REVIEW

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Methyl jasmonate signaling and signal crosstalk between methyl jasmonate and abscisic acid in guard cells

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Plants tightly control stomatal aperture in response to various environmental changes. A drought-inducible phytohormone, abscisic acid (ABA), triggers stomatal closure and ABA signaling pathway in guard cells has been well studied. Similar to ABA, methyl jasmonate (MeJA) induces stomatal closure in various plant species but MeJA signaling pathway is still far from clear. Recently we found that Arabidopsis calcium dependent protein kinase CPK6 functions as a positive regulator in guard cell MeJA signaling and provided new insights into cytosolic Ca^{2+}-dependent MeJA signaling. Here we discuss the MeJA signaling and also signal crosstalk between MeJA and ABA pathways in guard cells.

Stomata, which are formed by pairs of specialized cells called guard cells, control gas exchanges and transpirational water loss. Guard cells can shrink and swell in response to various physiological stimuli, resulting in stomatal closing and opening.[1,2] To optimize growth under various environmental conditions, plants have developed fine-tuned signal pathway in guard cells. Abscisic acid (ABA) is synthesized under drought stress and induces stomatal closure to reduce transpirational water loss.[2] ABA signal transduction in guard cells has been widely studied. ABA induces increases of various second messengers such as cytosolic Ca^{2+}, reactive oxygen species (ROS) and nitric oxide (NO) in guard cells. These early signal components finally evoke ion efflux through plasma membrane ion channels, resulting in reduction of guard cell turgor pressure.

Jasmonates are plant hormones synthesized via the octadecanoid pathway and regulate various physiological processes in plants such as pollen maturation, tendril coiling, senescence and responses to wounding and pathogen attacks.[3] Similar to ABA, jasmonates also trigger stomatal closure and the response is conserved among various plant species including *Arabidopsis thaliana*,[4] *Hordeum vulgare*,[5] *Commelina benghalensis*,[6] *Vicia faba*,[7] *Nicotiana glauca*,[8] *Paphiopedilum Supersuk*[9] and *Paphiopedilum tonsum*.[9] A volatile methyl ester of jasmonic acid (JA), methy jasmonate (MeJA), has been widely used for studying jasmonate signaling pathway. To date, pharmacological and reverse genetic approaches have revealed many important signal components involved in MeJA-induced stomatal closure and suggest a signal crosstalk between MeJA and ABA in guard cells. In this review, we mainly focus on the three important second messengers, ROS, NO and cytosolic Ca^{2+} and discuss recent advance about MeJA signaling and signal interaction between MeJA and ABA in guard cells.

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Roles of ROS and NO in Guard Cell MeJA Signaling

It has been shown that MeJA evokes ROS production in guard cells. MeJA-induced stomatal closure and ROS production are inhibited by an NAD(P)H oxidase inhibitor, diphenylene iodonium.[4,10] Suhita et al.[4] found that gene disruption of two Arabidopsis NAD(P)H oxidases, *AtrbohD* and *AtrbohF* causes impairment of MeJA-induced stomatal closure and ROS production. These findings indicate that the two NAD(P)H oxidases, AtrbohD and AtrbohF are major ROS sources in guard cell MeJA signaling. Beside NAD(P)H oxidases, other ROS producing enzymes also play important roles in various plant responses and some of them (e.g., cell wall bound-peroxidase and copper amine oxidase) have been shown to regulate stomatal movement.[11-13] However, roles of these ROS sources other than NAD(P)H oxidases in guard cell MeJA signaling have not been observed.

MeJA also elicits NO production for induction of stomatal closure.[10,14] In guard cells, NO stimulates Ca^{2+} release from intracellular stores.[15,16] It has been suggested that NO is generated by nitrate reductase (NR), NO synthase (NOS) and non-enzymatic system in plant cells.[17] Both NR and NOS are involved in NO production during stomatal closure induced by ABA.[18,19] A mammalian NOS inhibitor, N^G-nitro-L-Arg-methyl eater (L-NAME) represses JA-induced NO production and stomatal closure in *Vicia faba* guard cells but an NR inhibitor does not,[7] indicating that NOS-like enzyme is required for jasmonate signaling in guard cells but NR is not required.

It was shown that MeJA fails to induce production of ROS and NO in the Arabidopsis *rcn1* mutant, which has a mutation

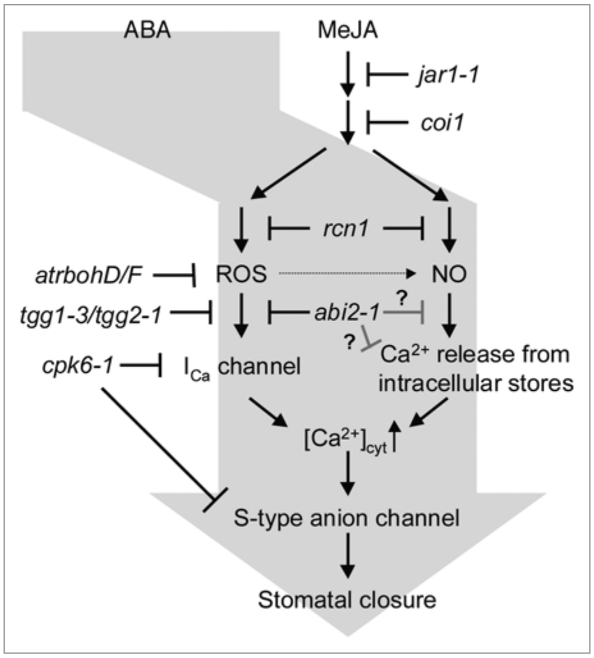

Figure 1. Proposed MeJA signaling pathway and signal crosstalk between MeJA and ABA in Arabidopsis guard cells. MeJA induces ROS and NO production in guard cells. RCN1-regulating PP2As are involved in this step. The major ROS sourses are NAD(P)H oxidases AtrbohD/F. NOS activity seems to be important for MeJA-induced NO production, but genes encoding NOS have not been indentified in plants. ROS and NO evoke guard cell $[Ca^{2+}]_{cyt}$ elevation by Ca^{2+} influx from apoplast and from intracellular stores, respectively. I_{Ca} channels mediate Ca^{2+} influx from apoplast. Elevated $[Ca^{2+}]_{cyt}$ is sensed by CDPKs including CPK6 and finally activates S-type anion channels. The *abi2-1* mutation disrupts ROS-mediated I_{Ca} channel activation. The *abi2-1* mutation also disrupts NO-dependent signal pathway, but the details are still unclear. Two myrosinases TGG1 and TGG2 are also involved in the signal crosstalk.

in a gene encoding a regulatory A subunit of protein phosphatase type 2A (PP2A).[14,20] This finding suggests that RCN1-regulating PP2As function upstream of ROS and NO production in guard cell MeJA signaling (**Fig. 1**). Changes of cytosolic pH (pH_{cyt}) in guard cells are also considered as an important step during MeJA-induced stomatal closure.[4,9] It has been suggested that MeJA evokes pH_{cyt} rise (alkalization) in guard cells and the alkalization is required for MeJA-induced ROS and NO production.[4,21] However, contrary to these reports, Islam et al.[22] suggested that pH_{cyt} changes function downstream of ROS production in guard cell signaling.

Roles of $[Ca^{2+}]_{cyt}$ in Guard Cell MeJA Signaling

Importance of $[Ca^{2+}]_{cyt}$ changes in guard cells during stomatal closure has been widely studied.[23] Similar to ABA and elicitors,[24-26] MeJA activates non selective Ca^{2+} permeable (I_{Ca}) channels of guard cell plasma membrane and evokes elevation of guard cell $[Ca^{2+}]_{cyt}$.[10,27] MeJA-induced stomatal closure is abolished by inhibition of plasma membrane Ca^{2+} channels or chelation of extracellular Ca^{2+},[8] suggesting that $[Ca^{2+}]_{cyt}$ elevation by Ca^{2+} influx across plasma membrane is required for MeJA-induced stomatal closure. Recently we identified an Arabidopsis calcium dependent protein kinase (CDPK), *CPK6*, as a positive regulator in guard cell MeJA signaling.[27] CDPKs function as important $[Ca^{2+}]_{cyt}$ sensors in many aspects of plant physiological processes.[28] CPK6 is also involved in guard cell ABA signaling and shares functional redundancy with CPK3.[29] In the *CPK6* gene disruption mutant, MeJA fails to activate I_{Ca} channels and induce $[Ca^{2+}]_{cyt}$ elevation.[27] CPK6 is also required for MeJA activation of slow-type (S-type) anion channels.[27] The new finding suggests that guard cell MeJA signaling is strongly dependent on $[Ca^{2+}]_{cyt}$ and a $[Ca^{2+}]_{cyt}$ sensor CPK6 is a central regulator of the signaling. Potato homologs of the Arabidopsis CPK6, StCDPK5 and StCDPK6 were shown to regulate NAD(P)H oxidase activity via direct phosphorylation.[30] However, in the *cpk6* mutant and *cpk3cpk6* double mutant, ABA- and MeJA-mediated ROS production is not reduced.

Signal Crosstalk between MeJA and ABA in Guard Cells

Signal crosstalk among different phytohormones is involved in many physiological responses and allows plants to respond adequately to environmental changes.[31] In guard cells, there is a signal crosstalk between MeJA and ABA. Reverse genetic approaches using Arabidopsis mutants have found out many signaling components involved in the crosstalk (**Fig. 1**). A regulatory A subunit of protein phosphatase type 2A (PP2A), RCN1 is involved in both MeJA and ABA signaling upstream of ROS and NO production.[14,20] Myrosinases, TGG1 and TGG2, function downstream of ROS production in guard cell MeJA and ABA signaling.[32] CPK6 is required for activation of I_{Ca} channels and S-type anion channels in both MeJA and ABA signaling.[27] In the ABA insensitive *abi2-1* mutant, which has a dominant negative mutation in *ABI2* encoding a protein phosphatase 2C (PP2C), MeJA fails to induce stomatal closure but production of ROS and NO induced by MeJA and ABA is still observed.[10,19,33] Recent findings revealed that PP2Cs participate in ABA core components together with ABA receptor PYR/PYL/RCAR.[34,35] Together, MeJA might affect regulation of the ABA receptor complexes.

Conclusions and Outlook

Here we reviewed MeJA signaling and signal crosstalk between MeJA and ABA in guard cells. MeJA employs ROS and NO to induce $[Ca^{2+}]_{cyt}$ elevation in guard cells. The elevated $[Ca^{2+}]_{cyt}$ is sensed by CPK6, resulting in activation of S-type anion channels. MeJA signaling is integrated into early ABA signaling and might affect ABA receptor complexes to regulate downstream common signal components. MeJA-induced stomatal closure has been observed in various plant species, indicating that it is one of the important plant responses. To date, however, physiological role of MeJA-induced stomatal closure is not so clear in comparison to that of ABA-induced stomatal closure. Moreover, some studies report that MeJA, other jasmonates and a phytotoxin coronatine, which mimics jasmonoyl-isoleucine and activates jasmonate signaling, induce stomatal opening instead of stomatal closure.[36-38] One of the reasons for the inconsistency could be difference of plant growth condition.[39] There is no doubt that MeJA and other jasmonates participate in regulation of stomatal movement accompanied with other phytohormones. However the signal pathway still remains a puzzle with many missing pieces.

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