Evidence for light perception in a bioluminescent organ

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Here we show that bioluminescent organs of the squid *Euprymna scolopes* possess the molecular, biochemical, and physiological capability for light detection. Transcriptome analyses revealed expression of genes encoding key visual transduction proteins in light-organ tissues, including the same isoform of opsin that occurs in the retina. Electroretinograms demonstrated that the organ responds physiologically to light, and immunocytochemistry experiments localized multiple proteins of visual transduction cascades to tissues housing light-producing bacterial symbionts. These data provide evidence that the light-organ tissues harboring the symbionts serve as extraocular photoreceptors, with the potential to perceive directly the bioluminescence produced by their bacterial partners.

Euprymna | evolutionary tinkering | extraocular photoreceptor | visual transduction

Extraocular photoreceptors are widespread across the animal kingdom. Opsin proteins typically mediate the associated phototransduction, although often through isoforms distinct from those produced in the retina (1–3). The complexity of such photoreceptors can vary from diffusely distributed photoreceptive cells, characteristic of dermal light sense, to complex organs in discrete locations on an animal's body (e.g., see ref. 4). Examples of the latter type are the elaborate photoreceptive vesicles (PSVs) (5) and nuchal organs (6) that occur in a wide array of cephalopod species. Although in most cases their functions remain unknown, the PSVs in certain bioluminescent squid species have been implicated in the perception and control of light emission, particularly in counterillumination (e.g., see refs. 7–9), a behavior in which the animal matches down-welling environmental light with ventrally emitted luminescence. The PSVs are not components of the light organs themselves but instead are some distance away and are thought to provide a feedback mechanism for the light-emitting tissues. Thus far, light organs themselves have not been reported to contain photoreceptive tissues. The studies presented here provide evidence that the counterilluminating squid *Euprymna scolopes* has additional photoreceptive tissue that occurs as an integral component of the host's bacterial light organ.

The light organ of *E. scolopes* has been studied for the past 20 years as a system for the analysis of tissues that interact with light and as a natural model of symbiosis (10, 11). *E. scolopes* houses populations of the luminous bacterial symbiont *Vibrio fischeri* in epithelium-lined crypts in the core of a bi-lobed light organ that is embedded in the animal's ink sac (12) (Fig. 1). This core has a set of surrounding tissues that modulate the intensity and direction of symbiont light emission (13, 14). The position and function of these tissues are analogous to the position and function of the tissues that modify light coming into the squid eye (Fig. 1). Specifically, similar to the choroid, tapetum, and iris of the eye, which surround the retina, the diverticula of the ink sac and a layer of reflective tissue envelop the bacteria-containing core of the light organ. Superficially, these tissues are covered by a thick transparent tissue, or "lens." These accessory tissues have been thought to function exclusively for the control of the intensity and direction of light output from the organ, with no role in light perception.

Our previous studies of the anatomy and biochemistry of the light-organ lens and reflector demonstrated dramatic biochemical convergences with those of eyes (13, 14). Similar to an eye lens, the "lens" of the *E. scolopes* light organ expresses a few proteins in very high concentration. The principal light-organ protein of this tissue is aldehyde dehydrogenase, 1 of 2 major proteins used by the squid eye lens to achieve high refractive index (14). The tissues surrounding the eye and those dorsal to the symbiont-containing crypts share the expression of a family of proteins, the reflectins (13). These proteins occur in stacks of platelets in arrangements that render the tissues reflective.

The morphology of the light organ, as well as behavioral studies, have suggested that the animal uses the light in counterillumination (15). A squid host lacking luminous symbionts is affected not only in its behavior but also in other features of the symbiosis. Studies of colonization with mutant *V. fischeri* strains defective in light emission (*lux* mutants) have demonstrated that symbiont luminescence somehow participates in the transformation of the organ from its juvenile morphology (16, 17), which promotes colonization (18), to the adult morphology, which mediates luminescence behavior. Further, *lux* mutants do not persist in the light organ (18). These findings have underscored the essential role for perception of symbiont light output for the effective functioning of the organ. However, the mechanisms by which *E. scolopes* perceives the light of its symbiont population have remained unexplored.

Recent studies of the transcriptome of the light organ of *E. scolopes* revealed the expression of several genes that encode proteins with sequence similarity to components of visual transduction cascades (19). These findings led us to hypothesize that the light organ may have the ability to perceive the luminescence that it produces. Here, we present several lines of evidence that the symbiotic light organ of the *E. scolopes* is capable of both generating and modulating luminescence and also of acting as a feedback system that directly senses the light that it produces. We

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database [Accession nos. EU344773 (opsin, eye), EU344774 (light organ), EU344775 (phospholipase C, eye), EU344776 (phospholipase C, light organ), EU344777 (rhodopsin kinase, eye), EU344778 (rhodopsin kinase, light organ), EU344779 (arrestin, eye), EU344780 (arrestin, light organ), EU344781 (visual G protein beta subunit, eye), EU344782 (visual G protein beta subunit, light organ), EU344783 (G alpha q subunit, eye), EU344784 (G alpha q subunit, light organ), EU344785 (G alpha i subunit, eye), EU344786 (G alpha i subunit, light organ), EU344787 (retinal-binding protein, eye), EU344788 (retinal-binding protein, light organ), EU344789 (Trp-C protein, eye), EU344790 (Trp-C protein, light organ), EU344791 (phosphodiesterase, light organ), and EU344792 (cyclic-nucleotide gated channel, light organ)].

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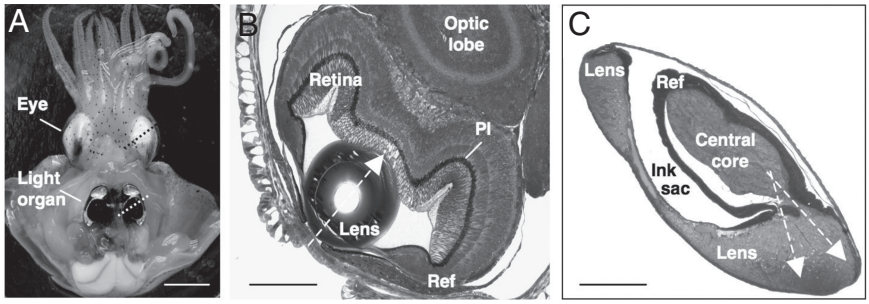

Fig. 1. The anatomy of the eye and light organ of *E. scolopes,* 2 complex organs that interact with light. (*A*) The locations of the light organ and the eye in an adult animal. The specimen has been dissected ventrally to reveal the position of the light organ in the center of the mantle cavity. (Scale bar, 1 cm.) (*B*) A light micrograph of a cross section of the eye (*black dotted line* in *A*). The lens focuses light (*dashed line*, *arrow*) onto the retina, which contains the proteins of visual transduction; the pigmented layer (Pl) and reflector (Ref) collect stray light and also form the iris, which modifies amount of light entering the eye. (Scale bar, 2 mm.) (*C*) A light micrograph of a cross section of the light organ (*white dotted line* in *A*). The ''lens'' acts as a diffusion filter, diffusing the light produced by the symbionts in the epithelial central core into the environment (*dashed line*, *arrow*). The reflector (Ref) reflects the luminescence ventrally, and the ink (lost in histological preparation) in the ink sac creates a pigmented layer that absorbs stray luminescence. As in the eye, the pigmented layer and reflector act as an iris, contracting and expanding to allow more or less light emission, respectively. (Scale bar, 2 mm.)

provide evidence that, to carry out this function, the light organ expresses phototransduction proteins that are homologous to those used in well-characterized visual systems. Thus, the light organ of *E. scolopes* seems to function as an extraocular photoreceptor.

Results

Genes of Visual Transduction Cascades Expressed in the Light Organ. An in-depth analysis of the Expressed Sequence Tags (EST) database generated from the light organs of juvenile *E. scolopes* (19), which contains nearly 14,000 unique clusters, revealed transcripts encoding proteins with high similarities to the key components of visual transduction cascades. We identified 11 such cDNAs (Fig. 2; Table S1), including the gene encoding the visual pigment opsin itself, as well as molecules involved in subsequent activation and deactivation of the cascades. To ensure that these cDNAs were host derived, we also examined the symbiont genome for similar sequences, and none were found. Full-length cDNA sequences of the ORFs for 9 of these genes were extended by rapid amplification of cDNA ends-PCR (RACE-PCR) (Table S2). Sequence alignments, motif characteristics, conservation of amino acids critical to function (BLASTP program, National Center for Biotechnology Information [NCBI]), and phylogenetic analyses (derived by maximum likelihood, see SI *Text*) demonstrated that the predicted proteins are highly similar to the phototransduction constituents

A

	Percent identity*	Clade (aLRT value)
R-Opsin	89 (*Lp*)	Cephalopod eye-expressed rhabdomeric 'rhodopsins' (1.00)
$G_q\alpha$	93 (*Lp*)	Cephalopod G-protein alpha (q) (1.00)
G_β	99 (*Lf*)	Cephalopod eye-expressed G-protein beta subunit 1 (0.98)
PLC	88 (*Lp*)	Cephalopod PLC (1.00)
TRP	88 (*Lf*)	Protostome TRP (0.99)
RK	99 (*Lf*)	Cephalopod 'rhodopsin kinase' (0.95)
Arr-β	80 (*Lp*)	Cephalopod visual arrestin (1.00)
RALBP	90 (*Tp*)	Cephalopod RALBP (1.00)
Gi	87 (*Ov*)	Cephalopod G_i-alpha (1.00)
PDE	70 (*Hs*)	Bilaterian cGMP PDE9A (0.97)
CNG	43 (*Aa*)	Bilaterian CNG-β (0.99)

B

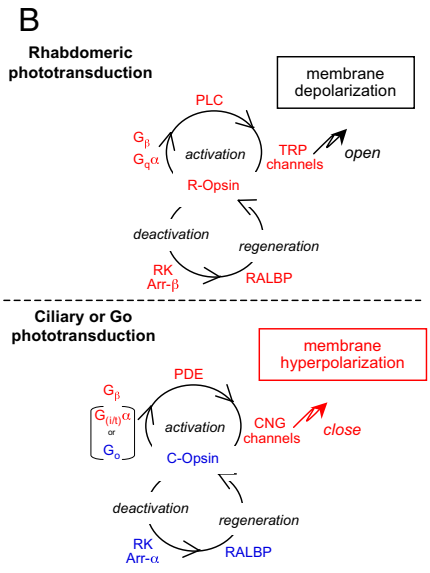

Fig. 2. Proteins of phototransduction encoded by genes expressed in the *E. scolopes* light organ. (*A*) Proteins predicted from derived amino acid sequences of the *E. scolopes* light organ cDNAs (red), their relatedness to the closest match in the NCBI database, and their clade, or phylogenetic relatedness to other sequences (confidence as aLRT values = approximate likelihood ratio test scores; see Fig. S1 and ref. 48). *, per cent identity to the light organ isoform. (*B*) Placement of the deduced light-organ proteins in diagrammatic representations of the phototransduction cascades typically associated with invertebrate rhabdomeric and vertebrate ciliary phototransduction in the visual system (red, components present in the light organ; blue, not detected). Biomolecule abbreviations: Arr-a, arrestin alpha; Arr-b, arrestin beta; cGMP, cyclic guanosine monophosphate; CNG, cyclic nucleotide-gated; C-opsin, ciliary opsin; $G_{i/ta}$, G protein alpha subunit i/t; G_o, G protein subunit o; G_q, G protein alpha subunit q; PDE, phosphodiesterase; PLC, phospholipase C; RALBP, retinal binding protein; RK rhodopsin kinase; R-opsin, rhabdomeric rhodopsin; TRP, transient receptor potential, photoreceptor-specific, Trp-C. Animal species abbreviations: *Aa, Aedes aegypti*; *Hs, Homo sapiens; Lp, Loligo pealei; Lf, L. forbesi; Ov, Octopus vulgaris; Tp, Todarodes pacificus*.

that are associated with rhabdomeric photoreception (Fig. 2 and Fig. S1, Table S1, and *SI Text*). In 5 instances (i.e., in the case of rhodopsin, arrestin, rhodopsin kinase, G alpha i, and G protein beta subunit 1), the same isoform seems to occur in both eye and light organ tissues. Also present were genes with similarities to those encoding proteins typically associated with ciliary photoreceptor cells, including the cyclic nucleotide-gated channel beta isoform (CNG-beta), G alpha i, and the phosphodiesterase 9A isoform, which functions to hydrolyze cGMP and close the CNG channel, resulting in the characteristic hyperpolarization of ciliary photoreceptors (20). These data demonstrate that light-organ tissues express genes that would enable phototransduction and suggest that, as in visual systems, these tissues also may be capable of responding electrophysiologically to light cues.

Electrophysiological Responses of the Light Organ. To test whether these tissues are photoreceptive and, if they are, to define the type of response, we performed electroretinograms (ERGs) (21, 22), which extracellularly record photoreceptor membrane potential, of both eyes and light organs of juvenile *E. scolopes*. To determine the effect of symbiosis on the light-organ ERGs, we analyzed the light organs both of animals that had been colonized by the symbiont at hatching and of those that had not been colonized. A characteristic rhabdomeric depolarization was observed in the electroretinographic spectra of the eyes, whereas the light-organ tissue responded with hyperpolarization (Figs. 2*B* and 3). The presence or absence of symbionts had no reproducible effect on the ERG pattern or the level of response. These data suggest that the provision of the proteins of phototransduction cascades is constitutive, i.e., that it is independent of the presence of symbiont luminescence. However, the level of response did increase with size of the light organ, suggesting an increase in the number of photoreceptive elements as the animal grows (Fig. 3).

Tissue-Specific Message Expression and Protein Production. Components of visual transduction cascades, including opsin, arrestin, and rhodopsin kinase, co-localize in the retina, because they function in concert during phototransduction (23, 24). To determine whether they also co-localize in light-organ tissues, we applied 2 independent analyses of these 3 components: localization of message by PCR and of protein by immunocytochemistry. First, using primers specific to the cDNA encoding each of these proteins, we performed RT-PCR on 7 tissues of the adult host, including those that are and those that are not dedicated to light modulation (Fig. 4*A*). Messenger RNA for all 3 genes was detected in both light-organ tissues and eyes, with eyes having relatively more message for these species. Although the arrestin message could be detected only in the eye and light organ, rhodopsin and rhodopsin kinase messages were present also in the optic lobe of the brain, and rhodopsin kinase was detectable at low levels in other tissues. In the light organs of adult animals, in which it is possible to isolate the symbiont-containing core of the light organ separately from the surrounding dioptrics, message levels were highest in the core portion of the organ (Fig. 4*B*). In a parallel approach, we used antibodies raised against cephalopod opsin, arrestin, and rhodopsin kinase (25, 26) to localize the protein in host tissues. Using juvenile animals, in which all light-organ tissues can be visualized simultaneously by confocal microscopy, we localized the protein to the crypt regions of the organ, which house the symbionts, and to the epithelia of the pore and duct regions, through which symbionts enter and exit the organ (Fig. 4*C*). Retinal tissues of *E. scolopes* were used as a positive control for the cross reactivity of these antibodies in regions known to produce these proteins (Fig. S2).

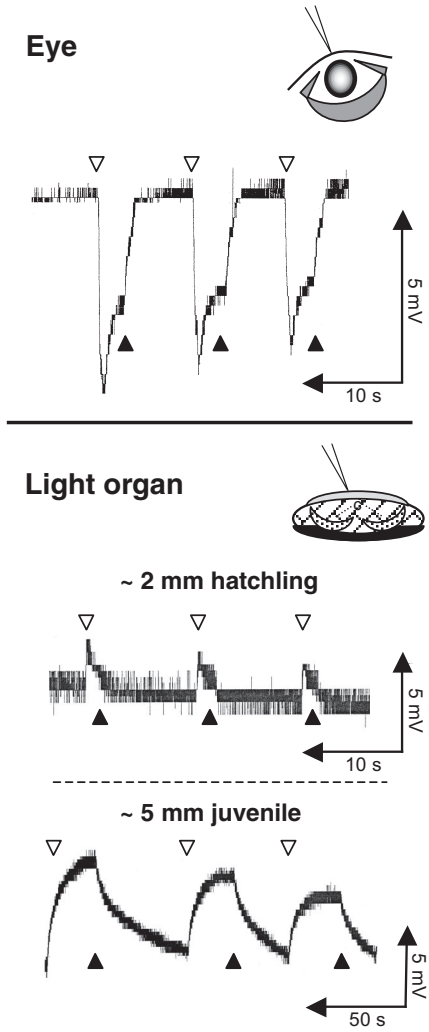

Fig. 3. Representative electroretinograms from the eyes (*Top*) and light organs (*Bottom*) of *E. scolopes*. Diagrams indicate the position of the electrodes on organ tissues. Light stimulus (*open triangles*) to the eyes ($n = 10$ animals) resulted in a rapid depolarization characteristic of invertebrate rhabdomeric phototransduction. The membrane potential rapidly returned to the resting potential with the cessation of the light stimulus (*closed triangles*). In contrast, the light organs ($n = 15$ animals) exhibited hyperpolarization with a markedly slower onset and recovery than the eyes. The light organs of both hatchling (*Bottom, Upper Panel*) and juvenile animals (*Bottom, Lower Panel*) displayed an increase in levels of hyperpolarization with organ growth.

Discussion

The results of this study provide evidence that the light organ of *E. scolopes* has the molecular machinery and physiological potential to respond to light cues. These data interface with a variety of fields of biology, including bioluminescence, symbiosis, phototransduction, and evolutionary biology.

No studies to determine the presence or absence of the genes encoding proteins of phototransduction cascades have been performed on other light organs. Thus, we cannot say whether their presence in the squid organ is unique or is an example of a more general feature of animal light organs. Such organs often are placed into 2 broad categories, bacterial or autogenic. Bacterial organs, in which the animal uses the light produced by symbionts, are restricted to representatives of only a few animal phyla (27–30), whereas autogenic light organs, in which the animal itself makes the enzymes and substrates for lumines-

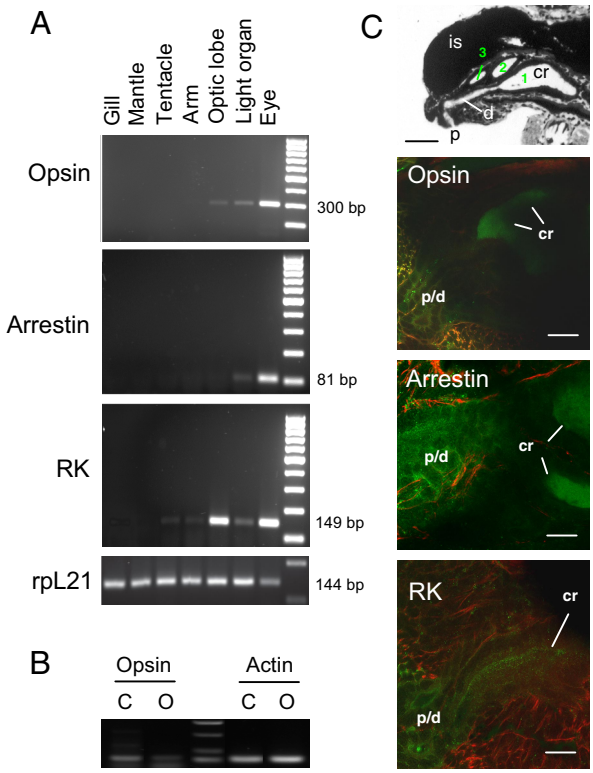

Fig. 4. Localization of mRNA and proteins of phototransduction. (*A*) RT-PCR analysis for mRNA detection in an array of tissues. The gene encoding the ribosomal protein L21 (rpL21) was determined to be a suitable control (20 ng total RNA/lane). (*B*) Opsin message in the adult light organ. The RNA of the organ's central epithelial ''core'' (C; see Fig. 1*C*), where symbionts reside, was extracted and analyzed separately from the other light-organ tissues (O) (20 ng total RNA/lane). (*C*) Localization of proteins of phototransduction in juvenile light-organ tissues. (*Upper image*) Histological section for orientation, showing a pore (p), a duct (d), and portions of 3 crypts (cr, 1, 2, 3). (*Lower 3 images*) Confocal images of light-organ immunocytochemistry revealed that the crypts, as well as the pore and duct regions, were cross-reactive for opsin, arrestin, and rhodopsin kinase (green, fluorescein isothiocyanate-labeled secondary antibodies) in both symbiont-colonized and uncolonized light organs. Sections were counterstained with rhodamine phalloidin (red), which labels filamentous actin. In control experiments, the retinal tissues of the eye labeled with these antibodies, and labeling was below background in controls excluding the primary antibody (see Fig. 2*S*). RK, rhodopsin kinase. (Scale bar, 20 μm.)

cence, occur in representatives of all animal phyla. In bacterial light organs, the animal host must control not only light emission but also the symbiont populations. This management of the symbionts by the host is essential to maintain symbiont number and to sanction cheaters (i.e., dark mutants that might arise and compromise the function of the organ). The finding that the *E. scolopes* juvenile does not allow dark mutants to persist in symbiosis (18, 31) demonstrates that the host must be able to sense the luminescence defect, and the extraocular photoreceptors in the organ tissue are likely candidates for this function. In the squid light organ, the photoreceptive tissues also may function to perceive environmental light, providing the animal with a mechanism to compare this exogenous light with symbiont light emission. Such a capacity might be important in mediating such behaviors as counterillumination, in which the animal matches down-welling light with luminescence. Should photoreceptive tissues also occur in autogenic organs, they may function similarly (i.e., as a feedback system to control light emission). Future characterization of the form and function of the nervous system in such bioluminescent tissues should provide insight into the underlying mechanisms of this type of feedback.

This study also presents some interesting opportunities for the study of phototransduction itself. Whereas the opsin expressed in the light organ is of the rhabdomeric type, and all key components of the rhabdomeric cascade are present in the light organ, the ERG spectra demonstrate hyperpolarization of the photoreceptor cell membranes. Caution must be used in interpreting these data, however, because ERGs are extracellular measures and as such are the summative response of multiple cells. Should these measurements be detecting the polarity of a specific photoreceptor cell type, then the light organ and eyes exhibit opposite physiological responses to light. Genes associated with hyperpolarizing ciliary phototransduction also are present in the light organ, and their sequence characteristics predict that they could be capable of driving the associated activities in ciliary phototransduction, but no ciliary opsin was detected. Although rhabdomeric opsin is the type of opsin principally associated with the eyes of invertebrates (32), the extraocular presence of ciliary opsin has been reported recently in a polychaete worm (33) and in the eye of a basal invertebrate, a cubozoan jellyfish (34); thus, to find ciliary opsin in a mollusk would not be unexpected. Further, because the co-occurrence of both ciliary and rhabdomeric phototransduction systems have been reported in other animals (32), including other mollusks (35), perhaps the light organ of the squid has both types of photoreceptor pathways. If ciliary phototransduction is present in the light organ, then (*i*) another opsin of the ciliary type may be present that we have not yet detected; (*ii*) the rhabdomeric opsin (r-opsin) that is present may be capable of participating in a cascade that results in hyperpolarization; or, (*iii*) this r-opsin may engage both rhabdomeric and ciliary phototransduction pathways. Although antagonistic signals from different opsins alternately depolarize or hyperpolarize the lizard parietal photoreceptors cells (36), a finding of hyperpolarization mediated by r-opsin would be unprecedented.

The finding of proteins of visual transduction cascades, coupled with previous studies of the biochemical similarities between the eye and light organ (13, 14), may have implications for the evolution of bacterial light organs in cephalopods. Although all squids have eyes, bacterial light organs occur as derived characters in only a few genera of loliginid and sepiolid squids. Such organs in the 2 groups have remarkably similar morphologies, although they have arisen independently and harbor different symbionts. Bacterial light organs in cephalopods may have arisen in part by co-opting the developmental and physiological mechanisms of the visual system. An evolutionary route to such novelty is the assembly of existing components in new combinations or contexts, a process that has been called genetic or evolutionary ''tinkering'' (37). Although the related mechanisms of tinkering and co-option have been applied to a variety of levels of biological organization (reviewed in ref. 38), it has been rare to document the acquisition of complex systems, such as whole organs, through the assembly of pre-existing genetic components. Should light organs have evolved by this mechanism, they would provide a particularly elaborate form of evolutionary tinkering. The possibility of this evolutionary trajectory remains to be explored fully. The eye specification gene *pax6* has been studied during development in *E. scolopes*, but no involvement in light-organ development was noted (39). Further studies of these 2 divergent photoreceptor types, eyes and light organs, are required to determine how their similarities and differences in form and function are achieved. Such studies promise to provide insight both into conserved mechanisms of modulating and perceiving light and into the evolution of novelty.

Methods

General Procedures. Specimens of *E. scolopes* were obtained from Oahu, Hawaii, transported to recirculating artificial seawater aquaria at the University of Wisconsin (Madison, WI), and maintained as described previously (40).

Rapid Amplification of cDNA Ends. Candidate sequences were identified by analyses of an EST database that had been constructed by using light organs of juvenile *E. scolopes* (19). RNA was extracted from, on average, 100 juvenile light organs using the MasterPure complete RNA purification kit (Epicentre Technologies Corporation) and RNeasy columns (Qiagen Inc.) according to the manufacturer's protocol. We performed 5′- and 3′-RACE PCR by using the GeneRacer cDNA amplification kit (Invitrogen) according to the manufacturer's instructions. We produced 5′- and 3′-RACE ready cDNA by using 1–5 μg total RNA from juvenile light organs. RACE primers (Table S2) were constructed from the EST sequences identified as having similarity to a transcript of interest. The resulting amplification products were gel purified by using the QIAquick gel extraction kit (Qiagen Inc.). Purified RACE products were ligated into pGEM-T Easy Vector (Promega Corp.) or into TOPO-pCR4 Vector (Invitrogen). PCR products were sequenced from the recombinant plasmid by using T7 and SP6 primers (Promega Corp.). The sequences were translated and compared with protein sequences by using the NCBI BLASTX program.

RT-PCR Analysis. Total RNA, extracted as described in a previous section, was purified from different tissues (gill, mantle, tentacle, arm, optic lobe, light organ, central core, and eye) of the adult squid and was converted into single-stranded cDNA using random primers. Specific primers were used to assess the abundance of a given transcript, (Table S2). All reactions were performed with a no-RT control to confirm that the reaction mixtures were not contaminated.

Phylogenetic Analyses. Using the cDNA sequences, we first used NCBI BLASTX analyses to identify the closest matches to the derived amino acid sequences (Table S1). In a more focused analysis (Fig. 2), we then used each target protein in a NCBI BLASTP program with the Blossum62 matrix to extract the most similar proteins from the Uniref50 and Uniref90 databases (uniprot.org). We aligned the sequences within each dataset using multiple sequence comparison by log-expectation (MUSCLE) (41). With each full dataset, we then performed maximum likelihood analysis implemented in phyML (42), assuming a JTT model of protein evolution (43). To assess nodal support on phylogenies, we report Approximate Likelihood Ratio Test (aLRT) scores, a statistical method to assess the robustness of the branching patterns of molecular phylogenies. Such scores have been shown to correlate with maximum likelihood bootstrap scores but require much less computational time (44).

Immunocytochemistry. We performed immunocytochemistry with 3 components of visual transduction cascades, the genes for which are expressed in the *E. scolopes* light organ: opsin, arrestin, and rhodopsin kinase. Polyclonal antibodies were generated in rats to a 14-mer, IPASEQTQETSPTD, which are amino acid residues 354–367 in the derived amino acid sequence of the *E. scolopes* opsin cDNA. The peptide was a mixture of phosphorylated and unphosphorylated serine in position #357, so that antibodies might be generated to both the activated and non-activated forms of the molecule. We used rabbit polyclonal antibodies to cephalopod arrestin or rhodopsin kinase, which had been generated to proteins derived from *Loligo pealei,* to localize these 2 protein species in *E. scolopes* tissues.

Tissues were prepared for immunocytochemistry as previously described (45, 46). Briefly, squid were anesthetized in a solution of 2% ethanol in filter-sterilized (0.2-μm pore size) seawater. After being incubated overnight at 4 °C in marine PBS (mPBS, 50 mM sodium phosphate buffer with 0.45 M NaCl, pH 7.4) containing 4% formaldehyde, the animals were washed 4 × 60 min in mPBS. The light organs and eyes then were dissected from the animal and were permeabilized in 1% Triton X-100 in mPBS for 2 d. After subsequent incubation with primary antibodies for 14 d (1:100 for opsin; 1:1000 for arrestin and rhodopsin kinase), the animals were stained with FITC-labeled goat anti-rat (opsin experiments) or goat anti-rabbit (arrestin and rhodopsin kinase experiments) secondary antibody (Jackson ImmunoResearch). The samples also were stained with rhodamine phalloidin (Sigma-Aldrich). For each stain, the light organs were incubated in the dark overnight, followed by washing 4 × 60 min in 1% Triton X-100 mPBS. The light organs or eyes were mounted individually on slides in VectaShield (Vector Labs), a mounting medium that slows the fading of fluorochromes. A Zeiss LSM510 laser-scanning confocal microscope was used to examine tissues and collect digital images, which were processed using Zeiss software.

Electroretinograms. ERGs were performed as previously described (47) to measure the change of membrane potential in the eyes and light organs of *E. scolopes* in response to light. The recording electrode was a glass micropipette filled with Ringer's solution; it was positioned with its tip on the surface of the eye or the ventral surface of the light organ (Fig. 3), and a reference electrode was placed on the mantle. The squid was illuminated with pulses of white light, and the responses were monitored using an Acq*Knowledge* 3.0 data acquisition system (BIOPAC Systems, Inc.).

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1. Foster RG, Soni BG (1998) Extraretinal photoreceptors and their regulation of temporal physiology. *Reviews of Reproduction* 3:145–150.
2. Terakita A (2005) The opsins. *Genome Biology* 6:213′.
3. Terakita A, et al. (2008) Expression and comparative characterization of Gq-coupled invertebrate visual pigments and melanopsin. *J Neurochem* 105A:883–890.
4. Messenger JB (1991) in *Evolution of the Eye and Visual Systems*, eds. Cronly-Dillon J, Gregory R (CRC Press, Boca Raton, FL), pp. 364–397.
5. Mauro A (1977) Extra-ocular photoreceptors of cephalopods. *Symposia of the Zoological Society of London* 38:287–308.
6. Parry M (2000) A description of the nuchal organ, a possible photoreceptor, in *Euprymna scolopes* and other cephalopods. *Journal of Zoology, London* 252:163–177.
7. Young RE (1977) Brain, behavior and evolution of cephalopods. *Symposia of the Zoological Society of London* 38:377–434.
8. Young RE, Roper CF (1976) Bioluminescent countershading in midwater animals: Evidence from living squid. *Science* 191:1046–1048.
9. Young RE, Roper CFE, Walters JF (1979) Eyes and extraocular photoreceptors in midwater cephalopods and fishes: Their roles in detecting downwelling light for counterillumination. *Mar Biol (Berlin)* 51:371–380.
10. Nyholm SV, McFall-Ngai MJ (2004) The winnowing: Establishing the squid-vibrio symbiosis. *Nature Reviews Microbiology* 2:632–642.
11. Visick KL, Ruby EG (2006) *Vibrio fischeri* and its host: It takes two to tango. *Current Opinion in Microbiology* 9:632–638.
12. McFall-Ngai MJ, Montgomery MK (1990) The anatomy and morphology of the adult bacterial light organ of *Euprymna scolopes* Berry (Cephalopoda: Sepiolidae). *Biol Bull (Woods Hole, Mass)* 179:332–339.
13. Crookes WJ, et al. (2004) Reflectins: The unusual proteins of squid reflective tissues. *Science* 303:235–238.
14. Montgomery MK, McFall-Ngai MJ (1992) The muscle-derived lens of a squid bioluminescent organ is biochemically convergent with the ocular lens. Evidence for recruitment of aldehyde dehydrogenase as a predominant structural protein. *J Biol Chem* 267:20999–21003.
15. Jones BW, Nishiguchi MK (2004) Counterillumination in the Hawaiian bobtail squid, *Euprymna scolopes* Berry (Mollusca: Cephalopoda). *Mar Biol (Berlin)* 144:1151–1155.
16. Montgomery MK, McFall-Ngai M (1994) Bacterial symbionts induce host organ morphogenesis during early postembryonic development of the squid *Euprymna scolopes*. *Development* (*Cambridge, UK)* 120:1719–1729.
17. Montgomery MK, McFall-Ngai MJ (1998) Late postembryonic development of the symbiotic light organ of *Euprymna scolopes* (Cephalopoda: Sepiolidae). *Biol Bull (Woods Hole, Mass)* 195:326–336.
18. Visick KL, Foster JF, Doino J, McFall-Ngai M, Ruby EG (2000) *Vibrio fischeri lux* genes play an important role in colonization and development of the host light organ. *J Bacteriol* 182:4578–4586.
19. Chun CK, et al. (2006) An annotated cDNA library of juvenile *Euprymna scolopes* with and without colonization by the symbiont *Vibrio fischeri*. *BMC Genomics* 7:154.
20. Kramer RH, Molokanova E (2001) Modulation of cyclic-nucleotide-gated channels and regulation of vertebrate phototransduction. *J Exp Biol* 204:2921–2931.
21. Chen DM, Stark WS (1994) Electroretinographic analysis of ultraviolet sensitivity in juvenile and adult goldfish retinas. *Vision Research* 34:2941–2944.
22. Rosenbaum EE, Hardie RC, Colley NJ (2006) Calnexin is essential for rhodopsin maturation, Ca2+ regulation, and photoreceptor cell survival. *Neuron* 49:229–241.
23. Hargrave PA, McDowell JH (1992) Rhodopsin and phototransduction: A model system for G protein-linked receptors. *FASEB J* 6:2323–2331.
24. Ridge KD, Abdulaev NG, Sousa M, Palczewski K (2003) Phototransduction: Crystal clear. *Trends Biochem Sci* 28:479–487.
25. Mayeenuddin LH, Mitchell J (2003) Squid visual arrestin: cDNA cloning and calcium-dependent phosphorylation by rhodopsin kinase (SQRK). *J Neurochem* 85:592–600.
26. Swardfager W, Mitchell J (2007) Purification of visual arrestin from squid photoreceptors and characterization of arrestin interaction with rhodopsin and rhodopsin kinase. *J Neurochem* 101:223–231.
27. Herring PJ (1978) in *Bioluminescence in Action*, ed. Herring PJ (Academic, London), pp. 199–240.
28. Herring PJ, Morin JG (1978) in *Bioluminescence in Action*, ed. Herring PJ (Academic, London), pp. 273–330.
29. McFall-Ngai MJ, Toller W (1991) in *Biochemistry and Molecular Biology of Fishes*, eds. Hochachka P, Mommsen T (Elsevier, New York), pp. 77–110.

30. Nealson KH, Hastings JW (1979) Bacterial bioluminescence: Its control and ecological significance. *Microbiol Rev* 43:496–518.
31. Visick KL, McFall-Ngai MJ (2000) An exclusive contract: Specificity in the *Vibrio fischeri-Euprymna scolopes* partnership. *J Bacteriol* 182:1779–1787.
32. Plachetzki DC, Serb JM, Oakley TH (2005) New insights into the evolutionary history of photoreceptor cells. *Trends Ecol Evol* 20:465–467.
33. Arendt D, Tessmar-Raible K, Snyman H, Dorresteijn AW, Wittbrodt J (2004) Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. *Science* 306:869–871.
34. Kozmik Z, et al. (2008) Assembly of the cnidarian camera-type eye from vertebrate-like components. *Proc Natl Acad Sci USA* 105:8989–8993.
35. Gomez MP, Nasi E (1994) The light-sensitive conductance of hyperpolarizing invertebrate photoreceptors: A patch-clamp study. *J Gen Physiol* 103:939–956.
36. Su CY, et al. (2006) Parietal-eye phototransduction components and their potential evolutionary implications. *Science* 311:1617–1621.
37. Jacob F (1977) Evolution and tinkering. *Science* 196:1161–1166.
38. True JR, Carroll SB (2002) Gene co-option in physiological and morphological evolution. *Annu Rev Cell Dev Biol* 18:53–80.
39. Hartmann B, et al. (2003) Pax6 in the sepiolid squid *Euprymna scolopes*: Evidence for a role in eye, sensory organ and brain development. *Mech Dev* 120:177–183.
40. Foster JS, McFall-Ngai MJ (1998) Induction of apoptosis by cooperative bacteria in the morphogenesis of host epithelial tissues. *Development Genes and Evolution* 208:295–303.
41. Edgar RC (2004) MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113.
42. Guidon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* 52:696–704.
43. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. *Computer Applications in the Biosciences* 8:275–282.
44. Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: A fast, accurate, and powerful alternative. *Systematic Biology* 55:539–552.
45. Davidson SK, Koropatnick TA, Kossmehl R, Sycuro L, McFall-Ngai MJ (2004) NO means 'yes' in the squid-vibrio symbiosis: Nitric oxide (NO) during the initial stages of a beneficial association. *Cellular Microbiology* 6:1139–1151.
46. Kimbell JR, McFall-Ngai MJ (2004) Symbiont-induced changes in host actin during the onset of a beneficial animal-bacterial association. *Appl Environ Microbiol* 70:1434–1441.
47. Larrivee DC, Conrad SK, Stephenson RS, Pak WL (1981) Mutation that selectively affects rhodopsin concentration in the peripheral photoreceptors of *Drosophila melanogaster*. *J Gen Physiol* 78:521–545.
48. Small AL, McFall-Ngai MJ (1999) Halide peroxidase in tissues that interact with bacteria in the host squid *Euprymna scolopes*. *J Cell Biochem* 72:445–457.