Mind the gap: Signal movement through plasmodesmata is critical for the manifestation of SAR

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Systemic acquired resistance (SAR) is a plant defense response in which an initial localized infection affords enhanced pathogen resistance to distant, uninfected leaves. SAR requires efficient long-distance signaling between the infected leaf, where SAR signals are generated, and the distant uninfected leaves that receive them. A growing body of evidence indicates that the lipid transfer protein DIR1 (Defective in Induced Resistance) is an important mediator of long-distance SAR signaling. In a recent publication, we investigated if cell-to-cell movement through plasmodesmata is required for long-distance movement of DIR1 during SAR. We determined that overexpression of Plasmodesmata-Located Proteins (PDLP1 and 5) negatively impacted long-distance DIR1 movement and SAR competence, suggesting that movement through plasmodesmata contributes to long-distance signal movement during SAR.

In Arabidopsis, mobile SAR signals are hypothesized to travel from induced to distant leaves via the phloem, as the establishment of SAR competence in distant leaves is predominately confined to the orthostichy (a collection of vascular bundles connecting vertically aligned leaves) of the induced leaf. However, other routes of transport cannot be ruled out, as upper leaves outside of the induced leaf orthostichy also become SAR competent.1 This suggests that SAR signals move cell-to-cell to access other orthostichies. While much of the SAR research to date has focused on screening for SAR-defective mutants and identifying mobile SAR signals, little is known about the physiological mechanisms and routes of signal movement. In plants, the cell-to-cell (symplastic) movement of macromolecules occurs via membrane lined cell-to-cell plasmodesmal junctions, which cytosolically connect neighboring cells.2 In the phloem, plasmodesmata provide an important conduit for cell-to-cell movement between companion cells and sieve elements, implicating plasmodesmata as important regulators of long-distance movement of macromolecules.

In healthy plants, the lipid transfer protein DIR1 is targeted to the cell wall3 and plasmodesmata.4 During the induction phase of SAR, DIR1 is activated to move from induced to distant leaves via the phloem to establish SAR competence.5 Other putative SAR mobile signals can be detected in phloem sap and some of these signals (azelaic acid,6 a glycerol-3-phosphate-derived molecule, 4 dehydroabietinal7) require functional DIR1 to contribute to SAR. Our knowledge of phloem loading and the detection of SAR mobile signals in the phloem led us to speculate that SAR mobile signals move through plasmodesmata to access the phloem for long-distance movement during SAR. To investigate if movement through plasmodesmata is important for SAR mobile signaling, we examined the impact of restricting plasmodesmatal pore size on long-distance movement of DIR1 during SAR.

We took advantage of transgenic lines that overexpress members of the Plasmodesmata-Located Protein (PDLP) family, which have been shown to accumulate at plasmodesmata and reduce cell-to-cell movement of fluorescent dyes (35S:PDLP58) and proteins (35S:PDLP19). Given that cell-to-cell movement through plasmodesmata is important for loading...
Pseudomonas bacteria have been observed in phloem cells, however it is not known if they form clusters in the apoplast near mesophyll. In locally infected leaves, the phloem symplastically through plasmodesmata. Our results suggest that PDLP overexpression suppresses the long-distance movement of DIR1 during SAR.

Our findings suggest that the regulation of plasmodesmatal pore size is important for long-distance SAR signaling. We hypothesize that movement of SAR signals into phloem cells is particularly sensitive to plasmodesmatal occlusion since proteins are thought to move into and out of the phloem symplastically through plasmodesmata. In locally infected leaves, Pseudomonas bacteria have been observed to form clusters in the apoplast near mesophyll cells, however it is not known if phloem parenchyma or companion cells are similarly infected. It is conceivable that mesophyll cells produce intra- and/or extracellular signals in response to SAR-inducing pathogens that move symplastically (via plasmodesmata) or apoplastically to access the vasculature. Our model in Figure 1 illustrates symplastic and apoplastic routes of SAR mobile signal movement starting from phloem parenchyma cells. Using a symplastic route, intracellular SAR signals in phloem parenchyma (PP) cells access companion cells (CC) via plasmodesmata, and then move from companion cells to sieve elements (SE) via plasmodesmata for long-distance transport. Alternatively, an apoplastic route could be used to access the phloem, where SAR signals would be loaded into companion cells or sieve elements from the apoplast by membrane-localized transporters. However, the evidence to date indicates that proteins and larger macromolecules such as RNA and viral genome complexes access the phloem symplastically from companion cells. Given the number of putative SAR signals, it is possible that some of these signals access the phloem via the symplastic route, while others may use an apoplastic route. Upon entering the phloem, mobile SAR signals move from locally infected to distant leaves. Currently, the mechanism of signal dissemination within distant leaves is unknown. We hypothesize that mobile SAR signals that arrive in distant leaves are symplastically unloaded from sieve elements to companion cells, and then from companion cells to phloem parenchyma, similar to other phloem mobile macromolecules. Once inside phloem parenchyma, long-distance SAR signals may move to other leaf cell types to induce SAR. Alternatively, the unloading of SAR signals in distant leaf vasculature may lead to the generation of secondary signals that communicate with leaf mesophyll cells, either through plasmodesmatal cell-to-cell movement or via the apoplast.

In conclusion, our work suggests that the regulation of plasmodesmatal pore size is essential for movement of SAR signals to distant leaves. Whether plasmodesmata are actively dilated to facilitate SAR signal movement is currently unknown and should be addressed in future investigations. Moreover, investigating the relative contributions of apoplastic and symplastic phloem loading will provide deeper insight into the regulation of SAR and long-distance transport processes in general.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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