

Minireview

Clocks, cryptochromes and Monarch migrations

Charalambos P Kyriacou

Address: Department of Genetics, University of Leicester, Leicester LE1 7RH, UK. Email: cpk@leicester.ac.uk

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Abstract

The annual migration of the Monarch butterfly (*Danaus plexippus*) from eastern North America to central Mexico is one of nature's most inspiring spectacles. Recent studies including one in *BMC Biology*, have begun to dissect the molecular and neurogenetic basis for this most complex behavior.

At the approach of fall in the northern United States and southern Canada, the Monarch butterfly, *Danaus plexippus* (Figure 1), starts to get restless. These butterflies usually represent the third or fourth generation that season, each previous generation having lived for around 6-8 weeks. The current generation senses the shorter photoperiod of autumn days and starts to shut down its reproductive system, lay down extra layers of fat, and in general become more physiologically resistant to environmental stresses such as colder temperatures. This state of diapause precedes one of nature's most glorious sights, the annual autumn migration of the Monarch from the northern United States to its overwintering grounds in central Mexico, where it settles in oyamel fir trees [1]. This alteration in the Monarch's physiological state, in readiness for the arduous 4,000-kilometer journey south, includes a three- to fourfold increase in longevity that will carry these individuals through to the following spring in Mexico. At that time they mate, lay the fertilized eggs on milkweed (*Asclepias*), and the few adults that survive begin their northern journey home. In two or three generations, each one laying eggs progressively further north as milkweed starts into new growth, the Monarchs finally reach their northern homes. In the autumn, the cycle begins again [1].

Butterfly migration and the circadian clock

This remarkable feat represents the longest annual insect migration known and has become a focus of study for the

circadian biologist Steven Reppert and his group at the University of Massachusetts in Worcester. In a series of papers over the past few years [2-4], they have confirmed that, like migrating birds, the Monarch depends on its circadian clock to find the right direction. It has a time-compensated sun compass, so if the clock is made arrhythmic (by placing the butterfly in constant bright light for a few days), or is phase-shifted by a few hours, the butterfly will lose its way, because it uses the time to tell it where the sun should be in the sky [2]. To do this, Monarchs use sky-light spectral gradients and polarized ultraviolet (UV) light, for which they have dedicated photoreceptors. Consequently, they can orient themselves towards Mexico in the southwest under a variety of meteorological conditions [2-4].

The likely neuroanatomical position of the circadian clock in the butterfly brain has been defined using antibodies against clock proteins (for example, PERIOD (PER), TIMELESS (TIM) and CLOCK (CLK)), as a region of the dorsolateral protocerebrum called the pars lateralis (PL) [4]. The polarized light receptor sits in the dorsal rim area of the retina, and, intriguingly, makes connections to the PL via nerve fibers that express CRY1 [4], a cryptochrome whose ortholog in *Drosophila* acts as a blue-light circadian photoreceptor [5]. Indeed, both in cell lines and *in vivo*, CRY1 in the Monarch displays some of the features one might expect of a photoreceptor [6]. CRY1-expressing fibers



Figure 1
The Monarch butterfly (*Danaus plexippus*).

also connect the PL to the pars intercerebralis (PI), which also expresses the clock proteins PER and TIM and is known to be important in insulin signaling, aging and diapause.

In addition, the Monarch expresses CRY2, which is orthologous to vertebrate CRY proteins, and appears to act, as in vertebrates, as the major negative regulator in the molecular feedback loop that defines the circadian mechanism (Figure 2) [6]. CRY2 is coexpressed in the neurons of the PL that express other clock proteins such as PER and TIM, and shows the type of nuclear translocation movements that one expects of a canonical clock protein. Furthermore, CRY2-expressing fibers show rhythmic oscillations in the level of CRY2 within the central complex region of the brain, a region that has been proposed to house the sun compass in locusts [7]. Thus we have a truly extraordinary and tantalizing set of results in which CRY1 connects the polarized-light-input pathway to the clock, and the clock to the PI (which is expected to respond to day length and initiate diapause, enhanced longevity and the associated migratory restlessness), while CRY2 might connect the clock output to the sun compass.

Following the milkweed trail north

One question Reppert and his team wished to answer was whether the spring and summer butterflies orient northwards (in the same way as their parents and (great)grandparents did southwards) in order to return north from Mexico, or whether they simply work their way north following the milkweed trail. In a study by the group published recently in *BMC Biology* [8], Zhu *et al.* treated diapausing fall butterflies with a juvenile hormone (JH) analog and showed that this stimulated reproductive development as expected. They observed, however, that the treated individuals were still able to fly directionally and point south towards Mexico. This means that although JH shutdown stimulates diapause and may initiate celestial orientation, JH deficiency is not required to maintain directionality, which can be independent of reproductive state.

In contrast, the majority of wild-caught summer butterflies did not show any directional response, confirming earlier reports [9]. These results therefore suggest, with some caveats, that the spring and summer butterflies may simply follow the milkweed back home rather than actively orienting with their compass. One wonders whether treating summer butterflies with a JH antagonist might initiate a stronger orienting response?

Genes associated with migration

To identify genes that might be involved in orientation, microarray experiments were carried out to compare the brain transcriptome of fall migrants and summer butterflies [8]. In addition, fall migrants treated with the JH agonist were studied, together with appropriate vehicle-injected migrant controls. Thus, the experimental design sought to identify transcripts that are differentially regulated between the summer group and the migrants (irrespective of the migrants' reproductive state). Forty transcripts were observed to show differences in expression, of which more than half had some annotation associated with them from other databases. The hits included a clock gene *vriille*, which regulates the *Clk* gene and the gene for tyramine beta hydroxylase (which is required for the biosynthesis of the neurotransmitter octopamine), as well as other genes involved in neural and behavioral plasticity. It remains to be seen how important any of these are to the orientation phenotype; this can only be assessed by direct manipulation of these molecules in the brain.

Monarch magnetism?

The receptors for polarized UV light in the butterfly's retinal dorsal rim and their input to the circadian time-oriented sun compass help point the way to Mexico. But many

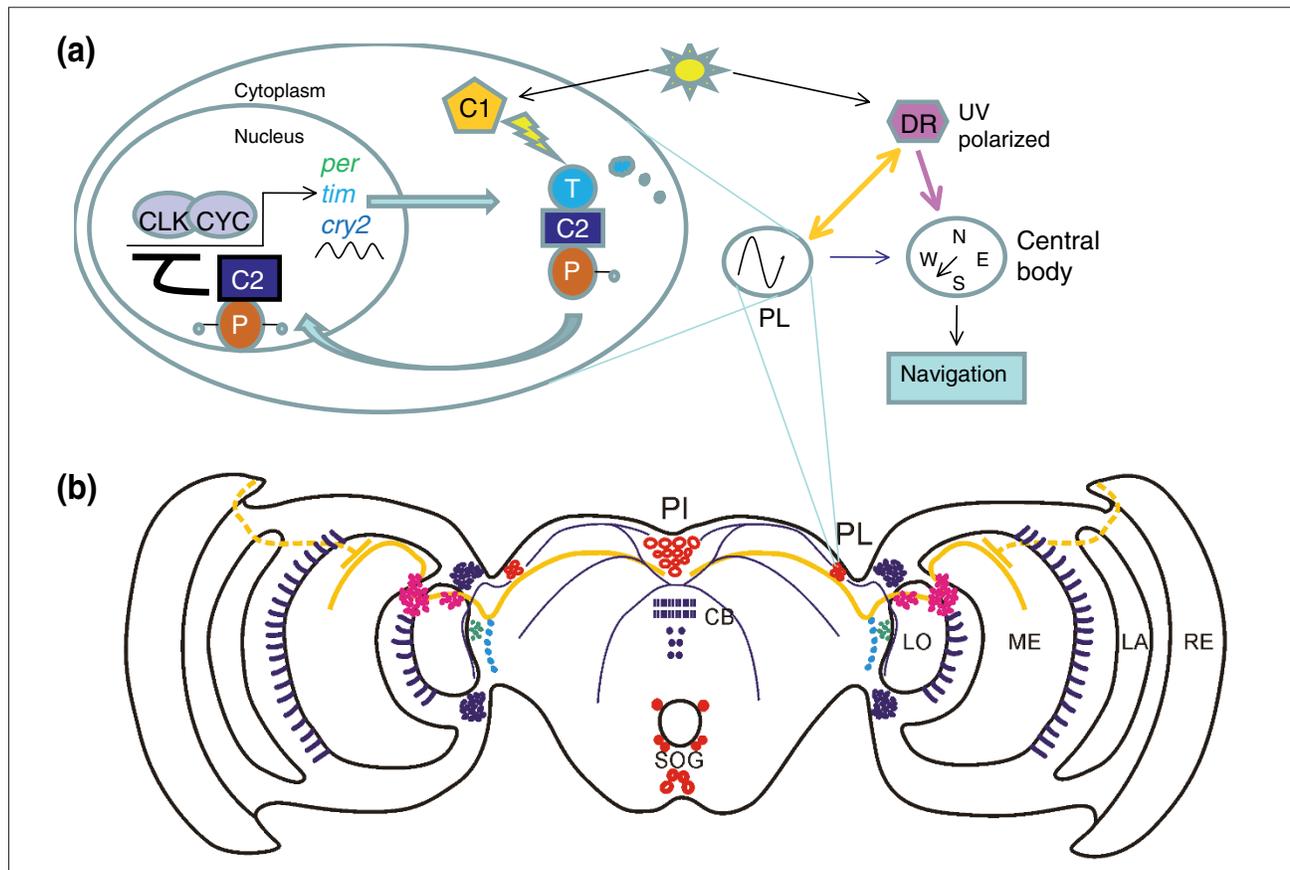


Figure 2

Connecting the circadian clock and the time-compensated sun compass to the brain of *Danaus plexippus*. **(a)** Top left: molecular mechanism that underlies the circadian clock in the Monarch within neurons of the *Pars lateralis* (PL). The positive transcription factors CLOCK (CLK) and CYCLE (CYC) bind to E-boxes (CACGTG) on the promoters of the *period* (*per*), and probably *timeless* (*tim*) and *cryptochrome 2* (*cry2*) genes and activate transcription. In the evening, the PER/CRY2/TIM (P/C2/T) proteins form a complex in the cytoplasm, with PER being phosphorylated during the night phase. Further phosphorylation leads to PER/CRY2 entering the nucleus late at night and CRY2 repressing CLK-CYC mediated transcription. At dawn, light activates the CRY1 blue light photoreceptor with resulting degradation of TIM in the cytoplasm. It is not known whether TIM enters the nucleus in these neurons as CRY1-mediated TIM degradation would also provide a derepression mechanism at dawn. However, CRY2 also cycles and is at low levels during the day so it is conceivable that CRY1 also acts in the nucleus to send a degradation signal to CRY2 at dawn. Top right: light stimulates receptors to UV polarized light in the dorsal rim (DR) of the retina. A CRY1 expressing pathway (double headed, orange) connects the DR to the circadian clock in the PL (and perhaps *vice-versa*), which in turn uses a CRY2 pathway (blue arrow) to connect with the central body (CB), which may house the sun compass that mediates navigation. There may also be a direct pathway (pink arrow) between the DR and the compass. **(b)** Schematic representation of neurons and fibers expressing different circadian clock proteins in monarch butterfly brain. Regions expressing TIM, PER, CRY1 and/or CRY2 are highlighted in red. In these areas the four clock proteins partially colocalize. Areas expressing TIM or CRY1 are indicated in green. In these regions the two clock proteins do not colocalize. CRY1 positive fibers are represented by continuous orange lines. Projections of dorsal rim area photoreceptors are indicated by dotted orange lines. Neurons and fibers expressing exclusively CRY2 are represented in blue and within the central body are shown as blue circles and blue hatching. Areas positive exclusively to TIM and PER are indicated in light blue and brown, respectively. PL, pars lateralis; PI, pars intercerebralis; SOG, subesophageal ganglion; CB, central body; LO, lobula; ME, medulla; LA, lamina; RE, retina (Modified from [4,6]).

animal compasses rely on magnetic fields, so how might magnetoreception be encoded within the Monarch? Reppert's group has also recently carried out a study of magnetoreception in *Drosophila melanogaster* [10]. Using flies trained to respond to a magnetic field, it was apparent that various fly strains showed a modest magnetosensitivity, but only when light in the near-blue region was included.

These wavelengths (around 420 nm) fit the action spectrum of *Drosophila* CRY, and indeed it has been speculated that the photoinducible electron-transfer reaction of this flavoprotein generates magnetosensitive radical pairs [11]. In support of this hypothesis, fly loss-of-function *cry* mutants were severely compromised in their magnetosensitivity [10].

These results suggest that CRY could be a magnetoreceptor, or if not, that it might act as a signaling component downstream of the true receptor. Either way, attention must now focus on the Monarch's CRY proteins, and whether one or both of these can provide magnetosensitivity. Interestingly, in the central body, the putative locality of the sun compass, nerve fibers expressing CRY2, not CRY1, are observed. So, although one would presume from the fly data that the *Drosophila*-like butterfly CRY1 protein would be the most relevant in any photoinducible radical-pair hypothesis, we should perhaps not rule out CRY2. An initial way forward would be to transform the butterfly *cry1* into the fly, and see whether it rescues magnetosensitivity in the *cry* mutant, and whether mutagenesis of the relevant radical-pair residues does not. This and many other experiments now suggest themselves, and there is little doubt that we will soon be treated to another breakthrough in the otherwise mystical phenomenon of Monarch migration.

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