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Jasmonates

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Arabidopsis is a superb model for the study of an important subgroup of oxylipins: the jasmonates. Jasmonates control many responses to cell damage and invasion and are essential for reproduction. Jasmonic acid (JA) is a prohormone and is conjugated to hydrophobic amino acids to produce regulatory ligands. The major receptor for active jasmonate ligands is closely related to auxin receptors and, as in auxin signaling, jasmonate signaling requires the destruction of repressor proteins. This chapter uses a frequently asked question (FAQ) approach and concludes with a practical section.

INTRODUCTION

What are oxylipins?

Oxylipins are oxygenated fatty acids containing one or more oxygen atoms other than those in the carboxyl group.

What are jasmonates?

Jasmonates form a family of oxylipins arising from the enzymatic oxygenation of 18 and 16-carbon triunsaturated fatty acids (Wasternack and Kombrink, 2010) and can also be made by a few fungi (Miersch et al., 1991). The best known jasmonates are jasmonic acid (JA), methyl jasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile). Oxo-phytodienoic acid (OPDA) and its 16-carbon homolog dinor-OPDA both have a cyclopentenone ring structure whereas JA and most of its derivatives are cyclopentanones. Cyclopentenones have a carbon-carbon double bond in the ring structure whereas this is saturated in cyclopentanones.

Which jasmonates are biologically active?

The jasmonates for which there is most evidence for biological activity are:

1. (+)-7-iso-jasmonoyl-L-isoleucine (JA-Ile; Fonseca et al., 2009b). This is the ligand that seems to play the major role in jasmonate signaling in Arabidopsis leaves and possibly flowers.
2. Jasmonoyl-L-tryptophan is an auxin signaling inhibitor active in Arabidopsis roots (Staswick, 2009).
3. OPDA and dinor-OPDA have signaling properties either independent of canonical jasmonate signaling (Stintzi et al., 2001; Taki et al., 2005) or using part of the jasmonate signaling machinery (Ribot et al., 2008).

What do jasmonates do in plant defense responses?

Herbivores: Jasmonates play major roles in plant defense at the interface of the primary and secondary trophic levels, i.e. interactions of plants and herbivores. Jasmonates control the expression of an estimated 67-85% of wound- and insect-regulated genes in Arabidopsis leaves. At least 1.3% of protein-coding genes respond to leaf damage via activation of jasmonate signaling (Reymond et al., 2004). These genes fall into functional categories including: jasmonate synthesis, jasmonate signaling components (e.g. transcription factors), defense genes (e.g. jacalin lectins, defensins and myrosinases involved in glucosinolate [secondary metabolite] production), resource relocalization genes, general stress response genes, etc.

In addition, jasmonate-controlled growth changes are initiated; new leaves have more trichomes and smaller petioles on wounded plants than on the leaves of unwounded plants (Yan et al., 2007; Zhang and Turner, 2008; Yoshida et al., 2009).

Necrotrophic pathogens: Jasmonates are important mediators of defense responses to necrotrophic pathogens (Kachroo and Kachroo, 2009).

Foliar biotrophs: Work on leaf pathogens such as powdery mildews is reviewed elsewhere (Lipka et al., 2008; Bari and Jones, 2009). Virulent *Pseudomonas syringae* strains often produce coronatine (COR), a potent and useful jasmonoyl-isoleucine mimic that acts as a virulence factor (Melotto et al., 2006).

Biotrophic root pathogens and symbionts: The place of jasmonates in plant interactions with nematodes, biotrophic pathogens and symbionts and with parasitic plants is reviewed in Gutjahr and Paszkowski (2009).

Detritivores: Without the ability to make JA living plants can be consumed by crustacean detritivores that normally feed on dead and decaying tissues (Farmer and Dubugnon, 2009).

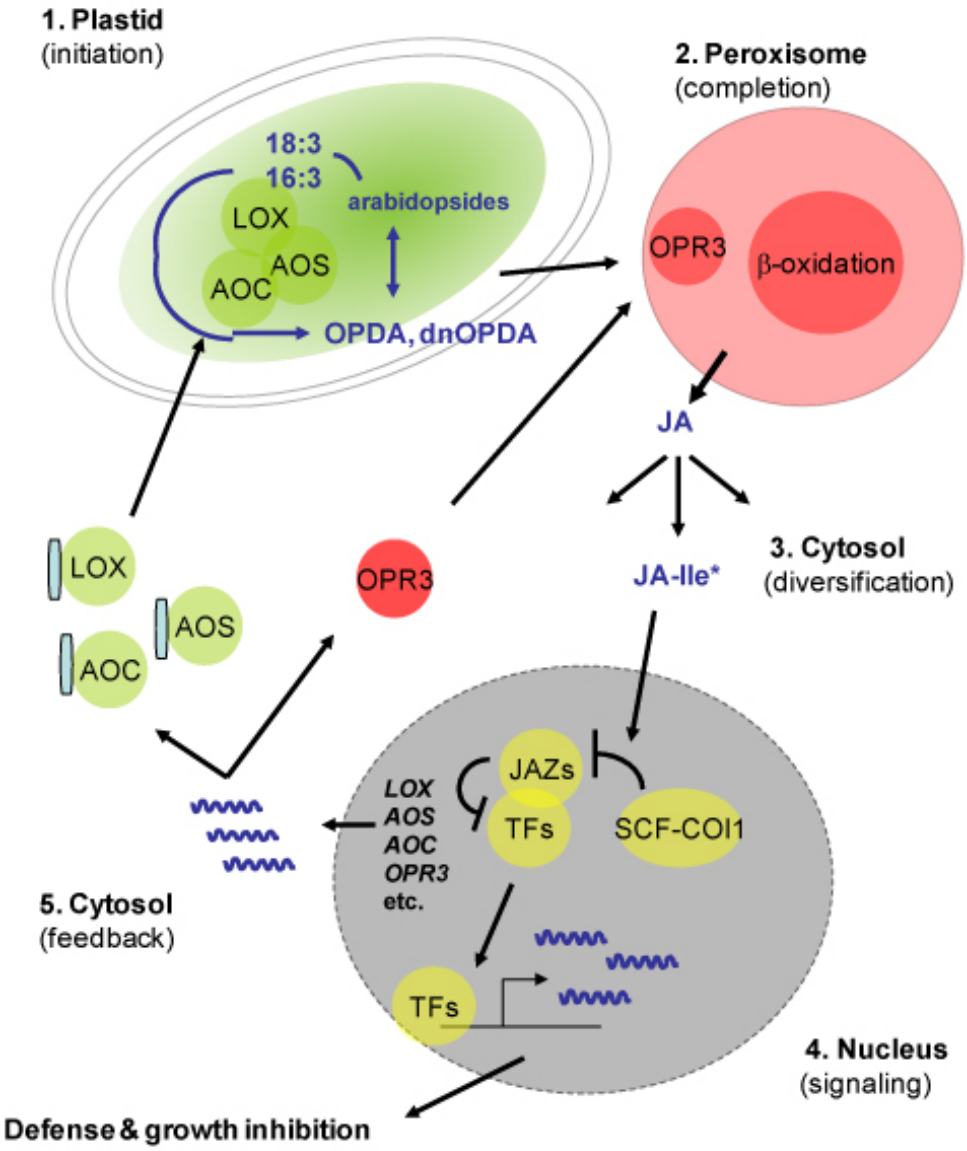


Figure 1. Cellular compartments in JA synthesis and signaling: an overview.

The five major steps of JA synthesis and signaling (see text) take place in the several cellular compartments. JA is made by the combined action of plastids and peroxisomes. Many of the participating enzymes are jasmonate-inducible and targeted to these organelles. In the case of the plastid-localized enzymes LOX, AOS and AOC their import sequences (blue boxes) are cleaved off. Plastids also harbor pools of the JA precursors OPDA and dn-OPDA esterified to galactolipids in compounds known as arabidopsides. Jasmonoyl isoleucine (JA-Ile) made in the cytosol from JA functions as a signal in the nucleus. Note that several other compartments such as the cell walls and vacuoles might contribute to JA signaling (not shown).

What, in a nutshell, do jasmonates do in Arabidopsis development?

Jasmonates are essential to complete the last steps of stamen development, specifically in three processes: a) Pollen maturation after the trinucleate stage to guarantee viability and fertility; b) Elongation of stamen filaments so that anthers reach the stigma in preparation for pollination; c) Dehiscence of anther locules for pollen release. Impairment of these functions in jasmonate synthesis or perception mutants results in male sterility (Feys et al., 1994; McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000; Ishiguro et al., 2001; Park et al., 2002; von Malek et al., 2002)

Jasmonates help to regulate petiole size even in the absence of wounding (Zhang and Turner, 2008) and as part of the wound response. They also control seed size (Farmer and Dubugnon, 2009) although the cell types affected in seeds are not yet identified. Jasmonate signaling limits petal size and affects vein structure (Brioudes et al., 2009).

FIVE MAJOR STEPS IN JASMONATE SYNTHESIS AND SIGNALING: AN OVERVIEW

JA synthesis and signaling are interlinked (Figure 1) by a positive feedback loop whereby jasmonates stimulate their own synthesis (e.g. Sasaki et al., 2001). The extent of activity of this positive feedback loop appears to differ in tissues near a wound where there is strong feedback leading to the production of high levels of JA, and distal tissues where JA levels increase but remain relatively low (Glauser et al., 2009).

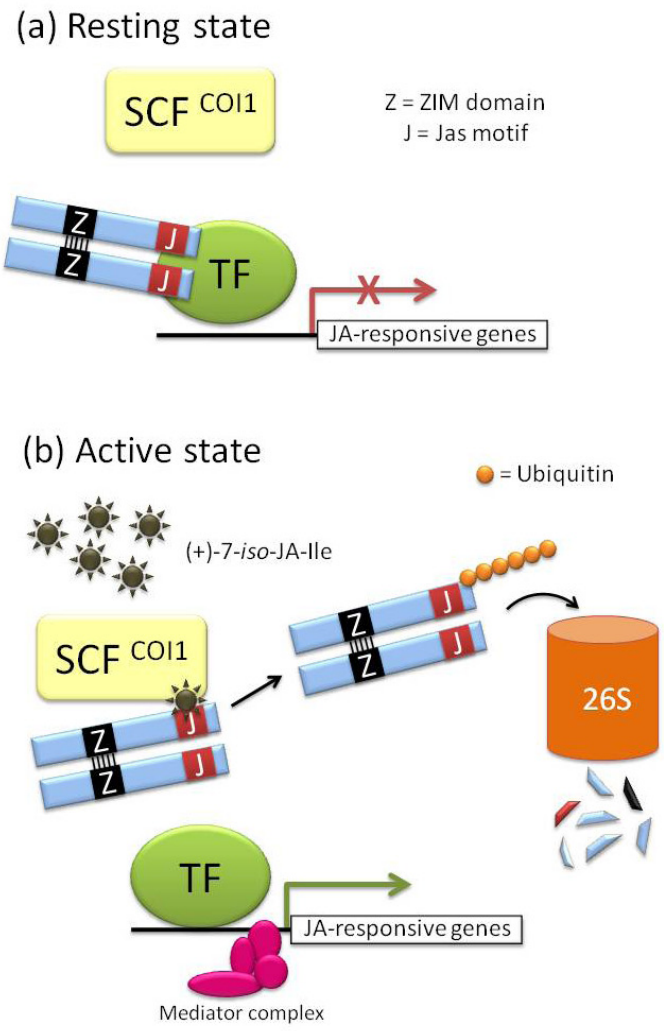
Step 1: Initiation of JA synthesis in plastids. LOXs oxygenate triunsaturated fatty acids (18:3 and 16:3) to produce 13-hydroperoxyfatty acids. These are substrates for AOS which generates unstable allene oxides that are cyclized by AOCs. The resulting compounds, OPDA and dinor-OPDA, are exported to the peroxisome.

Step 2: JA synthesis completion in peroxisomes. First, OPDA and dinor-OPDA are reduced from cyclopentenones to cyclopentanones by the peroxisomal enzyme OPR3. The resulting compounds, OPC8 and OPC6 respectively, are then subject to β-oxidation (3 rounds for OPC8 and 2 rounds for OPC6) to produce JA (Schaller and Stintzi, 2009). JA is exported by an unknown mechanism to the cytosol.

Step 3: Biochemical diversification. JA is the starting point for the synthesis of many compounds. JA-Ile (Staswick and Tiryaki, 2004) and probably JA-Trp (Staswick, 2009) are made in the cytosol. Both JA and JA-Ile undergo diverse modifications in vivo. For example, JA can be hydroxylated at the 11- and 12-positions. 12-hydroxyjasmonate can be sulfated (Gidda et al., 2003) or glucosylated (Świątek et al., 2004a).

Step 4: Signaling. This takes place in the nucleus where JA-Ile binds its receptor, the CORONATINE INSENSITIVE1 (COI1) protein which forms part of an SCF ubiquitin E3 ligase (Yan et al., 2009). This complex then interacts with JAZ proteins which are ubiquitinated. The destruction of JAZ proteins by 26S proteasomes liberates transcription factors and allows gene expression (Browse, 2009; Chung et al., 2009; Fonseca et al., 2009a; http://stke.sciencemag.org/cgi/cm/stkecm;CMP_13931).

Step 5: Production and targeting of JA synthesis enzymes. 13-Lipoxygenases (13-LOXs) are synthesized on cytosolic ribosomes and targeted to the plastid. The same is true of ALLENE OXIDE SYNTHASE (AOS) and ALLENE OXIDE CYCLASE (AOC). OXOPHYTODIENOIC ACID REDUCTAXSE3 (OPR3) is also encoded by a nuclear gene and is sent into the peroxisome (Schaller and Stintzi, 2009).



JASMONATE SIGNALING

Figure 2 displays the current model of jasmonate perception and signaling.

What is the jasmonate receptor?

The 18 leucine-rich repeat (LRR) protein COI1 is the receptor of (+)-7-*iso*-jasmonoyl-isoleucine (JA-Ile), a major biologically active jasmonate. Loss of COI1 function demonstrated its essential role for normal responsiveness to jasmonate in Arabidopsis (Feys et al., 1994). More recently, biochemical approaches have shown direct binding of JA-Ile (or its structural mimic coronatine) to COI1 (Yan et al., 2009). The JA-Ile binding site is formed by three surface loops situated in LRRs 2, 12 and 14. None of the 19 cysteine (Cys) residues in COI1 form sulfur bridges and a reducing environment is necessary for CO1 function: COI1 may be redox-regulated in vivo.

Figure 2. Model for jasmonate signaling

In the resting state **(a)**, when hormone concentrations are low or undetectable, JAZ proteins bind transcription factors such as MYC2 preventing their function as activators of jasmonate-responsive genes. In the active state **(b)**, certain external stimuli (e.g. wounding) or developmental programs (e.g. anther maturation) promote jasmonate biosynthesis leading to active hormone accumulation (e.g. jasmonate isoleucine [(+)-7-*iso*-JA-Ile]). The hormone is perceived by the protein COI1 which is the F-box subunit of an E3 ubiquitin ligase of the type SKP1-CUL1-F-box (SCF). Hormone recognition by COI1 favors binding of COI1 to JAZ proteins via their Jas motif. In this model the JAZ proteins can be seen as co-receptors acting with COI1. This promotes ubiquitination of JAZ proteins and their subsequent degradation by the 26S proteasome. The transcription factors are now relieved from JAZ-mediated repression and free to recruit the RNA polymerase II transcriptional machinery to the promoter of jasmonate-responsive genes. Recent evidence suggests that this recruitment occurs through universal adaptors such as the Mediator complex.

What are the components of the SCF-COI1 complex?

SCF complexes are a type of E3 ubiquitin ligase, multisubunit machines that specify and mediate protein ubiquitination for targeted degradation by the 26S proteasome. SCF ligases contain a variable F-box protein that directly binds the targets of the ubiquitination complex thereby conferring its specificity. The F-box protein COI1 defines an SCF complex specifically involved in jasmonate signaling (Devoto et al., 2002; Xu et al., 2002). COI1 binding to some members of the JAZ protein family and their subsequent proteasome-dependent degradation has been demonstrated (Chini et al., 2007; Thines et al., 2007; Chung and Howe, 2009).

The other components of the SCF-COI1 complex are:

a) Either ASK1 or ASK2 (for Arabidopsis SKP1, homologs of yeast's Suppressor of Kinetochore Protein 1), which serve as adaptors for COI1 to form the substrate-recognizing subunit.
b) RBX1 (Ring-Box Protein 1), a RING finger protein that recruits an E2 ubiquitin conjugating enzyme to bring it close to the substrate.
c) The scaffolding CULLIN1 protein that holds the substrate-recognizing portion of the complex at its N-terminus and RBX1 at its C-terminus.

RBX1 and CULLIN1 are necessary for normal jasmonate signaling (Xu et al., 2002; Ren et al., 2005) and so are other components known to interact with or regulate SCF complexes such as AXR1, the COP9 signalosome and SGT1b (Tiryaki and Staswick, 2002; Feng et al., 2003; Lorenzo and Solano, 2005).

What are JAZ proteins?

Jasmonate ZIM-Domain (JAZ) proteins are negative regulators of the transcription of jasmonate-responsive genes (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). This action is probably not performed by directly binding gene promoter sequences because JAZ proteins lack recognizable DNA-binding domains. However, their interaction with the transcription factor MYC2 (Chini et al., 2007; Melotto et al., 2008; Chini et al., 2009; Chung and Howe, 2009) suggests that JAZ proteins control jasmonate-related gene expression by preventing the function of transcriptional activators but the exact mechanism is still not known.

JAZ proteins in Arabidopsis are encoded by 12 different genes (JAZ1 through JAZ12), which are predicted to produce over 20 protein variants (http://arabidopsis.org), including two characterized alternative splice products, JAZ10.3 and JAZ10.4 (Yan et al., 2007; Chung and Howe, 2009). This level of redundancy suggests possible overlapping roles for the different JAZ proteins.

Interestingly, most *JAZ* genes are rapidly induced by MYC2 upon activation of jasmonate signaling (Mandaokar et al., 2006; Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung et al., 2008). In general, the promoters of *JAZ* genes contain putative MYC2-binding motifs that have been proven functional for *JAZ3* (Chini et al., 2007).

What are the Jas motif and the ZIM domain?

These are the two most conserved features between JAZ proteins. The Jas motif is towards the C-terminus and constitutes the binding site of COI1 in the presence of JA-Ile; therefore, the Jas motif is required for timely JAZ protein degradation upon jasmonate perception (Chini et al., 2007; Thines et al., 2007; Melotto et al., 2008; Chung and Howe, 2009). It is also necessary and sufficient for the interaction of JAZ proteins with MYC2 (Chini et al., 2007; Melotto et al., 2008; Chini et al., 2009). Finally, the Jas motif may be important for nuclear localization of JAZ proteins (Grunewald et al., 2009), even if its absence does not necessarily prevents their entry into the nucleus (e.g. Chung and Howe, 2009).

The ZIM domain sits around the center of the JAZ proteins and contains a conserved TIFY motif (TIFYXG). This domain mediates homo- and heteromeric interactions between many JAZ proteins, a capability that is critical for their function as negative regulators of jasmonate signaling (Chini et al., 2009; Chung and Howe, 2009). These interactions may be necessary to allow proper assembly of JAZ proteins into nuclear bodies (Grunewald et al., 2009), which may contain the macromolecular complexes regulating jasmonate responses.

What are the known functions of MYC2 in jasmonate responses?

MYC2 is a basic helix-loop-helix (bHLH) transcription factor that activates a first wave of gene transcription upon jasmonate perception, including the *JAZ* genes and the putative jasmonate biosynthesis gene LOX3 (Chini et al., 2007; Pauwels et al., 2008). MYC2 also has a dual role later in jasmonate-induced transcription: as an activator of wound response genes (e.g. *VSP2*) and, strikingly, as a negative regulator of pathogen-defence genes (e.g. *PDF1.2*) (Lorenzo et al., 2004). On the other hand, MYC2 is not the only transcription factor in early jasmonate signaling of processes such as stamen maturation. Moreover, MYC2 participates in other Arabidopsis functions such as abscisic acid signaling and photomorphogenesis (Abe et al., 2003; Yadav et al., 2005).

What is the nature of the MYC2 transcriptional complex?

This is not known yet beyond the interaction between JAZ proteins and MYC2. The answer to this question will help to explain how the diverse functions of MYC2 and other jasmonate-related transcription factors are regulated. As known for their animal counterparts, it is expected that plant bHLH transcription factors homo- and heterodimerize. There are at least three additional MYC2-like proteins encoded in the Arabidopsis genome (Heim et al., 2003) that may act redundantly (e.g. in stamen maturation) or forming heterodimers with MYC2.

Another layer of regulation may be provided by the formation of larger complexes with transcriptional coactivators, corepressors and other protein machineries. For example, proper recruitment of RNA polymerase II complexes by transcriptional activators requires the bridging complex Mediator (MED). In Arabidopsis, the subunits MED25 (aka PFT1) and MED8 of this complex are required for some jasmonate-signaled defense responses (Kidd et al., 2009). Changes in chromatin structure are another essential process during transcriptional activation. Accordingly, the chromatin remodeling ATPase SPLAYED and the chromatin modifiers HISTONE DEACETYLASE19 and HISTONE DEACETYLASE6

are also necessary for gene expression stimulated by jasmonate (Zhou et al., 2005; Wu et al., 2008). It remains to be seen if all these components are part of a MYC2 transcriptional complex.

How is jasmonate signaling restrained after the initial response?

As for any other hormone, jasmonate produces strong effects in cells (e.g. slowing down plant growth). It is important then to control hormone signaling after the initial response. In the case of jasmonates, one way to accomplish this is to metabolize the bioactive forms of the hormone to make them inactive (see below). The fact that *JAZ* gene transcription is rapidly induced upon jasmonate signaling suggests a negative feedback loop control mechanism, whereby newly made JAZ proteins would repress again their corresponding transcription factors. Moreover, to do this more efficiently, cells may rely on alternative splice variants of JAZ proteins such as JAZ10.3 and JAZ10.4 (Yan et al., 2007; Chung and Howe, 2009). Contrary to the full-length protein JAZ10.1, these two variants are missing part or the entire Jas motif. In consequence, they are not recognized efficiently by COI1 and are not subject to effective jasmonate-induced proteasome degradation (Chung and Howe, 2009). Rapid accumulation of these variants is predicted to desensitize cells to the presence of the hormone.

What are the commonalities between the perception mechanisms of jasmonate and auxin?

They both use as receptor an SCF-type E3 ubiquitin ligase with a specific F-box protein for each hormone, COI1 for jasmonate and TIR1 (and closely related proteins) for auxin (Dharmasiri et al., 2005). The other components and most accessory proteins of the SCF-COI1 complex are also used in the SCF-TIR1 complex; therefore, complete loss of function in some of these components impairs the responses to either hormone.

The similarities extend to the outcome of hormone perception. Auxin also promotes the binding of SCF-TIR1 to a set of negative regulators of auxin-responsive genes, the Aux-IAA proteins, which are then targeted for degradation by the proteasome (Dharmasiri et al., 2005; Kepinski and Leyser, 2005). Like the JAZ proteins in jasmonate signaling, Aux-IAA proteins act as repressors by binding the transcriptional regulators of auxin responses or ARFs (Auxin Response Factors). Aux-IAA degradation upon auxin perception releases ARFs to perform their function. JAZ and Aux-IAA proteins are not obviously related at the sequence level.

What are the interconnections between jasmonate and auxin signaling?

One type of cross-regulation may occur if the outcome of one hormone's signaling directly or indirectly affects the other's. For example, Auxin Response Factors ARF6 and ARF8 regulate normal jasmonate biosynthesis in Arabidopsis flowers (Nagpal et al., 2005), although the exact mechanism for this control remains unknown. Elsewhere, jasmonate promotes lateral root formation by directly inducing the auxin biosynthesis gene *anthranilate synthase* α*1* (ASA1) in root basal meristems; additionally, jasmonate reduces the levels of the proteins PIN-FORMED1 (PIN1) and PIN2 resulting in attenuated auxin transport (Sun et al., 2009).

As indicated before, several components and accessories of the SCF complex function in the perception machineries of both jasmonate and auxin. This might constitute another hypothetical scenario for crosstalk between the two hormones. For example, their perception machineries may compete for a limiting common protein; if hormone concentration and/or receptor-complex affinities tilt the balance, signaling may be favored for one of them while dampened for the other.

An intriguing variation on this idea is that jasmonate and auxin share other signaling proteins. Indeed, recent evidence indicates that the gene encoding the jasmonate repressor JAZ1 is also inducible by auxin independently of the jasmonate signaling pathway (Grunewald et al., 2009). The relevance of this phenomenon for the function of either or both hormones awaits further investigation.

Finally, a different type of cross-regulation may be mediated by tryptophan conjugates of jasmonic acid (JA-Trp) and auxin (IAA-Trp). These substances inhibit several auxin responses leading to the proposal that they modulate the sensitivity to auxin in planta (Staswick, 2009).

What is known about jasmonate/salicylate cross-regulation?

The plant hormone salicylic acid (SA) signals defense responses to biotrophic pathogens. JA and SA seem to act antagonistically since mutants with impaired signaling in one of these hormones display enhanced expression of marker genes of the other (Kloek et al., 2001; Glazebrook et al., 2003; Spoel et al., 2003). Furthermore, reduced JA signaling and responses are observed upon stimulation of SA signaling mediated by NPR1 (Nonexpressor of Pathogenesis-Related Genes 1; Spoel et al., 2003). However, the outcomes of JA and SA interplay vary with hormone concentrations and the type of stimuli (e.g. virulent vs. avirulent pathogen; Mur et al., 2006; Spoel et al., 2007).

Does jasmonate affect plant growth by influencing cell elongation or cell division?

Evidence from cell culture studies and wounding of Arabidopsis plants suggests that the plant growth retardation mediated by jasmonate occurs through a block in mitotic cell division that includes, for example, a reduction in the expression of *CyclinB1* genes; additionally, wounding does not seem to affect leaf cell size Świątek et al., 2002; Świątek et al., 2004b; Zhang and Turner, 2008).

The exact cellular/tissue responses to jasmonate during Arabidopsis male reproductive development are currently not understood. However, it has been shown that the requirement of jasmonate for pollen viability is not at the level of meiosis but at later stages (Devoto et al., 2002). While jasmonate slows down petiole growth upon wounding, it promotes stamen filament elongation in Arabidopsis. How are these two seemingly opposing responses mediated by the same hormone?

What is the connection between short, medium and long term effects of jasmonate?

Changes in gene expression mediated by jasmonate occur within a wide temporal range. This goes from minutes for the induction of genes encoding *JAZ* proteins, *MYC2* or jasmonate biosynthesis enzymes (Mandaokar et al., 2006; Chung et al., 2008; Pauwels et al., 2008) to hours for defense genes during wound responses (Reymond et al., 2000) or transcription factors during stamen maturation (Mandaokar et al., 2006). The morphological outcome of this reprogramming becomes visible after several days as in the case of growth inhibition (Yan et al., 2007; Zhang and Turner, 2008) or trichome formation (Yoshida et al., 2009).

The function of 'medium-term' factors induced by jasmonate to promote stamen fertility are starting to be elucidated, including several MYB-type transcriptional regulators (Mandaokar et al., 2006; Mandaokar and Browse, 2009). Some of the genes necessary for jasmonate-induced trichome development have recently been described (Yoshida et al., 2009). Other 'medium-term' targets of COI1-mediated jasmonate signaling are a set of transcription factors which in turn induce anthocyanin biosynthesis genes (Shan et al., 2009). We are still missing an understanding of the intermediate jasmonate-responsive factors controlling the phenomenon of wound-induced growth retardation.

As described above, MYC2 directly regulates the expression of early jasmonate-responsive genes. However, it is not known if the same transcriptional machinery directly regulates the expression of late jasmonate-responsive genes. It is more plausible that a different set of transcription factors, in turn induced during the early wave, are in charge of this function. Additionally, it has not been explored if jasmonate signaling results in changes in methylation patterns and other epigenetic processes.

What is the long-distance wound signal triggering JA synthesis distal to wounds?

The nature of the systemic signal leading to JA and JA-Ile synthesis in tissues distal to wounds is not clear. Work, mostly on other plants, implicates peptide signals acting early to stimulate the jasmonate response (Schilmiller and Howe, 2005). The signal may be propagated by long distance ion fluxes (Maffei et al., 2007). OPDA is not necessary for the export of the rapid long-distance signal from a wounded Arabidopsis leaf (Koo et al., 2009). The signal is estimated to move at an average of 3.4 to 4.5 cm/min through the plant to stimulate JA accumulation in distal leaves (Glauser et al., 2009). A similar speed (less than 2 cm per min) is reported for the long-distance wound-initiated signal leading to JA-Ile production in Arabidopsis (Koo et al., 2009; Koo and Howe, 2009).

JASMONATE BIOCHEMISTRY

Arabidopsis seems to be similar to other angiosperms (including monocots) with respect to jasmonates. However, the crucifer family contains the fatty acid '16:3' [7Z,10Z,13Z-hexadecatrienoic acid, an α-linolenic acid (18:3) homolog] and has 16:3-derived oxylipins including dinor-OPDA. The biochemical pathway of jasmonate synthesis is given in Figure 3 and can also be accessed at http://stke.sciencemag.org/cgi/cm/stkecm;CMP_7361. Quite a lot is known about the enzymes of jasmonate synthesis (Schaller and Stintzi, 2009).

What are the first enzymes of jasmonate synthesis?

In flowers the current model is that the enzyme DAD1 hydrolyzes 18:3 and/or 16:3 off lipids (either a phospholipid or a galactolipid)

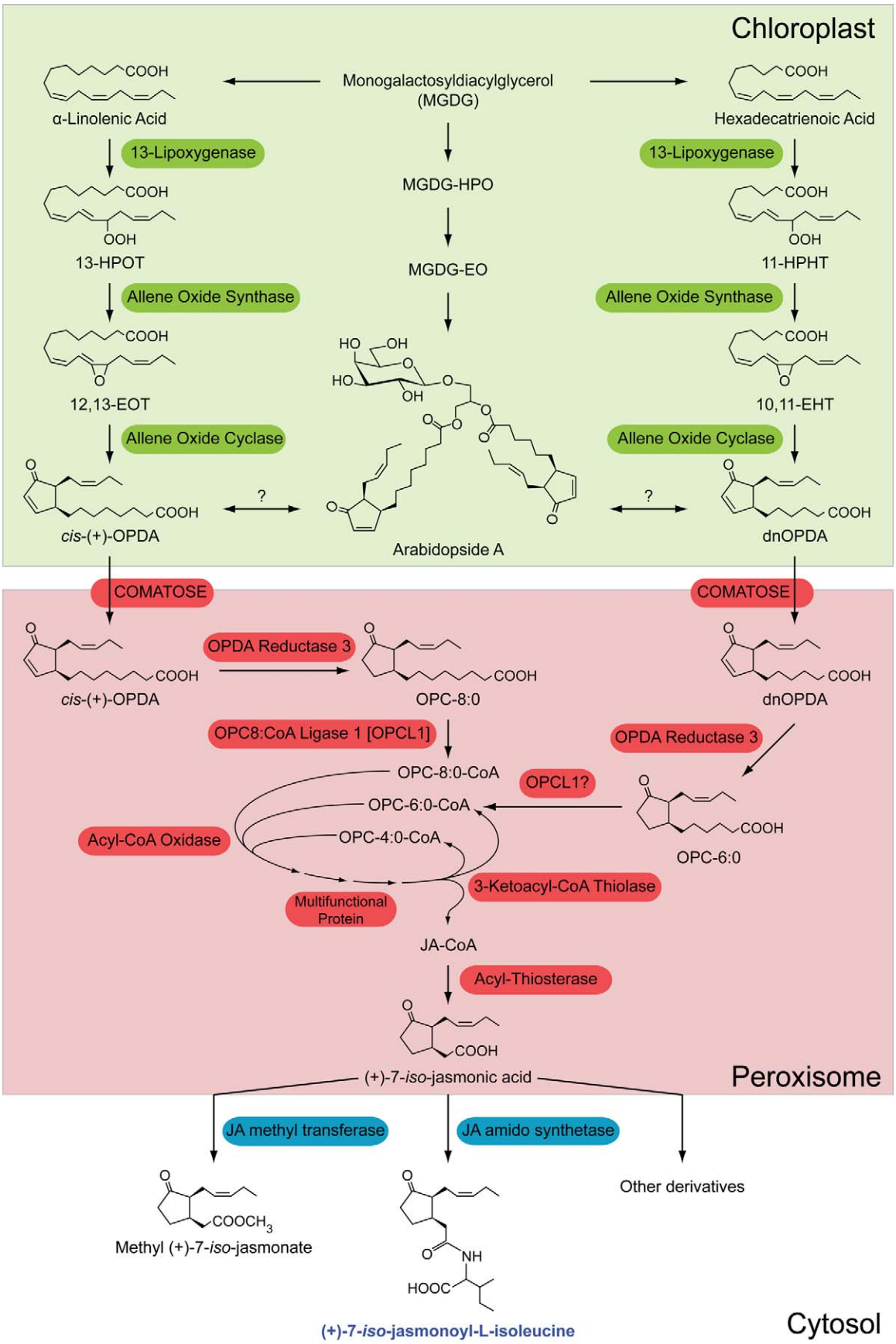
Figure 3. Jasmonic acid biosynthesis and metabolism in Arabidopsis

Jasmonic acid (JA) is derived from polyunsaturated fatty acids linolenic acid (18:3) and hexadecatrienoic acid (16:3) which are stored in plastids mostly esterified to monogalactosyldiacylglycerol (MGDG). 13-LOXs add molecular oxygen to 18:3 and 16:3 to form their corresponding hydroperoxides 13(*S*)-hydroperoxy-octadecatrienoic acid [13-HPOT] and 11(*S*)-hydroperoxy-hexadecatrienoic acid [11-HPHT]. AOS transforms these into the allene oxides (13*S*)-12,13-epoxy-octadecatrienoic acid [12,13-EOT] and (11*S*)-10,11-epoxy-octadecatrienoic acid [10,11-EOT]. AOC directs the formation of a cyclopentenone ring in these compounds to yield 12-oxo-phytodienoic acid (OPDA) and dinor-oxo-phytodienoic acid (dnOPDA), respectively. OPDA and dnOPDA are also found esterified to galactolipids in the form of arabidopsides, which to date have only been isolated from Arabidopsis and a few other related crucifers. It is possible that these arabidopsides are synthesized through the direct action of 13-LOX, AOS and AOC in MGDG.

OPDA and dnOPDA are transferred to the peroxisomes at least partially through the action of the ABC-type transporter COMATOSE (CTS). OPDA Reductase 3 catalyzes the reduction of the cyclopentenone ring in OPDA and dnOPDA to form 3-oxo-2-(2-(*Z*)-pentenyl)-cyclopentane-1-octanoic (OPC-8:0) and hexanoic (OPC-6:0) acids, respectively. The enzyme OPC-8:CoA ligase 1 (OPCL1) esterifies a CoA to the acyl group of OPC-8:0 and -presumably- OPC-6:0 in preparation for a series of ß-oxidation cycles that shorten the acidic chain of these compounds to yield jasmonic acid. ß-oxidation proceeds through three types of enzymes: acyl-CoA oxidase (ACX), multifunctional protein (MFP, which displays enoyl-CoA hydratase and ß-hydroxy-acyl-CoA dehydrogenase activities), and 3-ketoacyl-CoA thiolase (KAT). Finally, a presumed acyl-thioesterase (ACH) releases (+)-7-*iso*-jasmonic acid (JA) from its CoA ester.

Upon transport to the cytoplasm, JA undergoes further metabolism such as esterification into methyl (+)-7-iso-jasmonate or amino acid conjugation to form (+)-7-*iso*-jasmonoyl-isoleucine which is considered the active form of the hormone.

[Figure adapted from http://stke.sciencemag.org/cgi/cm/stkecm;CMP_7361 and Schaller and Stintzi (2009)]



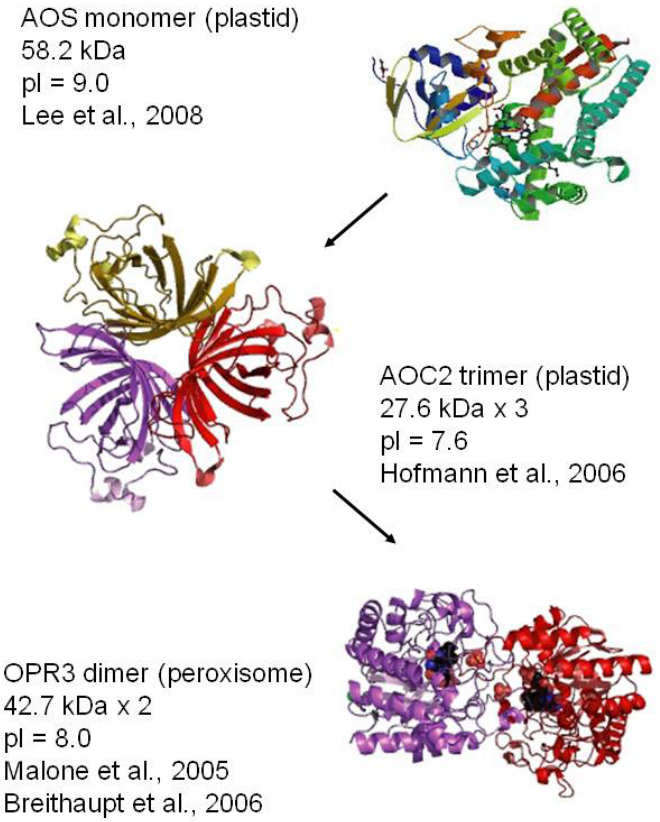


Figure 4. Structures of the three main enzymes of jasmonate synthesis

Allene oxide synthase (AOS) is a basic protein that catalyses the formation of allene oxides from fatty acid hydroperoxides generated by lipoxygenases (LOXs). AOS crystallizes as a monomer. Allene oxide cyclase 2 (AOC2) is one of four AOCs encoded in the Arabidopsis genome. AOC2 directs the correct folding pathway of allene oxides into the natural stereochemical form of OPDA. The enzyme crystallizes as a trimer. Oxo-phytodienoic acid reductase 3 (OPR3) is an NADPH dehydrogenase that catalyses the reduction of the cyclopentenone double bond in OPDA to produce the intermediate OPC8. Tomato OPR3 crystallizes as a dimer (Breithaupt et al., 2006). Dimer formation was not observed in Arabidopsis OPR3 crystals (Malone et al., 2005) but based on Breithaupt et al. (2006) dimer formation is likely for the Arabidopsis enzyme and is thus indicated in the figure. The molecular mass and pI data given for the proteins includes the plastid import sequences present on AOS and AOC2.

to allow the initiation of JA synthesis (Ishiguro et al., 2001). Gene products related to DAD1 are candidate hydrolases for 18:3 release in leaves (Hyun et al., 2008). Alternatively, it is possible that JA synthesis in leaves is initiated on esterified fatty acids. The first metabolic step in JA biosynthesis is the addition of molecular oxygen to 18:3 catalysed by 13-lipoxygenases (13-LOXs; Feussner and Wasternack, 2002). LOXs must first interact with divalent cations (usually Ca^{2+}) which make these otherwise soluble proteins become membrane-associated and catalytically active (Schneider et al., 2007). There are four 13-LOXs in Arabidopsis: LOXs 2, 3, 4 and 6 (Bannenberg et al., 2009a). LOX2 is necessary for the production of about 75% of JA made in the wounded leaf (Bell et al., 1995) and for a large proportion of the jasmonates found in arabidopsides (Glauser et al., 2009). Figure 3 shows how fatty acid hydroperoxides are transformed via the action of ALLENE OXIDE SYNTHASE (AOS) and ALLENE OXIDE CYCLASE (AOC) to OPDA.

What are the crystal structures of the enzymes of jasmonate synthesis?

Structures are known for AOS, AOC2 and OPR3 from Arabidopsis (Figure 4). AOS is a member of the cytochrome P-450 family CYP74. AOS crystallizes as a monomer and displays a large putative membrane-binding surface (Lee et al., 2008). Like other members of its family it has unique signature sequences that distinguish it from members of other families of CYPs. A single point mutation can change AOS into a hydroperoxide lyase (Lee et al., 2008). AOC2 is a member of the lipocalin family. The structure helped to explain how AOC2 funnels the rearrangement of allene oxide to OPDA along a strict stereochemical path. AOC2 is a trimer in vitro and in vivo (Hofmann et al., 2006). OPR3 is a member of a large family of proteins: flavoprotein oxidoreductases. OPR3 has been crystallized from Arabidopsis (Malone et al., 2005) and tomato (Breithaupt et al., 2006). The OPR3 structure from tomato revealed that the enzyme can dimerize to block access to its active site suggesting that dimerization can control OPDA generation. ACX1 crystallizes as a dimer (Pedersen and Henriksen, 2005).

Are the enzymes of β-oxidation specific for jasmonate synthesis?

Prior to β-oxidation OPDA is reduced to OPC8 by OPR3 in a reaction that appears to be specific to the jasmonate pathway (Sanders et al., 2000; Stintzi and Browse, 2000). After this step it is not clear how specific the steps of β-oxidation are for JA production. Several genetic studies are consistent with the enzymes of β-oxidation carrying out house-keeping functions in addition to making JA (Cruz Castillo et al., 2004; Koo et al., 2006; Delker et al., 2007; Schilmiller et al., 2007).

In which tissues are jasmonates made?

The levels of JA tend to be higher in leaves than in roots suggesting that cells with chloroplasts probably make the bulk of OPDA and JA. Nevertheless, the possibility that cells without chloroplasts (epidermal pavement cells, most root cells, etc.) make JA is open. Indeed, *LOX2*, a jasmonate-producing LOX (Bell et al., 1995) is expressed in the elongation zone of the root (Birnbaum et al., 2003)

How are active JA-amino acid conjugates deactivated?

Two processes are likely to inactivate biologically active JA-Ile in vivo. The first is epimerization whereby the biologically active ligand (+)-7-iso-JA-Ile is converted to (-)-7-JA-Ile (Fonseca et al.,

2009b) much as JA itself can be deactivated by epimerization (Farmer, 1994; Schulze et al., 2006). A second process is oxidation of JA-Ile to forms which are, apparently, no longer biologically active as jasmonates. These are 12-hydroxy-JA-Ile (Miersch et al., 2008) and the more polar compound 12-carboxy-JA-Ile (Glauser et al., 2008b).

What are the crucial time-points concerning JA synthesis and action after wounding?

First significant increase of JA in the wounded leaf: less than 30 sec after wounding (Glauser et al., 2009).

Rate of JA synthesis in the wounded leaf: JA levels double each 20 sec in the first min after wounding (Glauser et al., 2009).

First clear increase of JA in leaves distal to the wound: 2 min after wounding (Glauser et al., 2008b; Glauser et al., 2009).

First significant increase of JA-Ile in the wounded leaf: 5 min after wounding (Koo et al., 2009).

First increase of JA-Ile in leaves distal to the wound: 5 min after wounding (Koo et al., 2009).

First appearance of jasmonate-regulated transcripts in the wounded leaf: 5 min for *JAZ1* and 15-30 min for *JAZ7* and *MYC2* (Chung et al., 2008; Koo et al., 2009).

First appearance of jasmonate-regulated transcripts in leaves distal to the wound: 15 min for JAZ7 (Koo et al., 2009).

First appearance of the JA clearance products 11- and 12-hydroxyjasmonates and 12-carboxy-JA-Ile: approximately 45 min after wounding (Glauser et al., 2008b).

What are the levels of JA, OPDA and dinor-OPDA in Arabidopsis?

JA levels in very healthy arthropod-free Arabidopsis leaves are low, typically about 20-50 pmol per g fresh mass (Glauser et al., 2008b; Glauser et al., 2009). JA levels in the wounded leaf increase up to 500-fold, reaching about 10 nmol per g fresh mass (e.g. Reymond et al., 2000). Levels of JA in leaves distal to a wounded leaf can exceed 200 pmol per g fresh mass (Glauser et al., 2009). JA and JA-Ile levels for roots, shoots and flowers are reported in Suza and Staswick (2008). Flowers contain both JA and JA-Ile in a ratio of approximately 5 to 1. JA-Ile levels in wounded leaves reach less than 10% of the level of JA (Suza and Staswick, 2008). Rough handling of plants, the presence of insects in growth rooms, and even potassium starvation (Armengaud et al., 2004) activate JA production and jasmonate signaling. The most abundant jasmonate pools in the plant are found in arabidopsides. The levels of some arabidopsides, for example arabidopside D (OPDA-OPDA-digalactosyl diacylglycerol), increase up to 1000-fold in a leaf that has been wounded (Buseman et al., 2006).

What are arabidopsides?

Arabidopsides are plastid-localized galactolipid-derivatives containing esterified OPDA and dinor-OPDA. They are found in large quantities in *Arabidopsis thaliana* and in a few closely related crucifers such as *Arabidopsis arenosa* (Böttcher and Weiler, 2007). Arabidopsides are likely to play roles as 'secondary' defense compounds (Kourtchenko et al., 2007). Most characterized arabidopsides carry two cyclopentenones (either OPDA or dinor-OPDA; e.g. Buseman et al., 2006; Böttcher and Weiler, 2007). 'Lyso-arabidopsides' carrying one cyclopentenone jasmonate and having a free hydroxyl group in the glyceryl backbone also exist (Glauser et al., 2008a).

Are there big gaps in understanding jasmonate biochemistry?

How at the mechanistic level is JA biosynthesis initiated and how are enzyme activities regulated? A thorough knowledge of the cellular and intercellular transport of jasmonates is needed; for example, how is JA-Ile imported into the nucleus?

Besides jasmonates what other oxylipins does Arabidopsis make?

Arabidopsis makes a wide range of oxylipins including fatty acid ketodienes and ketotrienes, and hydroxyfatty acids (HOFAs) many of which are discussed in Mueller et al. (2006). HOFAs may be produced enzymatically by LOXs or non-enzymatically (Triantaphylidès et al., 2008) and by α-dioxygenases (Bannenberg et al., 2009b). Phytoprostanes are non-enzymatically derived from 18:3 (Mueller et al., 2006). 9-LOXs in Arabidopsis make 9-hydroxyoctadecatrienoic acid (9-HOT) that promotes root waving and inhibits lateral root formation (Vellosillo et al., 2007). The hydroperoxide lyase (HPL) pathway metabolizes fatty acid hydroperoxides creating at least one volatile fragment. For example, 13-HPL generates volatile 2-hexenal from 18:3 (Mirabella et al., 2008).

Should we rely solely on Arabidopsis to understand jasmonates?

No. New perspectives on jasmonate function will come from studying plants from different evolutionary groups from monocots to mosses. Ongoing research in tobacco and tomato should be followed. But, for the jasmonate community, Arabidopsis has many more secrets to yield…

PRACTICAL SECTION

JA is slightly soluble in water, whereas the solubility of MeJA and ODPA in water is considerably lower and JA-Ile has extremely low solubility in water. MeJA is volatile and this provides a simple way of treating plants (Farmer and Ryan, 1990).

How long can solutions containing JA and MeJA be stored?

When they are fresh JA and MeJA solutions are colorless. With aging they gradually go yellow. This is correlated with the non-enzymatic production of 11-hydroxyjasmonic acid as well as jas-

monate polymerization products (Glauser, Wolfender and Farmer, unpublished results). MeJA stored in aqueous solutions can be demethylated by microbial activity. Solutions should be stored at 4°C for no longer than 4 weeks.

What is the best way to test for altered sensitivity to jasmonate?

Root growth inhibition assays as originally developed by Staswick et al. (1992) are excellent. Seeds are germinated and grown vertically on agar medium in the presence and absence of MeJA (25 μM). MeJA inhibits the growth of the primary root and induces purple anthocyanins in cotyledons. Plants with reduced jasmonate sensitivity do not show these strong effects.

What is the best way to test for altered ability to make jasmonates?

This is done by gas chromatography (e.g. Mueller et al., 2006) or by liquid chromatography (e.g. Glauser et al., 2009; Koo et al., 2009). An alternative is to use 'Caldelari assays' to assess relative LOX and AOS activities. For this, freshly expressed plant juice (1 to 2 μL) is mixed with radiolabeled 18:3. The products are extracted and analyzed by thin-layer chromatography (Caldelari and Farmer, 1998; Bonaventure et al., 2007b).

What are good marker genes for jasmonate signaling activity?

Two JA-inducible transcripts are *JASMONATE ZIM-DOMAIN PROTEIN10* (JAZ10; At5g13320) and *VEGETATIVE STORAGE PROTEIN2* (*VSP2;* At5g24770).These are considered as 'early' and 'late' jasmonate-regulated genes (Chung et al., 2008). Ideally, two such markers are used as a first indication that the JA pathway might be active in a tissue. Gene identifier numbers and synonyms for jasmonate-related genes are given in Supplementary Table 1.

What are the most useful JA synthesis mutants?

The T-DNA insertion *aos* mutant of Park et al. (2002) is a strong biosynthesis mutant at the first committed step of the JA synthesis pathway. The mutant *opr3* (*dde2*) is also useful (Sanders et al., 2000; Stintzi and Browse, 2000). The *fad3-2 fad 7-2 fad 8* triple mutant (McConn and Browse, 1996) lacks all triunsaturated fatty acids. This mutant is very useful for removing the major proportion of oxylipins including jasmonates. All these mutants are sterile but fertility is restored by JA/MeJA treatments. Once flowering starts buds are dipped each two days in 0.01% (v/v) MeJA in water containing 0.1% (v/v) Tween-20. The mutation *jasmonate resistant1* (*jar1*; Staswick et al., 1992) reduces JA-Ile levels (Suza and Staswick, 2008) but the plants remain fertile. The mutant *fatty acid oxygenation2* (*fou2*) over-produces JA in response to wounding. Its transcriptome strongly resembles that of a plant being attacked by the chewing insect *Pieris rapae* (Bonaventure et al., 2007a, 2007b).

What are the most useful JA perception mutants?

The first *COI1* mutation, *coi1-1* (Feys et al., 1994) is a very useful null mutant in JA COI1-mediated perception. This and other strong JA perception mutants in Arabidopsis are male-sterile and cannot be rescued with JA/MeJA sprays. The seeds are maintained as heterozygotes and sown on 25 μM MeJA in Petri dishes. Root growth in the homozygotes is insensitive to MeJA and homozygous mutants are confirmed using a CAPS procedure (Xie et al., 1998). An alternative is plants that overexpress the gene *JAZ10.4* (Chung et al., 2009). These are dominant negatives: seeds can be produced by pollinating JAZ10.4 overexpressor females with wild-type pollen.

Have all agricultural applications of jasmonates been explored?

Far from it. The control of male fertility could be targeted for hybrid seed production (Stintzi and Browse, 2000). Since jasmonates control the production of many defensive traits, a great many new applications could be foreseen. If, for example, JA synthesis or perception were selectively down-regulated in fruits this might increase their palatability to humans or livestock.

This chapter replaces an earlier article on the Oxylipin Pathway in Arabidopsis by R.A. Creelman and R. Mulpuri. The earlier article has been archived and is available in PDF format at http://www.bioone.org/doi/suppl/10.1199/tab.0129/suppl_file/10.1199_tab.0012.pdf.

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