATP from the recording electrode [26]. Thus, it appears that amplification occurs at the levels of the G_q and PLC. The results by Hardie

et al. [26] also lend further support to the conclusion that DAG is an excitatory second messenger for the TRPC channels.

Ca²⁺-dependent termination of the photoresponse

Ca²⁺ is the critical second messenger that regulates termination of the photoresponse in *Drosophila* [4, 160, 161]. The central role for Ca²⁺ in response termination is illustrated by the observations that the termination time is increased either by removing or lowering extracellular Ca²⁺ from the bath solution during whole-cell recordings from photoreceptor cells [2, 4, 25] or by overexpressing the Na⁺/Ca²⁺ exchanger, CalX [157]. Conversely, the kinetics of termination is faster if the Ca²⁺ levels are elevated in the photoreceptor cells either by increasing extracellular Ca²⁺ during whole-cell recordings, or by genetically removing *calx* [2, 25, 157]. Several proteins that mediate the Ca²⁺-regulated termination have been identified, which include protein kinase C (PKC), CaM, and the myosin III referred to as NINAC.

Members of the PKC family of serine/threonine protein kinases are activated by Ca²⁺ and DAG, and an eye-enriched PKC encoded by the *inaC* (*inactivation nor afterpotential C*) locus [162] is localized to the rhabdomeres and is required for deactivation of the visual cascade [2, 163]. Ca²⁺ influx through the TRP channels is much higher in the *inaC* mutant than in the wild-type, indicating that INAC is required for inactivation of the TRP channels [161]. TRP is phosphorylated by INAC [164, 165] and a mutation that abolishes the primary INAC-dependent phosphorylation site in TRP causes slow termination of the light response [166]. These data suggest that INAC contributes to deactivation, at least in part by directly phosphorylating the TRP channel.

Deactivation in the *inaC;trp* double mutant is slower than in *trp* flies, indicating that TRP is not the only functional target of INAC [163]. The NINAC myosin III, which consists of linked protein kinase and myosin domains [23], is also phosphorylated by PKC in vitro [167]. Mutation of the PKC-dependent phosphorylation sites in NINAC results in an unusual defect in deactivation, suggesting that phosphorylation of NINAC by INAC is required for stable termination of visual cascade [167].

A universal regulator of Ca²⁺ signaling is the Ca²⁺-binding regulatory protein CaM, which in the photoreceptor cells is enriched in the rhabdomeres [168]. The subcellular distribution of CaM is dictated by the two NINAC isoforms, p174 and p132, which are localized specifically in the rhabdomeres and cell bodies, respectively [168, 169]. CaM is detected predominately in the cell bodies of photoreceptor cells that express only the p132 isoform and almost exclusively in the rhabdomeres of photoreceptor cells expressing just p174 [169]. A dramatic reduction in

rhabdomeral CaM is also observed if the CaM binding sites in p174 are mutated. NINAC p174 binds CaM through two sites, C1 and C2. The C1 site is common to both p132 and p174, whereas C2 is unique to p174. Elimination of either or both sites in p174 results in reduced levels of CaM in the rhabdomeres and slow termination of the photoresponse [168, 169]. These data suggest that the defect in response termination is a consequence of low rhabdomeral CaM. However, the results do not exclude that the defect in these flies reflects a role for p174 in response termination, independent of the effects on CaM distribution.

In *Drosophila*, CaM is encoded by a single gene, *cam* [170, 171], and null mutations in *cam* are lethal [172]. Mosaic flies missing *cam* from the eye display developmental defects. Therefore, the role of *cam* during the photoresponse was analyzed in homozygous viable hypomorphic alleles, which express a tenfold reduction of CaM [159]. Consistent with the studies on NINAC p174, the *cam* hypomorphs display a defect in response termination [159].

CaM is likely to regulate termination of the photoresponse through multiple mechanisms, including effects on the rhodopsin cycle (Fig. 4), and modulation of the cation influx channels. Both TRP [173] and TRPL [7, 174] bind CaM through C-terminal sequences in vitro. In the absence of TRP, expression of a TRPL isoform missing either of two CaM binding sites leads to remarkably slower termination than that observed in photoreceptors expressing wild-type TRPL only [159]. Thus, it appears that TRPL (and possibly TRP) are physiologically relevant targets for CaM during termination of the photoresponse.

A novel mechanism for Ca²⁺/CaM mediated response termination involves a CaM binding transcription factor, dCAMTA, and its transcriptional target, *dFbx14*, which encodes an F-box protein [175]. Mutations in *dcamta* result in slow termination of the photoresponse, and this phenotype is reversed by overexpression of *dFbx14* [175]. F-box proteins related to dFBX14 function in ubiquitination [176], and some GPCRs are regulated by ubiquitination [177]. Therefore, it is proposed that dFbx14 functions to ubiquitinate rhodopsin, which in turn interferes with activation of the Gα_q [175]. Loss-of-function mutations in *dFbx14* and the mechanisms that regulate its activity remain to be described.

Adaptation

Short-term adaptation due to inhibition of the TRPC channels

Light adaptation is the phenomenon by which photoreceptor cells alter their sensitivities to light in response to changes in light intensities. Such a phenomenon contributes to the ability

of photoreceptor cells to maintain a response over a broad range of light intensities. Certain forms of adaptation occur over a long time-scale of a few or many minutes, and one such type of long-term adaptation is described below. Adaptation also takes place over the course of a few seconds, or even on a milliseconds time-scale. It appears that short-term adaptation is highly Ca^{2+} -dependent and does not depend on the eye-enriched PKC [127]. Rather, short-term adaptation occurs subsequent to the activation of the PLC and results from Ca^{2+} -dependent inhibition of the TRP channels [127]. Since this form of adaptation is independent of PKC, it may occur through the CaM that binds to TRP and TRPL; however, this remains to be determined.

Long-term adaptation through Light-dependent translocation of signaling proteins

One mechanism for long-term adaptation in photoreceptors involves dynamic light-dependent movements of signaling proteins into and out of the rhabdomeres. If the flies are maintained in the dark ~80% of the major arrestin (Arr2) and nearly all of the minor arrestin (Arr1) are localized to the cell bodies; however, after ~5 min of light exposure, most of the arrestin moves into the rhabdomeres (Fig. 7a) [97, 99]. This phenomenon of light-dependent translocation of arrestin was first described in mammalian rods [178–181]. In animals kept in the dark most of the arrestin is in the inner segment, and after a few minutes of light exposure the arrestin is primarily present in the outer segment (Fig. 7b).

Two questions arise from the light-stimulated shuttling of arrestin. What are the mechanisms underlying these movements, and what are the functions? In the case of *Drosophila* Arr2, trafficking is dependent on interactions with phosphatidylinositol 3,4,5-trisphosphate (PIP_3). Mutations in genes that function in PI metabolism or trafficking, such as *rdgB*, *cds*, and *PTEN*, impair light-dependent translocation of Arr2 [182]. Arr2 binds to PIP_3 in vitro and disruption of the Arr2/PI interaction by mutation of the PIP_3 binding site (*arr2*^{3K/Q}) results in defective translocation [182]. Flies expressing *arr2*^{3K/Q} show impairment in long-term adaptation. In wild-type flies, termination of the photoresponse is accelerated by exposure to light. This light-dependent increase in termination kinetics is defective in *arr2*^{3K/Q} flies [182].

The $\text{G}\alpha_q$ -protein also undergoes light-dependent movements, but they are opposite in direction to the arrestin translocation. In the dark, most of the $\text{G}\alpha_q$ is in the rhabdomeres, and then it moves to the cell bodies in the light over the course of a few minutes (Fig. 7a) [118, 183]. In the dark, the $\text{G}\alpha_q$ subsequently returns to the membrane as an inactive GDP form. The same direction and kinetics of movement occur for the G-protein, referred to as transducin, in mammalian rods/cones [184]. Upon exposure to light, the

transducin moves from the outer to inner segment, then traffics back to the outer segment in the dark (Fig. 7b).

As expected, the light-dependent shuttling of the *Drosophila* $\text{G}\alpha_q$ is dependent on rhodopsin; however, it does not require other known signaling proteins, such as NORPA [118, 183], Arr2, PKC, TRP, and TRPL [183]. Thus, there may be other signaling proteins that remain to be identified, which interact with rhodopsin and promote the movement of the $\text{G}\alpha_q$. The movement of the $\text{G}\alpha_q$ may be dependent on interactions with $\text{G}\beta\gamma$ subunit, as the kinetics of the light-dependent translocation of $\text{G}\alpha_q$ and the subsequent return to the rhabdomeral membrane are markedly slowed down in the *Gβ_e* mutant [118].

The TRPL channel, but not TRP, also displays light-dependent trafficking between the rhabdomeres and cell body. During light stimulation, TRPL moves from the rhabdomeres to the cell bodies with a half-time of ~30 min and then translocates back into the rhabdomeres in the dark (Fig. 7a) [185]. If the rhabdomeral TRPL is depleted by maintaining the flies in the light for many hours, there is an ensuing 20-fold increase in rhabdomeral TRPL after 60 min in the dark [185]. Thus, wild-type flies maintained in the dark have higher levels of rhabdomeral TRPL than flies recently exposed to light. If the flies are kept for long periods in the light, the light-dependent conductance is due almost completely to TRP. Since the Ca^{2+} selectivity of TRP is much higher than TRPL [141], this change in ratio of TRP and TRPL has physiological consequences. Dark-raised wild-type flies, which have the maximal level of rhabdomeral TRPL, are more sensitive to dim background lights than wild-type flies kept in the light or *trpl* flies maintained in the dark [185]. These data indicate that TRPL translocation has effects on long-term adaptation.

The mechanism of TRPL translocation appears to be different from the arrestins and $\text{G}\alpha_q$ since TRPL is an integral membrane protein. It appears that there are two stages of light-dependent trafficking out of the rhabdomeres. The first stage involves movement of TRPL to the apical membrane adjacent to the rhabdomeres (apical stalk), within ~5 min [186]. The second and slower stage, which takes place over ~6 h, results in localization to the basolateral membrane.

Both stages of the TRPL translocation require rhodopsin activity; however, the mechanisms are otherwise different. The first stage depends on the NORPA PLC but not the TRP channel [186]. Conversely, the second stage requires the TRP channel as well as the PKC encoded by *inaC* [186]. In flies expressing a constitutively active derivative of TRP [187], TRPL is detected primarily in the basolateral membrane even in the dark [186, 188]. The localization is not due to an obstruction in the movement of TRPL to the rhabdomeres since TRPL is found in the rhabdomeres of very young *Trp*^{P365} flies.

Fig. 7 Light-dependent movements of signaling proteins.

a Translocation of signaling proteins in *Drosophila* photoreceptor cells. In the dark, arrestin1 and arrestin2 (*arrestin*) are mostly in the subrhabdomeral cell body, and $G\alpha_q$ and TRPL are primarily situated in the rhabdomeres. After a few minutes of exposure to light, the arrestins translocate to the rhabdomeres, and the $G\alpha_q$ and TRPL move to the subrhabdomeral cell bodies. The *large white ovals* represent the nuclei. **b** Translocation of signaling proteins in mammalian rods. In the dark, most of the arrestin is in the inner segment and the transducin (G_t) is the outer segment. In the light, the arrestin translocates to the outer segment, and the transducin to the inner segment. The *white ovals* represent nuclei

The dark-mediated re-entry of TRPL into the rhabdomeres may be dependent in part on arrestin since TRPL was mislocalized in an *arr2* mutant [186]. Furthermore, the mislocalized TRPL was found in a pattern similar to that observed after the first stage of light-dependent translocation out of the rhabdomeres [186]. Thus, shuttling of TRPL back to the rhabdomeres may also be a two step process. However, in a second study, elimination of both *arr1* and *arr2* caused a defect in light-induced movement out of the rhabdomeres, rather than dark-mediated shuttling into the rhabdomeres [188]. The basis for this difference is unclear.

Another controversial issue concerns the role of the NINAC myosin III in light-dependent translocations of

signaling proteins from the rhabdomeres. According to one report, the light-induced translocation of Arr2 into the rhabdomeres is impaired in the *ninaC* null mutant *ninaC^{P235}* and in mutant flies (*ninaC^{Δ132}*) missing the cell body-specific p132 isoform [189]. Elimination of p174 alone has no defect, but this rhabdomere-specific isoform also contributes to light-induced translocation since disruption of both p132 and p174 results in a stronger phenotype than that observed in *ninaC^{Δ132}* [189]. The *ninaC^{Δ132}* flies display a long-term adaptation defect, consistent with the role of arrestin in long-term adaptation. Moreover, Arr2 and NINAC interact both in vivo and in vitro; however, the association is indirect. As in the case for Arr2, NINAC also binds PIs and

the Arr2/NINAC interaction is PI-dependent [182, 189]. Amino acid substitutions in Arr2 that disrupt PI binding also abolishes Arr2/NINAC interaction. Therefore, light-dependent translocation of Arr2 into the rhabdomeres requires PI-mediated interactions between Arr2 and NINAC p132 [189]. According to another study, the light-dependent shuttling of neither Arr1 nor Arr2 is dependent on NINAC [99]. The basis for this latter observation is unclear but could be due to a variety of differences in the experimental protocols, which remain to be resolved. Nevertheless, the translocation of the $G\alpha_q$ from the cell bodies to the rhabdomeres is also dependent on NINAC [183]. In the case of the $G\alpha_q$, this movement takes place in the dark.

NINAC would be predicted to be required only for movement into the rhabdomeres, since it may be a plus-end-directed motor, based on analyses of a mammalian myosin III [190–192], and the barbed ends of the actin filaments are at the distal end of the rhabdomeres [193]. Consistent with this proposal, NINAC is required for translocation of the $G\alpha_q$ and Arr2 from the cell bodies to the rhabdomeres, but not for the movement out of the rhabdomeres [183, 189]. However, trafficking of TRPL-eGFP out of the rhabdomeres is reported to be disrupted in the *ninaC* null mutant [188]. While this could be due to a secondary effect of retinal degeneration in the null mutant flies, no retinal degeneration takes place in *ninaC*^{Δ132} flies [194], which exhibit a defect in translocation of Arr2 [189].

The signalplex

Many of the proteins that function in *Drosophila* phototransduction are organized into a supramolecular signaling complex (the signalplex; Fig. 8). The central protein in the signalplex is the scaffold protein, INAD, which is comprised of five PDZ protein interaction modules. The “core complex” [195] consists of INAD and three proteins that bind directly to INAD: TRP, INAC (PKC) and NORPA (PLC) [173, 196, 197]. Each of the three target proteins that are included in the core complex is expressed at similar levels [196] and may always be present in the complex as they depend on INAD for normal localization in the rhabdomeres and protein stability [173, 198].

It turns out that there is a reciprocal requirement between TRP and INAD for normal spatial distribution. In addition to TRP depending on INAD, the rhabdomere localization of INAD is severely disrupted either in a *trp* null mutant background or in flies expressing a TRP derivative missing the C-terminal four residues, which are necessary for INAD binding [195, 199]. Since INAD is required for localization of TRP, PKC and PLC, the entire core complex is destroyed if TRP is eliminated or if the INAD binding site in TRP is deleted [195, 199]. These findings demonstrate that in

addition to serving as a Ca^{2+} -permeable channel, TRP also plays an important role as a molecular anchor. This additional role may account for the observations that TRP is present in approximately stoichiometric proportions with INAD [196], which is a level tenfold higher than is necessary for a normal light-response [150]. The mutual requirement for TRP and INAD for localization reflects mutual roles in retention in the rhabdomeres, rather than for targeting [195, 199].

In addition to the core binding proteins, several other proteins bind INAD. These include TRPL, Rh1, NINAC, CaM [200, 201], and the immunophilin, FKBP59 [202]. Noncore binding proteins do not depend on INAD for localization in the rhabdomeres [200, 201]. Nevertheless, the INAD-binding site in NINAC is sufficient to target a heterologous protein (β -galactosidase) into the rhabdomeres [201]. This localization is dependent on INAD, since the β -galactosidase-NINAC fusion protein remains in the cell body in *inaD*¹ null mutant flies. It would appear that some of the noncore binding proteins may interact dynamically with INAD since TRPL undergoes light-dependent translocation to the cell bodies, and Rh1 is more abundant than INAD. Unlike the other INAD-binding proteins, CaM binds to a linker region between two PDZ domains (1 and 2), rather than directly to a PDZ module [200]. More proteins bind to INAD than there are PDZ domains. INAD has the capacity to nucleate a large array of target proteins since it self-assembles into a polymer, and does so through different interaction interfaces in two PDZ domains than those involved in binding other target proteins [200].

A central issue concerns the functions of the INAD/target protein interactions. As described above, the core-binding proteins depend on INAD for localization in the rhabdomeres and for protein stability [173, 198]. It also seems plausible that nucleation of an array of signaling proteins in a complex would promote rapid signaling. However, mutation of the INAD binding site in TRP, which disrupts direct interactions between TRP and INAD, has no major impact on activation or termination of the photoresponse [195].

Interactions of signaling proteins with INAD appear to function in rapid termination of phototransduction. Mutation of the INAD binding site in the eye-enriched PKC (INAC) causes slower response termination [203]. Since the interaction with INAD is required for the normal stability and localization of INAC, it cannot be excluded that the phenotype may simply reflect reduced levels of rhabdomeral INAC. Disruption of the NINAC/INAD interaction, due to mutation of the INAD binding site in NINAC, also causes delayed termination [201]. Given that NINAC does not depend on INAD for protein stability or rhabdomeral localization this phenotype indicates that binding to INAD is essential for rapid response termination.

An additional potential function for the signalplex is that INAD might serve to compartmentalize protein kinases and

Fig. 8 The signalplex. The “core complex” consists of INAD, TRP, NORPA (PLC) and INAC (PKC). Members of the core complex depend on the signalplex for normal rhabdomere localization. Other members of the signalplex include rhodopsin (Rh1), TRPL, NINAC, CaM and FKB59

substrates into the same complex reminiscent of the complexes containing receptors for activated C-kinase (RACK) [204] and A-kinase anchoring proteins (AKAPs) [205]. At least three members of the signalplex are phosphorylated by PKC: NINAC, INAD and TRP [164, 165]. In an in vitro assay, PKC phosphorylation of TRP is promoted by INAD and mutation of the major PKC phosphorylation in TRP site results in a slower deactivation kinetics [166]. Furthermore, the deactivation time is slower in flies expressing INAD^{P215}, which is a derivative of INAD that does not effectively bind TRP [197, 206]. Taken together, these results suggest that INAD facilitates PKC phosphorylation of TRP in vivo, which is necessary for rapid termination. However, the *InaD*^{P215} mutation might affect interactions of INAD with multiple target proteins, and the termination of the photo-response is normal in flies expressing a derivative of TRP that does not bind INAD [195]. Thus, although it seems likely, it remains unclear whether INAD functions as a RACK-like protein in vivo.

Retinal degeneration

The *Drosophila* visual system has proven to be a powerful model for dissecting the molecular mechanisms underlying retinal degeneration. Mutations in almost any gene that functions in phototransduction result in photoreceptor cell death and the majority of the retinal degenerations are light-dependent. The molecular bases underlying the retinal degenerations are diverse (Table 3).

Defective rhodopsin folding, transport or activity causes retinal degeneration

The first mutations linked to retinal degeneration were in *ninaE*, which encode the major rhodopsin, Rh1 [40, 41,

207, 208]. This observation turned out to have relevance to human retinal dystrophies, as mutations in human rhodopsin were shown subsequently to account for a large proportion of the cases of autosomal dominant retinitis pigmentosa disease (ADRP) [209–213].

Since Rh1 plays a structural role in photoreceptor cells [214], in addition to functioning as a light-receptor, most loss-of-function *ninaE* alleles result in light-independent retinal degeneration [208, 215]. The degeneration in *ninaE* is not dependent on the signal transduction cascade as the severity of photoreceptor cell death mutation is not reduced by disruption of the PLC (NORPA), which is required for phototransduction [215, 216]. The cell death is associated with accumulation of membranes in the subrhabdomeral regions, although there is considerable variation in the severities of the phenotypes.

Many of the mutations in *ninaE* are dominant [216, 217], as is the case for human ADRP disease resulting from mutations in rhodopsin [209, 210, 218]. The dominance may be attributable to misfolding of the rhodopsin derivatives, which in turn interferes with the posttranslational maturation of wild-type rhodopsin [216, 217, 219]. As a consequence, the levels of mature Rh1 are dramatically reduced.

The dominant *ninaE* mutations primarily affect the maturation and transport of Rh1 into the rhabdomeres [216, 217]. In *Drosophila* photoreceptor cells, rhodopsin is synthesized and core-glycosylated in the endoplasmic reticulum (ER), transported through the Golgi, and delivered to the rhabdomeres where it functions in phototransduction. Among the mutations in rhodopsin that affect maturation is one (N20I) that disrupts the N-linked glycosylations site of Rh1 [69]. The Rh1^{N20I} protein is retained in the secretory pathway, resulting in accumulation of ER cisternae and retinal degeneration.

Mutations in loci encoding molecular chaperones, which are necessary for rhodopsin maturation, also cause retinal

Table 3 Mutations that lead to retinal degeneration

Mutation	Light dependent	Possible mechanism
<i>arr1</i>	No	Endocytosis of Rh1/Arr2 complex
<i>arr2</i>	Yes	Increased Ca^{2+} levels
<i>calx</i>	Yes	Increased Ca^{2+} levels
<i>cds</i>	Yes	Disruption of PIP_2 regeneration
<i>cnx</i>	Yes	Increased Ca^{2+} levels and low rhodopsin
<i>crumbs</i>	Yes	NK
<i>dpis</i>	Yes	Disruption of PIP_2 regeneration
<i>inaF</i>	NT	Reduction in TRP-dependent Ca^{2+} influx
<i>laza</i>	Yes	Disruption of PI cycle
<i>ninaA</i>	No	Low rhodopsin, accumulation of ER
<i>ninaC</i>	Yes	Increased Ca^{2+} levels due to low CaM
<i>ninaE</i>	No	Low rhodopsin, accumulation of ER
<i>NinaE</i> (dominant)	No	Interference in rhodopsin maturation
<i>norpA</i>	Yes	Endocytosis of Rh1/Arr2 complexes
<i>rdgA</i>	No	Constitutively active channels; Ca^{2+} overload
<i>rdgB</i>	Yes	Disruption of PIP_2 regeneration
<i>rdgC</i>	Yes	Endocytosis of Rh1/Arr2 complexes
<i>sun</i>	Yes	Defective rhodopsin degradation
<i>trp</i>	Yes	Endocytosis of Rh1/Arr2 complexes
<i>Trp</i> (dominant)	No	Ca^{2+} overload

The light-dependence and the possible mechanisms for the retinal generations are indicated.

NT, not tested; NK, not known

degeneration. One chaperone, NINAA, is a cyclophilin-related protein, which is thought to promote the proper folding of Rh1 [57, 58, 60]. In the absence of NINAA, Rh1 accumulates in the ER and retinal degeneration ensues. Mutations in the gene encoding another rhodopsin chaperone, *calnexin* (*cnx*), result in a phenotype bearing some similarities to *ninaA* mutant flies [70]. Rh1 accumulates in the ER and there is retinal degeneration; however, in contrast to *ninaA*, the retinal degeneration in the *cnx* mutant is enhanced in the presence of light. This latter aspect of the *cnx* phenotype most likely reflects an additional role of calnexin as a Ca^{2+} buffer [70]. Rab6 also appears to be required for rhodopsin maturation, since expression of a dominant negative form of Rab6 causes defective rhodopsin maturation, and trafficking and triggers retinal degeneration [64]. Taken together, these findings demonstrate that disruption in rhodopsin maturation leads to retinal degeneration.

There are at least two possible mechanisms through which defective rhodopsin maturation leads to photoreceptor degeneration. In null or very strong *ninaE* alleles, the retinal degeneration is a consequence of the structural requirement for rhodopsin during morphogenesis. Rhodopsin may comprise the majority of total membrane protein in the rhabdomeres, and complete absence of Rh1 during development results in architectural defects that initiate during pupal

development [215, 217]. Production of even small amounts of Rh1 is sufficient for production of normal rhabdomeres [217, 220]. However, the level of Rh1 and the degree of retinal degeneration do not always correlate among the dominant *NinaE* alleles [216, 221]. Rather, retinal degeneration due to dominant *NinaE* mutations appears to result from inhibition of rhodopsin trafficking, which leads to accumulation of ER cisternae and unfolded rhodopsin in the ER.

Constitutive or uncontrolled activity of rhodopsin can also lead to retinal degeneration, such as in the dominant allele, *NinaE*^{PP100} [222]. This retinal degeneration is partially suppressed by mutations in *arr2* or *Gα_q*, whereas complete suppression is achieved by disrupting both *arr2* and the *Gα_q*. Mutation of *arr2* also leads to light-dependent retinal degeneration presumably due to uncontrolled activity of rhodopsin [95, 103]. Arrestin is necessary for deactivation of rhodopsin, and the cell death in the *arr2* mutant is blocked by the *dG_q*¹ mutation [95] suggesting that it results from excessive activation of the phototransduction cascade. Loss of *arr2* also leads to decreased endocytosis of Rh1 as reviewed in the next section.

Endocytosis of stable Rh1/Arr2 complexes leads to retinal degeneration

It appears that during and after light exposure, a small proportion of the rhodopsin pool is removed from the rhabdomeral membrane and degraded, possibly as a quality control mechanism to dispose of photodamaged or constitutively active rhodopsin [98]. Abnormal increases or decreases in the rhodopsin turnover pathway appear to cause retinal degeneration. In wild-type flies, removal of Rh1 is initiated by interactions with Arr1 [99] and Arr2 [223] and subsequent endocytosis of Rh1, which may then be trafficked to lysosomes through direct interactions with a tetraspanin, Sunglasses (Sun), present in the membranes of late endosomes and lysosomes [31]. Mutations in either *arr1*, *arr2*, or *sun* result in retinal degeneration because of decreased endocytosis or degradation of rhodopsin. Thus, a defect in degradation of internalized rhodopsin may be toxic. The cell death resulting from loss of *arr2* is countered by overexpressing ceramidase [224, 225], which cleaves ceramide to produce sphingosine. The ceramidase may decrease the toxicity resulting from absence of Arr2 by increasing endocytosis [224, 225]. Unlike the light-dependent cell death in *sun* [31] and *arr2* flies [95], the degeneration in *arr1* is light-independent [99]. However, no degeneration occurs in *arr1;arr2* double mutant flies [99]. It is proposed that Arr1 scavenges active rhodopsin by endocytosis and thereby counters toxic Arr2/Rh1 accumulation, as described below [99].

The converse of retinal degeneration resulting from too little endocytosis of rhodopsin is the cell death that occurs

from excessive endocytosis due to stable interactions between Rh1 and Arr2. Normally, the binding of Arr2 to Rh1 is transient, as light-induced Ca^{2+} influx activates CaM kinase II, which in turn leads to phosphorylation of Arr2 and release from Rh1 [103, 105]. Mutations such as those in *trp* and *norpA* that prevent the normal rise in Ca^{2+} after light stimulation result in stable Rh1/Arr2 complexes and retinal degeneration. Elimination of the rhodopsin phosphatase, RDGC, also promotes stable Rh1/Arr2 complexes [97]. Disruption of Rh1/Arr2 stable complexes either in *norpA*, *trp* or *rdgC*, by genetic removal of Arr2, suppresses the retinal degeneration.

For the stable Rh1/Arr2 complexes to cause massive cell death, the Rh1 needs to be internalized, and this is dependent on dynamin and the endocytic adaptor protein, AP-2. Mutation of the dynamin GTPase (*shibire*), which prevents clathrin-mediated endocytosis, suppresses the retinal degeneration in the *rdgC* mutant [97]. Loss of the α subunit of AP-2 adaptor complex has been shown to suppress the retinal degeneration in the *norpA* mutant [223]. Moreover, mutations in *arr2* that disrupt the interaction between Arr2 and AP-2 prevent the retinal degeneration in *norpA* photoreceptor cells [223].

Retinal degeneration due to unregulated Ca^{2+} levels

Precise control over Ca^{2+} levels is essential for photoreceptor cell survival [226]. Either high or low levels of Ca^{2+} are toxic to the cells. A demonstration that abnormally high Ca^{2+} levels lead to retinal degeneration is that loss of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger, CalX causes light-dependent retinal degeneration. The severity of the retinal degeneration is greatly suppressed in the *calx, trp* double mutant [150]. The Ca^{2+} levels are also elevated in dark-adapted *calx* flies, but no degeneration takes place under these conditions. Thus, abnormally high Ca^{2+} levels do not trigger photoreceptor cell death unless it occurs during phototransduction.

Trp^{P365} flies, which express a constitutively active TRP channel, undergo retinal degeneration. Unlike *calx*, the *Trp^{P365}* flies exhibit retinal degeneration in the dark, although the cell death is more rapid under a light/dark cycle [187]. The severity of the *Trp^{P365}* phenotype is reduced by overexpressing CalX [157], consistent with the conclusion that the retinal degeneration in *Trp^{P365}* is a consequence of Ca^{2+} overload. Loss of *calnexin* (*cnx*) also results in retinal degeneration and this phenotype is due to increased Ca^{2+} levels in addition to the defect in Rh1 maturation as described above, since Cnx serves to buffer Ca^{2+} in the photoreceptor cell body [70]. The light-dependent retinal degeneration that results from elimination of the NINAC p174 isoform (myosin III) [194] may be due to higher free Ca^{2+} levels, since elimination of p174 dramatically decreases the concentration of rhabdomeral

CaM [168, 169], which is normally ~ 0.5 mM [168] and serves as the major Ca^{2+} buffer in the photoreceptor cells. Excessive Ca^{2+} influx appears to underlie the very strong retinal degeneration in *rdgA* flies, which are missing the eye-enriched DAG kinase. The basis for the increased Ca^{2+} levels in *rdgA* is described in the following section, which focuses on mutations in the PIP_2 regeneration pathway.

Abnormally low light-stimulated rises in Ca^{2+} levels can also lead to retinal degeneration. The cell death in *trp* flies is primarily due to the low Ca^{2+} influx rather than loss of the molecular anchoring function since deletion of the INAD binding site in TRP leads to very mild degeneration, while a mutation (*trp¹⁴*) that specifically causes rapid channel inactivation causes retinal degeneration as severe as that caused by null mutations in *trp^{P343}* [150]. Both the *trp¹⁴* and *trp^{P343}* phenotypes are suppressed by loss-of-function mutation in *calx* and by mutations in *arr2* [150]. Thus, the loss of TRP channel function may lead to stable Rh1/Arr2 complexes, and this could result from a defect in CaM kinase II-dependent phosphorylation of Arr2, which is necessary for dissociation of Arr2 from Rh1 [103, 105].

Disruption of PI cycle leads to retinal degeneration

Mutations that interrupt the PIP_2 regeneration cycle in *Drosophila* photoreceptor cells (Fig. 6a) cause retinal degeneration. The most profound effects on photoreceptor cell survival result from disruption of the eye-enriched DAG kinase, RDGA, which normally catalyzes the conversion of DAG to PA [133, 227]. The failure to metabolize DAG in *rdgA* induces constitutive activity of TRP and TRPL and light-independent retinal degeneration [28, 136]. Overexpression of the eye-enriched PA phosphatase, Laza (Laza), which catalyzes dephosphorylation of PA and generation of DAG, enhances both the increased activation of the photoresponse and the retinal degeneration in *rdgA* mutant, whereas mutation of *laza* suppresses both aspects of the *rdgA* phenotype [32, 33]. Therefore, the increased DAG appears to be the major reason for the retinal degeneration in *rdgA*. Consistent with the concept that DAG contributes to excitation during the photoresponse, the retinal degeneration in the *rdgA* mutant is suppressed by mutations in *trp* or *norpA* [136, 228]. Thus, it is likely that the retinal degeneration in *rdgA* is caused by excessive Ca^{2+} influx through constitutively active TRP channels.

Operating in opposition to RDGA is Laza, and mutations that eliminate this PA phosphatase result in light-dependent retinal degeneration [32, 33]. Laza might function in concert with the PLD to generate DAG through the cleavage of PC to generate PA, which is subsequently converted to DAG (Fig. 6a). Overexpression of the PLD results in light-dependent retinal degeneration [137], which is suppressed by the *laza* mutation [33]. These data suggest that the retinal

degeneration resulting from overexpression of PLD is due to excessive production of DAG, which in turn leads to increase channel activity and Ca^{2+} influx.

Light-dependent retinal degeneration results from disruption of other proteins that function in the PIP_2 regeneration cycle, including RDGB (PI transfer protein), CDS (CDP-DAG synthase), and dPIS (PI synthase) [28, 29, 131, 132, 229]. Overexpression of PLD, which might lead to the generation of higher levels of PIP_2 through conversion of PC to PA, suppresses the retinal degeneration in the *rdgB* mutant [137]. Furthermore, overexpression of dPIS, which generates PI from CDP-DAG, suppresses the *rdgB* and *cds* retinal degeneration phenotypes [131]. Therefore, the retinal degeneration in both the *rdgB* and *cds* mutants is likely to be due to decreased PIP_2 levels.

The retinal degeneration associated with *rdgB*, *cds*, and *dpis* might result from decreased light-induced Ca^{2+} influx since decreased levels of PIP_2 would lead to lower channel activity. However, this possibility is counter to the observation that the retinal degeneration in *rdgB* is suppressed by the *trp* mutation but not by mutation of the eye-enriched PKC (INAC) [230], which results in slower TRP channel inactivation [166]. Moreover, chemical inhibition of the channels also inhibits the retinal degeneration phenotype of *rdgB* [231]. Therefore, lower light-induced Ca^{2+} levels do not appear to be the mode through which loss of *rdgB* leads to retinal degeneration.

Distinct effects of phototoxicity on the photoresponse and retinal survival

In addition to the retinal degeneration resulting from mutations in genes that function in phototransduction, many studies in mammals demonstrate that continuous long-term exposure to light of modest intensity can result in photoreceptor cell death and diminish the photoresponse [232]. The underlying basis of this phototoxicity is poorly understood and it has been presumed that the decrease in the visual response is a secondary consequence of the retinal degeneration. In flies, constant light also causes loss of the photoresponse and retinal degeneration, which is paralleled by a loss of Rh1 [233]. The reduction in Rh1 levels occurs through the formation of stable Rh1/Arr2 complexes.

The visual impairment and retinal degeneration resulting from continuous light occur through distinct mechanisms [233]. Mutations that suppress the decline in Rh1 and loss of the photoresponse, such as *arr2*, *sun*, and *Rh1*^{Δ356} (C-terminal truncation of Rh1), do not ameliorate the constant light-induced retinal degeneration. Conversely, mutations known to suppress most retinal degenerations do not protect against light-induced visual impairment. These results indicate that constant light leads to blindness through

the formation of stable rhodopsin/Arr2 complexes, which ultimately leads to degradation of rhodopsin.

Concluding remarks

Despite the diversity in mechanisms underlying photoreceptor cell death in *Drosophila*, many of the retinal degenerations show features of apoptosis, which is reminiscent of human retinal dystrophies [31, 98, 234, 235]. Although a lot of progress has been made over the past few years, there remain many unanswered questions regarding the mechanisms of retinal degeneration in *Drosophila*. The trigger that leads to the cell death resulting from endocytosis of Rh1/Arr2 complexes is still unknown. This issue may have bearing on human retinal dystrophies as the phenomenon of retinal degeneration resulting from stable rhodopsin/arrestin complexes has been documented in rodent animal models [236, 237]. Mutations in *Drosophila crumbs* and its human homolog lead to light-induced retinal degeneration, but the mechanisms underlying these retinal degenerations are poorly understood [238, 239]. The *Drosophila* retinal pigment cells appear to function similarly to human RPE cells in the generation of the chromophore [81], and many genetic defects in human RPE cells are known to cause photoreceptor cell degeneration. Thus, further characterization of the *Drosophila* retinal pigment cells is warranted. The work on phototoxicity in *Drosophila* raises the question as to whether the visual impairment in mammals that results from exposure to continuous light also is due to formation of stable rhodopsin/arrestin complexes and loss of rhodopsin. Finally, the recent indications that the ipRGCs operate through a visual cascade remarkably similar to that in *Drosophila* [11] have resulted in renewed interest in the mechanism of *Drosophila* phototransduction.

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