New checkpoints in stomatal defense

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Recent reports have revealed new guard cell signaling elements that function in stomatal defense in \textit{Arabidopsis thaliana} (\textit{Arabidopsis}). We discuss here the role of oxylipins, salicylic acid (SA), and abscisic acid (ABA) in stomatal immunity in response to the bacterial pathogen \textit{Pseudomonas syringae}.

Guard cell closure: one pathway for abiotic and biotic stress signaling

Stomata are microscopic pores in the epidermis of aerial organs of plants which ensure the gas exchanges of CO\textsubscript{2} and water vapor both of which are required for photosynthesis and water homeostasis. Plants control the size of stomata by regulating the osmotic pressure of the two guard cells that flank stomatal openings and ABA has been attributed a predominant role in this stomatal regulation in response to variations of the physical environmental conditions [1].

In 2006, Maeli Melotto et al. [2] also assigned stomata a function in the early phases of innate immunity. This pioneering work established that stomata close 1 to 2 h after bacterial recognition, thereby preventing the entry of microbes and host tissue colonization. This response is referred to as the stomatal defense [3] and can be triggered by surface inoculation of plants with not only plant, but also human bacterial pathogens such as \textit{Escherichia coli}. It was further demonstrated that microbial-associated molecular patterns (MAMPs), such as bacterial flagellin, flg22, a biologically active 22 amino acid long peptide of flagellin, and lipopolysaccharide (LPS), caused stomatal closure in \textit{Arabidopsis}. Moreover, it was found that flg22-induced stomatal closure depended on the plant FLAGELLIN-SENSING2 (FLS2) receptor. Based on the observations that \textit{Arabidopsis} mutants defective in ABA synthesis (\textit{aba3-1}) or ABA signaling (\textit{ost1-2}) were more susceptible to \textit{Pseudomonas syringae} p.v. \textit{tomato} (\textit{Pst}) upon surface inoculation and that \textit{Pst} or MAMPs were no longer able to trigger significant stomatal closure in these mutants, ABA was attributed a central role in stomatal defense [2]. SA has also been put forward as a key regulator in this signaling cascade [3,4]. SA induced stomatal closure and plants with reduced SA levels, such as \textit{eds5-1}, \textit{eds16}/\textit{sid2}, or \textit{nahG} lines, or plants affected in SA signaling, such as \textit{npr1}, were compromised in their ability to close stomata in response to either bacteria or MAMPs and displayed lower stomatal defense [2,4]. A causal link between SA and ABA has been further suggested based on the observation that the ABA-deficient mutant line \textit{aba2-I} no longer closed stomata in response to exogenously applied SA, whereas guard cells of SA-deficient \textit{sid2} and \textit{nahG} plants responded normally to ABA [4]. Together, these data suggested that both ABA and SA were required for stomatal closure and that SA action was upstream of that of ABA. Finally, it has been shown that the phytoalexin coronatine (COR) secreted by virulent strains of \textit{Pst}, enables these strains to reopen stomata, thereby circumventing host stomatal defense and enabling the bacteria to colonize plant tissues [2].

The biotic stress-signaling cascade in guard cells: new pieces of the jigsaw puzzle

Recent work has unraveled several new components of guard cell signaling in response to biotic stress signals. The analysis of the cell type-specific leaf transcriptome of \textit{Arabidopsis} allowed the identification of the guard cell 9-specific lipoxygenase LOX1 [5]. LOX1 was found to participate in stomatal defense and was required to trigger stomatal closure in response to both \textit{Pst} and flg22 [6]. 13- and 9-lipoxygenases use polyunsaturated fatty acids as substrates to produce a variety of oxylipins. The best known product of 13-LOX enzymes is jasmonic acid, but the biological action of 9-LOX products is less documented. Interestingly, among various candidates, fatty acid hydroperoxides and reactive electrophile species oxylipins (RES oxylipins) were found as likely products of LOX1 and could induce stomatal closure at nanomolar concentrations [6]. It was further shown that the mitogen-activated protein kinases MPK3 and MPK6 function upstream and SA acted downstream of these LOX1-derived oxylipins. Finally, COR blocked the biological activity of oxylipins in a COI1 (CORONATINE INSENSITIVE1)-dependent manner (Figure 1). Together, these findings reveal the functioning of a guard cell-specific oxylipin pathway in the plant immune response.

Does ABA regulate or modulate the biotic stress signaling in guard cells?

Recently, evidence has been provided that questions the central function of ABA in the biotic stress signaling cascade of \textit{Arabidopsis} guard cells [6]. The inability of the ABA mutants (\textit{ost1-2} or \textit{aba3-1}) to close stomata in
be assumed that ABA was unlikely produced immediately (10 min) after flg22 or oxylipin treatments. Strengthening the assumption that ABA is not produced very early after biotic stress, it was also demonstrated that over a period corresponding to the pathogen-induced stomatal closure (0.5–1 h), ABA-specific transcripts, such as RD29b, ABI1, and ABI2, were not produced following surface inoculation of plants with *Pst*. Even if ABA is not produced early after inoculation or MAMP treatment, ABA is essential in order to optimize stomatal closure in response to biotic stress and to enable plants to raise an efficient stomatal defense. To understand ABA-mediated modulation of stomatal response to biotic stresses, it must be considered that guard cell movements require the regulation of plasma membrane-located ion channels and H+-ATPases to modify the osmotic pressure of these cells. The synthesis of these proteins is known to be ABA-dependent [1] and in ABA-defective mutants, the basal amounts of these proteins are likely too low to maintain normal responses. This situation is illustrated by the fact that ABA induces lower stomatal closure in the *aba2-1* mutant than in wild type plants and this is also observed in response to flg22 or 13-KODE [6]. Therefore, ABA appears to prime the efficiency of both its own and that of other stimuli, such as biotic signals. Further evidence for an ABA-independent biotic stress signaling pathway comes from the analysis of the reactive oxygen species (ROS)-dependent step required for stomatal closure upon biotic and abiotic stimuli. Interestingly the response to biotic stress exclusively depends on the isoform RbohD [9], whereas the ABA-mediated stomatal closure requires the OST1-dependent activation of the NADPH oxidase RbohF [7,8].

**Concluding remarks and open questions**

In summary, it appears that guard cell closure in response to abiotic and biotic stresses is mediated by largely ABA-dependent and oxylipin pathways, respectively. Nevertheless, the mechanisms whereby biotic signals autonomously regulate these processes in guard cells will require further investigations. The present model envisions that downstream of biotic stress signal perception, the two MAPks, MPK3 and MPK6, are activated. Because these protein kinases display a large substrate range, it will be interesting to investigate an involvement of phosphorylation in the mechanism of the LOX1-dependent production of oxylipins. Structure–function analyses suggested that these oxylipins might covalently bind or alkylate thiol-containing target(s) to convey the signal towards SA accumulation. Downstream of this step, SA-mediated regulation of ABA synthesis could enable clarification of the function of ABA in the activation of stomatal defense. In this respect, it would be interesting to test whether genes involved in ABA synthesis or signaling are up-regulated in response to SA treatment. Apart from RES oxylipins, other naturally occurring thiol-reagents such as phenolics or isothiocyanates have been shown to display a potent activity on guard cells likely via covalent reactions with the RES oxylipin targets [6]. Hence, identification of these guard cell receptors of RES will undoubtedly help to understand how these cells sense and respond to different environmental stimuli. A recent approach, based on a random genetic
screen, identified Arabidopsis mutants that could rescue the virulence of COR-deficient mutant bacteria [3]. This work has contributed to characterize new genes essential for stomatal defense and should provide more insight into the key steps required for the plant immune response at the guard cell level.

One important conclusion that can be drawn from the latest findings is the existence of two guard cell pathways to mediate abiotic and biotic stress signaling. This offers independent strategies to improve plant stress resistance to pathogens without compromising tolerance to abiotic stresses, such as drought or heat, which belong to the most important economic challenges in agriculture. However, considering that other hormones and metabolites could also affect guard cell closure, our current model (Figure 1) is probably still far too simple to account for all regulatory mechanisms of guard cell behavior.

References
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