 doi:10.20944/preprints202209.0432.v1

Review

Deep Diversity: Extensive Variation in the Components of Complex Visual Systems across Animals

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Abstract: Understanding the molecular underpinnings of the evolution of complex (multi-part) systems is a fundamental topic in biology. One unanswered question is the extent to which similar or different genes and regulatory interactions underlie similar complex systems across species. Animal eyes and phototransduction (light detection) are outstanding systems to investigate this question because some of the genetics underlying these traits are well-characterized in model organisms. However, comparative studies using non-model organisms are also necessary to understand the diversity and evolution of these traits. Here, we compare the characteristics of photoreceptor cells, opsins, and phototransduction cascades in diverse taxa, with particular focus on cnidarians. In contrast to the common theme of deep homology, whereby similar traits develop mainly using homologous genes, comparisons of visual systems - especially in non-model organisms - are beginning to highlight a "deep diversity" of underlying components, illustrating how variation can underlie similar complex systems across taxa. Although using candidate genes from model organisms across diversity was a good starting point to understand the evolution of complex systems, unbiased genome-wide comparisons and subsequent functional validation will be necessary to uncover unique genes that comprise complex systems of non-model groups to better understand biodiversity and its evolution.

Keywords: eye evolution; opsin; photoreceptor; phototransduction; visual cycle

Introduction

A leading question in convergent evolution is: how similar or different are the genes and regulatory mechanisms underlying complex traits between species? Investigating the genetic basis for the evolution of complex systems over broad evolutionary timescales will inform us about conservation and convergence of genes and gene function, with fundamental implications for our ability to predict gene function across species and at different scales of biological organization. There are different mechanisms by which complex trait evolution might occur. One model proposes that complex traits evolve mainly by recruitment of homologous genes or existing genetic pathways (Monteiro and Podlaha 2009). Here, the genes and regulatory networks encoding complex traits are similar across organisms, in line with the idea of 'deep homology', where gene function is often conserved over vast evolutionary timescales (Shubin, Tabin, and Carroll 2009). In addition, traits may also evolve by co-opting from multiple existing genetic pathways and/or incorporating novel genes to form unique connections (Srivastava 2021). Here, rather different genes and gene networks may encode similar complex traits. These models are not mutually exclusive, and many traits could be encoded by a mix of homologous and non-homologous genes. Identifying the extent of constraint and co-option and finding general rules to explain the variety of results is still a major challenge in evolutionary biology.

Animal eyes are an excellent trait for investigating the evolution of complex systems for several reasons. First, eyes are diverse and vary in complexity, ranging from lens eyes,

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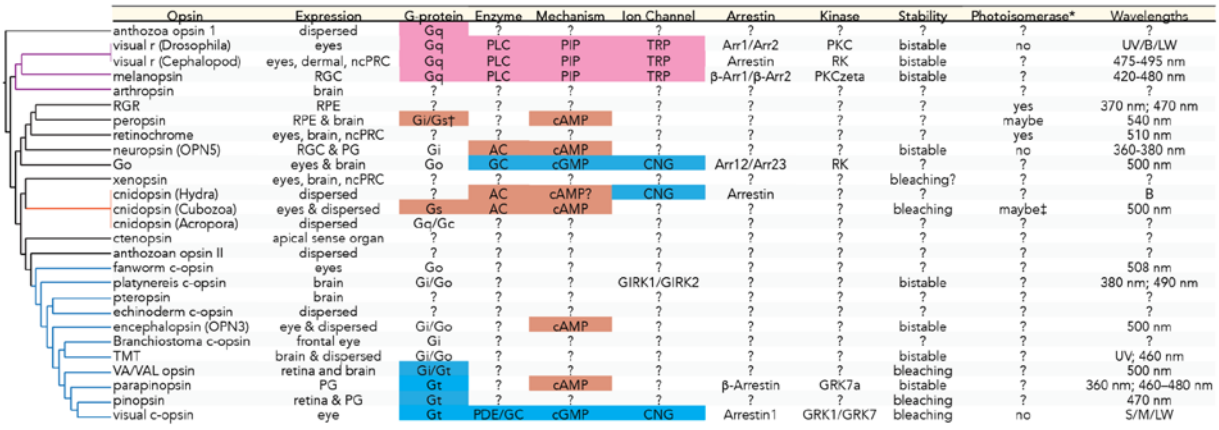
to compound eyes or simple eyespots. The evolutionary origin of eyes and photoreceptor cells has been a matter of ongoing debate aiming to determine whether they evolved multiple times independently, or share a common origin (L. V. Salvini-Plawen 2008; Goldsmith 2013; Serb, Porath-Krause, and Pairett 2013; Gehring 2014). Within animals, eyes may have evolved at least 40-60 times and some recent molecular studies suggest this number might be even higher (L. von Salvini-Plawen and Mayr 1977; Koenig and Gross 2020). Second, numerous studies have characterized many genes involved in eye development and phototransduction in model bilaterians (Hardie 2001; Trevor D. Lamb, Collin, and Pugh 2007; Fain, Hardie, and Laughlin 2010). These genes are used as a starting point to study visual systems of other animals. Yet, whether these models and candidate genes are predictive of biology and evolutionary trends in other organisms remains to be determined.

Here, we review literature on the evolution of eyes, photoreceptors, and opsins, as well as opsin expression and proposed functions, including mechanisms of phototransduction across animals (Fig 1). A close look at the literature reveals diversity that is overshadowed not only by the ease of looking only for candidate genes across species, but also by the traditional approach of classifying photoreceptor cells and opsins into ciliary cells employing 'c-opsins' and rhabdomeric cells employing 'r-opsins'. To emphasize the diversity that can underlie opsin-based light detection across animals, we highlight Cnidaria by mining existing data to uncover potential genes involved in their phototransduction and the visual cycle. As a sister group to Bilateria, studies in Cnidaria inform us about the conservation and repeatability of genes encoding for eye development and function. In particular, we explored expression of phototransduction and visual cycle gene homologs in four cnidarian species: *Hydra*, which lacks eyes, and *Aurelia*, *Tripedalia*, and *Sarsia* which each evolved eyes convergently (Picciani et al. 2018). Overall, we find that the representative phototransduction cascade of cnidopsin is distinct from the visual Gt and Gq opsins (Fig 1), some cnidopsins may serve photoisomerase roles with unknown cascades, and visual cnidopsins found in convergent eyes may have variation in their cascades.



Opsin	Expression	G protein	Enzyme	Mechanism	Ion Channel	Arrestin	Kinase	Stability	Photoisomerase*	Wavelengths
anthozoa opsin 1	dispersed	Gq	?	?	?	?	?	?	?	?
visual r (Drosophila)	eyes	Gq	PLC	PIP	TRP	Arr1/Arr2	PKC	bistable	no	UV/B/LW
visual r (Cephalopod)	eyes, dermal, ncPRC	Gq	PLC	PIP	TRP	Arrestin	RK	bistable	?	475-495 nm
melanopsin	RGC	Gq	PLC	PIP	TRP	β-Arr1/β-Arr2	PKCzeta	bistable	?	420-480 nm
arthropsin	brain	?	?	?	?	?	?	?	?	?
RGR	RPE	?	?	?	?	?	?	?	yes	370 nm; 470 nm
peropsin	RPE & brain	Gi/Gs†	?	cAMP	?	?	?	?	maybe	540 nm
retinochrome	eyes, brain, ncPRC	?	?	?	?	?	?	?	yes	510 nm
neuropsin (OPN5)	RGC & PG	Gi	AC	cAMP	?	?	?	bistable	no	360-380 nm
Go	eyes & brain	Go	GC	cGMP	CNG	Arr12/Arr23	RK	?	?	500 nm
xenopsin	eyes, brain, ncPRC	?	?	?	?	?	?	bleaching?	?	?
cnidopsin (Hydra)	dispersed	?	AC	cAMP?	CNG	Arrestin	?	?	?	B
cnidopsin (Cubozoa)	eyes & dispersed	Gs	AC	cAMP	?	?	?	bleaching	maybe‡	500 nm
cnidopsin (Acropora)	dispersed	Gα/Gc	?	?	?	?	?	?	?	?
ctenopsin	apical sense organ	?	?	?	?	?	?	?	?	?
anthozoan opsin II	dispersed	?	?	?	?	?	?	?	?	?
fanworm c-opsin	eyes	Go	?	?	?	?	?	?	?	508 nm
platynereis c-opsin	brain	Gi/Go	?	?	GIRK1/GIRK2	?	?	bistable	?	380 nm; 490 nm
pteropsin	brain	?	?	?	?	?	?	?	?	?
echinoderm c-opsin	dispersed	?	?	?	?	?	?	?	?	?
encephalopsin (OPN3)	eye & dispersed	Gi/Go	?	cAMP	?	?	?	bistable	?	500 nm
Branchiostoma c-opsin	frontal eye	Gi	?	?	?	?	?	?	?	?
TMT	brain & dispersed	Gi/Go	?	?	?	?	?	bistable	?	UV; 460 nm
VA/VAL opsin	retina and brain	Gi/Gt	?	?	?	?	?	bleaching	?	500 nm
parapinopsin	PG	Gt	?	cAMP	?	β-Arrestin	GRK7a	bistable	?	360 nm; 460–480 nm
pinopsin	retina & PG	Gt	?	?	?	?	?	bleaching	?	470 nm
visual c-opsin	eye	Gt	PDE/GC	cGMP	CNG	Arrestin1	GRK1/GRK7	bleaching	no	S/M/LW

Figure 1. Diversity of opsins and phototransduction cascades. Left: Opsin phylogeny with purple branches for traditional r-opsins, blue branches for c-opsins, orange branches for cnidopsins. Right: Table with opsin characteristics. Typical components of r-opsins are in pink boxes, of c-opsin in blue, and of cnidopsin in orange. *By photoisomerase activity we refer to chromophore isomerization from *trans* to *cis* and release to replenish rhodopsin. †Peropsin was mutated and signaled in the dark; it is likely dark activated. ‡A different cnidopsin from that used in the eyes for phototransduction. Details of evidence and references are in Supplemental Table 1. Abbreviations: RGC- retinal ganglion cells, RPE- retinal pigment epithelial, ncPRC- non-cephalic photoreceptor cells, PG- pineal gland, PLC- phospholipase C, AC- adenylyl cyclase, GC- guanylyl cyclase, PDE- phosphodiesterase, PIP- phosphatidylinositol 4,5-bisphosphate, TRP- transient receptor potential, CNG- cyclic nucleotide gated, GIRK- G protein gated inward rectifier potassium, Arr- Arrestin, PKC- protein kinase C,

 doi:10.20944/preprints202209.0432.v1

RK- rhodopsin kinase, GRK- G protein-coupled receptor kinase, UV- ultraviolet, B- blue, LW-long wavelength, S- short, M- medium.

Evolution of eyes and photoreceptor cell types

PRC evolution

Photoreceptor cells (PRC) are photosensitive neurons and the number of times these visual structures evolved is a matter for debate. Before the advent of molecular techniques, the main criteria to distinguish between PRCs was morphology (R. M. Eakin 1965). Most PRCs use cell surface enlargements to employ large amounts of the visual pigment rhodopsin to detect light (D. Arendt and Wittbrodt 2001; Detlev Arendt 2003; D. E. Nilsson 2009; D.-E. Nilsson 2013; Gehring 2014). Some PRCs have modified cilia, referred to as ciliary PRCs (Fig 2A). Others have microvilli, as in the arthropod rhabdom, leading to the name rhabdomeric PRCs (Fig 2B). Even though the distinction between ciliary and rhabdomeric PRCs is oversimplified, this differentiation has been widely used and led to different interpretations of their evolution (R. M. Eakin 1965; L. von Salvini-Plawen and Mayr 1977; Detlev Arendt 2003; L. V. Salvini-Plawen 2008). Eakin suggested a common evolutionary origin with two main lines of photoreceptors, a ciliary line in cnidarians and deuterostomes and a rhabdomeric line in protostomes (Richard M. Eakin 1982). Vanfleteren suggested a ciliary origin for all photoreceptors and an inductive function of cilia in rhabdomeric PRCs (Vanfleteren 1982). In contrast, von Salvini-Plawen & Mayr (1977) rejected common evolutionary origin and strongly promoted a polyphyletic origin of photoreceptors due to structural and cellular differences (L. von Salvini-Plawen and Mayr 1977). Some recent studies have uncovered diversity obscured by a binary division of PRCs. For example, it is not unusual to find a cilium, or at least remnants of a cilium, in otherwise rhabdomeric protostome eye PRCs (Schmidt-Rhaesa 2007) or PRCs with no cell surface enlargements at all (L. V. Salvini-Plawen 2008). Thus, to decipher the evolutionary history of PRCs, it will be important to understand these cell types in light of the cellular and molecular features that define their diversity and evolutionary history.

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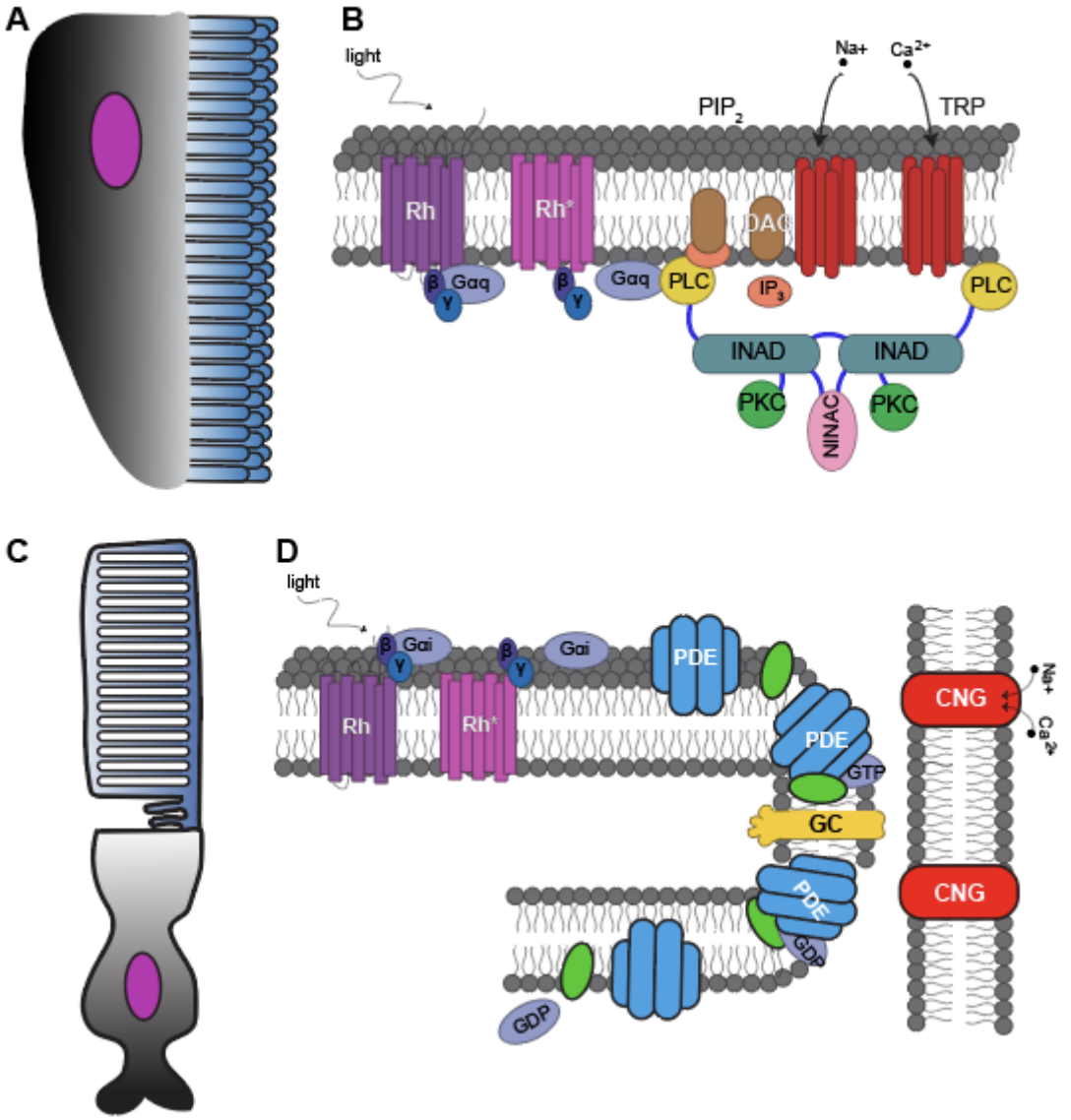

Figure 2. Schematic of rhabdomeric and ciliary receptors and cascades. A) Drawing of a rhabdomeric photoreceptor cell. B) Model of the phototransduction cascade in *Drosophila*. C) Drawing of a ciliary rod photoreceptor. D) Model of the phototransduction cascade in vertebrates. Figure adapted from Fain et al. 2010.

Genetics of eye development and PRC determination

Understanding the genetics of eye evolution first focused on candidate genes in model bilaterians, which overshadowed the true diversity we now understand. Early on, researchers identified a set of transcription factors (TF) which play important roles in eye determination and differentiation. Of particular importance was vertebrate *pax6* (homologous to *eyeless* and *twin of eyeless* in *Drosophila*), which can induce eye development in different body regions of *Drosophila* when misexpressed (Halder, Callaerts, and Gehring 1995; Gehring 2014). Even more astonishing, ectopic expression of squid or mouse *pax6* cDNA in *Drosophila* led to ectopic eyes morphologically similar to normal eyes (Halder, Callaerts, and Gehring 1995; Gehring 2014). These results put a new emphasis on the idea of a monophyletic origin of eyes under the control of the "master control gene" *pax6*. Later, scientists discovered many conserved TF genes are critically involved in eye development, including *dachshund*, *eyes absent*, *six1/2*, *six3/6* and *otx (Zagozewski et al. 2014)*. Similar to *pax6*, these TFs induced ectopic eyes when misexpressed and induced absence of eyes

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when knocked down. The idea of a monophyletic origin of eyes appeared to be a reasonable assumption based on homologous genes involved in eye development of different animal groups. However, a common evolutionary origin is not the only possible explanation because these similarities could also result from co-option events (i.e. eyes evolved independently by recruiting similar developmental genes) (Oakley 2003; L. V. Salvini-Plawen 2008; Serb, Porath-Krause, and Pairett 2013; Goldsmith 2013; Oakley and Speiser 2015).

Recent findings highlight an increasing diversity of visual system genetics and some plasticity in the genes and networks that carry out vision-related cell fate and development. Even though studies in non-model organisms do reveal some of the same gene families are used in eye development, many animal groups use paralogous genes, different combinations of genes, or different regulatory networks. As an example, members of the *pax*, *rax*, and *six* transcription factors are necessary for eye development, but the members of these gene families with a role in cell fate vary (Koenig and Gross 2020). In addition to different genes developing eyes themselves, PRC fate can also be driven by variation in gene regulation. In vertebrates, *Crx*, *Otx2* and *Rax* are expressed in all PRCs but different factors control the opsins expressed in rods (*Nrl*, *Nr2e3*, *Nr1d1*, *Pias 3*, and *Fiz1*) compared to cones (*Thbr* and *Rxrg* for Lws opsin; *Tbx2* for Sws1) (Musser and Arendt 2017). In insects, a set of genes (*Senseless*, *Prospero*, *Spalt*, and *Spineless*) is used for PRC specification, but variation in gene regulatory networks drive differences across a compound eye and between species (Wernet, Perry, and Desplan 2015; Perry et al. 2016; Miller et al. 2021).

Outside of Bilaterians, cnidarians have convergently evolved eyes at least 9 times and independent origins may use different genes for eye development (Picciani et al. 2018; Miranda and Collins 2019). For a review on cnidarian visual systems, light detection, and behavioral responses to light see Birch et al. (Birch et al. In Press). The genetics underlying convergently evolved cnidarian eyes are still not well understood, but studies characterized the expression of *eya*, *six*, and *pax* genes in some species. In the hydrozoan *Cladonema radiatum*, *eya* is expressed in eyes and gonads (Graziussi et al. 2012). Both *six1/2* and *six3/6* are expressed in the eyes of *Cladonema* and this expression increases during eye regeneration (Stierwald et al. 2004). Different members of the *pax* gene family also play a role in eye formation. For example, the cubozoan *Tripedalia* may use *paxB* for eye formation while the Hydrozoan *Cladonema* uses *paxA* (Suga et al. 2010). Moreover, in Cnidaria, opsins have undergone species-specific duplications and expansions (Suga, Schmid, and Gehring 2008; Macias-Munõz, Murad, and Mortazavi 2019) such that investigating the opsins and cascades used by these species will tell us more about the visual system diversity in this group.

Opsins and phototransduction cascades

Although the main functions of PRCs are detection and induction of a light response, they often employ different proteins to fulfill these functions (Fig 1). Phototransduction is initiated by rhodopsin, an opsin protein coupled to a vitamin A-derived chromophore molecule, usually retinal (A. Terakita 2005; Trevor D. Lamb, Collin, and Pugh 2007; Gehring 2014). When the chromophore absorbs light, it changes from an 11-*cis* to an all-*trans* conformation activating the opsin (Trevor D. Lamb, Arendt, and Collin 2009; Shichida and Matsuyama 2009). Opsins belong to a large family of G-protein coupled receptors, with a complex taxonomy based on different considerations, including the morphology of cells where they are expressed, the signal cascade they initiate, expression pattern, taxonomic scope, or their phylogenetic relationships (A. Terakita 2005; Trevor D. Lamb, Arendt, and Collin 2009; Fain, Hardie, and Laughlin 2010). Opsins are phylogenetically split into several (broadly accepted) major families: c-opsins, r-opsins, xenopsins, cnidopsin, anthozoan specific opsins, and tetraopsins (Fig 1) (Porter et al. 2011; Hering and Mayer 2014; Ramirez et al. 2016; Vöcking et al. 2017; Picciani et al. 2018; Gornik et al. 2020). However, the relationships within and among some of these groups remain unresolved (Porter et al. 2011; Feuda et al. 2012; Hering and Mayer 2014; Ramirez et al. 2016; Vöcking

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et al. 2017; Fleming et al. 2020). Depending on the opsin family, a different G-protein is activated, initiating different signaling cascades that result either in hyperpolarization or depolarization of the PRC (Yau and Hardie 2009; Fain, Hardie, and Laughlin 2010). More precisely, the Gα-subunit of the G-protein dissociates from the $\beta\gamma$-complex and acts as an effector for other enzymes of the cascade (Shichida and Matsuyama 2009). As a consequence, it is mainly the Gα-subunit which is referred to in studies of G-protein and opsin interactions. In this section we provide an overview of the major opsin families and their phototransduction components in more detail.

1. Gi/Gt opsins (traditionally c-opsins)

Visual c-opsins are the main visual opsin in vertebrate eyes, but phylogenetically related opsins are also present in brains of vertebrates and protostomes (Detlev Arendt et al. 2004; Velarde et al. 2005; A. Terakita 2005). These opsins are found in ciliary cells and function via the G-protein subunit Gi (Transducin (Gt) in vertebrates).

1.1. Gi/Transducin cascade

Phototransduction in rod cells of vertebrates is well-studied and used as a model for the c-opsin cascade (Fig 1; Fig 2). Rhodopsin activates the Gα subunit of Transducin (G-protein), which in turn activates a cGMP Phosphodiesterase (PDE). PDE hydrolyzes cGMP, leading to a decrease in cellular concentration of cGMP. The reduction of cGMP leads to closure of cyclic nucleotide gated channels (CNGs) preventing influx of positively charged Na^+ and Ca^{2+} ions. This reduces the electrical current of the PRC and by stopping an otherwise constant inward current, the PRC hyperpolarizes (Yau and Hardie 2009; Fain, Hardie, and Laughlin 2010; Kawamura and Tachibanaki 2014). In order to end signaling, rhodopsin is phosphorylated by rhodopsin kinase, followed by the binding of arrestin (Maeda, Imanishi, and Palczewski 2003).

1.2. Deuterostome Gi/Gt opsins

The most recognizable members of the Gi/Gt opsins are the visual opsins of vertebrates expressed in rods and cones of the retina. These are the by far best studied opsins, especially bovine rhodopsin. Depending on spectral sensitivity this group is further subdivided into at least 5 subgroups: LWS (long-wavelength sensitive) detects yellow/red, SWS1 (short-wavelength sensitive 1) detects UV, SWS2 (short-wavelength sensitive 2) detects blue, RH2 (rhodopsin-like) detects green, and RH1 (rhodopsin) the rod opsin has peak sensitivity in the blue/green (Baldwin and Ko 2020). These visual opsins have undergone multiple events of duplication, loss, and divergence (Rennison, Owens, and Taylor 2012; Lin et al. 2017). The expression of visual opsins also contributes to divergent and convergent evolution of color vision (Torres-Dowdall et al. 2021).

Closely related to vertebrate opsins used for color vision are vertebrate opsins traditionally referred to as non-visual, classically described as 'c-opsins' even though their transduction cascades remain largely unknown. This group includes pinopsins, expressed in the pineal organ of birds, reptiles and amphibians (Okano, Yoshizawa, and Fukada 1994; Taniguchi et al. 2001; Frigato et al. 2006), and recently found in the retina of non-teleost fish and frogs (Sato et al. 2018). Pinopsin is expressed in rod cells of the spotted gar and in rod and cone cells of the western clawed frog with peak absorbance at ~470 nm, and may function in dim-light detection (Sato et al. 2018). Parapinopsin is another non-visual opsin expressed in the pineal and parapineal organs of fish and amphibians (Blackshaw & Snyder 1997; Koyanagi et al. 2004). Teleost fish have two copies of parapinopsin one sensitive to UV, likely involved in wavelength discrimination, and the other sensitive to blue, hypothesized to function in melatonin secretion (Mitsumasa Koyanagi et al. 2015). Pinopsins and parapinopsin function via a Gt protein (Nakamura et al. 1999; Mitsumasa Koyanagi et al. 2017) but other components of their signaling cascade are yet to be described.

 doi:10.20944/preprints202209.0432.v1

Vertebrate ancient-opsins (VA-opsins), encephalopsins, and TMT-opsins also belong to the non-visual 'c-opsin' group. VA opsins are expressed in the bird hypothalamus and fish retinas and brains (Soni and Foster 1997; Philp et al. 2000; Santillo et al. 2006; Stephanie Halford et al. 2009). Encephalopsins are found in mice and humans and are broadly expressed in different tissues, primarily the brain, but also heart, lung, liver, kidneys, testes, and retina (Blackshaw and Snyder 1999; S. Halford et al. 2001; Ooka et al. 2010; Shichida and Matsuyama 2009; Nissilä et al. 2012). Despite broad expression, the function of encephalopsin remains unknown with suggested functions in circadian rhythm or melatonin production (Nissilä et al. 2012). TMT opsins, or teleost multiple tissue opsins, are expressed in neuronal and non-neuronal tissue in fish and may be associated with the adjustment of the inner circadian clock (Moutsaki et al. 2003; Shichida and Matsuyama 2009). TMT opsins can be divided into 3 groups: TMT1, TMT2, and TMT3. Both TMT1 and TMT2 are sensitive to blue light, bind all-trans-retinal, and function through the Gi and Go cascades (Mitsumasa Koyanagi et al. 2013; Sakai et al. 2015). TMT1 can also retain an 11-cis, 7-cis, and 9-cis chromophore (Sakai et al. 2015). Work in the medaka fish shows mutations in TMT1 result in increased avoidance behavior and increased average activity (Fontinha 2019).

While most research focused on vertebrates, they are not the only deuterostomes that have ciliary opsins. Sequences that group with traditional vertebrate c-opsins in phylogenetic analyses have been found in *Branchiostoma*, the ascidian *Ciona intestinalis*, several echinoderm species, and in hemichordates (Kusakabe et al. 2001; Ooka et al. 2010; Vopalensky et al. 2012; D'Aniello et al. 2015). In fact, in *Branchiostoma* the 'c-opsins' are expressed in the so called "frontal eye" (Vopalensky et al. 2012) and in *C. intestinalis* 'c-opsin' is expressed in the ocellus (Kusakabe et al. 2001).

1.3. Protostome Gi opsins

The ciliary and rhabdomeric PRC dichotomy led early researchers to believe that 'c-opsins' were only found in deuterostomes and r-opsins in protostomes thus the discovery of a c-opsin in a protostome was a breakthrough in photoreceptor research. The first ciliary opsin found in a non-vertebrate animal was the 'c-opsin' of the annelid *Platynereis dumerilii* expressed in the brain (Detlev Arendt et al. 2004). These opsins transduce signals using Gi/Go and may function in circadian photoentrainment (Detlev Arendt et al. 2004; Tsukamoto et al. 2017) and in larvae as a depth gauge (Verasztó et al. 2018). Closely following the discovery of the *Platynereis* 'c-opsin', pteropsin was found in the honeybee brain (Velarde et al. 2005). Pteropsin is expressed in the central nervous system and brain (not eyes) of other arthropods and may have a role in circadian clocks (Velarde et al. 2005; Eriksson et al. 2013; Collantes-Alegre et al. 2018). The only reported exception to date from a pattern of protostome 'c-opsins' expressed only outside eyes is PCR amplification of a c-opsin from brain and eye of an onychophoran (Eriksson et al. 2013). However, signal strength is weak in the eye sample and the results have not yet been confirmed by qPCR, in situ hybridization or antibody staining. The phototransduction cascade by which pteropsins signal is completely unknown.

2. Gq opsins (traditional r-opsins)

Traditional r-opsins are found in the main cells of the visual system in protostome eyes - microvillar PRCs - but can also be found in non-visual tissues and in deuterostomes. Where studied, r-opsins activate a Gq phototransduction cascade.

2.1. Gq cascade

The best studied phototransduction process in any invertebrate employing rhabdomeric opsins is from *Drosophila*, which often serves as the model for r-opsin cascades (Fig 1; Fig 2). Upon light detection, rhodopsin changes to its active conformation (metarhodop-

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sin), which activates Gq. Gq then activates the enzyme phospholipase C (PLC) that interacts with a membrane phospholipid (PIP_2) generating diacylglycerol (DAG) and inositol 1,4,5-tris-phosphate (IP_3). The exact function of DAG and IP_3 still needs to be determined but two classes of transient receptor potential channels (TRPC and TRPL) open. The opening of these channels allows an influx of positively charged Ca^{2+} ions leading to a depolarization of the PRC membrane. Termination happens when rhodopsin is deactivated (Hardie 2001; Yau and Hardie 2009; Montell 2012).

2.2. Protostome Gq opsins

Protostome visual Gq opsins (traditionally 'r-opsins' or 'canonical r-opsins') can be subdivided into at least three subgroups, depending on spectral sensitivity: short-wavelength or ultraviolet (UVRh), medium wavelength sometimes referred to as blue (BRh), and long wavelength (LWRh) (Henze and Oakley 2015). Gq opsins can also be found in non-ocular tissues, such as brain, ventral body region, skin, or bioluminescent organs of cephalopods (Shimizu et al. 2001; Tong et al. 2009; Backfisch et al. 2013; Collantes-Alegre et al. 2018). Accordingly, their main function in protostomes is vision, but they can also be used for other light-dependent functions such as circadian clocks, photo re-entrainement, and phototaxis (Spaethe and Briscoe 2005; Ogueta, Hardie, and Stanewsky 2018; Collantes-Alegre et al. 2018; S.-P. Chen et al. 2021). Moreover, these opsins can also have light independent functions such as sensing heat, taste, touch, and sound (Leung and Montell 2017). A closely related group to the visual Gq opsins was originally described in arthropods and named "arthropsins", but has since been found in lophotrochozoans and a few cephalochordates (Colbourne et al. 2011; Eriksson et al. 2013; Ramirez et al. 2016). The signaling cascade of arthropsins remains unknown.

2.3. Deuterostome Gq opsins

Similarly surprising to the finding of 'c-opsins' in protostomes was the discovery of an 'r-opsin' homolog in vertebrates, named melanopsin (Hattar et al. 2003; Panda et al. 2005). Melanopsin is expressed in the intrinsic photosensitive retinal ganglion cells of vertebrate eyes, but is not involved in image-forming vision (Hattar et al. 2003). Melanopsin is likely involved in circadian photoentrainment and functions through Gq transduction similar to protostome r-opsins, although isolated melanopsin can also activate transducin (Gi) (Brown and Robinson 2004; Chew et al. 2014). Several other r-opsin homologs besides melanopsin are found in different deuterostomes, including in *Branchiostoma (del Pilar Gomez, Angueyra, and Nasi 2009)* and echinoderms (D'Aniello et al. 2015; Lowe et al. 2018).

3. Tetraopsins

Tetraopsins or Group 4 opsins actually comprise three very distinct sub-groups: Go-opsins, neuropsins and the photoisomerase subfamily (A. Terakita 2005; Porter et al. 2011; Ramirez et al. 2016).

3.1. Go opsins

Go-opsin was first described in ciliary PRCs of the scallop *Patinopecten yessoensis*, so named because it activates G-alpha-o (Kojima et al. 1997). Go-opsin was also found in echinoderms, *Branchiostoma*, and *P. dumerilii* (Gomez and Nasi 2000; Mitsumasa Koyanagi et al. 2002; Holland 2008; D'Aniello et al. 2015; Gühmann et al. 2015; Randel and Jékely 2015). In *P. dumerilii* larval eyes, Go-opsin is co-expressed with an r-opsin and knocking it down reduces phototaxis to cyan light (Gühmann et al. 2015).

3.1.1. Go cascade

Phototransduction for Go-opsins leads to hyperpolarization similar to Gi/Gt opsins, but uses a different signaling cascade. The Go subunit activates a guanylate cyclase (GC)

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resulting in an increase of cGMP. This opens CNG channels that allow an influx of K^+ ions resulting in hyperpolarization (Kojima et al. 1997; Shichida and Matsuyama 2009; Fain, Hardie, and Laughlin 2010).

3.2. Neuropsins

Neuropsins (OPN5 in vertebrates) exist in both deuterostomes and protostomes but are poorly characterized. In vertebrates, neuropsins are expressed in spinal cord, testis, brain and eye (Tarttelin, Bellingham, Hankins, et al. 2003; Kumbalasiri and Provencio 2005) but it may be mainly a deep brain photoreceptor with the ability to detect UV light and interact with Gi (Nakane et al. 2010, 2014; Yamashita et al. 2010). In the marine annelid, *Capitella teleta*, neuropsins are expressed in brain regions but their function remains unknown (Neal, de Jong, and Seaver 2019). Transgenic studies in mice reveal that OPN5 has a role in skin circadian clocks and light sensitivity (Buhr et al. 2019).

3.3. Photoisomerases

Three opsin groups are summarized as the retinal photoisomerase subfamily including retinal G protein-coupled receptor (RGR), peropsins, and retinochromes (Shichida and Matsuyama 2009; Porter et al. 2011). Opsin photoisomerases are opsin proteins that may function in converting the activated all-*trans* retinal back into its reactive conformation of 11-*cis*. RGRs are found in vertebrates, retinochromes in invertebrates, and peropsin is found in both. Presumably, these opsins share the ability of light detection and retinal photoisomerization, and all of them preferentially bind retinal in all-*trans* conformations, even though they can also bind 11-*cis* conformations in vitro (Hao and Fong 1996; P. Chen et al. 2001; Mitsumasa Koyanagi et al. 2002). However, one of the key functions of opsins, activation of G-proteins, seems at least partly lost in this opsin group. While the characteristic Lysine residue K296 is present, some of the important amino acids for G-protein activation, such as the tripeptide and the NPxxY motif deviate in retinochromes and RGRs but are largely conserved in peropsins (Shichida and Matsuyama 2009; Vöcking, Leclère, and Hausen 2021). As a consequence, retinochrome and RGR are most likely not capable of initiating phototransduction, whereas peropsins at least preserved the potential capability of doing so. Where incapable of phototransduction, their main function seems to be photoisomerization (Vöcking, Leclère, and Hausen 2021).

3.3.1. RGRs

RGR was first found in the vertebrate retinal pigment epithelium and predicted to have sensitivity to blue and near-UV wavelengths (Hao and Fong 1996). RGRs are found in several vertebrate and invertebrate species (P. Chen et al. 2001; Díaz et al. 2017; Henze and Oakley 2015). Mutations of *RGR* in mice decrease 11-*cis* retinal and opsin activity, and increased accumulation for all-*trans* retinal (P. Chen et al. 2001). Similarly, in retinal ganglion cell cultures, knockdown of *RGR* results in significant differences of 11-*cis* and all-*trans* retinal in both light and dark adapted conditions (Díaz et al. 2017).

3.3.2. Peropsins

Peropsins are found in both vertebrates (chicken and mouse) and invertebrates (*Branchiostoma, Platynereis,* jumping spider) *(Sun et al. 1997; Mitsumasa Koyanagi et al. 2002; Tarttelin, Bellingham, Bibb, et al. 2003; Tosches et al. 2014)*. The expression of peropsin was only known from non-visual PRCs. For instance, it is present in the pineal organ of chickens (Bailey and Cassone 2004), the brain of *P. dumerilii* (Tosches et al. 2014), non-visual cells of the spider retina (Nagata et al. 2009), and cell surrounding photoreceptors in the horseshoe crab (Battelle et al. 2016). Recently, however, peropsin was found to be highly expressed in starfish eyes, but whether expression is in the photoreceptor or supporting cells is yet unknown (Lowe et al. 2018). Similar to RGR, mutations of peropsin in mice result in

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increased accumulation of all-*trans* retinal, suggesting a role for peropsin in light-dependent regulation of retinal (Cook et al. 2017).

3.3.3. Retinochromes

Retinochrome was first described in cephalopods and functions in chromophore photoisomerization (Tomiyuki Hara and Hara 1965; Akihisa Terakita, Hara, and Hara 1989; T. Hara and Hara 1991). Retinochrome sequences are found in multiple protostome species including gastropod and chiton mollusks, brachiopods, *P. dumerilii*, and arthropods (Ramirez et al. 2016). Retinochrome is expressed in the larval eyes of the chiton *Leptochiton asellus (Vöcking, Leclère, and Hausen 2021)* and a retinochrome-like gene is expressed in the eye pigment cells of the butterfly *Heliconius melpomene (Macias-Muñoz, Rangel Olguin, and Briscoe 2019)*. Yet, the role and function of retinochrome outside of mollusks has not been functionally demonstrated.

4. Xenopsin

Xenopsins, a recently described type of opsin, have been changing the conversation of PRC evolution as they have been found co-expressed with r-opsins and c-opsins (Vöcking et al. 2017; Rawlinson et al. 2019; Döring et al. 2020). Xenopsin, originally suggested to be a c-opsin, was first reported as expressed in the larval eye of *Terebratalia transversa* (Passamaneck et al. 2011). Phylogenetic analyses constantly find that these opsins form a clade separate from c-opsins (Ramirez et al. 2016; Vöcking et al. 2017; Rawlinson et al. 2019; Döring et al. 2020). Xenopsins exist in Lophotrochozoa and in Cnidaria as cnidopins (see below), and therefore were present before the Cnidaria and Bilateria split (Ramirez et al. 2016). In larvae of *Leptochiton asellus*, xenopsin and r-opsin are co-expressed in PRCs with both cilia and microvilli (Vöcking et al. 2017). In the larvae of tiger flatworm *Maritigrella crozieri,* both xenopsin and r-opsin are expressed in eyes, with xenopsin localized to cilia (Rawlinson et al. 2019). Xenopsin is also present in larvae of the annelid *Malacoceros fuliginosus* and Bryozoan *Tricellaria inopinata* localized to cilia (Döring et al. 2020). *Malacoceros* also has a c-opsin, indicating for the first time that xenopsin and c-opsin can occur together in a single genome (Döring et al. 2020). The G-protein by which xenopsins function remains unknown.

5. Cnidopsin

The first cnidopsins (originally named cnidops) were discovered by analyses of genomic data of cnidarians (David C. Plachetzki, Degnan, and Oakley 2007). Several studies confirm that many of these opsins form a phylogenetic clade on their own (Suga, Schmid, and Gehring 2008; Porter et al. 2011; Ramirez et al. 2016; Picciani et al. 2018; Gornik et al. 2020). Phylogenetically, these opsins appear to be a sister group to xenopsins (Fig 1) (Ramirez et al. 2016; Vöcking et al. 2017). Cnidopsins are quite multifaceted in terms of expression, with some being eye specific, while others are expressed in different tissues like gonads, tentacles, manubrium or umbrella (Suga, Schmid, and Gehring 2008; Liegertová et al. 2015; Macias-Munõz, Murad, and Mortazavi 2019).

5.1. Gs cascade

Where known, cnidopsins employ a Gs cascade (M. Koyanagi et al. 2008; Liegertová et al. 2015). In the box jellyfish *Carybdea rastonii*, Gs is co-localized with opsin in the outer segment of the cubozoan eye and responds to light by activating adenylyl cyclase (M. Koyanagi et al. 2008). Activation of Gs leads to a decrease in the cellular cAMP concentration (M. Koyanagi et al. 2008). The Gs pathway is also activated by two opsins upregulated in the rhopalia of the box jellyfish *Tripedalia*, but the signaling pathways of the other *Tripedalia* opsins remain unknown (Liegertová et al. 2015). In *Hydra*, opsin *HmOps2* is co-expressed with *CNG* and *Arrestin*, and based on pharmacological knockdown, *CNG* is

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necessary for response to light (D. C. Plachetzki, Fong, and Oakley 2010; David C. Plachetzki, Fong, and Oakley 2012).

5.2. Anthozoa opsins

In addition to cnidopsins, cnidarians in the group Anthozoa (sea anemones and corals) have two additional groups of opsins called anthozoa opsins I and anthozoa opsin II (ASO-I and ASO-II; Fig 1) (Hering and Mayer 2014). These opsins are divided into subtypes: ASO-I to subtypes 1 and 2, and ASO-II into subtypes I, 2.1, and 2.2 (Picciani et al. 2018; Gornik et al. 2020). In the sea anemone *Exaiptasia diaphana* expression of some of these ASOs varied between larval and adult stages and between symbiotic and aposymbiotic individuals (Gornik et al. 2020). Anthozoans are also unique among cnidarians in using cryptochrome to detect light (Gornik et al. 2020). Preliminary work suggests Anthozoans may possess multiple phototransduction cascades because heterologously expressed opsins from the coral *Acropora palmata* might initiate a Gq and 'Gc' (c for cnidarian) cascade (Mason et al. 2012).

5.3. Expression of bilaterian phototransduction genes in cnidarians

To investigate which genes may function in phototransduction across cnidarians with independently evolved eyes, we used bilaterian genes as candidates for functions in light detection. We hypothesized that genes used for light detection would be upregulated in jellyfish eye-bearing tissues (rhopalia of *Aurelia* and *Tripedalia* and tentacle bulbs of *Sarsia*; Fig 3). Because of their central importance in defining phototransduction cascades, we first looked for G-alpha genes in cnidarian genomes and transcriptomes to identify homologs of Gs, Gi, Gq, and Go (Table 1; Fig 3; Fig S1). We identified a potential duplication of Gi in *Aurelia* and two Gs-like genes in species excluding *Hydra* (Table 1; Fig S1). Gs, the phototransduction signaling protein in box jelly eyes, was in fact expressed at higher levels in *Tripedalia* and *Aurelia* rhopalia relative to other tissues. In *Sarsia* and *Hydra*, Gs was expressed at low levels in all tissues (Table 1; Fig S1). The next component that we surveyed was the G-protein subunit beta (GNB). We found two groups of GNB genes, *GNB5* orthologs and *GNB1-4-like* genes (Fig S2). We identified 3 copies of *GNB1-4-like* in *Hydra*, but two of them lacked or had low expression (Table 1; Fig S2). *GNB1-4-like* was expressed at higher levels in *Tripedalia* and *Aurelia* compared to *GNB5*, and had higher expression in the *Tripedalia* rhopalia compared to other tissues (Fig S2). In *Sarsia*, on the other hand, *GNB5* was expressed at higher levels but there was no significant difference in expression across tissues (Fig S2).

Two classes of ion channels are known in bilaterian phototransduction, TRP/TRPL used by *Drosophila* and CNG by vertebrates (Fig 2). In cnidarians, *TRPC* was present in all species with a potential duplication in *Aurelia* (Table 1; Fig S3). *TRP* had higher expression in the rhopalia of *Tripedalia* and *Aurelia* (both copies) (Fig S3). For *CNG*, a *CNG-like* gene was present in all species with two paralogs in *Aurelia*, one more highly expressed in the rhopalia (Table 1; Fig S4).

The enzyme proposed to function in cnidarian phototransduction is adenylyl cyclase (AC). We identified orthologs for 3 types of AC enzymes in cnidarians (AC-type2, AC-type5, and AC-type9) (Table 1; Fig S5). Only *AC-type9* had a duplication in *Hydra* (Table 1; Fig S5). *AC-type2* and *AC-type9* had higher expression in the *Tripedalia* rhopalia, but there was no significant difference of expression in the tissues of *Aurelia* and *Sarsia* (Fig S5). GC is the enzyme used in Gt opsin phototransduction, we found 3 GC genes in cnidarians (*GC-alpha*, *GC-beta*, and *GC88E*) (Table 1; Fig S6). We found two assembled transcripts for *GC-alpha* in *Sarsia* and *Tripedalia*, two for *GC-beta* in *Sarsia*, and *GC88E* had three copies in *Aurelia*, two in *Tripedalia*, but was absent in *Sarsia* and *Hydra* (Table 1; Fig S6). In terms of expression, *GC-beta* had higher expression in *Hydra* tentacles, all GC genes had higher expression in the *Aurelia* rhopalia, and most had higher expression in the *Tripedalia* rhopalia (Fig S6). For PLC, used by Gq opsins, we identified subunits beta1 and beta4

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(Table 1; Fig S7). *Hydra* has 3 copies of *PLC-B1* in tandem but one copy with no expression, and *PLC-B1* had higher expression in *Tripedalia* rhopalia. For PDE, we did not find an ortholog of PDE6 (the gene used by vertebrates) but we found a closely related group (Fig S8). We also identified *PDE5A* and *PDE11A* genes in cnidarians (Fig S8). These genes, for the most part, had similar expression in all tissues (Fig S8). G protein signaling is terminated by activated rhodopsin binding arrestin or being phosphorylated by rhodopsin kinase (Rhk). In cnidarians, we found one copy of *arrestin* and *Rhk* in all species (Fig S9-10). Both of these genes had higher expression in *Hydra* tentacles and *Tripedalia* rhopalia (Fig S9-S10).

From our observations, it seems as though there may be differences in the phototransduction cascades even within Cnidaria. We predicted that genes involved in phototransduction would be more highly expressed in eye bearing tissues. As predicted, there are instances where orthologs were highly expressed in rhopalia and tentacle bulbs implying similarity in cascades. However, there were other gene families where different paralogs were upregulated across the different species or there was no difference in expression across tissues. These results indicate that some cnidarian species may co-op paralogs or different genes in their phototransduction cascades. However, we cannot conclude without additional functional tests that the genes more highly expressed in the eye-bearing tissues have a direct role in phototransduction. The rhopalia are highly sensory tissues, so it could be that some of the G-protein components function in modulating other sensory modalities. Another caveat of this preliminary research is that without genomes for *Sarsia* and *Tripedalia* we cannot confidently conclude whether genes are duplicated or instead represent isoforms when we identify multiple transcripts. Additionally, lack of replication for *Tripedalia* and limited tissue sampling and replication in other species may bias expression results. For validation of the molecular evolution, expression, and function of these genes we need to: 1) assemble genomes for more cnidarian species, 2) increase replication and tissue sampling, 3) perform functional validations of genes highly expressed in eye-bearing tissues.

Table 1. Gene copies (potential duplications and loss).

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Gene name	Hydra	Sarsia*	Aurelia	Tripedalia*	Reference
Galphaq	1	1	1	1	Fig S1
Galphai	1	1	2	1	Fig S1
Galphao	1	1	1	1	Fig S1
GNB1-4-like	3	1	1	1	Fig S2
GNB5-like	1	1	1	1	Fig S2
CNGalpha-like	1	1	1	1	Fig S3
CNGbeta-like	0	0	1	0	Fig S3
TRP	1	1	2	1	Fig S4
AC-type2/3	1	1	1	1	Fig S5
AC-type5	1	1	1	1	Fig S5
AC-type9	2	1	1	1	Fig S5
GC alpha-like	1	2	1	2	Fig S6
GC beta	1	2	1	1	Fig S6
GC 88E	0	0	3	2	Fig S6
PLC beta1	3	1	1	1	Fig S7
PLC beta4	1	0	1	1	Fig S7
PDE5A	1	1	1	1	Fig S8
PDE6-like	2	1	2	1	Fig S8
PDE11A	1	0	1	1	Fig S8
Rhk	1	1	1	1	Fig S9
Arrestin	1	1	1	1	Fig S10
SEC14-2	1	1	1	2	Fig S11
SEC14-5	1	1	1	1	Fig S11
Clavesin/RLBP1	1	1	1	1	Fig S12
RLBP1-like	1	1	1	1	Fig S13
ALDHX	1	1	1	2	Fig S14
ALDH1/2/X	1	1	2	2	Fig S14

*These species do not have genomes. Numbers based on transcriptome assemblies.

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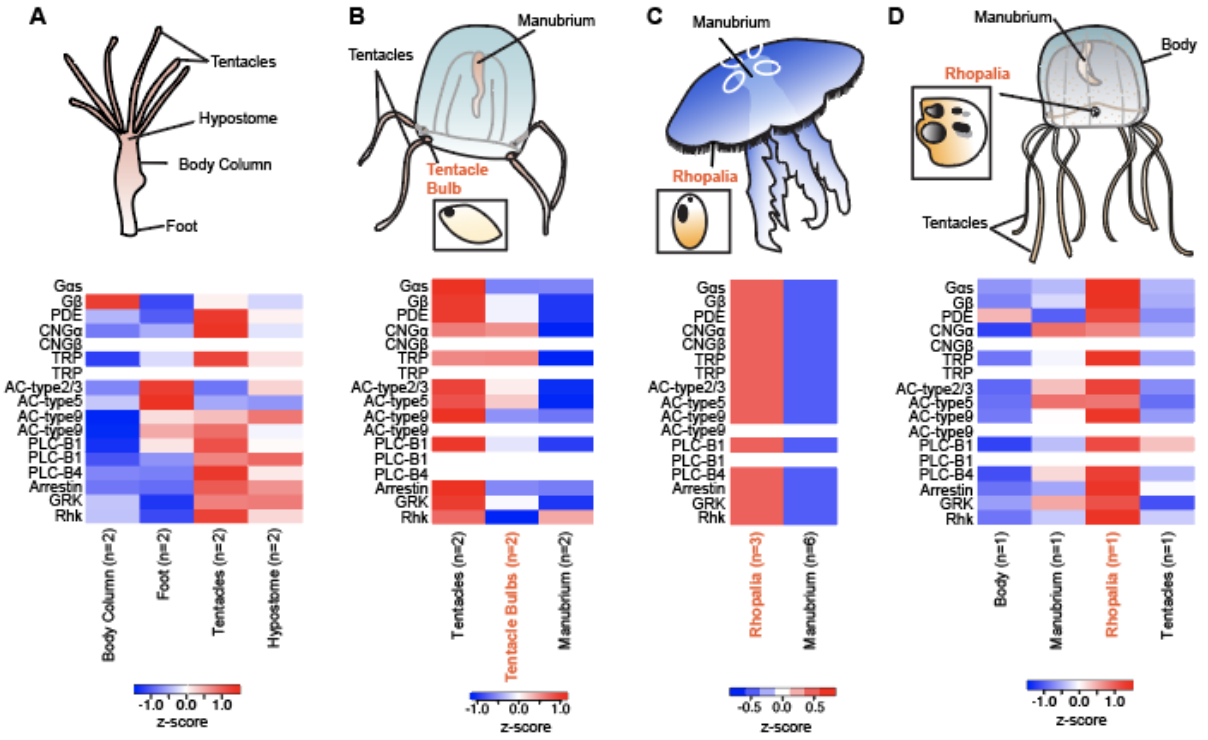

Figure 3. Expression of phototransduction gene homologs in 4 cnidarian species. A) Top: Diagram of a *Hydra vulgaris* polyp. Bottom: Heatmap showing relative expression of phhototransduction across tissues. Red indicates higher expression in that tissue and blue represents lower expression. White rows represent missing genes. B) Top: Diagram of *Sarsia tubulosa* and its tentacle bulb with one simple eye (ocellus). Bottom: Heatmap showing relative expression of phototransduction across tissues. Eye-bearing tissue (tentacle bulb) shown in orange. C) Top: Diagram of *Aurelia aurita* and its rhopalium with a large and small ocellus. D) Diagram of *Tripedalia cystophora* and its rhopalium (2 lens eye, 2 pit eyes and 2 slit eyes). Bottom: Heatmap showing relative expression of phototransduction across tissues.

6. Placopsins and ctenophore opsins

Placopsins, first reported in the *Trichoplax* genome (Srivastava et al. 2008), and a ctenophore opsin (*Mnemiopsis leidyi* 3) are proposed to be an outgroup to other animal opsins (Schnitzler et al. 2012; Feuda et al. 2012, 2014). *Mnemiopsis leidyi* possesses 3 opsins that do not form a phylogenetic group, but their relationship to other opsins varies under different models and with different outgroups (Schnitzler et al. 2012; Feuda et al. 2014). While homologs of traditional c-opsin and r-opsin cascades are expressed in the *M. leidyi*, their role in phototransduction or other sensory modalities have not been validated (Schnitzler et al. 2012).

Visual Cycle

In addition to different signaling cascades, opsins vary in the mechanisms used to recycle receptive rhodopsin, termed the visual cycle. One main difference between visual Gq and Gi/Gt opsins is that the retinal chromophore remains bound to Gq-opsins after absorbing light. Still bound to the Gq-opsin, retinal can absorb light of a different wavelength and change back to the 11-*cis* conformation. For visual Gt-opsins, on the other hand, retinal dissociates after the conformational change to all-*trans*. For this reason, Gt-opsins are referred to as monostable pigments, whereas Gq-opsins are called bistable pigments (Tsukamoto & Terakita 2010a). Yet, both visual systems have additional mechanisms to recycle retinal back to the 11-*cis* conformation.

Vertebrate visual cycle

 doi:10.20944/preprints202209.0432.v1

Vertebrates have light and dark visual cycles, and different visual cycles for their rod and cone PRCs (Wang and Kefalov 2011; Saari 2014; Choi et al. 2021). In the dark visual cycle of the rod PRCs, all-trans-retinal is reduced to all-trans-retinol by retinol dehydrogenases (RDHs), specifically RDH8 (and potentially additional RDHs) (Saari 2012). All-trans-retinol is transported through the interphotoreceptor matrix by chaperoning interphotoreceptor retinoid-binding protein (IRBP or RBP3) into the adjacent cells of the retinal pigment epithelium (RPE) where it is bound by the cellular retinol binding protein, CRBP1. While still bound to CRBP1 all-trans-retinol is esterified by the lecithin retinol acyltransferase (LRAT) to all-trans-retinyl esters (e.g. all-trans-retinyl palmitate). Consecutively, the isomerohydrolase RPE65 hydrolyzes and isomerizes the all-trans-retinyl ester to the respective fatty acid and 11-cis-retinol, which is then bound by the retinaldehyde binding protein RLBP1 (CRALBP). Subsequently, RDH5 catalyzes final oxidation to 11-cis-retinal before retinal is transported back to the rod PRC by IRBP to complete the cycle and regenerate rhodopsin (Kuksa et al. 2003; Radu et al. 2008; Saari 2012, 2014; Tang et al. 2013; Choi et al. 2021).

In contrast to rods (used in dim-light) that receive all their 11-cis-retinal exclusively from the RPE, cone PRCs (used in bright light) receive 11-cis-retinal from RPE and Müller cells. These Müller cells are capable of an alternative visual cycle, referred to as cone visual cycle. In mice, Müller cells require light, RGR, and RDH10 to recycle retinal (Morshedian et al. 2019). Before its function in Müller cells was discovered, RGR's photoisomerase function in the light visual cycle was predicted in the RPE (Jiang, Pandey, and Fong 1993; Maeda, Imanishi, and Palczewski 2003). In the RPE, RGR may function together with RDH5 and RPE65, but the cycle remains to be understood in its entirety (Van Hooser et al. 2002; Zhang et al. 2019; Choi et al. 2021). The exact function of this opsin has yet to be determined but a key role in rhodopsin regeneration under constant illumination, a storage for retinoids, and regulation of LRAT has been suggested (T. D. Lamb and Pugh 2004; Radu et al. 2008).

Invertebrate visual cycle

Due to the bistability of visual Gq opsins (traditionally r-opsins), it was assumed that invertebrates do not require visual cycling. However, it seems photoizomerization of metarhodopsin alone could not recover the high amount of receptive rhodopsin. Instead there must be another mechanism that ensures a constant supply of 11-cis-retinal (T. Hara and Hara 1991). The mollusk group of cephalopods have been the focus of this research for several decades and delivered first insights into invertebrate retinal recycling at a molecular level. In the eye PRCs of cephalopods, a second seven transmembran protein with great similarity to opsins was discovered (T. Hara and Hara 1991; Tomiyuki Hara and Hara 1965). This opsin preferentially bound retinal in the all-trans conformation and released it in the 11-cis conformation (Fig 4). It was given the name retinochrome, a sort of reverse opsin (Tomiyuki Hara and Hara 1965; T. Hara and Hara 1991; R. Hara, Hara, and Tokunaga 1981; Ozaki et al. 1983; Saari 2014). In squid, retinochrome is expressed in the inner segments of the PRCs and the basal regions of the outer segments, which is a contrast to r-opsins, which is only found in the rhabdomeric microvilli (T. Hara and Hara 1976; Ozaki et al. 1983; T. Hara and Hara 1991). As such, retinal transport is carried out by retinal binding protein (RALBP) that binds retinal in both conformations, 11-cis and all-trans (Ozaki et al. 1986; Akihisa Terakita, Hara, and Hara 1989; T. Hara and Hara 1991; Vöcking, Leclère, and Hausen 2021).

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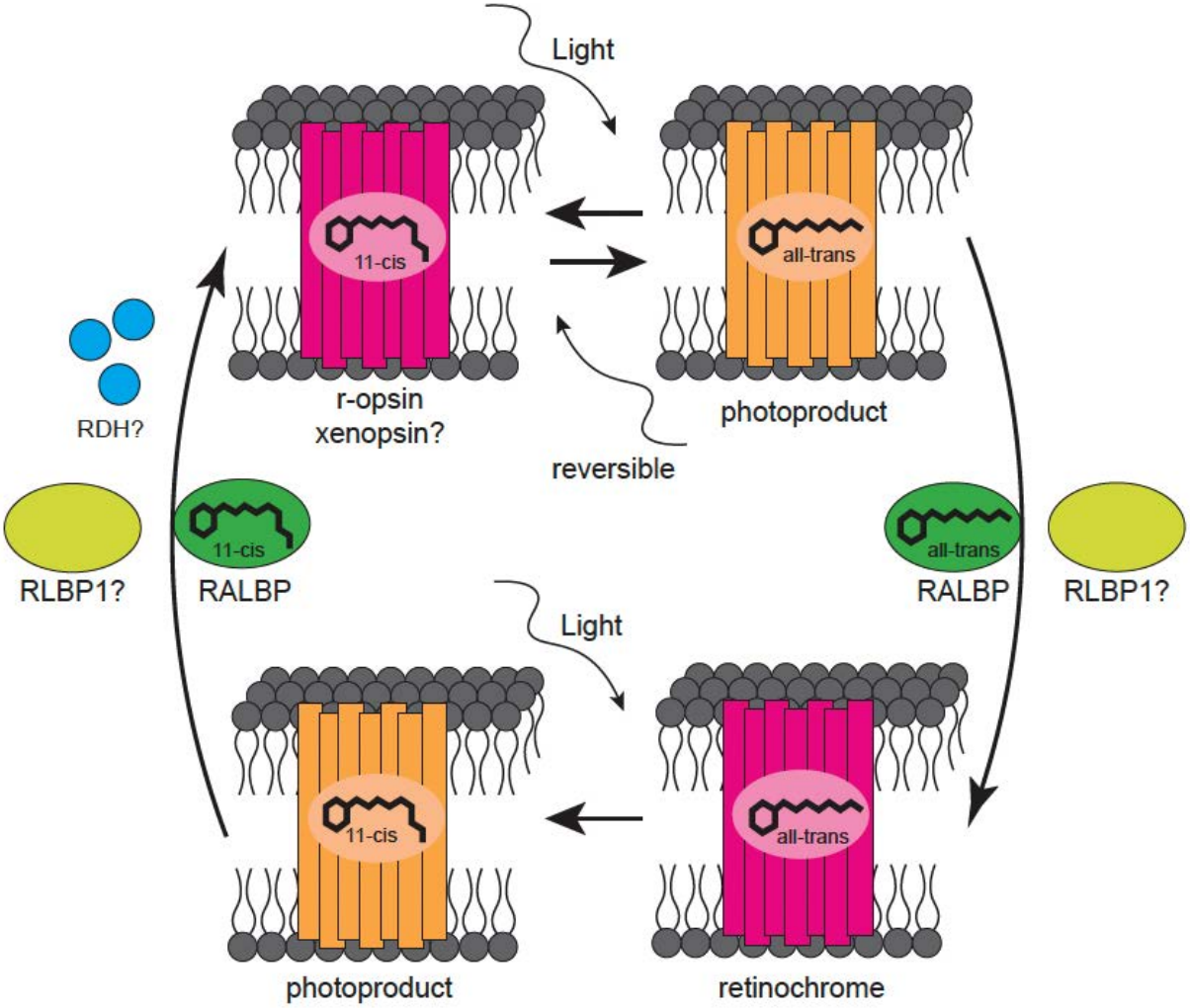

Figure 4. The rhodopsin-retinochrome system. Mostly known from cephalopods, in the rhodopsin-retinochrome system 11-cis retinal is bound to an r-opsin and changes its conformation upon light absorption to all-trans retinal. Then all-trans retinal either remains bound to the bistable r-opsin, or leaves the r-opsin and binds to RALBP, which transports it to retinochrome. Bound to retinochrome retinal changes back to the 11-cis conformation upon light detection. Finally, 11-cis retinal binds again to RALBP and is transported back to the r-opsin to complete the cycle. However, recent studies indicate more complexity, suggesting involvement of further proteins, including a potentially monostable xenopsin, the transporter protein RLBP1 and RDH enzymes. Though, their actual contributions remain speculative and still need to be determined by functional analyses (adapted from Koyanagi and Terakita 2014).

For a long time retinochrome was only known in cephalopod eyes and the rhodopsin-retinochrome system of cephalopods was the only retinal-reisomerization process (in addition to photoisomerization by bistable pigments) known from invertebrate species. However, retinochrome has now been found in the larval eyes of two additional mollusks: the chiton *L. asellus* and the gastropod *Conomurex luhuanus* (Ozaki et al. 1986; Vöcking, Leclère, and Hausen 2021). In chiton eyes, retinochrome is co-expressed with homologs to RLBP1, r-opsin, RALBP, and multiple retinal dehydrogenases (Vöcking, Leclère, and Hausen 2021). The chiton eyes also express xenopsin, whose sequence resembles a c-opsin in terms of its mono-stability, which might require a vertebrate-like visual cycle. Accordingly, the visual cycle in squids and chitons might be more complex than previously proposed.

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Cnidaria visual cycle

Cubozoan opsins, similar to vertebrate opsins, undergo bleaching suggesting presence of a visual cycle that has yet to be described (O'Connor et al. 2010). There is no cnidarian opsin that groups closely with known photoisomerases RGR or retinochrome (Picciani et al. 2018; Vöcking, Leclère, and Hausen 2021). It could be that a cnidopsin gene is co-opted as a photoisomerase, but that remains to be validated. In *Tripedalia*, an antibody against a cnidopsin detected protein in all eye types suggesting function as a photoisomerase (Garm et al. 2022). Although specific photoisomerase genes are yet unproven in cnidarians, cnidarians possess homologs of genes used in other animals for chromophore transport. We found two members of the *SEC14-like* family (protein 2 and protein 5) that have similar expression in all tissues (Table 1; Fig S11). In addition to finding homologs of *clavesin/RLBP1* (Fig S12), we also found a cnidarian-specific gene family whose top BLAST results matches *RLBP1* so we refer to these genes as *RLBP1-like* (Fig S13). These genes are expressed at higher levels in the rhopalia of *Aurelia* and *Tripedalia*, and *RLBP1-like* in the tentacles of *Hydra* and *Sarsia* (Fig S3). *RLBP1-like* was detected in cnidarians in a recent paper, but the amino acids necessary in other animals for retinal binding were lacking so it may not be involved in the visual cycle (Vöcking, Leclère, and Hausen 2021). For RDHs, we found a gene family matching aldehyde dehydrogenase (ALDH). Based on transcriptomes, this gene family may have duplications in *Aurelia* and *Tripedalia* (Fig S14). These genes were very lowly expressed in Hydra and similarly expressed across tissues in the other species (Fig S14).

Conclusions and Future Directions

Eyes are complex traits that require multiple independent components coming together to function and evolve. Across animals, eyes can vary in structural complexity and probably evolved convergently multiple times. Early studies of eye and PRC evolution focused on comparisons between *Drosophila* and mammals, overshadowing much diversity. Recent studies and our work in cnidarians have begun to explore these traits outside of model bilaterians to find that eyes can function using a much wider variety of components. The discussions and studies of eyes, PRCs, and opsin evolution now need to move away from traditional binary distinctions and candidate gene approaches to incorporate the diversity being uncovered. Future work should focus on genome-wide approaches in non-model organisms to uncover species-specific and additional genes that may be co-opted for eye and visual functions. Advances in technologies for genome manipulation will allow researchers to test the functions of eye development and phototransduction genes in model and non-model organisms to further validate the extent of conservation and diversity of these traits. Applying what we learned to complex traits more generally, visual research tells us that seemingly similar traits can have a deep diversity of underlying components.

Author Contributions: OV and AMM wrote the manuscript, SJ helped with figures, THO oversaw the project.

Data availability statement: The RNA-seq data presented here are available as follows: *Hydra* is from GEO accession GSE127279 (Murad et al. 2021), *Tripedalia* is from NCBI SRR8101518-SRR810526 (Khalturin et al. 2019), and *Sarsia* and *Aurelia* will be deposited to NCBI accompanying this paper.

Conflicts of Interest: The authors declare no conflict of interest.

References:

Arendt, Detlev. 2003. "Evolution of Eyes and Photoreceptor Cell Types." *The International Journal of Developmental Biology* 47: 563–71.

Arendt, Detlev, Kristin Tessmar-Raible, Heidi Snyman, Adriaan W. Dorresteijn, and Joachim Wittbrodt. 2004. "Ciliary Photoreceptors with a Vertebrate-Type Opsin in an Invertebrate Brain." *Science* 306 (5697): 869–71.

Arendt, D., and J. Wittbrodt. 2001. "Reconstructing the Eyes of Urbilateria." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 356 (1414): 1545–63.

Backfisch, Benjamin, Vinoth Babu Veedin Rajan, Ruth M. Fischer, Claudia Lohs, Enrique Arboleda, Kristin Tessmar-Raible, and Florian Raible. 2013. "Stable Transgenesis in the Marine Annelid Platynereis Dumerilii Sheds New Light on Photoreceptor Evolution." *Proceedings of the National Academy of Sciences of the United States of America* 110 (1): 193–98.

Bailey, Michael J., and Vincent M. Cassone. 2004. "Opsin Photoisomerases in the Chick Retina and Pineal Gland: Characterization, Localization, and Circadian Regulation." *Investigative Ophthalmology & Visual Science* 45 (3): 769–75.

Baldwin, Maude W., and Meng-Ching Ko. 2020. "Functional Evolution of Vertebrate Sensory Receptors." *Hormones and Behavior* 124 (August): 104771.

Battelle, Barbara Anne, Joseph F. Ryan, Karen E. Kempler, Spencer R. Saraf, Catherine E. Marten, Wesley C. Warren, Patrick J. Minx, et al. 2016. "Opsin Repertoire and Expression Patterns in Horseshoe Crabs: Evidence from the Genome of *Limulus Polyphemus* (Arthropoda: Chelicerata)." *Genome Biology and Evolution* 8 (5): 1571–89.

Blackshaw, S., and S. H. Snyder. 1999. "Encephalopsin: A Novel Mammalian Extraretinal Opsin Discretely Localized in the Brain." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 19 (10): 3681–90.

Brown, R. Lane, and Phyllis R. Robinson. 2004. "Melanopsin—Shedding Light on the Elusive Circadian Photopigment." *Chronobiology International* 21 (2): 189–204.

Buhr, Ethan D., Shruti Vemaraju, Nicolás Diaz, Richard A. Lang, and Russell N. Van Gelder. 2019. "Neuropsin (OPN5) Mediates Local Light-Dependent Induction of Circadian Clock Genes and Circadian Photoentrainment in Exposed Murine Skin." *Current Biology: CB* 29 (20): 3478–87.e4.

Chen, P., W. Hao, L. Rife, X. P. Wang, D. Shen, J. Chen, T. Ogden, et al. 2001. "A Photic Visual Cycle of Rhodopsin Regeneration Is Dependent on Rgr." *Nature Genetics* 28 (3): 256–60.

Chen, Shao-Ping, Zhao-Xia Liu, Yan-Ting Chen, Yue Wang, Jin-Zhi Chen, Shu Fu, Wei-Feng Ma, et al. 2021. "CRISPR/Cas9-Mediated Knockout of LW-Opsin Reduces the Efficiency of Phototaxis in the Diamondback Moth Plutella Xylostella." *Pest Management Science* 77 (7): 3519–28.

Chew, Kylie S., Tiffany M. Schmidt, Alan C. Rupp, Paulo Kofuji, and Jeffrey M. Trimarchi. 2014. "Loss of Gq/11 Genes Does Not Abolish Melanopsin Phototransduction." *PLoS ONE*. https://doi.org/10.1371/journal.pone.0098356.

Choi, Elliot H., Anahita Daruwalla, Susie Suh, Henri Leinonen, and Krzysztof Palczewski. 2021. "Retinoids in the Visual Cycle: Role of the Retinal G Protein-Coupled Receptor." *Journal of Lipid Research* 62 (February): 100040.

Colbourne, John K., Michael E. Pfrender, Donald Gilbert, W. Kelley Thomas, Abraham Tucker, Todd H. Oakley, Shinichi Tokishita, et al. 2011. "The Ecoresponsive Genome of Daphnia Pulex." *Science* 331 (6017): 555–61.

Collantes-Alegre, Jorge Mariano, Florian Mattenberger, Miquel Barberà, and David Martínez-Torres. 2018. "Characterisation, Analysis of Expression and Localisation of the Opsin Gene Repertoire from the Perspective of Photoperiodism in the Aphid Acyrthosiphon Pisum." *Journal of Insect Physiology* 104 (January): 48–59.

Cook, Jeremy D., Sze Yin Ng, Marcia Lloyd, Shannan Eddington, Hui Sun, Jeremy Nathans, Dean Bok, Roxana A. Radu, and Gabriel H. Travis. 2017. "Peropsin Modulates Transit of Vitamin A from Retina to Retinal Pigment Epithelium." *The Journal of Biological Chemistry* 292 (52): 21407–16.

D'Aniello, S., J. Delroisse, A. Valero-Gracia, E. K. Lowe, M. Byrne, J. T. Cannon, K. M. Halanych, et al. 2015. "Opsin Evolution in the Ambulacraria." *Marine Genomics*, 1–7.

Díaz, Nicolás M., Luis P. Morera, Tomas Tempesti, and Mario E. Guido. 2017. "The Visual Cycle in the Inner Retina of Chicken and the Involvement of Retinal G-Protein-Coupled Receptor (RGR)." *Molecular Neurobiology* 54 (4): 2507–17.

Döring, Clemens Christoph, Suman Kumar, Sharat Chandra Tumu, Ioannis Kourtesis, and Harald Hausen. 2020. "The Visual Pigment Xenopsin Is Widespread in Protostome Eyes and Impacts the View on Eye Evolution." *eLife* 9: 1–23.

Eakin, Richard M. 1982. "Continuity and Diversity in Photoreceptors." *Visual Cells in Evolution/editor, Jane A. Westfall*. https://agris.fao.org/agris-search/search.do?recordID=US201302622999.

Eakin, R. M. 1965. "Evolution of Photoreceptors." *Cold Spring Harbor Symposia on Quantitative Biology* 30: 363–70.

Eriksson, Bo Joakim, David Fredman, Gerhard Steiner, and Axel Schmid. 2013. "Characterisation and Localisation of the Opsin Protein Repertoire in the Brain and Retinas of a Spider and an Onychophoran." *BMC Evolutionary Biology* 13 (1): 186.

Fain, Gordon L., Roger Hardie, and Simon B. Laughlin. 2010. "Phototransduction and the Evolution of Photoreceptors." *Current Biology: CB* 20 (3): R114–24.

Feuda, Roberto, Sinead C. Hamilton, James O. McInerney, and Davide Pisani. 2012. "Metazoan Opsin Evolution Reveals a Simple Route to Animal Vision." *Proceedings of the National Academy of Sciences of the United States of America* 109 (46): 18868–72.

Feuda, Roberto, Omar Rota-Stabelli, Todd H. Oakley, and Davide Pisani. 2014. "The Comb Jelly Opsins and the Origins of Animal Phototransduction." *Genome Biology and Evolution* 6 (8): 1964–71.

Fleming, James F., Roberto Feuda, Nicholas W. Roberts, and Davide Pisani. 2020. "A Novel Approach to Investigate the Effect of Tree Reconstruction Artifacts in Single-Gene Analysis Clarifies Opsin Evolution in Nonbilaterian Metazoans." *Genome Biology and Evolution* 12 (2): 3906–16.

Fontinha, Bruno M. 2019. "TMT-Opsins Differentially Modulate Medaka Brain Function in a Context-Dependent Manner." *Intrinsic Activity*. https://doi.org/10.25006/ia.7.s1-a3.17.

Frigato, Elena, Daniela Vallone, Cristiano Bertolucci, and Nicholas S. Foulkes. 2006. "Isolation and Characterization of Melanopsin

and Pinopsin Expression within Photoreceptive Sites of Reptiles." *Die Naturwissenschaften* 93 (8): 379–85.

Garm, Anders, Jens-Erik Svaerke, Daniela Pontieri, and Todd H. Oakley. 2022. "Expression of Opsins of the Box Jellyfish Reveals the First Photopigment in Cnidarian Ocelli and Supports the Presence of Photoisomerases." *Frontiers in Neuroanatomy* 16 (August): 916510.

Gehring, Walter J. 2014. "The Evolution of Vision." *Wiley Interdisciplinary Reviews. Developmental Biology* 3 (1): 1–40.

Goldsmith, Timothy H. 2013. "Evolutionary Tinkering with Visual Photoreception" 30 (1-2): 21–37.

Gomez, M. P., and E. Nasi. 2000. "Light Transduction in Invertebrate Hyperpolarizing Photoreceptors: Possible Involvement of a Go-Regulated Guanylate Cyclase." *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience* 20 (14): 5254–63.

Gornik, Sebastian G., Bruno Gideon Bergheim, Benoit Morel, Alexandros Stamatakis, Nicholas S. Foulkes, and Annika Guse. 2020. "Photoreceptor Diversification Accompanies the Evolution of Anthozoa." *Molecular Biology and Evolution*. https://doi.org/10.1093/molbev/msaa304.

Graziussi, Daria Federica, Hiroshi Suga, Volker Schmid, and Walter Jakob Gehring. 2012. "The 'Eyes Absent' (eya) Gene in the Eye-Bearing Hydrozoan Jellyfish Cladonema Radiatum: Conservation of the Retinal Determination Network." *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution* 318 (4): 257–67.

Gühmann, Martin, Huiyong Jia, Nadine Randel, Csaba Verasztó, Luis A. Bezares-Calderón, Nico K. Michiels, Shozo Yokoyama, and Gáspár Jékely. 2015. "Spectral Tuning of Phototaxis by a Go-Opsin in the Rhabdomeric Eyes of Platynereis." *Current Biology: CB*, 1–7.

Halder, G., P. Callaerts, and W. Gehring. 1995. "Induction of Ectopic Eyes by Targeted Expression of the Eyeless Gene in Drosophila." *Science* 267 (5205): 1788–92.

Halford, S., M. S. Freedman, J. Bellingham, S. L. Inglis, S. Poopalasundaram, B. G. Soni, R. G. Foster, and D. M. Hunt. 2001. "Characterization of a Novel Human Opsin Gene with Wide Tissue Expression and Identification of Embedded and Flanking Genes on Chromosome 1q43." *Genomics* 72 (2): 203–8.

Halford, Stephanie, Susana S. Pires, Michael Turton, Lei Zheng, Irene González-Menéndez, Wayne L. Davies, Stuart N. Peirson, José M. García-Fernández, Mark W. Hankins, and Russell G. Foster. 2009. "VA Opsin-Based Photoreceptors in the Hypothalamus of Birds." *Current Biology: CB* 19 (16): 1396–1402.

Hao, W., and H. K. Fong. 1996. "Blue and Ultraviolet Light-Absorbing Opsin from the Retinal Pigment Epithelium." *Biochemistry* 35 (20): 6251–56.

Hara, Reiko, Tomiyuki Hara, and Fumio Tokunaga. 1981. "Photochemistry Retinochrome." *Photochemistry and Photobiology* 33: 883–91.

Hara, T., and R. Hara. 1976. "Distribution of Rhodopsin and Retinochrome in the Squid Retina." *The Journal of General Physiology* 67 (6): 791–805.

———. 1991. "Retinal-Binding Protein: Function in a Chromophore Exchange System in the Squid Visual Cell." *Progress in Retinal and Eye Research* 10: 179–206.

Hara, Tomiyuki, and Reiko Hara. 1965. "New Photosensitive Pigment Found in the Retina of the Squid Ommastrephes." *Nature* 206: 1331–34.

Hardie, Roger C. 2001. "Phototransduction in *Drosophila Melanogaster*." *The Journal of Experimental Biology* 204: 3403–9.

Hattar, Samer, Robert J. Lucas, N. Mrosovsky, S. Thompson, R. H. Douglas, Mark W. Hankins, J. Lem, et al. 2003. "Melanopsin and Rod-Cone Photoreceptive Systems Account for All Major Accessory Visual Functions in Mice." *Nature* 424 (6944): 76–81.

Henze, Miriam J., and Todd H. Oakley. 2015. "The Dynamic Evolutionary History of Pancrustacean Eyes and Opsins." *Integrative and Comparative Biology* 55 (5): 830–42.

Hering, L., and G. Mayer. 2014. "Analysis of the Opsin Repertoire in the Tardigrade Hypsibius Dujardini Provides Insights into the Evolution of Opsin Genes in Panarthropoda." *Genome Biology and Evolution* 6 (9): 2380–91.

Holland, L. Z. 2008. "The Amphioxus Genome Illuminates Vertebrate Origins and Cephalochordate Biology." *Genome Research* 18: 1100–1111.

Jiang, M., S. Pandey, and H. K. W. Fong. 1993. "An Opsin Homolog in the Retina and Pigment-Epithelium." *Investigative Ophthalmology & Visual Science* 34 (13): 3669–78.

Kawamura, Saturo, and Shuji Tachibanaki. 2014. "Phototransduction in Rods and Cones." In *Vertebrate Photoreceptors: Functional Molecular Bases*, 1:23–45.

Khalturin, Konstantin, Chuya Shinzato, Maria Khalturina, Mayuko Hamada, Manabu Fujie, Ryo Koyanagi, Miyuki Kanda, et al. 2019. "Medusozoan Genomes Inform the Evolution of the Jellyfish Body Plan." *Nature Ecology and Evolution* 3 (5): 811–22.

Koenig, Kristen M., and Jeffrey M. Gross. 2020. "Evolution and Development of Complex Eyes : A Celebration of Diversity." https://doi.org/10.1242/dev.182923.

Kojima, Daisuke, Akihisa Terakita, Toru Ishikawa, Yasuo Tsukahara, Akio Maeda, and Yoshinori Shichida. 1997. "A Novel G O -Mediated Phototransduction Cascade in Scallop Visual Cells." *The Journal of Biological Chemistry* 272 (37): 22979–82.

Koyanagi, Mitsumasa, Emi Kawano-Yamashita, Seiji Wada, and Akihisa Terakita. 2017. "Vertebrate Bistable Pigment Parapinopsin: Implications for Emergence of Visual Signaling and Neofunctionalization of Non-Visual Pigment." *Frontiers in Ecology and Evolution* 5. https://doi.org/10.3389/fevo.2017.00023.

Koyanagi, Mitsumasa, Eiichiro Takada, Takashi Nagata, Hisao Tsukamoto, and Akihisa Terakita. 2013. "Homologs of Vertebrate Opn3 Potentially Serve as a Light Sensor in Nonphotoreceptive Tissue." *Proceedings of the National Academy of Sciences of the United States of America* 110 (13): 4998–5003.

Koyanagi, Mitsumasa, Akihisa Terakita, Kaoru Kubokawa, and Yoshinori Shichida. 2002. "Amphioxus Homologs of Go-Coupled Rhodopsin and Peropsin Having 11- Cis - and All- Trans -Retinals as Their Chromophores" 531: 525–28.

Koyanagi, Mitsumasa, Seiji Wada, Emi Kawano-Yamashita, Yuichiro Hara, Shigehiro Kuraku, Shigeaki Kosaka, Koichi Kawakami, et al. 2015. "Diversification of Non-Visual Photopigment Parapinopsin in Spectral Sensitivity for Diverse Pineal Functions." *BMC Biology* 13 (September): 73.

Koyanagi, M., K. Takano, H. Tsukamoto, K. Ohtsu, F. Tokunaga, and A. Terakita. 2008. "Jellyfish Vision Starts with cAMP Signaling Mediated by Opsin-Gs Cascade." *Proceedings of the National Academy of Sciences* 105 (40): 15576–80.

Kuksa, Vladimir, Yoshikazu Imanishi, Matthew Batten, Krzysztof Palczewski, and Alexander R. Moise. 2003. "Retinoid Cycle in the Vertebrate Retina: Experimental Approaches and Mechanisms of Isomerization." *Vision Research* 43 (28): 2959–81.

Kumbalasiri, Tida, and Ignacio Provencio. 2005. "Melanopsin and Other Novel Mammalian Opsins." *Experimental Eye Research* 81 (4): 368–75.

Kusakabe, Takehiro, Rie Kusakabe, Isao Kawakami, Yutaka Satou, Nori Satoh, and Motoyuki Tsuda. 2001. "Ci-opsin1, a Vertebrate-Type Opsin Gene, Expressed in the Larval Ocellus of the Ascidian Ciona Intestinalis." *FEBS Letters* 506 (1): 69–72.

Lamb, T. D., and E. N. Pugh. 2004. "Dark Adaptation and the Retinoid Cycle of Vision." *Progress in Retinal and Eye Research* 23 (3): 307–80.

Lamb, Trevor D., Detlev Arendt, and Shaun P. Collin. 2009. "The Evolution of Phototransduction and Eyes." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 364 (1531): 2791–93.

Lamb, Trevor D., Shaun P. Collin, and Edward N. Pugh Jr. 2007. "Evolution of the Vertebrate Eye: Opsins, Photoreceptors, Retina and Eye Cup." *Nature Reviews. Neuroscience* 8 (12): 960–76.

Leung, Nicole Y., and Craig Montell. 2017. "Unconventional Roles of Opsins." *Annual Review of Cell and Developmental Biology* 33: 241–64.

Liegertová, Michaela, Jiří Pergner, Iryna Kozmiková, Peter Fabian, Antonio R. Pombinho, Hynek Strnad, Jan Pačes, Čestmír Vlček, Petr Bartůněk, and Zbyněk Kozmik. 2015. "Cubozoan Genome Illuminates Functional Diversification of Opsins and Photoreceptor Evolution." *Scientific Reports* 5 (1): 11885.

Lin, Jinn-Jy, Feng-Yu Wang, Wen-Hsiung Li, and Tzi-Yuan Wang. 2017. "The Rises and Falls of Opsin Genes in 59 Ray-Finned Fish Genomes and Their Implications for Environmental Adaptation." *Scientific Reports* 7 (1): 15568.

Lowe, Elijah K., Anders L. Garm, Esther Ullrich-Lüter, Claudia Cuomo, and Maria I. Arnone. 2018. "The Crowns Have Eyes: Multiple Opsins Found in the Eyes of the Crown-of-Thorns Starfish Acanthaster Planci." *BMC Evolutionary Biology* 18 (1): 168.

Macias-Munõz, Aide, Rabi Murad, and Ali Mortazavi. 2019. "Molecular Evolution and Expression of Opsin Genes in Hydra Vulgaris." *BMC Genomics* 20 (1): 1–19.

Macias-Muñoz, A., A. G. Rangel Olguin, and A. D. Briscoe. 2019. "Evolution of Phototransduction Genes in Lepidoptera." *bioRxiv*. https://doi.org/10.1101/577031.

Maeda, Tadao, Yoshikazu Imanishi, and Krzysztof Palczewski. 2003. "Rhodopsin Phosphorylation: 30 Years Later." *Progress in Retinal and Eye Research* 22 (4): 417–34.

Mason, Benjamin, Michael Schmale, Patrick Gibbs, Margaret W. Miller, Qiang Wang, Konstantin Levay, Valery Shestopalov, and Vladlen Z. Slepak. 2012. "Evidence for Multiple Phototransduction Pathways in a Reef-Building Coral." *PloS One* 7 (12): 1–9.

Miller, Adam C., Elizabeth A. Urban, Eric L. Lyons, Tory G. Herman, and Robert J. Johnston Jr. 2021. "Interdependent Regulation of Stereotyped and Stochastic Photoreceptor Fates in the Fly Eye." *Developmental Biology* 471 (March): 89–96.

Miranda, Lucília Souza, and Allen Gilbert Collins. 2019. "Eyes in Staurozoa (cnidaria): A Review." *PeerJ* 2019 (4): 1–13.

Monteiro, Antónia, and Ondrej Podlaha. 2009. "Wings, Horns, and Butterfly eyespots:How Do Complex Traits Evolve?" *PLoS Biology* 7 (2): 0209–16.

Montell, Craig. 2012. "*Drosophila* Visual Transduction." *Trends in Neurosciences* 35 (6): 356–63.

Morshedian, Ala, Joanna J. Kaylor, Sze Yin Ng, Avian Tsan, Rikard Frederiksen, Tongzhou Xu, Lily Yuan, et al. 2019. "Light-Driven Regeneration of Cone Visual Pigments through a Mechanism Involving RGR Opsin in Müller Glial Cells." *Neuron* 102 (6): 1172–83.e5.

Moutsaki, Paraskevi, David Whitmore, James Bellingham, Katsuhiko Sakamoto, Zoë K. David-Gray, and Russell G. Foster. 2003. "Teleost Multiple Tissue (tmt) Opsin: A Candidate Photopigment Regulating the Peripheral Clocks of Zebrafish?" *Molecular Brain Research* 112 (1-2): 135–45.

Murad, Rabi, Aide Macias-Muñoz, Ashley Wong, Xinyi Ma, and Ali Mortazavi. 2021. "Coordinated Gene Expression and Chromatin Regulation during Hydra Head Regeneration." *Genome Biology and Evolution* 13 (12). https://doi.org/10.1093/gbe/evab221.

Musser, Jacob M., and Detlev Arendt. 2017. "Loss and Gain of Cone Types in Vertebrate Ciliary Photoreceptor Evolution." *Developmental Biology* 431 (1): 26–35.

Nagata, Takashi, Mitsumasa Koyanagi, Hisao Tsukamoto, and Akihisa Terakita. 2009. "Identification and Characterization of a Protostome Homologue of Peropsin from a Jumping Spider." *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural,*

and Behavioral Physiology 196 (1): 51–59.

Nakamura, A., D. Kojima, H. Imai, A. Terakita, T. Okano, Y. Shichida, and Y. Fukada. 1999. "Chimeric Nature of Pinopsin between Rod and Cone Visual Pigments." *Biochemistry* 38 (45): 14738–45.

Nakane, Yusuke, Keisuke Ikegami, Hiroko Ono, Naoyuki Yamamoto, Shosei Yoshida, Kanjun Hirunagi, Shizufumi Ebihara, Yoshihiro Kubo, and Takashi Yoshimura. 2010. "A Mammalian Neural Tissue Opsin (Opsin 5) Is a Deep Brain Photoreceptor in Birds." *Proceedings of the National Academy of Sciences of the United States of America* 107 (34): 15264–68.

Nakane, Yusuke, Tsuyoshi Shimmura, Hideki Abe, and Takashi Yoshimura. 2014. "Intrinsic Photosensitivity of a Deep Brain Photoreceptor." *Current Biology: CB* 24 (13): R596–97.

Neal, S., D. M. de Jong, and E. C. Seaver. 2019. "CRISPR/CAS9 Mutagenesis of a Single R-Opsin Gene Blocks Phototaxis in a Marine Larva." *Proceedings. Biological Sciences / The Royal Society* 286 (1904): 20182491.

Nilsson, Dan-E. 2013. "Eye Evolution and Its Functional Basis." *Visual Neuroscience* 30 (1-2): 5–20.

Nilsson, Dan Eric. 2009. "The Evolution of Eyes and Visually Guided Behaviour." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 364 (1531): 2833–47.

Nissilä, Juuso, Satu Mänttäri, Terttu Särkioja, Hannu Tuominen, Timo Takala, Markku Timonen, and Seppo Saarela. 2012. "Encephalopsin (OPN3) Protein Abundance in the Adult Mouse Brain." *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 198 (11): 833–39.

Oakley, Todd H. 2003. "The Eye as a Replicating and Diverging, Modular Developmental Unit." *Trends in Ecology & Evolution* 18 (12): 623–27.

Oakley, Todd H., and Daniel I. Speiser. 2015. "How Complexity Originates: The Evolution of Animal Eyes." *Annual Review of Ecology, Evolution, and Systematics* 46 (1): 237–60.

O'Connor, Megan, Anders Garm, Justin N. Marshall, Nathan S. Hart, Peter Ekström, Charlotta Skogh, and Dan Eric Nilsson. 2010. "Visual Pigment in the Lens Eyes of the Box Ra Jellyfish Chiropsella Bronzie." *Proceedings of the Royal Society B: Biological Sciences* 277 (1689): 1843–48.

Ogueta, Maite, Roger C. Hardie, and Ralf Stanewsky. 2018. "Non-Canonical Phototransduction Mediates Synchronization of the Drosophila Melanogaster Circadian Clock and Retinal Light Responses." *Current Biology: CB* 28 (11): 1725–35.e3.

Okano, T., T. Yoshizawa, and Y. Fukada. 1994. "Pinopsin Is a Chicken Pineal Photoreceptive Molecule." *Nature*. https://doi.org/10.1038/372094a0.

Ooka, Shioh, Tomoko Katow, Shunsuke Yaguchi, Junko Yaguchi, and Hideki Katow. 2010. "Spatiotemporal Expression Pattern of an Encephalopsin Orthologue of the Sea Urchin Hemicentrotus Pulcherrimus during Early Development, and Its Potential Role in Larval Vertical Migration." *Development, Growth & Differentiation* 52 (2): 195–207.

Ozaki, K., R. Hara, T. Hara, and T. Kakitani. 1983. "Squid Retinochrome. Configurational Changes of the Retinal Chromophore." *Biophysical Journal* 44 (1): 127–37.

Ozaki, K., A. Terakita, R. Hara, and T. Hara. 1986. "Rhodopsin and Retinochrome in the Retina of a Marine Gastropod, Conomulex Luhuanus." *Vision Research* 26 (5): 691–705.

Panda, Satchidananda, Surendra K. Nayak, Brice Campo, John R. Walker, John B. Hogenesch, and Tim Jegla. 2005. "Illumination of the Melanopsin Signaling Pathway." *Science* 307 (5709): 600–604.

Passamaneck, Yale J., Nina Furchheim, Andreas Hejnol, Mark Q. Martindale, and Carsten Lüter. 2011. "Ciliary Photoreceptors in the Cerebral Eyes of a Protostome Larva." *EvoDevo* 2 (1): 6.

Perry, Michael, Michiyo Kinoshita, Giuseppe Saldi, Lucy Huo, Kentaro Arikawa, and Claude Desplan. 2016. "Molecular Logic behind the Three-Way Stochastic Choices That Expand Butterfly Colour Vision." *Nature*, 1–10.

Philp, A. R., J. M. Garcia-Fernandez, B. G. Soni, R. J. Lucas, J. Bellingham, and R. G. Foster. 2000. "Vertebrate Ancient (VA) Opsin and Extraretinal Photoreception in the Atlantic Salmon (Salmo Salar)." *The Journal of Experimental Biology* 203 (Pt 12): 1925–36.

Picciani, Natasha, Jamie R. Kerlin, Noemie Sierra, Andrew J. M. Swafford, M. Desmond Ramirez, Nickellaus G. Roberts, Johanna T. Cannon, Marymegan Daly, and Todd H. Oakley. 2018. "Prolific Origination of Eyes in Cnidaria with Co-Option of Non-Visual Opsins." *Current Biology: CB* 28 (15): 2413–19.e4.

Pilar Gomez, María del, Juan M. Angueyra, and Enrico Nasi. 2009. "Light-Transduction in Melanopsin-Expressing Photoreceptors of Amphioxus." *Proceedings of the National Academy of Sciences* 106 (22): 9081–86.

Plachetzki, David C., Bernard M. Degnan, and Todd H. Oakley. 2007. "The Origins of Novel Protein Interactions during Animal Opsin Evolution." *PloS One* 2 (10): e1054.

Plachetzki, David C., Caitlin R. Fong, and Todd H. Oakley. 2012. "Cnidocyte Discharge Is Regulated by Light and Opsin-Mediated Phototransduction." *BMC Biology* 10: 17.

Plachetzki, D. C., C. R. Fong, and T. H. Oakley. 2010. "The Evolution of Phototransduction from an Ancestral Cyclic Nucleotide Gated Pathway." *Proceedings of the Royal Society B: Biological Sciences* 277 (1690): 1963–69.

Porter, Megan L., Joseph R. Blasic, Michael J. Bok, Evan G. Cameron, Thomas Pringle, Thomas W. Cronin, and Phyllis R. Robinson. 2011. "Shedding New Light on Opsin Evolution." *Proceedings of the Royal Society B: Biological Sciences* 279 (1726): 3–14.

Radu, Roxana A., Jane Hu, Jennifer Peng, Dean Bok, Nathan L. Mata, and Gabriel H. Travis. 2008. "Retinal Pigment Epithelium-Retinal G Protein Receptor-Opsin Mediates Light-Dependent Translocation of All-Trans-Retinyl Esters for Synthesis of Visual

Chromophore in Retinal Pigment Epithelial Cells." *The Journal of Biological Chemistry* 283 (28): 19730–38.

Ramirez, M. Desmond, Autum N. Pairett, M. Sabrina Pankey, Jeanne M. Serb, Daniel I. Speiser, Andrew J. Swafford, and Todd H. Oakley. 2016. "The Last Common Ancestor of Most Bilaterian Animals Possessed at Least Nine Opsins." *Genome Biology and Evolution* 8 (12): 3640–52.

Randel, Nadine, and Gáspár Jékely. 2015. "Phototaxis and the Origin of Visual Eyes Main Text." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 371: 1–11.

Rawlinson, Kate A., Francois Lapraz, Edward R. Ballister, Mark Terasaki, Jessica Rodgers, Richard J. McDowell, Johannes Girstmair, et al. 2019. "Extraocular, Rod-like Photoreceptors in a Flatworm Express Xenopsin Photopigment." *eLife* 8 (October). https://doi.org/10.7554/eLife.45465.

Rennison, Diana J., Gregory L. Owens, and John S. Taylor. 2012. "Opsin Gene Duplication and Divergence in Ray-Finned Fish." *Molecular Phylogenetics and Evolution* 62 (3): 986–1008.

Saari, John C. 2012. "Vitamin A Metabolism in Rod and Cone Visual Cycles." *Annual Review of Nutrition* 32 (1): 125–45.

———. 2014. "Regeneration of 11-Cis-Retinal in Visual Systems with Monostable and Bistable Visual." In *Vertebrate Photoreceptors*, edited by Takahisa Furukawa, James B. Hurley, and Satoru Kawamura, 47–71. Tokyo: Springer Japan.

Sakai, Kazumi, Takahiro Yamashita, Yasushi Imamoto, and Yoshinori Shichida. 2015. "Diversity of Active States in TMT Opsins." *PloS One* 10 (10): e0141238.

Salvini-Plawen, Luitfried Von. 2008. "Photoreception and the Polyphyletic Evolution of Photoreceptors (with Special Reference to Mollusca)*." *American Malacological Bulletin* 26 (1-2): 83–100.

Salvini-Plawen, L. von, and Ernst Mayr. 1977. "On the Evolution of Photoreceptors and Eyes." *Journal of Evolutionary Biology*, 207–63.

Santillo, Silvia, Pierangelo Orlando, Luciano De Petrocellis, Luigia Cristino, Vittorio Guglielmotti, and Carlo Musio. 2006. "Evolving Visual Pigments: Hints from the Opsin-Based Proteins in a Phylogenetically Old 'Eyeless' Invertebrate." *Bio Systems* 86: 3–17.

Sato, Keita, Takahiro Yamashita, Keiichi Kojima, Kazumi Sakai, Yuki Matsutani, Masataka Yanagawa, Yumiko Yamano, et al. 2018. "Pinopsin Evolved as the Ancestral Dim-Light Visual Opsin in Vertebrates." *Communications Biology* 1 (October): 156.

Schmidt-Rhaesa, Andreas. 2007. *The Evolution of Organ Systems*. Oxford University Press.

Schnitzler, Christine E., Kevin Pang, Meghan L. Powers, Adam M. Reitzel, Joseph F. Ryan, David Simmons, Takashi Tada, et al. 2012. "Genomic Organization, Evolution, and Expression of Photoprotein and Opsin Genes in Mnemiopsis Leidyi: A New View of Ctenophore Photocytes." *BMC Biology* 10 (December): 107.

Serb, Jeanne M., Anita J. Porath-Krause, and Autum N. Pairett. 2013. "Uncovering a Gene Duplication of the Photoreceptive Protein, Opsin, in Scallops (bivalvia: Pectinidae)." *Integrative and Comparative Biology* 53 (1): 68–77.

Shichida, Y., and T. Matsuyama. 2009. "Evolution of Opsins and Phototransduction." *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 364 (1531): 2881–95.

Shimizu, I., Y. Yamakawa, Y. Shimazaki, and T. Iwasa. 2001. "Molecular Cloning of Bombyx Cerebral Opsin (Boceropsin) and Cellular Localization of Its Expression in the Silkworm Brain." *Biochemical and Biophysical Research Communications* 287 (1): 27–34.

Shubin, Neil, Cliff Tabin, and Sean Carroll. 2009. "Deep Homology and the Origins of Evolutionary Novelty." *Nature* 457 (7231): 818–23.

Soni, Bobby G., and Russell G. Foster. 1997. "A Novel and Ancient Vertebrate Opsin." *FEBS Letters* 406 (3): 279–83.

Spaethe, Johannes, and Adriana D. Briscoe. 2005. "Molecular Characterization and Expression of the UV Opsin in Bumblebees : Three Ommatidial Subtypes in the Retina and a New Photoreceptor Organ in the Lamina," 2347–61.

Srivastava, Mansi. 2021. "Beyond Casual Resemblance: Rigorous Frameworks for Comparing Regeneration Across Species." *Annual Review of Cell and Developmental Biology* 37 (October): 415–40.

Srivastava, Mansi, Emina Begovic, Jarrod Chapman, Nicholas H. Putnam, Uffe Hellsten, Takeshi Kawashima, Alan Kuo, et al. 2008. "The Trichoplax Genome and the Nature of Placozoans." *Nature* 454 (7207): 955–60.

Stierwald, Michael, Nathalie Yanze, Roky P. Bamert, Lars Kammermeier, and Volker Schmid. 2004. "The Sine oculis/Six Class Family of Homeobox Genes in Jellyfish with and without Eyes: Development and Eye Regeneration." *Developmental Biology* 274 (1): 70–81.

Suga, Hiroshi, Volker Schmid, and Walter J. Gehring. 2008. "Evolution and Functional Diversity of Jellyfish Opsins." *Current Biology: CB* 18 (1): 51–55.

Suga, Hiroshi, Patrick Tschopp, Daria F. Graziussi, Michael Stierwald, Volker Schmid, and Walter J. Gehring. 2010. "Flexibly Deployed Pax Genes in Eye Development at the Early Evolution of Animals Demonstrated by Studies on a Hydrozoan Jellyfish." *Proceedings of the National Academy of Sciences of the United States of America* 107 (32): 14263–68.

Sun, H., D. J. Gilbert, N. G. Copeland, N. a. Jenkins, and J. Nathans. 1997. "Peropsin, a Novel Visual Pigment-like Protein Located in the Apical Microvilli of the Retinal Pigment Epithelium." *Proceedings of the National Academy of Sciences of the United States of America* 94 (18): 9893–98.

Tang, Peter H., Masahiro Kono, Yiannis Koutalos, Zsolt Ablonczy, and Rosalie K. Crouch. 2013. "New Insights into Retinoid Metabolism and Cycling within the Retina." *Progress in Retinal and Eye Research* 32 (1): 48–63.

Taniguchi, Yuki, Osamu Hisatomi, Masao Yoshida, and Fumio Tokunaga. 2001. "Pinopsin Expressed in the Retinal Photoreceptors of a Diurnal Gecko." *FEBS Letters* 496 (2-3): 69–74.

Tarttelin, Emma E., James Bellingham, Lindsay C. Bibb, Russell G. Foster, Mark W. Hankins, Kevin Gregory-Evans, Cheryl Y. Gregory-Evans, Dominic J. Wells, and Robert J. Lucas. 2003. "Expression of Opsin Genes Early in Ocular Development of Humans and Mice." *Experimental Eye Research* 76 (3): 393–96.

Tarttelin, Emma E., James Bellingham, Mark W. Hankins, Russell G. Foster, and Robert J. Lucas. 2003. "Neuropsin (Opn5): A Novel Opsin Identified in Mammalian Neural Tissue." *FEBS Letters* 554 (3): 410–16.

Terakita, A. 2005. "The Opsins." *Genome Biology* 6 (3): 213.

Terakita, Akihisa, Reiko Hara, and Tomiyuki Hara. 1989. "Retinal-Binding Protein as a Shuttle for Retinal in the Rhodopsin-Retinochrome System of the Squid Visual Cells." *Vision Research* 29 (6): 639–52.

Tong, D., N. S. Rozas, T. H. Oakley, J. Mitchell, N. J. Colley, and M. J. McFall-Ngai. 2009. "Evidence for Light Perception in a Bioluminescent Organ." *Proceedings of the National Academy of Sciences* 106 (24): 9836–41.

Torres-Dowdall, Julián, Nidal Karagic, Andreas Härer, and Axel Meyer. 2021. "Diversity in Visual Sensitivity across Neotropical Cichlid Fishes via Differential Expression and Intraretinal Variation of Opsin Genes." *Molecular Ecology* 30 (8): 1880–91.

Tosches, Maria Antonietta, Daniel Bucher, Pavel Vopalensky, and Detlev Arendt. 2014. "Melatonin Signaling Controls Circadian Swimming Behavior in Marine Zooplankton." *Cell* 159 (1): 46–57.

Tsukamoto, Hisao, I-Shan Chen, Yoshihiro Kubo, and Yuji Furutani. 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." *The Journal of Biological Chemistry* 292 (31): 12971–80.

Vanfleteren, J. R. 1982. "Monophyletic Line of Evolution? Ciliary Induced Photoreceptor Membranes." *Visual Cells in Evolution/editor, Jane A. Westfall*. https://agris.fao.org/agris-search/search.do?recordID=US201302623000.

Van Hooser, J. Preston, Yan Liang, Tadao Maeda, Vladimir Kuksa, Geeng-Fu Jang, Yu-Guang He, Fred Rieke, Henry K. W. Fong, Peter B. Detwiler, and Krzysztof Palczewski. 2002. "Recovery of Visual Functions in a Mouse Model of Leber Congenital Amaurosis." *The Journal of Biological Chemistry* 277 (21): 19173–82.

Velarde, Rodrigo A., Colin D. Sauer, Kimberly K. O. Walden, Susan E. Fahrbach, and Hugh M. Robertson. 2005. "Pteropsin: A Vertebrate-like Non-Visual Opsin Expressed in the Honey Bee Brain." *Insect Biochemistry and Molecular Biology* 35 (12): 1367–77.

Verasztó, Csaba, Martin Gühmann, Huiyong Jia, Vinoth Babu Veedin Rajan, Luis A. Bezares-Calderón, Cristina Piñeiro Lopez, Nadine Randel, et al. 2018. "Ciliary and Rhabdomeric Photoreceptor-Cell Circuits Form a Spectral Depth Gauge in Marine Zooplankton." *bioRxiv*, 1–19.

Vöcking, Oliver, Ioannis Kourtesis, Sharat Tumu, and Harald Hausen. 2017. "Co-Expression of Xenopsin and Rhabdomeric Opsin in Photoreceptors Bearing Microvilli and Cilia." *eLife* 6: e23435.

Vöcking, Oliver, Lucas Leclère, and Harald Hausen. 2021. "The Rhodopsin-Retinochrome System for Retinal Re-Isomerization Predates the Origin of Cephalopod Eyes." *BMC Ecology and Evolution* 21 (1): 215.

Vopalensky, Pavel, Jiri Pergner, Michaela Liegertova, Elia Benito-Gutierrez, Detlev Arendt, and Zbynek Kozmik. 2012. "Molecular Analysis of the Amphioxus Frontal Eye Unravels the Evolutionary Origin of the Retina and Pigment Cells of the Vertebrate Eye." *Proceedings of the National Academy of Sciences of the United States of America* 109 (38): 15383–88.

Wang, Jin Shan, and Vladimir J. Kefalov. 2011. "The Cone-Specific Visual Cycle." *Progress in Retinal and Eye Research* 30 (2): 115–28.

Wernet, Mathias F., Michael W. Perry, and Claude Desplan. 2015. "The Evolutionary Diversity of Insect Retinal Mosaics: Common Design Principles and Emerging Molecular Logic." *Trends in Genetics*. https://doi.org/10.1016/j.tig.2015.04.006.

Yamashita, Takahiro, Hideyo Ohuchi, Sayuri Tomonari, Keiko Ikeda, Kazumi Sakai, and Yoshinori Shichida. 2010. "Opn5 Is a UV-Sensitive Bistable Pigment That Couples with Gi Subtype of G Protein." *Proceedings of the National Academy of Sciences of the United States of America* 107 (51): 22084–89.

Yau, King Wai, and Roger C. Hardie. 2009. "Phototransduction Motifs and Variations." *Cell* 139 (2): 246–64.

Zagozewski, Jamie L., Qi Zhang, Vanessa I. Pinto, Jeffrey T. Wigle, and David D. Eisenstat. 2014. "The Role of Homeobox Genes in Retinal Development and Disease." *Developmental Biology* 393 (2): 195–208.

Zhang, Jianye, Elliot H. Choi, Aleksander Tworak, David Salom, Henri Leinonen, Christopher L. Sander, Thanh V. Hoang, et al. 2019. "Photic Generation of 11-Cis-Retinal in Bovine Retinal Pigment Epithelium." *The Journal of Biological Chemistry* 294 (50): 19137–54.