Chapter 17
Jasmonate Biosynthesis and Signaling for Induced Plant Defense against Herbivory

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Jasmonates are a growing class of signaling molecules and plant hormones which are derived from polyunsaturated fatty acids via the octadecanoid pathway, and characterized by a pentacyclic ring structure. Until recently, jasmonic acid has been viewed as the end product of the pathway and as the bioactive hormone. It becomes increasingly clear, however, that biological activity is not limited to jasmonic acid, but extends to, and may even differ between its many metabolites and conjugates as well as its biosynthetic precursors. Like other plant hormones, jasmonates exhibit a broad spectrum of physiological activities, ranging from seed germination, over reproductive development, all the way to senescence. Jasmonates also serve important roles as signaling molecules in plant defense, particularly defense against insect herbivores and necrotrophic patghogens. In this chapter, we will briefly discuss each step of the octadecanoid pathway, emphasizing on those that are relevant for the regulation of jasmonic acid biosynthesis, and on insights derived from the recently solved crystal structures of two of the pathway's enzymes. With respect to jasmonate signaling, we will focus on their role as signal molecules in the systemic defense response against insect herbivores, and on jasmonate-dependent activation of defense gene expression.

17.1 Introduction

Since the identification of methyl jasmonate (MeJA) as a secondary metabolite in essential oils of jasmine in 1962 (Demole et al. 1962), many related compounds have been discovered in a wide range of plants, and are collectively referred to as jasmonates. Jasmonates share their biosynthetic origin from oxygenated polyunsaturated fatty acids, and a substituted pentacyclic ring as a common structural element. In the early 1980s, their widespread occurrence throughout the plant kingdom (Meyer et al. 1984), and their growth-inhibitory (Dathe et al. 1981), and

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senescence-promoting activities (Ueda and Kato 1980) were established. First indications for a role of jasmonates in the regulation of gene expression were obtained by Parthier and co-workers who observed the accumulation of jasmonate-inducible proteins (JIPs) in senescing barley leaves (Weidhase et al. 1987; Mueller-Uri et al. 1988). Seminal work of Farmer and Ryan subsequently demonstrated that MeJA and jasmonic acid (JA) induce the accumulation of proteinase inhibitors as a direct defense against insect herbivores (Farmer and Ryan 1990; Farmer et al. 1991). Shortly after, jasmonates were shown to mediate the elicitor-induced accumulation of antimicrobial phytoalexins in cell cultures (Gundlach et al. 1992), and the induction of vegetative storage proteins in soybean and *Arabidopsis* (Franceschi and Grimes 1991; Staswick et al. 1992). These findings greatly stimulated the interest in jasmonates as a new class of signaling molecules in plant defense against both insects and pathogens.

In subsequent years, numerous mutants were characterized that are impaired in either jasmonate synthesis or response clearly establishing their function as plant defense regulators. Mutants impaired in jasmonate perception and signaling, including the *coi1*, *jin1,* and *jai3* mutants in *Arabidopsis* (Feys et al. 1994; Berger et al. 1996; Lorenzo et al. 2004; Lorenzo and Solano 2005), *coi1* in *N. attenuata* (Paschold et al. 2007), and the *jai1* mutant in tomato (Li et al. 2004) fail to mount appropriate defense responses. Also compromised in the induction of defense responses are mutants that are deficient in the polyunsaturated fatty acid precursors of jasmonates (the *fad3fad7fad8* and the *spr2/LeFad7* mutants in *Arabidopsis* and tomato; McConn et al. 1997; Li et al. 2003), and mutants affected in the octadecanoid pathway for jasmonate biosynthesis, i.e. *dad1*, *aos*, *acx1/5*, *jar1* in *Arabidopsis* (Park et al. 2002; Staswick et al. 2002; von Malek et al. 2002; Schilmiller et al. 2007), *def1* in tomato (Howe et al. 1996) and *jar4* in *N. attenuata* (Kang et al. 2006).

In addition to their role as defense regulators, the characterization of biosynthesis and perception mutants established jasmonates as phytohormones in plant reproductive development. Many of the *Arabidopsis* mutants are male sterile due to defects in anther and pollen maturation (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; Schilmiller et al. 2007) while a defect in the maternal control of seed maturation appears to cause sterility in *jai1* in tomato (Li et al. 2004). Other jasmonate-regulated developmental processes include root growth (Staswick et al. 1992), glandular trichome development (Li et al. 2004), tuber formation (Yoshihara et al. 1989; Pelacho and Mingo-Castel 1991), laticifer differentiation (Hao and Wu 2000), seed germination (Corbineau et al. 1988; Finch-Savage et al. 1996), carbon/nitrogen allocation (Creelman and Mullet 1997), senescence (Ueda and Kato 1980; Creelman and Mullet 1997), and tendril coiling (Falkenstein et al. 1991).

A number of excellent review articles have recently been published on the biosynthesis of jasmonates and on their activity, particularly their contribution to the regulation of plant defense responses (Blee 2002; Liechti and Farmer 2002; Halitschke and Baldwin 2004; Howe 2004; Pozo et al. 2004; Schaller et al. 2004; Browse 2005; Lorenzo and Solano 2005; Schilmiller and Howe 2005; Delker

et al. 2006; Wasternack 2006; Cheong and Choi 2007; Wasternack 2007), and we refer the reader to these articles for a comprehensive discussion of the available literature. In this chapter, we will emphasize on the most recent findings with respect to jasmonate biosynthesis, and discuss those aspects of jasmonate signaling that are relevant for the systemic induction of herbivore defenses and defense gene activation.

17.2 Jasmonate Biosynthesis

The synthesis of jasmonates and many other oxylipins is initiated by lipoxygenases (LOXs), which catalyze the regio- and stereoselective dioxygenation of polyunsaturated fatty acids (reviewed by Blee 2002; Feussner and Wasternack 2002; Howe and Schilmiller 2002; Schaller et al. 2004; Wasternack 2007). Linoleic acid (18:2) and linolenic acid (18:3) are oxygenated by specific LOXs at C9 or C13 to result in the corresponding (9*S*)- or (13*S*)-hydroperoxy-octadecadi(tri)enoic acids, which feed into at least seven alternative pathways resulting in the formation of a large variety of oxylipins (Blee 2002; Feussner and Wasternack 2002). The first committed step in the two parallel pathways for JA biosynthesis (Fig. 17.1), i.e. the octadecanoid pathway from 18:3 and the hexadecanoid pathway from 16:3 (Weber et al. 1997), is performed by allene oxide synthase (AOS), an unusual cytochrome P450 which uses its hydroperoxide substrate as source for reducing equivalents and as oxygen donor, and is thus independent of molecular oxygen and NAD(P)H. AOS catalyzes the dehydration of 13(*S*)-hydroperoxy-octadecatrienoic acid (13-HPOT) to form an unstable allene oxide, 12,13(*S*)-epoxy-octadecatrienoic acid (12,13-EOT). In aqueous media, 12,13-EOT rapidly decomposes to α- and γ-ketols, or undergoes cyclization to form 12-oxo-phytodienoic acid (OPDA). As opposed to spontaneous cyclization which results in a racemic mixture of OPDA enantiomers, allene oxide cyclase (AOC) ensures the formation of the optically pure 9*S*,13*S* enantiomer. Dinor-OPDA (dnOPDA) is generated in the parallel pathway from 16:3 (Fig. 17.1). The short half-life of 12,13-EOT in aqueous media (26 s at 0°C and pH 6.7; Hamberg and Fahlstadius 1990; Ziegler et al. 1999) and the optical purity of endogenous OPDA (Laudert et al. 1997) suggest tight coupling of the AOS and AOC reactions in vivo. However, physical contact of AOS and AOC in an enzyme complex does not seem to be required for stereochemical control of the cyclization reaction (Zerbe et al. 2007).

Only 9*S*,13*S*-OPDA, i.e. one out of four possible OPDA stereoisomers, is a percursor for biologically active JA. AOC is thus crucially important to establish the enantiomeric structure of the cyclopentenone ring. The crystal structure of *Arabidopsis* AOC2 has recently been solved shedding light on how the enzyme exerts stereochemical control on the cyclization reaction (Hofmann et al. 2006). Considering the fact that cyclization occurs spontaneously in aqueous solution, AOC2 does not need to be much of a catalyst in terms of lowering the activation energy barrier. Indeed, binding of the substrate or the transition state does not involve any induced fit mechanism. The hydrophobic protein environment and very few ionic interactions with a glutamate residue (Glu23) and a tightly bound water molecule,

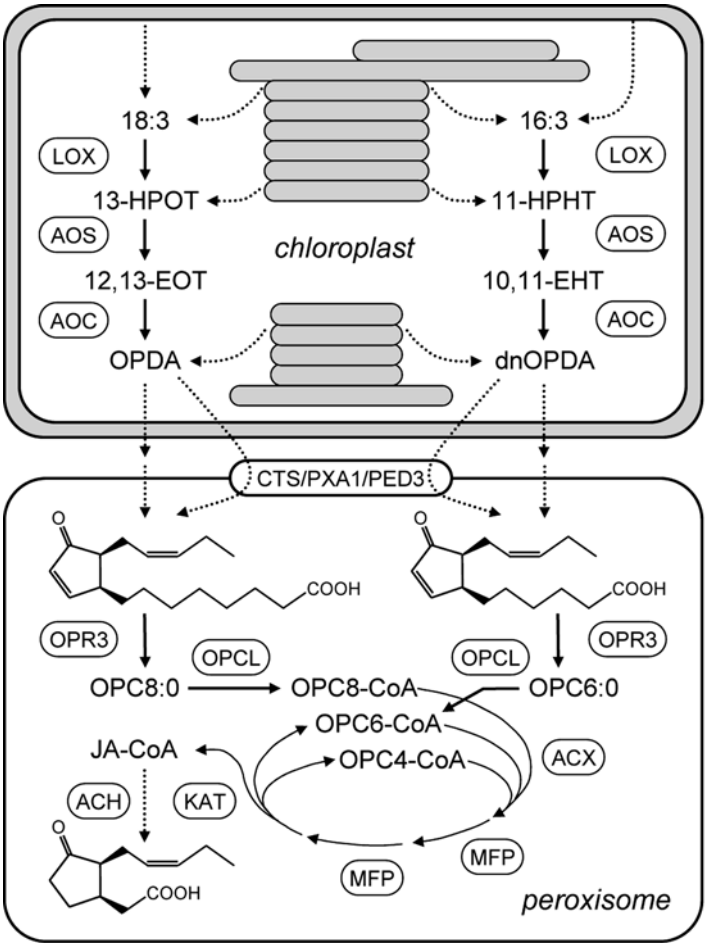

Fig. 17.1 Biosynthesis of jasmonic acid. Polyunsaturated fatty acids (18:3 and 16:3) are precursors for jasmonic acid biosynthesis via the octa- and hexadecanoid pathways, respectively. The first three steps are localized in plastids and lead to the formation of 12-oxophytodienoic acid (OPDA) and dinor 12-oxophytodienoic acid (dnOPDA). The subsequent steps, including the reduction of OPDA (dnOPDA) to OPC8:0 (OPC6:0) followed by three (two) cycles of β-oxidation result in the production of jasmonic acid in peroxisomes. Arrows and broken arrows are used to differentiate between well characterized reactions and those steps that are still hypothetical and for which the corresponding enzymes remain to be identified. Further detail is given in the text

ensure binding and correct positioning of the substrate 12,13-EOT. Steric restrictions imposed by the protein environment enforce the necessary conformational changes of the substrate's hydrocarbon tail resulting in the absolute stereoselectivity of the AOC2-mediated as opposed to the chemical cyclization reaction (Hofmann et al. 2006).

The passive role of AOC2 in the steroselective synthesis of 9*S*13*S*-OPDA is reminiscent of dirigent proteins and the way in which they promote stereoselective formation of lignans. Lignan formation involves the regio- and sterochemically controlled oxidative coupling of two phenols, e.g. the formation of (+)-pinoresinol from two molecules of coniferylalcohol (Davin et al. 1997). In absence of dirigent proteins, resonance-stabilized phenoxy radical intermediates couple randomly to form a mixture of racemic lignans. Dirigent proteins, which lack a catalytic center, are believed to bind and orient the free radical intermediates allowing stereoselective coupling to occur (Davin et al. 1997).

Interestingly, both dirigent proteins and AOC2 are distant members of the lipocalin family (Charron et al. 2005; Hofmann et al. 2006; Pleiss and Schaller unpublished observation). Lipocalins are β-barrel proteins comprising a central hydrophobic cavity for binding of small lipophilic molecules like steroids, pheromones, odorants, or retinoids. In fact most lipocalins do not function as enzymes but rather as binding proteins in olfaction, pheromone transport, retinol transport, and invertebrate cryptic coloration (Charron et al. 2005). Likewise, dirigent proteins and AOC2 act as binding proteins of the unstable lipophilic precursors (phenoxy radicals and allene oxides, respectively) to exert stereochemical control in the synthesis of lignans and OPDA.

The formation of 9*S*,13*S*-OPDA as the first member of the jasmonate family with signaling activity concludes the plastid-localized part of the octa(hexa)decanoid pathway. Little is known about how OPDA (and/or dnOPDA) is transferred from plastids to peroxisomes, where the final steps of JA biosynthesis occur. Recently, the ABC transporter COMATOSE (CTS, also known as PXA1 (Zolman et al. 2001) or PED3 (Hayashi et al. 2002)) was shown to be involved in this process (Theodoulou et al. 2005). CTS catalyzes the ATP-dependent import of fatty acids into peroxisomes as substrates for β-oxidation. The *cts* mutant has reduced levels of JA, is impaired in wound-induced JA accumulation, and expresses the JA-dependent *VSP1* gene at a lower level suggesting that CTS delivers substrates for JA synthesis into the peroxisomes, most likely (dn)OPDA, or the corresponding CoA esters (Theodoulou et al. 2005). However, other pathways for (dn)OPDA import must exist, as indicated by the residual levels of JA, and the lack of JA-deficiency symptoms (e.g., male sterility) in the *cts* mutant. Additional pathways for fatty acid import into peroxisomes, by diffusion or by an as yet unidentified mechanism, were also suggested by Fulda et al. (2004). Because of the higher pH in peroxisomes as compared to the cytoplasm, weak acids are predicted to be trapped in peroxisomes as the anion, and this may account for some of the (dn)OPDA import (Theodoulou et al. 2005).

Once within the peroxisomes, 9*S*,13*S*-OPDA is reduced by 12-oxophytodienoate reductase (OPR3) to yield 3-oxo-2-(2'(Z)-pentenyl)-cyclopentane-1-octanoic acid (OPC-8:0), and dnOPDA is reduced to the corresponding hexanoic acid derivative (OPC-6:0; Schaller et al. 2000; Stintzi and Browse 2000; Fig. 17.1). The signaling properties of jasmonates bearing a cyclopentenone ring (OPDA, dnOPDA) are clearly distinct from JA and its derivatives which are characterized by the reduced cyclopentanone ring (Blechert et al. 1999; Stintzi et al. 2001; Taki et al. 2005). OPR3 may therefore be particularly important for the regulation of the relative levels of these two classes of signaling molecules.

Even though OPR3 belongs to a small family of related flavin-dependent oxidoreductases (at least three in tomato, six genes in *Arabidopsis*, six in pea, eight in maize, ten in rice) it is functionally unique, as evident from JA-deficiency symptoms (male-sterility) of the *OPR3* loss-of-function mutant in *Arabidopsis* (Sanders et al. 2000; Stintzi and Browse 2000) and tomato (Schaller and Stintzi unpublished). The genetic evidence is consistent with biochemical and structural data which suggest that all OPRs catalyze the reduction of α,β-unsaturated carbonyls (conjugated enones) while only OPR3s and the rice ortholog OsOPR7 (Tani et al. 2007) are capable of reducing the 9*S*,13*S* enantiomer of OPDA (Schaller et al. 2000; Breithaupt et al. 2001, 2006; Strassner et al. 2002). Therefore, these are the only OPRs contributing to JA biosynthesis. The OPR1 isoforms from *Arabidopsis*, tomato, and rice, on the other hand, were shown to reduce numerous conjugated enones including 9*R*,13*R*-OPDA, but they do not accept 9*S*,13*S*-OPDA the precursor of biologically active JA (Schaller and Weiler 1997; Straßner et al. 1999; Schaller et al. 2000; Strassner et al. 2002; Sobajima et al. 2003; Tani et al. 2007).

Recent crystal structure analyses provided insights into the mechanisms of substrate reduction and the remarkable differences in stereospecificity between OPR1 and 3 (Breithaupt et al. 2001, 2006; Fox et al. 2005; Malone et al. 2005). Consistent with the reaction mechanism proposed for the related Old Yellow Enzyme from yeast, the carbonyl moiety of the substrate forms hydrogen bonds with two histidine residues (His187 and His197 in tomato OPR1) which leads to a polarization of the α,β-double bond. Consequently, hydride transfer from the reduced flavin cofactor to the substrate Cβ is facilitated, followed by a protonation of the Cα by a tyrosine residue (Breithaupt et al. 2001). A comparison of the OPR1 and OPR3 structures revealed a more open active site cavity in OPR3, explaining its more relaxed specificity allowing reduction of both the (9*S*,13*S*) and (9*R*,13*R*) isomers of OPDA. Two residues in OPR1, Tyr78 and Tyr246 seem to act as gatekeepers, narrowing the active site and blocking the entry of 9S,13S-OPDA (Breithaupt et al. 2001, 2006).

The shortening of the hexanoic and octanoic acid side chains in OPC-6:0 and OPC-8:0 to yield JA involves two or three rounds of β-oxidation, respectively. Prior to entry into the β-oxidation cycle, the carboxylic moiety needs to be activated as CoA ester. Co-expression with genes known to be involved in JA biosynthesis suggested At1g20510, a member of the ATP-dependent acyl-activating family of enzymes, as a candidate for the required Acyl-CoA ligase. The recombinant enzyme was found to activate OPDA and OPC-8:0 in vitro, as well as medium-to-long straight-chain fatty acids (Koo et al. 2006). Loss-of-function mutants in *Arabidopsis* accumulated less JA in response to mechanical wounding providing direct evidence for a role in JA biosynthesis. Hyper accumulation of OPC-8:0 in the null mutants supported OPC-8:0 as the physiological substrate, and the enzyme was thus named OPC-8:0 CoA Ligase1 (OPCL1; Koo et al. 2006; Fig 17.1). A closely related enzyme (At4g05160) was suggested by Schneider et al. (2005) as a candidate OPC-6:0-CoA ligase. Consistent with such a role, the enzyme is located in peroxisomes and its expression is induced by MeJA. However, the recombinant enzyme was found to prefer medium-chain fatty acids over OPC-6:0 as substrates in vitro (Schneider et al. 2005), and the corresponding loss-of-function mutant exhibited

wild-type levels of JA (Koo et al. 2006). Its contribution to JA biosynthesis is thus questionable.

Beta-oxidation itself involves three core enzymes, acyl-CoA oxidase (ACX), multifunctional protein (MFP; comprising enoyl-CoA hydratase and β-hydroxyacyl-CoA dehydrogenase activities), and 3-ketoacyl-CoA thiolase (Fig. 17.1). Despite early findings implicating β-oxidation in JA biosynthesis (Vick and Zimmerman 1984), direct evidence for the contribution of these enzymes is very recent. ACX1A was shown to catalyze the first step in the β-oxidation of OPC-8:0-CoA, and was found to be responsible for the bulk of wound-induced JA production in tomato. Consistant with its essential role in JA biosynthesis, the *acx1* tomato mutant was impaired in wound-induced defense gene activation and insect resistance (Li et al. 2005). In *Arabidopsis*, ACX1 is responsible for about 80% of JA production after wounding (Cruz Castillo et al. 2004; Schilmiller et al. 2007), and only the *acx1/5* double mutant showed hallmarks of severe JA deficiency, including impaired insect resistance and reduced male reproductive function (Schilmiller et al. 2007). Also the *aim1* mutant in *Arabidopsis*, which is disrupted in one of two MFP genes, is impaired in wound-induced accumulation of JA and expression of JA-dependent genes (Delker et al. 2007). Among the five 3-ketoacyl-CoA thiolase genes in *Arabidopsis*, *KAT2* appears to be the one most relevant for JA biosynthesis. In transgenic plants silenced for *KAT2* expression, wound-induced JA accumulation was markedly reduced (65%–80%), and the induction of a JA-dependent marker gene (*JR2*) impaired, both locally at the site of wounding and systemically (Cruz Castillo et al. 2004). As the final step in JA biosynthesis, the JA-CoA ester has to be hydrolyzed to release the free acid. Candidate acyl-thioesterases have been identified in *Arabidopsis*, two of which appear to be peroxisomal (*At*ACH1 and *At*ACH2; Tilton et al. 2000), but a direct involvement in JA biosynthesis remains to be shown.

Traditionally, JA has been viewed as the end product of the pathway and the bioactive hormone. The many different metabolites of JA and the enzymatic steps in their interconversion were thought to contribute to hormone homeostasis to sustain and control the levels of active JA. This view is changing, however, and E.E. Farmer takes the extreme position to consider JA as a mere precursor of bioactive hormones, i.e. JA conjugates (Farmer 2007). Indeed, there is clear evidence showing that some JA metabolites have unique signaling properties, and that some processes are not controlled by JA but rather by JA derivatives (reviewed by Wasternack 2007). For example, the nyctinastic leaf movement in *Albizzia* depends on a specific enantiomer of 12-OH-JA-*O*-glucoside (Nakamura et al. 2006a, b), 12-OH-JA (tuberonic acid) has long been implicated in potato tuber formation (Yoshihara et al. 1989), and in defense signaling, the active signal appears to be the amide-linked isoleucine conjugate JA-Ile, rather than JA itself (Staswick and Tiryaki 2004; Kang et al. 2006).

The relevance of JA-Ile as a signal in its own right was first demonstrated by Wasternack and co-workers who observed that JA and JA-Ile induce different responses when applied exogenously to barley leaves, and that they are both active without being inter-converted (Kramell et al. 1997; Wasternack et al. 1998). Biosynthesis of JA-Ile involves the adenylation of JA, followed by the exchange of

AMP with isoleucine, and is catalyzed by the amino acid conjugate synthetase JAR1 (Staswick et al. 2002; Staswick and Tiryaki 2004). The *Arabidopsis jar1* mutant is insensitive to exogenously applied JA with respect to root growth inhibition, induced resistance to *Phytium irregulare*, induced systemic resistance (ISR), and protection against ozone damage (Staswick and Tiryaki 2004, and references therein). Furthermore, silencing of the *JAR1* ortholog in *N. attenuata* compromised defense gene induction and resistance against *M. sexta* (Kang et al. 2006). Therefore, conjugation to Ile appears to be necessary for at least a subset of the jasmonate-regulated processes. Further support for the specific signaling function of JA-Ile is provided by the recent observation that unlike JA or OPDA, JA-Ile mediates the specific degradation of repressors of jasmonate-dependent gene expression (Thines et al. 2007, see below).

17.3 Jasmonate Signaling in the Systemic Induction of Herbivore Defenses

The systemic induction of defensive proteinase inhibitors in tomato plants in response to local wounding or herbivore attack was discovered by Ryan and coworkers 35 years ago and has since served as a model system to study long-range signaling processes in plants (Green and Ryan 1972). A number of chemical signals were identified in the Ryan lab that are intricately involved in the systemic induction of defense responses. This includes systemin peptides which are derived from larger precursor proteins by proteolytic processing (Pearce et al. 1991; McGurl et al. 1992; Ryan and Pearce 2003; Narváez-Vásquez and Orozco-Cárdenas this volume), oligogalacturonides which are generated by a polygalacturonase systemically induced after wounding (Bergey et al. 1999), and jasmonates which are derived from the octadecanoid pathway (Farmer and Ryan 1990; Farmer et al. 1991). Farmer and Ryan proposed a model according to which primary wound signals like oligogalacturonides and systemin trigger the activation of the octadecanoid pathway resulting in a burst of JA production which ultimately leads to the activation of defense genes (Farmer and Ryan 1992). The wound signaling pathway, its interaction with other hormone and defense signaling pathways, and the role of individual signaling molecules were subject of numerous recent reviews (Pieterse et al. 2006; Ryan and Pearce 2003; Stratmann 2003; Howe 2004; Lorenzo and Solano 2005; Schilmiller and Howe 2005; Wasternack et al. 2006; Cheong and Choi 2007), and systemic wound signaling is discussed in detail by Schaller and Howe (this volume). Here we will focus on the specific role of jasmonates in the activation of defense gene expression.

Since the discovery of the systemic wound response researchers have been fascinated by the question of what the long-distance signal might be (Ryan 1992; Bowles 1998). The search for the systemic wound signal in tomato plants led to the discovery of systemin in the Ryan lab, and its role as the systemically mobile signal was supported by numerous observations: Systemin was shown to be

necessary and sufficient for the systemic wound response, it triggers the synthesis and accumulation of JA, it is mobile within the phloem when applied to wound sites, and its precursor protein is specifically expressed in the vasculature. Based on these findings, a model had been proposed, according to which systemin is proteolytically released from its precursor upon wounding, loaded into sieve elements, and delivered along source-to-sink gradients into systemic tissues where it stimulates the synthesis of jasmonic acid as a secondary signal for the induction of defense gene expression (Ryan 2000).

However, this model is not entirely consistent with recent findings from the Howe lab, demonstrating that systemic signaling requires the activity of systemin and the synthesis of JA only in the wounded, not in the systemic leaves. Furthermore, the induction of defense genes in systemic tissues depends on JA perception and signaling, but not on the capacity to synthesize JA (Li et al. 2002, 2003; Schaller and Howe this volume). These findings are in complete agreement with the observation that expression of octadecanoid pathway genes is induced by wounding at the site of tissue damage but not systemically, and that there is a dramatic increase in JA levels in wounded leaves but not in unwounded tissues (Strassner et al. 2002). The data suggest that systemin acts at the site of tissue damage, to strengthen the systemic wound response by boosting the octadecanoid pathway, for the generation of the long-distance signal, maybe JA itself or one of its derivatives (Ryan and Moura 2002; Stratmann 2003; Schilmiller and Howe 2005; Schaller and Howe this volume).

The joint role of (pro)systemin and the octadecanoid pathway in the generation of a phloem-mobile signal for systemic induction of defense genes is consistent with their localization in the vasculature of tomato plants. Several of the octadecanoid pathway enzymes – LOX, AOS, and AOC – are located in the companion cell-sieve element complex (Hause et al. 2000, 2003a), whereas prosystemin accumulates in cells of the phloem parenchyma (Jacinto et al. 1997; Narváez-Vásquez and Ryan 2004; Narváez-Vásquez and Orozco-Cárdenas this volume). The presence of prosystemin and octadecanoid pathway enzymes in different cell types of the vascular bundle suggests a model in which systemin is released from phloem parenchyma cells in response to wounding, is then perceived at the cell surface of neighboring companion cells, where it triggers the activation of the octadecanoid pathway for the production of the systemic signal, and its release into the sieve elements for long-distance transport (Schilmiller and Howe 2005; Wasternack 2006).

The onset of JA accumulation at the site of tissue damage is almost instantaneous and peaks at around 1 hour after wounding (Doares et al. 1995; McConn et al. 1997; Ziegler et al. 2001; Strassner et al. 2002), and must therefore be independent from changes in gene expression. How the initial burst in jasmonate production is controlled, and how the production of jasmonates is limited in unstressed tissues are still open questions. It is obviously not the level of octadecanoid pathway enzymes, which are constitutively expressed and abundant in unstressed tomato leaves (Hause et al. 2003b; Stenzel et al. 2003b; Li et al. 2004). These enzymes do either not have access to their respective substrates, in this case substrate availability would be limiting for jasmonate production, and/or constitutively expressed octadecanoid pathway

enzymes may be inactive and require posttranslational modification for activation. Both hypotheses are consistent with the repeated observation that transgenic plants overexpressing octadecanoid pathway enzymes do not have elevated resting levels of JA, but show increased JA production after wounding (Wang et al. 1999; Laudert et al. 2000; Park et al. 2002; Stenzel et al. 2003a).

Primary substrates for the octa(hexa)decanoid pathway are polyunsaturated fatty acids (18:3, 16:3) which are abundant in chloroplast lipids, but not readily available as precursors for JA production in unwounded plants. Additional lipid-bound substrates may include fatty acid hydroperoxides, OPDA, and dnOPDA generated by LOX, AOS, and AOC from esterified rather than free fatty acids (Fig. 17.1; Buseman et al. 2006). The availability of these substrates as precursors for JA biosynthesis is controlled by lipolytic activities, which may include different types of phospholipases and acyl hydrolases (reviewed by Delker et al. 2006). However, in contrast to DAD1 which is required for JA production during male reproductive development (Ishiguro et al. 2001), the lipase(s) involved in the wound-induced burst of JA production have not been identified at the molecular level.

An interesting scenario of how post-translational modification may contribute to the regulation of octadecanoid pathway activity and the initial burst of JA production after wounding has recently been derived from the crystal structure of tomato OPR3. The enzyme was found to crystallize as a homodimer, in which each protomer blocks the active site of the other (Breithaupt et al. 2006). Dimerization of OPR3 concomitant with a loss of activity was also observed in solution, suggesting that OPR3 activity may be controlled in vivo by regulation of the monomer/dimer equilibrium. The crystallized OPR3 dimer was found to be stabilized by a sulfate ion at the dimer interface. Intriguingly, the sulfate is located close to Tyr364 and is positioned perfectly to mimic a phosphorylated tyrosine residue suggesting that dimerization and hence OPR3 activity may be regulated by phosphorylation of Tyr364 in vivo (Breithaupt et al. 2006). This scenario would be consistent with a proposed role for reversible protein phosphorylation in JA biosynthesis and the regulation of the wound response (Rojo et al. 1998; Schaller and Oecking 1999; Stratmann this volume).

Following the production of JA at the site of tissue damage and transmission of the long distance signal, the activation of defense responses in systemic tissues requires COI1 (Li et al. 2002, 2003). COI1 was identified many years ago as an essential component of the JA signaling pathway in a screen for insensitivity to coronatine, a structural analogue of JA-Ile (Feys et al. 1994; Xie et al. 1998). The finding that COI1 is part of the Skp/Cullin/Fbox $(SCF)^{COI1}$ complex, a type of E_3 ubiquitin ligase, suggested that JA signaling is controlled by a negative regulator that is ubiquinated specifically by SCF^{COI1}, targeted for destruction by the 26S proteasome, resulting in the activation of JA responses (Xu et al. 2002). The negative regulators of JA signaling have remained elusive until, very recently, two independent studies led to the identification of JAZ (jasmonate ZIM domain) proteins as targets of SCF^{COI1} (Chini et al. 2007; Thines et al. 2007).

JAI3 was identified in *Arabidopsis* as a member of the JAZ protein family which negatively regulates MYC2, the key transcriptional activator of JA-dependent genes

(Lorenzo et al. 2004; Chini et al. 2007). In the *jai3-1* mutant, a splicing acceptor site mutation results in the formation of a truncated JAI3 (JAZ3) protein, which is no longer subject to COI1-dependent degradation resulting in a JA-insensitive phenotype (Chini et al. 2007). Thines et al. (2007) identified the *JAZ* family in a search for transcripts rapidly upregulated by JA. JAZ1 was shown to be a negative regulator of JA-dependent genes, which is degraded in a COI1-dependent manner in response to JA. They further demonstrated that the physical interaction of COI1 and JAZ1 is promoted by JA-Ile, but not JA, MeJA, or OPDA. This interaction was independent of other protein factors suggesting that the COI1-JAZ1 complex is the site of JA-Ile perception (Thines et al. 2007). These exciting findings explain beautifully how jasmonate signals may activate defense responses in systemic tissues by triggering the COI1-dependent degradation of JAZ proteins, thus relieving MYC transcriptions factors from negative regulation, resulting in the transcriptional activation of defense genes.

17.4 Perspectives

Despite tremendous progress in recent years, many open questions remain with respect to jasmonate biosynthesis and signaling. As far as JA biosynthesis is concerned, surprisingly little is known of how it all starts. The question is not as trivial as it may seem. Obviously, plastid-localized 13-LOXs catalyze the formation of fatty acid hydroperoxides as the first step in JA biosynthesis, but LOX substrates may include free polyunsaturated fatty acids liberated from chloroplast lipids by unidentified lipases (Bachmann et al. 2002), or fatty acids that remain esterified in membrane lipids (Brash et al. 1987). Indeed, octa(hexa)decanoid pathway intermediates are present in complex lipids in *Arabidopsis*. Seventeen complex lipids have been identified already, including mono- and digalactosyldiacylglycerols (MGDGs and DGDGs), as well as phosphatidylglycerol, with OPDA and dnOPDA esterified in the *sn1* and/or *sn2* positions (Stelmach et al. 2001; Hisamatsu et al. 2003, 2005; Buseman et al. 2006). The very rapid (within 15 min) and very large (200–1000 fold) increase of complex lipids containing two oxylipin chains after wounding, as well as the relative abundance and positional specificity of OPDA and dnOPDA in the different galactolipid species which reflects the composition of galactolipids prior to wounding, suggest that they were generated in situ by direct conversion of esterified 18:3 and 16:3 (Buseman et al. 2006). Consistent with this notion, oxylipin-containing galactolipids and AOS are co-localized in the thylakoid membrane fraction of *Arabidopsis* chloroplasts (Böttcher and Weiler 2007). In the light of such a diversity of oxylipin-containing lipids in *Arabidopsis*, the identification of the lipases that are involved in their release, and of the mechanisms in control of their activities are urgent problems to be addressed in the future.

A long-standing question with respect to jasmonate signaling concerns the activities of different jasmonate family members. The traditional view of JA being the bioactive hormone was challenged by Seo et al. who suggested that defense signaling

relies on the formation of MeJA by a JA carboxyl methyltransferase (Seo et al. 2001). Furthermore, the JAR1-dependent conjugation to Ile was found to be required for (at least a subset of) JA responses (Staswick and Tiryaki 2004). The identification of the COI1-JAZ1 complex as the perception site for JA-Ile but not for JA, MeJA, or OPDA provided strong support for the importance of JA-Ile as a regulator of jasmonate response genes (Thines et al. 2007). However, this finding does not preclude the possibility that jasmonates other than JA-Ile may control subsets of COI1-dependent genes, as it was shown for OPDA (Stintzi et al. 2001). Conceivably, different members of the JAZ protein family may interact with different transcription factors, and thus, may each regulate part of the response to jasmonates. The degradation of these JAZ proteins and, consequently, transcriptional activation depend on their interaction with COI1, which in case of JAZ1 is stimulated by JA-Ile. Other jasmonates may promote the interaction of COI1 with different JAZ repressors, target them for degradation, and release inhibition of jasmonate-responsive genes.

Despite the progress made in recent years, there are still many open questions and discoveries waiting to be made concerning both, jasmonate biosynthesis and signaling, but also related to the transport of jasmonates and communication between organelles, plant organs, and organisms.

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