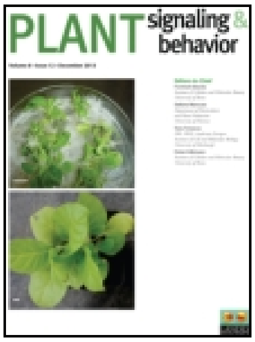
Title	Photoperiod sensitivity of the Arabidopsis circadian clock is tissue-specific.
Author(s)	Shimizu, Hanako; Araki, Takashi; Endo, Motomu
Citation	Plant signaling & behavior (2015), 10(6)
Issue Date	2015-07-15
URL	http://hdl.handle.net/2433/203054
Right	© 2015 The Author(s). Published with license by Taylor & Francis Group, LLC.; This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License http://creativecommons.org/licenses/by-nc/3.0/, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.; Permission is granted subject to the terms of the License under which the work was published. Please check the License conditions for the work which you wish to reuse. Full and appropriate attribution must be given. This permission does not cover any third party copyrighted material which may appear in the work requested.
Type	Journal Article
Textversion	publisher



Plant Signaling & Behavior

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

Photoperiod sensitivity of the Arabidopsis circadian clock is tissue-specific

Hanako Shimizu, Takashi Araki & Motomu Endo

To cite this article: Hanako Shimizu, Takashi Araki & Motomu Endo (2015) Photoperiod sensitivity of the Arabidopsis circadian clock is tissue-specific , Plant Signaling & Behavior, 10:6, e1010933, DOI: 10.1080/15592324.2015.1010933

To link to this article: http://dx.doi.org/10.1080/15592324.2015.1010933

Published online: 15 Jul 2015.

SHORT COMMUNICATION

Plant Signaling & Behavior 10:6, e1010933; June 2015; Published with license by Taylor & Francis Group, LLC

Photoperiod sensitivity of the *Arabidopsis* circadian clock is tissue-specific

Hanako Shimizu, Takashi Araki, and Motomu Endo*

Division of Integrated Life Science; Graduate School of Biostudies; Kyoto University; Kyoto, Japan

Keywords: ambient signal, arabidopsis, circadian clock, phase shift, tissue-specificity

Abbreviations: TOC1, TIMING OF CAB EXPRESSION 1; CAB2, CHLOROPHYLL A/B BINDING PROTEIN 2; CAT3, CATALASE 3.

Tissue-specific functions of the circadian clock in *Arabidopsis* have recently been revealed. The vasculature clock shows distinctive gene expression profiles compared to the clock in other tissues under light-dark cycles. However, it has not yet been established whether the vasculature clock also shows unique gene expression patterns that correlate with temperature cycles, another important environmental cue. Here, we detected diel phase of *TIMING OF CAB EXPRESSION 1* (*TOC1*) expression in the vasculature and whole leaf under long-day light-dark cycles and temperature cycles. We found that the vasculature clock had advanced *TOC1* phase under light-dark cycles but not under temperature cycles, suggesting that the vasculature clock has lower sensitivity against temperature signals. Furthermore, the phase advancement of *TOC1* was seen only under long-day condition but not under short-day condition. These results support our previous conclusion that the circadian clock in vasculature preferentially senses photoperiodic signals.

In many organisms, circadian clock systems are used for processing environmental stimuli and predicting daily and seasonal changes. The plant circadian clock system had long been assumed to process these signals cell-autonomously. Recently, we have revealed that there are tissue-specific functions of the circadian clock system in *Arabidopsis*, and demonstrated that vasculature has a unique clock system compared to other tissues.[1] We also uncovered a role of the vasculature clock in photoperiodic flowering. A previous study demonstrated that *CHLOROPHYLL A/B BINDING PROTEIN 2* (*CAB2*) and *CATALASE 3* (*CAT3*) have differential sensitivities to light and temperature signals.[2] The diel oscillation of CAB2 expression was entrainable by light-dark cycles but not temperature cycles. By contrast, the *CAT3* expression pattern was entrained by temperature cycles but not light-dark cycles. Since *CAB2* is known to be a mesophyll-specific gene, we hypothesized that dedicated tissue-specific circadian clock systems incorporate these different sensitivities of *CAB2* and *CAT3* against photoperiod and temperature cycles. However, little is known how photoperiod and temperature signals affect clock gene expression in vasculature.

To study the effects of photoperiod and temperature signals on circadian clock gene expression in the vasculature and whole leaf, plants were grown under long-day (16:8 light-dark cycles or temperature cycles) and short-day (8:16 light-dark cycles) conditions for 10 days. Then, the vasculature and whole leaf samples were collected every 2 to 4 hours and *TOC1* gene expression was monitored by quantitative real-time PCR.[1] In the whole leaf samples, the diel phase of *TOC1* expression showed almost the same phase both under long-day light-dark cycles and long-day temperature cycles. By contrast, the *TOC1* phase in the vasculature was slightly but significantly advanced compared to the whole leaf under long-day light-dark cycles, whereas no comparative phase advancement was observed under long-day temperature cycles (**Fig. 1A and B**). These results suggested that photoperiodic signals and temperature signals do not have equal effects on the vasculature clock entrainment. A previous study demonstrated that the diel phase of *TOC1* expression was sensitive to the day length in the whole leaf context.[3] Therefore, we tested whether the phase advancement of vasculature *TOC1* was detectable under short-day light-dark cycles (**Fig. 1A and C**). Interestingly, the phase advancement was not observed in

*Correspondence to: Motomu Endo; E-mail: moendo@lif.kyoto-u.ac.jp
Submitted: 12/08/2014; Revised: 01/04/2015; Accepted: 01/05/2015
http://dx.doi.org/10.1080/15592324.2015.1010933

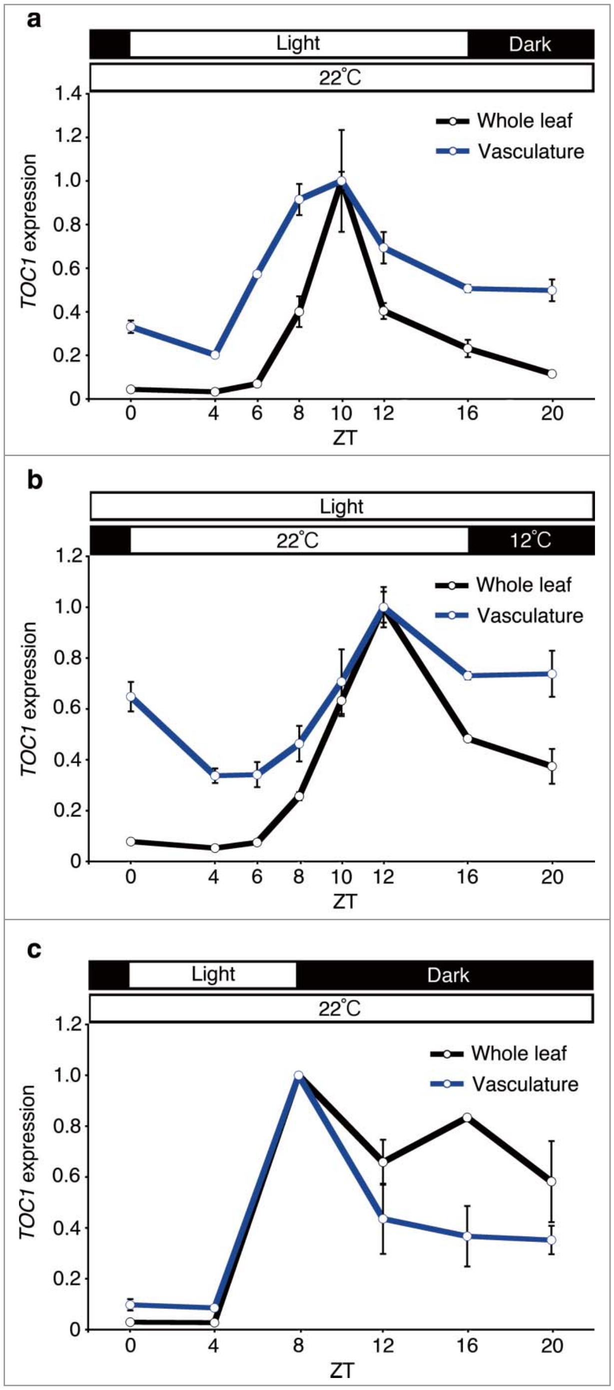


Figure 1. *TOC1* expression in the whole leaf and the vasculature under long-day light-dark cycles, temperature cycles and short-day light-dark cycles. Plants were grown under long-day light-dark cycles (16 hour light / 8 hour dark at 22°C) (**A**), temperature cycles (16 hour 22°C / 8 hour 12°C in continuous light) (**B**) and short-day light-dark cycles (8 hour light / 16 hour dark at 22°C) (**C**) for 10 days. ZT, zeitgeber time. Mean ± s.e. m. (long day, temperature cycles, n=3; and short day, n=2). Geometric mean of *IPP2* and *APA1* were used as internal controls.1

that condition, indicating that the phase advancement of *TOC1* operates in a long-day light-dark specific manner.

Despite the subtle phase advancement under long-day condition, the vasculature clock is rather insensitive to photoperiodic changes. The diel phase of *TOC1* expression in the whole leaf was drastically changed between long-day and short-day conditions. Since about 80% of mRNA in the whole leaf came from mesophyll cells1; therefore most of *TOC1* mRNA in whole leaves is expected to come from mesophyll cells. By contrast, the diel phase of *TOC1* expression was rather phase-locked. This feature of *TOC1* expression in vasculature might be beneficial for plants to register environmental light conditions, according to the external coincidence model,[4] since the internal clock gene expression pattern is less affected by photoperiodic changes. These results support our view that the vasculature clock is important for photoperiodic flowering.[5] More interestingly, we could not detect any phase advancement in the long-day temperature cycles. This result implies that there is another tissue in which temperature signals are processed. Since *CAT3* is expressed in both mesophyll and epidermis,[2] epidermis might be a candidate tissue for temperature sensing.

Cytosolic calcium oscillation is also reported as cell and stimulus-type specific.[6] Thus, as we demonstrated here, a tissue-specific approach in circadian clock studies will provide new insights into environmental signal sensing and processing by the circadian clock.

References

1. Endo M, Shimizu H, Nohales MA, Araki T, Kay SA. Tissue-specific clocks in arabidopsis show asymmetric coupling. Nature 2014; 515: 419–22; PMID: 25363766; http://dx.doi.org/10.1038/nature13919
2. Michael TP, Salome PA, McClung CR. Two arabidopsis circadian oscillators can be distinguished by differential temperature sensitivity. Proc Natl Acad Sci U S A 2003; 100: 6878–83; PMID: 12736379; http://dx.doi.org/10.1073/pnas.1131995100
3. Perales M, Más P. A functional link between rhythmic changes in chromatin structure and the arabidopsis biological clock. Plant Cell 2007; 19: 2111–23; PMID: 17616736; http://dx.doi.org/10.1105/tpc.107.050807
4. Sawa M, Nisinow DA, Kay SA, Imaizumi T. FKF1 and GIGANTEA complex formation is required for day length measurement in arabidopsis. Science 2007; 318: 261–65; PMID: 17872410; http://dx.doi.org/10.1126/science.1146994
5. Endo M, Mochizuki N, Suzuki T, Nagatani A. CRYPTOCHROME2 in vascular bundles regulates flowering in arabidopsis. Plant Cell 2007; 19: 84–93; PMID: 17259260; http://dx.doi.org/10.1105/tpc.106.048157
6. Cell-and stimulus type-specific intracellular free Ca^{2+} signals in Arabidopsis. Martí MC, stancombe MA, Webb AA. Plant Physiol 2013; 163: 625–34; PMID: 24027243; http://dx.doi.org/10.1104/pp.113.222901