Expression of a homologue of a vertebrate non-visual opsin Opn3 in the insect photoreceptors

Mitsumasa Koyanagi1,2,3, Hayato Honda2, Hirohisa Yokono4, Ryu Sato2, Takashi Nagata2 and Akihisa Terakita1,2,3

1Department of Biology, Graduate School of Science, Osaka Metropolitan University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan
2Department of Biology and Geosciences, Graduate School of Science, and 3The OCU Advanced Research Institute for Natural Science and Technology, Osaka City University, 3-3-138 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan
4Department of Earth and Space Science, Graduate School of Science, Osaka University, 1-1 Machikaneyama-cho, Toyonaka, Osaka 560-0043, Japan

Insect vision starts with light absorption by visual pigments based on opsins that drive Gq-type G protein-mediated phototransduction. Since Drosophila, the most studied insect in vision research, has only Gq-coupled opsins, the Gq-mediated phototransduction has been solely focused on insect vision for decades. However, genome projects on mosquitos uncovered non-canonical insect opsin genes, members of the Opn3 or c-opsin group composed of vertebrate and invertebrate non-visual opsins. Here, we report that a homologue of Opn3, MosOpn3 (Asop12) is expressed in eyes of a mosquito Anopheles stephensi. In situ hybridization analysis revealed that MosOpn3 is expressed in dorsal and ventral ommatidia, in which only R7 photoreceptor cells express MosOpn3. We also found that Asop9, a Gq-coupled visual opsin, exhibited co-localization with MosOpn3. Spectroscopic analysis revealed that Asop9 forms a blue-sensitive opsin-based pigment. Thus, the Gi/Go-coupled opsin MosOpn3, which forms a green-sensitive pigment, is co-localized with Asop9, a Gq-coupled opsin that forms a blue-sensitive visual pigment. Since these two opsin-based pigments trigger different phototransduction cascades, the R7 photoreceptors could generate complex photoreponses to blue to green light.

This article is part of the theme issue ‘Understanding colour vision: molecular, physiological, neuronal and behavioural studies in arthropods’.

1. Introduction

Insects are some of the most vision-dependent animals, and numerous studies on vision from molecular to ecological levels have been conducted to uncover its molecular basis, cellular and neural responses, physiological roles and evolution [1,2]. It is well known that opsins are photoreceptor proteins that form photosensitive pigments by binding the chromophore retinal—basically 11-cis retinal—as the basis of visual photoception in insects as well as in other animals, including vertebrates. Opsins are members of G protein-coupled receptors (GPCRs) and act as light-sensitive GPCRs. Thousands of opsins have been identified from various animals, including insects, and are phylogenetically and functionally classified into several groups, many of which are characterized by G protein (α subunit) selectivity, such as Gt-, Gq-, Go-, Gs-coupled opsins, and so on [3,4]. Activation of each G protein α subunit triggers specific signal transduction cascades to produce cellular responses. Interestingly, phototransductions initiated by visual opsins vary between animals. Arthropods including insects employ Gq-coupled opsins for
vision [5–9], whereas vertebrates and jellyfish employ Gt-coupled and Gs-coupled opsins, respectively [10–13]. Although many animals possess opsins belonging to multiple opsin groups, the fruit fly Drosophila melanogaster, the most studied insect in vision research, has seven opsins and they all belong to the Gq-coupled opsin group. This has resulted in Gq-mediated phototransduction being the sole focus of insect visual phototransduction research for decades [14]. However, the genome project researching the malaria mosquito Anopheles gambiae found that Anopheles has 12 opsins [15]: 10 opsins (Agop1–Agop10) belong to the Gq-coupled opsin group and two opsins (Agop11 and Agop12) belong to the Opn3 or c-opsin group composed of vertebrate and annelid non-visual opsins [16–20]. We have investigated the molecular properties of the Asop12, a homologue of Opn3 from another malaria mosquito Anopheles stephensi (MosOpn3), using spectroscopic and biochemical analysis of the recombinant photopigment to reveal that MosOpn3 forms a green-sensitive Gi/Go-coupled opsin-based pigment [21]. Remarkably, MosOpn3 formed photopigments by binding the 13-cis form as well as the 11-cis form of A1 retinal. Since 13-cis retinal is thermally generated from all-trans retinal, a retinal isomer present in every tissue, the 13-cis retinal-binding property allows for the phototransduction formation of MosOpn3 in ‘non-photorceptor’ tissues [21]. In fact, we have shown by RT-PCR that MosOpn3 is expressed in the body, suggesting the presence of MosOpn3 in extracellular tissues. The analysis also demonstrated that MosOpn3 is expressed in the head. Here, we investigated in detail the expression of MosOpn3 in the eyes of the mosquito A. stephensi to reveal that MosOpn3 is expressed specifically in a single kind of photoreceptor R7 of the dorsal and ventral ommatidia, where Asop9, which forms a blue-sensitive Gq-coupled visual pigment, is also expressed. These findings could suggest that this mosquito species employs two types of phototransduction cascades mediated by Gq and Gi/Go in R7, which possibly could affect Anopheles colour vision.

2. Material and methods

(a) Animals
The mosquitoes (Anopheles stephensi) were reared from eggs kindly provided by Hirotaka Kanuka (Jikei University School of Medicine, Tokyo) and maintained according to the protocol [22].

(b) cDNA cloning
Partial cDNAs of opsins (Asop1, Asop8 and Asop9) of A. stephensi were obtained from RNA isolated from the head by RT-PCR. The primers used for PCR amplification were designed based on the gene sequences found in the genome databases. The full-length cDNAs of the opsins were obtained by using the 5’ RACE and 3’ RACE systems (Invitrogen) as described previously [23].

(c) Phylogenetic tree inference
The multiple alignment of the amino acid sequences of opsins was performed with the aid of XCED software [24]. Phylogenetic tree inference was performed as described [25]. Briefly, the phylogenetic tree was inferred by the neighbour joining method, and bootstrap analysis was carried out for statistical evaluation. The INSDC accession numbers of the sequences used for analysis are as follows: Hasarius adansonii Rh1, AB251846; Hasarius adansonii Rh2, AB251847; Drosophila melanogaster ninaE, K02315; Drosophila melanogaster Rh2, M12896; Drosophila melanogaster Rh3, Z86118; Anopheles stephensi Asop5, XM_036037676; Anopheles stephensi Asop1, LC710138; Anopheles stephensi Asop6, XM_036037677; Anopheles stephensi Asop7, XM_036047970; Hasarius adansonii Rh3, AB251848; Hasarius adansonii Rh4, AB504662; Drosophila melanogaster Rh3, Y00043; Drosophila melanogaster Rh4, M17730; Anopheles stephensi Asop8, LC710139; Drosophila melanogaster Rh5, U67905; Anopheles stephensi Asop9, LC710140; Drosophila melanogaster Rh7, AA49949; Anopheles stephensi Asop10, XM_036056155; Branchiostoma belcheri melanoopsin, AB205400; Homo sapiens Opn4 (melanopsin), AF147788; Homo sapiens Opn3 (enchopalsin), NM_014322; Branchiostoma belcheri Ampihopia, AB504668; Platynereis dumerilii ciliary opsin, AX692353; Anopheles stephensi MosOpn3 (Asop12), AB753162; Anopheles stephensi Asop11, XM_036048022; Papilio xuthus Opn3, KM099103; Papilio xuthus Opn3, KM099102; Synaptetrum frequens pteropsin, LC009057; Photinus pyralis Opn3, XM_031484106; Tribolium castaneum c-opsin, XM_001145478; Halicnerphta halpe Opn3, XM_0144229876;Apis cerana pteropsin, XM_017061054; Aphis gossypii Opn3, XM_027982987; Homo sapiens red, AH005298; Homo sapiens green, AH005296; Homo sapiens blue, AH003620; Homo sapiens rhodopsin, U49742.

Figure 1. The expression pattern of the Opn3 homologue, MosOpn3 in the A. stephensi eye. (a) In situ hybridization analysis of MosOpn3 (Asop12) in the mosquito eye. The signals derived from MosOpn3 mRNA (arrowheads) were detected in a specific kind of photoreceptor cells in dorsal and ventral ommatidia. (b) Visualization of cell nuclei using the Hoechst stain (magenta) in the eye, in which MosOpn3-expressing cells were stained by in situ hybridization. The locations of MosOpn3-expressing cell nuclei (arrowheads) indicated that MosOpn3 is expressed in R7. The schematic drawing of mosquito ommatidia is also shown based on the previous report [32]. The lens is illustrated at the top of the ommatidium. R7 (blue) and other photoreceptor cells (yellow) with nuclei (magenta) are shown. Rhabdomeres were also indicated (grey). The scale bars represent 100 μm in (a) and 30 μm in (b).

(d) In situ hybridization
Preparation of the RNA probes and in situ hybridization were carried out as previously described [26]. Briefly, digoxigenin (DIG)- and biotin-labelled antisense and sense RNA probes for

Rh6, Z86118; Anopheles stephensi Asop5, XM_036037676; Anopheles stephensi Asop1, LC710138; Anopheles stephensi Asop6, XM_036037677; Anopheles stephensi Asop7, XM_036047970; Hasarius adansonii Rh3, AB251848; Hasarius adansonii Rh4, AB504662; Drosophila melanogaster Rh3, Y00043; Drosophila melanogaster Rh4, M17730; Anopheles stephensi Asop8, LC710139; Drosophila melanogaster Rh5, U67905; Anopheles stephensi Asop9, LC710140; Drosophila melanogaster Rh7, AA49949; Anopheles stephensi Asop10, XM_036056155; Branchiostoma belcheri melanoopsin, AB205400; Homo sapiens Opn4 (melanopsin), AF147788; Homo sapiens Opn3 (enchopalsin), NM_014322; Branchiostoma belcheri Ampihopia, AB504668; Platynereis dumerilii ciliary opsin, AX692353; Anopheles stephensi MosOpn3 (Asop12), AB753162; Anopheles stephensi Asop11, XM_036048022; Papilio xuthus Opn3, KM099103; Papilio xuthus Opn3, KM099102; Synaptetrum frequens pteropsin, LC009057; Photinus pyralis Opn3, XM_031484106; Tribolium castaneum c-opsin, XM_001145478; Halicnerphta halpe Opn3, XM_0144229876;Apis cerana pteropsin, XM_017061054; Aphis gossypii Opn3, XM_027982987; Homo sapiens red, AH005298; Homo sapiens green, AH005296; Homo sapiens blue, AH003620; Homo sapiens rhodopsin, U49742.
A. stephensi MosOpn3 (Asop12), Asop1, Asop8 and Asop9 mRNAs were synthesized using the DIG RNA-labelling kit and Biotin RNA-labelling kit (Roche), respectively. In double in situ hybridization, DIG-labelled probes for MosOpn3 and biotin-labelled probes for Asop1, Asop8 or Asop9 were used. The mosquito heads were fixed in 4% paraformaldehyde, cryoprotected in 0.1 M phosphate buffer containing 30% sucrose, frozen with OCT Compound (Sakura Finetechnical) and sectioned at 12 µm. DIG-labelled probes were visualized with an alkaline phosphatase-conjugated anti-DIG antibody (Roche), followed by a blue 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium colour reaction. Cell nuclei were stained with Hoechst.

(e) Expression of the opsin-based pigment and spectroscopy

The cDNA of Asop9 was tagged with the monoclonal antibody rho 1D4 epitope sequence (ETSVQAPA). The tagged cDNA was inserted into expression vectors, pcDNA3.1 (Invitrogen) and pUSRα [27], and the recombinant protein was expressed in HEK293S cells. The Asop9-based pigments were reconstituted with 11-cis retinal (A1 retinal) as the standard method and the pigments were purified as described [28,29]. Note that the chromophore in A. stephensi is not determined but similarity of the absorption maximum wavelength between opsin-based pigments bearing A1 retinal and 11-cis 3-hydroxyretinal (A3 retinal), the chromophore in many dipterans, has been demonstrated theoretically.
and experimentally in a butterfly [30,31]. The absorption spectra of the pigment were recorded at 4°C by using spectrophotometers (UV2450; Shimadzu, Japan, V-750 UV-VIS Spectrophotometer; JASCO International, Japan). Blue and yellow lights were supplied by a 1 kW halogen lamp (Philips) with a 420 nm interference filter and a Y50 glass cutoff filter (Toshiba), respectively.

3. Results

(a) MosOpn3 expression in the mosquito eye

In the malaria mosquito (Anopheles stephensi), we have previously shown the expression of MosOpn3 (Asop12) in the head and body by RT-PCR [21]. Then, we investigated detailed expression patterns of MosOpn3 in the head of the mosquito by in situ hybridization analysis. As a result, the expression of MosOpn3 mRNA was specifically detected in ommatidia located in dorsal and ventral areas of the compound eye in the frontal section (figure 1a). In the ommatidia, MosOpn3 expression was observed only in a specific photoreceptor cell. The mosquito ommatidium is composed of eight photoreceptor cells, R1–R8, and they are assembled in a specific pattern; R8 is surrounded by R1 to R6, and R7 is located outside the Rh1–Rh6 ring [32,33]. Among them, R7 is distinguishable from other photoreceptor cells by the unique location of its nucleus, which is isolated from those of R1–6 at the bottom of the ommatidium [32]. Visualization of cell nuclei revealed two photoreceptor nuclear layers, one near the lens and the other near the bottom of the ommatidium (figure 1b). Nuclei of MosOpn3-expressing photoreceptor cells are located between the two nuclear layers, which matches one of the criteria of R7. These observations indicated that MosOpn3 is specifically expressed in R7 in dorsal and ventral ommatidia in the mosquito.

(b) Co-localization of MosOpn3 and a canonical insect visual opsin in photoreceptor cells

We next compared the relationships between the expression of MosOpn3 with those of canonical visual opsins, which belong to the Gq-coupled opsin group. We focused on Asop1, Asop8 and Asop9 as representatives of insect long-wavelength-sensitive (LWS), ultraviolet-sensitive (UVS) and short-wavelength-sensitive (SWS) visual opsin subgroups, respectively [34,35] (figure 2) to examine their expression patterns by in situ hybridization analysis. We found that Asop1 (LWS visual opsin) is expressed in many ommatidia around the whole eye, including dorsal region (figure 3a). In the ommatidia, the expression of Asop1 was observed in multiple photoreceptor cells, similar to the case of fruit fly Rh1, in which it is expressed in R1 to R6 among eight photoreceptor cells [36]. Then, we compared the expression pattern of the Asop1 and MosOpn3 by double in situ hybridization analysis. In the transverse section of ommatidia, the Asop1-expressing cells form a ring composed of six photoreceptor cells, in which MosOpn3-expressing R7 was not included, showing the mutually exclusive expression of Asop1 and MosOpn3 as well as expression of Asop1 in R1–R6 (figure 3b,c). On the other hand, in situ hybridization analysis of Asop8 (UVS visual opsin) and Asop9 (SWS visual opsin), together with visualization of cell nuclei revealed that the Asop8 is expressed in R7 of lateral ommatidia (figure 4a,b) and the Asop9 is expressed in R7 of dorsal and ventral ommatidia (figure 4c,d). The expression of Asop8 in R7 of lateral ommatidia is consistent with the case of A. gambiae revealed by immunohistochemical analysis [32]. In A. gambiae, Asop2, a member of LWS opsins and closely related to Asop1 [37], is expressed in R7 of dorsal and ventral ommatidia, whereas in A. stephensi, the Asop1 is expressed in R1–R6 of ommatidia around the whole eye (figure 3). In Aedes aegypti, Asop9 is expressed in all R7 and a subset of R8, showing the variation among mosquito species [38].

Then, we compared the expression patterns between MosOpn3 and these visual opsins to understand contributions of MosOpn3 to the vision. Double in situ hybridization analysis clearly showed that the Asop8 and MosOpn3 are exclusively expressed in different cells (figure 4e,f), and the Asop9 and MosOpn3 are expressed in the same cells, indicating co-localization of the Asop9 and MosOpn3 in R7 of the dorsal and ventral ommatidia (figure 4g,h).
We then experimentally determined the absorption spectrum of Asop9, a SWS visual opsin deduced from the phylogenetic classification (figure 2). We expressed the Asop9 in mammalian cultured cells and successfully obtained the purified Asop9-based pigment reconstituted with A1 retinal as the chromophore. Spectroscopic analysis of the Asop9-based pigment revealed that Asop9 forms the blue-sensitive pigment having an absorption maximum at approximately 430 nm (figure 5a).

The result is consistent with the case of Aedes aegypti Aaop9.
Asop9 is a bistable opsin, like other insect visual opsins. The photoproduct to the dark state. These are typical photoresponses which were revealed to have an optimal response to 400–450 nm light by the electroretinogram transgenic Drosophila [38]. Blue light irradiation of the Asop9-based pigment resulted in increase and decrease of the absorbance around 500 nm and around 400 nm, respectively (figure 5b). The spectral change by the light irradiation is explained by the conversion of the dark state to the photoproduct having red-shifted absorption spectrum. In addition, subsequent yellow light irradiation caused the opposite reaction, which indicates the reverse conversion of the photoproduct to the dark state. These are typical photoreactions of bistable opsins whose photoproducts also have an absorption maximum in the visible region, showing that Asop9 is a bistable opsin, like other insect visual opsins and MosOpn3.

4. Discussions
Molecular basis of insect vision has been investigated mainly in the fruit fly, and the Gq-mediated phototransduction has been believed to be the sole visual phototransduction in insects; Gqα subunit activates PLCβ, and depletion of PIP2 caused by PLCβ leads to activation of TRP/TRPL channels and then to photoreceptor cell depolarizations [2,40]. That was reasonable because the fruit fly only has Gq-coupled opsins. In this paper, we focused on an Anopheles mosquito, which has an opsin, MosOpn3 (Asop12), previously reported to form a green-sensitive Gi/Go-coupled pigment, in addition to canonical Gq-coupled opsins [21]. We found that MosOpn3 is expressed in the mosquito eye, specifically in R7 photoreceptors among eight photoreceptors of dorsal and ventral ommatidia (figure 1), which is to our knowledge the first report in any animal of Opn3 expression in visual photoreceptor cells. We also showed that the blue-sensitive visual opsin (Asop9) is co-expressed with MosOpn3 in R7 of dorsal and ventral ommatidia, whereas in R7 of lateral ommatidia, the UVS visual opsin (Asop8) is expressed but MosOpn3 is not, suggesting functional division of the two regions in the mosquito eye (figures 4 and 5e). Understanding the region-dependent variation in physiology would provide a clue to the physiological meaning of the co-localization. In the fruit fly, R7 of all ommatidia express UVS opsin, R8a or R8b, and underlie colour vision together with R8 [41,42]. Colour vision of mosquito was recently reported but an involvement of R7 in the mosquito colour vision is still unknown [43]. Further investigations would be needed to clarify whether the mosquito R7 photoreceptors underlie colour vision like in fruit flies or alternatively, are involved in mosquito-specific unknown physiologies.

Coexistence of two opsins has been reported in several insects such as fruit fly, butterfly and red flour beetle [44–46]. In those cases, however, both opsins are canonical insect visual opsins, members of the Gq-coupled opsin group. Outside insects, coexistence of two opsins with different G protein selectivity has been known in the photoreceptor portion of a single photoreceptor cell of lizard parietal eyes and teleost pineal organs [47–49] and photoreceptor cells of some invertebrates [50–52]. In the well-studied pineal-related organs of lower vertebrates, the UVS opsin (parapinopsin) or the blue-sensitive opsin (pinopsin) and the green-sensitive opsin (parietopsin) light-dependently activate Gi to decrease and Go to increase the intracellular cGMP level, respectively. The antagonistic response generates colour opponency, which could allow for wavelength discrimination by detecting the ratio between UV/blue and green [47,49]. If MosOpn3 and Asop9 are co-localized in the photoreceptive portion, rhabdomere of R7 of dorsal and ventral ommatidia, wavelength discrimination with a single photoreceptor could be possible by an antagonistic regulation involving the Gq-mediated signalling and the Gi/Gi-coupled cAMP/cGMP signalling. Alternatively, according to a recent report that Gi-mediated signalling enhances the Gq-mediated signalling [53], the light response based on the blue-sensitive Asop9 could be enhanced in the blue to green region by the green-sensitive MosOpn3. These ideas, however, are plausible when both MosOpn3 and Asop9 are co-localized in the same rhabdomere but direct evidence that shows co-localizations of two opsins in the R7 rhabdomere still remains to be found.

Interestingly, our double in situ hybridization analysis showed a different distribution of their mRNA; Asop9 mRNA is located near the lens and rhabdomere and MosOpn3 mRNA is located apart from them and relatively close to the nerve terminal (figure 4h). Judging from the previous findings that the distribution of mRNA is correlated with that of the protein [54,55], the different mRNA distribution could suggest more likely that Asop9 is located at rhabdomeres, but MosOpn3 is not and rather at the cytoplasm or terminal of R7. The idea is intriguing because at Drosophila photoreceptor synapses,
histamine H3 receptors—which are Gq-coupled—receive a neurotransmitter histamine to generate feedback loops [56], indicating the presence of Gαi subunits at photoreceptor synapses. Moreover, in Drosophila R7, the Gi-mediated signaling triggered by activation of histamine H3 receptors is involved in processing colour opponency at the photoreceptor level [42]. The Gi-mediated signal transduction at the synapses could be light-dependently activated by MosOpn3, resulting in the contribution of MosOpn3 to the light response processed by R7. Further investigation of the cellular localization of MosOpn3 is required to precisely discuss its function.

Many insects possess Opn3 homologues in addition to canonical Gq-coupled opsins; in other words, ‘Opn3-less’ insects, including the fruit fly, are rare (figure 2). Therefore, the co-expression of Gq and Gi/Go-coupled opsins might occur in photoreceptors of other insects, potentially modifying their visual function.

Thousands of opsins identified from varied animals are categorized as c-opsins (also called ciliary opsins) or r-opsins (also called rhodopsin opsins). The former trigger cyclic nucleotide phototransductions via Gi, Gq/o or Gs in ciliary photoreceptor cells, and the latter trigger phosphoinositol phototransductions via Gq in rhabdomeric photoreceptor cells [13,17,57]. Opn3 is assigned to c-opsin based on the evidence that annelid worm Opn3 is expressed in ciliary photoreceptor cells in the brain [17], and several Opn3s including MosOpn3 trigger cyclic nucleotide phototransductions at least in vitro [21,58]. Together with the fact that insect visual cells are rhabdomeric-type, current findings that the Opn3 homologue is expressed in visual cells in the mosquito are exceptional, indicating the plasticity of the relationship between opsin and photoreceptor cell type.

Ethics. All experiments using animals were approved by the Osaka City University animal experiment committee.

Data accessibility. DNA sequences reported in this paper are available in public databases under the INSDC accession nos. LC710138–LC710140.

Authors’ contributions. M.K.: conceptualization, funding acquisition, investigation, project administration, resources, supervision, visualization, writing—original draft and writing—review and editing; H.H.: investigation, resources, visualization and writing—original draft; H.Y.: conceptualization, investigation and resources; R.S.: investigation and validation; T.N.: formal analysis, investigation, resources and supervision; A.T.: funding acquisition, project administration, resources, supervision, visualization, writing—original draft and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This work was supported by JSPS KAKENHI grant nos. JP18H02482, JP21H00435 (to M.K.), JP15H05777, JP20K21431 (to A.T.); Japan Science and Technology Agency (JST) Precursory Research for Embryonic Science and Technology (PRESTO) grant no. JPMJPR13A2 (to M.K.) and JST Core Research for Evolutional Science and Technology (CREST) Grant JPMJCR1753 (to A.T.).

Acknowledgements. We thank Robert S. Molday (University of British Columbia) for supplying rho 1D4-producing hybridoma.

References

1. Borst A. 2009 Drosophila’s view on insect vision. Curr. Biol. 19, R64–R67. (doi:10.1016/j.cub.2009.05.008)

2. Hardie RC, Juusola M. 2015 Phototransduction in Drosophila. Curr. Opin. Neurobiol. 34, 37–45. (doi:10.1016/j.conb.2015.01.008)

3. Terakita A. 2005 The opsins. Genome Biol. 6, 213. (doi:10.1186/gb-2005-6-3-213)

4. Koyanagi M, Terakita A. 2014 Diversity of animal opsin-based pigments and their opotogenic potential. Biochim. Biophys. Acta 1837, 710–716. (doi:10.1016/j.bjba.2013.09.003)

5. Terakita A, Hariyama T, Tsukahara Y, Katsukura Y, Tashiro H. 1993 Interaction of GTP-binding protein Gq with photoactivated rhodopsin in the photoreceptor membranes of crayfish. FEBS Lett. 330, 197–200. (doi:10.1016/0014-5793(93)80272-V)

6. Lee YJ, Shah S, Suzuki E, Zars T, O’Day PM, Hyde DR. 1994 The Drosophila dgr gene encodes a G alpha protein that mediates phototransduction. Neuron 13, 1143–1157. (doi:10.1016/0896-6273(94)90052-3)

7. Yarfitz S, Hurley JB. 1994 Transduction mechanisms of vertebrate and invertebrate photoreceptors. J. Biol. Chem. 269, 14329–14332. (doi:10.1016/S0021-9258(17)36260-6)

8. Hardie RC, Raghu P. 2001 Visual transduction in Drosophila. Nature 413, 186–193. (doi:10.1038/35093002)

9. Koyanagi M, Terakita A. 2008 Gq-coupled rhodopsin subfamily composed of invertebrate visual pigment and melanopsin. Photochem. Photobiol. 84, 1024–1036. (doi:10.1111/j.1757-1097.2008.00369.x)

10. Kuhn H. 1980 Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes. Nature 283, 587–589. (doi:10.1038/283587a0)

11. Stryer L. 1986 Cyclic GMP cascade of vision. Annu. Rev. Neurosci. 9, 87–119. (doi:10.1146/annurev.ne.09.031866.000511)

12. You KW, Baylor DA. 1989 Cyclic GMP-activated Conductance of retinal photoreceptor cells. Annu. Rev. Neurosci. 12, 289–327. (doi:10.1146/annurev.ne.12.030189.001445)

13. Koyanagi M, Takano K, Tsukamoto H, Ohtsu K, Tokunaga F, Fukumoto S, Kanuka H. 2011 The role of Gq-coupled rhodopsin in the brain. Proc. Natl Acad. Sci. USA 108, 1143–1157. (doi:10.1016/j.jneurosci.2011.02.017)

14. Adams MD et al. 2000 The genome sequence of Drosophila melanogaster. Science 287, 2185–2195. (doi:10.1126/science.28754612185)

15. Hill CA et al. 2002 G protein-coupled receptors in Anopheles gambiae. Science 298, 176–178. (doi:10.1126/science.1067169)

16. Blackshaw S, Snyder SH. 1999 Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain. J. Neurosci. 19, 3681–3690. (doi:10.1523/JNEUROSCI.19-10-03681.1999)

17. Arens D, Tessmar-Raible K, Snyman H, Doresteijn AW, Wittbrodt J. 2004 Ciliary photoreceptors with a vertebrate-type opsin in an invertebrate brain. Science 306, 869–871. (doi:10.1126/science.1099955)

18. Ozdeslik RN, Olinski LE, Trieu MM, Oprian DD, Oancea E. 2019 Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with melanocortin 1 receptor. Proc. Natl Acad. Sci. USA 116, 1108–1117. (doi:10.1073/pnas.1903285116)

19. Nayak G et al. 2020 Adaptive thermogenesis in mice is enhanced by opsin 3-dependent adipocyte light sensing. Cell Rep. 30, 672–686. (doi:10.1016/j.celrep.2019.12.043)

20. Sato M et al. 2020 Cell-autonomous light sensitivity via opsin3 regulates fuel utilization in brown adipocytes. PLoS Biol. 18, e3000630. (doi:10.1371/journal.pbio.3000630)

21. Koyanagi M, Takada E, Nagata T, Tsukamoto H, Terakita A. 2013 Homologs of vertebrate Opn3 potentially serve as a light sensor in nonphotoreceptive tissue. Proc. Natl Acad. Sci. USA 110, 4998–5003. (doi:10.1073/pnas.1219416110)

22. Maekawa E, Aonuma H, Nelson B, Yoshimura A, Tokunaga F, Fukumoto S, Kanuka H. 2011 The role of proboscis of the malaria vector mosquito Anopheles stephensi in host-seeking behavior. Parasit. Vectors 4, 10. (doi:10.1186/1756-3305-4-10)

23. Koyanagi M et al. 2015 Diversification of non-visual photopigment parapipopsin in spectral sensitivity for diverse pineal functions. BMC Biol. 13, 73. (doi:10.1186/s12915-015-0174-9)
24. Katoh K, Standley DM. 2013 MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. (doi:10.1093/molbev/msu010)

25. Koyanagi M, Kawano E, Kinugawa Y, Oishi T, Shichida Y, Tamotsu S, Terakita A. 2004 Bistable UV pigment in the lampry pineal. Proc. Natl Acad. Sci. USA 101, 6687–6691. (doi:10.1073/pnas.0406819101)

26. Koyanagi M, Kubokawa K, Tsukamoto H, Shichida Y, Terakita A. 2005 Cephalochordate melanopsin: evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. Curr. Biol. 15, 1065–1069. (doi:10.1016/j.cub.2005.04.063)

27. Kayada S, Hicatomi O, Tokunaga F. 1995 Cloning and expression of frog rhodopsin cDNA. Comp. Biochem. Physiol. B 110, 599–604. (doi:10.1016/0305-0491(94)90179-X)

28. Terakita A, Tsukamoto H, Koyanagi M, Sugahara M, Yamashita T, Shichida Y. 2008 Expression and comparative characterization of Gq-coupled invertebrate visual pigments and melanopsin. J. Neurochem. 105, 883–890. (doi:10.1111/j.1471-4159.2007.05184.x)

29. Saito T, Koyanagi M, Sugihara T, Nagata T, Arikawa K, Terakita A. 2019 Evolution and expression of frog rhodopsins in Drosophila photoreceptors. PLoS ONE 6, e23121. (doi:10.1371/journal.pone.023121)

30. Sekharan S, Yokoyama S, Morokuma K. 2011 Quantum mechanical/molecular mechanical structure, entaniosellectivity, and spectroscopy of hydroxynitrals and insights into the evolution of color vision in small white butterflies. J. Phys. Chem. B 115, 1380–1388. (doi:10.1021/jp208107r)

31. Spaethe J, Briscoe AD. 2004 Early duplication and coexpression of two visual pigments in a mosquito retina: implications for color vision in small white butterflies. J. Comp. Physiol. B 174, 380–391. (doi:10.1007/s003590050369)

32. Koyanagi M, Nagata T, Katoh K, Yamashita S, Tokunaga F. 2008 Molecular evolution of arthropod color vision deduced from multiple opsin genes of jumping spiders. J. Mol. Evol. 66, 130–137. (doi:10.1007/s00239-008-0905-9)

33. Nagata T et al. 2012 Depth perception from image defocus in a jumping spider. Science 335, 469–471. (doi:10.1126/science.1211667)

34. O’Toole JE, Baehe W, Martin RL, Hirsh J, Pak WL, Applebury ML. 1985 The Drosophila ninae gene encodes an opsin. Cell 40, 839–850. (doi:10.1016/0092-8674(85)90435-3)

35. Sneath A, Briscoe AD. 2004 Early duplication and functional diversification of the opsin gene family in insects. Mol. Biol. Evol. 21, 1583–1594. (doi:10.1093/molbev/msh162)

36. Spaethe J, Briscoe AD. 2004 Early duplication and functional diversification of the opsin gene family in insects. Mol. Biol. Evol. 21, 1583–1594. (doi:10.1093/molbev/msh162)

37. Hu X, Whaley MA, Stein MM, Mitchell BE, O’Toole JE. 2011 Coexpression of spectrally distinct rhodopsins in Aedea argyrostylis R7 photoreceptors. PLoS ONE 6, e23121. (doi:10.1371/journal.pone.023121)

38. Govardinovski VI, Fyhrquist N, Reuter T, Kuzmin DG, Donner K. 2000 In search of the visual pigment template. Vis. Neurosci. 17, 509–528. (doi:10.1017/S0952523800174036)

39. Hardie RC, Franke K. 2012 Photomechanical responses in Drosophila photoreceptors. Science 338, 260–263. (doi:10.1126/science.1222776)

40. Yamaguchi S, Desplan C, Heisenberg M. 2010 Contribution of photoreceptor subtypes to spectral wavelength preference in Drosophila. Proc. Natl Acad. Sci. USA 107, 5634–5639. (doi:10.1073/pnas.0809398107)

41. Schnaitmann C, Halkata V, Abraham E, Oberhauser V, Thestrup T, Griesbeck O, Reiff DF. 2018 Color processing in the eye of the butterfly Papilio xuthus. Proc. Natl Acad. Sci. USA 115, 11310–11315. (doi:10.1073/pnas.1802592115)

42. Vocking O, Kourtis I, Tumuc SC, Hausen H. 2017 Co-expression of xenopsin and rhodopsin cDNA in photoreceptors bearing microvilli and cilia. Elife 6, e23435. (doi:10.7554/elife.23435)

43. Matsu R, Koyanagi M, Nagata A, Matsu Y, Kinoshita M, Arikawa K, Terakita A. 2019 Co-expression of opsins in the eye photoreceptor cells of the terrestrial slug Limax maximus. J. Comp. Neurol. 527, 3073–3086. (doi:10.1002/cne.24732)

44. Doring CC, Kumar S, Tumuc SC, Kourtis I, Hausen H. 2020 The visual pigment xenopsin is widespread in protostome eyes and impacts the view on eye evolution. Elife 9, e55193. (doi:10.7554/eLife.55193)

45. Pfeil EM et al. 2020 Heterotrichomes G protein subunit galphq is a master switch for Gbetagamma-mediated calcium mobilization by Gi-coupled GPCRs. Mol. Cell 80, 940–954; e5. (doi:10.1016/j.molcel.2020.10.027)

46. Johnston DS. 1995 The intracellular localization of messenger RNAs. Cell 81, 161–170. (doi:10.1016/0092-8674(95)90324-0)

47. Ike K, Tadauchi T, Takazawa PA, Vale RD, Matsumoto K, Herskowitz I. 2002 The Khd1 protein, which has three KH RNA-binding motifs, is required for proper localization of ASH1 mRNA in yeast. EMBO J. 21, 1158–1167. (doi:10.1093/emboj/21.5.1158)

48. Gavin BA, Arruda SE, Dolph PJ. 2007 The role of carcino in signaling at the Drosophila photoreceptor synapse. PLoS Genet. 3, e206. (doi:10.1371/journal.pgen.0030206)

49. Yau KW, Hardie RC. 2009 Phototransduction motifs and variations. Cell 139, 246–264. (doi:10.1016/j.cell.2009.09.029)

50. Tsukamoto H, Chen IS, Kubo Y, Furutani Y. 2017 A ciliary opsin in the brain of a marine annelid zooplankton is ultraviolet-sensitive, and the sensitivity is tuned by a single amino acid residue. J. Biol. Chem. 292, 12 971–12 980. (doi:10.1074/jbc.M117.793539)