

# Melanopsin: an exciting photopigment

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**The discovery that mice lacking rods and cones are capable of regulating their circadian rhythms by light provided the conceptual framework for the discovery of an entirely new photoreceptor system within the mammalian eye. We now know that a small subset of retinal ganglion cells are directly photosensitive and utilize an opsin/vitamin A-based photopigment called melanopsin maximally sensitive in the blue part of the spectrum. We also know that these photosensitive retinal ganglion cells mediate a broad range of physiological responses to light, ranging from the regulation of circadian rhythms to pupil constriction. Most recently, it has become clear that the melanopsins are only distantly related to visual pigments and in terms of their biochemistry share more in common with invertebrate photopigments. Here we outline the discovery of this remarkable new photoreceptor system, review the structure of melanopsin and conclude with a working model of melanopsin phototransduction.**

## Introduction

The discovery of a third photoreceptor system in the mammalian retina, quite separate from rods and cones, arose from attempts to understand how endogenous 24 h body clocks (circadian clocks) are regulated by light. Circadian clocks are not exactly 24 h (hence the term: *circa/about* and *dies/day*) and must be synchronized to ensure that internal and local time coincide. This regulation is usually mediated by photoreceptors which detect changes in the quantity and quality of light over the 24 h dawn/dusk cycle, a process that has been termed photoentrainment [1].

The sensory task of photoentrainment differs markedly from image detection, requiring light stimuli of high irradiance and long duration. For example, the circadian system of the hamster requires light intensities some 200 times brighter than the intensities that would elicit a visual response. Furthermore, the clock is largely insensitive to stimulus durations of less than 30 s [2]. The non-mammalian vertebrates use a broad range of photoreceptors for photoentrainment including intracranial pineal organs and even photoreceptors buried deep within the brain. Although considerable amounts of light penetrate deep into the brain, the light reaching these extra-retinal photoreceptors is filtered and scattered by overlying tissues. This would preclude any form of image detection but make them ideally suited to detect gross

changes in environmental irradiance and hence time of day [3]. By contrast, photoentrainment in mammals relies exclusively upon ocular photoreceptors [4]. Why the mammalian lineage lost its extraocular photoreceptors remains speculative, but has been correlated with the early evolutionary history of mammals and their passage through what has been termed a nocturnal bottleneck [5,6]. It seems likely that the relatively poor colour vision, well-developed olfactory and auditory systems and loss of extra-retinal photoreceptors in modern mammals all reflect this nocturnal ancestry. Because multiple studies had shown that eye loss blocks photoentrainment in mammals, and because rods and cones were the only known ocular photoreceptors, all light responses were attributed to these cells. This gave rise to the straightforward question that framed much of the early research: how can rods and cones, which are so exquisitely adapted to build a visual representation of the world, also act as dawn/dusk detectors?

## Irradiance detection

In mammals, light information reaches the master circadian pacemaker, the suprachiasmatic nuclei (SCN), through a dedicated monosynaptic pathway originating in the retina and called the retinohypothalamic tract (RHT) [7]. Although the RHT had been defined by the early 1970s, the photoreceptor inputs to the SCN had not. Disentangling which retinal cells mediate photoentrainment was first addressed using animals with naturally occurring retinal mutations, such as mice homozygous for retinal degeneration (*rd/rd*). Although all functional rods are lost in the *rd/rd* retina, a small percentage (~5%) of cone cells survive beyond 18 months [8]. Despite this loss of photoreceptors, *rd/rd* mice show circadian responses to light that are indistinguishable from those of congenic mice with phenotypically normal retinas (*rd/+*; wild-type) [9]. These findings, along with studies on other rodent models such as the blind mole rat (*Spalax*) [10] and retinally degenerate humans [11,12], raised the possibility that perhaps the retina contained an additional specialized class of photoreceptor, analogous to the pineal and deep brain photoreceptors of non-mammals. Initially, this suggestion was greeted with considerable opposition based upon the argument that only a very small number of rods and/or cones are sufficient for normal photoentrainment [13]. To resolve this issue, mice were generated that lacked all functional rod and cone photoreceptors (*rd/rd cl*). Such animals were shown to be capable of normal photoentrainment of behavioural [14] and neuroendocrine rhythms [15]. Collectively, these results showed unambiguously that a third class

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### Box 1. The pupillary light response

The *rd/rd cl* mouse model also proved to be important in demonstrating that a range of other irradiance detection tasks are mediated by a non-rod, non-cone retinal photoreceptor. For example, in mammals, light-induced pupil constriction is regulated by both rod and cone photoreceptors, but is not abolished in animals that show a significant loss of these photoreceptors as a result of inherited retinal disease. Not unreasonably, it was assumed that the residual pupillary light response (PLR) was a result of the survival of a small number of rod and/or cone photoreceptors [58]. Detailed studies in *rd/rd cl* mice have shown that although there is diminished sensitivity, a full PLR can still occur in mice lacking rods and cones [19]. This suggests a complex interaction between visual and nonvisual photoreceptors in the regulation of pupil size. One hypothesis is that the PLR is a two-stage process: rapid and dim-light PLR is mediated by rods and cones, whereas nonvisual photoreceptors mediate the sustained PLR under relatively high environmental irradiances. A sensory task would be poorly suited to the rapidly adapting responses typical of rods and cones [19].

of photoreceptor must reside within the mammalian eye (Box 1).

### Melanopsin-based photosensitive retinal ganglion cells

If not the rods and cones, then what other retinal neurons are photoreceptive? Parallel studies in the rat and mouse addressed this question and identified a subset of photosensitive retinal ganglion cells (pRGCs) (Figure 1). In the rat, the approach involved injecting fluorescent microspheres into the SCN which then traveled down the axons of the RHT to retrogradely label retinal ganglion cells (RGCs). These RGCs showed a light-evoked depolarisation that persisted in the presence of a cocktail of drugs that blocked all retinal intercellular communication. Furthermore, the labeled RGCs still showed intrinsic light responses when dissected and isolated from the surrounding retinal tissue [16]. In mice, the approach utilized the isolated *rd/rd cl* retinae loaded with the  $\text{Ca}^{2+}$ -sensitive FURA-2AM dye. Fluorescent imaging identified light-induced  $\text{Ca}^{2+}$  changes in  $\sim 3.0\%$  of neurons within the RGC layer (Figure 1). Significantly, the gap junction blocker carbenoxolone reduced the number of RGCs responding to light to  $\sim 1.0\%$ . This suggests that pRGCs are coupled via gap junctions and form a syncytium of photosensitive and nonphotosensitive neurons. Three discrete classes of light-induced  $\text{Ca}^{2+}$  change have been identified in pRGCs, but the basis for these different responses remains unclear and it remains unknown whether specific pRGCs project differentially to all retinorecipient regions of the brain [17,18].

The *rd/rd cl* mouse also proved valuable in characterizing the photopigment of pRGCs. The known photopigments of animals consist of an opsin protein linked to a chromophore which is a specific form of vitamin A called 11-*cis* retinal. All opsin/vitamin A-based photopigments have a characteristic absorption profile that allows these photopigments to be identified on the basis of their spectral responses to light or action spectra. The process of generating an action spectrum involves the protracted task of measuring a series of full dose–response curves for a range of monochromatic stimuli. The first full action spectrum to define the nature of the non-rod, non-cone photopigment studied pupil constriction in *rd/rd cl* mice. The results

revealed a previously uncharacterized, opsin/vitamin A-based photopigment with peak sensitivity at 479 nm, which was tentatively named OP<sup>479</sup> (opsin photopigment  $\lambda_{\text{max}}$  479 nm) [19] (Figure 2). Subsequently, action spectra from mice to human have been deduced for a range of responses including the direct recording from pRGCs in rats [16] and macaque [20]. Additional action spectra are listed in Table 1. A clear consensus has emerged from these studies demonstrating the existence of a common single novel opsin photopigment with a  $\lambda_{\text{max}}$  of around 480 nm. Although the biochemistry of the photopigment was deduced by action spectroscopy, the molecular identity of OP<sup>479</sup> remained unknown.

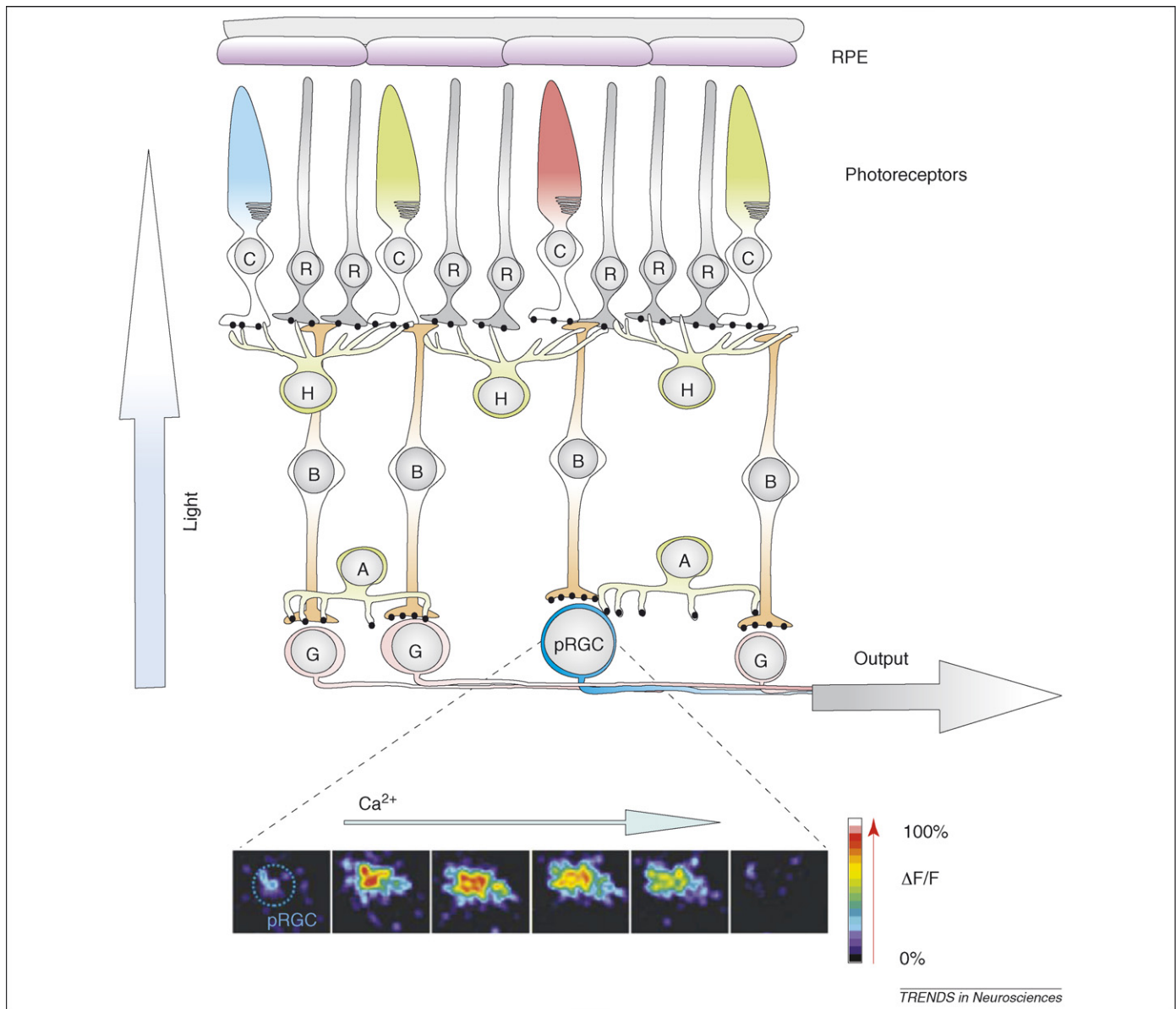
The discovery that the orphan opsin gene, melanopsin (now officially designated *Opn4*), is expressed within a subpopulation of RGCs generated considerable excitement because the anatomy and distribution of these melanopsin RGCs was remarkably similar to the RGCs that form the RHT [18,21–23]. Melanopsin gene ablation studies provided the definitive link between melanopsin and the capacity of the pRGCs to respond to light. Melanopsin knockout mice (*Opn4*<sup>-/-</sup>) show attenuated phase shifting; fail to show full pupil constriction; and show loss of direct photosensitivity in pRGCs [24–26]. Furthermore, if *Opn4*<sup>-/-</sup> mice are crossed with mice lacking functional rods and cones, all responses to light are lost [27,28]. These studies demonstrate that rods, cones and pRGCs can fully account for all light detection within the eye, and strongly implicate melanopsin as the photopigment molecule of pRGCs. These triple knockout studies also addressed the suggestion that cryptochrome (CRY) might act as a photopigment for photoentrainment [29,30]. The complete loss of light responses in these animals leaves little room for a CRY-base photopigment, and there is now a broad consensus that the CRYs do not form photopigments in mammals. For further discussion, see Refs [31,32].

### Rod, cone and pRGC interactions

The results from *rd/rd cl* mice demonstrate that rods and cones are not required for photoentrainment [14]. However, one cannot conclude from this that rods and cones play no role. Indeed, multiple lines of evidence have implicated an input from rods and cones [10,33–35], not least the finding that *Opn4*<sup>-/-</sup> mice still show circadian entrainment, albeit in an attenuated form [24–26]. Thus, melanopsin ablation studies show that rods and cones can

**Table 1. A range of the derived opsin  $\lambda_{\text{max}}$  values for irradiance detection tasks reported in various mammalian species**

Species	Measure	$\lambda_{\text{max}}$	Refs
Mouse <i>rd cl</i>	Pupillometry	479 nm	[19]
Mouse <i>rd cl</i>	Circadian phase shifting	481 nm	[27]
Rat WT	pRGC light response	484 nm	[16]
Macaque	pRGC light response	482 nm	[20]
Macaque	Pupillometry	482 nm	[59]
Human	Melatonin suppression	446–477 nm	[60]
Human	Melatonin suppression	459 nm	[61]
Human	Regulation of cone electroretinogram	483 nm	[62]
Human	Heterologous expression	420–440 nm	[38]
Mouse	Heterologous expression	420 nm	[37]
Mouse	Heterologous expression	479 nm	[39]
Mouse	Heterologous expression	480 nm	[40]



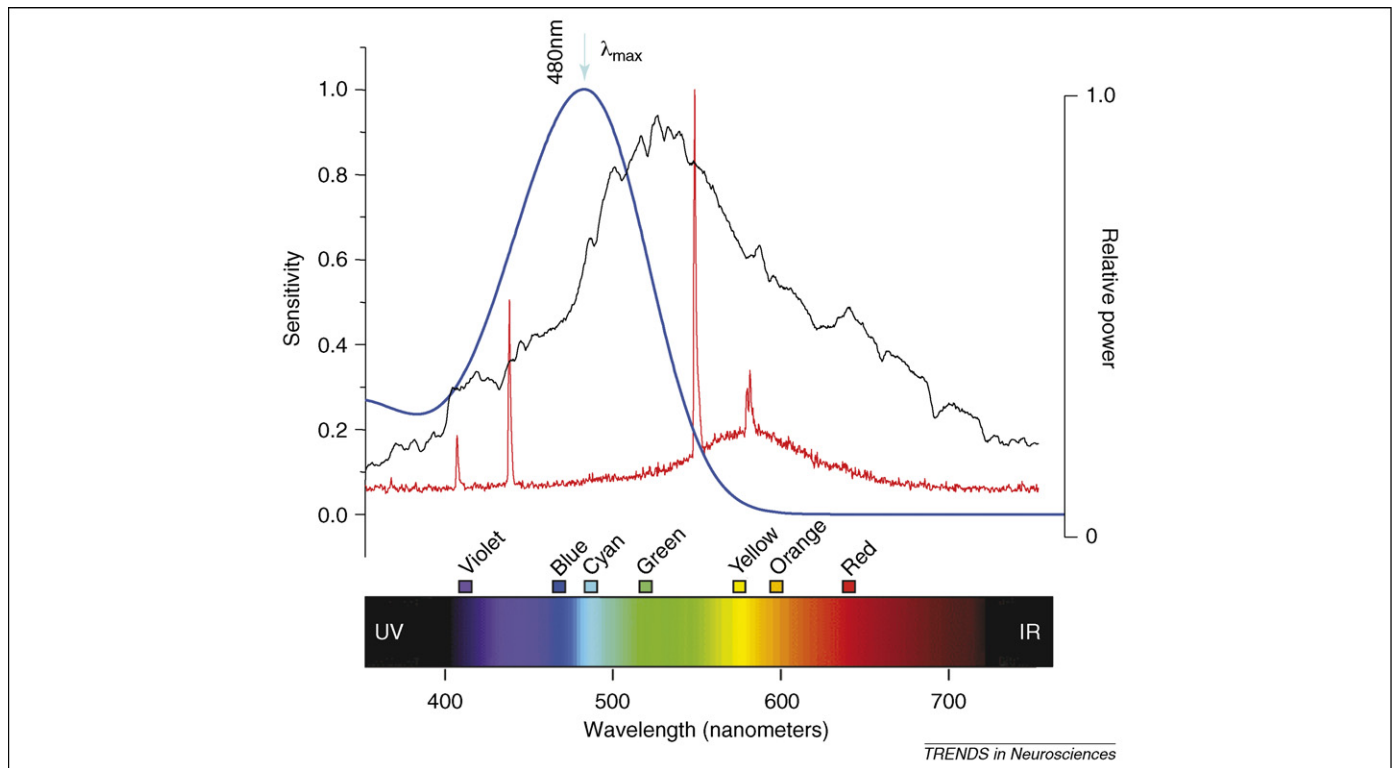
**Figure 1.** Light detection in the vertebrate retina. The rods (R) and cones (C) convey visual information to the ganglion cells (G) via the second-order bipolar cells (B). At the first synaptic layer, horizontal cells (H) facilitate lateral connectivity and feedback to the photoreceptors. At the second synaptic layer in the inner retina, amacrine cells (A) allow lateral connections between bipolar and ganglion cells. The optic nerve is formed from the axons of all the ganglion cells. A subset of ganglion cells, the photosensitive retinal ganglion cells (pRGCs), also detect light directly, utilizing the photopigment melanopsin. Calcium imaging (FURA2) in the rodless-coneless (*rd/rd cl*) mouse retina identified a population of pRGCs. These cells exhibited light responses that were classified into three distinct physiological categories: transient, sustained and repetitive [17]. Thus, photodetection in the retina occurs both in the outer and inner retina. In the vertebrate retina, light passes through the transparent ganglion layer to reach the rods and cones of the image-forming pathway.

partially compensate for the loss of pRGC photosensitivity, and the *rd/rd cl* data suggest that pRGCs can fully compensate for the loss of the rods and cones. Further evidence for rod, cone and pRGC interaction comes from recent studies on the macaque. Intracellular recording from melanopsin pRGCs has shown that short-wavelength cones attenuate the light responses of pRGCs, whereas the rods and medium-wavelength cones provide a complementary excitatory input [20]. The specific roles played by rods, cones and pRGCs in irradiance detection remain unresolved.

### Melanopsin expression studies

The observation that melanopsin ablation leads to the loss of photosensitivity of pRGCs served only to confirm that

Opn4 is a required component in the light response. Gene ablation could not formally preclude a vital accessory function such as a local photoisomerase, that is, generating chromophore for an unidentified opsin pigment [36]. This was addressed initially in COS cells, where it was shown that melanopsin could mediate light-dependent G-protein activation in a biochemical assay [37]. The photopigment capacity of this protein was ultimately addressed by three groups in parallel using heterologous expression of either human or mouse melanopsin in Neuro2A cells [38], HEK293 cells [39] and *Xenopus* oocytes [40]. Critically, all three studies demonstrated that melanopsin expression was fully sufficient to drive a retinal-dependent light cascade leading to a cellular membrane current. For example, in neuroblastoma (Neuro2A) cells the expression of



**Figure 2.** Action spectra. The functional properties of photopigments have been characterized through action spectroscopy. Irradiance-dependent responses from a range of species suggest a consensus opsin template with a  $\lambda_{max}$  (peak sensitivity) of around 480 nm (blue line). This is compared to the typical representative power spectra for natural daylight (black) and artificial fluorescent lighting (red). The range of the visible spectrum between the UV and IR is depicted on the x axis.

melanopsin, in the presence of retinal chromophore (9-*cis*-retinal or 11-*cis*-retinal), transformed a nonphotosensitive neuron into a functional irradiance detector [38]. Furthermore, these studies demonstrated that melanopsin appears to function as a bistable pigment, able to regenerate its chromophore utilizing all-*trans*-retinal and long-wavelength light in a manner reminiscent of the photoreversal observed in invertebrates [38]. In this respect, melanopsin is unique among the mammalian photopigments in forming a stable association with all-*trans*-retinal. The photoreversal capacity of melanopsin has also been observed with spectroscopic approaches in the case of amphioxus melanopsin [41]. Collectively, all of the expression studies have led to a consensus view that melanopsin forms a photopigment fully capable of coupling to ubiquitous cell signalling pathways. The ability to regenerate its own chromophore is most likely an important adaptation to its expression in retinal ganglion cells distant from the centre of retinoid recycling in the retinal pigment epithelium (Figure 1) [38].

Although all three expression studies showed broadly the same result, inconsistencies relating to the spectral sensitivity of expressed human and mouse melanopsin emerged. The spectral maxima of the pigments varied between 420 and 440 nm (human) [38] and 480 nm (mouse) [39,40]. The reason for this discrepancy still remains to be fully resolved, but presumably relates to varied experimental procedures, including the host cell environment and perhaps cell retinoid status. Classical spectroscopic approaches should resolve this issue.

### Melanopsin structure

Although melanopsin almost certainly forms the photopigment of pRGCs, very little is known about how this protein functions. Some deductions can be inferred from its structure and below we address a few of the structure/function relationships of the melanopsins. The first melanopsin gene isolated was from *Xenopus* dermal melanophores (hence the name, *melanopsin*), and it was recognized immediately that this opsin shared greater homology with invertebrate opsins, such as octopus rhodopsin (39%), than with the classical visual pigments (~27%) [42,43]. In addition, melanopsin shows several other features which resemble invertebrate opsins. These include the presence of a tyrosine instead of a glutamate counterion (E113); an insertion in the third cytoplasmic loop increasing its length; and, finally, an extended intracellular C terminus. Phylogenetic analysis also supported an affiliation with the invertebrate opsins, placing melanopsin in a clade closer to the invertebrate rather than vertebrate opsins [42]. The genomic structure of the melanopsins also differs markedly from the vertebrate visual pigments, possessing nine intron insertion sites. This strongly suggests that the melanopsin gene family has a different evolutionary lineage from the rod, cone, pineal and VA-opsin photopigments [43].

Recent studies have also shown that there are two melanopsin genes in the vertebrate lineage, the mammalian-like *Opn4m* and *Xenopus*-like *Opn4x* genes [44]. Non-mammalian vertebrates possess both *Opn4m* and *Opn4x* genes in separate chromosomal loci. However, the *Opn4x* gene is not present in either eutherian or marsupial



mammals [44,45], and preliminary analysis of the platypus and echidna suggests that the *Opn4x* gene is also missing from the monotremes (Pires, Halford, and Foster, unpublished). These results indicate that the *Opn4x* gene was lost as a result of chromosomal reorganisation relatively early in mammalian evolution. Both genes seem capable of forming photosensory pigments in the chicken, although the expression pattern of *Opn4m* is restricted to a subset of retinal ganglion cells whereas *Opn4x* appears to be expressed throughout the chicken retina [44]. The complementary function of these two forms of melanopsin remains completely unknown.

Whole amino acid sequence alignments of the various visual opsin classes show high levels of conservation (~40% identity [43]). By contrast, a comparison of melanopsins with the visual opsins produces very low levels of amino acid identity as a result of the highly variable N and C termini. When the more conserved transmembrane domains are considered alone, melanopsin still only shares ~27% identity with the visual opsins [43]. However, one problem with an overall comparison of sequence identities across the transmembrane regions is that it does not take into account the functional position of individual amino acids, in particular whether they are involved in the formation of the retinal/ligand binding pocket. To address this, a 35 amino acid vector termed the ligand pocket vector (LPV) of various melanopsins was deduced and aligned with other opsin sequences [46]. The LPV is shown schematically in Figure 3a. These core amino acids of the ligand binding pocket were then compared in rod, cone and melanopsins. The LPVs of visual pigment classes are highly conserved across species (Figure 3b, left). For example, within the rods, the sequences of the LPVs are 94%–100% identical. By contrast, the LPVs of the melanopsins across multiple species are considerably more variable, with 77%–100% identity (Figure 3b, right, top). However, when melanopsin sequences are segregated into *Opn4x* and *Opn4m*, these interspecies differences are markedly reduced, with *Opn4x* genes showing 89%–100% identity and *Opn4m* showing an even greater identity of 94%–100%. Collectively, this analysis shows that when the retinal binding pocket is compared within an opsin class, there is a remarkable degree of conservation. Indeed, the melanopsins are no more divergent in their LPV than the other opsin classes. This suggests that the melanopsins across species will interact with retinal in a similar manner, which may account for the conserved spectral maxima (~480 nm; Table 1). Based upon consensus sequences for each opsin class, *Opn4x* sequences share 40% amino acid identity with rod opsins, whereas *Opn4m* sequences share 46% identity (Figure 3b, lower right). These values are considerably higher than the level of overall sequence identity between rod opsins and melanopsins (~27%). This analysis emphasizes the importance of comparing functionally conserved regions of the different opsins rather than a simple comparison of overall sequence identity.

A consideration of the individual residues of the ligand binding pocket is beyond the scope of this review, but two key features are outlined below. (i) As noted above, the melanopsins have a glutamate-to-tyrosine substitution in the Schiff base counterion position (E113Y). It has been

suggested that E181 may serve as the counterion in melanopsin [47], as in amphioxus rhodopsin [48]. If true, then one would predict a corresponding change in the amino acids forming the binding pocket around this region. This seems to be the case: helices 1–4 show a much lower level of identity compared to helices 5–7 (Figure 3b, lower right). (ii) The second extracellular loop folds into the helical bundle and forms the extracellular boundary of the retinal binding pocket [49] and in melanopsin shows several non-conserved substitutions compared to the visual opsins. Differences in this region could influence the retinal binding site and/or the release of retinal following photoisomerisation [38].

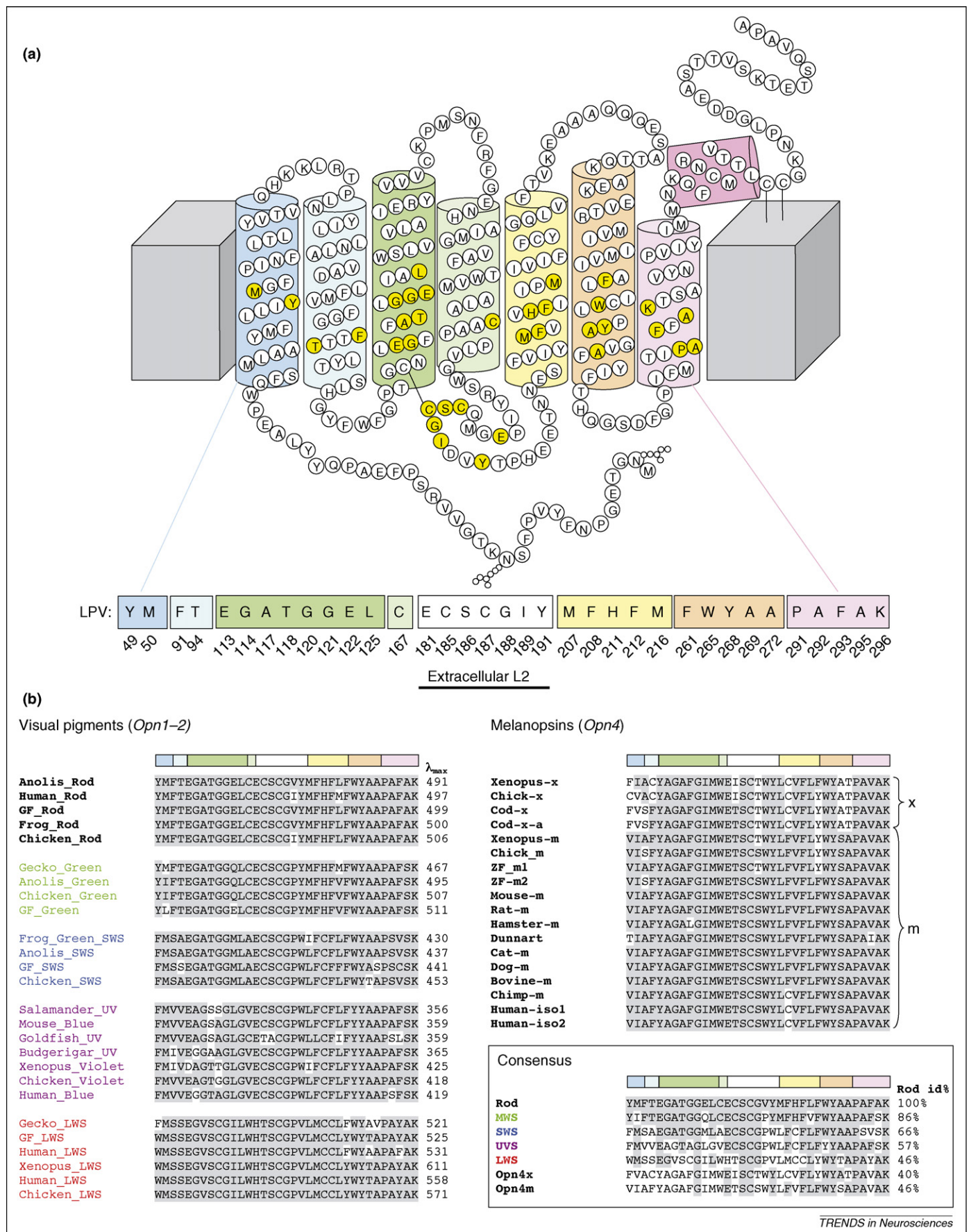
### Melanopsin phototransduction

The deduced structure of the melanopsins immediately suggested strong parallels with invertebrate light signalling systems. Invertebrate phototransduction has been characterized most extensively in *Drosophila* [50], and very broadly the rhabdomeric cascade involves activation of a G<sub>q</sub>/G<sub>11</sub>-type G protein, activation of phospholipase C (PLC) and subsequent opening of transient receptor potential channels (TRPCs) resulting in the depolarisation of the cell membrane. By contrast, the mechanisms of phototransduction utilized by the rod and cone opsins are quite different, involving the activation of transducin (G<sub>t</sub>), phosphodiesterase, hydrolysis of cGMP, closure of cyclic nucleotide gated (CNG) channels and a hyperpolarisation of the plasma membrane [50,51] (Figure 4).

In contrast to invertebrate and vertebrate visual phototransduction, our understanding of melanopsin signalling is only beginning to emerge. Nonnative heterologous cell systems have been used to express melanopsin and examine possible G-protein binding partners. The problem here is that G-protein-coupled receptors (GPCRs) can bind a variety of G proteins promiscuously and so the finding that a particular G protein can bind to a GPCR does not mean that this necessarily occurs in the native cell. For example, although it appears that melanopsin can activate the  $\alpha$  subunit of rod transducin in biochemical assays [37], this is unlikely to be a significant mode of action in pRGCs because ablation of this gene has no effect on cellular light responses [27].

Another approach has explored signalling in the pRGCs by combining pharmacology with electrophysiology or imaging in native cells. Although there have been advances using these techniques, progress has been limited because of the lack of a comprehensive pharmacological toolkit with which to probe this uncharacterized system. A further complication arises because melanopsin may activate multiple signalling channels in a semiredundant manner. Thus, the interpretation of drug action on pRGCs has to be suitably cautious.

The most recent approach, which has the advantage of assuming very little about the signalling systems, has utilized a microarray-based technique to investigate the transcriptional realignment that occurs in the *rd/rd cl* mouse eye following a light pulse. This has identified a number candidate genes/proteins that might be associated with the melanopsin cascade. This approach is also limited in that it tends to generate large numbers of candidates



**Figure 3.** Ligand pocket vector. Analysis of the putative retinal binding site of rod opsins, cone opsins and melanopsins. **(a)** Opsin peptide sequence illustrating major structural features. The amino acids of the ligand pocket vector (LPV) are highlighted in yellow. The 35 amino acid sequence (below) of the LPV is colour coded according to helix location. Amino acid numbering and sequence are based upon the crystal structure of bovine rhodopsin [49]. **(b)** The LPV of visual pigments (left column) and

and investigation of each individual gene is still relatively slow and laborious.

The consensus view is that melanopsin indeed signals through a G-protein-coupled system. For example, the disruption of GPCR coupling using suramin affects both expressed melanopsin and native cell responses to light. Primarily by analogy to rhabdomic transduction, it was first proposed that melanopsin might be specifically coupled to the  $G_q/G_{11}$  class of G protein and PLC [42]. There is support for this proposal from the heterologous expression studies. For example, melanopsin responses appear to be attenuated by antibodies to  $\alpha$  subunits of  $G_q/G_{11}$  [40]. In Neuro2A cells, the use of  $G_i/G_o$  blockers fails to inhibit melanopsin-dependent light responses [38], whereas putative  $G_q/G_{11}$  agonists fully blocked melanopsin-dependent light responses in HEK293-TRPC3 cells [39]. Although there is a degree of congruity in these data, it is important to remember that they only explore the broad potential of cascade coupling in a diverse range of host environments. PLC antagonists, such as U73122 or ET-18-OCH3, may be effective in some expression systems [39] but are without effect in Neuro2A cells [38]. It is hard to form solid conclusions from the current data sets, complicated by the real possibility of parallel transduction cascades in the native cells with some degree of functional redundancy. This possibility is supported by the finding that the light responses of these cells show a mixture of calcium currents that can be both sustained and transient [17].

Investigations of the channel, or indeed channels, involved in gating the melanopsin light response quickly centred upon the TRPC family. This was partially through analogy to the invertebrate cascades, but also because of the biophysical characteristics of the light current in native melanopsin-expressing cells [52]. Light induces a conductance increase in native pRGCs, and the photocurrent reverses close to zero mV, consistent with a nonspecific cationic channel. The mammalian canonical TRPCs can be divided into four families based upon sequence homology and pharmacological properties: TRPC1, TRPC2, TRPC3+6+7 and TRPC4+5 [53]. Furthermore, their functional diversity is increased because these channels can form heteromeric complexes with members of the same family (except TRPC1). TRPC proteins form nonspecific cationic channels with substantial  $Ca^{2+}$  permeability, matching known features of the pRGC light-activated channel [17]. Coexpression of melanopsin with TRPCs has been shown to result in a functional cascade [39,40]. Furthermore, evidence suggests that the light-evoked currents in rat pRGCs can be suppressed by TRPC blockers [54]. The expression profile of TRPC family members in

mouse pRGCs suggests the presence of both TRPC6 and TRPC7 (but not TRPC3) in the same cells that expressed melanopsin [55]. It has also been shown that 2-aminoethoxydiphenylborane (2-APB), which is both an antagonist at IP3 receptors and an inhibitor of TRP ion channels, is an extremely potent *in vitro* inhibitor of the light responses of pRGCs and that its effect is independent of store-dependent  $Ca^{2+}$  release [55]. Significantly, 2-APB is also effective *in vivo*, where it induces an acute knock-down of the pupillary light reflex, consistent with the silencing of melanopsin-dependent light detection [55].

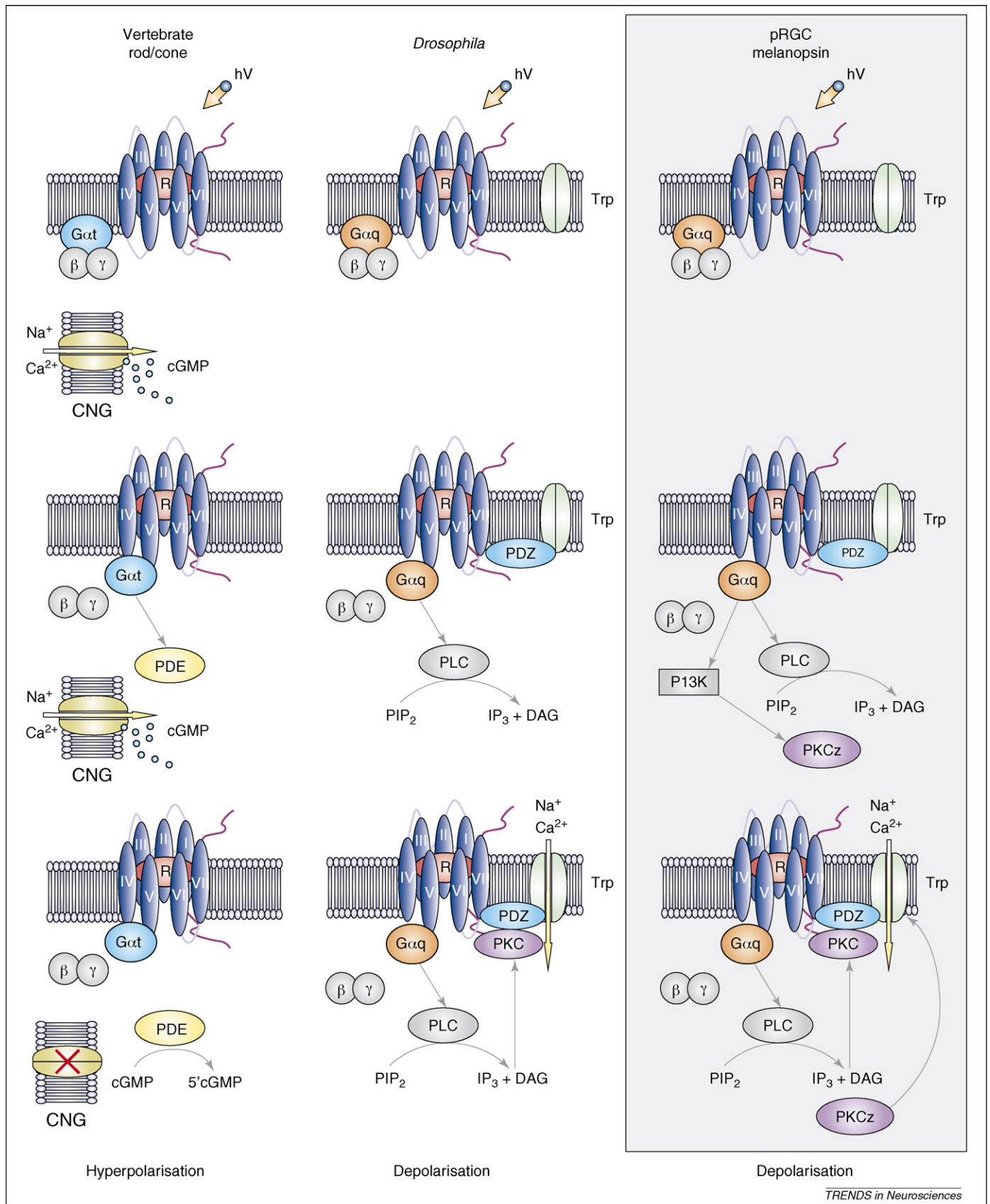
### A working model of melanopsin phototransduction

Based upon both expression studies and pharmacological approaches, an outline model of the melanopsin phototransduction cascade can be devised (Figure 4, right). Light-activated melanopsin seems to interact with  $G_q/G_{11}$ , which in turn activates a PLC- $\beta$ . PLC- $\beta$  generates  $Ins(1,4,5)P_3$  and diacylglycerol (DAG), which may ultimately modulate a TRPC6 or TRPC7 channel, possibly via a PKC. However, given the limits of the experimental data, virtually none of these components are confirmed beyond reasonable doubt. Furthermore, unlike the rod/cone and *Drosophila* cascades, we have virtually no knowledge regarding the likely modulatory and adaptation-dependent regulation of melanopsin signalling. Similarly, the deactivation stages of light current and receptor-specific issues such as potential phosphorylation sites wait to be explored.

This model of melanopsin phototransduction can be augmented substantially by very recent microarray-based approaches [56]. These experiments utilizing the *rd/rd cl* mouse demonstrated that ~30% of the ocular transcriptome is transiently regulated in response to nocturnal light exposure. From a selection of genes, laser capture microdissection demonstrated that *Gnas*, *Gnb2l1*, *Gnaq*, *Prkcz*, *Pik3r1*, *Inadl*, *Slc9a3r1* and *Drd1a* colocalized with melanopsin. The impact of genetic ablation of one of these genes, protein kinase C zeta (*Prkcz*), was assessed. Quite remarkably, *Prkcz*<sup>-/-</sup> mice were found to precisely phenocopy the extensively characterized melanopsin-ablated transgenic lines. They show attenuated circadian phase-shifting responses to light, reduced period lengthening under constant light and attenuated pupillary responses at high irradiances, as well as impaired light-induced gene expression in the suprachiasmatic nuclei (Figure 5). By analogy to the *Drosophila* phototransduction cascade, *Prkcz* may influence TRP ion-channel activity via participation in an INAD-like signalling complex (including PLC-, PKC-, and PDZ-domain-containing scaffolding proteins; Figure 4, right). Alternatively, it could act by regulating the activity

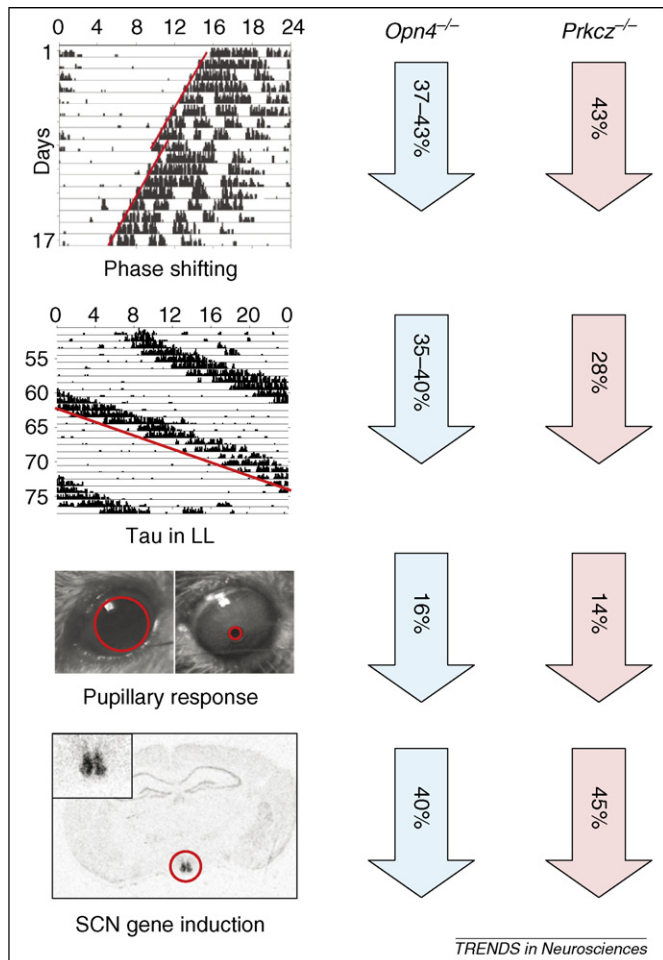
melanopsins (right column). Shaded regions show the consensus LPV sequence within each opsin class, highlighting the remarkable degree of conservation between species.  $\lambda_{max}$  values for the various pigments are taken from [43]. Consensus sequences for the various opsin classes (lower right) are shown. Grey shading illustrates the amino acid identity of the LPV with respect to the rod opsin consensus. GenBank accession numbers are as follows: *Anolis* RH1, L31503; Chicken Rod, D00702; Human Rod, U49742; Frog Rod, S79840; Goldfish Rod, L11863; *Anolis* RH2, AF134191; Chicken Green, M92038; Gecko P467, M92035; Goldfish Green1, L11865; *Anolis* SWS2, AF133907; Chicken Blue, M92037; Frog Green Rod, AB010085; Goldfish Blue, L11864; Budgerigar UV, Y11787; Chicken Violet, M92039; Mouse Blue, U92562; Human Blue, U53874; Salamander UV, AF038948; *Xenopus* Violet, U23463; Goldfish UV, D85863; Goldfish Red, L11867; *Xenopus* Red, U90895; Chicken Red, M62903; Human Green, K03494; Human Red, M13305; Gecko P521, M92036; *Xenopus* *Opn4x*, AF014797; Chicken *Opn4x*, AY036061; Cod *Opn4x-a*, AF385823; Cod *Opn4x-b*, AY126448; *Xenopus* *Opn4m*, DQ384639; Chicken *Opn4m*, AY882944; ZF *Opn4m1*, AY882945; ZF *Opn4m2*, AY078161; Mouse *Opn4m*, AF147789; Rat *Opn4m*, NM\_138860; Hamster *Opn4m*, Q5XXP2; Dunnart *Opn4m*, DQ383281; Cat *Opn4m*, NM\_001009325; Dog *Opn4m*, XM\_848642; Bovine *Opn4m*, XM\_593123; Chimp *Opn4m*, XM\_001135445; Human *Opn4m* isoform 1, NM\_033282; Human *Opn4m* isoform 2, NM\_001030015.





**Figure 4.** Phototransduction cascades. Comparison of the proposed phototransduction cascades associated with opsin-dependent light detection in a vertebrate (rod/cone), invertebrate (*Drosophila*) and in mammalian pRGCs. Detailed discussion of these cascades is provided in the text. In rods and cones, cells are depolarized in darkness and the effect of light is to close a channel in the plasma membrane resulting in hyperpolarisation. By contrast, in both *Drosophila* and pRGCs, light is coupled to the opening of a nonspecific cation channel that leads to cellular depolarisation. In each case, opsin photopigments are G-protein-coupled receptors. A photon of light ( $h\nu$ ) is absorbed by the chromophore, leading to a conformational change in the opsin. This leads to the activation of a multimeric G protein composed of an  $\alpha$ ,  $\beta$  and  $\gamma$  subunit. The  $\alpha$  subunit in the rod/cones is transducin (G $\alpha$ t); in *Drosophila* is G $\alpha$ q; and in the pRGCs it is believed to be G $\alpha$ q/11. Abbreviations: CNG, cyclic nucleotide gated channel; TRPC, transient





**Figure 5.** Protein kinase C zeta. Deletion of the *Prkcz* gene results in a comprehensive phenocopy of the *Opn4*<sup>-/-</sup> mouse, demonstrating that this gene is critical for melanopsin signalling. *Prkcz*<sup>-/-</sup> animals show attenuated phase-shifting responses to light and period lengthening under constant light (LL). These animals also show attenuated pupil responses to bright light but normal responses to lower-intensity stimuli. Finally, gene induction in the SCN in response to light is also reduced. The deficits in *Prkcz*<sup>-/-</sup> mice mirror those of *Opn4*<sup>-/-</sup> animals, suggesting that the lesion occurs at the level of the pRGCs. Data are taken from [56].

of some other critical component of the melanopsin signalling cascade.

### Conclusions and future studies

The study of the irradiance signalling system of the mammalian retina has advanced dramatically over a relatively short period of time. However, several critical areas remain only poorly understood. Although there is a general consensus regarding pRGC-dependent action spectra, there are some significant disparities in the spectral sensitivity of heterologous expressed melanopsin. The basis for this difference is obscure and classical spectroscopic analysis of the melanopsin photopigment is urgently required. We also know that non-mammalian vertebrates possess two melanopsin genes and that each encodes a fully functional photopigment [44]. Their differential roles

and the selection pressures that led to the loss of one of these genes in mammals remain entirely unclear. Previous predictions of melanopsin function based upon its sequence have suggested that this opsin family is only poorly conserved. This in turn led to the suggestion that melanopsins may subserve several different roles [43]. However, when the sequence analysis is restricted to the predicted ligand binding pocket, the results suggest that the apparent diversity has been considerably overestimated.

Clear parallels are also emerging between melanopsin and invertebrate phototransduction cascades; however, it would be unwise, based on analogy alone, to assume that the two systems map precisely onto one another. Our current knowledge of the cascade remains a skeleton work in progress. Microarray-based techniques have generated some additional candidates, but validating these genes in the absence of robust pharmacological tools remains a serious challenge. Although these issues can be partially approached with transgenesis and gene ablation, the study of this complex system will surely benefit from the application of *in vivo* RNA-interference approaches.

Finally, there remain many largely unexplored questions that relate to the modulatory and regulatory steps associated with melanopsin phototransduction. Insight into this sphere may possibly explain some of the unique properties of melanopsin signalling, including its relative insensitivity to light and a remarkable capacity to integrate photons over many minutes [57].

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