Topological Difference of Core Regulatory Networks Induces Different Entrainment Characteristics of Plant and Animal Circadian Clocks

Jeong-Rae Kim,* Won-Soung Bae,* Yeoin Yoon,† and Kwang-Hyun Cho*†
*Bio-MAX Institute, and †College of Medicine, Seoul National University, Seoul, Korea

ABSTRACT The plant circadian rhythm is quickly entrained to the change of a light stimulus but the mammalian circadian rhythm shows a relatively slow entrainment. Where does such a different entrainment feature of plants and mammals originate? To answer this question, we have investigated circadian regulatory networks of various species and identified the respective core structures of plants and animals. The core circadian regulatory network of plants is composed of two coupled negative feedback loops while the core network of animals consists of coupled negative and positive feedback loops. In addition, the way of regulation (gene transcription or protein degradation) induced by a light stimulus differs depending on species. Mathematical simulations revealed that the topological difference of the core regulatory networks as well as the different way of regulation induced by a light stimulus leads to the different entrainment characteristics of plant and animal circadian clocks.

Received for publication 13 February 2007 and in final form 12 April 2007.

Address reprint requests and inquiries to Kwang-Hyun Cho, Tel.: 82-2-887-2650; E-mail: ckh-sb@snu.ac.kr.

It has been well known that the response of a plant circadian clock to the change of a light period is fast (1–4) but that of a mammalian circadian clock is relatively slow (5,6). What does cause such a different feature of entrainment? To answer this question, we have investigated the circadian regulatory networks of various species and found that the core circadian regulatory network (CCRN)—the common structure of circadian regulatory networks—of animals is topologically different from that of plants.

The CCRN of plants is composed of two coupled negative feedback loops (NFLs). For instance, in *Arabidopsis thaliana*, the complex CCA1/LHY suppresses the transcription of *TOC1* whose product (protein) activates *LHY* and *CCA1* (7,8). These regulatory relations between TOC1 and LHY/CCA1 constitute an NFL. In addition, the complex CCA1/LHY induces the synthesis of PRR5, 7, 9 which repress the synthesis of CCA1 and LHY. As PRR5, 7, 9 show similar response/regulation patterns (7–9), we can consider these as one component. These regulations form another NFL. Many other plants have clock genes homologous to those of *Arabidopsis* and their expressions are regulated in a similar way (10–12). Hence, we can regard the CCRN of many plants as that of *Arabidopsis* (see Fig. 1).

On the other hand, the CCRN of animals consists of two NFLs coupled with one positive feedback loop (PFL). In *Drosophila melanogaster*, the complex CLK/CYC induces the transcription of *PER* and *TIM*, and the complex PER/TIM inhibits again the transcription of *PER* and *TIM* (8). This forms an NFL. Note that CLK is the limiting factor in constituting the CLK/CYC complex since CYC is usually highly abundant (13). In addition, the regulatory relations between PDP1 and CLK/CYC constitute a PFL since PDP1 activates the transcription of *CLK*; the regulatory relations between VRI and CLK/CYC form another NFL since VRI inhibits the transcription of *CLK* (13,14). As an example of mammals, let us consider the CCRN of a mouse (*Mus musculus*) that is composed of two NFLs coupled with one PFL as illustrated in Fig. 1. One NFL represents the regulatory relation between CLOCK/BMAL1 and PER/CRY, and the other NFL denotes the regulatory relation between CLOCK/BMAL1 and REV-ERBα. Moreover, the PFL indicates the regulatory relation between RORα and CLOCK/BMAL1 (15). By further investigating circadian regulatory networks of various other species such as *Homo sapiens, Bos taurus*, and *Rattus norvegicus* (16), it turns out that the CCRN of animals can be characterized by two NFLs coupled with one PFL as shown in Fig. 1.

A circadian rhythm produces an autonomous oscillation and periodic or aperiodic changes of an external stimulus affect the circadian oscillation. In particular, the shape of the external light-dark (LD) cycle and the interaction mechanism of the light with the circadian regulatory network affect the entrainment characteristics of circadian rhythms. In other words, the way of accommodating an external stimulus also plays an important role in circadian oscillations. In this regard, there is a different feature in the CCRNs of *Drosophila* and mammals (Fig. 2 *A*). A light stimulus activates the transcription of clock genes in mammals while it induces the degradation of clock proteins in *Drosophila* (8,17). Light acts in multiple ways in plants. For instance, in *Arabidopsis*, light induces the transcription of CCA1 and LHY through PHYs, CRYs, and ELF3 (8). Light also inhibits ZTL, which induces the degradation of TOC1. As TOC1 induces the transcription of CCA1 and LHY, light stimuli seem to play a

Editor: Herbert Levine.

doi: 10.1529/biophysj.107.106658

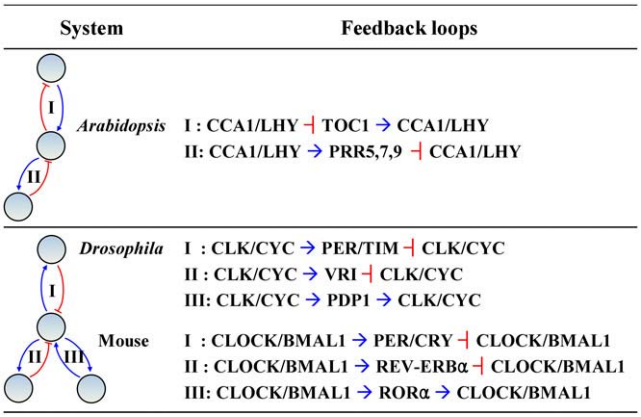

FIGURE 1 The CCRNs of plants and animals. Each node represents a clock protein or a complex of proteins. *I*, *II*, and *III* indicate the feedback loops in the CCRNs. The blue arrows (*red lines*) represent activations (inhibitions, respectively).

similar role in the two regulatory mechanisms. In this respect, we assumed that the main effect of light is to induce gene transcription in the plant CCRN model.

To examine how the topological difference of CCRNs affects the different feature of entrainments, we have constructed mathematical models of the plant CCRN and a modified plant (MP) CCRN, which is the plant CCRN with an additional positive feedback (see Fig. 2 *A* and Supplementary Material). Note that the topological structure of MP is the same as that of the mammalian CCRN except the interaction point of light. We applied various lengths of constant light stimuli to the two CCRN models after we made them entrained to the LD cycle of 12 h light and 12 h darkness (the 12:12 LD cycle), and measured the time for each CCRN to entrain to the 12:12 LD cycle (see Fig. 2 *B* for the stimulus pattern). The simulation results in Fig. 2 *C* show that the MP model takes much longer time to entrain to the 12:12 LD cycle after a constant light stimulus compared to that of the plant CCRN model. This implies that the added PFL elongated the entrainment time. This might be because a PFL in general amplifies an input stimulus and results in a slower response.

To examine the role of light in determining the entrainment time, we constructed mathematical models of *Drosophila* and mammals. The two mathematical models are same except the regulatory mechanism of light (gene transcription for mammals and protein degradation for *Drosophila*) (see Fig. 2 *A* and Supplementary Material). We applied various lengths of constant light stimuli to the two CCRN models after we made them entrained to the 12:12 LD cycle, and measured the time for each CCRN to entrain to the 12:12 LD cycle. The simulation results in Fig. 2 *D* show that the mammalian CCRN model is more slowly entrained to a light stimulus than the CCRN model of *Drosophila*, which is in accord with experimental results (5,6,18). Hence, we found that the protein degradation induced by light expedites the entrainment compared to the gene transcription. By comparing the simulation results of the MP model and the mammalian CCRN model, we also found that the different entrainment features are induced by the different interacting points of light (see Fig. 2).

We were intrigued why the response of a plant (and *Drosophila*) circadian clock to light changes is relatively fast compared to that of a mammalian circadian clock and explored this question through extensive computer simulations.

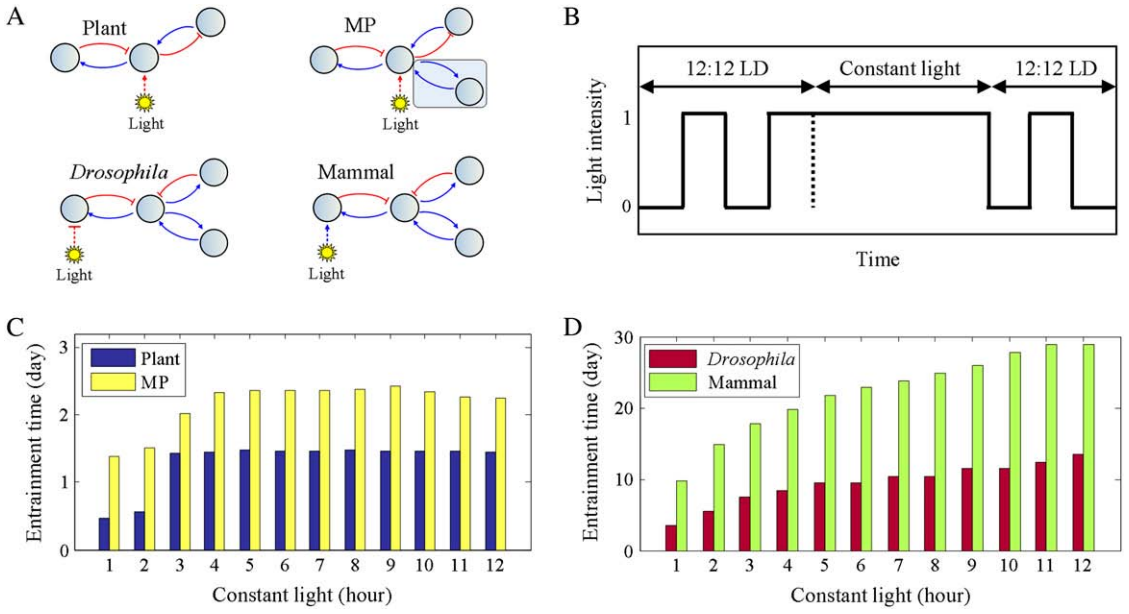

FIGURE 2 (*A*) The network models used in simulations. (*B*) The stimulus pattern. (*C*) The entrainments of the plant CCRN model and the modified plant (MP) CCRN model to the 12:12 LD cycle after various lengths of constant light stimuli. (*D*) The entrainments of the *Drosophila* CCRN model and the mammalian CCRN model to the 12:12 LD cycle after various lengths of the constant light stimuli.

Among the various factors determining the entrainment feature to light changes, we found that the topological structure of a CCRN, the regulatory mechanism induced by light, and the interacting point of light are important factors. In particular, the additional positive feedback in the coupled feedback structure seems to be responsible for the relatively slow entrainment in mammals compared to plants while the protein degradation induced by light in *Drosophila* might also contribute to its relatively fast entrainment. Investigating the relationship between the behavioral rhythms (e.g., overcoming jet lag) and the important factors we found in the entrainment of molecular clocks remains as a future study.

SUPPLEMENTARY MATERIAL

An online supplement to this letter can be found by visiting BJ Online at http://www.biophysj.org.

ACKNOWLEDGMENTS

This work was supported from the Korea Ministry of Science and Technology through the Korean Systems Biology Research grant (No. M10503010001-05N0301001111) and the 21C Frontier Microbial Genomics and Application Center Program (grant No. MG05-0204-3-0), and in part from Korea Ministry of Commerce, Industry & Energy through the Korea Bio-Hub program (No. 2005-B0000002). Y. Yoon and K.-H. Cho were supported by the second stage Brain Korea 21 Project in 2006. This work was also supported by Korea Ministry of Science and Technology through the Nuclear Research grant (No. M20708000001-07B0800-00110).

REFERENCES and FOOTNOTES

1. Alabadi, D., M. J. Yanovsky, P. Mas, S. L. Harmer, and S. A. Kay. 2002. Critical role for CCA1 and LHY in maintaining circadian rhythmicity in *Arabidopsis*. *Curr. Biol.* 12:757–761.
2. Kim, J. Y., H. R. Song, B. L. Taylor, and I. A. Carre. 2003. Light-regulated translation mediates gated induction of the *Arabidopsis* clock protein LHY. *EMBO J.* 22:935–944.
3. Millar, A. J. 2003. A suite of photoreceptors entrains the plant circadian clock. *J. Biol. Rhythms.* 18:217–226.
4. Yakir, E., D. Hilman, Y. Harir, and R. M. Green. 2007. Regulation of output from the plant circadian clock. *FEBS J.* 274:335–345.
5. Sharma, V. K. 2003. Effect of light intensity on the phase and period response curves in the nocturnal field mouse *Mus booduga*. *Chronobiol. Int.* 20:223–231.
6. Silva, M. M., A. M. Albuquerque, and J. F. Araujo. 2005. Light-dark cycle synchronization of circadian rhythm in blind primates. *J. Circadian Rhythms.* 3:10.
7. McClung, C. R. 2006. Plant circadian rhythms. *Plant Cell.* 18:792–803.
8. Wijnen, H., and M. W. Young. 2006. Interplay of circadian clocks and metabolic rhythms. *Annu. Rev. Genet.* 40:409–448.
9. Nakamichi, N., M. Kita, S. Ito, T. Yamashino, and T. Mizuno. 2005. Pseudo-response regulators, PRR9, PRR7 and PRR5, together play essential roles close to the circadian clock of *Arabidopsis thaliana*. *Plant Cell Physiol.* 46:686–698.
10. Boxall, S. F., J. M. Foster, H. J. Bohnert, J. C. Cushman, H. G. Nimmo, and J. Hartwell. 2005. Conservation and divergence of circadian clock operation in a stress-inducible Crassulacean acid metabolism species reveals clock compensation against stress. *Plant Physiol.* 137: 969–982.
11. Kaldis, A. D., P. Kousidis, K. Kesanopoulos, and A. Prombona. 2003. Light and circadian regulation in the expression of LHY and Lhcb genes in *Phaseolus vulgaris*. *Plant Mol. Biol.* 52:981–997.
12. Miwa, K., M. Serikawa, S. Suzuki, T. Kondo, and T. Oyama. 2006. Conserved expression profiles of circadian clock-related genes in two *Lemna* species showing long-day- and short-day photoperiodic flowering responses. *Plant Cell Physiol.* 47:601–612.
13. Bae, K., C. Lee, P. E. Hardin, and I. Edery. 2000. dCLOCK is present in limiting amounts and likely mediates daily interactions between the dCLOCK-CYC transcription factor and the PER-TIM complex. *J. Neurosci.* 20:1746–1753.
14. Cyran, S. A., A. M. Buchsbaum, K. L. Reddy, M. C. Lin, N. R. Glossop, P. E. Hardin, M. W. Young, R. V. Storti, and J. Blau. 2003. vrille, Pdp1, and dClock form a second feedback loop in the *Drosophila* circadian clock. *Cell.* 112:329–341.
15. Sato, T. K., S. Panda, L. J. Miraglia, T. M. Reyes, R. D. Rudic, P. McNamara, K. A. Naik, G. A. FitzGerald, S. A. Kay, and J. B. Hogenesch. 2004. A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron.* 43:527–537.
16. KEGG. Last updated: July 19, 2006. KEGG: Kyoto Encyclopedia of Genes and Genomes. Institute for Chemical Research, Kyoto University, Kyoto, Japan.
17. Shigeyoshi, Y., K. Taguchi, S. Yamamoto, S. Takekida, L. Yan, H. Tei, T. Moriya, S. Shibata, J. J. Loros, J. C. Dunlap, and H. Okamura. 1997. Light-induced resetting of a mammalian circadian clock is associated with rapid induction of the mPer1 transcript. *Cell.* 91:1043–1053.
18. Koh, K., X. Zheng, and A. Sehgal. 2006. JETLAG resets the *Drosophila* circadian clock by promoting light-induced degradation of TIMELESS. *Science.* 312:1809–1812.