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Article Addendum

MEK1/2 and p38-like MAP kinase successively mediate H_2O_2 signaling in *Vicia* guard cell

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Abbreviations: ROS, reactive oxygen species; ABA, abscisic acid; MAP kinase, mitogen-activated protein kinase; MEK, MAP kinase kinase; SA, salicylic acid

Key words: H_2O_2 signaling, ABA, p38-like MAP kinase, MEK1/2, guard cell

As a second messenger, H_2O_2 generation and signal transduction is subtly controlled and involves various signal elements, among which are the members of MAP kinase family. The increasing evidences indicate that both MEK1/2 and p38-like MAP protein kinase mediate ABA-induced H_2O_2 signaling in plant cells. Here we analyze the mechanisms of similarity and difference between MEK1/2 and p38-like MAP protein kinase in mediating ABA-induced H_2O_2 generation, inhibition of inward K^+ currents, and stomatal closure. These data suggest that activation of MEK1/2 is prior to p38-like protein kinase in *Vicia* guard cells.

An increasing number of literatures elucidate that reactive oxygen species (ROS), especially H_2O_2, is essential to plant growth and development in response to stresses,[1-4] and involves activation of various signaling events, among which are the MAP kinase cascades.[1-3,5] Typically, activation of MEK1/2 mediates NADPH oxidase-dependent ROS generation in response to stresses,[4,6-8] and the facts that MEK1/2 inhibits the expression and activation of antioxidant enzymes reveal how PD98059, the specific inhibitor of MEK1/2, abolishes abscisic acid (ABA)-induced H_2O_2 generation.[6,8,9] It has been indicated that PD98059 does not to intervene on salicylic acid (SA)-stimulated H_2O_2 signaling regardless of SA mimicking ABA in regulating stomatal closure.[2,6,8,10] Generally, activation of MEK1/2 promotes ABA-induced stomatal closure by elevating H_2O_2 generation in conjunction with inactivating anti-oxidases.

Moreover, activation of plant p38-like protein kinase, the putative counterpart of yeast or mammalian p38 MAP kinase, has been reported to participate in various stress responses and ROS signaling. It has been well documented that p38 MAP kinase is involved in stress-triggered ROS signaling in yeast or mammalian cells.[11-13] Similar to those of yeast and mammals, many studies showed the activation of p38-like protein kinase in response to stresses in various plants, including *Arabidopsis thaliana*,[14-16] *Pisum sativum*,[17] *Medicago sativa*[18] and tobacco.[19] The specific p38 kinase inhibitor SB203580 was found to modulate physiological processes in plant tissues or cells, such as wheat root cells,[20] tobacco tissue[21] and suspension-cultured *Oryza sativa* cells.[22] Recently, we investigate how activation of p38-like MAP kinase is involved in ABA-induced H_2O_2 signaling in guard cells. Our results show that SB203580 blocks ABA-induced stomatal closure by inhibiting ABA-induced H_2O_2 generation and decreasing K^+ influx across the plasma membrane of *Vicia* guard cells, contrasting greatly with its analog SB202474, which has no effect on these events.[23,24] This suggests that ABA integrate activation of p38-like MAP kinase and H_2O_2 signaling to regulate stomatal behavior. In conjunction with SB203580 mimicking PD98059 not to mediate SA-induced H_2O_2 signaling,[23,24] these results generally reveal that the activation of p38-like MAP kinase and MEK1/2 is similar in guard cells.

On the other hand, activation of p38-like MAP kinase[23,24] is not always identical to that of MEK1/2[8,25] in ABA-induced H_2O_2 signaling of *Vicia* guard cells. For example, H_2O_2- and ABA-induced stomatal closure was partially reversed by SB203580. The maximum inhibition of both regent-induced stomatal closure were observed at 2 h after treatment with SB203580, under which conditions the stomatal apertures were 89% and 70% of the control values, respectively. By contrast, when PD98059 was applied together with ABA or H_2O_2, the effects of both ABA- and H_2O_2-induced stomatal closure were completely abolished (Fig. 1). These data imply that the two members of MAP kinase family are efficient in H_2O_2-stimulated stomatal closure, but p38-like MAP kinase is less susceptive than MEK1/2 to ABA stimuli.

It has been reported that ABA or NaCl activate p38 MAP kinase in the chloronema cells of the moss *Funaria hygrometrica* in 2~10 min.[26] Similar to this, SB203580 improves H_2O_2-inhibited inward K^+ currents after 4 min and leads it to the control level (100%) during the following 8 min (Fig. 2). However, the activation of p38-like MAP kinase in response to ABA need more time, and

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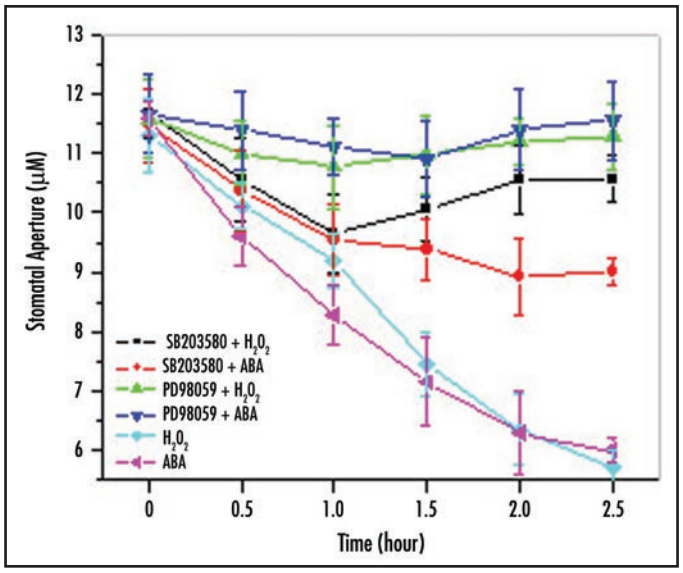

Figure 1. Effects of SB203580 and PD98059 on ABA- and H_2O_2-induced stomatal closure. The experimental procedure and data analysis are according to the previous publication.[8,23,24]

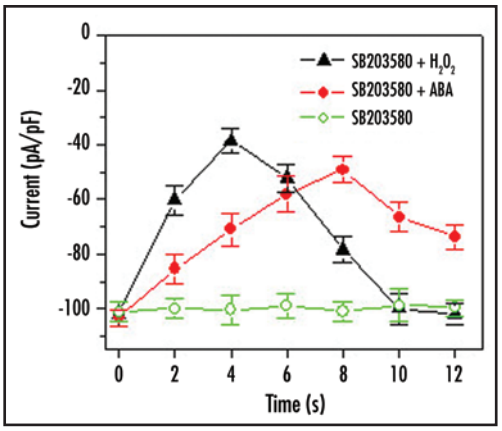

Figure 2. Effect of SB203580 on ABA- and H_2O_2-inhibited inward K^+ currents. The experimental procedure and data analysis are according to the previous publication.[24] SB203580 directs ABA- and H_2O_2-inactivated inward K^+ currents across plasma membrane of *Vicia* guard cells. Here the inward K^+ currents value is stimulated by -190 mV voltage.

only recovered to 75% of the control at 8 min of treatment (Fig. 2). These results suggest that control of H_2O_2 signaling is required for the various protein kinases including p38-like MAP kinase and MEK1/2 in guard cells,[1,2,8,23,24] and the ABA and H_2O_2 pathways diverge further downstream in their actions on the K^+ channels and, thus, on stomatal control. Other differences in action between ABA and H_2O_2 are known. For example, Köhler et al. (2001) reported that H_2O_2 inhibited the K^+ outward rectifier in guard cells shows that H_2O_2 does not mimic ABA action on guard cell ion channels as it acts on the K^+ outward rectifier in a manner entirely contrary to that of ABA.[27]

Based on the similarity and difference between PD98059 and SB203580 in interceding ABA and H_2O_2 signaling, we speculate the possible mechanism is that the member of MAP kinase family specially regulate signal event in ABA-triggered ROS signaling network,[1-4] and the signaling model as follows (Fig. 3).

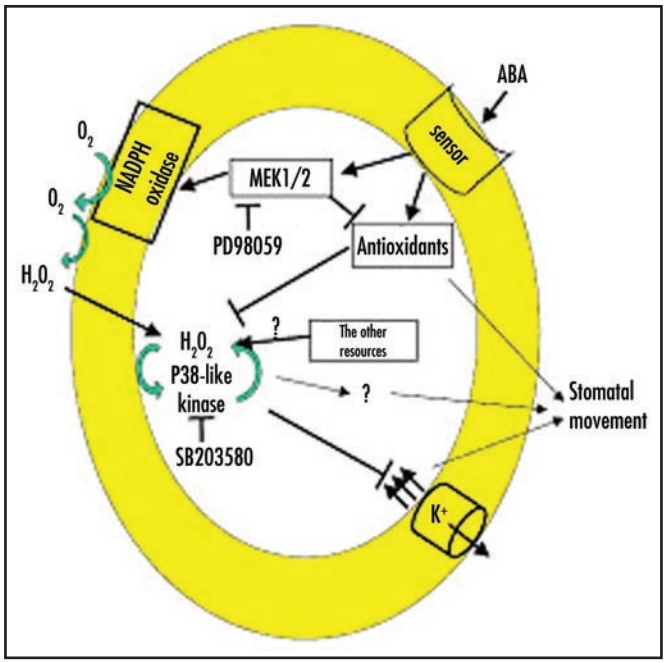

Figure 3. Schematic illustration of MAP kinase-mediated H_2O_2 signaling of guard cells. The arrows indicate activation. The line indicates enhancement and the bar denotes inhibition.

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