Recent Developments in Circadian Photoreception: More Than Meets the Eye

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Our perception of the world is so dominated by our sense of vision that we have been reluctant to accept the fact that the vertebrate eye mediates another, quite separate photosensory task—the detection of light for the regulation of biological time. In this short article, we outline some of the recent experimental findings that show that the absence of rod and cone photoreceptors does not block the effects of light on the circadian system. Furthermore, we review the progress to date in identifying the photopigments that may mediate the effects of light on the mammalian biological clock.

Most organisms do not merely respond to their environment but have the capacity to adjust physiology and behavior in anticipation of changing environmental conditions. Anticipation is required because it takes considerable time to bring about the complex realignment of physiological systems that permit an optimal response. For example, the full transition from sleep to wakefulness cannot be accomplished instantly but takes hours. Before we wake, our bodies are already preparing for activity: Our temperature begins to rise, glucose is released into the blood, and the neural and hormonal systems needed for activity are stimulated. By advance fine-tuning of physiological processes, we are already prepared for predictable changes in the environment by the time they occur. This capability requires some form of 24-hour circadian clock, and in mammals the master clock resides within the suprachiasmatic nuclei or SCN, a pair of small nuclei in the hypothalamus just above the optic nerve chiasma. In short, the function of the circadian system is to coordinate the phase of a biological event to a specific feature (or phase) of the 24-hour environmental cycle and to ensure that the phase of multiple rhythmic events within the organism are appropriately coupled. To achieve this timing, the circadian system must remain synchronized or entrained to the solar day, and most organisms have evolved to use the changes in the light environment at twilight as their primary zeitgeber (time giver) to bring about a change in circadian phase.

In humans, the most well-characterized markers of circadian phase are core body temperature and the nocturnal melatonin secretion by the pineal gland. If a person should be exposed to light during the night, pineal melatonin secretion is acutely suppressed.1 It remains unclear whether these photic responses are mediated by the same or different mechanisms. Until recently, all the experimental evidence suggested that the biological clock of mammals was entrained by photoreceptors within the eye. However, a recent report suggested that bright light applied to the skin behind the knee (popliteal illumination) of humans could shift circadian rhythms of body temperature and rhythms of the hormone melatonin.2 These results were widely reported in the press but remain highly controversial. No instances of circadian entrainment in humans who have undergone ocular enucleation have been reported,3 and popliteal illumination produces no effect whatsoever on the suppression of nocturnal levels of pineal melatonin4 (which may or may not be mediated by the same photoreceptors). Until and unless other experiments show that popliteal illumination can shift the human clock, this form of extraocular photoreception in humans and other mammals remains uncertain.

In addition, the vertebrate retina contains a circadian clock of its own. This oscillator is able to maintain the circadian rhythms in outer segment disc shedding and retinal melatonin rhythms in anticipation of changing environmental conditions. Anticipation is required because it takes considerable time to bring about the complex realignment of physiological systems that permit an optimal response. For example, the full transition from sleep to wakefulness cannot be accomplished instantly but takes hours. Before we wake, our bodies are already preparing for activity: Our temperature begins to rise, glucose is released into the blood, and the neural and hormonal systems needed for activity are stimulated. By advance fine-tuning of physiological processes, we are already prepared for predictable changes in the environment by the time they occur. This capability requires some form of 24-hour circadian clock, and in mammals the master clock resides within the suprachiasmatic nuclei or SCN, a pair of small nuclei in the hypothalamus just above the optic nerve chiasma. In short, the function of the circadian system is to coordinate the phase of a biological event to a specific feature (or phase) of the 24-hour environmental cycle and to ensure that the phase of multiple rhythmic events within the organism are appropriately coupled. To achieve this timing, the circadian system must remain synchronized or entrained to the solar day, and most organisms have evolved to use the changes in the light environment at twilight as their primary zeitgeber (time giver) to bring about a change in circadian phase.

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In addition, the vertebrate retina contains a circadian clock of its own. This oscillator is able to maintain the circadian rhythms in outer segment disc shedding and retinal melatonin synthesis, even in the absence of a cue from the SCN (reviewed in Reference 5). The presence of an auxiliary oscillator may be important for the retina’s function as a photoentrainment organ. The retinal clock is not, however, thought to have any direct impact on other tissues.

In mammals, light information from the eye reaches the SCN through the retinohypothalamic tract (RHT). This tract arises from a small subset of retinal ganglion cells and consists of a relatively small fraction of the optic nerve’s axons. For example, in the mouse retina, only 0.1% of all retinal ganglion cells project to the SCN.6 Although the ganglion cells that form the retinohypothalamic projection have been identified, the photoreceptors that communicate with the SCN through these cells have not. Part of the problem is that the photoentrainment pathway is obscured by the large number of photoreceptors and retinal neurons devoted to image formation. Disentangling which retinal cells mediate photoentrainment from the mass of neurons dedicated to image detection has been a major problem.

The initial and natural assumption was that the only known mammalian photoreceptors, rods and cones, which have undergone ocular enucleation (RGE, MvS); by National Science Foundation Grant IBN-9809916; and U. S. Public Health Service Grant RO7049.

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more recently, transgenic technology has been used to retain the ability to suppress melatonin levels in response to vision due to various types of ocular disease were found to already been known for more than a century. In more recent photoreceptors and photopigments other than those that had been studied, ocular enucleation abolished all responses to light. This introduced the possibility that the retina harbors cellular layers.

**FIGURE 1.** Schematic wiring diagram of the mammalian retina, indicating the localization of different opsin-like molecules in the different cellular layers.

issue, but the use of mice with naturally occurring genetic disorders of the eye has provided a partial answer. Mice homozygous for the retinal degeneration (rd/rd) gene provided the first clue that visual and circadian responses to light may in fact be profoundly different. Despite being completely blind because of total loss of rods and massive loss of cones, rd/rd mice appear to show completely normal shifts of circadian phase in response to light. As with all other mammals that have been studied, ocular enucleation abolished all responses to light. This introduced the possibility that the retina harbors photoreceptors and photopigments other than those that had already been known for more than a century. In more recent studies in humans, a subset of patients who had lost their vision due to various types of ocular disease were found to retain the ability to suppress melatonin levels in response to light. More recently, transgenic technology has been used to ablate both rods and cones in mice. In spite of the absence of functional visual photoreceptors, these animals exhibit completely normal circadian responses to light. The striking conclusion of these findings is that mammals are using some unidentified photoreceptor(s) and photopigment(s) other than the rods and cones and their opsins.

Although the requirement of rod and cone opsins in circadian light reception has thus been excluded, there is evidence for involvement of an opsin-based pigment in this response. This evidence is in the form of detailed action spectra for photoentrainment in the golden hamster and the mouse (summarized in Reference 11), which show a very close fit to the Dartnall nomogram for the spectral absorption of a vertebrate opsin-based photopigment. The fact that circadian photoreception works equally well without rod and cone photoreceptors directs the search for these photopigments to the inner layers of the retina. This radical conclusion is supported by the recent discovery of a novel opsin-based photopigment (VA opsin) in some amacrine and horizontal cells in the fish retina. Furthermore, a number of orphan putative photopigments have recently been identified in mammals. These include retinal-binding G protein-coupled receptor (RGR), peropsin, encephalopsin, and melanopsin. With the exception of encephalopsin, which is expressed in the testes and other parts of the central nervous system and therefore falls outside the scope of this review, they are all ocular and therefore, at first sight, are candidate circadian photopigments.

RGR, the first one to be identified, differs from rods and cones in that its preferred chromophore is all-trans-retinaldehyde rather than 11-cis-retinal. When exposed to light, RGR photoisomerizes the all-trans chromophore to the 11-cis configuration. Its likely function, therefore, is not that of a signaling photopigment but rather that of a photoisomerase. Furthermore, its localization to the retinal pigment epithelium (RPE) and Müller cells casts doubts on whether it could initiate a signal that would directly or indirectly communicate through the ganglion cells of the retinohypothalamic tract to the SCN. The next candidate, peropsin, is also localized to the RPE, but its function remains unknown. Phylogenetic analysis, however, places peropsin in the same branch as RGR and squid retinochrome, another proven photoisomerase, and it may therefore fall in the same functional group as RGR. The most recently discovered candidate, melanopsin, differs from all the other novel mammalian opsins in that it is expressed within the neural retina. Specifically, melanopsin is expressed in very few ganglion cells and even fewer cells within the amacrine cell layer in the mouse retina. Although its function is still unknown, its distribution is strikingly similar to the distribution of murine retinal ganglion cells known to participate in the retinohypothalamic tract.6 Localization of melanopsin within these ganglion cells would provide a direct route by which photic information collected in the eye could be communicated to the SCN. Because it is phylogenetically most similar to invertebrate opsins, it could be predicted that melanopsin would not require access to an auxiliary tissue such as the RPE for renewal of its chromophore.

An alternative hypothesis to the involvement of opsin-based photopigments is that the cryptochromes, a group of proteins initially suggested to be responsible for detecting light and mediating photoentrainment in phylogenetically diverse organisms, from the plant Arabidopsis to Drosophila and even mammals. In mammals, cryptochromes have been shown to be crucial components of the circadian pacemaker itself, operating in a light-independent manner. There is no evi-
dence that cryptochrome plays any role in circadian photoreception in mammals, and its spectral absorption characteristics make this very unlikely.\(^{21}\)

It has thus been shown that an unidentified photopigment within the mammalian eye is able to mediate photoentrainment in the absence of rods and cones. However, it cannot be excluded that rod and cone photoreceptors contribute at some level to this response in the normal retina.\(^{22}\) Whether such a contribution exists can only be established once the novel circadian photopigment(s) have been cloned and their expression ablated by knockout technology.

The past decade has provided quite a few challenges to our knowledge of the retina, which was thought to be well characterized in terms of its cell types and their interconnections. We now know that the retina hides at least one novel photoreceptor type involved in irradiance detection for the benefit of the circadian axis. We also know that this photoreceptor is most likely to be located in the inner layers of the retina. We predict that, with the current intensive interest in novel opsins, the identity of this novel photopigment will be established within the near future, opening up a whole new dimension to retinal research.

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**References**


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