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An Emerging Role for Chloroplasts in Disease and Defense

Pradeep Kachroo,[1] Tessa M. Burch-Smith,[2] and Murray Grant[3]

[1]Department of Plant Pathology, University of Kentucky, Lexington, Kentucky 40546, USA

[2]Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee 37996, USA

[3]School of Life Sciences, University of Warwick, Coventry, CV4 7AL, UK; email: m.grant@warwick.ac.uk

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Abstract

Chloroplasts are key players in plant immune signaling, contributing to not only de novo synthesis of defensive phytohormones but also the generation of reactive oxygen and nitrogen species following activation of pattern recognition receptors or resistance (R) proteins. The local hypersensitive response (HR) elicited by R proteins is underpinned by chloroplast-generated reactive oxygen species. HR-induced lipid peroxidation generates important chloroplast-derived signaling lipids essential to the establishment of systemic immunity. As a consequence of this pivotal role in immunity, pathogens deploy effector complements that directly or indirectly target chloroplasts to attenuate chloroplast immunity (CI). Our review summarizes the current knowledge of CI signaling and highlights common pathogen chloroplast targets and virulence strategies. We address emerging insights into chloroplast retrograde signaling in immune responses and gaps in our knowledge, including the importance of understanding chloroplast heterogeneity and chloroplast involvement in intraorganellular interactions in host immunity.

INTRODUCTION

Our understanding of plant immunity has progressed rapidly over the past 30 years. The cloning of plant disease resistance (R) proteins was followed shortly thereafter by the discovery of membrane-bound pattern recognition receptors (PRRs), which detect highly conserved pathogen-associated molecular patterns (PAMPs), activating PAMP-triggered immunity (PTI), the first line of active plant defenses. Adapted pathogens have evolved elaborate strategies to attenuate PTI through the actions of small molecule and proteinaceous effectors. In response, plants engage R proteins that directly or indirectly recognize effectors or cellular perturbations induced by their activities in a process termed effector-triggered immunity (ETI). These initial discoveries founded the classical zig-zag model of plant immunity (57). It is now becoming increasingly clear that this distinction is too simple, and there is compelling evidence for interdependency between PRR and R proteins for effective activation of immunity (80, 134).

Numerous studies have since characterized PRRs and their cognate coreceptors, in parallel identifying downstream mitogen-associated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) signal transduction pathways. Genomic approaches drove an effectoromics research wave, leading to the characterization of PRRs and their coreceptors, membrane-associated PTI signaling complexes, and downstream signaling pathways that effectors target to suppress immunity [effector-triggered susceptibility (ETS)]. Until recently, however, these studies have predominantly focused on the nucleus and cell wall and their associated immune components, including activated kinase signaling and transcriptional reprogramming. Our knowledge of the cellular processes and organellular contributions to effective immunity and how they are modulated by pathogens remains rudimentary.

A pivotal and previously underappreciated role for chloroplasts in elaborating a variety of plant immune responses has recently emerged. Oxygenic photosynthesis and primary metabolism are core chloroplast functions. However, chloroplasts are also capable of integrating, decoding, and responding to environmental signals through complex reprogramming of primary and secondary metabolite synthesis, balancing phytohormone cross talk and intracellular communication via chloroplast-associated retrograde signaling (CRS). The number, mobility, and spatial separation of chloroplasts across the cell facilitate the capacity for individual or subsets of chloroplasts to detect and respond to signals and interact with other organelles such as mitochondria or peroxisomes in an autonomous manner. Chloroplasts are probably best known as the site of synthesis of phytohormone precursors, which underpin resistance to pathogens (15, 117). Chloroplasts are also key contributors to redox homeostasis, including the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which in turn influence nuclear gene expression via retrograde signaling (RS). Perturbation in multiple chloroplast processes can generate reactive species that generally serve to positively reinforce host disease resistance.

As a consequence of their pivotal role in orchestrating defense responses, chloroplasts are direct or indirect targets for a diverse range of pathogens. This review covers the pervasive contribution of chloroplasts to plant immunity, summarizing our current understanding of key signaling processes, including the importance of calcium, reactive species, transcriptional reprogramming, and RS. In addition, we cover in detail the neglected but important roles of enzymatic and nonenzymatically derived chloroplast fatty acids (FAs) and lipid signaling molecules in elaborating local and systemic acquired resistance (SAR). Finally, we highlight key research challenges that will help us better understand chloroplast immunity (CI). The review focuses on poststomatal CI and its manipulation by pathogens, although it is important to recognize the critical role chloroplasts play in stomatal immunity (90). The reader is pointed to recent reviews with more detailed literature overviews of CI, including pathogen effectors directly targeting chloroplasts or interacting with chloroplast proteins and chloroplastic ROS (69, 77, 81).

CHLOROPLAST SIGNALS MODULATING DEFENSE RESPONSES

Calcium and Reactive Oxygen Species as Initiating Signals for Chloroplast Immunity

Pathogen-triggered apoplastic ROS accumulation can initiate organellular redox-sensing mechanisms; however, a more comprehensive understanding of the timing of these responses is required (93). Emerging evidence suggests a biphasic interaction comprising the generation of extremely rapid early PTI-mediated Ca^{2+}/ROS signals. This may prime the chloroplast for subsequent transcriptional and physiological reprogramming (124). Apoplastic Ca^{2+} concentrations (~1 mM) differ by ~10^4 from cytoplasmic levels, potentially facilitating rapid changes in cytoplasmic Ca^{2+}. Although stromal free Ca^{2+} concentrations are also very low, the chloroplast thylakoid lumen acts as a key intracellular Ca^{2+} store, with high-capacity Ca^{2+}-binding proteins and H^+/Ca^{2+} antiporters maintaining concentrations above 15 mM (reviewed in 109, 121, 124, 125). PAMPs such as flg22 (22-aa peptide derived from the flagellin N terminus of *Pseudomonas aeruginosa*), chitin, cryptogein, and oligogalacturonides evoke a long-lasting but transient elevation of the stromal Ca^{2+} concentrations in tobacco (*Nicotiana tabacum*) and *Arabidopsis* (86, 108). Calcium-sensing receptor (CAS), a low-affinity and high-capacity Ca^{2+}-binding protein localized on the thylakoid membranes (133, 143), is a key modulator of plant immune responses. CAS-mediated signaling restricts both virulent and avirulent bacterial growth, and consequently both PTI and ETI are impaired in the *cas* loss-of-function mutant. ETI and PTI in the *cas* mutant are associated with reduced accumulation of salicylic acid (SA) and callose and suppressed defense gene expression (108). Notably, the prolonged increases in stromal calcium were only partially abolished in *cas* mutants (108). Therefore, a feedback retrograde signal may be operational to amplify this initial perception, possibly utilizing other Ca^{2+} signaling components.

Another early signal associated with PTI is elevated chloroplastic ROS (cROS), although the dynamics of cROS accumulation are slower than that of Ca^{2+} in the chloroplast. A *Pseudomonas syringae* pv. *tomato* strain DC3000 (hereafter, DC3000) type III secretion–deficient mutant (DC3000*hrpA*) and *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) challenge rapidly induced H_2O_2 in chloroplasts of *Arabidopsis* and wild rice (*Oryza meyeriana*), respectively (28, 89). Notably, co-infiltration of DC3000*hrpA* with DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea], which blocks photosynthetic electron transport (PET) between photosystem II (PSII) and plastoquinone (92), abolished cROS, suggesting cROS is generated via oxygen photoreduction downstream of PSII-producing superoxide/H_2O_2 (98).

Significant transcriptional overlap between exogenous H_2O_2 treatment and flg22 application also implicates a direct signaling role for apoplastic respiratory burst oxygenase homologs D/F (RBOHD/F)-generated H_2O_2 in PTI (124). RBOHD/F-induced H_2O_2 can be imported via aquaporins (12) and potentially activate CI. flg22 application leads to a decrease in nonphotochemical quenching (NPQ) within ~20 min and may protect plants against damage resulting from ROS formation. NPQ decreases were correlated with a concomitant decrease in PSII subunit protein S (PsbS). This decrease activates protective mechanisms to prevent excessive energy transfer from light-harvesting complexes entering the PET chain at PSII (45), possibly accounting for cROS generation.

Direct evidence for cROS production as a central player in CI immunity comes from a study describing delayed cell death during nonhost pathogen challenge of tobacco (*N. tabacum*) plants overexpressing a chloroplast-targeted flavodoxin. Infiltration with the nonhost *Xanthomonas campestris* pv. *vesicatoria* restricted O_2 and H_2O_2 formation, leading to typical HR-like lesions but defense gene expression was unaffected (158).

Pathogen-Associated Molecular Patterns Induce Chloroplast Relocalization of Membrane Proteins

Early PTI signaling events are not just restricted to small molecules. It was recently demonstrated that PTI triggers certain plasma membrane (PM) components, encoding both an *N*-myristoylation site and a chloroplast transit peptide signal, to relocalize to chloroplasts (88). The PM-associated *Arabidopsis* calcium-dependent protein kinase 16 (CPK16) is phosphorylated and relocalizes to chloroplasts, enhancing PTI and mechanistically linking PM and chloroplast signaling components (88). Unsurprisingly, plant pathogens have evolved to co-opt this pathway to promote virulence. Phosphorylation-dependent relocalization of the geminivirus *Tomato yellow leaf curl virus* (TYLCV) C4 protein from the PM to chloroplast was triggered upon activation of defense by its cognate replication-associated viral protein (Rep) or via application of the bacterial PAMP flg22 or the DAMP Pep1. In the chloroplast, TYLCV C4 associated with CAS, and this interaction contributed to a reduced cytosolic Ca^{2+} burst, reduced callose deposition, and increased susceptibility to DC3000 (88).

CHLOROPLAST PROTEINS WITH CENTRAL ROLES IN IMMUNITY

CAS: A Major Player in Chloroplast Immunity

CAS is phosphorylated in a calcium-dependent manner, implicating involvement of chloroplastic kinases in CAS signaling (124, 125), possibly via the protein kinase STATE TRANSITION 8 (STN8), which can phosphorylate CAS following its activation under high light (133). Interestingly, CDPK-dependent genes show a significant overlap with both flg22- and CAS-dependent flg22-responsive genes (124). Thus, it will be interesting to determine whether PTI-induced CPK16 relocalization to the chloroplast (88) links to CAS phosphorylation. CAS appears to mediate the generation of RS, possibly via chloroplast-derived ROS, which upregulate nuclear-encoded defense-related genes and largely suppress chloroplast-related genes, such as those associated with photosynthesis and plastid sigma factors (124, 125). These reflect typical PTI transcriptional responses (28, 74), positioning CAS as a core integrator of PTI responses. CAS is a crucial component of the machinery driving cyclic electron flow (CEF) around photosystem I (PSI) in *Chlamydomonas reinhardtii* (130). Thus, PAMP-induced elevated stromal Ca^{2+} drives CAS-enhanced changes in CEF activity, leading to cROS-generated RS. We speculate that MAPK3 and MAPK6 may be targets of these RS, which subsequently impact transcriptional repression of nuclear-encoded genes involved in photosynthesis.

Stromal calcium signaling mediated via CAS plays an important role in mediating broad-spectrum immunity. In addition to restricting virulent and avirulent bacterial growth (108), CAS positively regulates defense against the broad host range plant-pathogenic fungus *Sclerotinia sclerotiorum* in a Ca^{2+}-dependent manner. Like the geminivirus C4 protein, the *S. sclerotiorum* integrin-like effector *Ss*ITL directly targets CAS to suppress immunity. Consistently, *CAS* overexpression or *SsITL*-expressing transgenic plants were, respectively, more resistant or susceptible to *S. sclerotiorum* (129).

Thylakoid Formation 1: A Negative Regulator of Cell Death

Thylakoid formation 1 (Thf1) is another key chloroplast hub protein mediating both PTI and ETI responses. Thf1 is targeted by diverse pathogens (necrotrophic, biotrophic, viral). *Pyrenophora tritici-repentis* ToxA, a 178-aa secreted effector protein found in a range of necrotrophic fungal pathogens (87, 118), targets the chloroplast and interacts with the chloroplast ToxA binding protein 1 (ToxABP1), a wheat homolog of *Arabidopsis* Thf1. ToxA-ToxABP1 interaction triggers

a decrease in PSI and PSII protein complex abundance and accumulation of ROS, leading to severe necrosis (38, 85). Silencing ToxABP1 or restricting ROS accumulation limits the severity of necrosis (85). *Arabidopsis* Thf1 plays a critical role in controlling PSII-LHCII dynamics during dark-induced senescence and light acclimation (54). Thf1 prevents photooxidation of chloroplast components by positively regulating the accumulation of the membrane-bound hetero-hexameric FtsH ATP-dependent Zn-metalloprotease complex, which mediates the proteolysis of damaged PSII components, primarily the D1 reaction center subunit (8, 54, 128). *Arabidopsis thf1* mutants or virus-induced gene silencing of the tomato Thf1 ortholog *SlALC* resulted in enhanced lesion formation upon DC3000 challenge.

Sensitivity to ToxA in wheat is governed by an NLR-like resistance gene encoding locus, *Tsn1*, implicating a role for NLRs (nucleotide-binding–leucine-rich repeat) resistance proteins in mediating ToxA recognition. Although no direct interaction between Tsn1 and ToxA was detected in yeast two-hybrid experiments (38), the coiled-coil (CC) domain of Thf1 interacts with the CC domain of Solanaceae I2-like class CC-NLRs (111). Activation of another I2 CC-NLR protein, N′, which recognizes tobamovirus [e.g., *Tomato mosaic virus* (ToMV)] coat protein (119), destabilizes Thf1 in a light-dependent manner (48). Interestingly, the N′-Thf1 interaction occurs in the cytosol, analogous to the interaction between N [confers resistance to *Tobacco mosaic virus* (TMV)] and the chloroplast-targeted NRIP (N receptor-interacting protein 1) (17). N′ destabilization of Thf1 diminishes FtsH2/5 levels, impacting PSII repair and leading to ROS accumulation and presumably HR cell death. This parallels the finding that a tobacco chloroplast FtsH protease is destabilized during N-mediated resistance against TMV (120).

Notably, Thf1 overexpression repressed HR induced by both the N′-CC domain and full-length N′ following activation by ToMV CP but did not repress HR induced by the R proteins Bs2 and AvrBs2 (48). Further experimentation is necessary to determine whether Thf1 actually negatively regulates I2-like CC-NLRs, thus acting as a host susceptibility factor, as predicted by its genetic interaction with *Tsn1* (38). Alternatively, Thf1 may function as a negative regulator of cell death, whereby depletion of Thf1 through interaction with I2-CCs destabilizes PSII and induces cell death. Regardless, Thf1 clearly sits in a nexus between host manipulation by pathogens such as *P. tritici-repentis* to support a necrotrophic lifestyle and R-mediated immunity. The complexity of the activation and interaction of chloroplast components during disease and defense is summarized in **Figure 1**.

Effector-Triggered Immunity–Induced Singlet Oxygen at Photosystem II Generates Key Chloroplastic Signals

R proteins activate the HR upon direct recognition of pathogen effectors or indirectly through detection of activity or perturbation of cellular processes. HR induced by ETI requires, or is enhanced by, light (5, 6, 131), leading to pathogen containment and activation of systemic immunity. The HR is widely thought to be triggered by $^1O_2$ generation (**Figure 1**) (79; reviewed in 69) and most likely nitric oxide (NO) production (154–156), which leads to lipid peroxidation (51, 99) generating important chloroplast-derived lipid signaling molecules essential to the establishment of systemic immunity (discussed in detail below).

During ETI, it is likely that the reduction in photosynthetic activity (10) and associated deconstruction of the photosynthetic apparatus leads to $^1O_2$-mediated lipid peroxidation (51) (**Figure 1**). For example, DC3000*avrRpm1*-induced HR is enhanced by increased light intensity and associated with PSII light-harvesting complex disruption and accumulation of the chlorophyll catabolite pheophorbide, a potent photosensitizer that generates $^1O_2$ (103).

A detailed lipidomics study of ETI elicited in *Arabidopsis* via a challenge with DC3000*avrRpm1* revealed complex lipid profiles. Significant levels of oxidatively modified plastid lipids were

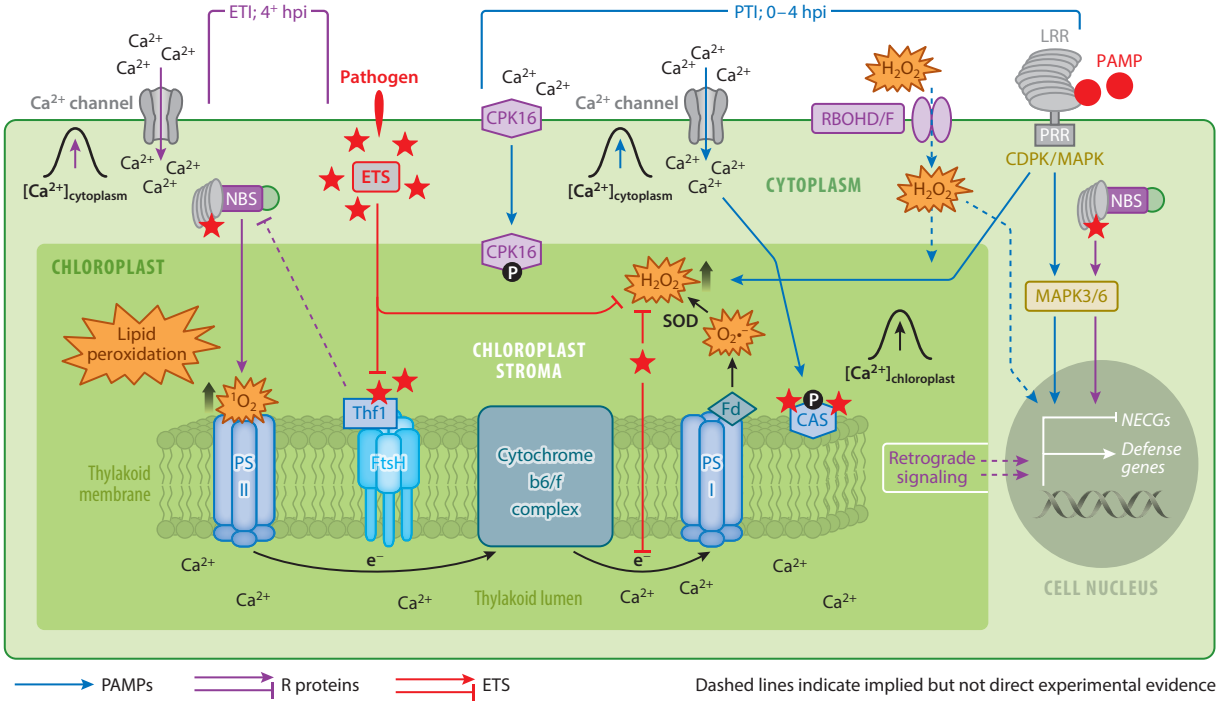

Figure 1

Schematic summary of immune responses impacting the chloroplast. PRR recognition of PAMPs (*blue arrows*) induces rapid local phosphorylation events and H_2O_2 production by RBOH and increases in cytosolic Ca^{2+} concentrations. An increased $[Ca^{2+}]_{cyt}$ stimulates CAS leading to its phosphorylation and increased $[Ca^{2+}]_{stromal}$. Increased $[H_2O_2]_{cyt}$ may also stimulate early CI immune events. Activated PTI induces a subset of membrane-associated proteins (e.g., CPK16) to translocate to the chloroplast. These collective events lead to a strong PTI induction of cROS. PTI also activates MAPK3/6 signaling, leading to repression of NECGs and induction of defense genes. Subsequently, effectors interact directly (e.g., via CAS and Thf1) or indirectly (e.g., modulating electron transport from PSII to PSI) target chloroplast function (ETS; *red arrows*) and attenuate cROS. Some effectors trigger R proteins (*purple arrows*), leading to a massive induction of cROS, most likely $^1O_2$ at PSII, which leads to lipid peroxidation and cell death. R proteins also stimulate sustained activation of MAPK3 and MAPK6 signaling to enhance defense gene expression. Lines ending with arrowheads indicate positive influence; lines ending with blunt stops indicate inhibitory influence. Abbreviations: CAS, calcium sensing; CDPK, calcium-dependent protein kinase; CI, chloroplast immunity; CPK16, calcium protein kinase 6; cROS, chloroplastic reactive oxygen species; e^-, electron; ETI, effector-triggered immunity; ETS, effector-triggered susceptibility; Fd, ferrodoxin; hpi, hours post-inoculation; FtsH, ATP-dependent zinc-metalloprotease; LRR, leucine-rich repeat; MAPK, mitogen-associated protein kinase; NBS, nucleotide-binding site; NECGs, nuclear-encoded chloroplast genes; $^1O_2$, singlet oxygen; $O_2^{\cdot-}$, superoxide; P, phosphate; PAMP, pathogen-associated molecular pattern; PRR, pattern recognition receptor; PSI/II, photosystem I/II; PTI, PAMP-triggered immunity; RBOHD/F, respiratory burst homolog D/F; SOD, superoxide dismutase; Thf1, thylakoid 1.

accompanied by a dramatic increase in nonoxidized lipids within 5–10 h, a timing consistent with earlier detection of DC3000*avrRpm1*-induced biophotons (9), which are indicative of lipid oxidation (52). Strikingly, and somewhat contradictory to traditional dogma associating jasmonic acid (JA) with antagonism of SA-mediated defenses, 13-LOX-derived JA increased dramatically (>75-fold) within 5–10 h of a DC3000*avrRpm1* challenge. Interestingly, AzA (azelaic acid) increased between 5 and 10 hpi (hours post-inoculation); however, its precursor, 9-oxo nonanoic acid (ONA), could not be detected and its increase was not compromised in the AzA-Induced 1 lipid transfer protein (LTP) mutant background (58, 152, 157).

Notably, the major oxidized lipids identified were predominantly confined to chloroplast-derived monogalactosyldiacylglycerols (MGDGs), digalactosyldiacylglycerols (DGDGs), and triacylglycerols, with substantial contributions from 13-lipoxygenase (LOX2), which was solely responsible for a massive increase in the plastid-localized galactolipid-derivative arabidopsides (3) and free radical–catalyzed lipid oxidation (138, 157). The importance of chloroplast nitric oxide and FA-derived lipids as immune signaling molecules has been afforded limited attention, and we address this in the following section.

CHLOROPLAST-DERIVED FATTY ACIDS AND LIPIDS: NEGLECTED BUT CRITICAL TO EFFECTIVE DEFENSE

Fatty Acids and Chloroplastic Lipids in Plant Defense

FAs are important energy reserves and essential components of membrane lipids that function as signaling molecules as well as precursors for other important bioactive molecules. Both JA and AzA are derived from FAs present on chloroplastic membrane lipids, highlighting the importance of chloroplastic lipids in signaling. AzA is derived from unsaturated 18-carbon (C) FAs that contain a double bond on C9 ($^{\Delta 9}$) (153). JA, a key member of the chemically diverse family of oxygenated FAs called oxylipins, is derived from hexadecenoic (16:3; C16 FA with 3 double bonds) or linolenic acid (18:3$^{\Delta 9,12,15}$). Other intermediates of the JA biosynthetic pathway, including oxo-phytodienoic acid (OPDA) and dinor (dn)-OPDA also function as signaling molecules. Along with 16:3 and 18:3, other common FAs in plants are palmitic (16:0), stearic (18:0), oleic (18:1$^{\Delta 9}$), and linoleic (18:2$^{\Delta 9,12}$) acids (96), of which several are involved in plant defense signaling (137). Chloroplastic trienoic acids are also required for ozone- and pathogen-induced accumulation of ROS. Conversely, 18:3 promotes the activity of the ROS-generating NADPH oxidase (149).

Phosphatidic acid (PA) produced from glycerol-3-phosphate (G3P) in the chloroplast is converted to phosphatidylglycerol and diacylglycerol, and the latter is a precursor for the synthesis of plastidal lipids MGDG and DGDG and sulfolipids. This chloroplastic pathway of glycerolipid synthesis is commonly referred to as the prokaryotic pathway. MGD1, located on the inner envelope of the chloroplasts, is one of three MGD isoforms in *Arabidopsis* accounting for most of the cellular pool of MGDG (4). MGDG is subsequently converted to DGDG via digalactosyl synthase (**Figure 2**).

Oleate- and Nitric Oxide–Regulated Signaling in Chloroplasts

Stearoyl acyl carrier protein (ACP) desaturases (SACPDs) catalyze the conversion of stearoyl-ACP to olealyl-ACP by introducing a *cis* double bond between C9 and C10 of the acyl chain. In *Arabidopsis*, mutations in one of the seven SACPD isoforms (SSI2/FAB2) result in constitutive defense activation and enhanced resistance to bacterial and oomycete pathogens, likely because SSI2 is the major isoform exhibiting the highest activity (62, 64, 65). SACPD enzymes are conserved between monocots and dicots and loss-of-function of SACPD is associated with constitutive defense activation in other plants (56, 59, 64). Characterization of *ssi2* suppressors has shown that the defense phenotypes in *ssi2* plants are associated with reduced 18:1 levels (59, 62, 146). Suppressor mutations in chloroplast-localized *ACT1* (encoding G3P acyltransferase), *GLY1* (encoding G3P dehydrogenase), and *ACP4* (encoding one of six ACP isoforms) increase 18:1 levels by disrupting 18:1 acylation of G3P and thereby restore wild type–like defense and morphological phenotypes in the *ssi2* background. Constitutive defense signaling in the *ssi2* mutant can also be restored by a mutation in the chloroplast-localized GTPase nitric oxide associated 1 (NOA1) (84, 97). In wild-type plants, 18:1 binding to NOA1 represses its GTPase activity and promotes its degradation in a protease-dependent manner (24, 84), ensuring that NO remains at basal

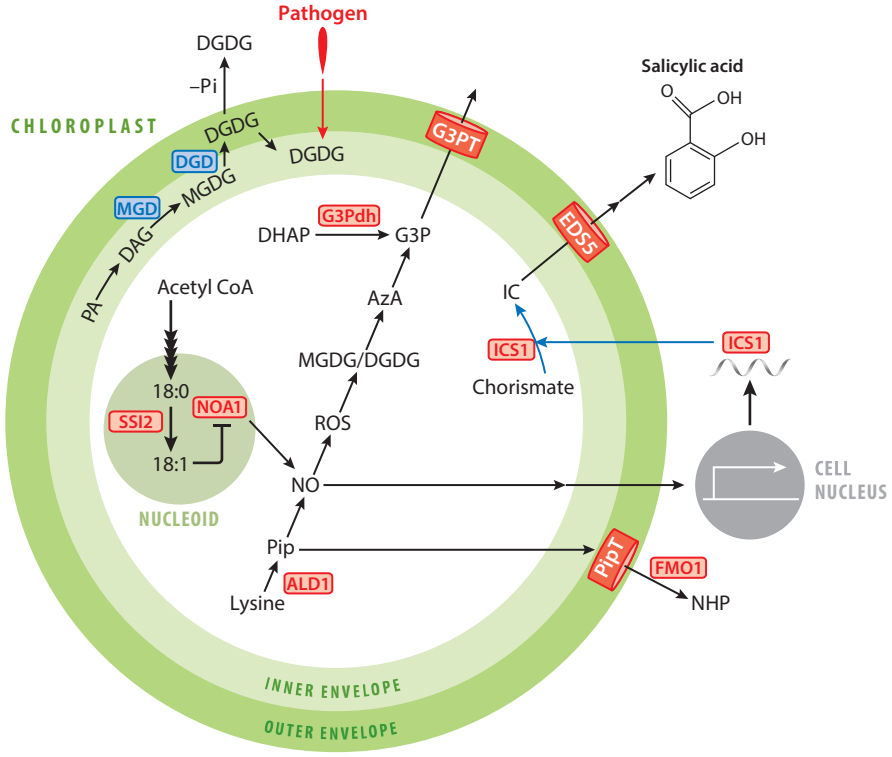

Figure 2

Schematic representation showing the relationships between fatty acids, lipids, and chemical signals involved in systemic acquired resistance (SAR). Monogalactosyl synthase (MGD) and digalactosyl synthase (DGD) are present in the inner and outer envelopes of chloroplasts, respectively, where they catalyze biosynthesis of monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG). DGDG is exported out of chloroplasts under phosphate-limiting conditions and substituted for phospholipids in extraplastidal membranes, thereby facilitating recycling of phosphate. DGDG is also required for pathogen-induced biosynthesis of nitric oxide (NO) and salicylic acid (SA). The *dgd1* mutant plants are unable to induce nuclear genes responsible for SA biosynthesis, suggesting that the DGDG is required for pathogen-induced retrograde signaling, leading to transcription of the SA biosynthesis gene isochorismate synthase (*ICS1*) in the nucleus (Nuc). The NO biosynthesis in chloroplasts is dependent on Nitric Oxide Associated 1 (NOA1), which localizes to chloroplastic nucleoids. NOA1 binds to oleic acid (18:1) and 18:1 negatively regulates the stability of NOA1. Consequently, a mutation in *SSI2*-encoded SACPD, which desaturates 18:0 to 18:1, is associated with constitutive accumulation of NO. An increase in pipecolic acid (Pip) also increases NO levels. The relationship between 18:1- and Pip-induced NO biosynthesis remains unclear. Pip is converted to *N*-hydroxy Pip (NHP) by FMO1 monooxygenase. NO triggers synthesis of reactive oxygen species (ROS), which in turn catalyze oxidation of $C18^{\Delta 9}$ unsaturated FAs present on MGDG and DGDG galactolipids to generate azelaic acid (AzA). AzA triggers biosynthesis of glycerol-3-phosphate (G3P) via upregulation of genes encoding G3P biosynthetic enzymes. Transport of G3P, Pip, and SA is mediated via membrane-localized transporters and of these only EDS5-mediated transport of the SA precursor isochorismate (IC) has been characterized thus far. Abbreviations: DAG, diacylglycerol; DHAP, dihydroxyacetone phosphate; G3Pdh, G3P dehydrogenase; G3PT, putative G3P transporter; PA, phosphatidic acid; Pi, phosphate; PipT, putative Pip transporter; SACPD, stearoyl acyl carrier protein desaturase.

levels. NOA1 is likely present in an 18:1-rich environment because NOA1 and SSI2 colocalize to nucleoids within the chloroplast (**Figure 2**). This suggests subcompartmentalization of the SSI2-generated 18:1 ACP4, which is then likely exported to the stroma to initiate lipid biosynthesis via ACT1-catalyzed acylation of G3P. The reduced 18:1 levels in *ssi2* plants remove repression of NOA1 activity and levels, resulting in chloroplastic NO accumulation followed by induction of NO-responsive nuclear gene expression, including multiple *R* genes (84). NO-mediated RS leading to constitutive defense activation of *ssi2* can be suppressed by simultaneous mutations in *ENHANCED DISEASE SUSCEPTIBILITY* (*EDS*) *1* and SA biosynthetic genes, which act redundantly (25, 135).

Chloroplasts Are an Important Hub for Systemic Acquired Resistance

SAR is intimately associated with activation of ETI and involves the systemic movement of signals from the site of primary infection to noninfected portions of the plant, which in turn confers broad-spectrum disease resistance against secondary infections (60, 63, 144). The onset of SAR involves the generation of a mobile signal(s) at the site of local infection, and this occurs within 4–6 h of primary infection (60). The signal(s) then translocates to the systemic tissues, where it primes for the activation of defense response in conjunction with secondary infections. Several chemicals have been identified as potential early SAR-inducing mobile signals and notably most of these signals are associated with chloroplasts. For instance, the C18 unsaturated FAs containing a double bond on C9 and present on MGDG and DGDG serve as a precursor for the SAR signal AzA (43, 153, 158). Notably, *dgd1* plants are unable to generate either SA or NO after pathogen infection and are impaired in the induction of the SA biosynthesis gene *ICS1* (43). A requirement for DGDG in pathogen-triggered induction of the nuclear gene *ICS1* suggests that DGDG is required for CRS. Interestingly, a mutation in CAS also impairs pathogen-mediated induction of *ICS1* (108), supporting the involvement of calcium signaling in CRS. Furthermore, CAS is also required for the full activation of MAPK3 and MAPK6 (47), which in turn are required for normal SAR but not for SA biosynthesis (7, 142). Together, these results suggest a possible link between CAS- and DGDG-mediated RS. Notably, the terminal galactose sugar on DGDG is essential for NO or SA accumulation, and replacing this galactose with a glucose moiety inhibits SAR (43, 94). Interestingly, petiole exudates from pathogen-infected *dgd1* plants confer SAR on wild-type plants, suggesting that these plants generate an early mobile signal(s) that confers SAR on plants with normal DGDG levels (79).

The nine-carbon FA AzA is generated by the oxidative cleavage of the Δ9 double bond on C18 FAs (153). This oxidation is facilitated by ROS, and different ROS species function additively to catalyze this cleavage to form monocarboxylic acid 9-ONA, an immediate precursor of AzA (145). As expected, increased accumulation of AzA after avirulent infection correlates with higher levels of 18:1, 18:2, and 18:3 free FAs (153). Because genetic studies showed that DC3000*avrRpm1*-elicited AzA increases were independent of 9-LOX and unaffected in the *fad3 fad7 fad8* triple mutant and the *azi1* LTP mutant (157), the source of AZA biosynthesis during ETI remains obscure.

Inoculation with an avirulent pathogen also increases chloroplastic NO levels, which are associated with a reduction in plastidal 18:1 levels (84). The SAR-associated pathogen-induced increase in NO is dependent on NOA1 and one of the nitrate reductases, NIA1 or NIA2, which act in a redundant manner. Thus, only *noa1 nia1* or *noa1 nia2* double mutant plants are compromised in pathogen-induced NO accumulation and SAR (84, 138). Together, these results suggest that specific changes in 18:1 levels in different subcellular and suborganellular compartments regulate pathogen-induced NO levels and thereby SAR. Mutations in *FAD2*, *3*, *4*, *5*, 7, and *8* encoding membrane-bound FA desaturases do not affect SAR (147), suggesting that desaturation of FAs on the membrane lipids does not contribute to SAR.

AzA contributes to SAR by inducing biosynthesis of G3P (153), which acts downstream of AzA and compensates for AzA deficiency in *dgd1* plants (43). G3P is synthesized via the glycerol kinase (GK)-mediated phosphorylation of glycerol (66) or the G3P dehydrogenase (G3Pdh)-mediated reduction of dihydroxyacetone phosphate (61). The *Arabidopsis* genome encodes multiple G3Pdh isoforms that localize to chloroplasts, mitochondria, or the cytosol. A mutation in either chloroplastic G3Pdh isoform GLY1 or the cytosolic enzyme GK impairs G3P biosynthesis and thereby SAR (21). Interestingly, G3P is also required for foliar resistance induced in response to rhizobia that cannot nodulate certain hosts because of genetic incompatibility (122). Furthermore, during SAR as well as rhizobia-mediated root-shoot-root signaling, G3P translocates primarily in the form of an unidentified derivative (21, 122). SAR-associated translocation of G3P is dependent on the LTP defective in resistance 1 (DIR1) (83), and intercellular transport of these occurs preferentially through the plasmodesmata (PD) (18, 75). Likewise, LTP-like AZI1, which associates with DIR1 and is required for G3P-mediated SAR, also localizes to the PD (19, 153). A small portion of AZI1 is also detected in the chloroplasts, and the chloroplastic AZI1 levels increase in response to pathogen infection (19, 70). Notably, AZI1 is dependent on PD-localizing proteins (PDLPs) 1 and 5 for its stability (see below) and localizes primarily to the chloroplast in SAR-compromised *pdlp1* or *pdlp5* mutant plants (75).

Along with SA, G3P, AzA, NO, and ROS, the nonprotein amino acid, pipecolic acid (Pip) is an important chloroplast-derived SAR inducer (105). In plants, biosynthesis of Pip is catalyzed by ALD1 (AGD2-like defense response protein)-encoded aminotransferase. ALD1 converts lysine to ε-amino-α-keto caproic acid, which then cyclizes to form Δ1-piperideine-2 carboxylic acid (P2C) (34, 49). P2C is subsequently converted to Pip by ornathine cyclodeaminase (encoded by SARD4). Notably, *sard4* plants accumulate Pip after infection, albeit at lower levels compared to wild-type plants. This suggests that a pathogen-inducible factor can generate Pip in the infected leaves of *sard4* mutant, and enzymes other than SARD4 are involved in conversion of P2C to Pip. Pip is converted to *N*-hydroxy Pip via flavin monooxygenase (FMO1) (22, 50), which in turn is conjugated to a glucoside derivative (*N*-OGlc-Pip) via an as yet unknown enzyme (22). Pip functions upstream of G3P and confers SAR by inducing the biosynthesis of free radicals (72, 138, 139). Notably, plants defective in NO, ROS, G3P, or SA biosynthesis contain reduced levels of Pip in their distal leaves, even though they accumulate wild type–like Pip in their infected leaves. This suggests that de novo biosynthesis of Pip in distal tissues is dependent on normal transport of SA and G3P.

Chloroplast Signaling Modulates Nuclear-Encoded Chloroplast Genes

Nuclear-encoded chloroplast genes (NECGs) are rapidly suppressed by activated MAPK3 and MAPK6 as part of a PTI response. This occurs after PTI induces Ca^{2+} signals but prior to detectable cROS and is evident within 2 hpi of a PTI inducing a DC3000*hrpA* challenge (13, 28, 74). Despite accounting for ∼13% of the *Arabidopsis* transcriptome, NECGs represent more than 30% of differentially suppressed genes up to 17.5 hpi, a subset of which are modulated by effectors delivered by DC3000 (28). Suppression of NECGs appears to be a common stress response. A meta-analysis of rice transcriptomic data sets monitoring biotic and abiotic stresses identified a core set of 85 photosynthesis-related genes suppressed across a diverse set of experiments (23). Such rapid transcriptional suppression of NECGs is most likely programmed through perception of a retrograde stress signal(s), given the few changes in photosynthetic parameters following DC3000*hrpA* challenge (28). It is currently unclear whether this is a strategy to maximize resource-use efficiency or reduce potential effector targets in the cytoplasm/chloroplast.

MAPKs are initially rapidly activated following PAMP recognition and subsequent apoplastic ROS burst (91) and may drive PTI-induced suppression of NECGs. It is possible that early

PTI-induced Ca^{2+} transients in the chloroplast may elicit a feedback RS to amplify initial PRR activation and MAPK3 and MAPK6 activity. There is an emerging body of evidence that sustained activation of the MAPK3 and MAPK6 pathways is also involved in modulating *NECG*s and orchestrating ETI responses following R-protein activation that leads to elevated cROS (79, 127) (**Figure 1**).

Chloroplast-to-Nucleus Retrograde Signaling

In response to environmental and developmental perturbations, chloroplasts generate signals that act to redirect nuclear gene expression (114). The outcome of the modified nuclear gene expression is often the production of proteins that are imported into the chloroplast to affect chloroplast development or physiology, a phenomenon called CRS (20, 26). It is now recognized that nuclear gene expression in response to chloroplast signaling is not limited to affecting chloroplast-associated proteins and that numerous facets of plant physiology and development, including pathogen defense, can be controlled by chloroplast-generated signals (132). Several of the molecules that initiate CRS are intermediates in chloroplast metabolism and a subset of these have been identified as mediating responses to pathogens. CRS may be classified as biogenic or operational. Here, we consider the stress-related operational CRSs, which are linked to CI, with a focus on the redox status of the chloroplast, protein movement from the chloroplast, and the isoprenoid precursor methylerythritol cyclodiphosphate (MEcPP) (148). We also briefly introduce the emerging areas of chloroplast-organellular and chloroplast-PD signaling, as summarized in **Figure 3**. Space constraints restrict covering emerging operational CRSs such as 3′-phospho-adenosine 5′-phosphate (36) or the oxidation products of carotenes such as the volatile β-cyclocitral (115), and readers are pointed toward recent reviews focused on RS (20, 26), including those dealing with possible roles for metabolites in immune signaling (39), for additional information.

Methylerythritol Cyclodiphosphate Links the Nucleus and Endoplasmic Reticulum in Chloroplast-to-Nucleus Retrograde Signaling

The methylerythritol (MEP) pathway in the chloroplasts of land plants is used for the synthesis of isoprenoids and eventually carotenoids, abscisic acid (ABA), and chlorophylls. In this pathway, the intermediate MEcPP is converted into hydroxymethylbutenyl diphosphate by the action of the enzyme 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (HDS). In *Arabidopsis*, MEcPP accumulates during abiotic stress, including high light, and induces the expression of nuclear stress–responsive genes indicative of a role in CRS (148). The *Arabidopsis ceh1* mutant lacking HDS and constitutively accumulating MEcPP has constitutively high levels of SA and enhanced resistance to *P. syringae*. Notably, MEcPP mediates induction of JA-responsive genes, even in the *ceh1* mutant, which accumulates high levels of SA and the JA precursor 12-OPDA but not free JA (71).

ceh1 leaves contain ER-derived structures called ER bodies that are associated with stress and wound responses, consistent with the constitutive expression of JA-responsive genes and general stress responses seen in this MEcPP-accumulating mutant (71, 136). Reinforcing the broad spectrum of systemic immune responses, ER bodies act as storage sites of enzymes involved in the breakdown of indole glucosinolates, a class of chloroplast-derived secondary metabolites (150). The altered indole glucosinolate profiles and accumulation of simple nitriles seen in the *ceh1* mutant (140) contribute to the defense response to herbivory (30, 102). Consistently, the related mutant *hds3* is highly resistant to the cabbage aphid (*Brevicoryne brassicae*), and *B. brassicae* feeding on *Arabidopsis* increased metabolic flux in the MEP pathway. This enhanced efflux of MEcPP from the plastid triggered CRS that induced genes contributing to aphid defense (110).

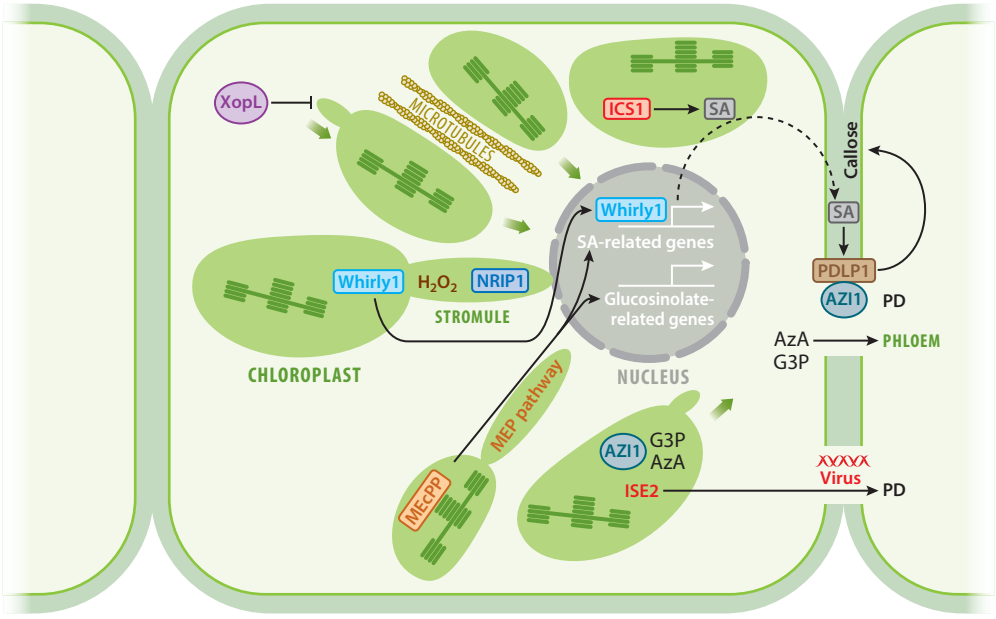

Figure 3

Chloroplast-associated retrograde signaling (CRS) in chloroplast immunity. The clustering of chloroplasts around nuclei is commonly observed during responses to pathogens. Chloroplast clustering depends on the cytoskeleton and stromule extension toward the nuclei and uses microtubules to guide perinuclear clustering. The bacterial effector XopL promotes chloroplast clustering but inhibits stromule formation. Stromules facilitate the trafficking of defense signaling molecules such as H_2O_2 and the protein NRIP1 to the nucleus. Chloroplast-localized Whirly1 (a single-stranded DNA binding protein) translocates to the nucleus to regulate expression of SA-related genes, although the involvement of stromules is unclear. The methylerythritol (MEP) pathway produces the chloroplast-to-nucleus retrograde signaling molecule methylerythritol cyclodiphosphate (MEcPP) in response to stress. MEcPP controls expression of SA-related and indole glucosinolate biosynthetic genes. In some species, the MEP pathway is associated with stromules. The chloroplast RNA helicase ISE2 is a negative regulator of intercellular trafficking via plasmodesmata (PD) through modulating CRS. Changes in *ISE2* expression lead to increased susceptibility to virus infection. PD are critical conduits and PD proteins are found in the intercellular trafficking of molecules like AzA and G3P, which are involved in SAR. The deposition of callose at PD to regulate intercellular trafficking is mediated by SA acting on PDLP1 even though it traffics via the apoplastic space. Abbreviations: ICS, isochorismate synthase; SA, salicylic acid.

Intracellular Signaling Involving Chloroplasts

From the discussion above, it is clear that the chloroplast does not act alone during defense but exerts its effects on defense responses by modulating the function of other organelles, including the ER and nucleus. For example, in addition to its primary roles in activating CI, ROS is thought to mediate CRS during defense by apparent translocation to the nucleus. The spatial and temporal dynamics of short-lived, highly reactive ROS as chloroplast-to-nucleus signals that allow them to act outside the chloroplast have long been puzzling. The ROS H_2O_2 moves from chloroplasts to the nucleus via stromules during defense responses in *N. benthamiana* (16). Stromules are tube-like extensions of the chloroplast envelope that enclose portions of the stroma (reviewed in 101). High light similarly promotes perinuclear clustering of chloroplasts. Analysis of a genetically encoded H_2O_2 protein biosensor targeted to the nucleus, stroma, and cytoplasm revealed that the accumulation of nuclear H_2O_2 was correlated with its initial appearance in the stroma. Scavenging H_2O_2 in the chloroplast, but not the cytoplasm, decreased the rate of nuclear accumulation of H_2O_2, providing compelling support for transport of cROS to the nucleus (37).

Chloroplast proteins have also been reported to translocate from the chloroplast to the nucleus during CRS to initiate defense signaling. One example is the multifunctional protein Whirly1, which was first identified as a nuclear transcription factor required for SA signaling during defense (31, 32). Whirly1 was subsequently recognized as a chloroplast protein (55, 67, 76) that was also important for various aspects of chloroplast and plant development and stress responses (82). Phosphorylation of Whirly1 by CIPK14 leads to its accumulation in the nucleus and a concomitant reduction of Whirly1 in the chloroplast (116). Partitioning of Whirly between the chloroplast and nucleus is thought to be part of a dynamic feedback mechanism that regulates SA accumulation and SA-mediated signaling during development and stress responses; chloroplast Whirly regulates expression of SA-related genes mediating stress responses, whereas nuclear Whirly is important for SA-mediated regulation of development (76).

Another well-studied protein that translocates from the chloroplast to the nucleus during defense is NRIP1 (see above). Recognition of the TMV p50 effector by the R protein N induces relocation from the chloroplast to cytosol and physical interaction of NRIP1 with N (16). Studies with NRIP1 have begun to illuminate how proteins traffic between subcellular compartments during defense. NRIP1 uses stromules to transit from chloroplasts to nuclei during defense in *N. benthamiana* (16). Although stromules are uncommon among chloroplasts in uninfected tissue that are photosynthesizing, their formation is induced by a variety of pathogens, including bacteria and viruses, suggesting they have important roles in plant defense (16). Subsequent work showed that these stromules extend along microtubules with anchor points on microfilaments and facilitate perinuclear clustering and potentially function in chloroplast nuclear ROS signaling to activate or reinforce ETI (68, 112). Interestingly, in Madagascar periwinkle (*Catharanthus roseus*), the enzymes of the MEP pathway can be detected in stromules (46). In this species, the MEP pathway is part of the synthesis of complex defense-related monoterpene indole alcohols, and it is associated with several specific cell types and subcellular localizations. The authors speculate that stromules are used as a means for specific delivery of metabolites between subcellular compartments. It remains to be determined whether stromules act as a highway for chloroplasts destined to translocate to the nucleus to participate in immune responses.

Related to the induction of stromules during infection is the relocalization of chloroplasts within the cell to nucleus-proximal positions during infection. The movement of chloroplasts is not dependent on the presence of pathogens but can be triggered by PTI/ETI and ROS (16, 112, 156). These defense-related chloroplast dynamics are dependent on the microtubule and actin components of the cytoskeleton (68), suggesting whole-cell-level coordination of this response and its importance to immune signaling. The recent identification of a bacterial effector that inhibits stromule formation highlights the importance of stromules for defense and demonstrates a causal link between perinuclear clustering and defense. When the XopL effector from *X. campestris* pv. *vesicatoria* was transiently expressed in *N. benthamiana* leaves, stromule formation was reduced and this suppressive effect required the E3 ubiquitin ligase activity of XopL (35). Curiously, although XopL inhibited stromule formation, it also induced the perinuclear localization of chloroplasts, suggesting that these two processes may be unrelated to each other. Chloroplast heterogeneity is often overlooked in discussions of chloroplast functions, but subpopulations of chloroplasts have been reported (40). A decrease in the number and size of chloroplasts occurs after high light and *Botrytis cinerea* infection (156). This suggests that functional specialization of chloroplasts could be a means of fine-tuning defense responses. This intriguing possibility requires further investigation.

In addition to interactions with the nucleus and ER, chloroplast signals also interact with PD to control intercellular trafficking during defense. The role of PD in trafficking of defense molecules is perhaps most clearly demonstrated for SAR. In the branch regulated by AzA and G3P, these two

signaling molecules are transported to distant parts of the plant in the phloem (75). Entry into the phloem uses the standard symplastic route provided by PD, and overexpression of the PD gating proteins PDLP1 and PDLP5 reduced plasmodesmal permeability, leading to compromised SAR (75). Further evidence for the role of PD in SAR is derived from the observation that AZI1, which is required for SAR, also interacts with PDLP1 (75). Besides facilitating the intercellular trafficking of chloroplast-generated defense molecules, PD are also directly and indirectly regulated by chloroplasts to mediate defense responses. Partial loss of *PDLP5* (previously named *HOPW1–1 INDUCED GENE 1* for its marked induction on infection with *P. syringae* expressing the effector Hopw1–1) led to increased susceptibility to *P. syringae*, whereas its overexpression led to enhanced resistance in an SA-dependent manner (70). Using *pdlp5* mutants, it was demonstrated that SA signaling can regulate PD permeability and intercellular trafficking, and SA mutants have defects in PD function (141). It was recently discovered that the association of PDLP5 with PD is dependent on the binding of phytosphinganine (t18:0), and mutants accumulating t18:0 were more resistant to the fungal pathogen *Verticillium dahliae* and DC3000 in a PDLP5-dependent manner (78). Thus, chloroplast products can directly regulate PD through their effects on PD-associated proteins. Work with the chloroplast RNA helicase ISE2 suggests that chloroplasts can indirectly regulate intercellular trafficking during defense, possibly via CRS (14). Decreased *ISE2* expression in *N. benthamiana* increased PD permeability, whereas increased *ISE2* expression decreased it (42, 126). Surprisingly, both conditions led to increased susceptibility to infection by *Turnip mosaic virus* (42), a perplexing result that suggests complex signaling between the chloroplast and PD and that viruses have evolved strategies for evading CI.

CHLOROPLASTS AS TARGETS FOR PATHOGENS: A STRATEGY FOR DEFEATING CHLOROPLAST IMMUNITY

Effectors Directly Targeting Chloroplasts

To circumvent and overcome CI for a successful infection, pathogens have evolved counterstrategies. This is perhaps best exemplified by viruses. Some viruses have adapted to use the chloroplast envelope membranes as sites for their replication and in this way disturb normal chloroplast function. More commonly, viruses that do not replicate in association with chloroplast membranes encode proteins that interact with specific chloroplast proteins. Many of the identified targets of these viral proteins are part of the photosynthetic machinery. For example, the replicase protein of TMV, which replicates in the host cytoplasm, has been found to interact with ATP-synthase γ-subunit (AtpC) and Rubisco activase (RCA) (11). Silencing of either *AtpC* or *RCA* in *N. benthamiana* conferred enhanced susceptibility to TMV, suggesting that AtpC and RCA were needed for an effective defense response. To date, there have been numerous reports of viruses interacting with chloroplast proteins to mitigate host defense.

Understanding how viruses perturb chloroplasts has set the stage for further investigation into disruption of chloroplast function by other pathogens. A major protein–protein interaction screen (100) provided tantalizing insight into potential chloroplast-localized pathogen effector targets. Of these, LSU (low sulfur) proteins represented a key hub for a diverse set of plant-pathogenic effectors. LSU1 interacts with, and stimulates, the chloroplastic superoxide dismutase FSD2 (44). *P. syringae* pv. *tomato* DC3000 virulence effectors interfere with this interaction by preventing LSU1 relocalization to chloroplasts, thus attenuating cROS production, leading to moderately enhanced virulence (44). LSU1 overexpressing lines showed an enhanced disease resistance phenotype. This is consistent with the increased superoxide production, lower PSII efficiency, and elevated NPQ exhibited in the *fsd2* mutant (41). LSU1 also interacts with RAF2/SDIRIP1, a protein involved in Rubisco assembly and in mediating ABA-dependent stress responses (106), highlighting a potentially multifaceted role in immune regulation.

Remarkably, a large proportion of the *P. syringae* type III effector complement physically localizes to chloroplasts, including HopI1, HopN1, HopK1, AvrRps4, HopR1, and HopO1–2, although direct targets are only known for HopI1 and HopN1 (reviewed in 77). The *Ralstonia* effector RipAL processes a DAD1-like lipase domain and localizes to chloroplasts, where it targets chloroplast lipids. Ectopic expression of RipAL suppressed PTI, inducing JA and JA-Ile as well as JA marker genes and concomitantly reducing both SA and SA-responsive gene expression. Accumulation of JA and suppression of SA were conditional on a functional DAD1-like lipase domain (104). Additional pathogen effectors localized to the chloroplast and their known targets are reviewed in References 77 and 81.

Pathogen-Induced Abscisic Acid Compromises Chloroplast Immunity

The chloroplast is the site of the synthesis of a range of phytohormone precursors, and these pathways are thus subject to direct or indirect manipulation by pathogen effectors (15, 117). A key challenge is to understand changes in hormone dynamics during pathogen infection. The *P. syringae* pathosystem allows comparative temporal monitoring of PTI, ETI, and ETS responses, enabling synchronous infections at specific inoculums, and type III secretion compromised mutants provide a readout of PTI. Given space constraints, we guide the reader to recent reviews on hormone modulation (15, 33) and instead highlight how DC3000 can hijack host ABA synthesis and signaling to attenuate PTI-induced cROS.

Treatment with the phytohormone ABA suppresses resistance to biotrophic and hemibiotrophic bacterial, fungal, and oomycete pathogens (53, 95), although ABA can act synergistically with JA to enhance resistance in necrotrophic pathogens (1). Genetic and transcriptomic studies have demonstrated that biotrophic pathogens hijack host ABA signaling to promote virulence and suppress SA signaling (27, 29, 113). DC3000 induces ABA biosynthetic genes leading to de novo ABA biosynthesis within 6 hpi. This occurs prior to DC3000 multiplication (27, 29) and coincides with suppression of the PAMP-triggered cROS burst (28) (**Figure 1**). Strikingly, like DCMU, co-infiltration of ABA with DC3000*hrpA* also attenuates PTI-triggered cROS generation and, moreover, facilitates significant growth of this nonpathogenic derivative (28). How ABA exerts its influence at its original site of synthesis remains obscure. ABA itself can suppress plastid gene expression via PP2C-dependent activation of nuclear genes (151), whereas *Xanthomonas* effectors repress chloroplast-localized PP2C transcripts during infection of *Arabidopsis* and rice (2).

WHERE TO NEXT?

CI is an emerging research field and is particularly challenging and complex. Chloroplast-derived ROS (and NO) underpin local CI but are also necessary to generate the lipid-based signaling pathways that underpin systemic immunity as well as CRS for communication and instigation of an effective defense. Although chloroplasts are often cited as the site of phytohormone biosynthesis whose manipulation is essential to the outcome of plant–microbe interactions, it is often neglected that the synthesis of many of these hormones is ultimately completed outside the chloroplast, e.g., ABA and SA in the cytoplasm and JA in the peroxisomes. Thus, although we are beginning to appreciate the critical role of chloroplasts in immune responses, we need to better understand the individual and collective contribution of other organelles to plant immune responses and immunity suppression.

The chloroplast is complex, and a detailed understanding of the contribution of PSI and PSII to induction of cROS during different phases of the immune response remains elusive. Similarly, the actual targets of pathogen virulence strategies leading to attenuated cROS via disassembly of the photosystems need to be identified. The fundamental challenge of understanding the relationship

between the production of ROS and their roles in signaling, not just by the chloroplast but other organelles during defense, is limited by the specificity of current ROS assays (123). Investigations of ROS locations, levels, and timing in the context of disease and defense responses will greatly benefit from new genetically encoded ROS reporters (107) and nanosensors (73), which provide greater chemical specificity and temporal-spatial resolution.

There are many other areas for which we currently lack fundamental knowledge with respect to chloroplasts and immune responses. These include the repositioning of chloroplasts, the role of stromules, the nature of the multiple retrograde chloroplast-to-nucleus signaling pathways, and retrograde signals targeting other organelles. Indeed, much greater detailed insight is necessary to understand both chloroplast intraorganellular interactions and chloroplast heterogeneity in immunity to answer fundamental questions. These include whether heterogeneity in size and positioning of chloroplasts in the cell reflects different metabolism and signaling roles in response to pathogens, and, simply, how many chloroplasts within a cell need to respond to confer effective immunity. Combining studies with genetically encoded reporters and plant lines with multiple fluorescently labeled organelles will greatly facilitate our understanding of ROS and intraorganellular dynamics during disease and defense.

Finally, identifying the chloroplast targets of effectors and characterizing their interaction will be in itself challenging but will provide important insights into how pathogens have evolved to target chloroplast components. Collectively, a better understanding of CI may help develop more resilient crops and even lead to new approaches for developing herbicide targets.

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Errata

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