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Vertebrate ancient opsin and melanopsin: divergent irradiance detectors†

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Both vertebrates and invertebrates respond to light by utilising a wide-ranging array of photosensory systems, with diverse photoreceptor organs expressing a characteristic photopigment, itself consisting of an opsin apoprotein linked to a light-sensitive retinoid chromophore based on vitamin A. In the eye, the pigments expressed in both cone and rod photoreceptors have been studied in great depth and mediate contrast perception, measurement of the spectral composition of environmental light, and thus classical image forming vision. By contrast, the molecular basis for non-visual and extraocular photoreception is far less understood; however, two photopigment genes have become the focus of much study, the *vertebrate ancient* (*va*) opsin and *melanopsin* (*opn4*). In this review, we discuss the history of discovery for each gene, as well as focusing on the evolution, expression profile, functional role and broader physiological significance of each photopigment. Recently, it has been suggested independently by Arendt *et al.* and Lamb that an ancestral opsin bifurcated in early metazoans and evolved into two quite different photopigments, one expressed in rhabdomeric photoreceptors and the other in ciliary photoreceptors. This interpretation of the evolution of the metazoan eye has provided a powerful framework for understanding photobiological organization. Their proposal, however, does not encompass all current experimental observations that would be consistent with what we term a central "*Evolution of Photosensory Opsins with Common Heredity (EPOCH)*" hypothesis to explain the complexity of animal photosensory systems. Clearly, many opsin genes (*e.g. va* opsin) simply do not fit neatly within this scheme. Thus, the review concludes with a discussion of these anomalies and their context regarding the phylogeny of photoreceptor and photopigment development.

1. A multiplicity of vertebrate photoreceptors, photopigments and photosensory tasks

Animals possess an extensive and diverse array of photoreceptors and photopigments that appear to mediate multiple responses to light.[1] In vertebrates, a number of different photoreceptor

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organs arise from the diencephalon and possess photoreceptor and photopigment systems of varying complexity. These have been classified as: (i) pineal (epiphysis cerebri) and parapineal organs, which reside within the cranium; (ii) extracranial "third eyes", variously called frontal organs (in frogs) and parietal eyes (in lizards); (iii) deep brain photoreceptors; and (iv) lateral eyes. In addition to well characterised cone and rod photoreceptors, lateral eyes also possess photosensitive retinal horizontal cells (*e.g.* in teleosts[2]), retinal ganglion cells (RGCs) (*e.g.* in rodents[3,4]), and almost certainly other photosensory cell types (*e.g.* retinal pigment epithelium (RPE)[5,6]). A variety of photoreceptor cell types have also been described in extraocular organs, ranging from the pineal organs of lampreys and fish, which possess photoreceptors that resemble cones,[7] to deep brain photoreceptors with no apparent cone-like or rod-like features.[8] In addition to the photoreceptors of diencephalic/forebrain origin, many vertebrates possess broad tissue photosensitivity (*e.g.* in teleosts[9]), dermal/melanophore photoreceptors (*e.g.* in amphibians[10]), and photosensitive iridocytes within the cornea (*e.g.* in teleosts[11]).

Generalisations are difficult, but overall the primary role of cone and rod photoreceptors are to mediate visual responses, including contrast and image detection tasks, whilst extraocular and non-cone, non-rod ocular photoreceptors mediate responses to gross changes in environmental light (irradiance) for the regulation of tasks that span 'time-of-day' cues for the control of sleep and circadian rhythms,[12] levels of behavioral arousal,[13] orientation and taxis,[14] body pigmentation and colouration,[15] temperature regulation,[16] and pupil size.[17] Until recently, the question of why vertebrates should possess both image forming visual photoreceptors and additional irradiance detectors was much debated. Initially, this multiplicity of photoreceptors made no sense. Parsimonious reasoning dominated the discussion – surely cones and rods can act as both image and irradiance light sensors, so why possess additional photoreceptors? Indeed, extraocular photoreceptors were often dismissed as vestigial organs, evolutionary baggage lost by "higher" mammals but retained in more "primitive" vertebrates. The discussion moved forward because of the growing appreciation that the sensory tasks of image detection and irradiance measurement are fundamentally different and difficult to combine with any level of sophistication in just one type of receptor.[18] The mammalian eye illustrates this point. Image detection requires a focused representation of the environment. The optical nature of the eye achieves this precision but in so doing makes irradiance detection more difficult. For reliable measurement of irradiance, light must be gathered from all directions and fall uniformly upon an array of photoreceptors. Because mammals lack extraretinal photoreceptors, they are faced with the sensory problem of measuring environmental irradiance using only ocular photoreceptors. The eye has resolved this problem by using parallel light detecting systems and projections into the brain. Very large numbers of densely packed cones and rods, often localised to different regions of the retina, collect light from a highly restricted region in space and then help construct a representation of the environment by maintaining a topographical map between the retina and the central visual sites within the brain. By contrast, relatively small numbers of photosensitive RGCs (pRGCs) are evenly distributed across the retina with large and overlapping light-sensitive dendritic fields and capture light from broad regions of space. Furthermore, the projections from pRGCs to central targets lack topographic order and are analogous to that achieved by a randomized fiber optic.[19] In this rather complex manner, the eye has achieved parallel image and irradiance detection in a single organ.

The advantages of separate extraocular irradiance detectors are obvious, and so the question arises: why have mammals lost their extraocular irradiance detectors? A partial explanation may be related to the early evolutionary history of mammals and their passage through a "nocturnal bottleneck". All mammals are derived from nocturnal insectivorous or omnivorous reptilian-like animals, the weasel-like therapids,[20] with an ancestry that dates back to at least 267 million years ago (MYA), with modern mammals first appearing in the fossil record about 225 MYA.[21] The "nocturnal bottleneck" proposal suggests that many early mammals were forced to adopt an increasingly nocturnal ecology in response to the dominance of the archosaurs in the diurnal realm.[22] Extraretinal photoreceptors would have been adequate for monitoring changes under bright diurnal light conditions but may not have been sufficiently sensitive to discriminate twilight changes in primitive mammals that spend the day hiding underground and emerging late in twilight. Extraocular photoreceptors and pRGCs were almost certainly both present in the diurnal mammal-like reptiles, but extraocular receptors were probably lost when early mammals began to exploit the nocturnal realm.[23]

The complexity in vertebrate photoreceptor cell types and organs is accompanied by a diversity of photopigments. These various photopigment families have been described in detail elsewhere,[24–29] and are listed in Fig. 1. Despite the multiplicity of photopigment types, there is remarkable conservation across the vertebrate photosensitive G protein-coupled receptors (GPCRs) in terms of their basic biochemistry. The dominant photopigment of the animal lineage is based upon an opsin protein associated with a light-absorbing molecule (11-*cis* retinal chromophore) based on vitamin A. The first stage of light detection involves the absorption of a photon by 11-*cis* retinal and the photoisomerisation of this molecule to the all-*trans* state. The conformational change of the chromophore alters the configuration of the opsin, allowing the activated photopigment to interact with a G protein and trigger a

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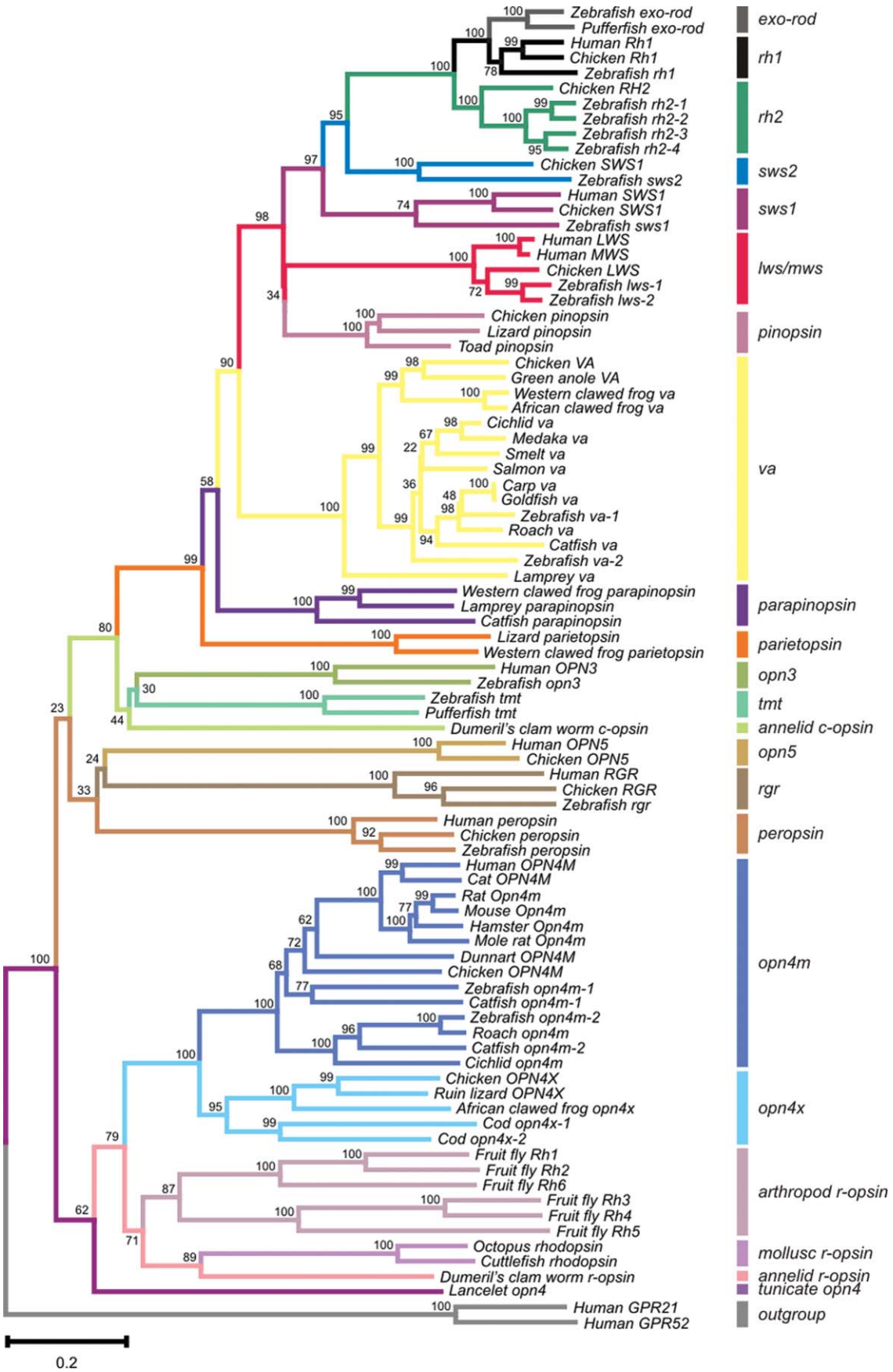


Fig. 1

phototransduction cascade, ultimately giving rise to a change in receptor membrane potential.[30] The isolation, characterisation and function of two vertebrate photopigments, vertebrate ancient (va) opsin and melanopsin (opn4) are considered below. Furthermore, the evolution and functional characteristics of these two opsins are compared. Ultimately, we hope that this review will provide a framework for considering the role of these recently discovered photopigments within the broader framework of light detection across the vertebrate lineage.

2. Identification of the *vertebrate ancient* (*va*) opsin gene

The va opsin was isolated originally from ocular cDNA from Atlantic salmon (*Salmo salar*).[31] The phylogenetic placement of this opsin suggested that it diverged from a common ancestor very early on in vertebrate evolution, hence the assignment of "*vertebrate ancient*" to this gene family. It is apparent from the phylogenetic tree of vertebrate opsins shown in Fig. 1 that whilst *va* opsins are likely to be ancestral to cone and rod visual pigments and pinopsins (P-opsin) (originally isolated from chicken pineal[32,33]), a number of additional opsin gene families (*e.g. parapinopsin*, *parietopsin*, *panopsin* (*opn3*), *teleost multiple tissue* (*tmt*) *opsin*, *neuropsin* (*opn5*), *RPE-specific rhodopsin homolog* (*rrh*) (*peropsin*), and *retinal G protein-coupled receptor* (*rgr*)) have been discovered that seem to pre-date the emergence of *va* opsin. With hindsight, the "*va*" designation may not be the most appropriate name for this gene family, and in this regard, the *va* opsins are similar to multiple other gene families. Following the isolation of *va* opsin from salmon, additional members of the *va* gene family were isolated in quick succession from a variety of teleost species. A second isoform of va opsin was identified in the common carp (*Cyprinus carpio*),[34] which is characterised by a very long carboxyl-terminus (85 amino acids) in comparison to salmon va opsin (19 amino acids). Independently, both long and short isoforms of va opsin were identified in zebrafish,[35] thus two isoforms of va opsin, val (long) and vas (short), exist. In addition, a second *va* gene (val2) was identified in the eyes and brain of zebrafish.[36] More recently, a 'medium' isoform of va opsin (vam) was isolated from a smelt fish (*Plecoglossus altivelis*), with a carboxyl-terminus of 43 amino acids.[37] However, as only two isoforms (long and medium) were identified in this species, it is unlikely that three splice variants of va exist amongst teleosts and smelt vam probably represents an elongated vas isoform. Variation in the carboxyl-tail is a noted feature of other non-cone, non-rod opsins (see below) and the significance of this observation remains to be determined. Interestingly, the *va* opsin gene is not restricted to teleosts, as *va* orthologues have been identified in non-teleosts as discussed below.[8] Comparisons between chicken full-length amino acid sequences show that VAL opsin shares approximately 35–40% amino acid identity with classical cone and rod opsins and 41% identity with P-opsin (see Table 1). A

Fig. 1 Phylogenetic analysis of photosensory pigments from representative vertebrates and invertebrates. Amino acid sequences were aligned using Clustal W[111] and manually manipulated to generate a codon-matched alignment. Evolutionary distances were calculated with the Poisson Correction (PC) method[112] and a neighbour-joining tree was constructed[112] with 1000 bootstrapping replications by using the MEGA Version 4 software.[113] The degree of support for internal branching is shown as a percentage at the base of each node and the scale bar indicates the number of amino acid substitutions per site. Amino acid sequences encoded by human (*Homo sapiens*) *GPR21* (GenBank Accession Number: NM005294) and *GPR52* (NM005684) were used as outgroups. The opsin sequences used for generating the tree are as follows: (a) exorhodopsin (exo-rod): zebrafish (*Danio rerio*), NM131212; pufferfish (*Takifugu rubripes*), AF201472; (b) rod opsin (rh1): human (*Homo sapiens*), NG009115; chicken (*Gallus gallus*), D00702; zebrafish (*Danio rerio*), NM131084; (c) rod opsin-like 2 (rh2): chicken (*Gallus gallus*), M92038; zebrafish (*Danio rerio*), NM131253 (Rh2-1), NM182891 (Rh2-2), NM182892 (Rh2-3), NM131254 (Rh2-4); (d) short-wavelength-sensitive 2 (sws2): chicken (*Gallus gallus*), M92037; zebrafish (*Danio rerio*), NM131192; (e) short-wavelength-sensitive 1 (sws1): human (*Homo sapiens*), NG009094; chicken (*Gallus gallus*), M92039; zebrafish (*Danio rerio*), NM131319; (f) long-wavelength-sensitive/middle-wavelength-sensitive (lws/mws): human (*Homo sapiens*), NG009105 (LWS), NG001606 (MWS); chicken (*Gallus gallus*), M62903; zebrafish (*Danio rerio*), NM131175 (lws-1), NM001002443 (lws-2); (g) pinopsin (p-opsin): chicken (*Gallus gallus*), U15762; lizard (*Uta stansburiana*), DQ100321; toad (*Bufo japonicus*), AF200433; (h) vertebrate ancient (va) opsin: chicken (*Gallus gallus*), GQ280390; green anole (*Anolis carolinensis*), *in silico* analysis; Western clawed frog (*Xenopus tropicalis*), *in silico* analysis; African clawed frog (*Xenopus laevis*), EU860403; cichlid (*Astatotilapia burtoni*), EU523854; medaka (*Oryzias latipes*), AB3831481; smelt (*Pleoglossus altivelis*), AB074483, salmon (*Salmo salar*), AF001499; carp (*Cyprinus carpio*), AF233520; goldfish (*Carassius auratus*), AB383149; zebrafish (*Danio rerio*), AB035276 (va-1), AY996588 (va-2); roach (*Rutilus rutilus*), AY116411; catfish (*Ictalurus punctatus*), FJ839436, lamprey (*Petromyzon marinus*), AH006524; (i) parapinopsin: Western clawed frog (*Xenopus tropicalis*), AB159672; catfish (*Ictalurus punctatus*), AF028014; lamprey (*Lampetra japonicum*), AB116380; (j) parietopsin: lizard (*Uta stansburiana*), DQ100320; Western clawed frog (*Xenopus tropicalis*), DQ284780; (k) panopsin (opn3): human (*Homo sapiens*), NM014322; zebrafish (*Danio rerio*), NM001111164; (l) teleost multiple tissue (tmt) opsin: zebrafish (*Danio rerio*), BC163681; pufferfish (*Takifugu rubripes*), AF402774; (m) annelid c-opsin: Dumeril's clam worm (*Platynereis dumerilii*), AY692353; (n) neuropsin (opn5): human (*Homo sapiens*), NM181744; chicken (*Gallus gallus*), AB368182; (o) retinal G protein-coupled receptor (rgr): human (*Homo sapiens*), NM002921; chicken (*Gallus gallus*), AY339627; zebrafish (*Danio rerio*), NM001017877; (p) retinal pigment epithelium-specific rhodopsin homolog (rrh) (peropsin): human (*Homo sapiens*), NM006583; chicken (*Gallus gallus*), AY339626; zebrafish (*Danio rerio*), NM001004654; (q) mammalian-like melanopsin (opn4m): human (*Homo sapiens*), NM033282; cat (*Felis catus*), AY382594; rat (*Rattus norvegicus*), NM138860; mouse (*Mus musculus*), EU303118; hamster (*Phodopus sungorus*), AY726733; mole rat (*Spalax ehrenbergi*), AM748539; dunnart (*Sminthopsis crassicaudata*), DQ383281; chicken (*Gallus gallus*), EU124632, zebrafish (*Danio rerio*), BC162681 (opn4m-1), AY078161 (opn4m-2); catfish (*Ictalurus punctatus*), FJ839437 (opn4m-1), FJ839438 (opn4m-2); roach (*Rutilus rutilus*), AY226847; cichlid (*Astatotilapia burtoni*), EU523855; (r) xenopus-like melanopsin (opn4x): chicken (*Gallus gallus*), EU124630; lizard (*Podarcis siculus*), DQ013043; African clawed frog (*Xenopus laevis*), AF014797; cod (*Gadus morhua*), AF385823 (opn4x-1), AY126448 (opn4x-2); (s) arthropod r-opsin: fruit fly (*Drosophila melanogaster*), NM079683 (Rh1/ninaE), NM079674 (Rh2), NM079687 (Rh3), NM057353 (Rh4), NM057748 (Rh5), NM079644 (Rh6); (t) mollusc r-opsin: octopus (*Enteroctopus dofleini*), X07797; cuttlefish (*Sepia officinalis*), AF000947; (u) annelid r-opsin: Dumeril's clam worm (*Platynereis dumerilii*), AJ316544; (v) chordate melanopsin (opn4): lancelet (*Branchiostoma belcheri*), AB205400. The gene nomenclature used follows the guidelines adopted by the Entrez Gene database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene). In brief, the genes of all terrestrial species are in uppercase, except for the mouse and rat, where only the first letter is capitalized. The genes of all aquatic species, including amphibians, are in lowercase.

Table 1 Percentage identity between known chicken opsin amino acid sequences, with comparisons made between full-length sequences (bold) and over the third cytoplasmic domain. Opsins discussed in the main text (*i.e.* VA, OPN4M, and OPN4X) are highlighted in italics

	RH1	*RH2*	*SWS2*	*SWS1*	*LWS*	*P-OPSIN*	*VAL*	*OPN5*	*RRH*	*RGR*	*OPN4M*	*OPN4X*
RH1		**72.5**	**49.2**	**45.6**	**39.7**	**42.7**	***35.1***	**22.1**	**24.0**	**21.3**	***17.7***	***16.8***
RH2	80.8		**51.8**	**48.0**	**41.5**	**43.5**	***39.9***	**22.1**	**21.5**	**18.0**	***17.7***	***16.8***
SWS2	57.7	65.4		**48.3**	**38.9**	**45.0**	***37.1***	**21.8**	**23.6**	**18.5**	***15.8***	***16.2***
SWS1	61.5	69.2	73.1		**41.0**	**43.5**	***38.4***	**19.8**	**20.0**	**18.4**	***19.7***	***16.4***
LWS	46.2	46.2	46.2	61.5		**42.2**	***36.8***	**20.9**	**23.3**	**18.5**	***18.0***	***18.5***
P-OPSIN	57.7	57.7	61.5	65.4	50.0		***41.4***	**22.9**	**25.1**	**20.5**	***17.4***	***17.7***
VAL	*23.1*	*34.6*	*42.3*	*38.5*	*19.2*	*30.8*		***23.2***	***26.2***	***19.2***	***17.9***	***17.4***
OPN5	16.7	23.3	13.3	13.3	6.7	20.0	*16.7*		**26.3**	**20.4**	***19.7***	***17.5***
RRH	17.9	3.6	7.1	3.6	10.7	10.7	*3.6*	10.0		**20.6**	***17.0***	***16.6***
RGR	7.7	7.7	3.8	3.8	3.8	3.8	*15.4*	13.3	7.1		***14.6***	***14.7***
OPN4M	*13.2*	*13.2*	*7.9*	*13.2*	*10.5*	*7.9*	*10.5*	*21.1*	*10.5*	*10.5*		***40.4***
OPN4X	*11.4*	*11.4*	*11.4*	*14.3*	*25.7*	*8.6*	*11.4*	*11.1*	*5.7*	*2.9*	*34.2*	

genomic sequence isolated from the sea lamprey (*Petromyzon marinus*) has been termed lamprey *P-opsin* due to its detection in the pineal gland of this agnathan.[38] However, whilst this gene bears some resemblance to the *pinopsins*, a detailed comparison of the genomic structure of lamprey *P-opsin*, coupled with its amino acid identity (lamprey P-opsin shares only 46–48% identity with the pinopsin class, but 61–65% with teleost va opsins), suggests that lamprey *P-opsin* is a member of the *va* opsin gene family. In view of the evolutionary position of lampreys, it seems likely that lamprey *P-opsin* is the evolutionary precursor of the fishes *va* opsin family[34] and, as such, the sea lamprey *P-opsin* gene should be renamed sea lamprey *va* opsin. When expressed *in vitro* and reconstituted with 11-*cis* retinal, teleost va opsins have been shown to form functional photopigments with λ_{max} values between 451 and 510 nm.[2,35,36] Functional expression of va opsins has been technically demanding and the broad spread in λ_{max} values between the few species that have been examined probably reflects technical artifacts, such as misfolding of over-expressed protein generated *in vitro* that may saturate cellular translation and quality control surveillance machineries, structure/function integrity based on membrane insertion and orientation, protein trafficking and post-translational modifications in non-native cells, pH balance that may influence both tuning (unprotonated verses protonated states) and the degree of protein denaturation, chromophore usage and entry/exit portals regarding the chromophore binding site, rather than any endogenous biochemistry of these photopigments.

The demonstration that teleost va opsins are expressed in subsets of neurones within the horizontal and ganglion cell layers of the retina provided the first unambiguous evidence that the retina possesses non-cone, non-rod photoreceptors,[2] and provided the conceptual framework to ask whether inner retinal photoreception might be a feature common to all vertebrates. Further studies on teleosts showed that *va* was also expressed within the pineal and in two bilateral columns of subependymal cells of the hypothalamus.[35,39] Subsequent electrophysiological studies showed that teleost retinal horizontal cells, probably expressing *va* opsin (and melanopsin), were directly photosensitive and utilise a rhodopsin photopigment with a λ_{max} near 477 nm,[40] results subsequently confirmed six years later by Cheng and colleagues.[41] Collectively the va opsin findings in fish provided new insights into both inner retinal photoreception[2] and the nature of deep brain photoreceptors.[39] Furthermore, the results showed that the teleost pineal is an even more complex photosensory organ than previously appreciated.[42,43] Unfortunately gene silencing techniques have still to be employed that link va opsin to a specific photosensory role.

The results in teleost fish prompted the search for va orthologues in other vertebrate classes, using PCR amplification approaches employing primers based upon the consensus sequences of multiple teleost va opsins. Multiple attempts by different laboratories over a period of almost ten years failed. This apparently restricted taxonomic distribution of *va* was a puzzle as most other opsin classes span several vertebrate taxa. The anomaly was finally resolved with the advent of genome databases from other vertebrate classes. The first successful BLAST searches used the chicken genome database. The nucleotide sequences of zebrafish va1l and va1s were used in searches to identify genomic sequences, followed by PCR amplification with chicken specific primers.[8] The full-length sequences of two isoforms of chicken VA (VAL and VAS) were isolated by PCR amplification from both ocular and hypothalamic cDNA. An *in vitro* heterologous expression system was then used to determine whether chicken VA isoforms could form functional photopigments.[44] Full-length chicken VAL and VAS were both cloned into a mammalian expression vector and transiently transfected into neuroblastoma Neuro2A cells. Whole-cell patch-clamp recordings from transfected fluorescent cells, expressing either chicken VAL or VAS, demonstrated light-dependent inward currents in the presence, but not the absence, of retinal chromophore.[8]

In order to correlate chicken VA opsin to a particular function, cellular localisation of the protein was undertaken using an antibody raised against a peptide corresponding to an amino-terminal region predicted to be extracellular and common to both chicken VA isoforms. VA opsin protein was only weakly detected in the pineal and eye of both chicken and quail and a clear cellular localization was not possible with this antibody. This was surprising as VA opsin mRNA is strongly expressed in these organs in both chicken and quail. By contrast, VA opsin mRNA expression within the hypothalamus was accompanied by strongly labelled VA opsin cell bodies. These perikarya are located throughout the anterior and mid-hypothalamus and send an extensive projection of fibers into the median eminence.[8] This distribution immediately suggested a role for these VA-labeled neurones. Reproduction and other seasonal phenomena in temperate birds are profoundly influenced by changes in day length (photoperiod). Studies in the 1930s demonstrated that birds possess photoreceptors located

within the hypothalamus that regulate photoperiodic responses to day length.[45,46] Most recently, studies on the Japanese quail have shown that the photoperiod alters the activity of thyrotrophs within the pars tuberalis (PT) to release thyrotrophin (TSH), which in turn triggers a cascade of events leading to a reproductive response.[47,48] Despite these significant findings, the cellular and molecular identity of hypothalamic photoreceptors has remained a mystery. Action spectra have suggested that they utilise an rhodopsin-based photopigment system,[49] but attempts to identify these photoreceptors using cone or rod opsin probes failed, suggesting the involvement of a novel opsin.[50] The demonstration of chicken VA opsin within neurones of the hypothalamus that project to the median eminence strongly implicate these neurones in mediating the photoperiodic responses of birds and perhaps other vertebrates.[8] However, a definite functional link between VA opsin and day length detection remains to be established and will be dependent upon the emerging gene silencing techniques being developed for birds.[51]

To date, *va* opsin gene orthologues have been identified in genome databases from the majority of vertebrate classes, such as the agnathans (*e.g.* sea lamprey), cartilaginous fishes (*e.g.* elephant shark), teleosts (*e.g.* zebrafish and many other bony fishes), amphibians (*e.g.* two species of clawed frogs), reptiles (*e.g.* green anole) and birds (*e.g.* chicken and zebrafinch).[8] By contrast, similar searches of genomes from the three main mammalian lineages, the protherian monotremes (*e.g.* platypus), the methatherian marsupials (*e.g.* opossum and tammar wallaby), and the eutherian placentals (*e.g.* mouse and human), have failed to identify *VA* opsin orthologues (W. L. Davies, unpublished work). This suggests that the *VA* gene was lost early in the evolution of modern mammals during the Carnian stage of the Upper Triassic epoch, some 225 MYA.[21,52] The eutherian mammals appear to have lost the *VA* gene, along with a significant number of additional non-cone, non-rod and visual opsin genes, such as *xenopus-like melanopsin* (*OPN4X*), *TMT*, *parietopsin*, *parapinopsin*, *pinopsin*, *short-wavelength-sensitive 2* (*SWS2*), *rod opsin-like 2* (*RH2*), and *exorhodopsin* (*exo-rod*) (see Fig. 1). Teleosts exhibit many opsin gene duplications and as such possess an increased repertoire on which to mediate complex mechanisms of visual and non-visual photosensitivity. In contrast, the number of visual pigment genes does not generally increase throughout mammalian evolution (the only exception is the development of trichromacy in primates with a duplication of the *long-wavelength-sensitive* (*LWS*) gene) but are considerably lost to form a more slim-lined inventory of opsins. Why the mammalian lineage has lost the *VA* gene but retained one of the melanopsins (see below) remains a fascinating question: perhaps these genetic changes are also associated with the evolutionary passage of the mammals through a "nocturnal bottleneck", or maybe melanopsin provided greater sensitivity in the dim-twilight world of the modern nocturnal mammalian lineage?

3. Photoreception mediated by melanopsin (opn4)

In an attempt to identify the photopigment responsible for light-induced melanosome aggregation and dermal photoreception in *Xenopus laevis*, a screen of a *X. laevis* dermal melanophore cDNA library isolated a clone that resembled an opsin. This opsin gene was termed "melanopsin", now also designated as *opn4*,[5] and was shown to be expressed within retinal horizontal cells, the RPE and iris. In the *X. laevis* brain, melanopsin was localised to the magnocellular preoptic nucleus and the suprachiasmatic nucleus,[19] all areas variously suggested as photoreceptive,[5] however, no corresponding biochemistry was undertaken to show that frog melanopsin was capable of forming a functional photopigment. Mammalian orthologues of melanopsin were subsequently isolated and expression was found to be restricted to a subset of human and mice pRGCs.[53,54] More recently orthologues of melanopsin have been isolated in a range of species from the chordate lineage, including the cephalochordate lancelets,[55] many bony fish (*e.g.* catfish,[41] African cichlid,[56] cod,[57] roach,[40] zebrafish[58]), amphibians (*e.g.* African clawed frog[5]), reptiles (*e.g.* ruin lizard[59]), birds (*e.g.* chicken[60,61]), marsupials (*e.g.* dunnart[62]), and a multitude of eutherian mammals (*e.g.* mouse,[63] rat,[64] hamster,[65] mole rat (AM748539), cat,[66] and human[53]).

Much of the functional analysis of melanopsin has been undertaken in mammals as the discovery of the *OPN4* gene provided a critical part of the jigsaw puzzle of understanding how mammals regulate their circadian rhythms by light. The "master" circadian clock of mammals is located within small paired nuclei of the anterior hypothalamus called the suprachiasmatic nuclei (SCN). This brain structure acts predicatively to "fine-tune" physiology and behaviour to the varying demands of the day/night cycle.[67] The SCN receives a direct retinal projection from the retinohypothalamic tract and changes in the quantity and quality of light at dawn and dusk act to entrain the molecular and cellular clockwork within the SCN. As eye loss in mammals blocks photoentrainment, the mammalian eye, therefore, performs two quite different sensory tasks: its familiar function is to collect photic information pertaining to light intensity and wavelengths and help generate a coloured three-dimensional image of the world, whilst its second function is to measure environmental irradiance at dawn and dusk to drive photoentrainment. In an attempt to define which ocular photoreceptors might perform these divergent tasks, circadian responses to light were examined in mutant mice with deficient rods and/or cones and lacking visual responses. Mice homozygous for the *retinal degeneration* gene (*rd/rd*), lacking all rods and most cones, were able to regulate their circadian rhythms with the same sensitivity as fully sighted animals.[68,69] These, and a host of subsequent experiments including studies in humans with genetic defects of the eye,[70] demonstrated that the processing of light information by the circadian and classical visual systems must be different and raised the possibility that the eye might contain an additional non-cone, non-rod photoreceptor. These data were far from conclusive, however, because there remained the possibility that only small numbers of rods and/or cones are necessary for normal photoentrainment. As a result, a mouse was engineered in which all cone and rod photoreceptors were ablated (*rd/rd cl*). The loss of all types of known photoreceptor had little effect on photoentrainment, although loss of the eyes abolished this capacity completely.[12] Thus, mammals had to possess another ocular photoreceptor. It also became apparent early on from work on the *rd/rd cl* mouse that these novel photoreceptors also contribute to a broad range of irradiance detection tasks, including melatonin synthesis[71] and pupil constriction.[17]

By 2001, it was clear that novel photoreceptors existed within the eye but the cellular identity remained a matter of speculation. Foster and colleagues[19] had implicated RGCs as being a candidate

for these photoreceptors, however, final proof came from two independent approaches, one in the rat and the other in *rd/rd cl* mice, that showed that the retina contains a small number of photosensitive retinal ganglion cells (pRGCs). In rats, a population of RGCs was labelled by retrograde dye injected into the SCN. The retina was removed and the electrical activity of individually labelled RGCs was recorded and shown to respond to bright light. This in itself was no surprise, because both cones and rods were present in the retina. However, light-evoked depolarisations continued in the presence of drugs that block intercellular communication and after labelled RGCs were surgically isolated from the surrounding retina.[3] The second approach utilised calcium (Ca^{2+}) imaging on the isolated *rd/rd cl* mouse retina. This study not only demonstrated an extensive network of pRGCs, but that three types of light-evoked Ca^{2+} influx were observed in these neurones: a sustained, a transient and a repetitive response.[4] The photopigment of these pRGCs was defined in the first instance by action spectroscopy. The first full action spectrum examined pupil constriction in *rd/rd cl* mice. The results described a previously uncharacterised, rhodopsin-based photopigment with a spectral sensitivity peak in the "blue" region of the spectrum near 480 nm.[17] Since 2001, a number of action spectra from mice to man were deduced for a range of irradiance responses to light, including the light responses of pRGCs in mice,[72] rats[3] and primates,[73] spanning pupil constriction, phase shifting circadian rhythms, plasma melatonin suppression, together with irradiance dependent regulation of human retinal cone function.[74] All these action spectra pointed to the existence of a coherent single novel opsin photopigment with a λ_{max} close to 480 nm. It remains unclear what ecological advantage this wavelength might confer, but one possibility is that pRGCs are tuned to the dominant wavelength of light at twilight. When the sun is close to the horizon, there is relative enrichment of "blue" light in the dome of the sky because of the preferential scattering of short wavelengths of light passing obliquely through the atmosphere.

Although the spectral properties of the photopigment of pRGCs were defined using action spectroscopy, the opsin gene itself remained unclear. Melanopsin surfaced as the strongest candidate based upon the observation that melanopsin was expressed in pRGCs,[3,4] and mice in which cones, rods and melanopsin were ablated, failed to show circadian or pupil responses to light, arguing that these three classes of photoreceptor could fully account for all light detection within the mammalian eye.[72,75–78] Although a powerful association, the melanopsin knock-out data could not demonstrate that melanopsin was the photopigment of the pRGCs, only that melanopsin was important. This issue was finally resolved by a number of different groups using heterologous expression of either human or murine melanopsin in Neuro2A cells,[44] HEK293 cells,[55,79,80] and *Xenopus* oocytes.[81] In each expression system, melanopsin expression was sufficient to drive a retinal-dependent phototransduction cascade. For example, in Neuro2A cells the expression of melanopsin, in the presence of retinal chromophore (11-*cis* retinal), transformed a non-photosensitive neurone into a photoreceptor. When bound to an 11-*cis* retinal chromophore, the spectral sensitivity of this photopigment (λ_{max} = 485 nm for amphioxus melanopsin[55] and 476–484 nm for chicken melanopsins[80]) was shown to be consistent with the expected absorbance peak of 480 nm determined from action spectrophotometry and electrophysiology. Furthermore, melanopsin (specifically from amphioxus and human) acted as a bistable pigment able to regenerate its chromophore (11-*cis* retinal), utilising all-*trans* retinal and long-wavelength light in a manner reminiscent of invertebrate photopigments.[44,55] Recent studies on the melanopsin phototransduction cascade suggest further similarities with invertebrate photopigments.

Invertebrate phototransduction, as elucidated in the fruit fly (*Drosophila melanogaster*), involves the interaction of an opsin with a G_q/G_{11}-type G protein, activation of phospholipase C (PLC), gating of transient receptor potential (TRP) channels and the depolarisation of the membrane potential. By contrast, the mechanism of phototransduction utilised by classical cone and rod opsins is quite different, involving the activation of isoform-specific transducins (G_i/G_o class), phosphodiesterases (*i.e.* PDE6), guanylyl cyclases and closure of cyclic nucleotide-gated (CNG) ion channels, resulting in a hyperpolarising membrane potential. In addition to bistablity, melanopsins share additional key characteristics with an invertebrate-like signal phototransduction pathway, with both pRGCs and cells transfected with melanopsin showing depolarising responses to light. In addition, melanopsin responses are greatly attenuated (although not blocked) by antibodies against G_q/G_{11} G proteins (but not by antibodies to G_i/G_0).[81] In Neuro2A cells, the use of G_i/G_0 blockers failed to inhibit melanopsin-dependent light responses,[44] whilst G_q/G_{11} agonists fully blocked melanopsin-dependent light responses in HEK293-TRPC3 cells.[79] More recently, *in vitro* biochemical analyses have shown that amphioxus melanopsin is able to couple and activate a G_q-type G protein, similar to photopigments expressed in invertebrates such as the honey bee and squid.[82] Downstream of the G protein, melanopsin-dependent light responses are greatly attenuated or blocked in *Xenopus* oocytes and HEK293-TRPC3 cells by PLC inhibitors.[79,81] Furthermore, coexpression of melanopsin with TRPC3 (similar to *D. melanogaster* TRP channels) in *Xenopus* oocytes shows that TRPC3 channels can generate a light-activated photocurrent in the presence of melanopsin.[79,81] More recently, combined pharmacological and morphological approaches suggest that the native pRGC cascade gates a TRPC7 channel in the plasma membrane.[83] Finally, the genetic ablation of the atypical protein kinase, *protein kinase C zeta* (*Prkcz*), mimics precisely the melanopsin knock-out phenotype in a battery of behavioural and pupillometric tests,[84] suggesting yet another element of similarity between vertebrate melanopsin and invertebrate phototransduction.

It is clear that the mammalian retina contains a population of melanopsin-based pRGCs that are used to measure environmental irradiance and modulate diverse physiological responses to light, including circadian physiology and pupil constriction. Nevertheless, it is worth stressing that there is complex crosstalk between pRGCs, cones and rods. Although the loss of cones, rods and pRGCs abolishes all responses to light,[72] the loss of melanopsin alone does not abolish circadian photosensitivity but only attenuates circadian photic responses. It seems that under these circumstances the cones and/or rods can partially compensate for pRGC loss of function. These results are consistent with previous studies suggesting the involvement of cone photoreceptors in rodent entrainment,[85,86] and from more recent studies on the macaque.[73] Intracellular recording from macaque pRGCs expressing melanopsin have shown that short-wavelength-sensitive (SWS) cones (λ_{max} ~435 nm) attenuate the light responses

of pRGCs, whilst the inputs from middle-wavelength-sensitive (MWS) (λ_{max} ~530 nm) and long-wavelength-sensitive (LWS) (λ_{max} ~560 nm) cones, and rods (λ_{max} ~500 nm), provide an excitatory input.[73] Recently, it was shown using a human LWS cone knock-in mouse model (where the murine *MWS* opsin gene on the X-chromosome was replaced with the human LWS opsin cDNA sequence),[87] that rods contribute to pRGC responses at dim (scotopic) light levels and at irradiances at which image forming vision relies heavily on cones. By contrast, cone input to irradiance responses dissipates following light adaptation to the extent that these receptors make a very limited contribution to circadian and pupillary light responses under these conditions.[88]

It is noteworthy that two melanopsin genes, *mammalian-like melanopsin* (*opn4m*) and *xenopus-like melanopsin* (*opn4x*), have been discovered in non-mammalian vertebrates.[61] PCR amplification analysis in zebrafish, frog, and chicken demonstrated expression of both *opn4m* and *opn4x* genes in tissues known to be photosensitive (*e.g.* eye, brain, and skin). In the chicken eye, OPN4M and OPN4X mRNA are broadly coexpressed within subsets of cells within the outer nuclear, inner nuclear, and ganglion cell layers of the retina. Significantly, both genes can encode a photosensitive pigment capable of activating G protein signalling cascades in a light- and retinaldehyde-dependent manner.[61] The differential function of these two melanopsin genes remains to be determined; however, the mammalian lineage appears to have lost the *OPN4X* gene. This analysis was initially based upon an *in silico* analysis of eutherian genomes. Subsequent analyses of two marsupials from Australia (*i.e.* dunnart) and South America (*i.e.* opossum) also failed to find evidence for the *OPN4X* gene, suggesting that *OPN4X* was lost from the mammalian lineage before the marsupial/eutherian split.[62] Finally, our most recent *in silico* analysis of the platypus genome identified an *OPN4M* gene but again no *OPN4X*, suggesting that the *OPN4X* gene was lost before the diversification of modern mammals in the Upper Triassic epoch (225 MYA) (W. L. Davies, unpublished work). Although the *OPN4X* gene has been lost from mammals, the mouse *Opn4m* gene encodes two mRNA isoforms (Opn4l and Opn4s), which are expressed within the RGC layer of the adult mouse retina.[63] Proteins encoded by Opn4l and Opn4s transcripts differ only in the length of their carboxyl-termini and so perhaps not surprisingly form fully functional photopigments. Antibodies raised against isoform-specific epitopes have identified discrete populations of retinal ganglion cells, with some ganglion cells expressing both Opn4l and Opn4s and others only Opn4l photopigments.[63] Whilst the functional significance of the carboxyl-terminal splice variants of Opn4 is unknown, it seems likely that they may play a role in determining the diversity of pRGC responses to light.[4,89]

4. Evolutionary and functional divergence of va and melanopsin (opn4) photopigments

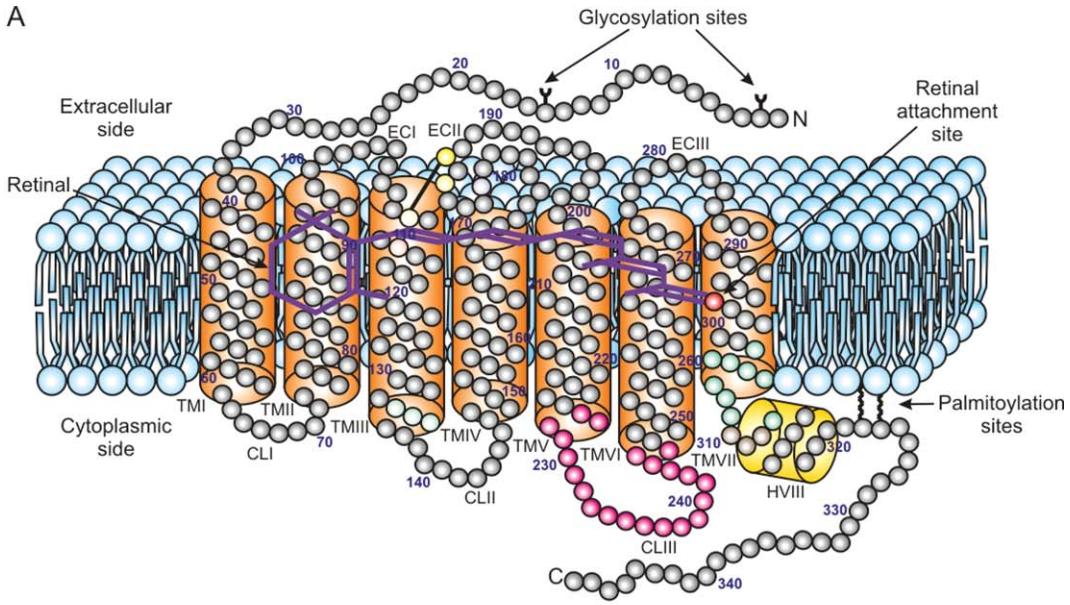
In addition to their highly conserved genomic structures,[24] the visual opsin genes (cone opsins: *lws*, *sws1*, *sws2*, *rh2*; rod opsin: *rh1*) share many other features. Using a numeration scheme based upon bovine rod opsin,[90] these features are discussed below and shown in Fig. 2. The most defining feature of all opsins is the presence of a retinal attachment site at K296 in the seventh transmembrane domain of the opsin apoprotein, which binds the chromophore covalently through a protonated (or unprotonated in UV-sensitive sws1 photopigments) Schiff base (azomethine) linkage.[91] The positive charge of the Schiff base is balanced by a negatively charged counterion, E113, in the third transmembrane domain.[91] Conformational integrity of the opsin is, in part, maintained by a disulfide bridge between C110 and C187 (and to a lesser extent C185) in the first and second extracellular loops, a feature highly conserved amongst GPCRs[92] (Fig. 2). An amino acid triad, the ERY motif ((E/D)R(Y/W/F)), is located between residues 134–136 and is highly conserved amongst GPCRs, where it appears to be critical for G protein interaction.[93] At the amino-terminus, two glycosylation sites are present, N2 and N15,[94] whilst at the carboxyl-terminus a pair of cysteine residues (C322 and C323) are palmitoylated and form a fourth intracellular loop[95] that links the $NPxxY(x)_{5,6}F$ motif (which works in conjunction with the ERY motif in controlling the structural changes accompanied with photopigment activation[96]) of the seventh transmembrane domain and an eighth cytoplasmic helix of unknown function.[97]

Similar to cone and rod photopigments, va opsins possess all of the critical amino acids discussed above, including a glutamate counterion at residue 113 (see Fig. 2B). In the majority of retinal and extraocular opsin classes, site 181 is either negatively charged (E or D) and may have a role in stabilising the positive charge of the Schiff base, especially when the E113 counterion is not present,[98] or positively charged (H) and shown to form a chloride binding site in photopigments with spectral peaks close to 560 nm.[99,100] In the latter case, a small number of visual pigments may replace histidine with an aromatic tyrosine at site 181, which results in a loss of chloride ion binding and a blue-shift in spectral sensitivity.[100,101] However, unique to va photopigments is the presence of S181, a neutral polar residue whose role in structural integrity and/or spectral tuning is currently unknown.

The melanopsins are very different from classical photopigments, as they possess a tyrosine instead of a glutamate at site 113.[24] In this case, the negatively charged counterion may be displaced to E181 as discussed above.[98] Overall, melanopsins share less than 25% identity (cone and rod photopigments show greater than 40% identity) with other known opsin photopigments[24] (Table 1), and the levels of identity between members of the melanopsin family are much lower than that found in other known vertebrate photopigment opsins. An alignment of the putative G protein interaction region of the third cytoplasmic loop in melanopsins shows very poor conservation, compared to a higher level of identity over this region in the va opsin class (Fig. 3), suggesting that different melanopsin photopigments may have species-specific G protein partners, or that melanopsin does not need to be as highly conserved as the known photosensory opsins to activate the phototransduction cascade. Finally, the melanopsins form a distinct branch of the rhodopsin-like branch (class A) of the GPCR superfamily tree and possess a unique genomic structure.[24,58] Collectively the evidence suggests that the melanopsins form a distinct evolutionary lineage and function very differently from the known photosensory opsins.

5. More questions than answers when comparing ciliary *vs.* rhabdomeric opsins

The evolution of vertebrate (deuterostome) and invertebrate (protostome) photoreception has been the subject of considerable



B

	110	113	134-136	181	185	187	296	302-313	310-312
exo-rod	C	E	ERY	E	C	C	K	NPxxY(x)$_{5,6}$F	NRQ
rh1	C	E	ERY	E	C	C	K	NPxxY(x)$_{5,6}$F	NKQ
rh2	C	E	ERY	E	C	C	K	NPxxY(x)$_{5,6}$F	NKQ
sws2	C	E	ERC	E	C	C	K	NPxxY(x)$_{5,6}$F	NKQ
sws1	C	E	ERY	E	C	C	K	NPxxY(x)$_{5,6}$F	NKQ
lws/mws	C	E	ERW	H	T	C	K	NPxxY(x)$_{5,6}$F	NRQ
pinopsin	C	E	ERY	E	T	C	K	NPxxY(x)$_{5,6}$F	NKQ
va	C	E	ERY	S	T	C	K	NPxxY(x)$_{5,6}$F	NKQ
parapinopsin	C	E	ERY	E	T	C	K	NPxxY(x)$_{5,6}$F	NRQ
parietopsin	C	Q	ERY	E	T	C	K	NPxxY(x)$_{5,6}$F	NKQ
opn3	C	D	ERY	E	L	C	K	NPxxY(x)$_{5,6}$F	SRK
tmt	C	Y	ERY	E	T	C	K	NPxxY(x)$_{5,6}$F	NKQ
annelid c-opsin	C	Y	VRC	E	T	C	K	NPxxY(x)$_{5,6}$F	NKQ
opn5	C	Y	DRY	E	T	C	K	NPxxY(x)$_{5,6}$F	DCK
rgr	C	H	DRY	E	T	C	K	NAxxY(x)$_{5,6}$Y	NEN
peropsin	C	Y	DRY	D	A	C	K	NPxxY(x)$_{5,6}$F	NKK
opn4	C	Y	DRY	E	T	C	K	NPxxY(x)$_{5,6}$Y	HPK
arthropod r-opsin	C	Y	DRY	E	T	C	K	NPxxY(x)$_{5,6}$Y	HPK
mollusc r-opsin	C	Y	DRY	E	T	C	K	NPxxY(x)$_{5,6}$F	HPK
annelid r-opsin	R	Y	DRF	E	T	C	K	NPxxY(x)$_{5,6}$F	HPK
tunicate opn4	C	Y	DRY	E	T	C	K	SPxxY(x)$_{5,6}$F	YPK
non-opsin outgroup	C	F	DRY	H	Q/E	C	I	NCxxY(x)$_{5,6}$F	NSV

Fig. 2 (A) Schematic of a vertebrate photopigment showing the presence of seven helical transmembrane domains (TMI-VII) (orange), three extracellular loops (ECI-III), three cytoplasmic loops (CLI-III) (with the third intracellular loop highlighted in pink), amino-terminus (N), carboxyl-terminus (C), an eighth cytoplasmic helix (HVIII) (gold), two amino-terminal glycosylation sites (residues 2 and 15),[94] and two carboxyl-terminal palmitoylation sites (residues 322 and 323),[95] based on bovine rod opsin (NP001014890) as determined by crystallography.[97] Additional residues identified as being critical for correct opsin protein conformation and function (as discussed in the main text) are shown. These include: (i) three conserved cysteine (C) residues at positions 110 (TMD3) (pale yellow), 185 (ECD2) (semi-conserved) (medium yellow) and 187 (ECD2) (dark yellow) that are involved in disulfide bond formation;[92] (ii) a conserved glutamate (E) (or tyrosine (Y) in many non-cone, non-rod opsins) at position 113 (TMD3) (pale pink) that provides the negative counterion to the proton of the Schiff base;[91] (iii) a conserved glutamate (E) at position 134 (TM3), located within a conserved ERY motif (134–136) (pale green), that provides a negative charge to stabilise the inactive opsin molecule;[93] (iv) a conserved charged residue at site 181 (pale purple) that may serve as a counterion in many non-cone, non-rod opsins[98] or affect spectral tuning;[99–101] (v) a conserved lysine (K) at position 296 (TM7) (red) that is covalently linked to the 11-*cis* retinal chromophore (dark purple) *via* a Schiff base;[91] (v) conservation of two cysteine (C) residues at palmitoylation positions 322 and 323;[95] and (vi) a conserved NPxxY(x)$_{5,6}$F motif (302–313) (dark green), containing a NKQ motif (310–312) (brown) that assists in maintaining structural integrity upon photopigment activation.[96] (B) Summary table showing the phylogenetic course of conserved structural and functional motifs throughout opsin evolution. For every opsin clade, a consensus sequence is shown for each motif discussed in the main text and above.

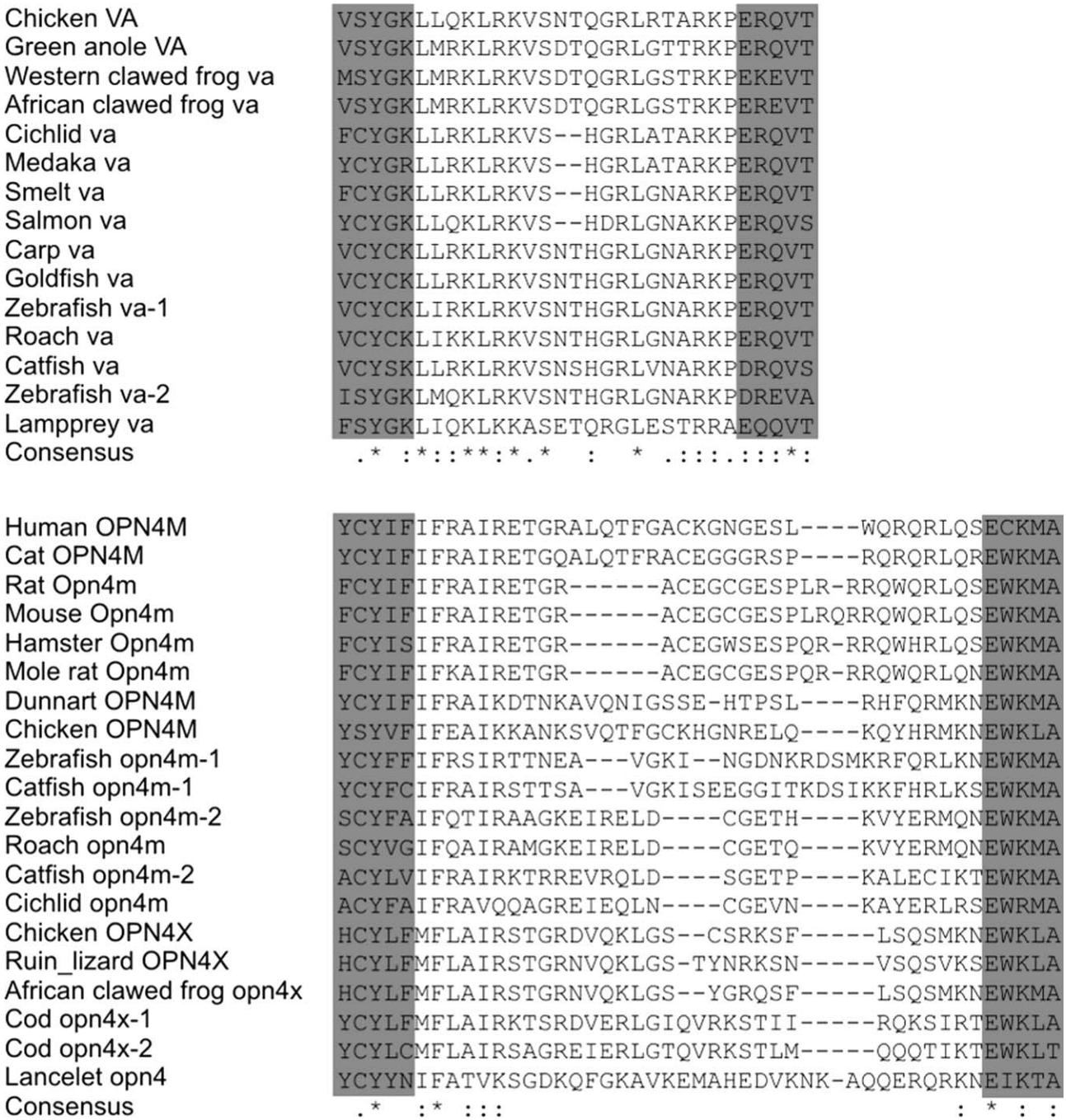


Fig. 3 Amino acid alignment of the third cytoplasmic loop (CLIII) of two irradiance detectors expressed in chordates, spanning from the cephalochordates (*e.g.* lancelet) to mammals (*e.g.* human), showing relatively high conservation of sequence identity across the va class of photopigments but not the melanopsins. (*) denotes an identical consensus residue between opsin sequences. (:) and (.) indicate a conserved or semi-conservative amino substitution, respectively, within the codon-matched protein alignment. Gaps were inserted to maintain a high degree of identity and are indicated by dashes (–). In both cases, orange shading denotes five amino acids from each transmembrane domain (TMV and TMVI) that flank the third cytoplasmic loop.

discussion. Until recently, these two animal lineages were broadly characterised as possessing two very different types of photoreceptors, with the deuterostomes/vertebrates employing ciliary photoreceptors, whilst the protostomes/invertebrates used microvilli rhabdomeric photoreceptors. Vertebrate ciliary photoreceptors are distinguished by possessing an inner segment containing a 9 + 0 cilium and expressing a ciliary opsin (c-opsin) bound to an 11-*cis* retinal chromophore. Upon absorption of a photon by 11-*cis* retinal, the chromophore undergoes a conformational change from 11-*cis* to all-*trans* retinal, followed by the release of the all-*trans* retinal chromophore from the opsin, with which it cannot form a stable association. These events alter the conformation of the c-opsin allowing it to bind to a member of the G_i/G_o-protein family (transducin) coupled to a phosphodiesterase (*i.e.* PDE6). As discussed above, in cone and rod photoreceptors, activation of PDE6 hydrolyses cyclic guanosine monophosphate (cGMP) to

GMP. The decrease in cGMP levels closes cGMP-gated Na^+/Ca^{2+} ion channels (*i.e.* CNG channels) and leads to hyperpolarisation of the cell. Regeneration of the released all-*trans* retinal back to the photosensitive 11-*cis* isomer involves the retinal/retinol chaperone carrier proteins of the visual cycle and the action of isomerases, all of which can be accomplished in the dark. By contrast, invertebrate rhabdomeric photoreceptors lack a 9 + 0 cilium and employ a rhabdomeric opsin (r-opsin) bound to an 11-*cis* retinal chromophore. Like c-opsins, absorption of a photon by 11-*cis* retinal found in the chromophore binding pocket of r-opsins causes an 11-*cis* to all-*trans* conformational change. The all-*trans* isomer, however, forms a stable association with the r-opsin and is not released, and it is this maintenance of the chromophore that may mediate bistability and the resistance to light bleaching observed with these photopigments. The conformational change exhibited by r-opsins allows the binding of a member of the G_q-protein family that in turn activates PLC and Ca^{2+} signaling, which in 'advanced' protostomes, involves TRP channels and depolarisation of the photoreceptor. Regeneration of all-*trans* retinal back to the photosensitive 11-*cis* isomer is dependent upon light and occurs either by the intrinsic isomerase activity of the r-opsin photopigment itself or by an accessory photoisomerase. In either case, the spectral sensitivity of the pigment undergoing photoisomerisation generally peaks at wavelengths longer (or sometimes identical or even shifted to shorter wavelengths) than the photosensory response.[28,55,82]

A number of researchers, but most notably Detlev Arendt in Heidelberg (Germany) and Trevor Lamb in Canberra (Australia), have proposed a series of evolutionary events that might account for the divergence of *c-opsin* and *r-opsin* genes.[28,102] As a result of these convergent ideas and other emerging alternative conjectures, we propose to introduce the collective noun "*Evolution of Photosensory Opsins with Common Heredity (EPOCH)*" as an umbrella term to describe any hypotheses that aim to explain the evolutionary origins that underlie the complexity of animal photosensory systems. Currently the most prominent hypotheses, assumes that ciliary and rhabdomeric photoreceptors are homologous and arose from a common ancestral photoreceptor in the early metazoans, which would have possessed a 9 + 0 cilium. The cilium was then subsequently lost in the rhabdomeric lineage. This initial sequence of events is proposed on the basis that the cnidarians (jellyfish), which pre-date the bilaterians, possess ciliary-like photoreceptors and express *c-opsin* genes.[103,104] Before the separation of the protostomes and deuterostomes, ancestral bilaterians would have possessed both ciliary and rhabdomeric photoreceptors. Lamb has suggested that the light-induced depolarising rhabdomeric photoreceptors might have signalled "light-on" whilst the light-induced hyperpolarisation of ciliary photoreceptors signalled "light-off" responses.[28] It is then argued that ancestral chordates moved away from the surface and into deeper waters of the sea, where light levels would have been lower. Under these conditions, the rhabdomeric photoreceptors would have been at a disadvantage because of the reduced levels of long-wavelength (or possibly short-wavelength) light needed for photoisomerisation of the all-*trans* chromophore back into the 11-*cis* isoform. This argument, however, may be flawed as the photopigments expressed within these early chordate rhabdomeric photoreceptors may utilise other wavelengths of light (that are transmissible at greater depths of the ocean) to activate chromophore isomerisation. The next suggestion is that ciliary photoreceptors developed synaptic contacts with rhabdomeric photoreceptors that possessed central axonal projections. Ultimately by 500 MYA and the evolution of bilateral image forming eyes, ancestral ciliary photoreceptors provided the lineage that would evolve into cones and ultimately rods, and possibly the bipolar cells (which lack a photoreceptor capacity but possess some features reminiscent of cones and rods), whilst the rhabdomeric photoreceptors evolved into the horizontal, amacrine and RGCs that lost their capacity to detect light – except for the pRGCs that express melanopsin.[102]

This depiction of the evolution of the vertebrate eye and the division of its cell types into a ciliary and rhabdomeric origin has formed a powerful framework for the consideration of photoreceptor evolution, but in its present form the va opsins do not fit comfortably within this scheme. The *va* opsins are clearly related to the *c-opsin* gene lineage but are expressed in retinal cells (horizontal cells and RGCs) that lack a cilium and are considered to be of a rhabdomeric origin. Furthermore, there is increasing evidence that the va opsins, at least in the teleost retina, are frequently coexpressed with melanopsin.[40,41] These findings raise a series of fascinating questions: (i) Perhaps the rhabdomeric origin hypothesis for horizontal and ganglion cell evolution is incorrect and, by extension, the entire argument relating to the evolution of ciliary *vs.* rhabdomeric cell types within the vertebrate eye? (ii) Alternatively, horizontal and ganglion neurons may represent cells of a very ancient lineage dating back to a time when *c-opsins* and *r-opsins* were coexpressed in primitive bilaterians? (iii) It is also possible that the *va* opsin gene may, for some reason, be "switched on" in rhabdomeric photoreceptors? Whatever the explanation for this anomaly, it is clear that the distinction that has been made between *c-opsin* and *r-opsin* expression within vertebrate photoreceptors of either a rhabdomeric or ciliary origin needs significant revision.

These sorts of questions also need to be addressed within the context of the extraocular photoreceptors. Multiple *c-opsin* genes (*e.g. rh1-like exorhodopsin*, cone opsins such as *lws* and *sws2*, *pinopsin*, *va*, *parapinopsin*) and a single *r-opsin* gene (*melanopsin*) are expressed within the pineal complex of non-mammalian vertebrates.[33,39,42,60] If the divergent ciliary and rhabdomeric origin of cell types within the vertebrate retina is correct then by extension the pineal will also consist of photoreceptors with a mixed ciliary and rhabdomeric origin. In addition to the cell types of the pineal complex, what is the evolutionary origin of deep brain photoreceptors? Like the simultaneous expression of *c-opsins* and *r-opsins* in pinealocytes, there is evidence for *opn4* and *c-opsin* (*e.g.* cone, rod, and *va* opsins) coexpression within the photosensitive deep brain regions of various vertebrates.[29] We also have to account for how *c-opsin* genes (*e.g. va* opsin) that are expressed within neurons of the inner retina, pineal gland and deep brain regions regenerate their photopigments. Do these photosensory neurons also possess the retinoid biochemistry found in the RPE and the capacity to regenerate their photopigments, or do they rely on the activity of adjacent cells to support this function. Müller cells within the vertebrate retina appear to have the capacity to synthesize 11-*cis* retinoids from all-*trans* retinol.[105,106] Perhaps these cells provide the source of chromophore for inner retinal photopigments? In the pineal complex, key retinoid transport and isomerase proteins have been identified,[107] but even this level of information is lacking for deep brain photoreceptors. Perhaps deep brain photoreception by va

photopigments use a system of 11-*cis* retinal regeneration similar to the urochordate tunicate *Ciona intestinalis*, which appears to involve both dark isomerisation and photoisomerisation? In *C. intestinalis*, the photoreceptor cells have non-photoreceptor neighboring cells that contain proteins thought to be involved in both dark isomerisation (*e.g. Ci-RPE65*, which is a possible orthologue of vertebrate *RPE65*) and photoisomerisation (*e.g. Ci-opsin3*, a possible orthologue of invertebrate retinochrome and vertebrate RGR opsins).[108] Immunocytochemical colocalisation approaches using antibodies against retinoid proteins and VA opsin in the chicken hypothalamus may provide a useful initial approach to address this question.

6. Conclusions

It seems likely that the different photosensory tasks of image detection and wavelength discrimination (vision) and irradiance detection have provided the selection pressure for the evolution of two markedly different photoreceptor systems. Whilst this makes intuitive sense, this does not explain why there is a plethora of photoreceptors and photopigment systems across the vertebrates and how these different systems might interact to extract photosensory information from the environment. It is worth emphasising that the sensory task of measuring environmental irradiance is deceptively complex and subject to multiple sources of noise. For example, variations in environmental light could result from cloud cover, seasonal changes, and extraneous sources of light such as starlight, moonlight, lightning, fire and in recent times artificial lighting, and may periodically compete with sunlight as a light stimulus. Temperature changes will also generate receptor noise, particularly in poikilothermic animals. In addition, an animal might experience considerable variation in its exposure to environmental light because of its location, behaviour and developmental state. Perhaps the information from multiple photoreceptor/photopigment channels, all sampling slightly different aspects of the light environment (*e.g.* light intensity, spectral composition, solar direction), is integrated within receptor organs and then within the CNS to reduce noise and extract reliable irradiance measurements?

Beyond the detailed discussion of the highly divergent va opsin and melanopsin photopigments, this review has focused on the general issue of why vertebrates have retained multiple photopigment systems of diverse evolutionary origin, apparently expressing both *c-opsin* and *r-opsin* genes. One could equally well ask why insects such as *D. melanogaster* appear to lack any orthologues of the *c-opsin* family. In this regard, perhaps the c-opsin irradiance detectors have been replaced by the cryptochrome (Cry) family of flavoproteins. The cry proteins of vertebrates (cry-2 family) are not photosensitive,[109,110] but in insects the Cry-1 proteins act as short-wavelength-sensitive photopigments and play an important role, along with r-opsins pigments, in regulating several irradiance responses to light, including photoentrainment. Perhaps during evolution invertebrates replaced their short-wavelength-sensitive c-opsins photopigments with Cry-1 photosensory receptors?

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