Transduction of wound and herbivory signals in plastids

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Plastids are the central orchestrators of the early and late responses to wounding and herbivory in plants. This organelle houses some of the most important enzymes involved in the biogenesis of intra and extracellular signals that mediate defense responses against these stresses. Among these enzymes are the ones initiating the biosynthesis of oxylipins [e.g., jasmonic acid (JA) and C6 volatiles], terpenoid volatiles and phenolic compounds, including both volatile [e.g., methylsalicylate (MeSA)] and non-volatile compounds [e.g., salicylic acid (SA)]. Plastids also play a major role in orchestrating changes in primary metabolism during herbivory and thereby in the reallocation of carbon and nitrogen to different functions in response to herbivory. How the primary stress signals generated by mechanical damage and herbivory reach the plastid to activate the rapid synthesis of these signal molecules is at present largely unknown.

Perception of Insect Herbivory and Induction of Plant Defense Responses

Plants have evolved elaborate defense systems to defend against or tolerate insect herbivores and some of these systems are constitutive whereas others are inducible. The induced defense responses are generally classified as direct and indirect. Direct defenses consist in the production of metabolites with are repellent or anti-digestive to the herbivore and those include (among others) glucosinolates, cyanogenic glucosides, alkaloids, phenolics and proteinase inhibitors. Which metabolites are produced depends largely on the plant species. Indirect defense responses include (among others) the production of organic volatiles (e.g., C6 compounds and terpenoids) and extrafloral nectar. These compounds play an important function as attractants of the herbivore’s natural enemies (e.g., predators or parasitoids).1,2

Because the induction of defense responses is energetically costly, plants use sophisticated regulatory mechanisms to perceive and trigger defense responses only when required. Insect herbivory is generally associated with tissue wounding, in particular for foraging insects, and therefore the plant’s responses to wounding overlap with those against herbivores. However, some responses are specific for a particular stimuli.3

One well-defined mechanism by which plants can perceive herbivore feeding is through the perception of components present in the oral secretion (OS) of the insect’s larvae. These factors can enhance some of the wound-induced response whereas others can suppress them. For example, N-linolenoyl-glutamate (18:3-Glu), a fatty acid amino acid conjugate (FAC) found in the OS of several lepidopteran larvae, can (among other responses) heighten the production of jasmonic acid (JA) and ethylene (ET) in Nicotiana attenuata.4 High glucose oxidase (GOX) activity in Spodoptera exigua OS is sufficient to elicit an enhanced SA burst in N. attenuata plants that can attenuate the FAC-mediated production of JA and ET in this plant species.5 Another example is the FAC volicitin [N-(17-hydroxy-linolenoyl)-Gln] originally found in the OS of Spodoptera exigua and which induces the differential production of volatiles in corn (Zea mays) plants.6 Thus, insect-derived compounds can modify the initial wound-associated cellular events resulting in reprogramming of the wound response that will ultimately have a strong impact on the differential activation of plastidial enzymes. However, not all plant species perceive FACs and therefore this mechanism for insect perception is not universal. For example, when lima beans (Phaseolus lunatus) are mechanically damaged by a robot (MecWorm) that mimics herbivore-caused tissue damage, they emit a volatile organic compound blend which is similar to that induced by herbivore feeding.7 Moreover, the level of leaf damage is linearly correlated with the accumulation of C6 volatiles and monoterpenes.8 Thus, in this case, mechanical damage alone is sufficient to mimic the emission of volatiles induced by insect herbivory. Recently, it has been proposed that plants can perceive ‘damaged-self recognition’, the perception of molecules derived from the plant and modified to indicate disintegrated plant cells.8

One example is the perception of inceptins, digestive fragments of an ATP synthase.9

The plastid reacts rapidly to wounding and herbivory by generating a broad range of different chemical signals

In green tissue, induced defense responses against insects and wounding relay largely on chloroplasts, which react almost immediately (within seconds) to these stimuli by the rapid generation of oxylipins and jasmonates.10,11 Direct defense responses to wounding require the biosynthesis of MeSA in the chloroplast, which is normally synthesized from UA with the help of an ATP synthase.9 This enzymatic activity therefore provides a rapid means of activating defense responses to wounding.11,12
of diverse chemical signals targeted to induce these responses both locally and systemically (Fig. 1). Among the first and most critical chemical signals to be generated by the plastids are the oxylipins. Oxylipins are lipid metabolites derived from the oxidation of unsaturated fatty acids [e.g., linoleic acid (18:2), α-linolenic acid (18:3) or roughanic acid (16:3)] and they include among others hydroperoxi-, hydroxy-, oxo- and keto-fatty acids, divinyl ethers, short-chain aldehydes and the phytohormone JA. The first step of oxylipin biosynthesis is the formation of fatty acid hydroperoxides, which can occur enzymatically (e.g., by lipoxigenases) or non-enzymatically by chemical oxidation of the fatty acid double bonds. Enzymatic conversion of hydroperoxides can occur by various pathways, including those initiated by allene oxide synthase (AOS), divinyl ether synthase (DES), hydroperoxide lyases (HPL), peroxycigenases (PXG) or epoxy alcohol synthase (EAS) among others. 

The signaling roles of some of the products of these pathways in defense responses against pathogens and herbivores have been extensively documented. As a result of biotic stresses, intracellular levels of reactive oxygen species (ROS) may rise rapidly. These ROS can induce the nonenzymatic oxidation of fatty acids to generate biologically active oxylipins. The plastid also produces the bulk of fatty acids in the cell and supplies these molecules for diverse biosynthetic and regulatory pathways. In addition to oxylipins, recent evidence indicates that structural fatty acids (e.g., 18:0, 18:1, 18:2 and 18:3) can also modulate defense-related responses in plants. For example, in Arabidopsis, a mutation in the major 18:0-ACP desaturase (FAB2, SSI2) alters defense signaling and resistance via mechanisms associated with the levels of 18:1. 

Plastid-derived C₆ volatiles are released from mechanically and herbivore damaged leaves immediately (0–5 min) after the stimulus. HPL catalyzes the cleavage of fatty acid hydroperoxides into o-oxo fatty acids and (3Z)-alkenals, generating C₆ volatiles that include (2E)-hexenal, (2E)-hexenol, (3Z)-hexenal, hexanal, hexanol and some of their esters and C₁₂ derivatives including traumatin and traumatic acid. The role of C₁₂ o-oxo fatty acids in the regulation of defense mechanisms requires further attention.

Plastids produce isoprenoids via the methyl-D-erythritol 4-phosphate (MEP) pathway and it is proposed that C₁₀ precursors of monoterpenes and C₂₀ precursors of diterpenes are predominantly synthesized within this organelle. Some monoterpenes are de novo synthesized after herbivore damage and released systemically from the whole plant. In contrast to the rapid release of C₆ volatiles, herbivore-induced monoterpenes start to be released in a diurnal cycle that is decoupled from short-term insect damage. It has been demonstrated that 1-deoxy-D-xylulose-5-phosphate synthase (DXS), the first enzyme of the MEP pathway, plays a major role in the regulation of the pathway. However, how plastids control the changes in substrate fluxes for the activation of monoterpene and diterpene biosynthesis and release after herbivory remains at present unclear.

Finally, the plastid reacts to some biotic stresses by the production of the 2-hydroxyl derivative of benzoic acid (BA), salicylic acid (SA). Two main SA biosynthetic pathways have been described in plants, one derived from trans-cinnamate and benzoic acid and one from chorismate via isochorismate synthase (ICS). The exact contribution of these two pathways is apparently species-specific. MeSA is emitted as a volatile and it can act as a defence compound against insects.

Mechanisms Conveying Primary Stress Signals to the Plastid

How the stresses of mechanical damage or herbivory are translated into changes in plastidial metabolic processes remains largely unknown. Recent studies performed with lima bean (Phaseolus lunatus) showed that among the early events occurring during herbivore attack and wounding are a strong depolarization of the plasmamembrane (ΔVm), an enhanced influx of Ca²⁺, and hydrogen peroxide (H₂O₂) generation. These studies suggest that early cellular signals in response to mechanical damage and herbivory are generated in the plasmamembrane environment. These early cellular mechanisms may induce the activation of downstream components such as protein MAPKs or calcium-dependent protein kinases (CDPKs) that will subsequently transduce the primary stress down into the chloroplast, where the targeted enzymes reside. For example, several extra-plastidial regulatory factors with a potential function upstream of JA biosynthesis have been identified in different plant species. Wounding and herbivory in tobacco species (Nicotiana spp) and tomato (Solanum lycopersicum) activate the mitogen activated protein kinases SIPK (salicylate-induced protein kinase) and WIPK (wound-induced protein kinase). The silencing of SIPK and WIPK expression in tobacco plants results in a substantial reduction of JA production after wounding and OS elicitation. The effect of SIPK on JA biosynthesis in N. attenuata leaves occurs most likely at the level of substrate supply, by stimulating the release of α-linolenic acid (18:3) via activation of GLA1 (glycerolipase 1; Fig. 2). In contrast, the effect of WIPK is at the level of conversion of 13-hydroperoxy-linolenic acid into 12-phytodienoic acid (OPDA) and therefore WIPK may affect the activity of AOS (allene oxide synthase) and/or AOC (allene oxide cyclase; Fig. 2). Another regulatory component that affects JA production in N. attenuata is NPR1 (Nonexpressor of PR-1), an essential component of the SA signal transduction pathway first identified in Arabidopsis. N. attenuata NPR1-silenced plants accumulate substantially lower JA levels after elicitation than WT and similar to SIPK, NPR1 most likely affect the release of 18:3 via GLA1 activation (Fig. 2). Other extraplasmoidal regulatory factors that affect JA accumulation in different plant species include the wound-induced receptor-like protein kinase (WRK), calcium-dependent protein kinases (CDPKs), MAPK KINASE 3-MAPK 6 and protein phosphatase 2C (AP2C1). How these factors affect JA formation is unknown however this diverse set of regulators suggests that a complex network of signals integrated by multiple transduction pathways convey the primary stress signal to the plastid to regulate JA biosynthesis.
Figure 1. Plastids play a central role in the generation of wounding and herbivory induced signals. After wounding and herbivory, several different cellular events take place at the plasmamembrane [e.g., increased ROS production, changes in ion fluxes and plasmamembrane potential, recognition of insect elicitors (e.g., FACs)]. The perception of pathogen elicitors by specific membrane receptors also activates some of these cellular processes. These events lead to the activation of downstream components in the cytosol (arrow 1) that transduce the primary stress signals to activate enzymes located in the plastid (arrow 2). Changes in ion fluxes between the cytosol and the tonoplast may also influence the activity of plastidial enzymes (arrow 3). The plastid reacts to these stimuli by activating the synthesis of fatty acid-derived signals (e.g., oxylipins and structural fatty acids), SA, and isoprenoid volatiles that regulate direct and indirect defense responses (dashed arrows). FAC, fatty-acid amino-acid conjugate; FAS, fatty acid synthase; ACP, acyl carrier protein; ROS, reactive oxygen species; GLA, glycerolipase A; LOX, lipoxygenase; AOS, allene oxide synthase; AOC, allene oxide cyclase; HPL, hydroperoxide lyase; SKP, shikimate pathway; ICS, isochorismate synthase; BA, benzoic acid (* formation of BA requires one cycle of β-oxidation that most likely takes place in the peroxisome); G3P, glyceraldehyde-3-phosphate; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; DXP, 1-deoxy-D-xylulose-5-phosphate; DXR, 1-deoxy-D-xylulose-5-phosphate reductoisomerase; MEP, methyl-D-erythritol 4-phosphate; MT, monoterpenes; DT, diterpenes.
In the case of SA biosynthesis, genetic evidence indicates that **ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1)**, **NONSPECIFIC DISEASE RESISTANCE 1 (NDR1)** and **PHYTOALEXIN DEFICIENT 4 (PAD4)** are components acting upstream of SA biosynthesis in basal resistance to biotrophic pathogens and that **Ca2+/calmodulin-dependent signaling** also regulates SA production. However, similar to JA biosynthesis, how these regulators signal the plastid to activate SA biosynthesis is at present also unclear (Fig. 1).

**Conclusions**

Although we understand the biosynthetic pathways leading to the formation of the signal molecules described in this review, the regulatory mechanisms underlying the activation of these plastidial pathways upon wounding and herbivory remain largely unknown. Thus, understanding how the primary stress signals generated by these stimuli are translated into the activation of specific plastidial enzymes controlling the flux of metabolites through signal-molecule-generating pathways is essential if we are to understand how plants control this important signaling system for defense responses.
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