Plant Signaling & Behavior

ISSN: (Print) 1559-2324 (Online) Journal homepage: http://www.tandfonline.com/loi/kpsb20

Diversity of plant circadian clocks: Insights from studies of *Chlamydomonas reinhardtii* and *Physcomitrella patens*

Masashi Ryo, Takuya Matsuo, Takafumi Yamashino, Mizuho Ichinose, Mamoru Sugita & Setsuyuki Aoki

To cite this article: Masashi Ryo, Takuya Matsuo, Takafumi Yamashino, Mizuho Ichinose, Mamoru Sugita & Setsuyuki Aoki (2016) Diversity of plant circadian clocks: Insights from studies of *Chlamydomonas reinhardtii* and *Physcomitrella patens* , Plant Signaling & Behavior, 11:1, e1116661, DOI: 10.1080/15592324.2015.1116661

To link to this article: https://doi.org/10.1080/15592324.2015.1116661

Accepted author version posted online: 08 Dec 2015.
Published online: 08 Dec 2015.

PLANT SIGNALING & BEHAVIOR
2016, VOL. 11, NO. 1, e1116661 (6 pages)
http://dx.doi.org/10.1080/15592324.2015.1116661

Taylor & Francis
Taylor & Francis Group

MINI-REVIEW

Diversity of plant circadian clocks: Insights from studies of *Chlamydomonas reinhardtii* and *Physcomitrella patens*

Masashi Ryo[a], Takuya Matsuo[b], Takafumi Yamashino[c], Mizuho Ichinose[b,d], Mamoru Sugita[b], and Setsuyuki Aoki[a]

[a]Graduate School of Information Science, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan; [b]Center for Gene Research, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan; [c]Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan; [d]Institute of Transformative Bio-Molecules, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-8601, Japan

ABSTRACT

Arabidopsis thaliana has long been the model plant of choice for elucidating the mechanisms of the circadian clock. Recently, relevant results have accumulated in other species of green plant lineages, including green algae. This mini-review describes a comparison of the mechanism of the *A. thaliana* clock to those of the green alga *Chlamydomonas reinhardtii* and the moss *Physcomitrella patens*, focusing on commonalities and divergences of subsystems of the clock. The potential of such an approach from an evolutionary viewpoint is discussed.

Abbreviations: LD, light-dark cycles; LL, continuous light; DD, continuous darkness; HK, histidine kinase.

ARTICLE HISTORY
Received 14 October 2015
Revised 30 October 2015
Accepted 30 October 2015

KEYWORDS
Circadian clock; circadian rhythm; clock gene; evolution; gene network

Circadian clocks, which are self-sustained oscillations with a period of approximately a day, drive circadian rhythms of a variety of processes in metabolism, growth and development.[1] A circadian system comprises 3 subsystems: 1) input pathways, through which environmental cues such as light and temperature reset the central clock; 2) the central clock, a self-sustained oscillation machinery; 3) output pathways, which transmit circadian timing information from the central clock to overt rhythms.[2] Many processes of plants show circadian rhythms and various dicotyledonous plants have been used to assess physiological aspects of the clock.[3,4] In the era of molecular genetics, the model dicot species *Arabidopsis thaliana* has been intensively studied to unravel the clock's mechanism, and many "clock genes," which encode components of the central clock machinery, have been identified and analyzed by genetic, biochemical and genomic techniques.[4] Consequently, molecular models of the *A. thaliana* circadian system with complex clock gene networks have been postulated.[5,6,7,8,9] These are briefly summarized in Fig. 1. In recent years, many homologs to *A. thaliana* clock genes were identified in various eudicots and monocots, and studies on them indicate that clock genes and their functions are broadly conserved in angiosperms.[10,11,12] This conservation may also extend, to some extent, even to the marine green alga *Ostreococcus tauri*, which has a *CCA1/LHY* homolog (*OtCCA1*) and a *TOC1* homolog (*OtTOC1*) (see the legend of Fig. 1 for abbreviations of gene/protein names).[13] The *O. tauri* clock was significantly compromised when either gene was overexpressed or when *OtTOC1* was knocked-down, and these 2 genes form a negative feedback loop.[13] These results indicate that both genes play central roles in the *O. tauri* clock. Moreover, the origin of angiosperm clocks can be traced back even to the branching point between higher plants and *O. tauri*.

Chlamydomonas reinhardtii, sometimes referred as "green yeast," is a model alga suitable for genetic and biochemical studies.[14] Clock mutants of this alga were isolated more than 40 y ago by monitoring circadian rhythm of phototactic behavior.[15,16] Four long period mutants, isolated and designated as "*per*" (*per-1* to *per-4*; but almost certainly unrelated to animal *per* genes), have mutations in 4 independent genomic loci and their period lengthening effects are additive.[16] A short period mutant of circadian phototaxis has also been isolated.[17] Unfortunately, the genes responsible for these mutants have not been identified yet. In recent years, findings related to the molecular components of the *Chlamydomonas* clock have accumulated rapidly. CHLAMY1 is a RNA-binding protein which binds to UG-repeat containing mRNAs in a night/subjective-night phase-specific manner.[18] Analyses of biochemically isolated CHLAMY1 revealed that it consists of a heteromer of C1 and C3 subunits containing lysine homology domains and RNA recognition motifs, respectively.[19] Since misexpression of genes encoding C1 and C3 induces abnormal circadian rhythms (arrhythmicity and advanced circadian phase, respectively), it is obvious that they are involved in the circadian clock of this alga.[20] In 2008, a large number of "*roc* (*rhythm of chloroplast*)" mutants were isolated by screening insertional mutants based on circadian phenotypes of a bioluminescence rhythm derived from the luciferase reporter gene transferred into the chloroplast genome.[21,22] The genes responsible for these mutants were termed "*ROC*" genes, and the proteins encoded by *ROC* genes were similar to higher plant proteins (Table 1): ROC15 and ROC75 have GARP DNA-binding motifs similar to that of

CONTACT Takuya Matsuo t-matsuo@gene.nagoya-u.ac.jp; Takafumi Yamashino yamasino@agr.nagoya-u.ac.jp; Setsuyuki Aoki aoki@is.nagoya-u.ac.jp

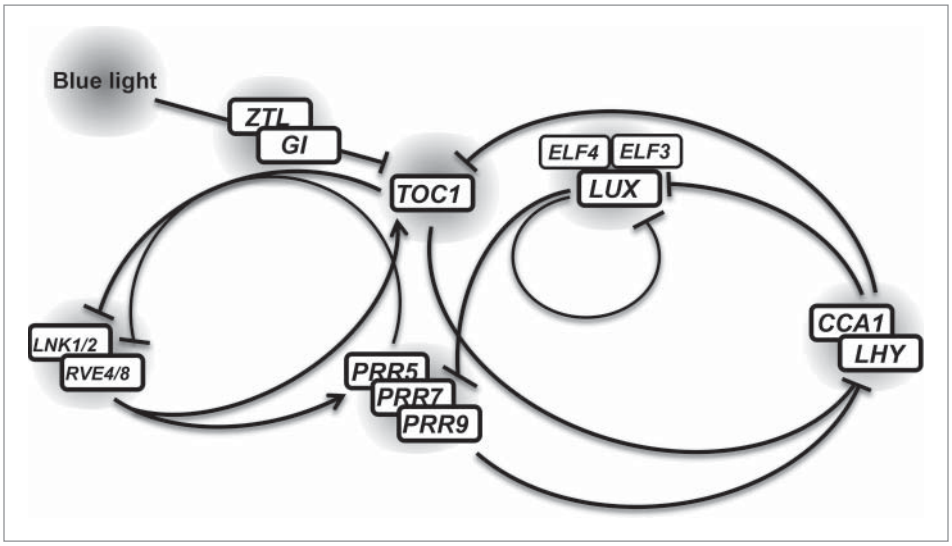

Figure 1. Model of the *A. thaliana* circadian clock. Arrows and T-shaped bars are regulatory links for the promotion and repression of gene transcription, respectively, except for the T-shaped bar from blue light, which indicates protein degradation. The *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) genes, which encode paralogous Myb-domain transcription factors, repress transcription of the *LUX ARRHYTHMO* (*LUX*) (also termed *PHYTOCLOCK 1* (*PCL1*)) and *EARLY FLOWERING 4* (*ELF4*) genes. LUX, ELF3 and ELF4 form the evening complex (EC), which represses transcription of *PRR7* and *PRR9*, both encoding "pseudo-response regulators" with a receiver-like domain and a CCT (CO/CO-Like/TOC1) domain. PRR7/9 and their paralog PRR5 repress *CCA1/LHY* transcription, thus closing a feedback loop. This tripartite circuitry appears to be essential for rhythmic expression patterns of its component genes: morning activation of *CCA1/LHY* immediately leads to repression of EC genes, thereby resulting in de-repression of direct target genes of EC, *PRR9/7* from early- to mid-daytime. The serial activation of PRR9/7/5, each of which represses *CCA1/LHY*, is important for enabling the sharp peaks of *CCA1/LHY* expression at dawn. On the other hand, CCA1/LHY repress transcription of *TOC1* (also termed *PRR1*), encoding another pseudo-response regulator, and oppositely TOC1 represses *CCA1/LHY* transcription, forming another loop. *Night light-inducible and clock-regulated gene 1* (*LNK1*) and *LNK2* encode transcriptional coactivators necessary for the expression of *PRR5* and *TOC1*, both of which repress *LNK1/2* in turn, forming yet another loop. Although there exist more links, *e.g.*, TOC1 and EC each represses the transcription of several other clock genes, they are not depicted in the figure for simplicity. Post-transcriptional/post-translational regulatory processes are also operating in the clock machinery. For example, ZEITLUPE (ZTL), a LOV domain-containing E3 ubiquitin ligase, mediates proteasome-dependent degradation of TOC1. ZTL is stabilized by a blue-light-enhanced interaction with rhythmically expressed GIGANTEA (GI) protein; this rhythmic stabilization of ZTL promotes high-amplitude rhythmicity of *TOC1* expression. Other post-transcriptional/post-translational processes, *e.g.*, alternative splicing, protein phosphorylation/dephosphorylation, and rhythmic chromatin regulation, are also involved in the clock mechanism,[48,49] though they are not described in this mini-review.

LUX(PCL1); ROC40 contains a Myb domain similar to those of CCA1/LHY; ROC66 contains an N-terminal B-box and a C-terminal CCT motif similar to those of CO/COL family proteins.[22] Although their sequence similarities do not extend beyond the domain regions, 4 genes positively identified by a forward genetic screen are similar to the *A. thaliana* clock/clock-associated flowering genes, indicating that the origin of the *C. reinhardtii* clock is, at least partially, shared with the *A. thaliana* clock. On the other hand, ROC55 and ROC114 do not share any significant similarity to clock proteins of not only higher plants but also other clock model organisms.[22] In addition to the above-mentioned factors, it is known that Casein kinase 1, N-terminal acetyltransferase 3, and Protein disulfide isomerase 2 are also involved in the *C. reinhardtii* clock.[23,24,25]

Table 1. Clock (-associated) genes and their homologs in *A. thaliana, C. reinhardtii* and *P. patens*. Shown in each row are numbers of the homologs that are present in the 3 species to the clock (-associated) gene(s) in the first column.

	A. thaliana	*C. reinhardtii*	*P. patens*
CCA1/LHY	2	2 (ROC40)	2
PRR/TOC1	5	2	4
LUX/BOA	2	3 (ROC15/75)	4
ELF3	1	0	3
ELF4/ELF4-like	5	0	1
GI	1	0	0
CHE	1	0	0
LOV-HK	0	1	2
ZTL	3	0	0
LNK	4	0	0
COL	17	3 (ROC66)	3

The *Chlamydomonas* clock is reset by light with a wide range of wavelengths including violet to red.[26,27] At the molecular level, light responses of several clock-related mRNAs have been demonstrated. The *c3* mRNA is strongly induced by blue, yellow, and red light.[28] In contrast, the *ROC15* and *ROC40* mRNAs are reduced.[28] aCRY, a *Chlamydomonas* homolog of animal cryptochrome/photolyase proteins, is able to absorb not only blue but also yellow and red light, and is involved in the responses of these mRNAs.[28] At the protein level, ROC15 undergoes phosphorylation and proteasomal degradation by blue, green, and particularly red light.[29] *ROC114*, encoding an F-box protein, is involved in this degradation.[29] Indeed, as expected, the *roc15* mutant exhibits abnormal phase responses of the clock to light.[29] *Chlamydomonas* Photolyase Homolog 1 (CPH1), another cryptochrome/photolyase homolog which is similar to the higher plant CRYs, also undergoes proteasomal degradation after blue and red light exposure.[30] Interestingly, a knockdown strain of CPH1 shows a larger phase response to blue light, indicating that CPH1 is involved in clock resetting as a negative regulator.[27] Information about external temperature is integrated into CHLAMY1.[31] Exposure to low temperature induces hyperphosphorylation of the C1 subunit and accumulation of the C3 subunit due to transcriptional activation of the *c3* gene *via* an E-box *cis*-element.[31]

Physcomitrella patens is a moss species, which diverged from vascular plant lineages at least 450 Ma.[32] The efficiency of gene

targeting by homologous recombination in *P. patens* is as high as those in yeasts,[33] facilitating the use of this moss for the study of gene functions. The *P. patens* genome[32] shows a suite of genes that are similar to the *A. thaliana* clock genes (Table 1). *P. patens* has 2 genes that are homologous to *CCA1/LHY* (*PpCCA1a* and *PpCCA1b*). *PpCCA1a* and *PpCCA1b* both showed, similar to *CCA1/LHY*, diurnal expression with peaks at dawn and circadian expression with peaks at subjective dawn in light-dark cycles (LD) and continuous darkness (DD), respectively.[34] *P. patens* also has 4 *PRR* gene homologs, *PpPRR1/2/3/4*,[35] all of which are expressed in a circadian manner, and whose phases are similar to the profiles of *PRR3* and *PRR5*.[35] Holm (2010)[36] presented a comparative overview of expression profiles and phylogenetic analyses for the *P. patens* genes homologous to *ELF3, ELF4* and *LUX* as well as *PpCCA1a/1b* and *PpPRR1/2/3/4*. They reported that expression profiles of most homologs are generally similar to their *A. thaliana* counterparts.[36] The double disruptant for *PpCCA1a/1b* showed circadian gene expression with a shorter period and a dampened amplitude compared to the wild-type (WT) strain,[34] similar to the *A. thaliana* null mutant for *CCA1/LHY*.[37] Interestingly, the receiver-like domain (RLD) of PpRPR2/3/4 exhibits a potential phosphoacceptor motif, aspartic acid-aspartic acid-lysine (DDK), which is distinct from the angiosperm PRRs, in which DDK motifs and probably their phosphoacceptor functions are not preserved.[35] Consistently, the PpPRR2 RLD had phosphoacceptor ability *in vitro*.[35] These observations suggest that PpPRR2 functions as a genuine response regulator, and not as a PSEUDO-response regulator, and that its upstream phosphorelay cascade with a counterpart histidine kinase (HK) is also preserved in *P. patens*.[35] This putative "circadian phosphorelay cascade" is predicted to have a regulatory function in the moss clock machinery because phosphorylation of a response regulator via a phosphorelay reaction switches its activity, generally resulting in activation or repression of downstream genes. An attempt to unravel an output pathway in *P. patens* was also made. The plastid sigma factor is the nuclear-encoded regulatory subunit of the plastid-encoded plastid RNA polymerase, which principally transcribes photosynthesis-related genes in plastids.[38] *P. patens* has at least 3 sigma factor genes, *PpSig1/2/5*, and of these, only *PpSig5* showed circadian expression in LD and DD.[38] The plastid gene *psbD*, which encodes the D2 protein of photosystem II, shows a diurnal expression rhythm in LD in *P. patens*,[38] and its amplitude was lowered when *PpSig5* was disrupted.[39] Therefore, PpSIG5 is a sigma factor that regulates *psbD* rhythm, possibly as an intermediate regulator of an output pathway.

The distribution of clock genes in *A. thaliana, C. reinhardtii* and *P. patens* is depicted as a Venn diagram in Fig. 2. In the peripheral regions of the Venn diagram, where factors found only in one or 2 species are indicated, there are several proteins that potentially mediate environmental signals to more centrally located factors. ZTL and GI, present only in *A. thaliana*, mediate a light signal to the clock by modifying TOC1 stability (Fig. 1). EC is likely involved in a temperature input pathway,[40,41] where ELF3 and ELF4, present only in *A. thaliana* and *P. patens*, possibly modify the activity of LUX as a transcription factor. CHLAMY1, which is only found in *C. reinhardtii* and is known to respond to both light and temperature,[28,31] might mediate these environmental cues to the central region, where ROC40 mRNA is a possible target molecule because its 3′-untranslated region contains an UG repeat, a binding motif of CHLAMY1.[22] Factors mediating a light signal that induces proteasomal degradation of ROC15 have not yet been identified.[29] ROC114, which is in a peripheral region

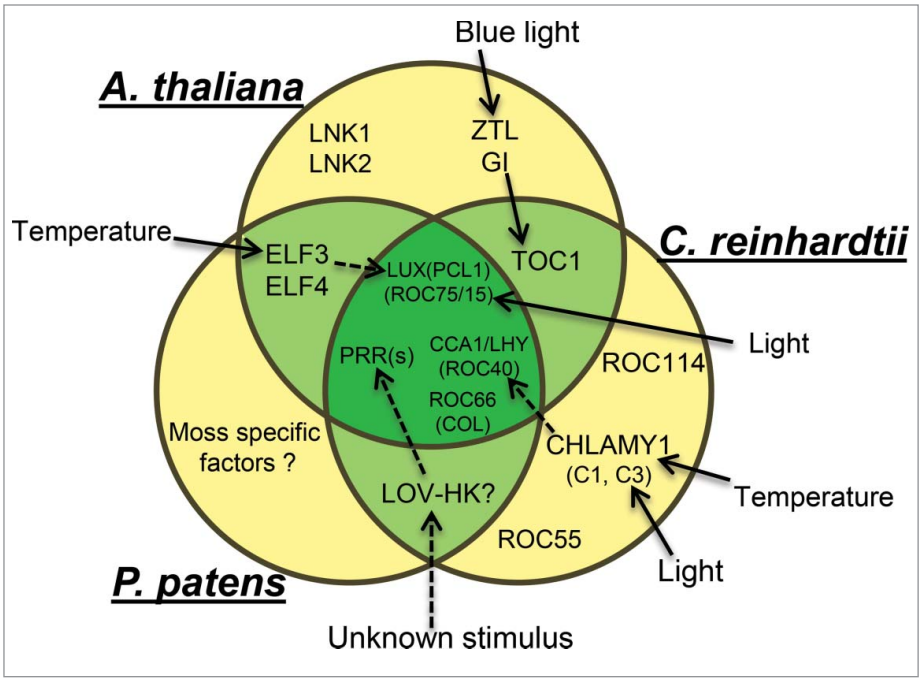

Figure 2. Distribution of clock genes (only factors predicted to have essential functions in the clock mechanisms are included) in *A. thaliana, C. reinhardtii* and *P. patens*. Arrows are flows that transmit environmental signals to the clock. Dashed lines have not yet been clarified.

of the diagram and is involved in ROC15 degradation, could be a potential candidate of such a factor.

PRRs with a DDK motif are only shared between *C. reinhardtii* and *P. patens* (not depicted in Fig. 2), suggesting that the putative circadian phosphorelay cascade is also shared between these 2 species, but not in *A. thaliana*. Indeed, no clock-related histidine kinase (HK) has been reported in *A. thaliana*. In *O. tauri*, which possesses a putative TOC1 homolog with a DDK motif,[13] a LOV (Light/Oxygen/Voltage) domain-containing HK has an important function for the operation of the clock under blue light.[42] Similar LOV-HK sequences are also found in the *P. patens* and *C. reinhardtii* genomes, but not in the *A. thaliana* genome (TY & TM, unpublished observations). Therefore, these LOV-HKs are promising candidates for HKs in the circadian phosphorelay cascade. The HK in the putative circadian phosphorelay cascade might mediate an environmental signal to the central region by modifying the activity of the moss PRRs. As for components of the output pathways (output factors), little is known in *C. reinhardtii* and *P. patens*. Noordally (2013)[43] demonstrated that *psbD* mRNAs show a robust circadian oscillation in continuous light (LL) in *A. thaliana*, and this rhythm is nullified in the *Sig5* (*AtSig5*) null mutants, indicating that SIG5 is a sigma factor that specializes as an output factor mediating the timing of circadian information from the clock to *psbD* in *A. thaliana*. Therefore, the function of this specialized sigma factor (Sig5) as an output factor seems to be shared between *A. thaliana* and *P. patens*. On the other hand, *C. reinhardtii* has only a single copy sigma-like gene, *PROD*, and its involvement in circadian regulation has not been clarified.[44] Thus, commonality/divergence between output factors of the 3 species remains a mystery.

Factors shared among the 3 species are LUX(ROC75/15), PRRs, CCA1/LHY(ROC40) and ROC66(COL). The three species have diverged considerably with great evolutionary distances between them. Despite this, they all preserve these factors, suggesting that these factors have indispensable functions in the operation of the clock. Consistently, LUX, PRRs and CCA1/LHY form the circuitry that seems to be essential in the generation of circadian oscillation.[6] In *P. patens, PpCCA1a/1b* feed back to *PpCCA1b* transcription and they also regulate *PpPRR1* transcription,[34] consistent with the regulatory interactions in the *A. thaliana* tripartite circuitry. However, in *C. reinhardtii*, the regulatory relationship between *ROC75* and *ROC40* may not be similar to that between LUX and CCA1/LHY (TM, unpublished observation); in addition, expression profiles of *ROC* genes are different from those of the *A. thaliana* counterparts.[22] These observations indicate that rewiring of the network structure took place after the divergence between *C. reinhardtii* and *A. thaliana*, while conserving the network components. ROC66 is supposed to be an essential clock gene because it considerably prolongs the period length of the clock (29.9 h) when mutated in *C. reinhardtii*.[22] In *A. thaliana*, the function of *COL* genes in the central clock machinery is unclear, although shortening of the period length of circadian rhythms of leaf movement and *CAB* gene expression were observed in *COL1* overexpressors.[45] *P. patens* has 3 *COL* genes but their functions have not been elucidated.[46,47] The position of ROC66 in the Venn diagram and the phenotype of the *ROC66* mutant suggest that some *COL* genes might also be important clock genes in *A. thaliana* and *P. patens*.

As suggested by McClung.[12] Comparisons of genomic data alone will only shed light on common factors between different species, and therefore, a small number of factors will become distinct when the genomes of phylogenetically distant species are compared. On the other hand, when the results of forward genetics are compared, as done with *C. reinhardtii* and *A. thaliana* in this mini-review, not only common factors but also species-specific factors are highlighted. Comparisons of clock genes, largely between these 2 species, suggest a possibility that clock components receiving environmental signals (directly or indirectly) may have diversified during evolution (Fig. 2). This idea is plausible because ambient cues affecting the performance of the clock could vary between species with different habitat conditions, though such proof awaits further investigations. Forward genetics has not been applied to *P. patens*,

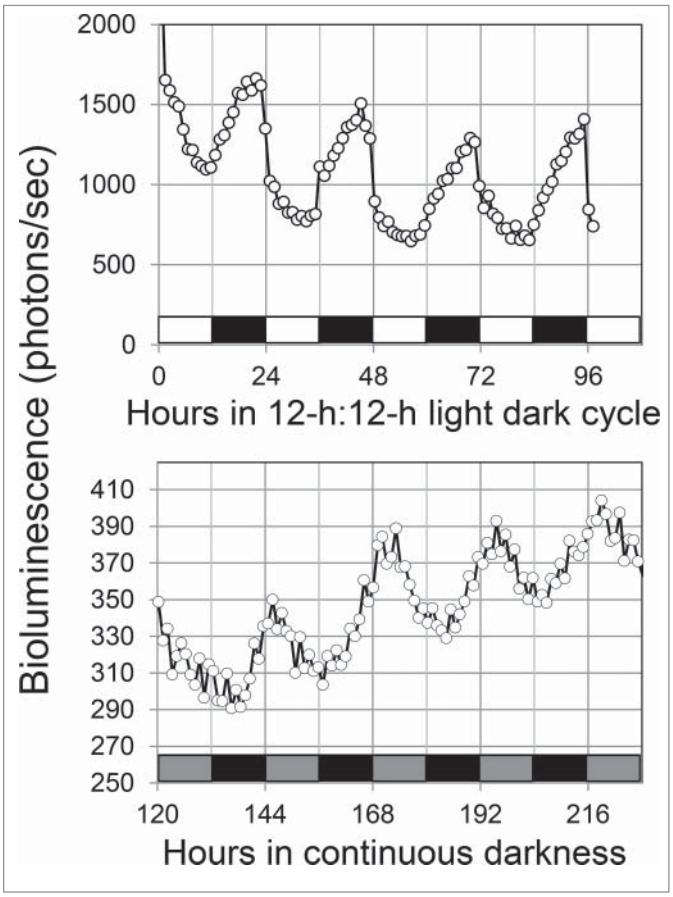

Figure 3. Bioluminescence rhythms from protonema tissues that transiently express a luciferase reporter construct. A DNA construct carrying a fusion of the promoter region of *PpCCA1b* and the firefly luciferase (*luc+*) gene was transferred to the moss protonema tissues by particle bombardment-mediated transformation,[34,50] entrained to 12-h:12-h light dark (LD) cycles, and released for bioluminescence monitoring into conditions shown below each graph. The peaks of the rhythm occurred immediately before the onsets of light periods in LD cycles, largely due to the rapid decline of bioluminescence caused by light. On the other hand, the peak times are shifted toward the beginnings of the subjective days in continuous darkness (DD), reflecting innate timing based on free-running of the clock. Additionally, the amplitudes of the rhythms in DD were always significantly lower than those in LD cycles for unknown reasons. The open and filled squares on the horizontal axes are light (50 μmol/m^2/sec) and dark periods, respectively. The gray squares are subjective days.

because monitoring of rhythms from small tissue colonies, which is essential for screening clock mutants, is still technically difficult (SA, unpublished observation). We are developing a method for monitoring the circadian expression of the luciferase reporter gene based on a particle bombardment-mediated transient gene expression assay (Fig. 3). This will facilitate, if combined with genomic analyses, effective screenings for new clock gene loci in *P. patens*. Another aspect that this mini-review highlights is that the data obtained with primitive groups, such as *C. reinhardtii* and *P. patens*, could be important because such ancient groups may still have prototypic gene circuitries, which were lost in higher plant lineages (such as the putative circadian phosphorelay cascade). If the clocks of more diverged species are investigated and systematically compared, the evolution, diversity and even origins of plant clocks will be clearer. Moreover, this may help to clarify the inherent complexity of the plant clock mechanisms.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed

Acknowledgments

This work was supported by JSPS KAKENHI (25440179 to TM, 26292051 to TY, 2529105 to MS, 24570007 to SA), Kato Memorial Bioscience Fondation (to TM) and Kurata Memorial Hitachi Science and Technology Foundation (to TM) and Naito Foundation (to TY).

References

1. Bünning E. The physiological clock: circadian rhythms and biological chronometry, 3rd ed. New York: Springer-Verlag; 1973.
2. Edmunds LN. Cellular and Molecular Bases of Biological Clocks. New York: Springer; 1988.
3. Sweeney BM. Rhythmic Phenomena in Plants, 2nd ed. San Diego: Academic Press; 1987.
4. McClung CR. Plant circadian rhythms. Plant Cell 2006; 18:792-803; PMID:16595397; http://dx.doi.org/10.1105/tpc.106.040980
5. Nagel DH, Kay SA. Complexity in the wiring and regulation of plant circadian networks. Curr Biol 2012; 22(16):R648-57; PMID:22917516; http://dx.doi.org/10.1016/j.cub.2012.07.025
6. Yamashino T. From a repressilator-based circadian clock mechanism to an external coincidence model responsible for photoperiod and temperature control of plant architecture in *Arabodopsis thaliana*. Biosci Biotechnol Biochem 2013; 77(1):10-6; PMID:23291766; http://dx.doi.org/10.1271/bbb.120765
7. Carré I, Veflingstad SR. Emerging design principles in the *Arabidopsis* circadian clock. Semin Cell Dev Biol 2013; 24(5):393-8; PMID:23597453; http://dx.doi.org/10.1016/j.semcdb.2013.03.011
8. McClung CR. Wheels within wheels: new transcriptional feedback loops in the Arabidopsis circadian clock. F1000Prime Rep 2014; 6:2; PMID:24592314; http://dx.doi.org/10.12703/P6-2
9. Hsu PY, Harmer SL. Wheels within wheels: the plant circadian system. Trends Plant Sci 2014; 19(4):240-9; PMID:24373845; http://dx.doi.org/10.1016/j.tplants.2013.11.007
10. McClung CR. A modern circadian clock in the common angiosperm ancestor of monocots and eudicots. BMC Biol 2010; 8:55; PMID:20459860; http://dx.doi.org/10.1186/1741-7007-8-55
11. Song YH, Ito S, Imaizumi T. Similarities in the circadian clock and photoperiodism in plants. Curr Opin Plant Biol 2010; 13(5):594-603; PMID:20620097; http://dx.doi.org/10.1016/j.pbi.2010.05.004
12. McClung CR. Beyond *Arabidopsis*: the circadian clock in non-model plant species. Semin Cell Dev Biol 2013; 24(5):430-6; PMID:23466287; http://dx.doi.org/10.1016/j.semcdb.2013.02.007
13. Corellou F, Schwartz C, Motta JP, Djouani-Tahri el B, Sanchez F, Bouget FY. Clocks in the green lineage: comparative functional analysis of the circadian architecture of the picoeukaryote *Ostreococcus*. Plant Cell 2009; 21(11):3436-49; PMID:19948792; http://dx.doi.org/10.1105/tpc.109.068825
14. Goodenough UW. Green yeast. Cell 1992; 70:533-8; PMID:1505022; http://dx.doi.org/10.1016/0092-8674(92)90424-B
15. Bruce VG. Mutants of the biological clock in *Chlamydomonas reinhardi*. Genetics 1972; 70:537-48; PMID:5034771
16. Bruce VG. Recombinants between clock mutants of *Chlamydomonas reinhardi*. Genetics 1974; 77:221-30; PMID:4847153
17. Mergenhagen D. Circadian clock: genetic characterization of a short period mutant of *Chlamydomonas reinhardii*. Eur J Cell Bio 1984; 33:13-8; PMID:6698035
18. Mittag M. Conserved circadian elements in phylogenetically diverse algae. Proc Natl Acad Sci U S A 1996; 93:14401-4; PMID:8962063; http://dx.doi.org/10.1073/pnas.93.25.14401
19. Zhao B, Schneid C, Iliev D, Schmidt E-M, Wagner V, Wollnik F, Mittag M. The circadian RNA-binding protein CHLAMY 1 represents a novel type heteromer of RNA recognition motif and lysine homology domain-containing subunits. Eukaryot Cell 2004; 3:815-25; PMID:15190002; http://dx.doi.org/10.1128/EC.3.3.815-825.2004
20. Iliev D, Voytsekh O, Schmidt E-M, Fiedler M, Nykytenko A, Mittag M. A heteromeric RNA-binding protein is involved in maintaining acrophase and period of the circadian clock. Plant Physiol 2006; 142:797-806; PMID:16920878; http://dx.doi.org/10.1104/pp.106.085944
21. Matsuo T, Onai K, Okamoto K, Minagawa J, Ishiura M. Real-time monitoring of chloroplast gene expression by a luciferase reporter: evidence for nuclear regulation of chloroplast circadian period. Mol Cell Biol 2006; 26:863-70; PMID:16428442; http://dx.doi.org/10.1128/MCB.26.3.863-870.2006
22. Matsuo T, Okamoto K, Onai K, Niwa Y, Shimogawara K, Ishiura M. A systematic forward genetic analysis identified components of the *Chlamydomonas* circadian system. Genes Dev 2008; 22:918-30; PMID:18334618; http://dx.doi.org/10.1101/gad.1650408
23. Schmidt M, Gessner G, Luff M, Heiland I, Wagner V, Kaminski M, Geimer S, Eitzinger N, Reissenweber T, Voytsekh O, et al. Proteomic analysis of the eyespot of *Chlamydomonas reinhardtii* provides novel insights into its components and tactic movements. Plant Cell 2006; 18:1908-30; PMID:16798888; http://dx.doi.org/10.1105/tpc.106.041749
24. Matsuo T, Iida T, Ishiura M. *N-terminal acetyltransferase 3* gene is essential for robust circadian rhythm of bioluminescence reporter in *Chlamydomonas reinhardtii*. Biochem Biophys Res Commun 2012; 418:342-6; PMID:22266323; http://dx.doi.org/10.1016/j.bbrc.2012.01.023
25. Filonova A, Haemsch P, Gebauer C, Weisheit W, Wagner V. Protein disulfide isomerase 2 of *Chlamydomonas reinhardtii* is involved in circadian rhythm regulation. Mol Plant 2013; 6:1503-17; PMID:23475997; http://dx.doi.org/10.1093/mp/sst048
26. Kondo T, Johnson CH, Hastings JW. Action spectrum for resetting the circadian phototaxis rhythm in the CW15 strain of *Chlamydomonas*: I. Cells in darkness. Plant Physiol 1991; 95:197-205; PMID:16667951; http://dx.doi.org/10.1104/pp.95.1.197
27. Forbes-Stovall J, Howton J, Young M, Davis G, Chandler T, Kessler B, Rinehart CA, Jacobshagen S. *Chlamydomonas reinhardtii* strain CC-124 is highly sensitive to blue light in addition to green and red light in resetting its circadian clock, with the blue-light photoreceptor plant cryptochrome likely acting as negative modulator. Plant Physiol Biochem 2014; 75:14-23; PMID:24361506; http://dx.doi.org/10.1016/j.plaphy.2013.12.002
28. Beel B, Prager K, Spexard M, Sasso S, Weiss D, Müller N, Heinnickel M, Dewez D, Ikoma D, Grossman AR, et al. A flavin binding cryptochrome photoreceptor responds to both blue and red light in *Chlamydomonas reinhardtii*. Plant Cell 2012; 24:2992-3008; PMID:22773746; http://dx.doi.org/10.1105/tpc.112.098947

29. Niwa Y, Matsuo T, Onai K, Kato D, Tachikawa M, Ishiura M. Phase-resetting mechanism of the circadian clock in *Chlamydomonas reinhardtii*. Proc Natl Acad Sci U S A 2013; 110:13666-71; PMID:23898163; http://dx.doi.org/10.1073/pnas.1220004110
30. Reisdorph NA, Small GD. The CPH1 gene of *Chlamydomonas reinhardtii* encodes two forms of cryptochrome whose levels are controlled by light-induced proteolysis. Plant Physiol 2004; 134:1546-54; PMID:15064387; http://dx.doi.org/10.1104/pp.103.031930
31. Voytsekh O, Seitz SB, Iliev D, Mittag M. Both subunits of the circadian RNA-binding protein CHLAMY1 can integrate temperature information. Plant Physiol 2008; 147:2179-93; PMID:18567830; http://dx.doi.org/10.1104/pp.108.118570
32. Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, et al. The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. Science 2008; 319(5859):64-9; PMID:18079367; http://dx.doi.org/10.1126/science.1150646
33. Schaefer DG, Zrÿd JP. Efficient gene targeting in the moss *Physcomitrella patens*. Plant J 1997; 11(6):1195-206; PMID:9225463; http://dx.doi.org/10.1046/j.1365-313X.1997.11061195.x
34. Okada R, Kondo S, Satbhai SB, Yamaguchi N, Tsukuda M, Aoki S. Functional characterization of CCA1/LHY homolog genes, *PpCCA1a* and *PpCCA1b*, in the moss *Physcomitrella patens*. Plant J 2009; 60 (3):551-63; PMID:19624471; http://dx.doi.org/10.1111/j.1365-313X.2009.03979.x
35. Satbhai SB, Yamashino T, Okada R, Nomoto Y, Mizuno T, Tezuka Y, Itoh T, Tomita M, Otsuki S, Aoki S. Pseudo-response regulator (PRR) homologues of the moss *Physcomitrella patens*: insights into the evolution of the PRR family in land plants. DNA Res 2011; 18(1):39-52; PMID:21186242; http://dx.doi.org/10.1093/dnares/dsq033
36. Holm K, Källman T, Gyllenstrand N, Hedman H, Lagercrantz U. Does the core circadian clock in the moss *Physcomitrella patens* (Bryophyta) comprise a single loop? BMC Plant Biol 2010; 10:109; PMID:20550695; http://dx.doi.org/10.1186/1471-2229-10-109
37. Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carré IA, Coupland G. *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in Arabidopsis. Dev Cell 2002; 2(5):629-41; PMID:12015970; http://dx.doi.org/10.1016/S1534-5807(02)00170-3
38. Ichikawa K, Sugita M, Imaizumi T, Wada M, Aoki S. Differential expression on a daily basis of plastid sigma factor genes from the moss *Physcomitrella patens*. Regulatory interactions among *PpSig5*, the circadian clock, and blue light signaling mediated by cryptochromes. Plant Physiol 2004; 136(4):4285-98; PMID:15563615; http://dx.doi.org/10.1104/pp.104.053033
39. Ichikawa K, Shimizu A, Okada R, Satbhai SB, Aoki S. The plastid sigma factor SIG5 is involved in the diurnal regulation of the chloroplast gene *psbD* in the moss *Physcomitrella patens*. FEBS Lett 2008; 582(3):405-9; PMID:18174028; http://dx.doi.org/10.1016/j.febslet.2007.12.034
40. Mizuno T, Nomoto Y, Oka H, Kitayama M, Takeuchi A, Tsubouchi M, Yamashino T. Ambient temperature signal feeds into the circadian clock transcriptional circuitry through the EC night-time repressor in *Arabidopsis thaliana*. Plant Cell Physiol 2014; 55(5):958-76; PMID:24500967; http://dx.doi.org/10.1093/pcp/pcu030
41. Box MS, Huang BE, Domijan M, Jaeger KE, Khattak AK, Yoo SJ, Sedivy EL, Jones DM, Hearn TJ, Webb AA, et al. ELF3 controls thermoresponsive growth in *Arabidopsis*. Curr Biol 2015; 25(2):194-9; PMID:25557663; http://dx.doi.org/10.1016/j.cub.2014.10.076
42. Djouani-Tahri el-B, Christie JM, Sanchez-Ferandin S, Sanchez F, Bouget FY, Corellou F. A eukaryotic LOV-histidine kinase with circadian clock function in the picoalga *Ostreococcus*. Plant J 2011; 65(4):578-88; PMID:21235644; http://dx.doi.org/10.1111/j.1365-313X.2010.04444.x
43. Noordally ZB, Ishii K, Atkins KA, Wetherill SJ, Kusakina J, Walton EJ, Kato M, Azuma M, Tanaka K, Hanaoka M, Dodd AN. Circadian control of chloroplast transcription by a nuclear-encoded timing signal. Science 2013; 339(6125):1316-9; PMID:23493713; http://dx.doi.org/10.1126/science.1230397
44. Kawazoe R, Mahan KM, Venghaus BE, Carter ML, Herrin DL. Circadian regulation of chloroplast transcription in *Chlamydomonas* is accompanied by little or no fluctuation in RPOD levels or core RNAP activity. Mol Biol Rep 2012; 39(12):10565-71; PMID:23053955; http://dx.doi.org/10.1007/s11033-012-1942-z
45. Ledger S, Strayer C, Ashton F, Kay SA, Putterill J. Analysis of the function of two circadian-regulated *CONSTANS-LIKE* genes. Plant J 2001; 26(1):15-22; PMID:11359606; http://dx.doi.org/10.1046/j.1365-313x.2001.01003.x
46. Shimizu M, Ichikawa K, Aoki S. Photoperiod-regulated expression of the *PpCOL1* gene encoding a homolog of CO/COL proteins in the moss *Physcomitrella patens*. Biochem Biophys Res Commun 2004; 324(4):1296-301; PMID:15504355; http://dx.doi.org/10.1016/j.bbrc.2004.09.194
47. Zobell O, Coupland G, Reiss B. The family of CONSTANS-like genes in *Physcomitrella patens*. Plant Biol (Stuttg) 2005; 7(3):266-75; PMID:15912446; http://dx.doi.org/10.1055/s-2005-865621
48. Henriques R, Mas P. Chromatin remodeling and alternative splicing: pre- and post-transcriptional regulation of the *Arabidopsis* circadian clock. Semin Cell Dev Biol 2013; 24(5):399-406; PMID:23499867 [http://dx.doi.org/10.1016/j.semcdb.2013.02.009
49. Seo PJ, Mas P. Multiple layers of posttranslational regulation refine circadian clock activity in *Arabidopsis*. Plant Cell 2014; 26(1):79-87; PMID:24481076; http://dx.doi.org/10.1105/tpc.113.119842
50. Tasaki E, Hattori M, Sugita M. The moss pentatricopeptide repeat protein with a DYW domain is responsible for RNA editing of mitochondrial *ccmFc* transcript. Plant J 2010; 62(4):560-70; PMID:20163555; http://dx.doi.org/10.1111/j.1365-313X.2010.04175.x