DOCTORAL THESIS

Mitogen activated protein kinase cascades mediate the regulation of antioxidant enzymes under abiotic stresses in arabidopsis

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Mitogen Activated Protein Kinase Cascades Mediate the Regulation of Antioxidant Enzymes under Abiotic Stresses in *Arabidopsis*

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Abstract

Catalase and H_2O_2 play important roles in plant adaptive responses to biotic and abiotic stresses, whereas little is known about their upstream signaling cascades leading to the gene expression of catalase and H_2O_2 production. We here report that catalase gene family regulated by AtMKK1, an *Arabidopsis* MAPK kinase, responded differently to ABA, drought and salt stress. *CAT1* expression was sensitive to ABA, drought and salt stresses, and at the mean time, AtMKK1 activity was also sensitively activated by all these stresses. Both *CAT1* expression and AtMKK1 activity could be highly inhibited by MAPK signaling inhibitor PD98059, suggesting that AtMKK1 might be involved in the stress-induced *CAT1* expression. The *AtMKK1* mutant, *mkk1*, totally blocked the stressed-induced *CAT1* expression, and interestingly, the stress-induced H_2O_2 production was also blocked. Over-expression of *AtMKK1* significantly promoted the stress-induced *CAT1* expression, and also promoted H_2O_2 production. These results conclusively indicate that stress-induced *CAT1* expression is mediated by AtMKK1, and further more, the triggering of H_2O_2 production might be involved in the process, as further proved by the observation that *CAT1* expression was sensitively induced by applied H_2O_2. Surprisingly, the signaling mechanisms for the stress-induced gene expression of *CAT2* and *CAT3* were observed to be rather different from *CAT1*. Except for drought stress, *CAT2* and *CAT3* expressions were not sensitive to ABA or salt stress, and AtMKK1 was not proved to be involved in the drought-induced *CAT2*, or *CAT3* expressions. Further studies showed that stomatal movement was much less sensitive to ABA in *mkk1*, and over-expression of *AtMKK1* in *Arabidopsis* increased the plant resistance to drought or salt stress, which further demonstrate that AtMKK1 is a crucial mediator in plant stress signal transduction.

In response to ABA treatment, *CAT1* expression was remarkably induced, and moreover, the *CAT1* expression and H_2O_2 production were both totally arrested in *mkk1*. Over-expression of *AtMKK1* significantly enhanced the ABA-induced *CAT1* expression and H_2O_2 production. Further studies showed that the ABA-induced *CAT1* expression and H_2O_2 production also were blocked in *mpk6* mutant plants, and by contrast, promoted in *AtMPK6* overexpressing plants. Moreover, the AtMPK6 kinase activity was observed to be activated by ABA in an AtMKK1-dependent manner. These data strongly suggest that the ABA-induced *CAT1* expression and H_2O_2 production are mediated by AtMKK1 via AtMPK6-coupled signaling. Further investigation showed that, compared to wild type plants, *mkk1* exhibited a much less sensitivity in germination to ABA, and a decreased tolerance to drought, whereas over-expression of *AtMKK1* exhibited a hyper-sensitivity to ABA in germination and an increased resistance to drought, suggesting that AtMKK1 is a key mediator in the ABA signaling cascades.

Superoxide dismutases (SODs) play important roles in plant adaptive response to biotic and abiotic stresses but little is known about their upstream signaling cascades leading to their gene expressions. We report that salt-induced gene expression of the

iron superoxide dismutases, *FSD2* and *FSD3*, were mediated by MKK5, one of *Arabidopsis* MAPK kinases. *FSD2* and *FSD3* expressions were remarkably increased in response to salt treatment but were blocked in *MKK5* null plants, *mkk5*. Using the transient expression assay in protoplasts, we found that MKK5 was also activated in response to salt stress. The over-expression of MKK5 in wild type plants enhanced the plant salt tolerance. In contrast, *mkk5* null mutant plants exhibited hypersensitivity to salt stress and in germination on salt-containing media. These data demonstrate that MKK5 is a key signal transducer of salt stress in *Arabidopsis*. Moreover, we identified that MPK6 was also involved in the MKK5-mediated iron superoxide dismutases (FSD) signaling pathway in salt stress. The kinase activity of MPK6 was totally arrested in MKK5 null plant-*mkk5*, whereas the activity of MPK3 was only partially blocked. MKK5 interacted with the AtMEKK1 protein that was also involved in the salt-induced FSD signaling pathway. These data strongly suggest that salt-induced FSD2 and FSD3 expressions are mediated by AtMEKK1 via MKK5-MPK6-coupled signaling. It is suggested that there is a complete MAP kinase cascade (MEKK1, MKK5 and MPK6) that mediates the salt-induced iron superoxide dismutases.

MKK5 is known involved in the oxidative stress-induced copper/zinc superoxide dismutases (Cu/Zn SODs) signaling pathway. Cu/Zn SODs transcripts can be induced in response to oxidative stress, but the regulatory mechanism of the induction is unknown. We found that *MKK5* expression was upregulated by oxidative stresses and this upregulation was important for *CSD1* and *CSD2* mRNA accumulation and oxidative stress tolerance. This should be an important role of MKK5 in oxidative stress-induced Cu/Zn SODs signaling. Our results showed that CSD1 and CSD2 expression was arrested in the MKK5 mutant, *mkk5*. Further more, transgenic *Arabidopsis* plants overexpressing *MKK5* accumulated more CSD1 and CSD2 mRNA than wild type plants and were consequently much more tolerant to high light, heavy metals and other oxidative stresses; whereas, the mutants of *mkk5*, *csd1* and *csd2* were more sensitive than wild type plants in response to oxidative stress. Contrast with the mutants of *mkk5*, *csd1* and *csd2*, double mutant *mkk5 csd1* was the most sensitive in response to the high light, heavy metal and other oxidative stresses. Results suggest that MKK5-guided upgrade of CSD1 and CSD2 in transgenic plants is an effective new approach to improve plant productivity under stress conditions.

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