

22. C. Janus, M. A. Chishti, D. Westway, *Biochim. Biophys. Acta* **1502**, 63 (2000).  
 23. R. Kayed et al., *Science* **300**, 486 (2003).  
 24. J. Ghanta, C.-L. Shen, L. L. Kiessling, R. M. Murphy, *J. Biol. Chem.* **271**, 29525 (1996).  
 25. J. C. Sacchettini, J. W. Kelly, *Nature Rev. Drug Discov.* **1**, 267 (2002).  
 26. F. E. Cohen, J. W. Kelly, *Nature* **426**, 905 (2003).  
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## Ciliary Photoreceptors with a Vertebrate-Type Opsin in an Invertebrate Brain

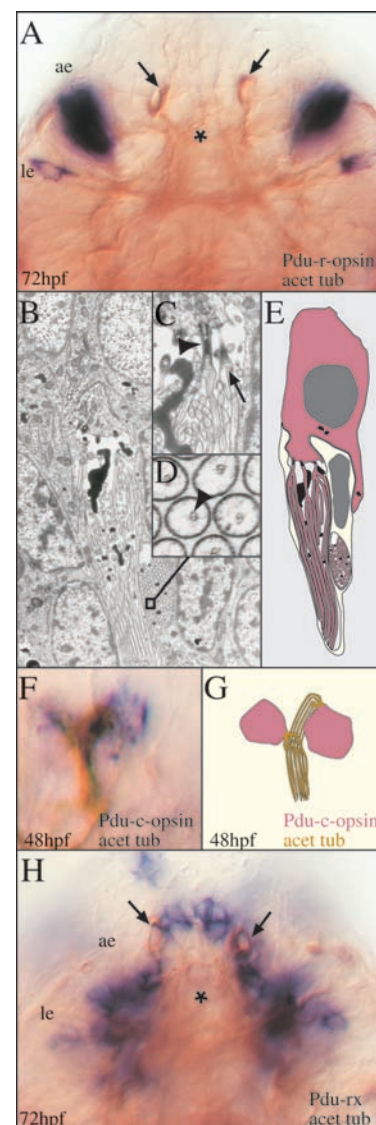
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For vision, insect and vertebrate eyes use rhabdomeric and ciliary photoreceptor cells, respectively. These cells show distinct architecture and transduce the light signal by different phototransducing cascades. In the marine ragworm *Platynereis*, we find both cell types: rhabdomeric photoreceptor cells in the eyes and ciliary photoreceptor cells in the brain. The latter use a photopigment closely related to vertebrate rod and cone opsins. Comparative analysis indicates that both types of photoreceptors, with distinct opsins, coexisted in Urbilateria, the last common ancestor of insects and vertebrates, and sheds new light on vertebrate eye evolution.

In animal photoreceptor cells (PRCs), the surface membrane is enlarged for the storage of opsin photopigment (1). Two major types of PRCs are recognized by electron microscopy (table S1). In rhabdomeric PRCs, the apical cell surface folds into microvilli. This is the predominant type of PRC used for vision in invertebrates. In contrast, in vertebrates, the rods and cones of the retina and the PRCs of the pineal eye, a light-sensitive structure in the dorsal diencephalon, are of the ciliary type. In ciliary PRCs, the membrane of the cilium is folded for surface enlargement. To elucidate the evolution of ciliary PRCs, we investigated the photosensitive system in *Platynereis dumerilii* (Polychaeta, Annelida, Lophotrochozoa) (2, 3). This species shows ancestral anat-

omy and development and an ancestral gene inventory (2, 4). In addition, polychaetes and vertebrates are evolutionarily far apart (2), and thus any feature specifically shared between them as a result of their common evolutionary heritage necessarily existed in Urbilateria, the last common ancestor of all animals with bilateral symmetry.

*Platynereis* develops different pairs of eyes (5), as demarcated by opsin expression in Fig. 1A: “larval eyes,” implicated in larval phototaxis, and “adult eyes,” active in adult vision. All these eyes use rhabdomeric PRCs. The two pairs of adult eyes originate from a single anlage that is not yet split at the stage of the analysis. To identify ciliary PRCs, we used an antibody directed against acetylated  $\alpha$ -tubulin (Fig. 1A) (3), which specifically labels stabilized microtubules in cilia and axons. In addition to the axonal scaffold, we detected two paired structures in the developing median brain, on the dorsal side of the apical organ (Fig. 1A, arrows). Electron microscopy (3) revealed that these structures represent multiple cilia (Fig. 1, B to E) that branch out into digits, each inheriting one of the peripheral microtubule doublets (Fig. 1, C and D). Such ciliated cells have been described for the brain of adult nereidids (6) and other polychaetes (7) and nemertines (8),



**Fig. 1.** *Pdu-c-opsin* and *Pdu-rx* expression in *Platynereis* ciliary photoreceptors (cPRCs). (A, F, H) Apical views of in situ hybridizations (blue) double-stained with an antibody to acetylated tubulin (brown). (A) *Pdu-r-opsin* expression (blue) 72 hours after fertilization (72 hpf), localized to larval and adult eyes (le, ae). (B) Electron micrograph of brain cPRCs. (C) Basal bodies of cilia (arrowhead), ramification in longitudinal section (arrow). (D) Ramifications in cross section with one doublet of microtubules (arrowhead). (E) Schematic based on (B). (F) *Pdu-c-opsin* expression (blue) in cPRCs (brown) at 48 hpf. (G) Schematic of (F). (H) Brain cPRCs (arrows) show high expression of *Pdu-rx*. Asterisks demarcate the apical organ. Magnification [(A) and (H)]  $\times 300$ , (B)  $\times 3000$ , (C)  $\times 3800$ , (D)  $\times 25,000$ , and (F)  $\times 1200$ .

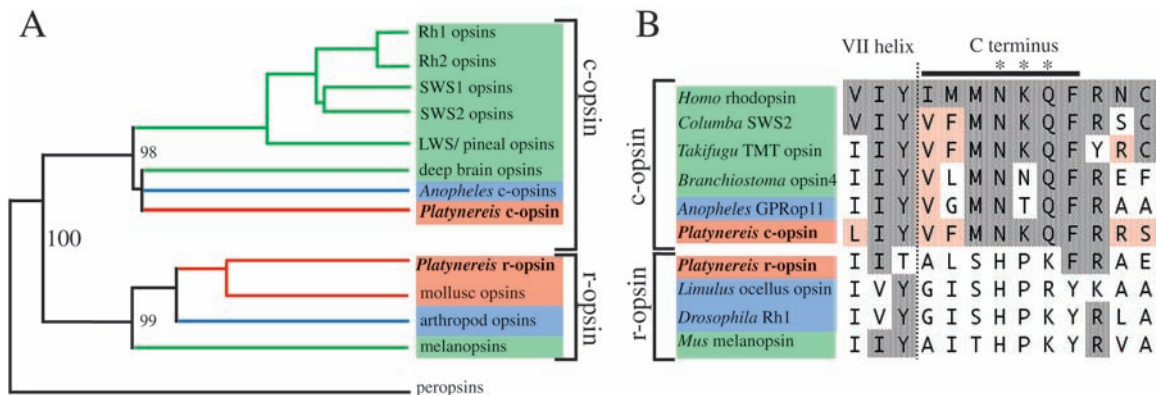
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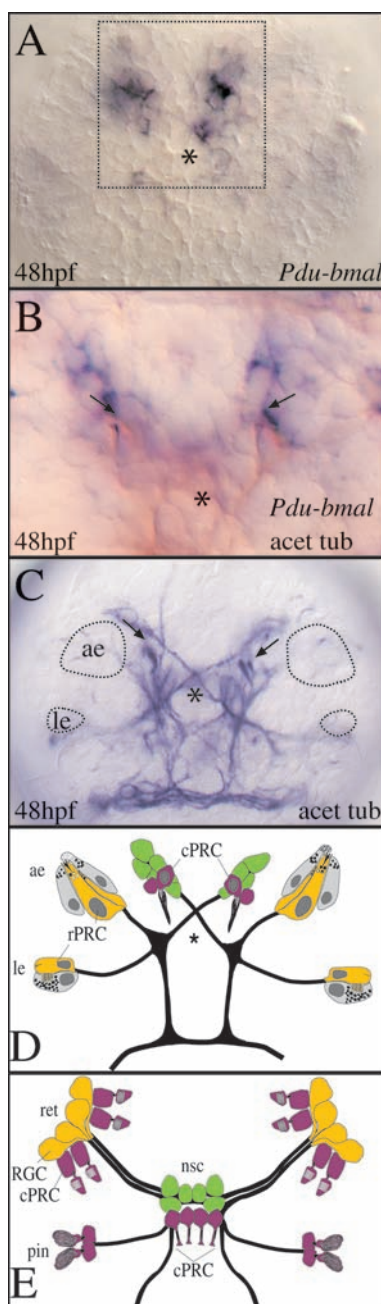
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**Fig. 2.** Rhabdomeric and ciliary opsins form distinct orthology groups. (A) Simplified tree of animal opsins as deduced from fig. S3. (B) Amino acid alignment at the transition from helix VII to the C-terminal tail. The bold line marks highly conserved sequence stretches. Asterisks mark tripeptide critical for G protein interaction. Gray shading denotes amino acids identical to human opsin2; red shading denotes amino acids identical to *Platynereis* c-opsin. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; and Y, Tyr.



**Fig. 3.** Comparison of photosensitive systems in the *Platynereis* and vertebrate brains. Double staining of in situ hybridizations as described in Fig. 1. Arrows in (B) and (C) demarcate cPRCs. (A) Apical views of *Pdu-bmal* expression in *Platynereis* brain at 48 hpf. Dotted box outlines area shown in (B). (B) Expression of *Pdu-bmal* in cPRCs at 48 hpf. (C) Axonal scaffold of *Platynereis* brain at 48 hpf, visualized by staining with an antibody to acetylated tubulin. (D and E) Schematic of cell types and axon tracts (black lines) in the polychaete (D) and vertebrate (E) developing brain [for vertebrates, see (25)]. Purple: ciliary PRCs; yellow: rhabdomeric photoreceptor/retinal ganglion cells; green: photoperiodicity neurons expressing *bmal*; gray: pigment cells. Abbreviations: ae: adult eye; cPRC: ciliary photoreceptor; le: larval eye; nsc: nucleus suprachiasmaticus; pin: pineal organ; ret: retina; RGC: retinal ganglion cell; rPRC: rhabdomeric photoreceptor. Asterisks demarcate the apical organ.



but not for the brain of *Drosophila* or *Caenorhabditis*. Given their vast ciliary surface extension, these cells had been considered sensory cells, possibly ciliary PRCs (6–8).

Photosensitivity of any of the presumed ciliary PRCs outside of chordates has not previously been addressed on the molecular level. To this end, and to determine whether the ciliated cells in the brain present a vertebrate-type molecular fingerprint, we searched for an opsin molecule and developmental control genes active in these cells. Because the previously characterized *Platynereis* opsin was not expressed in the ciliated cells (Fig. 1A, fig. S1A), we screened for additional *Platynereis* opsin molecules (3) and identified a second bona fide opsin gene encoding amino acids diagnostic for opsins (fig. S2). Expression analysis revealed that this opsin is indeed expressed in the ciliated cells in the brain, indicating that these cells function as photoreceptors (Fig. 1, F and G). Expression was highly specific. The gene was not detected in the rhabdomeric PRCs of the larval and adult eyes (fig. S1B).

Next we performed phylogenetic analysis of this second *Platynereis* opsin. Using neighbor-joining or maximum-likelihood methods, this opsin consistently clustered with the bulk of the vertebrate opsins and two opsins found in the *Anopheles* genome (3, 9) (Fig. 2A, figs. S3 and S4). This result indicates that two distinct opsin orthology groups exist in Bilateria: the ciliary opsins (c-opsins, active in ciliary PRCs in vertebrates and polychaetes) and the rhabdomeric opsins (r-opsins, active in rhabdomeric PRCs).

Orthology of *Platynereis* and vertebrate c-opsins is further supported by comparative sequence analysis (Fig. 2B, fig. S2). We detected a highly conserved stretch of amino acids (VFMNKQF) at the transition from transmembrane domain VII to the

cytoplasmic tail that is shared among the c-opsins but distinct in the r-opsins (Fig. 2B). This stretch plays an important role in heterotrimeric GTP-binding protein (G protein) interaction (10). All opsins catalytically activate heterotrimeric G proteins through interaction with the  $\alpha$  subunit, which is a  $G\alpha_q$ -subfamily member in the invertebrate rhabdomeric PRCs, distinct from the transducin  $G\alpha_t$  subunit active in the vertebrate rods and cones (table S1). In bovine opsin, alteration of the tripeptide NKQ (Fig. 2B, fig. S2) specifically diminishes the activation of transducin (10). A second stretch (REVLRM) with high conservation between *Platynereis* and vertebrate c-opsins, but not with r-opsins, lies at the transition between the third cytoplasmic loop and transmembrane domain VI. Again, this stretch overlaps another transducin activation site (fig. S2) (11). These findings unambiguously identify *Platynereis* c-opsin as a vertebrate-type ciliary opsin and suggest that it interacts with a transducin-like molecule.

If vertebrate and polychaete ciliary PRCs express orthologous opsins, do they also share the expression of transcription factors that specify their identity? In the differentiating vertebrate retina, expression of the *rx* (*retinal homeobox*) genes (12) is progressively restricted to the ciliary PRCs (rods and cones) in chick (*RaxL*) (13), frog (14), and fish (15). [Expression also persists in a small population of cells in the inner nuclear layer (13–16) but is absent from the retinal ganglion cells; see below.] Functional analysis in the chick retina shows that *rx* (*RaxL*) is selectively required for ciliary PRC development (13). Notably, *rx* is also expressed in the differentiating ciliary PRCs of the pineal eye (17). We have cloned a *Platynereis rx* ortholog, named *Pdu-rx* (3). This gene is expressed in the ciliary PRCs of the *Platynereis* brain, and in cells in and around the apical organ (Fig. 1H). Although we detected *Pdu-rx* in the ciliary PRCs, it was not expressed in the rhabdomeric PRCs of the larval and differentiating adult eyes (compare Fig. 1, A and H). Therefore, based on the shared expression of *rx* and *c-opsin* and on the shared morphology, we propose that polychaete and vertebrate ciliary PRCs are homologous cell types (18) (i.e., they evolved from the same precursor cell type in Urbilateria). The ciliary PRCs are conserved across Bilateria and are distinct from the rhabdomeric PRCs. In the vertebrate lineage, the ciliary PRCs diversified into a population of ciliary “deep brain photoreceptors” located in the ventral forebrain, the pineal PRCs, and the rods and cones of the retina (19) (Fig. 3E).

Because the *Platynereis* ciliary PRCs are not overtly associated with pigment cells, they might function in nondirectional photoreception such as the control of photoperiodic behavior, which is considered an ancestral characteristic of marine life cycles (20). To test this notion, we cloned and analyzed the *Platynereis bmal* gene (3), an ortholog of *Drosophila cycle* and vertebrate *bmal* genes encoding a key component of the circadian clock in flies and vertebrates (21). In *Platynereis*, *Pdu-bmal* is prominently expressed in the dorsal brain (Fig. 3, A and B), where it shows circadian rhythmicity in juvenile worms (22). The *Platynereis* ciliary PRCs are located within these *bmal*-positive dorsal brain regions, and they express *Pdu-bmal* (Fig. 3, B to D), consistent with a role in photoperiodicity. In line with this observation, in nonmammalian vertebrates, the light detected by the pineal ciliary PRCs directly entrains the circadian clock system located in these cells (23); in addition, the deep-brain photoreceptors form part of the *bmal*-expressing central circadian clock (19).

If the vertebrate ciliary PRCs belong to an ancestral bilaterian cell type distinct from the rhabdomeric PRCs, what happened to the rhabdomeric PRCs in the vertebrate line of evolution? Several lines of evidence suggest that they might have lost the rhabdomeric structure and persisted as retinal ganglion cells (RGCs). RGCs share with the invertebrate rhabdomeric PRCs the specific involvement of orthologous transcription factors in their differentiation (table S1, supporting online text). In addition, RGCs are photosensitive through the expression of melanopsins (24) that in phylogenetic trees cluster with the r-opsins of invertebrate rhabdomeric PRCs (Fig. 2, figs. S2 to S4). Melanopsins and r-opsins also share the highly conserved tripeptide HPK (in lieu of NKQ in c-opsins), as revealed by our comparison of functionally important G protein interaction sites (Fig. 2B, fig. S2).

We propose the following scenario for the evolution of animal PRCs and eyes. Early metazoans possessed a single type of precursor PRC that used an ancestral opsin for light detection and was involved in photoperiodicity control and possibly in phototaxis. In prebilaterian ancestors, the *opsin* gene then duplicated into two paralogs, *c-opsin* and *r-opsin*, allowing the diversification of the precursor PRC into ciliary and rhabdomeric sister cell types (table S1). The rhabdomeric PRCs associated with pigment cells to form simple eyes, whereas the ciliary PRCs formed part of the evolving brain, active in nondirectional photoreception. This ancestral setting

of Bilateria is still present in extant invertebrates such as *Platynereis* (Fig. 3D). In the evolutionary line leading to vertebrates, both photoreceptor types were incorporated into the evolving retina. The rhabdomeric PRCs transformed into ganglion cells, acquiring a new role in image processing. A distinctive feature of vertebrate eye evolution is that the ciliary (not rhabdomeric) PRCs became the main visual PRCs, the rods and cones (Fig. 3E). The vertebrate eye thus represents a composite structure, combining distinct types of light-sensitive cells with independent evolutionary histories.

## References and Notes

- L. v. Salvini-Plawen, E. Mayr, in *Evolutionary Biology*, M. K. Hecht, W. C. Steere, B. Wallace, Eds. (Plenum, New York, 1977), pp. 207–263.
- K. Tessmar-Raible, D. Arendt, *Curr. Opin. Genet. Dev.* **13**, 331 (2003).
- Materials and methods are available as supporting material on Science Online.
- F. Raible, D. Arendt, *Curr. Biol.* **14**, R106 (2004).
- D. Arendt, K. Tessmar, M. I. de Campos-Baptista, A. Dorrestein, J. Wittbrodt, *Development* **129**, 1143 (2002).
- N. Dhainaut-Courtois, *C. R. Acad. Sci. Paris* **261**, 1085 (1965).
- A. C. Whittle, D. W. Golding, *Cell Tissue Res.* **154**, 379 (1974).
- G. Vernet, *Ann. Sci. Nat. Zool.* **16**, 27 (1974).
- C. A. Hill *et al.*, *Science* **298**, 176 (2002).
- E. P. Marin *et al.*, *J. Biol. Chem.* **275**, 1930 (2000).
- R. R. Franke, T. P. Sakmar, R. M. Graham, H. G. Khorana, *J. Biol. Chem.* **267**, 14767 (1992).
- P. H. Mathers, A. Grinberg, K. A. Mahon, M. Jamrich, *Nature* **387**, 603 (1997).
- C. M. Chen, C. L. Cepko, *Development* **129**, 5363 (2002).
- M. Perron, S. Kanekar, M. L. Vetter, W. A. Harris, *Dev. Biol.* **199**, 185 (1998).
- J. C. Chuang, P. H. Mathers, P. A. Raymond, *Mech. Dev.* **84**, 195 (1999).
- K. Deschet, F. Bourrat, F. Ristoratore, D. Chourrout, J.-S. Joly, *Mech. Dev.* **83**, 179 (1999).
- S. Casarosa, M. Andreatzoli, A. Simeone, G. Barsacchi, *Mech. Dev.* **61**, 187 (1997).
- D. Arendt, *Int. J. Dev. Biol.* **47**, 563 (2003).
- B. Vigh *et al.*, *Histol. Histopathol.* **17**, 555 (2002).
- W. Gehring, M. Rosbash, *J. Mol. Evol.* **57** (suppl. 1), S286 (2003).
- R. N. Van Gelder, E. D. Herzog, W. J. Schwartz, P. H. Taghert, *Science* **300**, 1534 (2003).
- P. Edwards, P. W. H. Olive, personal communication.
- Y. Fukada, T. Okano, *Mol. Neurobiol.* **25**, 19 (2002).
- R. J. Lucas *et al.*, *Science* **299**, 245 (2003).
- S. W. Wilson, S. S. J. Easter, *Development* **112**, 723 (1991).
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## Supporting Online Material

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Materials and Methods

SOM Text

Figs. S1 to S4

Table S1

References

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