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Say "NO" to ABA signaling in guard cells by S-nitrosylation of OST1

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Nitric oxide (NO), a gaseous compound, plays important roles in plant immunity, abiotic stress response and plant development [1]. In plants, NO is synthesized through either oxidative or reductive route that is dependent on the nitrate reductases (NADH) 1 (NIA1) and NIA2. NO bioactivity is realized through redox-based post-translational protein modifications, such as S-nitrosylation, which involves the addition of an NO group to a protein cysteine thiol to generate an S-nitrosothiol (SNO). Protein S-nitrosylation is a major NO-mediated modification, and influences protein-protein interaction, protein localization and protein activity. As a second messenger, NO participates in signaling pathways of multiple plant hormones, such as salicylic acid (SA), abscisic acid (ABA) and cytokinin. ABA signaling pathways are extensively studied and are involved in many important biological processes, such as seed germination, plant growth and plant response to abiotic stresses [2]. In the presence of ABA, binding of ABA to receptors in the PYR1 (pyrabactin resistance 1)/PYL (Pyr1-Like)/RCAR (regulatory component of ABA receptor) protein family causes their conformational change. The activated receptors bind to and inhibit PP2Cs (clade A protein phosphatase 2Cs) to release SnRK2s (SNF1-related kinase 2s) from inhibition by the PP2Cs. Then downstream factors in ABA signaling are activated by protein phosphorylation catalyzed by the SnRK2s.

Under water stresses, ABA accumulates and induces stomatal closure, thus reducing transpirational water loss. It has been known that NO generation can be triggered by ABA. Previous studies showed that application of exogenous NO promotes ABA-mediated stomatal closure, suggesting a positive role of NO in ABA signaling [1]. However, it has also been found that *nia1nia2noa1* triple mutant with deficiency in NO generation is hypersensitive to ABA in stomatal closure, indicating a negative role of NO in ABA signaling [3]. To date, how NO functions in ABA-mediated stomatal closure is still not well understood.

A recent study by Wang et al. revealed that endogenous NO functions as a negative regulator of ABA signaling in guard cells through S-nitrosylation of OST1 (open stomata 1) [4]. OST1, also known as SnRK2.6, is an SnRK2 protein kinase preferentially expressed in guard cells, and is critical for ABA-mediated stomatal closure. Wang et al. showed that the NO donor GSNO (S-nitrosoglutathione) induced S-nitrosylation of OST1 and inhibited OST1 kinase activity *in vitro*. This inhibitory effect of GSNO on OST1 activity was abolished only by Cys137Ser mutation but not by other tested Cys-to-Ser mutations *in vitro*, consistent with mass spectrometry data showing that cysteine 137 is the S-nitrosylation target of GSNO. In addition, OST1 kinase activity was blocked by a Trp substitution at 137 that may mimic S-nitrosylation *in vitro*. Moreover, GSNO induced S-nitrosylation of OST1/SnRK2.6-GFP protein in transgen-

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ic plants in the *ost1-3* mutant background, and this induced S-nitrosylation was abolished by the *SnRK2.6*^{C137S}*-GFP* mutation. *SnRK2.6*^{C137W}*-GFP* transgenic plants did not exhibit any SnRK2.6 kinase activity. Together, these data suggest that NO induces S-nitrosylation of OST1 at Cys-137, thereby abolishing OST1 kinase activity. It has been known that dysfunction of OST1 in *ost1* mutant abolishes ABA induced stomatal closure and causes increased sensitivity to drought stress [5]. Consistent with biochemical data showing the importance of S-nitrosylation at C137 for OST1 activity, both water loss and stomatal closure phenotypes of *ost1-3* were rescued only by wild type SnRK2.6 but not by SnRK2.6^{C137W}.

OST1 is a positive regulator in ABA signaling in guard cells, and NO-mediated S-nitrosylation blocks OST1 kinase activity. In addition, ABA treatment induces NO generation, and Wang et al. found that in contrast to the very fast activation of OST1 by ABA, S-nitrosylation of OST1 *in vivo* occurs relatively late after ABA treatment, suggesting that NO plays a role in the negative feedback regulation of ABA signaling. The authors further investigated NO function using the NO overaccumulation mutant *gsnor1* (*GSNO reductase 1*)*-3*. Mutation of *GSNOR* caused endogenous NO overaccumulation and increased the level of S-nitrosylated OST1 compared to that in wild type plants. However, ABA treatment diminished the difference of S-nitrosylated OST1 between wild type and *gsnor1-3*. Similar to the *ost1* mutant, *gsnor1* mutants, *gsnor1-3*, *gsnor1-4* and *hot5-4*, were less sensitive to ABA induced stomatal closure, and showed faster transpirational water loss than the wild type. The stomatal closure and water loss phenotypes of *gsnor1* mutant plans were rescued by SnRK2.6^{C173S} but not by wild type SnRK2.6. These results suggest that NO overaccumulated in *gsnor1* mutants inhibits OST1 kinase activity by S-nitrosylation at C137, leading to insensitivity to ABA-induced stomatal closure and uncontrolled transpirational water loss.

Wang et al. revealed that cysteine 137 of OST1 is conserved in SNF1/AMPK-related kinases and glycogen synthase kinase 3 (GSK)/SKs in eukaryotes. Four of these kinases, HsBRSK1, AtBIN2, HsGSK3b and ScHsl1p were successfully expressed in *E. coli*, and their kinase activities were inhibited by GSNO. The authors further confirmed that S-nitrosylation of the conserved cysteine in AtBIN2 and HsGSK3b blocks their kinase activities, suggesting that NO-mediated S-nitrosylation at a cysteine near the catalytic center of protein kinases might be a general regulatory mechanism for kinase activities. SnRK2.2 and 2.3 are quite similar to SnRK2.6 in sequences and structures. In contrast to SnRK2.6 that is preferentially expressed in guard cells, SnRK2.2 and 2.3 are mainly expressed in seeds and seedlings, and function in ABA-mediated inhibition of seed germination and seedling development. Application of NO antagonizes the inhibition of ABA on seed germination and seedling development, suggesting a negative role of NO in ABA signaling during these developmental processes. It will be interesting to test whether SnRK2.2 and 2.3 can be S-nitrosylated by NO, and thus removing the inhibitory effects of ABA on seeds germination and seedling development.

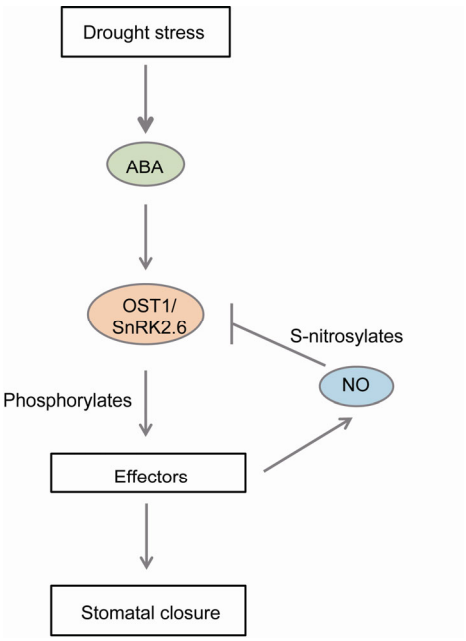

Figure 1 (color online) Model showing that NO negatively regulates ABA signaling through S-nitrosylation of OST1.

In summary, Wang et al. discovered that NO plays a negative role in ABA-mediated stomatal closure through S-nitrosylation of OST1 and S-nitrosylation may represent a general regulatory modification for kinase activities.

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