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

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Abstract

The “eyeless” animal *C. elegans* possesses the sense of light and engages in phototaxis behavior 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, here we show that phototransduction in ASJ is a G protein-mediated process and requires membrane-associated guanylate cyclases but not typical cGMP-cleaving phosphodiesterases (PDEs). In addition, we find that *C. elegans* phototransduction requires LITE-1, a candidate photoreceptor protein known to be a member of the invertebrate taste receptor family. Genetic, pharmacological and electrophysiological data suggest a model whereby LITE-1 transduces light signals in ASJ through G-protein signaling, which leads to up-regulation of the second messenger cGMP followed by opening of cGMP-sensitive CNG channels and thereby stimulation of photoreceptor cells. Our results identify a phototransduction cascade in *C. elegans* and implicate the function of a “taste receptor" in phototransduction.

Introduction

The sense of light is essential for the life of most organisms. In animals, photoreceptor cells in the eye detect light and transduce it into electrical responses through a process called

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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 transducin1. Light-activated transducin then turns on PDEs to cleave the second messenger cGMP, resulting in a decrease in cGMP level and hence closure of CNG channels1. 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 channels2. In both cases, guanylate cyclases (GCs) that produce cGMP, are constitutively active in the dark and thus play a rather passive role in phototransduction by providing substrates to PDEs1. In addition to this canonical phototransduction pathway, recent studies show that photosensitive retinal ganglion cells, which mediate non-image-forming visual functions, may employ a distinct pathway for phototransduction3; 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 (e.g. AWA and AWC) and gustatory neurons (e.g. ASE) to respond to chemical stimuli4, while reacting to mechanical forces through touch receptor neurons (e.g. ALM, AVM and PLM) and proprioceptor neurons (e.g. DVA)5, 6. However, worms were long thought to lack the sense of light, as these animals do not have eyes and live in the dark soil.

Recent work from us and others has demonstrated that despite the lack of eyes, the soil-dwelling *C. elegans* expresses the sense of light and engage in negative phototaxis behavior that allows the animal to avoid lethal doses of light and may also serve as a potential mechanism to retain the animal in the dark soil7, 8. We have also reported that worms sense light through a group of photoreceptor cells, some of which respond to light by opening cGMP-sensitive CNG channels7. These channels also mediate temperature-evoked currents in the thermosensory neuron AFD9. In addition, a previous study identified a receptor-like gene *lite-1* that is important for phototaxis behavior and has been suggested to encode a light-sensing molecule; however, it is not clear whether this gene has a role in phototransduction in photoreceptor cells8.

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)8. 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, does the *lite-1* gene play a role in phototransduction in photoreceptor cells?

Here 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 homologue LITE-1.

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 cell7, and recorded its activity in response to light by perforated whole-cell recording7. Classic whole-cell recording protocols are incapable of detecting light-induced currents (photocurrents) in this neuron7, probably because some components important for phototransduction are dialyzed out by the recording pipette. A similar phenomenon has been observed in recording vertebrate photoreceptor cells2.

To test whether G-protein signaling is required for phototransduction in ASJ, we checked the effect of mSIRK, a membrane-permeable peptide that dissociates Gα from Gβγ without affecting its GTPase activity, thereby exerting an inhibitory effect on GPCR-mediated activation of Gα10. mSIRK blocked the light-evoked conductance in ASJ (Fig. 1a,b). As a control, the cGMP-induced currents were not affected in ASJ (Fig. 1c,d,e). Thus, blocking G-protein signaling can inhibit phototransduction in ASJ, suggesting that G-protein signaling is required for phototranduction 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 analogue that activates G-proteins, into ASJ through the recording pipette. GTPγS stimulated ASJ by evoking an inward current in the dark (Fig. 1f). This dark 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. 1f)11–13. Therefore, stimulating G-protein signaling can stimulate photoreceptor cells, further 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 subfamily1. We thus tested the effect of mastoparan, a peptide that can activate Gi/o proteins14. Perfusion of mastoparan into ASJ elicited an inward current (Fig. 1g,h). Similarly, this current appears to be carried by CNG channels, as it can be blocked by L-cis-diltiazem and by mutations in *tax-2* and *tax-4* (Fig. 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 family15; in addition, many others are closely related to Gi/o16. We first tested the effect of pertussis toxin (PTX) that inhibits Gi/o function17. PTX blocked the photoresponse in ASJ, suggesting that Gi/o proteins are required for phototransduction in ASJ (Fig. 1i). As expected, PTX also blocked the ability of GTPγS in stimulating CNG channels in ASJ (Fig. 1j). As a control, direct application of cGMP can still efficiently activate CNG channels in ASJ (Fig. 1j), consistent with the view that CNG channels act downstream of G-proteins. These results together strongly suggest that phototransduction in ASJ is mediated by the Gi/o-family of G-proteins.

At least five *C. elegans* Gα genes are targets for PTX18. Among them, *goa-1, gpa-1* and *gpa-3* are known to be expressed in ASJ19. While photocurrents appeared normal in *goa-1, gpa-1* and *gpa-3* single mutants (Supplementary Fig. 1), the *goa-1;gpa-3* double mutant showed a severe defect in phototransduction in ASJ (Fig. 1k). In addition, GTPγS can no longer stimulate CNG channels in *goa-1;gpa-3* mutant worms (Fig. 1l,m). As a control, cGMP can still efficiently activate CNG channels in these mutant worms, indicating that the mutations did not affect the general health of the neuron (Fig. 1l,m). Thus, *goa-1* and *gpa-3* play 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 also play a role 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 (e.g. parietal eye photoreceptor cells) or stimulate PDEs (e.g. rods and cones), resulting in an increase or reduction in cGMP level and hence the opening or closing of CNG channels, respectively1, 2. Mice lacking the retina PDE (PDE6) are blind20. If *C. elegans* photoreceptor cells adopt such a mechanism, it would be similar to that in vertebrate parietal eye photoreceptor cells; namely, G-proteins may inhibit PDEs to up-regulate cGMP, thereby opening CNG channels. Thus, we decided to examine 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 homologue (Fig. 2a). PDE-4 and PDE-6 are highly homologous to human PDE4 and PDE8, respectively, both of which are cAMP-specific21. The other four PDEs (PDE-1, 2, 3 and 5) may cleave cGMP and hence could be involved in phototransduction. Thus, we isolated mutant alleles of all four *pde* genes and generated mutant strains lacking multiple PDEs. In the *pde-1,2,5* triple mutant, the photocurrent was not only present in ASJ, but also drastically potentiated, with a current density about five fold of that in wild-type worms (Fig. 2b–e). The same phenomenon was observed in the quadruple mutant devoid of all four PDEs (Fig. 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 showed normal photocurrents (Fig. 2d,e).

The photocurrent in the *pde-1,2,3,5* quadruple mutant exhibited very slow or no recovery after cessation of the light stimulus, consistent with a role for PDEs in down-regulating cGMP (Fig. 2c). Importantly, the input resistance in ASJ of the *pde* quadruple mutant (4.43 ± 0.66 GΩ; $n = 4$) was similar to that in wild-type (4.30 ± 0.60 GΩ; $n = 6$). This indicates that 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 cells2. This also suggests that guanylate cyclases (GCs) display 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 rather play a modulatory role in phototransduction in ASJ. It should be noted that, although we have

examined all 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 membrane-associated GCs

Alternatively, stimulation of GCs in principle may also up-regulate cGMP, leading to activation of CNG channels. There are two major types of GCs: soluble GCs and membrane-associated GCs22, 23. In *C. elegans*, soluble GCs are sensitive to O_2 and required for social feeding, whereas membrane-associated GCs are essential for chemotaxis and thermotaxis24–27. Notably, two membrane-associated GCs (*daf-11* and *odr-1*) are expressed in *C. elegans* photoreceptor cells, including ASJ, ASK and AWB 26, 28.

We thus tested *daf-11* and *odr-1* mutants. Two independent *daf-11* mutant alleles, *ks67* and *m47*, both lacked photocurrents in ASJ (Fig. 2f). *odr-1(n1936)* mutant worms also showed a severe reduction in the density of photocurrents (Fig. 2g,h and Supplementary Fig. 2). These results demonstrate that membrane-associated GCs are required for phototransduction in ASJ.

Supplement of non-saturating levels of cGMP did not restore photosensitivity in ASJ of *daf-11* mutant worms (Supplementary Fig. 3). This indicates that cGMP does not simply play a permissive role in phototransduction, providing additional evidence that cGMP is a second messenger for phototransduction in ASJ.

GC act downstream of G-protein but upstream of CNG channel

The above results suggest a model whereby G-protein activation may lead to up-regulation of cGMP level, leading to CNG channel activation. In other words, GCs may act downstream of G-proteins but upstream of CNG channels. If true, activation of G-proteins should no longer be able to stimulate CNG channels in the GC mutant background, while cGMP should retain the ability to open these channels in GC mutant worms.

Indeed, GTPγS failed to stimulate CNG channels in ASJ of *daf-11* mutant worms (Fig. 3a,b), while cGMP can still efficiently activate CNG channels in this mutant (Fig 3c,d). This observation suggests that GCs 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 only able to detect light-induced currents under the perforated but not classic whole-cell configuration. Due to this technical constraint, we can only test the effect of those few membrane-permeable chemicals on photocurrents by including them in the bath solution. This is because 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 under classic whole-cell configuration, we were able to detect photocurrents in *pde* mutant worms (Fig. 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 impact resulting from 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 tested the effect 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, providing further evidence that phototransduction requires G-protein signaling (Fig. 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. 4c,d). Light cannot further induce an inward current under these conditions, suggesting that light, GTPγS and cGMP all act on the same type of CNG channels and stimulate the same signaling cascade (Fig. 4c–e). This is also consistent with our phototransduction model whereby G-protein signaling up-regulates cGMP level, leading to CNG channel activation.

Phototransduction in photoreceptor cells require LITE-1

The *C. elegans* genome does not encode any closely-related homologue for opsins29, 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 thus conducted a forward genetic screen for mutants defective in phototaxis in hopes of identifying candidate photoreceptor genes. Three mutants (*xu7*, *xu8* and *xu10*) exhibited a strong defect in phototaxis behavior and failed to complement each other, suggesting that they harbor mutations in the same gene (Fig. 5a and data not shown). We mapped these mutations to the close proximity of *lite-1*, a gene identified by Edwards *et al*. in a screen for behavioral mutants defective in phototaxis8. Sequencing analysis shows that they all are alleles for *lite-1* (Fig. 5b). *lite-1* encodes a seven-transmembrane domain receptor-like protein and is a member of the invertebrate taste receptor family (Supplementary Fig. 4)8. This family was first identified in *Drosophila*30, 31. The *C. elegans* genome encodes a total of five such taste receptor genes (Supplementary Fig. 4).

The *lite-1* gene has been reported to be located in a large, complex operon, and GFP transgenic approaches appear to be unsuccessful in revealing its full expression pattern8. Although *lite-1* mutant worms show 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 compromise phototaxis behavior. Indeed, many mutants affecting synaptic transmission disrupt phototaxis behavior in a non-specific manner (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. 5c,d). Expression of wild-type LITE-1 specifically in ASJ fully rescued the photoresponse in ASJ (Fig. 5e,f). The same transgene was also sufficient to yield a rescuing

effect on *lite-1* phototaxis defect (Fig. 5g). These results suggest that LITE-1 functions in ASJ to mediate phototransduction.

We also recorded another putative photoreceptor cell ASK that expresses the same set of CNG channels and membrane-associated GCs as does ASJ12, 13, 26, 28. Light stimulation evoked an inward current in ASK of wild-type worms (Figs. 5f and Supplementary Fig. 5). This photoresponse also required CNG channels and membrane-associated GCs but not PDEs (Supplementary Fig. 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. 6). This is different from the case with ASJ, indicating that PDEs play a modulatory role in some but not all photoreceptor cells. Importantly, mutations in *lite-1* eliminated ASK photocurrents, and expression of wild-type LITE-1 specifically in ASK fully rescued this defect (Figs. 5f and Supplementary Fig. 5). The same transgene also showed a rescuing effect on *lite-1* phototaxis defect (Fig. 5g). Nevertheless, given the smaller amplitude and slower kinetics of ASK photocurrents compared to those recorded in ASJ (Supplementary Fig. 5), it remains possible that the recorded photocurrents in ASK may indirectly result from ASJ (ASJ synapses onto ASK) or other photoreceptor cells.

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. 6a–c). These results suggest that LITE-1 may be part of the photoreceptor complex or required for the function of this complex.

If LITE-1 is part of the photoreceptor complex, it should also function upstream of GCs and CNG channels. Mutations in the membrane-associated GC 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. 2e and unpublished observations). This can be explained by the fact that some other photoreceptor cells (e.g. ASH and ADL) do not express these genes and perhaps utilize distinct phototransduction mechanisms. Nonetheless, expression of wild-type LITE-1 in GCs/CNG channel-expressing photoreceptor cells, such as ASJ, ASK and AWB, was sufficient to rescue the phototaxis defect in *lite-1* mutant worms (Fig. 6d). Importantly, mutations in *daf-11* and *tax-4* can suppress the effect of the *lite-1* transgene on rescuing *lite-1* phototaxis defect (Fig. 6d). These results provide additional evidence that GCs 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. 7). The same ChR2 transgene also restored photosensitivity in ASJ of *daf-11*, *tax-2* and *tax-4* mutant worms (Supplementary Fig. 7). These results provide additional evidence that these mutations did

not affect the general health of the neuron. Consistent with the role of ChR2 as an ion channel that is directly gated by light independently of second messengers32, 33, the ChR2-dependent photocurrents in ASJ developed virtually instantaneously upon light stimulation without a detectable latency and also exhibited rapid activation kinetics (Supplementary Fig. 7; activation time constant τ_{act} = 8.95 ± 0.03 ms under 2 mW mm^{-2} of blue light). These features are in sharp contrast to those of the LITE-1-dependent intrinsic photocurrents in ASJ that exhibited a latency of hundreds of miliseconds and slow activation kinetics (latency: 356 ± 37 ms in ref 7; τ_{act} = 566 ± 2.6 ms), which are typical for a process requiring second-messengers. This is consistent with the model that 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. 8). Although it is unclear what mediates this ROS-induced current in ASJ, apparently it is not through the activation of LITE-1. This result suggests that the trace amount of ROS produced by light elimination, if any, cannot fully account for the activation of LITE-1.

LITE-1 confers photosensitivity to photo-insensitive cells

To provide further evidence, we sought to test the function of LITE-1 in heterologous systems. However, all attempts aimed at functionally expressing LITE-1 in cultured cell lines were unsuccessful (unpublished observations). LITE-1 has been ectopically expressed in muscles and found to induce muscle contraction8. 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 GCs.

We thus expressed LITE-1 as a transgene in the ASI neuron that also expresses the GC DAF-11 and the CNG channel TAX-2 and TAX-412, 13, 28. No photocurrent could be detected in ASI of wild-type worms, demonstrating that this neuron is photo-insensitive (Fig. 7a). Remarkably, expression of LITE-1 as a transgene in ASI rendered this neuron photosensitive (Fig. 7b). The LITE-1-dependent photocurrent in ASI also showed a latency of hundreds of miliseconds and slow activation kinetics (latency: 432 ± 66 ms; τ_{act} = 908 ± 3.4 ms), suggesting the involvement of second-messenger signaling. Indeed, as was the case with ASJ and ASK, the LITE-1-dependent photocurrent in ASI also required the GC DAF-11 and the CNG channel TAX-2 and TAX-4 (Fig. 7c–f). These results provide electrophysiological evidence that LITE-1 expression is sufficient to confer photosensitivity to photo-insensitive cells.

Discussion

A model for *C. elegans* phototransduction cascade is summarized in supplementary figure 9. Despite many remarkable 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. For example, they likely use distinct types of photoreceptor proteins (Supplementary Fig. 9). In addition, *C. elegans* phototransduction in ASJ requires membrane-associated GCs but not typical PDEs (Supplementary Fig. 9). Membrane-associated GCs are known to be activated by peptide ligands and GCAPs 22. Our results raise the possibility that G-protein signaling may modulate membrane-associated GCs, suggesting an unusual mechanism that regulates cGMP-sensitive CNG channels. It is unclear whether G-protein signaling directly or indirectly modulates GCs. Notably, it has been suggested that a similar mechanism may also function in some marine species to regulate K^+ channels34, 35; 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 GCs26, 28, but it is not known whether PDEs play a role in these processes. Thus, it is unclear whether chemosensation and thermosensation signal through GCs or PDEs in *C. elegans*4, since GCs might play a passive role by supplying substrates to PDEs for cleavage just like they do in vertebrate phototransduction. In fact, knockout mice lacking either GCs or PDE are blind1, 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.

Apparently, 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 (e.g. G-protein signaling) to transduce light signals. Despite this view, we do not exclude the possibility that LITE-1 might possess very small ion channel activity that is beyond the sensitivity of our detecting 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 topology36, our results point to the intriguing possibility that LITE-1 may represent a novel type of GPCRs. Nevertheless, it remains 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 like many membrane receptors. The observation that ROS-induced dark currents in ASJ do not depend on LITE-1 argues against a role for a light irradiation-induced byproduct in LITE-1 activation. However, it should be pointed out 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 it has not been successful to functionally express these receptors in heterologous systems. Whole-cell recording of taste neurons in *Drosophila* has not been reported, which poses a

challenge to directly interrogate the transduction mechanisms *in vivo*. Notwithstanding these technical challenges, genetic and behavioral studies have implicated G-protein signaling in *Drosophila* taste transduction37–39. 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 G-protein signaling may not be directly involved in the transduction pathway in taste neurons40. Nonetheless, more recent work shows that insect taste receptors and olfactory receptors have evolved along distinct paths during evolution and may employ distinct mechanisms for ligand recognition and signal transduction41. In light of this notion and the fact that LITE-1 and insect taste receptors belong to the same gene family, our results lend support to 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 (unpublished observations). Over sixty taste receptor genes have been identified in *Drosophila*42–44. Clearly, many of them function as taste receptors and are required for taste transduction42–44. Notably, some *Drosophila* taste receptor genes are expressed in many non-chemosensory neurons, suggesting that these receptors may adopt a distinct function in these neurons45. It will be interesting to test whether some of them have a role in photosensation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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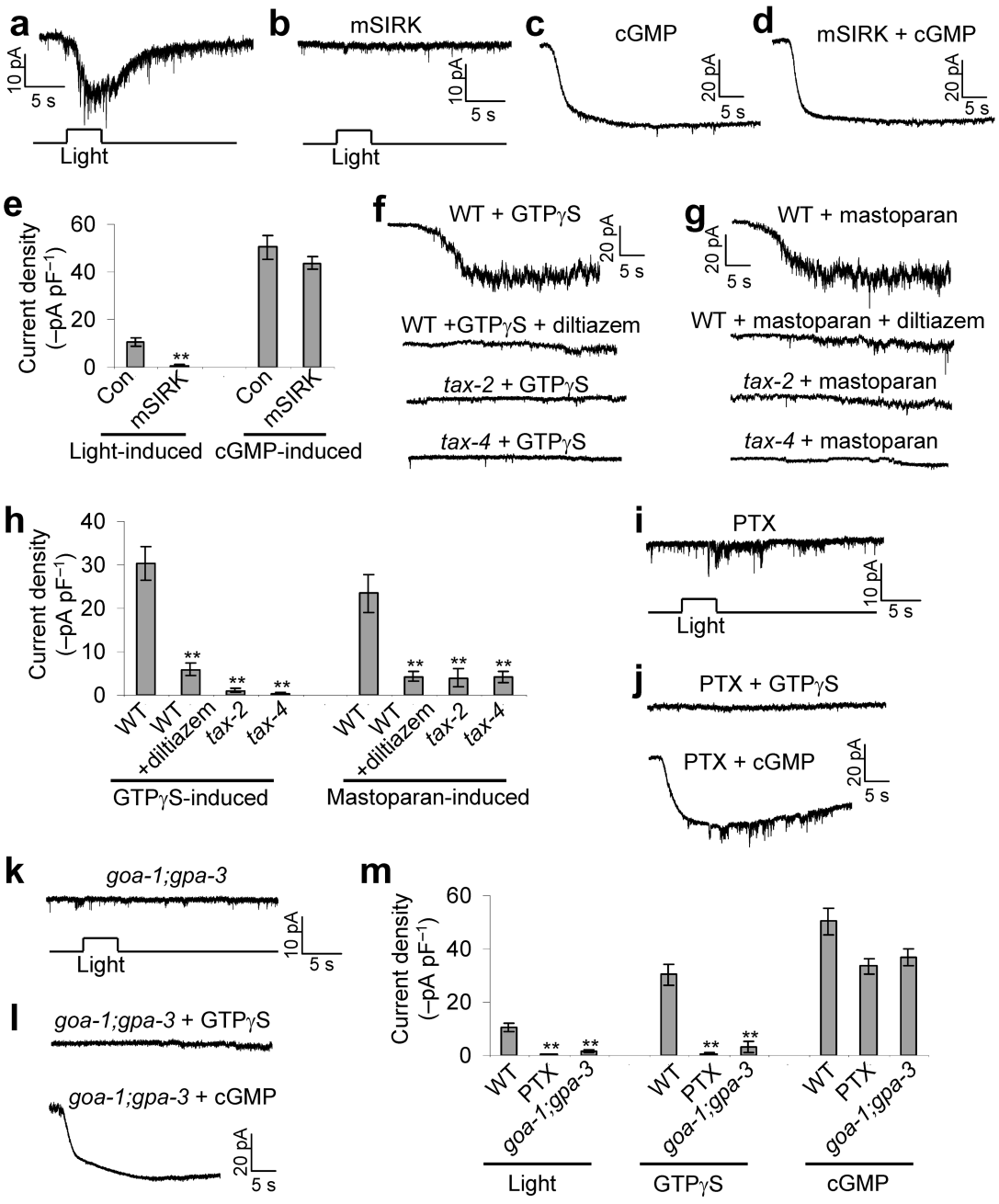

Figure 1. Phototransduction in ASJ is a G-protein-mediated process

(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 light7. The downward "spikes" in this trace and many others in the paper are typical for many worm neurons that are very small (~1 pF; ~2 μm in diameter) and exhibit high input resistance46.

(b) Blocking G-protein signaling blocked phototransduction. mSIRK (50 μM) is membrane-permeable.

(c–d) cGMP-evoked currents were not affected by mSIRK. cGMP: 1 mM.

(e) Bar graph summarizing the data in (a–d). $n \geq 6$ (photocurrents); $n \geq 4$ (cGMP-induced currents). Error bars: SEM. $^{**}P < 0.002$ (t test).
(f) Activation of G-proteins opened CNG channels in the dark. GTPγS: 100 μM. Alleles: *p671* for *tax-2*; *p678* for *tax-4*.
(g) Activation of Gi/o opened CNG channels in the dark. Mastoparon: 5 μM.
(h) Bar graph summarizing the data in (f–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- (top trace) but not cGMP-induced current (bottom trace).
(k) The *goa-1(n1134);gpa-3(pk35)* double mutant lacked photocurrents. See supplementary figure 1 for single mutant data.
(l) Mutations in *goa-1* and *gpa-3* blocked GTPγS- (top trace) but not cGMP-induced current (bottom trace).
(m) Histogram summarizing the data in (i–l). $n \geq 5$. Error bars: SEM. $^{**}P < 0.005$ (ANOVA with Dunnett test).

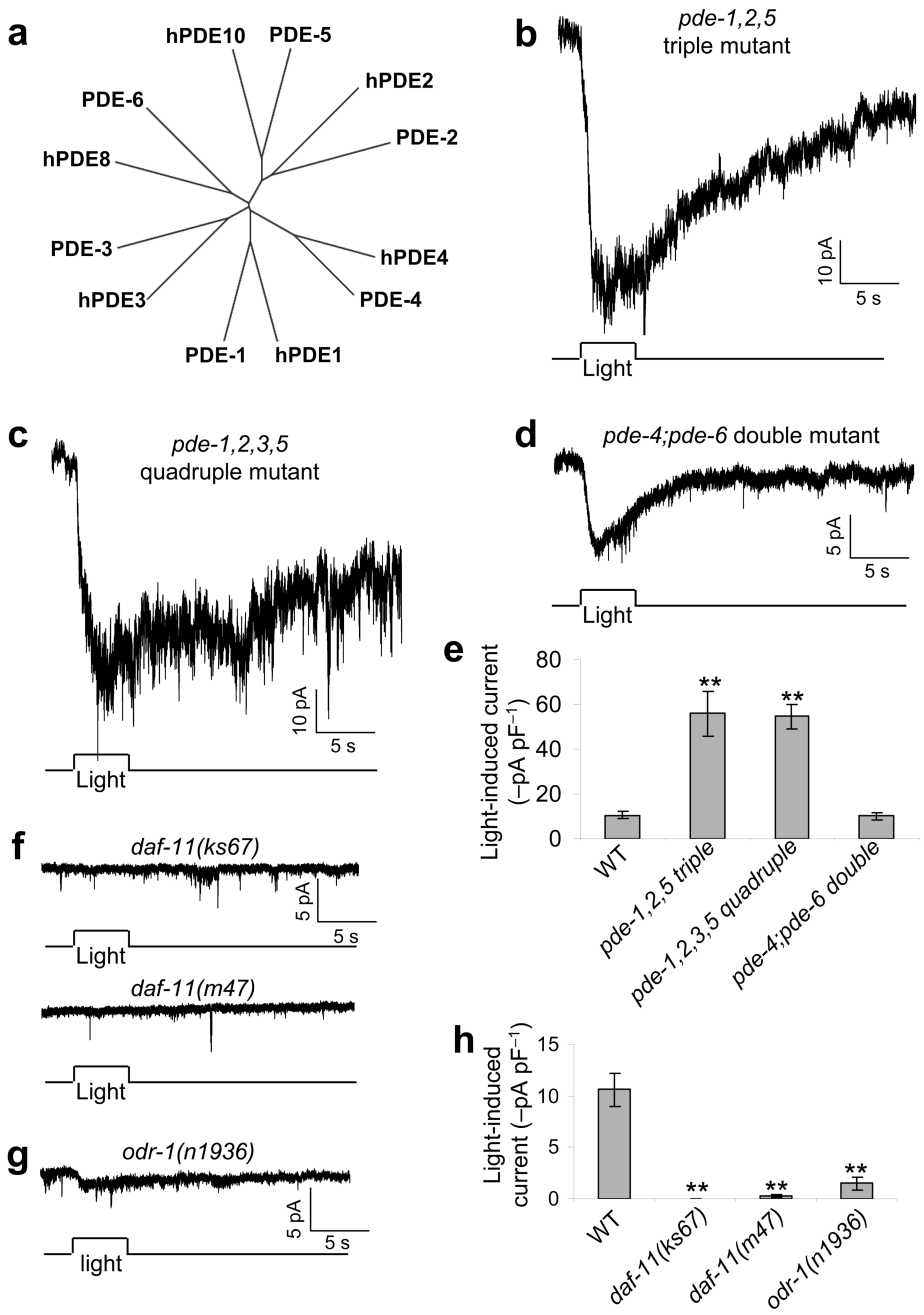

Figure 2. Phototransduction in ASJ requires membrane-associated GCs

(a) Dendrogram of *C. elegans* and human PDEs (hPDEs).

(b) The light-induced current was greatly potentiated in the *pde-1,2,5* triple mutant *pde-1(nj57)pde-5(nj49);pde-2(nj58).*

(c) The light-induced current was greatly potentiated in the *pde-1,2,4,5* 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 also 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: SEM. $^{**}P < 0.0001$ (ANOVA with Dunnett test; compared to WT).
(f) No light-induced current was detected in the GC mutants *daf-11(ks67)* and *daf-11(m47)*.
(g) The light-induced current in the GC mutant *odr-1(n1936)* was greatly reduced.
(h) Bar graph summarizing the data in (f–g). *daf-11(ks67)* is temperature-sensitive47, and all recordings involving this allele were done at 25°C in this study. 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: SEM. $^{**}P < 0.0005$ (ANOVA with Dunnett test; compared to WT).

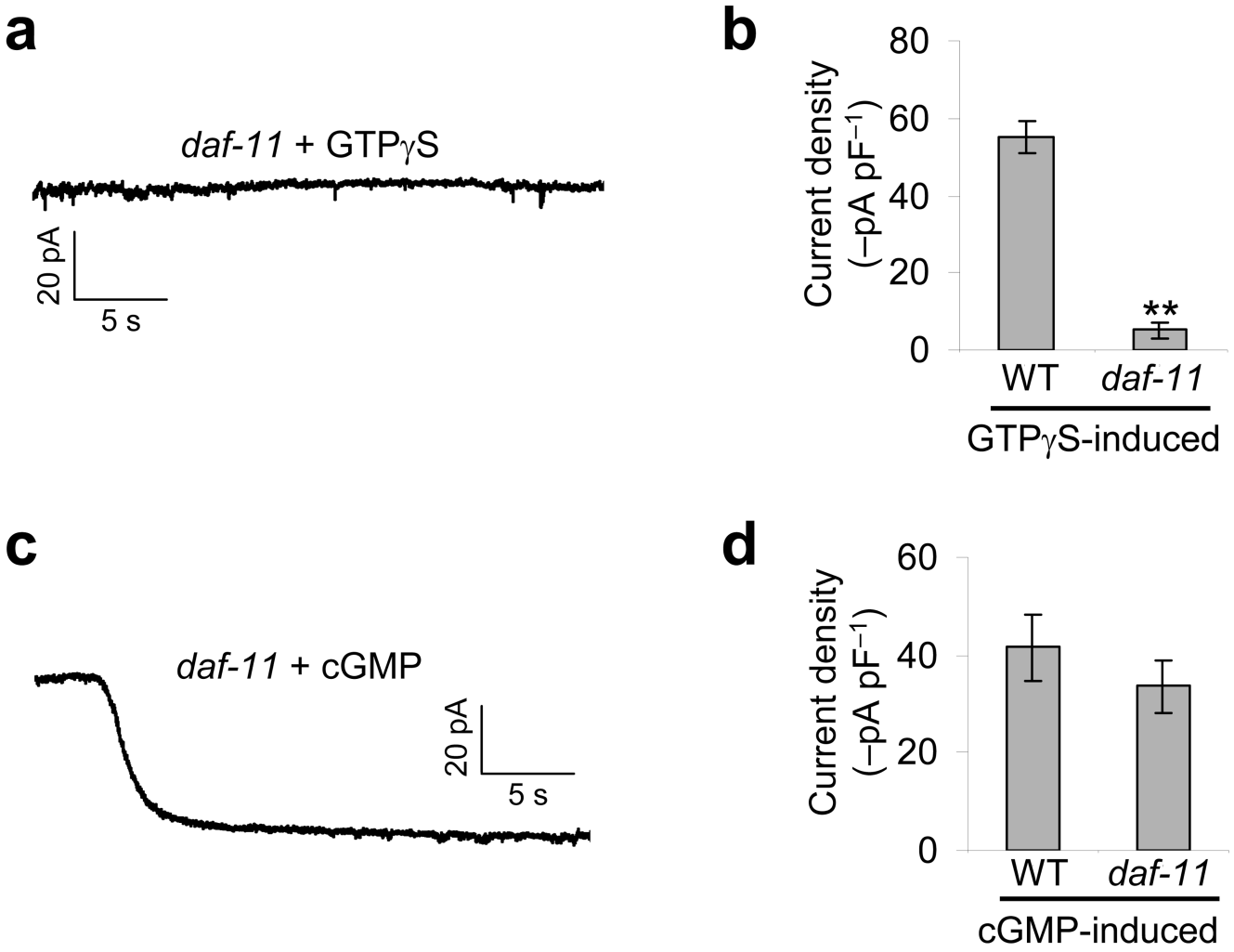

Figure 3. Guanylate cyclases act downstream of G-proteins and upstream of CNG channels to mediate phototransduction

(a–b) GC/DAF-11 acted downstream of G-proteins. Mutation in *daf-11* blocked the ability of GTPγS in stimulating ASJ. Shown in (a) is a sample trace. $n \geq 5$. Error bars represent SEM. $^{**}P < 0.00001$ (t test).

(c–d) GC/DAF-11 acted upstream of CNG channels. cGMP can efficiently open CNG channels in ASJ of *daf-11* mutant worms. Shown in (c) is a sample trace. $n \geq 5$. Error bars represent SEM.

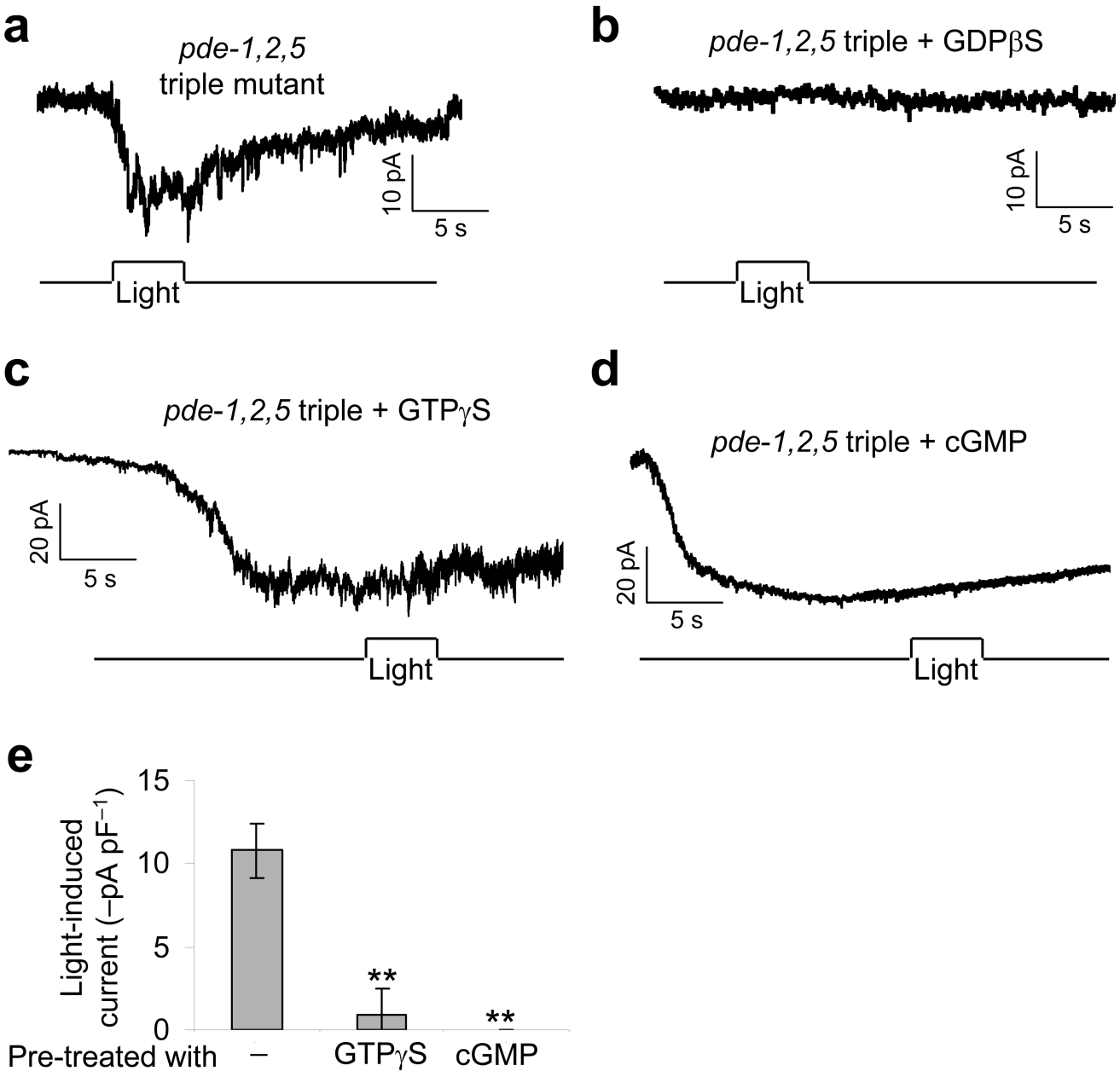

Figure 4. Light, GTPγS and cGMP activate the same type of CNG channels in photoreceptor cells

(a) Light evoked an inward current in the *pde-1,2,5* 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 cannot 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 cannot further induce an inward current.

(e) Bar graph summarizing the data in (a–d). $n \geq 6$. Error bars: SEM. $^{**}P < 0.0001$ (ANOVA with Dunnett test).

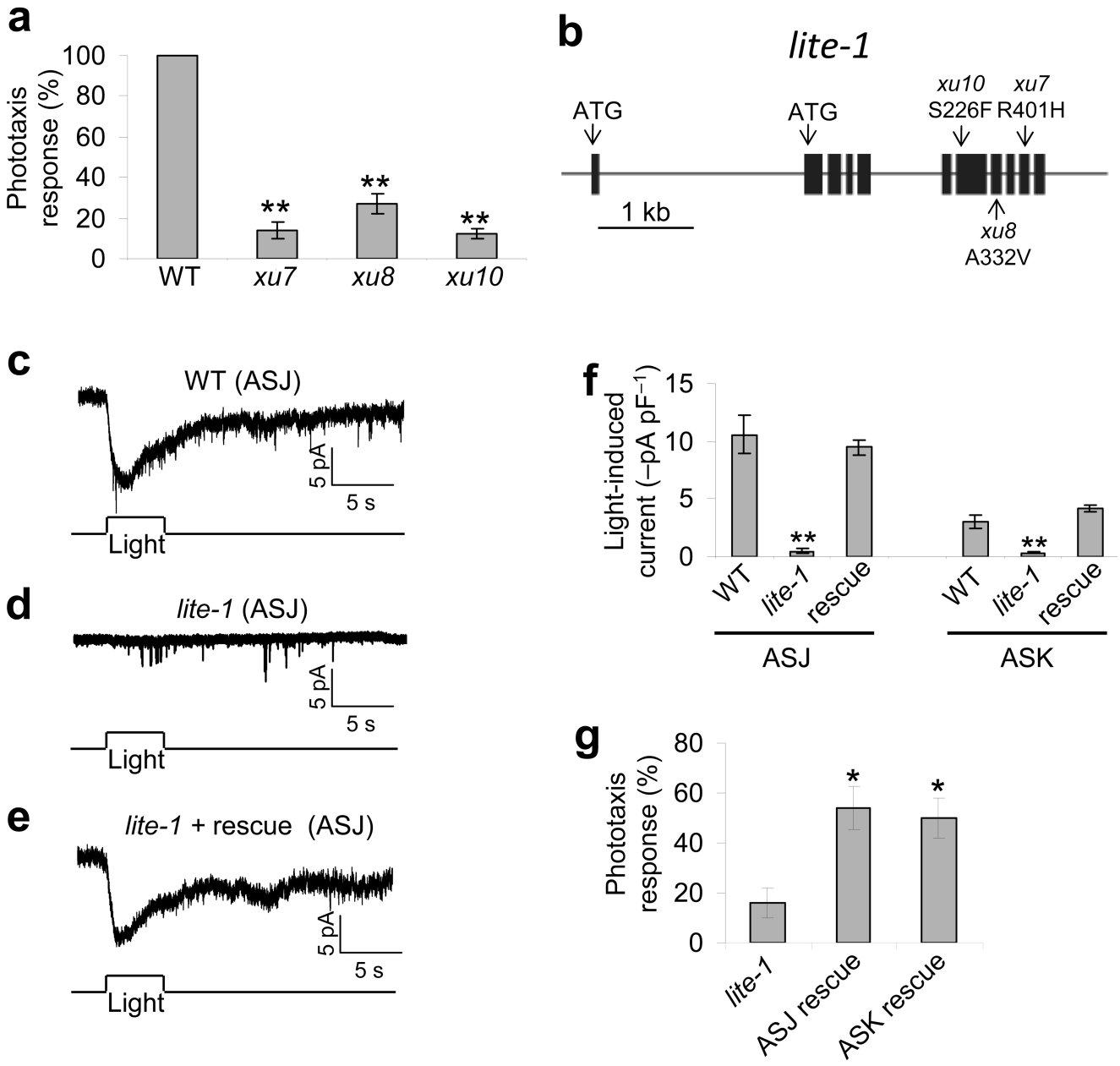

Figure 5. LITE-1 is required for phototransduction in photoreceptor cells

(a) Three mutants showed 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 described7, 48. The response rate in *xu7* and *xu10* was similar to that of no-light control and likely resulted from spontaneous reversals. $n \geq 10$. Error bars: SEM. $^{**}P < 0.00001$ (ANOVA with Dunnett test; compared to wild-type).

(b) *lite-1* genomic structure and mutations identified in *lite-1*. We have identified two *lite-1* isoforms. An SL1 sequence was found before the ATG in the second exon, indicating that it represents a short form of *lite-1*. This isoform was used in the current study.

(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 5 for ASK traces.

(f) Bar graph summarizing the data in (c–e). Error bars: SEM. $n \geq 7$. $^{**}P < 0.00002$ (ANOVA with Dunnett test; compared to WT).

(g) Expression of a wild-type *lite-1* transgene specifically in ASJ or ASK showed a rescuing effect on phototaxis behavioral defect in *lite-1(xu7)* mutant worms. The *trx-1* and *srg-8* promoter was used to drive expression of the transgene in ASJ and ASK, repectively49, 50. Error bars: SEM. $n \geq 10$. $^{*}P < 0.05$ (ANOVA with Bonferroni test; compared to *lite-1*).

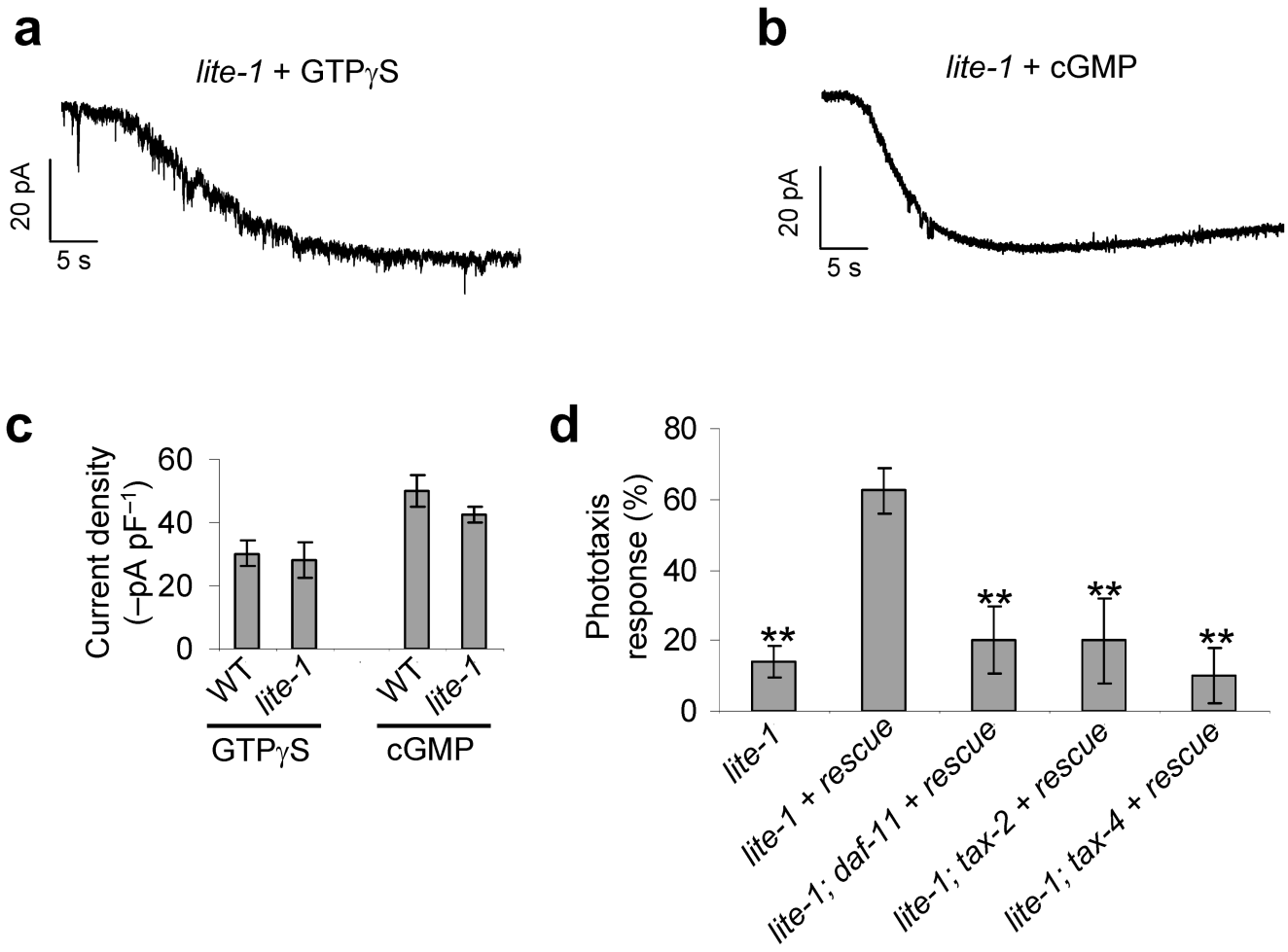

Figure 6. LITE-1 functions upstream of G-proteins

(a–b) LITE-1 acted upstream of G-proteins. GTPγS (a) and cGMP (b) can induce an inward current in ASJ of *lite-1(xu7)* mutant worms.

(c) Bar graph summarizing the data in (a–b). The density of GTPγS and cGMP induced currents in ASJ of *lite-1(xu7)* mutant worms are similar to those in wild-type. $n \geq 6$. Error bar: SEM.

(d) LITE-1 acted upstream of GCs 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 can rescue the phototaxis defect in *lite-1(xu7)* mutant worms. This rescuing effect requires the GC DAF-11 and CNG channel TAX-2 and TAX-4. $^{**}P < 0.001$ (ANOVA with Dunnett test; compared to the rescue). $n \geq 10$. Error bars: SEM.

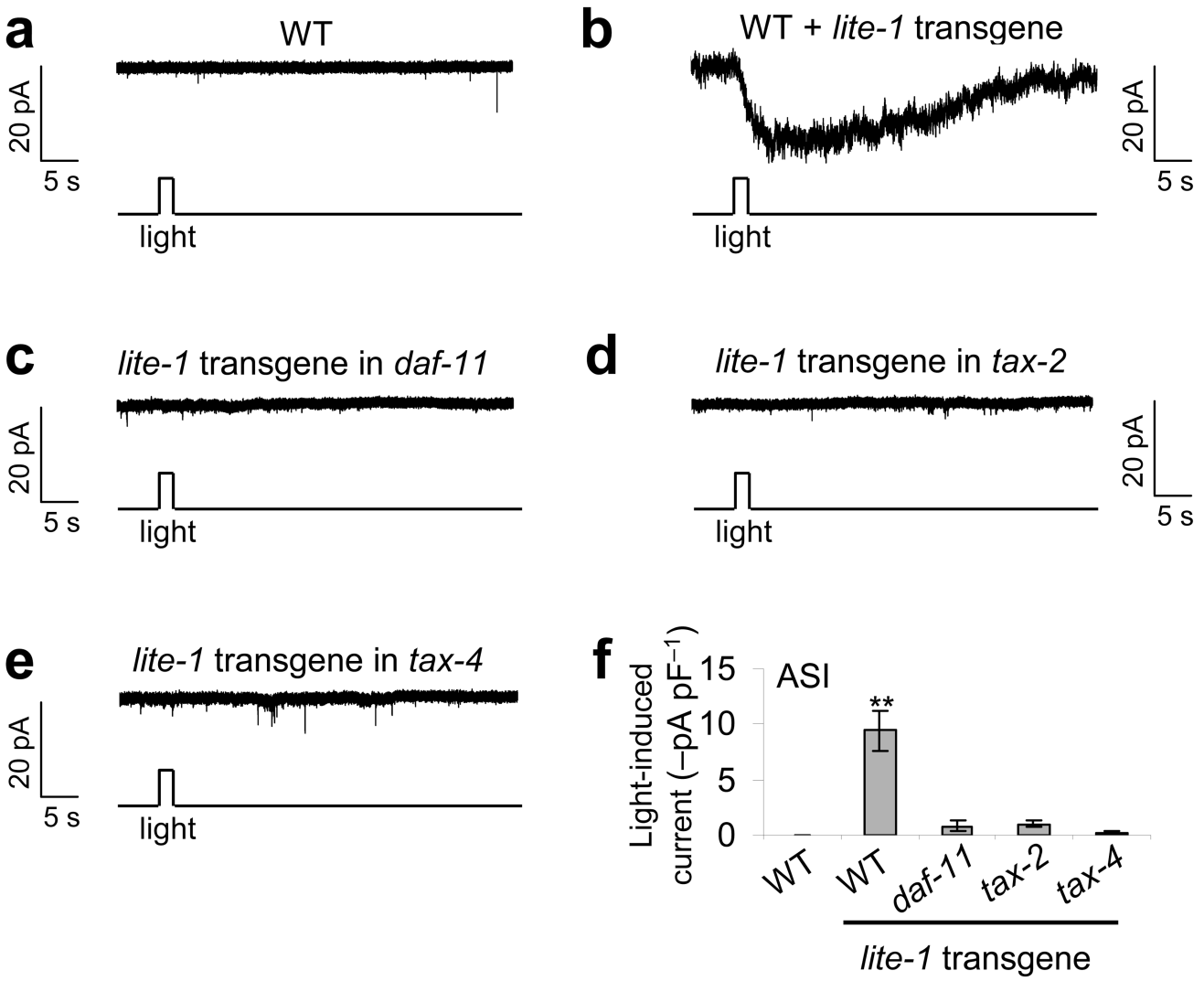

Figure 7. Transgenic expression of LITE-1 can confer photo-sensitivity to the photo-insensitive neuron ASI

(a) The ASI neuron was photo-insensitive. No photocurrent could be detected in ASI.

(b) Expression of LITE-1 in ASI turned it into photo-sensitive. LITE-1 was expressed as a transgene in ASI under the *sra-6* promoter that labels both ASI and ASH in the head 50. ASI recordings performed in ASH-ablated worms and non-ablated worms yielded similar results (9.1 ± 1.3 pA pF^{-1} vs. 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: SEM. $^{**}P < 0.00001$ (ANOVA with Dunnett test; all compared to WT without transgene).