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Article Addendum

Nitric oxide functions in both methyl jasmonate signaling and abscisic acid signaling in Arabidopsis guard cells

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Intracellular components in methyl jasmonate (MeJA) signaling remain largely unknown, to compare those in well-understood abscisic acid (ABA) signaling. We have reported that nitric oxide (NO) is a signaling component in MeJA-induced stomatal closure, as well as ABA-induced stomatal closure in the previous study. To gain further information about the role of NO in the guard cell signaling, NO production was examined in an ABA- and MeJA-insensitive Arabidopsis mutant, *rcn1*. Neither MeJA nor ABA induced NO production in *rcn1* guard cells. Our data suggest that NO functions downstream of the branch point of MeJA and ABA signaling in Arabidopsis guard cells.

Stomatal pores that are formed by pairs of guard cells respond to various environmental stimuli including plant hormones. Some signal components commonly function in MeJA- and ABA-induced stomatal closing signals,[1] such as cytosolic alkalization, ROS generation and cytosolic free calcium ion elevation. Recently, we demonstrated that NO functions in MeJA signaling, as well as ABA signaling in guard cells.[2]

NO production by nitric oxide synthase (NOS) and nitrate reductase (NR) plays important roles in physiological processes in plants.[3,4] It has been shown that NO functions downstream of ROS production in ABA signaling in guard cells.[5] NO mediates elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$), inactivation of inward-rectifying K^+ channels and activation of S-type anion channels,[6] which are known to be key factors in MeJA- and ABA-induced stomatal closure.[2,7-9]

It has been reported that ROS was not induced by MeJA and ABA in the MeJA- and ABA-insensitive mutant, *rcn1* in which the regulatory subunit A of protein phosphatase 2A, RCN1, is impaired.[7,10] We examined NO production induced by MeJA and ABA in *rcn1* guard cells (Fig. 1). NO production by MeJA and ABA was impaired in *rcn1* mutant (p = 0.87 and 0.25 for MeJA and ABA, respectively) in contrast to wild type. On the other hand, the NO donor, SNP induced stomatal closure both in wild type and *rcn1* mutant (data not shown). These results are consistent with our previous results, i.e., NO is involved in both MeJA- and ABA-induced stomatal closure and functions downstream of the branching point of MeJA and ABA signaling in Arabidopsis guard cells.[7] Our finding implies that protein phosphatase 2A might positively regulate NO levels in guard cells (Fig. 2).

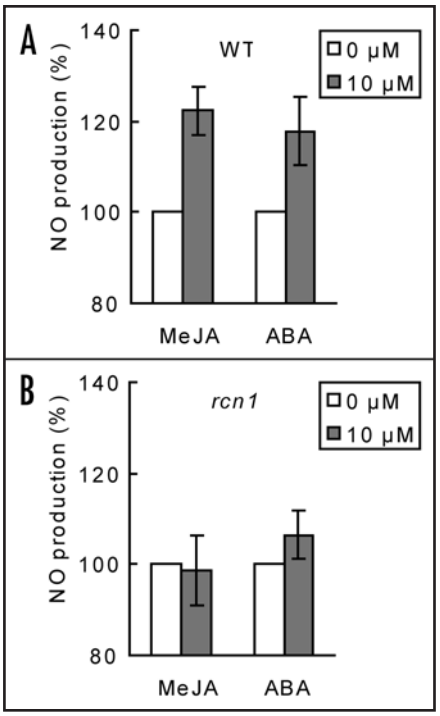

Figure 1. Impairment of MeJA- and ABA-induced NO production in rcn1 guard cells. (A) Effects of MeJA (n = 10) and ABA (n = 9) on NO production in wild-type guard cells. (B) Effects of MeJA (n = 7) and ABA (n = 7) on NO production in *rcn1* guard cells. The vertical scale represents the percentage of diaminofluorescein-2 diacetate (DAF-2 DA) fluorescent levels when fluorescent intensities of MeJA- or ABA-treated cells are normalized to control value taken as 100% for each experiment. Each datum was obtained from at least 30 guard cells. Error bars represent standard errors. Significance of differences between data sets was assessed by Student's t-test analysis in this paper. We regarded differences at the level of $p < 0.05$ as significant.

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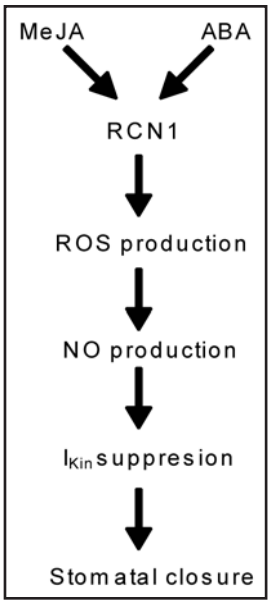

Figure 2. A model of signal interaction in MeJA-induced and ABA-induced stomatal closure. Neither MeJA nor ABA induces ROS production, NO production, I_{Kin} and stomatal closure in *rcn1* mutant. These results suggest that NO functions downstream of the branch point of MeJA signaling and ABA signaling in Arabidopsis guard cells.

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