

Mathematical models of circadian Ca^{2+} oscillations

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Ca^{2+} is a ubiquitous second messenger, involved in the transmission of inter- and intracellular signals. While short-term elevations of the concentration of cytosolic free Ca ($[\text{Ca}^{2+}]_{\text{cyt}}$) have been studied extensively in many organisms, little is known about the circadian regulation of its basal level. To establish the importance of this circadian control, we are characterizing the pathways that regulate $[\text{Ca}^{2+}]_{\text{cyt}}$ in the model plant *Arabidopsis thaliana*, both experimentally and mathematically. Existing mathematical models of Ca^{2+} signalling embrace the mechanistic details of Ca^{2+} -release channels, describing nonlinear dynamics on the timescale of seconds. In this research we show that the longer time-scale circadian control of $[\text{Ca}^{2+}]_{\text{cyt}}$ can be described by a linear system of ODE's, and show necessity for circadian-independent light activation.

The central oscillator gene *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* is required for oscillations of Ca^{2+} in constant light conditions. We find that circadian oscillations of $[\text{Ca}^{2+}]_{\text{cyt}}$ are not present in plants lacking a functional CCA1 protein (*cca1-1*) [1, 3], implicating *CCA1* as a necessary component for rhythmicity in constant conditions. The promoter activity of *CCA1* can be measured non-invasively using a *luciferase* reporter gene construct (*CCA1::luc*). These data (along with an assumed delay-step of τ hours) are used as a model input, to describe the central oscillator regulation of Ca^{2+} .

Standard systems identification [2] techniques are used to estimate model parameters from a single time-series experiment measuring $[\text{Ca}^{2+}]_{\text{cyt}}$ and *CCA1* promoter activity in diurnal (12 h light, 12 h dark) cycles and constant darkness. A single-input linear system requires a high order and large delay ($\tau \approx 8$), and is invalidated by other datasets (data not shown). However, providing a light input allows a close match to the estimation data (Fig. 1), with the clock delay of 3.3 hours. The resulting model is validated with three experiments conducted under alternative photoperiods. Key dynamical features are predicted by the model, such as the lower amplitude oscillations in constant light over diurnal cycles, and the phase of oscillation in long days (16 h light, 8 h dark; Fig. 2b) and short days (8 h light, 16 h dark; Fig. 2c). Constitutive over-expression of *CCA1* leads to a loss of rhythmicity in constant light and early-phased Ca^{2+} oscillations in long days. The equivalent simulation with constant (zero) *CCA1* input bears striking similarities to the measured data (Fig. 2d).

Linear systems identification has provided an accurate description of the dynamic regulation of basal $[\text{Ca}^{2+}]_{\text{cyt}}$. Our simulations have demonstrated that the transition of dark to light triggers the accumulation of Ca^{2+} in the cytosol, and this increase is prolonged by a slower-acting regulation by the central oscillator to achieve a peak between 7 and 9 hours after dawn. We have derived and quantified the contribution of two distinct regulators. This non-intuitive prediction suggests further experimental investigations should be carried to assess

the role of light signaling in regulating circadian and diurnal oscillations of $[Ca^{2+}]_{\text{cyt}}$.

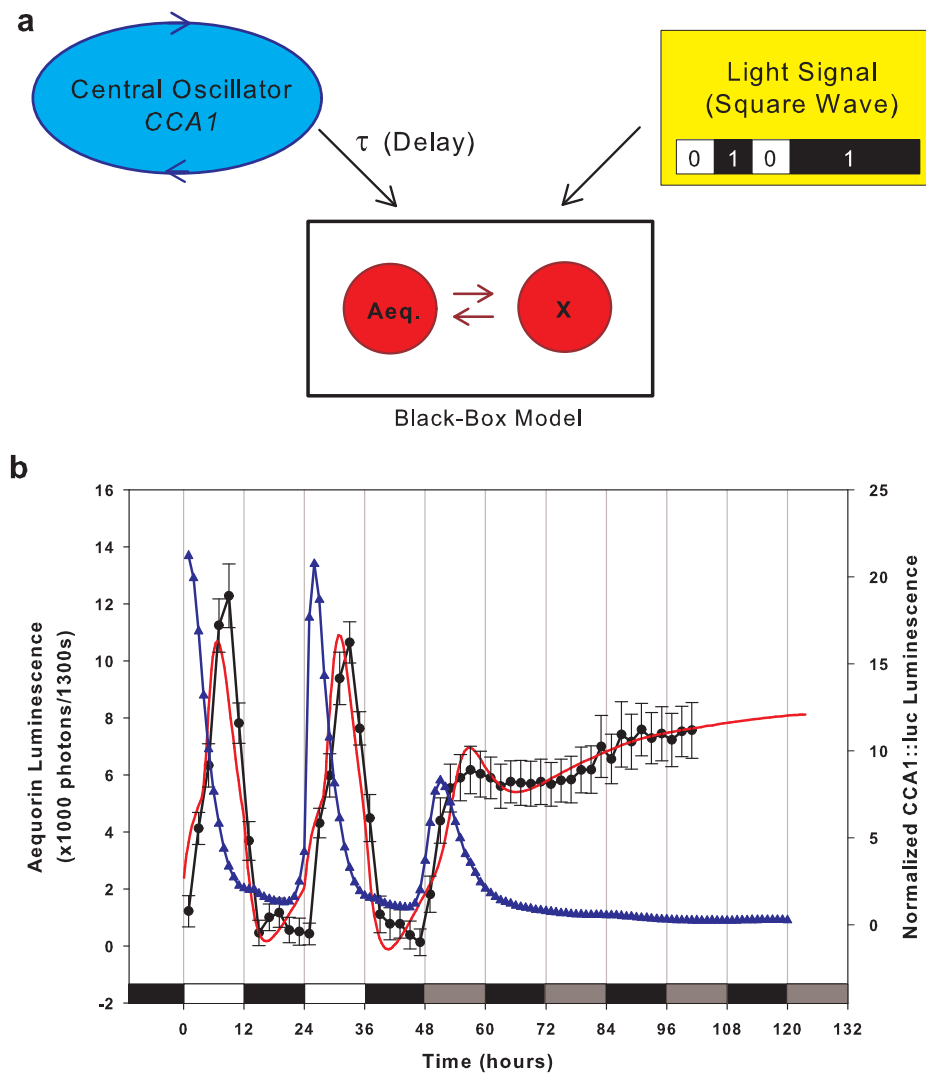


Figure 1: **Estimation.** a. Schematic representation of the 2nd order linear model of circadian Ca^{2+} oscillations. Aeq. (Aequorin luminescence) and X are the model states, regulated by the two inputs CCA1 (CCA1::luc luminescence) and the square wave light definition. b. Fit to the estimation data of the best choice of delay ($\tau = 3.3$ hours). Black circles are the measured aequorin luminescence with the minimum subtracted, and the error bars are for the standard error (SEM) of each data point. Solid red line is the model output. Blue triangles are measured levels of CCA1::luc luminescence, normalized to the minimum observed value. White and black bars indicate light and dark respectively, with grey bars indicating darkness in the subjective daytime.

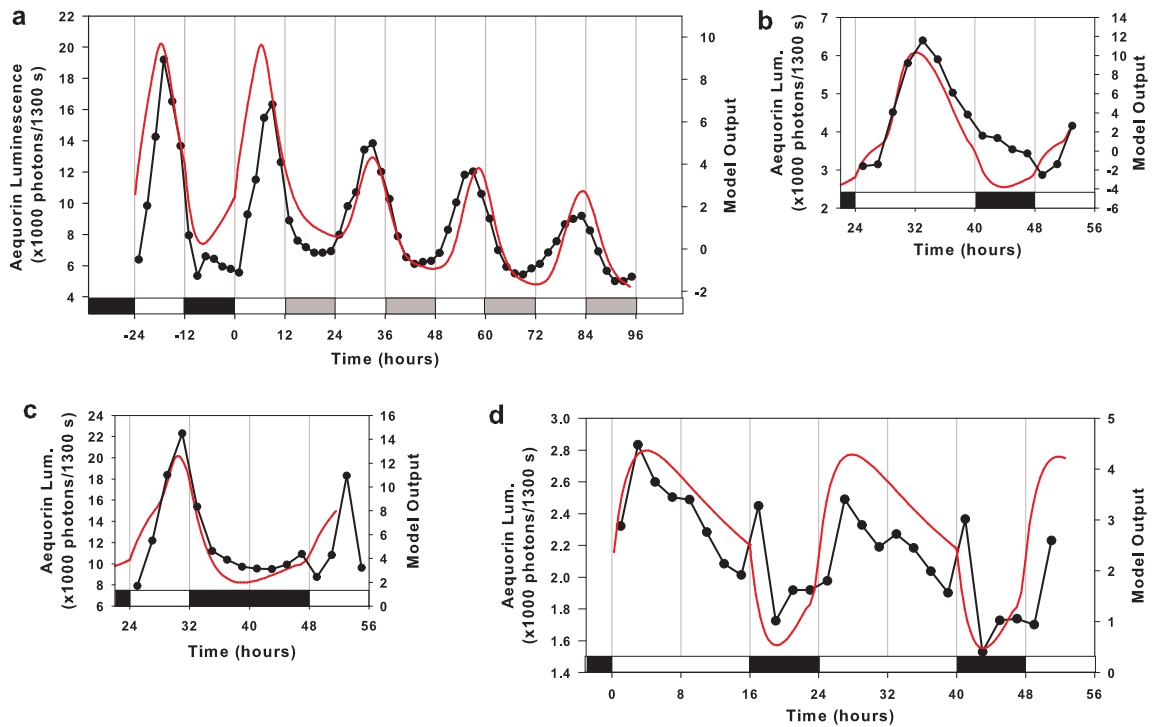


Figure 2: **Validation and Simulation.** Comparison of measured aequorin luminescence (black circles) in wild-type seedlings versus simulated levels in a. diurnal (12 h light, 12 h dark) cycles before transfer to constant light, b. long day (16 h light, 8 h dark), and c. short day (8 h light, 16 h dark) conditions. d. Simulation of $u_1 \equiv \text{constant}$ (arrhythmic CCA1) versus measured aequorin luminescence in CCA1-ox for long day cycles. White and black bars indicate presence and absence of light respectively, while grey bars imply light in the subjective night time (a).

References

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