

Japan Academy Prize to:

Takao KONDO
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University



for “Studies of Biological Time Measurement
in Cyanobacteria by Reconstitution of the
Circadian Clock”

Outline of the work:

To precisely fit their metabolic activities to day–night alternation of the environment, living organisms on the Earth have a biological clock (circadian clock) with a 24-hour period that originated by the rotation of the Earth. The question of the biological mechanism that generates a stable rhythm with a 24-hour period has fascinated researchers in several areas of the natural sciences. Dr. Takao Kondo devoted his graduate study to the circadian clock and, in the early 1990s, developed a new experimental system for studying the circadian clock in cyanobacteria. With this system, he isolated many clock mutants that enabled identification of the cyanobacterial clock genes *kaiA*, *kaiB*, and *kaiC*. By studying the expression of *kai* genes, the circadian clock model controlled by negative feedback of *kai* gene expression was proposed as the cyanobacterial circadian clock, which is similar to that for many eukaryotes.

However, Dr. Kondo recognized that it was difficult to explain how the 24-hour periodicity is defined and how it compensates against ambient temperature. Therefore, he focused on biochemical analyses of KaiC activity that could alter the period length. In 2005, his group found that the phosphorylation rhythm of KaiC persisted even under conditions when no transcriptional and translational activity was permitted. This finding generated a major impact to the conventional hypothesis. Further, they found that the 24-hour rhythm of KaiC phosphorylation occur autonomously just by mixing three Kai proteins and ATP in a test tube. The period of the rhythm of Kai proteins was circadian and temperature-compensated, and its phase could be reset by external temperature stimuli. Therefore, the reconstituted rhythm was almost identical to that found in the living cell. This finding was a novel and creative discovery, because we could study the circadian rhythm of living organisms *in vitro* using only three proteins and ATP.

Recent studies in Dr. Kondo’s group have indicated that KaiC consists of two ATPase domains, C-terminal domain and N-terminal domain. Serine and threonine residues in the C-terminal domain are phosphorylated cyclically by association of KaiA and KaiB. ATP hydrolysis by KaiC in the N-terminal domain is extremely weak but robustly temperature-compensated. Because the temperature-compensated ATPase activity is closely coupled with the frequency of the phosphorylation rhythm, they proposed a model of the cyanobacterial circadian clock in which the temperature-compensated ATPase activity functions as a circadian pacemaker to control the phosphorylation cycle.

The finding that the circadian rhythm of living organisms can be reconstituted by three proteins and ATP had a major impact on the field of chronobiology of all organisms. Moreover, the finding that proteins can measure time of day precisely have elucidated a completely novel function of proteins and introduced an unexpected turning point in biochronometry by providing a new concept for biological timing mechanisms. The finding also had a major impact on the fields of biochemistry and biophysics by introducing the concept

and possibility of proteins to measure time and to process information, influencing our view on substances.

Domestic and international evaluation of Dr. Kondo's contributions is extremely high, and he has been repeatedly invited to keynote lectures at many international conferences. Further elucidation of Kai protein function is expected in the future and there is no doubt that his research will continue to lead this international research field. Elucidation of the principle of the circadian clock would be an essential process not only for management of shift work and jet lag but also for psychiatry and clinical treatments (insomnia, depression). Dr. Kondo has elucidated the basic principle of the circadian clock, and his contribution will be important for understanding the human circadian clock.

Dr. Kondo's findings have appeared not only in academic journals but also in general magazines and TV programs, and have attracted general social attention. The Faculty of 1000 reviewers, who evaluate life science papers, have continued to award the paper of the reconstruction of the circadian clock in 2005 as the highest ratings in the circadian clock field. In addition, many prizes such as the Aschoff-Honma Prize (an international award in the circadian clock field); the Chunichi Culture Prize; the Asahi Prize; the Education, Culture, Sports, Science and Technology Minister's Commendation; Medal with Purple Ribbon, and prizes from academic societies (Botany, Plant Physiology and Genetics) have been awarded for his contributions. Therefore, these outstanding achievements of Dr. Kondo are appropriate for the Japan Academy Prize.

List of major publications

Dr. Kondo published 90 or more original papers and 40 or more review articles. The major publications are listed.

1. Kondo T, Strayer CA, Kulkarni RD, Taylor W, Ishiura M, Golden SS, Johnson CH (1993) Circadian rhythms in prokaryotes: Luciferase as a reporter of circadian gene expression in cyanobacteria. *Proc. Natl. Acad. Sci. USA* 90: 5672-5676.
2. Kondo T, Tsinoremas NF, Golden SS, Johnson CH, Kutsuna S, Ishiura M (1994) Circadian clock mutants of cyanobacteria. *Science* 266: 1233-1236.
3. Kondo T, Mori T, Lebedeva NV, Aoki S, Ishiura M, Golden SS (1997) Circadian rhythms in rapidly dividing cyanobacteria. *Science* 275: 224-227.
4. Ishiura M, Kutsuna S, Aoki S, Iwasaki H, Andersson, CA, Tanabe A, Golden SS, Johnson CH, Kondo T (1998) Expression of a gene cluster kaiABC as a circadian feedback process in cyanobacteria. *Science* 281: 1519-1523.
5. Iwasaki H, Williams SB, Kitayama Y, Ishiura M, Golden SS, Kondo T (2000) A KaiC-interacting sensory histidine kinase, SasA, necessary to sustain robust circadian oscillation in cyanobacteria. *Cell* 101: 223-233.
6. Nakahira Y, Katayama M, Miyashita H, Kutsuna S, Iwasaki H, Oyama T, Kondo T (2004) Global gene repression by KaiC as a master process of prokaryotic circadian system. *Proc. Natl. Acad. Sci. USA* 101: 881-885.
7. Tomita J, Nakajima M, Kondo T, Iwasaki H (2005) No transcription-translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 307: 251-254.
8. Nakajima M, Imai K, Ito H, Nishiwaki T, Murayama Y, Iwasaki H, Oyama T, Kondo T (2005) Reconstitution of circadian oscillation of cyanobacterial KaiC phosphorylation in vitro. *Science* 308: 414-415.
9. Nishiwaki T, Satomi Y, Kitayama Y, Terauchi K, Kiyohara R, Takao T, Kondo T (2007) A sequential program of dual phosphorylation of KaiC as a basis for circadian rhythm in cyanobacteria. *EMBO J.* 26: 4029-4037.

10. Terauchi K, Kitayama Y, Nishiwaki T, Miwa K, Murayama Y, Oyama T, Kondo T (2007) ATPase activity of KaiC determines the basic timing for circadian clock of cyanobacteria. *Proc. Natl. Acad. Sci. USA* 104: 16377-16381.
11. Ito H, Kageyama H, Mutsuda M, Nakajima M, Oyama T, Kondo T (2007) Autonomous synchronization of the circadian KaiC phosphorylation rhythm. *Nature Struct. Mol. Biol.* 14: 1084-1088.
12. Kondo T (2007) A cyanobacterial circadian clock based on the Kai oscillator. *Cold Spring Harb. Symp. Quant. Biol.* 72: 47-55.
13. Kitayama Y, Nishiwaki T, Terauchi K, Kondo T (2008) Dual KaiC-based oscillations constitute the circadian system of cyanobacteria, *Genes & Development* 22: 1513-1521.
14. Yoshida T, Murayama Y, Ito H, Kageyama H, Kondo T (2009) Nonparametric entrainment of the in vitro circadian phosphorylation rhythm of cyanobacterial KaiC by temperature cycle. *Proc. Natl. Acad. Sci. USA* 106: 1648-1653.
15. Murayama Y, Mukaiyama A, Imai K, Onoue Y, Tsunoda A, Nohara A, Ishida T, Maéda Y, Terauchi K, Kondo T, Akiyama S (2011) Visualizing the circadian ticking of cyanobacterial clock protein KaiC in solution. *EMBO J.* 30: 68-78.