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New insights into the early biochemical activation of jasmonic acid biosynthesis in leaves

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In plants, herbivore attack elicits the rapid accumulation of jasmonic acid (JA) which results from the activation of constitutively expressed biosynthetic enzymes. The molecular mechanisms controlling the activation of JA biosynthesis remain largely unknown however new research has elucidated some of the early regulatory components involved in this process. *Nicotiana attenuata* plants, a wild tobacco species, responds to fatty acid amino acid conjuguates (FAC) elicitors in the oral secretion of its natural herbivore, *Manduca sexta*, by triggering specific defense and tolerance responses against it; all of the defense responses known to date require the amplification of the wound-induced JA increase. We recently demonstrated that this FAC-elicited JA burst requires an increased flux of free linolenic acid (18:3) likely originating from the activation of a plastidial glycerolipase (GLA1) which is activated by an abundant FAC found in insect oral secretions, *N*-linolenoyl-glutamate (18:3-Glu). The lack of accumulation of free 18:3 after elicitation suggests a tight physical association between GLA1 and LOX3 in *N. attenuata* leaves. In addition, the salicylate-induced protein kinase (SIPK) and the nonexpressor of PR-1 (NPR1) participate in this activation mechanism that controls the supply of 18:3. In contrast, the wound-induced protein kinase (WIPK) does not but instead regulates the conversion of 13(S)-hydroperoxy-18:3 into 12-oxo-phytodienoic acid (OPDA). These results open new perspectives on the complex network of signals and regulatory components inducing the JA biosynthetic pathway.

Key words: jasmonic acid, lipase, lipoxygenase, wounding, plant-insect interactions, FAC

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An Overview of the Jasmonic Acid Biosynthesis Pathway

In non-elicited mature leaves, JA is maintained at very low levels however, upon wounding or herbivory, cellular mechanisms rapidly convey the primary signal to the plastid to activate the rapid production of JA. How plants convey these primary stress signals to the plastids remains at present largely unknown. Importantly, this rapid biosynthetic response results from the biochemical activation of constitutively expressed JA biosynthetic enzymes in unelicited leaf tissue most likely by substrate availability and post-translational modifications.

According to the canonical pathway, the first committed step in JA biosynthesis consists of the oxidation of free α-linolenic acid ($18:3^{\Delta 9,12,15}$, 18:3) by a plastidial 13-lipoxygenase (13-LOX) to form 13(*S*)-hydroperoxy-18:3 (13S-(OOH)-18:3). However, the identification of the *Arabidopsis thaliana DAD1* gene encoding a flower-specific lipase provided genetic evidence for the specific release of 18:3 from membrane glycerolipids as an earlier committed step in the activation of JA biosynthesis in flowers of this species.[1] 13S-(OOH)-18:3 is converted by allene oxide synthase (AOS) into the highly unstable allene oxide intermediate 12,13-epoxy-9Z, 11E, 15Z-18:3 which is processed by allene oxide cyclase (AOC) to yield (9*S*,13*S*)-12-oxo-phytodienoic acid (OPDA). OPDA is transported from the plastid into the peroxisome where it is reduced by OPDA reductase (OPR) and β-oxidized to (3*R*,7*S*)-JA. In contrast to *Arabidopsis thaliana* that accumulates

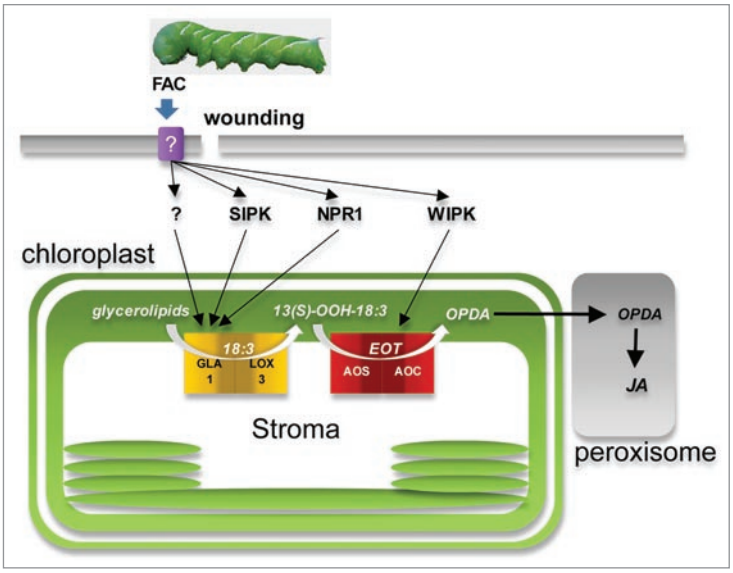

Figure 1. Schematic representation of the proposed enzymatic steps in the FAC-elicited JA biosynthesis pathway regulated by SIPK, WIPK and NPR1. During larval feeding, wounded plant tissue comes into contact with components of the oral secretion of insect larvae, such as fatty acid-amino acid conjugates (FAC). The perception of FACs by leaf cells elicits signaling mechanisms that differentially activate 18:3 release from membrane lipids via the activation of glycerolipase A1 (GLA1) in *N. attenuata*. SIPK and NPR1 participate in the activation of this enzymatic step. However unknown independent mechanisms (?) also operate. In contrast, WIPK-mediated mechanisms target the activation of OPDA biosynthesis via the regulation of allene oxide synthase (AOS) and/or allene oxide cyclase (AOC). Due to the instability of the AOS product *EOT*, AOS and AOC are thought to be in close contact. *EOT*: 12,13-epoxy-18:3; *OPDA*: (9S,13S)-12-oxo-phytodienoic acid.

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oxylipins esterified to galactolipids in leaves, most plant families do not accumulate these oxylipin conjugates in leaves and de novo JA biosynthesis depends primarily on free 18:3,[2,3] which based on their propensity to disrupt lipidic membranes, tend to accumulate to very low levels in leaf cells, levels which are too low to supply the JA biosynthesis pathway. Thus, 18:3 must be released from glycerolipids in the choloroplast membranes upon elicitation to feed this pathway. Previous studies reported that free fatty acid levels increase in leaves after mechanical damage however whether these changes reflect substrates available for JA biosynthesis or uncontrolled membrane degradation remains unknown.[4,5]

Due to the large number of enzymes and different cellular compartments involved in JA biosynthesis, it is likely that the pathway is regulated at multiple steps. Resolution of the structures of the tomato (*Lycopersicon esculentum*) OPR3 and Arabidopsis AOC2 and ACX1 has provided insights into potential regulatory mechanisms for these enzymes.[6-8] However, the molecular components of the signal transduction pathways that mediate post-translational changes in JA biosynthetic enzymes in response to elicitation remain unknown.

Biochemical Regulation of JA Biosynthesis

In *N. attenuata*, the wound-induced JA production is amplified by application of lepidopteran larvae (e.g., *Manduca sexta*) oral secretions (OS) to mechanical wounds. Major elicitors of the OS-mediated response are fatty acid-amino acid conjugates (FACs) which are sufficient to amplify JA production in leaves of this plant species.[9]

Among the regulatory components known to affect JA biosynthesis after wounding and herbivory in Nicotiana and Solanum species are the mitogen activated protein kinases (MAPKs) SIPK (salicylate-induced protein kinase) and WIPK (wound-induced protein kinase).[10-12] When the expression of these regulators is silenced, the plants accumulate significantly less JA after elicitation.[10,12] 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.[13] Upon activation in this plant species, NPR1 translocates from the cytosol into the nucleus where it regulates SA-mediated defense responses.[14] NPR1 also interacts with the JA and ethylene signaling cascades, and a cytosolic role for this factor in the regulation of JA dependent responses/biosynthesis has been proposed.[15] Additional examples of regulatory factors that affect JA accumulation in other plant species are the wound-induced receptor-like protein kinase (WRK) and calcium-dependent protein kinases (CDPKs) in tobacco and MAPK KINASE 3-MAPK 6 and protein phosphatase 2C (AP2C1) in Arabidopsis.[16-19] This diverse set of regulators suggests that a complex network of signals integrated by multiple transduction pathways mediate the regulation of FAC-elicited JA biosynthesis.[20]

During the induction of leukotriene biosynthesis in mammalian cells, activation of phospholipase A_2 (PLA_2) depends on Ca^{2+} binding, phosphorylation by a MAPK signaling cascade and association with FLAP (5-lipoxygenase-activating protein), a scaffold protein that also binds to 5-LOX upon Ca^{2+} binding.[21] The entire complex localizes to the nuclear membrane were arachidonic acid is oxidized and leukotriene biosynthesis is initiated.[21] Thus far, functional homologues of FLAP have not been identified in plants however, similar to mammals, most plant 13-LOXs conserve a Ca^{2+} binding domain.[22] The participation of Ca^{2+} in early responses to insect herbivory has been previously documented and genetic evidence for the participation of cations, including Ca^{2+}, in the regulation of JA biosynthesis comes from the identification of a gain-of-function allele of the Arabidopsis *Two Pore Channel 1* (*TPC1*) gene, a tonoplast membrane-localized cation channel which when deregulated, amplifies the accumulation of JA after wounding.[23,24] How Ca^{2+}-mediated signals affect JA biosynthesis is at present unknown, however the identification of cytosolic CDPKs affecting JA accumulation in tobacco leaves

suggests the possibility that these enzymes are involved in this process.[17]

New Insights into the Role of MAPKs and NPR1 in the Activation of JA Biosynthesis

In a recent study, Kallenbach et al. provided the first evidence for the participation of two MAPKs (SIPK and WIPK) and NPR1 in the activation of early enzymatic steps of the JA biosynthesis pathway after wounding and FAC elicitation in *N. attenuata* leaves.[25] Analysis of the early changes (within a few minutes) in the pools of JA precursors together with the identification of a plastidial glycerolipase (GLA1) essential for de novo JA biosynthesis in this plant species suggested that after wounding and FAC elicitation there is a rapid and specific flux of free 18:3 into the JA biosynthesis pathway. Moreover, the results suggested that 18:3 is channeled into LOX3 and therefore that a tight physical association exists between GLA1 and LOX3 in *N. attenuata* leaves (**Fig. 1**). This mechanism may resemble the mechanism that operates in mammalian cells during activation of leukotriene biosynthesis however whether plants have a functional FLAP analog or GLA1 and LOX3 that directly interact remain to be determined. Also similar to mammalian systems, LOX3 in *N. attenuata* plants may be recruited onto the membranes after activation to interact with GLA1. In Arabidopsis, AtLOX2 is found primarily in the stroma.[26]

The enhanced supply of 18:3 for JA biosynthesis via GLA1 activation is dependent on SIPK- and NPR1-mediated signal transduction mechanisms. In contrast, WIPK-mediated signal transduction mechanisms operate in the control of the biosynthetic steps that convert 13(S)-OOH-18:3 into OPDA, namely AOS and/or AOC (**Fig. 1**). This control is partially executed by the regulation of AOS activity at a post-transcriptional level.[25] WIPK, SIPK and NPR1 are thought to be extraplastidial proteins and therefore additional components must participate to convey the activating signal into the stroma of plastids, where the enzymes catalyzing the initial steps of JA biosynthesis reside. The further identification of signal transduction components and the elucidation of the mechanisms affecting enzyme activity would provide critical information for the understanding of how primary stress signals are translated into the activation of JA biosynthetic enzymes.

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