Current Biology 18, 51–55, January 8, 2008 ©2008 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.11.059

Report

Evolution and Functional Diversity of Jellyfish Opsins

Hiroshi Suga,[1] Volker Schmid,[1] and Walter J. Gehring[1,*]

[1]Department of Cell Biology
Biozentrum
University of Basel
Klingelbergstrasse 70
4056 Basel
Switzerland

Summary

Cnidaria are the most basal animal phylum possessing complex eyes [1]. Their eyes predominantly use ciliary photoreceptor cells (c-PRCs) like vertebrates, whereas insect eyes use rhabdomeric photoreceptor cells (r-PRCs) [1–4]. These two cell types show not only different cytoarchitectures but distinct phototransduction cascades, which are triggered by the respective types of opsins (e.g., [5]), ciliary opsins (c-opsins) and rhabdomeric opsins (r-opsins) [6]. Recent reports suggested that the c- and r-PRCs and their respective opsins diverged at least before the deuterostome-protostome split [7–9]. To study the earlier evolution of animal PRCs and opsins, we investigated two hydrozoan jellyfishes. We report here the first-characterized cnidarian opsins. Molecular phylogeny revealed that the cloned 20 jellyfish opsins, together with all the opsins from a hydra and some from a sea anemone, are more closely related to the c-opsins than to any other major opsin subfamily, indicating that the divergence of c- and r-opsins antedates the Cnidaria-Bilateria split. Possible scenarios of animal PRC evolution are discussed. Furthermore, *Cladonema* opsins show several distinct tissue- and stage-specific expression patterns. The expression of specific opsins in the eyes suggests a role in vision, whereas that in the gonads suggests a role in light-controlled release of gametes.

Results and Discussion

Jellyfish Opsin Genes

It is well known that light affects many behavioral activities of cnidarians, including diel vertical migration, responses to rapid changes in light intensity, and reproduction [1]. However, whether cnidarians use opsin (e.g., [5]) protein for perceiving light has remained unknown. Applying a polymerase chain reaction (PCR)-based approach on hydrozoan jellyfishes, we obtained as many as 18 full-length opsin complementary DNAs (cDNAs) from *Cladonema radiatum* (Figure 1A) with eyes (Figure 1B) and two from *Podocoryne carnea* (Figure 1C), which lacks eyes. All of the translated cDNAs showed overall sequence homologies to bilaterian rhodopsins. The conserved lysine, to which a chromophore 11-*cis*-retinal binds, was found in each of the cloned opsins, suggesting that they are indeed used for photoreception.

Molecular Phylogeny

To investigate the relationship between the cnidarian opsins and bilaterian opsins, we inferred a molecular phylogenetic tree by the maximum likelihood (ML) method (Figure 1D; see Figures S1 and S2 available online for the detail). By exploring the genomes of two other cnidarians, a hydra (J. Chapman, personal communication) and a sea anemone [10], we found 63 and 31 full-length opsins excluding pseudogenes, respectively. All of these opsins, except five from hydra (* in Figure S1), have the conserved lysine for retinal binding. On the basis of a preliminary phylogenetic analysis (Figure S1), we selected seven hydra and seven sea anemone opsins (# in Figure S1) for further analyses in order to simplify the tree. Figure 1D shows three distinct groups of cnidarian opsins: group 1, consisting of all the hydrozoan opsins and a subset of the sea anemone opsins, and groups 2 and 3, consisting of only sea anemone opsins. No opsins were found in the genomes of *Reniera sp.*, a sponge, and *Monosiga brevicollis*, a choanoflagellate (data not shown).

Intriguingly, the ML tree shows a close relationship of the cnidarian group 1 opsin cluster to the ciliary opsin (c-opsin) subfamily, with a local bootstrap probability of 79.4%. A Bayesian tree also supports this relationship, with a posterior probability of 0.84 (Figure S3). To validate this relationship, we statistically evaluated the branch position of the group 1 cluster by conducting another ML analysis on five different hypotheses: The group 1 cluster is the closest sister group to each of four major subfamilies, (hypothesis 1) c-opsin, (2) rhabdomeric opsin (r-opsin), (3) peropsin, and (4) G_o-coupled opsin (G_o-opsin), and (5) the group 1 cluster is the common ancestor of all four major subfamilies regardless of the topology among them. By any of the statistical criteria (log likelihood, Bayesian posterior probability [PP], usual bootstrap probability [BP], and approximately unbiased p value [AU] [11]), the first hypothesis, which claims the closest relationship between the group 1 cluster and the c-opsin subfamily, was supported most strongly (Table 1). Particularly, all of the other hypotheses were significantly rejected both by the $\Delta L_h / \sigma$ values and by the PP values ($\Delta L_h / \sigma \leq -1.0$ and $p < 0.05$). Although we cannot significantly reject any of the hypotheses by referring to the AU values ($p \geq 0.05$), the differences between the value of hypothesis 1 and those of hypotheses 2–5 are considerable.

We do not exclude the possibility that groups 2 and 3, novel opsin subfamilies consisting of sea anemone opsins, are closely related to any of the bilaterian opsin subfamilies. Their phylogenetic positions are, however, unclear even by more precise ML analyses, as done above for the group 1 (data not shown).

*Correspondence: walter.gehring@unibas.ch

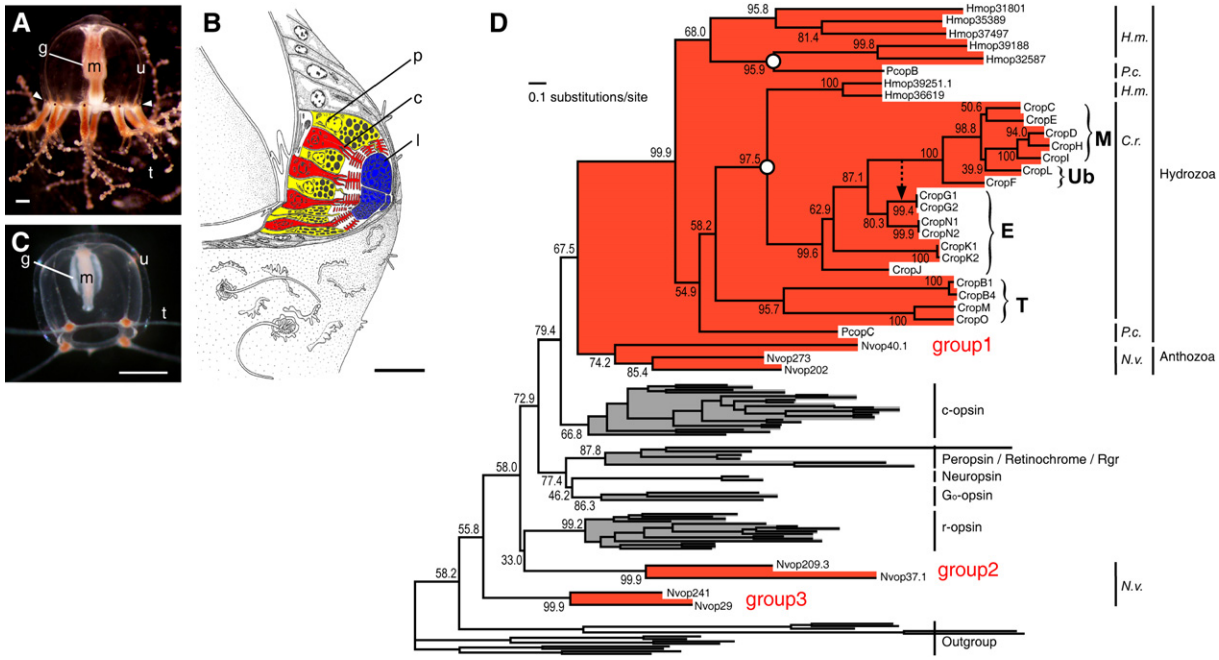

Figure 1. *Cladonema radiatum* and *Podocoryne carnea*

(A) Medusa of *Cladonema radiatum* with camera-type eyes located at the base of the tentacles (arrowheads).

(B) A schematic drawing of *Cladonema* eye (modified after [14]). Ciliary photoreceptor cells (c), pigment cells (p), and lens body (l) are highlighted in red, yellow, and blue, respectively.

(C) Medusa of *Podocoryne carnea*, which lacks eyes. The following abbreviations are used: gonads (g), manubrium (m), tentacle (t), and umbrella (u) (bell).

(D) Molecular phylogenetic tree of opsin family, including cnidarian sequences. Thirteen nonopsin G protein-coupled receptor sequences were used as an outgroup. Names of the genes are shown for the cnidarian sequences. Not all of the opsin genes found in the *Hydra* and *Nematostella* genomes were included; only the representative sequences for each group were included so that the tree could be simplified (see main text). The subtrees consisting of cnidarian sequences are shaded red, and each subfamily of bilaterian opsins is shaded gray. Body parts where the *Cladonema* opsins are expressed are indicated in bold letters (see main text): manubrium (M); ubiquitous (Ub); eye (E); and tentacle (T). An alternative branch position of the manubrium-specific (M) and the ubiquitously expressed (Ub) opsin group is indicated by a dashed arrow (see main text). The *Cladonema-Hydra* split and the *Podocoryne-Hydra* split are indicated by white circles. The detailed tree and accession numbers to the genes are in Figure S2. The following abbreviations are used: *Cladonema radiatum* (*C.r.*), *Hydra magnipapillata* (*H.m.*), *Nematostella vectensis* (*N.v.*), and *Podocoryne carnea* (*P.c.*).

Scale bars represent 300 μm in (A) and (C) and 20 μm in (B).

These data are in agreement with the fact that all known cnidarian photoreceptor cells (PRCs), except the one in *Tripedalia* larva [12], are of the ciliary type [1, 2, 4]. Furthermore, these data suggest an earlier divergence of the c- and r-opsin subfamilies than what has been suggested by recent reports [7–9]; it could have occurred even before the Cnidaria-Bilateria split. We propose here two possible scenarios for animal PRC type evolution. First, both the ciliary photoreceptor cell (c-PRC) and rhabdomeric photoreceptor cell (r-PRC) could have already been present in the common ancestor of cnidarians and bilaterians, and the r-PRC, as well as r-opsin, might have been lost in most of the cnidarian lineages (Figure S4A). Second, both c- and r-opsins and their respective downstream cascades could have been present in the ancestral PRC, which subsequently split into the two types of PRCs in the common ancestor of bilaterians (Figure S4B). The type of opsin used in the r-PRCs of *Tripedalia* larva has to be studied to refine the scenarios.

Figure 1D and Figure S1 show several gene duplications in the early evolution of Hydrozoa before the *Hydra-Cladonema* and *Hydra-Podocoryne* splits (white circles) and extensive gene duplications in each lineage after these splits. It is tempting to speculate that the large divergence of opsins might have contributed to the remarkable diversity of the photic behavior of cnidarians [1, 13].

Table 1. Evaluation of the Branch Position of Cnidarian Opsin Group 1

Hypotheses	$\Delta L_h \pm \sigma$ ($\Delta L_h / \sigma$)	PP	BP	AU
1. c-opsin	0.0 (0.0 : ML)	0.971	0.757	0.894
2. r-opsin	−5.6 ± 5.4 (−1.0)	0.003	0.014	0.141
3. Peropsin	−5.3 ± 4.8 (−1.1)	0.003	0.009	0.052
4. G_o-opsin	−6.5 ± 5.4 (−1.2)	0.002	0.006	0.101
5. Ancestral	−5.5 ± 5.5 (−1.0)	0.008	0.109	0.198

Five hypotheses regarding the branch position of the cnidarian opsin group 1 were statistically examined. ΔL_h and σ represent the difference in log likelihood relative to the ML tree and the standard error, respectively (see Supplemental Experimental Procedures for detail). The following abbreviations are used: Bayesian posterior probability calculated by the BIC approximation (PP), bootstrap probability calculated in the usual manner (BP), and p value of the approximately unbiased test (AU) [11]. The value is underlined when the hypothesis is not significantly rejected ($\Delta L_h / \sigma > -1.0$ or $p \geq 0.05$).

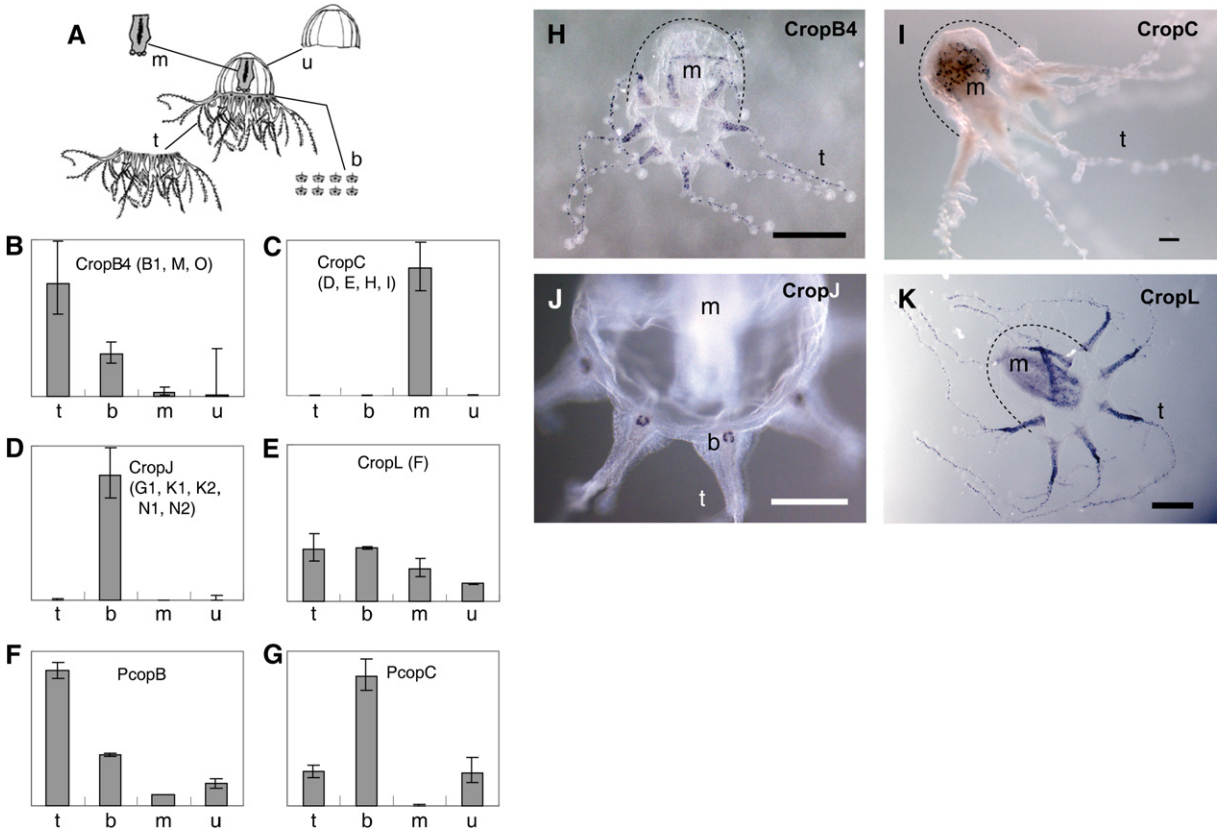

Figure 2. Expression Patterns of *Cladonema* and *Podocoryne* Opsin Genes

(A) For real-time PCR analysis, medusae were dissected into four body parts: tentacles (t), tentacle bulbs with eyes (b), manubrium (m), and umbrella (u) (bell).

(B–G) The cDNA from each part was analyzed by real-time PCR. The expression level of opsin in each body part relative to the whole body expression (1.0) was calculated and subsequently normalized to that of EF1α. The y axes are arbitrary. The expression of *Cladonema* opsins were categorized into four patterns (see text), and a typical example from each pattern is shown (B–E) even though all of the cloned genes, except CropG2, which is too closely related to CropG1, were analyzed (data not shown; only the names are shown in parentheses in the graph of their category representative). Expression patterns in male *Podocoryne* are shown in (F) and (G); female *Podocoryne* showed similar patterns to those of males (data not shown). Error bars represent standard deviations.

(H–K) In situ hybridization with antisense RNA probes of CropB4 (H), CropC (I), CropJ (J), and CropL (K). The original positions of the bells, which had shrunken during the hybridization procedures, are indicated by dashed lines (H, I, and K). Scale bars represent 200 μm.

Expression Patterns

To investigate the expression patterns of the hydrozoan opsins, we analyzed cDNAs from different body parts by real-time PCR (Figure 2A) and found that *Cladonema* opsins can be classified into four groups, according to their expressions: tentacle specific, manubrium specific, tentacle-bulb specific, and ubiquitous (Figures 2B–2E, respectively). These expression patterns were confirmed by whole mount in situ hybridization (Figures 2H–2K).

Two *Podocoryne* opsins show stronger expression in the tentacles and the tentacle bulbs than in the other body parts (Figures 2F and 2G). In particular, PcopC is primarily localized to the bulbs. Attempts to detect the expression of *Podocoryne* opsins by in situ hybridization were, however, not successful because of high background staining.

Eye-Specific Opsins of *Cladonema*

The expressions of eye-specific opsins were analyzed by in situ hybridization. CropG1 is expressed in the whole ocellus (Figure 3A). CropK1 and N1 are also expressed in a pattern similar to that of CropG1 (data not shown). In situ hybridization to paraffin sections revealed that CropG1 is expressed in the whole cluster of photoreceptor cell bodies behind the microvilli region (Figures 1B and 3B). Moreover, a real-time PCR analysis revealed that all of the eye-specific opsins are dramatically upregulated when the eyes are formed in medusa buds on sessile colonies (Figure 3C; see [14, 15] for the development of *Cladonema* eye). These data suggest that *Cladonema* possesses the eye-specific opsins for photoreception in the retina, like bilaterians.

In contrast, CropJ expression is restricted to several cells located at the boundary between retina and epidermal tissue (Figures 3D and 3E). We hypothesize that CropJ is expressed either (1) in a subset of PRCs that are newly made from epithelial cells at the edge of eye cup or (2) in neurons that hook up the retina to the rest of the nervous system. The former hypothesis is supported by an observation that CropJ is more strongly expressed in the eye of a just-detached (see Supplemental Experimental Procedures for definition) medusa

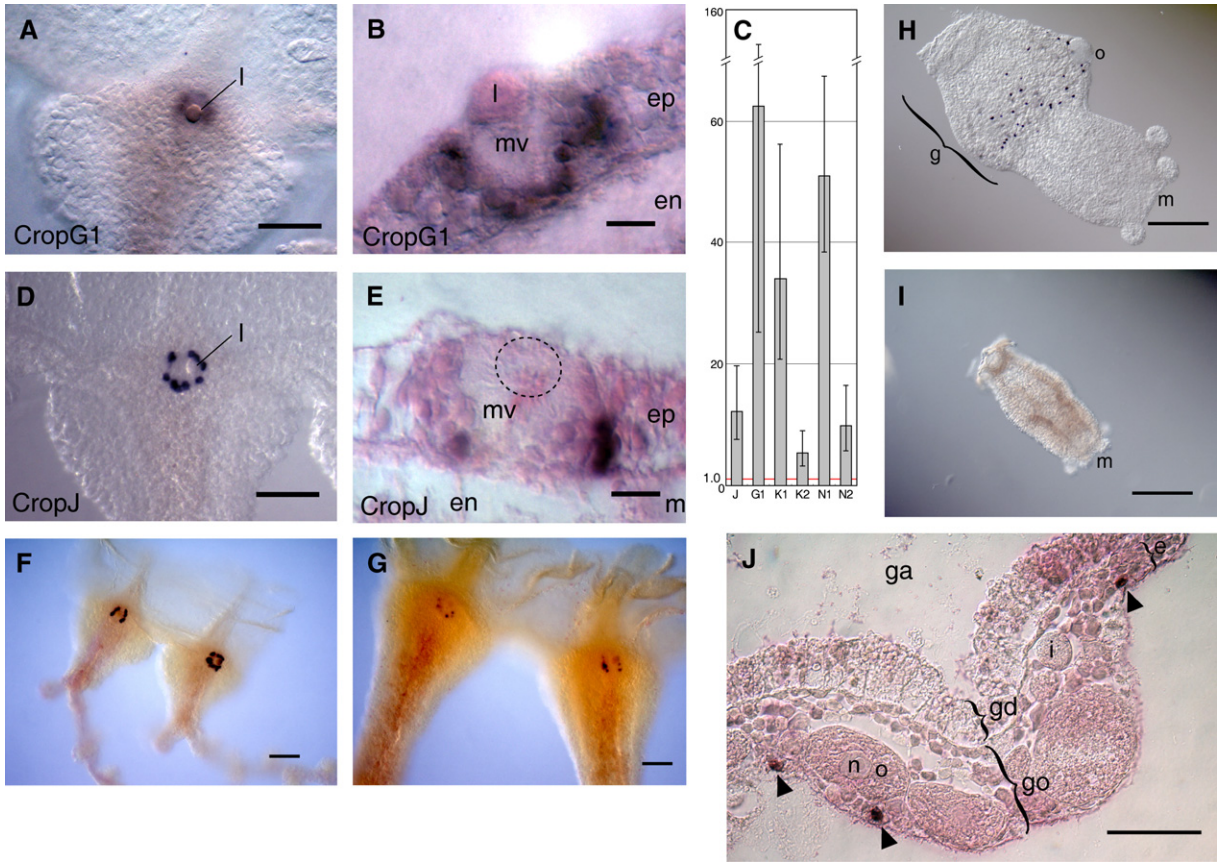

Figure 3. Expressions of Eye- and Manubrium-Specific Opsins

(A, B, D, and E) Whole-mount (A and D) and paraffin-section (B and E) in situ hybridizations were carried out with CropG1 (A and B) and CropJ (D and E) antisense RNA probes. The sections were counterstained with eosin solution. Note that the natural pigmentation in the eye had completely disappeared during the hybridization procedure. In (E), the position of the lens, which is not present in this section, is indicated by a dashed circle. The following abbreviations are used: endoderm (en), epidermis (ep), lens (l), mesogloea (m), and a region packed with microvillous processes of ciliary membranes of c-PRCs (mv) (see Figure 1B).

(C) Expression levels of the eye-specific opsins in medusa buds with eyes are compared with those in younger medusa buds that have not yet developed the eyes. The red line (1.0) indicates the average expression level in younger medusa buds without the eyes. Data are normalized to EF1α. Error bars represent standard deviations.

(F and G) In situ hybridization of CropJ to just-detached (F) and full-grown (G) medusae.

(H–J) Expression pattern of CropC. Whole-mount (H and I) and paraffin-section (J) in situ hybridizations were carried out on full-grown (H and J) and just-detached (I) medusae, and the manubriums were subsequently excised. The section was counterstained with eosin solution. Note that the gonads (g in [H]) with mature oocytes are not observed in just-detached medusae (I). In (J), the cells expressing CropC are indicated by arrowheads. The following abbreviations are used: epidermis (e), gonad part of manubrium (g), gastral cavity (ga), gastrodermis (gd), gonads (go), immature oocyte (i), mouth (m), nucleus (n), and mature oocyte (o).

Scale bars represent 50 μm in (A), (D), (F), (G), and (J), 10 μm in (B) and (E), and 200 μm in (H) and (I).

(Figure 3F) than a full-grown one (Figure 3G). A real-time PCR analysis has also confirmed it; in just-detached medusa, the CropJ expression is 14.5 times stronger on average than the full-grown one, whereas no such differences have been observed for the other eye-specific opsins, CropG1, K1, K2, N1, and N2 (1.0, 0.73, 0.33, 1.2, and 1.2 times, respectively; data are normalized to EF1α). The latter hypothesis is supported by positions of RFamide- (a neuronal marker) positive cells [15], which show a similar pattern in the ocellus to that of CropJ-expressing cells. Electron microscopy had also revealed a possible nerve cell, connected with the proximal end of PRC via a thin protrusion at the periphery of *Cladonema* retina [16]. If CropJ is not used for vision, then it might be used for adjusting the circadian rhythm, like vertebrate melanopsin in retinal ganglion cells [9].

Gonad-Specific Opsins of *Cladonema*

CropC is expressed in the cells that are scattered in the upper part of manubrium (Figure 3H), where the gonads develop [17]. Interestingly, CropC expression was not observed in the manubrium of just-detached medusae (Figure 3I), in which the gonads have not yet developed. CropD and CropE showed an identical expression pattern to the CropC (data not shown). A real-time PCR analysis has also demonstrated the very strong CropC expression in the full-grown medusae (120 times more on average than in the just-detached medusa; data are normalized to EF1α). Thin sections show that the CropC-expressing cells are located at the periphery of the gonads, from which gametes are directly released into the water, and all appear to have direct contacts with the thin epidermal cell layer that covers the ovaries (Figure 3J).

Many cnidarians, including *Cladonema* and *Podocoryne*, are triggered to spawn with the changing light conditions at dawn or dusk [1, 13]. Considering the position of the manubrium-specific-opsin-positive cells and the strong upregulation in the developing gonads, the manubrium-specific opsins in *Cladonema* are likely to be involved in the timing control of oogenesis or spawning process, possibly in cooperation with cryptochromes [18].

Figure 1D suggests that in *Cladonema*, the manubrium-specific opsins (M) had evolved from the eye-specific opsins (E) via ubiquitously-expressed opsins (Ub). This scenario has been validated by a more precise ML analysis, which significantly rejected all of the topologies ($\Delta L_h / \sigma \leq -1.0$; data not shown) except for two, the ML topology and the other topology indicated by a dashed arrow in Figure 1D. This observation might be in conflict with the fact that light-controlled spawning behavior is commonly observed in diverse lineages of Cnidaria [1, 13]. We speculate that in the *Cladonema* lineage, the manubrium-specific opsins have been recruited for controlling the spawning process after they branched from the eye-specific opsins, replacing the conventional opsins (not identified) that had controlled it before. Such flexibility in recruiting genes is often observed in evolution [19].

In this report, we have found as many as 18 opsins in a jellyfish *Cladonema* and two in *Podocoryne*. The molecular phylogenetic analyses indicated a very early divergence of c- and r-opsins before the Cnidaria-Bilateria split. Their expression patterns suggest two possible functions: a role in vision in the eye and that in the timing control of spawning. Our data shed some new light on the mechanisms of photic behavior of cnidarians.

Supplemental Data

Experimental Procedures and four figures are available at http://www.current-biology.com/cgi/content/full/18/1/51/DC1/.

Acknowledgments

We thank M. Koyanagi for discussion on the primer design and D. Graziussi and V. Charpignon for comments on the manuscript. We also thank J. Craig Venter institute for generating the sequences of cnidarian genomes, National Human Genome Research Institute for funding the genome project, and J. Chapman and the Rokhsar group for the assembly. H.S. was supported by Yamada Science Foundation for a long-term visit. The financial support of this project by the Swiss National Science Foundation and the Kantons of Basel-Stadt and Basel-Landschaft is gratefully acknowledged.

Received: September 5, 2007
Revised: November 11, 2007
Accepted: November 23, 2007
Published online: December 20, 2007

References

1. Martin, V.J. (2002). Photoreceptors of cnidarians. Can. J. Zool. *90*, 1703–1722.
2. Eakin, R.M., and Westfall, J.A. (1962). Fine structure of photoreceptors in the hydromedusan, *Polyorchis penicillatus*. Proc. Natl. Acad. Sci. USA *48*, 826–833.
3. Eakin, R.M. (1963). Lines of evolution of photoreceptors. In The General Physiology of Cell Specialization, D. Mazia and A. Tyler, eds. (New York: McGraw-Hill), pp. 393–425.
4. Singla, C.L. (1973). Ocelli of Hydromedusae. Cell Tissue Res. *149*, 413–429.
5. Terakita, A. (2005). The opsins. Genome Biol. *6*, 213.
6. Nilsson, D.E. (2004). Eye evolution: A question of genetic promiscuity. Curr. Opin. Neurobiol. *14*, 407–414.
7. Arendt, D., Tessmar-Raible, K., Snyman, H., Dorresteijn, A.W., and Wittbrodt, J. (2004). Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. Science *306*, 869–871.
8. Panda, S., Nayak, S.K., Campo, B., Walker, J.R., Hogenesch, J.B., and Jegla, T. (2005). Illumination of the melanopsin signaling pathway. Science *307*, 600–604.
9. Qiu, X., Kumbalasiri, T., Carlson, S.M., Wong, K.Y., Krishna, V., Provencio, I., and Berson, D.M. (2005). Induction of photosensitivity by heterologous expression of melanopsin. Nature *433*, 745–749.
10. Putnam, N.H., Srivastava, M., Hellsten, U., Dirks, B., Chapman, J., Salamov, A., Terry, A., Shapiro, H., Lindquist, E., Kapitonov, V.V., et al. (2007). Sea anemone genome reveals ancestral eumetazoan gene repertoire and genomic organization. Science *317*, 86–94.
11. Shimodaira, H., and Hasegawa, M. (2001). CONSEL: For assessing the confidence of phylogenetic tree selection. Bioinformatics *17*, 1246–1247.
12. Nordström, K., Wallén, R., Seymour, J., and Nilsson, D. (2003). A simple visual system without neurons in jellyfish larvae. Proc. Biol. Sci. *270*, 2349–2354.
13. Arkett, S.A. (1989). Hydromedusan photophysiology: An evolutionary perspective. In Evolution of the First Nervous Systems, P.A.V. Anderson, ed. (New York: Plenum Press), pp. 373–388.
14. Weber, C. (1981). Structure, histochemistry, ontogenetic development, and regeneration of the ocellus of *Cladonema radiatum* Dujardin (Cnidaria, Hydrozoa, Anthomedusae). J. Morphol. *167*, 313–331.
15. Stierwald, M., Yanze, N., Bamert, R.P., Kammermeier, L., and Schmid, V. (2004). The *Sine oculis/Six* class family of homeobox genes in jellyfish with and without eyes: Development and eye regeneration. Dev. Biol. *274*, 70–81.
16. Bouillon, J., and Nielsen, M. (1974). Etude de quelques organes sensoriels de Cnidaires. Arch. Biol. (Liege) *85*, 307–328.
17. Bouillon, J. (1994). Classe des hydrozoaires. In Traité de Zoologie, Cnidaires, Cténaires, Fascicule 2, Volume III, P.-P. Grassé, ed. (Paris: Masson).
18. Levy, O., Appelbaum, L., Leggat, W., Gothlif, Y., Hayward, D.C., Miller, D.J., and Hoegh-Guldberg, O. (2007). Light-responsive cryptochromes from a simple multicellular animal, the coral *Acropora millepora*. Science *318*, 467–470.
19. Miyata, T., and Suga, H. (2001). Divergence pattern of animal gene families and relationship with the Cambrian explosion. Bioessays *23*, 1018–1027.

Accession Numbers

Sequence data from this report have been deposited in GenBank/European Molecular Biology Laboratory Bank/DNA Data Bank of Japan databases under accession numbers AB332416–AB332435.