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CIRCADIAN CLOCKS

Who knows where the time goes

Plants contain several tissue-specific decentralized but communicating 'clocks'. These control developmental outputs in response to environmental change: the vasculature clock for photoperiodic control of flowering, and the epidermis clock for temperature-dependent elongation.

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The responses of many plant processes to environmental conditions are dependent on time of day and coordinated by internal circadian 'clock(s)'[1]. In particular, the system of interacting genes that constitute the circadian clock is a major regulator of flowering and growth. The photoperiodic induction of flowering entails expression of florigens such as FLOWERING LOCUS T (FT) in the phloem companion cells, and circadian clock activity in those cells is relevant to regulation of flowering[2]. However, considerable experimental evidence suggests that control of elongation growth resides in the epidermis[3]. In this issue of *Nature Plants* Shimizu *et al.*[4] show that circadian clock function in the epidermis, and not in the vasculature, controls the transduction of thermal and photoperiodic signals regulating elongation.

In order to ascertain the tissue(s) in which circadian clock function is required for proper temporal and photoperiodic regulation of flowering and growth, Endo and colleagues set out to disrupt the clock in specific tissues. To this end, they overexpressed GFP fusions of known clock genes, *CCA1* or *TOC1*, under different organ- or tissue-specific promoters. *CCA1* and *TOC1* are major components of 'morning' and 'evening' circuits of the clock, respectively. This analysis confirmed that overexpression of either gene is sufficient to disrupt the rhythmic oscillation of a representative circadian clock gene in the relevant tissue. Control of *CCA1* by its own promoter is sufficient to overexpress the protein throughout the plant, confer late flowering[2] and prevent the normal 12 h light/12 h dark photoperiod-dependent inhibition of hypocotyl elongation.

Overexpression of CCA1 in phloem companion cells using the *SUC2* promoter resulted in late flowering, demonstrating the relevance of circadian clock function in those cells to the proper photoperiodic production of the flowering inducers, CONSTANS (CO) and FT (ref. 2). However, overexpression of CCA1 in other vascular cells, including procambium and xylem, failed to delay flowering, confirming that CCA1 expression in phloem companion cells but not in other vascular cell types is critical to flowering time[4]. Similarly, overexpression of CCA1 in the epidermis under control of the *CER6* promoter did not affect flowering time[4]. CCA1 regulates the transcription of clock-controlled genes residing on output pathways from the circadian clock as well as the expression of core oscillator genes; therefore, it is possible that disruption of the output pathway rather than of the circadian oscillator itself was resulting in late flowering under long days. Shimizu *et al.* ruled out this possibility by demonstrating that disruption of circadian clock function, using the *SUC2* promoter to overexpress the second critical clock gene, *TOC1*, in phloem companion cells, also resulted in late flowering.

To determine in which tissues circadian clock function was required to regulate hypocotyl elongation, Shimizu *et al.* overexpressed either CCA1 or TOC1 in the epidermis using the epidermis-specific

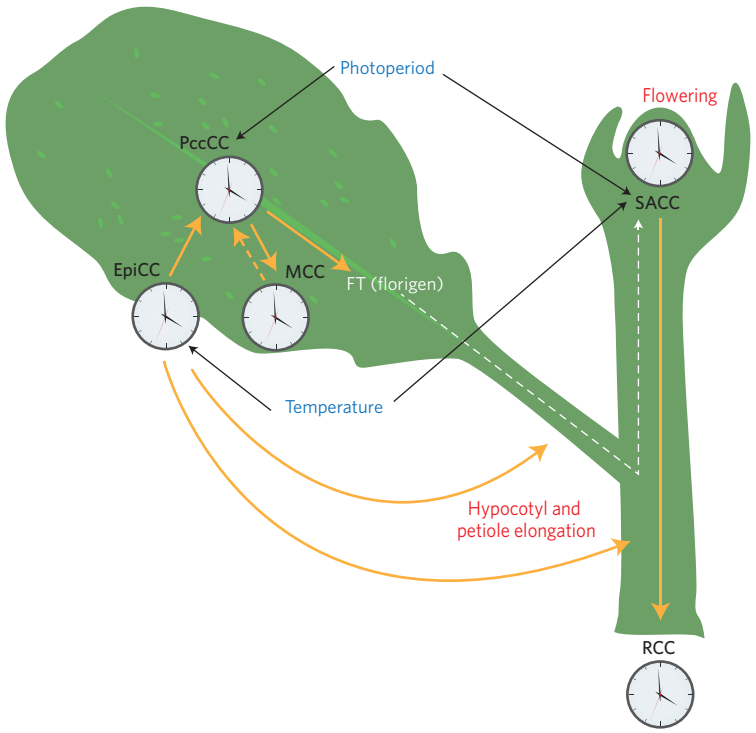

Figure 1 | The *Arabidopsis* circadian system is a hierarchical multi-oscillator network regulating growth and development. In the leaf, a photoperiod sensitive circadian clock in the phloem companion cells (PccCC) regulates the induction of a florigen, FT, which is translocated through the vasculature to the shoot apex to induce flowering. The PccCC also relays temporal information to the mesophyll circadian clock (MCC). An epidermal circadian clock (EpiCC), responsive to both photoperiod and temperature, regulates elongation growth in the petiole and hypocotyl. The EpiCC also relays thermal information to the PccCC. In the shoot apex, a photoperiod and temperature sensitive circadian clock (SACC) provides temporal information to help synchronize circadian clocks in distal organs, including (but not necessarily limited to) the roots (RCC).

CER6 promoter. This resulted in a long hypocotyl phenotype due to the disruption of photoperiod-dependent inhibition of hypocotyl elongation. Conversely, overexpressing either CCA1 or TOC1 in phloem companion cells with the *SUC2* promoter did not prevent photoperiod inhibition of hypocotyl elongation. Thus photoperiod-dependent control of hypocotyl cell elongation is exerted through the circadian clock of the epidermis and not of the phloem companion cells[4]. The authors then showed that overexpression of CCA1 throughout the plant or specifically in the epidermis, but not in the phloem companion cells, also caused a long petiole phenotype, thereby establishing that the epidermal rather than the vascular circadian clock function is essential for photoperiod-dependent regulation of petiole elongation.

A number of earlier studies have shown that circadian clock regulation of gene expression profiles differs between specific tissues or organs with varying sensitivities to environmental signals such as temperature[5–8]. The work of Shimizu, Endo and colleagues offers a mechanistic explanation of these findings, that tissue-specific circadian clocks each regulate distinct subsets of clock output pathways. The existence of such distinct clocks immediately raises the question of coupling and the possibility of asymmetry between clocks, in which the clock in one tissue influences clock function in a second tissue. Indeed, Endo *et al.*[2] showed that the phloem companion cell clock had a strong effect on mesophyll clock function, and James *et al.*[7] have independently shown that the 'shoot' clock strongly affects root clock function. In addition it has recently been shown that the circadian clock of the shoot apex is critical for shoot control of root circadian clock function[9]. The circadian clock in the shoot apex exhibits a high degree of synchrony among cells that is lost when cells are dispersed, indicating that the synchrony of the intact apex and its robustness against genetic and pharmacological perturbation result from intercellular coupling among cells[9]. Collectively, these results suggest that circadian clocks in plants operate as a hierarchical multi-oscillator system in which the shoot apex clock plays a dominant role, analogous to the suprachiasmatic nucleus in mammals[9]. ❐

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