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Wrzaczek, Michael

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A negative feedback loop controls ROS production in plant immunity

Michael Wrzaczek[1,2]

[1]Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, 370 05 České Budějovice, Czech Republic.

[2]Organismal and Evolutionary Biology Research Programme, Viikki Plant Science Centre, University of Helsinki, FI-00014 Helsinki, Finland.

Address for correspondence: michael.wrzaczek@umbr.cas.cz

Over the past two decades the view of reactive oxygen species (ROS) as merely cytotoxic compounds has changed considerably (Waszczak *et al*., 2018). While still reactive chemicals with potentially devastating detrimental effects, research has shown that plants and animals alike use ROS as messengers in numerous signaling networks including responses to biotic and abiotic environmental cues as well as developmental processes (Waszczak *et al.*, 2018; Zandalinas *et al*., 2020). The production of ROS as by-products of cellular metabolism as well as the active production of ROS in different subcellular localization is tightly controlled (Castro *et al*., 2021). However, mechanistic details on how ROS are sensed and how specificity in ROS signaling is achieved remain scarce. Few intracellular ROS sensors have been characterized on a molecular level and recently the first sensor for extracellular ROS, HPCA1, has been identified (Wu *et al*., 2020). However, the broad involvement of ROS in signal transduction in plants suggests the existence of a far larger and more diverse sensory array.

The activation of stimulus-induced ROS production in the extracellular space by plasma membrane-localized NADPH oxidases, referred to as respiratory burst oxidase homologs (RBOH) in plants, is an intensively investigated research area (Castro *et al.*, 2021). However, in contrast to the activation mechanisms, the inactivation or negative regulation of the ROS producing activity of plant RBOHs remains largely elusive. Phosphorylation by kinases, together with calcium-binding, is firmly established as activation mechanism for plant NADPH oxidases (Kadota *et al*., 2015). Few reports suggest that phosphorylation can also reduce the ROS producing activity and stability of RBOHs (Castro *et al.*, 2021; Lee et al., 2020). In addition, S-nitrosylation has been described as an element for the negative regulation of the ROS producing activity of RBOHD but how this modification is integrated into the regulatory circuitry surrounding RBOHD has remained elusive (Yun *et al*., 2011). In

the current issue of Molecular Plant, Chae and colleagues (Chae *et al*., 2021) now provide evidence, that ROS production is sensed by the plant quiescin sulfhydryl oxidase homolog (QSOX1) which subsequently provides negative regulation of S-nitrosoglutathione reductase (GSNOR). Thereby, QSOX1 links ROS and reactive nitrogen species (RNS) signalling to limit ROS production and consequently negatively regulate plant immunity (Figure 1).

Chae *et al.* (2021) started by mining the http://www.arabidopsis.org data based for redox proteins and identified a candidate with strong homology to mammalian QSOX. This protein contains two distinct redox-active domains: a PDI-like oxidoreductase with an active CxxC motif embedded in a thioredoxin (Trx)-like fold as well as a sulfhydryl oxidase mitochondrial ERV/ALR-related domain with two CxxC motifs and a flavin adenine dinucleotide (FAD)-binding motif. Electrons are transferred from the Trx domain with its low pKa via dithiol/disulfide relay to the EVR/ALR domain and FAD and subsequently to terminal electron acceptors. Via this mechanism, QSOX could be able to sense ROS and adjust the oxidation status of interacting proteins in response to a variety of stimuli.

Within hours following biotic stimuli or treatment with the defense hormones salicylic acid (SA) and jasmonic acid (JA), *QSOX1* transcript abundance increased. Chae *et al*. proceed to validate the oxidoreductase activity of QSOX1 and found that recombinant QSOX1 exhibited FAD-dependent activity *in vitro* towards artificial substrates. Their approach allowed Chae and colleagues to design mutant variants of QSOX1 with no detectable oxidoreductase activity. Intriguingly, in line with the results from transcriptional analyses of QSOX1, *qsox1* mutant plants displayed reduced susceptibility towards the bacterial pathogen *Pseudomonas syringae pv*. tomato DC3000 suggesting that the protein indeed participates in the coordination of plant defense responses. Variants of QSOX1 with mutations, that abate the enzymatic oxidoreductase activity, were not able to complement for the loss of QSOX1 highlighting that the oxidoreductase activity of QSOX1 is essential for its function.

ROS production is an integral part of the plant defense response (Castro *et al.*, 2021; Kimura *et al*., 2017). Chae *et al*. (2021) found that RBOHD-dependent pathogen-triggered ROS production was enhanced in the *qsox1* mutant. Surprisingly, QSOX1 did not interact with RBOHD directly, suggesting that QSOX1 would exert an indirect effect on the ROS-producing activity of RBOHD. During effector-triggered immunity there is not only accumulation of ROS, but also reactive nitrogen species (RNS; Bleau and Spoel, 2021), including S-nitrosoglutathione (GSNO). GSNO can react with RBOHD and S-nitrosylation

of a conserved cysteine residue leads to inactivation of RBOHD by ejection of FAD from the enzyme (Yun *et al.*, 2011). GSNOR catabolizes GSNO and thereby could provide a regulatory link to RBOHD. Thus, the authors hypothesized that QSOX1 could interact with GSNOR. Indeed, Chae *et al*. found an oxidoreductase activity-independent interaction between GSNOR and QSOX1 at the cell periphery. Both QSOX1 and GSNOR existed in a reduced state prior to pathogen application but became increasingly oxidized within hours of infection. In the *qsox1* mutant however, GSNOR was no longer oxidized and consequently not inhibited. This was reflected in the observation that GSNOR and NADPH oxidase activities were enhanced in *qsox1* mutants while SNO content was accordingly reduced.

The work of Chae *et al*. (2021) provides an excellent starting point for asking further research questions. One question concerns the temporal coordination of events. The ROS burst is a rather rapid response to pathogen infection or treatment with microbe-associated molecular patterns (MAMPs) occurring within minutes of signal perception. However, following the initial rapid ROS burst, a second peak of ROS production is typically observed within hours. With *QSOX1* transcript starting to accumulate only twelve hours after pathogen infection, it is unlikely that QSOX1 would participate in the negative regulation of the first ROS burst event. Here, it can be speculated that other post-translational mechanisms including phosphorylation and dephosphorylation events could participate in the negative regulation of RBOHD activity. However, QSOX1 could provide a safeguard mechanism that would prevent long-lasting and potentially damaging RBOHD-dependent ROS production (Figure 1) working in concert with other regulatory elements converging on RBOHD. It is also highly likely that GSNOR is not the only target of QSOX1 (Figure 1). Thus, based on the work of Chae *et al*. (2021) another interesting research direction will be to investigate, which other proteins are regulated by QSOX1 but also by the homolog QSOX2.

In summary, Chae *et al*. (2021) elegantly provide evidence for a regulatory circuit where pathogen-induced ROS production through RBOHD activates the sensor and regulator QSOX1 (Figure 1). QSOX1 subsequently deactivates GSNOR, thereby preventing it from catabolizing GSNO. The accumulating GSNO then leads to S-nitrosylation and inactivation of RBOHD. ROS and RNS are not only involved in the response to biotic stress. In the future, it will be exciting to test whether QSOX1 or QSOX2 also participate in maintaining the intricate balance of ROS and RNS in the responses to abiotic stimuli and regulation of developmental processes.

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Michael Wrzaczek

Institute of Plant Molecular Biology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic, and Organismal and Evolutionary Biology Research Programme, Viikki Plant Science Centre, Helsinki University, Finland.

Correspondence: michael.wrzaczek@umbr.cas.cz

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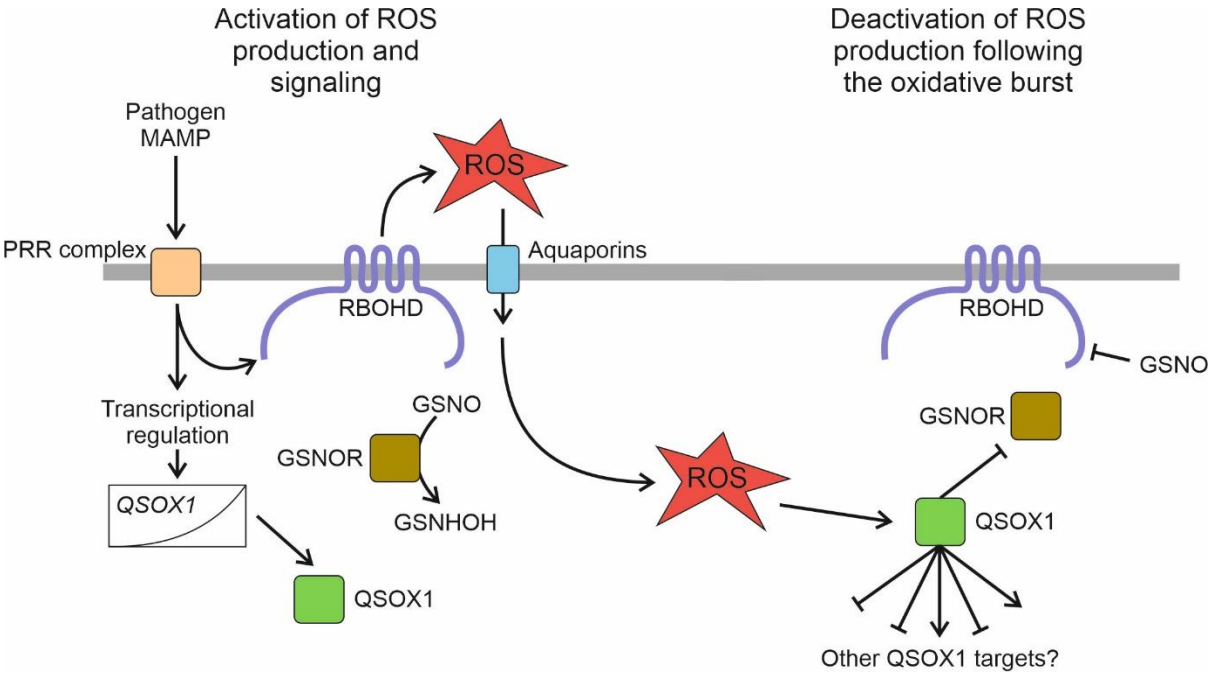

Figure 1. Schematic overview over the conceptual role of QSOX1 in the regulation of pathogen-induced ROS production.

Following perception of pathogen perception by the pattern-recognition receptor (PRR) complex, kinases phosphorylate and thereby activate RBOHD. Enhanced pathogen-triggered calcium influx contributes to the activation of RBOHD by calcium binding and activation of calcium-dependent protein kinases (CPKs). Active RBOHD produces superoxide in the apoplast which is dismutated to hydrogen peroxide (H_2O_2). H_2O_2 can enter the cytosol through aquaporins leading to ROS signaling inside the cell. GSNOR catabolizes GSNO preventing inactivation of RBOHD by S-nitrosylation. Pathogen perception also, through other signaling components, leads to transcriptional reprogramming, eventually resulting in the accumulation of *QSOX1* transcript and QSOX1 protein. QSOX1 is oxidized by elevated ROS levels in the cytosol and oxidizes and thereby inactivates GSNOR. The accumulation of GSNO then leads to S-nitrosylation and inactivation of RBOHD to prevent further ROS production in the apoplastic space.