# Assembly of the cnidarian camera-type eye from vertebrate-like components

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**Animal eyes are morphologically diverse. Their assembly, however, always relies on the same basic principle, i.e., photoreceptors located in the vicinity of dark shielding pigment. Cnidaria as the likely sister group to the Bilateria are the earliest branching phylum with a well developed visual system. Here, we show that camera-type eyes of the cubozoan jellyfish, *Tripedalia cystophora*, use genetic building blocks typical of vertebrate eyes, namely, a ciliary phototransduction cascade and melanogenic pathway. Our findings indicative of parallelism provide an insight into eye evolution. Combined, the available data favor the possibility that vertebrate and cubozoan eyes arose by independent recruitment of orthologous genes during evolution.**

evolution | gene | opsin | photoreceptor | cnidaria

The assembly of diverse animal eyes requires two fundamental building blocks, photoreceptors and dark shielding pigment. The function of photoreceptors is to convert light (stream of photons) into intracellular signaling. The photoreceptor cells (PRCs) are classified into two distinct types: rhabdomeric, characteristic of vision in invertebrate eyes; and ciliary, characteristic of vision in vertebrate eyes (1). In both ciliary and rhabdomeric PRCs, the seven-transmembrane receptor (opsin) associates with retinal to constitute a functional photosensitive pigment. Each photoreceptor type uses a separate phototransduction cascade. Rhabdomeric photoreceptors employ r-opsins and a phospholipase C cascade, whereas ciliary photoreceptors use c-opsins and a phosphodiesterase (PDE) cascade (2, 3). In general, the dark pigment reduces photon scatter and orients the direction optimally sensitive to light. The biochemical nature of the dark pigment appears more diverse than the phototransduction cascades used by the PRCs. Vertebrate eyes use melanin as their exclusive dark pigment. However, among invertebrates, pterins constitute the eye pigment in the polychaete *Platynereis dumerilii* (4), pterins and ommochromes are accumulated in eyes of *Drosophila* (5), and melanin is found rarely such as in the inverse cup-like eyes of the planarian, *Dugesia* (6).

Cnidaria, the likely sister group to the Bilateria, constitute the earliest branching phylum containing a well developed visual system. For example, Cubozoa (known as "box jellyfish") have camera-type eyes with cornea, lens, and retina; unexpectedly, the cubozoan retina has ciliated PRCs that are typical for vertebrate eyes (7–9). Cubomedusae are active swimmers that are able to make directional changes in response to visual stimuli (10). The cubozoan jellyfish, *Tripedalia cystophora* (Fig. 1*A*), has four sensory structures called rhopalia that are equally spaced around the bell. In addition to two camera-type lens containing eyes at right angles to one another, each rhopalium has two pit-shaped and two slit-shaped pigment cup eyes (Fig. 1*B*). Thus, with six eyes located on each rhopalium, *Tripedalia* has 24 eyes altogether. Because the visual fields of individual eyes of the rhopalium partly overlap, *Tripedalia* (like other Cubomedusae) has an almost complete view of its surroundings. The lens-containing *Tripedalia* eyes have sophisticated visual optics as do advanced bilaterian phyla (11).

In the present work, we characterize genes required for the assembly of camera-type eyes in *Tripedalia*. We show that the genetic building blocks typical of vertebrate eyes, namely ciliary opsin and the melanogenic pathway, are used by the cubozoan eyes. Although our findings of unsuspected parallelism are consistent with either an independent origin or common ancestry of cubozoan and vertebrate eyes, we believe the present data favor the former alternative.

## Results

**Ciliary Opsin Is Expressed in Camera-Type Eyes of *Tripedalia*.** We screened an expressed sequence tag (EST) library derived from rhopalia of *Tripedalia* to identify the jellyfish genes that are involved in vision; orthologues of other invertebrates and vertebrates were identified by phylogenetic analysis. Of the four opsin types present at the base of the bilaterians [rhabdomeric (r-opsins), ciliary (c-opsin), $G_o$-opsins, and peropsin/RGR (12–14)], the *Tripedalia* opsin EST clustered with the c-opsins, an orthology consistent with the conservation of the characteristic stretch of deduced amino acids between the transmembrane domain VII and cytoplasmic tail [supporting information (SI) Fig. S1]. This region includes the c-opsin fingerprint tripeptide NR/KQ (NRS in *Tripedalia*) that is critical for coupling to the downstream phototransduction cascade through interaction with a GTP-binding protein subunit $G\alpha_t$ in the vertebrate rods and cones (15). An antibody generated against *Tripedalia* c-opsin recognized a single electrophoretic band in protein extracts prepared from rhopalia and COS-7 cells transfected with c-opsin cDNA (Fig. 1*C*). Camera-type eyes of adult jellyfish (Fig. 1*D*) were immunostained with an anti-c-opsin antibody. The c-opsin localized in the retinal ciliated PRCs of both complex eyes (Fig. 1 *E* and *F*) in a pattern resembling that by staining with anti-acetylated tubulin antibody (Fig. 1*G*), which specifically labels stabilized microtubules in axons and cilia (13).

**Spectral Sensitivity of *Tripedalia* c-Opsin.** To address the question of whether the identified *Tripedalia* c-opsin can function as a true

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Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. EU310498 (*c-opsin*), EU310502 (*oca*), EU310499 (*mitf*), EU310500 (catalytic *pde*), EU310501 (inhibitory *pde6d*) and EU310503 (*guanylate cyclase*)].

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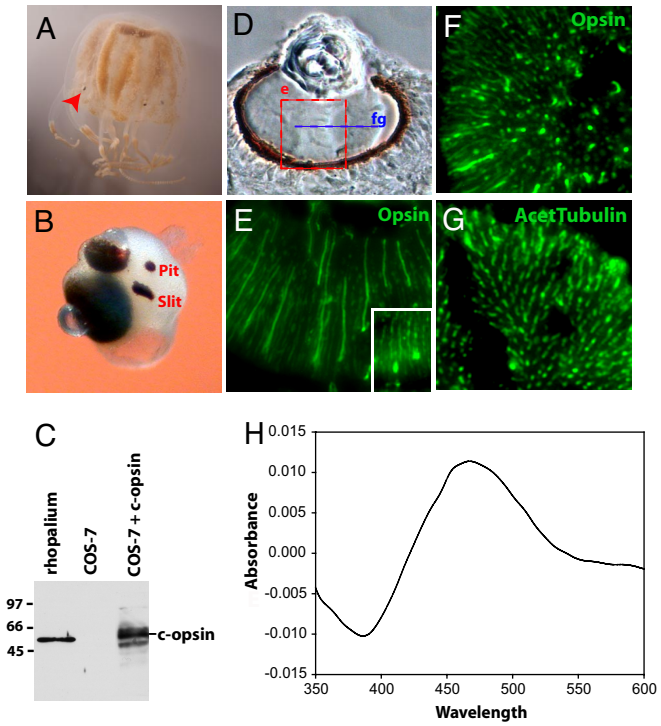


**Fig. 1.** Ciliary opsin is the functional photopigment of *Tripedalia* camera-type eyes. (*A*) *Tripedalia* medusae with rhopalia (arrowhead). (*B*) Rhopalium with two lens-containing camera-type eyes, a slit and a pit eye. Another set of slit and pit eyes is symmetrically located on the other side of the rhopalium. (*C*) Specificity of an anti-*Tripedalia* c-opsin antibody tested by Western blotting with protein extracts from rhopalia and COS-7 cells transiently transfected with *Tripedalia* c-opsin cDNA. (*D*) Bright-field section through the large camera-type eye. The area shown in *E* is boxed. The plane of sectioning in *F* and *G* is indicated (fg). (*E–G*) Immunohistochemical staining (green) of *Tripedalia* retina using antibodies to c-opsin (*E* and *F*) or acetylated tubulin (*G*). (*E Inset*) Immunohistochemical staining for c-opsin in the small camera-type eye. (*H*) Dark–light difference absorption spectrum of the reconstituted *Tripedalia* c-opsin photopigment. The photopigment reconstituted with 11-*cis*-retinal forms a functional photopigment most sensitive to blue–green light.

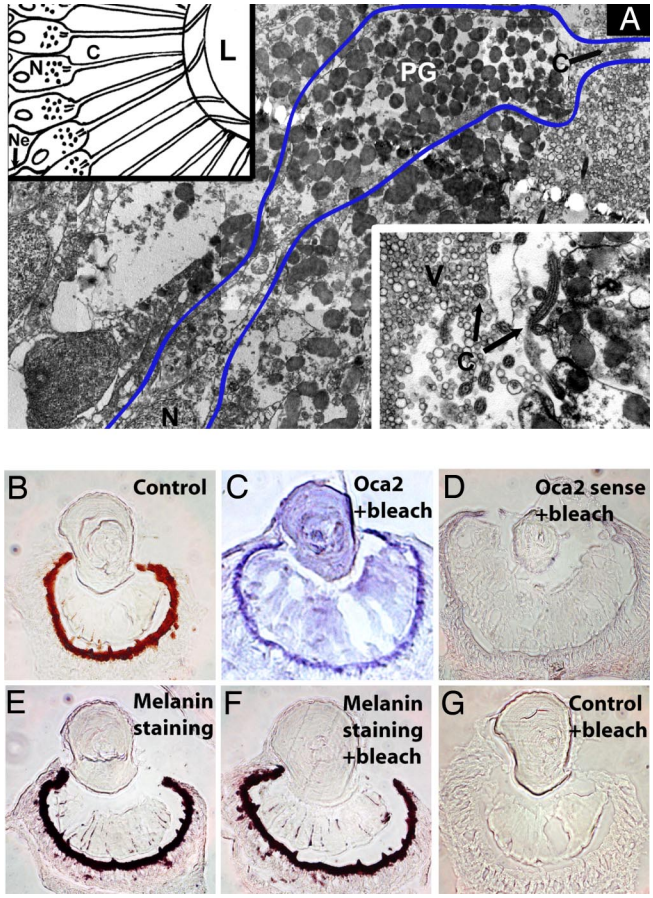


**Fig. 2.** Melanin is the shielding pigment of *Tripedalia* camera-type eyes. (*A*) Electron micrograph of camera-type eye PRCs. Blue line borders one PRC containing shielding pigment layer (PG) as well as photosensitive cilium (C). (*Upper Left Inset*) Diagram of PRCs with their cilia protruding to the lens capsule. Note that all PRC nuclei are located behind the shielding pigment. (*Lower Right Inset*) Apical end of a PRC with cilium. C, cilium; L, lens; N, nucleus; Ne, neurite; PG, pigment granules; V, vitreous body. (*B*) Bright-field cryosection of camera-type eye. (*C* and *D*) *In situ* hybridization (blue) using *oca2* antisense (*C*) or control sense (*D*) probes. (*E–G*) Melanin detection by Fontana–Masson staining. Tissue sections shown in *C*, *D*, *F*, and *G* were bleached to remove melanin.

visual opsin, we tested its photochemical properties. *Tripedalia* c-opsin was expressed in COS-1 cells and reconstituted as a functional photosensitive pigment with 11-*cis*- retinal. The reconstituted c-opsin was most sensitive to the blue–green region of the spectrum with a peak absorbance ($\lambda_{max}$) at 465–470 nm (Fig. 1*H*), in agreement with the spectral sensitivity of the *Tripedalia* electroretinogram (16). We conclude that *Tripedalia* c-opsin is a functional, vertebrate-like photopigment expressed in the PRCs of the camera-type cubozoan eye.

***Tripedalia* Orthologues of Vertebrate-Like Phototransduction Genes.** In vertebrates, activated heterotrimeric G proteins use cGMP PDE for signal transduction. In accordance with our identification of a vertebrate-like *c-opsin* in *Tripedalia*, we found that the catalytic subunit of *pde* expressed in the *Tripedalia* rhopalium phylogenetically clusters with the group of GAF domain-containing *PDEs* including vertebrate rod- and cone-specific *PDE6* (Fig. S2*A*). Furthermore, we identified other components of the ciliary-type cascade associated with deactivation or adaptation of phototransduction, such as the inhibitory subunit of phosphodiesterase (*PDE6D*), *phosducin* and *guanylate cyclase* (Fig. S2 *B–D*). Thus, the nature of the genes expressed in the rhopalia (detected by RT-PCR; Fig. S3) suggests that the camera-type eye of *Tripedalia* uses a ciliary-type phototransduction cascade similar to that of vertebrates.

**Melanin Granules in *Tripedalia* PRCs.** A conspicuous ring of dark shielding pigment surrounds the area of c-opsin expression (compare Fig. 1 *D* and *E*). Most if not all PRCs in the *Tripedalia* retina contain pigment granules (Fig. 2*A*). These PRCs thus resemble what one might imagine a prototypical ancestral cell combining photoreceptor and pigment functions to look like (17). The biochemical nature of the *Tripedalia* pigment was suggested by the identification of an orthologue of the vertebrate ocular and cutaneous albinism-2 (*Oca2*) gene in our EST library (Fig. S4*A*). *Oca2* (also known as *pink-eyed dilution*) is an essential gene for melanin biosynthesis. It is the most commonly mutated gene in cases of human albinism (18). In addition, mutations in *Oca2* are responsible for pigmentation defects in mouse (19), medaka (20), and independently arisen populations of the cave fish, *Astyanax* (21). *In situ* hybridization analysis revealed that *Tripedalia oca* is conspicuously expressed near the pigmented retina layer of PRCs (Fig. 2*C*). Control staining with an *oca* sense probe (Fig. 2*D*) did not yield a signal. The results of a direct chemical assay (Fontana–Masson) were consistent with melanin being the pigment in the *Tripedalia* retina (Fig. 2 *E–G*). In vertebrates, development of melanin-producing cells and specification of retinal pigment cells require the conserved

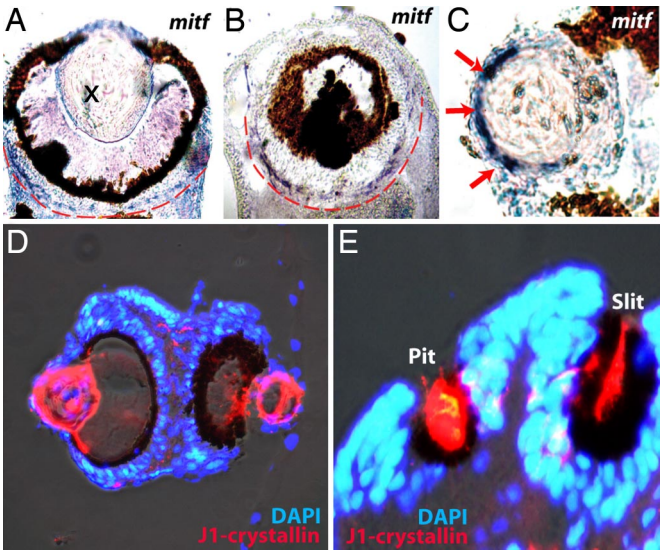


**Fig. 3.** Expression of *mitf* and *J1-crystallin* in *Tripedalia* eyes. (*A–C*) *In situ* hybridization (blue) detects *mitf* expression in the circle around pigment deposits (*A* and *B*) and in the lens (*C*, arrows). (*D* and *E*) Immunohistochemistry staining using an antibody to the major cubozoan lens crystallin, J1 (red). Nuclei of cells are visualized by DAPI staining (blue). J1-crystallin expression is localized to lenses of camera-type eyes (*D*) as well as to the slit and pit eyes (*E*).

microophthalmia-associated transcription factor, Mitf (22–24). Mitf regulates expression of *tyrosinase* and *tyrosinase-related protein-1* and *-2*, which are necessary for melanin biosynthesis (for review, see ref. 25). Here, we have cloned a *mitf* orthologue from *Tripedalia* (Fig. S4*B*) expressed in a ring-like pattern just outside of the melanin deposits (Fig. 3 *A* and *B*); this area contains the PRC nuclei (Figs. 2*A* and 3*D*). Thus, *mitf* is a conserved transcription factor with shared expression patterns in the complex eyes of *Tripedalia* and vertebrates.

***Mitf* Expression in Lens and Crystallin Expression in Pigmented PRCs of Nonlens Eyes of *Tripedalia*.** In addition to expression in the pigmented PRCs of camera-type eyes, *mitf* mRNA was detected in the outermost cells of the *Tripedalia* lens (Fig. 3*C*). Consequently, to investigate a possible relationship between the pigmented PRCs and the cellular lens we examined whether J1-crystallin, the major protein of the *Tripedalia* lens (26), is expressed in PRCs. The J1-crystallin antibody immunostained the slit and pit eyes as well as the cellular lens (Fig. 3 *D* and *E*). The presence of J1-crystallin in the slit and pit eyes of *Tripedalia* was unexpected because these cup-like eyes lack cellular lenses. They do, however, have pigmented PRCs, suggesting a relationship between the PRCs and cellular lens that warrants further study.

## Discussion

The present work reveals surprising similarities in the genetic components used for visual system development in vertebrates and cubozoan jellyfish. If Cubozoa and vertebrates express orthologous c-opsins in their PRCs and make use of the same pigmentation pathway including the key transcription factor Mitf, does this represent a parallel evolution or conservation of an ancestral "eye" program between those evolutionarily distant animal phyla (Fig. 4)? Although our data are formally consistent with both evolutionary scenarios, we believe that they favor the former.

Even though ciliary and rhabdomeric photoreceptive systems coexist throughout the animal taxa (1), the present evidence suggests that their evolutionary histories differ. For photodetection, all invertebrate PRCs examined employ $G_o$/*r-opsin*, and all vertebrate PRCs employ c-opsin (2, 3). Importantly, *c-opsin* is expressed in the ciliary PRCs in the brain of the polychaete worm, *P. dumerili*, whereas *r-opsin* is expressed in rhabdomeric PRCs in the eyes (13). Based on this result, Arendt *et al.* (13) have proposed that early metazoans possessed a single type of PRC with an ancestral opsin for light detection that later diversified into two distinct PRC and opsin types. The rhabdomeric PRCs (with r-opsin) were used in the eyes for photoreception, whereas the ciliary PRCs (with c-opsin) were incorporated into the evolving brain. These findings are consistent with vertebrates confining r-opsin to retinal ganglion cells apparently for photoperiodicity and using ciliary PRCs containing c-opsins exclusively for photoreception (rods and cones). Taken together, the data suggest that the ganglion cells of the vertebrate retina are the evolutionary descendents of rhabdomeric PRCs (3, 13). Moreover, because no identified *opsin* gene in cnidarians (27) contains a typical r-opsin fingerprint tripeptide HPK critical for coupling to the downstream phototransduction cascade, it was proposed that r-opsins are a bilaterian innovation that originated

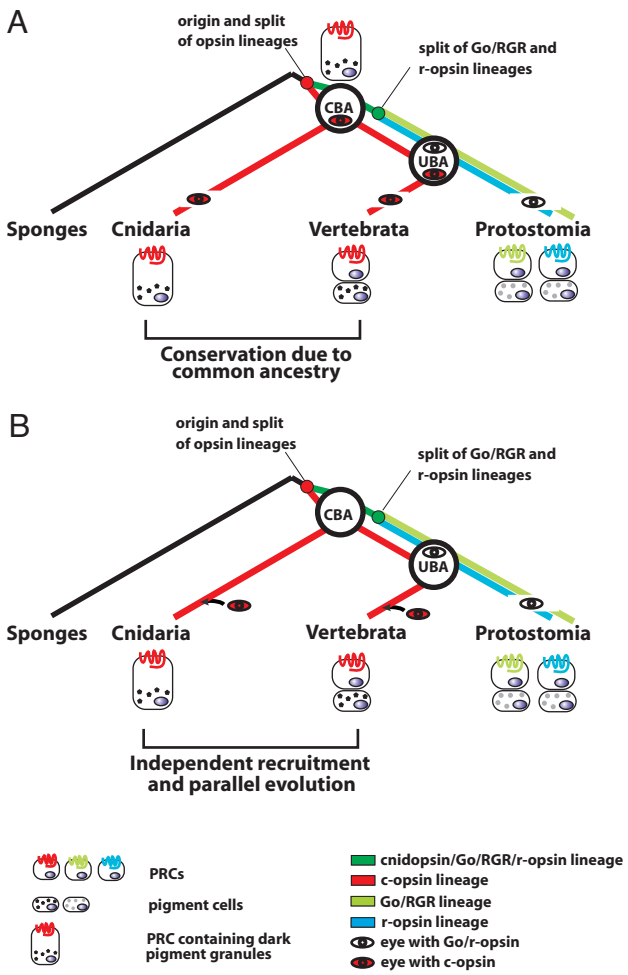


**Fig. 4.** Two scenarios for the use of ciliary phototransduction and melanogenic pathway in eye evolutionary history. A simplified view of the two evolutionary scenarios is compatible with the data in the present work. The use of similar genetic components in vertebrate and cubozoan eyes is either due to common ancestry (*A*) or independent parallel recruitments in cnidarian and vertebrate lineages (*B*). The *c-opsins* and $G_o$/*r-opsins* arose by duplication and diversification of an ancestral opsin in the early metazoans (27). In the schemes, only the visual (i.e., the eye-specific) PRCs and opsins are considered. Different shading of pigment granules indicates possible distinct chemical composition. CBA, cnidarian–bilaterian ancestor; UBA, urbilaterian ancestor.

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after the separation of the cnidarian and bilaterian lineages (Fig. 4) (27).

All eyes have shielding pigment typically found in cells adjacent to the PRCs. Melanin, the dark pigment of *Tripedalia* eyes, presumably performs the same function in vertebrate eyes as in the simple cup-like eyes of a basal lophotrochozoan, *Dugesia* (6). Interestingly, *Dugesia* uses another pigment, an ommochrome, as the body pigment (2, 6). Pterins constitute the dark eye pigment of the polychaete *P. dumerilii* (4), and pterins and ommochromes are the pigments in eyes of *Drosophila* (5). Thus, as with the opsins, *Tripedalia* shares the same dark pigment in the eye with vertebrates.

Unlike in the *Dugesia* eye, the camera-type *Tripedalia* eye combines the photoreceptor and pigment functions in the same cell consistent with an ancestral (basal) condition (Fig. 4*A*). However, the medusae stage of cubozoans may well be a derived rather than ancestral condition for Cnidaria, complicating discussions about the basal state of the cubozoan visual system (Anthozoans, for example, do not have eyes). Nevertheless, it remains possible that the pigmented PRCs in *Tripedalia* are descendants of one of the postulated ancient prototypical photosensitive cells diversified by natural selection (17, 28). However, this does not require a common origin for the eyes. It was estimated through computer-based modeling (29) that fewer than a half-million generations would be required under selective pressure to proceed from a cluster of light-sensitive cells to a sophisticated camera-type eye. In theory, this relatively short time interval would allow sophisticated eyes to have originated *de novo* several times during evolution (polyphyletic eye origin).

For the common-ancestry model to be true, the cnidarian-bilaterian ancestor (CBA) must have had the same genetic determinants as its descendants. The common-ancestry scenario for cubozoan and vertebrate eyes requires, however, that animals in many bilaterian phyla lost their eyes that were initially assembled by using the same building blocks as in present-day vertebrates and Cubozoa (*c-opsins*, *melanin*) to explain the exclusive occurrence of rhabdomeric PRCs in invertebrate eyes. There is no obvious explanation for such a specific selection against ciliary PRCs to be used for visual purposes. Eyes in general provide a freely moving animal with a tremendous advantage, and as such there should be a constant selection for eye maintenance, except in, for example, cave or underground animals.

Although not definitive, there are at least two additional complications to the common-ancestry model that arise if one invokes the developmental argument that similar transcription factor cascades may direct development of vertebrate and cubozoan eyes. The first is that PaxB, a Pax2/6/8-related transcription factor, is used in *Tripedalia* (30) instead of *Pax6* as in vertebrates (31) as well as flies (32, 33) and other species (34). The second is the apparent evolutionary "promiscuity" of developmental cascades in general; entire regulatory circuits can be co-opted for development of different cell types, tissues, or organs. For example, the *Pax–Six–Eya–Dach* gene regulatory network has a fundamental role in *Drosophila* visual system development but is also used for specification of muscle cells or placodes in vertebrates (35). Co-opting orthologous suites of genes for similar functions could be a possible explanation for independent or parallel evolution of cubozoan and vertebrate eyes with ciliary-type PRCs (Fig. 4*B*) (1, 36, 37). Independent derivation of *Tripedalia* and vertebrate eyes would also fit conceptually with the early idea that PRCs originated multiple times (38), although it does not address how many times PRCs themselves may have originated. That vertebrates and Cnidaria share many more genes than anticipated (39, 40), including *pax*, *mitf*, *c-opsin*, *pde*'s, *phosducin*, *guanylate cyclase*, and *oca2* (ref. 30 and this work), supports the notion that both animal groups use similar sets of genes to generate significantly different body plans. It follows that changes in gene regulation, rather than "new" genes, may drive novelties such as eyes during evolution. Finally, ectopic eye formation by misexpression of Pax6 provides an astounding example of how an eye might arise *de novo* in a foreign tissue environment (33, 34). The fact that ectopic eyes can be generated experimentally suggests that the same gene, *Pax*, used by various eyes of present-day animals could have been instrumental in creating eyes independently numerous times during evolution.

In addition to sharing the same genetic building blocks in their PRCs (ciliary phototransduction, melanogenic pathway), cubozoans and vertebrates both use a cellular lens to increase visual sensitivity and produce a sharp image in the desired plane of focus. The optical properties of cellular lenses are caused by the high-level expression of proteins collectively called crystallins (ref. 41 and this work). In striking contrast to the conservation of opsins as the visual pigments in the PRCs, the lens crystallins are diverse proteins that are often taxon-specific, i.e., entirely different proteins function as crystallins in different species. Similar transcription factors including those of the *Pax* gene family have been independently recruited for the regulation of nonhomologous crystallin genes in *Tripedalia* and vertebrates (30, 42, 43) to achieve a gradient of refractive index within their transparent lenses. The independent recruitment of lens crystallins is consistent with parallel evolution of cubozoan and vertebrate eyes and provides a striking example of the role of convergence in eye evolution.

Finally, the present findings of *mitf* in the lens and J1-crystallin in the pigmented slit and pit ocelli of *Tripedalia* support the idea that the cellular cubozoan lens arose from a pigmented cell ancestor. It is known that pigment cells may acquire the capacity to secrete lens-forming material (44). Combined, our data on J1-crystallin and *mitf* expression suggest that the cellular cubozoan lens with its remarkable ability to refract light without spherical aberration (11) originated from a pigment cell ancestor and that the primitive cup-like eyes located on the cubozoan rhopalia might be evolutionary forerunners of camera-type eyes.

In conclusion, the present study uncovers a surprising molecular parallelism in the eye design of vertebrates and cubozoan jellyfish. Although the current data do not distinguish unambiguously between the common-ancestry and independent-recruitment scenarios, we propose that they lean in the direction of the latter, favoring multiple independent reorganizations of common elements and independent recruitments of similar suites of genes during evolution of the diverse eyes.

## Materials and Methods

**Jellyfish Collection and Culture.** *T. cystophora* was collected and cultured as described in ref. 43.

**Isolation of Rhopalium-Expressed Genes and Phylogenetic Analysis.** An EST cDNA library was generated from rhopalia mRNA, and 2,433 individual clones from the library were sequenced by using an ABI capillary sequencer. The accession numbers for the clones are as follows: *c-opsin* (EU310498), *oca* (EU310502), *mitf* (EU310499), catalytic *pde* (EU310500), inhibitory *pde6d* (EU310501) and *guanylate cyclase* (EU310503). Details on phylogenetic analysis including the accession numbers of individual sequences are described in *SI Materials and Methods*.

**RNA *in Situ* Hybridization.** Jellyfish were fixed in 4% paraformaldehyde (PFA), cryoprotected in 30% sucrose overnight at 4°C, and embedded and frozen in OCT (Tissue Tek). RNA *in situ* hybridization was performed as described in ref. 43.

**Immunohistochemistry.** The cryosections were refixed in 4% PFA for 10 min, washed three times with PBS, permeabilized with PBT (PBS + 0.1% Tween 20) for 15 min, and blocked in 10% BSA in PBT for 30 min. The primary antibodies were diluted in 1% BSA in PBT, incubated overnight at room temperature, washed three times with PBS, and incubated with secondary antibodies in 1% BSA in PBT. The sections were counterstained with DAPI and mounted. Primary antibodies used were: anti-*Tripedalia* c-opsin, anti-*Tripedalia* J1-crystallin, and anti-acetylated tubulin (Sigma). The following secondary anti-

bodies were used: Alexa Fluor 488- or 594-conjugated goat anti-mouse or anti-rabbit IgG (Molecular Probes).

**Generation of Antibodies, COS-7 Cell Transfection, and Western Blotting.** Antibodies directed against *Tripedalia* c-opsin and J1-crystallin were prepared by immunization of rabbits as follows. The C-terminal region of *c-opsin* cDNA corresponding to amino acids 274–329 was cloned into the expression vector pET42, expressed in BL21(DE3)RIPL cells (Stratagene), and purified by using $His_6$ tag chromatography. The N-terminal peptide of J1-crystallin AAIVGSLVADAATQPVHK was attached to KLH via the C-terminal lysine and used for immunization. Monkey kidney COS-7 cells were transfected with CMV-c-opsin (amino acids 1–329) expression vector by using FuGENE6 reagent (Roche). Total extracts were prepared from c-opsin-transfected cells, mock-transfected cells, and rhopalia and were analyzed by Western blotting by using anti-c-opsin rabbit serum and chemiluminescent detection kit (Pierce). To avoid formation of multimeric opsin complexes, protein extracts from transfected cells were diluted and heated at low temperature (37°C) before SDS/PAGE.

**Fontana–Masson Method.** The cryosections were hydrated in distilled water and then incubated with Fontana silver nitrate working solution (2.5% silver nitrate) at 56°C for 1–2 h. After three washes in distilled water, sections were treated in 0.2% gold chloride at room temperature for 2 min, rinsed once in distilled water, placed in 5% sodium thiosulfate at room temperature for 1 min, washed again in water, and mounted.

**Melanin Bleach Procedure.** Bleaching was performed either after Fontana–Masson staining or RNA *in situ* hybridization. The sections were hydrated in distilled water and exposed to 0.25% potassium permanganate for 30 min at room temperature. The sections were treated with 5% oxalic acid for 5 min, washed with water, and mounted.

**Transmission Electron Microscopy.** Rhopalia excised from juvenile medusae were treated with Karnovsky fixative (2.5% glutaraldehyde, 2.5% paraformaldehyde in cacodylate buffer) for 24 h at 4°C. Fixed tissue was washed 12 h in 0.1% cacodylate buffer at 4°C. Karnovsky-fixed juvenile rhopalia and PFA-fixed adult rhopalia were postfixed in 2% $OsO_4$ for 2 h at 4°C and then washed in water. Samples were dehydrated in series of ethanol solutions, transferred to pure acetone, and embedded in Poly/Bed 812/Araldite 502 resin. Ultrathin sections (600–800 nm) were cut on Ultracut E (Reichert–Jung), placed on copper grids, and treated with 2.5% uranyl acetate for 1 h followed by lead citrate for 15 min. The material was examined by transmission electron microscopy (Jeol-1011), and images were taken with a MEGAview III Soft imaging system.

**Expression, Reconstitution, and Spectroscopic Analysis of *Tripedalia* c-opsin.** *Tripedalia* c-opsin cDNA was expressed in transfected COS-1 cells. Transfected cells were resuspended with 5 $\mu$M 11-*cis*-retinal, solubilized with 1% dodecyl maltoside, and the resulting c-opsin photopigment was purified by using immobilized 1D4 (Cell Culture Center, Minneapolis, MN). The UV-visible absorption spectrum was recorded for the c-opsin photopigment from 250 to 650 nm at 0.5-nm intervals by using the Hitachi U3010 dual-beam spectrometer at 20°C. Five replicates were performed in the dark and five more after 3 min of light exposure (with a $<$440-nm cut-off filter). The $\lambda_{max}$ value was taken from the dark–light difference spectrum.

For additional details, see *SI Materials and Methods*.

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