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Invited Review

Melanopsin and the Non-visual Photochemistry in the Inner Retina of Vertebrates†

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ABSTRACT

Melanopsin (Opn4), a member of the G-protein-coupled receptor family, is a vitamin A-based opsin in the vertebrate retina that has been shown to be involved in the synchronization of circadian rhythms, pupillary light reflexes, melatonin suppression and other light-regulated tasks. In nonmammalian vertebrates there are two Opn4 genes, Opn4m and Opn4x, the mammalian and *Xenopus* orthologs respectively. Opn4x is only expressed in nonmammalian vertebrates including reptiles, fish and birds, while Opn4m is found in a subset of retinal ganglion cells (RGCs), the intrinsically photosensitive (ip) RGCs of the inner retina of both mammals and nonmammalian vertebrates. All opsins described utilize retinaldehyde as chromophore, photoisomerized from 11-*cis*- to all-*trans*-retinal upon light exposure. Visual retinal photoreceptor cones and rods, responsible for day and night vision respectively, recycle retinoids through a process called the visual cycle that involves the retinal pigment epithelium or glial Müller cells. Although Opn4 has been characterized as a bistable photopigment, little is known about the mechanism/s involved in its chromophore regeneration. In this review, we will attempt to shed light on the visual cycle taking place in the inner retina and discuss the state of the art in the nonvisual photochemistry of vertebrates.

INTRODUCTION

The retina is a multilayer tissue that lies at the back of the eye and is composed of neurons (five classes: photoreceptors, horizontal cells [HC], bipolar cells, amacrine cells and ganglion cells) and glial cells (Müller cells) (1) (Fig. 1). Retinal cells are interconnected through a composite set of connections displaying a completely organized layered pattern involving three nuclear layers and two plexiform layers. The nuclear layers are the outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL) (Fig. 1), of which the ONL is closest close to the retinal pigment epithelium (RPE) at the posterior part of the eye. Photoreceptor cells (PRCs) form synapses with bipolar cells (BC) and HC in the outer plexiform layer (OPL), while BC, amacrine cells (AC) and retinal ganglion cells (RGCs) make synaptic contacts in the inner plexiform layer (IPL) (Fig. 1). Upon exposure, light travels through two nuclear cell layers (GCL and INL) and their processes (IPL and OPL) to finally reach the PRCs in most retinal areas. After photon capture, light information is vertically transmitted from PRCs to BC and to RGCs and additionally travels laterally, mediated by HC and AC in the OPL and IPL respectively, to regulate lateral interaction.

VISUAL PRCS

The visual PRCs in all vertebrate species, consisting of rods and cones specialized in nocturnal (black and white) and diurnal (colors) vision, respectively, comprise similar functional regions: the outer segment (OS), a region specialized in light detection situated at the distal retina surface; the inner segment, the cellular nucleus involving the biosynthesis machine located deeper within the retina; and a synaptic nerve ending that makes synapses with the target neighbor cells (Fig. 1). The OS are very specialized light detectors, made up of a number of stacked membrane discs containing opsin pigments that are subject to constant renewal (shedding). OS membranes exhibit a highly active turnover involving a very dynamic process of disc assembly at the bottom of the OS and disc shedding at the top of the segment, causing phagocytosis and digestion by the RPE (2,3). RPE cells are also implicated in a number of crucial retinal tasks such as the regulation of nutrient molecules, ion transport toward the PRCs and visual mechanisms involving vitamin A metabolism, storage, isomerization and recycling; they are also involved in absorbing excess light impacting the eye (4).

Interestingly, the vertebrate retina may contain a number of different photopigments which can be expressed in different layers, presumably involved in very precise image-forming and non-image-forming (NIF) tasks (5). In this respect, the visual pathways encode very complex information in terms of color, movement, spatial features (size, shape), contrasts, backgrounds, among others, while the NIF pathway related to photic synchronization of biological rhythms and other subconscious activities depends preferentially on information related to irradiance and duration of photic stimuli (6–8). Nevertheless, under physiological conditions visual and nonimage photoreceptors coexist in the retina and cooperate in photic detection, light synchronization of

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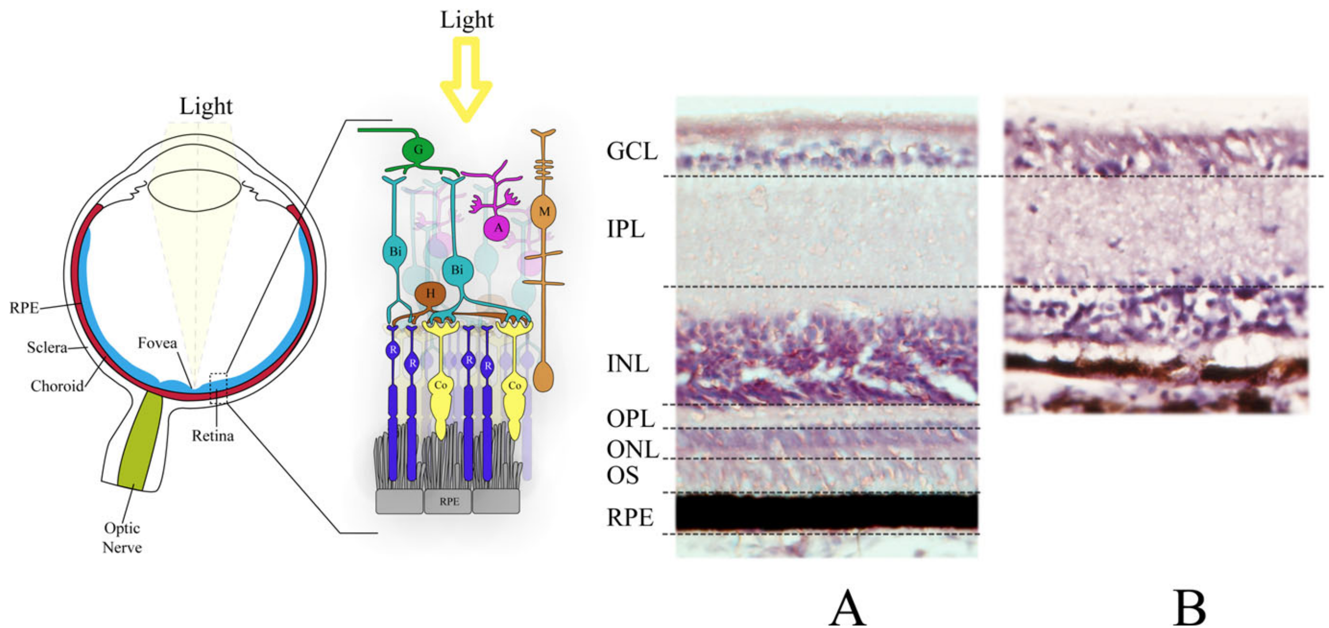

Figure 1. Schematic representation of the vertebrate eye and retina. The retina is a multilayered tissue which lies at the back of the eye and makes contact with the retinal pigment epithelium (RPE). The retina consists of: photoreceptors cells: cones (Co) and Rods (R) with outer segments (OS) and inner segments (IS). Their nuclei form the outer nuclear layer (ONL); this layer forms synapses in the outer plexiform layer (OPL) with the Inner nuclear layer (INL) which includes Horizontal cells (H), Amacrine cells (A), and Bipolar cells (Bi). The processes of the INL form the inner plexiform layer (IPL) which connects with the ganglion cells (G) at the ganglion cells layer (GCL). The axons of GC form the optic nerve. The main glial cells of the retina are the Müller cells (M) which support the neural retinal activity. (A, B) Histological staining of retinas from a wild-type animal (A) or from an animal exhibiting retinal degeneration (B) where there is a complete loss of visual photoreceptor cells.

circadian rhythms and modulation of other NIF functions. These findings are based on an important body of evidence from diverse retinal degeneration models (9–13) (Table 1).

INNER RETINAL PHOTORECEPTORS: THE INTRINSICALLY PHOTOSENSITIVE RGCS

Beyond the classical definition of the "eye" as simply "the organ of sight", recent decades have seen the emergence of another very important role for this organ whereby even under some conditions of blindness, the eye can still act as an ambient light detector, a process similar to the photometer in a photograph camera. A number of NIF functions such as daily entrainment of biological clocks, light-inhibition of activity, acute inhibition of melatonin synthesis in the pineal gland, and pupillary light reflexes (PLRs) are observed in animals that are blind as a consequence of mutations provoking a severe full or near-complete retinal degeneration in classical PRCs (rods and cones) as shown in Fig. 1B (9–11,13,14) (Table 1).

A number of core reports over the last decade have succeeded in identifying the photoreceptors involved in photoentrainment of mammals. It has been known since 1927 that blind rodless mice retain their PLRs (15) and in the 1980s, Ebihara's laboratory noticed that similar rodents suffering blindness were still able to synchronize the rhythm of their locomotor activity to the light–dark cycle (16). These observations strongly suggest that the eyes are essential for the photic regulation of the circadian system (7) and that the rods and cones appear not to be strictly required for the light entrainment of circadian rhythmicity in mammals.

In this connection, pioneer work by Foster's group clearly showed that the genetic ablation of these photoreceptors has no effect on the phase-shifting effects of light on the circadian responses and acute pineal melatonin suppression (5,9–11,17,18). In fact, these latter light responses are driven by other retinal photoreceptors, which is why animals lacking RGCs lose circadian photoresponses and PLR. In recent years, two major findings: (1) the identification of a novel photopigment called melanopsin (Opn4) (19) in mammalian RGCs and (2) the characterization of intrinsically photoresponsive retinal ganglion cells (ipRGCs) (20), have provided a very solid anatomical base to nonvisual phototransduction. Indeed, a number of reports point to a subpopulation of RGCs as being intrinsically photoresponsive and able to transmit light information related to ambient illumination situations to areas in the brain implicated in irradiance detection, as well as being involved in functions regarding adjustment of the circadian clock, PLR and regulation of pineal melatonin release by light (20–22). Strikingly, light during the dark phase inhibits high nocturnal plasma melatonin levels *via* a multi-synaptic circuit originating in the retino hypothalamic tract (23) and this acute melatonin inhibition is sustained even in nonrod, noncone mammalian models of retinal degeneration and in some blind human beings (24–26).

NOVEL PHOTOPIGMENTS FOR ANCIENT FUNCTIONS

One of the most conspicuous characteristics of the vertebrate retina is the presence of a variety of nonvisual opsins in addition to the classical opsins (Opn1 and Opn2), which have been shown to be expressed in the inner retina such as Opn3, Opn4,Opn5, retinal G-protein-coupled receptor (RGR) and peropsin (5,27). However, up to the present no defined functionality in the

Table 1. Gene mutations and animal models involved in the regulation of nonvisual functions.

Types	Characteristics	References
Retinal degeneration mutation (rd/rd)	Retinal degenerations mostly restricted to the retina (primary retinal degeneration) show a rapid loss of rod photoreceptors and a gradual loss of the majority of cone photoreceptors Photoreceptor neuronal degenerations are common and incurable causes of human blindness The circadian responses of rd/rd mice to photic stimuli do not differ significantly from wild-type controls	11,165–167
Retinal degeneration slow (rds)	Failure of rod and cone photoreceptor outer segments to develop, in homozygous mutant mice. Rds exhibits suppression of pineal melatonin levels in response to nocturnal light pulses	11,168,169
Null mutation in the photoreceptor guanylate cyclase (GC1) gene (GUCY1*)	The absence of GC1 in both the rod and cone photoreceptor cells leads to a major reduction in cGMP levels within these cells, which is too low to support phototransduction. In humans, GC1 frameshift and missense mutations have been shown to cause Leber congenital amaurosis type 1 (LCA1), a severe autosomal recessive disease that causes blindness at birth	170–178
$Opn4^{-/-}$,rd-cl	The residual photosensitivity of melanopsin $^{-/-}$ ($Opn4^{-/-}$) mice was abolished when outer retina photopigments were rendered nonfunctional (rd-cl), suggesting that at least one of the three outer retina photopigments must participate in the entrainment process	31,33,78,179,180
$RPE65^{-/-}$	RPE65 is an important component of the retinoid cycle that restores 11-*cis*-retinal after its photoisomerization to its all-*trans* form. Mutation of RPE65 can cause severe blindness from birth or early childhood	181,182
MW-opsin/$TRbeta^{-/-}$	A mice model specifically lacking mid-wavelength (MW)-cones, may play a significant role in light entrainment and phase shifting of circadian clocks mediated by light exposure of short duration and toward the longer wavelengths of the spectrum	183,184
$Gnat1^{-/-}$ (Tralfa) $Gnat1^{(-/-)}$, $Cnga3^{(-/-)}$ $Opn4^{(-/-)}$	$Gnat1^{(-/-)}$;$Cnga3^{(-/-)}$;$Opn4^{(-/-)}$ mice lack critical elements of each of these photoreceptive mechanisms *via* targeted disruption of genes encoding rod alpha transducin (Gnat1), the cone-specific alpha3 cyclic nucleotide-gated channel subunit (Cnga3) and melanopsin (Opn4)	161,185,186
$CNG3^{-/-}$	By deleting the cyclic nucleotide-gated channel CNG3, a mouse lacking any cone-mediated photoresponse was generated. The functional loss of cone function correlates with a progressive degeneration of cone photoreceptors but not of other retinal cell types. CNG3-deficient mice provide an animal model to dissect unequivocally the contribution of rod and cone pathways for normal retinal function	161,187

vertebrate inner retina has been reported for most of them. Melanopsin (Opn4) was first identified from the photosensitive dermal melanophores of *Xenopus laevis* (28) and is found in a broad variety of nonmammalian (Table 2) and mammalian vertebrates including humans. Melanopsin and invertebrate Gq-coupled visual opsins share a close phylogenetic relationship, both groups belonging to the Gq-coupled subfamily of opsins (29,30). Based on amino acid sequence, melanopsin exhibits higher levels of sequence similarity with invertebrate rhodopsin than with vertebrate visual opsins. Experiments carried out with knockout mice clearly showed that melanopsin acts as the photosensitive molecule in ipRGCs and is involved in NIF activities, *i.e.* PLRs, light-entrainment of circadian rhythm activity in mice and melatonin suppression (31–33). Moreover, ectopic expression of

Table 2. Non-mammalian melanopsins.

Organism	Isoform	Tissue expression	Absorption maximum (nm)	Light stability	Accession number	Reference
Amphioxus (*Branchiostoma belcheri*)	–	Joseph cells and photoreceptors of the dorsal ocelli	485	Bistable	AB205400	54
Lamprey (*Lethenteron camtschaticum*)	–	Horizontal cells and other cells of INL	480	Unknown	AB932626	56
Hagfish (*Eptatretus burgeri*)	–	Ganglion cells	Unknown	Unknown	AB932627	56
Zebrafish (*Danio rerio*)	Opn4m -1	Bipolar cells and amacrine cells	–	Bistable	GQ925715	59
	Opn4m -2	Photoreceptor cells and horizontal cells	484	Monostable	GQ925716	
	Opn4m-3	RPE, horizontal cells, bipolar cells, amacrine cells and ganglion cells	–	Bistable	GQ925717	
	Opn4x-1	Horizontal cells, bipolar cells, amacrine cells and ganglion cells	470	Monostable	GQ925718	
	Opn4x-2	Bipolar cells	–	Monostable	GQ925719	
Elephant shark (*Callorhinchus milii*)	Opn4m 1	Eye		Bistable	JQ172797	59
	Opn4m 2	Eye, brain, liver, skin, testis		Bistable	JQ172798	
	Opn4x	Eye, brain, liver, skin, testis		Monostable	JQ172799	
Clawed frog (*Xenopus laevis*)	Opn4m	–	–	Unknown	XP002937616	18,28
	Opn4 x	Melanophore, brain, RPE, inner retina	–	–	AF014797	
Chicken (*Gallus gallus*)	Opn4m	Ganglion cells, Brain and pineal gland	484	Unknown	AY882944	53,65,188
	Opn4 x	Inner retina, horizontal cells, brain, pineal gland.	476	Unknown	AY036061	

Opn4 reestablishes visual function in blind mice (34), whereas transient heterologous Opn4 expression in immortalized cultured cell lines renders nonretinal cells light-responsive (35,36).

In addition to NIF functions, melanopsin in the retina of mice and primates may also modulate visual processing, likely fine tuning visual pathways depending on the time of day (37,38). It was also reported that there is a variety of melanopsin (+) RGCs, conforming different subtypes with differential morphology and projections within the retina and to specific brain areas (39–43). Opn4 is expressed all through the plasma membrane of ipRGCs exhibiting sparsely branched dendritic arbors. Although ipRGCs were shown to be a small minority of mammalian RGCs (<10%) they conform an expansive photoreceptive "net" in the rodent inner retina (22); this bilayered photoreceptive net is anatomically distinct from the rod and cone photoreceptors of the outer retina. The family of mouse ipRGCs has been extended to include the formerly characterized M1 and M2 cells together with a bistratified M3 cell group (22,40,44) and M4 cells which exhibit a large soma size and dendritic field (39). The large, sparsely branched arbors of M1 cells have been shown to monostratify at the outer limit of the OFF sublayer while M2 cells also have large, monostratified dendritic arbors, but ramify within the ON sublayer of in the inner third of the IPL (40,41). Strikingly, some of these cells can support spatial visual perception in mice (39). In this respect, although ipRGCs were shown to regulate NIF tasks encompassing a number of subconscious functions and PLRs, there is increasing evidence that ipRGCs project to all major retinorecipient regions, and that their influence may extend to the regulation of perceptual vision. In light of these developments, ipRGCs can therefore be more appropriately designated not only as circadian/non visual photoreceptors but also as the origin of a particular type of environmentally related visual information that may be useful for multiple visual processes (45). However, it remains to be determined whether Opn4 plays a key role in vision. In this respect, AC and BC transmit inputs to ipRGC neurites, thereby providing an anatomical base by which ipRGCs may modulate visual pathways (46). Moreover, Opn4 appears to be involved in modulation of the cone visual pathway in humans in response to light exposures of long duration (47). It was shown that Opn4 (+) RGCs in primates (closely related to ipRGCs in rodents) combine with visual PRC mechanisms to encode irradiance over the whole visual spectra range (37). These observations strongly suggest that models of vision in the near future will have to consider the contribution of the Opn4 photoreceptive system. Moreover, a new role for Opn4 (+) RGCs has been reported in the mouse retina to modulate visual processing, contrast detection and light adaptation (48,49).

ipRGCs project directly to the SCN and also send projections to the intergeniculate leaflet and olivary pretectal nucleus which are both involved in modulation of circadian rhythms and PLR (21). Furthermore, it was shown that Opn4 (+) RGCs also project to brain areas implicated in sleep control and circadian locomotion: the ventral subparaventricular zone and the ventrolateral preoptic nucleus (50–52).

DIFFERENT MELANOPSINS, DIFFERENT FUNCTIONS?

It is noteworthy that most nonmammalian vertebrates have more than two kinds of melanopsins, classified into two groups within the phylogenetic tree: Opn4x, the *Xenopus* ortholog gene and Opn4m, the mammalian gene (53). Although nonmammalian vertebrates possess both, mammals have only one melanopsin gene. It has been suggested that during the course of evolution, as mammals entered the nocturnal niche, they lost some visual opsins and Opn4x, likely as a consequence of a chromosome re-arrangement (53). The physiological roles played by these two melanopsins in nonmammalian vertebrates require further investigation.

Melanopsin is highly conserved through evolution and was found in amphioxus and sea urchins, demonstrating that deuterostomes have a melanopsin gene(s) (30). When amphioxus melanopsin was expressed in cultured cells and its molecular properties investigated, it was found that this photopigment constitutes a blue-sensitive opsin with a maximum absorption at 485 nm (54). Based on this report, it can be inferred that the pigment exhibits a bistable nature and a light-mediated Gq activation with an efficiency rate closely related to that of Gq-coupled visual pigments of invertebrates (55). In addition, amphioxus Opn4 colocalized with the Gq protein in PRCs (29,30) which show a clear depolarization in response to light, like invertebrate rhabdomeric PRCs.

In 2014, two new melanopsins were described in cyclostomes (56). Lamprey and hagfish express the mRNA that encodes for the melanopsin protein. In accordance with the molecular phylogenetic tree of melanopsins, both the hagfish and the lamprey melanopsins were classified as belonging to the Opn4m group, mammalian orthologs. It was shown that lamprey melanopsin is a functional photopigment with a maximum absorption at 480 nm, which is very close to the sensitivity peak described.

In 2003, Hankins and collaborators (57) found that HC of teleosts are photosensitive independently of rods and cones; this result was confirmed by Chen *et al.* (2009) (58). Zebrafish express five genes for melanopsin (59), of which three correspond to melanopsin m (Opn4m -1, Opn4m -2 and Opn4m -3) and two to melanopsin x (opn4x-1 and opn4x-2). Melanopsin is widely expressed in zebrafish retina and other organs and all of the mentioned melanopsins are functional when expressed in the Neuro 2A cell line. Contrary to previous reports, three of five melanopsins were not functional when the chromophore provided was all-*trans*-retinal, a behavior typical of mono stable opsins like ciliaryopsins. In addition, the cartilaginous fish (Chondrichthyes) expresses three genes for melanopsin (59).

We and other laboratories have shown that the two genes for Opn4 (Opn4x and Opn4m) are expressed in the nonmammalian vertebrate retina at the level of mRNA (60–63) and protein (64,65). Moreover, the expression of Opn4 proteins was reported to vary in the chicken retina during development (65). In fact, Opn4m was shown to be exclusive to the GCL throughout development, whereas Opn4x was confined to the formation of GCL, axonal fibers and optic nerve during early development at embryonic day (E) 8, though by E15 its expression colocalized with Prox1, the main marker for HCs (65). Concomitantly with HC birth and migration between E10 and E15, immunoreactivity associated with Opn4x appeared in the cell somas of Prox1 (+) HC and showed a very robust immunolabeling of the lower OPL. Remarkably, these Opn4x (+) cells bore a morphological resemblance to typical HCs, some of them axonless candelabrum-shaped HCs found to mainly contact cone pedicles (66,67). Based on a number of specification markers, HC and AC in the inner retina could derive from a common ancestral photoreceptor progenitor with ipRGCs and be considered sister

cells (68). In fact more recently, when HCs were purified by a chemical gradient and cultured for several days (69), they were found to express Opn4x mRNA and protein, together with clock genes (*Bmal1* and *Per* 2), the melatonin synthesizing enzyme AA-NAT and components of the nonvisual photocascade (Gq protein) (70).

SPECTRAL SENSITIVITY AND THE IDENTIFICATION OF NON-VISUAL PHOTOPIGMENTS

Although studies involving an action spectrum for photic-induced phase-shifts of locomotor activity in the golden hamster indicated a maximum absorption at 500 nm (blue-green wavelength) (71) coinciding with the rod spectral peak, indicating strong involvement of these cells in the photic entrainment of activity rhythms, a later report using retinal degenerated (rd1) mice revealed a sensitivity peak at 480 nm (72) which did not match any of the classical visual PRCs so far described. Studies involving action spectra for other light responses such as pupillary reflexes (73) and the acute suppression of melatonin (74,75) strongly suggest that these nonvisual light-regulated responses were mediated at least in part by a different group of photoreceptors located in the inner retina and distinct from classical visual photoreceptors. In addition, the vast number of projections conveying photic information from ipRGCs may indicate a variety of functions as observed by melanopsin-null mice studies (31,33,48,49,76–82). A strong body of evidence shows that Opn4-containing ipRGCs play a major role in photoentrainment, regulation of the circadian period in response to constant illumination conditions, PLR, acute photoinhibition of nocturnal activity, and pineal melatonin synthesis suppression. Nevertheless, the loss of these responses was not complete in Opn4-null mice (32,78–80) it was only after the animals were crossed with mice lacking functional visual photoreceptors (rods and cones) that clear phenotypes of null photic responses were observed (31,33). In this connection, it is noteworthy that transgenic mice lacking functional visual PRCs (10,83) or mice homozygous for the rd1 allele (9) were able to regulate daily activity rhythms by light in a similar fashion to normal controls. In Opn4-null mice, the capacity to exhibit phase-shifting effects of light pulses on circadian rhythms was diminished (79,80) but a remnant capacity for phase-shifting effects of light persisted, while melanopsin KO mice lacking rods and cones were totally blind and thus unable to light-regulate the circadian clock (31,33).

ipRGCs are the earliest photoreceptors to appear during the development in both mammals (84) and birds (62,64). In mouse, rods and cones turn photosensitive around postnatal day 10 (P10) while ipRGCs are already photosensitive at birth, showing robust, intrinsically driven Ca^{2+} signaling and spikes in response to light stimulation (85). A similar observation was found in the chicken, since ipRGCs appear at least by E8 (62,64,65,70) whereas visual photoreceptors differentiate later and become functional by E14 (86). In terms of photosensitivity, ipRGCs are less sensitive than rods and cones requiring longer times of light exposure (seconds to minutes) and higher intensities with long lasting responses although the sensitivity to steady light conditions is considerably boosted (20,40,87). These observations allow inferring why Opn4-ko animals exhibit behavioral deficits preferentially in bright illumination conditions (31–33). Remarkably, the ipRGC responses to single photons are very slow with very prolonged integration times (20 times that of rods and over 100 times that of cones). Taking this into consideration, we can infer that a long integration time allows summation of photons arriving several seconds later whereas this feature makes the cell insensitive to rapid variations in light intensity. Overall, ipRGCs are well-matched to detecting ambient light in the surrounding environment over a long time window.

It has furthermore been shown in a pioneer report that photosensitivity mediated by inner retinal cells is also observed in nonmammalian vertebrates such as birds. In this respect, GUCY1* chickens, a model of blindness exhibiting a severe Leber Congenital Amaurosis–like retinopathy and lacking functional visual photoreceptors (63), show significant light responses in both PLR and photic synchronization of daily rhythms of food intake. The sensitivity spectrum for the PLR reveals that a single vitamin A2-based opsin photopigment with maximum absorption at 484 nm (resembling Opn4 photopigments) conducts these responses mediated by light. In fact, there were two Opn4 genes with five different isoforms identified in the normal (53,61,64,65,88) and GUCY1* chicken retinas (63).

Based on a more recent work from our laboratory, we can infer that the photoreception system that synchronizes the daily rhythms of food intake in chickens exhibits a high level of complexity, with retinal and extraretinal photoreceptor components contributing to significantly different degrees (89). These observations revealed that inner retinal photoreceptors (ipRGCs) are sufficient for the daily entrainment of feeding and that classical visual photoreceptor cones and rods, and those located in the pineal gland, play a major role by adjusting the phase and masking the clock under constant illumination conditions in which animals become arrhythmic. Moreover, encephalic photoreceptors operate under higher light intensities, presumably resulting in a basal contribution to entrainment of the circadian system under physiological conditions in non-mammalian vertebrates.

Overall, the reports on mammalian vertebrates and birds as summarized earlier support the idea that functional photoreceptors in the inner retina are responsible for driving diverse NIF activities through a nonvisual circuit involving ipRGCs. This pathway, present and functional in mammalian and nonmammalian vertebrates, would appear to be an evolutionarily conserved circuitry for sensing light intensity and duration to assure the time-
controlled organization of physiology and, ultimately, survival.

MELANOPSIN PHOTOCHEMISTRY

The first report about melanopsin photochemistry (90) utilized purified Opn4 that was modified with a tag and expressed in COS1 cells exhibited a maximum absorbance at 424 nm which differ from that of 480 nm previously published by (20). This difference can be due, at least in part, to the use of particular detergents to solubilize the opsin in the samples purified from COS1 cells and/or to modifications in the original opsin sequence which could cause the typical blue-shift observed.

In 2005, Koyanagi *et al.* (54) expressed and purified the amphioxus Opn4 (54), and by mean of UV spectroscopy they found a maximum absorbance at 485 nm which was closely related to the previous action spectra reported. Another interesting observation of this work was that light stimuli of longer

wavelengths alter the absorption spectra for melanopsin. In addition, the first work showing the photochemistry of the Opn4 chromophore used native melanopsin purified from mammalian ipRGCs (91). It was found a maximum absorbance at 500 nm and the presence of two photoproducts after stimulation with light of 480 nm. By using a truncated melanopsin it was seen that Opn4 exhibits a maximum absorbance at 476 nm and the presence of three photoproducts with different peaks of absorption (446, 467 and 476 nm) (92); one of them displays the ability to bind 7 *cis*-retinal and is induced by light of longer wavelengths. These observations are further supported by work of Walker *et al.* (91) and Koyanagi *et al.* (54) and show that the photochemistry of melanopsin is more complex than that for classical photoreceptors giving the ipRGCs the ability to conduct different functions than those driven by cones and rods.

PHOTOTRANSDUCTION CASCADE

Studies involving electrophysiological and pharmacological approaches with ipRGCs and Opn4- (+) cells in culture strongly suggest that the biochemical cascade of phototransduction involves a phosphoinositide (PIPs) signaling pathway similar to that of the invertebrate rhabdomeric photoreceptors acting through a Gq-protein-coupled opsin (36,62,64,93–95) (Fig. 2).

WHAT WE KNOW FROM THE INVERTEBRATE FIELD

After light stimulation, the photopigment of *Drosophila* rhabdomeric photoreceptors activates a Gq protein. This in turn activates a phospholipase Cβ (PLCβ), which provokes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P_2) in the membrane generating inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) (96,97) (Fig. 2). This process may in some way activate at least two classes of the large TRP ion channel superfamily (96,98,99). In ipRGCs, light presumably triggers signaling through Gq/11-class G-proteins involving the activation of PLC activity, an increase in cytoplasmic Ca^{2+} levels, and ultimately causing membrane depolarization (36,54,61–64, 70,93,94,100–104).

The phototransduction cascade triggered by melanopsin starts with activation of a G-protein of unknown identity. Using molecular approaches, a recent study on melanopsin phototransduction and melanopsin-evoked depolarization has implicated a G-protein of the Gq/11 family, consisting of Gna11, Gna14, Gnaq and Gna15 (105); however, the identity of the Gq/11 gene involved in this process requires further investigation.

We previously reported the presence of the Gq mRNA in primary cultures of chicken RGCs and that treatment with different PLC inhibitors abolished the light-suppressive effect on ^{3}H-melatonin synthesis (62). These findings were the first to demonstrate the biochemical components of the phototransduction cascade operating in vertebrate embryonic RGCs involving a Gq-protein, a distinct G-protein isoform expressed in these cells which may activate a PIP_2-phospholipase C. In fact, the blockade of PIP_2 hydrolysis with specific PLC inhibitors significantly inhibits the suppressive effects of light. Furthermore, the participation of TRP and TRPL channels, the Ca^{2+}-permeable light-sensitive channels, made clear in these experiments since treatments with a Ca^{2+} chelator or a TRP channel blocker were able to reduce the light effect.

Subsequently, in whole-cell recordings of dissociated mouse retinal cell cultures containing ipRGCs, specific blockers of PLC and Gq/11 class G-proteins abolish the light responses (100). Nevertheless, the complete phototransduction mechanism occurring in these cells is not fully understood and different approaches have attempted to elucidate whether PI(4,5)P_2 and/or DAG are associated with the light responses of ipRGCs. Studies from our laboratory and other colleagues strongly support a putative signal transduction cascade involving phosphoinositol signaling. Evidence from different laboratory shows that an invertebrate-like photocascade takes place in these cells involving a G-protein q, the activation of phospholipase C and calcium mobilization (35,36,62,64,93–95). In this connection, melanopsin in amphioxus drives a Gq-mediated signaling cascade after light stimulation, similar to invertebrate Gq-coupled visual opsins.

PHOSPHOINOSITIDE CYCLE ENZYMES

It is noteworthy that the activation of PLC enzyme activity causes the hydrolysis of PI(4,5)P_2 in the cellular membrane, generating two key second messengers: DAG and IP_3. DAG is transformed into PA, and PA into PI(4,5)P_2 through a multistep biosynthetic pathway involving the action of several enzymes

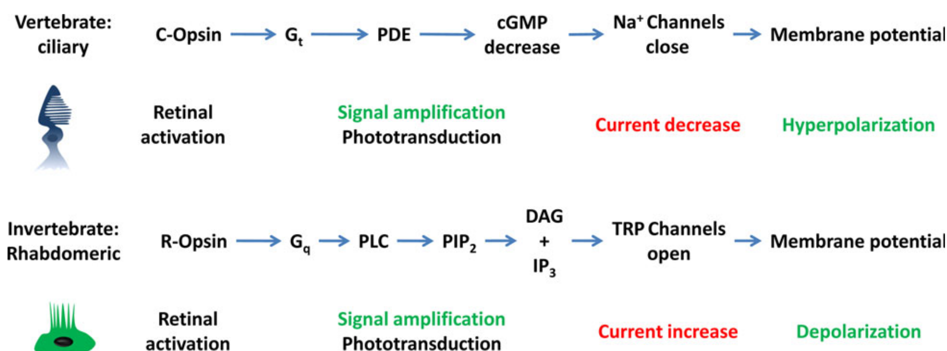

Figure 2. Phototransduction cascades in the animal kingdom. There are two known phototransduction pathways; in vertebrate ciliary photoreceptors (A), the activation of the opsin triggers the G-protein transducin (Gt) which activates a phosphodiesterase (PDE) that hydrolyzes cGMP. Thus, the decrease in cGMP closes sodium channels gated by cGMP. The decrease in the cation current leads to hyperpolarization of the photoreceptor cell. By contrast, in rhabdomeric photoreceptors (B), the pathway involves a Gq-protein. This G-protein activates a phospholipase C, which hydrolyzes phosphoinositides (PIP_2) producing two main second messengers, diacylglycerol (DAG) and inositol-triphosphaste (IP_3). These messengers activate TRP channels and increase the ionic current across the membrane, producing a cell depolarization.

such as DAGK, CDP-DAG synthase (CDS), PI synthase and two kinases, PIK and PIPK. Assessing changes in PI content alone is hampered by the ability of the cells to re-synthesize PI rapidly and therefore also PIP and PI(4,5)P_2 (98,102,106).

Upon light stimulation, a very rapid generation of inositol-phosphates occurred in RGC cultures by PLC activation (64,70). Specifically, there was a significant increase in radiolabeled IP_3 and IP in cultures exposed to bright white light compared with controls kept in the dark. Moreover, when DAGK, PIK and PIPK activities in chicken RGC cultures after brief light pulses ranging from 5 to 90 s were evaluated (64), the activation of DAGK and PIK activities was found to be significantly higher than in controls kept in the dark. The rapid increase in PA and PIP levels, taking only 5–15 s to peak, was transient and the activation disappeared after 10–30 s of stimulation. In addition, PIPK activity was light-stimulated after 5 s and PIP_2 levels showed a sustained increase after 30 s of stimulation. Overall, these observations demonstrate that the generation of PA and PIP takes place very rapidly during the first seconds of light exposure to restore the steady-state PIP levels in the membrane, with a very fast, transient and sequential activation of specific enzymatic PIP kinases. Furthermore, PLC may activate the opening of the light-gated channels by a membrane-associated process (100), with direct interaction between PI(4,5)P_2 and the channels, keeping them in a closed state during the absence of light. It is known that light activates PLC to hydrolyze PI(4,5)P_2 in *Drosophila* rhabdomeric photoreceptors, diminishing its levels and releasing the channels into an open state (99,107). Nevertheless, in ipRGCs it is unclear whether the phosphoinositol signaling cascade components IP_3, PI(4,5)P_2 or DAG play a key role in membrane depolarization. It can be hypothesized that renewal of PI(4,5)P_2 levels by the activation of PIP kinases may control the levels of PI(4,5)P_2 for modulating the channel state in a rapid and transient manner. These observations are in total agreement with other findings reported in *Drosophila melanogaster*, in which interruption of PI(4,5)P_2 renewal prevents the phototransduction events. In *Drosophila* phototransduction, the PI(4,5)P_2 regeneration cycle can be studied by mutations affecting the pathway that reduces or eliminates the generation of intermediates that produce PI(4,5)P_2 recycling. Mutation of DAGK (rdgA) does not abolish PA production since this can also be synthesized through a reaction catalyzed by the phospholipase D (108); however, this mutation induces the accumulation of DAG and affects protein kinase C and TRPC activity. Disruption of PI(4,5)P_2 regeneration by mutations in the genes encoding for CDS, required for catalyzing the subsequent enzymatic conversion of PA to CDP-DAG or for PI transfer protein, disrupts the phototransduction cascade and causes light-dependent retinal degeneration (109,110). Thus, mutations that affect PI(4,5)P_2 regeneration in vertebrates might interrupt photocascade signaling in the ipRGCs. Mechanisms that promote the renewal of PIP_2 levels in the membrane are therefore crucial for maintaining signaling ability. It has further been found that *Drosophila* mutants lacking TRP channels undergo light-dependent retinal degeneration as a consequence of the reduced Ca^{2+} influx (111). Overall, our findings (64,70) together with those from other laboratorys (93,100) strongly suggest that the biochemical phototransduction cascade occurring in chicken embryonic RGC cultures and summarized in Fig. 2, involves a PIP cascade.

VISUAL CYCLE IN THE OUTER RETINA

Retinal photopigments are composed of a protein portion—the opsin—which determines spectral sensitivity, and a chromophore, a vitamin A retinaldehyde, which is bound to a highly conserved lysine of the opsin *via* a Schiff base within a transmembrane domain (Fig. 3). These proteins of around 30−60 kDa were given the name “opsins”; in bacteria they function as cation and anion pumps whereas in higher organisms they are members of the G-protein-coupled receptor family (112–114).

Remarkably, most characterized opsins utilize a vitamin A-derivative as chromophore which shows a clear evolutionary convergence: although fish, amphibians and reptiles may use vitamin A1 (retinal) or A2 (3,4-didehydroretinal), or both, birds and mammals preferentially use vitamin A1- retinoids (115,116). For the same apoprotein and in the absence of any change in the primary amino acid sequence of the opsin, replacement of 11-*cis*-3,4-didehydroretinal by 11-*cis* retinal, along evolution, shifts the absorbance spectrum of the resulting new photopigment toward longer wavelengths.

Subsequent to light stimulation, visual PRCs, cones and rods, depend on the recycling of retinaldehyde in order to regenerate the visual photopigments. In the 1930s, the pioneer work of identifying the biochemical components of the visual system was carried out by George Wald (Nobel Prize, 1967) and his colleagues. Wald’s laboratory discovered that the chromophore giving rhodopsin its red color was a vitamin A-derivative named 11-*cis*-retinal. Moreover, he observed that photons impinging on the molecule of chromophore could induce a change in its configuration from an 11-*cis to* an all-*trans* state, through a series of photointermediates. Based on his studies, Wald was the first to propose a general scheme with the sequence of chemical reactions taking place after light exposure, coined “visual or retinoid cycle”, involving visual perception and chromophore recycling (117). John Dowling, a student of G. Wald at that time, determined that the retinal released from the photoactivated rhodopsin is rapidly taken up by the RPE and esterified during light exposure and dark adaptation. During adaptation to dark, the retinoid flow proceeds in a reverse current from the RPE to OS of photoreceptors for rhodopsin renewal to take place. These observations clearly demonstrate the role of the RPE in visual chromophore recycling (118).

It is now known that rods and cones use differential mechanisms of retinoid recycling after the photoconversion of 11-*cis*-retinal to all-*trans*-retinal (Fig. 4). The rod visual cycle requires both the retina and the RPE whereas the cone visual cycle does not totally depend on the presence of RPE, though it does require the support of Müller cells. Cones appeared earlier than rods during evolution, even along embryological development, since cones differentiate first (119). Depending on the proportion of rods to cones, retinas can be classified as rod-dominated retinas with rod dominance such as in nocturnal rodents and humans, cone-dominated retinas with a high proportion of cones such as in diurnal animals (chickens and ground squirrels) or duplex retinas with a similar rod/cone ratio as seen in fish and frogs. Along evolution, it is very likely that a separate cone visual cycle emerged in duplex retinas to provide cones with an autonomous mechanism for chromophore regeneration, thus avoiding competition with rods for the availability of 11-*cis*-retinal supplied by the RPE (120). Strikingly, cone-rich retinas

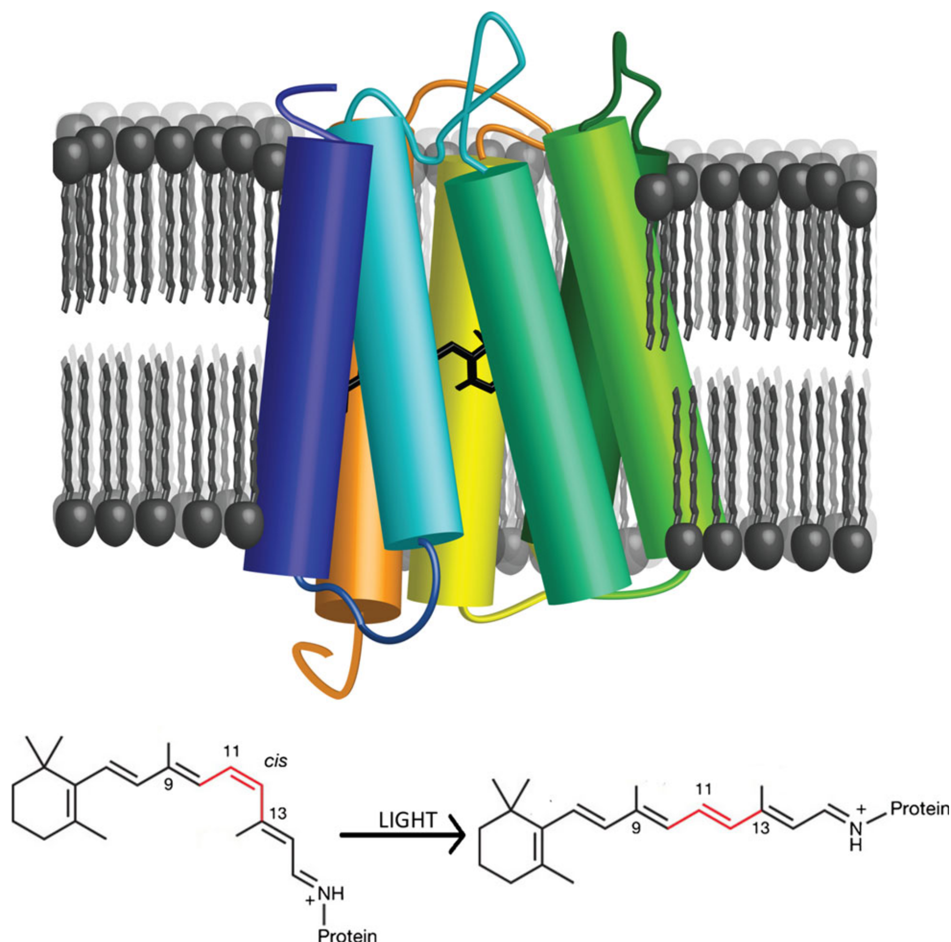

Figure 3. Representation of a typical opsin. A G-protein-coupled receptor with seven transmembrane domains, represented with different cylinders. All opsins described have a vitamin A derivative (retinaldehyde or retinal) bound to a lysine in the hydrophobic pocket. In the dark condition, retinaldehyde is present as 11-*cis* retinal which is photoisomerized to the all-*trans* form. The all-*trans* isomer changes the conformation of the opsin and activates a G-protein which in turn triggers a specific phototransduction cascade.

derived from duplex retinas consolidated this specific visual cycle regardless of the RPE. By contrast, rod-dominated retinas such as that of humans contain a highly cone-enriched fovea centralis and a cone-rich macula. Although the two visual cycles coexist in the vertebrate retina, the degree to which they interact under bright and dim-light exposures is still unknown.

In rods, after photobleaching the all-*trans*-retinal is released from the visual opsin and converted into all-*trans*-retinol by the action of multiple membrane-bound retinal dehydrogenases (RDH) such as RDH8 and RDH12 in the PRCs (121) and subsequent reactions (114,116) illustrated in Fig. 4.

It is to be noted that vitamin A-derivatives are lipid molecules requiring transporters able to bind retinoid pools present in the cytosolic or extracellular compartments and carry them from one site to another. Specific retinoid transporters have been shown to differentially bind 11-*cis* or all-*trans*-retinoids: cellular retinaldehyde-binding protein (CRALBP) and interphotoreceptor-binding protein (IRBP) bind 11-*cis*-retinoids while a cellular retinol-binding protein (CRBP) only binds all-*trans*-retinol; remarkably, CRBP and CRALBP are expressed in both RPE and Müller cells.

By contrast, the cone visual cycle can function regardless of the presence or not of the RPE. This process is observed mainly in cone-dominated retinas such as those of birds and amphibians which recover light sensitivity through cones in isolated retinas (125,126). It is known that a similar cone visual cycle independent of the RPE may occur in the mammalian retina. In contrast to rods, cones operate under very bright light exposure conditions without saturation, strongly indicating that the amount of visual chromophores released to these two cell types must be significantly different. Additionally, other reports have shown that the canonical retinoid cycle is too slow to supply sufficient chromophore to keep cone light-responses under bright light stimulation (120). The distribution of retinoids in cone-dominated retinas is considerably different from that of rod-dominated species, with clearly abundant 11-*cis*-retinyl esters present in the retina as opposed to the all-*trans*-retinyl ester stores in the RPE (127). Cones recover light sensitivity very quickly from either 11-*cis*-retinal or 11-*cis*-retinol, whereas rods do so only from 11-*cis*-retinal. This specific cone visual cycle occurs with the support of Müller cells (Fig. 4). Just as in the rod visual cycle, 11-*cis*-retinal bound to the cone pigment is photoisomerized to all-*trans*-retinal after light absorption and is then reduced to all-*trans*-retinol by means of a *trans*-RDH. All-*trans*-retinol is subsequently transferred from the cone OS to the Müller cells, likely transported by the IRBP across the interphotoreceptor matrix. Once all-*trans*-retinol is in the Müller cells, it is bound to CRBP and isomerized to 11-*cis*-retinol. A candidate enzyme was

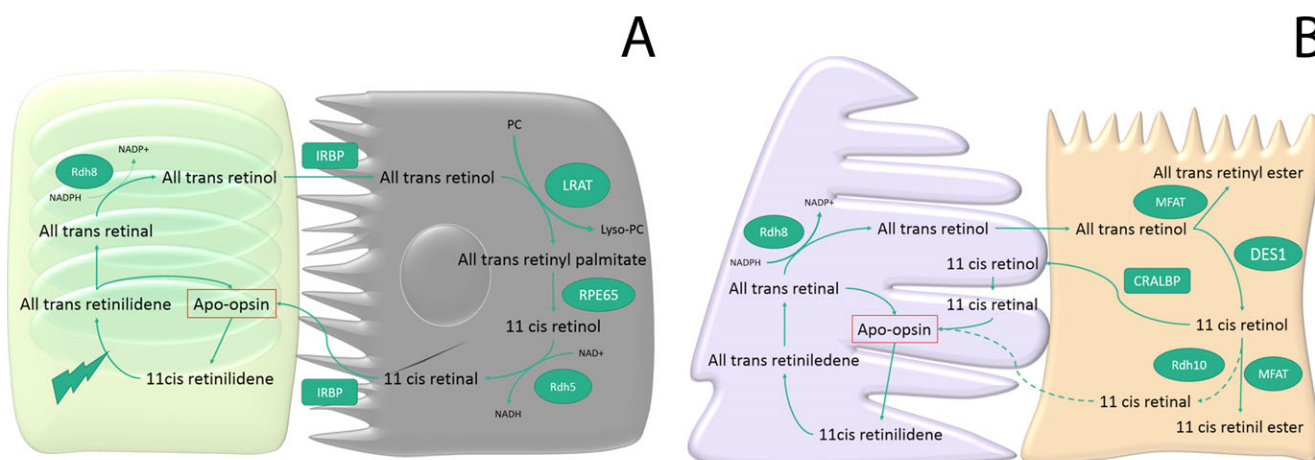

Figure 4. Chromophore recycling in the vertebrate retina. The opsin chromophore can be regenerated by two different visual cycles. The main cycle is through the retinal pigment ephithelium (RPE) (A); the regeneration starts when the 11-*cis* retinal is isomerized into all-*trans*-retinal in the outer segment of the photoreceptor cell (ROS) and then reduced to alcohol by multiple membrane bound retinal dehydrogenases (RDH) such as RDH5, RDH8 or RDH12 in the photoreceptor cell. The all-*trans*-retinol is then transferred into the RPE either by interphotoreceptor retinol-binding protein (IRBP), when released to the cytosol of rod outer segment, or by ABCA4 transporter once delivered to the intradiscal lumen where it is esterified by a lecitin:retinol: acyltransferase (LRAT) to form all-*trans*-retinyl esters. RPE65, also known as Isomerase I, catalyses the hydrolysis of all-*trans*-retinyl esters and uses the released energy to finally isomerize all-*trans*-retinol to 11-*cis*-retinol (122–124). The 11-*cis*-retinol recently isomerized is then chaperoned by cellular retinaldehyde-binding protein (CRALBP); its later oxidation to retinaldehyde is catalyzed by an 11-*cis*-specific retinol dehydrogenase (RDH5). The newly formed 11-*cis*-retinal is again taken up by CRALBP, transferred to the RPE apical membrane and returned to the photoreceptor cells *via* the interphotoreceptor-binding protein (IRBP), where it is eventually rebound to the photopigment to reconstitute a functional rhodopsin (121). In addition, the 11-*cis*-retinol can be esterified in the RPE by the action of LRAT to be stored as 11-*cis*-retinyl esters for later use. A second pathway to regenerate the chromophore (B) occurs between the cone photoreceptors and glial Müller cells. The conversion is very similar to the classical visual cycle, the difference lying in the substrate of the main enzyme of this pathway: whereas in the RPE visual cycle (A) the substrate is a retinyl ester, in the cone visual cycle (B) it is a retinyl alcohol. A retinol isomerase (a putative Isomerase II) named dihydroceramide desaturase-1 (DES-1) driving the alternative retinoid isomerization (all-*trans*-retinol to 11-*cis*-retinol) was recently described. The 11-*cis*-retinol formed can be stored as 11-*cis*-retinyl ester by action of the 11-*cis* ARAT and the 11-*cis*-retinyl ester can be later hydrolyzed to 11-*cis*-retinol. A new 11-*cis*-specific retinyl-ester synthase was found in retinal Müller cells, which is a multifunctional O-acyltransferase (MFAT). See text for further detail.

recently identified for the alternative retinoid isomerization (all-*trans*-retinol to 11-*cis*-retinol) activity: dihydroceramide desaturase-1, a member of the integral membrane hydroxylase/ desaturase enzyme family (128). This retinol isomerase (a putative isomerase II) was found in the retina of mammals and birds. The all-*trans*-retinol may also be esterified to all-*trans*-retinyl ester, and this can be isomerohydrolyzed to 11-*cis*-retinol by Isomerase I. The 11-*cis*-retinol formed in this manner can be stored as 11-*cis*-retinyl ester by action of the 11-*cis* acyl retinol acyl transferase (ARAT) and the 11-*cis*-retinyl ester can be later hydrolyzed to 11-*cis*-retinol for pigment regeneration. Recently a new 11-*cis*-specific retinyl-ester synthase was discovered in retinal Müller cells (129). This ester synthase is a multifunctional O-acyltransferase (MFAT). MFAT may act cooperatively with Isomerase II in glial Müller cells to conduct production of 11-*cis*-retinoids. Thus, the energy of retinoid isomerization comes from the hydrolysis of the thioester of an activated fatty acid through MFAT activity. Alternatively, 11-*cis*-retinol can be oxidized to 11-*cis*-retinal by a *cis*-RDH located either in the Müller cells or in the cone OS. When produced in Müller cells, 11-*cis*-retinal is bound to CRALBP for further protection and transferred from these glial cells to cones. It is then delivered to the interphotoreceptor matrix and transferred to the PRCs where it combines with the cone opsin to form the visual photopigment, thus completing the visual retinoid cycle. 11-*cis*-retinol provided to the cone photoreceptor has to be oxidized to retinal by a *cis*-RDH for pigment regeneration.

After light exposure of dark-adapted eyes, levels of 11-*cis*-retinyl esters are increased in the retina whereas those for all-*trans*-retinyl esters are accumulated in the RPE. Full recovery in darkness occurs within as little as 5 min in cone-dominated retinas (birds, squirrels, etc.) whereas it takes 100 min in albino rats.

Overall, three distinct enzymes are present in the photoreceptor membranes of cone-dominated retinas, all of which differ from those in rods: (1) one cone-specific ARAT which transfers palmitate in the presence of CRALBP and that is not pharmacologically inhibited by compounds that usually inhibit LRAT activity; (2) one cone-specific RDH that uses NADPH as cofactor instead of the NAD utilized by rods and that converts 11-*cis*-retinol to 11-*cis*-retinal; and (3) the recently identified cone-specific Isomerase II, which catalyzes the conversion of all-*trans*-retinol into 11-*cis*-retinol.

To add further complexity, the RGR is expressed in the RPE and Müller cells (130) and may interact with *cis*-RDH (RDH5) to isomerize all-*trans*-retinal to 11-*cis*-retinal under light conditions (121); this offers an alternative route for the regeneration of *cis*-retinoids in the visual cycle. Other roles have been proposed for this photoisomerase such as a potential negative regulation of all-*trans*-retinyl ester hydrolase and LRAT in light (131). For further details of the visual cycle in the outer retina, please see specialized reviews (125,132–134).

IS THERE A VISUAL CYCLE IN THE INNER RETINA?

The visual pigments coupled to Gq proteins are located in the microvilli of the rhabdomeric PRCs, which are morphologically

and biochemically different from the ciliary photoreceptors, rod and cone cells of vertebrates. These photopigments, in general, convert to thermally stable photoproducts with absorption maxima in the visible region of spectrum by light absorption, and exhibit a typical bistable nature (55,126,135,136). The photoproducts activate the Gq-type G-protein, which in turn stimulates phospholipase C, likely a PLCß isoform, resulting in the depolarization of the PRC membrane (137,138).

Work from several laboratories has suggested that mammalian melanopsin is a bistable photopigment with only one silent state (35,94,139); other reports have not detected any photoequilibration of melanopsin among stable states (36,87,140). These diverging findings can be explained at least in part by the recently described tristability feature of melanopsin (141). Melanopsin, unlike other opsins, possesses two silent states and one signaling state, so that when light distributes melanopsin in the ipRGCs across three states, photopigment tristability may produce the signal for physiological functions and behavior. Since the first description of melanopsin as a nonvisual photopigment, it was speculated that it is a bistable opsin (28). At present, several works have strongly suggested its bistable feature as observed in experiments involving its heterologous expression in cultured cells (35,36,94). In a series of studies knocking out specific components of the classical visual cycle, it was further shown that ipRGCs are independent of them as well as of the RPE contribution (142). Moreover, ipRGCs were shown to be highly resistant to photo-bleaching after bright and extended light stimulation (140,143) while recordings of light-evoked responses in the SCN, revealed that light of longer wavelengths further potentiates ipRGC responsiveness (144), suggesting that Opn4 is a bistable photopigment. Although these findings were not totally reproduced by another laboratory (145), in 2015, Emanuel and colleagues (141) were able to explain this behavior based on the mentioned specific feature of tristability. This triple state for Opn4 was formerly proposed by Matsuyama (92) by experiments with the purified photopigment. This unique Opn4 characteristic allows ipRGCs to integrate light stimuli in both time and wavelength (141) resulting in a more prolonged and sustained response to light.

It was initially proposed that ipRGC function was dependent upon the support of the RPE-process of the chromophore regeneration cycle (72,73). These observations were further supported by studies involving gene Knockouts for the two key enzymes in the RPE visual cycle, *Rpe65* and *Lrat*, whose KO animals show a 100-fold less sensitive PLR compared with wild-type controls. On the other hand, light sensitivity in ipRGCs, recorded in isolated retinas, was unchanged in these knockout mice (74). Moreover, the acute poisoning of the RPE visual cycle with all-*trans*-retinylamine to completely remove all traces of retinal, does not significantly affect PLR in mice with neither cones nor rods but only ipRGCs as functional photoreceptors (74). Taking reported observations into account, one main difference between ciliary and invertebrate (rhabdomeric) opsins, at the biochemical level, is the process of chromophore recycling (Fig. 5). In fact after light exposure, all-*trans*-retinal in rhabdomeric photopigments remains bound to the opsin, and for its recycling, the photopigment absorbs a second photon at another specific wavelength and re-isomerizes all-*trans*-retinal to 11-*cis*-retinal. Under these circumstances the photopigments are "bistable", displaying an activated form, or meta-state, a thermally stable-opsin form that lasts from seconds to minutes (75,76). Nevertheless, as has been described in the Drosophila by Montel, bistable pigments can still use an alternative mechanism of chromophore recycling that relies upon the activity of other enzymes (isomerases and RDH) (146) (Fig 5). Such alternative processes may take place within the PRC itself or could occur in a second cell-type closed to photoreceptive cells (146,147) such as the glial cells of the retina, which were shown to participate in the cone visual cycle (Fig. 4). The case with the photopigment melanopsin in ipRGCs could be similar, with the bistable mechanism at least partially supporting the resistance of ipRGCs subject to depleting systemic vitamin A conditions (148).

However, an alternative second cycle of chromophore regeneration cannot be discarded. It is worth noting that in heterologous cellular expression systems with the exogenous administration of retinoids, photic responses are dependent upon the administration of selected retinoids, mainly 11-*cis*-and 9-*cis*-retinal, which elicit the most significant photic responses. In this regard, the addition of all-*trans*-retinal evokes weaker responses to those obtained with *cis*-retinal; however, this diminished response increases with longer exposures to high illumination levels and at longer wavelengths (>540 nm), or illumination with the full visible spectra or the coexpression of the protein arrestin (35,94). Moreover, full ipRGC activity in response to very brief light pulses of only 1 min. at 480 nm is recovered after 5 min. of dark adaptation (149). Nevertheless, since the reestablishment of activity in the dark is not totally consistent with a purely bistable photopigment or with the opsin's capacity for tristability as described above (141), the results suggest other possibilities such as a second alternative cycle of support to regenerate the chromophore as seen in the *Drosophila*.

In addition to the rhabdomeric studies of invertebrate (146,147), recovery of activity in the dark needs a process in which all-*trans*-retinal is converted back to 11-*cis*-retinal as suggested in (55) using purified, recombinant amphioxus melanopsin. Furthermore, Müller glial cells exhibit closer contacts with ipRGCs and could act as the supporting second cell-type needed to carry out an alternative visual cycle in the inner retina. In this respect, based on their proximity, the presence of DES1 in the GCL (128) and the recovery after dark adaptation, we hypothesize that ipRGCs may use the Müller glial photocycle as cones do.

CIRCADIAN CLOCKS AND PHOTORECEPTORS

The vertebrate retina contains an autonomous circadian clock which temporally controls a whole variety of biochemical, genetic and physiological parameters. Retinal timekeepers are localized in cells of different retinal cell layers, and allow the organism to predict, anticipate and adapt to the billion-fold variations in the light intensity and duration taking place along a light–dark cycle (day/night alternance), thereby optimizing visual function for each photic situation. For further detail, see references (27,150).

In addition, a number of reports allow us to suggest the idea of convergence of circadian clocks and photoreceptors in the same retinal cell population; as previously shown, visual photoreceptors in the retina are photoresponsive, contain circadian clocks expressing clock genes and synthesize melatonin rhythmically just as well as some RGCs (27,62,64,70,150–153). These joint features may confer retinal cells with the capacity to tempo-

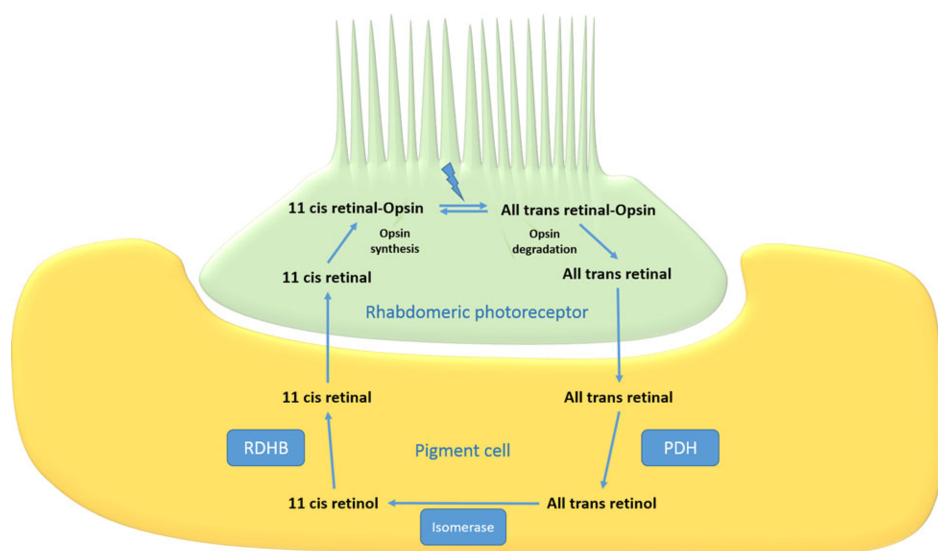

Figure 5. Chromophore recycling in the invertebrate photoreceptor. Rhabdomeric opsins are bistable photopigments with the ability to photoisomerize all-*trans*-retinal themselves upon the capture of a photon with a second wavelength. The structure of rhabdomeric opsins stabilizes the "meta" state of the opsin and facilitates the reisomerization to 11-*cis*-retinal by light. In pigmented cell of *Drosophila* there is also an alternative visual cycle dependent on enzyme activity which involves an isomerase and different deshydrogenases (PDH, RDHB) to finally provide the rhabdomeric photoreceptor with the 11-*cis*-retinal required for vision. A similar mechanism might take place in the inner retina of vertebrates to support chromophore regeneration in ipRGCs. See text for further detail.

rally fine-tune and adjust to ambient fluctuations taking place through the day-night cycles, adapting and optimizing cellular oscillators for phases of high/low metabolic rate activities, photic responses and sensitivities. As a clear example of this, it was recently shown that GUCY1 birds expressing Opn4-photoreceptive cells (63,65) display a circadian rhythmicity in the PLRs with higher sensitivity to white or blue light during midday (154). Overall, the expression of novel opsins such as Opn4 as well as that for different circadian markers such as the clock genes *Bmal1*, *Per* and *Cry* and the key melatonin synthesizing enzyme, AA-NAT, appears very early in development in RGCs and HCs, even before any sign of formal vision takes place (70,84). Inner retinal cells may therefore gain both the capacity to sense environmental light conditions and to measure time very early in development, which may support the synchronization of retinal physiology. In this context, melatonin may act as the nocturnal marker of physiology in the outer retina and together with dopamine as a daytime signal in the inner retina, modulate the function of local circuitries (27,155).

As mentioned above, a typical feature of mammals is that synchronization of the master circadian clock to day/night cycles is mediated exclusively through retinal photoreceptors, mostly ipRGCs, with the contribution of visual PRCs under physiological conditions. As also mentioned, the mammalian retina is a self-sustained circadian oscillator (150). Although the molecular circadian clock in the retina can be entrained by lighting cycles *in vitro*, a recent paper showed that rods, cones and Opn4 are not required for this entrainment (156). *In vivo*, retinas of Opn4-ko/rd1/rd1 mice entrain to light–dark cycles regardless of the phase of the master circadian pacemakers of the suprachiasmatic nuclei or the locomotor behavior of the animal. These observations strongly suggest that the retina uses a separate mechanism for local synchronization of its circadian pacemaker to that for entrainment of the whole organism. Further research is required to elaborate on this point. Keeping this in mind, other opsins such as encephalopsin/panopsin (*Opn3*) and neuropsin (*Opn5*) have been reported to be expressed in the vertebrate retina in addition to visual opsins and Opn4. Opn5 transcripts were seen to be expressed in mice testis, brain and eye, as well as in human retina and brain (157) and in rat eye (158). At the level of protein, OPN5 is specifically expressed within the mammalian inner retina, in cells of the INL and GCL and in processes of IPL (158). Recent publications have demonstrated that this opsin is a functional UV-sensitive Gi-coupled photopigment with maximum absorption at ~420 nm and a typical bistable nature. In addition, it is probably involved in the photoreception necessary for seasonal reproduction in birds (159,160). In the chicken, it was found to be expressed in the pineal organ and within the inner retina, in INL and GCL neurons (159) reflecting a retinal distribution closely related to that reported in the mammalian retina (158). Based on all these findings we may infer that OPN5 expression in the mammalian retina is responsible for the detection of some residual light such as observed in nonrod, noncone, nonmelanopsin animals (Gnat1$^{-/-}$ Cnga3$^{-/-}$ Opn4$^{-/-}$ mice) in which certain NIF tasks (a residual PLR) are still detected (31,161), or is responsible for the *in vitro* entraining of the retina to light–dark cycles.

CONCLUDING REMARKS

Since the time George Wald began investigating the biochemical processes taking place in cones and rods to regenerate the photopigment chromophore, which he coined "visual cycle", more than 80 years have passed. In the meantime numerous laboratories have carried out very extensive and detailed work in their efforts to elucidate the complexity of this cycle that involves the RPE and glial Müller cells (125,132–134,162). Even when nonvisual photoperception was shown to occur by Keeler (15) in the 1930's and later on by Ebihara's group (16), the two major discoveries to explain this phenomenon were reached only 15 years ago (19,20). Since then, we have learned the role of Opn4-expressing ipRGCs projecting to visual and nonvisual brain areas, the different NIF tasks encompassing a number of

subconscious and PLRs driven by these cells, and their potential involvement in vision and in time regulation of visual and nonvisual functions. Although Opn4 has been characterized as a bistable photopigment, little is known about the mechanism/s involved in its chromophore regeneration. Based on similarity with rhabdomeric photoreceptors in term of expression of specification markers, the biochemical nature of phosphoinositol-photocascade and opsin homology, among other common characteristics, we can infer that a novel visual cycle operates in Opn4-expressing ipRGCs which cooperates to regenerate the chromophore *via* a supplementary alternative process as described in *Drosophila* (98,147,163,164) possibly involving Müller cell end-feet that likely express the required enzymes. Future studies will address new insights into the nonvisual photochemistry occurring in the inner retina of vertebrates.

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REFERENCES

1. Tessier-Lavigne, M. (1991) Phototransduction and information processing in the Retina. In *Principles of Neural Science*, 3rd edn (Edited by E. R. Kandel, J. H. Schwartz and T. M. Jessell), pp. 401–418. Appleton & Lange, Norwalk.
2. Strauss, O. (2005) The retinal pigment epithelium in visual function. *Physiol. Rev.* **85**, 845–881.
3. Bobu, C. and D. Hicks (2009) Regulation of retinal photoreceptor phagocytosis in a diurnal mammal by circadian clocks and ambient lighting. *Invest. Ophthalmol. Vis. Sci.* **50**, 3495–3502.
4. Bok, D. (1985) Retinal photoreceptor-pigment epithelium interactions. Friedenwald lecture. *Invest. Ophthalmol. Vis. Sci.* **26**, 1659–1694.
5. Peirson, S. N., S. Halford and R. G. Foster (2009) The evolution of irradiance detection: Melanopsin and the non-visual opsins. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **364**, 2849–2865.
6. Devlin, P. F. and S. A. Kay (2001) Circadian photoperception. *Annu. Rev. Physiol.* **63**, 677–694.
7. Nelson, D. E. and J. S. Takahashi (1991) Sensitivity and integration in a visual pathway for circadian entrainment in the hamster (*Mesocricetus auratus*). *J. Physiol.* **439**, 115–145.
8. Roenneberg, T. and R. G. Foster (1997) Twilight times: Light and the circadian system. *Photochem. Photobiol.* **66**, 549–561.
9. Foster, R. G., I. Provencio, D. Hudson, S. Fiske, W. De Grip and M. Menaker (1991) Circadian photoreception in the retinally degenerate mouse (rd/rd). *J. Comp. Physiol. A.* **169**, 39–50.
10. Freedman, M. S., R. J. Lucas, B. Soni, M. von Schantz, M. Munoz, Z. David-Gray and R. Foster (1999) Regulation of mammalian circadian behavior by non-rod, non-cone, ocular photoreceptors. *Science* **284**, 502–504.
11. Lucas, R. J., M. S. Freedman, M. Munoz, J. M. Garcia-Fernandez and R. G. Foster (1999) Regulation of the mammalian pineal by non-rod, non-cone, ocular photoreceptors. *Science* **284**, 505–507.
12. Foster, R. and J. Bellingham (2002) Opsins and melanopsins. *Curr. Biol.* **12**, R543–R544.
13. Van Gelder, R. N., R. Wee, J. A. Lee and D. C. Tu (2003) Reduced pupillary light responses in mice lacking cryptochromes. *Science* **299**, 222.
14. Foster, R. G. and M. W. Hankins (2002) Non-rod, non-cone photoreception in the vertebrates. *Prog. Retin. Eye Res.* **21**, 507–527.
15. Keeler, C. E. (1927) Iris movements in blind mices. *Am. J. Physiol.* **81**, 107–112.
16. Ebihara, S. and K. Tsuji (1980) Entrainment of the circadian activity rhythm to the light cycle: Effective light intensity for a Zeitgeber in the retinal degenerate C3H mouse and the normal C57BL mouse. *Physiol. Behav.* **24**, 523–527.
17. Hankins, M. W., S. N. Peirson and R. G. Foster (2008) Melanopsin: An exciting photopigment. *Trends Neurosci.* **31**, 27–36.
18. Provencio, I., H. M. Cooper and R. G. Foster (1998) Retinal projections in mice with inherited retinal degeneration: Implications for circadian photoentrainment. *J. Comp. Neurol.* **395**, 417–439.
19. Provencio, I., I. R. Rodriguez, G. Jiang, W. P. Hayes, E. F. Moreira and M. D. Rollag (2000) A novel human opsin in the inner retina. *J. Neurosci.* **20**, 600–605.
20. Berson, D. M., F. A. Dunn and M. Takao (2002) Phototransduction by retinal ganglion cells that set the circadian clock. *Science* **295**, 1070–1073.
21. Hattar, S., H. W. Liao, M. Takao, D. M. Berson and K. W. Yau (2002) Melanopsin-containing retinal ganglion cells: Architecture, projections, and intrinsic photosensitivity. *Science* **295**, 1065–1070.
22. Provencio, I., M. D. Rollag and A. M. Castrucci (2002) Photoreceptive net in the mammalian retina. This mesh of cells may explain how some blind mice can still tell day from night. *Nature* **415**, 493.
23. Moore, R. Y. (1996) Neural control of the pineal gland. *Behav. Brain Res.* **73**, 125–130.
24. Hannibal, J., P. Hindersson, J. Ostergaard, B. Georg, S. Heegaard, P. J. Larsen and J. Fahrenkrug (2004) Melanopsin is expressed in PACAP-containing retinal ganglion cells of the human retinohypothalamic tract. *Invest. Ophthalmol. Vis. Sci.* **45**, 4202–4209.
25. Wee, R. and R. N. Van Gelder (2004) Sleep disturbances in young subjects with visual dysfunction. *Ophthalmology* **111**, 297–302; discussion 302–293.
26. Zeitzer, J. M., D. J. Dijk, R. Kronauer, E. Brown and C. Czeisler (2000) Sensitivity of the human circadian pacemaker to nocturnal light: Melatonin phase resetting and suppression. *J. Physiol.* **526** (Pt 3), 695–702.
27. Guido, M. E., E. Garbarino-Pico, M. A. Contin, D. J. Valdez, P. S. Nieto, D. M. Verra, V. A. Acosta-Rodriguez, N. de Zavalia and R. E. Rosenstein (2010) Inner retinal circadian clocks and non-visual photoreceptors: Novel players in the circadian system. *Prog. Neurobiol.* **92**, 484–504.
28. Provencio, I., G. Jiang, W. J. De Grip, W. P. Hayes and M. D. Rollag (1998) Melanopsin: An opsin in melanophores, brain, and eye. *Proc. Natl Acad. Sci. USA* **95**, 340–345.
29. Koyanagi, M. and A. Terakita (2008) Gq-coupled rhodopsin subfamily composed of invertebrate visual pigment and melanopsin. *Photochem. Photobiol.* **84**, 1024–1030.
30. Raible, F., K. Tessmar-Raible, E. Arboleda, T. Kaller, P. Bork, D. Arendt and M. I. Arnone (2006) Opsins and clusters of sensory G-protein-coupled receptors in the sea urchin genome. *Dev. Biol.* **300**, 461–475.
31. Hattar, S., R. J. Lucas, N. Mrosovsky, S. Thompson, R. H. Douglas, M. W. Hankins, J. Lem, M. Biel, F. Hofmann, R. G. Foster and K. W. Yau (2003) Melanopsin and rod-cone photoreceptive systems account for all major accessory visual functions in mice. *Nature* **424**, 76–81.
32. Lucas, R. J., S. Hattar, M. Takao, D. M. Berson, R. G. Foster and K. W. Yau (2003) Diminished pupillary light reflex at high irradiances in melanopsin-knockout mice. *Science* **299**, 245–247.
33. Panda, S., I. Provencio, D. C. Tu, S. S. Pires, M. D. Rollag, A. M. Castrucci, M. T. Pletcher, T. K. Sato, T. Wiltshire, M. Andahazy, S. A. Kay, R. N. Van Gelder and J. B. Hogenesch (2003) Melanopsin is required for non-image-forming photic responses in blind mice. *Science* **301**, 525–527.
34. Lin, B., A. Koizumi, N. Tanaka, S. Panda and R. H. Masland (2008) Restoration of visual function in retinal degeneration mice by ectopic expression of melanopsin. *Proc. Natl Acad. Sci. USA* **105**, 16009–16014.
35. Melyan, Z., E. E. Tarttelin, J. Bellingham, R. J. Lucas and M. W. Hankins (2005) Addition of human melanopsin renders mammalian cells photoresponsive. *Nature* **433**, 741–745.
36. Qiu, X., T. Kumbalasiri, S. M. Carlson, K. Y. Wong, V. Krishna, I. Provencio and D. M. Berson (2005) Induction of photosensitivity by heterologous expression of melanopsin. *Nature* **433**, 745–749.

37. Dacey, D. M., H. W. Liao, B. B. Peterson, F. R. Robinson, V. C. Smith, J. Pokorny, K. W. Yau and P. D. Gamlin (2005) Melanopsin-expressing ganglion cells in primate retina signal colour and irradiance and project to the LGN. *Nature* **433**, 749–754.
38. Barnard, A. R., S. Hattar, M. W. Hankins and R. J. Lucas (2006) Melanopsin regulates visual processing in the mouse retina. *Curr. Biol.* **16**, 389–395.
39. Ecker, J. L., O. N. Dumitrescu, K. Y. Wong, N. M. Alam, S. K. Chen, T. LeGates, J. M. Renna, G. T. Prusky, D. M. Berson and S. Hattar (2010) Melanopsin-expressing retinal ganglion-cell photoreceptors: Cellular diversity and role in pattern vision. *Neuron* **67**, 49–60.
40. Berson, D. M., A. M. Castrucci and I. Provencio (2010) Morphology and mosaics of melanopsin-expressing retinal ganglion cell types in mice. *J. Comp. Neurol.* **518**, 2405–2422.
41. Schmidt, T. M., S. K. Chen and S. Hattar (2011) Intrinsically photosensitive retinal ganglion cells: Many subtypes, diverse functions. *Trends Neurosci.* **34**, 572–580.
42. Brown, T. M., C. Gias, M. Hatori, S. R. Keding, M. Semo, P. J. Coffey, J. Gigg, H. D. Piggins, S. Panda and R. J. Lucas (2010) Melanopsin contributions to irradiance coding in the thalamo-cortical visual system. *PLoS Biol.* **8**, e1000558.
43. Badea, T. C., H. Cahill, J. Ecker, S. Hattar and J. Nathans (2009) Distinct roles of transcription factors brn3a and brn3b in controlling the development, morphology, and function of retinal ganglion cells. *Neuron* **61**, 852–864.
44. Schmidt, T. M. and P. Kofuji (2009) Functional and morphological differences among intrinsically photosensitive retinal ganglion cells. *J. Neurosci.* **29**, 476–482.
45. Lucas, R. J. (2013) Mammalian inner retinal photoreception. *Curr. Biol.* **23**, R125–R133.
46. Belenky, M. A., C. A. Smeraski, I. Provencio, P. J. Sollars and G. E. Pickard (2003) Melanopsin retinal ganglion cells receive bipolar and amacrine cell synapses. *J. Comp. Neurol.* **460**, 380–393.
47. Hankins, M. W. and R. J. Lucas (2002) The primary visual pathway in humans is regulated according to long-term light exposure through the action of a nonclassical photopigment. *Curr. Biol.* **12**, 191–198.
48. Schmidt, T. M., N. M. Alam, S. Chen, P. Kofuji, W. Li, G. T. Prusky and S. Hattar (2014) A role for melanopsin in alpha retinal ganglion cells and contrast detection. *Neuron* **82**, 781–788.
49. Allen, A. E., R. Storchi, F. P. Martial, R. S. Petersen, M. A. Montemurro, T. M. Brown and R. J. Lucas (2014) Melanopsin-driven light adaptation in mouse vision. *Curr. Biol.* **24**, 2481–2490.
50. Gooley, J. J., J. Lu, D. Fischer and C. B. Saper (2003) A broad role for melanopsin in nonvisual photoreception. *J. Neurosci.* **23**, 7093–7106.
51. Morin, L. P., J. H. Blanchard and I. Provencio (2003) Retinal ganglion cell projections to the hamster suprachiasmatic nucleus, intergeniculate leaflet, and visual midbrain: Bifurcation and melanopsin immunoreactivity. *J. Comp. Neurol.* **465**, 401–416.
52. Hattar, S., M. Kumar, A. Park, P. Tong, J. Tung, K. W. Yau and D. M. Berson (2006) Central projections of melanopsin-expressing retinal ganglion cells in the mouse. *J. Comp. Neurol.* **497**, 326–349.
53. Bellingham, J., S. S. Chaurasia, Z. Melyan, C. Liu, M. A. Cameron, E. E. Tarttelin, P. M. Iuvone, M. W. Hankins, G. Tosini and R. J. Lucas (2006) Evolution of melanopsin photoreceptors: Discovery and characterization of a new melanopsin in nonmammalian vertebrates. *PLoS Biol.* **4**, e254.
54. Koyanagi, M., K. Kubokawa, H. Tsukamoto, Y. Shichida and A. Terakita (2005) Cephalochordate melanopsin: Evolutionary linkage between invertebrate visual cells and vertebrate photosensitive retinal ganglion cells. *Curr. Biol.* **15**, 1065–1069.
55. Terakita, A., H. Tsukamoto, M. Koyanagi, M. Sugahara, T. Yamashita and Y. Shichida (2008) Expression and comparative characterization of Gq-coupled invertebrate visual pigments and melanopsin. *J. Neurochem.* **105**, 883–890.
56. Sun, L., E. Kawano-Yamashita, T. Nagata, H. Tsukamoto, Y. Furutani, M. Koyanagi and A. Terakita (2014) Distribution of mammalian-like melanopsin in cyclostome retinas exhibiting a different extent of visual functions. *PLoS One* **9**, e108209.
57. Jenkins, A., M. Munoz, E. E. Tarttelin, J. Bellingham, R. G. Foster and M. W. Hankins (2003) VA opsin, melanopsin, and an inherent light response within retinal interneurons. *Curr. Biol.* **13**, 1269–1278.
58. Cheng, N., T. Tsunenari and K. W. Yau (2009) Intrinsic light response of retinal horizontal cells of teleosts. *Nature* **460**, 899–903.
59. Davies, W. I., L. Zheng, S. Hughes, T. K. Tamai, M. Turton, S. Halford, R. G. Foster, D. Whitmore and M. W. Hankins (2011) Functional diversity of melanopsins and their global expression in the teleost retina. *Cell. Mol. Life Sci.* **68**, 4115–4132.
60. Bailey, M. J. and V. M. Cassone (2005) Melanopsin expression in the chick retina and pineal gland. *Brain Res. Mol. Brain Res.* **134**, 345–348.
61. Tomonari, S., A. Takagi, S. Noji and H. Ohuchi (2007) Expression pattern of the melanopsin-like (cOpn4m) and VA opsin-like genes in the developing chicken retina and neural tissues. *Gene Expr. Patterns* **7**, 746–753.
62. Contin, M. A., D. M. Verra and M. E. Guido (2006) An invertebrate-like phototransduction cascade mediates light detection in the chicken retinal ganglion cells. *FASEB J.* **20**, 2648–2650.
63. Valdez, D. J., P. S. Nieto, E. Garbarino-Pico, L. B. Avalle, H. Diaz-Fajreldines, C. Schurrer, K. M. Cheng and M. E. Guido (2009) A nonmammalian vertebrate model of blindness reveals functional photoreceptors in the inner retina. *FASEB J.* **23**, 1186–1195.
64. Contín, M. A., D. M. Verra, G. Salvador, M. Ilincheta, N. M. Giusto and M. E. Guido (2010) Light activation of the phosphoinositide cycle in intrinsically photosensitive chicken retinal ganglion cells. *Invest. Ophthalmol. Vis. Sci.* **51**, 5491–5498.
65. Verra, D. M., M. A. Contin, D. Hicks and M. E. Guido (2011) Early onset and differential temporospatial expression of melanopsin isoforms in the developing chicken retina. *Invest. Ophthalmol. Vis. Sci.* **52**, 5111–5120.
66. Araki, M. and H. Kimura (1991) GABA-like immunoreactivity in the developing chick retina: Differentiation of GABAergic horizontal cell and its possible contacts with photoreceptors. *J. Neurocytol.* **20**, 345–355.
67. Poche, R. A. and B. E. Reese (2009) Retinal horizontal cells: Challenging paradigms of neural development and cancer biology. *Development* **136**, 2141–2151.
68. Arendt, D. (2003) Evolution of eyes and photoreceptor cell types. *Int. J. Dev. Biol.* **47**, 563–571.
69. Morera, L. P., N. M. Diaz and M. E. Guido (2012) A novel method to prepare highly enriched primary cultures of chicken retinal horizontal cells. *Exp. Eye Res.* **101**, 44–48.
70. Diaz, N. M., L. P. Morera, D. M. Verra, M. A. Contin and M. E. Guido (2014) Early appearance of nonvisual and circadian markers in the developing inner retinal cells of chicken. *Biomed. Res. Int.* **2014**, 646847.
71. Takahashi, J. S., P. J. DeCoursey, L. Bauman and M. Menaker (1984) Spectral sensitivity of a novel photoreceptive system mediating entrainment of mammalian circadian rhythms. *Nature* **308**, 186–188.
72. Yoshimura, T. and S. Ebihara (1996) Spectral sensitivity of photoreceptors mediating phase-shifts of circadian rhythms in retinally degenerate CBA/J (rd/rd) and normal CBA/N (+/+)mice. *J. Comp. Physiol. A.* **178**, 797–802.
73. Lucas, R. J., R. H. Douglas and R. G. Foster (2001) Characterization of an ocular photopigment capable of driving pupillary constriction in mice. *Nat. Neurosci.* **4**, 621–626.
74. Brainard, G. C., J. P. Hanifin, J. M. Greeson, B. Byrne, G. Glickman, E. Gerner and M. D. Rollag (2001) Action spectrum for melatonin regulation in humans: Evidence for a novel circadian photoreceptor. *J. Neurosci.* **21**, 6405–6412.
75. Thapan, K., J. Arendt and D. J. Skene (2001) An action spectrum for melatonin suppression: Evidence for a novel non-rod, non-cone photoreceptor system in humans. *J. Physiol.* **535**, 261–267.
76. Guler, A. D., J. L. Ecker, G. S. Lall, S. Haq, C. M. Altimus, H. W. Liao, A. R. Barnard, H. Cahill, T. C. Badea, H. Zhao, M. W. Hankins, D. M. Berson, R. J. Lucas, K. W. Yau and S. Hattar (2008) Melanopsin cells are the principal conduits for rod-cone input to non-image-forming vision. *Nature* **453**, 102–105.
77. Hatori, M., H. Le, C. Vollmers, S. R. Keding, N. Tanaka, T. Buch, A. Waisman, C. Schmedt, T. Jegla and S. Panda (2008) Inducible ablation of melanopsin-expressing retinal ganglion cells reveals

their central role in non-image forming visual responses. *PLoS One* **3**, e2451.
78. Mrosovsky, N. and S. Hattar (2003) Impaired masking responses to light in melanopsin-knockout mice. *Chronobiol. Int.* **20**, 989–999.
79. Panda, S., T. K. Sato, A. M. Castrucci, M. D. Rollag, W. J. DeGrip, J. B. Hogenesch, I. Provencio and S. A. Kay (2002) Melanopsin (Opn4) requirement for normal light-induced circadian phase shifting. *Science* **298**, 2213–2216.
80. Ruby, N. F., T. J. Brennan, X. Xie, V. Cao, P. Franken, H. C. Heller and B. F. O'Hara (2002) Role of melanopsin in circadian responses to light. *Science* **298**, 2211–2213.
81. Sikka, G., G. P. Hussmann, D. Pandey, S. Cao, D. Hori, J. T. Park, J. Steppan, J. H. Kim, V. Barodka, A. C. Myers, L. Santhanam, D. Nyhan, M. K. Halushka, R. C. Koehler, S. H. Snyder, L. A. Shimoda and D. E. Berkowitz (2014) Melanopsin mediates light-dependent relaxation in blood vessels. *Proc. Natl Acad. Sci. USA* **111**, 17977–17982.
82. Kirkby, L. A. and M. B. Feller (2013) Intrinsically photosensitive ganglion cells contribute to plasticity in retinal wave circuits. *Proc. Natl Acad. Sci. USA* **110**, 12090–12095.
83. Barnard, A. R., J. M. Appleford, S. Sekaran, K. Chinthapalli, A. Jenkins, M. Seeliger, M. Biel, P. Humphries, R. H. Douglas, A. Wenzel, R. G. Foster, M. W. Hankins and R. J. Lucas (2004) Residual photosensitivity in mice lacking both rod opsin and cone photoreceptor cyclic nucleotide gated channel 3 alpha subunit. *Vis. Neurosci.* **21**, 675–683.
84. Sernagor, E. (2005) Retinal development: Second sight comes first. *Curr. Biol.* **15**, R556–R559.
85. Sekaran, S., D. Lupi, S. L. Jones, C. J. Sheely, S. Hattar, K. W. Yau, R. J. Lucas, R. G. Foster and M. W. Hankins (2005) Melanopsin-dependent photoreception provides earliest light detection in the mammalian retina. *Curr. Biol.* **15**, 1099–1107.
86. Mey, J. and S. Thanos (2000) Development of the visual system of the chick. I. Cell differentiation and histogenesis. *Brain Res. Brain Res. Rev.* **32**, 343–379.
87. Do, M. T., S. H. Kang, T. Xue, H. Zhong, H. W. Liao, D. E. Bergles and K. W. Yau (2009) Photon capture and signalling by melanopsin retinal ganglion cells. *Nature* **457**, 281–287.
88. Tomonari, S., A. Takagi, S. Akamatsu, S. Noji and H. Ohuchi (2005) A non-canonical photopigment, melanopsin, is expressed in the differentiating ganglion, horizontal, and bipolar cells of the chicken retina. *Dev. Dyn.* **234**, 783–790.
89. Valdez, D. J., P. S. Nieto, N. M. Diaz, E. Garbarino-Pico and M. E. Guido (2013) Differential regulation of feeding rhythms through a multiple-photoreceptor system in an avian model of blindness. *FASEB J.* **27**, 2702–2712.
90. Newman, L. A., M. T. Walker, R. L. Brown, T. W. Cronin and P. R. Robinson (2003) Melanopsin forms a functional short-wavelength photopigment. *Biochemistry* **42**, 12734–12738.
91. Walker, M. T., R. L. Brown, T. W. Cronin and P. R. Robinson (2008) Photochemistry of retinal chromophore in mouse melanopsin. *Proc. Natl Acad. Sci. USA* **105**, 8861–8865.
92. Matsuyama, T., T. Yamashita, Y. Imamoto and Y. Shichida (2012) Photochemical properties of mammalian melanopsin. *Biochemistry* **51**, 5454–5462.
93. Isoldi, M. C., M. D. Rollag, A. M. Castrucci and I. Provencio (2005) Rhabdomeric phototransduction initiated by the vertebrate photopigment melanopsin. *Proc. Natl Acad. Sci. USA* **102**, 1217–1221.
94. Panda, S., S. K. Nayak, B. Campo, J. R. Walker, J. B. Hogenesch and T. Jegla (2005) Illumination of the melanopsin signaling pathway. *Science* **307**, 600–604.
95. Berson, D. M. (2007) Phototransduction in ganglion-cell photoreceptors. *Pflugers Arch.* **454**, 849–855.
96. Hardie, R. C. and P. Raghu (2001) Visual transduction in Drosophila. *Nature* **413**, 186–193.
97. Lee, Y. J., S. Shah, E. Suzuki, T. Zars, P. M. O'Day and D. R. Hyde (1994) The Drosophila dgq gene encodes a G alpha protein that mediates phototransduction. *Neuron* **13**, 1143–1157.
98. Montell, C. (1999) Visual transduction in Drosophila. *Ann. Rev. Cell Dev. Biol.* **15**, 231–268.
99. Hardie, R. C. (2003) TRP channels in Drosophila photoreceptors: The lipid connection. *Cell Calcium* **33**, 385–393.
100. Graham, D. M., K. Y. Wong, P. Shapiro, C. Frederick, K. Pattabiraman and D. M. Berson (2008) Melanopsin ganglion cells use a membrane-associated rhabdomeric phototransduction cascade. *J. Neurophysiol.* **99**, 2522–2532.
101. Hardie, R. C., F. Martin, G. W. Cochrane, M. Juusola, P. Georgiev and P. Raghu (2002) Molecular basis of amplification in Drosophila phototransduction: Roles for G protein, phospholipase C, and diacylglycerol kinase. *Neuron* **36**, 689–701.
102. Fisher, S. K., A. M. Heacock and B. W. Agranoff (1992) Inositol lipids and signal transduction in the nervous system: An update. *J. Neurochem.* **58**, 18–38.
103. Sekaran, S., R. G. Foster, R. J. Lucas and M. W. Hankins (2003) Calcium imaging reveals a network of intrinsically light-sensitive inner-retinal neurons. *Curr. Biol.* **13**, 1290–1298.
104. Berson, D. M. (2003) Strange vision: Ganglion cells as circadian photoreceptors. *Trends Neurosci.* **26**, 314–320.
105. Hughes, S., A. Jagannath, D. Hickey, S. Gatti, M. Wood, S. N. Peirson, R. G. Foster and M. W. Hankins (2015) Using siRNA to define functional interactions between melanopsin and multiple G Protein partners. *Cell. Mol. Life Sci.* **72**, 165–179.
106. Hardie, R. C. (2001) Phototransduction in *Drosophila melanogaster*. *J. Exp. Biol.* **204**, 3403–3409.
107. Suh, B. C., T. Inoue, T. Meyer and B. Hille (2006) Rapid chemically induced changes of PtdIns(4,5)P2 gate KCNQ ion channels. *Science* **314**, 1454–1457.
108. LaLonde, M. M., H. Janssens, E. Rosenbaum, S. Y. Choi, J. P. Gergen, N. J. Colley, W. S. Stark and M. A. Frohman (2005) Regulation of phototransduction responsiveness and retinal degeneration by a phospholipase D-generated signaling lipid. *J. Cell Biol.* **169**, 471–479.
109. Wu, L., B. Niemeyer, N. Colley, M. Socolich and C. S. Zuker (1995) Regulation of PLC-mediated signalling *in vivo* by CDP-diacylglycerol synthase. *Nature* **373**, 216–222.
110. Harris, W. A. and W. S. Stark (1977) Hereditary retinal degeneration in Drosophila melanogaster. A mutant defect associated with the phototransduction process. *J. Gen. Physiol.* **69**, 261–291.
111. Sengupta, S., T. R. Barber, H. Xia, D. F. Ready and R. C. Hardie (2013) Depletion of PtdIns(4,5)P(2) underlies retinal degeneration in Drosophila trp mutants. *J. Cell Sci.* **126**, 1247–1259.
112. Terakita, A. and T. Nagata (2014) Functional properties of opsins and their contribution to light-sensing physiology. *Zoolog. Sci.* **31**, 653–659.
113. Davies, W. L., M. W. Hankins and R. G. Foster (2010) Vertebrate ancient opsin and melanopsin: Divergent irradiance detectors. *Photochem. Photobiol. Sci.* **9**, 1444–1457.
114. Purschwitz, J., S. Muller, C. Kastner and R. Fischer (2006) Seeing the rainbow: Light sensing in fungi. *Curr. Opin. Microbiol.* **9**, 566–571.
115. Provencio, I., E. R. Loew and R. G. Foster (1992) Vitamin A2-based visual pigments in fully terrestrial vertebrates. *Vision. Res.* **32**, 2201–2208.
116. Provencio, I. and R. G. Foster (1993) Vitamin A2-based photopigments within the pineal gland of a fully terrestrial vertebrate. *Neurosci. Lett.* **155**, 223–226.
117. Wald, G. (1968) Molecular basis of visual excitation. *Science* **162**, 230–239.
118. Dowling, J. E. (1960) Chemistry of visual adaptation in the rat. *Nature* **188**, 114–118.
119. Reichenbach, A. and S. R. Robinson (1995) Phylogenetic constraints on retinal organisation and development. *Prog. Retin. Eye Res.* **15**, 139–171.
120. Mata, N. L., R. A. Radu, R. C. Clemmons and G. H. Travis (2002) Isomerization and oxidation of vitamin a in cone-dominant retinas: A novel pathway for visual-pigment regeneration in daylight. *Neuron* **36**, 69–80.
121. Chen, P., T. D. Lee and H. K. Fong (2001) Interaction of 11-cis-retinol dehydrogenase with the chromophore of retinal g protein-coupled receptor opsin. *J. Biol. Chem.* **276**, 21098–21104.
122. Redmond, T. M., E. Poliakov, S. Yu, J. Y. Tsai, Z. Lu and S. Gentleman (2005) Mutation of key residues of RPE65 abolishes its enzymatic role as isomerohydrolase in the visual cycle. *Proc. Natl Acad. Sci. USA* **102**, 13658–13663.
123. Jin, M., S. Li, W. N. Moghrabi, H. Sun and G. H. Travis (2005) Rpe65 is the retinoid isomerase in bovine retinal pigment epithelium. *Cell* **122**, 449–459.

124. Moiseyev, G., Y. Chen, Y. Takahashi, B. X. Wu and J. X. Ma (2005) RPE65 is the isomerohydrolase in the retinoid visual cycle. *Proc. Natl Acad. Sci. USA* **102**, 12413–12418.
125. Kiser, P. D., M. Golczak, A. Maeda and K. Palczewski (2012) Key enzymes of the retinoid (visual) cycle in vertebrate retina. *Biochim. Biophys. Acta* **1821**, 137–151.
126. Hillman, P. (1979) Bistable and sensitizing pigments in vision. *Biophys. Struct. Mech.* **5**, 111–112.
127. Rodriguez, K. A. and A. T. Tsin (1989) Retinyl esters in the vertebrate neuroretina. *Am. J. Physiol.* **256**, R255–R258.
128. Kaylor, J. J., Q. Yuan, J. Cook, S. Sarfare, J. Makshanoff, A. Miu, A. Kim, P. Kim, S. Habib, C. N. Roybal, T. Xu, S. Nusinowitz and G. H. Travis (2013) Identification of DES1 as a vitamin A isomerase in Muller glial cells of the retina. *Nat. Chem. Biol.* **9**, 30–36.
129. Kaylor, J. J., J. D. Cook, J. Makshanoff, N. Bischoff, J. Yong and G. H. Travis (2014) Identification of the 11-cis-specific retinyl-ester synthase in retinal Muller cells as multifunctional O-acyltransferase (MFAT). *Proc. Natl Acad. Sci. USA* **111**, 7302–7307.
130. Pandey, S., J. C. Blanks, C. Spee, M. Jiang and H. K. Fong (1994) Cytoplasmic retinal localization of an evolutionary homolog of the visual pigments. *Exp. Eye Res.* **58**, 605–613.
131. Radu, R. A., J. Hu, J. Peng, D. Bok, N. L. Mata and G. H. Travis (2008) Retinal pigment epithelium-retinal G protein receptor-opsin mediates light-dependent translocation of all-trans-retinyl esters for synthesis of visual chromophore in retinal pigment epithelial cells. *J. Biol. Chem.* **283**, 19730–19738.
132. Muniz, A., E. T. Villazana-Espinoza, B. Thackeray and A. T. Tsin (2006) 11-cis-Acyl-CoA:retinol O-acyltransferase activity in the primary culture of chicken Muller cells. *Biochemistry* **45**, 12265–12273.
133. Wang, J. S. and V. J. Kefalov (2011) The cone-specific visual cycle. *Prog. Retin. Eye Res.* **30**, 115–128.
134. Kefalov, V. J. (2012) Rod and cone visual pigments and phototransduction through pharmacological, genetic, and physiological approaches. *J. Biol. Chem.* **287**, 1635–1641.
135. Tsukamoto, H. and A. Terakita (2010) Diversity and functional properties of bistable pigments. *Photochem. Photobiol. Sci.* **9**, 1435–1443.
136. Hillman, P. (1979) Introduction to the symposium on bistable and sensitizing pigments in vision. *Biophys Struct Mech* **5**, 113–116.
137. Yarfitz, S. and J. B. Hurley (1994) Transduction mechanisms of vertebrate and invertebrate photoreceptors. *J. Biol. Chem.* **269**, 14329–14332.
138. Yau, K. W. and R. C. Hardie (2009) Phototransduction motifs and variations. *Cell* **139**, 246–264.
139. Shirzad-Wasei, N., J. van Oostrum, P. H. Bovee-Geurts, M. Wasserman, G. J. Bosman and W. J. Degrip (2013) Large scale expression and purification of mouse melanopsin-L in the baculovirus expression system. *Protein Expr. Purif.* **91**, 134–146.
140. Sexton, T. J., M. Golczak, K. Palczewski and R. N. Van Gelder (2012) Melanopsin is highly resistant to light and chemical bleaching *in vivo*. *J. Biol. Chem.* **287**, 20888–20897.
141. Emanuel, A. J. and M. T. Do (2015) Melanopsin tristability for sustained and broadband phototransduction. *Neuron* **85**, 1043–1055.
142. Tu, D. C., L. A. Owens, L. Anderson, M. Golczak, S. E. Doyle, M. McCall, M. Menaker, K. Palczewski and R. N. Van Gelder (2006) Inner retinal photoreception independent of the visual retinoid cycle. *Proc. Natl Acad. Sci. USA* **103**, 10426–10431.
143. Zhu, Y., D. C. Tu, D. Denner, T. Shane, C. M. Fitzgerald and R. N. Van Gelder (2007) Melanopsin-dependent persistence and photopotentiation of murine pupillary light responses. *Invest. Ophthalmol. Vis. Sci.* **48**, 1268–1275.
144. Mure, L. S., C. Rieux, S. Hattar and H. M. Cooper (2007) Melanopsin-dependent nonvisual responses: Evidence for photopigment bistability *in vivo*. *J. Biol. Rhythms* **22**, 411–424.
145. Mawad, K. and R. N. Van Gelder (2008) Absence of long-wavelength photic potentiation of murine intrinsically photosensitive retinal ganglion cell firing *in vitro*. *J. Biol. Rhythms* **23**, 387–391.
146. Wang, X., T. Wang, Y. Jiao, J. von Lintig and C. Montell (2010) Requirement for an enzymatic visual cycle in Drosophila. *Curr. Biol.* **20**, 93–102.
147. Wang, X., T. Wang, J. D. Ni, J. von Lintig and C. Montell (2012) The Drosophila visual cycle and *de novo* chromophore synthesis depends on rdhB. *J. Neurosci.* **32**, 3485–3491.
148. Thompson, C. L., C. P. Selby, R. N. Van Gelder, W. S. Blaner, J. Lee, L. Quadro, K. Lai, M. E. Gottesman and A. Sancar (2004) Effect of vitamin A depletion on nonvisual phototransduction pathways in cryptochromeless mice. *J. Biol. Rhythms* **19**, 504–517.
149. Tu, D. C., D. Zhang, J. Demas, E. B. Slutsky, I. Provencio, T. E. Holy and R. N. Van Gelder (2005) Physiologic diversity and development of intrinsically photosensitive retinal ganglion cells. *Neuron* **48**, 987–999.
150. McMahon, D. G., P. M. Iuvone and G. Tosini (2014) Circadian organization of the mammalian retina: From gene regulation to physiology and diseases. *Prog. Retin. Eye Res.* **39**, 58–76.
151. Cahill, G. M. and J.C. Besharse (1993) Circadian clock functions localized in xenopus retinal photoreceptors. *Neuron* **10**, 573–577.
152. Garbarino-Pico, E., A. R. Carpentieri, P. I. Castagnet, S. J. Pasquare, N. M. Giusto, B. L. Caputto and M. E. Guido (2004) Synthesis of retinal ganglion cell phospholipids is under control of an endogenous circadian clock: Daily variations in phospholipid-synthesizing enzyme activities. *J. Neurosci. Res.* **76**, 642–652.
153. Iuvone, P. M., G. Tosini, N. Pozdeyev, R. Haque, D. C. Klein and S. S. Chaurasia (2005) Circadian clocks, clock networks, arylalkylamine N-acetyltransferase, and melatonin in the retina. *Prog. Retin. Eye Res.* **24**, 433–456.
154. Valdez, D. J., P. S. Nieto, N. S. Della Costa, C. Schurrer and M. E. Guido (2015) Circadian control of the pupillary light responses in an avian model of blindness, the GUCY1* chickens. *Invest. Ophthalmol. Vis. Sci.* **56**, 730–737.
155. Valdez, D. J., E. Garbarino-Pico, N. M. Diaz, D. C. Silvestre and M. E. Guido (2012) Differential regulation of arylalkylamine N-acetyltransferase activity in chicken retinal ganglion cells by light and circadian clock. *Chronobiol. Int.* **29**, 1011–1020.
156. Buhr, E. D. and R. N. Van Gelder (2014) Local photic entrainment of the retinal circadian oscillator in the absence of rods, cones, and melanopsin. *Proc. Natl Acad. Sci. USA* **111**, 8625–8630.
157. Tarttelin, E. E., J. Bellingham, L. C. Bibb, R. G. Foster, M. W. Hankins, K. Gregory-Evans, C. Y. Gregory-Evans, D. J. Wells and R. J. Lucas (2003) Expression of opsin genes early in ocular development of humans and mice. *Exp. Eye Res.* **76**, 393–396.
158. Nieto, P. S., D. J. Valdez, V. A. Acosta-Rodriguez and M. E. Guido (2011) Expression of novel opsins and intrinsic light responses in the mammalian retinal ganglion cell line RGC-5. Presence of OPN5 in the rat retina. *PLoS One* **6**, e26417.
159. Yamashita, T., H. Ohuchi, S. Tomonari, K. Ikeda, K. Sakai and Y. Shichida (2010) Opn5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. *Proc. Natl Acad. Sci. USA* **107**, 22084–22089.
160. Nakane, Y., K. Ikegami, H. Ono, N. Yamamoto, S. Yoshida, K. Hirunagi, S. Ebihara, Y. Kubo and T. Yoshimura (2010) A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds. *Proc. Natl Acad. Sci. USA* **107**, 15264–15268.
161. Allen, A. E., T. M. Brown and R. J. Lucas (2011) A distinct contribution of short-wavelength-sensitive cones to light-evoked activity in the mouse pretectal olivary nucleus. *J. Neurosci.* **31**, 16833–16843.
162. Palczewski, K. (2012) Chemistry and biology of vision. *J. Biol. Chem.* **287**, 1612–1619.
163. Montell, C. (2001) An end in sight to a long TRP. *Neuron* **30**, 3–5.
164. Montell, C. (2005) The TRP superfamily of cation channels. *Sci. STKE* **2005**, re3. Review.
165. Carter-Dawson, L. D., M. M. LaVail and R. L. Sidman (1978) Differential effect of the rd mutation on rods and cones in the mouse retina. *Invest. Ophthalmol. Vis. Sci.* **17**, 489–498.
166. Bowes, C., T. Li, M. Danciger, L. C. Baxter, M. L. Applebury and D. B. Farber (1990) Retinal degeneration in the rd mouse is caused by a defect in the beta subunit of rod cGMP-phosphodiesterase. *Nature* **347**, 677–680.
167. Mysore, N., J. Koenekoop, S. Li, H. Ren, V. Keser, I. Lopez-Solache and R. K. Koenekoop (2014) A review of secondary photoreceptor degenerations in systemic disease. *Cold Spring Harb. Perspect Med.* **11**, 4–5.

168. Sanyal, S., A. De Ruiter and R. K. Hawkins (1980) Development and degeneration of retina in rds mutant mice: Light microscopy. *J. Comp. Neurol.* **194**, 193–207.
169. Sanyal, S. and G. H. Zeilmaker (1984) Development and degeneration of retina in rds mutant mice: Light and electron microscopic observations in experimental chimaeras. *Exp. Eye Res.* **39**, 231–246.
170. Cheng, K. M., R. N. Shoffner, K. N. Gelatt, G. G. Gum, J. S. Otis and J. J. Bitgood (1980) An autosomal recessive blind mutant in the chicken. *Poult. Sci.* **59**, 2179–2181.
171. Ulshafer, R. J. and C. B. Allen (1985) Hereditary retinal degeneration in the Rhode Island Red chicken: Ultrastructural analysis. *Exp. Eye Res.* **40**, 865–877.
172. Semple-Rowland, S. L. (1991) Expression of glial fibrillary acidic protein by Muller cells in rd chick retina. *J. Comp. Neurol.* **305**, 582–590.
173. Semple-Rowland, S. L. and W. W. Dawson (1987) Cyclic light intensity threshold for retinal damage in albino rats raised under 6lx. *Exp. Eye Res.* **44**, 643–661.
174. Semple-Rowland, S. L., K. S. Eccles and E. J. Humberstone (2007) Targeted expression of two proteins in neural retina using self-inactivating, insulated lentiviral vectors carrying two internal independent promoters. *Mol. Vis.* **13**, 2001–2011.
175. Semple-Rowland, S. L. and D. A. Green (1994) Molecular and biochemical analyses of iodopsin in rd chick retina. *Invest. Ophthalmol. Vis. Sci.* **35**, 2550–2557.
176. Semple-Rowland, S. L., P. Larkin, J. D. Bronson, K. Nykamp, W. J. Streit and W. Baehr (1999) Characterization of the chicken GCAP gene array and analyses of GCAP1, GCAP2, and GC1 gene expression in normal and rd chicken pineal. *Mol. Vis.* **5**, 14.
177. Larkin, P., W. Baehr and S. L. Semple-Rowland (1999) Circadian regulation of iodopsin and clock is altered in the retinal degeneration chicken retina. *Brain Res. Mol. Brain Res.* **70**, 253–263.
178. Semple-Rowland, S. L., W. A. Gorczyca, J. Buczylko, B. S. Helekar, C. C. Ruiz, I. Subbaraya, K. Palczewski and W. Baehr (1996) Expression of GCAP1 and GCAP2 in the retinal degeneration (rd) mutant chicken retina. *FEBS Lett.* **385**, 47–52.
179. Allen, A. E., M. A. Cameron, T. M. Brown, A. A. Vugler and R. J. Lucas (2010) Visual responses in mice lacking critical components of all known retinal phototransduction cascades. *PLoS One* **5**, e15063.
180. Rollag, M. D., D. M. Berson and I. Provencio (2003) Melanopsin, ganglion-cell photoreceptors, and mammalian photoentrainment. *J. Biol. Rhythms* **18**, 227–234.
181. Mo, G., Q. Ding, Z. Chen, Y. Li, M. Yan, L. Bu, Y. Song and G. Yin (2014) A novel mutation in the RPE65 gene causing Leber congenital amaurosis and its transcriptional expression *in vitro*. *PLoS One* **9**, e112400.
182. Ripamonti, C., G. B. Henning, R. R. Ali, J. W. Bainbridge, S. J. Robbie, V. Sundaram, V. A. Luong, L. I. van den Born, I. Casteels, T. J. de Ravel, A. T. Moore and A. Stockman (2014) Nature of the visual loss in observers with Leber's congenital amaurosis caused by specific mutations in RPE65. *Invest. Ophthalmol. Vis. Sci.* **55**, 6817–6828.
183. Dkhissi-Benyahya, O., C. Gronfier, W. De Vanssay, F. Flamant and H. M. Cooper (2007) Modeling the role of mid-wavelength cones in circadian responses to light. *Neuron* **53**, 677–687.
184. Panda, S. (2007) Multiple photopigments entrain the Mammalian circadian oscillator. *Neuron* **53**, 619–621.
185. Calvert, M. J., D. G. Ward, H. R. Trayer and I. P. Trayer (2000) The importance of the carboxyl-terminal domain of cardiac troponin C in Ca2+-sensitive muscle regulation. *J. Biol. Chem.* **275**, 32508–32515.
186. Calvert, P. D., N. V. Krasnoperova, A. L. Lyubarsky, T. Isayama, M. Nicolo, B. Kosaras, G. Wong, K. S. Gannon, R. F. Margolskee, R. L. Sidman, E. N. Jr Pugh, C. L. Makino and J. Lem (2000) Phototransduction in transgenic mice after targeted deletion of the rod transducin alpha -subunit. *Proc. Natl Acad. Sci. USA* **97**, 13913–13918.
187. Biel, M., M. Seeliger, A. Pfeifer, K. Kohler, A. Gerstner, A. Ludwig, G. Jaissle, S. Fauser, E. Zrenner and F. Hofmann (1999) Selective loss of cone function in mice lacking the cyclic nucleotide-gated channel CNG3. *Proc. Natl Acad. Sci. USA* **96**, 7553–7557.
188. Torii, M., D. Kojima, T. Okano, A. Nakamura, A. Terakita, Y. Shichida, A. Wada and Y. Fukada (2007) Two isoforms of chicken melanopsins show blue light sensitivity. *FEBS Lett.* **581**, 5327–5331.

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