Redox rhythms reinforce the plant circadian clock: New insights into coupled biological oscillators

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***Short Abstract* — Multiple oscillators often co-exist within the same cell. Are there mechanisms and regulatory principles to ensure functional harmony between these oscillators? Here, we show that salicylic acid, a plant immune signaling hormone, uniquely perturbs the cellular circadian redox rhythm to reinforce the expression of core circadian clock genes through the master immune regulator NPR1. Mathematical modeling and subsequent experiments demonstrated that NPR1 targets both morning and evening genes of the circadian clock. This balanced network architecture ensures the maintenance of period and reinforcement of the circadian clock amplitude by simultaneous regulation of differently phased components.**

***Keywords* — Circadian Clock, Redox Rhythm, Immunity, Arabidopsis.**

I. Introduction

RECENT studies have shown that many organisms, including *Arabidopsis,* have a circadian redox rhythm driven by the organism's metabolic activities [1,2]. It has been hypothesized that the redox rhythm in plants is linked to the circadian clock, but the mechanism of this link remains largely unknown. Our experimental work shows that the master immune regulator NPR1 of *Arabidopsis* is a sensor of the plant's redox state and regulates transcription of core circadian clock gene *TOC1* through TGA transcription factors. Strikingly, acute perturbation in the redox status triggered by the immune hormone salicylic acid (SA) leads to the reinforcement rather than perturbation of oscillations in *TOC1* expression in an NPR1-dependent manner. Mutation of NPR1 resulted in lower *TOC1* expression with the same period of oscillations. Because the levels of TOC1 are known to regulate the period of the circadian clock [3], our results suggest that NPR1 couples to other clock genes, in addition to the evening-phased *TOC1*.

II. Quantitative Model of Plant Circadian Clock

To systematically search for other possible clock components that are regulated by redox rhythms, we explored the effect of adding NPR1 regulation to a mathematical model of the plant circadian clock that includes most of the known components of the *Arabidopsis* circadian clock [4].

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A. Fitting procedure

Based on our data, we made the assumption that NPR1 is a transcriptional activator of other clock genes. We systematically coupled NPR1 to *TOC1* and two other circadian clock genes, X and Y. For each X, Y pair, we used nonlinear least squares fitting to find NPR1 parameters that best fit our *TOC1p:LUC* time-series. We repeated this procedure for all X, Y pairwise combinations of the circadian clock genes.

B. Results

Our modeling showed that NPR1 must also activate the expression of the morning-phased *PRR7* and *LHY* genes. We experimentally confirmed these new regulatory links using qPCR of *LHY* and *PRR7* transcripts in *npr1* mutants and under SA-induction. We show how a balanced network architecture converts a redox perturbation into reinforcement of the circadian clock with no change in period.

III. Biological Significance

We further showed that TOC1 is a repressor of plant immunity. Because morning phased LHY positively regulates plant immunity [5], we hypothesized that the reinforced circadian clock helps gate plant immunity to be more responsive to induction in the morning and less responsive at night to avoid diverting scarce resources from plant growth at night. Our microarray analysis revealed that, indeed, plants have a greater immune response in the morning upon SA induction, and suffer a larger penalty on growth at night upon SA induction. Last, we showed that the expression of catalase *CAT3* (*CATALASE3*) is also upregulated by SA. This may help the circadian clock restore a circadian redox rhythm after pathogen challenge and SA induction.

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