Multiple Photopigments Entrain the Mammalian Circadian Oscillator

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Circadian rhythms are entrained to the natural day:night cycle. Melanopsin expressed in retinal ganglion cells partially accounts for circadian photoentrainment. Dkhissi-Benyahya et al. demonstrate that medium wavelength opsin (MW-opsin) also plays an important role in the process. Furthermore, they develop a model explaining wavelength-dependent photoentrainment by melanopsin and MW-opsin.

An endogenous circadian clock synchronizes rhythmic behaviors and physiologies to the appropriate time of day. With the changing seasonal photoperiod, the clock needs some adjustment to maintain a constant phase relationship between the day-night cycle and its rhythmic outputs, including the activity-rest cycle. It is well known that retina photoreceptors play a dominant role in entraining the circadian oscillator to the ambient light:dark cycle, but the relative contributions of different photopigments in circadian photoentrainment are unclear. In this issue of *Neuron*, Dkhissi-Benyahya et al. (2007) demonstrate a specific role of medium wavelength opsins (MW-opsin) in circadian photoentrainment and develop a model that explains how mammals under various spectral environments recruit MW-opsin and melanopsin to entrain their circadian clock.

The approach of Dkhissi-Benyahya et al. highlights the complexity of assessing the effect of specific light quality on the circadian clock and offers new ways to address this issue. Rodents have been extensively used in delineating the molecular bases of circadian oscillator in mammals. Under a standard light:dark (LD) cycle of 12 hr of bright light and 12 hr of darkness, nocturnal rodents restrict their activity to the night time. The nocturnal activity is a result of both the circadian consolidation of activity to the night as well as light suppression of activity (masking) during the day. To filter out the masking phenomenon, and to accurately measure the effect of light on the circadian oscillator, researchers can manipulate the LD cycle in various ways including reducing the light intensity during daytime, changing the phase of the LD cycle or, more frequently, assessing the efficacy of a brief light pulse in shifting the phase of the activity-rest cycle under constant darkness (DD). Rodents held under DD continue to exhibit ~24 hr rhythm in activity-rest cycle with activity consolidated to the subjective night. A few minutes pulse of light, delivered at a variety of intensities and wavelengths, administered during the subjective late evening can cause a stable, quantifiable shift in the phase of the activity-rest cycle.

In mammals, the action spectrum of circadian photoentrainment shows striking similarity to the absorption spectrum of vitamin-A-based, opsin class of photopigments (Takahashi et al., 1984), suggesting involvement of opsin-based photopigment(s) from the retina. Mammals express three major functional opsins in the outer retina—rhodopsin with peak sensitivity ~500 nm that can detect low levels of light (scotopic vision), cone opsin with peak sensitivity above 500 nm (MW-opsin), and short wavelength opsin (SW-opsin) with peak sensitivity less than 500 nm (reviewed in Nathans, 1999). The peak sensitivity of circadian photoentrainment in rodents is around 500 nm (Takahashi et al., 1984), but mice lacking a majority of functional outer retina photopigments can still entrain to bright LD cycle (Foster et al., 1991). Furthermore, under DD, the photosensitivity of the circadian clock to bright white light pulses in these mice is undistinguishable from that of wild-type (wt) mice. This raised the possibility that a novel inner retina photopigment may play a dominant role in circadian photoentrainment.

The surprising discovery of a novel photopigment, melanopsin (Opn4) in a small subset of retinal ganglion cells of the inner retina (Provencio et al., 2000) and subsequent genetic experiments established that melanopsin plays an important role in circadian photoentrainment. Melanopsin containing RGCs are intrinsically photosensitive (mRGCs or ipRGCs) with peak sensitivity around 480 nm and primarily project to the hypothalamic brain center suprachiasmatic nucleus (SCN) that harbors the master circadian oscillator (Berson et al., 2002; Hattar et al., 2002). Although the melanopsin-deficient (*Opn4*$^{-/-}$) mice entrain normally to bright light:dark cycle, under constant darkness their oscillator is less sensitive to discrete pulses of light, thus establishing an important role of melanopsin in circadian photoentrainment (Panda et al., 2002; Ruby et al., 2002). However, the significant residual circadian photosensitivity of *Opn4*$^{-/-}$ mice was abolished when nearly all outer retina photopigments were rendered nonfunctional, thus suggesting that at least one of the three outer retina photopigments must participate in the entrainment process (Hattar et al., 2003; Panda et al., 2003).

Dkhissi-Benyahya et al. were ingenious in selecting which candidate

outer retina photopigment is involved in the entrainment process. In rodless/coneless mice the peak circadian photosensitivity is ~480 nm (Hattar et al., 2003), which matches the peak spectral sensitivity of the ipRGCs (Berson et al., 2002). However, the sensitivity shifts to ~500 nm in rodents with functional outer-retina photopigments (e.g., Takahashi et al., 1984). Such shift toward a longer wavelength naturally argues against the SW-opsin being a prime player, leaving rhodopsin and MW-opsin as the two likely candidates. Dkhissi-Benyahya et al. compared the relative sensitivity of these two photopigments and noticed that the phase shifting effect of light in mammals is more pronounced under irradiance levels exceeding the saturating amount for rhodopsin, thus leaving MW-opsin as the prime outer-retina candidate.

To test their hypothesis, Dkhissi-Benyahya et al. used recently characterized mice deficient in both splice variants of the thyroid hormone receptor β ($TR\beta^{-/-}$). The TRβ gene is essential for the development of MW cones, therefore the $TR\beta^{-/-}$ mice selectively lack MW-opsin, but retain other functional retina photopigments (Dkhissi-Benyahya et al., 2007) and references therein. They assessed the circadian photosensitivity of $TR\beta^{-/-}$ mice under two different paradigms: (a) ability of these mice to maintain a stable phase relationship between dark onset and activity onset and (b) the phase shifting effect of monochromatic lights on mice held under DD. When the light cycle was shifted by 6 hr and, simultaneously, the light was dimmed from 100 lux to 10 lux, the $TR\beta^{-/-}$ mice took longer than their wt littermates to lock their nocturnal activity onset to the new dark onset time. The $TR\beta^{-/-}$ mice performed even worse when the light level was lowered to ~1 lux. Furthermore, under DD conditions, the mice exhibited almost normal phase shift in response to monochromatic light pulses of 480 nm or shorter wavelength. However, their circadian oscillator was less sensitive to light of 530 nm. Such selective attenuation of circadian phase shift in the $TR\beta^{-/-}$ mice in response to a longer wavelength of light can only be ascribed to the loss of MW-opsin.

Having established a role of MW-opsin in entraining the clock, the authors developed a mathematical model integrating the contribution of melanopsin and MW-opsin in this process. The model quantitatively predicts the wavelength specific participation of these two photopigments, such that at <490 nm contribution of melanopsin predominates, while at >490 nm MW-opsin primarily entrains the clock. Amazingly, the prediction of the model is accurately confirmed in additional experiments involving $Opn4^{-/-}$ and $TR\beta^{-/-}$ mice. The $Opn4^{-/-}$ mice with functional MW-opsin exhibit almost normal phase shift in response to 530 nm light, while their sensitivity to 480 nm light is severely attenuated confirming the predicted dominance of melanopsin in wavelengths <490 nm. Conversely, a light pulse of 560 nm caused significant phase shift in wt, but not in $TR\beta^{-/-}$ mice.

The modeling of circadian photosensitivity incorporating peak sensitivities of the two photopigments is an important step in unraveling the sophistication of mammalian circadian photoentrainment. As we learn more about the spectral properties of melanopsin photopigment, it may add further complexities. Melanopsin has been suggested to possess an intrinsic photoisomerase activity to regenerate its own retinal based chromophore (Panda et al., 2005). In a heterologous expression system, the putative photoisomerase activity of melanopsin is augmented by illumination of >500 nm wavelength of light (Melyan et al., 2005). If melanopsin photopigment in the inner retina exhibits similar photopotentiation by prior illumination of longer wavelengths, entrainment even with these two photopigment may be more complicated. Additionally, as the authors emphasize, other outer retina photopigments may participate in the entrainment process. It is known that the mRGCs projecting to the SCN also receive both rod and cone inputs (Dacey et al., 2005), thus establishing a clear framework for conveying both rod/cone input to the master circadian oscillator. Although no direct rod input to circadian photoentrainment has been conclusively established, a strong modulatory role of rods on ipRGC function has been implied (Doyle et al., 2006).

Does recruitment of multiple photopigments for circadian entrainment have an adaptive advantage? The phase of the circadian oscillator in many organisms including mammals is primarily reset either at dawn or dusk. These twilight times are marked by extreme changes in both the ratio of blue- versus red-shifted spectra and the irradiance level as the organism transitions between photopic and scotopic ambience. Furthermore, incidence angle, polarization, and diffraction of light in different geological or meteorological niches can impose further variations in spectral quality during twilight times. To ensure appropriate entrainment to twilight zones, organisms appear to recruit at least two photopigments with photopic sensitivity, one with blue-shifted and one with red-shifted peak sensitivity. As shown by this manuscript and others, loss of a single photopigment may still enable the organism to entrain to a standard laboratory white light regime, but in nature, the organism may be unable to entrain to suboptimal spectral conditions, thus potentially exposing it to predators or to unfavorable temporal niches.

Finally, the experimental approach used by Dkhissi-Benyahya et al. (2007) and their novel finding opens up fronts for the discovery of additional photopigment(s) for circadian photoentrainment and understanding how interactions among these photopigments fine-tune the phase of the circadian clock.

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Two Oscillators Are Better Than One: A Circadian Pacemaker Escapes from the Light

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Continuous light exposure can suppress circadian rhythms. In this issue of *Neuron*, Murad et al. demonstrate that, under certain genetic conditions, a novel cluster of pacemaker neurons can drive rhythmic behavior in constant light. Surprisingly, these neurons are distinct from those thought to drive rhythms in constant darkness.

Circadian clocks are imperfect timekeepers, running with a periodicity a little slower or faster than 24 hr. The daily rhythm of sunlight synchronizes these clocks to the 24 hr environment. Unfortunately, you can get too much of a good thing. Continuous light exposure (constant light; LL) can suppress circadian rhythms in many organisms, including mammals and insects. Here, Murad and colleagues examine how *Drosophila* circadian clocks can "escape" from this light suppression (Murad et al., 2007).

To understand the destructive aspects of light, one first needs to understand the inner workings of the circadian clock. Genetic studies in fruit flies and mice have revealed a remarkably conserved, cell-autonomous molecular clock. In *Drosophila*, clocks consist of a primary transcriptional feedback loop in which the CLOCK/CYCLE dimer activates *period* (*per*) and *timeless* (*tim*) transcription (reviewed in Hardin, 2005). PER represses CLK/CYC, leading to robust transcriptional oscillations. Phosphorylation of these components, most notably PER, appears to contribute to protein stability and feedback repression to modulate the periodicity of molecular and behavioral oscillations.

How does light impact this molecular circuit? Unlike their mammalian counterparts, flies have a cell-autonomous photoreceptor, CRYPTOCHROME (CRY). Cryptochromes are blue-light photoreceptors related to UV-dependent DNA repair enzymes (photolyases). Light triggers both CRY and CRY-dependent TIM degradation, resetting the molecular clock (see Busza et al., 2004, and references within). Under constant light conditions, *cry* mutants are rhythmic (Emery et al., 2000a), although reports of splitting, i.e., two rhythmic components with different periods, have also been made (Yoshii et al., 2004). In mammals, CRYs (CRY1 and CRY2) appear to be the principal transcriptional repressors rather than photoreceptors.

The work of Murad et al. highlights the role of a network of clock neurons in the fly brain. While *Drosophila* is revered for its arsenal of molecular genetic tools, tremendous progress has been made in revealing the neuronal network that rhythmically modulates fly behavior. In mammals, circadian behavior is driven by the hypothalamic suprachiasmatic nuclei, a complex and heterogeneous network consisting of approximately 20,000 neurons. The fly circadian pacemaker is a model of efficiency, accomplishing comparable timekeeping tasks with only about 100 pacemaker neurons, and under certain genetic conditions, behavioral rhythms are observed with just a small fraction of functional pacemaker