F. THE ROLES OF HORMONES IN DEFENSE AGAINST INSECTS AND DISEASE

F1. Jasmonates

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INTRODUCTION

Jasmonic acid (JA[1]) and its volatile methyl ester, MeJA, are fatty acid-derived cyclopentanones that occur ubiquitously in the plant kingdom. Since the discovery of jasmonates (JAs) in plants over 40 years ago, our understanding of the biosynthesis and physiological function of these compounds has been marked by several major developments. Experiments performed in the 1980s elucidated the JA biosynthetic pathway and demonstrated that exogenous JAs exert effects on a wide range of physiological processes. The discovery in the early 1990s that JAs act as potent signals for the expression of defensive proteinase inhibitors (PIs) aroused intense interest in the function of hormonally active JAs in plant defense. Research in the past decade has led to several key developments, including identification of genes encoding most of the JA biosynthetic enzymes and discovery of novel biologically active JAs. Identification of a large collection of JA biosynthesis and response mutants has provided important tools to assess the role of JAs in plant developmental and defense-related processes. The widespread occurrence of JAs in plants and some lower eukaryotes, together with their capacity to regulate physiological

[1] Abbreviations: 13-HPOT, 13-hydroperoxy linolenic acid; EST, Expressed Sequence Tag; PA, Phosphatidic acid; SA, Salicylic acid; ISR, Induced systemic resistance; FAA, Fatty acid amide; JAs, Jasmonates; MeJA, Methyl jasmonate; MAP, Mitogen-activated protein; PPO, Polyphenol oxidase; COX, cyclooxygenase; DOX, dioxygenase; OPDA, 12-oxo-phytodienoic acid; MFP, Multifunctional protein; COR , Coronatine; PI, Proteinase inhibitor; SCF, Skp1 Cullin F-box.

processes in animals (e.g., insects), reinforces the notion that JAs are of general biological interest.

JASMONATE BIOSYNTHESIS

Oxylipin Metabolism in Plants and Animals

Jasmonates belong to the family of oxygenated fatty acid derivatives, collectively called oxylipins, which are produced via the oxidative metabolism of polyunsaturated fatty acids. In animals, members of the eicosanoid (C_{20}) group of lipid mediators are synthesized from arachidonic acid and function as regulators of cell differentiation, immune responses, and homeostasis. In plants, oxygenated compounds derived mainly from C_{18} α-linolenic acid (18:3) control a similarly broad spectrum of developmental and defense-related processes (4, 13, 19, 30, 47, 48). The biochemical logic underlying the synthesis of oxylipins in plants and animals is remarkably similar (Fig. 1). Species in both kingdoms utilize cytochromes P450, lipoxygenase (LOX), and cyclooxygenase or cyclooxygenase-like (e.g., plant α-dioxygenase) activities to oxidize polyunsaturated fatty acid substrates (3). The resulting oxygenated fatty acids are further metabolized by various enzymatic and non-enzymatic systems to an array of intermediates and end products. These compounds are typically synthesized *de novo* in specific cell types upon activation of lipases that release fatty acids from membrane lipids.

Plant and animal pathways for oxylipin biosynthesis may have evolved from an ancestral lipid-based signaling system (2). Alternatively, oxylipin-based signaling in plants and animals may have evolved independently, with natural selection acting on intrinsic features that allow these compounds to transduce cellular information: (i) large supply of precursors in the form of membrane lipids, (ii) rapid synthesis in response to extracellular cues, (iii) structural diversity that permits functional specificity, (iv) physical properties that facilitate intra- and intercellular transport, and (v) rapid turnover or

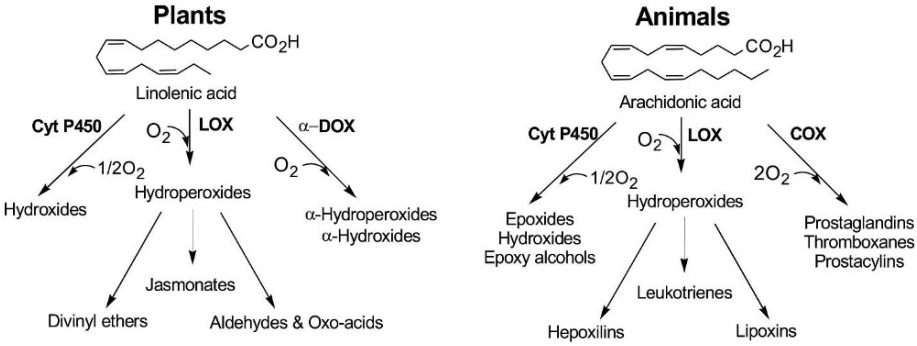

Figure 1. Oxylipin metabolism in plants and animals. LOX; lipoxygenase; COX, cyclo-oxygenase; DOX, dioxygenase.

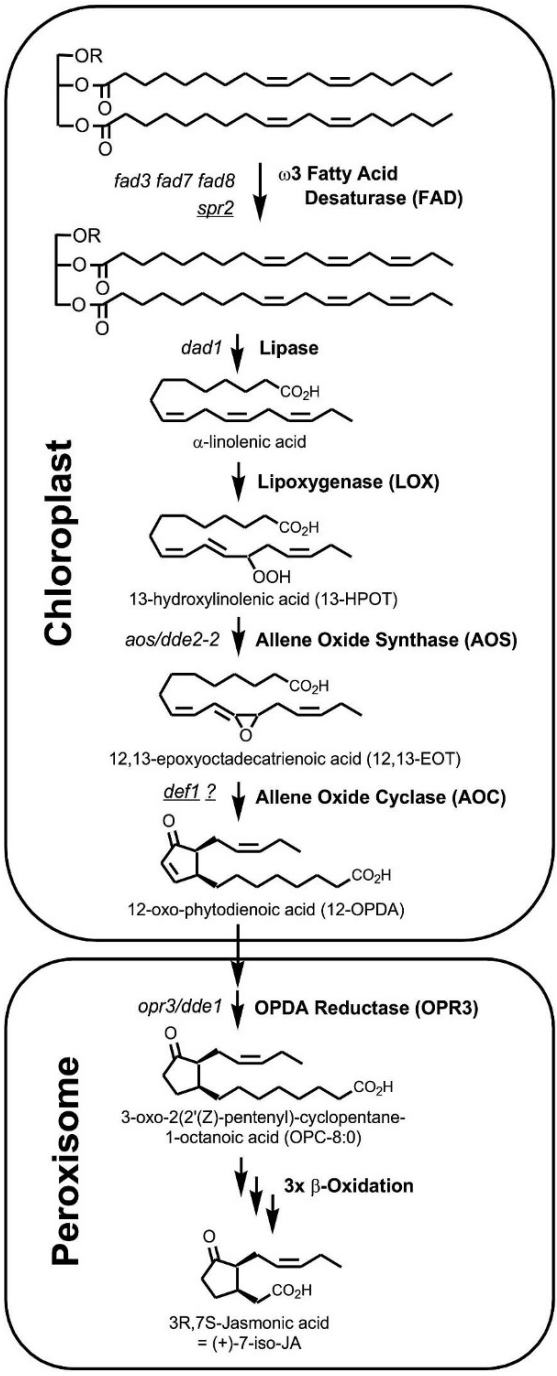

Figure 2. .Octadecanoid pathway for JA biosynthesis. Mutations that block JA biosynthesis in Arabidopsis and tomato (underlined) are indicated to the left of the arrows.

inactivation. A greater understanding of JA biosynthesis and action promises to provide insight into the evolutionary origins of fatty acid-based signaling pathways in diverse biological systems.

The Octadecanoid Pathway

Elucidation of the JA biosynthetic pathway was largely the result of research conducted by Vick and Zimmermann in the 1980s (45). As shown in Figure 2, the pathway is initiated in the chloroplast by lipoxygenase (13-LOX)-catalyzed addition of molecular oxygen to the C_{13} position of 18:3. The resulting 13-hydroperoxy derivative (13-HPOT) is converted to an unstable allene oxide by the action of allene oxide synthase (AOS). AOS is a member of the CYP74 family of cytochromes P450 that appears to have evolved specifically for the metabolism of hydroperoxy fatty acids (13, 19). Allene oxide cyclase (AOC) transforms the AOS reaction product to the first cyclic compound in the pathway, namely the cyclopentenone 12-oxo-phytodienoic acid (OPDA). The two chiral centers of OPDA allow for four possible stereoisomers. An important feature of

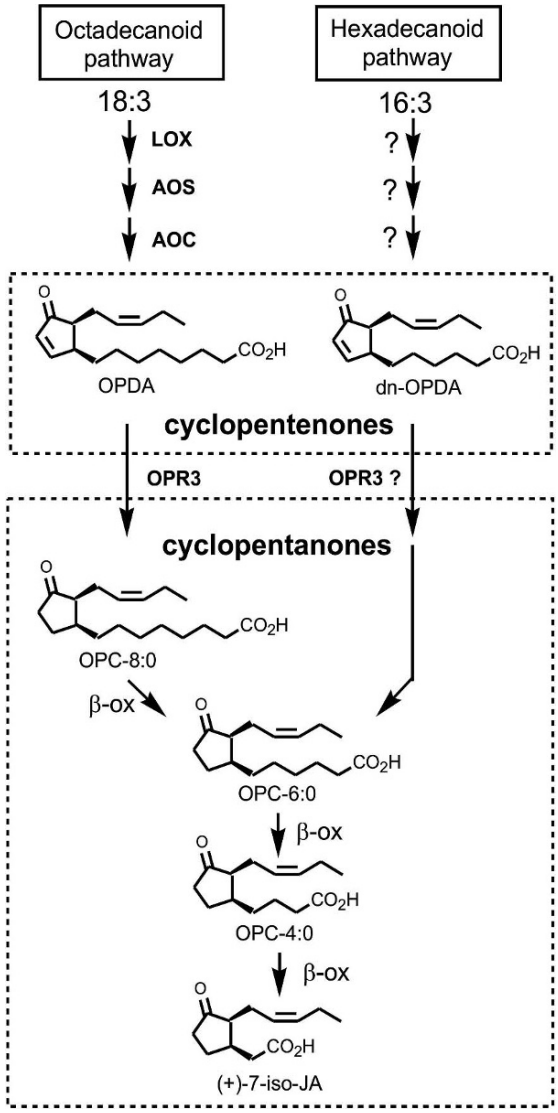

Figure 3. The octadecanoid and hexadecanoid pathways for jasmonate biosynthesis.

AOC is its ability to produce only the 9*S*,13*S* isomer of OPDA. This stereochemistry is maintained throughout the remaining biosynthetic steps. The terminal reactions of JA synthesis occur in peroxisomes, which are the site of fatty acid β-oxidation in plants. First, the cyclopentenone ring of OPDA is reduced by OPDA reductase (OPR3) to yield OPC-8:0. Three cycles of β-oxidation remove six carbons from the carboxyl side chain, completing the biosyntheis of JA.

Because JA is derived from 18:3 (octadecatrienoic acid), the series of enzymatic reactions leading to JA biosynthesis is often referred to as the octadecanoid pathway. Recent analysis of the oxylipin content in Arabidopsis leaves revealed the existence of a novel C_{16} cyclopentenone called dn-OPDA (48). The structural similarity of this compound to OPDA led to the proposal that dn-OPDA is produced via a "hexadecanoid pathway" starting from 16:3 (Fig. 3). Assuming that dn-OPDA is a substrate for OPR3, this C_{16} compound could be further metabolized to JA by β-oxidation enzymes in the peroxisome. For the purposes of this discussion, the term "jasmonate" is used to include biologically active cyclopentenones (e.g., OPDA) and cyclopentanones (e.g., JA/MeJA) that are derived from oxygenated polyunsaturated fatty acids. Other authors (47) have used the terms octadecanoids and jasmonates to refer more specifically to C_{18} and C_{12} products, respectively, of the pathway.

Tissue and Cell Type-Specific Location of JA Biosynthesis

Endogenous JA levels can vary over several orders of magnitude depending on the environmental conditions and the specific tissues and cell-types under consideration. In unstressed plants, JA levels are typically higher in young sink tissues than in older vegetative tissues (4, 47). Developing reproductive structures such as fruit and flowers accumulate very high levels of JAs, which is consistent with evidence that JAs play an important role in reproductive development (see below). Additional work is needed to determine how intracellular levels of JAs are regulated in different cell types and tissues.

It is well established that JA levels in healthy leaf tissues increase rapidly and transiently in response to wounding, herbivory, elicitor treatments, and other biotic and abiotic stimuli (4, 47). Genes encoding many JA biosynthetic enzymes are preferentially expressed in vascular bundle cells of leaf tissue. This observation is in agreement with the finding that JA and OPDA accumulation in tomato leaves is enriched in veins compared to the leaf lamina (39). Preferential accumulation of JAs in vascular tissues may account for the cell type-specific expression of JA-regulated vegetative storage proteins in soybean (4). In tomato leaves, JA-regulated PIs are expressed predominantly in leaf mesophyll cells. In this case, restriction of octadecanoid pathway enzymes to vascular bundles suggests that JAs produced in one cell type exert effects on neighboring cells (35, 39).

Identification of Genes Encoding JA Biosynthetic Enzymes

Work performed in many laboratories during the past 10 years has identified genes encoding nearly all enzymes involved in JA biosynthesis. Much of this work has involved the use of traditional biochemical approaches for enzyme purification, followed by use of appropriate molecular techniques to identify the corresponding cDNAs. Heterologous expression systems, usually *E. coli*, have been used to verify the enzymatic activity of cDNA products and to determine the substrate specificity and kinetic parameters of these enzymes. This strategy was successfully employed for the first identification of *AOS*, *AOC*, and *OPR* cDNAs from flax, tomato, and Arabidopsis, respectively (36, 47). The discovery of these "pioneer" genes of the octadecanoid pathway has provided the necessary DNA sequence information to identify homologous genes in a wide variety of plant species.

The recent completion of plant genome sequencing projects has also provided insight into the number of genes that encode various pathway enzymes. For those pathway enzymes encoded by a multigene family, a future challenge will be to determine the physiological function of each isoform in JA homeostasis. As illustrated by an increasing collection of JA biosynthetic mutants (Fig. 2), genetic approaches are expected to play an important role in addressing this question. The following discussion highlights the ways in which combined genetic and biochemical approaches

have advanced our understanding of the molecular basis of JA biosynthesis. Additional details about specific enzymes and their role in the regulation of JA biosynthesis can be found in recent reviews (5, 13, 36, 43).

Production of linolenic acid

Important insight into the role of JA in plant growth and development has come from genetic analysis of trienoic fatty acid biosynthesis in Arabidopsis (46). The Arabidopsis genome encodes three ω3 fatty acid desaturases (FAD3, FAD7, and FAD8) that convert dienoic fatty acids (16:2 and 18:2) to trienoic fatty acids (16:3 and 18:3). The *FAD3* gene product is an endoplasmic reticulum-localized enzyme that desaturates 18:2, whereas the *FAD7* and *FAD8* gene products desaturate both 16:2 and 18:2 in the chloroplast. Because of functional redundancy between these enzymes and the trafficking of lipids between the chloroplast and extrachloroplast membranes, mutations in any one of these genes have only modest effects on trienoic fatty acid accumulation and JA levels. However, a *fad3fad7fad8* "triple" mutant produces no trienoic fatty acids and, as a consequence, is completely deficient in JA (46). With the exception of being deficient in JA-mediated defense, this mutant exhibited normal vegetative growth. Interestingly, however, attempts to grow the mutant to maturity revealed that it was male sterile. This observation, together with the ability of exogenous 18:3 and JA to restore fertility to the mutant, provided the first conclusive evidence that JAs play an essential role in male reproductive development. The strict dependence of male fertility on JA has been exploited as a convenient assay to identify additional JA biosynthetic mutants of Arabidopsis (see below).

Additional insight into the role of ω3-FADs in JA biosynthesis has come from the characterization of the *spr2* mutant of tomato (25). This mutant was originally isolated in a genetic screen for plants that are defective in anti-herbivore defenses in response to wounding and the peptide wound signal, systemin. The *Spr2* gene encodes a chloroplast fatty acid desaturase (LeFAD7) that is homologous to the Arabidopsis *FAD7*/*8* genes. *spr2* mutant plants are severely deficient in 18:3/16:3 and JA accumulation and, as expected, are compromised in defense against insect attack (see below). These results indicate that chloroplast pools of 18:3 are required for wound- and systemin-induced JA synthesis and subsequent activation of defense responses in tomato leaves. The *spr2* mutant exhibits normal growth, development, and reproduction, indicating that LeFAD7 is not required for fertility in tomato. The availability of ω3-FAD mutants in tomato and Arabidopsis should assist future work to determine the function of JAs in diverse plant species.

Release of linolenic acid from membrane lipids

Jasmonate accumulation in plant tissues appears to be regulated at several steps along the octadecanoid pathway (39, 47). Nevertheless, recent evidence indicates that phospholipases (PLs) that release fatty acid precursors from

membrane lipids play a critical role in this regulation. A significant advance in this area came from the characterization of the male sterile *delayed in anther dehiscence1* (*dad1*) mutant of Arabidopsis that is deficient in JA accumulation in floral tissues (20). *DAD1* encodes a PLA_1 that liberates 18:3 from the *sn1* position of membrane lipids (Fig. 2). Localization of DAD1 to the chloroplast is consistent with the notion that it generates fatty acid substrate for the plastid-localized enzymes of the octadecanoid pathway. Analysis of substrate specificity showed that recombinant DAD1 was highly active against phospholipids such as phosphatidylcholine. The enzyme was somewhat less active against galactolipids that are abundant in the chloroplast envelope membrane where the initial steps of JA biosynthesis are thought to occur (47). Nevertheless, the ability of DAD1 to utilize galactolipids is interesting in the context of the recent discovery that a large pool of OPDA is esterified to chloroplast galactolipids (19, 48). This finding raises the possibility that DAD1 may be involved in releasing OPDA from the plastid for subsequent metabolism in the peroxisome. Substantiation of this hypothesis will require experiments aimed at determining the precursor-product relationship between OPDA-containing galactolipids and JA.

The capacity of *dad1* mutant plants to accumulate normal levels of JA in wounded leaves (20) indicates that other lipases are involved in JA biosynthesis in response to insect and pathogen attack. Other members of the *DAD1* gene family, which includes several putative chloroplast isozymes, are candidates for such a lipase. There is also evidence for the involvement of PLA_2 and PLD in wound-induced JA biosynthesis (5, 19, 43). For example, depletion of PLDα1 in Arabidopsis resulted in reduced JA accumulation and reduced expression of JA-regulated genes in response to wounding. Given that PLD is an extrachloroplastic lipase that generates phosphatidic acid (PA) rather than free fatty acids, it appears that PLD plays an indirect role in generating precursor 18:3 for JA synthesis. For example, PA may serve as a substrate for PLA, or as an intracellular regulator of the lipase directly involved in wound-induced JA biosynthesis.

Chloroplast reactions

Genes encoding LOX, AOS, and AOC, which catalyze the three "core" chloroplastic reactions in the octadecanoid pathway, have now been identified in several plant species. Localization studies using biochemical fractionation and immunocytochemical and *in vitro* chloroplast import assays have demonstrated a chloroplast location for these enzymes, and further indicate that LOX and AOS may be associated with the chloroplast envelope (47). A precise understanding of the contribution of the chloroplast reactions to JA homeostasis is complicated by the presence of multiple isozymes for these enzymes. For example, the completed sequence of the Arabidopsis genome indicates the existence of four genes for both 13-LOX and AOC (5, 13, 17, 47). Antisense expression of one 13-LOX isoform (LOX2) resulted in decreased JA accumulation in response to wounding, but did not affect male fertility (4, 13). This phenotype may reflect incomplete loss of function of

LOX2 in the antisense lines, or functional compensation by another 13-LOX. It is possible that individual isoforms of 13-LOX and AOC modulate JA levels in specific tissue or cell types. Further insight into the function of individual isoforms in JA homeostasis will likely come from the characterization of the corresponding T-DNA knock-out lines of Arabidopsis. In contrast to LOX and AOC, Arabidopsis has a single gene encoding AOS. Consistent with this, T-DNA-mediated disruption of *AOS* resulted in decreased JA accumulation and male sterility (33).

Forward genetic analysis has identified three mutants of tomato that are defective in JA biosynthesis. These include the *spr2* ω3-FAD mutant described above and two mutant lines (JL1 and JL5) that were identified in a genetic screen for plants that fail to express PIs in response to wounding (26). Characterization of the latter (renamed *def1*) showed that this mutant is deficient in wound- and systemin-induced JA accumulation and more susceptible to herbivore attack than wild-type plants. As expected for a block in JA synthesis, treatment of *def1* plants with exogenous JAs induced the expression of defense-related genes and restored resistance to herbivory (28). Available data suggest that *Def1* does not correspond to any known octadecanoid pathway gene in tomato. Rather, it appears that this locus is involved in the regulation of JA synthesis, perhaps by affecting the activity of AOC (28, 39). A defect in JA biosynthesis in the JL1 mutant line is indicated by a severe deficiency in wound-induced JA accumulation and the ability of the mutant to respond to exogenous JA (G.I. Lee and G.A. Howe, unpublished). The specific biosynthetic step affected in JL1 remains to be determined.

Peroxisomal reactions

Purification of enzymatically active OPR from cell suspension cultures of *Corydalis sempervirens* led to the isolation of the first OPR-encoding cDNA from Arabidopsis (36). The deduced amino acid sequence of this cDNA showed significant similarity to the Old Yellow Enzyme of yeast. This flavin-containing protein catalyzes the reduction of the olefinic bond of α,β-unsaturated carbonyls, such as the cyclopentenone ring of OPDA. Subsequent characterization of *OPR* genes showed that both Arabidopsis and tomato contain three highly related OPR genes (*OPR1*, *OPR2*, and *OPR3*). Biochemical analysis of recombinant OPR enzymes, however, revealed that only OPR3 is capable of reducing the 9*S*,13*S* stereoisomer of OPDA, which is the physiologically relevant precursor of JA. This finding is consistent with the demonstration that OPR3 is targeted to peroxisomes, whereas OPR1 and OPR2 are cytosolic isoforms (41). The conclusion that OPR3 is the only OPR isoform capable of initiating the transformation of 9*S*,13*S*-OPDA to JA was confirmed in studies demonstrating that an *opr3* null mutant (also known as *dde1*) lacks JA and is male sterile (46).

An important question raised by the organization of the octadecanoid pathway into two distinct compartments is the mechanism by which OPDA is transported between chloroplasts and peroxisomes. By analogy to what is

known about peroxisomal β-oxidation of straight-chain fatty acids, it is conceivable that interorganellar trafficking of OPDA involves formation of an OPDA-CoA intermediate (Fig. 4). Although very little is known about specific transport systems for fatty acyl-CoAs, recent evidence suggests that ABC transporters may play a role in the trafficking of these compounds to the peroxisome (15). Additional work is needed to address this question and to determine whether OPR3 accepts OPDA-CoA as a substrate. Metabolic labeling experiments (45) demonstrated that OPDA is reduced to OPC-8:0 prior to β-oxidation. The three core enzymes of the β-oxidation cycle are acyl-CoA oxidase, the multifunctional protein (MFP, containing 2-*trans*-enoyl-CoA hydratase and L-3-hydroxyacyl-CoA dehydrogenase activities), and 3-ketoacyl-CoA thiolase (Fig. 4). In Arabidopsis, these enzymes are encoded by small gene families, and mutations in some of these genes have been identified (15). In the context of JA biosynthesis, it is noteworthy that isoforms of some β-oxidation enzymes (e.g., acyl-CoA oxidase) exhibit specificity for fatty acids of a particular chain length. Whether or not specific β-oxidation isozymes mediate the conversion of OPC-8:0 to JA remains to be determined. Analysis of JA levels in various β-oxidation mutants will be useful to address this question.

JASMONATE METABOLISM

The balance between the *de novo* formation of JA and its further metabolism is likely to play a critical role in controlling the activity of the hormone. The simplest transformation is epimerization of newly synthesized (+)-7-iso-JA to the more stable (-)-JA isomer in which the side-chains at positions 3 and 7 are in the thermodynamically more stable *trans* configuration (Fig. 5). Commercially available JA typically consists of a mixture (approximately 9:1) of these two isomers. Although the extent to which epimerization occurs *in vivo* is unknown, it is generally assumed that (+)-7-iso-JA is the biologically relevant and more active form of JA. Support for this idea comes from the observation that the phytotoxin coronatine (see below) and synthetic indanoyl conjugates, which exhibit greater activity than JA in some bioassays, possess cyclopentanone ring constituents that are held rigidly within a six-membered ring structure (Fig. 5; 24).

Newly synthesized JA is subject to a variety of enzymatic transformations that profoundly affect the range of signaling activities of the molecule. As illustrated in Figure 6, the major metabolic routes include: (i) methylation of C_1, yielding MeJA; (ii) hydroxylation at C_{11} or C_{12}, yielding tuberonic acid-related derivatives; (iii) conjugation of the carboxy terminus to amino acids or other adducts; (iv) reduction of C_6, yielding cucurbic acid-related derivatives; and (v) degradation of C_1 to form (*Z*)-jasmone. Products of the reduction and hydroxylation pathways may be glucosylated to give the corresponding O-glucosides. Most of these metabolites have been shown to occur naturally (47).

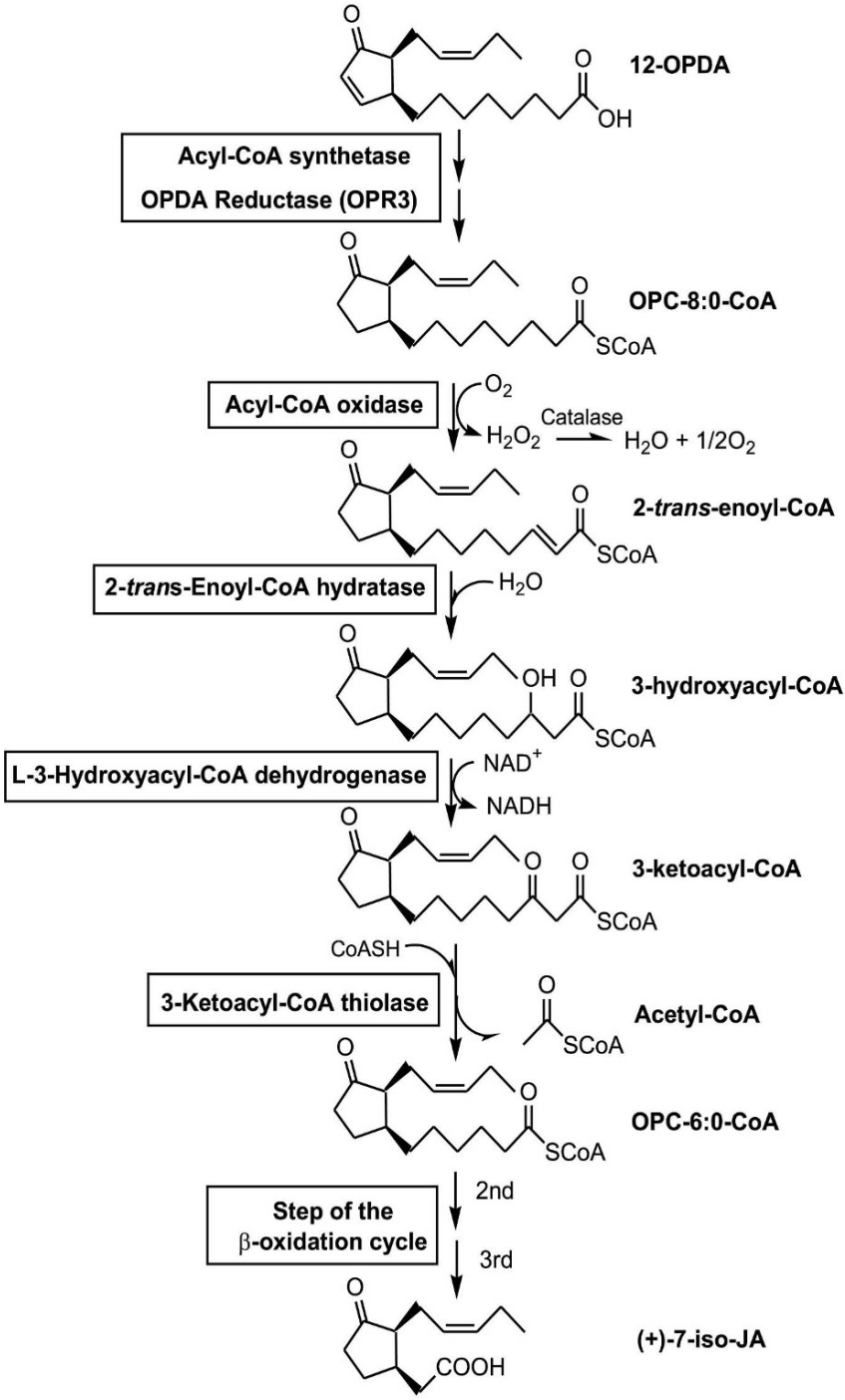

Figure 4. Hypothetical pathway for conversion of OPDA to JA in peroxisomes. The relative order in which Acyl-CoA synthetase and OPR3 act in the pathway is unknown, and may be reversed from that shown. See text for details.

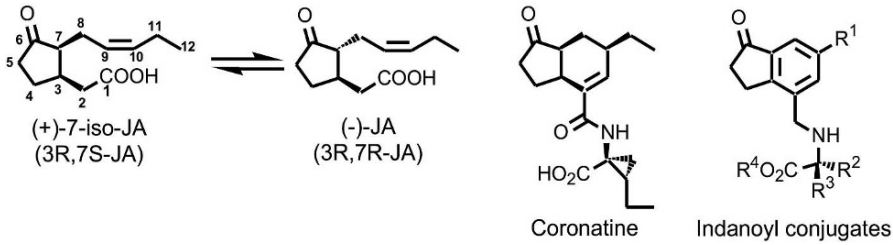

Figure 5. Chemical structures of JA isomers, coronatine, and indanoyl conjugates.

A significant recent advance in our understanding of JA metabolism was the identification of genes encoding various JA-metabolizing enzymes. Two examples of this are genes encoding a JA-specific methyl transferase (JMT) and a sulfotransferase (ST2a) catalyzing the sulfonation of 12-OH-JA (Fig. 6). Transgenic studies in Arabidopsis have provided insight into the physiological function of these metabolic tranformations. For example, overproduction of JMT resulted in increased levels of MeJA, constitutive expression of JA-responsive genes, and enhanced resistance to a fungal pathogen (37). These results suggest that methylation of JA is an important regulatory step in jasmonate-signaled defenses. Overexpression of *St2a* in Arabidopsis resulted in decreased levels of 12-OH-JA and delayed onset in flowering time (47). This provocative finding suggests that 12-OH-JA may function as a component of the inductive signal for flowering.

Characterization of the Arabidopsis *jar1* mutant that is defective in JA-signaled root growth inhibition led to the discovery that the *JAR1* gene encodes an adenylate-forming enzyme that catalyzes ATP-dependent

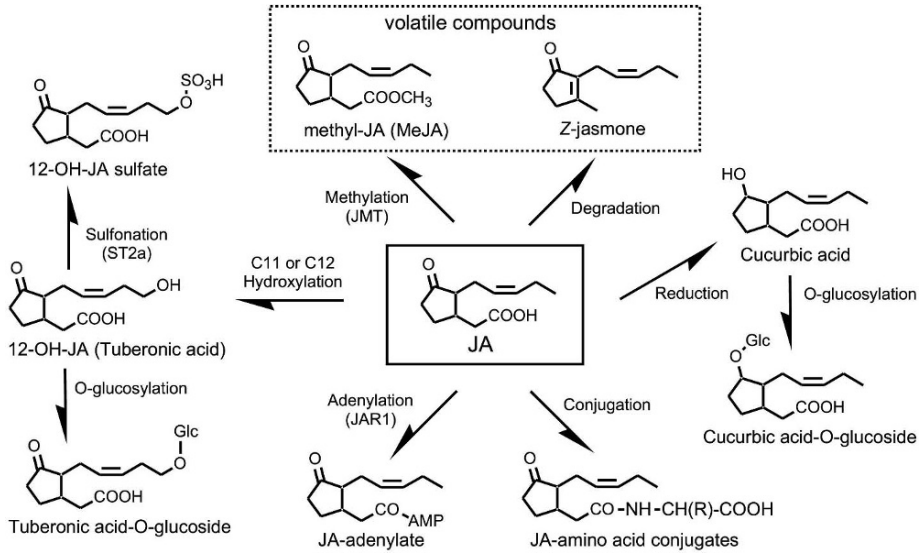

Figure 6. Pathways for JA metabolism.

adenylation of JA (Fig. 6; 38). JAR1 belongs to a superfamily of enzymes that activate a wide array of carboxylic acids. Although the precise biochemical function of JAR1 in JA metabolism remains to be determined, the activity of the enzyme suggests a role in conjugation to amino acids or other adducts, transport of JA to specific cellular locations, or metabolism of JA to an as-yet-unidentified product. Significantly, other members of the JAR1 family of enzymes were shown to catalyze adenylation of salicylic acid (SA) and indole-3-acetic acid (IAA) (38). This observation is important because it suggests that adenylation is a general feature of plant hormone metabolism. In this context, the insensitivity of *jar1* plants to JA indicates that adenylation promotes some JA-signaled responses (e.g., inhibition of root growth). Additional work should clarify the role of JAR1 in the JA signal transduction pathway.

JASMONATE ACTION

Regulation of Gene Expression

The discovery that MeJA and JA activate the expression of wound-inducible PIs in tomato aroused intense interest in the role of these compounds as regulatory signals for plant defense (9, 10). It is now widely recognized that JAs are among the most potent and important signals for the regulation of defense-related genes in species throughout the plant kingdom. Recent developments in technology to assess genome-wide patterns of gene expression have prompted research aimed at defining the size and function of the jasmonate transcriptome (14, 22, 30, 50). These studies have demonstrated that JA production in response to some biotic stress conditions (e.g., wounding) results in large-scale changes in the transcription of genes, many of which function in the reconfiguration of metabolism and the elaboration of diverse defense traits. Interestingly, JAs also regulate gene expression in herbivorous insects (29). Jasmonate perception by insects may allow these herbivores to "eavesdrop" on host plant defensive systems, and to rapidly mount counter-defenses against JA-induced phytotoxins. These findings raise the possibility that components of the JA signaling pathway may be conserved between the plant and animal kingdoms.

Despite extensive knowledge about the effects of JAs on gene expression, our understanding of the signaling events that couple the production of JA and other bioactive oxylipins to the activation of downstream target genes is still in its infancy. Systematic analysis of mutants obtained from forward and reverse genetic approaches is beginning to yield valuable insight into this question. For example, studies of the interaction of the *opr3* mutant of Arabidopsis with insect and fungal pests led to the discovery that OPDA promotes defense responses in the absence of its conversion to JA (40). This finding is consistent with previous studies showing that OPDA, rather than JA, is the physiologically relevant signal for the tendril coiling response of *Bryonia* (47, 48). The general theme that

emerges from these studies is that individual cyclopentenone (e.g., OPDA) and cyclopentanone (e.g., JA) signals work together to optimize expression of specific downstream responses.

One of the more remarkable effects of JAs on plant cells is increased production of secondary metabolites (e.g., phytoalexins) that play a known or suspected role in plant defense. Zenk and colleagues demonstrated that JAs are an integral part of the signaling cascade that couples elicitor action to the activation of genes for the biosynthesis of phytoalexins in plant cell cultures (16). This pioneering study of plant metabolism has spurred research aimed at using JAs to enhance the production of various phytochemicals. A survey of the literature shows that the accumulation of compounds belonging to nearly all of the major classes of plant secondary metabolites is enhanced in response to applied JAs (Table 1). The phenomenon of JA-induced secondary metabolism is interesting not only for what it reveals about how metabolism is reconfigured in response to stress signals, but may also have practical importance for the production of economically useful compounds (14). For example, MeJA was shown to significantly enhance production of the anti-cancer drug, taxol, in *Taxus* cell suspension cultures (49).

The ability of JAs to promote the accumulation of diverse defensive phytochemicals implies the existence of JA-responsive regulatory factors that coordinate the expression of numerous biosynthetic genes within a particular pathway. Support for this has come from two lines of investigation. The first is the use of gene expression profiling techniques to simultaneously monitor the expression of hundreds or thousands of genes in response to conditions that induce the JA pathway (14, 22, 50). The second approach has been the investigation of the underlying transcription factors. Analysis of JA-induced secondary metabolism in *Catharanthus roseus* (periwinkle) led to the identification of ORCA3, a jasmonate-responsive member of the AP2/ERF-domain family of plant transcription factors (32). ORCA3 specifically binds to the promoter region of several JA-responsive genes, including those involved in the synthesis of terpenoid indole alkaloids, to regulate their expression. Increasing evidence indicates that regulation of defense-related genes by JAs requires the action of additional signals. For example, several defense responses in Arabidopsis and tomato have been shown to require both JA and ethylene (7, 23). A recent breakthrough in our understanding of the molecular basis of this phenomenon was the identification of ERF1, an AP2-domain transcription factor that regulates ethylene/JA-dependent defense responses in Arabidopsis (31). Transgenic studies support the idea that ERF1 plays a pivotal role in integrating defense responses to both signals. The observation that *ERF1* expression is rapidly up-regulated by JA and ethylene indicates the involvement of transcription factors that act upstream of ERF1.