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Commentary

Calcium-Mediated Modulation of GC Switch Regulates Peroxisomal H_2O_2 Levels in Response to Wounding in Plants

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Abstract: Ca^{2+} and H_2O_2 interact with each other to regulate plant systemic responses. However, their precise mechanism is not fully understood. A recent study revealed that the Ca^{2+} regulates the glycolate oxidase-catalase (GC) switch-mediated photorespiratory H_2O_2 during wounding. Glutamate-receptor-like (GLR) Ca^{2+} channels (GLR 3.3 and GLR3.6) are responsible for Ca^{2+} influx during injury for regulation of the GC switch. Mechanical injury quickly shifts the GC switch to a highly interactive state in the systemic leaves that ultimately results in the reduced peroxisomal H_2O_2. However, the mechanism of H_2O_2 reduction in peroxisome remains elusive.

Keywords: Ca^{2+}; H_2O_2; GC switch; peroxisome; wounding

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1. Introduction

Plants are consistently challenged by adverse environmental conditions such as light stress, thermal stress, mechanical injury and pathogen attack. In these circumstances, the local tissues detect the stimuli and transmit signals to other plant tissues through rapid systemic signaling. Plant systemic signaling results in systemic acquired acclimation (SAA), which helps in their survival during stress conditions [1,2]. Hydrogen peroxide (H_2O_2) and calcium (Ca^{2+}) ions are key signaling molecules that interplay to mediate plant systemic signaling and the systemic wound response (SWR). H_2O_2 is produced in various compartments of a cell in response to different environmental stimuli and acts as a signaling molecule to trigger downstream responses. Photorespiratory H_2O_2 produced in the peroxisome has a significant role in activating defense mechanisms in plants. Photorespiratory H_2O_2 is generated during the oxidation of glycolate to glyoxylate by enzyme glycolate oxidase (GLO). H_2O_2 is further catalyzed by catalase (CAT) into water (H_2O) and oxygen (O_2). GLO and CAT together serve as a dynamic molecular switch called a GC switch, which balances photorespiratory H_2O_2 during mechanical injury [3].

Ca^{2+} signaling plays a significant role in mitigating a variety of stress conditions. It is involved in a wide range of physiological processes including gene expression, cell division, and differentiation as well as biotic and abiotic stress responses [4,5]. Plants respond to mechanical injury by generating a rapid and systemic increase in cytosolic calcium $[Ca^{2+}]_{cyt}$ concentration [6]. $[Ca^{2+}]_{cyt}$ fluctuations are among the primary events associated with the rapid systemic signaling of plants [7]. Ca^{2+} signals travel long distances during mechanical stress to propagate and modify local signals into a systemic defense response system [8].

Ca^{2+} and H_2O_2 interact with each other to regulate plant systemic responses. However, the exact mechanisms have not been fully revealed. Li et al. [9] in a recent study revealed a partial mechanism by which plants respond to mechanical injury through Ca^{2+}-H_2O_2 crosstalk (Figure 1). The study mainly focused on the importance of GC switch-mediated modulation in photorespiratory H_2O_2 during wounding. The authors shed light on the role of Ca^{2+} in modulating photorespiratory H_2O_2 for systemic adaptation by employing different sets of experiments and analyses in Arabidopsis and rice. Understanding these mechanisms is crucial for developing strategies to improve plant growth and stress tolerance, which is of great importance for sustainable agriculture and food security.

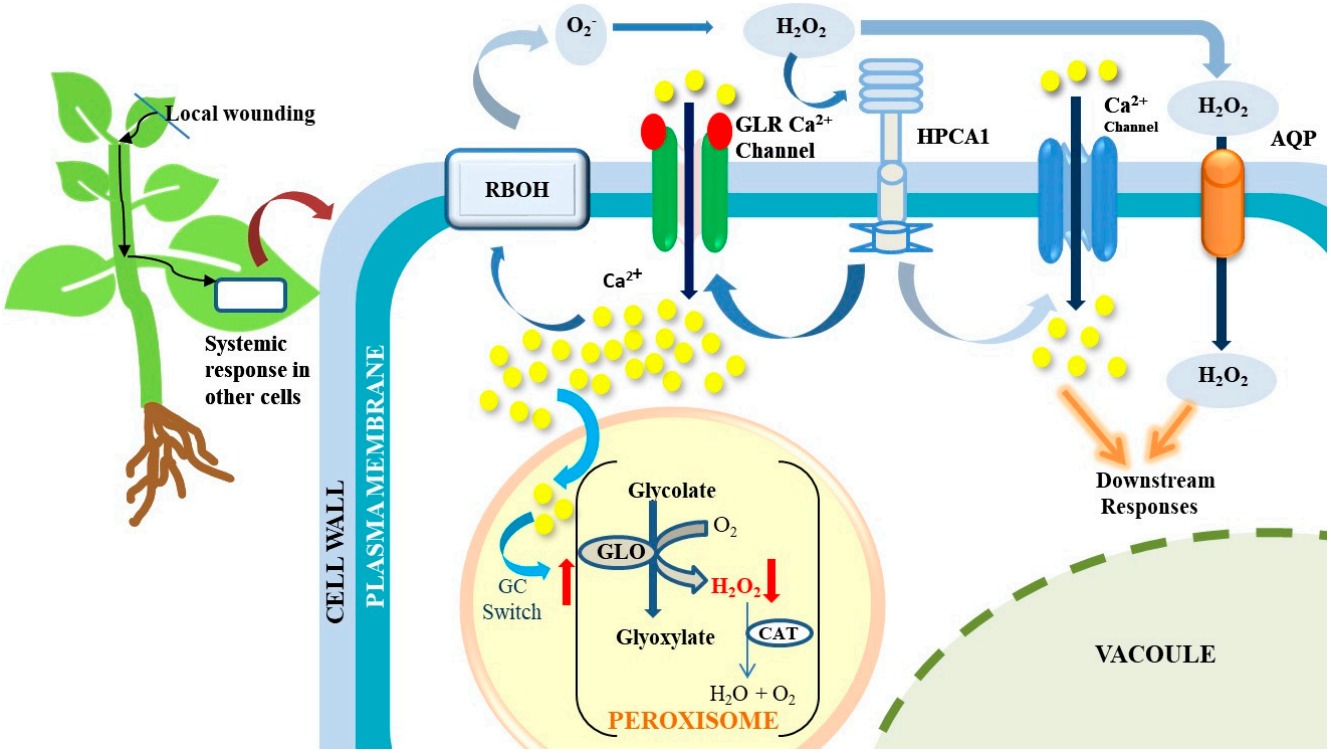

Figure 1. A model showing Ca^{2+}–mediated regulation of GC switch. Upon local wounding RBOH triggers initial apoplastic H_2O_2 production in systemic leaves. Downstream H_2O_2 targets include receptor kinases (HPCA1) that activate glutamate-like receptors (GLR) Ca^{2+} channels, allowing Ca^{2+} entry into the cytosol which further enhances RBOH activity and increases apoplastic H_2O_2 levels. The apoplastic H_2O_2 enters into the cytosol through aquaporins and activates downstream stress-responsive signaling. The Ca^{2+} influx leads to an increase in cytoplasmic Ca^{2+} concentration that further elevates peroxisomal Ca^{2+} concentration. The elevated peroxisomal Ca^{2+} modulates the glycolate oxidase-catalase (GC) switch to a more interactive state, leading to a decrease in peroxisomal H_2O_2 levels. The figure is based on the study of Li et al. [9] and drawn by the authors.

2. GC Switch Facilitates Peroxisomal H_2O_2 Decrease during Wounding

The key photorespiration enzymes in Arabidopsis are GOX1, GOX2, and CAT2, which are analogous to GLO1, GLO4, and CATC in rice [10]. Arabidopsis GOXs also interact with CAT, equivalent to the GLO-CAT complex in rice. GLO and CAT interactions dynamically modulate H_2O_2 levels through metabolic pathways [3,11]. The GC switch primarily exists in three states: highly dissociated, intermediate and highly interactive [3]. Wounding triggers the GC switch to change to a highly interactive state, resulting in peroxisomal H_2O_2 reduction. Li et al. [9] performed a bimolecular fluorescence complementation (BiFC) assay to investigate the interaction between GLO and CAT by fusing them to the N- and C-terminal of a yellow fluorescent protein (YFP) and expressing them in Arabidopsis protoplasts. The results demonstrated that GLO and CAT interact with each other, which is further confirmed by split nano luciferase (Nluc) complementation (SLC) and co-immunoprecipitation (Co-IP) assays. Additionally, mechanical injury generates an immediate accumulation of jasmonic acid (JA), which is assisted by the activation of JA-related defense genes. Moreover, the Nluc-based SLC assay demonstrated that methyl jasmonic acid (MeJA) shifts the GC switch to a more interactive state [9].

The authors used ratiometric redox-sensitive green fluorescent protein 2 (roGFP2) fused with peroxisomal target signal 1 (SKL) as a substitute for the detection of photorespiratory H_2O_2 dynamics [10,12,13]. Additionally, two peroxisome-specific H_2O_2 reporter genes HSP17 and SAP12 were also employed to detect peroxisomal H_2O_2 reduction. The extent of decline of peroxisomal H_2O_2 during wounding is significantly reduced by a-

hydroxyl-2-pyridinemethanesulfonic acid (HPMS), a GLO activity inhibitor [3]. Moreover, wounding contributes to an elevation in apoplastic H_2O_2 produced by the respiratory burst oxidase homolog (RBOH) [14]. The zinc finger reporter protein ZAT12 and 20,70 -dichlorofluorescein diacetate (H_2DCFDA) [15] are used to detect changes in the RBOH-derived accumulation of H_2O_2 in systemic leaves. The results indicated a rise in apoplastic H_2O_2 levels in response to mechanical injury. Additionally, an inhibition assay using diphenyleneiodonium (DPI) demonstrated that RBOH-derived H_2O_2 has no effect on peroxisomal H_2O_2 reduction. The RBOH-derived H_2O_2 is sensed by H_2O_2-induced Ca^{2+} increase 1 (HPCA1). HPCA1 induces systemic cell-to-cell Ca^{2+} signals and elevates $[Ca^{2+}]_{cyt}$ by activating Ca^{2+} influx channels [16,17], which might be responsible for the Ca-ROS hub that elevates systemic signaling [18]. These findings suggest that the GC switch quickly shifts to a highly interactive state upon local injury and triggers a reduction in peroxisomal H_2O_2 levels compared to a burst of RBOH-derived H_2O_2. These results suggest that mechanical injury activates two different H_2O_2 signaling pathways at the same time: (i) the GC switch-mediated peroxisomal H_2O_2 reduction, and (ii) the RBOH-derived apoplastic H_2O_2 burst. However, the functional significance of GC switch-mediated photorespiratory H_2O_2 needs to be investigated further. In addition, the status of H_2O_2 in the cytoplasm remains elusive, which would be interesting to investigate in future research.

3. GLR-Mediated Ca^{2+} Influx Regulates the GC Switch

It has been well established that the long-distance transmission of a Ca^{2+} signal plays a crucial role in regulating systemic adaptations in plants [19]. Mechanical injury triggers a rapid influx of apoplastic Ca^{2+} into the cytosol, resulting in the elevation of $[Ca^{2+}]_{cyt}$ [20]. The authors found that the elevated $[Ca^{2+}]_{cyt}$ further leads to the transition of GC switch to a highly interactive state by increasing peroxisomal Ca^{2+} ($[Ca^{2+}]_{per}$). However, the exact mechanism of Ca^{2+} entry into peroxisomes is not fully understood. Li et al. [9] analyzed $[Ca^{2+}]_{per}$ using the fluorescence probe fluo-4/AM and further validated the process with the peroxisome-targeted Ca^{2+} sensor GCaMP3-SKL. The inductive effect of $[Ca^{2+}]_{cyt}$ on the GC switch was also confirmed by a surface plasmon resonance (SPR/Biacore) assay, a Glutathione S-transferase (GST) pull-down assay, a Co-IP assay and exogenous Ca^{2+} treatment. In addition, the authors also used lanthanum chloride ($LaCl_3$) to block the influx of Ca^{2+} ions into the cytosol. The results revealed that both the GC switch transition and reduction in peroxisomal H_2O_2 levels almost disappeared suggesting that Ca^{2+} influx is required for both the processes. Moreover, it has previously been reported that the increased cellular Ca^{2+} concentration during local wounding does not have any impact on CAT activity in systemic leaves [20]. Hence, the reduction in peroxisomal H_2O_2 levels can not be related to the CAT activity. Therefore, it is still unclear how peroxisomal H_2O_2 declines without an elevation in CAT activity, which would be interesting to investigate in future studies.

GLR3.3 and GLR3.6 are glutamate-receptor-like (GLR) Ca^{2+} channels present on the plasma membrane which are involved in increasing $[Ca^{2+}]_{cyt}$ upon wounding [1,14]. To validate this, Li et al. [9] generated a double-knockout mutant for *glr3.3* and *glr3.6* in the GCR-Arabidopsis and Col-0 backgrounds. GCR-Arabidopsis shows real-time states of the GC switch, providing a non-invasive method for monitoring plant signaling. The authors reported that the knocking out of *glr3.3* and *glr3.6* inhibited the influx of $[Ca^{2+}]_{cyt}$ and the systemic wound response of the GC switch, thereby causing a consequent reduction in peroxisomal H_2O_2 levels. The result suggests that GC switch-mediated H_2O_2 reduction is dependent on GLR-dependent Ca^{2+} signaling. Moreover, it is imperative to study the impact of other Ca^{2+} channels using overexpression or knockout approaches because apoplastic H_2O_2-mediated activation of HPCA1 triggers some other Ca^{2+} channels for Ca-ROS hub-mediated systemic signaling [17,18,21].

4. Conclusions and Future Perspectives

This study provides valuable insights into the molecular mechanisms regulating plant systemic signaling in response to wounding by elucidating the interplay between Ca^{2+} ions and H_2O_2. Ca^{2+} and H_2O_2 form a feedback loop in systemic parts of the plant during wounding. Mechanical injury increases $[Ca^{2+}]_{cyt}$ influx in systemic leaves via GLR3.3/GLR3.6 Ca^{2+} channels in contrast to an increase in RBOH-derived apoplastic H_2O_2. Enhanced $[Ca^{2+}]_{cyt}$ further elevates $[Ca^{2+}]_{per}$ that shifts the GC switch to an interactive state, which further leads to a decrease in peroxisomal H_2O_2. The interplay between the above two processes, apoplastic H_2O_2 outburst and peroxisomal H_2O_2 reduction, form an optimized H_2O_2 wave that prepare the plants for biotic and abiotic stress tolerance. This study provides insights into how Ca^{2+} regulates the GC switch in peroxisomes, however, the precise mechanism remains elusive. The mechanism of Ca^{2+} entry into peroxisomes is also not explained. How does peroxisomal H_2O_2 decline without an elevation in CAT activity demands further research. It must be determined whether the CAT has been transported to the cytoplasm or if its production has been reduced. In addition, the role of the GC switch in other stresses and the involvement of other Ca^{2+} channels in GC switch modulation as well as in wounding remain to be addressed in future studies.

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