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News and Perspectives

The discoveries of molecular mechanisms for the circadian rhythm: The 2017 Nobel Prize in Physiology or Medicine

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ABSTRACT

Circadian clocks evolved to allow plants and animals to adapt their behaviors to the 24-hr change in the external environment due to the Earth's rotation. While the first scientific observation of circadian rhythm in the plant leaf movement may be dated back to the early 18th century, it took 200 years to realize that the leaf movement is controlled by an endogenous circadian clock. The cloning and characterization of the first *Drosophila* clock gene *period* in the early 1980s, independently by Jeffery C. Hall and Michael Rosbash at Brandeis University and Michael Young at Rockefeller University, paved the way for their further discoveries of additional genes and proteins, culminating in establishing the so-called transcriptional translational feedback loop (TTFL) model for the generation of autonomous oscillator with a period of ~24 h. The 2017 Nobel Prize in Physiology or Medicine was awarded to honor their discoveries of molecular mechanisms controlling the circadian rhythm.

Circadian rhythms are generated by endogenous oscillators to allow organisms to change their behaviors with a period of ~24 h in anticipation for the changing environment of day–night cycle brought about by the Earth's rotation. The term "circadian" (circa, ~; dies, a day) is used because in constant conditions (free from external time cues) the free-running period may be longer or shorter than, but not exactly, 24 h.

The first scientific observation of circadian rhythm was made in 1729 by the French astronomer Jean Jacques d'Ortous de Mairan, who placed the mimosa plant in a light-tight dark room and observed that the plant continued to unfold its leaves in the morning and close them in the evening [1,2]. Two hundred years later in the 1930s the German biologist Erwin Bünning determined that the bean plant leaf movement has a period of 24.4, but not 24, hr in the constant light condition and that the trait can be inherited, thereby establishing that the plant photoperiodism is controlled by an endogenous clock that can be synchronized by external stimuli [1,2].

In the 1960s, single genes controlling physical appearance of traits have been firmly established in the fruit fly *Drosophila*,

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but Seymour Benzer went steps further to contemplate that single genes may also control specific behaviors [1]. By using mutagens to treat the fly and screening for abnormalities in the circadian rhythm of pupal eclosion and locomotor activity, Konopka and Benzer identified three mutants, one arrhythmic, another a shorter period of 19 h, and the third a longer period of 28 h [3]. This landmark discovery marked the beginning of a long quest for the discoveries of molecular mechanisms for the circadian rhythm.

Molecular mechanisms for controlling the circadian rhythm

It would wait until the early 1980s when the rapid progress of recombinant DNA made it feasible to clone a gene and then identify it by genetic rescue [4]. In 1984, the first clock gene *period* (*per*) was identified when Jeffery C. Hall and Michael Rosbash at Brandeis University and Michael Young at Rockefeller University independently cloned and rescued *Drosophila period* [5–8]. The cloning of *period* did not, however, automatically reveal its molecular mechanism for the circadian clock and the following years before 1988 marked a state of confusion about the function of its protein product PER [2,4].

The first hint of a possible role of PER as a transcription factor came in 1988 with the identification of the *Drosophila single-minded* gene, which encodes a nuclear protein with sequence similarity to the *period* gene product PER [4,9]. Hall and Rosbash then made a series of breakthroughs beginning in 1988 with the discovery of a free-running circadian rhythm in the abundance of PER protein in the fly visual system [10]. Two years later they further found in the fly head a free-running circadian rhythm in the levels of *per* mRNA, which peak in the early night, several hours earlier than the peak PER protein abundance [11]. Importantly, the *per* nonsense mutation abolishes the oscillation in mRNA levels, which is rescued by the addition of wild-type PER protein. Furthermore, the *per* missense mutations identically affect both the phase of mRNA oscillation and behavioral rhythm. The results prompted Hall the Rosbash to propose a feedback model of PER protein directly affecting its own gene expression. Their subsequent findings of PER being a nuclear protein shuttling between the nucleus and the cytoplasm [12] and its overexpression lowering *per* mRNA levels [13] are consistent with the transcriptional translational feedback loop (TTFL) model.

It remains to be determined how the PER protein enters the nucleus to act as a transcription factor. The discovery of the second clock gene *timeless* (*tim*) by Young in 1990s provided an answer to the question [14,15]. Young's group found that the *tim* mRNA levels oscillate in phase with *per* mRNA [15] and that the *tim* mutant suppresses the rhythm of *per* mRNA levels and abolishes both rhythmic pupal eclosion and locomotor activity [16]. Importantly, the TIM protein encoded by the *tim* gene interacts with PER to allow nuclear entry of PER [17]; the *tim* mutant suppresses the PER levels and blocks nuclear localization of PER protein as well as the circadian oscillations in both PER abundance and phosphorylation [18,19]. Together the results indicate that the cyclic expression of *tim* dictates the cyclic accumulation and nuclear localization of PER protein, further supporting the TTFL model.

To sustain an autonomous oscillation, however, requires a positive input to fuel the transcription of *tim* and *per*. The discovery of the *Clock* gene in mouse by Joseph Takahashi [20–22] and subsequently its partner BMAL1 [23] establishes that CLOCK-BMAL1 heterodimers binding to the enhancer E-box serves as the positive input component to drive *per* transcriptional oscillations. Importantly, PER and TIM inhibit *Drosophila* CLOCK activity, thereby closing the circadian feedback loop [24]. Hall and Rosbash went on to discover the *Drosophila Clk* and *Cyc*, orthlogs of mammalian *Clock* and *Bmal1*, respectively, as positive transcription factors for *per* and *tim* [25,26]. Putting together into the core TTFL model of circadian rhythms, the core transcriptional activator (*Clk* and *Cyc* in *Drosophila* and *Clock* and *Bmal1* in mammals) drives the expression of their own negative regulators (*Per* and *tim* in *Drosophila* and *Per* 1–3 and *Cry* 1–2 in mammals) (Fig. 1).

However, the biochemical processes involved in transcription and translation are generally rapid and a delayed formation of PER/TIM is required to ensure a period of ~24 h [15]. Young's discovery of another essential clock component the *doubletime* (*dbt*) gene provides the needed delay [27,28]. The *dbt* gene encodes a kinase (casein kinase 1) that binds to and phosphorylates PER for degradation, and as such DBT reduces the stability and accumulation of PER, thereby promoting a delay between *per*/*tim* transcription and PER/TIM nuclear function (see Fig. 1). In hamster, the short-period *tau* mutant CKIε (casein kinase 1 epsilon), the mammalian orthlogs of *Drosophila* DBT, has markedly reduced maximal velocity and autophosphorylation state [29].

Perspectives

The core TTFL model, PER/TIM binds to and inhibits their own gene transcription by CLOCK/CYCLE, established in *Drosophila* is considered to be the canonical model for circadian clocks. Although the core proteins may not be conserved across species, meaning that circadian clocks may have evolved multiple times, the core TTFL structure is very similar in mammals [30], plants [31], the filamentous fungus *Neurospora crassa* [32], and even in the cyanobacterium *Synecchococcus aureus* [33] (see ref. [34] for review). Interestingly, a temperature-compensated circadian oscillation of KaiC phosphorylation in the cyanobacterium can be reconstituted in the test tube even in the absence of transcription and translation by simply adding recombinant proteins and ATP [35]. Recent studies also indicate the presence of transcription-independent circadian oscillations in the oxidation state of peroxiredoxin proteins in human red blood cells, algae, and in all domains of life [36–38].

In conclusion, the nearly ubiquitous presence of circadian clocks in all life forms suggests evolutionary advantage to being able to anticipate and adapt to the daily changing environments. Indeed, the clock genes have since greatly expanded along with parallel feedback loops added to allow mutual interaction between circadian clocks and various aspects of physiology, attesting to a role beyond simple timekeeping. The 2017 Nobel Prize in Physiology or Medicine was awarded to honor the three Nobel Laureates for their

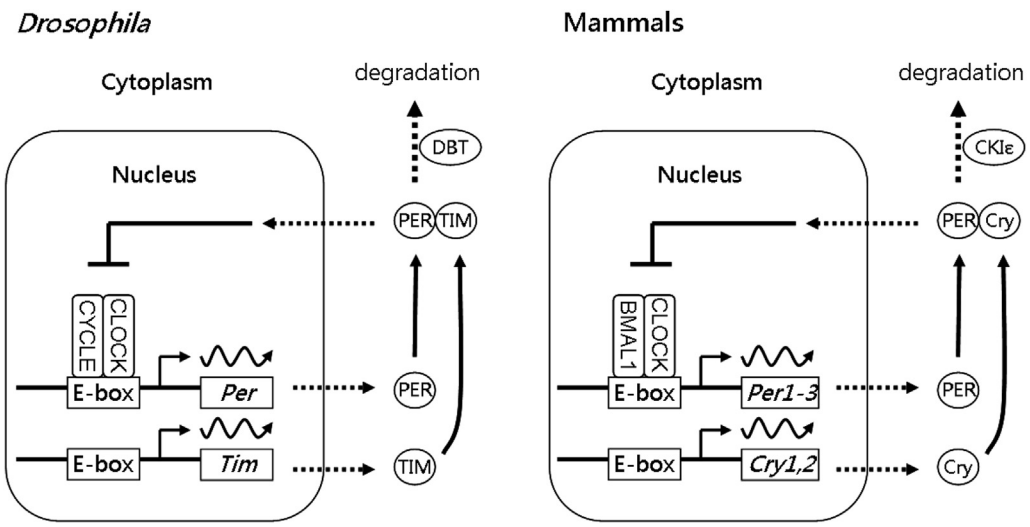

Fig. 1 Oversimplified schematic drawing showing the core transcriptional translational feedback loop (TTFL) clockwork mechanism in *Drosophila* (left) and mammals (right).

discoveries of molecular mechanisms controlling the circadian rhythm.

Conflicts of interest

The author declares that he has no competing interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.bj.2018.02.003.

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