CURRENT REVIEW

Guarding the Green: Pathways to Stomatal Immunity

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Guard cells regulate plant gas exchange and transpiration by modulation of stomatal aperture upon integrating external cues like photosynthetic effective illumination, CO₂ levels and water availability and internal signals like abscisic acid (ABA). Being pores, stomata constitute a natural entry site for potentially harmful microbes. To prevent microbial invasion, stomata close upon perception of microbe-associated molecular patterns (MAMPs), and this represents an important layer of active immunity at the preinvasive level. The signaling pathways leading to stomatal closure triggered by biotic and abiotic stresses employ several common components, such as reactive oxygen species, calcium, kinases, and hormones, suggesting considerable intersection between MAMP- and ABA-induced stomatal closures, which we will discuss in this review.

A significant number of microbes—bacteria, oomycetes, and fungi—of the phyllosphere habitat use stomata to invade plant tissues. For example, uredospore and basidiospore germlings of the obligate biotrophic rust fungi have long been described to target stomatal pores as plant entry sites (Mendgen et al. 2006; Moldenhauer et al. 2006; Staples 2000), by which the germ tube specifically recognizes physical features of the outer ledge of stomatal pores (Dickinson 1949; Maheshwari et al. 1967). Likewise, germ tubes from zoospores of the oomycete *Plasmopara viticola* grow towards open stomata and penetrate into the substomatal space, where they form an infection structure (Espino and Nesbitt 1982; Kiefer et al. 2002). For completion of its life cycle, *P. viticola* also co-opts stomata, and sporangiophores emerge through the stomatal pores four to five days after infection. Bacterial pathogens rely on natural openings and wound sites to gain access to plant tissues, and stomatal pores provide a major entry route into leaves (Hugouvieux et al. 1998). *Pseudomonas syringae* pv. *tomato* DC3000 selectively moves towards and enters open stomata on *Arabidopsis thaliana* leaves (Melotto et al. 2006). Confocal microscopy demonstrated distinct clustering of green fluorescent protein-tagged *Salmonella enterica* serovar *Typhimurium* within the guard cell pore complex of lettuce leaves, in particular the substomatal space and the apoplast of the spongy parenchyma (Kroupitski et al. 2009, 2011). *Salmonella* bacteria were able to penetrate stomata only in light, which is

caused by chemo-attraction through photosynthetic sugars (Kroupitski et al. 2009). Dark-induced stomatal closure prior to infection abolished *Salmonella* penetration, and bacteria were confined to the leaf surface.

Similarly, stomatal closure induced by dark-incubation or by abscisic acid (ABA) decreased the disease incidence and severity of *Lycopersicon* spp.-infected with *Xanthomonas campestris* pv. *vesicatoria* (Ramos and Volin 1987). In comparative experiments in which plants were syringe-infiltrated neither dark-incubation nor agents triggering stomatal closure showed any significant difference in susceptibility. Leaf-inoculation with *P. syringae* pv. *tomato* DC3000 or *Escherichia coli* O157:H7 decreased stomatal aperture within 1 to 2 h after inoculation (Melotto et al. 2006). *P. syringae* pv. *tomato* DC3000-inoculated plants, however, reopened their stomata after 3 h (Melotto et al. 2006). Taken together, these findings reinforce the importance of the guard cell pore complex as an active layer of plant immunity at the preinvasive level. The data further indicate that plant pathogens have developed mechanisms to prevent stomatal closure. In this review, we discuss current knowledge of microbe-induced stomatal closure and the overlap with abiotic stress signaling pathways regulating stomatal apertures.

MAMPs trigger the closure of stomata.

Plants recognize potentially harmful microbes by the perception of so-called microbe or pathogen-associated molecular patterns (MAMPs or PAMPs), conserved molecules present across microbial species, which elicit a series of defense responses ultimately resulting in plant immunity referred to as PAMP-triggered immunity (PTI). The restriction of microbial entry by stomatal closure, preinvasive or stomatal immunity, is one of the PTI responses that can be detected within minutes (Cao et al. 2011; Melotto et al. 2006). Chemically diverse MAMPs, such as flg22 (a conserved peptide of the bacterial flagellum), elf18 (a peptide comprising of the first 18 amino acids of the bacterial elongation factor Tu), bacterial lipopolysaccharides (LPS), chitin (a component of the fungal cell wall), yeast elicitor, and oligogalacturonic acid (a degradation product of the plant cell wall) are all capable of inducing stomatal closure, and for flg22, it was further demonstrated that this limits bacterial colonization and disease breakout (Casabuono et al. 2011; Lee et al. 1999; Keinath et al. 2010; Klüsener et al. 2002; Melotto et al. 2006; Zeng and He 2010; Zipfel et al. 2004).

Microbe-induced stomatal closure depends on the perception of MAMPs by pattern recognition receptors (PRR) (Fig. 1). Global transcript analyses of guard cells indicate expression of the cognate PRR for detecting flg22, FLAGELLIN SENSING 2 (FLS2), and chitin, CHITIN ELICITOR RECEPTOR KINASE

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1 (Liu et al. 2009; Mersmann et al. 2010; Yang et al. 2008). Mutant *fls2* plants are impaired in stomatal closure in response to flg22 and show increased susceptibility to *P. syringae* pv. *tomato* DC3000 when sprayed onto the leaf surface but not when infiltrated into leaves (Chinchilla et al. 2006; Gómez-Gómez and Boller 2000; Zeng and He 2010; Zipfel et al. 2004). Interestingly, MAMP-induced stomatal closure is not restricted to MAMP perception in leaves. Root inoculation with the rhizobacterium strain *Bacillus subtilis* FB17 not only reduces the average stomatal aperture but, also, decreases the percentage of open stomata in an FLS2-dependent manner (Kumar et al. 2012). Likewise, stomatal closure was observed when flg22 and LPS were applied to roots. As a result, *B. subtilis* FB17-treated plants were more resistant to *P. syringae* pv. *tomato* DC3000 infection. These findings suggest that MAMP recognition in roots elicits a systemic signal provoking stomatal closure or that the MAMPs themselves could travel between tissues. As stomata respond in a cell-autonomous manner, not all stomata of a leaf close under MAMP stimulus (Melotto et al. 2006). It is, therefore, possible that the presence of a few nonresponsive stomata might be sufficient for highly virulent bacteria to enter the leaf and cause disease while, for less-virulent bacteria, more open stomata might be required for successful infection (Speth et al. 2009).

Reactive oxygen species (ROS), Ca^{2+} , and nitric oxide (NO) regulate stomatal apertures.

ROS, NO, and cytosolic calcium elevations ($[\text{Ca}^{2+}]_{\text{cyt}}$) function in both MAMP- and ABA-induced stomatal closure. Production of H_2O_2 , a major ROS, in response to ABA and different MAMPs is achieved only in part through overlapping components. ABA and flg22 trigger H_2O_2 production via the activation of plasma membrane-resident NADPH oxidases (Kwak et al. 2003; Mersmann et al. 2010). ABA-induced stomatal closure is largely impaired in mutant plants of the NADPH oxidase RESPIRATORY BURST OXIDASE HOMOLOGUE F (RBOHF) and is fully abolished in *rbohD rbohF* double mutants (Kwak et al. 2003). ROS production in response to MAMPs on the other hand, relies on RBOHD as the primary NADPH oxidase, and

flg22- and elf18-dependent stomatal closure is abolished in the *rbohD* mutant (Macho et al. 2012; Mersmann et al. 2010). In addition, ASPARTATE OXIDASE, the enzyme catalyzing the first step in nicotinamide adenine dinucleotide (NAD) biosynthesis, is required for elf18- and flg22-elicited ROS burst and RBOHD-dependent stomatal closure without impacting the ABA-induced RBOHF-mediated ROS burst or ABA-induced stomatal closure (Macho et al. 2012). Chitin, yeast elicitor, and salicylic acid (SA) trigger ROS production independently of NADPH oxidases via activation of cell-wall peroxidases (Khokon et al. 2010a and b; Manthe et al. 1992). Though both biotic and abiotic stress-induced stomatal closures depend on ROS, these findings highlight different mechanisms of ROS production between ABA and individual MAMPs.

NO functions as a secondary messenger in ABA-dependent stomatal closure downstream of ROS production (Garcia-Mata and Lamattina 2007; Khokon et al. 2010a; Neill et al. 2002; Saito et al. 2009). The MAMPs flg22, LPS, chitin, and yeast elicitor were shown to also induce guard cell-localized NO production (Khokon et al. 2010a; Ma et al. 2008; Melotto et al. 2006; Neill et al. 2002; Srivastava et al. 2009). The pathway of NO synthesis in plants as well as the role of NO in stomatal closure, however, is still under debate (Bellin et al. 2012; Yu et al. 2012). Because NO can covalently attach to cysteines to form *S*-nitrosothiol, a redox-based post-translational modification that can act as a molecular switch (Yu et al. 2012) and RBOHD is inactivated by *S*-nitrosylation (Bellin et al. 2012; Yun et al. 2011), this might represent a negative feedback loop during stomatal closure.

Perhaps the most important secondary messenger in MAMP- and ABA-dependent stomatal closure is calcium. In a ROS-dependent manner, ABA induces activation of a yet-unidentified plasma membrane hyperpolarization-activated (I_{Ca}) channel, thereby eliciting Ca^{2+} elevations. Inhibition of Ca^{2+} elevations using the Ca^{2+} chelator BAPTA reduced ABA-triggered stomatal closure by 70% (Siegel et al. 2009). MAMPs, including LPS, chitin, and yeast elicitor, likewise provoke I_{Ca} currents, and application of Ca^{2+} chelators as well as inhibitors of ROS generation prevented the chitin-induced stomatal closure in

Fig. 1. Guard cell signaling in response to pathogen attack. Perception of microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRR) triggers calcium channel (I_{Ca}) activation, increase of Ca^{2+} and in turn activates calcium-dependent protein kinases (CDPK). CDPK and Ca^{2+} binding subsequently activate NADPH oxidases (respiratory burst homologues, RBOHs) and induce reactive oxygen species (ROS) production. CDPK also activates anion channels, as was shown in ABA-induced stomatal closure. This facilitates K^+ efflux through the function of outward rectifying channels (K^+_{out}) and promotes decrease in guard cell turgor leading to stomatal closure. Microbe-induced stomatal closure involves ABA and SA as well as the Snf1-related protein kinase SNRK2.6/OST1 that mediates ABA-induced stomatal closure by activating RBOH oxidases and anion channels in a Ca^{2+} -independent manner. The pathogen effectors coronatine and fusicoccin activate the plasma membrane H^+ -ATPase AHA1/OST2, which in turn activates an inward rectifying K^+ channel (K^+_{in}) and promotes stomatal reopening. Coronatine also impairs SA production via CORONATINE INSENSITIVE 1 (COI1).

Pisum sativum (Ali et al. 2007; Gan et al. 2012; Garcia-Mata and Lamattina 2007; Srivastava et al. 2009; Zeidler et al. 2004). In mesophyll cells, both, flg22 and elf18 trigger Ca^{2+} elevations that are independent of a prior ROS burst (Jeworutzki et al. 2010). This was, however, not tested in guard cells. Flg22-triggered membrane depolarization is inhibited by the calcium channel blocker La^{3+} , placing Ca^{2+} elevations upstream of ion channel regulation (Jeworutzki et al. 2010).

Stomatal closure and opening requires channels and transporters.

In response to MAMPs and ABA, the turgor of guard cells is reduced by efflux of anions and potassium ions, promoting the closure of stomata (Hedrich 2012). ABA activates two types of anion channels via phosphorylation, the slow-activating sustained (S-type) and the rapid transient (R-type) anion channels (Hedrich et al. 1990; Keller et al. 1989; Linder and Raschke 1992; Schroeder and Hagiwara 1989; Schroeder and Keller 1992), and the S-type anion channels SLAC1 and SLAH3 were reported to be regulated by ABA-induced guard cell signaling in *Arabidopsis thaliana* (Geiger et al. 2011; Negi et al. 2008; Vahisalu et al. 2008). Though similar in function, SLAH3 is strictly activated in a Ca^{2+} -dependent manner while SLAC1 can also be activated independently of Ca^{2+} elevations (discussed below). For mediating stomatal closure, anion efflux causes membrane depolarization, which, in turn, drives potassium ions across the plasma membrane of the guard cells through outward-rectifying potassium channels (Ache et al. 2000; Hosy et al. 2003; Schroeder and Hagiwara 1989; Schroeder et al. 1984), water passively follows the flow of solutes and, eventually, the stomata close.

Like ABA, MAMP perception triggers membrane depolarization and increases the extracellular pH (Felix et al. 1993, 1999; Felle et al. 2004; Jeworutzki et al. 2010; Kuchitsu et al. 1997; Pelissier et al. 1986). In mesophyll cells, flg22- and elf18-triggered membrane depolarization is achieved by activation of a nitrate and chloride permeable channel in a Ca^{2+} -dependent manner (Garcia-Brugger et al. 2006; Jeworutzki et al. 2010). S-type anion channels are activated in guard cells in response to *Blumeria graminis* infection as well as chitin treatment, (Geiger et al. 2009, 2010; Koers et al. 2011), but neither the respective channels for the response to fungal chitin nor to the bacterial MAMPs flg22 and elf18 have been identified so far, which is an important question for future research (discussed below).

In addition to channels, ion fluxes across the plasma membrane are regulated by transporters including ATPases, some of which are differentially phosphorylated in response to MAMPs (Benschop et al. 2007). Plants expressing constitutive active variants of the *Arabidopsis* H^{+} -ATPase 1 (AHA1/OST2) are impaired in stomatal closure induced by ABA or MAMPs (Merlot et al. 2007; Liu et al. 2009). AHA1 and its close homologue AHA2 are positively regulated by RPM1 INTERACTING PROTEIN 4 (RIN4), which is an important component of effector-triggered immunity and negatively regulates late PTI responses (Kim et al. 2005; Mackey et al. 2002). Mutant *rin4* plants show decreased ATPase activity affecting the reopening of stomata in response to *P. syringae* pv. *tomato* DC3000 (Liu et al. 2009). RIN4 is a prerequisite for stomatal reopening by the bacterial effector coronatine (discussed below). In a similar manner, the fungus *Fusicoccum amygdale* produces the toxin fusicoccin that causes the activation of plasma membrane H^{+} -ATPases and thereby rapidly induces stomatal reopening (Baunsgaard et al. 1998; Oecking et al. 1997). Thus, one mode of action of some pathogens to counteract MAMP-induced stomatal closure is the activation of plasma membrane H^{+} -ATPases, thereby triggering stomatal opening.

Kinase signaling pathways mediating stomatal closure.

The SNF1-RELATED KINASE 2.6 (SNRK2.6/OST1) is a key component of the signaling pathway regulating stomatal apertures (Mustilli et al. 2002; Yoshida et al. 2006). In a Ca^{2+} -independent manner, SNRK2.6 directly activates the S-type anion channel SLAC1 through phosphorylation that, in turn, mediates the movement of stomata (Geiger et al. 2009). Likewise, SNRK2.6 phosphorylates the NADPH oxidase RBOHF in vitro, suggesting a direct activation and as a result, ABA-induced ROS production is disrupted in guard cells of *open stomata 1* (*ost1*) mutants (Mustilli et al. 2002; Sirichandra et al. 2009). A phosphoproteomic analysis revealed that RBOHD is phosphorylated on multiple sites in response to flg22 that are homologous to the RBOHF phosphorylation sites by SNRK2.6 (Benschop et al. 2007). Phosphorylation of RBOHB in *Solanum tuberosum* occurs in a similar region compared with RBOHF and RBOHD from *Arabidopsis* but at different sites and is mediated by CALCIUM-DEPENDENT PROTEIN KINASES (CDPK) (Kobayashi et al. 2007). In *Arabidopsis*, four CDPK function in both ABA and MAMP signaling pathways (CPK4, CPK6, and CPK11) and, based on initial experiments monitoring the induction of flg22-responsive genes, this would also expand to CPK3 (Boudsocq et al. 2010; Mori et al. 2006; Zhu et al. 2007). Mutant *cpk3*, *cpk6*, and *cpk3 cpk6* plants are impaired in the ROS-mediated I_{Ca} channel activation upon ABA treatment (Mori et al. 2006). This defect can be rescued by exogenous application of H_2O_2 , which positions CDPK signaling upstream of H_2O_2 generation (Mori et al. 2006). Likewise, the flg22-induced ROS burst is down-regulated in *cpk5 cpk6* mutants and is further reduced in *cpk5 cpk6 cpk11 cpk4^{vigs}* quadruple mutants (Boudsocq et al. 2010), pointing at CDPK signaling required for MAMP-triggered ROS production upstream of stomatal closure. Taken together, these findings provide evidence that parallel kinase-dependent signaling pathways mediate ROS production in a Ca^{2+} -dependent and Ca^{2+} -independent manner (Hubbard et al., 2012): i) ABA activates RBOHF via phosphorylation by the Ca^{2+} -independent kinase SNRK2.6. The resulting ROS burst activates the plasma membrane-localized I_{Ca} channel, generating Ca^{2+} elevations. Ca^{2+} in turn binds to the EF hands of RBOHF, leading to further activation of this NADPH oxidase (Kimura et al. 2012; Ogasawara et al. 2008); and ii) the Ca^{2+} elevations trigger CDPK-mediated RBOHF phosphorylation that further activates ROS production.

In parallel, CDPK target anion channels for mediating stomatal closure. *Arabidopsis* CPK6, CPK21, CPK23, and the constitutive active CPK3~~deIF~~ variant phosphorylate and activate SLAC1 in oocytes (Brandt et al. 2012; Geiger et al. 2010; Scherzer et al. 2012). However, these CDPK target SLAC1 in a differential manner. Whereas CPK6 and CPK23 activate SLAC1 at resting Ca^{2+} levels (80 to 100 μM), CPK3 and CPK21 require increased Ca^{2+} levels (Scherzer et al. 2012). As a result, guard-cell protoplasts from *cpk6* and *cpk23* single mutants show reduced S-type anion channel activity in response to elevated Ca^{2+} levels (Geiger et al. 2010; Mori et al. 2006). S-type anion channel activity upon ABA treatment was not affected in *cpk6* single mutants but in *cpk3 cpk6* double mutants, revealing redundancy of CDPK signaling pathways in the ABA response (Mori et al. 2006).

Mitogen-activated protein kinases (MAPK) also play key roles in MAMP- and ABA-induced signaling pathways mediating stomatal closure and have long been associated with PTI and drought stress responses (Liu et al. 2010). MPK9 and MPK12 positively regulate ABA signaling in both stomatal closure and inhibition of light-induced stomatal opening (Jammes et al. 2009). Because ROS production and Ca^{2+} oscillation are unaffected in *mpk9 mpk12* mutants, this supports a

downstream role for MPK9 and MPK12 in the signaling pathways for stomatal movements. In addition, *mpk9 mpk12* mutants are impaired in stomatal closure triggered by chitin and yeast elicitor, the likely cause of the observed increased susceptibility to *P. syringae* pv. *tomato* DC3000 infection (Jammes et al. 2011; Salam et al. 2012a and b). The major MAPK activated by flg22 in *Arabidopsis* are MPK3 and MPK6, which function downstream of ROS production and are also highly responsive to drought stress (Asai et al. 2002; Kovtun et al. 2000; Liu et al. 2010; Tsugama et al. 2012). Though these findings point at partially overlapping MAMP- and ABA-activated MAPK signaling pathways, guard cell-specific *mpk3* antisense lines are affected in closing stomata when triggered with bacteria or LPS but not ABA (Gudesblat et al. 2009), which suggests a specific requirement for MPK3 in MAMP-induced stomatal closure.

In addition to SNRK-, CDPK- and MAPK-type kinases, the AUTHENTIC HISTIDINE KINASE 5 (AHK5) is involved in regulating stomatal closure in response to MAMPs and ABA. Mutant *ahk5* plants display reduced stomatal closing upon treatments with H₂O₂, ethylene, darkness, NO, flg22, *P. syringae* pv. *tomato* DC3000, and *Botrytis cinerea* (Choi et al. 2010). Interestingly, H₂O₂ production was strongly affected in *ahk5* mutants, in particular, in response to flg22 and ethylene. AHK5 therefore seems to be involved in both H₂O₂ production and signaling downstream of NO, possibly via a positive feedback loop. To conclude, the signaling pathways regulating stomatal apertures during biotic and abiotic stresses engage a range of different kinases functioning at different levels of the signaling cascade and partially acting redundantly. This suggests a tight interplay within and between the signaling pathways induced by MAMPs and ABA.

Hormone crosstalk involved in stomatal movements.

The plant hormones ABA, jasmonic acid (JA), and SA all function in MAMP-triggered stomatal closure. In preinvasive stomatal immunity, ABA acts as a positive regulator of stomatal closure (Asselbergh et al. 2008; Cao et al. 2011). Melotto and coworkers (2006) presented results indicating that pathogen-triggered stomatal closure in *Arabidopsis* is impaired in the ABA biosynthesis mutant *aba deficient 3* (*aba3-1*) (Melotto et al. 2006). Whether this reflects a requirement for MAMP-induced ABA synthesis during stomatal closure or basal ABA levels remains to be addressed. To fully understand the intersection between ABA signaling and pathogen-triggered stomatal movements, it is important to take the different stages of the infection cycle into consideration. At 3 to 4 h postinoculation, *P. syringae* pv. *tomato* DC3000-inoculated plants reopen their stomata and ABA-induced gene regulation and stomatal closure are also partially impaired (Kim et al. 2011; Melotto et al. 2006). At later time points, *P. syringae* pv. *tomato* DC3000 increases in planta ABA levels, renders *Arabidopsis* plants hypersensitive towards ABA, and thereby suppresses defense responses, all of which involves the function of secreted effectors HopAM1 and HopAB2 (de Torres-Zabala et al. 2007; Goel et al. 2008). Taken together, this provides evidences that, through the action of effectors, pathogens adjust the plant's physiological state associated with their infection cycle, keeping stomata open at the beginning of the infection phase and closing stomata later on to reduce further water loss. However, this needs to be further substantiated by detailed time-course experiments of stomatal movements and infection cycles, and it will be interesting to learn whether ABA acts in a guard cell-autonomous manner, as recently shown for stomatal responses to low air humidity (Bauer et al. 2013).

JA triggers the closure of stomata in a Ca²⁺-, ROS-, and NO-dependent manner and requires the activation of K⁺- and S-type

anion channels (Evans 2003; Munemasa et al. 2007; Suhita et al. 2003, 2004). ABA-mediated stomatal closure is decreased in the JA-insensitive mutants *jasmonate resistance 1* and *coronatine insensitive 1*. Conversely, JA-induced stomatal closure is reduced in the ABA-insensitive protein phosphatase 2C mutant *aba insensitive 2*, in the ABA-hyposensitive mutant *ost1* and in *cpk6* mutants (Munemasa et al. 2007, 2011; Suhita et al. 2004), while low JA concentrations prevent ABA-induced stomatal closure (Speth et al. 2009). This highlights a tight interplay between the different hormones engaged in regulating stomatal apertures and provides evidence for overlapping ABA and JA signaling pathways upstream of ROS production. Coronatine, a *P. syringae* pv. *tomato* DC3000-secreted effector, structurally mimics JA-isoleucine (Melotto et al. 2006; Mittal and Davis 1995), thereby binding to the JA receptor complex consisting of CORONATINE INSENSITIVE 1 (COI1), an F-box protein, and its substrate JASMONATE ZIM DOMAIN (JAZ) proteins (Yan et al. 2009). Binding of coronatine triggers COI1-dependent degradation of JAZ proteins, causing the degradation of SA and inhibition of SA biosynthesis, which, in turn, triggers the reopening of stomata (Melotto et al. 2006; Zheng et al. 2012). This mechanism enables *P. syringae* pv. *tomato* DC3000 to overcome PTI at the level of stomatal closure and to invade leaf tissues (Melotto et al. 2006; Zheng et al. 2012). Using the coronatine-deficient bacterial strain *P. syringae* pv. *tomato* DC3118, aspartate oxidase and SNRK2.6 as well as the SA biosynthesis gene *ENHANCED DISEASE SUSCEPTIBILITY 5* were revealed as components of MAMP-induced stomatal closure (Macho et al. 2012; Zeng et al. 2011).

SA-induced stomatal closure requires ROS generated by peroxidases, which is downstream of NO production and inactivation of K⁺_{in} channels (Khokon et al. 2011; Lee 1998; Manthe et al. 1992; Mori et al. 2001). SA-deficient plants, either overexpressing the SA hydroxylase *nahG* or mutant for *SA induction deficient 2* (*sid2*) are impaired in MAMP- and bacteria-induced stomatal closure, highlighting the importance of this hormone in stomatal immunity (Melotto et al. 2006). Mutant *sid1/sid2* and *nahG* plants close stomata in response to exogenous ABA, whereas exogenous SA does not induce stomatal closure in the ABA-biosynthesis mutant *aba2-1* (Zeng and He 2010). These findings position SA upstream of ABA signaling for mediating stomatal closure (Zeng and He 2010).

Summary.

Plants actively close stomata in response to pathogens via the perception of MAMPs by PRR like FLS2. MAMP-triggered stomatal closure requires the activation of anion channels, membrane depolarization, and subsequent activation of potassium channels. Stomatal closure induced by MAMPs engages a range of kinase signaling pathways (SNRK, CDPK, MAPK, AHK) and is tightly intersected with hormone signaling (ABA, JA, SA). Guard-cell signaling seems to branch between MAMP- and ABA-induced pathways at the point of SNRK2.6. Thereafter each pathway targets different members of the NADPH oxidases, CDPK, MAPK, and AHK. While we have gained some knowledge about individual aspects and the molecular components of stomatal responses to MAMPs and effectors, we lack a comprehensive view of the signaling pathways and the mechanisms by which microbes trigger stomatal closure and successful pathogens can antagonize this response. A number of questions need to be addressed. i) What are the molecular components of guard cell signaling induced by microbes? In particular, what are the ion channels mediating stomatal closure and opening upon biotic stress? Are they the same as in ABA-induced stomatal closure? How are they regulated? ii) What is the role of SNRK2.6 during MAMP-induced stomatal closure? In particular, is SNRK2.6 biochemically acti-

vated in response to MAMPs and thereby regulating SLAC1 activity? Does SNRK2.6 not phosphorylate RBOHF when activated by MAMPs? iii) Do MAMP signaling pathways differ between guard cells and the whole plant scale? Future works addressing these questions and, specifically, the interconnection between the signaling pathways of MAMP- and ABA-triggered stomatal closure will further our understanding about how plants integrate biotic and abiotic stresses.

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NOTE ADDED IN PROOF

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