Friend or Foe: Hybrid proline-rich proteins determine how plants interact with and respond to beneficial and pathogenic microbes

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Short title: HyPRPs control microbe induced growth and defense

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Abstract

Plant plastids generate signals, including some derived from lipids, that need to be mobilized to effect signaling. We used informatics to discover potential plastid membrane proteins involved in microbial responses. Among these are proteins co-regulated with the systemic immunity component AZI1, a hybrid proline-rich protein (HyPRP) and HyPRP superfamily members. HyPRPs have a transmembrane domain, a proline-rich region (PRR) and a lipid transfer protein domain. The precise subcellular location(s) and function(s) is unknown for most HyPRP family members. As predicted by informatics, a subset of HyPRPs have a pool of protein that targets plastid outer envelope membranes (OEMs) via a mechanism that requires the PRR. Additionally, two HyPRPs may be associated with thylakoid membranes. Most of the plastid and non-plastid localized family members also have pools that localize to endoplasmic reticulum, plasma membrane or plasmodesmata. HyPRPs with plastid pools regulate, positively or negatively, systemic immunity against the pathogen *Pseudomonas syringae*. HyPRPs also regulate the interaction with the plant growth promoting rhizobacteria *Pseudomonas simiae* WCS417 in the roots to influence colonization, root system architecture and/or biomass. Thus, HyPRPs have broad and distinct roles in immune, development and growth responses to microbes and reside at sites that may facilitate signal molecule transport.

Introduction

Plants have evolved strategies to provide immunity against a broad range of pathogens. The recognition of pathogen-derived molecules at the surface or inside the cell leads to the establishment of local or systemic immunity (Albert et al., 2020; Fu and Dong, 2013; Zipfel, 2008). During systemic immunity, the host plant responds to a primary infection to provide a faster response against a broad range of secondary infections by a phenomenon called priming (Jaskiewicz et al., 2011; Jung et al., 2009; Pieterse et al., 2014). The recognition of pathogens/pathogen-derived molecules in the leaves induces systemic acquired resistance (SAR), a broad spectrum program that suppresses diverse pathogens at sites distal to a primary infection (Fu and Dong, 2013). Colonization of roots with beneficial bacteria can lead to induced-systemic resistance (ISR) in the aboveground part of the plant (Pieterse et al., 2014). These systemic immune programs require long distance transport of mobile signal(s) from the immunized tissue (Carella, 2020; Cecchini et al., 2019b; Kachroo and Kachroo, 2020; Pieterse et al., 2014). Many candidate signal molecules, including azelaic acid (AZA), have been identified as mobile signals for SAR. These signals may act together to confer SAR (Jung et al., 2009; Kachroo and Kachroo, 2020). Although there are differences in the mechanism of induction, some of the signaling components for SAR and ISR overlap (Cecchini et al., 2019b, 2015; Pieterse et al., 1996, 1998; Beckers and Conrath, 2007; Beckers et al., 2009). Beneficial rhizobacteria not only induce ISR, but also stimulate the growth and productivity of plants and impact the root system architecture (Berendsen et al., 2012; Efthimiadou et al., 2020). A crosstalk between the signaling pathways of rhizosphere-induced growth promotion and ISR remains unknown. However, some degree of shared signaling seems plausible (Bulgarelli et al., 2013; Haney et al., 2015).

Plastids coordinate many cellular responses to infections, generating signals that activate defenses (Bobik and Burch-Smith, 2015). Several key defense molecules including hormones and second messengers are synthesized in plastids (Bobik and Burch-Smith, 2015; Dempsey et al., 2011; Mittler et al., 2004). More importantly, signals needed for SAR, such as salicylic acid, AZA and pipecolic acid are produced at least in part within plastids (Dempsey et al., 2011; Hartmann et al., 2017; Zoeller et al., 2012; Rekhter et al., 2019). These molecules (or others regulated by them) should be mobilized from their site(s) of synthesis for long-distance transport. The SA precursor isochorismate synthesized in the chloroplasts is thought to be exported by ENHANCED DISEASE SUSCEPTIBILITY5 (EDS5) (Rekhter et al., 2019; Serrano et al., 2013). Similarly, pools of AZELAIC ACID INDUCED 1 (AZI1) and its paralog EARLY ARABIDOPSIS ALUMINUM INDUCED 1 (EARLI1) are localized in the plastid envelopes and help in the mobilization of plastid-produced AZA (Cecchini et al., 2015; Zoeller et al., 2012). These proteins also localize to the endoplasmic reticulum (ER), and plasma membrane (PM), including plasmodesmata (PD) (Cecchini et al., 2015). Pathogen infection or treatment with the microbe (pathogen)-associated molecular pattern MAMP/PAMP flg22, an epitope of bacterial flagellin, induces AZI1 and EARLI to become enriched at chloroplasts (Cecchini et al., 2015, 2020). The plastids together with other membranous organelles like nucleus, ER and PM (including PD), facilitate the efficient relay of inter-organellar signaling to achieve whole-cell immunity (Helle et al., 2013; Toulmay and Prinz, 2011; Caplan et al., 2015; Lee, 2015). AZI1 and EARLI1 are essential for SAR, MAMP/PAMP-triggered SAR, ISR and AZA-induced systemic disease resistance (Cecchini et al., 2019b, 2015). AZI1 is a signal-anchored protein that has an N-terminal bipartite signal composed of a signal peptide (SP)-like hydrophobic domain followed by a charged protein region (CPR, consisting of at least three Lys and/or Arg residues,

within eight amino acids at the C-terminal side of TMD) and proline rich region (PRR) for the plastid targeting (Cecchini et al., 2015, 2020; Lee et al., 2011). The SP-like region acts as a transmembrane domain (TMD) anchoring AZI1 to the membranes, whereas the PRR guides its plastid association (Cecchini et al., 2020). The superfamily of proteins to which AZI1 belongs are called hybrid proline-rich proteins (HyPRPs). Invariant features include the SP-like region and a C-terminal 8-cysteine motif (8CM)/lipid transfer protein-like (LTP) domain. Most members of the family also have a PRR between these regions that varies in length. Although AZI1 uses a signal-anchored mechanism for plastid targeting, it does not conform to targeting features of classical signal anchor proteins whose N-termini have low hydrophobicity scores on the Wimley-White hydrophobicity scale (Lee et al., 2011).

In this work, we used bioinformatic analyses to identify proteins that, like AZI1, have a pool of protein that localizes to plastid membranes, but which are not classical signal anchored proteins. Included in the screen are proteins whose transcripts are co-regulated with AZI1 and members of the HyPRP superfamily, respectively. Herein, we show the utility of our bioinformatics approach for finding proteins with a pool that localizes to plastid membranes and show that some of them, in the HyPRP superfamily, have different positive or negative roles in systemic immunity, development and growth responses to microbes in roots and shoots.

Results

In silico analysis of subcellular targeting of the Arabidopsis proteome

Well-known targeting algorithms (TargetP, ChloroP and SignalP; (Emanuelsson et al., 1999, 2000; Nielsen et al., 1997) predict AZI1 to be a secreted protein. However, a pool of AZI1 (and that of close paralogs) localizes to plastid envelopes using an N-terminal bipartite signal

(SP-like/TMD + PRR; (Cecchini et al., 2015)) AZI1 has similar properties to apicomplexan proteins that target apicoplasts (non-photosynthetic plastids) using N-terminal bipartite signal sequences (**Figure 1**; (Cecchini et al., 2020)). The PATS algorithm, trained using apicomplexan plastid proteins, can successfully predict plastid localization of AZI1 and paralogs by searching for this bipartite signal (Cecchini et al., 2015, 2020; Zuegge et al., 2001). We used PATS to predict N-terminal bipartite signal sequence and plastid localization of the total Arabidopsis proteome. Overall, the PATS-generated prediction output (positive and negative selections) showed that approximately 7-8% proteins coded by each chromosome may localize to plastids (**Figure 1B**). The Arabidopsis proteins predicted to target to the plastids by PATS are listed in **Supplementary Dataset S1**. Interestingly, the gene ontology (GO) functional characterization (biological processes) of these proteins revealed that among others, the GO terms related to cell communication, response to biotic stimulus, defense response, protein phosphorylation, cell wall organization and cell-cell signaling were significantly overrepresented (**Supplementary Figure S1A**).

To test our predictions, we selected six PATS-positive proteins whose transcripts showed similar expression profiles and clustered together with AZI1 as assessed by the GENVESTIGATOR[®] Hierarchical clustering tool (biological context: Perturbations; (Hruz et al., 2008)). As a secondary screen, we chose proteins that did not conform to a key characteristic of classical signal-anchored plastid OEM proteins. Specifically, their SP-like N-terminal regions lacked low hydrophobicity scores (<0.4) on the Wimley-White hydrophobicity scale (Lee et al., 2011). Instead, the scores (calculated with the MPEx algorithm (<http://blanco.biomol.uci.edu/mpex>; (Jayasinghe et al., 2001; Snider et al., 2009)) were characteristic of ER-targeted proteins (>0.4): AT5g54170: 0.58, AT1G22890/STMP2: 0.63,

AT2G45570.1/CYP76C2: 0.90, AT1G65500/STMP6: 0.63, AT1G33720/CYP76C6: 0.81, AT3G04210.1/TN13: 0.50. We then assessed protein localization by confocal microscopy of C-terminal GFP fusions expressed under dexamethasone (Dex) control in *Nicotiana benthamiana*. Three candidates encoded by At5g54170, At1g65500/STMP6 and At2g45570, respectively, showed GFP signals that co-localized to varying extents with plastid signals (**Figure 1C, Left panel**). The subcellular locations of the At5g54170-encoded protein and STMP6 were further corroborated by fractionation and immunoblotting (**Figure 1C, Right panel**). This shows that the PATS algorithm can identify some plastid proteins besides AZI1 and EARLI1 from Arabidopsis.

***In silico* analysis of subcellular targeting of the Arabidopsis HyPRPs**

PATS also predicted that seven HyPRP members, besides AZI1 and close paralogs, are plastid localized (**Figure 1D**). Arabidopsis HyPRPs are present in gene clusters in the genome (**Supplementary Figure S1B**). Some members of the HyPRPs family were previously named (AZI1, EARLI1, AZI3-AZI7, DEG27, DRN1, DHyPRP1, AIR1, AIR1B, ELP and CWLP); we named the remaining HyPRPs as AZL (AZI1-LIKE) (**Figure 1D**). Considering that the PATS algorithm was trained using apicomplexa proteins, we also implemented another approach to identify AZI1-like plastid-targeting signals in Arabidopsis HyPRPs. TargetP and SignalP can efficiently recognize SP-like sequences and their cleavage sites in plant proteins. Therefore, we manually removed the N-terminal SP-like sequences identified by these algorithms. These SP-like-minus sequences of HyPRPs were then reanalyzed by TargetP and ChloroP (referred to herein as iterative TargetP, iTargetP or iChloroP) customized for plants with default cutoffs. By using SP-like-depleted sequences, the algorithms can analyze the N-terminal transit peptide-like

sequences (called cTPs) for targeting specificity. About 70% of the Arabidopsis HyPRPs were predicted to localize to plastids (**Figure 1D, Supplementary Table S1**). Moreover, the PATS output had a high overlap with that of iTargetP/iChloroP, suggesting that apicoplast or iterative chloroplast predictors can be successfully deployed to find candidates of plant plastid membrane proteins with SP-like motifs.

Arabidopsis HyPRPs localize to the expected sites for the defense signal mobilization

The expected sites of defense signal movement are plastids, ER, PM including PD, and the multiple membrane contact sites formed by them (Cecchini et al., 2015; Lee, 2015; Lim et al., 2016). Using confocal microscopy, eighteen HyPRP-GFP fusion proteins produced in *N. benthamiana* were found to target the expected sites of defense signal transport (**Figure 2**). Based on comparison with our previous study of AZI1 localization (Cecchini et al., 2015), these patterns of GFP signals could be grouped as: (1) ER and PM including foci similar to PD (AIR1, AIR1B, AZL1, AZL5, AZL7, AZL9, AZL10, AZL11, AZL12), (2) chloroplasts plus ER (AZL13), and (3) chloroplasts plus ER and PM including foci similar to PD (ELP, AZL2, CWLP and AZL14). Initially, we cautiously assigned DRN1 to group three, because although there were some chloroplast signals in the micrographs (**Figure 2A**), in many cases GFP was detected in nuclei (**Supplementary Figure S1C**). This raised the possibility that DRN1-GFP was proteolyzed. Indeed, GFP was cleaved from a large pool of the protein (**Supplementary Figure S1C**). Two previous mass spectrometry experiments assigned DRN1 to plastid membranes (Peltier et al., 2004; Tomizioli et al., 2014), providing additional confidence that the protein is plastid-associated.

Generally, the chloroplast-targeted proteins showed a ring-like pattern of fluorescent signal around them. In one case (AZL3), punctate GFP signals around many chloroplasts in addition to PM signals were observed. While AZL6 showed similar localization to AZL3, the chloroplast-associated punctate signals were less prominent. Finally, DHYPRP1 showed ER, PM/PD and incomplete chloroplast rings (**Figure 2A**). The patterns showing ER, PM/PD and chloroplast localization of HyPRPs were largely similar to the family member AZI1 and close paralogs (Cecchini et al., 2015, 2020).

Evaluation by immunoblotting of chloroplast fractions from *N. benthamiana* leaves transiently expressing many HyPRP-GFP fusion proteins largely corroborated the microscopy (**Figure 2B**). We were unsuccessful in using immunoblotting to detect AZL3-GFP after fractionation due to interference from the rubisco large subunit. Instead, we used a proteomics approach and found AZL3 to be differentially enriched in chloroplast membranes of a constitutively defense active Arabidopsis line (*ACD6-IHA*, (Lu et al., 2005)) relative to WT (**Figure 2C**). Prior proteomics studies detected AZL3 in the thylakoid and envelope fractions, respectively (Tomizioli et al., 2014; Kleffmann et al., 2004).

We next analyzed the localization patterns of Dex-inducible GFP fusions of the HyPRPs AZL3, AZL13, AZL14 and ELP in stable Arabidopsis transgenic lines. Each transgene was expressed in the respective mutant background. A portion of the GFP signal of these four fusion proteins colocalized with chloroplast autofluorescence (**Figure 3**). Interestingly, while the GFP signals for AZL13, AZL14 and ELP each showed a ring-like pattern surrounding the chloroplasts, AZL3 had a more diffuse signal. This was further confirmed by analyzing the fluorescence intensity profiles of the merged images (**Figure 3, right most panel**) and is consistent with its import and association with thylakoid membranes (Tomizioli et al., 2014).

Inspection of the features of the PRR for AZL3 compared with other HyPRPs that show the ring-like pattern revealed that the AZL3 PRR lacks a KP motif present in others and also possesses a unique repeated sequence (**Supplementary Table S1**). These and/or other differences in the PRR composition (e.g. abundance of prolines relative to charged amino acid residues) may account for differences in the suborganellar plastid localization patterns. Altogether, our data show that for several HyPRPs, a pool of these proteins localizes to chloroplasts, as predicted.

Several chloroplast-localized HyPRPs are associated with OEMs

Except for AZL1 and AZL9, N-terminal regions of all HyPRPs have hydrophobicity scores >0.4 on the Wimley-White scale (**Supplementary Table S1**). Thus, they do not have features of classical signal-anchored OEM plastid proteins (Lee et al., 2011). Moreover, AZL1 and AZL9 (TMD hydrophobicity < 0.4; plastidial score) did not target to the chloroplasts as indicated by confocal microscopy (**Figure 2A**). This suggests that HyPRPs follow a non-canonical mechanism of targeting to chloroplast membranes, similar to the family member AZI1 (Cecchini et al., 2020).

To study the above possibility, we analyzed the chloroplast membrane association of HyPRPs that showed ring-like localization around chloroplasts. We isolated chloroplasts from *N. benthamiana* leaves transiently expressing GFP-tagged AZL2, AZL13, AZL14 and ELP. The chloroplasts were then partitioned into pellet (membrane) and soluble fractions. Interestingly, all these GFP-tagged HyPRPs were found to be associated with pellets (chloroplast membranes) as evaluated by immunoblotting (**Figure 4**). Furthermore, intact chloroplasts from plants transiently producing HyPRP-GFP fusion proteins treated with thermolysin resulted in HyPRP-GFP proteolysis, consistent with localization to the OEMs. In contrast, the intermembrane space-

located POTRA domains of OEM protein Toc75 and stromal HSP70 were fully protected from thermolysin digestion (**Figure 4B**; (Chen et al., 2016; Seguel et al., 2018)). These experiments indicate that several chloroplast-targeted HyPRPs reside in OEMs and that their C-terminal regions topologically face the cytosol.

The proline-rich region is required for the chloroplast targeting of HyPRPs

Multiple sequence alignment of Arabidopsis HyPRPs revealed conserved N-terminal SP-like hydrophobic and C-terminal 8CM/LTP domains, and highly variable PRRs, variable both in terms of the number of Pro residues and the length of each PRR (**Supplementary Figure S2, Supplementary Table S1**). AZI1 uses a non-canonical signal-anchored targeting mechanism that depends on the PRR for a pool of AZI1 to target chloroplasts (Cecchini et al., 2020). To determine whether this is true for other members, we deleted the PRRs (**Figure 5**) of AZL2, AZL13, AZL14, and ELP. We observed GFP cleavage for AZL14 Δ PRR (AZL14 Δ^{26-44}), which was circumvented by deleting a portion of its LTP together with the PRR (AZL14 Δ^{24-101}). The deletion of PRRs prevented all these HyPRP-GFP fusion proteins from targeting to chloroplasts, consistent with a role for the PRRs in promoting the association of some Arabidopsis HyPRPs with chloroplasts (**Figure 5A-C**).

In AZI1 and the closely related paralogs EARLI1 and AZI3, the C-terminal 8CM/LTP domains did not contribute to chloroplast targeting (Cecchini et al., 2015, 2020). To check if this domain is similarly dispensable for chloroplast targeting of a more distantly related HyPRP, we generated ELP Δ LTP-GFP. This truncated version of ELP associated with chloroplasts (**Figure 5D**). These data show that the PRRs play key roles in chloroplast targeting and are consistent with the N-terminal region of HyPRPs being sufficient to confer this targeting.

Expression of *HyPRPs* is regulated in response to microbes

The *HyPRPs* AZI1, EARLI1 and DRN1 are required for defense against pathogens and their transcripts are modulated during infection (Cecchini et al., 2015; Dhar et al., 2020). Mining of the literature and public data indicated that many *HyPRP* transcripts are differentially regulated by pathogens or pathogen-derived molecules (e.g. the PAMP flg22), suggesting that they may play a role during plant defense responses (**Table 1**). Moreover, the expression of many *HyPRPs* was also responsive to inoculation of roots with the beneficial microbe *P. simiae* WCS417 (Stringlis et al., 2018). Analysis of publicly available gene expression data also revealed that a number of *HyPRPs* are co-regulated to different degrees, suggesting they may be involved in the same processes (**Figure 6**). The *HyPRPs* with the highest co-regulation values were encoded by genes with strong chromosomal linkage (**Supplementary Figure S1B**).

We analyzed the transcript levels of several *HyPRPs* in WT after infection with a pathogen capable of inducing strong local and systemic defenses, *Pseudomonas cannabina* pv. *alisalensis* carrying AvrRpt2 (avirulent strain *Pma*DG6). Most *HyPRP* transcripts assayed were downregulated by 18h post infection (**Figure 6B**). Because AZL3 showed increased protein levels in the plastid membrane fraction of the constitutively defense-active line ACD6-1HA (**Figure 2C**), we also assayed its transcript levels in these plants. Interestingly, the AZL3 transcript level was reduced in the ACD6-1HA line relative to WT (**Figure 6C**). Thus, AZL3 may be subject to post transcriptional regulation.

Together, previously published and our data show that the *HyPRPs* are differentially regulated during biotic interactions (or simulated infection conditions provided by the ACD6-1HA line) and thus may play a role, positive or negative, during interactions with microbes.

Several HyPRPs regulate SAR in Arabidopsis

Since AZI1 and EARLI1 are required for systemic immunity, other HyPRPs with chloroplast-associated pools might be similarly required. To test this, we evaluated local foliar pathogen resistance and SAR using *azl3*, *azl13*, *azl14* and *elp* T-DNA mutants. We used an anti-sense line AZL2-AS (Jülke and Ludwig-Müller, 2015) for SAR experiments, since no *azl2* mutant was available.

In all mutants tested, the local (basal) resistance against a SAR-inducing (avirulent) as well as a virulent *Pseudomonas* strain (*PmaDG6* and *PmaDG3*, SAR-inducing and virulent *Pseudomonas cannabina* pv. *alisalensis*, respectively) was similar to WT (**Supplementary Figure S3**). In contrast, the *azl3* and *azl13* mutants and the AZL2-AS plants were SAR-deficient, whereas the *azl14* mutant still showed robust SAR, similar to WT (**Figure 7**). SAR response gain values (Jiang et al., 2021) of the different genotypes supported this conclusion (**Figure 7B, D**). Interestingly, the *elp* mutant showed significantly higher resistance levels to *PmaDG3* in systemic leaves during SAR and an increased response gain compared to the WT (**Figure 7A, B**). This unusual “super-SAR” phenotype was confirmed in *elp-2*, another T-DNA allele (**Supplementary Figure S4A**).

We also analyzed the induction of SAR marker PR1 in the uninfected systemic leaves of WT, *azl3*, *azl13*, *azl14*, and *elp* mutants after local immunization with *PmaDG6* or mock treatment. Consistent with the observed bacterial growth (**Figure 7A**), there was increased accumulation of PR1 in systemic leaves of *elp* compared to WT, 72 hours post local *PmaDG6* infection (**Figure 7E, F**). Moreover, *azl3* and *azl13* mutants showed significantly lower PR1 than WT in systemic leaves at both 48 and 72 hpi (**Figure 7E, F**).

We performed complementation experiments (**Figure 7G**) using the transgenic plants in which GFP fusions were studied in **Figure 3**. The expression of HyPRPs was induced by spraying whole plants with Dex plus 0.04% Tween 20, 21h before infection. In addition to visualizing GFP (**Figure 3**), transgene expression was confirmed by RT-PCR (**Figure 7H**). Dex:AZL3#1 and Dex:AZL13#1 transgenic lines were complemented for SAR and were indistinguishable from WT plants (**Figure 7G, I**). Independent Dex:AZL3#2 and Dex:AZL13#2 lines were also complemented for SAR (**Supplementary Figure S4 B, C**). The Dex:ELP#1 line was completely SAR-deficient, indicating that the dosage of ELP (overexpression) strongly affects SAR (**Figure 7G, I**). The contrasting phenotypes of the mutant (super-SAR) and Dex:ELP#1 line (SAR-deficient) strongly position this particular HyPRP as a negative regulator of SAR.

HyPRPs are required for ISR

Many HyPRPs are differentially expressed in roots, particularly during the interaction of roots with *P. simiae* WCS417 (**Table 1**; (Stringlis et al., 2018)). Therefore, we tested the role of several HyPRPs with plastid-associated pools in ISR. As shown in **Figure 8**, *azl3*, *azl13* and *azl14* mutants were unable to mount ISR. In contrast, only WT and *elp* showed significant (and similar) ISR (**Figure 8A, B**). These data indicate that several newly characterized HyPRPs have positive roles in promoting ISR.

HyPRPs determine colonization, developmental and/or growth-promoting effects of root-associated bacteria

Strain *P. simiae* WCS417, used to stimulate ISR, also promotes plant growth and lateral root formation in Arabidopsis (Haney et al., 2015; Pieterse et al., 2014). Unlike WT, upon treatment of roots with *P. simiae* WCS417, the *azl13* and *azl14* mutants showed no significant increase in biomass or lateral root numbers. The *azl3* mutant also failed to show a biomass increase, but did display increased lateral root numbers (even higher than WT, **Figure 9**). As was seen with the SAR assay, the *elp* mutant was hyper-responsive, showing both biomass and lateral root numbers that exceeded that seen in WT upon *P. simiae* WCS417 inoculation.

We noticed that *azl14* and to a lesser extent *azl3* and *azl13* had shorter primary root lengths than WT in the absence of bacteria (**Figure 9D**). However, *azl3* and *azl13* showed similar increased primary root growth as WT in response to WCS417. In contrast, the *elp* mutant failed to show increased primary root growth and *azl14* showed reduced growth in response to *P. simiae* WCS417.

Differences in *P. simiae*-induced phenotypes may be related to altered levels of root colonization (Haney et al., 2015). Therefore, we analyzed the root bacteria attachment in WT and mutant plants after growing the different genotypes hydroponically in 48-well plates on a Teflon mesh (**Figure 9E**). Seedlings were initially grown with 2% sucrose to avoid differences in root development between genotypes and thus allowing subsequent comparisons of colonization of roots in media without sucrose (see Methods). Interestingly, although there was no significant difference in the bacterial growth (thought to be stimulated by root exudation) in the well media in which plants were growing, *azl3*, *azl13* and *azl14* mutant roots supported less *P. simiae* colonization compared to WT or *elp* mutant plants (**Figure 9F**).

Altogether, these data suggest that rhizosphere-mediated differences in *P. simiae*-induced responses may partly depend on the host's ability to support bacterial growth on the roots.

Importantly, these results suggest that HyPRPs have varied positive and negative role(s) in the roots including supporting beneficial microbe colonization and impacting root architecture and growth promotion.

Discussion

Most HyPRP family proteins have SP-like/HD domains at their N termini followed by varying lengths of unstructured PRRs. We previously proposed that the N-terminal bipartite signal formed by SP-like/HD (TMD) + PRR define the subcellular targeting of AZI1 and paralogs to plastids (Cecchini et al., 2015, 2020). By using algorithms (PATS, iTargetP/iChloroP) that recognize the N-terminal signal (SP-like/TMD + PRR/cTP or just PRR/cTP), we predicted with about 80% success the subcellular targeting of many members of the HyPRP family in Arabidopsis. Predictions that were not correct largely correlated with HyPRPs harboring shorter PRRs. Importantly, the PATS algorithm also identified plausible bipartite signals in a large number of Arabidopsis proteins. PATs correctly predicted $\geq 33\%$ of the non-HyPRP proteins that we tested. The non-HyPRP plastid protein STMP6 is among a group of proteins recently proposed to be secreted (Yu et al., 2020). Many proteins in this group, regulated in response to pathogens, are PATS-positive and are thus also good candidates for having a pool that localizes to plastids.

Plastid targeting signals are diverse, consisting of multiple sequence motifs that are distinct or conserved (Bruce, 2001; Lee et al., 2008). Of note is that Pro residues in cTPs mediate efficient translocation of TMD-containing proteins through the chloroplast envelopes (Lee et al., 2018). A Ser/Pro-rich domain targets or facilitate targeting/anchoring of other inner envelope

proteins (Küchler et al., 2002; Tripp et al., 2007). Longer PRRs together with the TMDs may similarly help in chloroplast membrane-anchoring of HyPRPs (Cecchini et al., 2020). Charged amino acids (intrinsic charge or those caused by post translational modifications) may play a role in targeting, given their prevalence in PRRs (**Supplementary Table S1**). Considering that AZI1 has a non-cleavable N-terminal signal sequence, these domains in other HyPRPs also are unlikely to be cleaved during their import (Cecchini et al., 2020). This is consistent with the finding that most known proteins in the outer envelope lack a cleavable targeting signal (Hofmann and Theg, 2005). Thus, it is plausible that other plastid-membrane targeted HyPRPs follow the targeting mechanism similar to AZI1, with SP-like/TMD + PRR guiding their plastid association.

We showed that four HyPRPs (AZL2, AZL13, AZL14 and ELP) are OEM plastid proteins. Two HyPRPs (AZL3 and DRN1) were previously suggested to be thylakoid and/or envelope proteins, as evidenced by their presence in proteomics datasets (Peltier et al., 2004; Tomizioli et al., 2014; Kleffmann et al., 2004). While thylakoid samples could be contaminated with plastid envelope proteins, our microscopy of AZL3 in Arabidopsis is consistent with a thylakoid location. This suggests that bipartite signals, depending on their composition, may permit the targeting of proteins to plastid envelopes or the internal plastid thylakoid membrane.

Figure 10 summarizes the localization patterns of HyPRPs, and by analogy to AZI1/EARLI1, proposes that they are needed for mobilizing signals. The multiple roles of HyPRPs in microbial responses that were found previously (Cecchini et al., 2015, 2019b; Dhar et al., 2020) and herein are shown in **Figure 10B**. ELP is unique among the HyPRPs in how it impacts responses to microbes, as it is a negative regulator of both SAR and growth/development responses to the root-colonizing microbe WCS417. The *elp* mutant shows increased response

gain and PR1 accumulation during SAR, but does not affect basal resistance, suggesting increased signaling output to distal tissue. This mutant also has normal root colonization, but has increased plant growth and lateral root numbers relative to WT, further supporting enhanced signaling. However, ISR is not affected in the *elp* mutant, indicating distinct requirements for the developmental responses and ISR. This is consistent with certain ISR-defective mutants showing robust growth and developmental responses when co-cultivated with WCS417 (Zamioudis et al., 2013).

Some mutants in this study (*azl3* and *azl13*) are affected in both SAR and ISR, similar to the *azi1* and *earli1* mutants of HyPRPs (Jung et al., 2009; Cecchini et al., 2015). For the SAR phenotypes, the defects are linked to long distance signaling, since the growth of the immunizing pathogen is normal. However, the *azl3*, *azl13* and *azl14* mutants show possible local signaling defects evidenced by lower colonization of roots with WCS417 relative to that seen in WT. It is possible that other root-related defects in these mutants arise from low root colonization. A certain threshold of rhizobacterial colonization may be needed to induce the downstream root development, plant growth and ISR phenotypes. However, *azl3* still shows lateral root induction, indicating that there is enough colonization to cause signaling for this phenotypic output. Lateral root induction in *azl3* may occur due to the presence of other HyPRPs (AZL13, AZL14, AZI1 and/or EARLI1 (Cecchini et al., 2019b)). This would implicate other HyPRPs in regulating both colonization as well as the downstream signaling for lateral root induction.

It seems possible that different phenotypic outputs (growth promotion, ISR and lateral root number increases) require different thresholds of bacterial colonization of the roots. Alternatively, all outputs may require the same threshold, but the certain HyPRPs may be differentially needed for multiple steps: colonization and downstream signaling to cause growth,

ISR and/or lateral root production. A genome-wide association mapping study (Wintermans et al., 2016) identified different markers that were independently associated with shoot growth promotion, lateral root formation and primary root length changes in response to WCS417. This finding supports the idea that there are separate pathways (or branches of a pathway) that independently regulate the different phenotypic outputs. Interestingly, two HyPRPs (DEG27, AZL2) are within 5-10 KB of a marker associated with regulating the lateral root changes.

Despite having positive roles in regulating interactions with/responses to microbes, transcript levels of many HyPRPs are down regulated by microbe-related signals. This could reflect negative feedback to modulate signaling. However, in plants with constitutive defenses (ACD6-1HA) the transcript level of AZL3 is low, while the plastid membrane-associated protein level is high relative to WT. This suggests that there is complexity to the regulation of HyPRPs. Some HyPRPs may become stabilized by post translational modification, since they have kinase motifs (SP/TP, **Supplementary Table S1**). AZI1's PRR is a kinase substrate *in vitro* (Pitzschke et al., 2014).

HyPRPs are similar and they locate to several types of membranes. This could permit the formation of membrane contact sites and complexes with other HyPRPs/LTPs in different places. Multiple contact sites between plastids, ER and PM, comprising of LTPs, are the sites of exchange of small molecule signals (Andersson et al., 2007; Li et al., 2020; Toulmay and Prinz, 2011). HyPRPs are likely enriched in these sites for the mobilization/transport of immune and/or developmental and growth signals. Supporting this, AZI1 and EARLI1 can interact and mobilize the SAR signal AZA, which also can trigger AZI1-/EARLI1-dependent root architecture changes (Cecchini et al., 2019b, 2015). Moreover, because AZI1/EARLI1 physically interact with non-HyPRP LTP superfamily member (i.e. DIR1) in membrane contact sites to modulate systemic

responses (Cecchini et al., 2015; Yu et al., 2013), it is also possible that other positive regulatory HyPRPs similarly form active homo or hetero-oligomeric LTP complexes at the key sites/structures for transmission of developmental, growth and/or defense signals to regulate systemic defense responses. The presence of so many HyPRPs may allow the movement of signals at different rates (to reach specific thresholds) or enable movement of various signals that require different membrane-paths to move. For example, to reach the xylem to move signals aboveground may require the use of different contact sites compared to signals reaching parenchyma for lateral root stimulation. Thus, different signals (or the amount of signal moved) could affect and explain different specific phenotypes.

Methods

Plants, bacteria and plasmids

All the *Arabidopsis thaliana* plants used were Columbia-0 (Col-0) ecotype. The plants used for infection were grown in soil (Berger, BM1:BM2; 50:50 mix) or Jiffy-7[®] peat pellets (for ISR, Hummert International, # 14-23700) at 12h day/night cycle as previously described (Cecchini et al., 2015). The following *Arabidopsis* accessions were obtained from ABRC: *At1g12090* (*elp*, SALK_147582C; *elp-2*, SAIL_532_C09), *At2g10940* (*azl3*, SALK_083118C), *At5g46890* (*azl13*, SALK_065132), *At5g46900* (*azl14*, SALK_096750C). *At1g62510* AZL2-AS line was described previously (Jülke and Ludwig-Müller, 2015). The transgenic line ACD6-1HA (Lu et al., 2005) used for proteomic analysis was grown at 16h day/8h night cycle. pt-gk (cTP-GFP) transgenic line from ABRC (CS16266) was used as a chloroplast marker. *Arabidopsis* HyPRP complementation lines were generated by floral dip method using *Agrobacterium tumefaciens*

C58C1 suspensions (Clough and Bent, 1998). *N. benthamiana* used for localization studies were grown in soil at 24°C under 16h light/8h night regime. For *A. tumefaciens*-mediated transient assays, 4–5-week-old *N. benthamiana* plants were used.

Bacterial strains used for infections or colonization were virulent *Pseudomonas cannabina* pv. *alisalensis* (formerly *Pseudomonas syringae* pv. *maculicola* strain ES4326) carrying an empty vector (*Pma*DG3), the avirulent isogenic strain carrying *avrRpt2* (*Pma*DG6) and *Pseudomonas simiae* WCS417 (formerly *Pseudomonas fluorescens* WCS417r).

The full-length coding sequences or the truncated versions of HyPRPs were amplified by PCR and cloned by Gateway procedure into Dexamethasone (Dex)-inducible plant expression vectors pBAV150 (C-terminal GFP tag) or pBAV154 (C-terminal HA tag) (Cecchini et al., 2015). Full-length coding sequences of HyPRPs in pBAV150 vector were used to transform *Arabidopsis* to generate complementation lines. To generate Δ PRR variants, proper fragments were generated with overlapping primers and linked by PCR. Δ LTP variants were generated using non-overlapping primers in PCR. All the primers and constructs used in this study are listed in (Supplementary Table S2).

***In silico* analysis of subcellular targeting**

For bipartite plastid targeting signal prediction, we analyzed the full-length sequences of *Arabidopsis* proteome (ATpepTAIR10) by using PATS software (<http://modlabcadd.ethz.ch/software/pats/>) or an iterative method (iTargetP/iChloroP). In the iterative method, N-terminal SP-like regions as determined by TargetP (<http://www.cbs.dtu.dk/services/TargetP-1.1/>) or SignalP (<http://www.cbs.dtu.dk/services/SignalP/>), varying between 22 and 27 amino acids (except for

AZI7 with 30 amino acids as SP-like) were removed *in silico* and the remaining sequences were analyzed by TargetP/ChloroP to determine the presence of chloroplast transit peptide following the SP-like region.

The GO analysis was done as described (Bonnot et al., 2019). The list of representative enriched GO terms was obtained using Panther (https://www.arabidopsis.org/tools/go_term_enrichment.jsp) and REVIGO (<http://revigo.irb.hr>) tools.

Confocal Imaging

9-12 day-old Arabidopsis seedlings or 4-week old *N. benthamiana* leaves expressing the different HyPRP versions fused at C-terminal with GFP were visualized by confocal microscopy. Zeiss LSM710 and LSM800 laser scanning confocal microscopes were used to visualize GFP fluorescence (excitation = 488 nm, emission = 509 nm) and chlorophyll autofluorescence (excitation = 631 nm, emission = 647 nm). LD C-Apochromat 40x/1.1 W Korr (LSM710) and EC Plan-Neofluor 40x/1.3 (LSM800) objectives were used for taking images. The images are optical sections captured at 1024 x 1024 pixels scanning resolution in maximum speed mode. Fluorescence of GFP and chlorophyll was acquired in sequential acquisition mode. Plant tissues were mounted in perfluorodecalin (Strem Chemicals, Inc.) for optical enhancement. Images were processed by ImageJ (Fiji) and Adobe photoshop software.

Fractionation

N. benthamiana leaves, transiently transformed with *A. tumefaciens* harboring different constructs, were used to isolate chloroplasts as described in (Cecchini et al., 2015) with minor

modifications. Briefly, leaves (0.5-1g) were homogenized in XpI buffer (0.33 M sorbitol, 50 mM HEPES pH 7.5, 2 mM EDTA, 1 mM MgCl₂, 0.25% BSA, 0.1% sodium ascorbate and protease inhibitors) using polytron (Kinematica) and filtered through two layers of Miracloth (Calbiochem, # 475855). The filtrate was centrifuged at 5000 rpm for 5 minutes and the pellet was resuspended in 0.4ml of XpI buffer. The resuspended pellet was loaded on Percoll (GE, # 17089101) gradient (80% and 40%) and centrifuged at 13,000 rpm to isolate the intact chloroplasts. The recovered intact chloroplasts at the interphase were resuspended in 0.5ml XpI buffer and centrifuged at 3000 rpm for 5 minutes to remove the Percoll impurities. The purity of the chloroplast fractions was checked using specific markers by immunoblotting.

To obtain enriched membrane and soluble fractions, the intact chloroplasts were partitioned into membrane (pellet) and soluble fractions by centrifugation. To do this, the plastids were resuspended in protease free ice-cold water containing protease inhibitor cocktail (Thermo Scientific, #A32965). For complete lysis, the chloroplasts were incubated on ice for 45-60 min followed by incubation at -80°C O/N. The samples were then thawed on ice and centrifuged at 13,000 rpm. The soluble fraction was recovered, and the pellet was resuspended in 1XPBS (+1% SDS and protease inhibitor cocktail).

Thermolysin assay

Proteolysis by thermolysin was performed as described in (Cecchini et al., 2015). Briefly, intact chloroplast fractions were resuspended in 1X import buffer (0.33 M sorbitol, 50 mM HEPES, pH 8.0) and treated with thermolysin (to a final concentration of 0.1mg/ml). The reaction was quenched by adding 10mM EDTA. The chloroplasts were carefully separated on 40% Percoll cushion.

Immunoblotting

Equal amounts of total proteins were separated on 12% SDS-PAGE and transferred to PVDF membranes (Millipore, #IPVH00010). The concentration of proteins was determined by Bradford assay. The following primary antibodies were used in this study: PR1 antibody (Agrisera, #AS10687; 1:2,500), GFP monoclonal antibody (Takara, # 632375; 1:2,000), Bip2 antibody (Agrisera, #AS09481; 1:4,000), Tic110 antibody (a gift from Masato Nakai; 1:3,000), chloroplast Hsp70 (a gift from Thomas Leustek; 1:12,000), Toc75 (Agrisera, #AS08351, 1:3000). Horseradish peroxidase-conjugated anti-mouse (Invitrogen, #SA1-100; 1:4,000), anti-guinea pig (Sigma, #A5545; 1:100,000), or anti-rabbit (Thermo Scientific, #32460; 1:5,00) secondary antibodies were used. The bands were detected by using SuperSignal pico/femto chemiluminescence kits (Thermo Scientific; #s1859022/3 and 1859674/5).

Transcript analysis

Total RNA was isolated with the RNeasy Plant Mini Kit (Qiagen) and cDNA was synthesized using the Reverse Transcriptase SuperScript III and Oligo (dT)₂₀ primers (ThermoScientific) according to the manufacturer's instructions. *HyPRP* expression (in WT/ACD6-1HA) was analyzed by quantitative RT-PCR using SYBR Premix Ex Taq (Takara) in a Bio-Rad Real-Time CFX96TM C1000 system (Bio-Rad) according to the manufacturer's instructions. For Dex:AZL lines, relative transcript levels were checked by RT-PCR (optimized for cycle #s). EF1 α was used as a reference for normalization.

Pathogen growth bioassays

The evaluation of bacterial pathogen growth was carried as described (Jung et al., 2009). Briefly, 26-28 day-old wild-type and *HyPRP* mutant plants were syringe-infiltrated with *PmaDG3* or *PmaDG6* ($OD_{600}=0.0001$). Growth was quantified using eight equal sized leaf discs from different plants 3 days after bacterial inoculation. All graphs of colony forming units (cfus) were plotted as $\log_{10}$ values.

For SAR assays (Jiang et al., 2021), local leaves of 26–28-day old plants were syringe-infiltrated with mock (10mM $MgSO_4$) or *PmaDG6/avrRpt2* ($OD_{600} = 0.01$). Two days after the primary infection, systemic leaves were infiltrated with *PmaDG3* ($OD_{600}= 0.0001$). The local leaves were removed from the plants prior to the secondary challenge and the plants were covered with plastic domes following the secondary challenge. Three days post-secondary challenge, equal sized leaf discs were collected and ground in 10mM $MgSO_4$. The ISR assay was performed exactly as described (Cecchini et al., 2019a) with *PmaDG3* OD_{600} of 0.0001-0.0002. The response gain of SAR or ISR was calculated as described (Jiang et al., 2021).

Plant growth promotion assays of whole plants and roots

Arabidopsis were grown vertically at $\sim 20^{\circ}C$ with 16h day/8h night on small square plates containing MS media (GibcoBRL, Life Technologies) without sugar as previously described (Haney et al., 2015). Briefly, ten seeds were germinated on plates and five days after plating, seedlings were thinned to five seedlings per plate and roots inoculated with 1 μ l 10mM $MgSO_4$ (Mock) or *Pseudomonas simiae* WCS417 ($OD_{600}=0.001$). After 10 days, scanned (Epson) plant images were used to count lateral roots and measure primary root lengths. Subsequently the fresh weights were determined.

Root colonization assay

Root colonization assay was performed in 48-well plates as described (Haney et al., 2015). 250 µl MS media (Sigma, #M5519-10L) containing 2% sucrose was added to the wells of 48-well tissue cultures plates (BD Falcon). Round Teflon mesh disks (McMaster- Carr #1100t41) were sterilized by autoclaving and placed into the wells. A single surface sterilized and imbibed seed was placed at the center of each disc. Seedlings were subsequently grown at ~20°C with 16 h day/8 h dark for 10 days. The MS media was replaced with 270 µl of fresh MS media without sugar. Two days after the media change, wells were inoculated with 30 µl (final OD₆₀₀ = 0.00002) of *P. simiae* WCS417 suspended in water. After 7 days, seedlings were removed, and roots and well media analyzed for bacterial amount (six seedlings were combined per data point). The root extracts prepared by grinding and the corresponding well media were plated by serial dilution to determine cfus.

LC-MS/MS

Approximately 15g Arabidopsis shoots of WT and ACD6-1HA were homogenized and the chloroplasts separated on Percoll gradients (80% and 40%). The total plastid membrane fraction obtained after centrifugation was used for LC-MS/MS. Total plastid membrane proteins were analyzed by Q-Exactive Orbitrap mass spectrometer. Relative quantification was performed using Maxquant software. The raw data was searched against the *Arabidopsis thaliana* database, as well as reversed protein sequences, and common contaminants. The search results were filtered to be within 1% false discovery rate and label-free quantification using normalized protein abundances was performed to assess differential protein expression between WT and the transgenic expressing *ACD6-1HA*.

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Plotting and statistical analysis.

The graphs were plotted in R studio (v.3.6.1/4.1) using ggplot2 package (v3.3.2) and all statistics were done using agricolae package (v1.3.3).

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Author contributions

J.T.G., N.M.C. and Z.Z.B. designed the experiments. Z.Z.B, N.M.C, A.T.S., C.T.H., R.C.F., and E.A. performed experiments. Z.Z.B, N.M.C., A.T.S. and J.T.G. performed bioinformatic analysis or data mining. Z.Z.B, N.M.C and J.T.G. wrote the manuscript.

Supplemental data

Supplementary Figure S1. Over-represented GO terms for biological processes in PATS plastid

predicted proteins, location of *HyPRPs* in Arabidopsis genome and GFP cleavage in DRN1.

Supplementary Figure S2. ClustalW alignment of Arabidopsis *HyPRPs*.

Supplementary Figure S3. Local disease resistance of *HyPRP* mutants against *Pseudomonas*

syringae pv. *maculicola* strains.

Supplementary Figure S4. SAR in *elp-2* allelic mutant and Dex-inducible complementation

lines.

Supplementary Dataset S1. List of all PATS positive proteins of Arabidopsis.

Supplementary Table S1. CPRs, PRRs, WWH scores, experimental and predicted plastid

targeting of Arabidopsis *HyPRPs*.

Supplementary Table S2. List of primers and constructs used in this study.

References

Albert, I., Hua, C., Nürnberger, T., Pruitt, R.N., and Zhang, L. (2020). Surface Sensor Systems in Plant Immunity. *Plant Physiol* 182: 1582–1596.

Andersson, M.X., Goksör, M., and Sandelius, A.S. (2007). Membrane contact sites: physical attachment between chloroplasts and endoplasmic reticulum revealed by optical manipulation. *Plant Signal Behav* 2: 185–187.

Ascencio-Ibáñez, J.T., Sozzani, R., Lee, T.-J., Chu, T.-M., Wolfinger, R.D., Cella, R., and Hanley-Bowdoin, L. (2008). Global Analysis of Arabidopsis Gene Expression Uncovers a Complex Array of Changes Impacting Pathogen Response and Cell Cycle during Geminivirus Infection. *Plant Physiol.* 148: 436–454.

Baum, S., Reimer-Michalski, E.-M., Bolger, A., Mantai, A.J., Benes, V., Usadel, B., and Conrath, U. (2019). Isolation of Open Chromatin Identifies Regulators of Systemic Acquired Resistance. *Plant Physiol.* 181: 817–833.

Beckers, G.J.M. and Conrath, U. (2007). Priming for stress resistance: from the lab to the field. *Curr Opin Plant Biol* 10: 425–431.

641 Beckers, G.J.M., Jaskiewicz, M., Liu, Y., Underwood, W.R., He, S.Y., Zhang, S., and Conrath,
642 U. (2009). Mitogen-activated protein kinases 3 and 6 are required for full priming of
643 stress responses in *Arabidopsis thaliana*. *Plant Cell* 21: 944–953.

644 Berendsen, R.L., Pieterse, C.M.J., and Bakker, P.A.H.M. (2012). The rhizosphere microbiome
645 and plant health. *Trends Plant Sci* 17: 478–486.

646 Bobik, K. and Burch-Smith, T.M. (2015). Chloroplast signaling within, between and beyond
647 cells. *Front Plant Sci* 6: 781.

648 Bonnot, T., Gillard, M., and Nagel, D. (2019). A Simple Protocol for Informative Visualization
649 of Enriched Gene Ontology Terms. *BIO-PROTOCOL* 9.

650 Bruce, B.D. (2001). The paradox of plastid transit peptides: conservation of function despite
651 divergence in primary structure. *Biochimica et Biophysica Acta (BBA) - Molecular Cell*
652 *Research* 1541: 2–21.

653 Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., and Schulze-Lefert, P.
654 (2013). Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol*
655 64: 807–838.

656 Caplan, J.L., Kumar, A.S., Park, E., Padmanabhan, M.S., Hoban, K., Modla, S., Czymmek, K.,
657 and Dinesh-Kumar, S.P. (2015). Chloroplast Stromules Function during Innate Immunity.
658 *Dev Cell* 34: 45–57.

659 Carella, P. (2020). Xylem-Mobile Oxylipins Are Critical Regulators of Induced Systemic
660 Resistance in Maize. *Plant Cell* 32: 13–14.

661 Cecchini, N., Song, Y., Roychoudhry, S., Greenberg, J., and Haney, C. (2019a). An Improved
662 Bioassay to Study *Arabidopsis* Induced Systemic Resistance (ISR) Against Bacterial
663 Pathogens and Insect Pests. *BIO-PROTOCOL* 9.

664 Cecchini, N.M., Roychoudhry, S., Speed, D.J., Steffes, K., Tambe, A., Zodrow, K.,
665 Konstantinoff, K., Jung, H.W., Engle, N.L., Tschaplinski, T.J., and Greenberg, J.T.
666 (2019b). Underground Azelaic Acid–Conferred Resistance to *Pseudomonas syringae* in
667 *Arabidopsis*. *MPMI* 32: 86–94.

668 Cecchini, N.M., Speed, D.J., Roychoudhry, S., and Greenberg, J.T. (2020). Kinases and protein
669 motifs required for AZI1 plastid localization and trafficking during plant defense
670 induction. *Plant J*.

671 Cecchini, N.M., Steffes, K., Schläppi, M.R., Gifford, A.N., and Greenberg, J.T. (2015).
672 *Arabidopsis* AZI1 family proteins mediate signal mobilization for systemic defence
673 priming. *Nat Commun* 6: 7658.

674 Chen, Y.-L., Chen, L.-J., and Li, H.-M. (2016). Polypeptide Transport-Associated Domains of
675 the Toc75 Channel Protein Are Located in the Intermembrane Space of Chloroplasts.
676 *Plant Physiol* 172: 235–243.

677 Clough, S.J. and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated
678 transformation of *Arabidopsis thaliana*. *Plant J* 16: 735–743.

679 Dempsey, D.A., Vlot, A.C., Wildermuth, M.C., and Klessig, D.F. (2011). Salicylic Acid
680 biosynthesis and metabolism. *Arabidopsis Book* 9: e0156.

681 Dhar, N., Caruana, J., Erdem, I., and Raina, R. (2020). An *Arabidopsis* DISEASE RELATED
682 NONSPECIFIC LIPID TRANSFER PROTEIN 1 is required for resistance against
683 various phytopathogens and tolerance to salt stress. *Gene*: 144802.

684 Efthimiadou, A., Katsenios, N., Chanioti, S., Giannoglou, M., Djordjevic, N., and Katsaros, G.
685 (2020). Effect of foliar and soil application of plant growth promoting bacteria on
686 growth, physiology, yield and seed quality of maize under Mediterranean conditions. *Sci*
687 *Rep* 10: 21060.

688 Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000). Predicting Subcellular
689 Localization of Proteins Based on their N-terminal Amino Acid Sequence. *Journal of*
690 *Molecular Biology* 300: 1005–1016.

691 Emanuelsson, O., Nielsen, H., and Heijne, G.V. (1999). ChloroP, a neural network-based method
692 for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci.* 8: 978–
693 984.

694 Fu, Z.Q. and Dong, X. (2013). Systemic acquired resistance: turning local infection into global
695 defense. *Annu Rev Plant Biol* 64: 839–863.

696 Gupta, A. and Senthil-Kumar, M. (2017). Transcriptome changes in *Arabidopsis thaliana*
697 infected with *Pseudomonas syringae* during drought recovery. *Sci Rep* 7: 9124.

698 Haney, C.H., Samuel, B.S., Bush, J., and Ausubel, F.M. (2015). Associations with rhizosphere
699 bacteria can confer an adaptive advantage to plants. *Nature Plants* 1: 15051.

700 Hartmann, M., Kim, D., Bernsdorff, F., Ajami-Rashidi, Z., Scholten, N., Schreiber, S., Zeier, T.,
701 Schuck, S., Reichel-Deland, V., and Zeier, J. (2017). Biochemical Principles and
702 Functional Aspects of Picecolic Acid Biosynthesis in Plant Immunity. *Plant Physiol.* 174:
703 124–153.

704 Helle, S.C.J., Kanfer, G., Kolar, K., Lang, A., Michel, A.H., and Kornmann, B. (2013).
705 Organization and function of membrane contact sites. *Biochim Biophys Acta* 1833:
706 2526–2541.

707 Hofmann, N.R. and Theg, S.M. (2005). Chloroplast outer membrane protein targeting and
708 insertion. *Trends Plant Sci* 10: 450–457.

709 Howard, B.E., Hu, Q., Babaoglu, A.C., Chandra, M., Borghi, M., Tan, X., He, L., Winter-
710 Sederoff, H., Gassmann, W., Veronese, P., and Heber, S. (2013). High-throughput RNA
711 sequencing of *pseudomonas*-infected *Arabidopsis* reveals hidden transcriptome
712 complexity and novel splice variants. *PLoS One* 8: e74183.

713 Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem,
714 W., and Zimmermann, P. (2008). Genevestigator v3: a reference expression database for
715 the meta-analysis of transcriptomes. *Adv Bioinformatics* 2008: 420747.

716 Jaskiewicz, M., Conrath, U., and Peterhänsel, C. (2011). Chromatin modification acts as a
717 memory for systemic acquired resistance in the plant stress response. *EMBO Rep* 12: 50–
718 55.

719 Jayasinghe, S., Hristova, K., and White, S.H. (2001). Energetics, stability, and prediction of
720 transmembrane helices11 Edited by G. von Heijne. *Journal of Molecular Biology* 312:
721 927–934.

722 Jiang, S.-C., Engle, N.L., Banday, Z.Z., Cecchini, N.M., Jung, H.W., Tschaplinski, T.J., and
723 Greenberg, J.T. (2021). ALD1 accumulation in *Arabidopsis* epidermal plastids confers
724 local and nonautonomous disease resistance. *J Exp Bot*.

725 Jülke, S. and Ludwig-Müller, J. (2015). Response of *Arabidopsis thaliana* Roots with Altered
726 Lipid Transfer Protein (LTP) Gene Expression to the Clubroot Disease and Salt Stress.
727 *Plants* 5: 2.

728 Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., and Greenberg, J.T. (2009). Priming in
729 Systemic Plant Immunity. *Science* 324: 89–91.

730 Kachroo, A. and Kachroo, P. (2020). Mobile signals in systemic acquired resistance. *Curr Opin*
731 *Plant Biol* 58: 41–47.

732 Kleffmann, T., Russenberger, D., von Zychlinski, A., Christopher, W., Sjölander, K., Gruissem,
733 W., and Baginsky, S. (2004). The *Arabidopsis thaliana* Chloroplast Proteome Reveals
734 Pathway Abundance and Novel Protein Functions. *Current Biology* 14: 354–362.

735 Küchler, M., Decker, S., Hörmann, F., Soll, J., and Heins, L. (2002). Protein import into
736 chloroplasts involves redox-regulated proteins. *EMBO J* 21: 6136–6145.

737 Lee, D.W., Kim, J.K., Lee, S., Choi, S., Kim, S., and Hwang, I. (2008). *Arabidopsis* Nuclear-
738 Encoded Plastid Transit Peptides Contain Multiple Sequence Subgroups with Distinctive
739 Chloroplast-Targeting Sequence Motifs. *Plant Cell* 20: 1603–1622.

740 Lee, D.W., Yoo, Y.-J., Razzak, Md.A., and Hwang, I. (2018). Prolines in Transit Peptides Are
741 Crucial for Efficient Preprotein Translocation into Chloroplasts. *Plant Physiol.* 176: 663–
742 677.

743 Lee, J., Lee, H., Kim, J., Lee, S., Kim, D.H., Kim, S., and Hwang, I. (2011). Both the
744 Hydrophobicity and a Positively Charged Region Flanking the C-Terminal Region of the
745 Transmembrane Domain of Signal-Anchored Proteins Play Critical Roles in Determining
746 Their Targeting Specificity to the Endoplasmic Reticulum or Endosymbiotic Organelles
747 in *Arabidopsis* Cells. *Plant Cell* 23: 1588–1607.

748 Lee, J.-Y. (2015). Plasmodesmata: a signaling hub at the cellular boundary. *Curr Opin Plant Biol*
749 27: 133–140.

750 Li, B.-C., Zhang, C., Chai, Q.-X., Han, Y.-Y., Wang, X.-Y., Liu, M.-X., Feng, H., and Xu, Z.-Q.
751 (2014). Plasmalemma localisation of DOUBLE HYBRID PROLINE-RICH PROTEIN 1
752 and its function in systemic acquired resistance of *Arabidopsis thaliana*. *Functional Plant*
753 *Biol.* 41: 768.

754 Li, T., Xiao, Z., Li, H., Liu, C., Shen, W., and Gao, C. (2020). A Combinatorial Reporter Set to
755 Visualize the Membrane Contact Sites Between Endoplasmic Reticulum and Other
756 Organelles in Plant Cell. *Front. Plant Sci.* 11: 1280.

757 Lim, G.-H., Shine, M.B., de Lorenzo, L., Yu, K., Cui, W., Navarre, D., Hunt, A.G., Lee, J.-Y.,
758 Kachroo, A., and Kachroo, P. (2016). Plasmodesmata Localizing Proteins Regulate
759 Transport and Signaling during Systemic Acquired Immunity in Plants. *Cell Host*
760 *Microbe* 19: 541–549.

761 Lu, H., Liu, Y., and Greenberg, J.T. (2005). Structure-function analysis of the plasma
762 membrane- localized *Arabidopsis* defense component ACD6. *Plant J* 44: 798–809.

763 Mészáros, T., Helfer, A., Hatzimasoura, E., Magyar, Z., Serazetdinova, L., Rios, G., Bardóczy,
764 V., Teige, M., Koncz, C., Peck, S., and Bögre, L. (2006). The *Arabidopsis* MAP kinase
765 kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. *The*
766 *Plant Journal* 48: 485–498.

767 Mine, A., Seyfferth, C., Kracher, B., Berens, M.L., Becker, D., and Tsuda, K. (2018). The
768 Defense Phytohormone Signaling Network Enables Rapid, High-Amplitude
769 Transcriptional Reprogramming during Effector-Triggered Immunity. *Plant Cell* 30:
770 1199–1219.

771 Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen
772 gene network of plants. *Trends in Plant Science* 9: 490–498.

773 Mohr, P.G. and Cahill, D.M. (2007). Suppression by ABA of salicylic acid and lignin
774 accumulation and the expression of multiple genes, in *Arabidopsis* infected with
775 *Pseudomonas syringae* pv. tomato. *Funct Integr Genomics* 7: 181–191.

776 Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997). Identification of prokaryotic
777 and eukaryotic signal peptides and prediction of their cleavage sites. *Protein Eng* 10: 1–6.

778 Peltier, J.-B., Ytterberg, A.J., Sun, Q., and van Wijk, K.J. (2004). New functions of the thylakoid
779 membrane proteome of *Arabidopsis thaliana* revealed by a simple, fast, and versatile
780 fractionation strategy. *J Biol Chem* 279: 49367–49383.

781 Pieterse, C.M., van Wees, S.C., Hoffland, E., van Pelt, J.A., and van Loon, L.C. (1996).
782 Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of
783 salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* 8: 1225–
784 1237.

785 Pieterse, C.M., van Wees, S.C., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek,
786 P.J., and van Loon, L.C. (1998). A novel signaling pathway controlling induced systemic
787 resistance in Arabidopsis. *Plant Cell* 10: 1571–1580.

788 Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., and Bakker,
789 P.A.H.M. (2014). Induced systemic resistance by beneficial microbes. *Annu Rev*
790 *Phytopathol* 52: 347–375.

791 Pitzschke, A., Datta, S., and Persak, H. (2014). Salt stress in Arabidopsis: lipid transfer protein
792 AZI1 and its control by mitogen-activated protein kinase MPK3. *Mol Plant* 7: 722–738.

793 Qutob, D. et al. (2006). Phytotoxicity and Innate Immune Responses Induced by Nep1-Like
794 Proteins. *Plant Cell* 18: 3721–3744.

795 Rekhter, D., Lüdke, D., Ding, Y., Feussner, K., Zienkiewicz, K., Lipka, V., Wiermer, M., Zhang,
796 Y., and Feussner, I. (2019). Isochorismate-derived biosynthesis of the plant stress
797 hormone salicylic acid. *Science* 365: 498–502.

798 Seguel, A., Jelenska, J., Herrera-Vásquez, A., Marr, S.K., Joyce, M.B., Gagesch, K.R., Shakoor,
799 N., Jiang, S.-C., Fonseca, A., Wildermuth, M.C., Greenberg, J.T., and Holuigue, L.
800 (2018). PROHIBITIN3 Forms Complexes with ISOCHORISMATE SYNTHASE1 to
801 Regulate Stress-Induced Salicylic Acid Biosynthesis in Arabidopsis. *Plant Physiol* 176:
802 2515–2531.

803 Serrano, M., Wang, B., Aryal, B., Garcion, C., Abou-Mansour, E., Heck, S., Geisler, M., Mauch,
804 F., Nawrath, C., and Métraux, J.-P. (2013). Export of Salicylic Acid from the Chloroplast
805 Requires the Multidrug and Toxin Extrusion-Like Transporter EDS5. *Plant Physiol.* 162:
806 1815–1821.

807 Siemens, J., Keller, I., Sarx, J., Kunz, S., Schuller, A., Nagel, W., Schmölling, T., Parniske, M.,
808 and Ludwig-Müller, J. (2006). Transcriptome Analysis of *Arabidopsis* Clubroots Indicate
809 a Key Role for Cytokinins in Disease Development. *MPMI* 19: 480–494.

810 Snider, C., Jayasinghe, S., Hristova, K., and White, S.H. (2009). MPEx: A tool for exploring
811 membrane proteins. *Protein Science* 18: 2624–2628.

812 Stringlis, I.A., Proietti, S., Hickman, R., Van Verk, M.C., Zamioudis, C., and Pieterse, C.M.J.
813 (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial
814 immune elicitors reveal signatures of adaptation to mutualists. *Plant J* 93: 166–180.

815 Thilmony, R., Underwood, W., and He, S.Y. (2006). Genome-wide transcriptional analysis of
816 the *Arabidopsis thaliana* interaction with the plant pathogen *Pseudomonas syringae* pv.
817 *tomato* DC3000 and the human pathogen *Escherichia coli* O157:H7. *The Plant Journal*
818 46: 34–53.

819 Tomizioli, M. et al. (2014). Deciphering Thylakoid Sub-compartments using a Mass
820 Spectrometry-based Approach. *Mol Cell Proteomics* 13: 2147–2167.

- Toulmay, A. and Prinz, W.A. (2011). Lipid transfer and signaling at organelle contact sites: the tip of the iceberg. *Curr Opin Cell Biol* 23: 458–463.
- Tripp, J., Inoue, K., Keegstra, K., and Froehlich, J.E. (2007). A novel serine/proline-rich domain in combination with a transmembrane domain is required for the insertion of AtTic40 into the inner envelope membrane of chloroplasts. *Plant J.* 52: 824–838.
- Wintermans, P.C.A., Bakker, P.A.H.M., and Pieterse, C.M.J. (2016). Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. *Plant Mol Biol* 90: 623–634.
- Yu, K., Soares, J.M., Mandal, M.K., Wang, C., Chanda, B., Gifford, A.N., Fowler, J.S., Navarre, D., Kachroo, A., and Kachroo, P. (2013). A Feedback Regulatory Loop between G3P and Lipid Transfer Proteins DIR1 and AZI1 Mediates Azelaic-Acid-Induced Systemic Immunity. *Cell Reports* 3: 1266–1278.
- Yu, Z. et al. (2020). The Brassicaceae-specific secreted peptides, STMPs, function in plant growth and pathogen defense. *J Integr Plant Biol* 62: 403–420.
- Zamioudis, C., Hanson, J., and Pieterse, C.M.J. (2014). β -Glucosidase BGLU42 is a MYB72-dependent key regulator of rhizobacteria-induced systemic resistance and modulates iron deficiency responses in Arabidopsis roots. *New Phytol* 204: 368–379.
- Zamioudis, C., Mastranesti, P., Dhonukshe, P., Blilou, I., and Pieterse, C.M.J. (2013). Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiol* 162: 304–318.
- Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Curr. Opin. Immunol.* 20: 10–16.
- Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S., and Mueller, M.J. (2012). Lipid Profiling of the Arabidopsis Hypersensitive Response Reveals Specific Lipid Peroxidation and Fragmentation Processes: Biogenesis of Pimelic and Azelaic Acid. *Plant Physiol.* 160: 365–378.
- Zuegge, J., Ralph, S., Schmuker, M., McFadden, G.I., and Schneider, G. (2001). Deciphering apicoplast targeting signals – feature extraction from nuclear-encoded precursors of *Plasmodium falciparum* apicoplast proteins. *Gene* 280: 19–26.

Figure legends

Figure 1. Targeting of Arabidopsis bipartite signal proteins to plastids. (A) Schematic of N-terminal bipartite signal of Arabidopsis HyPRPs/Apicomplexan proteins predicted by PATS. SP-like is signal-peptide like and cTP-like is chloroplast transit-peptide like sequence. (B) The percentage prediction output of PATS algorithm for each chromosome of Arabidopsis. The full-length sequences of Arabidopsis proteome (ATpepTAIR10) were analyzed by PATS for the predicted localization (%) to plastids. (C) Left panels: Laser scanning confocal micrographs of epidermal cells showing localization of non-HyPRP:GFP proteins encoded by the indicated genes transiently expressed in *N. benthamiana* leaves under Dex control. Samples were imaged 21-24h after infiltration with 30μM Dex. White arrowheads indicate chloroplasts (shown in insets). Scale bar is 20μm. Right Panel: Immunoblots of total (T) and chloroplast (Chl) fractions of *N. benthamiana* leaves transiently expressing At5g54170-encoded and STMP6 GFP-tagged proteins. Chloroplast fractions were isolated from leaves 21h after treatment with 30μM Dex. Signal was observed using anti-GFP antibody. ns is non-specific band; arrows indicate specific band. Coomassie blue (CB) staining shows the protein loading. (D) Targeting prediction of Arabidopsis HyPRPs by iterative TargetP/ChloroP and PATS algorithms. For iTargetP/iChloroP, the sequences without N-terminal SP-like region were used. The proteins with targeting studied herein are in bold. The asterisk indicates AZI1, a variant of signal-anchored protein, where SP-like domain is a transmembrane (TMD) (Cecchini et al., 2020). HyPRPs not predicted by either method to be plastid-localized are in the lower section of the panel.

Figure 2. Subcellular localization of HyPRP:GFPs in *N. benthamiana*. (A) Laser scanning confocal micrographs of epidermal cells showing localization of various HyPRP:GFP proteins transiently expressed in *N. benthamiana* leaves under Dex-inducible promoter. Samples were

imaged 21-24h after infiltration with 30μM Dex. White arrowheads indicate chloroplasts (shown in insets), red arrowheads show plasmodesmata-like structures/plasma membrane and yellow arrowheads show dense GFP signal in perinuclear ER. Scale bars are 20μm. GFP, green; chloroplast autofluorescence, blue. **(B)** Immunoblots of total (T) and chloroplast (chl) fractions of *N. benthamiana* leaves transiently expressing a subset of HyPRP:GFP proteins shown in **(A)**. Chloroplast fractions were isolated from leaves 21h after treatment with 30μM Dex. Signal was observed using anti-GFP or anti-HA antibody. Coomassie blue (CB) or Ponceau S (PS) staining shows the protein loading. **(C)** Normalized total spectra of peptides matching AZL3 in chloroplast membranes of constitutively defense active *ACD6-1HA* and WT. PHB3, a known chloroplast envelope protein is shown as control (Seguel et al., 2018). Total spectra normalization was analyzed by Scaffold software for each sample (WT or *ACD6-1HA*).

Figure 3. Chloroplast localization of HyPRP:GFP proteins in Arabidopsis transgenic lines.

Laser scanning confocal micrographs of epidermal cells showing chloroplast localization of indicated GFP tagged HyPRPs in 9-12 day-old Arabidopsis transgenic seedlings (hypocotyl). Plants were sprayed with 30μM Dex plus 0.04% Tween 20, 21-24h prior to imaging. Fluorescence intensity profiles in the merged images show the overlap of GFP (green) and chlorophyll (Chyll, blue) signal intensities along solid white lines (indicated by the red arrowheads). Note the overlap of GFP (green) and chlorophyll (Chyll, blue) intensities along chloroplast peripheries. The plastid-targeted GFP line (cTP-GFP) (Jiang et al., 2021) and untransformed WT (Col-0) serve as positive and negative controls, respectively, for this experiment. The images were analyzed by ImageJ (Fiji). Similar results were observed in two independent experiments. White arrowheads indicate chloroplast localization. Scale bar is 5μm. GFP, green; chloroplast autofluorescence, blue.

Figure 4. Chloroplast OEM localization of Arabidopsis HyPRPs in *N. benthamiana*. (A)

Immunoblots of total (T), membrane (M) and soluble (S) fractions of isolated chloroplasts from *N. benthamiana* leaves transiently expressing AZL14, AZL13, AZL2 and ELP tagged with GFP at C-terminal under Dex-inducible promoter. HyPRPs were detected with α -GFP antibody. α -Tic110 and α -cpHSP70 antibodies were used to detect the markers of inner envelope membrane, Tic110 and stroma, HSP70. (B) Immunoblots of intact chloroplast fractions expressing HyPRP:GFPs as in (A) treated with buffer (-) or thermolysin (+). The digestion of HyPRP:GFPs by thermolysin was evaluated by α -GFP antibody. Due to the higher observed expression of ELP:GFP compared to other HyPRP:GFPs, 1/4X total protein was loaded for this sample. A low exposure for ELP:GFP with α -GFP antibody is shown. The antibodies for OEM protein Toc75 and stromal HSP70 were used to detect the non-digestible controls. These experiments were repeated two-three times with similar results.

Figure 5. Subcellular localization of the truncated versions of HyPRP:GFPs in *N.*

benthamiana. (A) Schematic of a HyPRP showing N-terminal SP-like or HD, PRR and the C-terminal 8CM/LTP domain. The Δ PRR and Δ LTP versions are also depicted. (B) Confocal micrographs showing the localization of Δ PRR mutants of GFP-tagged HyPRPs in epidermal cells under Dex-inducible promoter. Lower panels show the enlarged views of chloroplasts. (C) Immunoblots of total (T) and chloroplast (Chl) fractions from *N. benthamiana* leaves transiently expressing HyPRP:GFP proteins as in (B). GFP-tagged HyPRPs were detected by α -GFP antibody. ns is non-specific band; arrow indicates specific band. The same blots stained with Coomassie blue (CB) show protein loading. Similar results were observed in two or more independent experiments for AZL14, AZL13 and ELP. For AZL2, the result of one experiment is shown. (D) Confocal micrograph of epidermal cells and immunoblot of total (T) and

chloroplast (Chl) fraction showing the subcellular localization of GFP-tagged ELP:ΔLTP. Inset shows signal associated with chloroplast. For the micrographs, GFP is green; chloroplast autofluorescence is blue. All scale bars are 20μm.

Figure 6. Expression, predicted co-expression and plastid envelope localization of *HyPRPs*.

(A) Network co-expression map of the *HyPRP* genes using ATTED-II (version 11.0; <https://atted.jp/>). Solid lines indicate co-expression between the genes included within the top 20 with higher score (Logit Score). Line thickness indicates the co-expression score. Condition-independent and condition-specific co-expression data is depicted. Green circles indicate *HyPRPs* targeted to the plastid envelope found in this and our previous (Cecchini et al., 2015) study. Dotted green circle indicates DRN1 plastid targeting evidenced in this and other studies (Peltier et al., 2004; Tomizioli et al., 2014). Light green circles indicate partial targeting to plastids. Inside the dashed box are the genes with no co-expression or a Logit score lower than 3. (B) Transcript levels of *HyPRPs* in *PmaDG6*-infected WT (Col-0) leaves 18hpi. PR1 induction shows that the defense response was activated in these plants. (C) Transcript levels of AZL3 in constitutively defense active mutant *ACD6-1HA*. In (B and C) *EF1α* was used as an internal reference. Error bars indicate SEM from two biological replicates and two technical replicates. *p<0.05, student's t-test.

Figure 7. SAR in Arabidopsis *HyPRP* mutants. (A, C) Growth of virulent bacteria *PmaDG3* in systemic leaves of WT (Col-0) and indicated mutants three days after infection. The plants were previously immunized in local leaves with avirulent *PmaDG6* or mock treated, 2 days prior to the secondary infection. The mean c.f.u. of two-three independent experiments are plotted. (B) Response gain of SAR due to *PmaDG6* immunization in (A). (D) Response gain of SAR due to *PmaDG6* immunization in (C). (E) PR1 induction at the indicated time points in the uninfected

systemic leaves of plants that were locally mock treated or immunized with *Pma*DG6. (F) Densitometric quantification of PR1 levels in immunized samples shown in (E). The PR1 levels are relative to the non-specific band in Coomassie blue (CB) stained membrane. The mean from two replicates is shown. Quantification was done by ImageJ (Fiji). (G) Growth of virulent bacteria *Pma*DG3 in systemic leaves of WT (Col-0) and Dex-inducible complementation lines, three days after infection. The plants were mock treated or immunized as in (A and C). 30μM Dex plus 0.04% Tween 20 was sprayed on local and systemic leaves 21h before local and distal infection, respectively. (H) RT-PCR of the indicated *HyPRP* transcripts in Dex-inducible complementation lines. Leaves were collected 21h after treatment with 30μM Dex (+) or no treatment (-). Expression of *EFLα* was used as control. (I) Response gain of SAR due to *Pma*DG6 immunization in (G). In the graphs showing ‘response gain’, error bars indicate uncertainties in the experiment; in all other graphs error bars show SEM. Different letters above the bars indicate statistically significant difference. Anova, SNK test or Student’s test, $p < 0.05$

Figure 8. ISR in *HyPRPs* mutants in Arabidopsis. (A) ISR was assayed by quantification of virulent bacteria *Pma*DG3 in leaves of plants, three days after infection. The roots of the plants were mock-treated or inoculated with *P. simiae* WCS417, 15-18 days before challenging the leaves with *Pma*DG3. Error bars show SEM. (B) Response gain of ISR due to *P. simiae* WCS417 immunization in roots of plants in (A). Error bars indicate uncertainties in the experiment. The mean c.f.u. of two independent experiments are plotted. Different letters above the bars indicate statistically significant difference by anova, SNK test, $p < 0.05$

Figure 9. Growth promotion and root-colonization of *HyPRP* mutants by *P. simiae* WCS417. (A) Representative images of WT (Col-0) and indicated mutants grown on agar plates without sugar and inoculated with mock or *P. simiae* WCS417 (WCS417). 15 day-old seedlings,

10dpi with WCS417 or mock are shown. **(B)** Fresh weight, **(C)** number of lateral roots and **(D)** primary root length in plants inoculated with mock or WCS417 as in **(A)**, n=15-20 plants. Combined data from three independent experiments is plotted. **(E)** Representative images of WT (Col-0) plants grown hydroponically in 48-well plates before (12 day-old) and 7dpi (19 day-old) with WCS417. **(F)** Number of c.f.u. of WCS417 in the wells or attached to the roots of WT and mutants. ‘No plant’ represents the number of c.f.u. of WCS417 in the well-media without any plant present. n=20-24 plants. In all graphs error bars are SEM and different letters above bars indicate statistical differences by anova, SNK test. p<0.05.

Figure 10. Speculative model and summary of roles of HyPRPs in microbial interactions.

(A) Cellular localization and proposed roles of HyPRPs in signal mobilization. Green color indicates organelles and structures in cells where HyPRPs are found. Arrows show the potential mobilization of signals by HyPRPs, either within a cell or locally near the infection (dashed line and *) or the long-distance mobilization of signals to distal tissue. Although leaf epidermal cells are depicted, similar localization patterns are expected for root cells. HyPRPs with * have roles in local responses to microbes (root or leaf colonization); most also have roles in longer distance responses as well (see part B). Data from this and previous work (Cecchini et al., 2015, 2020; Peltier et al., 2004; Tomizioli et al., 2014) is summarized. The cell on the left shows HyPRPs that likely locate to plastid thylakoids and/or OEMs as well as ER and PM/PD. HyPRPs that fit the left pattern, AZL3* and DRN1*, have positive roles. The cell on the right shows HyPRPs that locate to plastid OEMs as well as ER and/or PM/PDs. HyPRPs that fit the right pattern have positive roles (AZI1, EARLI1, AZL2, AZL13, and AZL14), a mainly negative role (ELP) or have unknown roles (CWLP, DHyPRP, AZI3, AZI4) in microbial responses. **(B)** Schematic of the steps at the whole plant level that are affected by one or more HyPRPs: 1, leaf colonization

by pathogen (DRN1, positive role); 2, increased immunity to distal infections after an immunizing infection at site 1 (SAR: AZI1, EARLI1, AZL2, AZL3, AZL13, positive roles; ELP, negative role); 3, growth of beneficial bacteria in association with roots (AZL3, AZL13, AZL14, positive roles); 4, stimulation of primary root growth in response to beneficial bacteria colonization of the root (ELP, AZL14, positive roles); 5, stimulation of lateral roots in response to beneficial bacteria colonization of the root (AZL13, AZL14, positive roles; ELP, negative role); 6, stimulation of whole plant growth by root colonization by beneficial bacteria (AZL3, AZL13, AZL14, positive roles; ELP, negative role); 7, increased immunity of aerial tissue (ISR after root colonization by beneficial bacteria: AZI1, EARLI1, AZL3, AZL13, AZL14, positive roles).

Supplementary Figure Legends

Supplementary Figure S1. Over-represented GO terms for biological processes in PATS plastid predicted proteins, location of HyPRPs in Arabidopsis genome and GFP cleavage in DRN1. (A) The most representative and significant GO terms sorted by fold enrichment are plotted. The size of dot indicates the number of genes associated with the process and the color of dot indicates the significance of the enrichment $\{-\log_{10}(\text{FDR-corrected } P\text{-values})\}$. The vertical dashed line represents a fold enrichment of 1. (B) Location of HyPRP genes depicted in Arabidopsis genome, made with the TAIR chromosome map tool (<https://www.arabidopsis.org/jsp/ChromosomeMap/tool.jsp>). (C) Confocal micrograph of epidermal cells and immunoblotting of total (T) fraction of *N. benthamiana* leaves transiently expressing DRN1-GFP showing cleavage of GFP. White arrowhead shows cytoplasm and white

arrow indicates nucleus. Scale bar is 20µm. Asterisk shows the free GFP detected by anti-GFP antibody. rbcL is the non-specific rubisco large subunit.

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Supplementary Figure S2. ClustalW alignment of Arabidopsis HyPRPs. The bars above the alignment indicate the putative SP-like or HD, PRR and 8CM/LTP domain. Black and grey boxes indicate identical or similar sequences, respectively. Red asterisks show the eight conserved Cys residues. AZL4 and AZL8 were excluded due to relatively larger sizes. ClustalW and BoxShade (Expasy tools) were used for the alignment.

Supplementary Figure S3. Local disease resistance of *HyPRP* mutants against *Pseudomonas syringae* pv. *maculicola* strains. Wild-type (Col-0) and indicated *HyPRP* mutants were infected with (A) *PmaDG3* (virulent strain) and (B) *PmaDG6* containing *avrRpt2* (SAR-inducing strain) at OD₆₀₀=0.0001 and growth measured 3 days post infiltration. Error bars indicate SEM. Figures represent the combined data from two independent experiments with n=7-8 for each replicate. Anova (SNK test) showed no statistically significant difference between WT and *HyPRP* mutants.

Supplementary Figure S4. SAR in *elp-2* allelic mutant and Dex-inducible complementation lines. SAR assay showing the growth of virulent bacteria *PmaDG3* in systemic leaves of (A) *elp-2* mutants and (B) Dex-inducible complementation lines (#2), three days after infection. The plants were immunized in local leaves with avirulent *PmaDG6* or mock treated 2 days prior to secondary challenge. The mean c.f.u. of one experiment is shown. (C) RT-PCR of the indicated

1036 Dex line (#2). Leaves were collected 21h after treatment with 30μM Dex plus 0.04% Tween 20
 1037 (+) or no treatment (-). Expression of *EFLα* was used as control. In (**A**, **B**), error bars show SEM.
 1038 Different letters above the bars indicate statistically significant difference by anova, SNK test,
 1039 p<0.05.
 1040

1041 **Table 1.** Expression changes of Arabidopsis *HyPRPs* to WCS417, pathogens and PAMPs

HyPRP	Pathogen/PAMP/Beneficial microbe-related	References
At1g12090 (ELP)	<i>CalCuV</i> ↓, <i>Pst</i> ↓, PAMPs↓, <i>Psm</i> ↓, flg22↓(roots)	(Ascencio-Ibáñez et al., 2008; Baum et al., 2019; Stringlis et al., 2018; Thilmony et al., 2006)
At1g12100 (AZL1)	flg22↓(roots)	(Stringlis et al., 2018)
At1g62500 (DEG27)	WCS417↑(roots), flg22↓(roots)	(Stringlis et al., 2018)
At1g62510 (AZL2)	<i>CalCuV</i> ↓, <i>Pst</i> ↓, PAMPs↓, <i>P. brassicae</i> ↑, WCS417↓ (roots), flg22 ↓ (roots)	(Ascencio-Ibáñez et al., 2008; Siemens et al., 2006; Stringlis et al., 2018; Thilmony et al., 2006; Zamioudis et al., 2014)
At2g10940 (AZL3)	<i>CalCuV</i> ↓, <i>Pst</i> ↓, PAMPs↓, <i>Psm</i> ↓, WCS417↓ (roots), flg22↓(roots)	(Ascencio-Ibáñez et al., 2008; Baum et al., 2019; Stringlis et al., 2018; Thilmony et al., 2006)
At2g45180 (DRN1)	<i>CalCuV</i> ↓, <i>Pst</i> ↓, PAMPs↓, <i>Psm</i> ↓, <i>B. cinerea</i> ↓, flg22↓(roots)	(Ascencio-Ibáñez et al., 2008; Dhar et al., 2020; Stringlis et al., 2018; Thilmony et al., 2006)
At3g22120 (CWLP)	<i>CalCuV</i> ↓, PAMPs↓, <i>Pst</i> DC3000/avrRpt2↓	(Ascencio-Ibáñez et al., 2008; Thilmony et al., 2006; Mine et al., 2018)
At3g22142 (AZL4)	WCS417↓ (roots), flg22↓(roots)	(Stringlis et al., 2018)
At4g00165 (AZL5)	WCS417↓ (roots), flg22↓(roots)	(Stringlis et al., 2018)
At4g12470 (AZI1)	<i>Psm</i> ↑, PAMPs ↑, WCS417↑ (roots) flg22PA ↑(roots)	(Cecchini et al., 2020; Jung et al., 2009; Stringlis et al., 2018; Zamioudis et al., 2014)
At4g12480 (EARLI1)	<i>CalCuV</i> ↑, <i>Pst</i> ↑, PAMPs ↑ flg22PA ↑ (1h roots)	(Ascencio-Ibáñez et al., 2008; Gupta and Senthil-Kumar, 2017; Stringlis et al., 2018; Thilmony et al., 2006)
At4g12490 (AZI3)	<i>CalCuV</i> ↑, PAMPs ↑, WCS417↑(roots), flg22 ↑(roots)	(Ascencio-Ibáñez et al., 2008; Stringlis et al., 2018; Thilmony et al., 2006; Zamioudis et al., 2014)
At4g12500 (AZI4)	PAMPs ↑, <i>Pst</i> ↑, flg22 ↑, Oomycete NLP↑	(Mohr and Cahill, 2007; Qutob et al., 2006; Thilmony et al., 2006)
At4g12510 (AZI5)	<i>CalCuV</i> ↓, WCS417↓ (roots), flg22↓ (roots)	(Ascencio-Ibáñez et al., 2008; Stringlis et al., 2018)
At4g12520 (AZI6)	WCS417↓ (roots), flg22↓ (roots), Chitin ↓ (roots)	(Stringlis et al., 2018)
At4g12530 (AZI7)	<i>Pst</i> ↓	(Howard et al., 2013)
At4g12545 (AIR1B)	flg22↓ (roots), Chitin↓(roots)	(Stringlis et al., 2018)
At4g12550 (AIR1)	flg22↓(roots), Chitin ↓ (roots)	(Stringlis et al., 2018)
At4g15160 (AZL6)	flg22↓ (roots)	(Stringlis et al., 2018)
At4g22460 (AZL7)	WCS417↓ (roots), flg22↓ (roots)	(Stringlis et al., 2018)

At4g22470 (DHYPRP1)	<i>Pst</i> ↑, flg22 ↑, WCS417↑ (roots), flg22↑(roots), flg22-PA↑(roots), Chitin ↑(roots)	(Li et al., 2014; Mészáros et al., 2006; Stringlis et al., 2018)
At4g22485 (AZL8)	No known change	
At4g22490 (AZL9)	<i>Pst</i> ↑	(Gupta and Senthil-Kumar, 2017)
At4g22517 (AZL10)	No known change	
At4g22520 (AZL11)	No known change	
At4g22610 (AZL12)	WCS417↓ (roots), flg22↓ (roots), Chitin ↑(roots)	(Stringlis et al., 2018)
At5g46890 (AZL13)	WCS417↓ (roots), flg22-417↓ (roots), flg22-PA↓ (roots)	(Stringlis et al., 2018)
At5g46900 (AZL14)	WCS417↓ (roots), flg22-417↓(roots), flg22-PA↓ (roots)	(Stringlis et al., 2018)

CalCuV is Cabbage leaf curl virus, *Psm* is *P. syringae* pv *maculicola* ES4326, *Pst* is *P. syringae* pv *tomato* DC3000, WCS417 is *P. simiae* WCS417, *B. cinerea* is *Botrytis cinerea*, PAMPs is pathogen-associated molecular patterns, *P. brassicae* is *Plasmodiophora brassicae*, flg22-PA is flg22 from *P. aeruginosa*, NLP is (Nep1)-like protein

C

Figure 1. Targeting of Arabidopsis bipartite signal proteins to plastids. (A) Schematic of N-terminal bipartite signal of Arabidopsis HyPRPs/Apicomplexan proteins predicted by PATS. SP-like is signal-peptide like and cTP-like is chloroplast transit-peptide like sequence. **(B)** The percentage prediction output of PATS algorithm for each chromosome of Arabidopsis. The full-length sequences of Arabidopsis proteome (ATpepTAIR10) were analyzed by PATS for the predicted localization (%) to plastids. **(C)** Left panels: Laser scanning confocal micrographs of epidermal cells showing localization of non-HyPRP:GFP proteins encoded by the indicated genes transiently expressed in *N. benthamiana* leaves under Dex control. Samples were imaged 21-24h after infiltration with 30μM Dex. White arrowheads indicate chloroplasts (shown in insets). Scale bar is 20μm. Right Panel: Immunoblots of total (T) and chloroplast (Chl) fractions of *N. benthamiana* leaves transiently expressing At5g54170-encoded and STMP6 GFP-tagged proteins. Chloroplast fractions were isolated from leaves 21h after treatment with 30μM Dex. Signal was observed using anti-GFP antibody. ns is non-specific band; arrows indicate specific band. Coomassie blue (CB) staining shows the protein loading. **(D)** Targeting prediction of Arabidopsis HyPRPs by iterative TargetP/ChloroP and PATS algorithms. For iTargetP/iChloroP, the sequences without N-terminal SP-like region were used. The proteins with targeting studied herein are in bold. The asterisk indicates AZI1, a variant of signal-anchored protein, where SP-like domain is a transmembrane (TMD) (Cecchini et al., 2020). HyPRPs not predicted by either method to be plastid-localized are in the lower section of the panel.

A **B** **C**

Figure 2. Subcellular localization of HyPRP:GFPs in *N. benthamiana*. (A) Laser scanning confocal micrographs of epidermal cells showing localization of various HyPRP:GFP proteins transiently expressed in *N. benthamiana* leaves under Dex-inducible promoter. Samples were imaged 21-24h after infiltration with 30μM Dex. White arrowheads indicate chloroplasts (shown in insets), red arrowheads show plasmodesmata-like structures/plasma membrane and yellow arrowheads show dense GFP signal in perinuclear ER. Scale bars are 20μm. GFP, green; chloroplast autofluorescence, blue. (B) Immunoblots of total (T) and chloroplast (chl) fractions of *N. benthamiana* leaves transiently expressing a subset of HyPRP:GFP proteins shown in (A). Chloroplast fractions were isolated from leaves 21h after treatment with 30μM Dex. Signal was observed using anti-GFP or anti-HA antibody. Coomassie blue (CB) or Ponceau S (PS) staining shows the protein loading. (C) Normalized total spectra of peptides matching AZL3 in chloroplast membranes of constitutively defense active *ACD6-1HA* and WT. PHB3, a known chloroplast envelope protein is shown as control (Seguel et al., 2018). Total spectra normalization was analyzed by Scaffold software for each sample (WT or *ACD6-1HA*).

Figure 3. Chloroplast localization of HyPRP:GFP proteins in *Arabidopsis* transgenic lines. Laser scanning confocal micrographs of epidermal cells showing chloroplast localization of indicated GFP tagged HyPRPs in 9-12 day-old *Arabidopsis* transgenic seedlings (hypocotyl). Plants were sprayed with 30µM Dex plus 0.04% Tween 20, 21-24h prior to imaging. Fluorescence intensity profiles in the merged images show the overlap of GFP (green) and chlorophyll (Chyll, blue) signal intensities along solid white lines (indicated by the red arrowheads). Note the overlap of GFP (green) and chlorophyll (Chyll, blue) intensities along chloroplast peripheries. The plastid-targeted GFP line (cTP-GFP) (Jiang et al., 2021) and untransformed WT (Col-0) serve as positive and negative controls, respectively, for this experiment. The images were analyzed by ImageJ (Fiji). Similar results were observed in two independent experiments. White arrowheads indicate chloroplast localization. Scale bar is 5µm. GFP, green; chloroplast autofluorescence, blue.

Figure 4. Chloroplast OEM localization of Arabidopsis HyPRPs in *N. benthamiana*. (A) Immunoblots of total (T), membrane (M) and soluble (S) fractions of isolated chloroplasts from *N. benthamiana* leaves transiently expressing AZL14, AZL13, AZL2 and ELP tagged with GFP at C-terminal under Dex-inducible promoter. HyPRPs were detected with α -GFP antibody. α -Tic110 and α -cpHSP70 antibodies were used to detect the markers of inner envelope membrane, Tic110 and stroma, HSP70. (B) Immunoblots of intact chloroplast fractions expressing HyPRP:GFPs as in (A) treated with buffer (-) or thermolysin (+). The digestion of HyPRP:GFPs by thermolysin was evaluated by α -GFP antibody. Due to the higher observed expression of ELP:GFP compared to other HyPRP:GFPs, 1/4X total protein was loaded for this sample. A low exposure for ELP:GFP with α -GFP antibody is shown. The antibodies for OEM protein Toc75 and stromal HSP70 were used to detect the non-digestible controls. These experiments were repeated two-three times with similar results.

Figure 5. Subcellular localization of the truncated versions of HyPRP:GFPs in *N. benthamiana*. (A) Schematic of a HyPRP showing N-terminal SP-like or HD, PRR and the C-terminal 8CM/LTP domain. The Δ PRR and Δ LTP versions are also depicted. (B) Confocal micrographs showing the localization of Δ PRR mutants of GFP-tagged HyPRPs in epidermal cells under Dex-inducible promoter. Lower panels show the enlarged views of chloroplasts. (C) Immunoblots of total (T) and chloroplast (Chl) fractions from *N. benthamiana* leaves transiently expressing HyPRP:GFP proteins as in (B). GFP-tagged HyPRPs were detected by α -GFP antibody. ns is non-specific band; arrow indicates specific band. The same blots stained with Coomassie blue (CB) show protein loading. Similar results were observed in two or more independent experiments for AZL14, AZL13 and ELP. For AZL2, the result of one experiment is shown. (D) Confocal micrograph of epidermal cells and immunoblot of total (T) and chloroplast (Chl) fraction showing the subcellular localization of GFP-tagged ELP: Δ LTP. Inset shows signal associated with chloroplast. For the micrographs, GFP is green; chloroplast autofluorescence is blue. All Scale bars are 20 μ m.

Figure 6. Expression, predicted co-expression and plastid envelope localization of *HyPRPs*. (A) Network co-expression map of the *HyPRP* genes using ATTED-II (version 11.0; <https://atted.jp/>). Solid lines indicate co-expression between the genes included within the top 20 with higher score (Logit Score). Line thickness indicates the co-expression score. Condition-independent and condition-specific co-expression data is depicted. Green circles indicate *HyPRPs* targeted to the plastid envelope found in this and our previous (Cecchini et al., 2015) study. Dotted green circle indicates DRN1 plastid targeting evidenced in this and other studies (Peltier et al., 2004; Tomizioli et al., 2014). Light green circles indicate partial targeting to plastids. Inside the dashed box are the genes with no co-expression or a Logit score lower than 3. (B) Transcript levels of *HyPRPs* in *PmaDG6*-infected WT (Col-0) leaves 18hpi. PR1 induction shows that the defense response was activated in these plants. (C) Transcript levels of AZL3 in constitutively defense active mutant *ACD6-1HA*. In (B and C) *EF1α* was used as an internal reference. Error bars indicate SEM from two biological replicates and two technical replicates. * $p < 0.05$, student's t-test.

A **B** **C** **D** **E** **F** **G** **H** **I**

Figure 7. SAR in Arabidopsis *HyPRP* mutants. (A, C) Growth of virulent bacteria *PmaDG3* in systemic leaves of WT (Col-0) and indicated mutants three days after infection. The plants were previously immunized in local leaves with avirulent *PmaDG6* or mock treated, 2 days prior to the secondary infection. The mean c.f.u. of two-three independent experiments are plotted. (B) Response gain of SAR due to *PmaDG6* immunization in (A). (D) Response gain of SAR due to *PmaDG6* immunization in (C). (E) PR1 induction at the indicated time points in the uninfected systemic leaves of plants that were locally mock treated or immunized with *PmaDG6*. (F) Densitometric quantification of PR1 levels in immunized samples shown in (E). The PR1 levels are relative to the non-specific band in Coomassie blue (CB) stained membrane. The mean from two replicates is shown. Quantification was done by ImageJ (Fiji). (G) Growth of virulent bacteria *PmaDG3* in systemic leaves of WT (Col-0) and Dex-inducible complementation lines, three days after infection. The plants were mock treated or immunized as in (A and C). 30μM Dex plus 0.04% Tween 20 was sprayed on local and systemic leaves 21h before local and distal infection, respectively. (H) RT-PCR of the indicated *HyPRP* transcripts in Dex-inducible complementation lines. Leaves were collected 21h after treatment with 30μM Dex (+) or no treatment (-). Expression of *EF1α* was used as control. (I) Response gain of SAR due to *PmaDG6* immunization in (G). In the graphs showing 'response gain', error bars indicate uncertainties in the experiment; in all other graphs error bars show SEM. Different letters above the bars indicate statistically significant difference. Anova, SNK test or Student's test, $p < 0.05$

Figure 8. ISR in *HyPRPs* mutants in *Arabidopsis*. (A) ISR was assayed by quantification of virulent bacteria *PmaDG3* in leaves of plants, three days after infection. The roots of the plants were mock-treated or inoculated with *P. simiae* WCS417, 15-18 days before challenging the leaves with *PmaDG3*. Error bars show SEM. (B) Response gain of ISR due to *P. simiae* WCS417 immunization in roots of plants in (A). Error bars indicate uncertainties in the experiment. The mean c.f.u. of two independent experiments are plotted. Different letters above the bars indicate statistically significant difference by anova, SNK test, $p < 0.05$

Figure 9. Growth promotion and root-colonization of *HyPRP* mutants by *P. simiae* WCS417. (A) Representative images of WT (Col-0) and indicated mutants grown on agar plates without sugar and inoculated with mock or *P. simiae* WCS417 (WCS417). 15 day-old seedlings, 10dpi with WCS417 or mock are shown. (B) Fresh weight, (C) number of lateral roots and (D) primary root length in plants inoculated with mock or WCS417 as in (A), n=15-20 plants. Combined data from three independent experiments is plotted. (E) Representative images of WT (Col-0) plants grown hydroponically in 48-well plates before (12 day-old) and 7dpi (19 day-old) with WCS417. (F) Number of c.f.u. of WCS417 in the wells or attached to the roots of WT and mutants. 'No plant' represents the number of c.f.u. of WCS417 in the well-media without any plant present. n=20-24 plants. In all graphs error bars are SEM and different letters above bars indicate statistical differences by anova, SNK test. $p < 0.05$.

Figure 10. Speculative model and summary of roles of HyPRPs in microbial interactions. (A) Cellular localization and proposed roles of HyPRPs in signal mobilization. Green color indicates organelles and structures in cells where HyPRPs are found. Arrows show the potential mobilization of signals by HyPRPs, either within a cell or locally near the infection (dashed line and *) or the long-distance mobilization of signals to distal tissue. Although leaf epidermal cells are depicted, similar localization patterns are expected for root cells. HyPRPs with * have roles in local responses to microbes (root or leaf colonization); most also have roles in longer distance responses as well (see part B). Data from this and previous work (Cecchini et al., 2015, 2020; Peltier et al., 2004; Tomizioli et al., 2014) is summarized. The cell on the left shows HyPRPs that likely locate to plastid thylakoids and/or OEMs as well as ER and PM/PD. HyPRPs that fit the left pattern, AZL3* and DRN1*, have positive roles. The cell on the right shows HyPRPs that locate to plastid OEMs as well as ER and/or PM/PDs. HyPRPs that fit the right pattern have positive roles (AZI1, EARLI1, AZL2, AZL13, and AZL14), a mainly negative role (ELP) or have unknown roles (CWLP, DHyPRP, AZI3, AZI4) in microbial responses. (B) Schematic of the steps at the whole plant level that are affected by one or more HyPRPs: 1, leaf colonization by pathogen (DRN1, positive role); 2, increased immunity to distal infections after an immunizing infection at site 1 (SAR: AZI1, EARLI1, AZL2, AZL3, AZL13, positive roles; ELP, negative role); 3, growth of beneficial bacteria in association with roots (AZL3, AZL13, AZL14, positive roles); 4, stimulation of primary root growth in response to beneficial bacteria colonization of the root (ELP, AZL14, positive roles); 5, stimulation of lateral roots in response to beneficial bacteria colonization of the root (AZL13, AZL14, positive roles; ELP, negative role); 6, stimulation of whole plant growth by root colonization by beneficial bacteria (AZL3, AZL13, AZL14, positive roles; ELP, negative role); 7, increased immunity of aerial tissue (ISR after root colonization by beneficial bacteria: AZI1, EARLI1, AZL3, AZL13, AZL14, positive roles).

Parsed Citations

- Albert, I., Hua, C., Nürnberger, T., Pruitt, R.N., and Zhang, L. (2020).** Surface Sensor Systems in Plant Immunity. *Plant Physiol* 182: 1582–1596.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Andersson, M.X., Goksör, M., and Sandelius, A.S. (2007).** Membrane contact sites: physical attachment between chloroplasts and endoplasmic reticulum revealed by optical manipulation. *Plant Signal Behav* 2: 185–187.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ascencio-Ibáñez, J.T., Sozzani, R., Lee, T.-J., Chu, T.-M., Wolfinger, R.D., Cella, R., and Hanley-Bowdoin, L. (2008).** Global Analysis of Arabidopsis Gene Expression Uncovers a Complex Array of Changes Impacting Pathogen Response and Cell Cycle during Geminivirus Infection. *Plant Physiol.* 148: 436–454.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Baum, S., Reimer-Michalski, E.-M., Bolger, A., Mantai, A.J., Benes, V., Usadel, B., and Conrath, U. (2019).** Isolation of Open Chromatin Identifies Regulators of Systemic Acquired Resistance. *Plant Physiol.* 181: 817–833.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Beckers, G.J.M. and Conrath, U. (2007).** Priming for stress resistance: from the lab to the field. *Curr Opin Plant Biol* 10: 425–431.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Beckers, G.J.M., Jaskiewicz, M., Liu, Y., Underwood, W.R., He, S.Y., Zhang, S., and Conrath, U. (2009).** Mitogen-activated protein kinases 3 and 6 are required for full priming of stress responses in Arabidopsis thaliana. *Plant Cell* 21: 944–953.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Berendsen, R.L., Pieterse, C.M.J., and Bakker, P.A.H.M. (2012).** The rhizosphere microbiome and plant health. *Trends Plant Sci* 17: 478–486.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bobik, K. and Burch-Smith, T.M. (2015).** Chloroplast signaling within, between and beyond cells. *Front Plant Sci* 6: 781.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bonnot, T., Gillard, M., and Nagel, D. (2019).** A Simple Protocol for Informative Visualization of Enriched Gene Ontology Terms. *BIO-PROTOCOL* 9.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bruce, B.D. (2001).** The paradox of plastid transit peptides: conservation of function despite divergence in primary structure. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1541: 2–21.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Bulgarelli, D., Schlaeppi, K., Spaepen, S., Ver Loren van Themaat, E., and Schulze-Lefert, P. (2013).** Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol* 64: 807–838.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Caplan, J.L., Kumar, A.S., Park, E., Padmanabhan, M.S., Hoban, K., Modla, S., Czymmek, K., and Dinesh-Kumar, S.P. (2015).** Chloroplast Stromules Function during Innate Immunity. *Dev Cell* 34: 45–57.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Carella, P. (2020).** Xylem-Mobile Oxylipins Are Critical Regulators of Induced Systemic Resistance in Maize. *Plant Cell* 32: 13–14.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cecchini, N., Song, Y., Roychoudhry, S., Greenberg, J., and Haney, C. (2019a).** An Improved Bioassay to Study Arabidopsis Induced Systemic Resistance (ISR) Against Bacterial Pathogens and Insect Pests. *BIO-PROTOCOL* 9.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cecchini, N.M., Roychoudhry, S., Speed, D.J., Steffes, K., Tambe, A., Zodrow, K., Konstantinoff, K., Jung, H.W., Engle, N.L., Tschaplinski, T.J., and Greenberg, J.T. (2019b).** Underground Azelaic Acid–Conferred Resistance to Pseudomonas syringae in Arabidopsis. *MPMI* 32: 86–94.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cecchini, N.M., Speed, D.J., Roychoudhry, S., and Greenberg, J.T. (2020).** Kinases and protein motifs required for AZ1 plastid localization and trafficking during plant defense induction. *Plant J.*
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Cecchini, N.M., Steffes, K., Schläppi, M.R., Gifford, A.N., and Greenberg, J.T. (2015).** Arabidopsis AZ1 family proteins mediate signal mobilization for systemic defence priming. *Nat Commun* 6: 7658.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Chen, Y.-L., Chen, L.-J., and Li, H.-M. (2016).** Polypeptide Transport-Associated Domains of the Toc75 Channel Protein Are Located in the Intermembrane Space of Chloroplasts. *Plant Physiol* 172: 235–243.
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Clough, S.J. and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735–743.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Dempsey, D.A., Vlot, A.C., Wildermuth, M.C., and Klessig, D.F. (2011). Salicylic Acid biosynthesis and metabolism. *Arabidopsis Book* 9: e0156.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Dhar, N., Caruana, J., Erdem, I., and Raina, R. (2020). An *Arabidopsis* DISEASE RELATED NONSPECIFIC LIPID TRANSFER PROTEIN 1 is required for resistance against various phytopathogens and tolerance to salt stress. *Gene*: 144802.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Efthimiadou, A., Katsenios, N., Chanioti, S., Giannoglou, M., Djordjevic, N., and Katsaros, G. (2020). Effect of foliar and soil application of plant growth promoting bacteria on growth, physiology, yield and seed quality of maize under Mediterranean conditions. *Sci Rep* 10: 21060.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Emanuelsson, O., Nielsen, H., Brunak, S., and von Heijne, G. (2000). Predicting Subcellular Localization of Proteins Based on their N-terminal Amino Acid Sequence. *Journal of Molecular Biology* 300: 1005–1016.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Emanuelsson, O., Nielsen, H., and Heijne, G.V. (1999). ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci.* 8: 978–984.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Fu, Z.Q. and Dong, X. (2013). Systemic acquired resistance: turning local infection into global defense. *Annu Rev Plant Biol* 64: 839–863.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Gupta, A and Senthil-Kumar, M. (2017). Transcriptome changes in *Arabidopsis thaliana* infected with *Pseudomonas syringae* during drought recovery. *Sci Rep* 7: 9124.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Haney, C.H., Samuel, B.S., Bush, J., and Ausubel, F.M. (2015). Associations with rhizosphere bacteria can confer an adaptive advantage to plants. *Nature Plants* 1: 15051.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hartmann, M., Kim, D., Bernsdorff, F., Ajami-Rashidi, Z., Scholten, N., Schreiber, S., Zeier, T., Schuck, S., Reichel-Deland, V., and Zeier, J. (2017). Biochemical Principles and Functional Aspects of Picecolic Acid Biosynthesis in Plant Immunity. *Plant Physiol.* 174: 124–153.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Helle, S.C.J., Kanfer, G., Kolar, K., Lang, A., Michel, A.H., and Kornmann, B. (2013). Organization and function of membrane contact sites. *Biochim Biophys Acta* 1833: 2526–2541.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hofmann, N.R. and Theg, S.M. (2005). Chloroplast outer membrane protein targeting and insertion. *Trends Plant Sci* 10: 450–457.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Howard, B.E., Hu, Q., Babaoglu, A.C., Chandra, M., Borghi, M., Tan, X., He, L., Winter-Sederoff, H., Gassmann, W., Veronese, P., and Heber, S. (2013). High-throughput RNA sequencing of pseudomonas-infected *Arabidopsis* reveals hidden transcriptome complexity and novel splice variants. *PLoS One* 8: e74183.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W., and Zimmermann, P. (2008). Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinformatics* 2008: 420747.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jaskiewicz, M., Conrath, U., and Peterhansel, C. (2011). Chromatin modification acts as a memory for systemic acquired resistance in the plant stress response. *EMBO Rep* 12: 50–55.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jayasinghe, S., Hristova, K., and White, S.H. (2001). Energetics, stability, and prediction of transmembrane helices¹¹ Edited by G. von Heijne. *Journal of Molecular Biology* 312: 927–934.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jiang, S.-C., Engle, N.L., Banday, Z.Z., Cecchini, N.M., Jung, H.W., Tschaplinski, T.J., and Greenberg, J.T. (2021). ALD1 accumulation in *Arabidopsis* epidermal plastids confers local and nonautonomous disease resistance. *J Exp Bot*.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jülke, S. and Ludwig-Müller, J. (2015). Response of *Arabidopsis thaliana* Roots with Altered Lipid Transfer Protein (LTP) Gene Expression to the Clubroot Disease and Salt Stress. *Plants* 5: 2.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Jung, H.W., Tschaplinski, T.J., Wang, L., Glazebrook, J., and Greenberg, J.T. (2009). Priming in Systemic Plant Immunity. *Science* 324: 89–91.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kachroo, A and Kachroo, P. (2020). Mobile signals in systemic acquired resistance. Curr Opin Plant Biol 58: 41–47.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kleffmann, T., Russenberger, D., von Zychlinski, A., Christopher, W., Sjölander, K., Gruissem, W., and Baginsky, S. (2004). The Arabidopsis thaliana Chloroplast Proteome Reveals Pathway Abundance and Novel Protein Functions. Current Biology 14: 354–362.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Küchler, M., Decker, S., Hörmann, F., Soll, J., and Heins, L. (2002). Protein import into chloroplasts involves redox-regulated proteins. EMBO J 21: 6136–6145.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee, D.W., Kim, J.K., Lee, S., Choi, S., Kim, S., and Hwang, I. (2008). Arabidopsis Nuclear-Encoded Plastid Transit Peptides Contain Multiple Sequence Subgroups with Distinctive Chloroplast-Targeting Sequence Motifs. Plant Cell 20: 1603–1622.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee, D.W., Yoo, Y.-J., Razzak, Md.A., and Hwang, I. (2018). Prolines in Transit Peptides Are Crucial for Efficient Preprotein Translocation into Chloroplasts. Plant Physiol. 176: 663–677.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee, J., Lee, H., Kim, J., Lee, S., Kim, D.H., Kim, S., and Hwang, I. (2011). Both the Hydrophobicity and a Positively Charged Region Flanking the C-Terminal Region of the Transmembrane Domain of Signal-Anchored Proteins Play Critical Roles in Determining Their Targeting Specificity to the Endoplasmic Reticulum or Endosymbiotic Organelles in Arabidopsis Cells. Plant Cell 23: 1588–1607.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lee, J.-Y. (2015). Plasmodesmata: a signaling hub at the cellular boundary. Curr Opin Plant Biol 27: 133–140.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li, B.-C., Zhang, C., Chai, Q.-X., Han, Y.-Y., Wang, X.-Y., Liu, M.-X., Feng, H., and Xu, Z.-Q. (2014). Plasmalemma localisation of DOUBLE HYBRID PROLINE-RICH PROTEIN 1 and its function in systemic acquired resistance of Arabidopsis thaliana. Functional Plant Biol. 41: 768.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Li, T., Xiao, Z., Li, H., Liu, C., Shen, W., and Gao, C. (2020). A Combinatorial Reporter Set to Visualize the Membrane Contact Sites Between Endoplasmic Reticulum and Other Organelles in Plant Cell. Front. Plant Sci. 11: 1280.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lim, G.-H., Shine, M.B., de Lorenzo, L., Yu, K., Cui, W., Navarre, D., Hunt, A.G., Lee, J.-Y., Kachroo, A., and Kachroo, P. (2016). Plasmodesmata Localizing Proteins Regulate Transport and Signaling during Systemic Acquired Immunity in Plants. Cell Host Microbe 19: 541–549.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Lu, H., Liu, Y., and Greenberg, J.T. (2005). Structure-function analysis of the plasma membrane- localized Arabidopsis defense component ACD6. Plant J 44: 798–809.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mészáros, T., Helfer, A., Hatzimasoura, E., Magyar, Z., Serazetdinova, L., Rios, G., Bardóczy, V., Teige, M., Koncz, C., Peck, S., and Bögre, L. (2006). The Arabidopsis MAP kinase kinase MKK1 participates in defence responses to the bacterial elicitor flagellin. The Plant Journal 48: 485–498.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mine, A., Seyfferth, C., Kracher, B., Berens, M.L., Becker, D., and Tsuda, K. (2018). The Defense Phytohormone Signaling Network Enables Rapid, High-Amplitude Transcriptional Reprogramming during Effector-Triggered Immunity. Plant Cell 30: 1199–1219.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. Trends in Plant Science 9: 490–498.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mohr, P.G. and Cahill, D.M. (2007). Suppression by ABA of salicylic acid and lignin accumulation and the expression of multiple genes, in Arabidopsis infected with Pseudomonas syringae pv. tomato. Funct Integr Genomics 7: 181–191.

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Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997). Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Eng 10: 1–6.

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Peltier, J.-B., Ytterberg, A.J., Sun, Q., and van Wijk, K.J. (2004). New functions of the thylakoid membrane proteome of Arabidopsis thaliana revealed by a simple, fast, and versatile fractionation strategy. J Biol Chem 279: 49367–49383.

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Pieterse, C.M., van Wees, S.C., Hoffland, E., van Pelt, J.A., and van Loon, L.C. (1996). Systemic resistance in Arabidopsis induced by

biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* 8: 1225–1237.

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Pieterse, C.M., van Wees, S.C., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J., and van Loon, L.C. (1998). A novel signaling pathway controlling induced systemic resistance in Arabidopsis. *Plant Cell* 10: 1571–1580.

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Pieterse, C.M.J., Zamioudis, C., Berendsen, R.L., Weller, D.M., Van Wees, S.C.M., and Bakker, P.A.H.M. (2014). Induced systemic resistance by beneficial microbes. *Annu Rev Phytopathol* 52: 347–375.

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Pitzschke, A., Datta, S., and Persak, H. (2014). Salt stress in Arabidopsis: lipid transfer protein AZ1 and its control by mitogen-activated protein kinase MPK3. *Mol Plant* 7: 722–738.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Qutob, D. et al. (2006). Phytotoxicity and Innate Immune Responses Induced by Nep1-Like Proteins. *Plant Cell* 18: 3721–3744.

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Rekhter, D., Lüdke, D., Ding, Y., Feussner, K., Zienkiewicz, K., Lipka, V., Wiermer, M., Zhang, Y., and Feussner, I. (2019). Isochorismate-derived biosynthesis of the plant stress hormone salicylic acid. *Science* 365: 498–502.

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Seguel, A., Jelenska, J., Herrera-Vásquez, A., Marr, S.K., Joyce, M.B., Gagesch, K.R., Shakoor, N., Jiang, S.-C., Fonseca, A., Wildermuth, M.C., Greenberg, J.T., and Holuigue, L. (2018). PROHIBITIN3 Forms Complexes with ISOCHORISMATE SYNTHASE1 to Regulate Stress-Induced Salicylic Acid Biosynthesis in Arabidopsis. *Plant Physiol* 176: 2515–2531.

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Serrano, M., Wang, B., Aryal, B., Garcion, C., Abou-Mansour, E., Heck, S., Geisler, M., Mauch, F., Nawrath, C., and Métraux, J.-P. (2013). Export of Salicylic Acid from the Chloroplast Requires the Multidrug and Toxin Extrusion-Like Transporter EDS5. *Plant Physiol* 162: 1815–1821.

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Siemens, J., Keller, I., Sarx, J., Kunz, S., Schuller, A., Nagel, W., Schmölling, T., Parniske, M., and Ludwig-Müller, J. (2006). Transcriptome Analysis of Arabidopsis Clubroots Indicate a Key Role for Cytokinins in Disease Development. *MPMI* 19: 480–494.

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Snider, C., Jayasinghe, S., Hristova, K., and White, S.H. (2009). MPEX: A tool for exploring membrane proteins. *Protein Science* 18: 2624–2628.

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Stringlis, I.A., Proietti, S., Hickman, R., Van Verk, M.C., Zamioudis, C., and Pieterse, C.M.J. (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. *Plant J* 93: 166–180.

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Thilmony, R., Underwood, W., and He, S.Y. (2006). Genome-wide transcriptional analysis of the Arabidopsis thaliana interaction with the plant pathogen Pseudomonas syringae pv. tomato DC3000 and the human pathogen Escherichia coli O157:H7. *The Plant Journal* 46: 34–53.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Tomizioli, M. et al. (2014). Deciphering Thylakoid Sub-compartments using a Mass Spectrometry-based Approach. *Mol Cell Proteomics* 13: 2147–2167.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Toulmay, A. and Prinz, W.A. (2011). Lipid transfer and signaling at organelle contact sites: the tip of the iceberg. *Curr Opin Cell Biol* 23: 458–463.

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Tripp, J., Inoue, K., Keegstra, K., and Froehlich, J.E. (2007). A novel serine/proline-rich domain in combination with a transmembrane domain is required for the insertion of AtTic40 into the inner envelope membrane of chloroplasts. *Plant J* 52: 824–838.

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Wintermans, P.C.A., Bakker, P.A.H.M., and Pieterse, C.M.J. (2016). Natural genetic variation in Arabidopsis for responsiveness to plant growth-promoting rhizobacteria. *Plant Mol Biol* 90: 623–634.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu, K., Soares, J.M., Mandal, M.K., Wang, C., Chanda, B., Gifford, A.N., Fowler, J.S., Navarre, D., Kachroo, A., and Kachroo, P. (2013). A Feedback Regulatory Loop between G3P and Lipid Transfer Proteins DIR1 and AZ1 Mediates Azelaic-Acid-Induced Systemic Immunity. *Cell Reports* 3: 1266–1278.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yu, Z. et al. (2020). The Brassicaceae-specific secreted peptides, STMPs, function in plant growth and pathogen defense. *J Integr Plant Biol* 62: 403–420.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zamioudis, C., Hanson, J., and Pieterse, C.M.J. (2014). β -Glucosidase BGLU42 is a MYB72-dependent key regulator of rhizobacteria-induced systemic resistance and modulates iron deficiency responses in Arabidopsis roots. *New Phytol* 204: 368–379.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zamioudis, C., Mastranesti, P., Dhonukshe, P., Blilou, I., and Pieterse, C.M.J. (2013). Unraveling root developmental programs initiated by beneficial *Pseudomonas* spp. bacteria. *Plant Physiol* 162: 304–318.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zipfel, C. (2008). Pattern-recognition receptors in plant innate immunity. *Curr. Opin. Immunol.* 20: 10–16.

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Zoeller, M., Stingl, N., Krischke, M., Fekete, A., Waller, F., Berger, S., and Mueller, M.J. (2012). Lipid Profiling of the Arabidopsis Hypersensitive Response Reveals Specific Lipid Peroxidation and Fragmentation Processes: Biogenesis of Pimelic and Azelaic Acid. *Plant Physiol.* 160: 365–378.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zuegge, J., Ralph, S., Schmuker, M., McFadden, G.I., and Schneider, G. (2001). Deciphering apicoplast targeting signals – feature extraction from nuclear-encoded precursors of *Plasmodium falciparum* apicoplast proteins. *Gene* 280: 19–26.

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