THE VALUE OF INTERDISCIPLINARY RESEARCH: LESSONS FROM THE 2017 NOBEL PRIZE IN CHRONOBIOLOGY

JADWIGA M. GIEBULTOWICZ A–F
Department of Integrative Biology, Oregon State University, Corvallis, OR, USA

A – study design, B – data collection, C – statistical analysis, D – interpretation of data, E – manuscript preparation, F – literature review, G – sourcing of funding

ABSTRACT

Since 1901, the Nobel Prize has been awarded to scientists who have made the most important discoveries for the benefit of humanity. The 2017 Nobel Prize in Physiology or Medicine was awarded jointly to Jeffrey C. Hall, Michael Rosbash and Michael W. Young “for their discoveries of molecular mechanisms controlling the circadian rhythm.” It may be surprising to learn that those three scientists dedicated their entire careers to research on the fruit fly, Drosophila melanogaster. However, as their studies progressed, it became increasingly clear that the mechanism of the biological clock that they discovered in Drosophila is very similar to a timekeeping mechanism present in mammals, including humans. Through interdisciplinary work between scientists performing basic research on model organisms and doctors working in medical schools, we have learned over time that daily rhythms support human health while disruption of these rhythms is associated with a range of pathological disorders such as cardiovascular problems, metabolic, neurological, and many other diseases. This short review will highlight critical milestones on the way to understanding biological clocks, focusing on the roles played by the three Nobel Prize winners.

KEYWORDS: circadian rhythms, biological clock, Drosophila, human health

Most animals lead rhythmic lives; some are active at night and sleep during the day while others are diurnal being active during daytime. These rhythms are not merely a response to a daylight or darkness at nighttime. When animals are placed in constant conditions such as constant darkness, they do not lose a sense of time but rather maintain rhythmicity that has a period of about or “circa” 24 hours; therefore, these cycles are called circadian rhythms. Even humans isolated from a solar day and left to schedule their own activities maintain a clear circadian rhythm of sleep and wakefulness. These types of experiments demonstrate that organisms have evolved their own internal timekeeping mechanism, which is synchronized (entrained) daily to a 24h solar cycle. The internal sense of time allows us to anticipate cyclically occurring daily events. For example, before we wake up, our internal clock orchestrates an increase in blood pressure and levels of cortisol to prepare us for activities of the day. The understanding of how our clocks operate at the molecular level emerged from basic research involving organisms approximately 10 million times lighter than human.

The discovery of the first clock gene

The mechanism of circadian clocks remained a total mystery until researchers working with fruit flies, Drosophila melanogaster, used the knowledge about fly rhythmic behavior to demonstrate that the fly clock has genetic basis. Fruit flies have been used as a genetic model for over a hundred years, as they possess a short life cycle of 10 days from egg to adult, a high reproduction rate, and there are well-established methods to induce and map mutations in their genome. It was known that adult fruit flies emerge from their pupal cases in a rhythmic fashion (in the morning) and a free-running rhythm of adult emergence persists in constant darkness. The experimental approach was to mutate hundreds of flies and test whether any of them would emerge at the “wrong” time, suggesting that they could carry a circadian timing mutation. Indeed, the authors of this study, Ron Konopka and Seymour Benzer, discovered that a single genomic locus named period (per) carried three different mutations [1]. One mutant completely lost the emergence rhythm (per0), another mutation shortened the free-running rhythm from circa 24h to 19h (per short), and the third mutation produced long-period rhythms of 29h (per long) of adult emergence. Excitingly, the same mutations caused corresponding changes in the locomotor activity rhythms of individual flies, indicating that the period...
gene is part of the clock controlling different behavioral rhythms.

**Work of Nobel Laureates on the Clock Mechanism**

The discovery that the gene *period* was necessary for circadian rhythms in flies was the first milestone on the way to understanding the mechanism of biological clocks. However, the sequence and function of *period* remained unknown until the mid-80s, when novel genetic and molecular tools were developed in *Drosophila* allowing DNA sequencing and introducing pieces of cloned DNA into the fly genome. An interdisciplinary team led by behavioral geneticist Jeffrey Hall and molecular biologist Michael Rosbash at Brandeis University used these tools to characterize the *period* gene. Meanwhile, another scientist Michael Young at Rockefeller University also attempted to sequence period DNA and determine whether it was indeed a part of the circadian clock. The Brandeis and Rockefeller teams independently demonstrated in 1984 that the introduction of *period* genomic fragments into an arrhythmic per-1 mutant caused rescue of both adult emergence rhythm and locomotor activity rhythm [2,3]. Further studies in the labs of J. Hall and M. Rosbash showed that PER protein [4] and *per* mRNA [5] undergo daily oscillations and suggested that clock may consist of a negative feedback loop with the PER protein acting as a repressor [5]. Meanwhile, another mutant that abolished circadian rhythms in flies was uncovered in the laboratory of M. Young [6]. This second clock gene was named *timeless* (*tim*) and the TIM protein turned out to be a partner of PER, necessary for its stability and nuclear entry [7,8].

Although it was evident that PER and TIM proteins somehow affected transcription of their own genes, the mechanism was not clear owing to the fact that these proteins did not possess DNA-binding domains; therefore, they could not directly affect transcription of their own or other genes. Fortunately, a search for more arrhythmic mutants in the labs of J. Hall and M. Rosbash revealed two genes encoding transcription factors CLOCK [9] and CYCLE [10] that had known DNA-binding domains and could bind to *per* and *tim* promoter region and activate their transcription. Interestingly, the *Clock* gene was first identified as part of the mammalian timing mechanism [11], and interdisciplinary communication between fly and mouse researchers greatly facilitated the progress in the understanding of the circadian clock.

**Human Circadian Clocks Are Remarkably Similar to Fly Clocks**

By the turn of the century, it was clear that the fly clock operates as a negative feedback loop involving transcription and translation of several clock genes. A simplified version of the core feedback loop in *Drosophila* was reviewed recently [12] and is shown in Figure 1. Two transcription factors encoded by the genes *Clock* (*Clk*) and *cycle* (*cyc*) act as the positive limb of the clock, whereby CLK-CYC form heterodimers, which bind to the E-box sequences in the promoters of *per* and *tim* genes, stimulating their transcription in the early night. PER and TIM proteins act as the negative limb of the clock when they accumulate in the cell nuclei late at night and repress CLK-CYC activity. This results in the suppression of *per* and *tim* transcription until the repressive PER and TIM are degraded. Degradation of TIM is initiated by light via the photoreceptive CRY protein encoded by the *cryptochrome* (*cry*) gene characterized in *Drosophila* by J. Hall and M. Rosbash [13,14]. Upon activation by light, CRY binds to TIM protein leading to its degradation. Because TIM stabilizes PER, the latter is also degraded within few hours of lights-on. The progress in the understanding of the fly clock was followed closely by discovery that the negative feed-
back loop is at the core of the circadian clock in mammals, including humans. Mammalian clocks operate by the same mechanisms and contain mostly homologous genes as Drosophila clocks. A major difference between fly and mammalian clocks is the use of CRY, rather than TIM, as the PER partner. Mammalian CRY lost light sensitivity and gained a function as the circadian repressor.

Based on early observations of behavioral rhythms in sleep/activity, feeding, and cognitive functions, it was assumed that the clock would reside in specialized neurons. Indeed, the circadian clocks regulating behavior have been identified in the specific brain neurons of mammals and insects using perturbation of locomotor activity rhythms as a readout of clock function. However, it is now well established that animals possess multi-oscillatory circadian systems with master clocks residing in the central nervous system and peripheral clocks present in cells forming most other tissues. The existence of peripheral clocks that can function independently of the brain was first demonstrated in moths [15], then in Drosophila [16] and finally in mammals [17]. Clocks that exist in cells making up most body organs in flies and mammals provide the temporal framework to organize activity of different tissues, allowing synchronization of compatible and separation of incompatible processes. The molecular rhythms generated by the tissue-specific clocks contribute to rhythmic physiology such as daily fluctuations in the levels of hormones, enzymes, and various metabolites. In fact, nearly all aspects of metabolism vary with time of day, at both cellular and systemic levels [18]. These rhythms are tightly connected to daily cycles of food intake, digestion, motor, and cognitive activities that are followed by sleep associated with fasting and cellular repair.

Circadian clocks are important for human health

Studies in model organisms show that disruption of circadian rhythms may have pathological consequences. Laboratory mammals with genetically engineered defects in their circadian clocks show many pathologies including obesity, diabetes, steatosis, cardiomyopathy, and atherosclerosis [18]. There is also accumulating evidence that age-related disruptions of normal circadian rhythms and sleep cycles can affect neuronal health and contribute to pathogenesis of neurodegenerative diseases, such as Alzheimer’s disease [19]. A Nobel Prize for the discovery of the circadian clock mechanism may increase the awareness of “circadian hygiene” that humans should maintain to stay healthy. Eating, working, and sleeping at the right time of the solar day supports human health and wellbeing, while disrupting these natural rhythms may be associated with a host of pathological problems. Increasingly, modern humans tend to impair their natural circadian rhythms by shift work and travel across time zones. In addition, irregular eating habits and prolonged exposure to artificial light emitted by electronic devices disturbs our clocks and reduces sleep, which has detrimental effects on attention and learning.

Concluding remarks.

The discovery of the circadian clock was driven by the curiosity of scientists coming from different fields of study and collaborating by putting together their respective expertise. Such interdisciplinary approach is always evident at the meetings of the Society for Research on Biological Rhythms, which brings together researchers working on clocks in bacteria, plants, and animals as well as clinicians. They can learn from each other because most molecular pathways are conserved in evolution and human cells function and divide by the same mechanisms as in flies. The Nobel Prize for three fly scientists highlights the unity of fundamental life processes and underscores the value of basic research on simple model organisms for the understanding of our own physiology and for making progress in preventing and treating various human diseases.

Acknowledgements

The author thanks Eileen Chow for editing the manuscript. Polish-American Fulbright Commission is acknowledged for financial support granted to JMG to teach and do research in Poland.

References

1. Konopka Rj, Benzer S. Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci USA 1971; 68: 2112–2116.
2. Bargiello TA, Young MW. Molecular genetics of a biological clock in Drosophila. Proc Natl Acad Sci USA 1984; 81: 2142–2146.
3. Reddy P, Zehring WA, Wheeler DA, Pirrotta V, Hadfield C, et al. Molecular analysis of the period locus in Drosophila melanogaster and identification of a transcript involved in biological rhythms. Cell 1984; 38: 701–710.
4. Siwicki KK, Eastman C, Petersen G, Rosbash M, Hall JC. Antibodies to the period gene product of Drosophila reveal diverse tissue distribution and rhythmic changes in the visual system.
5. Neuron 1988; 1: 141–150.
6. Hardin PE, Hall JC, Rosbash M. Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. Nature 1990; 343: 536–540.
7. Sehgal A, Price J, Man B, Youngs M. Loss of circadian behavioral rhythms and per RNA oscillations in the Drosophila mutant timeless. Science 1994; 263: 1603–1606.
8. Gekakis N, Saiz L, Sehgal A, Young M, Weitz C. Isolation of timeless by PER protein interaction: defective interaction between timeless protein and long-period mutant PER. Science 1995; 270: 811–815.
8. Vosshall L, Price J, Sehgal A, Saez L, Young M. Block in nuclear localization of period protein by a second clock mutation, timeless. Science 1994; 263: 1606–1609.
9. Allada R, White NE, So WV, Hall JC, Rosbash M. A mutant Drosophila homolog of mammalian Clock disrupts circadian rhythms and transcription of period and timeless. Cell 1998; 93: 791–804.
10. Rutilla JE, Suri V, Le M, So V, Rosbash M, et al. CYCLE is a second bHLH-PAS Clock protein essential for circadian rhythmicity and transcription of Drosophila period and timeless. Cell 1998; 93: 805–814.
11. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, et al. Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. Science 1994; 264: 719–725.
12. Giebultowicz JM. The circadian system and aging of Drosophila. In: Jazwinski S, Belancio V, Hill S, ed. Circadian Rhythms and Their Impact on Aging, Volume 7. Cham, Switzerland: Springer International Publishing AG; 2017: 129-145.
13. Emery P, So V, Kaneko M, Hall JC, Rosbash M. CRY, a Drosophila clock and light-regulated cryptochrome, is a major contributor to circadian rhythm resetting and photosensitivity. Cell 1998; 95: 669–679.
14. Stanewsky R, Kaneko M, Emery P, Beretta B, Wager-Smith K, et al. The cry' mutation identifies cryptochrome as a circadian photoreceptor in Drosophila. Cell 1998; 95: 681–692.
15. Giebultowicz JM, Riemann JG, Raina AK, Ridgway RL. Circadian system controlling release of sperm in the insect testes. Science 1989; 245: 1098–1100.
16. Hege DM, Stanewsky R, Hall JC, Giebultowicz JM. Rhythmic expression of a PER-reporter in the Malpighian tubules of decapitated Drosophila: evidence for a brain-independent circadian clock. J Biol Rhythms 1997; 12: 300–308.
17. Balsalobre A, Daniola F, Schibler U. A serum shock induces circadian gene expression in mammalian tissue culture cells. Cell 1998; 93: 929–937.
18. Brown SA. Circadian Metabolism: From Mechanisms to Metabolomics and Medicine. Trends Endocrinol Metab 2016; 27: 415–426.
19. Musiek ES, Holtzman DM. Mechanisms linking circadian clocks, sleep, and neurodegeneration. Science 2016; 354: 1004–1008.