Evolutionary Constraint on Visual and Nonvisual Mammalian Opsins

Brian A. Upton*,†‡§, Nicolás M. Díaz||, Shannon A. Gordon||, Russell N. Van Gelder||§, Ethan D. Buhr|| and Richard A. Lang*,†,#,**,1

*Visual Systems Group, Abrahamson Pediatric Eye Institute, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, †Center for Chronobiology, Division of Pediatric Ophthalmology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, ‡Molecular & Developmental Biology Graduate Program, University of Cincinnati College of Medicine, Cincinnati, Ohio, §Medical Scientist Training Program, University of Cincinnati College of Medicine, Cincinnati, Ohio, ||Department of Ophthalmology, University of Washington School of Medicine, Seattle, Washington, ‡Department of Ophthalmology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, and #Division of Developmental Biology, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio

Abstract Animals have evolved light-sensitive G protein–coupled receptors, known as opsins, to detect coherent and ambient light for visual and nonvisual functions. These opsins have evolved to satisfy the particular lighting niches of the organisms that express them. While many unique patterns of evolution have been identified in mammals for rod and cone opsins, far less is known about the atypical mammalian opsins. Using genomic data from over 400 mammalian species from 22 orders, unique patterns of evolution for each mammalian opsins were identified, including photoisomerases, RGR-opsin (RGR) and peropsin (RRH), as well as atypical opsins, encephalopsin (OPN3), melanopsin (OPN4), and neuropsin (OPN5). The results demonstrate that OPN5 and rho-dopsin show extreme conservation across all mammalian lineages. The cone opsins, SWS1 and LWS, and the nonvisual opsins, OPN3 and RRH, demonstrate a moderate degree of sequence conservation relative to other opsins, with some instances of lineage-specific gene loss. Finally, the photoisomerase, RGR, and the best-studied atypical opsin, OPN4, have high sequence diversity within mammals. These conservation patterns are maintained in human populations. Importantly, all mammalian opsins retain key amino acid residues important for conjugation to retinal-based chromophores, permitting light sensitivity. These patterns of evolution are discussed along with known functions of each atypical opsin, such as in circadian or metabolic physiology, to provide insight into the observed patterns of evolutionary constraint.

Keywords encephalopsin, melanopsin, neuropsin, RGR-opsin, peropsin, atypical

1. To whom all correspondence should be addressed: Richard A. Lang, Center for Chronobiology, Division of Pediatric Ophthalmology, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229-3026, USA; e-mail: richard.lang@cchmc.org.
Life has evolved in the presence of consistent rhythms of light and dark. This light has been used for adaptive advantage by allowing an organism to interact with its external world, through focusing and detection of coherent, image-forming light, as well as to synchronize to daily and seasonal rhythms via the detection of ambient, scattered light. While several mechanisms of light detection are known, all animals use a single family of retinal-based G protein–coupled receptors (GPCRs), known as opsins, for light detection. For their light-detecting function, energy from an incident photon isomerizes a retinal-based chromophore, resulting in a structural change within the opsin protein that initiates a G protein signaling event. This protein family evolved prior to the evolution of animal visual systems, suggesting that the first functions for opsins were nonvisual, such as detecting time of day information based on light intensity.

Research over the past two decades has strongly indicated that several of these opsin classes play specific and crucial roles in circadian entrainment in mammals. While other classes of photopigments, such as cryptochromes, function as circadian photoreceptors in insects (Emery et al., 1998; Stanewsky et al., 1998), in vertebrates all known circadian photopigments are opsin-based. In particular, recent work has suggested that the nonvisual opsins OPN4 (melanopsin), OPN5 (neuropsin), and OPN3 (encephalopsin) each have unique functions for circadian rhythmicity in the mammal. Hence, an analysis of the evolution of these photoreceptive proteins may provide insights into the evolution of circadian rhythmicity in animals.

Opsins can be broadly classified as visual opsins, atypical opsins (i.e., nonvisual), or photoisomerases. This functional classification largely mirrors the evolutionary divergence of these opsins and has been analyzed based on the exon-intron structure of each opsin gene (Bellingham et al., 2003). The visual opsins and encephalopsins are referred to as C-type or ciliary opsins (regardless of the expression in cilia). In addition, a second class of opsins with a more distant divergence is the R-type or rhodomeric opsins (regardless of the association with rhabdomeres), which include the atypical opsin OPN4 and invertebrate visual opsins. Cnidopsins are another class of opsins that are not found in vertebrate lineages (Plachetzki et al., 2007). Finally, Group 4 (RGR/G.) opsins share a common evolutionary lineage and consist of photoisomerases and the atypical opsin, OPN5. While each of these 4 classes have distinct genomic organization, they all evolved from duplication events from a single melatonin receptor-like GPCR in stem eumetazoans prior to the Cambrian explosion and subsequently diversified prior to the vertebrate evolution (Feuda et al., 2012; Fleming et al., 2020). Thus, all vertebrates contain a similar repertoire of opsin genes. Despite the vast array of opsins found in the animal kingdom, the most recent common ancestor to all mammals likely had just 10 (Figure 1a) (Gerkema et al., 2013). Loss of opsin genes in stem mammals is often attributed to a “nocturnal bottleneck” during the Mesozoic era, affecting both visual and nonvisual opsins (Gerkema et al., 2013). Which opsins persist is dependent on the function of each opsin and the lighting environment in which the species inhabits. Ecological niches that promote diversification of the light-detecting mechanisms commonly include marine or subterranean habitats or strictly nocturnal lifestyles. The following analysis describes the mutational rate, relative site-by-site variation, and unique deviations in opsin evolution, as well as a brief summary of the currently known functions for each opsin that has contributed to the observed pattern of evolution.

MATERIALS AND METHODS

Opsin Sequence Acquisition

Protein sequences used in this study were acquired from multiple publicly available resources, namely, NCBI (Sayers et al., 2009), UCSC Genome Browser (Haeussler et al., 2019), UniProt (UniProt Consortium, 2019), Ensembl (Cunningham et al., 2019), Bat1KT (Jebb et al., 2020; Teeling et al., 2018), and the Ruminant Genome Database (L. Chen et al., 2019), as well as select publications (Meredith et al., 2013; Newman and Robinson, 2006; Sadier et al., 2018). Accession numbers and assemblies used for each species’ opsin sequence can be found in supplemental data 1. Fragmented and incomplete sequences were not used in analyses. Splice variants were excluded from the analysis.

Substitutions/Site and Evolutionary Rate

Amino acid sequences were aligned using the MUSCLE algorithm. Substitutions/site was calculated in MEGA-X (Kumar et al., 2018) using the Poisson correction model (Zuckerandl and Pauling, 1965). The shape parameter for the discrete gamma distribution was first estimated under the Jones-Taylor-Thornton (JTT) model (Jones et al., 1992). Species list was entered into TimeTree (Kumar et al., 2017) to generate a phylogenetic tree and determine estimates of divergence between species (Boc et al., 2012). Evolutionary rate was determined by pairwise
substitutions/site divided by evolutionary distance (i.e., twice the time since species divergence).

**Similarity Identity Matrices**

Similarity and identity matrices were constructed using MatGat (Campanella et al., 2003) using the BLOSUM62 substitution matrix and opsin protein sequences.

**Site-by-Site Relative Mutational Rate**

The relative mutational rate for each amino acid position was determined under the JTT model using a discrete gamma distribution with 8 categories, allowing some sites to stay invariant (Jones et al., 1992; Kumar et al., 2018). Rates were scaled such that the average evolutionary rate for each protein was 1. The color scale was set such that the minimum rate for each protein was gray, the average rate (1) was yellow, and the maximum rate corresponded to the maximum absorption of each opsin. Only sites that were present in the ancestral amino acid sequence were used for analysis to prevent skewing by lineage-specific insertions.

**Ancestral Sequence Reconstruction**

Stem mammalian ancestral sequences were reconstructed using MUSCLE-aligned sequences and phylogenetic trees generated from each species using TimeTree (Kumar et al., 2017) within Mega-X (Kumar et al., 2018) based on maximum likelihood using the JTT model (Jones et al., 1992). Available opsin sequences from Gallus, Alligator mississippiensis, and Anolis carolinensis were used as outgroup sequences.

**PER2::LUCIFERASE Rhythms**

All animal experiments were carried out according to Institutional Animal Care and Use Committee guidelines at University of Washington, Seattle, WA. Wild-type: Per2Luciferase/Luciferase or Opn3lacZlacZ, Per2Luciferase/Luciferase mice were euthanized by CO₂ asphyxiation followed by cervical dislocation. The dorsal interscapular fat pads and inguinal white adipose tissue were dissected into cold Hank’s Balanced Salt Solution (Gibco). Interscapular white fat was dissected away from brown fat under a dissecting microscope. Small (~1 mm³) pieces of adipose tissue were placed on cell culture inserts (Millipore, PICMORC50) and cultured in sealed dishes in Dulbecco’s Modified Eagle Medium (Cellgro) containing B27 serum-free supplement (Life Technologies), 0.1 mM D-Luciferin potassium salt (Biosynth), 352.5 μg/mL sodium bicarbonate, 10 mM HEPES, and 25 units/mL penicillin and 25 μg streptomycin (Life Technologies). Bioluminescence was monitored at 10-min intervals for 2.5 days using a Lumicycle 32 photometer device (Actimetrics, www.actimetrics.com) contained within a 36 °C incubator.
RESULTS AND DISCUSSION

Visual Opsins

Rhodopsin (RHO) is the dim light–sensitive opsin expressed in rod cells in the mammalian retina that is necessary for scotopic vision (λ_{max} ~500 nm) (Imai et al., 2007). RHO is well-conserved in all mammals assessed. This sequence conservation includes animals living in virtually lightless environments, such as subterrestrial mammals, or limited-light environments, such as nocturnal and marine mammals (Figures 1a and 2a). RHO is even well-conserved in species of mole rats, which often possess a rudimentary eye that lacks a lens and is covered in fur and skin (Emerling and Springer, 2014a; Hendriks et al., 1987). Despite these limitations, mole rats are still able to detect breaches in tunnels and possess negative phototaxis. This ability demonstrates that even if conventional image-forming vision has been lost, there is continued selective pressure for opsin-mediated photodetection (Cooper et al., 1993; Kott et al., 2010). In addition, blind mole rats can entrain to a light-dark cycle, a nonvisual function of the retina that can be mediated via RHO, cone opsins, or OPN4, which remain present and functional in this fossorial mammal (David-Gray et al., 1998, 1999; Esquiva et al., 2016; Hannibal et al., 2002; Janssen et al., 2000). The RHO sequences that have diverged the most from the predicted ancestral sequence belong to the squirrel family of rodents (Sciuridae) and tree shrews (order Scandentia) (Figure 3a and Suppl. Fig. S2A), both of which, intriguingly, possess cone-dominant retinas (Kryger et al., 1998; Müller and Peichl, 1989).

Unlike RHO, the cone opsins are remarkably diverse within the mammalian class (Figure 3b and 3c). There are 4 different mammalian cone opsins: short-wavelength-sensitive 1 (SWS1; λ_{max} ~430 nm), short-wavelength-sensitive 2 (SWS2; λ_{max} ~440 nm), medium-wavelength-sensitive (MWS; λ_{max} ~530 nm), and long-wavelength-sensitive (LWS1; λ_{max} ~560 nm) (Merbs and Nathans, 1992; Wakefield et al., 2008). Most mammals possess 2 cone opsins, SWS1 and MWS/LWS, for dichromatic vision. There was likely redundant function between the two SWS opsins in early mammals, as monotremes (i.e., egg-laying mammals) retained SWS2 and lost SWS1, whereas all other mammals reciprocally retained SWS1 and lost SWS2 (Figure 1a) (Wakefield et al., 2008). Notably, trichromacy is only found within primates, as monotremes (i.e., egg-laying mammals) retained SWS2 and lost SWS1, whereas all other mammals reciprocally retained SWS1 and lost SWS2 (Figure 1a) (Wakefield et al., 2008). In primates, trichromacy arose independently in old world monkeys, apes, and humans (parvorder Catarhini) and in new world howler monkeys (genus Alouatta) through a duplication event of LWS and sequence divergence in the former and unequal crossover of polymorphic alleles in the latter. By contrast, in trichromatic marsupials, there is no genetic evidence of an additional
opsin gene. Due to the limited number of species expressing SWS2 and MWS opsins, they were not included in further analyses. The remaining 2 cone opsins, SWS1 and LWS, have evolved with a moderate degree of sequence conservation relative to other mammalian opsins (Figure 2b and 2c). Each has been lost in specific lineages, always occurring in animals with restricted light availability, such as marine, nocturnal, or subterrestrial mammals. For example, SWS1 has been lost in all cetaceans (toothed and baleen whales) (Fasick and Robinson, 2016; Meredith et al., 2013; Peichl et al., 2001), all pinnipeds (seals) (Levenson et al., 2006; Peichl et al., 2001; Peichl and Moutairou, 1998), all xenarthrans (armadillos, sloths, and anteaters) (Emerling and Springer, 2014b), and night monkeys (family Aotidae) (Jacobs et al., 1993). Similarly, LWS function has been lost in many whale species (most baleen whales, some toothed whales) (Meredith et al., 2013), all xenarthrans (armadillos, sloths, and anteaters) (Emerling and Springer, 2014b), and naked mole rats (Kim et al., 2011), and star-nosed and golden moles (Emerling and Springer, 2014a).

Numerous amino acid residues have been identified in RHO, SWS1, and LWS that shift the spectral sensitivity of the opsins to shorter or longer wavelengths (Bowmaker, 2008; Lin et al., 2017; Musilova et al., 2019; Nathans, 1990; Yokoyama, 2008) (Figure 2c). While these mutations have been best studied in RHO homologues, they are broadly conserved across species. The absorption spectrum of retinal within an opsin is altered by these amino acid residues, altering the energetics of retinal isomerization. Notable residues for cone opsin spectral tuning include five which red-shift MWS/LWS (permitting trichromacy when both MWS and LWS are present) and F2.53 (in Ballesteros-Weinstein nomenclature, corresponding to Phe-86 in bovine RHO) in SWS1, which shifts the $\lambda_{\text{max}}$ of SWS1 into the UV spectrum (Cowing et al., 2002; Hunt et al., 2007; Yokoyama, 2000; Yokoyama and Radlwimmer, 1998). The predicted mammalian ancestral sequence for SWS1 contains this substitution, indicating that stem mammals had visual perception into the UV range, although it is a locus of increased variability (Suppl. Fig. S3B). While these loci are the best predictors for visual opsins, they may still be informative in spectral tuning of the remaining opsins, although such predictions should be determined experimentally.

In addition to the variation in amino acid residues necessary for spectral tuning, increased sequence variation in the amino acid residues necessary for light sensitivity, such as the seventh transmembrane
lysine residue, is seen in SWS1 and LWS. This observation is due to the inclusion of organisms that have lost the opsin protein’s photosensitive functions and therefore have reduced constraint on these key amino acid residues but have retained an identifiable genomic sequence (Figure 2c). These patterns of evolution indicate the variable need for color vision in distinctly illuminated environments. In addition, environmental lighting does not only impact the visual function of rod and cone opsins but also influences nonvisual functions of these opsins. Within the retina, visual opsins in rods and cones can signal to intrinsically photosensitive retinal ganglion cells to mediate nonvisual responses such as circadian entrainment and the pupillary light response, independent of OPN4, although in the absence of OPN4, these responses are not sustained as well (Altimus et al., 2008; Güler et al., 2008; Lucas et al., 2003; Panda et al., 2002, 2003). Visual opsins are also found in extraretinal tissues, potentially suggesting additional nonvisual roles. Overall, when evolutionary pressure favors cone opsin function, they are conserved with moderate sequence constraint relative to other mammalian opsins; however, their necessity understandably varies with the environmental light of the animal. While these results largely recapitulate previous findings on mammalian opsin evolution, they serve as an important point of reference for the subsequent analysis into the mammalian evolution of nonvisual opsin evolution.

Encephalopsins

Encephalopsins are a unique, yet poorly understood class of opsins. While several encephalopsins are present in teleost and sauropsid genomes, most mammals have only retained the blue light–sensitive OPN3, although monotrems and marsupials have retained teleost multiple tissue opsin 2 (TMT2, $\lambda_{\text{max}} \sim 470 \text{ nm}$) (Kato et al., 2016; Sakai et al., 2015). Their expression is intriguingly not limited to the eye, as they are found throughout the body in a variety of cell types (Blackshaw and Snyder, 1999; Halford et al., 2001). Among the mammalian opsins, OPN3 stands out as the only opsin that has not yet been expressed as a functional photopigment in vitro. While zebrafish OPN3 has been shown to function as a blue light photopigment with $\lambda_{\text{max}}$ of 465 nm and murine OPN3 has been shown to bind the retinaldehyde pigment, reconstitution of functional photopigment has remained elusive (Ozdeslik et al., 2019; Sugihara et al., 2016). Whether this is due to technical issues with heterologous expression or whether OPN3 has limited photoreceptive function is currently unclear (Olinski et al., 2020).

Outside of mammals, encephalopsins appear to function to synchronize peripheral clock gene rhythmicity to the external light-dark cycle (Cavallari et al., 2011; Moutsaki et al., 2003; Poletini et al., 2015). In mammals, in tissues such as the retina and skin, OPN3 appears neither necessary nor sufficient for local light entrainment (Buhr et al., 2015, 2019). The most apparent role to date for OPN3 in circadian function is in setting the amplitude of core clock gene oscillation. Retinal clock gene amplitude is diminished in the absence of OPN3 (Buhr et al., 2015). This phenomenon appears to occur in other OPN3-expressing tissues, as loss of Opn3 dampens clock gene amplitude in interscapular and inguinal adipocytes (Figure 4a and 4b). These data suggest that OPN3 has dedicated functions within the circadian clock but leave open the question of whether it can serve as a photopigment for entrainment or phase shifting.

Lipolysis, or the mobilization of lipids from adipocytes, occurs with a circadian pattern that is controlled, in part, by clock genes within adipocytes. Mice with specific conditional loss of the clock gene Bmal1 in adipocytes have disrupted lipolysis, increased weight gain in response to high-fat diet, increased adipocyte size, and a reduced metabolic rate (Paschos et al., 2012; Shostak et al., 2013). Loss of Opn3 from adipocytes also results in impaired lipolysis, increased weight gain in response to high-fat diet, increased adipocyte size, and a reduced metabolic rate (Nayak et al., 2020; Sato et al., 2020). Interestingly, OPN3 is downregulated in adipocytes in response to high-fat diet (Choi et al., 2015). The findings that OPN3 facilitates clock gene rhythmicity in adipocytes help to reconcile these metabolic phenotypes. However, OPN3 and light were also demonstrated to interdependently modulate adaptive thermogenesis and glucose uptake in adipocytes, suggesting that OPN3 also functions in the photoreceptive pathway. Additional photoreceptive functions suggested for OPN3 include photorelaxation of smooth muscle within the uterus and airway (Barreto Ortiz et al., 2018; Wu et al., 2020; Yim et al., 2019, 2020). A non-light-dependent role for OPN3 has also been proposed in epidermal melanocytes, where it heterodimerizes with melanocortin 1 receptor to regulate pigmentation (Ozdeslik et al., 2019). A splice variant of OPN3, which excludes the second exon and subsequently truncates the protein within the helix, is unlikely to function as a light-sensitive protein or even as a GPCR, but may still function as a negative modulator of other proteins (Haltaufderhyde et al., 2015). Taken together, these diverse functions are expected to produce different patterns of evolution than seen with visual opsins.
In Euarchontoglires (e.g., primates and rodents), OPN3 is well-conserved with little sequence divergence (Figures 2a and 3d). However, in the laurasiatherian superorder, OPN3 remains well-conserved in carnivora, but has been lost in the Vespertilionidae family of bats, camelids, pigs, and ruminants (Figure 3d and Suppl. Fig. S2D). In xenarthrans, no complete sequences were identified for OPN3, but between sloths and armadillos all regions of the sequence could be identified, indicating either poor sequence coverage of OPN3 or, less likely, a recent loss in both branches of xenarthrans. Finally, OPN3 could not be found in the platypus genome, suggesting additional loss in monotremes. Altogether, these results suggest that the lighting environment may play some role in OPN3 sequence constraint (e.g., bats and early monotremes, but not subterrestrial rodents). It is possible, however, that other factors such as food and water availability or metabolic rate may place additional demands on sequence conservation (e.g., even-toed ungulates). An important caveat to genomic analysis of OPN3 is its high GC content in the first exon, leading to poor sequence coverage (Wang et al., 2011). In addition, domesticated animals (goat, cattle) serve as reference genomes for other artiodactyla species. Potential selection against the metabolic effects of OPN3 during domestication, similar to the absence of the thermogenic gene UCP1 from adipocytes in pigs (Berg et al., 2006), may bias the analysis of nondomesticated OPN3 artiodactyla sequences due to poor alignment for the reference genomes.

Melanopsin

OPN4 is a blue light–sensitive (λ_max ~480 nm) atypical opsin expressed in intrinsically photosensitive retinal ganglion cells (ipRGCs) in the retina that mediates a range of nonvisual functions during development through adulthood. These functions include negative phototaxis, retinal vascular development in neonates, circadian photoentrainment of the suprachiasmatic nucleus, and the pupillary light response (Do, 2019; Hattar et al., 2002, 2003; Johnson et al., 2010; Lucas et al., 2003; Panda et al., 2002; Provencio et al., 1998; Rao et al., 2013; Ruby et al., 2002). Our analysis demonstrates that OPN4 shows the most sequence diversity within mammals relative to other opsins (Figure 2a). A previous study has demonstrated that OPN4 sequence variation is greater between 2 Euarchontoglire species than between 2 marsupials that diverged at similar evolutionary times (Pires et al., 2007). Our results demonstrate that the source of this variation is due to increased mutation rates in rodents, rather than increased mutations in primates or increased genetic constraint in marsupials (Suppl. Fig. S2E). Across all mammals, most of the observed diversity arises from mutations in the N- and C-termini of the protein (Suppl. Fig. S3E). These changes commonly alter the number of phosphorylatable serine and threonine residues on the C-terminus, which is known to alter the deactivation kinetics of OPN4 (Blasic et al., 2012a, 2012b; Blasic et al., 2014; Somasundaram et al., 2017). Proximal C-terminal serine and threonine residues appear more likely to regulate protein function. Reduced serine and threonine residue sites have been suggested to preserve rod function in animals occupying low-light environments, such as armadillos (Fasick and Robinson, 2016), by modulating the kinetics of the pupillary light response (Fasick and Robinson, 2016; Somasundaram et al., 2017). Our mammalian classwide analysis of the C-terminus of OPN4 is consistent with this, although notably whales were not observed to have a decrease in phosphorylatable residues, whereas mouse-like rodents were found to have an increase (Figure 5). When separating these sites of phosphorylation into previously described clusters (P-I through P-IV) (Valdez-Lopez et al., 2020), there is an elevation of phosphorylatable sites in rodents in P-II, the cluster with the most influence on...
OPN4 deactivation (Figure 5). The cluster with the most diversity across mammals is P-IV (Figure 5), which has minimal influence on OPN4 deactivation (Valdez-Lopez et al., 2020). Interestingly, we did not find any mammalian lineages with a loss of OPN4, indicating its importance for all mammalian life independent of the lighting environment.

Increased sequence variation in OPN4 does not include contribution from various isoforms of OPN4. OPN4 has 2 isoforms, a short (OPN4S) and a long form (OPN4L) differing only in the distal C-terminus (Hughes et al., 2012; Pires et al., 2009). OPN4S is the predominant isoform and is found in the M1 subtype of ipRGCs, whereas OPN4L is found in both M1 and non-M1 ipRGCs (Pires et al., 2009). Both isoforms have similar spectral tuning and response amplitudes in vitro; however, differences in the C-terminus may alter post-translational regulation of the protein in vivo (Pires et al., 2009). The functions of these isoforms have reflected their relative expression and subtype specificity, in which OPN4S exclusively functions in pupillary light response and OPN4L exclusively functions in negative masking, whereas both OPN4S and OPN4L function in phase shifting and sleep induction in mice (Jagannath et al., 2015). The developmental roles of OPN4 are likely limited to OPN4S, as it is the first of the two isoforms to be expressed in the retina (Hughes et al., 2012).

Unlike the previously discussed opsins, OPN4 (and other rhabdomeric opsins such as arthropod opsins) can undergo a process known as photoreversal. During photoreversal, the all-trans-retinal remains bound to the opsin and a subsequent photon isomerizes retinal back to a cis-configuration, eliminating the
need for extrinsic regeneration of the chromophore, as is present in the visual cycle. This characteristic likely involves numerous amino acid residues stabilizing all-trans-retinal within the opsin and is not unique to rhabdomeric opsins, as non-eutherian OPN5 (discussed in the next section) and lamprey parapinopsin (a ciliary opsin) also possess photoreversal (Koyanagi et al., 2004; Yamashita et al., 2010). Distinct from other mammalian opsins, OPN4 is tristable, forming stable complexes with 11-cis-, all-trans-, and 7-cis-retinal, and this property of OPN4 allows for sustained signaling and broad absorption which is beneficial for its nonvisual functions (Emanuel and Do, 2015). This stability is consistent with a view that retinal remains covalently bound to melanopsin, preventing bleaching observed in other pigments (Sexton et al., 2012).

**Neuropsin**

OPN5 is a violet light–sensitive ($\lambda_{max}$ ~380 nm) opsin expressed in the retina, cornea, skin, brain, and testes (Kojima et al., 2011; Tarttelin et al., 2003; Yamashita et al., 2010). While OPN5 in birds and amphibians is bistable, binding all-trans-retinal with a second absorption peak in blue wavelengths, mammalian OPN5 does not bind to all-trans-retinal and lacks this second absorption peak (Yamashita et al., 2010). A single mutation is responsible for this shift at location 4.57 (corresponding to A168 in bovine RHO) with an alanine residue conferring bistability and a threonine residue, present in most mammals, lacking bistability (Yamashita et al., 2014). Interestingly, monotremae and marsupials have A4.57, suggesting that monostable OPN5 evolved in eutherian mammals and was not present in stem mammals (Suppl. Fig. S2F). Only recently have functions been identified for mammalian OPN5. OPN5 functions to regulate perinatal vascular development and entrains local retinal, corneal, and skin clock gene rhythmicity independent of the suprachiasmatic nucleus; it also plays a minor role in behavioral entrainment/phase shifting (Buhr et al., 2015, 2019; Díaz et al., 2020; Nguyen et al., 2019; Ota et al., 2018). In the mouse, OPN5 functions in the medial preoptic nucleus of the hypothalamus to regulate energy metabolism and thermogenesis (Zhang et al., 2020). Importantly, all of these known functions of OPN5 are dependent on light. Consistent with this finding, ex vivo phase shifting of the skin and cornea has an action spectrum that matches the in vivo absorption spectrum of OPN5 (Buhr et al., 2019; Díaz et al., 2020).

In birds, OPN5 is expressed in tanyocytes that line the third ventricle, where it detects seasonal changes in light to regulate circannual physiology and behavior independent of the retina (Nakane et al., 2010). However, in the murine hypothalamus, OPN5 is not expressed in tanyocytes (unpublished data). Interestingly, numerous studies have converged on a mammalian $\text{Opn5}^+$/Lepr$^{-}$/Ptger3$^+$/Bdnf$^+$/Adcyap1$^+$/Qrfp$^+$ preoptic population as a potent regulator of energy metabolism and thermogenesis that also functions in regulating entry to torpor (Hrvatin et al., 2020; Moffitt et al., 2018; Takahashi et al., 2020; Tan et al., 2016; Yu et al., 2016; Zhang et al., 2020). The functions of these marker genes provide insight into the role of this OPN5-expressing cell type, as leptin receptor (Lepr) signaling conveys information about the energy status of an organism, prostaglandin receptor EP3 signaling mediates pyrexia, and Qrfp regulates activity and food intake. It is conceivable that this neuronal population serves as a central hub, integrating temperature, seasonal lighting, and food availability to regulate seasonal changes in metabolism in mammals.

Despite the seemingly disjoint functions of OPN5, it is the most conserved mammalian opsin (Figure 2a). We were unable to identify any mammalian species that had lost OPN5 or that had greatly deviated from the ancestral sequence (Figure 3f and Suppl. Fig. S2F). Most mutations occur in the N- or C-termini of the protein (Suppl. Fig. S3F), while all residues necessary for GPCR or photoreceptive function remained intact (Figure 2c). Most of the residues associated with spectral tuning in the visual opsins show little variation, suggesting that there is little deviation from its known violet absorption (Suppl. Fig. S3F). In addition, there are several potential isoforms of OPN5; however, no functions or tissue-specific expression of these isoforms has been reported. It is likely that extraretinal functions of OPN5 may mediate this extreme sequence conservation in mammals, as mice lacking hypothalamic OPN5 have profound metabolic dysregulation.

**Photoisomerases**

Photoisomerases bind all-trans retinal and convert it to 11-cis-retinal upon light stimulation, recycling the chromophore to maintain the visual cycle in parallel with retinal isomerase (Chen et al., 2001; Morshedian et al., 2019; Radu et al., 2008). RGR and RRH are both expressed within the retinal pigmented epithelium, while RGR is additionally expressed in Müller glia within the retina (Morshedian et al., 2019; Shen et al., 1994; Sun et al., 1997). It should be noted that RGR expression in Müller glia in diurnal animals, such as the human and cattle, is far greater than in nocturnal animals, such as the mouse (Zhang et al., 2019). This observation is consistent with an important role of RGR as a photoisomerase in the cone visual cycle, which occurs in photopic light conditions during the
RGR is poorly conserved relative to other opsins (Figure 2a) and notably is absent in marsupial genomes (Figure 1a). In addition, decreased genetic constraint is seen in hystricomorphs, the rodent suborder containing guinea pigs, mole rats, chinchillas, and degus (Figure 3g and Suppl. Fig. S2G). RGR likely does not function as a signaling GPCR due to accumulated mutations in the ionic lock switch, a domain necessary for receptor activation and coupling to G proteins upon activation (Figure 2c) (Vogel et al., 2008). For example, Euarchontoglires, laurasiatherians, and xenarthrans all have accumulated mutations in the highly conserved E/DRY motif within the third helix, while all mammals lack the highly conserved glutamate (E5.30) located within the sixth helix that serves as a counterion to the switch in the inactive state (Palczewski et al., 2000; Vogel et al., 2008). However, other features, such as those necessary for GPCR structure and retinal binding, remain conserved in mammals, supporting RGR as a photoreceptive but nonsignaling GPCR. A splice variant, in which 4 amino acids in the first extracellular loop are inserted, greatly reduces photoisomerization, while a second splice variant, in which 38 amino acids are excluded from a region spanning the sixth and seventh transmembrane helices, abolishes the ability of RGR to regenerate 11-cis retinal (Zhang et al., 2019). It is currently unclear what the consequences are from the loss of RGR in marsupials or mutations in hystricomorphs, but in mice, loss of RGR results in cones losing sensitivity during light exposure and reduced electroretinogram b-wave amplitude following constant light exposure (Chen et al., 2001; Morshedian et al., 2019). It is conceivable that diurnal animals with high visual acuity would be under increased genetic constraint relative to nocturnal mammals or animals that do not rely heavily on the visual system without additional adaptations to regenerate chromophore for the cone visual cycle.

Peropsin is moderately conserved (Figure 2a) with no identifiable loss in mammalian lineages. Increased sequence variation is seen in 2 groups: mouse-like rodents (i.e., myomorpha) and the order eulipotyphla (e.g., shews, hedgehogs) (Figure 3h and Suppl. Fig. S2H). Unlike RGR, RRH has retained the DRY sequence within the ionic lock switch as well as other GPCR features necessary for structure, G protein signaling, and retinal binding (Figure 2c). While no functions for RRH have been established in mammals beyond photoisomerization and retinal transport, it is interesting that arachnid RRH is in an active, potentially signaling state in the dark and is suppressed by light (Cook et al., 2017; Nagata et al., 2018). Not only do these results suggest that RRH may have G protein signaling functions, but that the functions of RRH are conserved in all mammals, regardless of their lighting environment although decreased sequence constraint in subterrestrial/nocturnal mammals demonstrate a spectrum of biological selection.

Genetic Constraint in the Human Population

While evolutionary analysis provides insight into genetic constraint on homozygous variants across time, they provide little evidence for ongoing constraint in human populations. Fortunately, available genomic data on allelic variants from more than 140,000 individuals from the Genome Aggregation Database (gnomAD) allow us to understand continued selective pressure on hemizygous opsin sequence variants as determined by the ratio of observed-to-predicted variants for each gene (Karczewski et al., 2020). Of note, MWS and LWS cannot be analyzed with this method due to their location on the X chromosome (Vollrath et al., 1988). No opsins demonstrate selection against synonymous variants (i.e., changes in nucleotides that do not change amino acid sequence), indicating that the sequences are not located in hypomutated regions of the genome (Siepel et al., 2005) and that any constraint is not due to the primary DNA sequence, as might be the case for non-coding regulatory RNA elements (e.g., microRNA [miRNA], long noncoding RNAs [lncRNA]) (Morris and Mattick, 2014) (Figure 6a). However, OPN3 and OPN5 demonstrate reduced variation to missense variants (i.e., mutations that result in a change in amino acid sequence) (Figure 6b). Finally, SWS1, OPN3, and OPN5 all demonstrate selection against nonsense variants (i.e., premature stop codons, frameshift mutations, mutations at splice junctions) (Figure 6c). Thus, there is continued selective pressure against hemizygous variants for the two atypical opsins, OPN3 and OPN5, and the visual opsin, SWS1, in the modern human population.

It is important to consider that although not constrained against hemizygous variants at the population level, the remaining opsins RHO, OPN4, RGR, and RRH are likely still under selective pressure as variants of RHO and RGR are known to cause ocular diseases, such as retinitis pigmentosa (Dryja, McGee, Hahn, et al., 1990; Dryja, McGee, Reichel, et al., 1990; Li et al., 2016; Morimura et al., 1999), and OPN4 polymorphisms have been associated with seasonal affective disorder (Roecklein et al., 2009). Mutations in SWS1, MWS, LWS, and the locus control region for MWS/LWS are known to cause a variety of types of color blindness (Neitz and Neitz, 2011). Consistent with its role in photorelaxation of airway smooth muscles, OPN3 has been identified as a risk locus for
asthma susceptibility (White et al., 2008). To date, no human diseases have been associated with OPN5.

**Nonphotic Stimulation of Opsins**

Conjugation to a chromophore, such as 11-cis-retinal, is necessary for animal opsins to detect and respond to light, and this conjugation is dependent on a crucial lysine residue within the seventh helix (K7.43) and a counterion in the third helix (commonly E3.28 or Y3.28) (Yan et al., 2003). An important caveat to the above analysis is that any stimulus that results in isomerization of retinal is sufficient for opsin G protein signaling (Kandori et al., 2001; Kukura et al., 2005). While light is the primary source of this isomerization, thermal (Pérez-Cerezales et al., 2015; Roy et al., 2020; Shen et al., 2011; Sokabe et al., 2016; Yau et al., 1979) and mechanical (Senthilan et al., 2012; Zanini et al., 2018) forces may also isomerize opsin-conjugated retinal, although the role of the chromophore in these functions may be restricted to opsin trafficking rather than direct detection of stimuli (Leung and Montell, 2017). Current data on OPN5 suggest that it functions only via light-mediated stimulation in the retina, skin, and hypothalamus (Buhr et al., 2015, 2019; Nguyen et al., 2019). Recent studies in smooth muscle and adipocytes demonstrate that OPN3 may also function in a light-dependent manner (Nayak et al., 2020; Sato et al., 2020; Wu et al., 2020; Yim et al., 2019, 2020); however, it has also been proposed that OPN3 has light-independent functions in skin, mediated via conjugation to other GPCRs such as the melanocortin 1 receptor (Ozdeslik et al., 2019). Unlike RHO (Jastrzebska et al., 2015; Ploier et al., 2016; Zhang et al., 2016), OPN3 does not appear to homodimerize (Felce et al., 2017). Whether OPN3 heterodimerization is limited to melanocortin 1 receptor or whether it can conjugate to other GPCRs remains to be determined. It is also currently unclear whether GPCR dimerization plays a role in light-mediated functions of OPN3.

Given that opsins can respond to stimuli other than light and can serve functions other than G protein signaling, such as vitamin A transport, it is important to outline criteria that should be met when attributing a photosensitive function to an opsin. (1) A light-dependent biological response should have a wavelength-dependent action spectrum. This action spectrum could be narrow if involving a single opsin or broad if multiple opsins are involved in the response. To reduce thermal isomerization of retinal, this spectrum should be measured in a temperature-controlled manner since light sources (such as LEDs) can have different thermal efficiencies. (2) An opsin with an absorption spectrum corresponding to the biological response should be expressed within the biological system. Finally, (3) functional loss of the opsin should alter or abolish the action spectrum of the biological response. If the response is mediated by a single opsin, the response should be lost entirely; however if multiple opsins are functioning together, the wavelengths and kinetics that the opsin functions

---

Figure 6. Genetic constraint in human populations. Genetic variation in opsins and clock-related genes from 141,456 humans accessed from the Genome Aggregation Database (Karczewski et al., 2020). Ratio of observed-to-expected genetic variants for (a) synonymous, (b) missense, and (c) loss-of-function variants. Green indicates that the confidence interval of observed-to-expected variants does not exceed the null hypothesis. Yellow, orange, and red indicate increasing constraint against variants. Abbreviations: RHO = rhodopsin; SWS = short-wavelength-sensitive; OPN3 = encephalopsin; OPN4 = melanopsin; OPN5 = neuropsin; RGR = RGR-opsin; RRH = peropsin.
within will be disrupted. For example, the pupillary light response can be mediated via rod or cone input to ipRGCs, such that in the absence of a single opsin, the biological effect would remain intact; however, the kinetics of this response or wavelengths at which it occurs differ. One of the most sensitive ways to determine this effect would be to selectively mutate the seventh transmembrane lysine, leaving the remainder of the opsin intact. Currently, chromophore conjugation to this lysine residue is necessary for all known light-mediated opsin functions. In addition, selective mutations to the E/DRY sequence would be insightful to determine light-dependent signaling events from other potential opsin functions. Currently available gene-editing techniques make this feasible and could also be incorporated into cell type–specific and temporally controlled methods. Together, these criteria would indicate that a given light-sensitive response is opsin-dependent.

CONCLUSION

Loss of visual opsins follows predictable patterns based on limited-light environments of nocturnal, subterrrestrial, or marine mammals. While this factor has shaped the evolution of nonvisual opsins, such as loss of OPN3 in vespertilionid bats, phosphorylation sites of OPN4, or reduced constraint in RGR and RRH sequences in rodents, other factors are likely in play, which is unsurprising given the diverse functions of these atypical opsins. OPN3 and, to a greater extent, OPN5 are well-conserved across mammals, and this conservation continues in modern humans, whereas OPN4 appears to be the most diverse of the mammalian opsins. Better understanding of the functions of these opsins in the variety of tissues that express them will provide us with new insight into the physiology and behavior of organisms that have reduced constraint of these nonvisual opsins.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (R01EY027077, R01EY027711 to R.A.L, R01 GM124246 to E.D.B, NIH R01 EY026921 to R.V.G.) and [5T32GM063483-16 to UC MSTP]. We thank Shane D’Souza for comments and discussion while preparing this manuscript. This work was supported by NEI R01s EY027711 and EY027077 (to R.A.L.), NIGMS 5T32GM063483 (to University of Cincinnati MSTP), funds from the Goldman Chair of the Abrahamson Pediatric Eye Institute at CCHMC, NIH R01 GM124246 (to E.D.B.), and NIH R01 EY026921 (to R.V.G.).

REFERENCES

Altimus CM, Güler AD, Villa KL, McNeill DS, LeGates TA, and Hattar S (2008) Rods-cones and melanopsin detect light and dark to modulate sleep independent of image formation. Proc Natl Acad Sci U S A 105:19998-20003.
Arrese CA, Hart NS, Thomas N, Beazley LD, and Shand J (2002) Trichromacy in Australian marsupials. Curr Biol 12:657-660.
Arrese CA, Oddy AY, Runham PB, Hart NS, Shand J, Hunt DM, and Beazley LD (2005) Cone topography and spectral sensitivity in two potentially trichromatic marsupials, the quokka (Setonix brachyurus) and quenda (Isoodon obesulus). Proc R Soc B Biol Sci 272:791-796.
Barreto Ortiz S, Hori D, Nomura Y, Yun X, Jiang H, Yong H, Chen J, Paek S, Pandey D, Sikka G, et al. (2018) Opsin 3 and 4 mediate light-induced pulmonary vasorelaxation that is potentiated by G protein-coupled receptor kinase 2 inhibition. Am J Physiol Lung Cell Mol Physiol 314:L93-L106.
Bellingham J, Wells DJ, and Foster RG (2003) In silico characterisation and chromosomal localisation of human RRH (peropsin): implications for opsin evolution. BMC Genomics 4:3.
Berg F, Gustafson U, and Andersson L (2006) The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: a genetic explanation for poor thermoregulation in piglets. PLoS Genet 2:e129.
Blackshaw S and Snyder SH (1999) Encephalopsin: a novel mammalian extraretinal opsin discretely localized in the brain. J Neurosci 19:3681-3690.
Blasic JR, Brown RL, and Robinson PR (2012a) Light-dependent phosphorylation of the carboxy tail of mouse melanopsin. Cell Mol Life Sci 69:1551-1562.
Blasic JR, Brown RL, and Robinson PR (2012b) Phosphorylation of mouse melanopsin by protein kinase A. PLoS One 7:e45387.
Blastic JR, Matos-Cruz V, Ujla D, Cameron EG, Hattar S, Halpern ME, and Robinson PR (2014) Identification of critical phosphorylation sites on the carboxy tail of melanopsin. Biochemistry 53:2644-2649.

Boc A, Diallo AB, and Makarenkov V (2012) T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. Nucleic Acids Res 40:W573-W579.

Bowmaker JK (2008) Evolution of vertebrate visual pigments. Vision Res 48:2022-2041.

Buhr ED, Vemaraju S, Diaz N, Lang RA, and Van Gelder RN (2019) Neuropsin (OPN5) mediates local light-dependent induction of circadian clock genes and circadian photoentrainment in exposed murine skin. Curr Biol 29:F3478-3478.

Buhr ED, Yue WWS, Ren X, Jiang Z, Liao H-WR, Mei X, Vemaraju S, Nguyen M-T, Reed RR, Lang RA, et al. (2015) Neuropsin (OPN5)-mediated photoentrainment of local circadian oscillators in mammalian retina and cornea. Proc Natl Acad Sci 112:13093-13098.

Campanella JJ, Bitincka L, and Smalley J (2003) MatGAT: an application that generates similarity/identity matrices using protein or DNA sequences. BMC Bioinformatics 4:29.

Cavallari N, Frigato E, Vallone D, Fröhlich N, Lopez-Olmeda JF, Foà A, Berti R, Sánchez-Vázquez FJ, Bertolucci C, and Foulkes NS (2011) A blind circadian clock in cavefish reveals that opsins mediate peripheral clock photoentrainment. PLoS Biol 9:e1001142.

Chen P, Hao W, Rife L, Wang XP, Shen D, Chen J, Ogden T, Van Boemel GB, Wu L, Yang M, et al. (2001) A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat Genet 28:256-260.

Chen L, Qiu Q, Jiang Y, Wang K, Lin Z, Li Z, Bibi F, Yang Y, Wang J, Nie W, et al. (2019) Large-scale ruminant genome sequencing provides insights into their evolution and distinct traits. Science 364:eav6202.

Chen P, Hao W, Rife L, Wang XP, Shen D, Chen J, Ogden T, Van Boemel GB, Wu L, Yang M, et al. (2001) A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat Genet 28:256-260.

Cunningham F, Achuthan P, Akhani W, Allen J, Amode MR, Armean IM, Bennett R, Bhai J, Billis K, Boddu S, et al. (2019) Ensembl 2019. Nucleic Acids Res 47:D745-D751.

David-Gray ZK, Cooper HM, Janssen JWH, Nevo E, and Foster RG (1999) Spectral tuning of a circadian photopigment in a subterranean “blind” mammal (Spalax ehrenbergi). FEBS Lett 461:343-347.

David-Gray ZK, Janssen JWH, Degrip WJ, Nevo E, and Foster RG (1998) Light detection in a “blind” mammal. Nat Neurosci 1:655-656.

Diaz NM, Lang RA, van Gelder RN, and Buhr ED (2020) Wounding induces facultative opn5-dependent circadian photocoreception in the murine cornea. Investig Ophthalmol Vis Sci 61:37.

Do MTH (2019) Melanopsin and the intrinsically photosensitive retinal ganglion cells: biophysics to behavior. Neuron 104:205-226.

Dryja TP, McGee TL, Hahn LB, Cowley GS, Olsson JE, Reichel E, Sandberg MA, and Berson EL (1990) Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. N Engl J Med 323:1302-1307.

Dryja TP, McGee TL, Reichel E, Hahn LB, Cowley GS, Yandell DW, Sandberg MA, and Berson EL (1990) A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature 343:364-366.

Emanuel AJ and Do MTH (2015) Melanopsin tristability for sustained and broadband phototransduction. Neuron 85:1043-1055.

Emerling CA and Springer MS (2014a) Eyes underground: regression of visual protein networks in subterranean mammals. Mol Phylogenet Evol 78:260-270.

Emerling CA and Springer MS (2014b) Genomic evidence for rod monochromacy in sloths and armadillos suggests early subterranean history for xenarthra. Proc R Soc B Biol Sci 282. 20142192.

Emery P, So WV, Kaneko M, Hall JC, and Rosbash M (1998) Cry, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 95:669-679.

Esquiva G, Avivi A, and Hannibal J (2016) Non-image forming light detection by melanopsin, rhodopsin, and long-middlewave (L/W) cone opsins in the subterranean blind mole rat, Spalax ehrenbergi: immunohistochemical characterization, distribution, and connectivity. Front Neuroanat 10:61.

Esquiva G, Avivi A, and Hannibal J (2016) Non-image forming light detection by melanopsin, rhodopsin, and long-middlewave (L/W) cone opsins in the subterranean blind mole rat, Spalax ehrenbergi: immunohistochemical characterization, distribution, and connectivity. Front Neuroanat 10:61.

Facick JI and Robinson PR (2016) Adaptations of cetacean retinal pigments to aquatic environments. Front Ecol Evol 4:70.

Felce JH, Latty SL, Knox RG, Mattick SR, Lui Y, Lee SF, Klenerman D, and Davis SJ (2017) Receptor quaternary organization explains G protein-coupled receptor family structure. Cell Rep 20:2654-2665.

Feuda R, Hamilton SC, McNerney JO, and Pisani D (2012) Metazoan opsin evolution reveals a simple route to animal vision. Proc Natl Acad Sci U S A 109:18868-18872.
Fleming JF, Feuda R, Roberts NW, and Pisani D (2020) A novel approach to investigate the effect of tree reconstruction artifacts in single-gene analysis clarifies opsin evolution in nonbilaterian metazoans. Genome Biol Evol 12:3906-3916.

Gerkema MP, Davies WIL, Foster RG, Menaker M, and Hut RA (2013) The nocturnal bottleneck and the evolution of activity patterns in mammals. Proc R Soc B Biol Sci 280:20130508.

Güler AD, Ecker JL, Lall GS, Haq S, Altimus CM, Liao HW, Barnard AR, Cahill H, Badea TC, Zhao H, et al. (2008) Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. Nature 453:102-105.

Haeussler M, Zweig AS, Tyner C, Speir ML, Rosenbloom KR, Raney BJ, Lee CM, Lee BT, Hinrichs AS, Gonzalez JN, et al. (2019) The UCSC genome browser database: 2019 update. Nucleic Acids Res 47:D853-D858.

Halford S, Freedman MS, Bellingham J, Inglis SL, Poopalasundaram S, Soni BG, Foster RG, and Hunt DM (2001) Characterization of a novel human opsin gene with wide tissue expression and identification of embedded and flanking genes on chromosome 1q43. Genomics 72:203-208.

Haltaufderhyde K, Ozdeslik RN, Wicks NL, Najera JA, and Halford S, Freedman MS, Bellingham J, Inglis SL, Poopalasundaram S, Soni BG, Foster RG, and Hunt DM (2001) Characterization of a novel human opsin gene with wide tissue expression and identification of embedded and flanking genes on chromosome 1q43. Genomics 72:203-208.

Hannibal J, Hindersson P, Nevo E, and Fahrenkrug J (2002) The circadian photopigment melanopsin is expressed in the blind subterranean mole rat, Spalax. Neureport 13:1411-1414.

Hattar S, Liao HW, Takao M, Berson DM, and Yau KW (2002) Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. Science 295:1065-1070.

Hattar S, Lucas RJ, Mosovskiy N, Thompson S, Douglas RH, Hankins MW, Lem J, Biel M, Hofmann F, Foster RG, et al. (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. Nature 424:75-81.

Hendriks W, Leunissen J, Nevo E, Bloemendal H, and de Jong WW (1987) The lens protein alpha A-crystallin of the blind mole rat, Spalax ehrenbergi: evolutionary change and functional constraints. Proc Natl Acad Sci U S A 84:5320-5324.

Hrvasin S, Sun S, Wilcox OF, Yao H, Lavin-Peter AJ, Cicconet M, Assad EG, Palmer ME, Aronson S, Banks AS, et al. (2020) Neurons that regulate mouse torpor. Nature 583:115-121.

Hughes S, Welsh L, Katti C, González-Menéndez I, Turton M, Halford S, Sekaran S, Peirson SN, Hankins MW, and Foster RG (2012) Differential expression of melanopsin isoforms Opn4L and Opn4S during postnatal development of the mouse retina. PLoS One 7:e34531.

Hunt DM, Carvalho LS, Cowing JA, Parry JWL, Wilkie SE, Davies WL, and Bowmaker JK (2007) Spectral tuning of shortwave-sensitive visual pigments in vertebrates. Photochem Photobiol 83:303-310.

Hunt DM, Dulai KS, Cowing JA, Julliot C, Mollon JD, Bowmaker JK, Li WH, and Hewett-Emmett D (1998) Molecular evolution of trichromacy in primates. Vision Res 38:3299-3306.

Imai H, Kefalov V, Sakurai K, Chisaka O, Ueda Y, Onishi A, Morizumi T, Fu Y, Ichikawa K, Nakatani K, et al. (2007) Molecular properties of rhodopsin and rod function. J Biol Chem 282:6677-6684.

Jacobs GH, Deegan JF, Neitz J, Crognaele MA, and Neitz M (1993) Photopigments and color vision in the nocturnal monkey, Aotus. Vision Res 33:1773-1783.

Jagannath A, Hughes S, Abdélgany A, Pothecary CA, Di Pretoro S, Pires SS, Vachtsevanos A, Pilorz V, Brown LA, Hossbach M, et al. (2015) Isoforms of melanopsin mediate different behavioral responses to light. Curr Biol 25:2430-2434.

Janssen JWH, Bovee-Geurts PHM, Peeters ZPA, Bowmaker JK, Cooper HM, David-Gray ZK, Nevo E, and DeGrip WJ (2000) A fully functional rod visual pigment in a blind mammal: a case for adaptive functional reorganization? J Biol Chem 275:38674-38679.

Jastrzebska B, Chen Y, Orban T, Jin H, Hofmann L, and Palczewski K (2015) Disruption of rhodopsin dimerization with synthetic peptides targeting an interaction interface. J Biol Chem 290:25728-25744.

Jebb D, Huang Z, Pippel M, Hughes GM, Lavrichenko K, Devanna P, Winkler S, Jermiin LS, Skirmuntt EC, Katzourakis A, et al. (2020) Six reference-quality genomes reveal evolution of bat adaptations. Nature 583:578-584.

Johnson J, Wu V, Donovan M, Majumdar S, Renteria RC, Porco T, Van Gelder RN, and Copenhagen DR (2010) Melanopsin-dependent light avoidance in neonatal mice. Proc Natl Acad Sci U S A 107:17374-17378.

Jones DT, Taylor WR, and Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Bioinformatics 8:275-282.

Kandori H, Shichida Y, and Yoshizawa T (2001) Photosomerization in rhodopsin. Biochem 66:1197-1209.

Karczewski KJ, Francioli LC, Tao G, Cummings BB, Alföldi J, Wang Q, Collins RL, Laricchia KM, Ganna A, Birnbaum DP, et al. (2020) The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 581:434-443.

Kato M, Sugiyama T, Sakai K, Yamashita T, Fujita H, Sato K, Tomonari S, Shichida Y, and Ohuchi H (2016) Two opsin 3-related proteins in the chicken retina and brain: a TMT-type opsin 3 is a blue-light sensor in retinal horizontal cells, hypothalamus, and cerebellum. PLoS One 11:e0163925.

Kim EB, Fang X, Fushan AA, Huang Z, Lobanov AV, Han L, Marino SM, Sun X, Turanov AA, Yang P, et al. (2011) Genome sequencing reveals insights into physiology and longevity of the naked mole rat. Nature 479:223-227.
Kojima D, Mori S, Torii M, Wada A, Morishita R, and Fukada Y (2011) UV-sensitive photoreceptor protein OPR5 in humans and mice. PLoS One 6:e26388.

Kott O, Šumbera R, and Némec P (2010) Light perception in two strictly subterranean rodents: life in the dark or blue? PLoS One 5:e11810.

Koyanagi M, Kawano E, Kinugawa Y, Oishi T, Shichida Y, Tamotsu S, and Terakita A (2004) Bistable UV pigment in the lamprey pineal. Proc Natl Acad Sci U S A 101:6687-6691.

Kryger Z, Galli-Resta L, Jacobs GH, and Reese BE (1998) The topography of rod and cone photoreceptors in the retina of the ground squirrel. Vis Neurosci 15:685-691.

Kukura P, McCamant DW, Yoon S, Wandschneider DB, and Mathies RA (2005) Chemistry: structural observation of the primary isomerization in vision with femtosecond-stimulated Raman. Science 310:1006-1009.

Kumar S, Stecher G, Li M, Knyaz C, and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35:1547-1549.

Kumar S, Stecher G, Suleski M, and Hedges SB (2017) TimeTree: a resource for timelines, time trees, and divergence times. Mol Biol Evol 34:1812-1819.

Leung NY and Montel C (2017) Unconventional roles of opsins. Annu Rev Cell Dev Biol 33:241-264.

Levenson DH, Ponganis PJ, Crogna MA, Deegan JF, Dizon A, and Jacobs GH (2006) Visual pigments of marine carnivores: pinnipeds, polar bear, and sea otter. J Comp Physiol A Neuroethol Sens Neural Behav Physiol 192:833-843.

Li J, Xiao X, Li S, Jia X, Guo X, and Zhang Q (2016) RGR variants in different forms of retinal diseases: the under-termined role of truncation mutations. Mol Med Rep 14:4811-4815.

Lin JJ, Wang FY, Li WH, and Wang TY (2017) The rises and falls of opsin genes in 59 ray-finned fish genomes and their implications for environmental adaptation. Sci Rep 7:15568.

Lucas RJ, Hattar S, Takao M, Berson DM, Foster RG, and Yau KW (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. Science 299:245-247.

Merbs SL and Nathans J (1992) Absorption spectra of human cone pigments. Nature 356:433-435.

Meredith RW, Gatesy J, Emerling CA, York VM, and Springer MS (2013) Rod monochromacy and the coevolution of cetacean retinal opsins. PLoS Genet 9:e1003432.

Moffitt JR, Bambah-Mukku D, Eichhorn SW, Vaughn E, Shekhar K, Perez JD, Rubinstein ND, Hao J, Regev A, Dulac C, and et al. (2018) Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science 362: eaau5324.

Morimura H, Saindelle-Ribeaudeau F, Berson EL, and Dryja TP (1999) Mutations in RGR, encoding a light-sensitive opsin homologue, in patients with retinitis pigmentosa. Nat Genet 23:393-394.

Morris KV and Mattick JS (2014) The rise of regulatory RNA. Nat Rev Genet 15:423-437.

Morshead A, Kaylor JJ, Ng SY, Tsan A, Frederiksen R, Xu T, Yuan L, Sampath AP, Radu RA, Fain GL, et al. (2019) Light-driven regeneration of cone visual pigments through a mechanism involving RGR opsin in Müller glial cells. Neuron 102:1172-1183.

Moutsaki P, Whitmore D, Bellingham J, Sakamoto K, David-Gray ZK, and Foster RG (2003) Teleost multiple tissue (TMT) opsin: a candidate photopigment regulating the peripheral clocks of zebrafish? Mol Brain Res 112:135-145.

Müller B and Peichl L (1989) Topography of cones and rods in the tree shrew retina. J Comp Neurol 282:581-594.

Musilova Z, Cortesi F, Matschiner M, Davies WIL, Patel JS, Stieb SM, De Busscherelles F, Malmstrom M, Torresen OK, Brown CJ, et al. (2019) Vision using multiple distinct rod opsins in deep-sea fishes. Science 364:588-592.

Nagata T, Koyanagi M, Lucas R, and Terakita A (2018) An all-trans-retinal-binding opsin peropsin as a potential dark-active and light-inactivated G protein-coupled receptor. Sci Rep 8:3535.

Nakane Y, Ikegami K, Ono H, Yamamoto N, Yoshida S, Hirunagi K, Ebihara S, Kubo Y, and Yoshimura T (2010) A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds. Proc Natl Acad Sci 107:15264-15268.

Nathans J (1990) Determinants of visual pigment absorbance: identification of the retinylidene Schiff’s base counterion in bovine rhodopsin. Biochemistry 29: 9746-9752.

Nayak G, Zhang KX, Vemaraju S, Odaka Y, Buhr ED, Holt-Jones A, Kernodle S, Smith AN, Upton BA, D’Souza S, et al. (2020) Adaptive thermogenesis in mice is enhanced by opsin 3-dependent adipocyte light sensing. Cell Rep 30:672-686.

Neitz J and Neitz M (2011) The genetics of normal and defective color vision. Vision Res 51:633-651.

Newman LA and Robinson PR (2006) The visual pigments of the West Indian manatee (Trichechus manatus). Vision Res 46:3326-3330.

Nguyen MTT, Vemaraju S, Odaka Y, Buhr ED, Alonzo N, Tran U, Batie M, Upton BA, Darvas M, et al. (2019) An opsin 5-dopamine pathway mediates light-dependent vascular development in the eye. Nat Cell Biol 21:420-429.

Olimski LE, Lin EM, and Oancea E (2020) Illuminating insights into opsin 3 function in the skin. Adv Biol Regul 75:100668.

Ota W, Nakane Y, Hattar S, and Yoshimura T (2018) Impaired circadian photoentrainment in Opm5-null mice. iScience 6:299-305.

Ozdeslik RN, Olimski LE, Trieu MM, Oprian DD, and Oancea E (2019) Human nonvisual opsin 3 regulates pigmentation of epidermal melanocytes through functional interaction with melanocortin 1 receptor. Proc Natl Acad Sci U S A 116:11508-11517.
Palczewski K and Kiser PD (2020) Shedding new light on the generation of the visual chromophore. Proc Natl Acad Sci U S A 117:19629-19638.

Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, et al. (2000) Crystal structure of rhodopsin: a G protein-coupled receptor. Science 289:739-745.

Panda S, Provencio I, Tu DC, Pires SS, Rollag MD, Castrucci AM, Pletcher MT, Sato TK, Wiltshire T, Andahazy M, et al. (2003) Melanopsin is required for non-image-forming photic responses in blind mice. Science 301:525-527.

Panda S, Sato TK, Castrucci AM, Rollag MD, DeGrip WJ, Hogenessh JB, Provencio I, and Kay SA (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. Science 298:2213-2216.

Paschos GK, Ibrahim S, Song WL, Kunieda T, Grant G, Reyes TM, Bradfield CA, Vaughan CH, Eiden M, Masoodi M, et al. (2012) Obesity in mice with dipocyt-specific deletion of clock component Arntl. Nat Med 18:1768-1777.

Peichl L and Moutairou K (1998) Absence of short-wavelength sensitive cones in the retina of seals (Carnivora) and African giant rats (Rodentia). Eur J Neurosci 10:2586-2594.

Peichl L, Behrmann G, and Kröger RHH (2001) For whales and seals the ocean is not blue: a visual pigment loss in marine mammals. Eur J Neurosci 13:1520-1528.

Pérez-Cerezales S, Boryshpolets S, Afanzar O, Brandis A, Nevo R, Kiss V, and Eisenbach M (2015) Involvement of opsins in mammalian sperm thermotaxis. Sci Rep 5:16146.

Pires SS, Hughes S, Turton M, Melyan Z, Peirson SN, Zheng L, Kosmaoglou M, Bellingham J, Cheetham ME, Lucas RJ, et al. (2009) Differential expression of two distinct functional isofoms of melanopsin (Opn4) in the mammalian retina. J Neurosci 29:12332-12342.

Pires SS, Shand J, Bellingham J, Arrese C, Turton M, Peirson S, Foster RG, and Halford S (2007) Isolation and characterization of melanopsin (Opn4) from the Australian marsupial Sminthopsis crassicaudata (fat-tailed dunnart). Proc R Soc B Biol Sci 274:2791-2799.

Plachetzki DC, Degnan BM, and Oakley TH (2007) The origins of novel protein interactions during animal opsin evolution. PLoS One 2:e1054.

Ploier B, Caro LN, Morizumi T, Pandey K, Pearring JN, Goren MA, Finnemann SC, Graumann J, Arshavsky VY, Dittman JS, et al. (2016) Dimerization deficiency of enigmatic retinitis pigmentosa-linked rhodopsin mutants. Nat Commun 7:12832.

Poletini MO, Ramos BC, Moraes MN, and Castrucci AML (2015) Nonvisual opsins and the regulation of peripheral clocks by light and hormones. Photochem Photobiol 91:1046-1055.

Provencio I, Jiang G, De Grip WJ, Hayes WP, and Rollag MD (1998) Melanopsin: an opsin in melanophores, brain, and eye. Proc Natl Acad Sci 95:340-345.

Radu RA, Hu J, Peng J, Bok D, Mata NL, and Travis GH (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. J Biol Chem 283:19730-19738.

Rao S, Chun C, Fan J, Kofron JM, Yang MB, Hegde RS, Ferrara N, Copenhagen DR, and Lang RA (2013) A direct and melanopsin-dependent fetal light response regulates mouse eye development. Nature 494:243-246.

Roecklein KA, Rohan KJ, Duncan WC, Rollag MD, Rosenthal NE, Lipsky RH, and Provencio I (2009) A missense variant (P10L) of the melanopsin (OPN4) gene in seasonal affective disorder. J Affect Disord 114:279-285.

Roy D, Levi K, Kiss V, Nevo R, and Eisenbach M (2020) Rhodopsin and melanopsin coexist in mammalian sperm cells and activate different signaling pathways for thermotaxis. Sci Rep 10:1112.

Ruby NF, Brennan TJ, Xie X, Cao V, Franken P, Heller HC, and O’Hara BF (2002) Role of melanopsin in circadian responses to light. Science 298:2211-2213.

Sadier A, Davies KTJ, Yohe LR, Yun K, Donat P, Hedrick BP, Dumont ER, Dávalos LM, Rossiter SJ, and Sears KE (2018) Multifactorial processes underlie parallel opsin loss in neotropical bats. eLife 7:e37412.

Sakai K, Yamashita T, Imamoto Y, and Shichida Y (2015) Diversity of active states in TMT opsins. PLoS One 10:e0141238.

Sato M, Tsuji T, Yang K, Ren X, Dreyfuss JM, Huang TL, Wang CH, Shamsi F, Leiria LO, Lynes MD, et al. (2020) Cell-autonomous light sensitivity via Opsi3 regulates fuel utilization in brown adipocytes. PLoS Biol 18:e3000630.

Sayers EW, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, Dicuccio M, Edgar R, Federhen S, et al. (2009) Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 37:D5-D15.

Senthilan PR, Piepenbrock D, Ovezmyradov G, Nadrowski B, Bechstedt S, Pauls S, Winkler M, Möbius W, Howard J, and Göpfert MC (2012) Drosophila auditory organ genes and genetic hearing defects. Cell 150:1042-1054.

Sexton TJ, Golczak M, Palczewski K, and Van Gelder RN (2012) Melanopsin is highly resistant to light and chemical bleaching in vivo. J Biol Chem 287:20888-20897.

Shen D, Jiang M, Hao W, Li T, Salazar M, and Fong HKW (1994) A human opsin-related gene that encodes a retinaldehyde-binding protein. Biochemistry 33:13117-13125.

Shen WL, Kwon Y, Adegbola AA, Luo J, Chess A, and Montell C (2011) Function of rhodopsin in temperature discrimination in Drosophila. Science 331:1333-1336.
Yokoyama S (2008) Evolution of dim-light and color vision pigments. Annu Rev Genomics Hum Genet 9:259-282.
Yokoyama S and Radlwimmer FB (1998) The “five-sites” rule and the evolution of red and green color vision in mammals. Mol Biol Evol 15:560-567.
Yu S, Qualls-Creekmore E, Rezai-Zadeh K, Jiang Y, Berthoud HR, Morrison CD, Derbenev AV, Zsombok A, and Münzberg H (2016) Glutamatergic preoptic area neurons that express leptin receptors drive temperature-dependent body weight homeostasis. J Neurosci 36:5034-5046.
Zanini D, Giraldo D, Warren B, Katana R, Andrés M, Reddy S, Pauls S, Schwedhelm-Domeyer N, Geurten BRH, and Göpfert MC (2018) Proprioceptive opsin functions in Drosophila larval locomotion. Neuron 98:67-74.
Zhang J, Choi EH, Tworak A, Salom D, Leinonen H, Sander CL, Hoang TV, Handa JT, Seth Blackshaw X, Palczewska G, et al. (2019) Photic generation of 11-cis-retinal in bovine retinal pigment epithelium. J Biol Chem 294:19137-19154.
Zhang KX, D’Souza S, Upton BA, Kernodle S, Vemaraju S, Nayak G, Gaitonde KD, Holt AL, Linne CD, Smith AN, et al. (2020) Violet-light suppression of thermogenesis by opsin 5 hypothalamic neurons. Nature 585:420-425.
Zhang T, Cao LH, Kumar S, Enemchukwu NO, Zhang N, Lambert A, Zhao X, Jones A, Wang S, Dennis EM, et al. (2016) Dimerization of visual pigments in vivo. Proc Natl Acad Sci U S A 113:9093-9098.
Zuckerkandl E and Pauling L (1965) Evolutionary divergence and convergence in proteins. In: Bryson V and Vogel HJ editors. Evolving Genes and Proteins. p. 97-166. DOI: 10.1016/b978-1-4832-2734-4.50017-6.