Jellyfish vision starts with cAMP signaling mediated by opsin-Gs cascade

Mitsumasa Koyanagi1†, Kosuke Takano*‡, Hisao Tsukamoto*, Kohzoh Ohtsu*, Fumio Tokunaga*, and Akihisa Terakita*‡

*Department of Biology and Geosciences, Graduate School of Science, Osaka City University, 3-3-138 Sugimoto-cho, Sumiyoshi-ku, Osaka 558-8585, Japan; ‡Oki Marine Biological Station, Faculty of Life and Environmental Science, Shimane University, 194 Kamo, Okinoshima-cho, Oki, Shimane 685-0024, Japan; †Department of Earth and Space Science, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan

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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_s-type G protein in insect and molluscan rhabdomeric-type visual cells and vertebrate phototransductive retinal ganglion cells, and by G_q-type G protein in scallop ciliary-type visual cells. Here, we investigated the phototransduction cascade of a prebilateral 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_s-type G protein-mediated phototransduction cascade in the ciliary-type visual cells of the box jellyfish eye. We also demonstrated the light-dependent cAMP increase in the jellyfish visual cells and HEK293S cells expressing the jellyfish opsin. The first identified prebilateral 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 prebilateral to vertebrate.

G protein | phototransduction | visual cell | visual pigment | rhodopsin

Many animals sense light signals for vision and nonvisual photoreceptions. Light is captured by an opsin-based photopigment in a photoreceptor cell and leads to cellular light response through a G protein-mediated phototransduction cascade. Three kinds of phototransduction cascades have been found thus far (1). In vertebrate rods and cones, the light is absorbed by visual pigment and the information is relayed via transducin (G_t) (2), causing the decrease of intracellular cGMP concentration to close cyclic nucleotide-gated channels (3, 4). In rhabdomeric-type visual cells of higher invertebrates, such as arthropods and molluscs, G_t-type G protein passes the light information to the phosphoinositol signaling cascade (5–9), which is also found in vertebrate phototransductive retinal ganglion cells (10). In addition, we reported that the G_s-type G protein-mediated phototransduction cascade (11) involving in the cGMP increase as a second messenger exists in scallop ciliary visual cells (12). Recently, the G_s-mediated phototransduction cascade was also found in the ciliary photoreceptor cells of lizard parietal eyes (13). These varied phototransduction cascades are, respectively, driven by particular opsins, which belong to phylogenetically distinct opsin subfamilies (1). Because vision has evolved with phototransduction cascades and has diverged in different species, the opsin-based pigment and signaling cascade of lower invertebrates, such as prebilateral animals, is important to understand the evolution of phototransduction, especially the origin of vertebrate vision.

The prebilateral cnidaria is the most basal phylum having a visual system with specialized sensory organs (eyes), and, in particular, cubozoans or box jellyfish are distinguished from all other cnidarians by possessing elaborate lens eyes, which resemble those of higher animals (14, 15). In addition, visual cells in the box jellyfish eyes have ciliary morphology as do vertebrate rods and cones. Therefore, it has been of great interest for more than a century whether the box jellyfish visual system is similar to that of vertebrate at the molecular level (16). However, the underlying molecular mechanisms of the jellyfish vision, including the particular photopigment and signal transduction cascade, remain to be elucidated. Because opsin sequences were recently found in other classes of cnidarians, a sea anemone (anthozoan), hydra (17), and hydrozoan jellyfish (hydrozoan) (18), opsin is a candidate for the photoreceptive pigment in cnidianian vision.

Here, we investigated the box jellyfish visual system to elucidate the prebilateral phototransduction cascade and to understand the phototransduction evolution throughout the animal kingdom. We showed that the opsin-based pigment functions as a green-sensitive visual pigment and triggers G_s-type G protein-mediated phototransduction cascade in the ciliary-type visual cells of the box jellyfish lens eyes. We also demonstrated that the opsin–Gs cascade causes a light-dependent cAMP increase in the jellyfish visual cells. The first identified prebilateral cascade was

Fig. 1. Box jellyfish opsin as a photosensitive pigment. (A) Absorption spectra of the box jellyfish opsin-based pigment in the dark state (solid curve) and after irradiation with green-light (dotted curve). (B) Chromophore configurations of the pigment in the dark (Upper) and after irradiation (Lower) analyzed by HPLC as retinal oximes (syn- and anti-forms of 11-cis- and all-trans-retinal oximes). Relative absorbances, which were normalized with absorbance of syn-form of 11-cis-retinal oxime in the dark state, are indicated.

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1M.K. and K.T. contributed equally to this work.

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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 \( \sim 500 \text{ nm} \). The absorption spectrum agreed with the spectral property, which is involved in the G protein activation, to the photosensitization profile and with differences in the photoproduct. Photosensitive pigments exhibit (10, 21). The chromophore configurations of the jellyfish opsin and its photoproduct were revealed to be 11-cis and all-trans forms, respectively, by HPLC analyses of the jellyfish opsin-based pigment expressed in HEK293S cells (Fig. 1B). These results may suggest that the jellyfish visual cells contain a chromophore retinal-replacement system from the all-trans to 11-cis form, to restore the photosensitivity of the pigment, as found in the dark regeneration of bistable pigments (22). Therefore, we concluded that the box jellyfish opsin forms a photosensitive pigment with similar characteristics in photosensitization profile and with differences in the photoproduction property, which is involved in the G protein activation, to the visual pigments of higher animals, such as insects, molluscs, and vertebrates.

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. 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 and Fig. S2A). 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 and Fig. S3B). 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 pigments, as found in the dark regeneration of bistable pigments (22). Therefore, we concluded that the box jellyfish opsin forms a photosensitive pigment with similar characteristics in photosensitization profile and with differences in the photoproduction property, which is involved in the G protein activation, to the visual pigments of higher animals, such as insects, molluscs, and vertebrates.

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Fig. 2. Colocalization of the opsin and Gs in the box jellyfish visual cells. (A) Nomarski image of the large lens eye of the box jellyfish. L, lens; OS, outer segment; Pg, pigment granule-rich region; IS, inner segment. (B and C) Immunofluorescence labeling of outer segments of box jellyfish visual cells with the anti-box jellyfish opsin antibody (B, green) and the anti-Gs antibody (C, magenta). (D) Merged image showing colocalization of the opsin and Gs in the visual cells. (Scale bars: 100 \( \mu \text{m} \).)

Fig. 3. Light-dependent cAMP increase mediated by the opsin-based pigment. (A) A cAMP increase caused by light irradiation was observed in HEK293S cells transfected with the box jellyfish opsin cDNA (jellyfish opsin) but not in cells transfected with expression vector alone (mock). \( n = 3 \). As a control, the cAMP increase caused by the agonist isoproterenol (ISO) in HEK293S cells transfected with human \( \beta_2 \)-adrenergic receptor cDNA was measured \( \( n = 3 \)). (B) Light-dependent cAMP increase in the box jellyfish eyes. The amounts of cAMP extracted from dark-adapted (Dark) and irradiated rhopalia containing eyes (Light) were compared \( \( n = 3 \). Error bars represent SEM. (C) Immunofluorescence labeling of outer segments of the box jellyfish visual cells with the anti-adenyl cyclase antibody (magenta). (Scale bar: 100 \( \mu \text{m} \).)
ducted PCR-based screening of the that they contain a novel phototransduction cascade. We con-
did not label the visual cells (Fig. S4).

irradiated cells was linked immunoassay showed that the cAMP concentration in light-dependent increase in intracellular cAMP. An enzyme-
the box jellyfish opsin in HEK293S cells and analyzed the cAMP elevation in

According to studies on G protein-mediated signal transduc-
tion in higher animals, G, Gq, and Go, which mediate phototransduction in higher animals, exist in the jellyfish visual cells. Unexpectedly, the anti-α subunit of Gi (Go), anti-Gq, and anti-Go, antibodies did not label the visual cells (Fig. S4 A–C), raising the possibility that they contain a novel phototransduction cascade. We con-
ducted PCR-based screening of the α subunit of G protein against the cDNAs derived from the jellyfish lens eyes and obtained cDNAs encoding Go, Go12, and Go11. We performed immu

phosphoinositol

hybridization 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 Gi, Gq, and Go, which mediate phototransduction in higher animals, exist in the jellyfish visual cells. Unexpectedly, the anti-α subunit of Gi (Go), anti-Gq, and anti-Go, antibodies did not label the visual cells (Fig. S4 A–C), raising the possibility that they contain a novel phototransduction cascade. We con-
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Discussion

We have shown here that opsin functions as a visual pigment in cnidarians, and its photopigment property differs from that of bilaterian visual pigments. In addition, we have clearly demonstrated that the box jellyfish opsin triggers a Gq-adenyl cyclase signal transduction cascade and consequently elicits a light-
dependent increase in cAMP in the visual cells. Recent phylo-
genetic 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 Gq. We emphasize that the “opsin–Gq–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 G_{i}-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 rhodocentral-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 rhodocentral-type cells and their relatives, which are found in many higher invertebrate visual cells and vertebrate photoreceptors, cilia and rhabdodermal-type cells with membranes of modified cilia and rhabdodermal photoreceptor cell containing phosphoinositol signaling cascade via G_{i} (7, 9, 10). Therefore, rhodocentral-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 transducin (referred to as G_{i}-coupled opsin) (3), and its related pigment ephoxepinopin or o-copin is found in ciliary cells in the marine worm brain (26). In addition, G_{i}-coupled opsin, which is phyleogenetically distinct from G_{i}-coupled opsin, couples with G_{o} in scallop ciliary visual cells (11, 12). Recently, parietopsin, which is closely related to G_{i}-coupled opsins, was also shown to couple with G_{o} in the ciliary photoreceptor cells of lizard paretial eyes (13).

Here, we demonstrated that jellyfish ciliary-type visual cells contain a G_{i}-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 G_{i}-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 G_{i}-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 cDNA encoding a channel, which fell into the CNG subfamily including vertebrate rod, cone, and olfactory CNGs, from the box jellyfish lens eyes (Fig. S4B). These observations, together with the fact that all known CNGs respond to both cAMP and cGMP (29), suggest a monophyletic group of animal phototransduction cascades characterized by employing cyclic nucleotides in phototransduction signaling, presenting an evolutionary linkage from prebilaterian phototransduction to vertebrate phototransduction. Therefore, we propose a classification of animal photoreceptor cells, including the previous reports (11, 25–27), the rhodocentral photoreceptor cell containing photopin, and the ciliary photoreceptor cell containing cyclic nucleotide signaling mediated by G_{o}, by G_{i}, or by G_{o} (Fig. 4).

Surprisingly, the G_{i}-mediated phototransduction cascade in jellyfish visual cells exhibits overall similarities with the vertebrate olfactory signaling cascade, which is composed of Golf (a kind of G_{i}) and adenylyl cyclase type III, and elicits increases in cAMP and activation of CNG channels (32). Thus, it may be an intriguing hypothesis that the vertebrate olfactory sensory neuron, which also has cilary morphology, shares an evolutionarily common origin with the ciliary photoreceptor cells.

Since submission of this paper, Kozmik et al. (33) reported the photosensitivity of the opsin-based pigment of other box jellyfish species, based on the difference spectrum, which does not provide the absolute absorption spectrum of the pigment or its photoreception properties, basically. They also reported existence of some signal transduction-related molecules other than G_{i} and adenyl cyclase in the rhopalia. It would be interesting to investigate their relation to the opsin–G_{i}–adenylyl cyclase cascade.

Materials and Methods

Animals. Box jellyfish (C. rastonii) were collected in the Japanese Sea around the Oki islands, Japan. The jellyfish were kept in the dark overnight before experiments. The rhopalia, which contain the lens eyes, were dissected under dim red light.

cDNA Cloning. The opsin, G_{o}, G_{i}, and CNG cDNAs of the box jellyfish were isolated from the rhopalia by RT-PCR with following degenerated primers: 5'-GCITITYTIGITGGCAGNCNCTA-3' as the sense primer and 5'-GAATCATCIRGRTATIAINGGR-3' as the antisense primer for cloning of the opsin designed based on FLAVATP and NPIIYAL, respectively; 5'-GTIAACARAT-GAARATHATHCA-3' as the sense primer and 5'-TIGAICKYTIGICCCICNA-CRTC-3' as the antisense primer for cloning of the G protein, designed based on VKMQMIIM and DVGQGQSE, respectively; and 5'-YTIACITIACNAC-NATHGG-3' as the sense primer and 5'-ATRATTICICNGNGNAGARA-3' as the antisense primer for cloning of the CNG channel, designed based on LTTLTG and FPSPGYI, respectively. PCR amplifications were carried out with annealing temperatures of 40 or 50°C. The 3' and 5' ends of the cDNAs were obtained by using the 3'-RACE and 5'-RACE systems, respectively (Invitrogen).

Expression of Opsin-Based Pigment and Spectroscopy. The box jellyfish opsin was expressed in HEK293S cells and purified as described in ref. 35. To reconstitute the pigment, the expressed proteins were incubated with an excess of 11-cis-retinal. The absorption spectra of purified samples were recorded at 4°C with a Shimadzu UV2450 spectrophotometer. Green light was supplied by a 1-kW halogen lamp (Philips) with a 500-nm interference filter (Toshiba).

HPLC Analysis. The chromophore configurations of irradiated and nonirradiated purified box jellyfish opsin were analyzed by HPLC as described in ref. 35.

Antibodies. The anti-box jellyfish opsin and anti-G_{i} antibodies were generated against the C-terminal region of the box jellyfish opsin and the helical domain of G, respectively, by using the PMAL protein fusion and purification system (New England BioIabs) according to the method reported (27). The anti-G_{o} and anti-G_{i} antibodies were a generous gift from Tatso Suzuki (Hyogo College of Medicine) (36, 37), and the anti-G_{i} antibodies were commercially obtained (MBL; Santa Cruz Biotechnology).

Immunohistochemistry. The dissected rhopalia of the box jellyfish were immersion-fixed in 4% paraformaldehyde, cryoprotected in 0.1 M phosphate buffer containing 15% sucrose, frozen with OCT medium (Sakura), and sectioned at 12 μm. The sections were incubated with 1:500 diluted antisera incubated with Alexa Fluor 488 anti-mouse IgG or 594 anti-rabbit IgG (Molecular Probes) for immunofluorescent detections.

cAMP Assay. The cAMP content of HEK293S cells and rhopalia of the box jellyfish was measured with an enzyme-linked immunoassay system (Amersham Biosciences) according to the manufacturer's protocol. Cells transfected with the box jellyfish opsin cDNA and mock-transfected cells were incubated with 11-cis-retinal in the dark followed by irradiation with white light for 30 s (as a light stimulus) before lysis. Cells transfected with human β_{2}-adrenergic receptor cDNA were incubated with 10 nM isoproterenol for 20 min to induce cAMP formation. To prevent the degradation of cAMP by intrinsic cAMP phosphodiesterase activity, cells were treated with Hesper-buff ered saline containing 1 mM 3-isobutyl-1-methylxanthine, an inhibitor of cAMP phosphodiesterase, before stimulation. To measure the light-dependent cAMP increase in the jellyfish eyes, eight rhopalia from two jellyfish were used for one experiment. Half of them were kept in the dark, and the other half were irradiated with white light for 2 min followed by immediate lysis. A 320-W halogen lamp (Cabin) was used for sample irradiation.

Phylogenetic Analyses. Phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39). The phylogenetic tree inferences were carried out as described in ref. 38. Multiple alignments of the amino acid sequences including the box jellyfish genes were calculated by using the XCED software (39).
Supporting Information

Koyanagi et al. 10.1073/pnas.0806215105

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); hydrozoo 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, AF012270; 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; scollop G, 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.138781; 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–Gₛ–adenylyl 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-Gₛ antibody (C, magenta), and anti-adenylyl cyclase antibody (D, magenta). (Scale bars: 100 μm.)
Fig. S4. Immunohistochemical analyses of the box jellyfish lens eye using Gα11 (A), Gα12 (B), Gα13 (C) and Gα12 (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.)