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Circadian Clock Photo-Entrainment: The Unexpected Role of Opn5/neuropsin in the Retina [Version 1, 2 Approved]

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Abstract

Circadian clocks are entrained by light signals from the environment or photo-entrained. A pair of recent papers has identified the visual pigment required for photo-entrainment of the circadian clock in the retina, reporting surprising results: neither the conventional photoreceptors, rods and cones, nor the photopigment Opn4/melanospin are required. However the orphan opsin Opn5/neuropsin appears to be necessary and sufficient to do the job.

Keywords: Circadian Clocks; Visual Pigment; Opn5/Neuropsin; Retina; Photoreception; Photo-entrainment; Circadian Rhythms; Visual Processes

Circadian clocks are endogenous time-keeper mechanisms that orchestrate most aspects of our physiology and behavior on a daily basis [1]. The key property of circadian clocks is that their activity persists in the absence of any time cue, including in constant darkness, with an intrinsic period close to 24 h [1]. For obvious survival reasons, the rhythm of clock activity needs to be constantly adjusted to match that of the environment, and this is achieved through a process called *entrainment* or *synchronization* [1]. Of the many environmental cues, light is the most predicable and powerful synchronizer [1].

The only light sensitive organ in mammals, the retina is the entry to a circadian system that is built around a central clock in the suprachiasmatic nucleus of the hypothalamus (SCN) [1]. Light-entrainment in the SCN relies on intrinsically photosensitive retinal ganglion cells (ipRGCs) that express the visual pigment Opn4/melanopsin [2] (Figure 1). Conventional photoreceptors, namely rods and cones, also participate in photo-entrainment of the SCN clock through their inputs to ipRGCs, but ipRGCs appear to be the sole conduit to the SCN [2]. The retina is also a circadian clock and functions and entrains to light independently of the SCN [3]. Until recently, it was not known which photoreceptor or visual pigment was required for photo-entrainment of the retinal clock. In a series of two papers [4,5], Russel van Gelder and collaborators addressed this question and made the surprising discovery that rods, cones, or Opn4/melanopsin are not required for photo-entrainment of the retinal clock [4]. Rather, they propose that Opn5/neuropsin, which was until now an orphan opsin, is the visual pigment responsible for photoentrainment of the retinal clock [5]. Thus the retina and the SCN clocks use different visual pigments for photo-entrainment.

Circadian rhythms in the retina were first reported more than 30 years ago. Jo Besharse and collaborators used the *Xenopus* eyecup preparation to show that rhythms in melatonin production persisted *in vitro* in the dark for several days and, thereby, established that a circadian clock intrinsic to the retina controls melatonin synthesis [6]. Similar observations were later made in mammals [7]. Since the melatonin rhythm persists *in vitro* when the photoreceptor layer (PRL) is isolated from the rest of the retina, the dogma that gradually emerged was that the retinal clock is localized in the PRL and uses melatonin as a major output to control retinal physiology and function on a daily basis. In addition, as the melatonin rhythm in isolated retina or isolated PRL is sensitive to light [6,7], there must be an intracellular mechanism within rods and/or cones that links light absorption and entrainment of the clock. Why would researchers be looking for another photoreceptor or visual pigment responsible for photo-entrainment when the clock mechanism and its output effector are all in the same photosensitive cells?

Since the pioneering discovery of the clock-controlled melatonin rhythm in the retina, the molecular nature of the mammalian clock mechanism has been revealed and the clock genes identified [1].

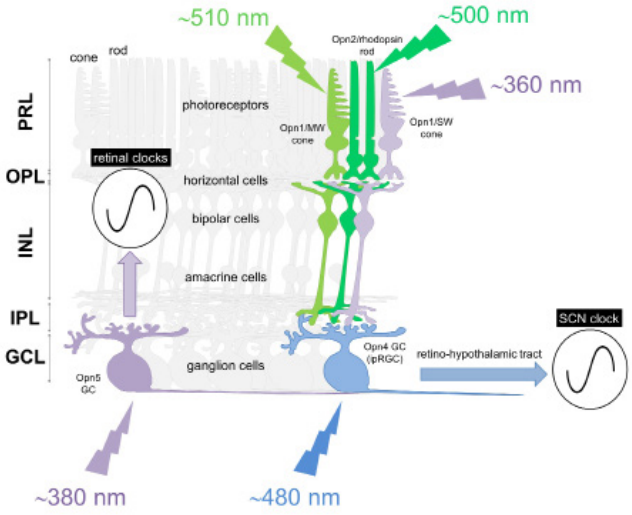

Figure 1: Different photo-entrainment pathways for different clocks. Most cells in the mammalian retina--perhaps with the exception of the rods-- contain a circadian clock. Clock activity and entrainment to light in the retina occur independently of the clock located in the suprachiasmatic nucleus of the hypothalamus (SCN). Photo-entrainment of the SCN clock is mediated through photoreception in the retina and a subset of ganglion cells that express the photopigment Opn4/melanopsin and are intrinsically photosensitive (ipRGCs). The visual pigment responsible for photo-entrainment of the retinal clock has remained unknown. Recent evidence indicates that Opn5/neuropsin is required for light entrainment of the retinal clock. Opn5 is expressed in a subset of ganglion cells. Note that ipRGCs receive rod and cone inputs whereas Opn5 ganglion cells may not. PRL: photoreceptor layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; GC: ganglion cell.

Surprisingly, clock gene expression in the retina is not restricted to the PRL but is widespread in all retinal layers [3]. Thus, the retina does not contain *a* clock but rather an entire circadian system built on a myriad of clocks present not only in the PRL but also in the inner nuclear layer (INL) and ganglion cell layer (GCL), including ipRGCs [3]. What these clocks do in the inner retina remains the focus of intense ongoing research, but their discovery triggered interesting questions of physiology: are all clocks photosensitive cells? And if not, how do non-photosensitive clock cells remain in phase with the light/dark cycle? Could Opn4/melanopsin and/or ipRGCs be involved in photo-entrainment of the clocks in the inner retina?

The first answers came from the laboratory of Douglas McMahon at Vanderbilt University. They used the *mPer2Luc* mouse, in which continuous measurements of the mPER2::LUC fusion protein luminescence in real-time can be used as a proxy of molecular circadian rhythms [8]. mPER2::LUC rhythms persist for weeks in explanted retinas cultured in the dark, and their phase can be reset by pulses of light or the modulator dopamine [9,10]. Importantly, the resetting effects of light can be blocked by prior application of a dopamine receptor antagonist [10]. Dopamine is a well-established light-adaptive modulator in the retina [11]. Dopamine is synthesized and released by a specific type of amacrine cell and known to act on most retinal neurons through synaptic release or diffusion in the extracellu-

lar space [11]. Dopamine release is triggered by light, and rods, cones, and ipRGCs have all been hypothesized to be involved in this process [2,11]. Thus dopamine appeared to be an ideal candidate that could mediate the entraining effects of light on most clock cells in the retina through control of its release by known retinal photoreceptors.

In a surprising twist, Van Gelder's team reported that rods, cones, or Opn4/melanopsin are not required for light entrainment of the retinal clock [4]. The investigators created *mPer-2Luc* mice that lack Opn4/melanopsin and in which rods and cones have degenerated (i.e. *mPer2Luc;opn4*$^{-/-}$*;rd1/rd1* mice). The team developed an elegant approach to study photo-entrainment *in vitro*: the two retinas from the same animal are cultured together but exposed to antiphasic light/dark cycles. If the retinal clock is able to follow the light/dark cycle, then clock activity rhythms in the two retinas would become oppositely phased from each other after a few days. If the rhythms cannot be entrained by the light/dark cycle, they would free run with the same period and therefore display similar phases. The difference in phase between the two rhythms can be used to quantify photo-entrainment on a scale from 0° (no entrainment) to 180° (total entrainment). In retina pairs from mice that lacked rods, cones, and Opn4/melanopsin, the mPER2::LUC rhythms became out of phase invariably, indicating that they entrained normally and that neither conventional photoreceptors nor Opn4/melanopsin are required for photo-entrainment [4]. If rods, cones, and Opn4/melanopsin are not involved in photo-entrainment of the retinal clock, then which visual pigment fulfills this function?

The Van Gelder group sought to identify this pigment [5]. The team first established the spectral sensitivity of photo-entrainment of the retinal clock. Using light of specific wavelengths instead of the broad-spectrum white light they used in the first study [4], they showed that photo-entrainment of the retinal clock is most sensitive to UV-A and violet light in the range 370-417 nm [5]. The spectral sensitivity of photo-entrainment, therefore, does not match that of rhodopsin (Opn2/rhodopsin; ~ 500 nm), middle-wavelength/green opsin (Opn1/MW; ~ 510 nm), or Opn4/melanopsin (~ 480 nm). However, the increased sensitivity to UV-A/violet light matches that of the cone UV/blue opsin (Opn1/SW; ~ 360 nm). It is known that a few cones remain in adult *rd1* retinas [12]. Could these remaining cones be responsible for photo-entrainment of the retinal clock in *rd1* retinas? In addition, two other opsins, Opn3/encephalopsin [13] and opn5/neuropsin [14], have been found in retinal cells. The function of these opsins and the spectral sensitivity of Opn3/encephalopsin remain unknown, but Opn5/neuropsin shows a maximal absorption ~ 380 nm when heterologously expressed [15]. Could Opn1/SW, Opn3/encephalopsin and/or Opn5/neuropsin be responsible for photo-entrainment of the retinal clock?

To answer this question, the Van Gelder group teamed up with King-Way Yau at Johns Hopkins University, whose lab lab developed Opn3/encephalopsin and Opn5/neuropsin knock out mouse lines. Additionally, the Neitz lab at the University of Washington provided Opn1/SW knock out mice. Mice from these 3 lines were bred into the *mPer2::Luc* line, and the retinas tested for *in vitro* photoentrainment. The genetic approach gave a clear answer: retinas from Opn1/SW- or Opn3/encephalopsindeficient animals are able to photo-entrain normally whereas those from Opn5/neuropsin-deficient mice are not [5]. Preliminary localization of Opn5/neuropsin indicates that it is expressed in a subset of retinal ganglion cells [5] (Figure 1).

These two studies reveal that Opn5/neuropsin is required and sufficient for photo-entrainment of the retinal clock. Although these results are without any doubt new and interesting, they raise many new important questions: i) if Opn5/neuropsin is only expressed in a small subset of ganglion cells (≤ 5% of all ganglion cells [5]), how are light signals transmitted from Opn5/neuropsin cells to the other rhythmic cells within the ganglion cell layer (GCL) and in the other retinal layers?; ii) from McMahon's work [10], we would have expected that the light-triggered release of dopamine would be sufficient to photo-entrain the retinal clock in the absence of Opn5/neuropsin; is dopamine function normal in Opn5/neuropsindeficient retinas?; and iii) the data from Buhr et al. [5] suggest that rods and cones do not contribute to photo-entrainment since the retinal clock cannot entrain to light bright enough to activate rods and cones in the absence of Opn5/neuropsin; are Opn5/neuropsin ganglion cells an unconventional type of ganglion cell that are functionally isolated from the mainstream light signaling pathways?

Although the answers to these questions remain unknown, the work from the Van Gelder lab presented here represents an important advance. Not only does it shed light on the mechanisms involved in light entrainment of the retinal clock, but it also proposes a role for Opn5/neuropsin in the mammalian retina, which until now had remained elusive. Interestingly, Opn5/neuropsin is also expressed in other areas of the brain [14]. Could some brain cells be intrinsically sensitive to UV light? This possibility has been proposed in birds, where Opn5/neuropsin appears to be a deep-brain photopigment that contributes to seasonal reproduction [16]. To date there is no evidence that some neurons in the mammalian brain are sensitive to light, but the relative insensitivity to light and peculiar sensitivity to the UV-A/violet part of the spectrum of Opn5/neuropsin reported by Buhr et al. should lead neuroscientists to reinvestigate this possibility with a bluer and brighter light. The retina may not be the only light-sensitive organ in mammals, after all…

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