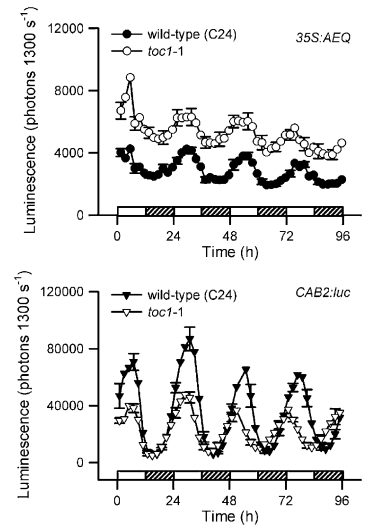


IN BRIEF

Circadian Regulation of Cytosolic Ca²⁺

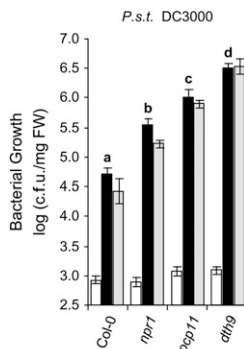
Cytosolic free Ca²⁺ [Ca²⁺]_{cyt} shows circadian oscillations in plants, which might play a role in clock control of circadian rhythms of various processes, such as photosynthesis and photo-periodic control of flowering. **Xu et al. (pages 3474–3490)** undertook an extensive analysis of circadian [Ca²⁺]_{cyt} oscillations under a variety of light/dark conditions in a number of *Arabidopsis* photoreceptor and circadian clock mutants carrying the bioluminescent Ca²⁺ reporter aequorin. The results showed that the circadian oscillation of [Ca²⁺]_{cyt} in *Arabidopsis* is regulated by red and blue light through CRY1, CRY2, PHYB, and, possibly, PHVA. They further suggest a role for PHYB in cooperation with CRY1 and/or CRY2 in blue light signaling that regulates the amplitude of clock-controlled [Ca²⁺]_{cyt} oscillations.

Experiments with various circadian clock mutants expressing the reporter gene *CAB2:luciferase* and aequorin suggested that (1) [Ca²⁺]_{cyt} oscillations function as output, rather than input, of the circadian system, and light input into the oscillator controlling [Ca²⁺]_{cyt} rhythms is gated by ELF3, and (2) multiple circadian oscillators are present that may be located in different cell types. In the *toc1-1* mutant, the period of the [Ca²⁺]_{cyt} (35S:AEQ) rhythm is unaltered, but the *CAB2:luc* period is shortened (see figure), suggesting the presence of multiple circadian oscillators.



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Gene Silencing and Resistance to Bacterial Pathogens



The *ocp11/ago4-2* mutant shows enhanced susceptibility to *P. syringae* pv *tomato* DC3000 comparable to enhanced susceptibility mutants *npr1* and *dth9*.

Agorio and Vera (pages 3778–3790) characterize an *ocp* (for *overexpressor of cationic peroxidase*) mutant that overexpresses the H₂O₂-responsive Ep5C promoter fused to the β-glucuronidase reporter gene. The *ocp11* mutant exhibits enhanced disease susceptibility to several virulent and avirulent strains of the bacterial pathogen *Pseudomonas syringae*. *OCP11* was cloned and found to encode ARGONAUTE4 (AGO4), a component of the pathway that mediates the transcriptional gene silencing associated with siRNA. Another mutant allele, *ago4-1*, was examined and likewise found to be compromised in resistance to *P. syringae*. Surprisingly, AGO4 was found to function independently of other components of the AGO4-dependent gene silencing pathway in conferring resistance to *P. syringae* strains, possibly due to functional redundancy of the other components. In

addition, the authors found that transcriptional activation of the reporter gene was correlated with demethylation of specific sites in the promoter region and that methylation of this promoter was altered following bacterial infection. This suggests that that demethylation of specific (as yet unidentified) host genes might be an important component of *AGO4* involvement in modulating disease resistance. This work provides additional insight into and support for the notion that the small RNA gene silencing pathway is involved in resistance to bacterial pathogens.

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