

Scientists discover molecular basis of monarch butterfly migration

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Since its discovery, the annual migration of eastern North American monarch butterflies has captivated the human imagination and spirit. That millions of butterflies annually fly a few thousand miles to reach a cluster of pine groves in central Mexico comprising just 70 square miles is, for many, an awesome and mysterious occurrence. However, over the past two decades, scientists have begun to unveil the journey for what it is: a spectacular result of biology, driven by an intricate molecular mechanism in a tiny cluster of cells in the butterfly brain.

University of Massachusetts Medical School Professor and Chair of Neurobiology Steven M. Reppert, MD, has been a pioneering force in the effort to demystify the migration of the monarch. His previous research has demonstrated that the butterflies use a time-compensated sun compass and daylight cues to help them navigate to the pine groves. His studies have shown that time compensation is provided by the butterfly's circadian clock, which allows the monarch to continually correct its flight direction to maintain a fixed flight bearing even as the sun moves across the sky.

Now, in two papers that will be published this week in two journals of the open-access publisher Public Library of Science (PLoS), Dr. Reppert and colleagues describe in detail the monarch butterfly circadian clock for the first time, and identify and characterize an entirely new clock gene that provides insight into not only the biology of the butterfly and its migration, but also the evolution of circadian clocks in general.

In “Cryptochromes Define a Novel Circadian Clock Mechanism in Monarch Butterflies That May Underlie Sun Compass Navigation,” published in *PLoS Biology*, Reppert and colleagues reveal that the circadian clock of the monarch uses a novel molecular mechanism, heretofore not found in any other insect or mammal.

By studying the clock in two other organisms—the fruit fly and the mouse—scientists thought that they had very good models for an understanding of the insect clock and the mammalian clock, respectively. Through these studies, scientists had described a clock mechanism that is essentially a loop where proteins are made and destroyed over a cycle that takes approximately 24 hours to complete. Further, investigators identified those factors that work together to drive this process.

Reppert and colleagues were particularly interested in one of these factors: CRY, a cryptochrome protein that was initially discovered in plants and was subsequently found in the fly and the mouse. In the fly, CRY functions as a blue light photoreceptor, allowing light access to clock-containing cells. This enables the resetting of the clock by the light-dark cycle. In the mouse, CRY does not function to absorb light; rather, it is one of the essential components that power the central clockwork enabling the feedback loop to continue. (In the mouse, light enters the clock through the animal’s eyes.)

Given the function of CRY in flies and the role of light in migration, scientists presumed that the monarch’s clock would resemble that of the fly. Reppert and his collaborators were stunned and elated to find that the clock of the butterfly was as spectacular as its migration. Genetic studies revealed that the monarch had not only the fly-like CRY, but also another cryptochrome that further tests identified as a new clock molecule in the butterfly. Surprisingly, this cryptochrome, dubbed CRY2, is more similar in structure to vertebrate CRY than to that of the

fruit fly.

Notably, the scientists also found that the core components of the monarch clock resembled those of the mammalian clock. As in the mouse, CRY2 functions in the butterfly to maintain the feedback loop, while CRY1 still allows light to access the cells, as in the fly.

“This is a very interesting realignment of how one thinks about insect clock models. There was no reason to suspect that the butterfly clock would be different from that of *Drosophila*. That it is different has already told us something about how circadian clocks have evolved,” explained Reppert. “What we have in the butterfly is an astounding clock mechanism, one that is more similar to our own circadian clock and less similar to the clock of the fly! The presence and function of two distinct CRYs suggest that the monarch’s is an ancestral clock; a clock that, over the course of evolution, has changed differently in other insects and mammals.”

Reppert and colleagues not only discovered the function of CRY2 in the monarch clock, but they also found that CRY2 may function to mark a critical neural pathway from the circadian clock to the sun compass. This clock-to-compass pathway provides an essential link between the clock and the sun compass, as both are necessary for successful orientation and navigation. As Reppert explains, “CRY2 appears to have a dual function— as a core clock component and as an output molecule, linking the clock to the compass.”

Concurrent with their studies of the monarch clock and relevant to the identification of CRY2, Reppert and colleagues have been working to create a butterfly genomics resource.

In “Chasing Migration Genes: A Brain Expressed Sequence Tag Resource for Summer and Migratory Monarch Butterflies (*Danaus*

plexippus),” published in *PLoS ONE*, Reppert and his collaborators describe a brain expressed sequence tag (EST) resource, used to identify genes involved in migratory behaviors by comparing the gene expression in the brains of migrating butterflies to those of non-migrating butterflies. They have already identified ~10,000 ESTs that likely represent over 50 percent of the genes that make up the monarch genome. The ESTs, which represent expression units of genes in the butterfly brain, are currently being analyzed and catalogued and Reppert hopes that the genetic information will be of wide use to scientists around the world.

“This information, along with genetic markers identified in the study, will help us distinguish genetic differences between populations or even between butterflies that are migratory and not migratory” Reppert said, adding, “This information sets the stage for the cloning of the butterfly genome.”

In fact, Reppert and his fellow investigators recently initiated a collaborative agreement with SymBio Corporation (www.sym-bio.com) of Menlo Park, CA to sequence the entire butterfly genome. According to Robert A. Feldman, President and CEO of SymBio, “We are very excited about the prospect of sequencing the monarch genome. The information gained will not only help elucidate the molecular basis of butterfly migration, but will also add substantial knowledge to comparative genomic studies.” SymBio specializes in sequencing the genomes of a wide range of organisms, from bacteria to mammals.

Ultimately, the Reppert laboratory will continue to work to understand how the monarch clock “talks” to the sun compass, with a focus on CRY2. The goal of the researchers’ studies is to understand the molecular mechanism and anatomical mechanisms for clock-compass interactions that enable migrants to maintain a set flight bearing as the sun moves across the sky during the day.

Dr. Reppert also states, “The monarch provides a fascinating animal model for the study of neurobiology. By understanding more about the way the circadian clock and the sun compass interact to allow the monarch to fulfill its biological destiny, we will gain valuable insights into how the brain functions to incorporate information about time and space, which has relevance far beyond the butterfly.”

Citation: Zhu H, Sauman I, Yuan Q, Casselman A, Emery-Le M, et al. (2008) Cryptochromes define a novel circadian clock mechanism in monarch butterflies that may underlie sun compass navigation. PLoS Biol 6(1): e4. doi:10.1371/journal.pbio.0060004

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