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

Rong-Chi Huang

A R T I C L E   I N F O

Article history:
Received 16 January 2018
Accepted 7 February 2018
Available online 29 March 2018

Keywords:
Circadian clocks
Circadian rhythms
Clock genes
TTFL model
2017 Nobel Prize

A B S T R A C T

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,
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 and 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 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* **Ck** and **Cyc**, orthologs 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 (**Ck** 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 **Cki** (casein kinase 1 epsilon), the mammalian orthologs 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 *Synechococcus 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
discoveries of molecular mechanisms controlling the circadian rhythm.

Conflicts of interest

The author declares that he has no competing interest.

Acknowledgements

I am grateful to Neuroscience Research Center of Chang Gung Memorial Hospital, Linkou, Taiwan. This work was supported by Chang Gung Medical Foundation (CMRPD1G0051, CMRPD1H0071; R.C.H) and by Taiwan Ministry of Science and Technology (MOST103-2320-B-182-007; R.C.H).

Appendix A. Supplementary data

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

 REFERENCES

[1] Foster RG, Kreitzman L. Rhythms of life. The biological clocks that control the daily lives of every living thing. New Haven and London: Yale University Press; 2004.
[2] Ibanez C. The 2017 Nobel prize in physiology or medicine – advanced information: discoveries of molecular mechanisms controlling the circadian rhythm. Nobelpriize.org. Nobel Media AB 2014. http://www.nobelpriize.org/nobel_prizes/medicine/laureates/2017/advanced.html. [Accessed 10 January 2018].
[3] Konopka RJ, Benzer S. Clock mutants of Drosophila melanogaster. Proc Natl Acad Sci USA 1971;68:2112–6.
[4] Rosbash MA. 50-year personal journey: location, gene expression, and circadian rhythms. Cold Spring Harb Perspect Biol 2017;9:a032516.
[5] Bargiello TA, Young MW. Molecular genetics of a biological clock in Drosophila. Proc Natl Acad Sci USA 1984;81:2142–6.
[6] Bargiello TA, Jackson FR, Young MW. Restoration of circadian behavioural rhythms by gene transfer in Drosophila. Nature 1984;312:752–4.
[7] Reddy P, Zehring WA, Wheeler DA, Pirrotta V, Hadfield C, Hall JC, et al. Molecular analysis of the period locus in Drosophila melanogaster and identification of a transcript involved in biological rhythms. Cell 1984;38:701–10.
[8] Zehring WA, Wheeler DA, Reddy P, Konopka RJ, Kyriacou CP, Rosbash M, et al. P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic Drosophila melanogaster. Cell 1984;39:369–76.
[9] Crews ST, Thomas JB, Goodman CS. The Drosophila single-minded gene encodes a nuclear protein with sequence similarity to the per gene product. Cell 1988;52:143–52.
[10] 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. Neuron 1988;1:141–50.
[11] Hardin PE, Hall JC, Rosbash M. Feedback of the Drosophila period gene product on circadian cycling of its messenger RNA levels. Nature 1990;343:536–40.
[12] Liu X, Zwiebel LJ, Hinton D, Benzer S, Hall JC, Rosbash M. The period gene encodes a predominantly nuclear protein in adult Drosophila. J Neurosci 1992;12:2735–44.
[13] Zeng H, Hardin PE, Rosbash M. Constitutive overexpression of the Drosophila period protein inhibits period mRNA cycling. EMBO J 1994;13:3590–8.
[14] Myers MP, Wager-Smith K, Wesley CS, Young MW, Sehgal A. Positional cloning and sequence analysis of the Drosophila clock gene timeless. Science 1995;270:805–8.
[15] Sehgal A, Rothenfluh-Hilfiker A, Hunter-Ensor M, Chen Y, Myers M, Young MW. Rhythmic expression of timeless: a basis for promoting circadian cycles in period gene autoregulation. Science 1995;270:808–10.
[16] Sehgal A, Price J, Man B, Young M. Loss of circadian behavioral rhythms and per RNA oscillations in the Drosophila mutant timeless. Science 1994;263:1603–6.

Fig. 1 Oversimplified schematic drawing showing the core transcriptional translational feedback loop (TTFL) clockwork mechanism in Drosophila (left) and mammals (right).
[17] Gekakis N, Saez L, Delahaye-Brown A, Myers MP, Sehgal A, Young MW, et al. Isolation of \textit{timeless} by PER protein interactions: defective interaction between \textit{timeless} protein and long-period mutant PERL. Science 1995;270:811–5.

[18] Vosshall LB, Price JL, Sehgal A, Saez L, Young MW. Block in nuclear localization of \textit{period} protein by a second clock mutation, \textit{timeless}. Science 1994;263:1606–9.

[19] Price JL, Dembinska ME, Young MW, Rosbash M. Suppression of \textit{PERIOD} protein abundance and circadian cycling by the \textit{Drosophila} clock mutation \textit{timeless}. EMBO J 1995;14:4044–9.

[20] Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, et al. Mutagenesis and mapping of a mouse gene, \textit{Clock}, essential for circadian behavior. Science 1994;264:719–25.

[21] King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, et al. Positional cloning of the mouse circadian clock gene. Cell 1997;89:641–53.

[22] Antoch MP, Song EH, Chang AM, Vitaterna MH, Zhao Y, Wilsbacher LD, et al. Functional identification of the mouse circadian clock gene by transgenic BAC rescue. Cell 1997;89:655–67.

[23] Gekakis N, Staknis D, Nguyen HB, Davis CF, Wilsbacher LD, King DP, et al. Role of the \textit{CLOCK} protein in the mammalian circadian mechanism. Science 1998;280:1564–9.

[24] Darlington TK, Wage-Smith K, Ceriani MF, Staknis D, Gekakis N, Steeves TDL, et al. Closing the circadian feedback loop: \textit{CLOCK}-induced transcription of its own inhibitors, \textit{period} and \textit{timeless}. Science 1998;280:1599–603.

[25] Allada R, White NE, So WV, Hall JC, Rosbash M. A mutant \textit{Drosophila} homolog of mammalian \textit{CLOCK} disrupts circadian rhythms and transcription of \textit{period} and \textit{timeless}. Cell 1998;93:791–804.

[26] Rutila JE, Suri V, Le M, So WV, Rosbash M, Hall JC. \textit{CYCLE} is a second \textit{bHLH-PAS} clock protein essential for circadian rhythmicity and transcription of \textit{Drosophila} \textit{period} and \textit{timeless}. Cell 1998;93:805–14.

[27] Price JL, Blau J, Rothenfluh A, Abeodeely M, Kloss B, Young MW. \textit{double-time} is a novel \textit{Drosophila} clock gene that regulates \textit{PERIOD} protein accumulation. Cell 1998;94:83–95.

[28] Kloss B, Price JL, Saez L, Blau J, Rothenfluh A, Wesley CS, et al. The \textit{Drosophila} clock gene \textit{double-time} encodes a protein closely related to human casein kinase \textit{I}. Cell 1998;94:97–107.

[29] Lowrey PL, Shimomura K, Antoch MP, Yamazaki S, Zemelides PD, Ralph MR, et al. Positional syntenic cloning and functional characterization of the mammalian circadian mutation \textit{tau}. Science 2000;288:483–92.

[30] Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 2001;63:647–76.

[31] Nohales MA, Kay SA. Molecular mechanisms at the core of the plant circadian oscillator. Nat Struct Mol Biol 2016;23:1061–9.

[32] Baker CL, Loros JJ, Dunlap JC. The circadian clock of \textit{Neurospora crassa}. FEMS Microbiol Rev 2011;36:95–110.

[33] Ishiura M, Kutsuna S, Aoki S, Iwasaki H, Andersson CR, Tanabe A, et al. Expression of a gene cluster \textit{kaiABC} as a circadian feedback process in cyanobacteria. Science 1998;281:1519–23.

[34] Brown SA, Kowalska E, Dallmann R. \textit{(Re)inventing} the circadian feedback loop. Dev Cell 2012;13:477–87.

[35] Nakajima M, Imai K, Ito H, Nishiwaki T, Murayama Y, Iwasaki H, et al. Reconstitution of circadian oscillation of cyanobacterial \textit{KaiC} phosphorylation in vitro. Science 2005;308:414–5.

[36] O'Neill JS, Reddy AB. Circadian clocks in human red blood cells. Nature 2011;469:498–503.

[37] O'Neill JS, van Ooijen G, Dixon LE, Troein C, Corellou F, Bouget FY, et al. Circadian rhythms persist without transcription in a eukaryote. Nature 2011;469:554–8.

[38] Edgar RS, Green EW, Zhao Y, van Ooijen G, Olmedo M, Qin X, et al. Peroxiredoxins are conserved markers of circadian rhythms. Nature 2012;485:459–64.