Phototaxis and Phototransduction Mechanisms in the Model System *C. elegans*

by

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Contributions:

Chapter 2. Alex Ward (A.W.), Jie Liu (J.L.), Zhaoyang Feng (Z.F.), and X.Z. Shawn Xu (X.Z.S.X) were the authors for data in Chapter 2. A.W. and J.L. contributed equally to this work. A.W. conducted the experiments and analyzed the data in Figures 2.1–2.4. J.L. conducted the experiments and analyzed the data in Figures 2.5 and 2.6. Z.F. developed tools to acquire and analyze behavioral data. X.Z.S.X. supervised the project and wrote the manuscript.

Chapter 3. Jie Liu (J.L.), Alex Ward (A.W.), Jingwei Gao (J.G.), Yongming Dong (Y.D.), Nana Nishio (N.N.), Hitoshi Inada (H.I.), Lijun Kang (L.K.), Yong Yu (Y.Y.), Di Ma (D.M.), Tao Xu (T.X.), Ikue Mori (I.M.), Zhixiong Xie (Z.X.) and X.Z. Shawn Xu (X.Z.S.X) were the authors for data in Chapter 3. A.W. and J.L. contributed equally to this work. J.L. performed most of the electrophysiological recordings and analyzed the data. A.W. carried out most of the molecular biology, genetic and behavioral experiments and analyzed the data. J.G. and Z.X. performed some of the molecular biology, genetic and behavioral experiments. Y.D. and L.K. carried out some of the recordings. N.N., H.I. and I.M. isolated *pde* mutants. A.W. and D.M. isolated *lite-1* mutants. A.W., Y.Y. and T.X. mapped *lite-1* mutants. X.Z.S.X. supervised the project and wrote the paper with help from all of the other authors.

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Abstract

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C. elegans has become an increasingly popular model system for the study of sensory systems, in particular olfactory transduction and mechanotransduction. However, *C. elegans* is eyeless and lives in darkness (i.e. soil), and this organism has generally been presumed to be photoinsensitive. The ability to sense light is crucial to the survival of many organisms. In my thesis work I challenged the assumption that *C. elegans* is photoinsensitive, reasoning that light might serve functions other than “vision” per se. For instance, negative phototaxis behavior in *C. elegans* could function to retain worms in soil, or protect them from harmful effects of UV light. In my thesis research, I found that light stimuli, indeed, elicit avoidance behavior in *C. elegans*, and that prolonged light stimulation is lethal to worms. We also identified a group of ciliary sensory neurons as candidate photoreceptor cells. In a subset of these neurons (ASJ and ASK), we showed that light evokes a depolarizing conductance mediated by cyclic guanosine monophosphate (cGMP)-sensitive cyclic nucleotide-gated (CNG) channels.

By recording the photoreceptor neuron ASJ and ASK in wild-type and various mutant worms, we found that phototransduction is a G protein–mediated process and requires membrane-associated guanylate cyclases, but not typical phosphodiesterases. In addition, we found that *C. elegans* phototransduction requires LITE-1, a candidate photoreceptor protein known to be a member of the invertebrate taste receptor family. Our genetic, pharmacological and electrophysiological data suggest a model in which LITE-1 transduces light signals via G protein signaling, which leads to upregulation of the second messenger cGMP, followed by opening of cGMP-sensitive CNG channels and stimulation of photoreceptor cells. Our results identify a phototransduction cascade in *C. elegans* and implicate the function of a ‘taste receptor’ in phototransduction.

Chapter 1

Introduction

An overview of phototransduction

In the animal kingdom there are numerous examples of the eye that range in complexity−from the simple mirror eye of marine mollusks and the lensed eye of the box jellyfish to the elaborate insect compound eye and vertebrate camera eye. It has long been postulated that the earliest example of the eye in evolution would be a proto-eye composed only of two cells: a photoreceptor cell and a pigment cell to provide shading and directionality to light (Darwin, 1859). Indeed, eye development is highly conserved and is regulated by a group of transcription factors called the retinal determination gene network (RDGN), and in particular the master-switch gene family Eyeless/PAX6 (Gehring and Ikeo, 1999; Silver and Rebay, 2005).

Based on morphological and mechanistic distinctions, there are two main classifications of photoreceptors in the animal kingdom which include ciliary and rhabdomeric. Ciliary photoreceptors are microtubule-based and are formed by folding of a modified cilium. Conversely, rhabdomeric photoreceptors are actin-based and are derived from folding of the apical cell surface (Arendt, 2003; Fu and Yau, 2007; Lamb et al., 2007; Wang and Montell, 2007). In addition, there

are simple photoreceptors lacking cilia or microvilli found in certain invertebrate organims including crayfish, *Aplysia*, *Onchidium*, and *Helix* (Gotow and Nishi, 2008), as well as the more recently discovered intrinsically photosensitive retinal ganglion cells (ipRGCs) of the mammalian retina (Berson, 2007).

In general, ciliary photoreceptors are associated with deuterostomes, whereas rhabdomeric photoreceptors are dominant in the invertebrate protostomes. However, there are examples of protostomes (i.e. Scallop) and deuterostomes (i.e. the Cephalochordate amphioxus) having both types of photoreceptor cells. Thus, it is generally believed that ciliary and rhabdomeric photoreceptor cells coexisted in urbilatarians, the common ancestor of all bilatarians before the deuterostome/protostome split nearly 550 million years ago. Most known photoreceptors utilize a photopigment consisting of an opsin and a vitamin A-based chromophore. Opsins are prototypical seven-transmembrane domain G protein-coupled receptors (GPCRs) that are photo-coupled via the chromophore to a G protein-mediated phototransduction cascade. In the animal kingdom, over 1000 opsins are known to exist, all of which are believed to originate from a common ancestor (Arendt, 2003; Terakita, 2005). The opsins are largely divided into two major groups called c-opsin and r-opsin. This division is based on molecular homology and the photoreceptor type with which they associate: c-opsins are found in ciliary photoreceptors and r-opsins in rhabdomeric photoreceptors. Minor groups of opsins also exist, these are G0-opsin, peropsin, neuropsin, encepholopsin/teleost multiple tissue (tmt) opsin, and photoisomerases (Terakita, 2005). Recent work from our lab suggests

C. elegans may utilize a chemoreceptor-like protein LITE-1 to sense light (Liu et al., 2010). The role of LITE-1 in *C. elegans* phototransduction will be discussed in detail in Chapter 3.

Ciliated photoreceptor cells

The best studied ciliated photoreceptor cells are the vertebrate rods and cones (Fu and Yau, 2007). Examples of ciliated photoreceptors in lower organisms exist, including the ciliary retinal layer photoreceptors of the scallop (del Pilar Gomez and Nasi, 1995; Gomez and Nasi, 2000), the ciliated neurons comprising the lensed eye of the jellyfish (although direct physiological responses to light in these cells have not been demonstrated) (Koyanagi et al., 2008; Suga et al., 2008), as well as the ciliated photosensitive neurons of the nematode *C. elegans*, which will be described in detail in the results section of this thesis (Ward et al., 2008; Liu et al., 2010). The fundamental motif of ciliary photoreceptors is their coupling of G protein signaling to modulate levels of cyclic neucleotides to open/close cyclic nucleotide-gated (CNG) cation channels.

Almost all known cases of ciliated photoreceptors utilize a vitamin A-based chromophore (e.g. retinal) and a 7-transmembrane domain opsin, which together are referred to as the photopigment (Terakita, 2005). Vertebrate rods, which utilize the c-opsin Rhodopsin (Rh), function in low-light vision and are capable of detecting a single photon of light with a ~1 pA response (Hecht et al., 1942; Baylor, 1987). Conversely, cones mediate daylight vision, have a higher

threshold for activation, a greater temporal resolution, and mediate color vision. Cone cells express the S- and M-cone opsins.

The rod and cone motif of phototransduction is shown in Fig. 1.1. In short, the vertebrate opsins couple to a G_t-type G-protein (transducin). Rods and cones are depolarized (so called “dark current”) in the dark because guanlyate cyclase (GC) is constitutively active producing steady states of cyclic-guanosine monophosphate (cGMP). Continuous production of cGMP in rods and cones gates CNGs producing the dark current. Upon stimulation by light, a transducin-mediated cascade in vertebrate rods and cones activates phosophodiesterase (PDE) which cleaves cGMP, having the net effect of closing CNGs (hyperpolarizing these cells). Details of rod and cone phototransduction are discussed in the next section.

Activation of the photoresponse in vertebrate rods and cones

The photopigment of most ciliated photoreceptor cells consists of an opsin and a vitamin A based chromophore that is covalently linked to the opsin by a protonated Schiff-base linkage to a conserved lysine residue in the seventh transmembrane segment (Fu and Yau, 2007). The chromophore of the majority of vertebrate photoreceptor cells is 11-*cis*-retinal, however, some amphibians and aquatic organisms use 11-*cis*-3,4 dehydroretinal. In the case of vertebrate rods and cones, 11-*cis*-retinal retains the opsin in an essentially inactivated state. Indeed, free opsin can activate the phototransduction cascade (Cornwall and Fain, 1994; Cornwall et al., 1995; Surya et al., 1995) and even 11-*cis*-retinal

bound Rhodopsin can be spontaneously activated in the dark by thermal energy (Baylor et al., 1980).

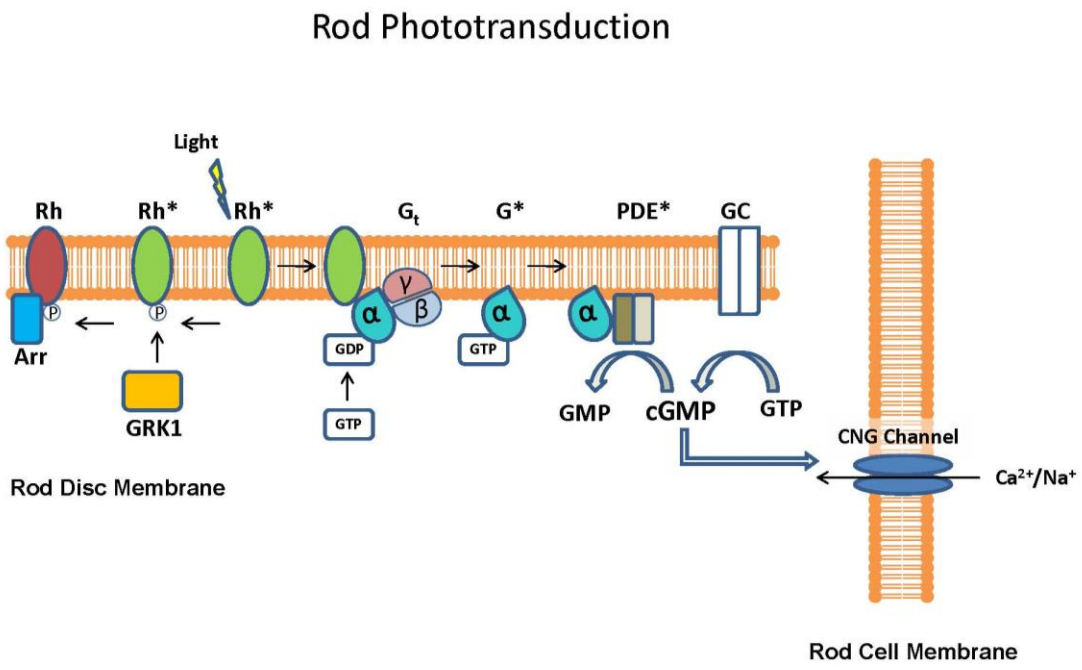


Figure 1.1 Schematic of rod phototransduction

Light-induced activation of rod and cone photoreceptor cells occurs when a photon of light is absorbed by 11-*cis*-retinal which isomerizes to all-*trans*-retinal (see Fig. 1.1). The all-*trans*-retinal choromophore is an agonist for activation of the photopigment, whereby Rhodopsin undergoes a series of conformational shifts within a few milliseconds leading to the active form metarhodopsin II (Meta II or R*) (Okada et al., 2001). R*/Meta II decays to the inactive Meta III, whereupon the covalent linkage between opsin and chromophore is hydrolyzed yielding free all-*trans*-retinal. Regeneration of the chromophore involves a pigment cycle in which all-*trans*-retinal is converted to all-*trans*-retinol then

shuttled to the retinal pigment epithelial cell layer where a series of enzymes convert it to the active form 11-*cis*-retinal (Rando, 2001).

In the next step of activation, R* binds transducin and catalyzes the exchange of GTP for GDP on the G_{α} subunit to make $G_{\alpha t}$-GTP (G*). Free R* was originally estimated to activate >100 $G_{\alpha t}$ in mouse rods (Pugh et al., 1999), however, recent estimates based on an 80 ms R* lifetime and an activation rate of 240 s^{-1} indicate R* activates only ~20 $G_{\alpha t}$ (Krispel et al., 2006). R* activation of $G_{\alpha t}$ represents the first amplification step in the rod phototransduction cascade. Active G* dissociates from the R* and $G_{\beta\gamma}$ complex, whereupon it interacts with the gamma subunit of a cGMP-specific PDE (PDEγ), which frees the catalytic PDEαβ subunits to hydrolyze cGMP to make GMP (Fu and Yau, 2007; Yau and Hardie, 2009). PDE has a high catalytic rate (Leskov et al., 2000), but only a single PDE is estimated to be activated by G* (Fu and Yau, 2007). The catalytic activity of PDE represents the second amplification step in rod and cone phototransduction.

In the dark, constitutive activity of a cGMP guanylate cyclase maintains a concentration of one to several micromolar (Fu and Yau, 2007). This basal cGMP level opens a subset of the cGMP-gated CNGs in rods and cones, thus producing depolarizing currents in the dark. The drop in cGMP concentration upon light-dependent activation of PDEαβ results in rapid (sub-millisecond) closure of the rod and cone CNGs (Karpen et al., 1988).

Not only do rods and cones use different opsins, they also have distinct isoforms of transducin, PDE and the CNG transduction channel (Fu and Yau, 2007; Yau and Hardie, 2009). The rod transducin isoform is $G_{\alpha t1}G_{\beta 1\gamma 1}$ and the cone isoform is $G_{\alpha t2}G_{\beta 3\gamma 8}$. Rod and cone PDEs are tetrameric proteins, however, rod photoreceptors have two catalytic domains, P_{α} and P_{β}, and two identical P_{γ} domains, whereas cone PDE is composed of only α and γ subunits in a ratio of $2P_{\alpha}$:$2P_{\gamma}$. The CNG nonselective cation channel is also tetrameric and is composed of A and B subunits. Rod CNGs adopt an asymmetrical composition with a 3CNGA1:1CNGB1 stoichiometry (Zhong et al., 2002), while cone CNGs are symmetrical having a 2CNGA1:2CNGB1 stoichiometry (Peng et al., 2004).

Termination of the photoresponse in vertebrate rods and cones

Termination of the photoresponse culminates from the multiple inactivations of R*, G*, and PDE*, as well as Ca^{2+}/GCAP-modulated restoration of cGMP levels. These processes enable the photoreceptor cell to regain its responsiveness to subsequent light stimulation and, therefore, timing of inactivation in part dictates the temporal aspects of rod and cone photosensitivity.

Inactivation of R* is a two-step process. The first step occurs with the phosphorylation of R* by the Rhodopsin kinase GRK1 at multiple serine/threonine residues at the C-terminus of the protein (the “cone pigment kinase” GRK7 is present in some vertebrate cones, including in humans). Phosphorylation of R* lowers the activity of the opsin. The second step in

inactivation occurs when arrestin binds phosphorylated R*, which in turn shuts-down the remaining activity of the opsin. These will each be discussed in order.

Rhodopsin and cone pigments have multiple phosphorylation sites at the C-terminus of the protein which together contribute to the shutoff of activity by GRK phosphorylation (Fu and Yau, 2007). In the case that phophorylation of R* is removed altogether, the response amplitude of rods is twice that of wild type animals and decays to baseline considerably more slowly (Chen et al., 1995; Chen et al., 1999; Mendez et al., 2000). Further, it has been shown in mouse rods that all six rhodopsin phosphorylation sites must be phosphorylated in order to get complete inhibition of the R* activity (Mendez et al., 2000). In a separate study, it was demonstrated that the single photon response in rod photoreceptor cells is modified in a graded manner in relation to the number of mutated phosphorylated sites, but that the identity of those sites was not important (Doan et al., 2006).

While less is known about the specific relationship between phophorylation events and cone photoreceptor activity, evidence suggests a similar two-step inactivation mechanism (Fu and Yau, 2007). Interestingly, the cone pigment GRK7 has been shown to have a much higher activity than GRK1, and is expressed in fish cones at higher levels than GRK1 is in fish rods (Tachibanaki et al., 2005; Wada et al., 2006), possibly explaining the differences between rods and cones with respect to shutoff kinetics and sensitivity (Tachibanaki et al., 2005; Fu and Yau, 2007; Torisawa et al., 2008).

In the second step of R* termination, the protein arrestin (a member of a family of cytoplasmic proteins that bind GPCRs) appears to function in mediating the falling phase of the photoresponse. Rods and cones each express their own arrestin. The photoresponse of rods from arrestin-knockout (*Arr*$^{-/-}$) mice to dim-flash light displays a normal current amplitude and a rapid partial recovery, however, a prolonged final phase which decays to baseline much slower than wild type animals suggests that arrestin functions in quenching the residual activity of phosphorylated R* (Xu et al., 1997a). Another study looked at rods from *Grk1*$^{-/-}$ *Arr*$^{-/-}$ double mutant mice. Like the GRK1$^{-/-}$ single mutants, rods from *Grk1*$^{-/-}$ *Arr*$^{-/-}$ double mutants have potentiated response amplitudes. Surprisingly, it was shown that rods from *Grk1*$^{-/-}$ *Arr*$^{-/-}$ and *Arr*$^{-/-}$ animals each have a slower second phase decay compared to the *Grk1*$^{-/-}$ single mutants, suggesting that inhibition by arrestin does not require phosphorylated R* (Burns et al., 2006).

Recent studies have begun to unravel the mechanisms underlying G* and PDE* termination in vertebrate rods and cones (Fu and Yau, 2007). G* is active when bound to GTP (G*-GTP), and only becomes inactivated upon hydrolysis of GTP to GDP through its intrinsic GTPase activity. It has been shown that the intrinsic GTPase activity of transducin is enhanced by a GAP complex of proteins which includes a regulator-of-G-protein-signaling (RGS9-1), the long form of Gβ5 (Gβ5-L), and a membrane anchor protein (R9AP) (Fu and Yau, 2007). Upon hydrolysis of GTP, GDP-bound transducin dissociates from PDEγ, thus freeing the inhibitory PDEγ subunit to sequester the catalytic PDEαβ subunits.

Regulation of cGMP production in vertebrate rods and cones

The vertebrate rods and cones express guanylate cyclases (GCs) which synthesize cGMP from GTP, enabling the photoreceptor to recover cGMP levels depleted by the photoresponse. There are two different GCs present in mouse rods and cones (each are membrane-associated GCs): retGC1 and retGC2. Whereas retGC1 is expressed in rods and cones (Liu et al., 1994; Cooper et al., 1995), retGC2 is expressed only in rods (Lowe et al., 1995).

GC activity in mouse rods and cones is highly dependent on Ca^{2+} levels, a process mediated by the Ca^{2+}-binding guanylate-cyclase activating proteins (GCAPs) (Palczewski et al., 2004). Two different GCAPs are expressed in mouse retinas, GCAP1 and GCAP2, and while GCAP1 and GCAP2 are each expressed in mouse rods, GCAP1 is the primary form in mouse cones (Cuenca et al., 1998; Howes et al., 1998). When bound to Ca^{2+} via the GCAP EF-hand domains, GCAP inhibits GC, thus providing a negative feedback mechanism for regulating GC activity. Ca^{2+} levels in rods and cones are relatively high in darkness, and so GCAP and GC activity is low. However, following light-stimulation Ca^{2+} levels decrease sharply, which relieves inhibition of GCAP to activate GC. In mice lacking both GCAP1 and GCAP2, the rod single photon response is much greater than wild type animals (Mendez et al., 2001), indicating that Ca^{2+}-mediated activation of GC is crucial to limiting the sensitivity of photoreceptor cells. Further, GCAP-mediated inhibition is found to take effect within ~40ms of flash onset (Burns et al., 2002), significantly faster than what is estimated for R* termination (80-100ms) (Chen et al., 1999; Krispel et al., 2006).

Light-dependent adaptation of the photoresponse in vertebrate rods and cones

An important feature of vertebrate rods and cones is their ability to adapt sensitivity to different light conditions through various mechanisms mediated predominantly by Ca^{2+}. Under dark conditions, the vertebrate rods and cones have elevated Ca^{2+}-levels because CNGs are permeable to Ca^{2+}, which comprises approximately 15% of CNG “dark currents” (Yau and Hardie, 2009). Ca^{2+} Levels in the dark are kept in equilibrium by a Na,Ca,K exchanger (NCKX) which couples Na^{+} influx with Ca^{2+} and K^{+} efflux (Schnetkamp, 2004). Upon light stimulation, Ca^{2+} influx decreases due to the closing of CNGs, yet because NCKX continues to extrude Ca^{2+} from the cell the net effect is intracellular Ca^{2+} decreases sharply.

Ca^{2+} has two main effectors that regulate adaptation of the photoresponse in light conditions. 1) As discussed in the previous section, Ca^{2+} regulates the activity of GCAPs via its EF-hand domains (Palczewski et al., 2004). Low intracellular Ca^{2+} disinhibits the GCAP, which in turn activates GC, thus replenishing cGMP levels depleted by photo-stimulated PDE activity. This is considered to be the dominant mechanism governing adaptation in low and intermediate light intensities (Yau and Hardie, 2009). 2) Another effector of Ca^{2+} in mediating adaptation is recoverin which also binds Ca^{2+} via EF-hand domains (Kawamura and Tachibanaki, 2002). Ca^{2+}-bound recoverin associates with GRK1 and inhibits R* phosphorylation. Conversely, the absence of Ca^{2+} promotes dissociation of recoverin-GRK1, thus freeing GRK1 to phosphorylate

R*. Under dim light conditions GRK1/arrestin-mediated termination of R* is relatively slow, however, in progressively brighter conditions recoverin plays an increasing role in facilitating R* phosphorylation. By lowering the active lifetime of R* through this Ca^{2+}-mediated feedback mechanism, recoverin plays a major role in adaptation under bright light conditions (Yau and Hardie, 2009).

Ca^{2+}-independent mechanisms also mediate light adaptation in vertebrate rods and cones. Under steady light conditions basal PDE activity is higher than in dim light, and so the fractional effect of absorbing additional photons is less, in effect raising the threshold for photostimulation. Furthermore, GC continues to be active in light adapted photoreceptors. As a result, the light response is less sensitive, and cGMP recovery is faster, in light adapted rods and cones (Pugh et al., 1999; Yau and Hardie, 2009).

Recent work has focused on the role of light-dependent translocation of proteins required for phototransduction. This is believed to be a form of slow adaptation proceeding over the course of many minutes (Calvert et al., 2006). In rods, transducin is shuttled from the outer segment to the inner segment during prolonged light stimulation (Brann and Cohen, 1987; Philp et al., 1987; Whelan and McGinnis, 1988). Arrestin is also translocated in a light-dependent manner, but moves in the opposite direction from inner to outer segment (Broekhuyse et al., 1985). Finally, recoverin also translocates in response to light, but moves from the outer segment to rod synapses (Strissel et al., 2005). Light-driven

protein translocation is also present in cones (Mirshahi et al., 1994; Zhu et al., 2002; Zhang et al., 2003).

Invertebrate GC-based ciliary phototransduction motifs

Not all ciliary photoreceptor cells utilize a PDE-dependent mechanism to signal the light response. The scallop retina has two retinal layers, a ciliated photoreceptor layer and a rhabdomeric layer. Light currents in the ciliated photoreceptor cell layer are mediated by cGMP-sensitive K^+ channels (del Pilar Gomez and Nasi, 1995). These cells have been shown to express an opsin photopigment SCOP2 and a G_o-type G-alpha protein, suggesting SCOP2 couples to G_o in mediating the light response in scallops (Kojima et al., 1997). Additionally, it was shown that light responses in these cells is blocked using the $G_{i/o}$-specific inhibitor of G protein function, pertussis toxin (PTX), and the $G_{i/o}$-specific activator mastoparan evokes outward currents (Gomez and Nasi, 2000). Based on the finding that light currents can be reduced with the GC inhibitor LY83583, Gomez and colleagues proposed a model whereby G_o signals through GC to raise the level of cGMP and activate outward K^+-selective currents (Gomez and Nasi, 2000).

As will be discussed later in this chapter (and which is the topic of this thesis), *C. elegans* were recently shown to exhibit light-avoidance behavior (Ward et al., 2008). C. elegans photoreceptor cells utilize a G protein-mediated cGMP/CNG-based mechanism of phototransduction (Ward et al., 2008; Liu et al., 2010). Interestingly, our results demonstrate that photosignaling in these neurons

requires a novel taste receptor-like protein LITE-1 (Edwards et al., 2008), which functions upstream of G protein signaling (Liu et al., 2010). Our data suggest LITE-1 may serve as a photopigment coupling to G proteins in the phototransduction cascade. Please refer to the section titled “Other *C. elegans* Sensory Systems” in Chapter 1 for a summary of *C. elegans* phototransduction, and to Chapters 2 and 3 for detailed results of our studies on *C. elegans* phototransduction.

Rhabdomeric phototransduction

Rhabdomeric photoreceptors differ from their ciliated photoreceptor counterparts both with respect to their structure (i.e. microvillar versus ciliary morphology) and the molecular details of the phototransduction cascade. Much of our knowledge about phototransduction in rhabdomeric photoreceptors has resulted from experiments using the fruit fly *Drosophila* (Wang and Montell, 2007). More recently, however, functional rhabdomeric photoreceptors were identified in the mammalian retina, aptly named intrinsically photosensitive retinal ganglion cells (ipRGCs) (Berson, 2007). The hallmark of the rhabdomeric phototransduction motif is signaling through phoshpolipase-C (PLC) to activate transient receptor potential (TRP) channel-mediated photocurrents. While many species of varying complexity are known to utilize this PLC/TRP photosignaling motif, the discussion here will focus on our current understanding of phototransduction in *Drosophila*.

Drosophila vision and the compound eye

Drosophila has a compound eye, so called because it is made up of ~800 hexagonal repeating units, called “ommatidia”, each representing a simple eye. The ommatidia is comprised of 20 cells, eight of which are photoreceptor cells, the others being pigment cells, mechanosensory bristles, etc.. Of the eight photoreceptor cells, six (R1-R6) are peripheral and extend the entire depth of the ommatidia, while the remaining two are central and restricted to the distal (R7) and proximal (R8) portion of ommatidia.

Six opsins have been found to be expressed in *Drosophila* (Rh1-Rh6). Rh1 is a blue-green absorbing opsin (λ_{max} ~486nm) and is the sole opsin expressed in R1-R6 (O'Tousa et al., 1985; Zuker et al., 1985). The ultraviolet-absorbing opsins Rh3 (λ_{max} ~331) and Rh4 (λ_{max} ~355) are expressed in the R7 photoreceptor (Fryxell and Meyerowitz, 1987; Montell et al., 1987; Zuker et al., 1987; Fortini and Rubin, 1990), whereas Rh5 (λ_{max} ~442) and Rh6 (λ_{max} ~515), blue and green absorbing opsins, respectively, are expressed in R8 (Chou et al., 1996; Papatsenko et al., 1997; Salcedo et al., 1999). R7 and R8 therefore mediate color vision in *Drosophila*, analogous to cones in vertebrate retinas. The violet absorbing opsin Rh2 (λ_{max} ~418) is not expressed in the ommatidia but rather in photoreceptors in the ocelli (Cowman et al., 1986; Feiler et al., 1988; Pollock and Benzer, 1988; Zuker et al., 1988).

Drosophila opsins are covalently linked via a Schiff base linkage to the chromophore 11-*cis* 3-hydroxyretinal. Many of the genes required for production of 11-*cis* 3-hydroxyretinal are known revealing a relatively detailed model for chromophore biosynthesis in flies (Wang and Montell, 2007). In brief, the *Drosophila* diet includes β-carotene which is shuttled from the gut to head neurons where it is converted to all-*trans*-retinol (Vitamin A) by the ninaB gene product β β'-carotene 15,15'-monoxygenase (BCO) (Stephenson et al., 1983; von Lintig and Vogt, 2000; von Lintig et al., 2001). Two other genes are required for production of Vitamin A: *ninaD* (Stephenson et al., 1983; Kiefer et al., 2002) and *santa maria* (Wang et al., 2007), each encoding proteins proposed to be involved in the transport of β-carotene to the head neurons (Kiefer et al., 2002; Voolstra et al., 2006; Wang et al., 2007). Vitamin A is then transported to the retina where a retinoid binding protein encoded by the *pinta* gene (Wang and Montell, 2005) and an oxidoreductase encoded by the ninaG gene (Sarfare et al., 2005; Ahmad et al., 2006) are required for production of the chromophore.

In vertebrate rods and cones, an enzymatic pigment cycle is required to regenerate the chromophore. In the case of *Drosophila*, however, the covalent linkage between the opsin and the chromophore is stable in the all-*trans* metarhodopsin configuration (M-state) and so the chromophore is regenerated while still attached to the opsin. This is achieved via a photoisomerization step in which a photon of orange light (λ_{max} ~570) is absorbed by the all-*trans* chromophore to reform 11-*cis* 3-hydroxyretinal (Wang and Montell, 2007).

Activation of the *Drosophila* photoresponse

The *Drosophila* phototransduction cascade is significantly different from the cGMP-mediated photosignaling in vertebrate rods and cones (see Fig. 1.3). *Drosophila* photoreceptors utilize G_q-type G protein signaling to regulate PLCβ, coupled via an unknown mechanism to TRP channel-mediated photocurrents. Evidence for this model is discussed in detail in this section.

Initiation of the phototransduction cascade occurs upon absorption of a photon of light by 11-*cis* 3-hydroxyretinal, thereby isomerizing 11-*cis* 3-hydroxyredinal to the all-*trans* conformation. This photoisomerization triggers a conformational change in the opsin to activated metarhodopsin (M-state, or Rh*).

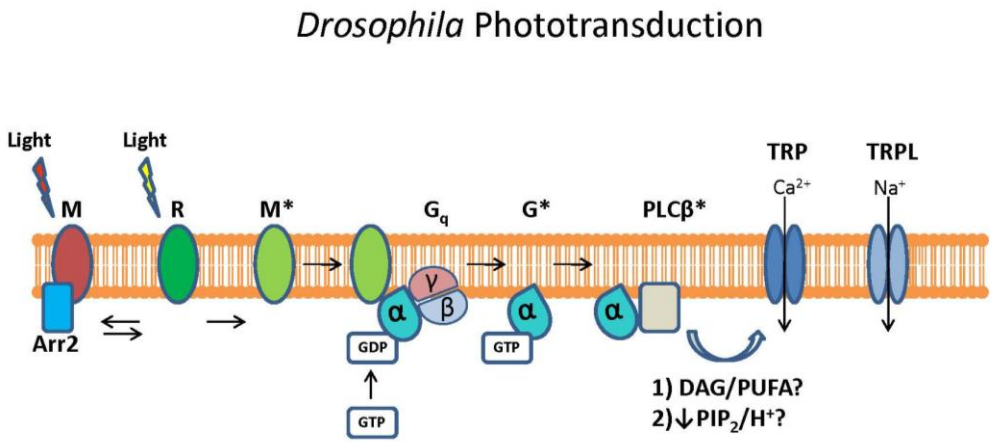


Figure 1.2 Schematic of *Drosophila* phototransduction

Rh* couples to G-protein mediated signaling (Emeis et al., 1982; Kibelbek et al., 1991; Kiselev and Subramaniam, 1994), in particular, to a the G_q–type

heteromeric G protein encoded by the genes *dGq* (Lee et al., 1990; Lee et al., 1994), *Gβe* (Yarfitz et al., 1991; Dolph et al., 1994), and *Gγe* (Schulz et al., 1999; Schillo et al., 2004). The catalytic $G\alpha_q$ is required for the photoresponse as dGq mutants are 1,000 fold less sensitive to light (Scott et al., 1995). Upon Rh*-mediated exchange of GDP for GTP, $G\alpha_q$–GTP is freed from the regulatory Gβγ complex and interacts with (and activates) phospholipase Cβ (PLCβ/NORPA) (Bahner et al., 2000) encoded by the norpA gene (Bloomquist et al., 1988). Interestingly, the Gβ and Gγ subunits are also required for normal photoresponses (Dolph et al., 1994; Schillo et al., 2004), but likely play an indirect role. Indeed, it has been shown that Gβγ does not interact with NORPA (Bahner et al., 2000), and that Gβγ are required for targeting of $G\alpha_q$ to the membrane (Kosloff et al., 2003; Elia et al., 2005).

Photocurrents in *Drosophila* are known to be mediated by both TRP and TRPL, each members of the transient receptor potential canonical (TRPC) family of TRP channels. TRP was the first cloned channel in the TRP superfamily (Montell et al., 1985; Montell and Rubin, 1989); the TRP name derives from the observation that mutants have a transient response to light with a decreased Ca^{2+} influx (Cosens and Manning, 1969; Hardie and Minke, 1992). *trpl* mutants have defective light-responses, however, the defect is not as severe as *trp* mutants (Leung et al., 2000). *trp*/*trpl* double mutants are completely photoinsensitive (Niemeyer et al., 1996; Reuss et al., 1997). Based on *trp* and *trpl* single mutant studies these individual channels have different permeability characteristics and might function as homomeric tetramers (Reuss et al., 1997).

However, TRP and TRPL coimmunoprecipitate in fly extracts, and have been shown to interact in heterologous cell systems suggesting these channels might form heteromultimers (Xu et al., 1997b). However, the sum of the *trp* and *trpl* single mutant light conductances is equal to the wild type conductance, which argues against a third heteromeric channel conductance (Reuss et al., 1997). It remains to be shown whether TRP and TRPL form a heteromeric channel in vivo.

How light-dependent PLC activation leads to opening of TRPC channels remains a major unresolved question. The lipase activity of NORPA, which hydrolyzes phosphatidyl 4,5-biphosphate (PIP_2) to produce inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), is required for photoresponses in *Drosophila* (Bloomquist et al., 1988; Toyoshima et al., 1990). In principle, increased IP_3 or DAG, or alternatively, PIP_2 depletion (or a combination of these), could be required for activation of the photoresponse. Several studies argue against an involvement of IP_3. Release of caged IP_3 does not “phenocopy” the photoresponse (Hardie, 1995), and mutations in the only *Drosophila* IP_3 receptor does not alter phototransduction (Acharya et al., 1997; Raghu et al., 2000b).

There is support for a role of DAG in triggering the photoresponse. DAG and other PUFAs are capable of activating *Drosophila* photoreceptor cells (Chyb et al., 1999). DAG kinase (DGK) phosphorylates DAG to make phosphatidic acid (PA), thus in a DGK mutant background the DAG level is increased. In the DGK mutant *rdgA*, TRPC channels are constitutively active (Raghu et al., 2000a). Likewise, the phosphatidic acid phosphatase (PAP) mutant *laza*, which converts

PA to DAG, has reduced photocurrents and faster termination kinetics (Garcia-Murillas et al., 2006; Kwon and Montell, 2006).

It has been argued that PIP_2 depletion on its own is not a sufficient mechanism for channel gating, since a mutation in the only *Drosophila* phosphoinositide (PI) synthase (i.e. which is required for making PIP_2) results in the absence of photoresponses (Wang and Montell, 2006), rather than the predicted constitutive activation of TRPC. More recent work, however, suggests PIP_2 does have a role in channel gating (Huang et al., 2010). Huang and colleagues reasoned that hydrolysis of PIP_2, in addition to producing IP_3 and DAG, also generates a proton, and they showed that light-stimulation causes acidification of the cytosolic surface of *Drosophila* photoreceptor cells (Huang et al., 2010). Importantly, they demonstrated that manipulations which deplete the PIP_2 level resulted in greater sensitivity of *Drosophila* photoreceptors to the lipophilic protonophore 2-4 dinitrophenol (DNP), which produces PLC-dependent activation of light-sensitive currents. Indeed, it has been suggested that TRPC channel gating arises from biophysical changes in the plasma membrane of the photoreceptor cell (Katz and Minke, 2009), therefore, a PIP_2-depletion/acidification mechanism is plausible. In all likelihood, *Drosophila* photocurrents are activated by a combination of mechanisms involving DAG/PUFA and PIP_2-depletion/acidification, consistent with current ideas regarding a polymodal scheme of TRP channel gating (Rohacs and Nilius, 2007).

Termination of the *Drosophila* photoresponse

Much like the vertebrate rod and cone system, the crucial steps in termination of the *Drosophila* phototransduction cascade involves a two-step process: 1) phosphorylation of Rh*, and 2) arrestin binding of Rh* which displaces $G\alpha_q$. The *Drosophila* receptor kinase responsible for Rh* phosphorylation is G-protein-coupled Kinase 1 (GPRK1). Surprisingly, GPRK1 has greater homology with adrenergic receptor kinases than it does with mammalian rhodopsin kinases (Lee et al., 2004). GPRK1 phosphorylates Rh* at key serine and threonine residues on the rhodopsin C-terminus (Matsumoto and Pak, 1984; Cassill et al., 1991; Lee et al., 2004). Phosphorylation of Rh1 by GPRK1 has been shown to modulate the phototransduction cascade: overexpression of GPRK1 results in small amplitude light responses, whereas overexpression of a “kinase dead” GPRK1 yields large responses (Lee et al., 2004).

The *Drosophila* genome encodes two arrestin genes, arrestin1 (Arr2) and arrestin2 (Arr2) (Hyde et al., 1990; Smith et al., 1990; Toyoshima et al., 1990). Arr2 is more abundant than Arr1 (Matsumoto and Yamada, 1991), and *arr2* mutants display severe defects in light response deactivation compared to *arr1* mutants which are only modestly defective (Dolph et al., 1993). Indeed, the kinetics of response termination was shown to be dependent on the rate of arrestin binding (Ranganathan and Stevens, 1995). Interestingly, unlike in the vertebrate system, arrestin binding does not require phosphorylation of Rh1 (Vinos et al., 1997; Alloway et al., 2000; Kiselev et al., 2000).

The *Drosophila* PLCβ NORPA also has a role in termination of the light response. As was shown to be the case with mammalian PLCβ (Berstein et al., 1992), NORPA has dual activity as a lipase and a GTPase activating protein (GAP) (Cook et al., 2000). Flies with low NORPA levels have both reduced light responses and slow response termination (Cook et al., 2000). This latter defect indicates the GAP activity of NORPA is required for deactivation of the photoresponse.

Ca^{2+} also plays an important role in regulating light response termination in *Drosophila* (Wang and Montell, 2007). The dynamic range of Ca^{2+} within the microvilli of the rhabdomere is large with measurements of ~160 nM at rest to greater than 200 µM upon light stimulation (Hardie, 1996; Oberwinkler and Stavenga, 1998, 2000). This large photoactivated Ca^{2+} influx is countered by the Na^{+}/Ca^{2+} exchanger CalX, which extrudes one Ca^{2+} for every three Na^{+} that enter (Hryshko et al., 1996; Ruknudin et al., 1997; Schwarz and Benzer, 1997). Experiments in which CalX is overexpressed result in a slower termination of the photoresponse (Wang et al., 2005), as is the case in recordings from photoreceptor cells in low Ca^{2+} bath solution (Hardie, 1991; Ranganathan et al., 1991; Henderson et al., 2000).

A protein kinase C (PKC) encoded by the *inaC* (*inactivation nor afterpotential C*) gene (Schaeffer et al., 1989) has a central role in terminating the photoresponse (Ranganathan et al., 1991; Smith et al., 1991). PKCs are serine/threonine kinases activated by DAG and Ca^{2+}. An indication that INAC is

involved in deactivation of the photoresponse was that TRP-mediated Ca^{2+} influx in response to light is greater in *inaC* mutants compared to wild type animals (Hardie and Minke, 1994). It was later shown that INAC phosphorylates TRP channels (Huber et al., 1998; Liu et al., 2000) and that a key INAC-phosphorylation site on TRP is required for a normal photoresponse termination rate (Popescu et al., 2006).

Termination of the light-response is slower in *inaC/trp* double mutants than in *trp* single mutants (Smith et al., 1991), suggesting that INAC has additional targets which regulate the phototransduction cascade. In support of this, the kinase/myosin domain containing protein NINAC is phosphorylated by PKC in vitro (Li et al., 1998). Further, mutation of the key consesnsus PKC phosphorylation sites in NINAC results in oscillations at the termination of the photoresponse (Li et al., 1998), suggesting phosphorylation of NINAC by INAC is involved in preventing reactivation of the photoreceptor.

The Ca^{2+}-binding protein CaM, a calcium-modulated regulator of signaling pathways, also plays a role in termination of the phototransduction cascade (Wang and Montell, 2007). *Drosophila* CaM is encoded by the *cam* gene (Yamanaka et al., 1987; Doyle et al., 1990) and *cam* hypomorphs have severe defects in light response deactivation (Scott et al., 1997). CaM localizes to both the rhabdomeres and cell bodies of *Drosophila* photoreceptors (Porter et al., 1993; Porter et al., 1995) and localization to the rhabdomere is dependent on the presence of the rhabdomere-specific isoform of NINAC p174 (Porter et al., 1995).

NINAC has two CaM binding sites, C1 and C2: mutation of these sites in NINAC p174 reduces CaM levels at the rhabdomere and slows termination of the light response (Porter et al., 1993; Porter et al., 1995).

Ca^{2+}/CaM is known to regulate response termination via at least three different mechanisms. 1) Both TRP (Chevesich et al., 1997) and TRPL (Phillips et al., 1992; Warr and Kelly, 1996) have been shown to bind CaM in vitro, and in vivo the interaction between TRPL and CaM appears to be required for normal response termination (Scott et al., 1997). It remains to be shown whether TRP interacts with CaM *in vivo*. 2) CaM regulates steps required for regeneration of rhodopsin. Following the photoisomerization of the all-*trans* chromophore to 11-*cis* 3-hydroxyretinal, metarhodopshin releases Arr2 and is dephosphorylated. In order for this to happen, Arr2 is itself phosphorylated by Ca^{2+}/CaM-dependent kinase II which in turn is required for dissociation of Arr2 from rhodopsin (Matsumoto and Pak, 1984; Byk et al., 1993; Matsumoto et al., 1994; Kahn and Matsumoto, 1997; Alloway and Dolph, 1999). Following release of Arr2, rhodopsin is dephosphorylated by the serine/threonine phosphatase retinal degeneration C (*rdgC*) (Steele and O'Tousa, 1990; Steele et al., 1992; Byk et al., 1993; Vinos et al., 1997), the activity of which is dependent on interaction with Ca^{2+}/CaM (Lee and Montell, 2001). In *rdgC* mutants rhodopshin is hyperphosphorylated resulting in slowed response termination (Lee and Montell, 2001). 3) The CaM binding transcription factor dCAMPTA has been shown to have a role in response termination. Photoresponse termination in *dcampta* mutants is slow and can be compensated by overexpressing the transcriptional

target of dCAMPTA dFbx14 (Han et al., 2006). The *dfbx14* gene encodes an F-box protein which has been proposed to function in ubiquitinating rhodopsin (Han et al., 2006).

Light-dependent adaptation in *Drosophila*

As is the case with vertebrate rods and cones, the *Drosophila* photoresponse adapts to varying light conditions via a Ca^{2+}-dependent mechanism. Indeed, it was shown that artificially raising intracellular Ca^{2+} levels in dark adapted photoreceptor cells is sufficient to mimic the light adapted response (Gu et al., 2005). Exactly how Ca^{2+} mediates light adaptation in *Drosophila* photoreceptors is still not clearly understood. The mechanism was shown to be PKC-independent, to be downstream of PLC, and is likely to be at the level of the transduction channel (Gu et al., 2005).

Drosophila photoreceptors, like their vertebrate rod and cone counterparts, also display slow forms of adaptation involving light-dependent translocation of proteins. For example, switching animals from dark conditions to light over a period of several minutes results in Arr1 and Arr2 translocation from the cell bodies, where it localizes in dark adapted animals, almost entirely to the rhabdomeres (Kiselev et al., 2000; Satoh and Ready, 2005). Light-dependent translocation of Arr2 has been shown to involve interactions with the phosphatidylinositol 3,4,5-triphosphate (PIP_3). Arr2 mutants lacking a PIP_3 binding site ($arr2^{3K/Q}$) have defective light-dependent translocation of Arr2 and impaired adaptation (Lee et al., 2003). The myosin III kinase NINAC has been

reported to play a role in light-dependent arrestin translocation (Lee and Montell, 2004), although this has been contradicted by another study (Satoh and Ready, 2005). Arr2 and NINAC interact indirectly in vivo, and indeed this interaction has been shown to be PI-dependent (Lee et al., 2003; Lee and Montell, 2004).

$G\alpha_q$ (Kosloff et al., 2003; Cronin et al., 2004) and TRPL (Bahner et al., 2002) also translocate in a light-dependent fashion, but move in the opposite direction shuttling from rhabdomere to cell body. Light-dependent translocation of $G\alpha_q$ is dependent on rhodopsin (Kosloff et al., 2003; Cronin et al., 2004) and may also be dependent on interactions with Gβγ (Kosloff et al., 2003). TRPL translocation involves a two stage process (Cronin et al., 2006). The first stage of TRPL translocation involves shutting the channel to the apical membrane over a period of several minutes (Cronin et al., 2006). Translocation to the apical membrane requires the PLCβ NORPA (Cronin et al., 2006). The second stage of TRPL translocation takes longer (~6 hours), involves movement of TRPL to the basolateral membrane, and is both TRP and PKC-dependent (Cronin et al., 2006).

Mammalian intrinsically photosensitive retinal ganglion cells (ipRGCs)

In recent years, a small subset of vertebrate retinal ganglion cells were shown to be directly photosensitive and have been proposed to utilize a PLC-based phototransduction mechanism similar to *Drosophila* photoreceptors (Berson, 2007). The intrinsically photosensitive retinal ganglion cells (ipRGCs) function in regulating such things as circadian rhythms, pupillary responses, and

melatonin release (Berson, 2007). A key observation that led to discovery of ipRGCs was that a small subset of retinal ganglion cells expresses melanopsin (Provencio et al., 1998; Provencio et al., 2000; Gooley et al., 2001; Hannibal et al., 2002), and it was later shown that melanopsin is required for ipRGC photoresponses (Lucas et al., 2003) and forms a functional photopigment in purified extracts (Newman et al., 2003). Light-stimuli induce relatively slow depolarizing currents (onset to peak amplitude takes several seconds), consistent with a role in detecting steady illumination by ambient light (Berson, 2007). ipRGCs have relatively low sensitivity, and lack specialized membrane structures that function in other photoreceptors types to boost surface expression of opsin, such as disks in rods and cones and microvilli in *Drosophila* rhabdomeres (Berson, 2007).

Evidence supports involvement of a PLC-dependent/TRP-mediated phototransduction mechanism in ipRGCs. Melanopsin has greater homology to r-opsins than it does to c-opsins (Provencio et al., 1998; Provencio et al., 2000; Arendt, 2003; Koyanagi et al., 2005; Terakita, 2005), and in heterologous systems melanopsin can trigger light-sensitive currents by coupling to G_q and PLC (Panda et al., 2005; Qiu et al., 2005). Furthermore, in cultured ipRGCs, light-induced increases in intracellular Ca^{2+} can be blocked with TRPC inhibitors (Hartwick et al., 2007). Additional indirect evidence also suggests a PLC/TRPC pathway in ipRGCs (Berson, 2007).

An overview of *C. elegans* sensory systems

C. elegans is a nematode that lives in soil and feeds on bacteria and fungi. This simple model organism reproduces primarily asexually, however, males are present in populations at low frequencies (1 for every 1000 hermaphrodites) and allow researchers the advantage of cross-fertilization. For many aspects of *C. elegans* biology, the major exception being mating, experimentation focuses on hermpaphrodites. In hermaphrodites, 959 somatic cells make up the hypodermis, muscle, digestive tract and nervous system (Sulston and Horvitz, 1977; Sulston et al., 1983). The simplicity of *C. elegans* makes it a useful tool in neurobiology, particularly in the case of the nervous system which is significantly stereotyped. Serial section electron micrograph (EM) studies have shown the position of neuronal nuclei to be essentially invariant and the neural connectivity is ~75% similar across worms (White et al., 1986). Neural connectivity in *C. elegans* consists of 5000 chemical synapses, 600 gap junctions and 2000 neuromuscular junctions. Hermaphrodites have 302 neurons which are subdivided into 118 classes based on neuroanatomical criteria (Ward et al., 1975; Ware et al., 1975). These include 39 classes of sensory neurons, 27 classes of interneurons, and 62 classes of motorneurons. These designations are somewhat arbitrary as many sensory neurons receive tremendous synaptic input suggesting they function in processing (White et al., 1986), and interneurons have been shown to play a sensory role as is the case with the proprioceptive neuron DVA (Li et al., 2006; Bounoutas and Chalfie, 2007).

C. elegans sensory neurons have distinct morphologies which make them specialized for their respective function. For example, many chemosensory neurons have ciliated endings exposed to the external environment through openings created by socket and sheath cells, whereby they sample the animal's chemical surroundings (Inglis et al., 2007). Such ciliated neurons are identifiable by fluoroscein dye-filling. The ciliated sensory neurons take dye up through their exposed ciliated endings (Hedgecock et al., 1985; Perkins et al., 1986), facilitating genetic screens for cilia mutants (Perkins et al., 1986). Of the 39 classes of sensory neurons, 21 are ciliated (Ward et al., 1975; Ware et al., 1975). Another example is the mechanoreceptor neuron (MRN) PVD, which has non-ciliated dendritic endings that form elaborate branching networks enmeshing the body wall of the animal. The extensive dendritic coverage of PVD makes it well-suited for touch sensitivity (Halevi et al., 2002; Tsalik et al., 2003).

In their natural environment, worms must navigate a 3-dimensional soil medium with its concomitant mechanical forces in order to locate food sources and mating partners. Not surprisingly, therefore, two very important, and well-studied, sensory systems in *C. elegans* are chemosensation and mechanosensation. *C. elegans* hermaphrodites have 32 putative chemosensory neurons and 30 putative mechanosensory neurons. Males have an additional 52 sensory neurons thought to be dedicated to mechanosensory functions related to mating. The sensory neurons, in most cases, form left right pairs positioned bilaterally to the worm midline. Because worms have relatively few neurons,

many sensory neurons subserve multiple sensory functions, a property best illustrated by the polymodal ASH neuron (discussed later in this chapter).

C. elegans chemosensory systems

C. elegans chemosensory neurons are ciliated, and with the exception of AWA, AWB, AWC, and AFD, have ciliated dendritic endings directly exposed to the worm exterior via the amphid and phasmid pores. The molecules required for sensory transduction are localized to the ciliated endings where they sample the external chemical milieu, such as salts and other water soluble compounds, volatile odors, oxygen, and secreted hormones. *C. elegans* exhibits chemotaxis behavior in response to both chemical attractants and repellents. Attractants are detected by positive valence sensory neurons, such as ASE, AWA and AWC, which trigger an attractive behavioral response moving the animal closer to a chemical source. Chemotaxis toward attractants has been described with a “pirouette model” of locomotion (Pierce-Shimomura et al., 1999), such that animals respond to changes in attractant concentration with pirouettes (changes in direction) as the worm moves down the attractant gradient, and with long unhindered forward movement when moving up the gradient. Chemical repellents are detected by negative valence sensory neurons, such as ASH and AWB, and trigger repulsive locomotor responses. When the worm encounters a chemical repellent it immediately reverses and then initiates forward locomotor bursts in a new direction (Culotti and Russell, 1978).

C. elegans responds to salts, cyclic nucleotides, amino acids and other water soluble attractants largely with the gustatory ASE neuron (Ward, 1973; Dusenbery, 1974; Dusenberry, 1980; Bargmann and Horvitz, 1991b), although a number of other sensory neurons are required for normal behavioral responses to these attractants. The ASE neuron is unique in that it displays asymmetrical expression of guanylate cyclases in the left and right neurons (Yu et al., 1997). ASER (right) expresses GCY-5 and detects Cl^- and K^+, whereas ASEL (left) expresses GCY-6 and GCY-7 and preferentially senses Na^+ (Yu et al., 1997; Pierce-Shimomura et al., 2001). *C. elegans* also exhibits aversive responses to a number of chemical repellents, including high osmolarity, heavy metals (i.e. Cu^{2+}), detergents, bitter alkaloids (i.e. quinine), acid pH, and some organic odors. Avoidance of these chemical repellents requires the polymodal ASH neurons (Ward, 1973; Dusenbery, 1974; Culotti and Russell, 1978; Colbert et al., 1997; Hilliard et al., 2002; Hilliard et al., 2004).

Volatile odorants such as alcohols, ketones, aldehydes, esters, amines, sulfhydryls, organic acids, aromatic and heterocyclic compounds are sensed with the olfactory neurons AWA, AWB and AWC (Bargmann et al., 1993). Many of these odorants are attractive at low concentrations, and are sensed by AWA and AWC. However, attractive odorants can become repulsive at high concentrations, best exemplified by 2-nonanone which is detected at high concentrations by the AWB neuron and triggers avoidance behavior (Troemel et al., 1997). It was shown that the AWA-specific GPCR for the attractant diacetyl (ODR-10) can be misexpressed in AWB making diacetyl repulsive (Troemel et al., 1997) and in

AWC making it attractive (Wes and Bargmann, 2001). These misexpression studies suggest odorant receptors can couple with machinery in other sensory cell types to regulate various *C. elegans* behaviors.

C. elegans chemosensory neurons are also required for detecting pheromones that signal population density (Golden and Riddle, 1982, 1984) and mating cues (Srinivasan et al., 2008). It was originally shown that worms detect a pheromone termed “daumone” which is secreted by all animals and which signals entry into the dauer state (Golden and Riddle, 1982, 1984). Daumone has been purified and was found to be a complex of ascarosides (Jeong et al., 2005; Butcher et al., 2007). Killing the ADF, ASI and ASG neurons causes constitutive dauer formation suggesting that these neurons detect ascarides and other cues that regulate dauer formation (Bargmann and Horvitz, 1991a). These same ascarosides (at low concentrations) were found to attract males to hermaphrodites, a sensory response which requires the ASK and male-specific CEM neurons (Srinivasan et al., 2008). Ascarosides have also been implicated in social feeding behavior (Macosko et al., 2009).

C. elegans is aerobic and prefers intermediate oxygen concentrations between 5-12% (Dusenbery, 1980; Gray et al., 2004). Aerotaxis behavior, in which animals clump in areas of intermediate oxygen concentrations, is mediated by the soluble guanylate cyclases GCY-35 and GCY-36 and requires the URX, AQR and PQR neurons (Cheung et al., 2004; Gray et al., 2004). While URX sends ciliated endings to the nose tip, the ciliated endings of AQR and PQR

terminate in the ceolomic cavity, suggesting these three neurons together sample both internal and external oxygen levels (Coates and de Bono, 2002).

Chemotransduction molecules in *C. elegans*

C. elegans has an estimated 500 expressed genes predicted to be chemosensory receptors (Bargmann, 2006a). These putative chemoreceptors all have predicted seven transmembrane domains and share distant homology with rhodopsin-related GPCRs. *C. elegans* chemoreceptors are typically expressed in only a single pair of sensory neurons, however, each neuron often expresses multiple chemoreceptor genes (Troemel et al., 1995; Colosimo et al., 2004; Chen et al., 2005; McCarroll et al., 2005). As mentioned previously, one of the best studied chemoreceptors is ODR-10 which is required for sensing the volatile odor diacetyl and is expressed in the cilia of the diacetyl-sensing neuron AWA (Sengupta et al., 1996).

The *C. elegans* genome encodes 21 G protein alpha subunits, including at least one member of each of the four mammalian G-alphpa classes (Gs, Gq, Go, and G12) (Jansen et al., 1999), and two additional nematode-specific expansions (O'Halloran et al., 2006). 14 of the *C. elegans* G-alpha proteins are expressed in sensory neurons, and like the chemoreceptor proteins, multiple G-alphas are often expressed in a single chemoreceptor neuron (Jansen et al., 1999). The G-alpha proteins exhibit both positive and negative regulatory functions in olfactory responses (Lans et al., 2004). For example, the G-alpha ODR-3 is expressed in both AWA and AWC, and *odr-3* mutants are defective in chemotaxis to both

AWA and AWC-specific odorants (Roayaie et al., 1998; Lans et al., 2004). The G-alpha GPA-3 is also expressed in AWA and AWC, and appears to function redundantly with ODR-3 because *odr-3*;*gpa-3* double mutants lack the residual chemotaxis response to AWA and AWC-specific odorants exhibited by *odr-3* single mutants (Jansen et al., 1999). Conversely, GPA-5 appears to have a negative regulatory function in olfactory responses since the chemotaxis defect towards AWA-specific odorants in *odr-3* single mutants is suppressed in *odr-3*;*gpa-5* double mutants (Jansen et al., 1999; Lans et al., 2004). The G-alpha GPA-2 has a similar negative regulatory role in olfactory responses to AWC-specific odorants (Lans et al., 2004). Gα proteins can also regulate diverse functions in the same sensory neuron. ODR-3 and GPA-3 are both expressed in the polymodal ASH neuron, but have distinct roles in ASH-mediated sensory responses: ODR-3 mediates osmotic avoidance and nose touch, whereas GPA-3 has a role in copper and quinine avoidance (Roayaie et al., 1998; Hilliard et al., 2004; Lans et al., 2004).

C. elegans chemosensory neurons express CNGs (Coburn and Bargmann, 1996; Komatsu et al., 1996) and TRPs (Colbert et al., 1997; Tobin et al., 2002) which are likely to be the chemotransduction channels downstream of G protein signaling. The *C. elegans* genome encodes six CNG channel homologs (Cho et al., 2005) and 17 TRP family proteins, with representatives from each of the seven TRP subfamilies (Xiao and Xu, 2009). The best-studied *C. elegans* CNGs are TAX-2 and TAX-4 which encode CNG beta and alpha subunits, respectively, and are required for the function of many worm

chemosensory neurons (Coburn and Bargmann, 1996; Komatsu et al., 1996). Likewise, the OSM-9 and OCR-2 proteins form a putative TRP channel required in the remaining chemosensory neurons that do not express TAX-2/TAX-4 (Colbert et al., 1997; Tobin et al., 2002).

TAX-2/TAX-4 has been expressed in a heterologous cell system and shown to form a functional heteromeric cGMP-sensitive channel, but has little sensitivity to cAMP (Komatsu et al., 1999). TAX-4 on its own can also produce a functional homomeric channel with cGMP-sensitivity (Komatsu et al., 1996), but TAX-2 alone is not functional in culture (Komatsu et al., 1999). The *C. elegans* genome encodes 34 guanylate cyclases (GCs) (Ortiz et al., 2006) and six phosphodiesterases (PDEs) (Liu et al., 2010), and each could potentially modulate the cGMP level to regulate CNGs. The GCs are divided into two main classes: 1) a membrane-associated form which functions in neurons important for olfaction and thermosensation (Vowels and Thomas, 1994; Birnby et al., 2000; L'Etoile and Bargmann, 2000; Inada et al., 2006), and 2) a soluble form that is intracellular, members of which are required for O_2 sensation and social feeding (Cheung et al., 2004; Gray et al., 2004; Rogers et al., 2006). DAF-11 (Vowels and Thomas, 1994; Birnby et al., 2000) and ODR-1 (L'Etoile and Bargmann, 2000) are membrane-associated GCs expressed in many of the same sensory neurons as TAX-2 and TAX-4. It has been proposed that DAF-11 and ODR-1 form a heterodimer (Morton, 2004) and function downstream of G protein signaling in olfaction (Bargmann, 2006a). However, there is no direct evidence that G protein-mediated olfactory responses activate DAF-11/ODR-1 to

regulate cGMP/CNG channels. The soluble GCs GCY-35 and GCY-36 are expressed in AQR, PQR, and URX (also TAX-2/TAX-4 neurons) and are required for aerotaxis behaviors (Cheung et al., 2004; Gray et al., 2004; Rogers et al., 2006). GCY-35 has been shown to bind oxygen with its heme group indicating it might function as a primary oxygen sensor (Gray et al., 2004).

OSM-9 and OCR-2 are required for all AWA and ASH-mediated chemosensory responses (Colbert et al., 1997; Tobin et al., 2002). Since G proteins are also required for AWA and ASH-mediated chemosensory responses (Roayaie et al., 1998; Hilliard et al., 2004; Lans et al., 2004), OSM-9/OCR-2 are proposed to function as a transduction channel downstream of G protein signaling. OSM-9 and OCR-2 depend on each other for localization at the AWA and ASH cilia endings, indicating they might form a heteromeric channel (Tobin et al., 2002). Calcium-imaging with Chameleon (Miyawaki et al., 1997) in the ASH neuron has demonstrated Ca^{2+}-transients in response to various nociceptive stimuli, which are absent in *osm-9* null mutants (Hilliard et al., 2005). PUFAs are proposed to be second-messengers linking GPCRs to TRP channel activation in AWA and ASH (Kahn-Kirby et al., 2004; Bargmann, 2006a). AWA and ASH-mediated responses are defective in *fat-3* mutants (Kahn-Kirby et al., 2004), a gene which encodes a Δ6 desaturase involved in lipid synthesis of long-chain polyunsaturated fatty acids (PUFAs). The mechanisms underlying PUFA mobilization in AWA and ASH have yet to be worked out, and therefore, more work is required to validate this model of TRP channel activation in AWA and ASH.

C. elegans Mechanosensory systems

The MRNs are made up of both ciliated and non-ciliated neurons. Each of the long processes of the non-ciliated ALM, AVM, PLM, and PVM neurons cover approximately half of the animal's body length and are filled with specialized 15-protofilament microtubules, encoded by *mec-7* (Savage et al., 1989) and *mec-12* (Fukushige et al., 1999), which blanket the cell membrane (Chalfie and Thomson, 1979; Chalfie and Sulston, 1981). These touch receptor neurons adhere to the worm cuticle through extracellular matrix (ECM) and hypodermal cells making them sensitive to forces encountered along the worm's body (Chalfie and Sulston, 1981). Conversely, the ciliated mechanosensitive neuron CEP sends dendritic processes to the mouth, and in combination with the ciliated ADE and PDE sensory neurons, it is presumed to function in detecting the mechanical aspects of bacteria and other food sources (Sawin et al., 2000). Another ciliated MRN is the polymodal ASH neuron which, in addition to its chemosensory role, functions in detecting osmotic (Bargmann et al., 1990) and mechanical forces (Kaplan and Horvitz, 1993).

Worms respond to both weak and strong mechanical stimuli. The response to weak stimuli, termed the "gentle touch" response, is assayed using an eyebrow hair to prod either the anterior, posterior, or nose of the worm. Anterior gentle touch to a forward moving worm causes the worm to move backwards, whereas posterior gentle touch to a reversing animal results in forward movement (Chalfie et al., 1985). The anterior gentle touch response requires the neurons ALM and AVM, while the posterior response requires PLM

(Chalfie et al., 1985) (Wicks et al., 1996). The nose touch response causes animals to reverse and requires the ASH neuron (Kaplan and Horvitz, 1993). Stronger mechanical stimuli delivered with a wire pick (termed “harsh touch”) also evoke an avoidance response. The harsh touch response requires, in addition to the gentle touch neurons, the MEC-3 expressing high-threshold MRN PVD (Way and Chalfie, 1989). Another mechanosensory assay is the plate tap response, which probably activates multiple gentle touch neurons simultaneously. Worms display habituation to repeated plate tap, and mutations that disrupt dopamine and glutamate (*cat-2* and *eat-2*, respectively) accelerate short-term plate tap habituation suggesting these neurotransmitter systems play a role in the plasticity of mechanosensory responses (Rankin and Wicks, 2000; Sanyal et al., 2004).

Worms slow their locomotion rate upon encountering a lawn of bacteria, a phenomenon referred to as the “basal slowing response” (Sawin et al., 2000). The basal slowing response requires intact dopamine neurotransmitter systems and the dopaminergic CEP, ADE and PDE neurons (Sawin et al., 2000). Interestingly, it was shown that basal slowing involves a mechanosensory response to the tiny forces of bacteria on the cuticle because the response can be phenocopied using Sephadex beads (Sawin et al., 2000).

Male worms have 42 male-specific MRNs that innervate structures throughout the tail region. These male-specific MRNs are required for stereotyped and well-coordinated mating behaviors exhibited by males upon

encountering hermaphrodites (Liu and Sternberg, 1995). These MRNs include: 1) HOA and HOB innervate the hook sensilla and are involved in locating the vulva; 2) SPD and SPV innervate the spicule and are thought to function in spicule insertion; and 3) 18 RnA and 18 RnB neurons innervate the dorsal and ventral surface of the sensory rays and mediate response to contact with the hermaphrodite (Liu and Sternberg, 1995).

Mechanotransduction molecules in *C. elegans*

The ciliated and non-ciliated MRNs have different classes of putative mechanotransduction channels. The ciliated MRNs express genes predicted to encode members of the TRP channel superfamily, whereas the non-ciliated MRNs express members of the DEG/ENaC superfamily (Goodman and Schwarz, 2003). As mentioned previously, the *C. elegans* genome encodes 17 TRP family proteins with at least one member of all seven TRP subfamilies (Xiao and Xu, 2009). The worm genome also encodes 23 DEG/ENaCs (Bounoutas and Chalfie, 2007).

The *C. elegans* TRPV channels OSM-9 and OCR-2 are required for the ASH-mediated nose touch response (Colbert et al., 1997; Tobin et al., 2002). As mentioned earlier, it has been proposed that OSM-9 and OCR-2 might form a heteromeric transduction channel since they depend on each other for proper localization at the ASH cilia endings (Tobin et al., 2002). Indeed, it has been shown that *osm-9* null mutants lack nose-touch evoked Ca^{2+}-transients seen in wild-type animals (Hilliard et al., 2005). OSM-9 is also expressed in the OLQ,

PVD and FLP neurons, and might function in mechanosensation in these MRNs. It has been suggested that activation of OSM-9/OCR-2 requires G protein-mediated lipid mobilization since mutants involved in lipid synthesis which have reduced levels of long-chain polyunsaturated fatty acids (PUFAs) are defective in ASH-mediated responses (Kahn-Kirby et al., 2004). TRP channels also appear to function in the male-specific MRNs. The *C. elegans* TRPP genes *pkd-2* and *lov-1* are co-expressed in the male-specific ray sensory neurons and HOB neuron, and are required for the tail response to contact with hermaphrodites and for spicule insertion, respectively (Barr and Sternberg, 1999; Barr et al., 2001).

The DEG/ENaCs receive their name from 1) founding family members identified in *C. elegans* which when mutated cause swelling and degeneration (DEG) (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994), and 2) epithelial amiloride-sensitive Na^+ channels (ENaC) (Chalfie and Wolinsky, 1990; Canessa et al., 1993; Canessa et al., 1994). The *C. elegans* DEG/ENaCs UNC-105, MEC-4 and MEC-10 have been shown to form functional channels when expressed in heterologous cells (Garcia-Anoveros et al., 1998; Goodman et al., 2002). The MEC-4 and MEC-10 channels are expressed in touch receptor neurons, and together with the accessory proteins MEC-2 and MEC-6, form a mechanotransduction complex (Chelur et al., 2002; Goodman et al., 2002) that is required for touch-evoked Ca^{2+} transients (Suzuki et al., 2003), and mechanoreceptor currents (O'Hagan et al., 2005). Unlike the TRP proteins which are localized to the cilia endings, MEC-2 and MEC-4 have been shown to be distributed along the entire length of the touch receptor neuron processes in a

punctate pattern (Chelur et al., 2002). This punctate expression pattern is disrupted in *mec-6* mutants, and in extracellular matrix (ECM) mutants *mec-1*, *mec-5*, and *mec-9*, but is unperturbed in the 15-p microtubule mutant mec-7, indicating associations with the ECM are required for correct localization of the mechanotransduction complex (Chelur et al., 2002; Emtage et al., 2004; Zhang et al., 2004).

Other *C. elegans* sensory modalites

In addition to chemosensory and mechanosensory systems, *C. elegans* also possesses neurons that function in thermotaxis, electrotaxis, and phototaxis (the topic of this thesis). Thermotaxis was demonstrated in the early days of *C. elegans* research, when it was observed that worms migrate to their cultivation temperature (between 16° to 20°C) on a thermal gradient and remain there (Hedgecock and Russell, 1975). This behavior was termed “isothermal tracking.” *C. elegans* thermotaxis behavior was also shown to exhibit plasticity, since worms adapt to new preferred temperatures within several hours, and avoid temperatures associated with starvation (Hedgecock and Russell, 1975). The sensory neuron AFD was later shown to be required for isothermal tracking (Mori and Ohshima, 1995). Thermosensory transduction mechanisms have been predicted based on genetics data (Mori, 1999; Mori et al., 2007). For instance, thermotaxis is defective in the CNG mutants *tax-2* and *tax-4* (Hedgecock and Russell, 1975; Komatsu et al., 1996), and the membrane GC mutants *gcy-8*, *gcy-18*, and *gcy-23* (Inada et al., 2006). Furthermore, TAX-2/TAX-4 channels have been shown to mediate temperature-evoked currents in AFD (Ramot et al.,

2008). These findings suggest thermosensory transduction in AFD utilizes membrane GCs to modulate the cGMP level in gating CNG channels.

C. elegans also exhibits electrotaxis behavior, such that animals placed in an electric field migrate toward the negative pole (Sukul and Croll, 1978). However, not until recently was the neuroanatomical basis of electrotaxis behavior studied in detail (Gabel et al., 2007). Using a laser ablation approach, Gabel and colleagues identified the ASJ and ASH sensory neurons as being important for normal thermotaxis behavior. Interestingly, these two sensory neurons are also required for normal phototaxis behavior in worms (Ward et al., 2008). In the same study, Gabel and colleagues screened a panel of candidate mutants, and found that several chemotaxis (che) and osmosensation (osm) defective mutants are also defective in thermotaxis behavior (Gabel et al., 2007). However, the mechanisms underlying electrosensory transduction are completely unknown, and indeed, electrotaxis in *C. elegans* remains an understudied phenomenon.

C. elegans are photosensitive and utilize a cGMP/CNG-based phototransduction mechanism

C. elegans inhabit a dark environment within soil, which might suggest the organism has no requirement for vision. In our studies, we reasoned that phototransduction might serve functions other than “seeing,” namely that sensing light could be useful for photoavoidance behaviors. In this thesis work, we developed *C. elegans* as a model for phototransduction. We found that, despite the lack of specialized light-sensing organs, worms engage in negative

phototaxis behavior, which is important for survival and might provide a potential mechanism for retaining worms in soil.

The work presented in this thesis demonstrates that *C. elegans*, a creature previously assumed photoinsensitive, exhibits light avoidance behavior (Ward et al., 2008). We show that a group of amphid ciliated sensory neurons in *C. elegans* are light-sensitive and are required for normal light-avoidance behavior (Ward et al., 2008). *C. elegans* photoresponses are most sensitive to UV light (Edwards et al., 2008; Ward et al., 2008), and in fact, we observed that prolonged exposure to UV, purple, and blue light is lethal to worms, suggesting that light-avoidance may be a protective mechanism (Ward et al., 2008).

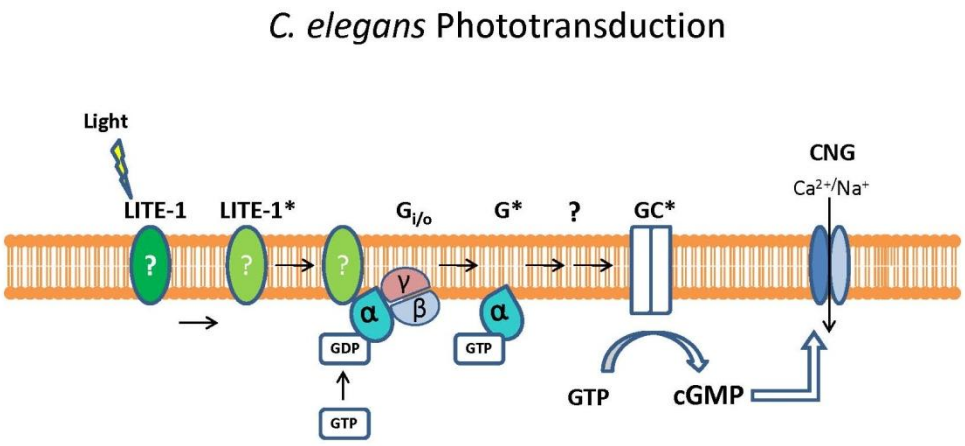


Figure 1.3 Schematic of *C. elegans* phototransduction

Photocurrents in *C. elegans*, which are inward/depolarizing, are mediated by CNG non-selective cation channels, and are cGMP-dependent (Ward et al., 2008) (see Fig. 1.2). Light-dependent production of cGMP in *C. elegans*

photoresponses was shown to require the genes *daf-11* and *odr-1* (Liu et al., 2010), which encode membrane GCs required for normal chemotaxis behavior (Birnby et al., 2000; L'Etoile and Bargmann, 2000). This thesis also shows that phototransduction requires $G_{i/o}$-type G protein signaling, because *C. elegans* photoresponses are PTX-sensitive (Liu et al., 2010). A novel non-opsin taste receptor-like gene *lite-1* was identified in a screen for photoinsensitive mutants (Edwards et al., 2008; Liu et al., 2010), and the LITE-1 protein was shown to be required upstream of G protein signaling in the *C. elegans* phototransduction pathway (Liu et al., 2010). LITE-1 has homology to the *Drosophila* family of gustatory receptors. Currently, it is not known how *Drosophila* taste receptors function *in vivo*. As taste receptors are related to odorant receptors in insects, it has been suggested that taste receptors may function as ion channels and that G protein signaling may not be directly involved in the transduction pathway in taste neurons (Sato et al., 2008). Recent work, however, has found that insect taste receptors and olfactory receptors have evolved along distinct paths during evolution and may employ distinct mechanisms for ligand recognition and signal transduction (Gardiner et al., 2009; Yao and Carlson, 2010). In light of this notion and the fact that LITE-1 and insect taste receptors belong to the same gene family, our results support the view that some *Drosophila* taste receptors may recruit G protein signaling in the transduction pathway. These results and conclusions will be discussed in detail in Chapters 2, 3 and 4.

Chapter 2

Light-sensitive neurons and channels mediate phototaxis in *C. elegans*

Summary

Phototaxis behavior is commonly observed in animals with light-sensing organs. *C. elegans*, however, is generally believed to lack phototaxis, as this animal lives in darkness (soil) and does not possess eyes. Here, we found that light stimuli elicited negative phototaxis in *C. elegans* and that this behavior is important for survival. We identified a group of ciliary sensory neurons as candidate photoreceptor cells for mediating phototaxis. Furthermore, we found that light excited photoreceptor cells by evoking a depolarizing conductance carried by cyclic guanosine monophosphate (cGMP)-sensitive cyclic nucleotide–gated (CNG) channels, revealing a conservation in phototransduction between worms and vertebrates. These results identify a new sensory modality in *C. elegans* and suggest that animals living in dark environments without light-sensing organs may not be presumed to be light insensitive. We propose that urbilaterians, the last common ancestor of bilaterians, might have already evolved a visual system that employs CNG channels and the second messenger cGMP for phototransduction.

Introduction

The ability to sense and react to environmental stimuli is essential for animal survival (Kandel et al., 2000). Among the most common stimuli are chemicals, mechanical forces and light. Animals have evolved specialized sensory systems (for example, olfactory, gustatory, auditory and visual systems) to detect these stimuli. Although the morphology of sensory organs is highly diverse among different organisms, the cellular and molecular mechanisms underlying sensory perception, transduction and processing have similarities across phylogeny (Bargmann, 2006b). As such, invertebrate organisms have been widely used as genetic models for the study of sensory physiology.

Light sensation is a universal phenomenon found in most organisms. In vertebrates and insects, light is detected by photoreceptor cells in the retina, which mediates image-forming vision (Fu and Yau, 2007; Wang and Montell, 2007). Photoreceptor cells also mediate non–image-forming functions, such as phototaxis and circadian rhythm (Kelber et al., 2003; Berson, 2007). Notably, retinal photoreceptor cells in vertebrates (for example, cones and rods) and insects adopt distinct morphologies, with the former being ciliated and the latter bearing microvillar structures (Fu and Yau, 2007; Wang and Montell, 2007). The phototransduction cascades in these two types of photoreceptor cells are also distinct, although both types of cells detect light with the rhodopsin family of G protein–coupled receptors (Fu and Yau, 2007; Wang and Montell, 2007). Specifically, vertebrate rods and cones transduce light signals into electrical responses by opening/closing CNG channels using cGMP as a second

messenger (Fu and Yau, 2007). In contrast, *Drosophila* photoreceptor cells employ transient receptor potential (TRP) family channels and an unknown second messenger for phototransduction (Fu and Yau, 2007). It is not known how these two distinct modes of phototransduction have evolved in vertebrates and insects during evolution.

The nematode *C. elegans* has emerged as an increasingly popular genetic model organism for the study of sensory transduction, including olfactory transduction and mechanotransduction (Bargmann, 2006a; Bounoutas and Chalfie, 2007). Here, we developed *C. elegans* as a model for phototransduction. We found that, despite the lack of specialized light-sensing organs, worms engage in phototaxis behavior that is mediated by light-sensitive neurons and requires cGMP/CNG channel–dependent phototransduction. This behavior is important for survival and might provide a potential mechanism for retaining worms in soil.

Experimental Procedures

Behavioral and statistical analysis

Phototaxis was tested on day 1 adult worms unless otherwise indicated. Worms were transferred to nematode growth medium plates (one worm per plate) covered with a thin layer of freshly spread OP50 bacteria 2–5 min before the test. To quantify the percent responding, we tested each worm five times with an 8–10-min interval between each test and tabulated a percentage score for each worm. To quantify response delay, response amplitude and response

duration, we tested each worm only once. The number of head swings was determined according to the definition created in a previous study (Gray et al., 2005). Light pulses from an Arc lamp (EXFOXcite) were delivered to the worm head via a 10X objective in combination with a 1–8X zoom lens on a Zeiss microscope (Zeiss Discovery) and the entire event was recorded with a digital camera (Cohu 7800) at 16 frames per s. To direct light to the worm head, we manually moved the stage (plate) such that only the head of the worm appeared in the field of view. A positive response was scored if the worm stopped forward movement within 3 s after the cessation of light illumination and also initiated backward movement that lasted at least half of a head swing. In most cases, a 2-s light pulse was used to trigger responses unless otherwise indicated.When light was directed to the worm tail or body, it usually stimulated forward movement. Light intensity was determined with a radiometric sensor head (268S for UV-A light and 268LP for visible light) coupled to an optometer (S471, UDT Instruments). The intensities of UV-A, violet, blue, green-1, green-2 and yellow light were sampled at 340, 430, 470, 500, 550 and 580 nm, respectively. The background light used to visualize worms was filtered into red with a red filter. I_o was set as 20 $mWmm^{-2}$ for all wavelengths. A software package was developed in the laboratory by modifying one reported previously to control the shutter and the camera, as well as to process images and quantify behavioral parameters (Feng et al., 2006; Li et al., 2006). Laser ablation was performed on L2 worms using standard protocols (Bargmann and Avery, 1995) and phototaxis was analyzed at day 1 or 2 adulthood. A GFP transgene under the control of the *tax-*

2Δ promoter was expressed in the worm to aid laser ablation (Coburn and Bargmann, 1996).

Statistical analysis was carried out using the Statistica (StatSoft). P values were generated by ANOVA using the Bonferroni test. $P < 0.05$ was considered to be significant.

Genetics and molecular biology

To generate transgenic worms expressing the wild-type *tax-2* genes in specific neurons, we directly injected plasmids encoding *tax-2* cDNA under the control of the *trx-1* (ASJ), *str-1* (AWB) and *srg-8* (ASK) promoters into *tax-2*(*p671*) worms (Troemel et al., 1995; Miranda-Vizuete et al., 2006). Plasmids encoding DsRed driven by the same cell-specific promoters were used as a co-injection marker to facilitate selection of the worms carrying the transgene in the neuron of interest for behavioral tests. The *srg-8*::*tax-2* transgene appeared to get silenced after more than two passages, and the worms were thus assayed at the F2 generation

Electrophysiology

Patch-clamp recordings were carried out under an Olympus microscope (BX51WI) with an EPC-10 amplifier and the Pulse software (HEKA) using a protocol modified from previous studies (Richmond and Jorgensen, 1999; Brockie et al., 2001). In brief, worms were glued to a sylgard-coated coverglass covered with bath solution and a small piece of cuticle in the worm head was cut

open and pinned down to the coverglass to expose the cells. The ASJ neuron was identified by an mCherry fluorescence marker expressed as a transgene driven by the trx-1 promoter. mCherry was excited by orange light (590 ± 10 nm). Background light was filtered into red with a red filter. Light pulses (0.5 s) were delivered from an Arc lamp (EXFO Xcite) coupled to a mechanical shutter (Sutter) triggered by the amplifier. Recording pipettes were pulled from borosilicate glass and firepolished. The bath solution contains 145 mM NaCl, 5 mM KCl, 1 mM CaCl2, 5 mMMgCl2, 11 mM dextrose and 5 mM HEPES (330 mOsm, pH adjusted to 7.3). The pipette solution for perforated patch clamp contained 115 mM potassium gluconate, 15 mM KCl, 5 mM MgCl2, 10 mM HEPES, 0.25 mM CaCl2, 20 mM sucrose, 5 mM EGTA and 50 μg ml^{-1} nystatin (315 mOsm, pH adjusted to 7.2). We included 5 mM Na_2ATP and 0.5 mM Na_2GTP in the pipette solution during classic whole-cell recording. When acquiring voltage-ramp traces, potassium gluconate was replaced with CsCl in the pipette solution. Nystatin was included in the pipette solution only during perforated whole-cell recording. Several other ionophores were also tested for perforated patch clamp (for example, β-escin, amphotericin B and gramicidin), and nystatin was found to be the most efficient under our conditions. Voltages were clamped at –70 mV. Current data were sampled at 5 kHz. Series resistance and membrane capacitance were both compensated for during recording.

Results

Light stimuli evoke negative phototactic responses

Animals living in dark environments without light-sensing organs are generally believed to have not evolved or to have lost sensitivity to light during evolution. However, we reasoned that there must be a mechanism(s) that acts to keep such animals in the dark. One possibility is that when the animal approaches a light environment, light may trigger negative phototactic responses that would drive the animal back to a dark environment.

We tested this hypothesis in *C. elegans*, an organism that lives in soil and lacks morphologically distinct light-sensing organs (Brenner, 1974).We found that light stimuli elicited robust avoidance responses in worms. Specifically, when a flash of light was focused on the head of a worm moving forward, the animal quickly responded by stopping forward movement and initiating reversals (Fig. 2.1a). Similarly, when a light pulse was directed to the tail or body of a worm moving backwards, the animal stopped its backward movement and began to move forward. As a result of these behavioral responses, the animals were able to avoid light. This negative phototaxis behavior might serve as a potential mechanism for keeping worms in soil.

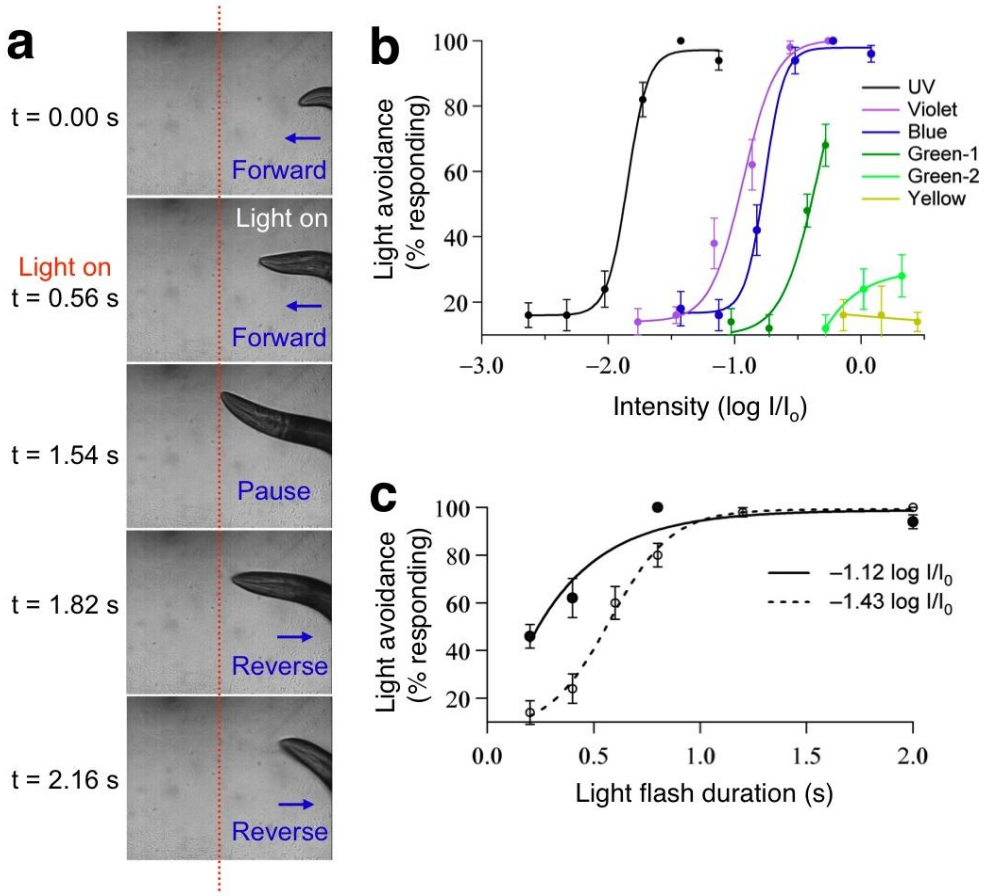
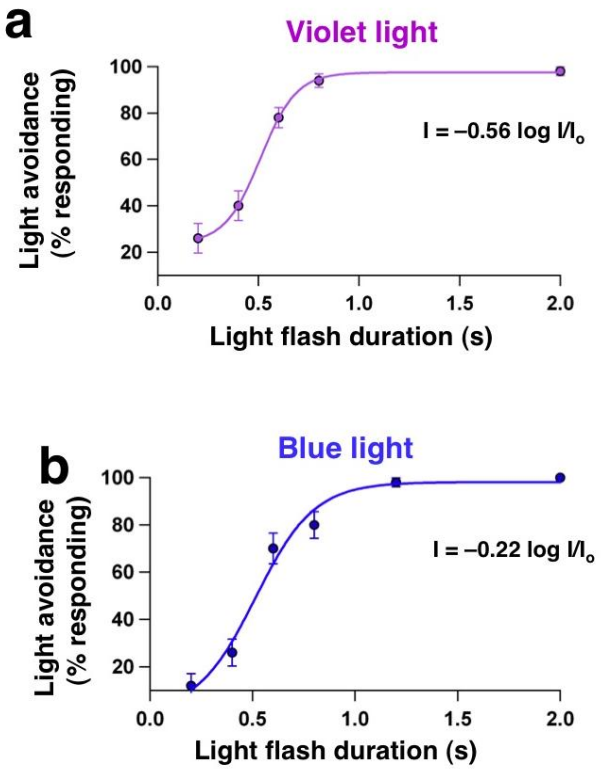


Figure 2.1 Light evokes avoidance responses in *C. elegans* in a dose-dependent manner. (**a**) Snapshot images showing that a flash of light triggered an avoidance response in a worm moving forward. A flash of light (2 s, UV-A) was delivered by an objective to the head of a worm moving forward under a microscope. The animal quickly responded by stopping forward movement and initiating reversals. The dotted red line indicates the position of the worm in the field. (**b**) Worms responded to light in an intensity-dependent manner and were most sensitive to UV-A light. Light pulses (2 s) of varying intensity were tested for the head avoidance response and the percentage of worms that responded was scored (I_o = 20 mW mm^{-2}, n = 10). Error bars represent s.e.m. (**c**) Worms responded to light in a duration-dependent manner. Light pulses of varying duration were tested for the head avoidance response. Two different intensities of UV-A light were tested (–1.12log I/I_o and –1.43log I/I_o, n = 10). We also examined violet and blue light (**Supplementary Fig. 2.1**). Error bars represent s.e.m.



Supplementary figure 2.1. **Worms respond to light in a duration-dependent manner.** Light pulses of varying duration were tested for the avoidance response. Shown here are data for violet light **(a)** and blue light **(b)**. Please see data for UV light in figure 1c. n=10. Error bars: SEM.

Worms respond to light in a dose-dependent manner

To characterize phototaxis behavior, we focused on the head avoidance response, as it is relatively easy to quantify this response. We found that worms responded to light stimulation in a dose-dependent manner (Fig. 2.1b,c and Supplementary Fig. 2.1). The percentage of worms that responded increased as the intensity of the stimulus increased (Fig. 2.1b). A similar phenomenon was observed when we extended the duration of the stimulus (Fig. 2.1c and

Supplementary Fig. 2.1). We also quantified the response delay and found that worms initiated reversals as soon as 1 s after the onset of light illumination, depending on the light intensity (Fig. 2.2a). To quantify the response amplitude and duration, we measured the distance (that is, the number of head swings) and the duration of backward movement (Fig. 2.2b,c). The distance and duration of backward movement increased with the intensity of the stimulus (Fig. 2.2b,c). These results demonstrate that behavioral responses to light in *C. elegans* are dose dependent.

Notably, we found that worms showed the highest sensitivity to UV-A light (long ultraviolet; 350 ± 25 nm), followed by violet (435 ± 10 nm) and blue light (470 ± 20 nm) (Fig. 1b). UV-B (280–315 nm) and UV-C (<280 nm) light were not tested because of technical reasons. In contrast, worms were rather insensitive to green-1 light (500 ± 10 nm; Fig. 2.1b). Very little, if any, response was induced by green-2 (545 ± 15 nm) or yellow light (575 ± 25 nm), the wavelengths shown to have subtle effects on worm movement (Burr, 1985) (Fig. 2.1b). These results indicate that the observed avoidance responses resulted from light rather than

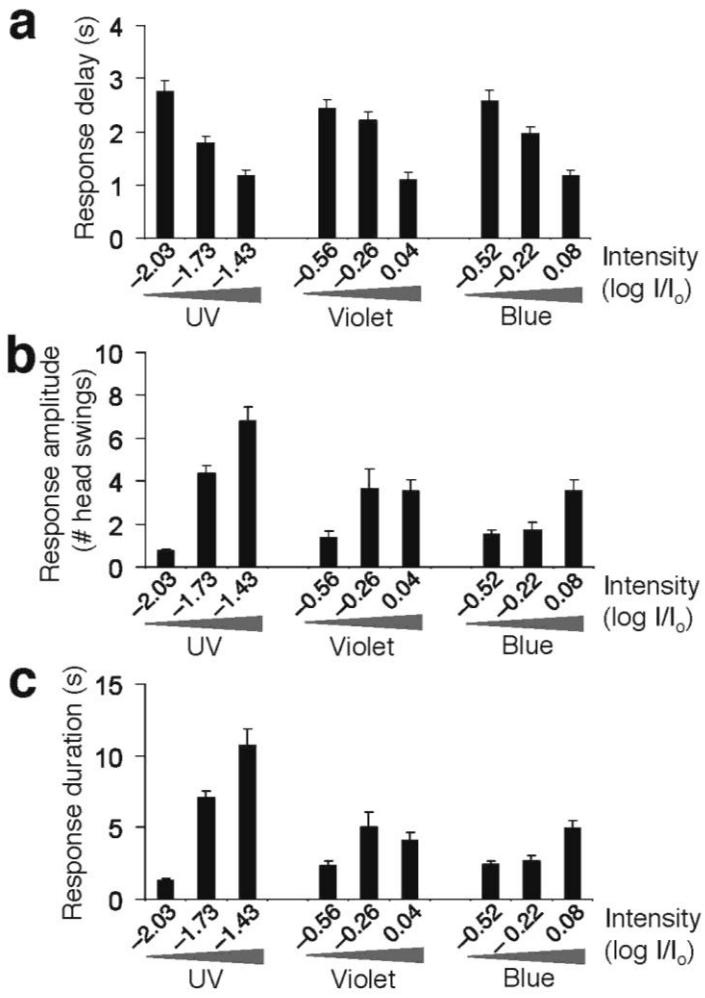
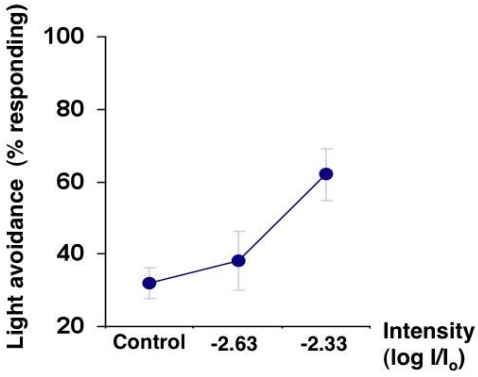


Figure 2.2 Behavioral quantification of phototactic responses. (**a**) Quantification of the response delay. Worms responded to a flash of light by initiating reversals in as short as ~1 s, depending on the light intensity. The response delay was quantified as the time interval between the onset of light illumination and the time point at which the animal initiated backward movement. We tested three different intensities of UV-A, violet and blue light pulses (2 s, *n* = 10). Error bars represent s.e.m. (**b**) Quantification of the response amplitude. The assay was performed as described in **a**, and the number of head swings during backward movement was quantified (*n* = 10). Error bars represent s.e.m. (**c**) Quantification of the response duration. The assay was performed as described in **a**, and the duration of backward movement was quantified (*n* = 10). Error bars represent s.e.m.

heat, as green and yellow light produce more heat than ultraviolet, violet and blue light. Although it is always difficult to compare conditions in the laboratory with those in the natural environment, the ultraviolet components in sunlight might potentially induce a negative phototactic response in worms (Supplementary Fig. 2.2). Phototaxis to ultraviolet light has also been observed in other organisms, including the fruit fly *Drosophila* (Harris et al., 1976).



Supplementary figure 2.2. **The threshold of UV-A light intensity in inducing an avoidance response in worms.** Using a slightly longer duration of UV-A pulses (5 s instead of 2 s), we began to observe phototactic responses at an intensity of -2.63 log I/I_o (control: no light). This intensity is equivalent to 47 mW/mm^2, which would probably become lower if the stimulus duration is further increased. The UV-A component (310-400 nm) in the sunlight at a summer day (e.g. mid-June) in the U.S. can reach up to ~74 mW/mm^2 (Langley-Calibrated irradiance) in Manna Loa of Hawaii, ~64 mW/mm^2 in Homestead of Florida, and ~55 mW/mm^2 in Pellston of Michigan based on the data monitored by the U.S. observatories sponsored by the USDA (raw data are available at its website and were integrated across 310-400 nm). Thus, while it is always difficult to compare conditions in the laboratory and those in the natural environment, it remains possible that the UV-A component alone in the sunlight could be sufficient to induce an avoidance response in worms. UV-B light is also present in the sunlight and may further contribute to evoke a response. In addition, violet and blue light in the sunlight may also further contribute.

Phototaxis is essential for survival

Phototaxis behavior may also serve as a protective mechanism for C. *elegans*, as prolonged light exposure paralyzed and killed the animal (Fig. 2.3).

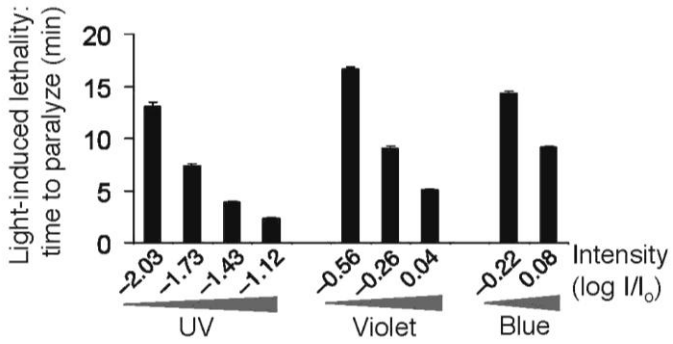
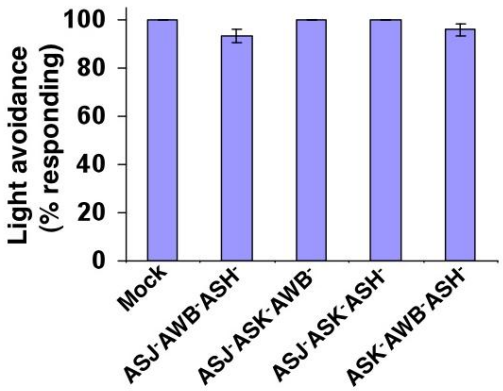


Figure 2.3 Prolonged light exposure induces paralysis/lethality in worms. Worms were exposed to prolonged light illumination until death and the elapsed time was recorded. To keep the animal exposed to light continuously, we manually moved the stage to follow the animal to keep it in the field of illumination. Under this condition, worms were usually hyperactive at the beginning, but eventually ceased movement and pharyngeal pumping (n = 10). Error bars represent s.e.m.

Thus, it seems that the ability to avoid light is essential for survival. The paralysis induced by prolonged light exposure and the phototactic responses triggered by acute light pulses are probably mediated by different mechanisms, as mutants lacking phototaxis can still be paralyzed by light (A.W. and X.Z.S.X., unpublished observations). As observed with phototaxis, UV-A light was also more efficient at paralyzing worms than violet and blue light (Fig. 2.3). Green and yellow light did not induce paralysis in worms in 20min under our conditions. As worms showed the highest sensitivity to UV-A light, we chose to focus on UV-A light for further characterization.

Identification of candidate photoreceptor cells

In the vertebrate retina, light is first detected by photoreceptor cells (for example, rods and cones) (Fu and Yau, 2007). To identify candidate photoreceptor cells in *C. elegans*, we used a laser ablation approach to determine which sensory neurons are required for mediating the light-induced head avoidance response. Laser ablation of a combination of seven neurons (ASJ, AWB, ASK, ASH, ASI, AWC and ADL) abrogated the head avoidance response (Fig. 2.4a). All of these neurons are ciliated neurons (White et al., 1986).



Supplementary figure 2.3. **Additional laser ablation data.**

Laser ablation of different combinations of sensory neurons. No severe defect in light-induced avoidance responses was observed in these combinations. A 2 s light pulse (UV-A, -1.43 log I/I_o) was used in the test. $n \geq 5$. Error bars: SEM.

We further narrowed down the list to four neurons (ASJ, AWB, ASK and ASH) that, when killed together, led to a severe defect in the head avoidance response (Fig. 2.4a). A similar group of neurons have been found to be important for electrotaxis (Gabel et al., 2007). Ablation of these neurons individually or in different combinations did not yield a severe defect (Fig. 2.3a and Supplementary Fig. 2.3), revealing the presence of functional redundancy among these

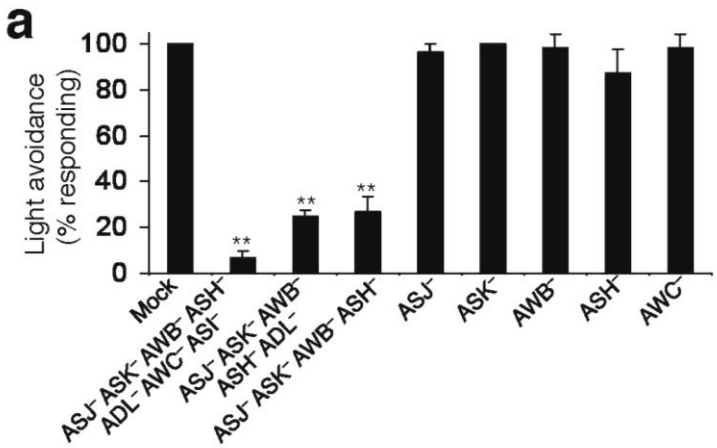
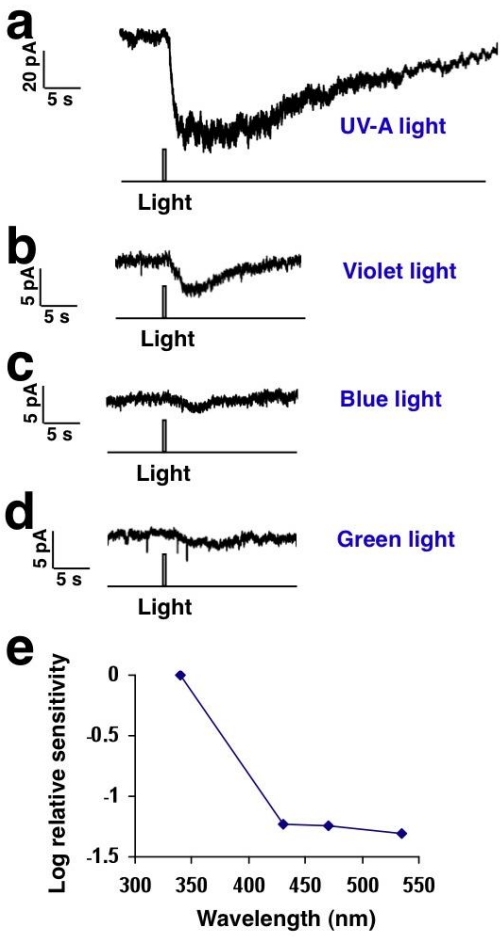


Figure 2.4 Phototaxis in *C. elegans* requires ciliary sensory neurons. (**a**) Phototaxis in *C. elegans* required ciliary sensory neurons. Laser ablation of a group of ciliary sensory neurons led to a severe defect in light-induced avoidance responses. A 2-s light pulse (UV-A, –1.43log I/I_0) was used. $^{**}P < 0.0002$ compared with mock, $n = 4$. Error bars represent s.e.m.

neurons for mediating phototaxis. Although we cannot rule out the possibility that other neurons may also be light sensitive, our results identify these neurons as candidate photoreceptor cells that are important for phototaxis in *C. elegans*.

Light evokes an inward current carried by CNG channels

To obtain direct evidence that the identified candidate photoreceptor cells are light sensitive, we sought to record the activity of these neurons in response to light by patch clamp. Calcium imaging approaches were not chosen, as worms are sensitive to violet and blue light, which overlap with the spectrum of all of the genetically encoded calcium sensors that are currently available. We decided to focus on the ASJ neuron. However, initial attempts to record this neuron using classic whole-cell recording protocols failed to detect light-induced currents in ASJ. This might result from some potential physical damage to the neuron that was caused by the recording protocol. Alternatively, some component(s) that are essential for phototransduction might have been dialyzed out by the recording pipette. To overcome this difficulty, we developed a protocol to record ASJ in situ in dissected live worms by perforated whole-cell recording. We found that a flash of light evoked an inward current in ASJ, which developed in milliseconds (356 ± 37 ms, n = 12) after the onset of light illumination (Fig. 2.5a). In vertebrate photoreceptors from the parietal eye, light can also evoke an inward current by opening CNG channels, although in those from lateral eyes light elicits an outward current (Finn et al., 1997). Consistent with our behavioral data, UV-A light is more efficient in inducing a light conductance than are violet, blue and green light (Supplementary Fig. 2.4). The light-induced current in ASJ was slightly outward rectifying, with a reversal potential near zero (Fig. 2.5), a feature similar to that observed in vertebrate photoreceptors (Kaupp and Seifert, 2002).



Supplementary figure 2.4. **ASJ is more sensitive to UV light than to violet, blue and green light. (a)** ASJ was recorded by perforated whole-cell patch-clamp. A 0.5 s of light pulse (UV-A, -1 log I/I_o) was used to simulate ASJ. The trace is a duplicate of figure 5a. **(b-d)** ASJ respond to violet, blue and green-2 light but with a lower sensitivity. **(e)** Log relative sensitivity of the ASJ neuron to UV-A, violet, blue and green light.

In vertebrate rods and cones, light signals are transduced into electrical responses in a process called phototransduction, which requires CNG channels (Kaupp and Seifert, 2002; Fu and Yau, 2007). We thus wondered whether CNG channels were also involved in mediating phototaxis in *C. elegans*. The worm genome encodes a total of six CNG channel homologs, four of which have

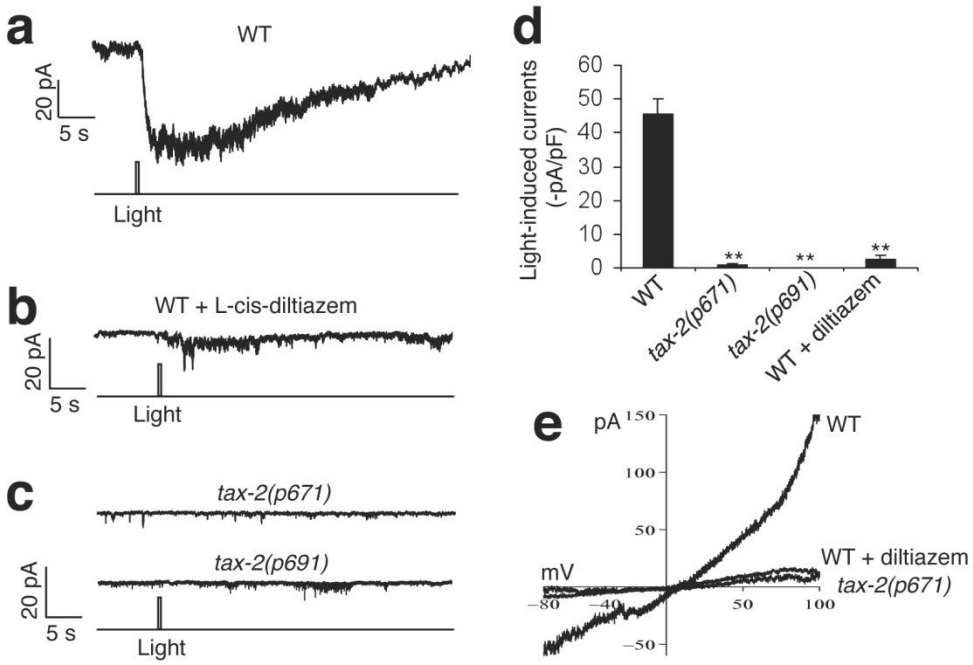
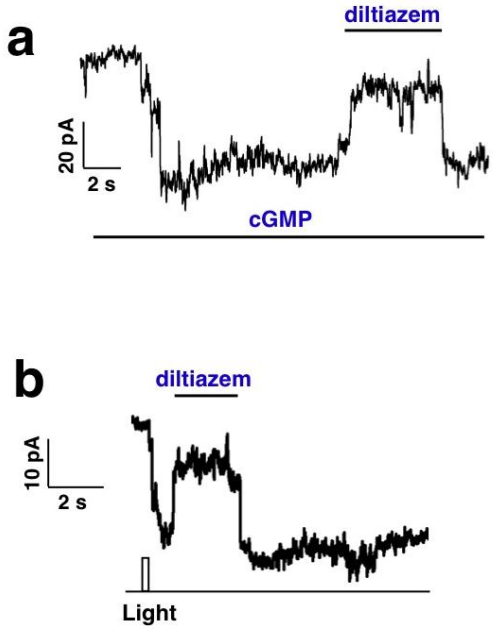


Figure 2.5 Light stimulates the photoreceptor neuron ASJ by evoking an inward current carried by CNG channels. (**a**) Light evoked an inward current in the ASJ neuron of wild-type worms. The ASJ neuron from acutely dissected live worms was recorded by perforated voltage clamp (–70 mV). A flash of UV-A light (0.5 s, –1log I/I_o) was used to stimulate the neuron. The same intensity and duration of UV-A light was used during the rest recordings unless otherwise indicated. Shown is a representative trace. (**b**) The light-induced current was sensitive to the CNG-channel inhibitor L-*cis*-diltiazem. Recording was performed as described in **a**. L-*cis*-diltiazem (100 µM) is membrane-permeable and was included in the bath solution. The inhibitory effect of this drug was reversible (**Supplementary Fig. 2.5**). Shown is a representative trace. (**c**) The light-induced current was absent in mutant worms lacking the CNG-channel homolog TAX-2. Recording was performed as in **a**. Two different *tax-2* mutant alleles (*p671* and *p691*) were examined. (**d**) Bar graph summarizing the data in **a**–**c**. ***P* < 0.00001 compared with wild type, *n* = 9. Error bars represent s.e.m. (**e**) *I-V* relations of the light-induced conductance. Shown are voltage-ramp traces recorded from wild-type worms with and without L-*cis*-diltiazem and from *tax-2*(*p671*) mutant worms.

known mutant alleles available for study (*cng-1*, *cng-2*, *tax-2* and *tax-4*) (Cho et al., 2005). Some of these genes have also been shown to function as CNG channels in heterologous systems (Komatsu et al., 1999). Notably, a previous

study showed that *tax-2* is expressed in a number of ciliary sensory neurons, including ASJ, AWB and ASK, that we identified as candidate photoreceptor cells by laser ablation (Coburn and Bargmann, 1996). We found the light-induced current in ASJ was sensitive to L-*cis*-diltiazem, a CNG channel-specific inhibitor that blocks light-induced currents in vertebrate rods and cones (Stern et al., 1986) (Fig. 2.5b,d and Supplementary Fig. 2.5). To provide further evidence for a critical role of CNG channels in mediating the light conductance in ASJ, we recorded this neuron from mutant animals lacking the CNG-channel homolog TAX-2 (Fig. 2.5c). No notable light-induced current was observed in ASJ of *tax-2* mutant worms (Fig. 2.5c–e). This observation, together with the electrophysiological and pharmacological evidence described above, strongly suggests that the light-induced conductance in ASJ is mediated by CNG channels.



Supplementary figure 2.5. **The inhibitory effect of L-*cis*-diltiazem on the light- and cGMP-induced currents is reversible. (a)** cGMP (1mM) was dialyzed into ASJ by the recording pipette. After the development of an inward current, L-*cis*-diltiazem (100 mM) was briefly (~5 s) perfused toward ASJ via a pressurized rapid perfusion system (i.e. puffing). **(b)** ASJ was recorded by perforated whole-cell patch-clamp. A 0.5 s of light pulse (UV-A) was used to simulate ASJ. After the appearance of an inward current, L-*cis*-diltiazem (100 mM) was then very briefly (~2 s) perfused toward ASJ via a pressurized rapid perfusion system. Rapid local perfusion often causes loss of giga-seal during recording.

cGMP is a second messenger for phototransduction in ASJ

In vertebrate rods and cones, the light-sensitive CNG channels are gated by the second messenger cGMP, but are rather insensitive to cAMP (Kaupp and Seifert, 2002). In contrast, the olfactory transduction CNG channels in vertebrate olfactory receptor neurons can be activated by both cAMP and cGMP, although their native ligand is cAMP (Kaupp and Seifert, 2002). We thus asked whether the light-sensitive CNG channels in worm photoreceptor neurons depend on

cGMP and/or cAMP. Dialysis of cGMP into the ASJ neuron elicited an inward current, the amplitude of which showed a dose dependence on cGMP concentration (Fig. 2.6). Notably, cAMP evoked very little, if any, current in ASJ at concentrations of up to 2mM (Fig. 2.6b,e), demonstrating that cGMP, rather than cAMP, is the preferred ligand for the CNG channels in ASJ, a property that is shared by those in vertebrate rods and cones (Kaupp and Seifert, 2002). As was the case with the light-induced current, the cGMP-induced current in ASJ was also sensitive to L-*cis*-diltiazem, a CNG channel–specific inhibitor (Stern et al., 1986) (Fig. 2.6c,f and Supplementary Fig. 2.5). In addition, both types of currents shared a nearly identical I-V relationship, that is, slightly outward-rectifying with a reversal potential near zero (Fig. 2.6g).

Normalized I-V traces from both channels extensively overlap (Fig. 2.6h). Furthermore, similar to the light-induced current, the cGMP-dependent current also required the CNG-channel homolog TAX-2, as no current was induced by cGMP in the ASJ neuron recorded from *tax-2* mutant worms (Fig. 2.6d,f). Taken together, these observations strongly suggest that the light- and cGMP-induced currents were carried by the same type of CNG channels. These data also suggest that cGMP may be a second messenger for transducing light signals into electric responses in the photoreceptor neuron ASJ.

If cGMP is a second messenger mediating phototransduction in ASJ, as suggested above, then blocking the production of cGMP should block phototransduction. cGMP is produced by guanylate cyclases. The worm genome

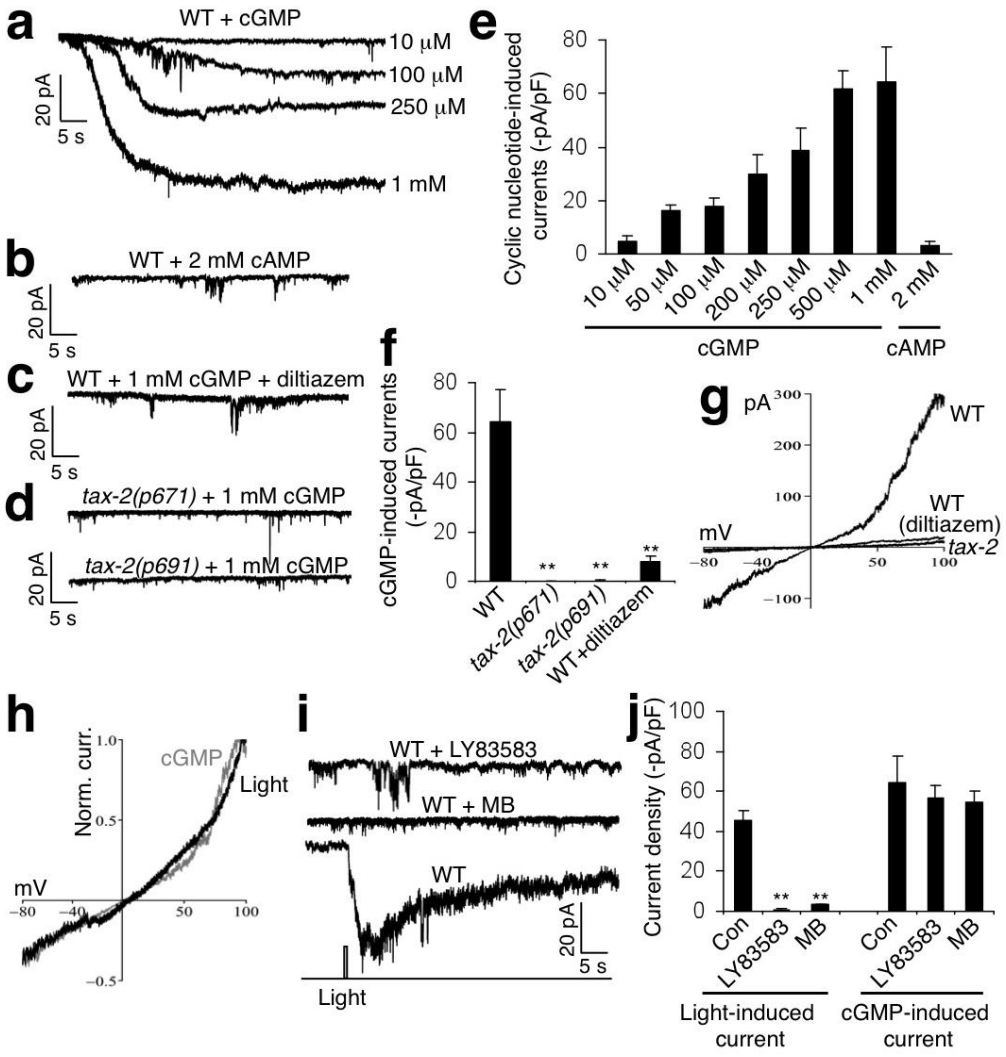


Figure 2.6 The light-sensitive CNG channels in the photoreceptor neuron ASJ are sensitive to cGMP. (**a**) cGMP induced an inward current in ASJ in a concentration-dependent manner. We dialyzed cGMP at varying concentrations into ASJ with the recording pipette. (**b**) cAMP failed to evoke an inward current in ASJ at concentrations of up to 2 mM. (**c**) The cGMP-induced current was sensitive to L-*cis*-diltiazem. The drug (100 μM) was included in the bath solution. (**d**) The cGMP-induced current was absent in *tax-2* mutants. (**e**) Bar graph summarizing the cGMP- and cAMP-induced currents recorded from wild-type worms ($n = 5$). (**f**) Bar graph summarizing the cGMP-induced currents recorded from *tax-2* mutant worms. $^{**}P < 0.0001$ compared with wild type, $n = 5$. (**g**) *I-V* relations of the cGMP-induced conductance. Shown are voltage-ramp traces recorded from wild-type worms with and without L-*cis*-diltiazem and *tax-2*(*p671*) mutant worms. (**h**) The light-induced and the cGMP-induced conductance shared a nearly identical *I-V* relationship. The voltage-ramp traces from **g** and **Figure**

2.5e were normalized and superimposed. (**i**) The light-induced current was blocked by the guanylate cyclase inhibitors LY83583 and methylene blue (MB). LY83583 (100 µM) and MB (10 µM) were included in the bath solution. A control trace (drug free) is also shown. (**j**) Bar graph summarizing the effects of the guanlynate cyclase inhibitors on the light- and cGMP-induced currents. LY83853 and MB blocked the light-induced current, but had no significant effect on the cGMP-induced current. ***P* < 0.0003 compared with control, *n* = 5. All error bars represent s.e.m.

encodes over 30 guanylate cyclase genes (Yu et al., 1997). To overcome the potential functional redundancy, we tested LY83857, a known guanylate cyclase inhibitor (Mulsch et al., 1989), and found that it suppressed the light-induced current in ASJ (Fig. 2.6i,j). As a control, this drug did not have a significant effect on the cGMP-induced current in ASJ (P > 0.50; Fig. 2.6j). To obtain additional evidence, we tested another known guanylate cyclase inhibitor, methylene blue (Danziger et al., 1993), and found that methylene blue also suppressed the light-induced current in ASJ (Fig. 2.6i,j). These results demonstrate that cGMP has a critical role in phototransduction and strongly suggest that cGMP is a second messenger for mediating phototransduction in the photoreceptor cell ASJ.

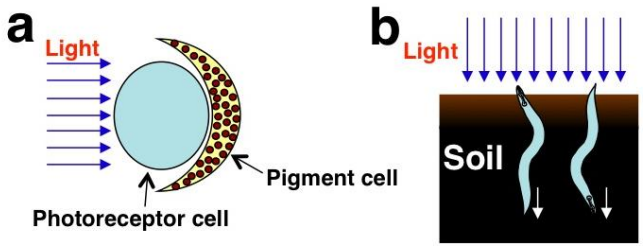
Discussion

C. elegans reacts to a wide variety of chemical (for example, odorants, tastants and oxygen, etc.) and mechanical (for example, body and nose touch) stimuli and is commonly used as a model for the study of sensory transduction (Ward, 1973; Chalfie et al., 1985; Bargmann et al., 1993; Kaplan and Horvitz, 1993; Gray et al., 2004; Cheung et al., 2005). In this study, we found that phototaxis behavior is present in *C. elegans*, a soil-dwelling organism that lacks

specialized light-sensing organs. This behavior is essential for survival and might provide a potential mechanism for retaining worms in soil, their natural environment. It thus appears that organisms living in dark environments without light-sensing organs may not be presumed to be completely blind. Our studies identify a new sensory modality in *C. elegans* and indicate that *C. elegans* could be a suitable model organism for the study of phototransduction.

Classic anatomical analyses indicate that, in light of the wide diversity of eye structure, eyes in vertebrates and invertebrates must have evolved independently (Salvini-Plawen and Mayr, 1961), although genetic studies of eye development have cast doubt on this view (Gehring and Ikeo, 1999). On the contrary, Charles Darwin postulated a monophyletic origin of eye evolution in his book, The Origin of Species, and suggested that all complex eyes may have evolved from a prototype eye that comprised only two cells: a photoreceptor cell (optic nerve) and a pigment cell(s), which were covered by translucent skin without any lens or other refractive body (depicted in Supplementary Fig. 2.6). The photoreceptor cell senses light and the pigment cell shades light such that light is only detected by the photoreceptor cell at certain directions (Supplementary Fig. 2.6). This type of primitive eye has been suggested to be present in a number of invertebrate organisms, including some planarians and annelid larva (Arendt et al., 2002; Gehring, 2005). It would be interesting to test whether the proposed photoreceptor cells are light sensitive.

In the case of *C. elegans*, clearly no pigment cells have been identified that may act to shade light from the photoreceptor cells. Nevertheless, it is



Supplementary figure 2.6. **Schematic models. (a)** A schematic illustrating Darwin's prototype eye. Light shed from the right was not drawn, but would be blocked by the pigment cell, such that only the light from the left would be sensed by the photoreceptor cell. **(b)** A schematic showing that a worm living in soil approaches the surface of the ground with its head or tail. Light would only be shed from top but not from underneath.

Under this scenario, light would trigger an avoidance response, and the worm would be driven back to soil. important to consider that worms live in soil (depicted in Supplementary Fig. 2.6), an environment that is distinct from that above ground where light would be detected from all directions. It is conceivable that when a worm approaches or emerges from the surface of the ground, light would be projected from top but not underneath, which would trigger a negative phototactic response in the animal (Supplementary Fig. 2.6). Under this scenario, soil shades light, acting as a surrogate pigment cell (Supplementary Fig. 2.6). We thus propose that the photoreceptor cells in worms are capable of assuming the proposed function of Darwin's primitive eyes. It is possible that pigment cells have been lost in *C. elegans* during evolution since its ancestors began to live in soil. Indeed, some marine and freshwater nematodes do have pigments in the head and are phototactic, although no photoreceptor cell has been functionally

identified in these species (Chitwood and Murphy, 1964; Croll, 1966). It is also possible that pigment cells have evolved independently of photoreceptor cells and have been recruited as needed during evolution.

There are two major types of photoreceptor cells in metazoans: the ciliary photoreceptors represented by vertebrate rods and cones (Fu and Yau, 2007) and the rhabdomeric photoreceptors, exemplified by those from *Drosophila* ommatidia (Montell, 1999). Although these two types of photoreceptors both detect light with the rhodopsin family of G protein-coupled receptors, the downstream phototransduction cascades in the two cell types are distinct (Montell, 1999; Fu and Yau, 2007). Specifically, vertebrate rods and cones employ light sensitive CNG channels and the second messenger cGMP for phototransduction (Fu and Yau, 2007), whereas *Drosophila* phototransduction is mediated by light-sensitive TRP channels and an unknown second messenger(s) (possibly DAG or its metabolites) (Montell, 1999). Thus, the question arises as to whether these two distinct phototransduction cascades have evolved separately in vertebrates and insects after their ancestors split from urbilaterians, the last common ancestor of all bilaterians (Adoutte et al., 1999). Alternatively, one or both types of phototransduction may have already been present in urbilaterians. Our studies indicate that *C. elegans* photoreceptor cells also employ CNG channels and the second messenger cGMP for phototransduction. Thus, the cGMP/CNG channel-mediated phototransduction seems to be an ancient pathway. We propose that urbilaterians might have already evolved a visual system that employs the cGMP/CNG channel-mediated signaling for

phototransduction. Considering that *C. elegans* and *Drosophila* both belong to the same superphylum, Ecdysozoa (Adoutte et al., 1999), it is possible that *Drosophila* might have lost this mode of phototransduction during evolution; alternatively, this pathway may exist in some *Drosophila* photoreceptors that have not yet been functionally identified. Future work is needed to address the evolutionary origin of TRP channel-mediated phototransduction.

Chapter 3

C. elegans phototransduction requires a G protein–dependent cGMP pathway and a taste receptor homolog

Summary

The eyeless animal *C. elegans* is able to sense light and engages in phototaxis behavior that is mediated by photoreceptor cells. However, the molecular and cellular mechanisms underlying phototransduction in *C. elegans* remain largely unclear. By recording the photoreceptor neuron ASJ in wild-type and various mutant worms, we found that phototransduction in ASJ is a G protein–mediated process and requires membrane-associated guanylate cyclases, but not typical phosphodiesterases. In addition, we found that *C. elegans* phototransduction requires LITE-1, a candidate photoreceptor protein known to be a member of the invertebrate taste receptor family. Our genetic, pharmacological and electrophysiological data suggest a model in which LITE-1 transduces light signals in ASJ via G protein signaling, which leads to upregulation of the second messenger cGMP, followed by opening of cGMP-sensitive CNG channels and stimulation of photoreceptor cells. Our results identify a phototransduction cascade in *C. elegans* and implicate the function of a 'taste receptor' in phototransduction.

Introduction

Being able to sense light is essential for the survival of most organisms. In animals, photoreceptor cells in the eye detect light and transduce it into electrical responses through a process called phototransduction. Among the best-characterized photoreceptor cells are vertebrate rods and cones, a group of ciliated sensory neurons in the retina. In these photoreceptor cells, light is absorbed by the rhodopsin family of GPCRs, which activate the G-protein transducin (Fu and Yau, 2007). Light-activated transducin then turns on phosphodiesterases (PDEs) to cleave the second messenger cGMP, resulting in a decrease in cGMP level and hence closure of CNG channels (Fu and Yau, 2007). In vertebrate parietal eye photoreceptor cells, however, light-activated G proteins can inhibit PDEs, leading to an increase in cGMP level and opening of CNG channels (Xiong et al., 1998). In both cases, membrane-associated guanylate cyclases that produce cGMP in these photoreceptor cells are constitutively active in the dark and therefore have a passive role in phototransduction by providing substrates to PDEs (Fu and Yau, 2007). In addition to this canonical phototransduction pathway, recent studies have found that photosensitive retinal ganglion cells, which mediate non–image forming visual functions, may employ a distinct pathway for phototransduction (Berson, 2007); nevertheless, the exact mechanisms remain unclear.

The nematode *C. elegans* has been widely used as a model for the study of sensory transduction. Among the three major sensory stimuli are chemicals, mechanical forces and light. Worms rely on olfactory neurons (for example, AWA

and AWC) and gustatory neurons (for example, ASE) to respond to chemical stimuli (Bargmann, 2006a), while reacting to mechanical forces via touch receptor neurons (for example, ALM, AVM and PLM) and proprioceptor neurons (for example, DVA) (Li et al., 2006; Bounoutas and Chalfie, 2007). However, worms were long thought to be unable the sense of light, as they do not have eyes and live in dark soil.

Recent work from us and others has shown that, despite lacking eyes, the soil-dwelling *C. elegans* is able to sense light and engages in negative phototaxis behavior that allows it to avoid lethal doses of light (Edwards et al., 2008; Ward et al., 2008). We suggested that this behavior may also provide a potential mechanism for retaining worms in the dark soil (Ward et al., 2008). We also reported that worms sense light through a group of photoreceptor cells, some of which respond to light by opening cGMP-sensitive CNG channels (Ward et al., 2008). These channels also mediate temperature-evoked currents in the thermosensory neuron AFD (Ramot et al., 2008). In addition, a genetic screen identified *lite-1*, a taste receptor-like gene that is important for phototaxis behavior and has been suggested to encode a light-sensing molecule (Edwards et al., 2008); however, it is not clear whether this gene is involved in phototransduction in photoreceptor cells.

Nevertheless, numerous unanswered questions remain. In particular, the phototransduction cascade in worm photoreceptor cells has not been elucidated. First, phototaxis behavior appears to persist in some G protein–signaling mutants

(Gq and Gs signaling) (Edwards et al., 2008). Does this indicate that *C. elegans* phototransduction is independent of G protein signaling? Second, do *C. elegans* photoreceptor cells also employ PDEs rather than guanylate cyclases for phototransduction? Third, is the *lite-1* gene involved in phototransduction in photoreceptor cells?

We conducted a comprehensive dissection of the phototransduction cascade in *C. elegans* using a combination of electrophysiological, pharmacological and genetic approaches. We found that phototransduction in the photoreceptor cell ASJ required a G protein–dependent cGMP pathway and the taste receptor homolog LITE-1.

Experimental Procedures

Behavioral analysis

Phototaxis behavior was analyzed exactly as previously described (Ward et al., 2008). In brief, day 1 worms were tested for head avoidance response to UV-A light on NGM plates freshly seeded with a thin layer of OP50 bacteria. Each worm was tested five times, and a percentage score was tabulated for each worm. Light pulses (UV-A, 350±25 nm, 2 s, -1.43 log I/Io) were delivered from an Arc lamp (EXFO) to the head of a worm slowly moving forward. UV light is most efficient in triggering phototaxis responses (Ward et al., 2008). Background light was filtered into red. Io was set as 20 mW/mm^2 in all cases. A positive response was scored if the animal stopped forward movement within 3 sec after the cessation of light illumination and also initiated backward movement that lasted

at least half a head swing. The whole event was recorded by a digital camera (Cohu 7800) at 16 Hz. A laboratory developed software package was used to control the light source and the camera and for image processing (Feng et al., 2006; Li et al., 2006).

Electrophysiology

Photocurrents were recorded by perforated whole-cell patch-clamp, a configuration that does not allow for dialysis of chemicals (with the exception of monovalent ions) into the recorded cell through the recording pipette. All other types of currents were recorded by classic whole-cell recording protocols that permit perfusion of chemicals into the patched cell through the recording pipette. Recordings were performed on an upright Olympus microscope (BX51WI) with an EPC-10 amplifier (HEKA), a micromanipulator (Sutter) and the Patchmaster (HEKA) software as previously described (Ward et al., 2008). Worms were glued on the surface of a sylgard-coated cover glass. A small piece of cuticle in the worm head was cut open and pinned down to the cover glass to expose the neurons of interest for recording. Background light was filtered into red. Light flashes were delivered to neurons from an Arc lamp (EXFO) controlled by a mechanical shutter (Sutter) triggered by the amplifier. Bath solution: 145 mM NaCl, 5 mM KCl, 1 mM CaCl2, 5 mM MgCl2, 11 mM dextrose, and 5 mM HEPES (330 mOsm; pH adjusted to 7.3). Pipette solution (perforated patch): 115 mM K-gluconate, 15 mM KCl, 5 mM MgCl2, 10 mM HEPES, 0.25 mM CaCl2, 20 mM sucrose, 5 mM EGTA, and 50 µg/ml nystatin (315 mOsm; pH adjusted to 7.2). Neurons were identified for recording by an mCherry fluorescence marker

expressed as a transgene (Ward et al., 2008). During classic whole-cell recordings, we included 5 mM Na2ATP and 0.25 mM NaGTP in the pipette solution. Recording pipettes (~10 MΩ) were pulled from borosilicate glass and fire-polished. Voltages were clamped at -70 mV. Series resistance and membrane capacitance were both compensated during recording.

Genetics and molecular biology

lite-1 mutants (*xu7*, *xu8* and *xu10*) were isolated in an F1 clonal EMS mutagenesis screen for mutants defective in phototaxis behavior. Standard SNP mapping protocols were used to position *xu7* to the close proximity of the SNP marker uCE6-981 (-4.03 cM) on the X-chromosome, which is very close to the location of *lite-1*. All three alleles failed to complement each other and *ce314*. Molecular lesions in the *lite-1* gene in all three alleles were identified by sequencing PCR products amplified from genomic DNA. Mutants were extensively outcrossed (e.g. six times for *xu7*) to N2 prior to behavioral and electrophysiological analysis.

pde deletion mutants were isolated by TMP/UV-based mutagenesis screens and were outcrossed at least six times to N2 prior to behavioral and electrophysiological analysis. Deletions cause frame shift and/or disruption of the catalytic domain. Primers used in deletion screens and the deleted segments are listed below:

pde-1(*nj57*): CCACCTGAAATCGCAGAACT (forward primer), TTCAAGGATAAATTTGCCGC (reverse primer) with a deletion in exon 5 and 6.

pde-2(*nj58*): TCGTTGTCGTTGTCGTCT TC (forward primer), GATAATGACGTGGCAATGAGG (reverser primer) with a deletion in exon 2. *pde-3*(*nj59*): CACCACAATTGACGGACAAC (forward primer), ACTTCACGGGAAACAAATGC (reverse primer), with a deletion in exon 3 and 4. *pde-4*(*nj60*): GGGATATCACGTGGCTTTGGAG (forward primer), CCTTGACGCTAACACCGAACAC (reverse primer), with a deletion in exon 6. *pde-5*(*nj49*): CGGATCTATCAATGAAGCGGAG (forward primer), CCAATTGTGGTAGGCAACTCGG (reverse primer) with a deletion spanning exon 4-9 (Bargmann, 2006a; Li et al., 2006; Bounoutas and Chalfie, 2007; Edwards et al., 2008; Ramot et al., 2008; Ward et al., 2008).

Standard protocols were used to generate transgenic lines. The *myo-3* promoter was used to express *lite-1* cDNA in muscle cells. The *trx-1* and *srg-8* promoter was used to express *lite-1* cDNA specifically in the ASJ and ASK neuron, respectively, to rescue mutant phenotypes (Troemel et al., 1995; Miranda-Vizuete et al., 2006). A fragment of the *tax-2* promoter (*tax-2Δ*) was also used to express *lite-1* cDNA in a subset of CNG neurons including the photoreceptor cells ASJ, ASK and AWB to rescue the behavioral phenotype of *lite-1* (Coburn and Bargmann, 1996).

Results

Phototransduction in ASJ requires G protein signaling

We first asked whether phototransduction in *C. elegans* photoreceptor cells requires G protein signaling. We focused on ASJ, the best characterized

photoreceptor cell (Ward et al., 2008), and recorded its activity in response to light by perforated whole-cell recording (Ward et al., 2008). Classic whole-cell recording protocols are incapable of detecting light-induced currents (photo-currents) in this neuron (Ward et al., 2008), probably because some components that are important for phototransduction are dialyzed out by the recording pipette. A similar phenomenon has been observed in recording vertebrate photoreceptor cells (Xiong et al., 1998).

To test whether G protein signaling is required for phototransduction in ASJ, we examined the effect of mSIRK, a membrane-permeable peptide that dissociates Gα from Gβγ without affecting its GTPase activity and thereby exerting an inhibitory effect on GPCR-mediated activation of Gα (Goubaeva et al., 2003). mSIRK blocked the light-evoked conductance in ASJ (Fig. 3.1a,b). As a control, the cGMP-induced currents were not affected in ASJ (Fig. 3.1c–e). Thus, blocking G protein signaling can inhibit phototransduction in ASJ, suggesting that G protein signaling is required for phototransduction in *C. elegans* photoreceptor cells.

If G protein signaling mediates phototransduction, then stimulating G protein signaling should stimulate photoreceptor cells. To test this, we perfused GTPγS, a non–hydrolyzable GTP analog that activates G proteins, into ASJ through the recording pipette. GTPγS stimulated ASJ by evoking an inward current in the dark (Fig. 3.1f). This current was apparently carried by CNG channels, as it can be blocked by the CNG channel–specific inhibitor l-*cis*-

diltiazem and was absent in the CNG channel mutants *tax-2* and *tax-4* (Fig. 3.1f) (Stern et al., 1986; Coburn and Bargmann, 1996; Komatsu et al., 1996). Therefore, stimulating G protein signaling can stimulate photoreceptor cells, suggesting that phototransduction in ASJ is a G protein–mediated process. These results also suggest that CNG channels act downstream of G proteins.

We next asked which type of G protein mediates phototransduction in *C. elegans* photoreceptor cells. Phototransduction in vertebrate rods and cones requires transducin, a Gα protein that belongs to the Gi/o subfamily (Fu and Yau, 2007). We tested the effect of mastoparan, a peptide that can activate Gi/o proteins (Leyte et al., 1992). Perfusion of mastoparan into ASJ elicited an inward current (Fig. 3.1g,h). Similarly, this current appeared to be carried by CNG

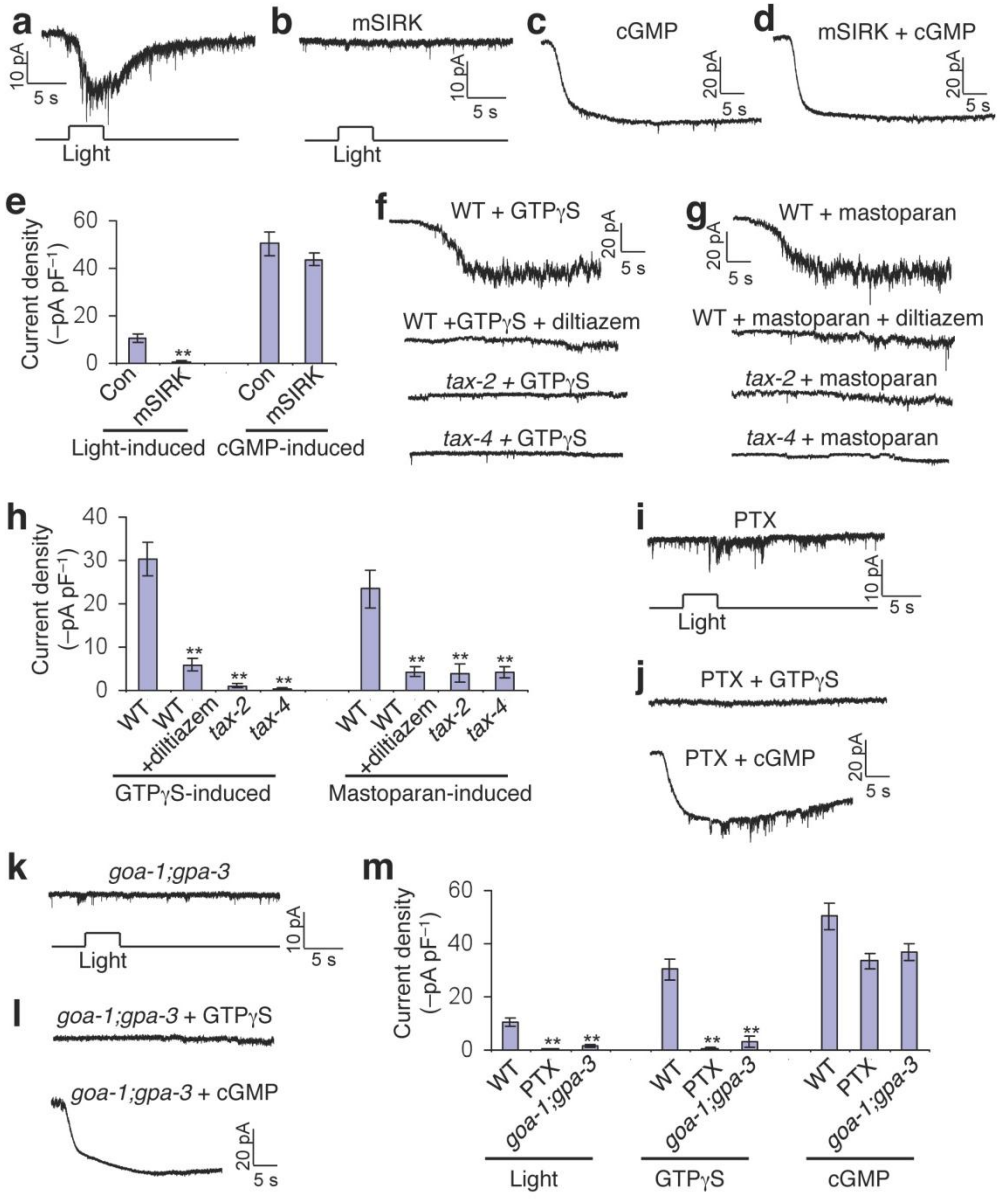
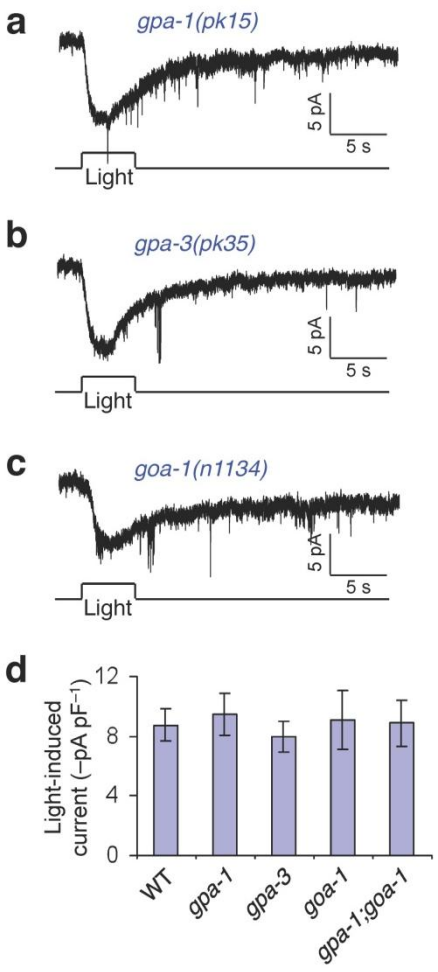


Figure 3.1 Phototransduction in ASJ is a G protein–mediated process. (**a**) Light-induced conductance in ASJ (clamping voltage, −70 mV; light stimulus, 350 ± 25 nm, 5 s, −1.75 log *I*/*I*o). Worm photoreceptor cells are most sensitive to UV-A light (Ward et al., 2008). The downward spikes in this trace and in other figures are typical for many worm neurons that are very small (~1 pF, ~2 μm in diameter) and exhibit high input resistance (Goodman et al., 1998). (**b**) Blocking G protein signaling blocked phototransduction. mSIRK (50 μM) is membrane permeable. (**c**,**d**) cGMP-evoked currents were not affected by mSIRK (1 mM cGMP). (**e**) Bar graph summarizing the data in **a**–**d** (*n* ≥ 6, photocurrents; *n* ≥ 4, cGMP-induced

currents). Error bars represent ± s.e.m. **$P < 0.002$ (*t* test). (**f**) Activation of G proteins opened CNG channels in the dark (100 μM GTPγS). We used the *tax-2* allele *p671* and the *tax-4* allele *p678*. WT, wild type. (**g**) Activation of Gi/o opened CNG channels in the dark (5 μM mastoparon). (**h**) Bar graph summarizing the data in **f** and **g** ($n \geq 6$). **$P < 0.0003$ (ANOVA with Dunnett test). (**i**) Blocking Gi/o blocked phototransduction. PTX was expressed as a transgene in ASJ. (**j**) PTX blocked GTPγS-induced (top), but not cGMP-induced (bottom), current. (**k**) The *goa-1(n1134); gpa-3(pk35)* double mutant lacked photocurrents. See **Supplementary Figure 3.1** for single mutant data. (**l**) Mutations in *goa-1* and *gpa-3* blocked GTPγS-induced (top), but not cGMP-induced (bottom), current. (**m**) Bar graph summarizing the data in **i–l** ($n \geq 5$). Error bars represent ± s.e.m. **$P < 0.005$ (ANOVA with Dunnett test).

channels, as we were able to block it with L-*cis*-diltiazem and mutations in *tax-2* and *tax-4* (Fig. 3.1g,h). Thus, activation of Gi/o can lead to the opening of CNG channels.

To provide additional evidence, we sought to block the function of Gi/o. The worm genome encodes 21 Gα proteins, at least three of which belong to the Gi/o family (O'Halloran et al., 2006); in addition, many others are closely related to Gi/o (Roayaie et al., 1998). We first tested the effect of pertussis toxin (PTX), which inhibits Gi/o function (Darby and Falkow, 2001). PTX blocked the photoresponse in ASJ, suggesting that Gi/o proteins are required for phototransduction in ASJ (Fig. 3.1i). As expected, PTX also blocked the ability of GTPγS to stimulate CNG channels in ASJ (Fig. 3.1j). As a control, direct application of cGMP was still able to efficiently activate CNG channels in ASJ (Fig. 3.1j), consistent with the view that CNG channels act downstream of G proteins. These results strongly suggest that phototransduction in ASJ is mediated by the Gi/o family of G proteins.



Supplementary figure 3.1. *gpa-1, gpa-3* and *goa-1* single mutants do not show a noticeable defect in phototransduction in ASJ. (a–c) Sample traces recorded from *gpa-1, gpa-3* and *goa-1* mutants. (d) Bar graph. $n \geq 5$. Error bars: SEM.

At least five *C. elegans* Gα genes are targets for PTX (Tanis et al., 2008). Among them, *goa-1*, *gpa-1* and *gpa-3* are known to be expressed in ASJ (Jansen et al., 1999). Although photocurrents appeared to be normal in *goa-1*, *gpa-1* and *gpa-3* single mutants (Supplementary Fig. 3.1), the *goa-1; gpa-3* double mutant had a severe defect in phototransduction in ASJ (Fig. 3.1k). In addition, GTPγS

could no longer stimulate CNG channels in *goa-1; gpa-1* mutant worms (Fig. 3.1l,m). As a control, cGMP could still efficiently activate CNG channels in these mutant worms, indicating that the mutations did not affect the general health of the neuron (Fig. 3.1l,m). Thus, *goa-1* and *gpa-3* have a redundant role in mediating phototransduction in ASJ. Nevertheless, as the known expression patterns for Gα genes could be incomplete; it is possible that other Gα genes may be involved in phototransduction in ASJ. It is also possible that other photoreceptor cells may depend on different sets of Gα genes for phototransduction.

Phototransduction in ASJ does not require typical PDEs

How does G protein activation lead to the opening of CNG channels? In vertebrate photoreceptor cells, light-activated G proteins either inhibit PDEs (for example, parietal eye photoreceptor cells) or stimulate PDEs (for example, rods and cones), resulting in an increase or reduction in cGMP level and thus the opening or closing of CNG channels, respectively (Xiong et al., 1998; Fu and Yau, 2007). Mice lacking the retina PDE (PDE-6) are blind (Bowes et al., 1990). If *C. elegans* photoreceptor cells use such a mechanism, it would be similar to that in vertebrate parietal eye photoreceptor cells; namely, G proteins upregulate cGMP by inhibiting PDEs, thereby opening CNG channels. Thus, we examined the role of PDEs in worm phototransduction.

The *C. elegans* genome encodes six PDEs, PDE-1–6, each of which has a closely related human homolog (Fig. 3.2a). PDE-4 and PDE-6 are highly

homologous to human PDE-4 and PDE-8, respectively, both of which are cAMP specific (Omori and Kotera, 2007). The other four PDEs (PDE-1, 2, 3 and 5) may cleave cGMP and could therefore be involved in phototransduction. We isolated mutant alleles of all these four *pde* genes and generated mutant strains lacking multiple PDEs. In the *pde-1*, *2* and *5* triple mutant, the photocurrent was not only present in ASJ, but also markedly potentiated, with a current density about fivefold greater than that in wild-type worms (Fig. 3.2b–e). The same phenomenon was observed in quadruple mutant strains devoid of all four PDEs (Fig. 3.2c,e). We also generated a *pde-4; pde-6* double mutant strain lacking the two putative cAMP-specific PDEs and found that these worms had normal photocurrents (Fig. 3.2d,e).

The photocurrent in the *pde-1*, *2*, *3* and *5* quadruple mutant exhibited very slow or no recovery after cessation of the light stimulus, consistent with a role for PDEs in downregulating cGMP (Fig. 3.2c). Notably, the input resistance in ASJ of the *pde* quadruple mutant (4.43 ± 0.66 GΩ, $n = 4$) was similar to that in the wild type (4.30 ± 0.60 GΩ, $n = 6$). This indicates that a loss of PDE function did not lead to the opening of additional channels in the dark, the opposite of which has been observed in vertebrate parietal eye photoreceptor cells (Xiong et al., 1998).

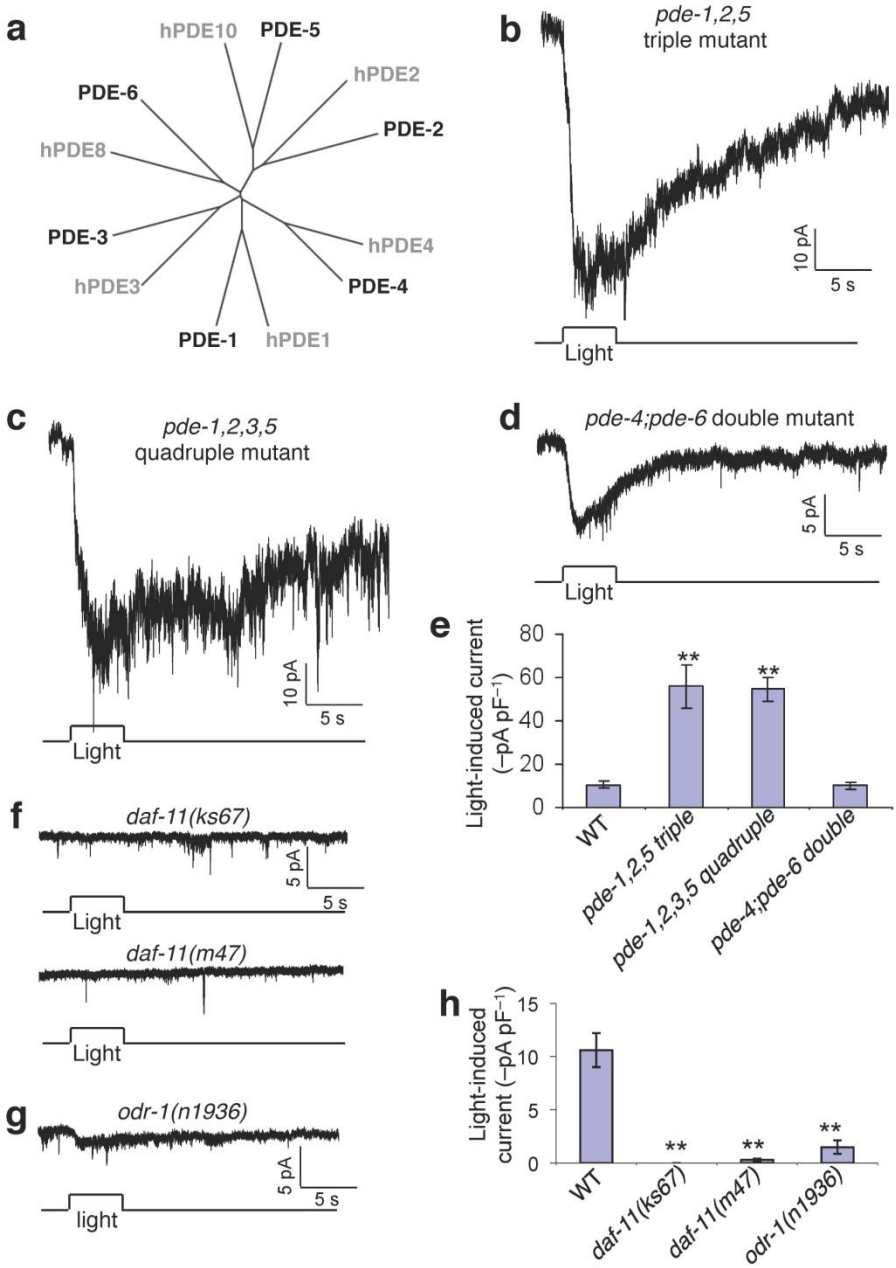


Figure 3.2 Phototransduction in ASJ requires membrane-associated guanylate cyclases. (**a**) Dendrogram of *C. elegans* and human PDEs (hPDEs). (**b**) The light-induced current was greatly potentiated in the *pde-1*, *2* and *5* triple mutant *pde-1(nj57)pde-5(nj49); pde-2(nj58)*. (**c**) The light-induced current was greatly potentiated in the *pde-1*, *2*, *4* and *5* quadruple mutant *pde-1(nj57);pde-5(nj49); pde-3(nj59);pde-2(nj58)*. A similar result (51.7 ± 3.28 pA pF−1, *n* = 5) was obtained with another quadruple mutant strain, *pde-1(nj57);pde-5(nj49);*

pde-3(nj59);pde-2(tm3098). (**d**) The light-induced current was normal in the *pde-4(nj60);pde-6(ok3410)* double mutant. (**e**) Bar graphs summarizing the data in **b**–**d** ($n \geq 7$). Error bars represent ± s.e.m. $^{**}P < 0.0001$ (ANOVA with Dunnett test, compared with wild type). (**f**) No light-induced current was detected in the guanylate cyclase mutants *daf-11(ks67)* and *daf-11(m47)*. (**g**) The light-induced current in the guanylate cyclase mutant *odr-1(n1936)* was greatly reduced. (**h**) Bar graph summarizing the data in **f**–**g**. *daf-11(ks67)* is temperature sensitive (Murakami et al., 2001) and all recordings involving this allele were carried out at 25 °C. All other recordings were performed at 20 °C. The photocurrent density in wild-type recorded at 25 °C was similar to that at 20 °C (data not shown; $n \geq 7$). Error bars represent ± s.e.m. $^{**}P < 0.0005$ (ANOVA with Dunnett test, compared with wild type).

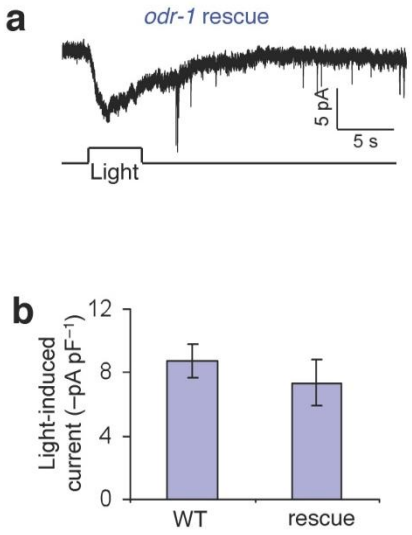
This also suggests that guanylate cyclases have very low activity in the dark in ASJ, a feature that is distinct from that observed in vertebrate photoreceptor cells. Taken together, these results suggest that PDEs may not be required for phototransduction, but are instead involved in modulation of phototransduction in ASJ. It should be noted that, although we examined all of the predicted *pde* genes, we cannot rule out the possibility that some unknown type of PDEs, which do not show homology to known PDEs, may act in phototransduction.

Phototransduction in ASJ requires guanylate cyclases

Alternatively, stimulation of guanylate cyclases in principle may also upregulate cGMP, leading to activation of CNG channels. There are two major types of guanylate cyclases: soluble guanylate cyclases and membrane-associated guanylate cyclases (Palczewski et al., 1994; Tremblay et al., 2002). In *C. elegans*, soluble guanylate cyclases are sensitive to O_2 and are required for social feeding, whereas membrane-associated guanylate cyclases are essential for chemotaxis and thermotaxis (L'Etoile and Bargmann, 2000; Gray et al., 2004;

Inada et al., 2006; Rogers et al., 2006). Notably, two membrane-associated guanylate cyclases (*daf-11* and *odr-1*) are expressed in *C. elegans* photoreceptor cells, including ASJ, ASK and AWB (Birnby et al., 2000; L'Etoile and Bargmann, 2000).

We examined *daf-11* and *odr-1* mutants. There were no photocurrents in ASJ from *ks67* and *m47* mutants, which are two independent alleles of *daf-11* (Fig. 3.2f). *odr-1(n1936)* mutant worms also had a severe reduction in the density of photocurrents (Figs. 3.2g,h and Supplementary Fig. 3.2). These results indicate that membrane-associated guanylate cyclases are required for phototransduction in ASJ. Supplementing *daf-11* mutant worms with non–saturating levels of cGMP did not restore photosensitivity in ASJ (Supplementary Fig. 3.3). This indicates that cGMP does not simply have a permissive role in phototransduction, providing additional evidence that cGMP is a second messenger for phototransduction in ASJ.



Supplementary figure 3.2. Rescue of *odr-1* phototransduction defect. As we only analyzed one *odr-1* allele (only one allele is available at CGC), we rescued the mutant phenotype. Expression of wild-type *odr-1* gene under its own promoter restored photocurrents in ASJ (*n* = 14). *odr-1* is expressed in a subset of photoreceptor cells, including ASJ, ASK and AWB (ref 26). Error bars: SEM.

Guanylate cyclases act downstream of G proteins

These results suggest a model in which G protein activation leads to upregulation of cGMP level, which in turn causes CNG channel activation. In other words, guanylate cyclases act downstream of G proteins, but upstream of CNG channels. If this is true, activation of G proteins should no longer be able to stimulate CNG channels in guanylate cyclase mutant worms, but cGMP should still be able to open these channels.

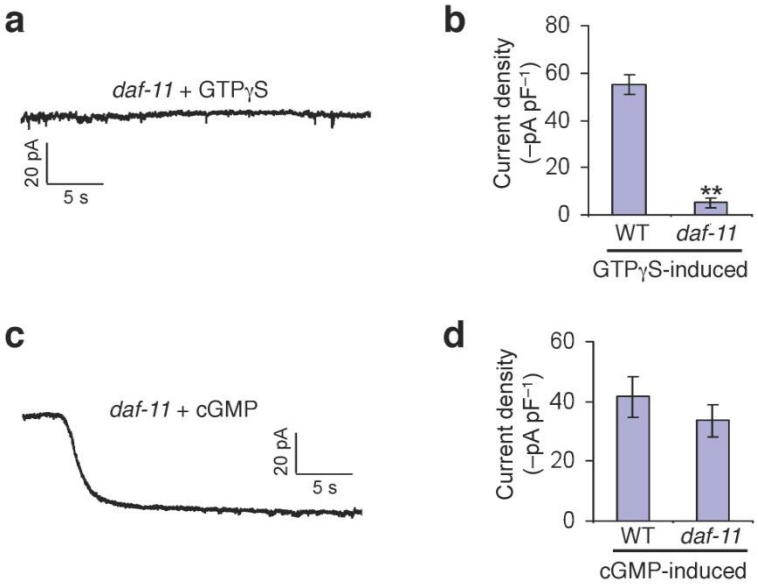


Figure 3.3 Guanylate cyclases act downstream of G proteins and upstream of CNG channels to mediate phototransduction. (**a**,**b**) Guanylate cyclase/DAF-11 acted downstream of G proteins. The *Ks67* mutation in *daf-11* blocked the ability of GTPγS in stimulating ASJ. A sample trace is shown in **a** ($n \geq 5$). Error bars represent ± s.e.m. $^{**}P < 0.00001$ (*t* test). (**c**,**d**) Guanylate cyclase/DAF-11 acted upstream of CNG channels. cGMP efficiently opened CNG channels in ASJ of *daf-11* (*Ks67*) mutant worms. A sample trace is shown in **c** ($n \geq 5$). Error bars represent ± s.e.m.

To test this model, we examined the effects of GTPγS and cGMP on CNG channels in *daf-11* mutant worms. Indeed, GTPγS failed to stimulate CNG channels in ASJ of *daf-11* mutant worms (Fig. 3.3a,b), whereas cGMP was still able to efficiently activate CNG channels in this mutant (Fig. 3.3c,d). This observation suggests that guanylate cyclases act downstream of G proteins, but upstream of CNG channels, to mediate phototransduction in ASJ.

pde mutants allow further testing of the proposed model

In wild-type worms, we were able to detect light-induced currents under the perforated, but not classic, whole-cell configuration. As a result of this technical constraint, we can only test the effect of membrane-permeable chemicals on photocurrents by including them in the bath solution. Unlike classic whole-cell configuration, perforated patch does not allow for dialyzing most membrane-impermeable chemicals into photoreceptor cells through the recording pipette. We were surprised to find that we were able to detect photocurrents in *pde* mutant worms under classic whole-cell configuration (Fig. 3.4a). The exact mechanism underlying this observation is not known, but it is probably because the loss of PDEs potentiated cGMP level under light stimulation, which may offset the negative effect of the wash-out by the recording pipette of some phototransduction-promoting factors. This offers us a unique opportunity to gather further evidence supporting the proposed phototransduction model.

We first examined the effects of GDPβS (membrane impermeable), one of the most commonly used G protein–signaling blockers. Dialysis of GDPβS into ASJ of *pde* mutant worms through the recording pipette abolished photocurrents, indicating that phototransduction requires G protein signaling (Fig. 3.4b). In another experiment, we first activated CNG channels in ASJ of *pde* mutants by dialyzing GTPγS or cGMP (both membrane impermeable) into ASJ and then stimulated ASJ with light (Fig. 3.4c,d). Light could not further induce an inward current under these conditions, suggesting that light, GTPγS and cGMP all act on

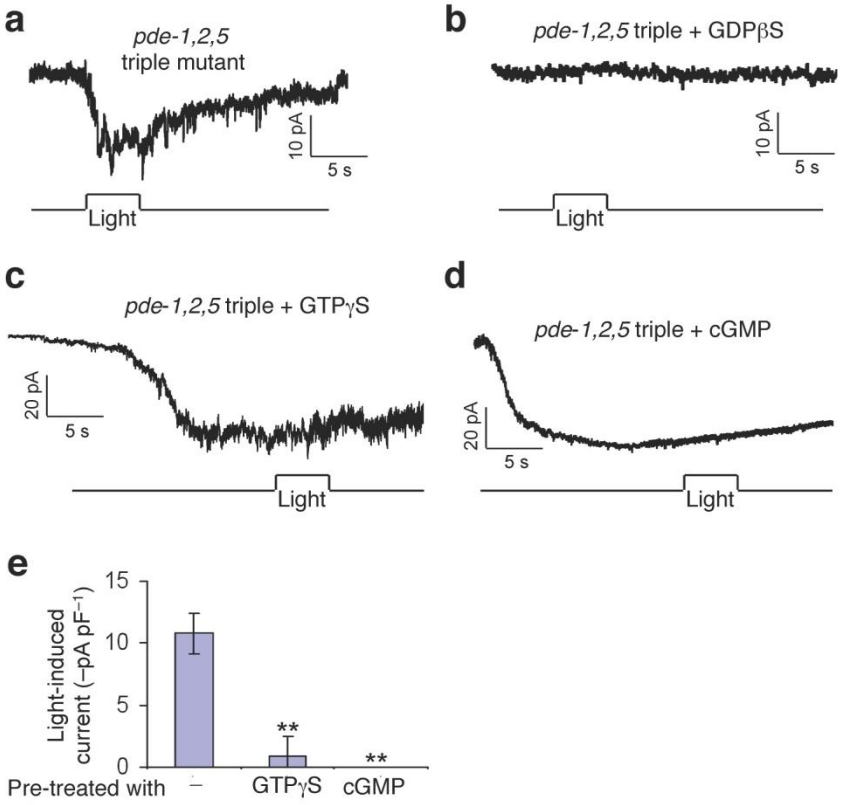
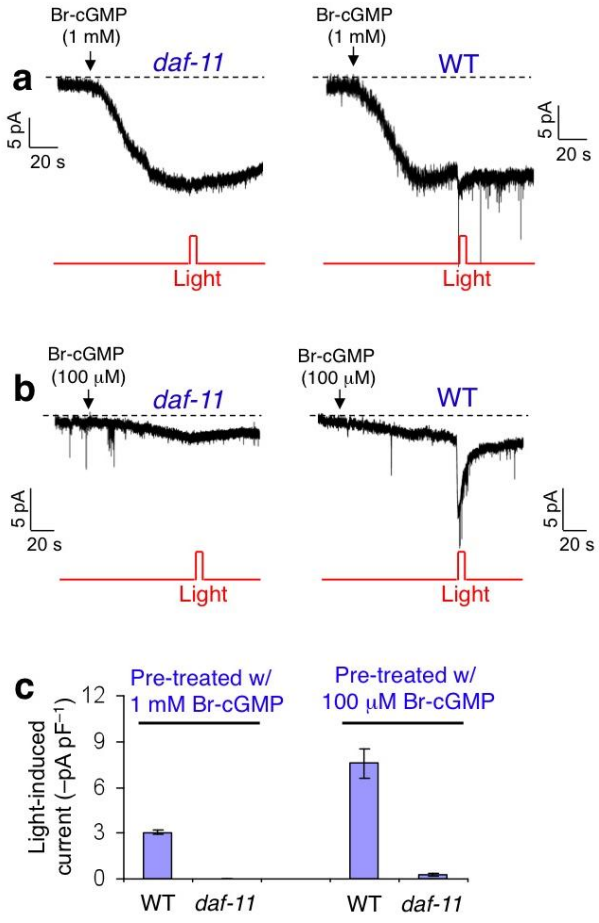


Figure 3.4 Light, GTPγS and cGMP activate the same type of CNG channels in photoreceptor cells. (**a**) Light evoked an inward current in the *pde-1*, *2* and *5* triple mutant under the classic whole-cell mode. (**b**) GDPβS blocked phototransduction. GDPβS (100 μM) was dialyzed into ASJ through the recording pipette. (**c**) Light and GTPγS acted on the same type of CNG channels. In the *pde* triple mutant, once CNG channels were activated by GTPγS, light did not further induce an inward current. (**d**) Light and cGMP activated the same type of CNG channels. In the *pde* triple mutant, once CNG channels were activated by cGMP, light did not further induce an inward current. (**e**) Bar graph summarizing the data in **a**–**d** ($n \geq 6$). Error bars represent ± s.e.m. $^{**}P < 0.0001$ (ANOVA with Dunnett test).

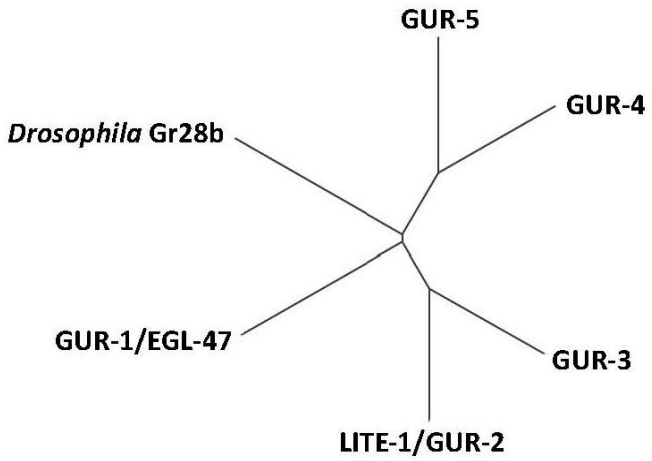
the same type of CNG channels and stimulate the same signaling cascade (Fig. 3.4c–e). This is also consistent with our phototransduction model in which G protein signaling upregulates cGMP levels, leading to CNG channel activation.



Supplementary figure 3.3. Supplement of cGMP does not restore photosensitivity in ASJ of *daf-11* mutant worms. ASJ was first perfused with the membrane-permeable Br-cGMP at non-saturating levels to evoke a small inward dark current with an amplitude of ~5 pA (100 mM Br-cGMP) shown in (b) and ~20 pA (1mM Br-cGMP) shown in (a). Complete activation of CNG channels by cGMP would lead to a dark current of ~40 pA (fig. 3c,d). Subsequently, ASJ was stimulated with light. While wild-type worms showed photocurrents in ASJ under both Br-cGMP conditions, *daf-11(m47)* mutant worms did not. This shows that cGMP does not simply play a permissive role in phototransduction, providing further support for its role as a second messenger for phototransduction.

Phototransduction in photoreceptor cells require LITE-1

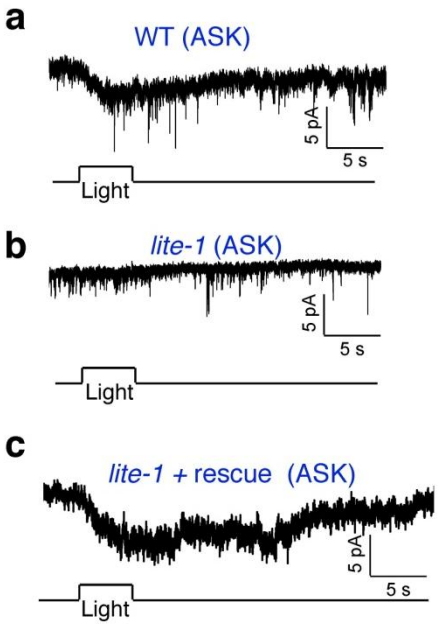
The *C. elegans* genome does not encode any closely related homologs for opsins (Terakita, 2005), a group of GPCRs that represent the most common photoreceptor proteins in metazoan photoreceptor cells. This suggests that *C. elegans* photoreceptor cells may adopt an opsin-independent mechanism for phototransduction. We carried out a forward genetic screen for mutants defective in phototaxis in hopes of identifying candidate photoreceptor genes. Three mutants (*xu7*, *xu8* and *xu10*) had a strong defect in phototaxis behavior and failed to complement each other, suggesting that the mutations occur in the same gene (Fig. 3.5a and data not shown). Using SNP (single nucleotide polymorphism) mapping, we found that these mutations were in the close proximity to *lite-1* and sequencing analysis revealed that they all were alleles for *lite-1* (Fig. 3.5b) (Edwards et al., 2008). *lite-1* encodes a seven transmembrane domain receptor-like protein and is a member of the invertebrate taste receptor family (Supplementary Fig. 3.4) (Edwards et al., 2008). This family was first identified in *Drosophila* (Clyne et al., 2000; Scott et al., 2001). The *C. elegans* genome encodes a total of five such taste receptor genes (Supplementary Fig. 3.4).



Supplementary figure 3.4. Dendrogram of *C. elegans* taste receptors. Five gustatory receptor (GUR) genes are encoded by the *C. elegans* genome (www.wormbase.org). The closely-related *Drosophila* gustatory receptor Gr28b is also included for analysis.

The *lite-1* gene has been reported to be located in a large, complex operon, and GFP transgenic approaches appear to be unsuccessful at revealing its full expression pattern (Edwards et al., 2008). Although *lite-1* mutant worms have a strong defect in phototaxis behavior, it is not clear whether *lite-1* has a role in phototransduction in photoreceptor cells. Mutations in *lite-1* may simply disrupt synaptic transmission in motor circuits or the function of interneurons and/or motor neurons that act downstream of photoreceptor cells to induce phototaxis behavior. Indeed, many mutants that affect synaptic transmission disrupt phototaxis behavior in a nonspecific manner (A.W., D.M. and X.Z.S.X., unpublished observations).

To determine whether LITE-1 participates in phototransduction in photoreceptor cells, we recorded the photoresponse in ASJ of *lite-1* mutant worms. Light failed to elicit an inward current in mutant neurons, indicating that LITE-1 is required for phototransduction in ASJ (Fig. 3.5c,d). Expression of wild-type LITE-1 specifically in ASJ fully rescued the photoresponse in ASJ (Fig. 3.5e,f). The same transgene also rescued *lite-1* phototaxis defect (Fig. 3.5g). These results suggest that LITE-1 functions in ASJ to mediate phototransduction.



Supplementary figure 3.5. LITE-1 is also required for phototransduction in the photoreceptor cell ASK. Shown are sample ASK traces for figure 5f. (a) light-induced current in ASK of wild-type. (b) *lite-1(xu7)*. (c) *lite-1(xu7)* expressing a wild-type *lite-1* transgene under the control of the ASK-specific promoter *srg-8*.

We also recorded another putative photoreceptor cell, ASK, which expresses the same set of CNG channels and membrane-associated guanylate cyclases as ASJ (Coburn and Bargmann, 1996; Komatsu et al., 1996; Birnby et al., 2000; L'Etoile and Bargmann, 2000). Light stimulation evoked an inward current in ASK of wild-type worms (Fig. 3.5f and Supplementary Fig. 3.5). This

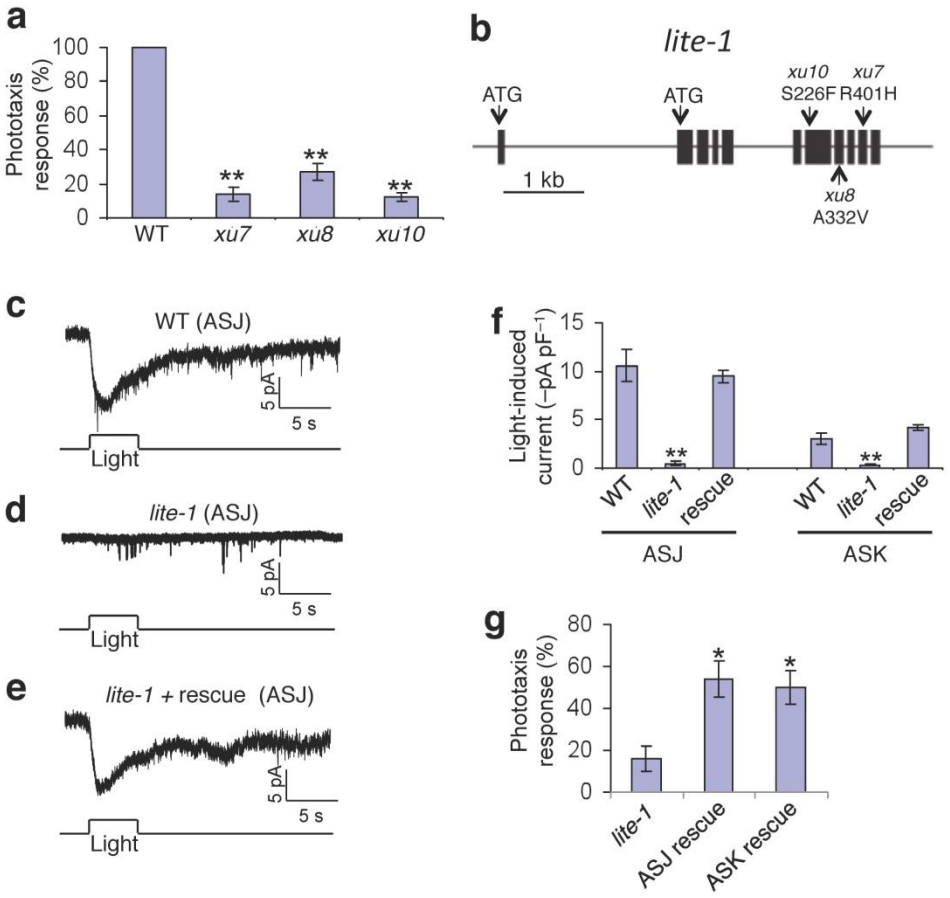
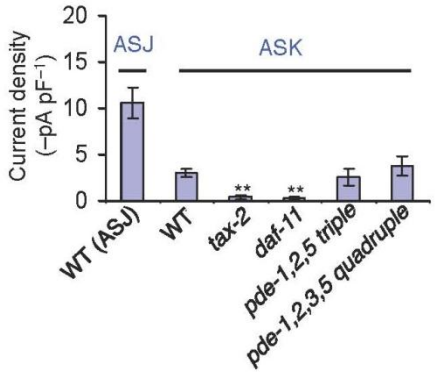


Figure 3.5 LITE-1 is required for phototransduction in photoreceptor cells. (**a**) Three mutants had a strong defect in phototaxis behavior. Head avoidance response to UV-A light (2 s, −1.43 log *I*/*I*o) was scored as previously described (Feng et al., 2006; Ward et al., 2008). The response rate in *xu7* and *xu10* was similar to that of a no-light control and likely resulted from spontaneous reversals ($n \geq 10$). Error bars represent ± s.e.m. $^{**}P < 0.00001$ (ANOVA with Dunnett test,

compared with wild type). (**b**) *lite-1* genomic structure and mutations identified in *lite-1*. We identified two *lite-1* isoforms. There is an SL1 sequence before the ATG in the second exon, indicating that there is a short form of *lite-1*, which we used here. (**c**–**e**) LITE-1 was required for phototransduction in ASJ. Shown are sample traces of ASJ in wild type (**c**), *lite-1(xu7)* (**d**) and *lite-1(xu7)* expressing a wild-type *lite-1* transgene specifically in ASJ under the *trx-1* promoter49 (**e**). See **Supplementary Figure 3.5** for ASK traces. (**f**) Bar graph summarizing the data in **c**–**e**. Error bars represent ± s.e.m. ($n \geq 7$). $^{**}P < 0.00002$ (ANOVA with Dunnett test, compared with wild type). (**g**) Expression of a wild-type *lite-1* transgene specifically in ASJ or ASK had a rescuing effect on the phototaxis behavioral defect in *lite-1(xu7)* mutant worms. The *trx-1* and *srg-8* promoters were used to drive expression of the transgene in ASJ and ASK, respectively49,50. Error bars represent ± s.e.m. ($n \geq 10$). $^{*}P < 0.05$ (ANOVA with Bonferroni test, compared with *lite-1*).

photoresponse required CNG channels and membrane-associated guanylate cyclases, but not PDEs (Supplementary Fig. 3.6). Notably, although *pde* mutants retained photocurrents in ASK, the current density in these mutants was not higher than that in wild type (Supplementary Fig. 3.6). This is different from the case with ASJ, indicating that PDEs have a modulatory role in some, but not all, photoreceptor cells. Mutations in *lite-1* eliminated ASK photocurrents, and expression of wild-type LITE-1 specifically in ASK fully rescued this defect (Fig. 3.5f and Supplementary Fig. 3.5). The same transgene also rescued the phototaxis defect of *lite-1* mutants (Fig. 3.5g). Nevertheless, given the smaller amplitude and slower kinetics of ASK photocurrents compared with those recorded in ASJ (Supplementary Fig. 3.5), it remains possible that the recorded photocurrents in ASK may indirectly result from ASJ (ASJ synapses onto ASK) or other photoreceptor cells.



Supplementary figure 3.6. The photoreceptor cell ASK requires CNG channels and membrane-associated GCs but not PDEs for phototransduction. Though ASK exhibits a relatively smaller photocurrent than ASJ, it requires a similar set of genes for phototransduction. Mutations in the CNG channel TAX-2 and the membrane associated GC DAF-11 block the photoresponse in ASK. In contrast, the photocurrent density in the *pde* triple and quadruple mutants is similar to that in wild-type, suggesting that PDEs may not play a modulatory role in ASK. $^{**}P < 0.005$ (ANOVA with Dunnett test). $n \geq 5$. Error bars: SEM.

LITE-1 acts upstream of G proteins in phototransduction

We next sought to place LITE-1 in the phototransduction cascade. We reasoned that if LITE-1 functions upstream of G proteins, we would expect that both GTPγS- and cGMP-elicited currents in *lite-1* mutants are similar to those in wild type. This is indeed the case. In *lite-1* mutant worms, both GTPγS and cGMP can efficiently stimulate CNG channels in ASJ, indicating that LITE-1 acts upstream of G proteins (Fig. 3.6a–c). These results suggest that LITE-1 may be part of the photoreceptor complex or required for the function of this complex.

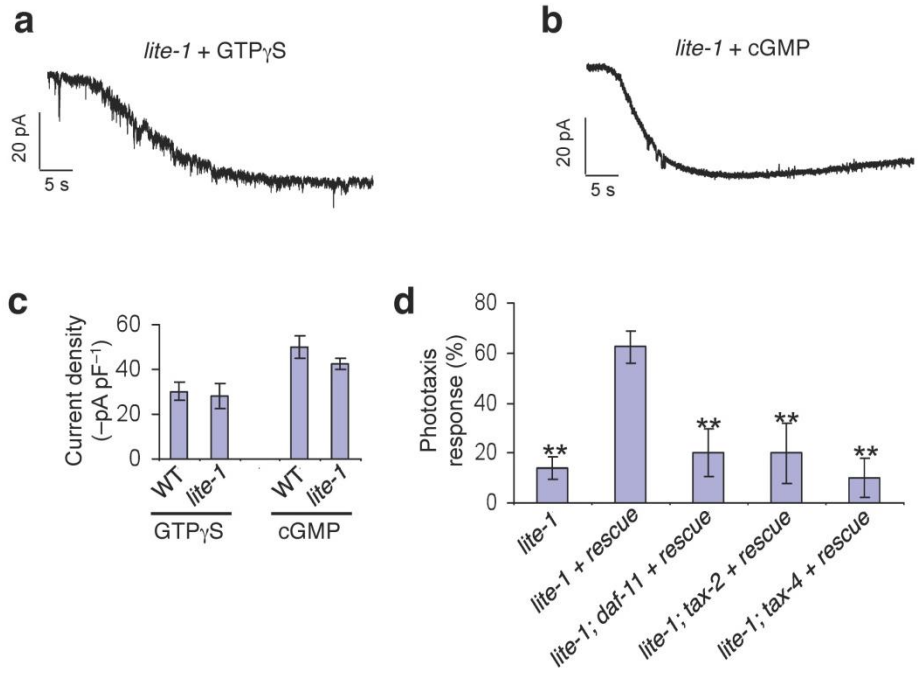


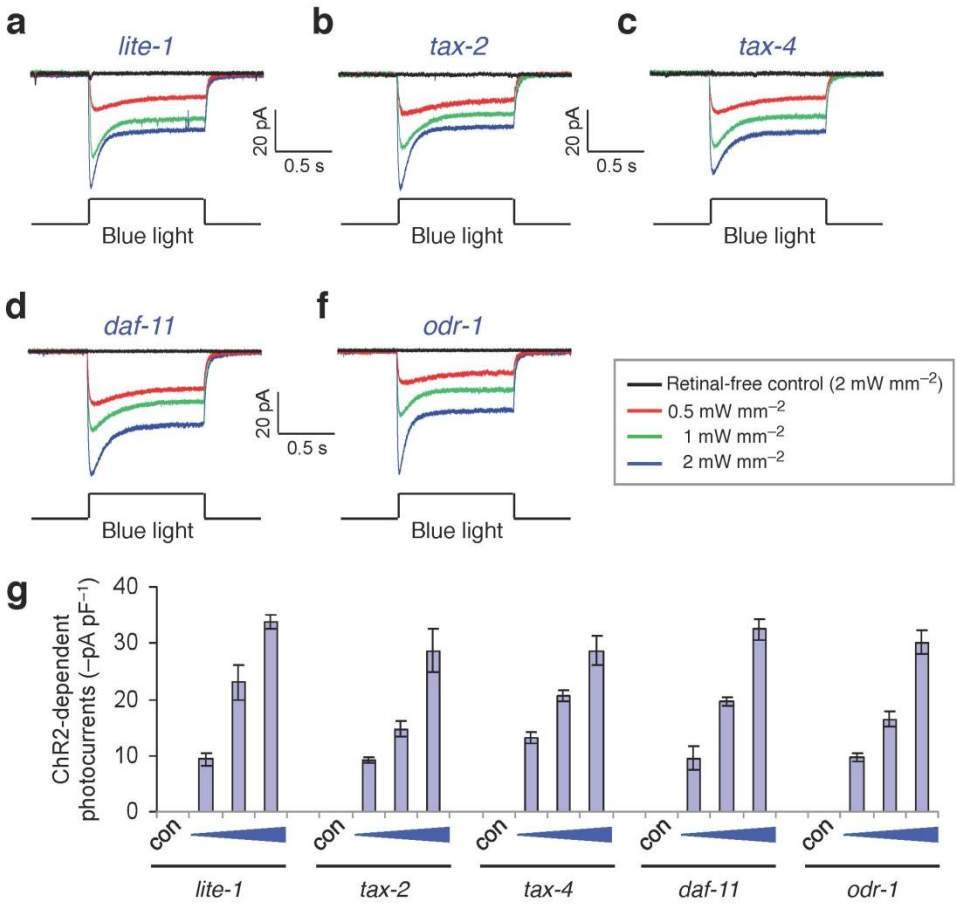
Figure 3.6 LITE-1 functions upstream of G proteins. (**a**,**b**) LITE-1 acted upstream of G proteins. GTPγS (**a**) and cGMP (**b**) induced an inward current in ASJ of *lite-1(xu7)* mutant worms. (**c**) Bar graph summarizing the data in **a** and **b**. The densities of GTPγS- and cGMP-induced currents in ASJ of *lite-1(xu7)* mutant worms were similar to those in wild type ($n \geq 6$). Error bars represent ± s.e.m. (**d**) LITE-1 acted upstream of guanylate cyclases and CNG channels. Wild-type *lite-1* was expressed as a transgene under the *tax-2*Δ promoter in the photoreceptor cells ASJ, ASK and AWB. This transgene rescued the phototaxis defect in *lite-1(xu7)* mutant worms. This rescuing effect required the guanylate cyclase DAF-11 and CNG channels TAX-2 and TAX-4. $^{**}P < 0.001$ (ANOVA with Dunnett test, compared with the rescue). Error bars represent ± s.e.m. ($n \geq 10$).

If LITE-1 is part of the photoreceptor complex, it should also function upstream of guanylate cyclases and CNG channels. Mutations in the membrane-associated guanylate cyclase DAF-11 and CNG channel subunit TAX-4 abrogated the photoresponse in ASJ and ASK, but these mutants did not exhibit a strong phenotype in phototaxis behavior (Fig. 3.2e and unpublished

observations from A.W. and X.Z.S.X.). This can be explained by the fact that some other photoreceptor cells (for example, ASH and ADL) do not express these genes and perhaps utilize distinct phototransduction mechanisms. Nonetheless, expression of wild-type LITE-1 in guanylate cyclases/CNG channel-expressing photoreceptor cells, such as ASJ, ASK and AWB, was sufficient to rescue the phototaxis defect in *lite-1* mutant worms (Fig. 3.6d). Notably, mutations in *daf-11* and *tax-4* can suppress the effect of the *lite-1* transgene on rescuing *lite-1* phototaxis defect (Fig. 3.6d). These results provide additional evidence that guanylate cyclases and CNG channels function downstream of LITE-1 in phototransduction.

ChR2 restores photosensitivity in *lite-1* mutant worms

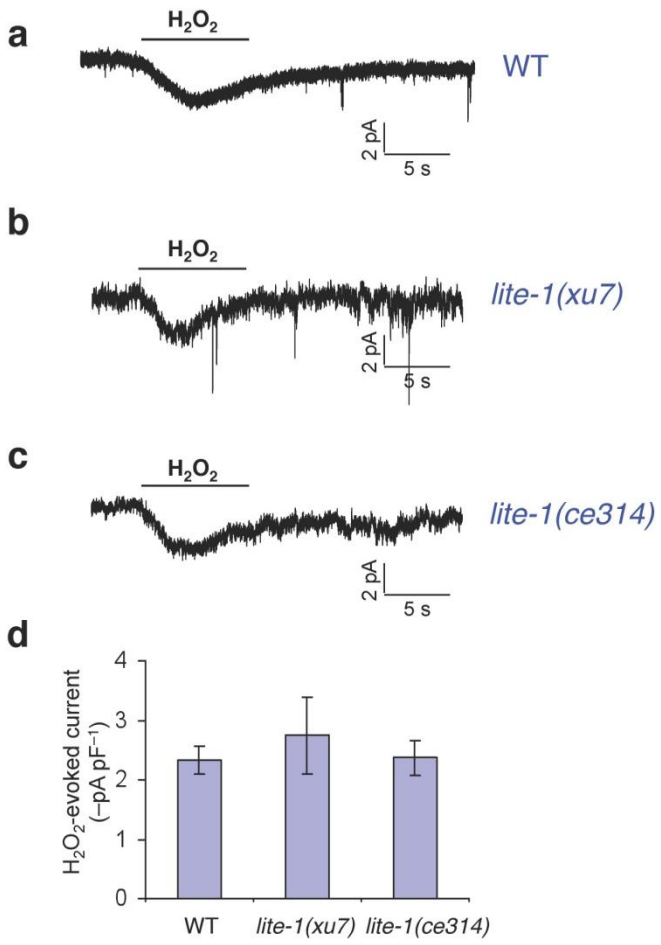
Expression of the light-gated ion channel channelrhodopsin-2 (ChR2) specifically in ASJ of *lite-1* mutant worms rendered ASJ photosensitive (Supplementary Fig. 3.7). The same ChR2 transgene also restored photosensitivity in ASJ of *daf-11*, *tax-2* and *tax-4* mutant worms (Supplementary Fig. 3.7). These results indicate that these mutations did not affect the general health of the neuron. Consistent with the idea that ChR2 is an ion channel that is directly gated by light independently of second messengers (Boyden et al., 2005; Nagel et al., 2005), the ChR2-dependent photocurrents in ASJ developed virtually instantaneously on light stimulation, without a detectable latency, and also exhibited rapid activation kinetics (activation time constant τ_{act} = 8.95 ± 0.03



Supplementary figure 3.7. The light-gated channel channelrhodopsin-2 (ChR2) is functional in *lite-1, daf-11, odr-1, tax-2* and *tax-4* mutant worms. (a-f) sample traces. (g) bar graph. Three different intensities (0.5, 1, and 2 mW mm^{-2}) of blue light were used to stimulate ChR2 expressed as a transgene specifically in ASJ under the *trx-1* promoter. Photocurrents developed virtually instantaneously without detectable latency, indicating that no second messenger is involved. The ChR2-dependent photocurrents also showed fast activation kinetics (activation time constant t = 8.95 ± 0.03 ms under 2 mW mm^{-2}). This is consistent with the fact that ChR2 is directly gated by light. These features are in sharp contrast to those of the intrinsic photocurrents in ASJ that depend on *lite-1*, GCs and CNG channels, which show a very long latency and slow activation kinetics 356 ± 37 ms in ref[7]; activation time constant t = 566 ± 2.6 ms). Long latency and slow kinetics of the intrinsic photocurrents in ASJ are consistent with the requirement of the second messenger cGMP.

ms under 2 mW mm^{-2} of blue light; Supplementary Fig. 3.7). These features are in sharp contrast with those of the LITE-1–dependent intrinsic photocurrents in ASJ, which exhibited a latency of hundreds of milliseconds and slow activation kinetics (latency = 356 ± 37 ms in ref. 7, τ_{act} = 566 ± 2.6 ms). Such a long latency and slow activation kinetics are typical for a process requiring second messengers. This is consistent with a model in which LITE-1 acts as a receptor protein that requires G protein signaling and the second messenger cGMP to transduce light signals in ASJ. This is also consistent with the fact that the LITE-1–dependent intrinsic photocurrents in ASJ are carried by downstream CNG channels.

We also tested whether reactive oxygen species (ROS) can activate LITE-1. Perfusion of hydrogen peroxide evoked a small inward current in ASJ. However, this current persisted in *lite-1* mutant worms (Supplementary Fig. 3.8). Although it is unclear what mediates this ROS-induced current in ASJ, it apparently does not occur through the activation of LITE-1. This result suggests that the trace amount of ROS produced by light illumination, if any, cannot fully account for the activation of LITE-1.



Supplementary figure 3.8. Reactive oxygen species (ROS) evoke an inward current in ASJ independently of LITE-1. Perfusion of 1 mM hydrogen peroxide (H_2O_2) towards ASJ evoked an inward current in wild-type worms (a), but this current persisted in *lite-1* mutant worms (b–d). These data do not support the possibility that the trace amount of ROS induced by photo-oxidation, if any, fully accounts for the activation of LITE-1. $n \geq 5$. Error bars: SEM.

LITE-1 confers photosensitivity to photo-insensitive cells

We sought to test the function of LITE-1 in heterologous systems. However, all of our attempts to functionally express LITE-1 in cultured cell lines were unsuccessful (L.K. and X.Z.S.X., unpublished observations). LITE-1 has

been ectopically expressed in worm muscles and found to induce muscle contraction (Edwards et al., 2008). However, we only detected a tiny, if any, photocurrent in muscle cells expressing *lite-1* transgenes by whole-cell recording (0.46 ± 0.1 pA pF^{-1}, n = 15). This may be caused by the fact that muscle cells lack some standard components in the phototransduction machinery, such as CNG channels and guanylate cyclases.

We thus expressed LITE-1 as a transgene in the ASI neuron that also expresses the guanylate cyclase DAF-11 and the CNG channels TAX-2 and TAX-4 (Coburn and Bargmann, 1996; Komatsu et al., 1996; Birnby et al., 2000). No photocurrent could be detected in ASI of wild-type worms, indicating that this neuron is photo-insensitive (Fig. 3.7a). Notably, expression of LITE-1 as a transgene in ASI rendered this neuron photosensitive (Fig. 3.7b). The LITE-1–dependent photocurrent in ASI also showed a latency of hundreds of milliseconds and slow activation kinetics (latency = 432 ± 66 ms, τ_{act} = 908 ± 3.4 ms), suggesting that second-messenger signaling was involved. Indeed, as was the case with ASJ and ASK, the LITE-1–dependent photocurrent in ASI also required the guanylate cyclase DAF-11 and the CNG channels TAX-2 and TAX-4 (***Fig. 3.7c–f***). These results provide electrophysiological evidence that LITE-1 expression is sufficient to confer photosensitivity to photo-insensitive cells.

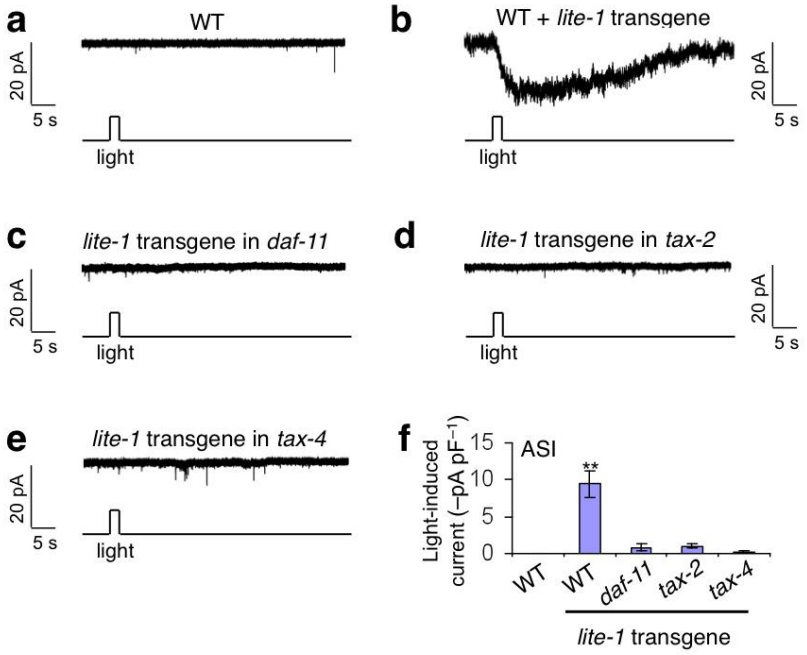
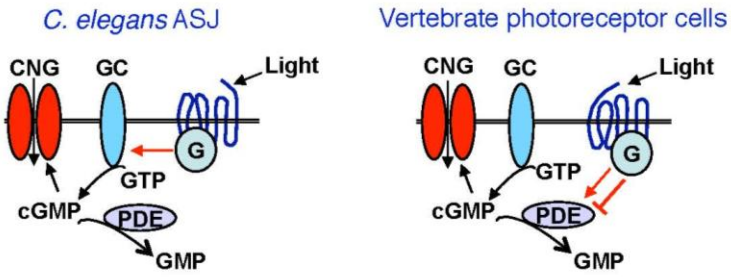


Figure 3.7 Transgenic expression of LITE-1 can confer photo-sensitivity to the photo-insensitive neuron ASI. (**a**) The ASI neuron was photo-insensitive. No photocurrent could be detected in ASI. (**b**) Expression of LITE-1 in ASI made it photo-sensitive. LITE-1 was expressed as a transgene in ASI under the *sra-6* promoter, which labels both ASI and ASH in the head50. ASI recordings performed in ASH-ablated worms and non-ablated worms yielded similar results (9.1 ± 1.3 pA pF^{-1} versus 9.4 ± 1.8 pA pF^{-1}, $n = 5$). (**c**–**e**) The function of LITE-1 in ASI also required *daf-11*, *tax-2* and *tax-4*, as mutations in these genes blocked LITE-1–dependent photocurrents in ASI. (**f**) Bar graph summarizing the data in **a**–**e** ($n \geq 5$). Error bars represent ± s.e.m. $^{**}P < 0.00001$ (ANOVA with Dunnett test, all compared with wild type without transgene).

Discussion

Despite many similarities between *C. elegans* and vertebrate photoreceptor cells (both are ciliated neurons and depend on G protein signaling, the second messenger cGMP and CNG channels for phototransduction), there are clear differences between the two (a model for *C. elegans* phototransduction



Supplementary figure 3.9. A model for *C. elegans* phototransduction cascade in the photoreceptor cell ASJ. The phototransduction cascade in vertebrate photoreceptor cells is shown for comparison. In vertebrate photoreceptor cells, G-proteins may stimulate PDE (rods and cones) or inhibit PDE (parietal eye photoreceptor cells), leading to down- or up-regulation of cGMP followed by closure or opening of CNG channels, respectively. In the *C. elegans* ASJ neuron, G-proteins (GOA-1 and GPA-3) may be coupled to the guanylate cylcases (GC) DAF-11/ODR-1, leading to up-regulation of cGMP and opening of the CNG channel TAX-2/TAX-4. Note that other *C. elegans* photoreceptor cells may employ different sets of signaling genes or even adopt different transduction mechanisms.

cascade is summarized in Supplementary Fig. 3.9). For example, they likely use distinct types of photoreceptor proteins (Supplementary Fig. 3.9). In addition, *C. elegans* phototransduction in ASJ requires membrane-associated guanylate cyclases, but not typical PDEs (Supplementary Fig. 3.9). Membrane-associated guanylate cyclases are known to be activated by peptide ligands and calmodulin-like guanylate cyclase–activating proteins (Tremblay et al., 2002). Our results raise the possibility that G protein signaling may modulate membrane-associated guanylate cyclases, suggesting an unusual mechanism for regulating cGMP-sensitive CNG channels. It is unclear whether G protein signaling directly or indirectly modulates guanylate cyclases. Notably, it has been suggested that a similar mechanism may also function in some marine species to regulate K^+

channels (Gomez and Nasi, 2000; Gotow and Nishi, 2008); however, the molecular and genetic evidence supporting its presence in organisms other than *C. elegans* has been lacking.

Chemotaxis to some odorants and thermosensation in AFD neurons in *C. elegans* also require membrane-associated guanylate cyclases (Birnby et al., 2000; L'Etoile and Bargmann, 2000), but it is not known whether PDEs are involved in these processes. Thus, it is unclear whether chemosensation and thermosensation signal through guanylate cyclases or PDEs in *C. elegans* (Bargmann, 2006a), as guanylate cyclases might have a passive role by supplying substrates to PDEs for cleavage, just as they do in vertebrate phototransduction. In fact, knockout mice lacking either membrane-associated guanylate cyclases or PDE are blind (Fu and Yau, 2007), indicating that a requirement at the genetic level does not provide adequate information to assess the role of these genes in the transduction pathway. Thus, the transduction mechanisms underlying chemosensation and thermosensation in *C. elegans* remain to be determined.

Worm photoreceptor cells do not seem to utilize opsins, but instead require LITE-1, a taste receptor-like protein, for phototransduction. LITE-1 acts upstream of G proteins and ectopic expression of LITE-1 in photo-insensitive cells can endow them with photosensitivity. These data suggest that LITE-1 may be part of the photoreceptor in worm photoreceptor cells. Unlike light-gated ion channels, such as ChR2, LITE-1 most likely functions as a receptor protein that

requires downstream signaling events (for example, G protein signaling) to transduce light signals. Despite this view, we do not exclude the possibility that LITE-1 might possess a very small ion channel activity that is beyond the sensitivity of our detection method; however, such activity, if any, does not have a noticeable contribution to the photocurrent in ASJ. As LITE-1 shows no strong homology to known GPCRs and may adopt a reversed membrane topology (Benton et al., 2006), our results suggest the intriguing possibility that LITE-1 may represent a previously unknown type of GPCRs. Nevertheless, it is possible that LITE-1 may be indirectly coupled to G protein signaling.

LITE-1 may function on its own or form a complex with other proteins, similar to many membrane receptors. The observation that ROS-induced dark currents in ASJ did not depend on LITE-1 argues against a role for a light irradiation-induced byproduct in LITE-1 activation. However, it should be noted that such a possibility cannot be completely ruled out and a definitive role for LITE-1 as a photoreceptor requires biochemical validation.

LITE-1 is a member of the invertebrate taste receptor family that was first identified in *Drosophila*. Currently, it is not known how *Drosophila* taste receptors function *in vivo* and these receptors have not been functionally expressed in heterologous systems. Whole-cell recording of taste neurons in *Drosophila* has not been reported, which makes it challenging to directly interrogate the transduction mechanisms *in vivo*. Notwithstanding these technical challenges, genetic and behavioral studies have implicated G protein signaling in *Drosophila*

taste transduction (Ishimoto et al., 2005; Ueno et al., 2006; Ueno and Kidokoro, 2008). However, this view has recently been questioned. As taste receptors are related to odorant receptors in insects, it has been suggested that these taste receptors may function as ion channels and that G protein signaling may not be directly involved in the transduction pathway in taste neurons (Sato et al., 2008). Nonetheless, more recent work has found that insect taste receptors and olfactory receptors have evolved along distinct paths during evolution and may employ distinct mechanisms for ligand recognition and signal transduction (Gardiner et al., 2009). In light of this notion and the fact that LITE-1 and insect taste receptors belong to the same gene family, our results support the view that some *Drosophila* taste receptors may recruit G protein signaling in the transduction pathway.

LITE-1 is probably not the only member in the invertebrate taste receptor family that has a role in phototransduction. Ectopic expression of GUR-3, another *C. elegans* member of this family, can also confer photosensitivity to photo-insensitive cells (A.W. and X.Z.S.X., unpublished observations). Over sixty taste receptor genes have been identified in *Drosophila* (Scott, 2005; Hallem et al., 2006; Ebbs and Amrein, 2007). Clearly, many of them function as taste receptors and are required for taste transduction (Scott, 2005; Hallem et al., 2006; Ebbs and Amrein, 2007). Notably, some *Drosophila* taste receptor genes are expressed in many non-chemosensory neurons, suggesting that these receptors may adopt a distinct function in these neurons (Thorne and Amrein, 2008). It will be interesting to determine whether some of them have a role in photo-sensation.

Chapter 4

Conclusion

The ability to sense light is crucial to the survival of many organisms, including species inhabiting both well-lit environments and dark conditions, such as soil- and deep sea-dwelling creatures. Vertebrates and insects have image-forming vision that is mediated by photoreceptors in the retina (Fu and Yau, 2007; Wang and Montell, 2007). Phototransduction is also important in non-image forming vision, which underlies such functions as phototaxis and circadian rhythm (Kelber et al., 2003; Berson, 2007). Vertebrates and insects have distinct phototransduction mechanisms. The vertebrate rods and cones utilize ciliated photoreceptors with a cGMP/CNG-mediated phototransduction pathway (Fu and Yau, 2007), whereas *Drosophila* has rhabdomeric photoreceptors with TRP channel-mediated photocurrents (Wang and Montell, 2007). Examples of invertebrates with cGMP/CNG-mediated phototransduction also exist (Yau and Hardie, 2009), however, details of the signaling pathways in these species remain largely unknown.

C. elegans has become an increasingly popular model system for the study of sensory systems, in particular olfactory transduction and mechanotransduction (Bargmann, 2006a; Bounoutas and Chalfie, 2007). Importantly, *C. elegans* has many of the same neurotransmitters, synaptic

machinery, transporters, ion channels, and signal transduction mechanisms present in more complex organisms, such as insects and mammals (Bargmann, 1998). Founding members of the DEG/ENaC family were originally identified in *C. elegans* (Driscoll and Chalfie, 1991; Huang and Chalfie, 1994), and TRP channels have been shown to function in worm osmosensation (Colbert et al., 1997) and proprioception (Li et al., 2006). *C. elegans* lives in dark soil and lacks eyes. However, we hypothesized that light-sensing in *C. elegans* could serve an important function, namely, to retain worms in dark soil. Perhaps light could trigger an avoidance response in worms, such that they would avoid the surface of the soil? Additionally, C. *elegans* is translucent, and so negative phototaxis would enable them to avoid the potentially damaging effects of UV radiation.

In this thesis work we have established *C. elegans* as a model system for the study of phototransduction. This work has led to several interesting discoveries that impact not only the worm field, but which contribute to our understanding of sensory transduction mechanisms in general. Namely, we identified a novel gene *lite-1* which is required for phototransduction in *C. elegans* photoreceptors. The LITE-1 protein has homology to a family of *Drosophila* gustatory receptors, and in our studies we found that LITE-1 functions upstream of G protein signaling in the worm phototransduction cascade. A recent study found that *Drosophila* olfactory receptor (Or) molecules expressed heterologously can form heteromultimers that elicit odour and pheromone ligand-gated cation non-selective currents in *Xenopus* oocytes (Sato et al., 2008). The authors suggested that Or ionic conductances may serve a general mechanism

for olfactory transduction in *Drosophila*, and which may be extended to gustatory receptors (Gr) given the homology between the seven-transmembrane domain Ors and Grs. Our findings argue against this hypothesis. Our work clearly shows that LITE-1 functions upstream of G protein signaling, and is required for activation of CNG-mediated light currents in *C. elegans* photoreceptor cells. This might suggest that Grs in Drosophila taste neurons also couple to G proteins in mediating taste responses. A recent study supports the idea that *Drosophila* Grs couple to G protein signaling (Yao and Carlson, 2010), however, direct evidence showing exactly how Grs function *in vivo* in *Drosophila* taste neurons is lacking.

Our research also found *C. elegans* utilizes an unusual GC-based mechanism for regulating the cGMP level in light responses. This mechanism is similar to the proposed phototransduction cascade in scallops (del Pilar Gomez and Nasi, 1995; Gomez and Nasi, 2000). The specifics of how GCs are activated in the *C. elegans* photoresponse remains to be shown, and raises the question of whether or not G proteins couple directly to GCs in the cascade. The following sections provide a comprehensive summary and conclusions of our findings in Chapters 2 and 3, and offer several interesting avenues for future research.

C. elegans **exhibits light-avoidance behavior**

We have shown the *C. elegans* photo-avoidance response to be most sensitive to UV-A light (340 nm), however, worms also avoid light wavelengths in the visual spectrum (i.e. 430 to 500 nm). To better understand the rationale for negative phototaxis behavior in *C. elegans*, we tested the effects of prolonged

light stimulation. Surprisingly, in all wavelengths tested (340, 430 and 470 nm), we found that prolonged light stimulus is lethal to worms, indicating that photoavoidance could serve a protective function in *C. elegans*. Future studies should test the role of phototaxis as a mechanism for retaining worms in soil (or another suitable dark medium).

We identified the ciliated sensory neurons ASJ, ASK, AWB and ASH as candidate photoreceptor cells using a laser ablation approach. Laser ablation of

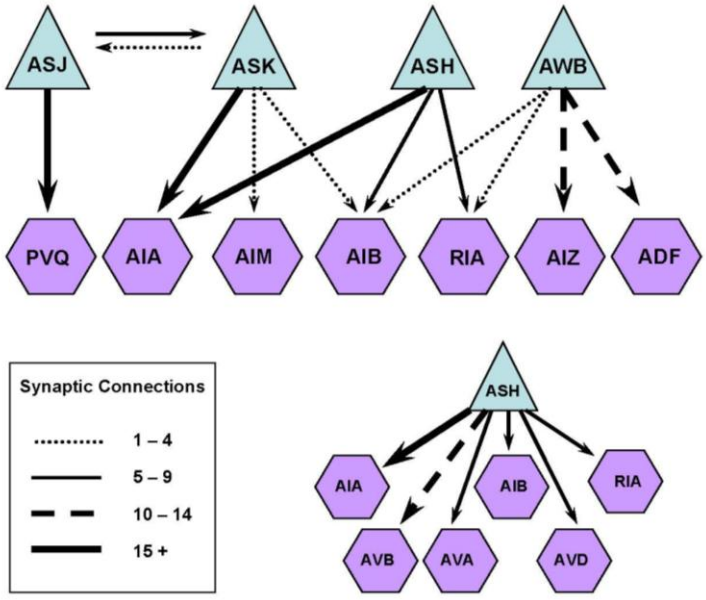


Figure 4.1 Schematic of *C. elegans* photosensory neurons and the synaptic partners. Based on EM wiring reconstructions at WormAtlas.org (White et al., 1986; Hall and Russell, 1991; Altun et al., 2002-2010).

ASJ, ASK, AWB and ASH causes a severe defect in the head response to light. These four sensory neurons are known to be involved in chemosensation and

mechanosensation (Ward, 1973; Dusenbery, 1974; Culotti and Russell, 1978; Bargmann et al., 1990; Bargmann and Horvitz, 1991b; Kaplan and Horvitz, 1993; Colbert et al., 1997; Troemel et al., 1997; Hilliard et al., 2002; Hilliard et al., 2004; Bargmann, 2006a; Srinivasan et al., 2008; Macosko et al., 2009), and thus represent polymodal sensory neurons. ASJ, ASK and ASH are particularly interesting because they are interconnected via chemical and electrical synapses (see Fig. 4.1), and ASH makes direct synaptic contact with AVA, a key interneuron that regulates backward movement (Chalfie et al., 1985). ASJ, ASK and AWB all express the CNG non-selective cation channel genes *tax-2* and *tax-4* (Coburn and Bargmann, 1996; Komatsu et al., 1996), whereas ASH expresses the TRP channel genes *osm-9* and *ocr-2* (Colbert et al., 1997; Tobin et al., 2002). A distributed nexus of polymodal sensory neurons might have interesting functional consequences: 1) to reduce noise in the response; and 2) the integration of multiple coincident sensory stimuli.

UV Light evokes inward depolarizing cGMP/CNG-mediated photocurrents in ASJ and ASK

To study light responses in the candidate photoreceptor neurons from our laser ablation experiments, we developed an *in situ* recording protocol in dissected live worms by perforated whole-cell recording. This research project focused on the *tax-2*/*tax-4* expressing neurons ASJ, ASK and AWB because our goal was to explore the possible role of CNGs in *C. elegans* photoresponses. We found that light flash evokes inward depolarizing currents in ASJ, ASK, and AWB

(although responses in AWB are significantly smaller than those in ASJ and ASK (J.L. and X.Z.S.X., unpublished observations). Consistent with our behavioral studies, light responses in ASJ and ASK are most sensitive to UV-A light, however, responses to purple, blue and green light were also observed.

We used genetics and pharmacology to further explore the nature of the photocurrents in ASJ and ASK. The CNG channel-specific blocker L-*cis*-diltiazem blocked light-induced currents in ASJ and ASK, and photocurrents are absent in the CNG channel mutants *tax-2* and *tax-4*. These results suggest photocurrents in ASJ and ASK are carried by CNGs. We also showed that application of cGMP, but not cAMP, induces currents in ASJ and ASK. In another experiment, we showed that light cannot further induce an inward current in ASJ following cGMP application, indicating that light stimulation and cGMP act on the same type of CNG channels. Our findings illustrate several important similarities and differences between *C. elegans* photocurrents and those of vertebrate rods and cones. Light currents in both systems are mediated by the 2nd messenger cGMP which acts on CNG channels. However, in the case of *C. elegans* photoreceptor neurons light increases cGMP levels and is depolarizing, whereas in vertebrate rods and cones cGMP production is shut-off and is hyperpolarizing. The parietal eye of some lizards and amphibians also exhibit CNG-mediated depolarizing photocurrents.

Light activates a G protein-dependent pathway which requires membrane guanylate cyclases

All examples of light-signaling in vertebrate and invertebrate photoreceptors utilize G proteins. The vertebrate rods and cones signal through transducin (G_t), whereas insect photoreceptors use Gq-type G proteins (the *dgq* gene in *Drosophila*) (Fu and Yau, 2007; Wang and Montell, 2007). Our pharmacology data suggest G protein signaling is required for *C. elegans* photoresponses, and more specifically, that Gi/o-type Gα mediates phototransduction since the light response could be blocked with pertussis toxin (PTX). Although the worm genome encodes 21 Gα proteins, only five *C. elegans* Gα genes are potential targets for PTX (Tanis et al., 2008), and among them, *goa-1*, *gpa-1* and *gpa-3* have been shown to be expressed in ASJ (Jansen et al., 1999). By testing these three Gα mutants individually and in combinations we found that *goa-1* and *gpa-3* have a redundant role in mediating photoresponses in ASJ.

This raised the question of how G protein-mediated light signaling might regulate the cGMP level in *C. elegans* photoresponses. In vertebrate photoreceptor cells, light-activated G proteins either inhibit PDEs (for example, parietal eye photoreceptor cells) or stimulate PDEs (for example, rods and cones), resulting in an increase or reduction in cGMP level and thus the opening or closing of CNG channels, respectively (Xiong et al., 1998; Fu and Yau, 2007). Alternatively, G protein-mediated stimulation of GCs could upregulate cGMP, leading to activation of CNG channels. Indeed, membrane GCs have been

shown to play a constitutive role in producing cGMP in vertebrate rods and cones (Palczewski et al., 1994). Based on our genetics data, light signaling requires the *C. elegans* membrane GCs DAF-11 and ODR-1. Furthermore, using GTPγS we were able to show that DAF-11 and ODR-1 function downstream of G protein signaling. We also tested PDE mutants individually and in combination and showed they are not required for light response activation. Our results indicate that G protein-mediated light-signaling modulates membrane-associated guanylate cyclases, suggesting an unusual mechanism for regulating cGMP-sensitive CNG channels. It remains to be shown whether GC activation by G_{α} is direct or indirect.

This raises some interesting questions concerning the role of GCs and PDEs in other *C. elegans* sensory systems. Chemotaxis to some odorants and thermosensation in AFD neurons in *C. elegans* also require membrane-associated guanylate cyclases (Birnby et al., 2000; L'Etoile and Bargmann, 2000; Inada et al., 2006), but it is not known whether PDEs are involved in these processes. Thus, it is unclear whether chemosensation and thermosensation signal through guanylate cyclases or PDEs in *C. elegans* (Bargmann, 2006a), as guanylate cyclases might have a passive role by supplying substrates to PDEs for cleavage, just as they do in vertebrate phototransduction. In fact, knockout mice lacking either membrane-associated guanylate cyclases or PDE are blind (Fu and Yau, 2007), indicating that a requirement at the genetic level does not provide adequate information to assess the role of these genes in the

transduction pathway. Thus, the transduction mechanisms underlying chemosensation and thermosensation in *C. elegans* remain to be determined.

The taste receptor-like protein LITE-1 is required for light responses and functions upstream of G protein signaling

The *C. elegans* genome does not encode any closely related opsin homologues, and so we relied on forward genetic screening to identify potential photoreceptor genes. Using this approach we found a candidate gene *lite-1*, a *Drosophila* taste-receptor homologue, which is required for *C. elegans* photo-avoidance behavior. We showed that LITE-1 functions upstream of G protein signaling in ASJ and ASK light responses, and ectopic expression of LITE-1 in photo-insensitive cells can endow them with photosensitivity. These data suggest that LITE-1 may be part of the photoreceptor in worm photoreceptor cells. Unlike light-gated ion channels, such as ChR2, LITE-1 most likely functions as a receptor protein that requires downstream signaling events (for example, G protein signaling) to transduce light signals. Despite this view, we do not exclude the possibility that LITE-1 might possess a very small ion channel activity that is beyond the sensitivity of our detection method; however, such activity, if any, does not have a noticeable contribution to the photocurrent in ASJ. As LITE-1 shows no strong homology to known GPCRs and may adopt a reversed membrane topology (Benton et al., 2006), our results suggest the intriguing possibility that LITE-1 may represent a previously unknown type of GPCRs. Nevertheless, it is possible that LITE-1 may be indirectly coupled to G protein signaling.

LITE-1 is a member of the invertebrate taste receptor family that was first identified in *Drosophila*. Currently, the details of how *Drosophila* taste receptors function *in vivo* remains unknown. As taste receptors are related to odorant receptors in insects, it has been suggested that taste receptors may function as ion channels and that G protein signaling may not be directly involved in the transduction pathway in taste neurons (Sato et al., 2008). Recent work, however, has found that insect taste receptors and olfactory receptors have evolved along distinct paths during evolution and may employ distinct mechanisms for ligand recognition and signal transduction (Gardiner et al., 2009; Yao and Carlson, 2010). In light of this notion and the fact that LITE-1 and insect taste receptors belong to the same gene family, our results support the view that some *Drosophila* taste receptors may recruit G protein signaling in the transduction pathway.

Future Directions

Is LITE-1 directly activated by light?

A major unresolved question in this work is whether LITE-1 serves as a photoreceptor molecule. LITE-1 may function on its own or form a complex with other proteins, similar to many membrane receptors. Alternatively, it could be argued that LITE-1 might serve as a receptor for a light-evoked humoral response (although the kinetics of the light response in ASJ suggests otherwise). To address this question it should be shown biochemically that purified LITE-1 protein forms a photosensitive pigment. Recently, lizard parietopsin and box jellyfish opsin were overexpressed and purified from heterologous cell systems

and shown to have spectral absorbance properties (Su et al., 2006; Koyanagi et al., 2008). LITE-1 could be overexpressed in heterologous cells, or alternatively it could be overexpressed in a native tissue (such as *C. elegans* muscle cells) if presence of a worm-specific cofactor is required for LITE-1 function. We would expect LITE-1 to absorb most strongly in the UV range, and possibly to see peaks in the visual spectrum range. Biochemical proof that LITE-1 absorbs UV light would also definitively show that ASJ and ASK are photoreceptor cells. The observation that ROS-induced dark currents in ASJ did not depend on LITE-1 argues against a role for a light irradiation-induced byproduct in LITE-1 activation. LITE-1 is probably not the only member in the invertebrate taste receptor family that has a role in phototransduction. Ectopic expression of GUR-3, another *C. elegans* member of this family, can also confer photosensitivity to photo-insensitive cells (A.W. and X.Z.S.X., unpublished observations). Further studies examining the role of the remaining *C. elegans* GUR-family genes in photoresponses are required.

Is ASH a photoreceptor cell?

Our laser ablation experiments identified a subset of four neurons that are required for normal head responses to light. Of these four neurons, ASH is unique in that it does not express TAX-2 and TAX-4. Preliminary studies show that ASH also displays inward depolarizing currents in response to UV light flash (Y.M.D. and X.Z.S.X., unpublished observations). TRP channels such as OSM-9

and OCR-2 are expressed in ASH raising the question of whether photocurrents in ASH are TRP-channel dependent, as is the case in *Drosophila* rhabdomeric photoreceptor cells (Wang and Montell, 2007). Alternatively, ASH might use an atypical light-channel, as is the case in ciliated scallop photoreceptors (del Pilar Gomez and Nasi, 1995; Gomez and Nasi, 2000). Is the ASH phototransduction pathway LITE-1/$G_{i/o}$ protein-dependent? Pharmacology and genetic approaches similar to those used in dissecting the ASJ/ASK phototransduction pathway will be helpful in understanding the nature of ASH photoresponses.

What neurons mediate the tail response to light?

Our studies focused on neurons regulating avoidance responses to head-directed light stimuli. However, we noted in our work that light directed at the worm tail also evokes an avoidance response, causing the animal to move forward. This suggests the presence of light-sensing cell(s)/neuronal processes in the tail of worms. A laser ablation approach, perhaps focusing on phasmid sensory neurons in the tail, is warranted. Interestingly, LITE-1 worms are insensitive to light stimuli of both the head and tail, indicating LITE-1 probably functions in the tail response. A complete LITE-1 expression profile, therefore, would be useful in identifying photoreceptor cells mediating tail responses. Another important issue relates to how the head and tail stimuli are integrated, for instance when the entire body of the worm is illuminated. The light-sensitive behaviors in *C. elegans* provide a useful entry point for studying the wiring

mechanisms in worms, in particular, how these photosensitive neurons integrate and feed into the locomotor circuitry.

Detailed analysis of G protein function in *C. elegans* phototransduction

We identified two different $G_{i/o}$-type G alpha proteins, GOA-1 and GPA-3, which function redundantly in ASJ phototransduction? The *C. elegans* genome encodes a total of 21 G protein alpha subunits, including at least one member of each of the four mammalian G-alpha classes (Gs, Gq, Go, and G12) (Jansen et al., 1999), and two additional nematode-specific types (O'Halloran et al., 2006). Fourteen of the 21 *C. elegans* G-alpha proteins are expressed in sensory neurons, and like the chemoreceptor proteins, multiple G-alphas are often expressed in a single chemoreceptor neuron (Jansen et al., 1999). It has been shown previously that G-alpha proteins have both positive and negative regulatory functions in olfactory responses (Lans et al., 2004). For example, the G-alpha proteins ODR-3 and GPA-3 are both expressed in the olfactory neurons AWA and AWC and function redundantly in chemotaxis to AWA- and AWC-specific odorants (Lans et al., 2004). Conversely, the G-alpha proteins GPA-2 and GPA-5 are thought to negatively regulate chemotaxis to AWC- and AWA-specific odorants, respectively (Jansen et al., 1999; Lans et al., 2004). This raises the question of whether G-alphas function as both positive and negative regulators of *C. elegans* photoresponses. A more detailed exploration of the role of non-$G_{i/o}$-type G proteins in *C. elegans* phototransduction may yield new insights into mechanisms regulating the phototransduction cascade in ASJ and ASK.

How is the photoresponse terminated in *C. elegans*?

Termination of the photoresponse in vertebrate rods and cones arises from multiple inactivations of R*, G*, and PDE*, as well as Ca^{2+}/GCAP-mediated regulation of GC activity (Fu and Yau, 2007). The key steps in rod and cone inactivation is phosphorylation of R* followed by arrestin binding. *Drosophila* photoreceptors have a similar two-step phosphorylation/arrestin-dependent mechanism of R* inactivation, and Ca^{2+} also plays an important role in regulating light-response termination in flies (Wang and Montell, 2007). The surprise finding that neurons from *C. elegans* PDE mutants have potentiated photoresponses suggests involvement of PDEs (at least PDE-1, PDE-2 and PDE-5) in light response termination in worms. Alternatively, PDEs might function to regulate sensitivity of the photoreceptor. In mice, GCAPs/Ca^{2+}-mediated regulation of GC activity functions to dampen the sensitivity of rods and cones (Mendez et al., 2001). Future studies might explore whether PDEs play an analogous role in *C. elegans* photoreceptors. Additionally, the *C. elegans* genome encodes two G protein receptor kinases (GRKs), *grk-1* and *grk-2*, and one arrestin, *arr-1* (Fukuto et al., 2004). GRK2 and ARR1 are expressed throughout the nervous system, and *grk-2* mutants are defective in chemotaxis to diacetyl and octanol (Fukuto et al., 2004). It remains to be seen whether these genes function in modulation of *C. elegans* photoresponses.

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