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Stomatal regulation: Role of H_2S-induced persulfidation in ABA signaling

Hydrogen sulfide (H_2S) is an important signaling molecule in plants. It is a small gasotransmitter with high permeativity through lipid membranes. H_2S can convert the mercapto groups (Cys–SH) of the amino acid cysteine into hydropersulfide groups (–Cys–SSH). This process is called persulfidation. Post-translational modifications such as persulfidation can play an important role in protein functionality, altering protein conformation and/or activity. In plants, H_2S can be generated within guard cells by CYSTEINE-DESULFHYDRASES, such as DES1, by degradation of cysteines into H_2S, ammonia, and pyruvate (Zhang et al., 2020). H_2S was reported to take part in physiological processes, such as stress response, and in the regulation of the functions of phytohormones such as salicylic acid and abscisic acid (ABA) (Pandey and Gautam, 2020). Although the mechanism of how H_2S affects ABA signaling pathways is not well understood, two recent studies by Chen et al. (2020) and Zhou et al. (2021) shed light on this topic, reporting how H_2S-induced persulfidation regulates the stomatal ABA signaling pathway.

PERSULFIDATION OF SnRK2.6 CONNECTS ABA AND H_2S SIGNALING AT THE POST-TRANSLATIONAL LEVEL

Chen et al. (2020) identified the effect of cysteine persulfidation on ABA-mediated stomatal closure. SnRK2.6 (SUCROSE NONFERMENTING 1-RELATED PROTEIN KINASE 2.6), which is a major player in the regulation of stomatal movement, was identified as a persulfidation target. Signaling by the phytohormone ABA is of paramount importance in plants, and stomatal closure is one of the important processes governed by ABA. In the presence of ABA, the ABA receptors PYRABACTIN RESISTANCE/PYR-LIKE/REGULATORY COMPONENT OF ABA RECEPTOR (PYR/PYL/RCAR) bind to the negative regulator PP2C (protein phosphatase type 2C), thereby removing the inhibition of SnRK2.6. As a result, SnRK2.6, also known as Open Stomata 1 (OST1), is phosphorylated and activated by PYR/PYL/RCAR. Activated SnRK2.6 induces reactive oxygen species (ROS) production and Ca^{2+} influx into guard cells, thereby triggering stomatal closure (Figure 1).

This new study identified persulfidation of SnRK2.6 at two Cys residues, Cys131 and Cys137. Persulfidation resulted in elevated SnRK2.6 kinase activity and its ability to interact with ABA response element-binding factor 2 (ABF2). Treatment of wild-type SnRK2.6 with H_2S *in vitro* and *in vivo* resulted in increased phosphorylation and binding affinity toward its substrate ABF2, whereas mutation of both Cys sites resulted in loss of phosphorylation and ABF2 binding activity. Transgenic *ost1-3*/SnRK2.6^{C131SC137S} plants showed also decreased drought tolerance due to the lack of SnRK2.6-induced Ca^{2+} influx into guard cells and therefore their inability to close stomata. Interestingly, Cys137 of SnRK2.6 was described previously as an S-nitrosylation site, which inhibited kinase activity (Wang et al., 2015). This finding implies that persulfidation and nitrosylation on Cys137 affect SnRK2.6 activity in competitive and opposite manners. Taken together, the results reported by Chen et al. (2020) provide new insights into the link between H_2S and the ABA signaling pathway (Figure 1).

PERSULFIDATION OF ABI4 CONNECTS H_2S WITH ABA SIGNALING AT THE TRANSCRIPTIONAL LEVEL

Zhou et al. (2021) revealed recently another example how H_2S links with ABA signaling. Persulfidation of abscisic acid insensitive 4 (ABI4) was observed to affect ABA-mediated responses in plants. The transcription factor ABI4 regulates a variety of stimuli and their signaling pathways. It acts as inhibitor as well as activator of the expression of its downstream targets (Wind et al., 2013). ABI4 harbors several cysteines. Zhou et al. (2021) report the reversible persulfidation of ABI4 *in vivo* and *in vitro* upon ABA and NaHS treatments. Wild-type ABI4 was necessary for NaHS-induced phenotypes, like inhibition of seed germination, seedling establishment, primary root growth, and stomatal closure. Similar results were obtained with ABI4^{Cys250Ala} mutants, which performed like *abi4* knockout lines and could not rescue ABA-responsive plant phenotypes. Non-modified Cys250 was needed for ABI4 autoactivation activity, explaining the observed mutant phenotype.

Persulfidation of ABI4 also affected the expression of its downstream target MAPKKK18, which regulates the ABA-mediating MKK3-MPK1/2/7/14 MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) pathway (de Zelicourt et al., 2016). *MAPKKK18* expression is reduced in *abi4* plants or ABI4^{Cys250Ala} plants in the *abi4* background, indicating that ABI4 is an upstream regulator of MAPKKK18 (Zhou et al., 2021). Moreover, SnRK2 induces RAV1 phosphorylation and thereby inhibits ABI4 expression (Feng et al., 2014). Analysis of the ABI4 binding site revealed its high affinity toward the CACCG motif, which is present in the *MAPKKK18* promoter, and its interaction was proven *in vivo* and *in vitro*. The binding ability of ABI4 was enhanced by NaHS and ABA, while mutation of Cys250 abolished the binding and ABI4^{Cys150Ala} was unable to bind to the promoter of *MAPKKK18*. Further work also identified DES1 as an ABI4 target that also harbors a CACCG motif in its

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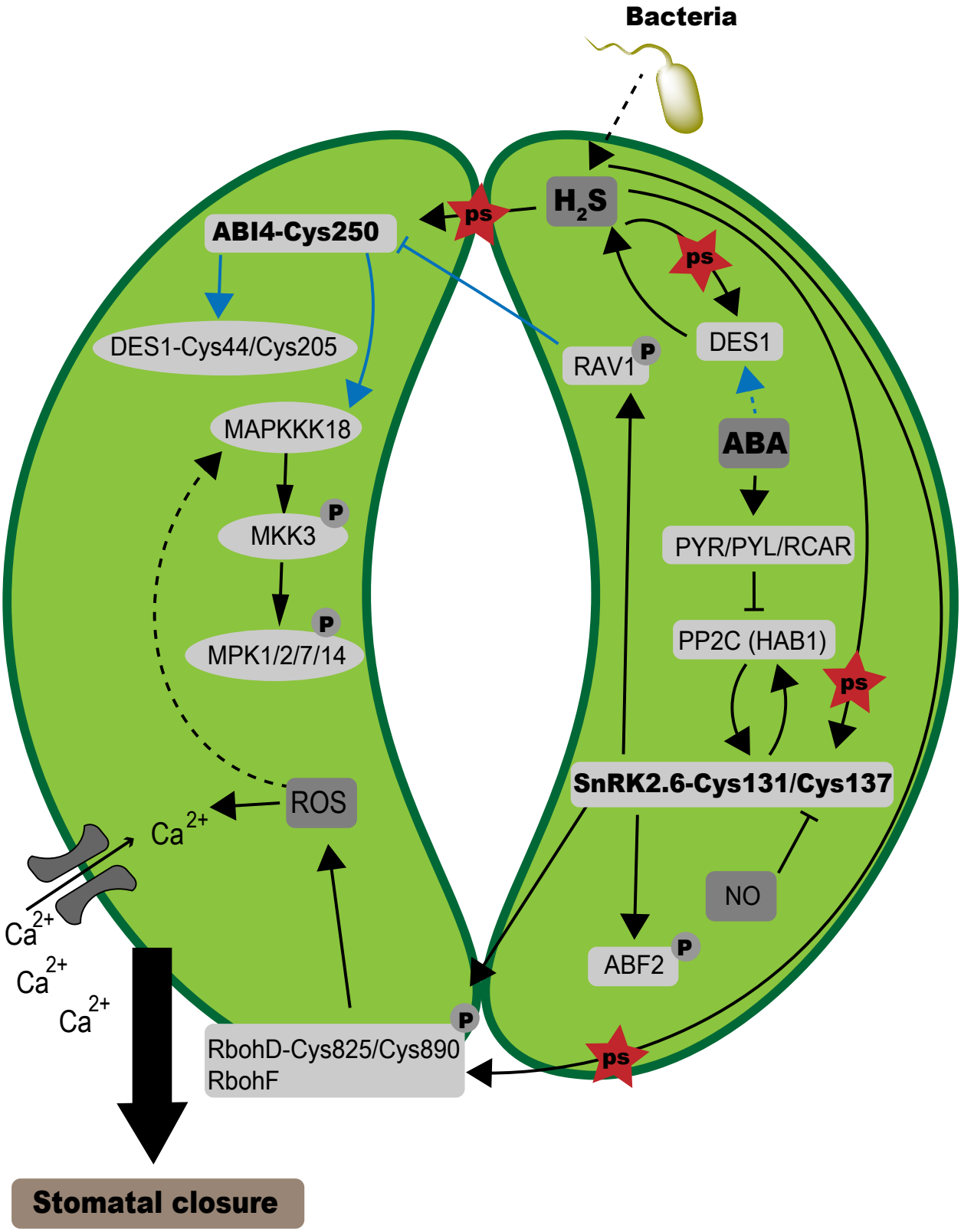

Figure 1. Model of persulfidation events in ABA signaling cascade.

Under conditions triggering ABA signaling, inhibition of SnRK2 by PP2Cs is abolished. SnRK2-induced RAV1 phosphorylation inhibits ABI4 expression and activation of the MAPKKK18 MAPK pathway. SnRK2.6 also activates RbohD/F to enhance ROS levels, leading to Ca^{2+} influx. Simultaneously, DES1-regulated H_2S production affects stomatal ABA signaling via activation of SnRK2.6, RbOHD/F, ABI4, and DES1 itself. Red stars with "ps" represent persulfidation; solid lines represent direct effects; dotted lines represent indirect effects; arrow-headed lines represent promoting effects; bar-headed lines represent inhibiting effects; blue lines represent transcriptional effects; black lines indicate post-translational effects.

promoter and was observed to be bound by ABI4. ABI4 and DES1 seem to be subject to a mutual regulation, in which DES1 expression is regulated by ABI4 and ABA-induced ABI4 expression is DES1 dependent. *Des1* mutants showed impaired enhancement of *ABI4* transcripts after ABA treatment, whereas NaHS induced ABI4 expression. As an upstream factor, DES1 not only affected ABI4 expression but also induced ABA-triggered persulfidation of ABI4. To summarize, these results reveal an interesting regulation of ABI4 activity by DES1-regulated persulfidation on Cys250 and its effect on downstream transcription targets (Figure 1).

H_2S-INDUCED PERSULFIDATION IN THE ABA SIGNALING PATHWAY AND POSSIBLE INTERPLAY WITH OTHER FACTORS

Cysteines are not only persulfidation targets. They are also subjected to oxidation by ROS. In fact, oxidation of Cys–thiol groups into sulfenic acid by H_2O_2 is a requirement for H_2S-induced persulfidation (Zhang et al., 2021). The NADPH oxidases RESPIRATORY BURST OXIDASE (RbohF, RbohD) produce ROS upon ABA signaling, inducing stomatal closure (Postiglione and Muday, 2020). Evidence was reported that ROS can affect ABA signaling in a regulatory feedback mechanism. HAB1, RbohD, and DES1 are examples of ABA signaling molecules controlled by ROS on Cys side chains, oxidation of which induces ABA desensitivity, implying a feedback inhibition (Sridharamurthy et al., 2014; Shen et al., 2020). These findings emphasize the strong correlation between ROS and H_2S in ABA signaling. It remains to be elucidated how ROS and H_2S interact and compete for cysteines, thereby regulating ABA signaling.

So far, research has been focused on the molecular mechanisms underlying the responses to plant-derived H_2S. However, H_2S is also produced by plant-associated bacteria, such as the recently reported beneficial bacterial *Cronobacter muytjensii* strain JZ38, which secretes H_2S and induces plant resistance to abiotic and biotic stress (Eida et al., 2020). These findings raise the possibility that H_2S of both plant and microbial origin might be involved in regulating plant signaling pathways, promising further insights into plant stress signaling.

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