REVIEW

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Jasmonates: Structural Requirements for Lipid-Derived Signals Active in Plant Stress Responses and Development

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ABSTRACT Jasmonates are lipid-derived signals that mediate plant stress responses and development processes. Enzymes participating in biosynthesis of jasmonic acid (JA) (**1**, **2**) and components of JA signaling have been extensively characterized by biochemical and molecular-genetic tools. Mutants of *Arabidopsis* and tomato have helped to define the pathway for synthesis of jasmonoyl-isoleucine (JA-Ile), the active form of JA, and to identify the F-box protein COI1 as central regulatory unit. However, details of the molecular mechanism of JA signaling have only recently been unraveled by the discovery of JAZ proteins that function in transcriptional repression. The emerging picture of JA perception and signaling cascade implies the SCF^{COI1} complex operating as E3 ubiquitin ligase that upon binding of JA-Ile targets JAZ repressors for degradation by the 26S-proteasome pathway, thereby allowing the transcription factor MYC2 to activate gene expression. The fact that only one particular stereoisomer, (+)-7-*iso*-JA-L-Ile (**4**), shows high biological activity suggests that epimerization between active and inactive diastereomers could be a mechanism for turning JA signaling on or off. The recent demonstration that COI1 directly binds (+)-7-*iso*-JA-L-Ile (**4**) and thus functions as JA receptor revealed that formation of the ternary complex COI1-JA-Ile-JAZ is an ordered process. The pronounced differences in biological activity of JA stereoisomers also imply strict stereospecific control of product formation along the JA biosynthetic pathway. The pathway of JA biosynthesis has been unraveled, and most of the participating enzymes are well-characterized. For key enzymes of JA biosynthesis the crystal structures have been established, allowing insight into the mechanisms of catalysis and modes of substrate binding that lead to formation of stereospecific products.

Plants are sessile organisms and therefore have to continuously adjust to environmental changes. The plant hormones, structurally unrelated small molecules such as auxin, abscisic acid, cytokinin, gibberellin, ethylene, as well as the more recently recognized signaling compounds, brassinosteroid, salicylic acid, nitric oxide, strigolactone and jasmonic acid (JA) (**1**, **2**), not only are involved in plant growth and development but also mediate plant responses to the environment (*1*). Among these compounds, JA and its metabolites, collectively called jasmonates, are lipid-derived signals that share notable structural and functional properties with prostaglandins found in animals. Both types of compounds originate from lipid oxidation pathways, which in plants comprise at least seven different branches giving rise to numerous products, such as leaf aldehydes, leaf alcohols, peroxy fatty acids, keto fatty acids, hydroxy fatty acids, epoxides, ketoles, divinyl ethers, and jasmonates (*2, 3*). Jasmonates are generated *via* one specific branch of oxylipin biosynthesis, the AOS branch of the so-called LOX pathway. Stress responses that depend on jasmonate signaling include defense response against insects and pathogens (*4*–*7*) but also responses to ozone, UV light, wounding, and other abiotic stresses (*8*). In addition, jasmonates modulate root growth, flower development, tendril coiling, senescence, and carbon partioning in healthy plants (*9, 10*), and jasmonate responses are associated with extensive reprogramming of gene expression as revealed by transcriptional profiling (*11*–*14*). The analysis of mutants, mainly from *Arabidopsis thaliana* and tomato (*Solanum lycopersicum*), that are deficient in jasmonate biosynthesis or jasmonate signaling has been extremely useful for dissecting jasmonate function (*10, 15, 16*). Among these, the *Arabidopsis* mutant *coi1-1*, which was already isolated

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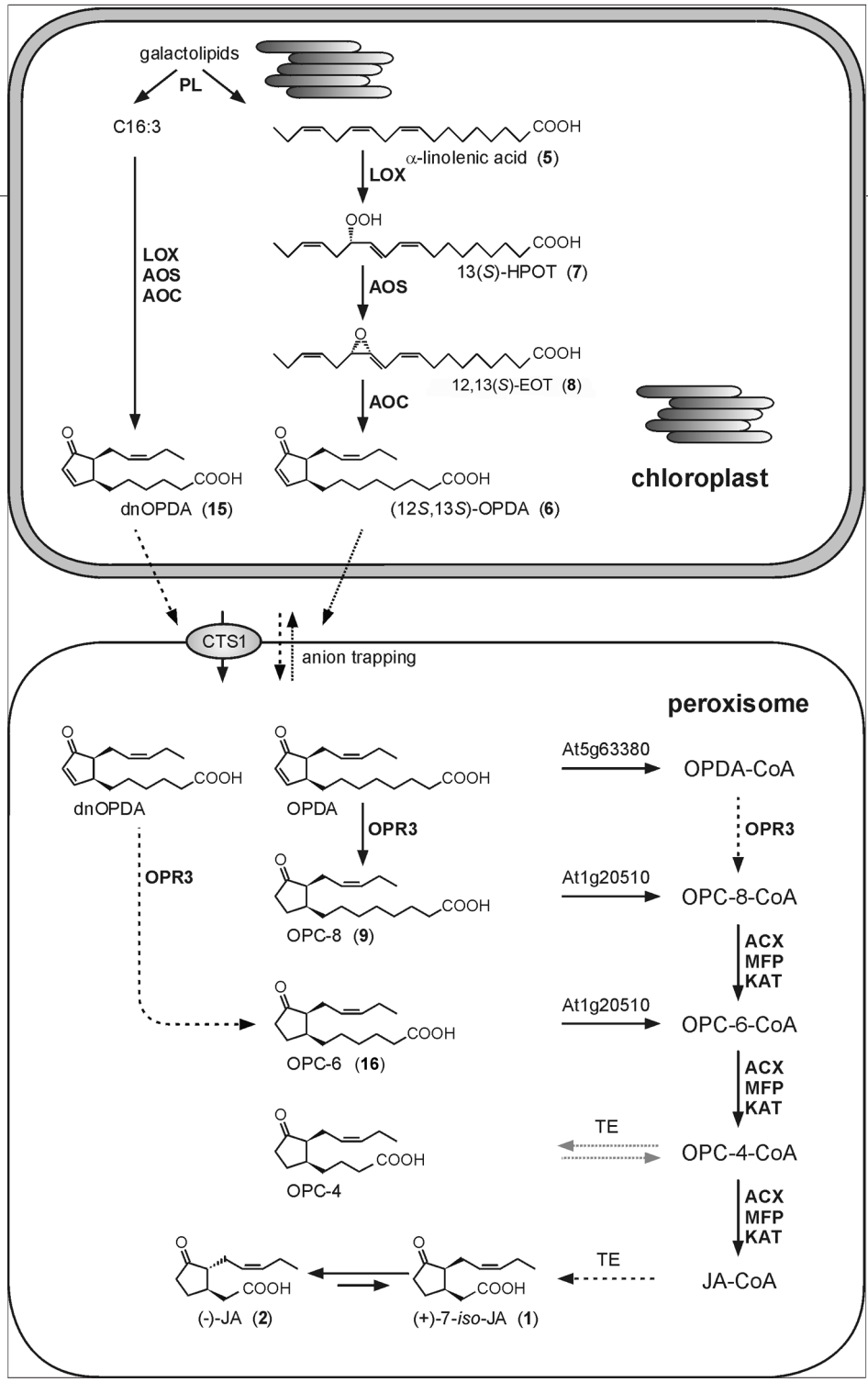

Figure 1. Biosynthesis of JA in the chloroplast and the peroxisome. Upon release of α-linolenic acid (C18:3) (5) by phospholipases (PL), the hydroperoxy compound 13(*S*)-HPOT (7) is formed by 13-lipoxygenase (LOX). The unstable allene oxide 12,13(*S*)-EOT (8) is generated by allene oxide synthase (AOS) and further converted to (9*S*,13*S*)-OPDA (6) by allene oxide cyclase (AOC). In a parallel pathway dinor-OPDA (15) can be generated from hexadecatrienonic acid (C16:3) by the same set of enzymes. The export mechanism of OPDA and dinor-OPDA from the chloroplast is not known, whereas their import into peroxisomes is mediated by the ABC transporter COMATOSE (CTS1) and/or an ion trapping mechanism. OPDA (6) is reduced to OPC-8 (9) by OPDA reductase 3 (OPR3). Subsequent activation to the corresponding CoA esters by OPC-8:CoA ligase (OPCL1, At1g20510) allows shortening of the carboxylic acid side chain *via* the fatty acid β-oxidation machinery, comprising acyl-CoA oxidase (ACX), multifunctional protein (MFP), and L-3-ketoacyl CoA thiolase (KAT). The endproduct, jasmonoyl-CoA, is cleaved by a putative thioesterase (TE) yielding (+)-7-*iso*-JA (1), which equilibrates with the more stable (−)-JA (2). Dashed arrows indicate putative but not yet demonstrated reactions. Reaction scheme modified after ref 32.

in 1994 (*17*), has been exceptional, because it is deficient in nearly all jasmonate responses (*12, 17*), and after a long and winding road, jasmonate research has come full circle by the recent discovery that the COI1 protein is a jasmonate receptor (*18*).

Likewise, numerous obstacles have long hampered the search for the plant endogenous, bioactive, and physiologically relevant jasmonate. JA carries two chiral centers, and thus four stereoisomers are possible (although not all occur in nature), which are subject to numerous metabolic conversions giving rise to a large diversity of compounds that may grossly differ in abundance and bioactivity (*8, 19*). Although extensive structure−activity studies defined structural elements required for jasmonate function, the discovery during the past two years that core components of the JA perception module are two proteins (COI1, JAZ), which form a ternary complex with an amino acid conjugate of JA (*20, 21*), allowed new experimental strategies for studying jasmonate activity *in vitro*. The pronounced differences in biological activity of different jasmonate stereoisomers raise two important questions: (1) How is the stereospecificity of the product(s) controlled along the biosynthetic pathway? (2) Is epimerization (at one or two of the chiral centers) a possible control mechanism for turning JA signaling on or off? This Review will concentrate on the structural requirements for biosynthesis and perception of jasmonates, as revealed by protein−protein and protein−ligand interaction studies and from the crystal structures that were resolved for several JA biosyn-

thesis enzymes. For additional aspects of jasmonate synthesis and signaling we refer to other excellent recent reviews ((*8–10, 22–25*); *cf.* special issue of *Phytochemistry* (2009) Vol. *70*, issue 13−14), and we apologize for references not cited due to space limitation.

Jasmonate Biosynthesis. The pathway of JA biosynthesis has been unraveled; it comprises at least nine mostly well-characterized enzymatic reactions that are outlined in Figure 1 (*8, 26*). The initial substrates are α-linolenic acid (α-LeA; C18:3) (**5**) or hexadecatrienoic acid (C16:3) released from plastidial galactolipids by phospholipases such as DAD1 or GDL, which are active in flowers or leaves, respectively (*27, 28*). Following the oxidation of α-LeA by LOX to 13(*S*)-hydroperoxy-octadecatrienoic acid (13(*S*)-HPOT) (**7**), the first committed step of JA biosynthesis is the conversion of the LOX product to the allene oxide 12,13(*S*)-epoxy-octadecatrienoic acid (12,13(*S*)-EOT) (**8**) by AOS. This unstable allylic epoxide can decompose to different products, such as α- and γ-ketols, or spontaneously rearrange to racemic 12-oxophytodienoic acid (*29*), or it can be enzymatically cyclized by allene oxide cyclase (AOC) to optically pure *cis*-(+)-12-oxophytodienoic acid ((9*S*,13*S*)-OPDA) (**6**). This compound is the endproduct of the plastid-localized part of the JA biosynthesis pathway and carries the same stereochemical configuration as the naturally occurring (+)-*7-iso*-JA ((3*R*,7*S*)-JA) (**1**). An open question is how OPDA is released from the chloroplast; specific transporters have not been identified yet.

Translocation of OPDA into peroxisomes, where the second half of JA biosynthesis occurs, is mediated by the ABC transporter COMATOSE (CTS1, also known as PXA1/PED3) and/or an ion trapping mechanism (*30*). OPDA reductase 3 (OPR3) catalyzes the reduction of OPDA and dnOPDA, originating from C16:3, to 3-oxo-2-(2′[*Z*]-pentenyl)-cyclopentan-1-octanoic acid (OPC-8) (**9**) and 3-oxo-2-(2′[*Z*]-pentenyl)-cyclopentan-1-hexanoic acid (OPC-6) (**16**), respectively (Figure 1). In *Arabidopsis* a new group of acyl-coenzyme A synthetase has been identified that activates various JA precursors (*e.g.*, OPDA, dnOPDA, OPC-8, OPC-6) to the corresponding CoA esters, and thereby allows shortening of the carboxylic acid side chain *via* the fatty acid β-oxidation machinery (*31, 32*). Two or three rounds of β-oxidation catalyzed by acyl-CoA oxidase (ACX), multifunctional protein (MFP), and L-3-ketoacyl-CoA thiolase (KAT) lead to jasmonoyl-CoA, from which a yet unknown thioesterase releases (+)-7-*iso*-JA ((3*R*,7*S*)-JA) (**1**) that equilibrates to the more stable (−)-JA ((3*R*,7*R*)-JA) (**2**). Most enzymes of JA biosynthesis have been cloned from different plant species; with few exceptions they occur in small gene families. For key enzymes of JA biosynthesis the crystal structures have been determined, allowing insights into the mechanisms of catalysis and modes of substrate binding that give rise to stereospecific products.

Structural Specificities in Catalysis of Enzymes Active in Jasmonate Biosynthesis. *Lipoxygenase (LOX).* In plants the oxidation of α-LeA (and other polyunsaturated fatty acids) is catalyzed by lipoxygenases (LOX), a family of nonheme iron containing dioxygenases, which insert molecular oxygen into polyunsaturated fatty acids in regio- and stereospecific manner, thereby creating the first chiral center in the JA precursor, 13(*S*)-HPOT (Figure 1) (*2, 33*). Plant LOXs have been classified by different criteria, and functionally two types can be distinguished: enzymes that oxygenate α-LeA at C9 or C13, yielding 9(*S*)-HPOT or 13(*S*)-HPOT, respectively. Importantly, all plastid-localized LOXs synthesize 13(*S*)-HPOT (**7**) and thus are members of the 13-LOX family, whereas 9-LOXs are ubiquitously found in the cytoplasm (*2, 33, 34*). In *Arabidopsis*, LOX2, one of four 13-LOX isoforms, has been demonstrated to participate in wound-induced JA biosynthesis (*35*).

The establishment of crystal structures for several LOXs in combination with molecular modeling and extensive biochemical characterization was essential to identify the mechanism of catalysis and to create a comprehensive model for regio- and stereospecificity of LOX activity (*2, 33, 36, 37*). Essentially, stereoselective hydrogen abstraction at C11 followed by antarafacial dioxygen insertion at position −2 (C9) or +2 (C13) of the rearranged fatty acid radical intermediate gives rise to the products, 9(*S*)-HPOT or 13(*S*)-HPOT, respectively. In support of the model, a number of critical amino acids have been identified at the active site of selected LOX isozymes, which determine the orientation and depth of substrate penetration into the substrate cavity or shield either C9 or C13 against oxygen attack; correspondingly, single amino acid substitutions have been sufficient to invert regio- or stereospecificity of specific isoforms (*33, 36–38*).

Allene Oxide Synthase (AOS). The 13(*S*)-HPOT arising from LOX activity is further metabolized by at least six different enzymes, including AOS and hydroperoxide

lyase (HPL), resulting in the formation of different classes of oxylipins (*2, 3, 16, 39*). AOS catalyzes the formation of the unstable product 12,13(*S*)-EOT (Figure 1), whereas HPL catalyzes the cleavage of hydroperoxides into oxo-fatty acids and alkenals, also referred to as green leaf volatiles (GLVs) (*3, 39*). AOS and HPL are unusual cytochrome P450-containing enzymes (thiolate-coordinated heme proteins of the CYP74 family), which do not function as monooxygenases but instead rearrange fatty acid hydroperoxides into structurally different products (*3, 39, 40*). All known AOS are localized in the chloroplast where the substrate is generated; an exception is AOS from guayule (*Parthenium argentatum*), which is localized in the cytoplasm (*41–43*). The essential function of AOS in JA biosynthesis is evident from the *Arabidopsis aos* mutant (single gene loss-of-function), which is deficient in JA (*8, 44, 45*). However, many aspects of AOS biochemistry and catalysis remained enigmatic. The recently resolved crystal structure of *Arabidopsis* AOS provided exciting insight into the evolutionary paths of oxylipin biosynthetic enzymes and the distinctive features of their catalytic mechanisms (*46*).

KEYWORDS

COI1: An F-box protein of the SCF (Skip−Cullin−F-box) complex that, in response to jasmonates, targets JAZ proteins for degradation; named after the *Arabidopsis* mutant *coi1* (for coronatine insensitive 1).

Coronatine: A phytotoxin produced by *Pseudomonas syringae*, which is a structural and functional jasmonate mimic causing hormone imbalance in plants and thereby impairs plant defense responses against bacterial colonization.

Jasmonates: A group of compounds synthesized from the poly-unsaturated fatty acid α-linolenic acid that act as plant hormones (signaling molecules) mediating stress responses and developmental processes.

(+)-7-*iso*-Jasmonoyl isoleucine: The most active naturally occuring jasmonate synthesized by plants; a conjugate between one of the four jasmonic acid stereoisomers, (3*R*,7*S*)-jasmonic acid, and L-isoleucine.

JAZ protein: Jasmonate-ZIM-domain proteins are members of a larger family of TIFY proteins that is defined by the highly conserved signature sequence TIF[F/Y]XG located in the so-called ZIM (for Zinc-finger protein expressed in Inflorescence Meristem) domain. JAZs are distinguished from other TIFY proteins by the presence of the additional Jas motif located near the C-terminus and their involvement in jasmonate signaling.

The structural basis of substrate recognition is apparent from the co-crystals of AOS with the substrate analogue 13(*S*)-hydroxy-octadecantrienoic acid (13(*S*)-HOT) or the putative reaction intermediate (12*S*,13*S*)-vernolic acid (Figure 2, panels a and b). Both fatty acids nicely conform to the shape and physicochemical properties of the AOS active site, with the carboxyl groups hydrogen-bonding with Thr389, the aliphatic segments maintaining favorable hydrophobic contacts with neighboring nonpolar side chains (Figure 2, panel a). The oxygen of the hydroxy or peroxy group of the substrate side chain ligates the heme, while having productive interaction with the Asn321 residue and C11 of the substrate is positioned in proximity of the aromatic face of Phe137 (Figure 2, panel b). Asn321 is the catalytic residue in both AOS and HPL, whereas Phe137 is strictly conserved in all AOS suggesting an essential function (*46*).

The reaction mechanism deduced from these crystal structures and previous enzymological studies is depicted in Figure 2, panel c. In both AOS and HPL, the terminal hydroperoxide oxygen coordinates with the ferric heme iron, while Asn321 is catalytically important for homolytic cleavage of the O−O bond of the hydroperoxide, giving rise to an alkoxyl radical (RO•) at C11 and a protonated ferryl-species (S-Fe(IV)-OH). Essential for the AOS-specific reaction is the stabilization of the epoxyallylic radical intermediate (C•), allowing its oxidation by Fe(IV)-OH, and of the incipient carbocation (C+) by the neighboring active site residue Phe137 (*46*). In HPL, leucine takes the place of Phe137 with the consequence that the epoxyallylic radical is not stabilized and undergoes a different bond rearrangement, giving rise to different products *via* hemiacetal formation (Figure 2, panel c). The proposed reaction mechanism was verified by site-directed mutagenesis. Substitution of Asn321 abolished activity, whereas the substitution of Phe137 by leucine in *Arabidopsis* and rice AOS not only severely compromised AOS activity but also created robust HPL activity (*46*).

Allene Oxide Cyclase (AOC). The allene oxides produced by AOS, *e.g.*, 12,13(*S*)-EOT (**8**), are unstable compounds, with a half-life <30 s in water (*29*), and spontaneous hydrolysis or cyclization gives rise to different products, including α- and γ-ketols and racemic mixtures of *cis*(+)- and *cis*(−)-OPDA. The accumulation of optically pure (9*S*,13*S*)-OPDA (*cis*(+)-OPDA) (**6**) in plant tissues suggests a tight coupling of the AOS and AOC reactions, possibly in a synthase−cyclase complex, to avoid spontaneous chemical decomposition of the instable intermediate. Thus, it is thought that AOC is needed not so much as catalyst for lowering the activation energy of the reaction, but rather as a scaffold to enforce steric restrictions for substrate cyclization (*47*). The recently resolved crystal structure of *Arabidopsis* AOC2 is in accordance with such mode of catalysis. Binding and correct positioning of the substrate, 12,13(*S*)-EOT (**8**), is facilitated by the hydrophobic nature of the binding pocket; the active site residue Glu23 initiates delocalization of the C15 double bond, fol-

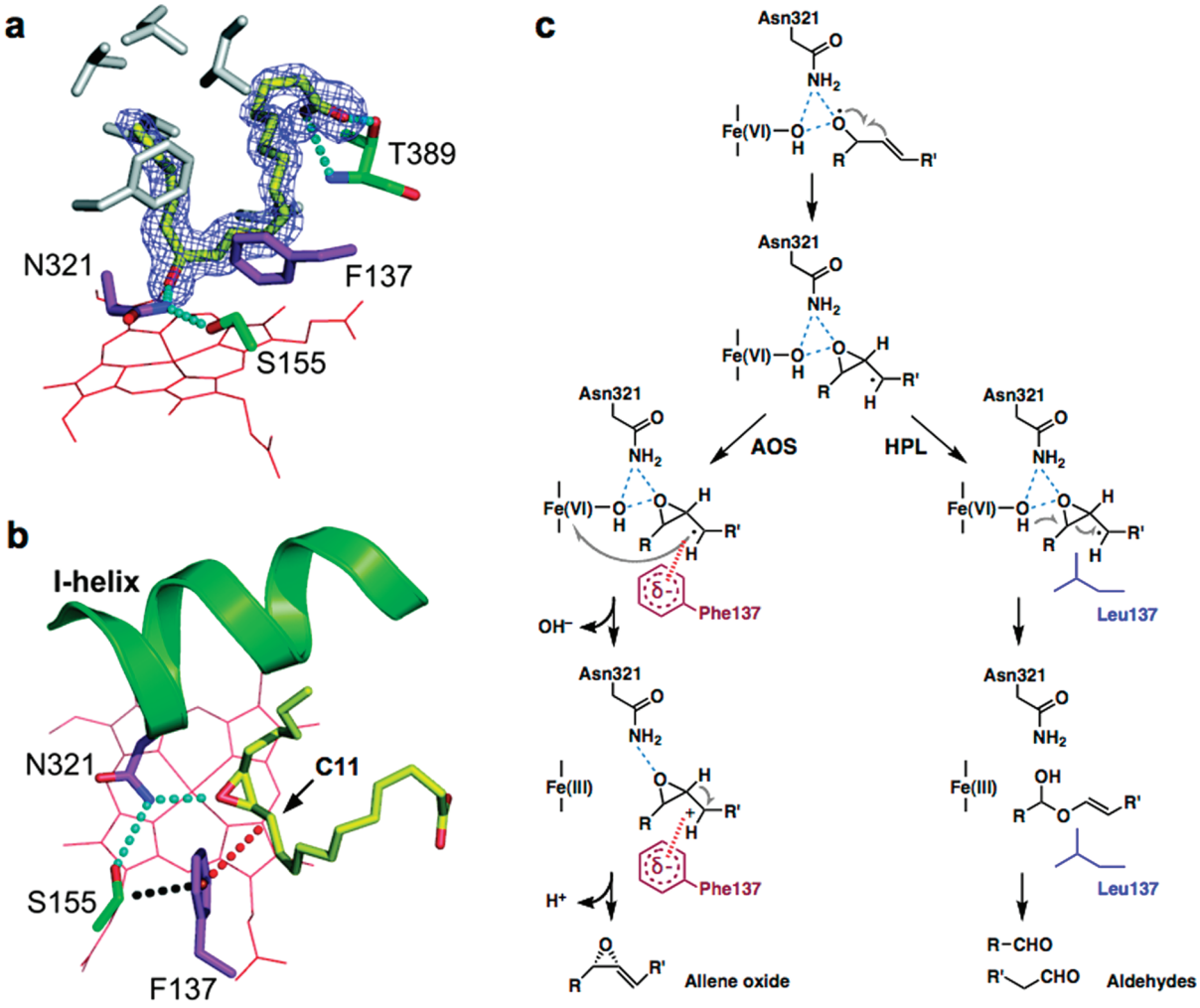

Figure 2. Structure of the substrate-binding site of allene oxide synthase (AOS) and proposed reaction mechanisms for AOS and hydroperoxide lyase (HPL). a) Active site configuration of AOS with 13(*S*)-hydroxyoctadecatrienoic acid (13(*S*)-HOT) (yellow), a close substrate mimic containing a OH group instead of the OOH as the natural substrate 13(*S*)-HPOT (with electron density contoured at 3δ shown in blue). The substrate carboxyl group coordinates with Thr386 (T389), and the oxygen of the side chain with Asn321 (N321). The strictly conserved Phe137 (F137) of AOSs is marked in purple, the nonpolar amino acid side chains are in gray, and the heme is in red. b) AOS in complex with the putative reaction intermediate analogue (−)-vernolic acid (*cis*-(12*R*,13*S*)-epoxy-octadec-*cis*-9-enoic acid) (yellow), demonstrating the close proximity of Phe137 (F137) to C11 where the alkoxy radical (RO•) is formed (*cf.* panel c). c) Reaction path for AOS and HPL activity as deduced from structural studies (*46*). The intermediate epoxyallylic radical formed at C11 can either undergo one-electron oxidation followed by proton loss (AOS) or oxygen rebound (HPL). Phe137 stabilizes the carbocation at C11 in the AOS-catalyzed reaction, allowing formation of the allene oxide. The peroxy fatty acid substrate is abbreviated to highlight the region undergoing chemical reaction. Dashed lines illustrate hydrogen bonds (blue) or ionic interactions (red). Scheme modified after ref 46; illustrations in panels a and b were kindly provided by Pierre Nioche and C. S. Raman.

lowed by opening of the epoxy ring, possibly stabilized by the neighboring Phe53 residue, allowing *trans–cis* isomerization around C10−C11 and stereospecific ring closure (*47*, *48*).

In *Arabidopsis* AOC is encoded by a small gene family (AOC1−4) (*49*); all isoforms are localized in chloroplasts and therefore may contribute to JA biosynthesis (*50*). In contrast to AOS, which produces allene oxides

from several 13(*S*)-hydroperoxy fatty acids (C18:3 and C18:2), AOC (from corn) accepts only 12,13(*S*)-epoxylinolenic acid (C18:3) and not 12,13(*S*)-epoxylinoleic acid (C18:2) as substrate (*51*). It thus appears that the enzyme confers additional specificity to the octadecanoid biosynthetic pathway. In fact, the only natural diastereomer arising from the combined action of AOS and AOC *in vivo* is (9*S*,13*S*)-OPDA (*cis*(+)-OPDA), representing the first cyclic compound and biologically active jasmonate of the pathway (*8*).

Oxophytodienoic Acid Reductase (OPR). Following translocation to the peroxisome, (9*S*,13*S*)-OPDA is reduced to OPC-8 (**9**) by OPR3 (Figure 1). OPR3 is a flavin-dependent oxidoreductase, which is encoded by small gene families in all plants studied so far (*26*). However, in *Arabidopsis* and tomato only the isoform OPR3 is found in the peroxisome, and the JA-deficient phenotype of the *opr3* mutant indicates that other isoforms do not contribute to JA biosynthesis (*52, 53*). Consistent with the genetic data, the biochemical characterization of recombinant OPRs revealed that only OPR3 orthologs (*Arabidopsis*, tomato, rice) shared the unique capacity to reduce natural (9*S*,13*S*)-OPDA as well as enantiomeric (9*R*,13*R*)-OPDA, whereas OPR1 and OPR2 selectively converted only (9*R*,13*R*)-OPDA (*54–56*).

The high-resolution crystal structures of OPR1 and OPR3 provide insight into the active-site architecture of the enzymes and their remarkable stereoselectivity (*57–60*). Essentially, access to the active site cavity, which is located above the noncovalently bound flavin mononucleotide (FMN), is gated in OPR1 by two large amino acid residues, Tyr246 and Tyr78, which in OPR3 are substituted by the smaller residues, His244 and Phe74. Thus, OPR3 imposes less restriction on substrates entry into the active site cavity, resulting in relaxed substrate specificity in comparison to that of OPR1 (*58*). That this mechanism is responsible for stereoselectivity of substrate conversion by OPR1 and OPR3 is supported by single amino acid substitutions (*59*).

Intriguingly, OPRs occur abundantly in plants, yeast, and bacteria (but not animals), but in contrast to OPR3, the function(s) of other isoenzymes remains enigmatic. Their common feature is that they reduce a broad range of α,β-unsaturated aldehydes, ketones, maleimides, and nitroalkenes (*61–63*). Several studies indicate that OPRs generally function as antioxidants, detoxifying breakdown products of lipid peroxidation and other toxic electrophilic compounds and xenobiotics, including drugs, the explosive trinitrotoluene (TNT), or acrolein (*63–66*).

β-Oxidation Machinery. Peroxisomes harbor the complete set of enzymes for fatty acid degradation, and three cycles of β-oxidation are also required for JA biosynthesis from OPC-8 (*43*). β-Oxidation is initiated by activation of the corresponding acid to the CoA-ester, and among the 63 predicted acyl-activating enzymes in *Arabidopsis*, a new family of peroxisomal fatty acyl-CoA synthetase has been identified, comprising four family members with the capacity to activate biosynthetic precursors of JA (*31, 32*). Significantly, OPC-8:CoA ligase (OPCL1, At1g20510) showed relaxed substrate specificity and activated a range of substrates, including OPC-8, OPDA, dnOPDA, and OPC-6; in fact, it was the only isoform active toward OPC-8 (*32*). By contrast, the other enzymes activating JA precursors (*i.e.*, At5g63380, At4g05160, At1g20500) showed specificity toward OPDA or OPC-6. Despite these different *in vitro* activities, OPCL1 (At1g20510) is the only acyl-CoA synthetase for which an essential *in vivo* contribution to JA biosynthesis has been demonstrated; the *Arabidopsis opcl1* mutant accumulated ~50% lower levels of JA in response to wounding when compared to wild-type plants (*32, 67*). However, the identity of alternative acyl-CoA synthetases contributing to JA biosynthesis remains unknown, because mutants defective for the other enzymes that activate JA precursors, in particular, At5g63380, At4g05160, At1g20500, and various combinations of double and triple mutants, did not display any significant alteration in wound-induced JA accumulation (*32, 67*). Likewise, the structural basis for the pronounced substrate selectivity of acyl-CoA synthetases remains unknown, although molecular modeling of the closely related 4-coumarate:CoA ligase uncovered a signature motif determining substrate specificity (*68*).

For the core enzymes of β-oxidation (ACX, MFP, KAT; *cf.* Figure 1), it has also been demonstrated that specific isoforms participate in wound-induced JA biosynthesis, *i.e.*, ACX1, ACX5, and KAT2/PED1/PKT3 in *Arabidopsis* and ACX1A in tomato (*6, 69–71*). ACXs catalyze the conversion of acyl-CoA to *trans*-2-enoyl-CoA, the first and rate-limiting step of peroxisomal β-oxidation, and members of the ACX family differ in their specificity with respect to type and chain-length of their preferred acyl-CoA substrate but collectively encompass the oxidation of the full range of acyl chains present *in vivo* (*72–74*). The crystal structures of *Arabidopsis* ACX1, ACX4, KAT2

and tomato ACX1 have been resolved (*75–78*). Although these structures provide insight into structural determinants of substrate specificity and uncovered amino acid residues that are essential for catalysis or cofactor-binding, the β-oxidative shortening of the side chain has no impact on the stereoisomeric configuration of the final product (3*R*,7*S*)-JA ((+)-7-*iso*-JA), which is released by an unknown mechanism to the cytoplasm for the synthesis of the bioactive variant (+)-7-*iso*-JA-Ile.

Structure–Activity Relationship of Jasmonates and Other Oxylipins. Since the first discovery of JA in plants, numerous JA metabolites were also shown to be constituents in various plant species and tissues or to accumulate in response to stress (Figure 3) (*8, 19*). Among these metabolites are the methyl ester and amino acid conjugates of JA, cucurbic acid, *cis*-jasmone, the glucose ester of JA, the 12-OH-JA and its sulfated, glucosylated and amino acid conjugated derivatives; even the conjugate with the ethylene precursor aminocylopropane carboxylic acid (ACC) and the glucose ester of OPC-4 were found (Figure 3). The development of new, sensitive analytical techniques and isolation methods has led to an ever-increasing number of identified JA derivatives (*19, 79, 80*). However, only a few enzymes active in JA metabolism have been characterized and cloned so far, including JA methyltransferase (JMT), JA amino acid conjugate synthase (JAR), and a sulfotransferase converting 12-OH-JA into 12-HSO_4-JA (*81–83*). The abundance and bioactivity of different metabolites may vary considerably (see below) (*84*).

The first physiological plant responses reported for jasmonates were promotion of senescence and growth inhibition (*85, 86*). Subsequently, the diversity of identified jasmonates and their pronounced differences in bioactivity led to a growing interest in structure–activity relationships. Distinct structural requirements for JA activity were defined on the basis of various biological responses caused by JA treatment, such as tuber formation, root growth inhibition, tendril coiling, alkaloid formation, or expression of JA responsive genes (*87–91*). The following observations provided insight into the structural requirements for JA bioactivity (*8*):

1. A cyclopentanone ring carrying a keto group at C-6 is essential.
2. An intact pentenyl side chain is required for activity.

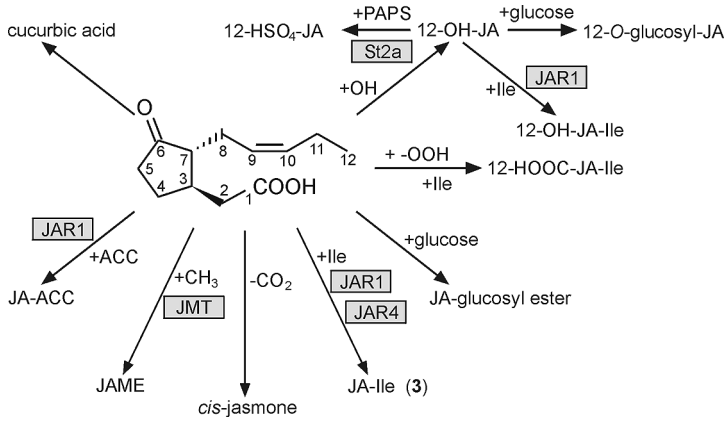

Figure 3. Metabolism of JA. Methylation of JA at the carboxyl group by JA methyltransferase (JMT) and conjugation of JA to isoleucine by JA amino acid conjugate synthase (JAR1 in *Arabidopsis*, JAR4 in tobacco) are common reactions in JA metabolism. Less important is conjugation of JA to other amino acids or aminocyclopropane carboxylic acid (ACC; presumably also catalyzed by JAR1), glucosyl ester formation with JA, conjugation of 12-HOOC-JA with isoleucine, and hydroxylation of JA at C12. 12-OH-JA can be glucosylated to 12-*O*-glucosyl-JA, conjugated with isoleucine to 12-OH-JA-Ile (presumable by JAR1), or sulfated by a sulfotransferase (ST2a) to 12-HSO_4-JA. Reduction of the keto group at C6 to a hydroxy group leads to cucurbic acid. Enzymes that have been cloned are boxed.

3. The carboxylic acid side chain retains activity only with an even number of C-atoms.
4. Formation of methyl ester or amino acids conjugates generally increases bioactivity,
5. whereas hydroxylation at C-11 or C-12 or reduction between C-11 and C-12 impairs biological activity (*cf.* below).
6. (−)-JA (**2**) and its derivatives are more active than (+)-JA (**1**) and its derivatives.

Initially, bioactivity of jasmonates was exclusively determined in direct application experiments, and the results were obviously dependent on the concentration and lipophilicity of the compound, duration of treatment, and the potential metabolic conversion to a more or less active derivative. Therefore, such data provided only first hints of the functional significance of particular derivatives or distinct structural units. Some of the JA metabolites, such as 12-OH-JA, 12-HSO_4-JA, and 12-O-Glc-JA (Figure 3), may even occur in distinct tissues or organs of some plants (*e.g.*, *Zea mays*, *Glycine max*) at concentrations that are orders of magnitude higher than those of JA, suggesting a function as an inactivated storage form of JA (*84*). Indeed, it has been demonstrated that formation of 12-OH-JA and 12-HSO_4-JA was accom-

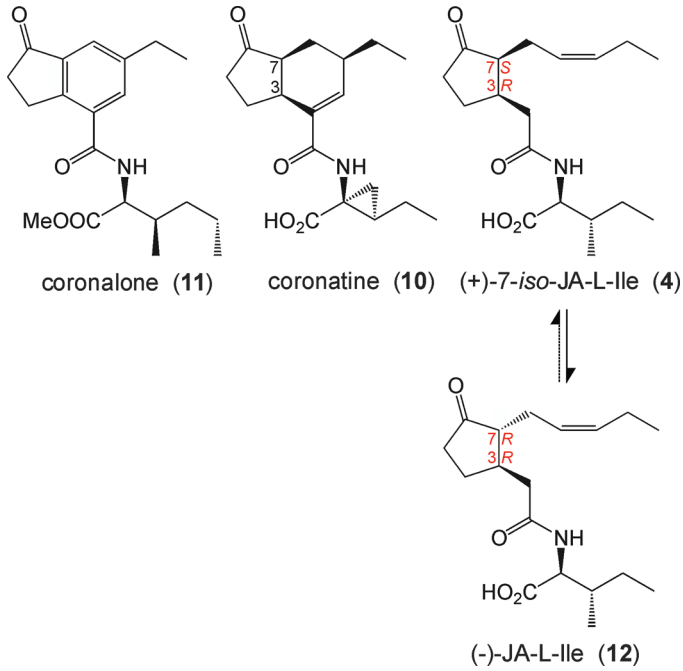

Figure 4. Structures of coronalon (11), coronatine (10), (+)-7-*iso*-JA-L-Ile (4), and (−)-JA-L-Ile (12). The absolute (*R*,*S*) configuration at the chiral centers C-3 and C-7 of the JA-Ile stereoisomers (+)-7-*iso*-JA-L-Ile ((3*R*,7*S*)-JA-L-Ile) (4) and (−)-JA-L-Ile ((3*R*,7*R*)-JA-L-Ile) (12) is indicated. In coronatine epimerization is not possible, because the configuration is fixed by the second ring structure. Coronalone contains a planar, aromatic structure without chiral centers.

panied with a partial suppression of JA signaling (*84*). Likewise, accumulation of several glucosides of JA and JA derivatives has been observed upon wound-induced JA biosynthesis, and collectively, these data indicate a role of JA metabolism in turning off JA signaling by conversion of active jasmonates into metabolites that are at least partially inactive (*79, 80, 84*).

Coronatine (**10**), a phytotoxin produced by several strains of *Pseudomonas syringae*, is a structural and functional JA mimic with extremely high biological activity (Figure 4). It was successfully used to screen for JA-insensitive mutants of *Arabidopis thaliana*, with *coronatine insensitive 1* (*coi1-1*) being the most informative (*17, 18, 92*). Similar high activity was found for structurally related, synthetic compounds, such as coronalon (**11**) (*93*). The defined stereochemical structures of these highly active compounds and the differential bioactivity of defined stereoisomers of JA or JA methyl ester observed in earlier studies revealed the importance of the chiral centers at C-3 and C-7 (*87, 94*). Similarly, stereochemically restricted JA analogs carrying additional substitutions at C-7 showed activity lower than that of the parent compounds (*94, 95*). The presence of two chiral centers allows four possible stereoisomers of jasmonates; however, only the (3*R*,7*S*) and (3*R*,7*R*) configurations occur in natural compounds, *e.g.*, JA or JA-Ile (**4**, **12**, **13**, **14**), since isomerization at C-3 apparently does not occur (Supplementary Figure S1). Furthermore, compounds with a (3*R*,7*S*) configuration were generally more active than (3*R*,7*R*)-isomers (*87, 94, 96*). These structural requirements for jasmonate activity are further supported by modeling and binding studies of the interaction between jasmonates and the recently identified JA receptor (*18*).

Jasmonate Receptor—Structural Specificity of JA Amino Acid Conjugate Binding. *COI1-JAZ-JA-Ile Complex.* Ubiquitin-dependent proteolysis is a central regulatory function in many biological processes (*1, 97*). Target proteins acting as negative regulators, *e.g.*, transcriptional repressors, are ubiquitinated by the Skp1-Cullin-F-box protein (SCF) complex, to which the F-box protein confers specificity by directly interacting with the target protein (Figure 5). The identification of the *Arabidopsis* COI1 protein as F-box protein in 1998 was a milestone for our current understanding of JA signaling (*98*). Recent evidence has well established that JA signaling indeed operates *via* the SCF^{COI1} complex, in particular, the discovery of the so-called jasmonate-ZIM-domain (JAZ) proteins in 2007. JAZ proteins function in repression of JA responsive genes and are encoded by a gene family of 12 members in *Arabidopsis* (*99*). They were identified by three different experimental approaches, their rapid JA-induced expression in stamens of *Arabidopsis* flowers (*20*), by cloning of the dominant JA-resistant *Arabidopsis* mutant *jai3-1* (*21*), and identification and cloning of a gene acting as repressor of JA-inhibited root growth in *Arabidopsis*, which turned out to represent a splice variant of JAZ10 (*14*). All JAZ proteins contain a ZIM domain of 27 amino acids in their central part and a Jas domain near the C-terminus; they are translocated into the nucleus *via* a nuclear localization signal present in the Jas domain (*9, 99, 100*). Beside the sheer number of different JAZ proteins, their functional diversity may be further increased by the occurrence of different splice variants, *e.g.*, as demonstrated for *JAZ10*, which may contribute to the sensitiv-

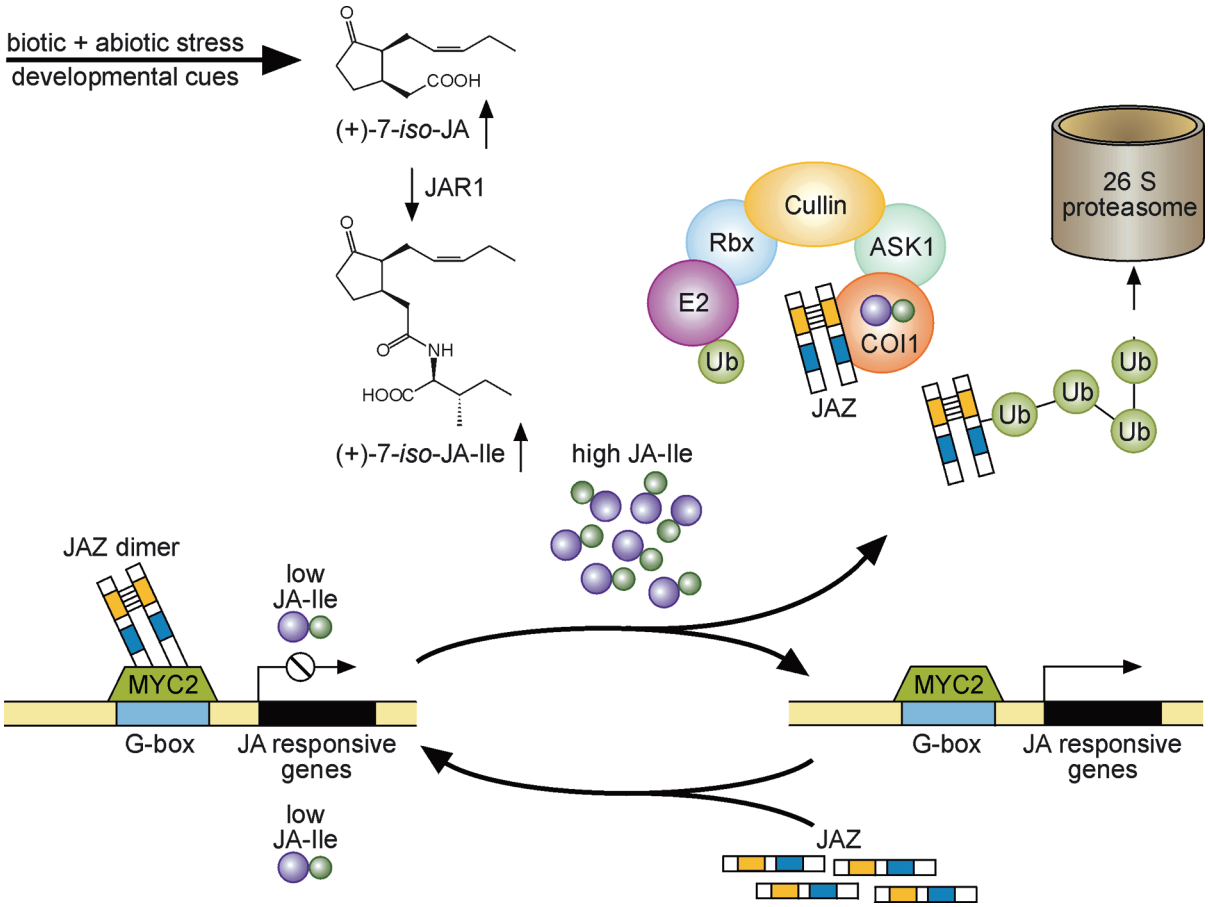

Figure 5. Model of jasmonate perception and regulation of jasmonate responsive gene expression. At low level of JA-Ile, transcription factors (*e.g.*, MYC2) are bound to their target sequence at the promoter of jasmonate-responsive gene (*e.g.*, G-box), but their activity is repressed by homo- or heterodimers of JAZ proteins. Upon stress or developmental cues, (+)-7-*iso*-JA (1) is synthesized *de novo* and conjugated with isoleucine by JAR1. The resulting high level of (+)-7-*iso*-JA-L-Ile (4) ((3*R*,7*S*)-JA-L-Ile) leads to binding by the jasmonate receptor COI1 (F-box protein with LRR domains), which is part of the SCF^{COI1} complex, comprising the protein components ASK1, Cullin, Rbx, and the E2 ubiquitin-conjugating enzyme. Subsequently, JAZ proteins are recruited from their initial binding site to the COI1-JA-Ile unit of the SCF^{COI1} complex, which acts as an E3 ubiquitin ligase transferring ubiquitin (Ub) from the E2 ubiquitin-conjugating enzyme to the COI1-interacting JAZ proteins. Upon polyubiquitination, JAZ proteins are directed to the 26S proteasome for degradation, releasing the repression of MYC2, thereby activating expression of JA responsive genes, including those encoding JAZ proteins. Synthesis of new JAZ repressors results in termination of JA gene expression.

ity and specificity of JA signaling (*14, 101*). Another regulatory role seems to be provided by homo- and heterodimerization of particular JAZ proteins, and the demonstration of 38 combinatorial interactions among the 66 possible heterodimeric combinations clearly underscores the potential regulatory flexibility in JA signaling (*101, 102*). The output of JAZ protein action is dependent on the specific transcription factors affected by JAZ proteins. In JA signaling, the basic-loop-helix-leucine zipper transcription factor JIN1/MYC2 is a central regulator acting both positively and negatively on JA-responsive gene expression (*103, 104*). Finally, establishing the function of JAR1 as JA amino acid conjugate synthase and providing evidence that the JAR1 product, JA-Ile, which is deficient in the *jar1* mutant and therefore responsible for its JA-resistant phenotype (*82*), is the preferred ligand binding to the COI1-JAZ protein pair and thereby stabilizing their interaction, provided the last pieces for understanding the JA signaling puzzle (*20, 21, 82, 92*). With all of these components, the scenario of

JA signaling *via* the SCF^{COI1} complex can be summarized as shown in Figure 5.

A rapid increase in endogenous JA levels, resulting from environmental stimuli or developmental programs, will lead to a concomitant increase in JA-Ile *via* JAR1 activity (*22, 105, 106*). Such a rise in JA-Ile occurs very rapidly, within 5 min after wounding (*106*). In the presence of a high JA-Ile level, the F-box protein COI1 recruits its targets, JAZ proteins, from its initial binding site, *e.g.*, MYC2, by physical interaction. This interaction is strongly potentiated by JA-Ile but much less by conjugates of JA with other amino acids (*20, 21, 23, 92*). The functional output is the degradation of the JAZ repressor(s) *via* the 26S proteasome, thereby allowing transcription factors such as MYC2 to activate expression of JA-responsive genes. This model is supported by a wealth of experimental evidence that was generated by yeast-to-hybrid protein interaction studies, expression analyses, binding assays, pull-down experiments, and analyses employing mutants and overexpression lines for various signaling components presented (*cf.* reviews (*9, 22–25, 99*)). The mechanism of JA signaling *via* SCF complex-dependent proteasomal degradation exhibits striking similarities to the signaling mechanisms by auxin, ethylene and gibberellin, where similar components participate (*1, 24*). With respect to jasmonate perception by the SCF^{COI1} complex, two important questions were open until recently: (1) which of the numerous jasmonate derivatives/stereoisomers are native and functionally important ligands, and (2) which of the participating proteins (COI1 or JAZ) is the actual JA receptor?

*(3R,7S)-JA-L-Ile (**4**), the Most Active Jasmonate.* As outlined above, distinct structural requirements for bioactivity of jasmonates have long been known. The identification of the COI1-JAZ module as site for interaction with JA-Ile, allowed development of a cell-free test system for analyzing the bioactivity of jasmonates more precisely. Essentially, jasmonate-dependent COI1-JAZ interaction is detected *in vitro* by co-immunoprecipitation (pull-down) experiments (*96*). Obviously, it was of particular interest to unravel which of the remarkable number of naturally occurring JA precursors and derivatives (*cf.* Figures 1 and Figure 3) activate the jasmonate perception module. Initially, only JA-Ile and not other jasmonate was found to promote COI1-JAZ interactions and hence was suggested to represent the active jasmonate derivative (*20, 92*). In view of the fact that the ratio of the stereoisomers, (+)-7-*iso*-JA [(3*R*,7*S*)-JA] (**1**) and (−)-JA [(3*R*,7*R*)-JA] (**2**), can shift from 20:80 in control plants (resting conditions) to 80:20 in wounding tissue (*107*) and that the jasmonate mimics coronatine (**10**) and coronalon (**11**), in which epimerization is not possible, show extremely high bioactivity, a search for the native, most active stereoisomer of different JA amino acid conjugates and other jasmonates was initiated (*96*). A prerequisite for this approach was the stereospecific synthesis of amino acid conjugates from different JA stereoisomers (*96, 108*). Among 40 different JA derivatives tested, only (+)-7-*iso*-JA-L-Ile (**4**) was exceptionally active; most other compounds showed only weak or no activity (*96*). This also included the epimer (−)-JA-L-Ile (**12**), and its previously reported activity (*20, 92*) could be attributed to low, residual contamination by the active epimer (+)-7-*iso*-JA-L-Ile (**4**). Other JA amino acid conjugates, such as (−)-JA-L-Val, (−)-JA-L-Leu, (−)-JA-L-Ile, (−)-JA-D-Val, (−)-JA-D-Ile, and (−)-JA-ACC were likewise only weakly active or completely inactive.

The large differences in bioactivity of (3*R*,7*S*)-JA-L-Ile ((+)-7-*iso*-JA-L-Ile) (**4**) and (3*R*,7*R*)-JA-L-Ile ((−)-JA-Ile) (**12**) suggested that epimerization at C7 could be a mechanism to activate or inactivate JA signaling by generating or destroying the native ligand of the jasmonate perception module. Although such mechanism was already suggested from earlier work (*109, 110*), now first evidence and experimental tools for testing this hypothesis have been provided (*96*). Although (3*R*,7*S*)-JA-L-Ile ((+)-7-*iso*-JA-L-Ile) (**4**) was stable at acidic or neutral pH, alkaline conditions or high temperature promoted rapid epimerization into the thermodynamically more stable *trans* isomer (3*R*,7*R*)-JA-L-Ile ((−)-JA-Ile) (**12**) (*96*). Thus, *in vivo*, at cellular pH, epimerization could be a regulated process that is subject to enzymatic control, but participating components still need to be identified. Another open question concerns the biosynthesis of (+)-7-*iso*-JA-L-Ile (**4**) and the substrate specificity of JAR1. The recombinant protein showed highest activity toward (−)-JA (**2**) when compared to (+)-JA or other JA derivatives (*111*). However, (+)-7-*iso*-JA-L-Ile (**4**) has not yet been tested.

COI1 Is a Jasmonate Receptor. The results obtained so far strongly suggest that the COI1-JAZ-JA-Ile complex is an essential component in jasmonate perception and signaling. Although the structural requirements for a bioactive jasmonate, (+)-7-*iso*-JA-L-Ile (**4**), were clearly de-

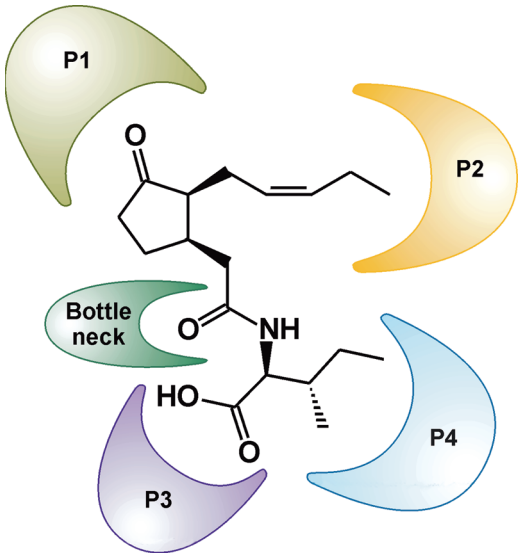

Figure 6. Model of JA-Ile bound to its receptor COI1. Three functional groups of the bioactive jasmonate (+)-7-*iso*-JA-L-Ile (4) are thought to mediate binding to COI1 surface pocket: (1) the keto group of the cyclopentanone ring forms hydrogen bonds to positively charged amino acids in pocket 1 (P1); (2) the pentenyl side chain builds van der Waal interactions to hydrophobic amino acids in pocket 2 (P2); and (3) the oxygen atom of the amide group forms hydrogen bonds with two tyrosine residues of the so-called bottleneck region, which is located between the lower (P1, P2) and the upper part (P3, P4) of the surface pocket. Anchoring of JA-Ile into COI1 may lead to reorientation of amino acid positions in P3 and P4, thereby generating a new interface for interaction with JAZ proteins. Figure modified after ref 18.

fined and binding of labeled coronatine to COI1-JAZ was also demonstrated (*92, 96*), final proof that this complex constitutes the JA receptor was still lacking, and the participation of additional components could not be excluded, because experiments were performed with crude extracts or partially purified COI1 and JAZ proteins. It also remained unknown in which order the COI1-JAZ-JA-Ile complex assembled. Now, COI1 was identified as a JA receptor (*18*). Starting from a high quality structural model of COI1, a surface pocket was identified that could function as potential binding site for JA-Ile. Molecular modeling and docking simulations of COI1-jasmonate interactions clearly indicated highest binding affinity for (+)-7-*iso*-JA-L-Ile (**4**) and its molecular mimic coronatine (**10**) when compared to (−)-JA-L-Ile and other jasmonates (*18*). From the derived model it was suggested that (+)-7-*iso*-JA-L-Ile binds to the surface pocket *via* the keto group of the cyclopentanone ring, the pentenyl side chain, and the oxygen atom of the amide group in the JA moiety (Figure 6).

Experimental evidence for direct binding of JA-Ile (**4**, **12**) or coronatine (**10**) by COI1 was also provided by three independent approaches: (1) retention of COI1 from crude plant extracts by JA-Ile immobilized on sepharose beads, (2) surface plasmon resonance measurements with purified COI1 and JAZ1 protein to reveal the interactions among COI1, JA-Ile, and JAZ1, and (3) binding of recombinant COI1 to the coronatine residue of a biologically active biotin-tagged photoaffinity probe generated from coronatine (**10**) (*18*).

Taken together, these results demonstrate that COI1 directly binds to (+)-7-*iso*-JA-L-Ile (**4**) and coronatine (**10**) and serves as a receptor for jasmonate. The derived model for jasmonate signaling thus implies a defined order for assembly of the COI1-JAZ-JA-Ile complex (*18*). Once JA-Ile is anchored to COI1 *via* the three important functional groups mentioned above, the Ile moiety of JA-Ile, by binding to the surface pocket, may form new interfaces for interactions with JAZ proteins (Figure 6). Eventually, the crystal structure of the JA receptor will provide insight into the molecular mechanisms and give answers to still open questions.

Conclusions. Over the past few years, the field of plant hormone biology in general and jasmonate research in particular has seen exciting developments, and our knowledge of hormone biosynthesis, metabolism, transport, perception, signaling, and plant response has drastically increased. However, despite the seminal discovery of the SCF^{COI1}-JAZ complex (and the JAZ interaction with MYC2) as essential JA signaling module, the identification of COI1 as a jasmonate receptor and (+)-7-*iso*-JA-L-Ile (**4**) is its ligand, or the mechanistic details of catalysis and substrate selection of enzymes participating in JA biosynthesis, several important questions remain to be answered, for example: (1) How can substrate binding of JA biosynthetic enzymes attribute to specificity and regulatory signal output *in vivo*? (2) Does JA biosynthesis include substrate channeling? (3) What are the cellular conditions for epimerization of (+)-7-*iso*-JA-L-Ile, and is this an enzyme-catalyzed process? (4) Which transcription factors, other than MYC2, are targets of JAZ proteins? (5) What is the contribution of homo- and heterodimers of JAZ proteins to differential JA perception and regulatory signal out-

put? (6) What is the perception mechanisms for other bioactive oxylipins, such as OPDA, which operate in an COI1-independent manner? From these and other open questions, *e.g.*, the crystal structure of the JA receptor in complex with different ligands and interactors, groundbreaking discoveries can be expected in the upcoming years, while other tasks such as *in planta* detection of the bioactive stereoisomers of JA and JA-Ile or the cell-type specific detection of jasmonates will require the development of new experimental tools.

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Supporting Information Available: This material is available free of charge *via* the Internet at http://pubs.acs.org.

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