Light-sensitive coupling of rhodopsin and melanopsin to G_{i/o} and G_{q} signal transduction in *Caenorhabditis elegans*

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**ABSTRACT** Activation of G-protein-coupled receptors (GPCRs) initiates signal transduction cascades that affect many physiological responses. The worm *Caenorhabditis elegans* expresses \( \sim 1000 \) of these receptors along with their cognate heterotrimeric G proteins. Here, we report properties of 9-cis-retinal regenerated bovine opsin [(b)isoRho] and human melanopsin [(h)Mo], two light-activated, heterologously expressed GPCRs in the nervous system of *C. elegans* with various genetically engineered alterations. Profound transient photoactivation of G_{i/o} signaling by (b)isoRho led to a sudden and transient loss of worm motility dependent on cyclic adenosine monophosphate, whereas transient photoactivation of G_{q} signaling by (h)Mo enhanced worm locomotion dependent on phospholipase C{\beta}. These transgenic *C. elegans* models provide a unique way to study the consequences of G_{i/o} and G_{q} signaling in vivo with temporal and spatial precision and, by analogy, their relationship to human neuromotor function.—Cao, P., Sun, W., Kramp, K., Zheng, M., Salom, D., Jastrzebska, B., Jin, H., Palczewski, K., Feng, Z. Light-sensitive coupling of rhodopsin and melanopsin to G_{i/o} and G_{q} signal transduction in *Caenorhabditis elegans*. FASEB J. 26, 480–491 (2012). www.fasebj.org

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Membrane-bound G-protein-coupled receptors (GPCRs) transduce extracellular signals into intracellular physiological responses via their functional heterotrimeric G-protein partners. In the nervous system, these G-protein signals mediate neurotransmission that affects subsequent animal behaviors. However, the underlying molecular and cellular mechanisms of G-protein signaling in vivo are still poorly understood.

The soil-dwelling nematode *Caenorhabditis elegans* uses \(~ 1000\) GPCRs (5\% of its genome; ref. 1) expressed in its neurons to respond to environmental chemical, mechanical, and thermal stimuli, mediate synaptic function, reshape neural circuits, and modulate muscle activity, all of which affect its motor behavior. Components of the anciently evolved heterotrimeric G-protein-signaling pathways are highly conserved in *C. elegans* with respect to their protein sequences, functions, and signaling mechanisms. For example, there are \( 21 \) G_{a}, \( 2 \) G_{q}, and \( 2 \) G_{s} subunits in G proteins of *C. elegans*. Among the \( 21 \) \( \alpha \) subunits, GSA-1, GOA-1, EGL-30, and GPA-12 are orthologs of the corresponding mammalian G_{a} families G_{a}, G_{i/o}, G_{q} and G_{12}, respectively (2, 3). Together with its fully described shape, position, and connectivity of \( > 300 \) neurons, *C. elegans* provides a unique model to study the cellular and molecular mechanisms of G-protein signaling (4, 5).

Because of its relatively simple genetics, *C. elegans* has been used to develop several models that allowed us to identify, quantify, and analyze many signaling components that regulate neuromuscular behaviors (6–8). In recent years, bacterial photoactivated channels (channel rhodopsin or ChR2; refs. 9–12), ion pumps (halorhodopsin or Halo/NpHR; refs. 13–16), and enzymes (photoactivated adenyl cyclase or PAC; refs. 17, 18) were introduced into *C. elegans* to exert spatiotemporal control over excitation and inhibition of neurons or the onset of intra- and intercellular processes affecting specific behaviors. Approaches that introduced engineered protein chimeras of mammalian Rho and GPCRs (optoXRs; ref. 19), vertebrate rhodopsin (vRh; ref. 20), and a synthetic optogenetic transcription device (21) into mice were also used to control GPCR-mediated physiological processes. These optogenetic tools provide additional convenient experimental means and unparalleled opportunities to dissect cellular and molecular mechanisms regulating such behaviors (for review, see ref. 22). However, some intrinsic properties of native or engineered photoreceptive proteins can also limit their applications. For example, ChR2 and NpHR

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directly depolarize or hyperpolarize host neurons rather than indirectly affecting neuronal activity through other cellular processes. Whether optogenetic studies of PAC-induced increase in cytosolic concentrations of cyclic adenosine monophosphate (cAMP)- and OptoXR-stimulated Gε or Gq signaling apply to endogenous signaling pathways in live host organisms has yet to be shown. Therefore, establishing optogenetic approaches that can directly monitor heterogeneous GPCRs that functionally couple to specific C. elegans G proteins to affect downstream motor behavior would be highly desirable.

The mammalian opsin family members rhodopsin (Rho) and melanopsin (Mo) are photoreceptive GPCRs found in specialized rod cells or photoreceptive ganglion cells (ipGCs), respectively (23). On photoactivation, Rho couples to transducin (GT) for visual signal transduction in vivo (24) and also to Gι/o in vitro (25), both of which belong to the Gι/o subfamily. In contrast, Mo is believed to couple to Gq for signaling that regulates circadian rhythms (23). Although C. elegans avoids lethal exposure to short-wavelength light (26, 27), this soil-inhabiting nematode does not possess vision. There are no known orthologs of Rho, Mo, or Gq in the genome of C. elegans. Because Gι/o and Gq are conserved in the transparent body of C. elegans, it is possible to heterologously express photoreceptors and directly activate endogenous Gι/o and Gq pathways in these live organism, thus identifying G protein signaling pathway components with high spatial and temporal precision.

Here, we expressed bovine (b)opsin and human (h)Mo in the nervous system of C. elegans and used optogenetic tools to directly monitor (b)Rho and (h)Mo coupling to Gι/o and Gq signaling in vivo. We found that exposure to a pulse of low-dose visible light sufficed to trigger a sudden and transient loss of motility in (b)opsin-expressing animals, or initiate increased locomotion of (h)Mo-expressing worms. Both light-mediated motor behaviors depended on added 9-cis-retinal, an active chromophore of Rho, and required the presence of endogenous worm Gι/o and Gq-signaling components.

MATERIALS AND METHODS

C. elegans strains and maintenance

Bristol N2 strain C. elegans worms were used for this study. The loss-of-function mutants goa-1(sa734), egl-8(md1971), egl-30(md186), gsa-1(pk75), gpa-12(pk322), gpa-3(pk35), pde-6(ok3410), tax-4(p678), cng-1(jh111), and cng-3(jh113), the triple mutant cng-1(jh111); cng-3(jh113); tax-4(p678), and the quadruple mutant pde-1(nj57); pde-2(tm3098); pde-3(nj59); pde-5(nj49) were obtained from the Caenorhabditis Genetics Center (CGC; University of Minnesota, Minneapolis, MN, USA). pde-4(nj60) was generated by crossing wild-type (WT) worms with the double-mutant pde-4(nj60); pde-6(ok3410) (provided by Dr. X. Z. Xu, University of Michigan, Ann Arbor, MI, USA). The quadruple mutant cng-1(jh111); cng-3(jh113); tax-2(ok3403); tax-4(p678) was generated by crossing the tax-2(ok3403) mutant with the triple mutant cng-1(jh111); cng-3(jh113); tax-4(p678). Each mutant worm line was crossed 3 times with WT if it had not been reported to be outcrossed extensively by the CGC.

Primers used in mutation screens and the mutated segments were as follows: goa-1(sa734); GCTGCACTACAGTGAGTGA (forward), ACGAATATTTCCGACGC (reverse), with an early stop mutation in aa52; egl-30(md186): CTGGCCGTGACGACTATCT (forward), TGGTTAAACGACGATCATCT (reverse), with a D to E mutation in aa201; gsa-12(pk322): TCTGAAAGACGCTGCGA (forward), GTCTGTCCGAAATCACTGTA (reverse), with a deletion spanning exon 2–8; gpa-3(pk35): CCGATTCTGTCCGAAGAGGC (forward), CATTCCGACTGTTGGAAC (reverse), with a deletion spanning exon 2–6 and a transposon insertion, T1c: pde-4(nj60): GGATATCGTGGCAGTCTTGAG (forward), ACTTACGAGTCTTTGGA (reverse), with a deletion of exon 4 (isofrom a) causing a frameshift; tax-2(ok3403): CAACTTATACGACCACCG (forward), GCTGCTGAAAGAGG (forward), GAGTCCGAGCATTCTTTGAAA (reverse), with a deletion of ~400 bp; pde-4(p678): TGCACTACGAGGCACGTAG (forward), GTAACACTACAGCTTGAC (forward), with an amber stop mutation in aa82; cng-1(jh111): TCCGCTTGAGCACTGGAAT (forward), GAGTCTACAAGAGGGAGCATACA (reverse), with a deletion spanning exon 8–13; cng-3(jh113): CAGTGACACTTTGGC (forward), TTACCAGGAAATGACCG (reverse), with a deletion spanning exon 4–8; gsa-1(pk75): TCTCAGTCTTGCGGAAAGCACA (forward), GGAGAATGACTGCTGTTTTT (reverse), with a transposon-induced deletion of exon 7 causing a frameshift; The egl-8(md1971) mutant expressing (h)Mo in neurons was obtained by screening for the specific egl-8 phenotype.

Worms were maintained by standard methods that included culture on nematode growth medium (NGM; 0.25% peptone, 51 mM NaCl, 25 mM K3PO4,5.1 mM KH2PO4, 1 mM MgCl2, and 1 mM CaCl2) with OP50 bacteria, cryostorage, and recovery from stocks. Compositions of media and solutions, as well as detailed protocols for their use, were previously described (28).

GPCR gene constructs and generation of transgenic (TG) worm lines

For GPCR expression constructs, the promoter of myo-3 (29) or H20 (30) was inserted into a pBluscript KS(+) vector at HindIII/XbaI or PstI, respectively. Synthesized (Genescr ipt USA, Piscataway, NJ, USA) codon-optimized (31) cDNAs encoding (b)opsin and (h)Mo or unoptimized cDNA encoding (h)5HT4Rb were engineered between NosI and XbaI. A Rho tag (32) was linked to the C-terminal of (h)Mo and (h)5HT4Rb. The ptx gene (provided by Dr. X. Z. Xu, University of Michigan, ref. 33) was subcloned into a Pptx construct with BanHI/NodI. The entire GPCR fusion protein coding region of each construct was sequenced to confirm the presence of the GPCR and the absence of random mutations. Sequence references for GPCRs are as follows: (b)opsin, NP_001014890; (h)Mo, NP_150598.1; and 5HT4Rb, NP_000861.

TG worm lines transiently expressing GPCRs and selected by DsRed visualization were generated by injecting GPCR constructs (10 ng/ml) and DNA encoding coral-derived red fluorescent protein DsRed (3 ng/ml) under control of the same promoter (either Pmyo-3 or P H20). The ptx gene was coexpressed with green fluorescent protein driven by the same
promoter (P<sub>H20</sub>). To integrate GPCR cDNA into the worm genome, identified TG worm lines were exposed to 350 × 100 μl/cm² ultraviolet light (Spectrolinker XL-1500; Spectronics Corp., Westbury, NY, USA) and F3 progeny of integrated TG lines were then screened and backcrossed to WT worms 3 times.

**In vivo light-response assays**

One day before these experiments, larval stage 4 (L4) animals raised at 20 °C were transferred onto NGM plates seeded with 100 μl OP50 bacteria culture containing either DMSO vehicle control (no retinal), 10 μM 9-cis-retinal, or 10 μM all-trans-retinal (Toronto Research Chemicals, Toronto, ON, Canada). The resulting plates were wrapped with aluminum foil and stored in a cardboard box overnight at 20 °C. Light-response experiments were carried out at 22 °C in a dark room by using a Zeiss Stemi SV11-Apo microscope (Carl Zeiss, Oberkochen, Germany) mounted with a Kramer Universal Stereo Fluorescence Attachment and Cubes (USFAC) unit (Kramer Scientific, Amesbury, MA, USA), an Andor iXon camera (Andor, South Windsor, CT, USA), and a ProScan II H117 motorized stage (Prior Scientific, Rockland, MA, USA). A ×1.6 objective lens in combination with a ×2.5 magnifying lens and 7 lux of transmitted white light was used for (b)opsin-expressing animals, or 5 lux of such light for (h)Mo-expressing animals, at the above hardware settings to visualize and track worms during experiments. For each light-response assay, a d 1 worm with an embedded platinum wire (an L4 worm raised overnight, becomes a young adult) was transferred onto an unseeded NGM plate (tracking plate); worms crawled vigorously under these conditions (34).

To measure the motor response to light of worms expressing (b)opsin in neurons, 1000 lux blue light (488±20 nm) was delivered after ~5 s of control imaging to animals from a metal halide short arc bulb housed in an EXFO X-Cite 120PC-Q unit (Lumen Dynamics, Mississauga, ON, Canada) through a Kramer USFAC for 1 s, and animals were continuously imaged after another 6 min. To measure the light response of thrashing worms, 2 μl dH₂O was used to immerse animals, a procedure that initiated thrashing within 1 s (35). To measure the light response during reversals of direction, an anterior nose touch with a platinum wire was applied to trigger this response (36), if a spontaneous reversal did not occur. Before measuring the light response of worms expressing (h)Mo, animals on tracking plates (seeded with OP50 bacteria with or without 9-cis-retinal) were kept in the dark for 2 h. Worms then were imaged for 1 min, exposed to blue light (1000 lux, 488±20 nm) for 15 s, and then tracked for another 5 min. To measure the light response of egl-30 mutant worms expressing control, (b)opsin, or (h)Mo, such worms were pretreated with 2.5 μM PMA (LC Laboratories, Woburn, MA, USA) for 2 h before tracking. Worm locomotion before and after illumination was recorded in AVI movies at 30 Hz with a self-developed software package to capture images, control the onset and duration of illumination, and integrate this information. Light intensity output of the EXFO unit was calibrated to reach a targeted intensity (~5%) at the microscopic field, measured with a Macam L203 Photometer (MacamPhotometrics, Livingston, UK). Worm locomotion velocities were computed by a previously published algorithm (37). To score the degree of motor activity loss, images were analyzed frame by frame. A light-response index was defined and used as follows: 5 = complete lack of motion; 4 = complete lack of motion; 3 = lack of motion for head shaking; 2 = lack of motion ≤2 s; 1 = changed locomotion speed or direction; and 0 = no change noted in motor activity.

**Spectrum of isoRho and G<sub>t</sub> coupling assays**

Reconstitution and purification of isoRho are described in the accompanying article (38). Purified recombiant (b)isoRho was scanned in a quartz cuvette with a Varian Cary 50 Bio UV-vis spectrophotometer (Varian, Santa Clara, CA, USA). The function of (b)isoRho purified from bovine retinas or worms was evaluated by a G<sub>t</sub> activation fluorescence assay. At respective concentrations of 250 and 25 nM, the molar ratio of G<sub>t</sub> to Rho was 10:1. Protein samples were diluted in 20 mM bis-tris-propane (BTP) buffer (pH 7.0), 120 mM NaCl, 2 mM MgCl<sub>2</sub>, and 1 mM α-dodecyl-β-D-maltoside (DDM), and then exposed to light for 15 s with a fiber light covered with a bandpass wavelength filter (480–520 nm). Reactions were carried out at 20 °C in a continuously stirred cuvette. After 300 s of incubation, 5 μM GTPγS was added. Pseudo-first order kinetic rates (~k) were derived from the function A(t) = A<sub>max</sub>(1 − exp(−kt)), where A<sub>max</sub> is the maximal G<sub>t</sub> fluorescence change, and A(t) is the relative fluorescence change at time t. The intrinsic fluorescence increase emanating from G<sub>t</sub> was measured with a Perkin Elmer L55 luminescence spectrophotometer (Perkin Elmer, Wellesley, MA, USA), by using excitation and emission wavelengths of 300 and 345 nm, respectively (39-41). No changes in tryptophan fluorescence were detected in control experiments without GTPγS.

**Immunohistochemistry (IHC)**

Age-synchronized d 1 or L4 animals from TG worm lines were sandwiched between two cover glasses, buried in dry ice for 30 min, and then fixed with 100% methanol (10 min), followed by 100% acetone (10 min). Worms then were washed with PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>HPO<sub>4</sub>, and 1.76 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4) for 0.5 h and incubated overnight at 4 °C with PBS containing Alexa-488-conjugated anti-Rho 1D4 antibody and 0.1% Triton X-100. Stained worms were subsequently washed 3 times with PBS before examination by confocal microscopy. All experiments were done with a Leica TCS SP2 confocal microscope (Leica Microsystems, Bannockburn, IL, USA). Either live worms immobilized with 10 mM NaN<sub>3</sub> on 2% agarose pads or methanol/acetone-fixed worms were used. Stains employed were DsRed (λ<sub>ex</sub>=543 nm; λ<sub>em</sub>=580–630 nm) and Alexa-488 (λ<sub>ex</sub>=488 nm; λ<sub>em</sub>=510–530 nm).

**Statistical analyses**

Statistical significance was analyzed with Statistica software (StatSoft, Tulsa, OK, USA), using t tests and ANOVA with Bonferroni corrections or Dunnet’s post hoc analyses, as indicated in the figure legends.

**RESULTS**

Light induces a 9-cis-retinal-dependent sudden and transient loss of motility in C. elegans expressing (b)opsin in neurons

Because Rho and Mo are known to activate G<sub>i/o</sub> and G<sub>q</sub> in vitro, we introduced these photoreceptors into the nervous system of C. elegans, to determine whether worm G<sub>i/o</sub> and G<sub>q</sub> can couple to these ectopically expressed GPCRs in vitro. To detect directly any light-induced behavioral response of these GPCR TG ani-
mals, we modified the automated quantitative analysis of behavior of nematode (AQUABN) system, which simultaneously quantifies many aspects of worm locomotion (6–8), to facilitate delivery of light at a specific wavelength, intensity, and duration, and record worm motor activity under extremely dim illumination (Fig. 1).

TG worms expressing (b)opsin in neurons ([N] (b)opsin) were used initially to examine worm locomotion behavior. As described in the accompanying article (38), expression of (b)opsin was confirmed by IHC and immunoblots of worm cell lysates. Similar to WT animals, when these worms were touched with a platinum wire applied to their anterior or posterior, they responded with a vigorous reversal of direction (anterior) or increased forward locomotion (posterior), respectively (36). We then carried out a behavioral analysis to determine whether these TG worms exhibit any in vivo behavioral phenotype in response to light. Blue light (488 ± 20 nm) was chosen as the stimulus because (b)Rho (11-cis-retinal-bound opsin) and (b)isoRho (9-cis-retinal-bound opsin) have a maximum absorbance at ~500 nm and 485 nm, respectively (42). Moreover, it had been reported that exposure to low-energy 1000-lux blue light (~1.5 × 10^-3 mW/mm^2) per se did not modify normal worm behavior (26, 27). Therefore, any behavioral change of TG worms in response to this stimulus should result from their expression of (b)opsin.

Before reaching the young adult d 1 stage, TG worms expressing (b)opsin in neurons were preincubated overnight in the dark with either 10 μM 9-cis-retinal (an active chromophore for opsin), 10 μM all-trans-retinal (the photoactivated isomerized product) or vehicle control (no retinal). Worms then were transferred to new NGM tracking plates for examination. Similar to WT animals that exhibit an initial locomotion acclimation period after changing conditions (7), these worms moved rapidly on transfer to the tracking plates and then slowed down. Strikingly, immediately after exposure to 1000 lux blue light for 1 s, fast-moving worms preincubated with 9-cis-retinal instantly were immobilized at their last body position on the plates and remained stationary for 20 min (Supplemental Movie S1A). This light-induced complete lack of motor activity and slow recovery was observed even when worms were either moving rapidly after stimulation by harsh touch before light exposure, crawling forward or backward, or changing direction.

To quantify this behavioral phenotype, we used our response index to quantify the degree of lost motor function (Fig. 2A). If an animal completely ceased moving and held its posture for >10 s after light exposure, it was scored as a 5. If it stopped moving for >10 s except for wiggling its head, it was ranked as a 4. An animal that did not move for >2 but ≤10 s was given a 3, and a brief pause ≤2 s was scored as a 2. An animal that exhibited a change in motor behavior (speed or direction) but did not cease moving within 2 s after light exposure was rated as 1. Animals with no detectable motility change within 2 s were scored as 0. Compared to those with 9-cis-retinal pretreatment, TG worms expressing (b)opsin in neurons preincubated with control vehicle (no retinal) did not respond to blue light exposure (Fig. 2B and Supplemental Movie S1B). Moreover, worms from the same line preincubated with all-trans-retinal or those expressing (b)opsin in muscles preincubated with 9-cis-retinal failed to cease locomotion after light exposure (Fig. 2B). These results indicate that the sudden loss of motility in worms expressing (b)opsin was dependent on preincubation with 9-cis-retinal and specific to neurons. Moreover, worms expressing human serotonin receptor subtype 4 in neurons ([N] (h)5HT4R) preincubated with 9-cis-retinal did not display any obvious motor response under the same experimental conditions (Fig. 2B and Supplemental Movie S1C). Expression of (h)5HT4R in neurons was confirmed by immunoblotting of worm cell lysates (see accompanying article; ref. 38). This result further indicates that the sudden loss of worm motility was specifically due to the expression of (b)opsin rather than other GPCRs in neurons.

To determine the relationship between the degree of light exposure and the loss of mobility, TG worms expressing (b)opsin in neurons preincubated with 9-cis-

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**Figure 1.** Diagram of the modified AQUABN system used to quantify the motor behavior of heterologous (b)Rho- and (h)Mo-expressing *C. elegans*. Light-response experiments were done with a Zeiss Stemi SV11-Apo fluorescent dissecting microscope mounted with a Kramer USFAC, an Andor iXon DV897 EMCCD camera, and a ProScan II H117 motorized stage. A ×1.6 objective lens combined with ×2.5 magnification lens, and 5 or 7 lux of transmitted white light was used with the listed hardware to visualize and track worms for motor functional analyses. Illumination of selected intensity and wavelength was delivered from a metal halide short-arc bulb housed in an EXFO X-Cite 120PC-Q unit through the Kramer USFAC. Worm movements before and after illumination were recorded as AVI movies at 30 Hz with a self-developed software package used to capture images, control the onset and duration of illumination, and integrate this information. The camera and stage were connected with peripheral component interconnect (PCI) cards located in the computer, and the light source was connected via a recommended standard (RS) 232 port.
retinal were exposed to blue light with illuminance ranging from 10 to 1000 lux for 1 s, and their motor responses were scored. As shown in Supplemental Fig. S1A, the loss of motility of these worms positively correlated with increasing blue light intensity, such that intensities approaching 1000 lux caused complete cessation of movement. In addition, preincubation with 10 μM 9-cis-retinal for a minimum of 40–80 min was sufficient to produce this maximal response to 1000 lux blue light (Supplemental Fig. S1B, C). These studies further support the relationship between the light-induced behavior of TG worms and the photoactivation of (b)isoRho, thereby establishing an experimental paradigm for subsequent mechanistic studies.

*Caenorhabditis elegans* exhibits two fundamentally different types of motor activity: crawling on a solid surface with an S-shaped movement pattern (slow muscle activity) and thrashing (swimming) in liquid medium with a C-shaped movement (fast muscle activity) (35). The underlying mechanisms controlling these two types of muscle activity are unknown. We found that blue light (1000 lux, 488±20 nm, 1 s) also effectively triggered an instant cessation of thrashing in TG worms expressing (b)opsin in neurons after their preincubation with 9-cis-retinal, but not with the vehicle control (no retinal; Fig. 2C and Supplemental Movie S1D, E). These data indicate that the sudden loss of motility exhibited by these TG worms is independent of their initial type of motor activity.

Both contracted or relaxed muscles can lead to apparent loss of muscle activity but by distinctly different underlying physiological and molecular mechanisms. Crawling worms expressing (b)opsin in neurons and preincubated with 9-cis-retinal promptly ceased motor activity upon light exposure and maintained their S-shaped body posture on the solid agar support (Fig. 2D, left panel). Whether this behavior resulted from muscle rigidity or relaxation was unclear. However, worms in liquid medium that promptly lost their thrashing motor behavior on light exposure gradually straightened and lengthened (Fig. 2D, right panel, and Supplemental Movie S1D). The latter behavior indicates that light-induced loss of motility of these TG worms in liquid medium resulted from muscle relaxation.

To learn whether these light-exposed inactive TG worms were seriously injured or even dead, we continuously observed completely inactive TG worms after 1 s blue light exposure every 10 min for total of 80 min under 7 lux transmitted white light. These animals started to shake their heads (scored as 1 by the recovery index) within 20 min, then wiggled their bodies (scored as 2), evidenced noticeable movement (scored as 3), exhibited uncoordinated locomotion (scored as 4), and finally, displayed normal locomotion (scored as 5) by 80 min (Fig. 2E, F, and Supplemental Movie S1A). Fully recovered worms also exhibited a touch response similar to WT animals, and no obvious behavioral,
developmental, or reproductive defect was noted for the rest of their lives. Therefore, the light-induced sudden loss of motility in these TG worms was transient. Taken together, worms expressing (b)opsin in neurons, when preincubated with 9-cis-retinal, exhibited a striking cessation of motility when exposed to 1000 lux blue light. This sudden loss of motility was transient and independent of the initial type of motor activity.

**Characterization of (b)isoRho in vivo and in vitro**

The above rapid motor response of 9-cis-retinal-pre-treated TG worms expressing (b)opsin in neurons to blue light suggested that functional (b)isoRho was present in worm neurons. This encouraged us to characterize [N] (b)isoRho further by comparing its biochemical properties with those of (b)isoRho from its native source. To determine the wavelength that triggered the maximum light response, TG worms expressing (b)opsin in neurons and preincubated with 9-cis-retinal were exposed to 250 lux light at wavelengths ranging from 300–700 nm for 1 s, and their neuromotor responses were scored. The light-induced motility defect displayed a bell-shaped curve with a maximum at a wavelength of ~490 nm, matching the absorbance spectrum of (b)isoRho purified from bovine retina (Fig. 3A).

In rod cells, 11-cis-retinal binds to opsin to form ground-state Rho in the dark (24). Absorption of a photon of light by Rho causes photoisomerization of 11-cis-retinal to all-trans-retinal, resulting in a major conformational change of the photopigment that leads to activation of G, within seconds and release of all-trans-retinal in minutes (43, 44). Within hours, the resulting apo-(b)opsin binds 11-cis-retinal produced by the retinoid cycle, regenerating Rho (24). To further test whether worm [N] (b)isoRho can be regenerated after light exposure, TG worms expressing (b)opsin in neurons and preincubated with 9-cis-retinal were exposed to blue light (1000 lux, 488±20 nm) for up to 10 s and then transferred to freshly seeded NGM plates without a retinal supplement for 2 h, to allow complete recovery of locomotor function. These recovered TG worms were next exposed to blue light again for 1 s, and their motor responses were scored. As shown in Fig. 3B, this light-response index displayed an inverse relationship to their period of previous exposure to blue light. That is, a 10 s previous exposure to light completely abolished a locomotion response to the second brief light stimulus. This finding indicates that once a TG worm takes up 9-cis-retinal and converts it to all-trans-retinal in response to light, it is unable to convert this retinoid back into the photosensitive cis form under these experimental conditions. Consistent with this observation, if worms recovered from the first light exposure in plates that contained 9-cis-retinal, but not all-trans-retinal, they did respond to the second light stimulus (Fig. 3C). These findings further confirm that (b)opsin expressed in worms is fully functional in vivo.

As described in the accompanying article (38), [N] (b)isoRho can be purified from TG worms. To examine whether [N] (b)isoRho is functional in vitro, the absorption spectrum of purified worm recombinant [N] (b)isoRho was determined and found to display its expected ~485-nm maximum absorbance (Fig. 3D). Because purified (b)isoRho from bovine retina can activate G, (45), a fluorescence-based assay was used to

**Figure 3. In vivo and in vitro functional analysis of recombinant (b)isoRho.** A) Vigorously crawling TG animals expressing (b)opsin in neurons and preincubated with 10 μM 9-cis-retinal were exposed to light (250 lux) at indicated wavelengths, and their motor responses were recorded and scored (y axis). Data were derived from 3 independent experiments with 5–10 animals each. B) TG animals expressing (b)opsin in neurons and preincubated with 10 μM 9-cis-retinal were exposed to 250 lux light at wavelengths ranging from 300–700 nm for 1 s, and their neuromotor responses were scored. Data were derived from 3 independent experiments with 5–10 animals each. C) TG animals expressing (b)opsin in neurons and preincubated with 10 μM 9-cis-retinal were exposed to blue light (1000 lux, 488±20 nm) for 1 s, and their light responses were recorded as shown. Data were derived from 3 independent experiments with 5–8 animals each. D) TG animals expressing (b)opsin in neurons and preincubated with 10 μM 9-cis-retinal were exposed to blue light (1000 lux, 488±20 nm) for 10 s. Motionless animals then were transferred to a OP50 bacteria seeded NGM plate containing 10 μM 9-cis-retinal, 10 μM all-trans-retinal, or no retinal to recover for 2 h. Recovered animals were tested for their responses to blue light (1000 lux, 488±20 nm, 1 s). Data were derived from 3 independent experiments with 5–10 animals each. D) Absorbance spectrum of recombinant (b)isoRho purified from TG worms expressing (b)opsin in neurons ([N] (b)isoRho). E) Fluorescence assay of G, activation by (b)isoRho and [N] (b)isoRho purified from bovine retina or from TG worms expressing (b)opsin in neurons, respectively. Fluorescence change was normalized for both traces. Error bars indicate means ± se. ***P < 0.001; 1-way ANOVA with Bonferroni correction.
monitor guanyl-nucleotide exchange in the α subunit of G, that occurs on its activation. As shown in Fig. 3E, recombinant [N] (b)isoRho purified from worms did activate G. Moreover, the rates of G activation promoted by reconstituted/purified (b)isoRho from bovine retina and recombinant [N] (b)isoRho from worms were similar, \( k = 2.64 \pm 0.08 \times 10^{-3} \cdot s^{-1} \) and \( 2.07 \pm 0.06 \times 10^{-3} \cdot s^{-1} \), respectively. These observations strongly indicate that the (b)isoRho expressed in worm neurons was functional in vivo and in vitro.

Photoactivated (b)isoRho activates G\(_{i/o}\) in vivo

*Caenorhabditis elegans* contains only one known ortholog of the mammalian G\(_{i/o}\) α subunit, GOA-1 (NP_492108), which shares an 83% identical amino acid sequence with both the a and b isoforms of human G\(_o\) subunit α (GNAO1, NP_066268.1, and NP_620073.2, respectively). Thus, we hypothesized that expressed (b)isoRho would activate endogenous GOA-1, leading to a sudden and transient loss of worm motility.

To test this idea, we first determined whether blocking GOA-1 function would inhibit the light-induced motility response. cDNA encoding pertussis toxin (PTX), a peptide that selectively inhibits G\(_{i/o}\) function (33, 46), was introduced into the nervous system of TG worms expressing (b)opsin. PTX incorporation then activate endogenous GOA-1, leading to a sudden and transient loss of worm motility.

To further examine whether this signaling occurred specifically through G\(_{i/o}\), the [N] (b)opsin TG worm line was crossed with loss-of-function mutants of goa-1, egl-30 (47, 48), gpa-12 (49, 50), and gsa-1 (51), with gene products that encode the worm α subunits of G\(_o\), G\(_q\), G\(_{12}\), and G\(_s\), respectively. Because the homozygous gsa-1 loss-of-function mutant is a larval lethal mutant, a heterozygous (gsa-1\(^+/−\)) worm was produced instead. The resulting [N] (b)opsin-expressing-heterozygous (gsa-1) and homozygous (goa-1, egl-30, and gpa-12) worms were then tested for their responses to light. Because worms bearing the loss-of-function egl-30 mutation were lethargic, they were supplemented with phorbol 12-myristate 13-acetate (PMA), an activator of G\(_q\) downstream protein kinase C (PKC), 2 h before tracking (52) to promote WT- or near WT-locomotive behavior. As expected, [N] (b)opsin-expressing-goa-1 mutant worms did not respond to the light stimulus, indicating that disruption of G\(_{i/o}\) function inhibited (b)isoRho signaling (Fig. 4B and Supplemental Movie S1). In contrast, light induced a marked and robust loss of motility in [N] (b)opsin-expressing egl-30, gsa-1, and gpa-12 mutant animals, comparable to that seen in (b)opsin-expressing WT worms (Fig. 4B). This observation supports the idea that G\(_{i/o}\) signaling was specifically activated. Additional experiments revealed that worms expressing (b)opsin in loss-of-function mutant gpa-3, whose gene product encodes a weak mammalian G\(_{i/o}\) α subunit homologue that mediates the response to lethal doses of short-wavelength light (33), responded to light similarly to WT animals (Fig. 4B). The above pharmacological and genetic evidence indicates that photoactivation of (b)isoRho in neurons specifically activates worm G\(_{i/o}\), and not other G proteins.

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*Figure 4.* Light induces activation of G\(_{i/o}\) and cAMP-specific PDEs in TG worms expressing (b)opsin in neurons. A) TG animals ([N] (b)opsin) and control vector (−) were pretreated with 10 \( \mu \)M 9-cis-retinal and tested for their motor response to blue light (1 s, 1000 lux, 488±20 nm). Data were derived from 3 independent experiments with 10 animals each. ***\( p < 0.001; \) t test. B) TG animals ([N] (b)opsin) were pretreated with 10 \( \mu \)M 9-cis-retinal and tested for their response to light. Because worms bearing the loss-of-function egl-30 mutation were lethargic, they were supplemented with phorbol 12-myristate 13-acetate (PMA), an activator of G\(_q\) downstream protein kinase C (PKC), 2 h before tracking (52) to promote WT- or near WT-locomotive behavior. As expected, [N] (b)opsin-expressing-goa-1 mutant worms did not respond to the light stimulus, indicating that disruption of G\(_{i/o}\) function inhibited (b)isoRho signaling (Fig. 4B and Supplemental Movie S1). C) TG animals with transient pan-neuronal expression of (b)opsin in WT. ***\( p < 0.001; \) 1-way ANOVA with Dunnett’s correction.

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cAMP and cyclic guanosine monophosphate (cGMP) are important second messengers that enable downstream G-protein signaling. Levels of cAMP and cGMP are regulated by phosphodiesterases (PDEs) with activities that are, in turn, regulated by G proteins. In photoreceptor cells, Rho activates G, which then stimulates PDEs to cleave cGMP, thereby causing decreased levels of cGMP that then lead to closure of cyclic nucleotide-gated (CNG) channels (53).

The pathway G employed for downstream signaling in [N] (b)opsin expressing TG worms had not been identified. In C. elegans, there are 4 cGMP-specific PDEs (PDE-1, PDE-2, PDE-3, and PDE-5) and 2 cAMP-specific PDEs (PDE-4 and PDE-6), all of which share high identity with human PDEs (33, 54). To examine which pathway is responsible for G-mediated signaling in these TG worms, a quadruple (pde-1; pde-2; pde-3; pde-5) loss-of-function mutant, a double (pde-4; pde-6) loss-of-function mutant, and a pde-6 loss-of-function mutant worm line, each expressing (b)opsin in its nervous system, were generated. This was accomplished by injecting the (b)opsin DNA construct into double and quadruple mutants, or by genetic crossing the single mutants with the neuronal (b)opsin expressing [N] (b)opsin loss-of-function mutant, and a (b)opsin expressing quadruple mutant (circles; A, B) or with loss-of-function mutants tax-2 (diamonds; A), tax-4 (squares; A), cng-1 (diamonds; B) or cng-3 (squares; B) expressed in neurons were preincubated with 9-cis-retinal and tested for their responses to blue light (1 s, 1000 lux, 488 ± 20 nm). Data were derived from 3 independent experiments with 5–11 animals each. WT worms and quadruple loss-of-function mutant of cng-1; cng-3; tax-2; tax-4 worms, both with pan-neuronal expression of (b)opsin, were tested for their response to blue light (1 s, 1000 lux, 488 ± 20 nm). Data represent 3 independent experiments with 5–10 animals each. Error bars indicate means ± se. ***P < 0.001; 2-way ANOVA.

Enhanced light-induced locomotion is mediated by G in worms expressing (h)Mo in neurons

By simply monitoring this on and off phenotypic motility switch, we were able to identify heterotrimetric G proteins in live worms that directly coupled to (b)isoRho activated by light. In addition, this protocol allowed us to dissect the involved G downstream signaling pathways in vivo. To examine whether the same optogenetic tool was sufficiently sensitive to carry out functional studies of other heterologously expressed GPCRs, we then tested the light-induced responses of TG worms expressing (h)Mo, a GPCR in the opsin family believed to couple to Gq. Similar to (b)opsin, (h)Mo was expressed in the C. elegans nervous system under control of P and in muscles under control of P (Fig. 6A, B). TG worms expressing (h)Mo in neurons were slightly hyperactive in locomotion, whereas TG worms expressing (h)Mo in muscles did not display a phenotype obviously different from WT controls. To determine the light-induced response, L4 TG larvae expressing (h)Mo in neurons were preincubated with either 10 μM 9-cis-retinal, 10 μM all-trans-retinal, or control vehicle (no retinal) overnight in the dark and then transferred to a new NGM tracking plate.
Unlike worms expressing (b)opsin in neurons that ceased motor activity in response to blue light, TG worms expressing (h)Mo in neurons and preincubated with 9-cis-retinal failed to show any significant change in their rapid locomotion on light exposure. However, if these TG worms were incubated on the tracking plates seeded with OP50 bacteria for 2 h in dark before light stimulation, most worms slowed their motor activity during this period. On stimulation by blue light (1000 lux, 488 ± 20 nm), locomotion of worms preincubated with 10 μM 9-cis-retinal but not control vehicle (no retinal), was enhanced (Fig. 6D, E) compared to [N] (h)Mo worms. [M] (h)Mo and [N] (h)5HT4R worms preincubated with 9-cis-retinal did not respond to the light stimulus under these experimental conditions (Fig. 6D, E). Locomotion ratios of WT and loss-of-function egl-30, egl-8, and goa-1 mutant animals expressing Mo in neurons and preincubated with 10 μM 9-cis-retinal. Data were derived from 3 independent experiments with 4–6 animals each. Error bars indicate means ± se. **P < 0.01, ***P < 0.001; t test.

**DISCUSSION**

In this study, (b)opsin and (h)Mo were expressed in the nervous system of C. elegans, and activation of these photoreceptors led to their coupling to G_\text{\(i/o\)} and G_q signaling pathways, respectively, with temporal precision. Activation of G_\text{\(i/o\)} signaling in worm neurons resulted in a sudden but transient loss of motility of rapidly moving TG animals, whereas activation of G_q signaling in neurons enhanced locomotion.
Optogenetic control of G-protein signaling pathways

Our optogenetic tools combined with behavioral, genetic, and biophysiological evidence demonstrated conservation of photochemical and signaling mechanisms in *C. elegans*. For example, the so-called Rho cycle of visual transduction could be detected in [N] opsin-expressing worms by analysis of their motor function *in vivo* and by the absorbance spectrum and G protein coupling experiments with recombinant (h)isoRho purified from these worms *in vitro*. In [N] (h)Mo-expressing TG worms, 9-cis-retinal was also observed to be required for robust Mo-mediated initiation of locomotion. This finding is consistent with a recent report that Mo obtained from murine ipGGs in the dark state bound to 11-cis-retinal, which was converted into all-trans-retinal after light exposure. The functional output induced by endogenous Gi/o or Gq signaling in host animals was tightly associated with precise coupling of the G protein with the ectopically expressed photoreceptor. Thus, we conclude that photoreceptive Rho and Mo can be used to elicit specific Gi/o or Gq signaling pathways in *C. elegans*.

More than 800 human GPCRs are involved in many key biological processes. However, humans only have 16 distinct Ga genes encoding 23 different isoforms that belong to 4 major classes of heterotrimeric G proteins, Gi/o, Gq, G12, and G13. A common GPCR-activated mechanism is shared by all heterotrimeric G proteins, although each Ga protein engages distinct downstream effectors. Recent studies indicate that the exact downstream effectors and the physiological outputs of the same G-protein signaling pathway can vary, depending on the physiological properties of the cells where G-protein signals are elicited, as well as the cellular/signaling connectivity of these cells. Identification of downstream effectors and the consequent physiological output of a specific Ga signal in a given context is critical for understanding how a variety of GPCRs regulate distinct biological processes through a limited number of heterotrimeric G proteins. *C. elegans* uses >1000 GPCRs to sense a variety of environmental stimuli, mediate synaptic function, reshape neural circuits and modulate muscle activity. Similar to mammals, worms have only 21 Ga proteins that presumably couple to all these GPCRs. Although worms with loss-of-function or gain-of-function mutations in genes encoding components of Gi/o and Gq pathways provide useful models to study signaling by these highly conserved G proteins, transient and specific activation of their pathways with spatiotemporal precision provides an unparalleled approach to dissect the mechanisms of GPCR and G protein signaling at both cellular and molecular levels. Our optogenetic tools present just such an opportunity. In the future, we will express mammalian opsins in specific *C. elegans* cells to manipulate neuromuscular activity. Combined with classical genetics and electrophysiological methods, this approach will greatly facilitate understanding of G-protein signaling mechanisms.

Here we found that exogenous 9-cis-retinal, but not all-trans-retinal, is required for a full light-mediated neuromuscular response in both [N] (h)opsin and [N] (h)Mo-expressing TG worms. Therefore, it appears unlikely that worms contain visual cycle components that convert all-trans-retinal to a retinal with a cis double bond. But unlike Rho, the photochemistry of Mo, an important retinylidene GPCR that regulates circadian rhythms, the pupillary light reflex and other nonvisual responses to light, is poorly understood. Our [N] Mo-expressing TG worms and neuromotor quantification paradigm provide an *in vivo* experimental means to investigate certain photochemical aspects of [N] Mo signaling. For example, this model could be used to test the hypothesis that Mo is a bistable photopigment with intrinsic photoisomerase activity that uses light to convert trans-cis and cis-trans isomers of the chromophore. Partial activity of [N] (h)Mo-expressing TG animals treated with all-trans-retinal could indicate that (h)Mo is a bistable photopigment. Recombinant (h)Mo expressed in *C. elegans* could be purified to test this possibility as well as to pursue other biophysical studies (see accompanying article; ref. 38).

Notably, the onset of Gi/o and downstream cAMP signaling in worm neurons resulted in a dramatic, sudden, and transient loss of motility. This on-and-off behavior was independent of the previous type of motor activity. More studies are needed to characterize further the molecular and cellular mechanisms underlying this intriguing phenotype. Genetic and pharmacological screens will also be performed to identify downstream Gi/o signaling components that regulate this motor activity along with small molecules that affect it.

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