Jellyfish vision starts with cAMP signaling mediated by opsin-G<sub>S</sub> cascade

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Light sensing starts with phototransduction in photoreceptor cells. The phototransduction cascade has diverged in different species, such as those mediated by transducin in vertebrate rods and cones, by G<sub>S</sub>-type G protein in insect and molluscan rhodopsin-type visual cells and vertebrate photosensitive retinal ganglion cells, and by G<sub>q</sub>-type G protein in scallop ciliary-type visual cells. Here, we investigated the phototransduction cascade of a prebilaterian box jellyfish, the most basal animal having eyes containing lens and ciliary-type visual cells similar to vertebrate eyes, to examine the similarity at the molecular level and to obtain an implication of the origin of the vertebrate phototransduction cascade. We showed that the opsin-based pigment functions as a green-sensitive visual pigment and triggers the G<sub>S</sub>-type G protein-mediated phototransduction cascade in the ciliary-type visual cells of the box jellyfish lens eyes. We also demonstrated the light-dependent cAMP increase in the jellyfish visual cells and HEK293S cells expressing the jellyfish opsin. The first identified prebilaterian cascade was distinct from known phototransduction cascades but exhibited significant partial similarity with those in vertebrate and molluscan ciliary-type visual cells, because all involved cyclic nucleotide signaling. These similarities imply a monophyletic origin of ciliary phototransduction cascades distributed from prebilaterian to vertebrate.

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distinct from any known phototransduction cascades, but it exhibited significant partial similarity to those in vertebrate and molluscan ciliary-type visual cells because all involved cyclic nucleotide signaling. Based on these similarities, we discussed evolutionary linkage among ciliary phototransduction cascades distributed from prebilaterian to vertebrate.

Results

To obtain the direct evidence of the involvement of an opsin-based pigment in cnidarian vision and to characterize its molecular basis, we isolated a cDNA encoding opsin from rhopalia, which contain lens eyes, of the box jellyfish Carybdea rastonii [supporting information (SI) Fig. S1 A and B]. The amino acid sequence of the box jellyfish opsin exhibited typical features of opsins (1), such as seven putative membrane-spanning domains and a lysine residue in the seventh membrane-spanning domain that binds the retinal chromophore (Fig. S1C). In the phylogenetic tree of the opsin family, the box jellyfish opsin fell into the cnidarian opsins, clustering with the hydrozoan opsins, which was consistent with the relationship among cnidarian classes (19) (Fig. S2A). We then expressed the box jellyfish opsin in HEK293S cells, to demonstrate that the jellyfish opsin forms a photosensitive pigment, and we succeeded in obtaining the purified pigment from these cells. Fig. 1A shows the spectroscopic features of the opsin-based pigment, demonstrating that it is a green-light-sensitive pigment with an absorption maximum at ~500 nm. The absorption spectrum agreed with the spectral absorption maximum at 500 nm of an electroretinogram of the jellyfish opsin-based pigment expressed in HEK293S cells (Fig. S1B). The lens eyes contain ciliary-type visual cells composed of three parts: an outer segment derived from a modified cilium (putative photoreceptive region), a pigment granule-rich region, and an inner segment (14) (Fig. 2A).

Each rhopalium of the box jellyfish contains two highly sophisticated lens eyes, upper small lens eye and lower large lens eye, in addition to two pairs of simple pit eyes (14, 16, 23) (Fig. 2B). The lens eyes contain ciliary-type visual cells composed of three parts: an outer segment derived from a modified cilium, a pigment granule-rich region, and an inner segment (14) (Fig. 2A and Fig. S3A). We raised a polyclonal antibody specific for the box jellyfish opsin (Fig. S1D) and conducted immunohistochemical staining of the jellyfish eyes to determine the cellular localization of the opsin. In the lens eyes, the antibody strongly labeled all of the outer segments of visual cells, demonstrating abundant expression of the opsin (Fig. 2B). In situ hybridization analysis confirmed the specific expression of the box jellyfish opsin in visual cells on the nucleotide level (Fig. S1 E and F). These localizations, together with the matching of the absorption spectrum of the box
Conducted PCR-based screening of the genome from lens eyes (20), demonstrate that the opsin is the visual pigment of the jellyfish.

Next, we investigated which phototransduction cascade the box jellyfish opsin triggers in vivo. We first examined the possibility that $G_\gamma$, $G_\beta$, and $G_\alpha$, which mediate phototransduction in higher animals, exist in the jellyfish visual cells. Unexpectedly, the anti-$\alpha$ subunit of $G_\gamma$ ($G_{\alpha\gamma}$), anti-$G_\beta$, and anti-$G_\alpha$ antibodies did not label the visual cells (Fig. S4 A–C), raising the possibility that they contain a novel phototransduction cascade. We conducted PCR-based screening of the $\alpha$ subunit of $G$ protein against the cDNAs derived from the jellyfish lens eyes and obtained cDNAs encoding $G_{\alpha\gamma}$ and $G_{\alpha12}$. We performed immunohistochemical analyses with antibodies against $G_{\alpha\gamma}$ and $G_{\alpha12}$ and observed strong fluorescent signals in outer segments of visual cells when employing the anti-$G_{\alpha\gamma}$ antibody (Fig. 2C and Fig. S3C), whereas no signal was obtained by using the anti-$G_{\alpha12}$ antibody (Fig. S4D). A merged image of immunofluorescent labeling with the anti-jellyfish opsin antibody and with the anti-$G_{\alpha\gamma}$ antibody showed colocalization of the opsin and $G_\gamma$ in the jellyfish visual cells (Fig. 2D). These results suggest that the jellyfish opsin triggers a $G_\gamma$-mediated signal transduction cascade in vivo and that the phototransduction cascade in box jellyfish ciliary visual cells differs from that in higher animal photoreceptor cells.

According to studies on $G$ protein-mediated signal transduction in higher animals, $G_\alpha$ activates adenylyl cyclase, which elevates intracellular cAMP (24). Therefore, to obtain evidence that the jellyfish opsin activates $G_\alpha$, we heterologously expressed the box jellyfish opsin in HEK293S cells and analyzed the light-dependent increase in intracellular cAMP. An enzyme-linked immunoassay showed that the cAMP concentration in irradiated cells was 10-fold higher than that of nonirradiated cells and was comparable with the level of agonist-induced cAMP elevation in $\beta_2$-adrenergic receptor-expressing cells (Fig. 3A). Irradiation of mock-transfected cells did not increase cAMP, demonstrating that box jellyfish opsin triggers the $G_\alpha$–adenyl cyclase cascade in a light-dependent manner. Furthermore, we investigated whether the light-dependent cAMP increase also occurs in vivo. We measured the amount of cAMP in dark-adapted and irradiated rhopalia of the box jellyfish. Fig. 3B shows that the levels of cAMP in irradiated rhopalia were significantly higher than those in dark-adapted rhopalia, which provides clear evidence that a light-dependent increase of cAMP concentration occurred in the jellyfish eyes. We also confirmed the existence of an adenylyl cyclase in the box jellyfish visual cells by immunohistochemical analysis by using the anti-adenyl cyclase antibody that recognizes most members of adenylyl cyclase family. The antibody labeled the outer segment of the jellyfish visual cells, as do the antibodies against the opsin and $G_\alpha$ (Fig. 3C and Fig. S3D). Because such abundant expression of adenylyl cyclase was not observed in any parts of the rhopalia other than the visual cells (data not shown), we concluded that the light-dependent increase of cAMP shown here occurred in these cells. These results demonstrate that the jellyfish phototransduction cascade is composed of opsin, $G_\alpha$, and adenylyl cyclase.

**Discussion**

We have shown here that opsin functions as a visual pigment in cnidarians, and its photoproduction property differs from that of bilaterian visual pigments. In addition, we have clearly demonstrated that the box jellyfish opsin triggers a $G_\alpha$–adenyl cyclase signal transduction cascade and consequently elicits a light-dependent increase in cAMP in the visual cells. Recent phylogenetic analyses, including genome data of cnidarian opsins, showed that most cnidarian opsins formed a single group (17), and we showed that the box jellyfish opsin belonged to the cnidarian opsin group (Fig. S2A and Fig. 4). Our findings suggest that other members of the cnidarian opsin group can also function as photopigments and activate $G_\alpha$. We emphasize that the “opsin–$G_\alpha$–adenyl cyclase” cascade that we report here is
direct evidence not only of phototransduction but also of G protein-mediated signal transduction in the lower animals, the prebilaterians. Furthermore, the discovery of Gs-coupled opsin is also important because it provides an elegant method to investigate G protein-coupled receptor-linked cAMP-dependent cellular or physiological responses by using light instead of chemical ligands as a trigger (Fig. 3.4).

Two morphologically distinct photoreceptor cell types, ciliary-type cells with membranes of modified cilia and rhombodermic-type cells with apical microvilli, exist in animals (25), and their relationships to the photopigment and signal transduction cascade have been analyzed (11, 26, 27). In rhombodermic-type cells and their relatives, which are found in many higher invertebrate visual cells and vertebrate photosensitive retinal ganglion cells, Gs-coupled opsin or r-opsin functions as a photopigment (1, 28); that is, it triggers the inositol phospholipid signaling cascade via Gi (7, 9, 10). Therefore, rhombodermic-type photoreceptor cells appear to be of monophyletic origin from both morphological and functional viewpoints (Fig. 4). By contrast, several types of opsins and phototransduction cascades are found in ciliary-type photoreceptor cells (1) (Fig. 4). In vertebrate rods and cones, visual pigment triggers transduction (referred to as Gs-coupled opsin) (3), and its related pigment eucaryophotin or c-opsin is found in ciliary cells in the marine worm brain (26). In addition, Gs-coupled opsin, which is phylogenetically distinct from Gs-coupled opsin, couples with Ga in scallop ciliary visual cells (11, 12). Recently, parietopsin, which is closely related to Gs-coupled opsin, was also shown to couple with Ga in the ciliary photoreceptor cells of lizard pialretial eyes (13).

Here, we demonstrated that jellyfish ciliary-type visual cells contain a Gs-mediated phototransduction cascade (Figs. 2 and 3). Accordingly, judging from the G protein subtype that mediates light information, the ciliary-type photoreceptor cells seem to have polyphyletic origins (Fig. 4). However, despite these differences, all of these cells employ cyclic nucleotides (cGMP or cAMP) as the second messenger in the phototransduction signaling cascade. In the Gs-mediated phototransduction of vertebrate rods and cones, it has been shown that cyclic nucleotide-gated (CNG) channels are used for generating cellular responses (4, 29). Similarly, in the Gs-mediated phototransduction of molluscan photoreceptors, the functioning of CNG channels for generating cellular responses was inferred from pharmacological experiments (30, 31). Interestingly, we also isolated a Gs-coupled phototransduction cascade (Figs. 2 and 3). Accordingly, judging from the G protein subtype that mediates light information, the ciliary-type photoreceptor cells seem to have polyphyletic origins (Fig. 4).

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Supporting Information

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SI Materials and Methods

Amino Acid Sequences. The accession numbers of amino acid sequences used for analyses are as follows: Box jellyfish opsin, AB435549; sea anemone opsin, BR000662; hydra opsin, Contig39347:487820–488855 (http://hydrazome.metazome.net); hydrozoan jellyfish opsin, AB332435; human encephalopsin, AF140242; ragworm c-opsin, AY692353; human rhodopsin, U49742; human blue, M13299; human red, Z68193; lamprey parapinopsin, AB116380; lizard parietopsin, DQ100320; amphioxus peropsin, AB050610; human peropsin, AF12270; human rgr, U15790; squid retinochrome, X57143; human melanopsin, AF147788; amphioxus melanopsin, AB205400; squid rhodopsin, X70498; fruit fly Rh1, K02315; human neuropsin, AY377391; amphioxus rhodopsin, AB050606; scallop G_{o}-rhodopsin, AB006455; human muscarinic acetylcholine receptor M1 (CHRM1), NM.000738; human melatonin receptor IA (MTNR1A), NM.005958; Arabidopsis AKT1, U06745; Arabidopsis AKT2, U40154; fruit fly elk, U04246; human KCNH2, BC001914; fruit fly eag, M61157; human KCNH1, NM.002238; fruit fly HCN, AF124300; human HCN1, NM.021072; fruit fly CNGB, NM.137763; human CNGB1, NM.001297; human CNGB3, NM.019098; box jellyfish CNG, AB435552; fruit fly CNG4, NM.167441; fruit fly CNG3, NM.137871; human CNGA4, NM.001037329; fruit fly CNGA, NM.057768; human CNGA1, NM.000087; human CNGA2, NM.005140; human CNGA3, NM.001298.

Western Blot Analysis. The proteins extracted from half a rhopalia were separated by 12% SDS/PAGE, transferred onto a PVDF membrane, and incubated with 1:500 diluted anti-box jellyfish opsin antiserum. Visualization was carried out by the ABC method (Vectastain) and by staining with 3,3′-diaminobenzidine (Sigma).

In Situ Hybridization. Digoxigenin-labeled antisense and sense RNA probes for the box jellyfish opsin were synthesized by using the DIG RNA labeling kit (Roche). Sections were pretreated with proteinase K and hybridized with each RNA probe. The probe on the sections was detected with alkaline phosphatase-conjugated antidigoxigenin (Roche) by using a blue 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium color reaction.
Fig. S1. Specific expression of box jellyfish opsin in the lens eye. (A) Box jellyfish Carybdea rastonii and the position of the rhopalia (arrowheads). A high-magnification image of the rhopalium is also shown (lateral view). (B) Rhopalium containing two highly sophisticated lens eyes, an upper small lens eye (arrowhead), and a lower large lens eye (arrow). (C) Schematic secondary structure of box jellyfish opsin based on homology with the bovine rhodopsin. Characteristic residues of opsin are highlighted (see Results in main text). (D) High specificity of the anti-box jellyfish antibody. Western blot analysis with the anti-box jellyfish opsin antibody against proteins extracted from rhopalia (Eye) showed that it recognized a 36-kDa protein (arrowhead), which is in good agreement with the expected molecular mass of the jellyfish opsin. (E and F) In situ hybridization with antisense (E) and sense (F) probes of the box jellyfish opsin. In the high-magnification image of the area boxed in E, specific blue signals (arrowheads) are seen just below the pigment granule-rich region. (Scale bars: 100 μm.)
Fig. S2. Molecular phylogenetic trees of the opsin family (A) and cyclic nucleotide-gated channel family (B). (A) The amino acid sequences of opsins that were revealed to function as photopigments and their apparent homologs were included in the phylogenetic analysis. The human CHRM1 and MTNR1A were used as an outgroup. The relationships among subfamilies (black circles) are ambiguous. See Fig. 4. (B) Plant K-channels were used as an outgroup. The subfamily names of animal cyclic nucleotide-gated channels are shown on the right side of each cluster. eag, ether-a-gogo; HCN, hyperpolarization-activated and cyclic nucleotide-gated; CNG, cyclic nucleotide-gated. Box jellyfish sequences are indicated by red letters. Bootstrap probabilities higher than 80% are indicated at each branch node. (Scale bars: 0.1 substitutions per site.)
Fig. S3. Opsin–G<sub>i</sub>–adenyl cyclase cascade in the small lens eye of the box jellyfish. (A) Nomarski image of the small lens eye of the box jellyfish. L, lens; OS, outer segment; Pg, pigment granule-rich region; IS, inner segment. (B–D) Immunofluorescence labeling of outer segments of box jellyfish visual cells with the anti-box jellyfish opsin antibody (B, green), the anti-G<sub>i</sub> antibody (C, magenta), and anti-adenyl cyclase antibody (D, magenta). (Scale bars: 100 μm.)
**Fig. S4.** Immunohistochemical analyses of the box jellyfish lens eye using $G_{11}$ (A), $G_{10}$ (B), $G_{14}$ (C) and $G_{12}$ (D). Fluorescent signals derived from these G proteins are not observed in the outer segments of visual cells (dotted traces) where the jellyfish opsin is localized (E). (Scale bars: 100 μm.)