

Review

Role of a novel photopigment, melanopsin, in behavioral adaptation to light

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Abstract. Adaptation to changes in the ambient light is of critical importance to life. In mammals, three principal photoadaptation mechanisms depend on ocular photoreception and exhibit spectral sensitivity suggestive of the opsin class of photopigment(s). These include rapid adaptation of the visual system to the ambient light by pupil constriction, direct modulation of neuroendocrine function and entrainment of the circadian clock to the day:night cycle. Surprisingly, these processes can largely function independent of classical rod/cone photorecep-

tors, suggesting a novel opsin-based signaling mechanism. They appear to involve a recently discovered network of intrinsically photosensitive retinal ganglion cells that make direct or indirect axonal connections to brain centers regulating photoadaptive behaviors. The discovery of a novel opsin, melanopsin, in these cells has offered an exciting entry point to explore, at the molecular level, how mammals adapt to their light environment. There is now genetic proof of a principal role for melanopsin in all three major photoadaptation processes.

Keywords. Melanopsin, circadian rhythm, SCN, phototransduction.

Introduction

Photoadaptation in mammals consists of three predominant types: control of retinal illumination by pupillary light reflex (PLR), direct light modulation of the neuroendocrine system leading to modulation of sleep or activity, and entrainment of the circadian timekeeping system to the ambient light:dark cycle. Intriguingly, photoadaptive responses are left intact or are only partially attenuated in many human patients and in several animal models with complete loss of rod/cone function [1, 2]. Experimental bilateral enucleation in animals, however, abolishes all ocular light responses, suggesting participation of a novel class of ocular photoreceptor(s) [3]. Until recently the

photoreceptor(s) have remained elusive. Efforts to understand circadian photoentrainment have led to the discovery of a small population of intrinsically photosensitive retinal ganglion cells (ipRGCs) expressing a novel opsin photopigment, melanopsin (Opn4) [4]. This finding has triggered a series of studies that have yielded a wealth of knowledge about adaptive photic responses in the vertebrate retina. In this review we will summarize the discovery of melanopsin and ipRGCs from a historical perspective, the functional properties of ipRGCs and genetic proof for the role of melanopsin in adaptive photoresponses mediated by ipRGCs. Furthermore, we will discuss the current state of knowledge regarding the photochemical properties of melanopsin, the melanopsin phototransduction pathway and the evolutionary conservation of melanopsin function in photoadaptation in vertebrates.

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Circadian rhythms and the search for a novel photoreceptor

Circadian rhythms in mammals are near 24-h cycles in behavior and physiology that are regulated by an endogenous oscillator resident in 10,000–20,000 neurons of the hypothalamic suprachiasmatic nucleus (SCN) [5]. When animals are maintained under constant darkness, the SCN-generated behavioral rhythms persist or ‘free-run’ with a periodicity that is slightly different from that of the planetary 24-h day:night cycle [6]. Such a discrepancy between the intrinsic circadian period length and the planetary day:night cycle of 24 h necessitates daily resetting of the oscillator to the ambient 24-h light:dark cycle. Additional resetting is necessary for adjustment of the phase of the oscillator with the seasonal changes in day length. Chronic deficiency in the mechanism of entrainment is presumed to underlie several circadian-related disorders, such as shift-work syndrome, seasonal affective disorders and depression.

Ocular photoreception serves as the major entraining stimulus for the SCN oscillator [3]; however, intact circadian photoentrainment in outer retinal rod/cone-deficient rodents suggested a role for a novel inner retina photoreceptor(s). Additional observations supported this possibility and alluded to the characteristics of the suggested new photoreceptor. For instance, the action spectra of circadian photoentrainment and PLR in rodents can be fit to an opsin nomogram with peak sensitivity around 480 nm [7, 8], which is distinct from the absorption spectra of rod and cone opsins. Physiological and neuroanatomic studies further unraveled crucial spectroscopic and cellular properties of the inner retinal photoreceptor(s). Partial optic tract ablation studies revealed that a small number of RGCs project directly to hypothalamic regions, including the SCN, and constitute what is termed as the retinohypothalamic tract (RHT) [9]. The RGCs constituting the RHT contain glutamate and the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) [10, 11]. PACAP and glutamate are potent mediators of circadian entrainment of the SCN [12, 13], and PACAP is expressed exclusively in RHT RGCs [10]. Furthermore, PACAP-containing RGCs exhibit *c-fos* immunoreactivity in response to light [14]. Hence, the putative photopigment(s) was predicted to be an opsin-like molecule with a peak absorbance near 480 nm and either was expressed in the PACAP-positive cells or in cells which make synaptic contact with them.

A detour through amphibians

A candidate for the inner retinal photoreceptor emerged from studies of amphibian skin melanosomes, which exhibit exquisite photosensitivity. Under bright light the melanosomes disperse and function as a natural sunscreen.

The action spectrum of this response matches closely with an opsin nomogram [15]. Provencio et al. screened for opsin-like molecules in *Xenopus laevis* melanophores and were able to identify a novel opsin, which they named melanopsin [16]. *Xenopus* melanopsin is also found in the brain and inner retina. A mammalian melanopsin ortholog was soon identified in mice and was shown to be expressed in a few retinal ganglion cells of the inner retina [17].

Intrinsically photosensitive RGCs express melanopsin

Berson et al. hypothesized that the SCN-innervating RGCs would be intrinsically photosensitive. To test their hypothesis, they identified the SCN-projecting RGCs by retrograde labeling and made whole-cell recordings from these neurons [18]. In a breakthrough discovery, the labeled neurons responded to illumination with large depolarization and superimposed fast action potentials. The light responses were resistant to synaptic blockade, and persist in physically isolated labeled neurons, implying the RGCs are intrinsically photosensitive (ip). The ipRGCs turned out to be the same RGCs that express melanopsin [4].

Photoresponses of the melanopsin-containing ipRGCs exhibit some unique properties. At threshold illumination intensity, the ipRGCs exhibit extremely long response latency [4, 18–20]. With increasing light intensity, there is a corresponding decrease in response latency and an increase in the magnitude of depolarizing current. Coupled with the observation that ipRGCs could be repeatedly photoactivated without any significant bleaching or supplementation of exogenous retinoid, these features imply that ipRGCs tonically encode light intensity. Their sluggish response especially at low illumination levels is highly consistent with observations that circadian photoentrainment is far less sensitive than normal vision, requiring seconds to several minutes of illumination for clock resetting. Importantly, the action spectra of light responses in the ipRGCs closely match those of circadian photoentrainment.

Interestingly, ipRGC photosensitivity exhibits both light and dark adaptation. Under constant illumination the background photoresponse gradually decays, while the response to superimposed light flashes increases. After dark incubation, the ipRGCs regain full light sensitivity [21]. Such adaptations may further fine-tune organisms’ ability to sense changing light conditions.

Ontogeny, anatomy and heterogeneity of ipRGCs

Melanopsin immunostaining in mice is almost entirely restricted to the ipRGCs. The protein is detected from

embryonic day 18 [22], and light responses in ipRGCs are already evident at birth (P0) [23, 24], long before detectable rod/cone photosensitivity first appears on postnatal day P10 [25]. Any functional significance of such early photosensitivity in melanopsin-containing cells is currently unknown, but could reflect an adaptive advantage to circadian photoentrainment at a time when vision is unnecessary.

The distribution of melanopsin-staining cells in adult rodents and human is almost uniform across the retina [4, 26, 27], although, in nonhuman primates, sparse presence in the fovea has been reported [28]. The somata of melanopsin-positive RGCs in rodents are roughly 15 μm in diameter, intermediate in diameter among RGCs [29] and have sparsely branching dendrites that are relatively long (up to several hundred microns) [4, 18, 26]. Most of the dendrites arborize and terminate in the OFF sublayer of the inner plexiform layer (IPL) [18, 19]. The dendritic fields of these RGCs have an average diameter of 500 μm , and melanopsin immunoreactivity is found throughout the dendrites, soma and axons [18, 19]. Thus, despite the limited expression of melanopsin in only 1–2% of RGCs, these RGCs form a diffuse photosensitive web that covers virtually the entire retina [26].

The subcellular localization of melanopsin in ipRGCs differs strikingly from that of image-forming photoreceptors. In both vertebrate rods/cones and invertebrate rhabdomeric photoreceptors, the photopigment and signal transduction machinery is concentrated in highly specialized membrane structures (reviewed in [30, 31]), likely facilitating enhanced photosensitivity and rapid response. In contrast, melanopsin immunostaining is almost uniformly distributed throughout the plasma membrane of the dendrites, soma and axons [26, 32].

The use of multielectrode array recording to simultaneously measure electrical activity from a population of ipRGCs has uncovered a fascinating heterogeneity in murine ipRGC populations with respect to speed of onset, sensitivity and offset kinetics [20]. In newborn mice, three major subtypes of ipRGCs are identified up to postnatal day 8: (i) slow onset, sensitive, fast off (type I); (ii) slow onset, insensitive, slow off (type II); and (iii) rapid onset, sensitive, very slow off (type III). As the mice age, the number of melanopsin-positive cells decline, and the heterogeneity also changes. Type I cells are almost undetectable in adult retina, while type II and type III cells become predominant. Photoresponses in all three types of ipRGCs are mediated by melanopsin and exhibit peak sensitivity ~ 480 nm. The cause and functional significance of ipRGC heterogeneity is currently unknown. A possible mechanism for heterogeneity in activation and deactivation kinetics may lie in the downstream signaling pathway. Therefore, a better understanding of the melanopsin phototransduction pathway is necessary to understand how melanopsin can mediate diverse responses in distinct cells.

Projections of the ipRGCs

The projections of the ipRGCs have been studied in rodents using reporter transgenic mice [4], the fortuitous tropism of the AAV2 serotype of adeno-associated virus for melanopsin-containing RGCs [33] and from immunostaining for axonal projections of PACAP-expressing RGCs [34]. Results from these experiments have been extensively discussed in a recent manuscript [35] and a review [36], and will only be summarized here. While most melanopsin-positive RGCs project to the SCN (Fig. 1), some also project to hypothalamic and thalamic regions such as the ventrolateral preoptic (VLPO) nuclei regulating sleep, the olivary pretectal nucleus (OPN) regulating pupil constriction and lateral geniculate nucleus (LGN) [4, 28, 33, 34]. Although these projections have only been extensively studied in nocturnal rodents, many are expected to be conserved in diurnal animals, with a few small differences. For example, the direct contact between ipRGCs and the sleep-active neurons of the VLPO may explain the sleep-promoting effect of light in nocturnal animals [33]. However, light is known to enhance alertness in diurnal animals, and it will be worth examining the nature of contact between melanopsin-RGCs and sleep-active neurons of the VLPO in diurnal animals. In summary, melanopsin-containing ipRGCs predominantly project to brain regions regulating photoadaptive responses, and hence the neuroanatomy supports the genetic proof (discussed below) for its role in these responses.

Melanopsin and rod/cone photoreceptors mediate adaptive photoresponses

Mouse genetics has established the role of melanopsin in adaptive photoresponses. The circadian activity rhythm of melanopsin-deficient (*opn4*^{−/−}) mice exhibits a reduced sensitivity to light. Specifically, the phase of circadian activity in *opn4*^{−/−} mice is less perturbed by a brief pulse of light than that in wild-type (wt) littermates [37, 38]. Other adaptive photoresponses, such as PLR and light suppression of activity, are significantly attenuated in *opn4*^{−/−} mice under specific irradiance conditions. For example, at low to intermediate fluence level, no significant difference in pupil constriction is observed between WT and *opn4*^{−/−} mice. However, at high irradiance level the pupil of *opn4*^{−/−} mice is less constricted than that in the WT mice [39]. Similarly, *opn4*^{−/−} mice exhibit less suppression of activity by light (negative masking) under prolonged illumination [40]. For the first few minutes of illumination during nighttime, both WT and *opn4*^{−/−} mice usually reduce their overall activity. However, during extended illumination, the *opn4*^{−/−} mice regain activity, and 2 h into illumination, they have almost 50% of the activity of mice housed in the dark. Activity of WT mice is

suppressed during the entire period of illumination. This demonstrates a unique role for melanopsin in long-term light adaptation [40] and possibly for promoting sleep in nocturnal animals. Importantly, attenuation of photoadaptive responses in *opn4*^{-/-} mice correlates with the loss of intrinsic photosensitivity of the ipRGCs, without any apparent effects on the overall anatomy and distribution of these cells [39].

The residual photoadaptive responses in *opn4*^{-/-} mice raised an additional set of questions. Is there another putative photosensor, such as cryptochrome in the inner retina [41], or does light information collected by rods and cones account for the residual responses? Mice with outer retina degeneration or with genetic ablation of rod/cone function exhibit intact photoentrainment of the circadian clock [42, 43]. However, mice deficient in both rod/cone and melanopsin function lose all ocular photoresponses. They fail to entrain their activity to imposed light/dark cycles, and free-run with a <24-h periodicity, as if they were under constant darkness. Other adaptive photoresponses, notably PLR, light suppression of pineal melatonin synthesis enzymes and light suppression of activity, are completely abolished in these mice [44, 45]. Such genetic models conclusively established that both rod/cone photoreceptors and melanopsin account for all ocular light responses in mammals. These experiments also point to important differences in the relative contribution of the rod/cone and melanopsin phototransduction pathways to photoadaptive responses at various fluence levels and length of illumination.

Photoresponses from the rod/cone photoreceptors and the ipRGCs have significant differences with regard to latency to activation, deactivation kinetics and bleaching. These differences are reflected in their relative contributions to various photoadaptive processes. Two hallmark features of ipRGC photosensitivity are in stark contrast to rod/cone photoreceptors: long latency to photoactivation and resistance to bleaching [18]. This allows for faithful reporting of the length and intensity of illumination while buffering the system from low levels of illumination noise. Therefore, melanopsin's roles in physiological processes are most evident at high light intensity or under prolonged illumination. Conversely, the contribution of rod/cone pigments is most evident at low irradiance and/or rapid responses. For example, a robust circadian oscillator requires protection from occasional photic noises, such as lightning and moonlight. Accordingly, melanopsin plays a dominant role in circadian photoentrainment. In contrast, the pupillary light reflex requires a rapid response that is well matched to the sensitivity and speed of the rod/cone pathway. Thus, the contribution of melanopsin to pupillary light reflex is more subtle. While the loss of rod/cone photopigments cause ~1.5 log unit reduction in sensitivity of pupil constriction over low to intermediate light intensity [8], loss of melanopsin causes

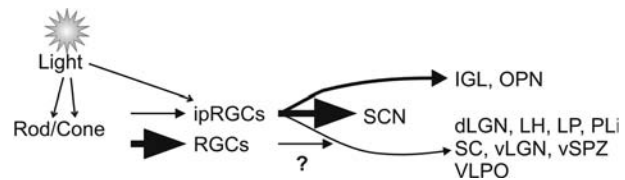


Figure 1. Neuroanatomic circuitry of melanopsin-containing RGCs. Only a small subset of RGCs is intrinsically photosensitive and expresses melanopsin. These cells also receive both rod and cone cell input. A majority of melanopsin-containing RGCs (ipRGCs) project to the hypothalamic circadian center suprachiasmatic nucleus (SCN), but significant projections to intergeniculate leaflet (IGL) and olivary pretectal nucleus (OPN) also exist. A few ipRGCs send axons to the dorsal lateral geniculate nucleus (dLGN), lateral hypothalamus (LH), lateral posterior thalamic nucleus (LP), posterior limitans thalamic nucleus (PLi), superior colliculus (SC), ventral lateral geniculate nucleus (vLGN), ventral subparaventricular zone (vSPZ) and ventrolateral preoptic nucleus (VLPO). Thickness of the arrows roughly implies the relative preponderance of the neuronal connection.

only a small loss of photosensitivity at very high intensity light [39, 45].

ipRGCs as potential site of signal integration

The complementary contribution of both the rod/cone and the melanopsin pathways to photoadaptive responses suggest that signals from the two pathways must be integrated at the cellular and molecular level at some stage of processing. Although integration at the target brain region is highly likely, the properties of melanopsin-containing ipRGCs are well suited to be an important hub for signal integration. The ipRGCs constitute the dominant portion of efferent projections to several brain centers regulating adaptive behaviors [33, 35], and there is growing evidence indicating ipRGCs do receive input from the rod/cone pathway (Fig. 1). Immunoelectron micrographs of the mouse retina stained for melanopsin have indicated that ipRGCs receive synaptic contacts from both rod and cone bipolar cells [32]. Intracellular recordings from ipRGCs of rodents and primates also exhibit signs of synaptic input from the rod/cone pathway [28, 46]. Targeted ablation or ‘electrical silencing’ of the ipRGCs will potentially answer how much signal integration occurs in these cells. If the ipRGCs constitute the major conduit for transmission of rod/cone-initiated photic signal to brain regions involved in photoadaptation, it raises further questions about how these synaptic signals are integrated with intrinsic melanopsin-based signals. Electrical signals generated by the two pathways could simply be additive, or there could be significant cross-talk at the molecular level. Exploring the latter possibility requires a much greater understanding of the signaling events that occur downstream of melanopsin activation.

Melanopsin exhibits functional similarity to invertebrate opsins

Several properties of the photosensitivity of ipRGCs and features of the melanopsin sequence (Fig. 2) suggest the melanopsin phototransduction pathway may be similar to that of invertebrate opsins. The molecular basis of how photoactivation leads to changes in membrane potential is well understood for both vertebrate rod/cone and invertebrate opsins such as the dominant *Drosophila* visual photopigment Rh1 opsin. In vertebrate rods and cones, photoactivated opsins activate photoreceptor-specific members of the pertussis toxin-sensitive $G\alpha_i/G\alpha_o$ class of G-proteins called transducins ($G\alpha_t$). Transducins, in turn, activate phosphodiesterases, which degrade cyclic nucleotides, primarily cyclic GMP (cGMP). This leads to the closure of cyclic nucleotide-gated cation channels. These nonselective cation channels endow rods and cones with a tonically depolarized resting potential; photoactivation of opsins thus leads to hyperpolarization (reviewed in [47]). In contrast, invertebrate opsins activate the pertussis toxin-insensitive $G\alpha_q$ pathway, leading to PLC activation and the production of diacylglycerol (DAG). DAG or a metabolite is the likely messenger which activates TRP family non-selective cation channels. The resultant increase in cation permeability leads to photoreceptor depolarization (Fig. 3 and reviewed in [31]). As discussed previously, ipRGCs depolarize in response to light, suggesting similarity to the invertebrate photoresponse [18, 31]. Furthermore, voltage clamp of ipRGCs has revealed that the light-activated current is a nonselective cation channel that could possibly be generated by (a) TRP family member(s) [19].

Melanopsin shares greater sequence similarity with *Drosophila* Rh1 opsin than with mammalian rod/cone opsins (Fig. 2). Notably, melanopsin contains invertebrate-like sequence signatures in regions presumed to be important in G-protein binding and activation. The cytoplasmic loops of rhodopsin have been found to play important roles in the activation of the G-protein [48–50], and melanopsin is highly similar to Rh1 in these regions (Fig. 2). Melanopsin has an unusual insertion in the third loop that is reminiscent of many GPCRs shown to signal through $G\alpha_q$ [51, 52]. Combined with the photocurrent properties of the ipRGCs, these sequence features suggested that melanopsin-based phototransduction in vertebrates might have functional ties to invertebrate vision.

Candidate signaling mechanisms identified by heterologous expression of melanopsin

Given the scarcity of melanopsin-expressing RGCs, molecular characterization of the native phototransduction pathway in mammals still faces overwhelming obstacles. Thus ectopic expression of melanopsin in mammalian

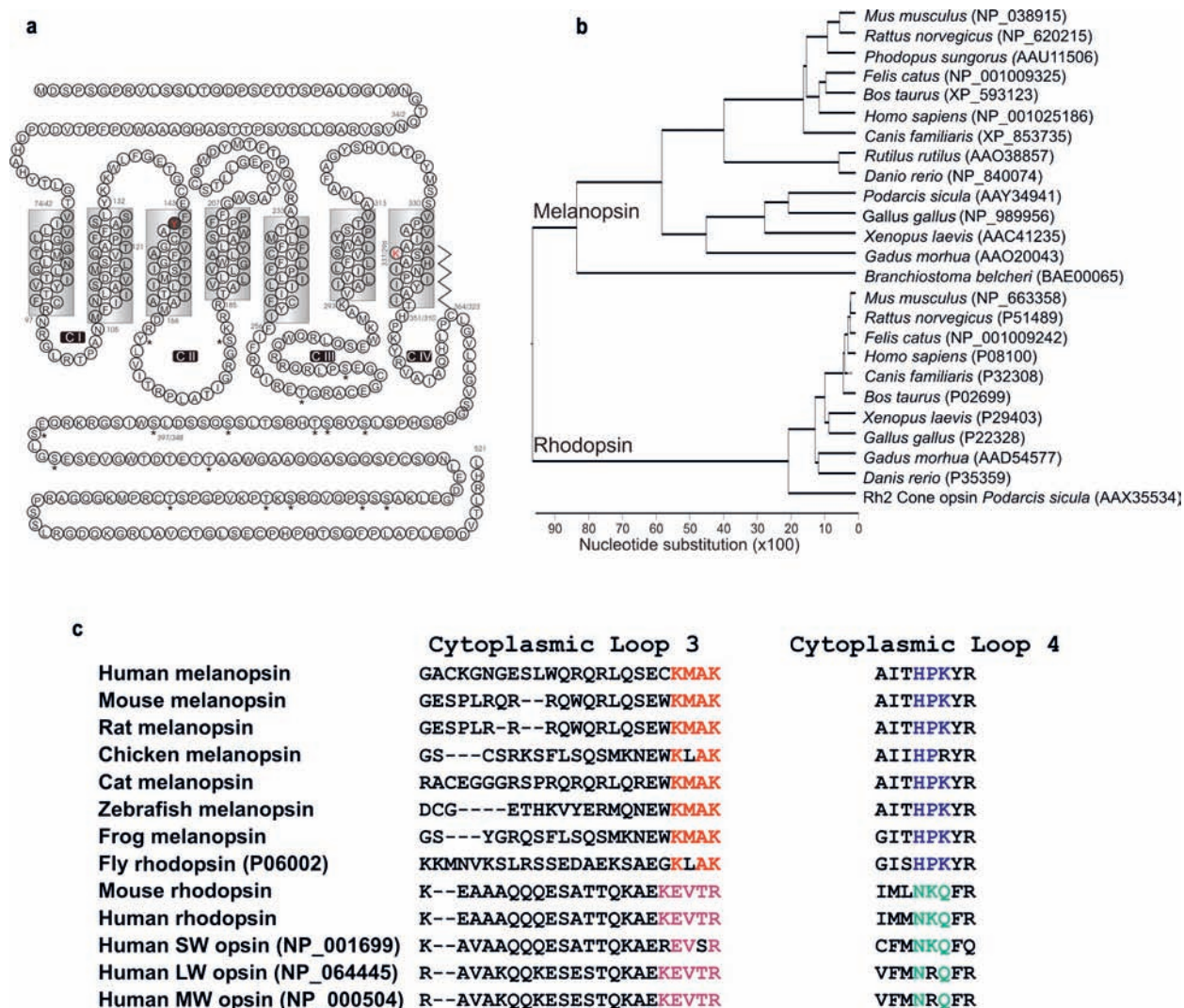
cells and *Xenopus* oocytes has been invaluable in characterizing its function. Studies of melanopsin in *Xenopus* melanophores, where it was first discovered, have also proved useful. Melanopsin photopigment, expressed and purified from COS cells, exhibits a typical opsin absorbance spectra and can activate a G-protein [53]. This initial experiment indicated direct photoexcitation by melanopsin and its ability to activate a G-protein in a cell-free system. The nature of the effector G-protein and the downstream signaling cascade, however, seems to be dependent on the cell type and the experimental conditions.

Photoresponses in *Xenopus* melanophores are mediated by a pertussis toxin-insensitive G-protein which activates phospholipase C- β and ultimately causes an increase in intracellular calcium levels [54]. This implies involvement of a $G\alpha_q$ -class of G-proteins. Similarly, photoactivated mouse melanopsin expressed in *Xenopus* oocytes or HEK293 cells can trigger activation of the $G\alpha_q/G\alpha_{11}$ class of G-proteins and PLC- β [55, 56]. However, photocurrent generated by ectopically expressed human melanopsin in the Neura-2a cell line is resistant to blockade by a PLC inhibitor; instead, cGMP and intracellular calcium release are suggested as possible messengers [57]. It is likely that the relative expression level of various G-proteins and other cellular factors can influence the downstream signaling cascade in heterologous expression systems.

Heterologous expression systems have also been a useful tool to test which candidate channels can be activated by melanopsin photoactivation. Coexpression of mouse melanopsin and TRPC3, a vertebrate paralog of the *Drosophila* phototransduction channels TRP and TRPL, in HEK293 cells or in *Xenopus* oocytes produces depolarizing photocurrent similar to that of ipRGCs [55, 56]. More distantly related TRP channels, including TRPV1 and TRPA1, were not activated [56]. In summary, a majority of these studies indicate the melanopsin phototransduction pathway may function similarly to that of *Drosophila* Rh1 opsin. However, many disagreements exist on the specific molecular pathways. Ultimately, genetic ablation of candidate components may be required to persuasively establish the exact nature of the phototransduction mechanism.

Photochemical and spectral properties of melanopsin

The action spectra of several melanopsin-mediated responses in mammals, and of photoresponses from ipRGCs, can be fit to an opsin nomogram with peak sensitivity around 480 nm. This implies melanopsin might use a retinal-based chromophore and exhibit absorption spectra with peak sensitivity ~480 nm. Melanopsin photoresponses in some heterologous expression systems are



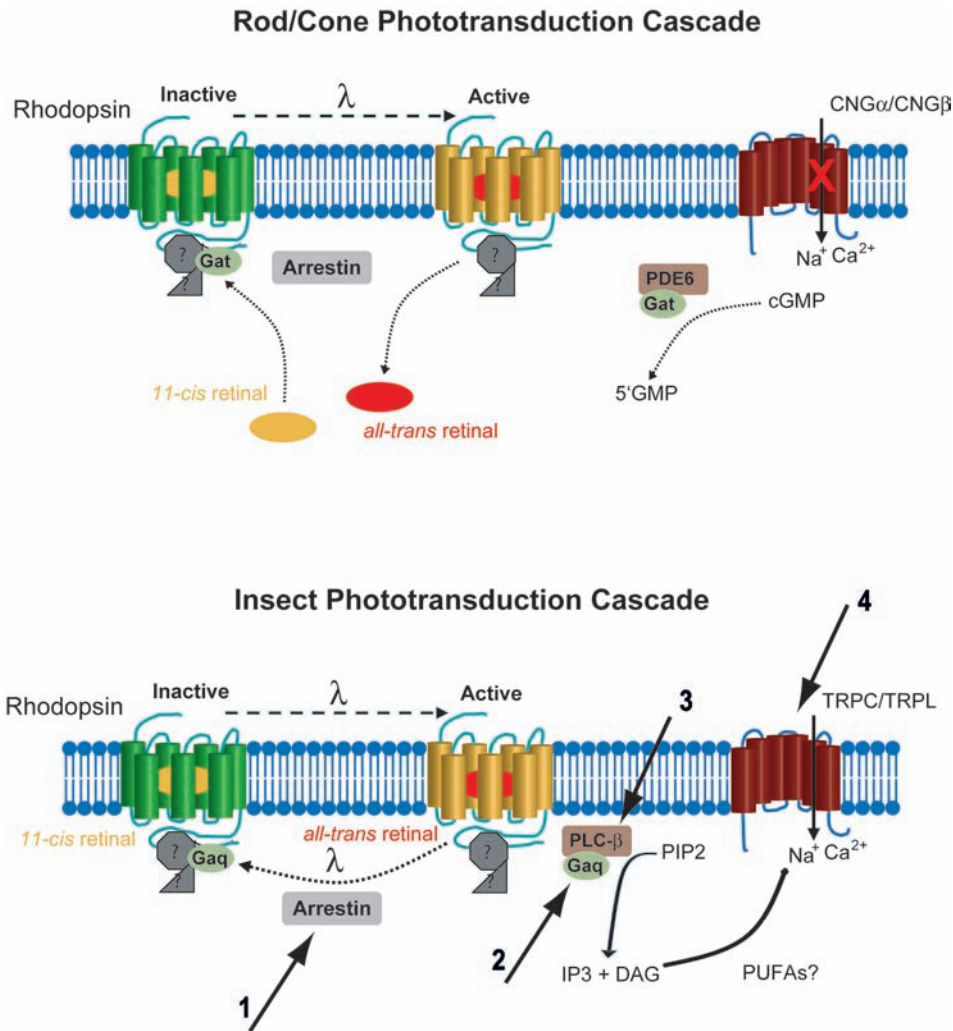


Figure 3. Phototransduction cascades of vertebrate and insect image-forming photoreceptors. The vertebrate rod/cone cell phototransduction cascade is shown at the top. Light (λ) activates rod/cone opsins through isomerization of the 11-*cis*-retinal chromophore to the all-*trans* form and releases transducin (G α_t), a Gi/Go-class G-protein α -subunit. Transducin, in turn, activates a phosphodiesterase (PDE6 β), leading to rapid degradation of cGMP and subsequent closing of cyclic nucleotide-gated cation channels (CNG α /CNG β). The reduction in cation influx causes hyperpolarization of the photoreceptor. Deactivation of the opsin involves binding of arrestin and possibly exchange of the all-*trans* retinal chromophore for 11-*cis*-retinal. The insect rhabdomeric photoreceptor transduction cascade is shown in the bottom panel. In this case, opsins activate G α_q -family G-proteins, leading to phospholipase C (PLC) activation, and production of inositol triphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate (PIP2). DAG or a metabolite polyunsaturated fatty acid (PUFA) then activates TRP channels leading to cation influx and depolarization. Arrestin mediates deactivation of rhodopsin and likely plays a key role in photoisomerization of the all-*trans*-retinal chromophore to the 11-*cis* form. Arrows 1–4 indicate steps of the insect phototransduction cascade that have been implicated in melanopsin signaling: Gq-activation, PLC activation, TRP channel activation and arrestin-dependent deactivation.

in *Xenopus* oocytes large photocurrents could also be induced with all-*trans*-retinal by coexpression of melanopsin with arrestin [56]. In *Drosophila*, arrestin is presumed to promote photoisomerization of the opsin-all-*trans*-retinal complex back to the photoactivable 11-*cis*-retinal complex by an unknown mechanism. If a similar mechanism is conserved in melanopsin function, it may explain the ability of the ipRGCs to be repeatedly photoactivated without addition of supplemental retinal.

The spectral property of ectopically expressed melanopsin shows some variability. COS-cell-expressed melanopsin exhibits absorption spectra with peak sensitivity around 420 nm [53] upon purification and reconstitution with 11-*cis* retinal. Similarly, photoresponses from Neura-2a cells expressing human melanopsin is blue shifted to 380–420 nm when 11-*cis* retinal is used as a chromophore [57]. However, the peak sensitivity of photoresponses from HEK293 cells or from *Xenopus* oocytes expressing melanopsin and supplemented with 11-*cis* ret-

inal is around 480 nm [55]. It is likely that the variation in spectral property in different experiments may reflect different spectral states of melanopsin or differences in experimental conditions.

In summary, these experiments highlight the challenges and difficulties in characterizing the photochemical properties of melanopsin, identifying the native chromophore of melanopsin and how this chromophore is delivered and regenerated in the ipRGCs.

Regulation of melanopsin expression

The developmental programs for ipRGC specificity or the transcription factors regulating melanopsin expression are yet to be understood. However, observations in adult rodents suggest the potential for complex species-specific regulation of melanopsin expression by light and circadian rhythm. While in mice no significant change in melanopsin expression in the presence or absence of functional outer retinal photopigment has been reported, in rats melanopsin expression is both circadian and light regulated. It is dependent on functional outer retinal photopigments. Rat melanopsin messenger RNA (mRNA) exhibits rhythmic expression both under light:dark cycle and under constant darkness, with its peak level during the subjective evening. Under prolonged darkness, rat melanopsin expression gradually increases for up to 5 days, with immunostaining progressively accumulating in the distal dendrites. Conversely, constant illumination causes gradual loss of melanopsin staining from the distal dendrites [61]. Intriguingly, in rats with progressive photoreceptor degeneration, melanopsin mRNA and protein levels gradually decline and become almost undetectable, even though expression of the ganglion cell marker Thy1 and the ipRGC marker PACAP are unaffected [62]. A possible mechanism for rod/cone regulation of melanopsin expression is via dopamine signaling. Rod and cone photoreceptors regulate dopamine synthesis and release in the retina, and dopamine in turn upregulates melanopsin expression [63]. However, under constant light, the loss of melanopsin staining may be mediated by arrestin dependent internalization and degradation of GPCRs [64]. The functional significance of the regulation of melanopsin expression in rats on behavioral adaptation is yet to be analyzed in detail, and it is not yet understood how melanopsin expression is regulated in primates.

Melanopsin in nonmammalian vertebrates

Expression of melanopsin in several tissue types in amphibians and birds differs from mammals, but still implies a conserved role in photoadaptation. In *Xenopus*, melanopsin mRNA is detected in photosensitive melano-

phores and the iris, where it is presumed to modulate light adaptation by melanosome migration and iris constriction, respectively [16]. Melanopsin expression extends into brain areas involved in photic function in both *Xenopus* and chicken. Significant RNA expression is detected in hypothalamic regions of *Xenopus* tadpoles, including magnocellular cells of the preoptic nucleus and in some cells of the SCN [16]. In chicken, melanopsin expression is detected in several thalamic and hypothalamic nuclei, most notably in the pineal gland [65, 66], which harbors a major circadian oscillator, and is intrinsically photosensitive. Some of the pineal photoresponses in chicken are pertussis toxin-sensitive, while others are insensitive [67]. It is likely that these two types of responses are mediated by two different opsins, pinopsin [68] and perhaps melanopsin, respectively. Despite the significant differences in the extent of melanopsin expression among mammals, birds and amphibians, strong colocalization of melanopsin with circadian oscillator or light input tissue in animals suggests an evolutionary conservation of melanopsin as a circadian photopigment.

Although electrophysiological characterization of melanopsin-expressing cells in nonmammals has not yet been achieved, studies on temporal regulation of melanopsin expression in these species has revealed some evolutionary conservation. Particularly in chicken [65, 66], melanopsin mRNA levels in the ganglion cell layer exhibit a diurnal rhythm with peak expression during the nighttime that persists under constant darkness. The circadian and light:dark regulation of melanopsin reflects an evolutionary conservation in circadian regulation of the photoentrainment pathway. In fungus, plants and flies the bona fide circadian photoreceptors are also under oscillator control [69–72], which may partly account for diurnal variation in circadian photosensitivity in most species. For instance, the mammalian circadian oscillator is more sensitive to light resetting when the light is administered during subjective night and less sensitive during the subjective day [7]. In summary, both spatial and temporal regulation of melanopsin expression in various species suggest an evolutionary trend to recruit the melanopsin system for adaptation to ambient light.

Future prospects

Progress in the last few years has conclusively established that multiple photoreceptors are recruited to regulate adaptive photoresponses in mammals. Genetic perturbation studies have conclusively established the role of melanopsin and rod/cone photoreceptors in adaptive photoresponses in mammals. However, the relative contributions of the individual rod and cone pathways are still unclear. Combinatorial genetic perturbation among melanopsin, rod and cone function will be useful in com-

prehensively elucidating the relative roles of these three photoreceptors in regulating adaptive photic processes in mammals. Furthermore, while loss-of-function mutation of melanopsin has established its molecular role in photoperception, the cellular role of melanopsin-positive ipRGCs in transmitting rod/cone pathway information to key brain centers has yet to be conclusively established. New molecular genetic tools to specifically ablate or silence ipRGCs will be essential for answering this question. If ablation of ipRGCs abolishes photoentrainment, including rod and cone components, then it would signify a dominant role for these neurons in transmission of rod/cone light signals to the SCN.

In parallel, transgenic reporter gene labeling and knowledge of the gene expression profile of the ipRGCs will shed light on the molecular bases of their ontogeny, distinct axon guidance cues to different brain targets, phototransduction and likely integration of rod/cone signals. Currently, genomics techniques allow amplification and unbiased detection of expressed genes of the entire genome from few cells. However, current genetic resources and labeling techniques to specifically label and harvest melanopsin-expressing RGCs for gene expression studies are limiting. Establishment of a transgenic rodent line specifically expressing a green fluorescent protein (GFP)-based fluorescent tag in melanopsin RGCs would be an invaluable tool in expression-profiling these cells. Results from such studies will likely yield candidate GPCR signaling pathway components, channels, neurotransmitters and molecules determining neuronal guidance. Such molecular characterization of the ipRGCs would be a catalyst in advancing our understanding of how these cells function and how they specifically target brain regions involved in photoadaptive responses.

Expression of melanopsin in heterologous systems indicates a role for $G\alpha_q$ -class G-proteins, arrestins and TRPC channels in the melanopsin phototransduction pathway. All of these protein classes are represented by multigene families in mammals. Thus, if this preliminary model of melanopsin phototransduction is correct, identifying the specific gene family members involved in melanopsin phototransduction within ipRGCs will be a major step forward. If past studies of phototransduction serve as a guide, we can also expect the discovery of a host of additional proteins involved in organizing the macromolecular structure of the melanopsin signal transduction complex and in regulating each specific step of the phototransduction cascade.

Dissection of the native melanopsin phototransduction cascade will also be necessary to understand how rod/cone signals are integrated with intrinsic photoresponses in ipRGCs. While integration could simply involve summation of distinct excitatory signals, it may also involve more complex cross-regulation at the level of signal transduction intermediates or end effectors. Identification of

the neurotransmitter receptors in ipRGCs that collect input from rod/cone bipolar cells could help answer this question. It will also be important to determine whether both rod/cone-initiated responses and melanopsin-initiated responses involve modulation of the same or different ion channel(s).

Finally, the relevance of the melanopsin phototransduction mechanism in human circadian-related diseases is yet to be established. Just as for the complex image-forming phototransduction process, mutations in components involved in any of these steps could modulate melanopsin phototransduction and, thus, could be a genetic risk factor for circadian disorders, including sleep disorders and depression.

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