INVITED REVIEW

Jasmonates: An Update on Biosynthesis, Signal Transduction and Action in Plant Stress Response, Growth and Development

C. WASTERNACK*

Department of Natural Product Biotechnology, Leibniz-Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle (Saale), Germany

• *Background* Jasmonates are ubiquitously occurring lipid-derived compounds with signal functions in plant responses to abiotic and biotic stresses, as well as in plant growth and development. Jasmonic acid and its various metabolites are members of the oxylipin family. Many of them alter gene expression positively or negatively in a regulatory network with synergistic and antagonistic effects in relation to other plant hormones such as salicylate, auxin, ethylene and abscisic acid.
• *Scope* This review summarizes biosynthesis and signal transduction of jasmonates with emphasis on new findings in relation to enzymes, their crystal structure, new compounds detected in the oxylipin and jasmonate families, and newly found functions.
• *Conclusions* Crystal structure of enzymes in jasmonate biosynthesis, increasing number of jasmonate metabolites and newly identified components of the jasmonate signal-transduction pathway, including specifically acting transcription factors, have led to new insights into jasmonate action, but its receptor(s) is/are still missing, in contrast to all other plant hormones.

Key words: Oxylipins, jasmonic acid, jasmonate metabolites, enzymes in biosynthesis and metabolism, signal function.

INTRODUCTION

In recent years, large scale analyses of gene sequences (genomics), expression (transcriptomics), protein patterns (proteomics), metabolite profiles (metabolomics) and lipid compounds (lipidomics) have been performed and remain the focus of many studies. The tremendous amount of accumulated data has led to new insights into how plants respond to biotic or abiotic stress and how a growth and developmental program takes place in the plant life cycle. An essential link within such programming is provided by small molecules, such as plant hormones. Among these, jasmonates have attracted much interest over the last two decades.

In 1962, jasmonic acid methyl ester (JAME; see Appendix for a list of abbreviations) was isolated for the first time from the essential oil of *Jasminum grandiflorum* (Demole *et al.*, 1962). The first physiological effects of JAME, however, and its free acid (JA) were only observed two decades later. In 1980, Ueda's group in Osaka, Japan, described a senescence-promoting effect of JA, and Sembdner's group in Halle, Germany, isolated and characterized these compounds as growth inhibitors (Ueda and Kato, 1980; Dathe *et al.*, 1981). Inhibition of root growth became the most prominent assay for screening of mutants affected in respect of JA signalling. The biosynthesis of JA was elucidated by Vick and Zimmermann (1984). The functional analysis of the mode of action was initiated by the observation in Parthier's group in Halle that application of JA or its methyl ester to barley leaves leads to an altered protein pattern. The synthesis of abundantly accumulating proteins, so-called jasmonate-induced proteins (JIPs) and degradation of housekeeping proteins such as Rubisco were described for the first time in 1987 (Weidhase *et al.*, 1987*a*, *b*). In 1990, another dramatic alteration of gene expression by JAME was observed in tomato plants (Farmer and Ryan, 1990). Here, a proteinase inhibitor (PIN2) was found to accumulate upon wounding (such as that caused by herbivore attack) or by treatment with airborne JAME. Subsequently, JA-induced alteration of gene expression and metabolite patterns became the focus of studies examining accumulation of vegetative storage proteins in Staswick's group (Staswick *et al.*, 1992) and alkaloids in Zenk's group (Gundlach *et al.*, 1992). In the latter case, an endogenous rise of JA was demonstrated upon elicitation of cell suspension cultures, thus indicating JA as a putative signal between the external stimulus and the response by cells of alkaloid formation. Other breakthroughs were the first cloning of a JA biosynthetic enzyme, allene oxide synthase (AOS) in Brash's lab (Song *et al.*, 1993) and isolation of the first JA-insensitive mutant in Turner's lab (Feys *et al.*, 1994). This mutant was screened by use of coronatine, a bacterial toxin with structural and functional similarity to JA (*co*ronatine-*i*nsensitive1, *coi1*). Four years later, Turner's lab were able to clone the corresponding gene. *COI1* codes for an F-box protein, which is part of the ubiquitin-mediated proteasome pathway (Xie *et al.*, 1998). In the same year, the corresponding F-box protein in auxin signaling, TIR1 was cloned. Consequently, much effort was spent in trying to understand hormone signalling in molecular terms and this led to the identification of TIR1 as one of the auxin receptors

* For correspondence. E-mail cwastern@ipb-halle.de

(Dharmasiri *et al.*, 2005; Kepinski and Leyser, 2005). The JA receptor, however, is still unknown. Other important steps in understanding the action of jasmonates were the identification of mutants affected in JA-biosynthesis (cf. Turner *et al.*, 2002), the identification of the first transcription factors regulating JA-responsive gene expression by Memelink's group (van der Fits and Memelink, 2001), the proof that fatty acid β-oxidation acts in JA biosynthesis (Li *et al.*, 2005), and the cloning of JA amino acid conjugate synthase (JAR1) by Staswick's lab (Staswick *et al.*, 2002; Staswick and Tiryaki, 2004). Subsequent to the latter, interest was shifted to JA metabolism and the action of JA metabolites.

A number of reviews emphasizing different aspects of JA physiology have appeared in recent years (e.g. Walling, 2000; Blée, 2002; Feussner and Wasternack, 2002; Wasternack and Hause, 2002; Farmer *et al.*, 2003; Halitschke and Baldwin, 2004; Howe, 2004; Mithöfer *et al.*, 2004; Pauw and Memelink, 2004; Pozo *et al.*, 2004; Browse, 2005; Rosahl and Feussner, 2005; Schilmiller and Howe, 2005; Wasternack, 2006). The following review will cover some recent results in biosynthesis, metabolism, signal transduction and action of jasmonates. I apologize for references not cited due to space limitations.

THE LIPOXYGENASE PATHWAY

Lipid-derived signals

Membrane lipids consist of polyunsaturated fatty acids (PUFAs) esterified to glycerol in the *sn1* and *sn2* positions. The third position of glycerol is linked via an inorganic ester linkage to a phosphate group. The resulting lipid molecule phosphatidic acid (PA), and the cleavage product of phosphoinositol bisphosphate, inositol triphosphate (IP_3), are known as signals in Ca^{2+} signalling and stress responses, respectively (Bargmann and Munnik, 2006). However, it is not only the main group of this class of lipids that may serve as secondary messengers, but their fatty acid residues, for example PUFAs, are also the source of further signals, such as oxylipins, which include jasmonates. Oxylipins originate from α-linolenic acid (18 : 3) (α-LeA) released from chloroplast membranes. Lipid hydrolyzing enzymes belong to at least five different families (Dörmann, 2005): (1) phospholipase A_1, which cleaves in the *sn*1 position; (2) phospholipase A_2, which cleaves in the *sn*2 position; (3) patatin-like acyl hydrolases with phospholipase and glycolipase activity; (4) DAD-like lipases, which degrade phospholipids and galactolipids; and (5) *SAG* (senescence-associated gene) 101-like acyl hydrolases.

So far a link to JA biosynthesis has only been shown for the wound-induced phospholipase A_2 (Narváez-Vásquez *et al.*, 1999) and a DAD-like phospholipase A_1. The *Arabidopsis* mutant *dad1* exhibits delayed anther dehiscence, shorter filament length and JA deficiency in flowers, indicating role of DAD1 in JA biosynthesis and of JA in filament elongation (Ishiguro *et al.*, 2001; see below). The corresponding DAD-like lipases of leaves are unknown.

Oxylipins generated in the lipoxygenase pathway

α-LeA released by lipase activity on chloroplast membranes is the substrate for numerous oxygenated compounds collectively called oxylipins including jasmonates, which comprise JA, JAME, JA amino acid conjugates and further JA metabolites (see below). The generation of oxylipins is initiated by lipoxygenases (LOXs), which form hydroperoxides from α-LeA (18 : 3) or linoleic acid (18 : 2) (Feussner and Wasternack, 2002). With α-LeA as the substrate, (13*S*)-hydroperoxyoctadecatrienoic acid (13-HPOT) or (9*S*)-hydroperoxyoctadecatrienoic acid (9-HPOT) are formed (Fig. 1). The corresponding products with linoleic acid as the substrate are (13*S*)-hydroperoxyoctadecadienoic acid (13-HPOD) and (9*S*)-hydroperoxyoctadecadienoic acid (9-HPOD). Although non-enzymatic oxygenation can take place by formation of phytoprostanes (Mueller, 2004), the LOX-catalysed reaction is indicated by the exclusive formation of the *S*-isomer. The increasing amount of *LOX* sequence data has allowed the construction of phylogenetic trees, which show gene families of different size for many plant species (Feussner and Wasternack, 2002). Analysis of the substrate-binding pockets by site-directed mutagenesis has revealed their role in the positional specificity of 9-LOXs and 13-LOXs (Liavonchanka and Feussner, 2006). A change in the histidine residues in the binding pocket of a cucumber lipid-body 13-LOX led to a 9-LOX activity (Hornung *et al.*, 1999). In addition to these structural requirements for positional specificity, clear differences were found for the expression of genes coding for 9-LOXs and 13-LOXs. Consequently, a concept for discrete 9-LOX and 13-LOX pathways was proposed (Howe and Schilmiller, 2002) and may explain the occurrence of numerous oxylipins (Fig. 1). The LOX products 13-HPOT and 13-HPOD represent branch points within the LOX pathway. At least seven different groups of compounds are formed by individual enzyme families (Feussner and Wasternack, 2002).

(1) Octadecanoids and jasmonates originating from 13-allene oxide synthase (13-AOSs) activity.
(2) Epoxyhydroxy-PUFAs formed by peroxygenases.
(3) Aldehydes, ω-oxo fatty acids and alcohols formed by hydroperoxy lyases (13-HPLs).
(4) Divinyl ether-containing PUFAs synthesized by divinyl ether synthases.
(5) Epoxyhydroxy PUFAs formed by epoxy alcohol synthases.
(6) Keto-PUFAs formed by LOXs.
(7) Hydroxy PUFAs formed by reductases.

Increasing examples are known where there are corresponding reactions with the 9-LOX products 9-HPOT and 9-HPOD. Subsequently acting enzymes should be specific for 9-LOX and 13-LOX products. Indeed, 9-HPLs and 13-HPLs as well as 9-AOSs and 13-AOS have been characterized, thus supporting the concept of discrete 9-LOX and 13-LOX pathways (Stumpe and Feussner, 2006). In potato, there are two 13-AOSs and one 9-AOS (AOS3), which is
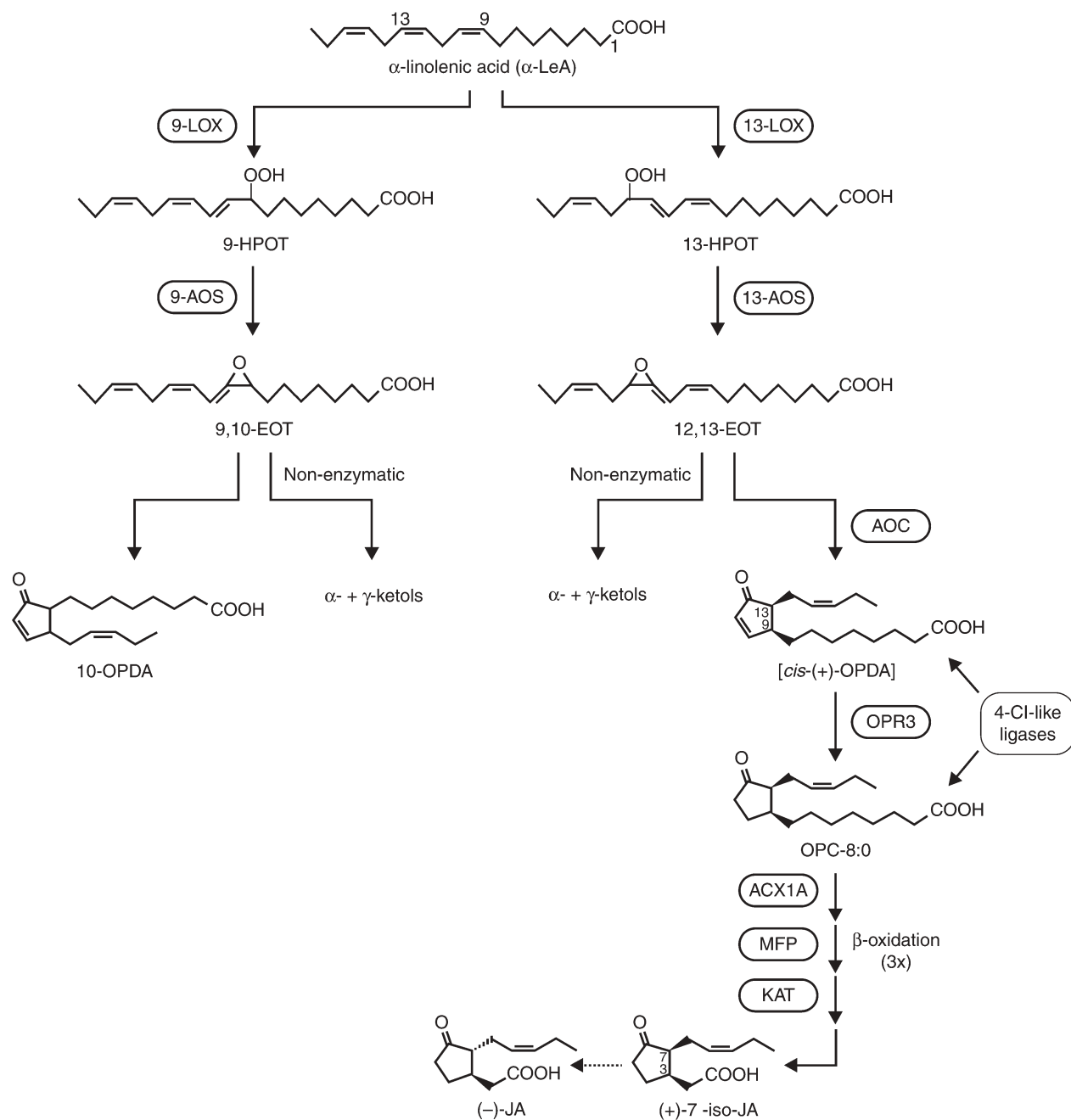
FIG. 1. The 9-LOX and 13-LOX pathways, and JA biosynthesis (modified after Schilmiller and Howe, 2005; Wasternack, 2006).

highly specific for 9-hydroperoxides and leads *in vitro* to α- and γ-ketol formation (Stumpe *et al.*, 2006). In below-ground organs, *AOS3* is expressed in sprouting eyes, stolons, tubers and roots leading to α-ketol formation *in vivo*.

JA BIOSYNTHESIS

The AOS branch in the LOX pathway: JA biosynthesis

Conversion of 13-HPOT, the first step in JA biosynthesis, is carried out by 13-AOS, leading to an unstable allene oxide

(Vick and Zimmermann, 1987). AOSs belong to the family of CYP74A enzymes, which are independent from molecular oxygen and NADPH, exhibit low affinity to CO and use the hydroperoxide group as a source for reducing equivalents and oxygen (Song *et al.*, 1993; Feussner and Wasternack, 2002; Howe and Schilmiller, 2002). Based on structural information on AOS, an effective and selective inhibitor, JM-8686, has been designed (Oh *et al.*, 2006). First cloned from flax seeds, more than 20 *AOS* sequences have been included in a phylogenetic tree analysis in which *9-AOSs* and *13-AOSs* were grouped separately (Stumpe and Feussner, 2006). With the exceptions of the AOS of

guayule (Pan *et al.*, 1995) and barley (Maucher *et al.*, 2000), all of the 13-AOSs carry a plastid transit peptide. Plastid localization has been shown by immunolocalization and import studies, even for the barley AOSs that lack the transit peptides (Maucher *et al.*, 2000; Froehlich *et al.*, 2001). This compartmentation corresponds to the plastid location of 13-LOX, whereas 9-LOX activity was found in the cytosol (Feussner and Wasternack, 2002) (Fig. 2). The picture is, however, more complicated. The recently cloned potato 9-AOS is associated with amyloplasts and leucoplasts (Stumpe *et al.*, 2006), and the recombinant AOS of *A. thaliana* exhibits dual 9-/13-specificity regulated by monomer-micelle association (Hughes *et al.*, 2006). This raises the possibility that *in vivo* an AOS can be a versatile enzyme in respect to 'substrate-specific' membrane association and oligomeric state (Hughes *et al.*, 2006).

The subsequent enzyme of the AOS branch, the allene oxide cyclase (AOC) is also plastid-located (Ziegler *et al.*, 2000). Using the highly unstable allene oxide, the AOC establishes the enantiomeric structure at the cyclopentenone ring that occurs in JA. Non-enzymatic side-reactions in the absence of AOC are the formation of racemic OPDA and the cleavage to α- and γ-ketol (Fig. 1). Due to the instability of the allene oxide and the common location of AOS and AOC, both enzymes were thought to be in close contact. But so far there is no proof on an AOS–AOC protein interaction (Zerbe *et al.*, 2007). The AOC product *cis*(+)-12-oxophytodienoic acid (OPDA) is the final product of the plastid-located part of JA biosynthesis. The subsequent step, reduction of the cyclopentenone ring, is catalysed by a peroxisomal OPDA reductase (OPR; Strassner *et al.*, 2002). In *Arabidopsis* and tomato there are more than three enzymes, but only OPR3 exhibits specificity for *cis*(+)-OPDA (Schaller *et al.*, 2000; Strassner *et al.*, 2002).

OPRs reduce conjugated enone structures including that occurring in OPDA. For OPR1 there are hints that the enzyme might be responsible for the formation of 9-ketodienes, such as 9-KODE (Tani *et al.*, 2006). The role of *OPR3* in JA biosynthesis was shown by genetic evidence provided by the JA-deficient mutants *opr3* and *dde1*, both affected in *OPR3* (Sanders *et al.*, 2000; Stintzi and Browse, 2000). All OPR3s cloned so far carry a peroxisomal target sequence, and a peroxisomal location of the OPR protein was shown by immunocytochemical techniques and by analysis of expression of *OPR3* fused to a reporter gene (Strassner *et al.*, 2002). Conversion of OPDA by the peroxisomal OPR3 raises the question as to how OPDA is released from the chloroplast and imported into peroxisomes. To date, an OPDA transporter of chloroplast membranes is unknown. For the import of OPDA or its CoA ester into peroxisomes, there is some evidence for transport by the ABC transporter COMATOSE (CTS), covering the PXA1 activity. The CTS mutant is partially JA-deficient, but is not male-sterile, a characteristic phenotype of complete JA-deficiency. Consequently, a parallel pathway of OPDA import by ion trapping has been suggested (Theodoulou *et al.*, 2005).

Initial experiments with labelled OPDA revealed β-oxidative side-chain shortening as essential steps in JA biosynthesis (Miersch and Wasternack, 2000). More recently, for *Arabidopsis* and tomato genetic evidence has been described showing that enzymes of fatty acid β-oxidation, such as acyl-CoA oxidase of tomato (ACX1), a multifunctional protein (MFP) and a L-3-ketoacyl CoA thiolase all have a function in JA biosynthesis (Fig. 1) (Cruz Castillo *et al.*, 2005; Li *et al.*, 2005; Wasternack *et al.*, 2006). Participation of *ACX1* in JA biosynthesis was clearly shown by isolation of an *acx1* mutant of tomato exhibiting JA deficiency but accumulating OPDA upon wounding (Li *et al.*, 2005). In *Arabidopsis* only the double mutant *acx1/5* shows less JA formation upon wounding, indicating redundancy among the five *ACX* genes (Schilmiller *et al.*, 2007). Further proof for role of fatty acid β-oxidation enzymes in JA biosynthesis has come from analysis of the *aim1* mutant, affecting one of the MFPs, and the *pex6* mutant, affecting peroxisome biogenesis (C. Delker *et al.*, unpubl. res.; Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany). β-oxidative steps take place only with the corresponding CoA ester. The formation of the OPDA–CoA ester in the chloroplast has been suggested, but not proven experimentally. There are, however, several indications for synthesis of OPDA–CoA ester in peroxisomes by 4-coumarate:CoA ligase-like (4-Cl-like) enzymes encoded by a small gene family in *A. thaliana* (Schneider *et al.*, 2005). Interestingly, this type of enzyme is also able to activate downstream intermediates in JA biosynthesis such as OPC-8 (Schneider *et al.*, 2005; Koo *et al.*, 2006) or OPC-6 (E. Kombrink, pers. comm.; Max Planck Institute for Plant Breeding Research, Cologne, Germany). These data suggest different import reactions (free OPDA versus OPDA–CoA) and activation of OPDA (or its products) where it carrys a different carboxylic acid side-chain to the corresponding CoA ester. It is, however, still unclear whether OPR3 can convert the OPDA–CoA ester. The existence of gene families for 4-CL-like enzymes and for genes encoding enzymes in β-oxidation, such as acyl CoA oxidase, suggest distinct and/or overlapping roles in β-oxidation of fatty acids and in the final steps of biosynthesis of JA. Similar β-oxidation steps were found for the final steps in auxin biosynthesis. Consequently, mutant analysis requires double or triple mutants in order to dissect the role in JA-dependent, auxin-dependent or β-oxidation (development)-dependent processes. These aspects have been recently reviewed (Baker *et al.*, 2006).

Regulation of JA biosynthesis

All genes encoding enzymes of JA biosynthesis are JA-inducible (Wasternack, 2006), and promoters analysed so far increase their activity upon JA treatment (Kubigsteltig *et al.*, 1999; I. Stenzel and C. Wasternack, unpubl. res.). This has led to the suggestion that JA biosynthesis is regulated by a positive feedback. Further experimental evidence for this has come from the following observations. (1) Mutants with constitutively elevated JA levels, such as *cev*, exhibit a phenotype characteristic for JA treatment (Ellis *et al.*, 2002) and show elevated *AOC* expression (I. Stenzel and C. Wasternack, unpubl. res.). (2) JA-deficient mutants, such as *opr3* or *coi1*, show
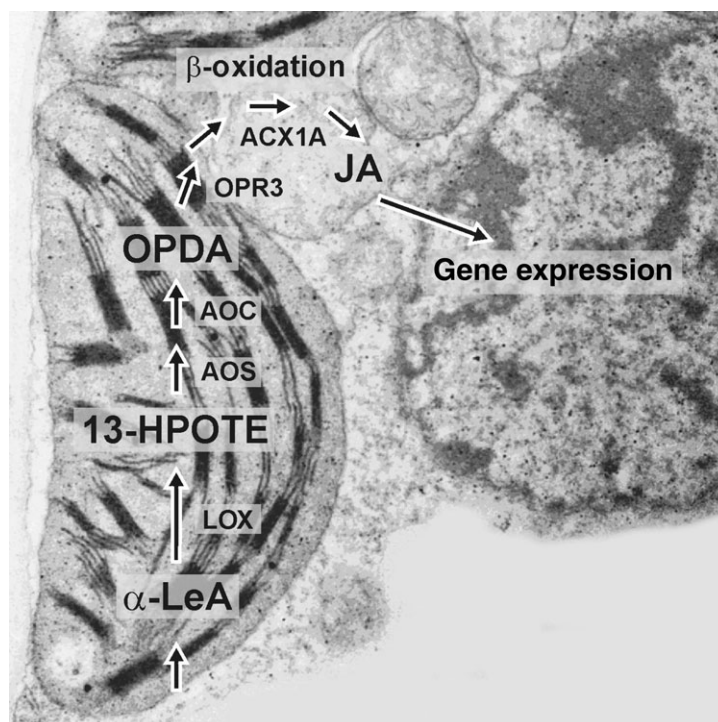
F[IG]. 2. Intracellular location of enzymes and intermediates in JA biosynthesis, illustrated on a SEM of a barley mesophyll cell showing the associated cellular compartments (photograph: B. Hause).

decreased *AOC* expression (Stenzel *et al.*, 2003*b*; I. Stenzel and C. Wasternack, unpubl. res.). Interestingly, as shown for tomato leaves by feeding experiments with deuterated OPDA, JA biosynthesis is not induced by endogenous jasmonates, which suggests extracellular perception of JA (Miersch and Wasternack, 2000). This has been substantiated by an autoradiographic study where applied labelled-JA was detected exclusively in the apoplast of tomato leaves (Bücking *et al.*, 2004).

Gene expression data are only part of the information necessary to understand regulation of JA biosynthesis. For example, the fully developed leaves of *A. thaliana* carry LOX, AOS and AOC proteins abundantly (Stenzel *et al.*, 2003*b*); however, JA-formation takes place only upon external stimuli, such as wounding. Furthermore, the wound-induced rise in JA is transient and appears before expression of *LOX*, *AOS* and *AOC* (Howe *et al.*, 2000; Stenzel *et al.*, 2003*a*, *b*), and plants over-expressing *AOS* or *AOC* constitutively do not show elevated JA levels before wounding or other stimuli (Laudert *et al.*, 2000; Stenzel *et al.*, 2003*a*). These data clearly show that JA biosynthesis is regulated by substrate availability. Finally, the tissue-specific location of JA biosynthetic enzymes contribute to the outcome in JA biosynthesis. In contrast to *A. thaliana* (Stenzel *et al.*, 2003*b*), in tomato AOC is confined to vascular bundles (Hause *et al.*, 2000) and even the sieve elements carry AOC protein (Hause *et al.*, 2003); consequently, cell- and tissue-specific formation of JA has been considered to have a role in wound signalling (Schilmiller and Howe, 2005; Wasternack *et al.*, 2006).

Arabidopsides: esterified oxylipins and protein-oxylipin adducts

Most of the substrates of many enzymes within the LOX pathway occur in free and esterified form. As well as the 18 : 2 and 18 : 3 PUFAs, their 9- and 13-hydroperoxides and 9- and 13-hydroxides have been found esterified in *A. thaliana* leaves and tomato flower organs (Stenzel *et al.*, 2003*b*; Miersch *et al.*, 2004).

Recently, esterified OPDA has received particular attention. First, esterified OPDA was found in galactolipids (MGDG) in the *sn-1* position in untreated *A. thaliana* leaves (Stelmach *et al.*, 2001). Subsequently, so-called arabidopsides A and B, derived from MGDG, and arabidopsides C and D, derived from DGDG, were found containing OPDA and dinor-OPDA, respectively (Hisamatsu *et al.*, 2003, 2005). Finally, 13-OPDA-, 18- and 16-carbon ketol-containing MGDG, DGDG and PC species were found (Buseman *et al.*, 2006). The diversity of esterified OPDA and the large amount found in lipid membranes (Stelmach *et al.*, 2001) raise the question as to its biological functions. OPDA exhibits individual signalling in plant defence reactions (Stintzi *et al.*, 2001), and there is a specific subset of genes that is specifically expressed in response to OPDA (Taki *et al.*, 2005). Hence release of esterified OPDA would greatly influence defence reactions. Indeed, wounding leads to a dramatic increase of free OPDA (Stelmach *et al.*, 2001; Buseman *et al.*, 2006). A specific function in the hypersensitive response of *A. thaliana* was found for a new esterified

OPDA, arabidopside E. Here, OPDA is bound to the *sn*1 and the *sn*2 position, and one dinor-OPDA to the galactose of MDGD (Andersson *et al.*, 2006). This compound accumulates up to 7–8 % of the total lipid content in a race-specific defence reaction against bacterial pathogens and inhibits bacterial growth *in vitro* (Andersson *et al.*, 2006). This suggests an important role of the 13-LOX pathway in the hypersensitive response- (HR) associated cell death. Furthermore, there was an increase in non-enzymatically formed hydroxy fatty acids and F_1-phytoprostanes (reviewed by Mueller, 2004) being esterified in membranes upon infection with a virulent and with an avirulent strain of *Pseudomonas syringae* that was up to 30-fold greater in level than that of the corresponding free compounds (Grun *et al.*, 2007).

Whilst induction of plant senescence has been known for a long time as a characteristic property of JA (Ueda and Kato, 1980; Parthier, 1990; Wasternack, 2004), recently a much stronger senescence-promotion effect was shown for arabidopside A (Hisamatsu *et al.*, 2006). It will be interesting to see whether arabidopsides occur in species other than *Arabidopsis thaliana* and which other JA-related processes are affected by these compounds.

Many oxylipins generated by the LOX pathway display cell toxicity and antimicrobial activity (Rusterucci *et al.*, 1999; Prost *et al.*, 2005). Therefore, the observation that oxylipins can be trapped by binding to lipid transfer proteins (LTPs) is an exciting result in helping to understand cell injury and the role of LTPs (Bakan *et al.*, 2006). LTPs are ubiquitously occurring plant lipid-binding proteins known for a long time to be associated with developmental and stress responses. But their function is still unknown. Direct inhibitory activity of LTPs on microbes was initially hypothesized, but the role in stress signalling is now the preferred suggestion. This is strongly supported by the identification of an abundantly occurring LTP1 in germinating barley seeds, which is covalently modified by the α-ketol 9-hydroxy-10-oxo-12(*Z*)-octadecanoic acid. This reactive oxylipin is formed by 9-LOX and AOS activity and is specifically trapped by covalent linkage to LTP1 (Bakan *et al.*, 2006). It will be interesting to know whether other LTPs form adducts with other oxylipins or hydrophobic molecules. Another putative candidate is a JA-binding LTP occurring in JA-loaded cells (Buhot *et al.*, 2004)

First crystal structures of enzymes in JA biosynthesis

AOS. So far, there is no crystal structure determined for a higher plant AOS. Interestingly, in corals a fusion protein with LOX and AOS domains has been found (Koljak *et al.*, 1997). These organisms form eicosanoid compounds similar to mammals. The AOS domain has weak activity and a sequence homology to a catalase. Recent crystal-structure analysis has revealed that in the coral AOS the switch from activity of a catalase to that of an AOS is facilitated (Oldham *et al.*, 2005). It is possible that the LOX–AOS fusion protein has been evolved by successive mutations in discrete but interacting LOX and AOS proteins.

AOC. Recently the first crystal structure has been described for the AOC2 of *A. thaliana* (Hofmann *et al.*, 2006). This is of special interest for understanding JA biosynthesis, since it is in the AOC reaction that the correct enantiomeric form of naturally occurring jasmonates is established. Knowledge of the crystal structure has led to insights into the oxylipin cyclization reaction. The stereoselectivity occurring in AOC catalysis was found to be a steric restriction for the essential substrate isomerization. Surprisingly, AOC2 of *A. thaliana* was detected as a trimeric protein in crystals and *in planta*, and forms a hydrophobic binding cavity with two distinct polar patches (Hofmann *et al.*, 2006). The folding pattern and sequence homology suggest that AOC2 is a member of the lipocalin family. This type of protein represents a widely distributed protein family characterized by a six- or eight-stranded β-barrel and a highly conserved motif with functions in the transport of small, hydrophobic molecules (Grzyb *et al.*, 2006). Consequently, implications for functions in cell growth, membrane biogenesis, induction of apoptosis, environmental stress responses and regulation of immune stress responses have been suggested (Charron *et al.*, 2005). The identification of AOC2 as a member of plant lipocalins is exciting and sheds new light on the diverse functions of lipid-based signalling and jasmonates.

OPR1 and OPR3. OPRs belong to a family of flavoproteins identified first with Warburg's old yellow enzyme (OYE). OYEs reduce the C=C double bond of α-,β-unsaturated carbonyl compounds, but only for OPR3 has a physiological function been identified. This enzyme reduces all four 1-stereoisomers of OPDA with preference for the only intermediate in JA biosynthesis, (9*S*,13*S*)-OPDA, whereas OPR1 reduces (9*R*,13*R*)-OPDA (Schaller *et al.*, 1998, 2000). This specificity for enantiomers has now been explained by the crystal structures of OPR1 (Breithaupt *et al.*, 2001) and OPR3 (Breithaupt *et al.*, 2006). The overall structures of OPR1 and OPR3 are very similarly characterized by a (β/α) barrel fold. The substrate-binding cavity, however, reveals a narrow entrance for OPR1 and a more 'released' cavity for OPR3 (Breithaupt *et al.*, 2006). Surprisingly, OPR3 was found as an extraordinary self-inhibited dimer in the crystal structure. A weak association *in vitro* but a strong dimerization *in vivo* by a putative phosphorylation at Tyr-364 has been suggested as a regulatory mechanism for OPR3 (Breithaupt *et al.*, 2006). This scenario accords with an activation of pre-existing JA biosynthetic enzymes leading to the early rise in JA before expression of JA biosynthetic genes (Stenzel *et al.*, 2003*a*, *b*).

ACX1. In the recently isolated tomato mutant *acx1*, a single point mutation of the conserved residue Thr138 is substituted by isoleucine (Li *et al.*, 2005). The crystal structure of tomato ACX1 showed an unusual packing arrangement compared to other ACX enzymes (Powers *et al.*, 2006); in tomato ACX1, three monomers form an asymmetric unit.

METABOLITES OF JA

Diversity among naturally occurring jasmonates and structure–activity relationships

Since the first isolation of JAME as a constituent of the oil of *Jasminum grandiflorum* (Demole *et al.*, 1962), numerous jasmonate compounds have been detected in diverse plant phyla (Meyer *et al.*, 1984). Jasmonates occur in algae, mosses, fungi, gymnosperms and angiosperms. The capacity to form or to convert various jasmonates is remarkably high in fungi; for example, more than 20 jasmonates were detected in culture filtrate of *Fusarium oxysporum* (Miersch *et al.*, 1999*a*), whilst *Aspergillus niger* grown on liquid medium could form more than 25 jasmonate compounds upon application of JA, 9,10-dihydro-JA and their methyl esters (Miersch *et al.*, 1999*c*). Some fungi, such as *Botryodiplodia theobromae*, are able to accumulate up to 500 μg mL^{-1} of culture medium of (+)-7-*iso*-JA, the initial product in JA biosynthesis (Miersch *et al.*, 1989). Whereas in fungi the biological function of various jasmonates is unknown, application of jasmonates and their structural analogs to higher plants led to the first insights into their structural requirements for biological activity. Based on different assays, such as alkaloid formation (Blechert *et al.*, 1995), tendril coiling (Blechert *et al.*, 1999), tuber formation (Koda, 1992), or gene expression (Miersch *et al.*, 1999*b*), the following structural requirements have been found (Fig. 3).

(1) (−)-JA and its derivatives are more active than (+)-derivatives.
(2) An intact pentenyl side-chain is most active, whereas hydroxylation at C-11 or C-12 and reduction between C-11 and C-12 reduce biological activity.
(3) Chain elongation of the carboxylic acid side-chain retains activity only with an even number of C-atoms.
(4) Activity is retained or increased by methylation or conjugation to amino acids.
(5) A cyclopentanone ring carrying a keto group at C-6 is essential.

More recently, highly active JA compounds such as coronalon (Schüler *et al.*, 2004) and other 6-substituted 4-oxo-indanoyl-isoleucine conjugates have been synthesized (Mithöfer *et al.*, 2004). The construction of these compounds was deduced by modelling based on the structure of the bacterial toxin coronatine. The extremely high biological activity of coronatine has been used as a tool and has led to isolation of the first JA-insensitive mutant of *A. thaliana*, *coi1* (Feys *et al.*, 1994). Recent data have revealed distinct responses for JA and coronatine (Uppalapati *et al.*, 2005).

Metabolic fate of JA

Homoestasis between various metabolites is a common mechanism in plants to sustain the level of active hormones such as auxins, cytokinines, gibberellins and jasmonates. In the case of JA, seven different metabolic routes have been suggested by identification of the corresponding products, but only a few enzymes have been cloned so far (Fig. 4).
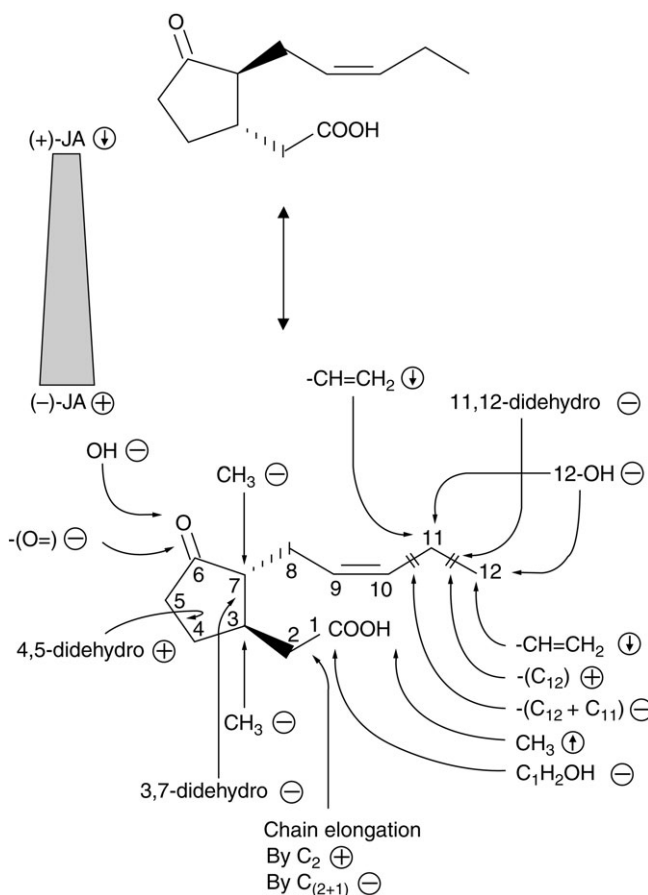

FIG. 3. Structure–activity relationships among jasmonates. The various structural analogs and enantiomers were tested with respect to JA-induced gene expression in barley (data from Miersch *et al.*, 1999*b*). $\downarrow$, decreased expression; $\uparrow$, increased expression; −, inactive; +, active in gene expression.

(1) Formation of amino acid conjugates by a JA conjugate synthase (JAR1) (Staswick and Tiryaki, 2004) upon adenylation at the carboxylic acid side-chain of JA by the AMP-transferase activity of JAR1.
(2) Methylation of JA by a JA-specific methyl transferase (Seo *et al.*, 2001).
(3) Hydroxylation at C-11 or C-12 of the pentenyl side-chain and subsequent *O*-glucosylation (Sembdner and Parthier, 1993; Swiatek *et al.*, 2004) or sulfation (Gidda *et al.*, 2003).
(4) Decarboxylation of JA to *cis*-jasmone (Koch *et al.*, 1997).
(5) Formation of cucurbic acids by reduction of the keto group of the cyclopentanone ring (Sembdner and Parthier, 1993).
(6) Formation of jasmonoyl-1-β-glucose, jasmonoyl-1-β-gentiobiose and hydroxyjasmonoyl-1-β-glucose (Swiatek *et al.*, 2004).
(7) Conjugation of the ethylene precursor ACC to JA (Staswick and Tiryaki, 2004).

JA amino acid conjugates are permanent constituents of plant tissues (Sembdner and Parthier, 1993) and their levels increase upon osmotic stress (Kramell *et al.*, 1995).
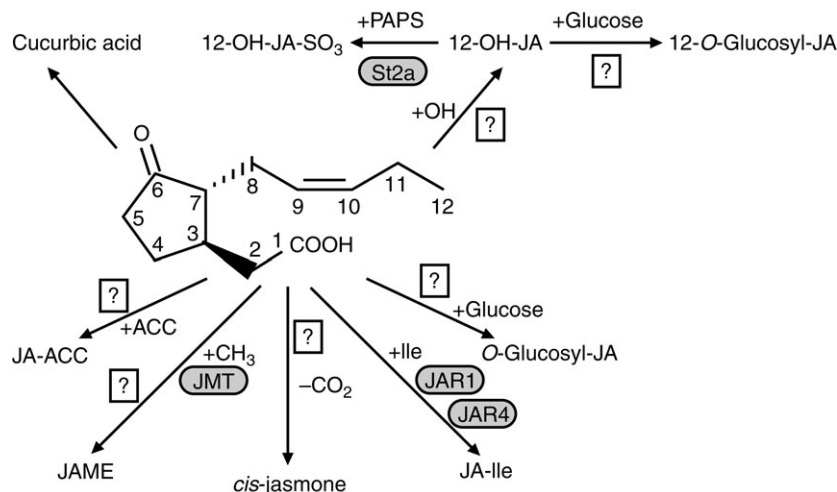
FIG. 4. Metabolic fate of jasmonic acid. The carboxylic acid side-chain can be conjugated to the ethylene precursor 1-amino cyclopropane-1-carboxylic acid (ACC), methylated by JA methyl transferase (JMT), decarboxylated to *cis*-jasmone, conjugated to amino acids such as Ile by JA amino acid synthase (*Arabidopsis*, JAR1; tobacco, JAR4) or glucosylated. The pentenyl side-chain can be hydroxylated in positions C-11 or C-12. In the case of 12-OH-JA, glucosylation or sulfation are subsequent reactions. Reduction of the keto group of the pentenone ring can lead to cucurbic acid.

In barley leaves applied JA amino acid conjugates are active *per se* without cleavage (Kramell *et al.*, 1997). Many of them induce a subset of genes compared with JA or JAME (Kramell *et al.*, 2000). Among the various conjugates, JA-Ile mainly accumulates in leaves (Kramell *et al.*, 1997), flowers (Hause *et al.*, 2000) and mycorrhizal roots (Hause *et al.*, 2002, 2007). This preference is reflected by the enzymatic properties of the JA amino acid conjugate synthase (JAR1), and the *jar1* mutant can be complemented by JA-Ile (Staswick and Tiryaki, 2004). *JAR1* belongs to a large gene family encoding enzymes that adenylate a carboxylic acid-containing substrate followed by exchange with a second substrate. In the case of JAR1, AMP is exchanged with an amino acid (Staswick *et al.*, 2002; Staswick and Tyriaki, 2004). Similar reactions are catalysed by auxin conjugate synthase (Staswick *et al.*, 2005) and 4-CL-like CoA ligases activating OPDA or OPC-8 to the corresponding CoA esters (Schneider *et al.*, 2005; Koo *et al.*, 2006). The tobacco homologue of *JAR1* has recently been cloned as *JAR4* from *N. attenuata* (Kang *et al.*, 2006). Interestingly, *JAR4*-silenced plants were shown to be more susceptible to *Manduca sexta* attack, indicating the role of JA-Ile in plant defence. Another JA metabolite highly active in plant–insect interactions via emission of volatiles is *cis*-jasmone (Birkett *et al.*, 2000; Bruce *et al.*, 2003).

ACTION OF JASMONATES

Jasmonates as novel anti-cancer agents

Whilst growth-inhibitory effects of salicylate on various types of cancer have been known for a long time, only recently has it been shown that cell lines of a wide spectrum of malignancies can exhibit selective cytotoxic effects of JAME (Flescher, 2005). These effects were independent of transcription, translation and p53 expression. Mitochondria seem to be one target. In hep3B hepatoma cells,

JAME-induced swelling of mitochondria and release of cytochrome *c* was observed, this being characteristic for an apoptotic pathway (Kim *et al.*, 2004; Rotem *et al.*, 2005). The selectivity of jasmonates on cancer cells was surprisingly high. Another target of jasmonates was detected in human myeloid leukemia cells. Here, jasmonates exert a growth-inhibitory and differentiation-induced effect by activating a NO-activated protein kinase (MAPK) pathway in a cAMP-independent manner (Ishi *et al.*, 2004). Among the various jasmonates tested, a methyl-4,5-didehydrojasmonate was 30-times more active than JAME. Jasmonates have not been detected so far in mammalian cells, but they are ubiquitous in plants. Some plant extracts have for a long time been used as therapeutic agents in cancer therapy. It will be interesting to see whether such plants contain high levels of jasmonates.

Jasmonates as signals in biotic and abiotic stresses

Solanaceae. One of the best-studied signal-transduction pathways of jasmonates is that of wound-response, which was initially studied mainly in tomato. Briefly, a sequential action of the 18-aa peptide systemin, cleaved from prosystemin upon local wounding, activates JA biosynthetic enzymes such as AOC and leads to local rise in JA (Fig. 5). An amplification in JA formation has been suggested due to JA-dependent *PROSYSTEMIN* expression, a systemin-dependent *AOC* expression, and their common location in vascular bundles (Stenzel *et al.*, 2003*a*; Narváez-Vásquez and Ryan, 2004). It is possible that JA acts as a systemic signal, leading also to systemic expression of genes encoding proteinase inhibitors (PINs) and other foliar compounds with negative effects on herbivore performance. Consequently the plant becomes immunized against a subsequent herbivore attack. Grafting experiments with JA-deficient and JA-signalling mutants in G. Howe's laboratory have shown that JA
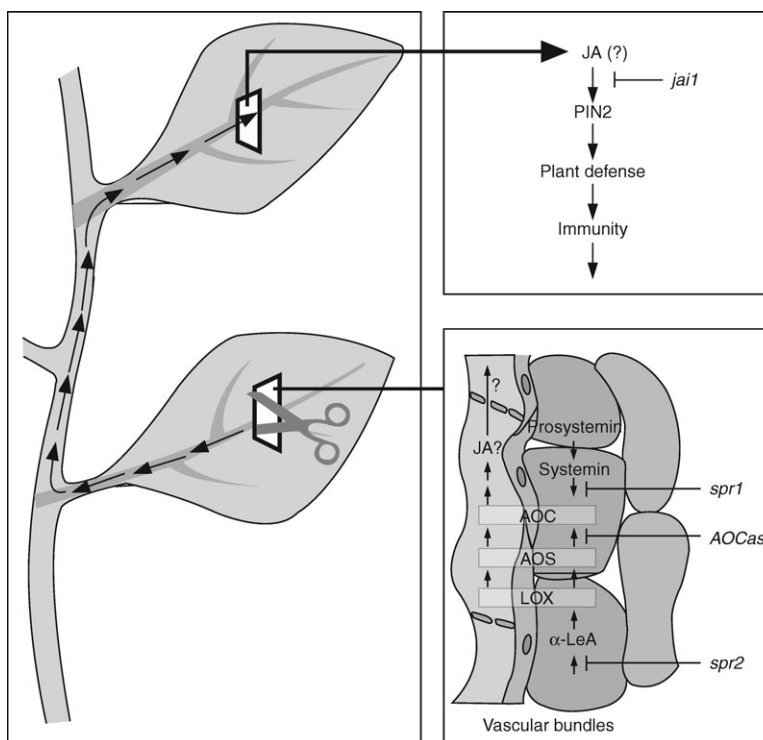
F<small>IG</small>. 5. Local and systemic wound response in tomato and cell-type-specific occurrence of AOC, AOS and LOX (after Hause *et al.*, 2003; modified from Wasternack *et al.*, 2006).

signalling – but not JA biosynthesis – is necessary in the systemic leaf. The numerous data on the wound response pathway have been reviewed repeatedly, including discussions on cross-talk to other signals, further elements of the wound-response pathway such as MAP kinases, and additional herbivore-induced compounds such as volatiles and their consequences for diverse plant–insect interactions, together with overlap to plant pathogen–interactions (Farmer *et al.*, 2003, Howe, 2004; Schilmiller and Howe, 2005; Wasternack, 2006). Here, I want to mention only few important new developments.

For a long time, an active systemin was found only in tomato (*Solanum lycopersicon*), but not in tobacco. Even in the closely related *Solanum nigrum* the systemin-homolog of *Solanum lycopersicon* is not active in defence responses (Schmidt and Baldwin, 2006). In tobacco, hydroxyproline-rich homologs of tomato systemin have been found that do not induce *PIN* expression in tomato (Pearce *et al.*, 2001; Pearce and Ryan, 2003). Recently, peptides active in wound-signalling have been identified outside the Solanaceaen species. In *A. thaliana* a 23-aa peptide (AtPEP1) processed from a 92-aa precursor is part of the innate immune response (Huffaker *et al.*, 2006). *PROPEP1* expression is induced by wounding, JA and ethylene leading to H_2O_2 formation and *PDF1·2* expression. *Arabidopsis thaliana* plants over-expressing *PROPEP1* show altered root development and enhanced resistance to the root pathogen *Pythium irregulare*. *PROPEP1* belongs to a small gene family, and AtPEP1 exhibits striking similarities in structure and function to tomato systemin (Huffaker *et al.*, 2006). The receptor of

AtPEP1 has been isolated and characterized as a functional LRR receptor, which is active even if transformed in tobacco (Yamaguchi *et al.*, 2006). The link between defence against pathogens and wound signalling provided by *AtPEP1* suggests that receptor-mediated defence-signalling peptides generated upon wounding may amplify defence against pathogens in *A. thaliana* (De Vos *et al.*, 2006). Resistance against pathogens can also be increased by modified emission of green-leaf volatiles initially released upon herbivory (Shiojiri *et al.*, 2006).

The JA-mediated wound response following herbivore attack leads to indirect (volatile emissions) and direct (formation of defence proteins and small compounds) defence (cf. reviews by Halitschke and Baldwin, 2004; Howe, 2004; Pieterse *et al.*, 2006), and even mechanical wounding is sufficient to give a volatile pattern similar to that caused by herbivore attack (Mithöfer *et al.*, 2005). Among the defence proteins formed in tomato there are PINs that are thought to inhibit proteolytic degradation in the midgut of herbivores. Herbivore-induced proteins in tomato also include leucine aminopeptidases, threonine deaminase (TD) and arginase (Walling, 2000; Chen *et al.*, 2004). Their direct role in the digestive tract of herbivores has recently been shown (Chen *et al.*, 2005). All these wound-induced plant proteins were detected in the midgut, and TD showed extremely high activity (Chen *et al.*, 2005). TD catalyzes the initial step in isoleucine biosynthesis of plants and bacteria and is negatively regulated by Ile via a C-terminal regulatory domain. Interestingly, the TD occurring in the midgut of a herbivore following infestation on tomato leaves lacked its regulatory domain. Consequently,

threonine degradation could take place in the presence of high Ile concentrations. Correspondingly, larvae infested on transgenic lines over-expressing arginase exhibited high arginase activity in their midgut and produced much less weight (Chen *et al.*, 2005). Obviously, catabolism of higher amino acids in the midgut of herbivores following their infestation on leaves acts synergistically to the deterrent role of PINs. The appearance within the midgut of a distinct set of proteins with altered properties, as in case of TD, and their anti-nutritional effects by perturbing amino acid homoestasis may be evolved in plants such as *Solanacean* species attacked by herbivores. Dual strategies seem to occur. The TD can exert an anti-nutritive defence in the midgut of herbivores, and within the plant contributes to the formation of Ile as an essential substrate for synthesis of JA-Ile, a highly active wound-response signal. In TD-silenced tobacco plants, JA-Ile-dependent defences are impaired (Kang *et al.*, 2006). It is possible that chemically based and developmentally based defence strategies co-evolved: the central element of JA-regulated gene expression, COI1, an F-box protein functioning in the SCF proteasome pathway, is essential for proper trichome development of tomato (Li *et al.*, 2004), and PIN2 of *Solanum americanum* is constitutively expressed in glandular trichomes and positively regulates trichome density (Liu *et al.*, 2006).

Arabidopsis. Analyses of transcript accumulation upon wounding or pathogenic attack have greatly improved our understanding of plant responses to biotic and abiotic stress (Pieterse *et al.*, 2006). Mutants in JA biosynthesis and JA signalling have become an important tool in dissecting signalling pathways and in determining signalling properties of JA and related compounds (Turner *et al.*, 2002; Lorenzo and Solano, 2005; Delker *et al.*, 2006; Wasternack, 2006). Initial expression analyses with the JA-deficient but OPDA-accumulating *opr3* mutant revealed OPDA-specific gene expression (Stintzi *et al.*, 2001). Following the first large-scale transcription analysis, genes could be grouped into COI1-dependent and COI1-independent types (Reymond *et al.*, 2000, 2004). Recent transcription analyses have shown distinct and overlapping groups of genes being expressed dependently and independently with respect to COI1, OPDA and JA (Devoto *et al.*, 2005; Taki *et al.*, 2005). Similar differences and overlaps in action have been found at the level of transcription factors acting in JA-/OPDA-dependent processes (Fig. 6). Among transcription factors acting downstream of JA in stress responses are the ethylene response factor 1 (ERF1), the bHLHzip-type transcription factor ATMYC2 (Lorenzo *et al.*, 2004), WRKY70 and the newly found family of ORAs (identified by J. Memelink's group). ORAs are the *Arabidospis* homologs of ORCAs initially identified in *Catharanthus roseus* cell suspension cultures (Memelink *et al.*, 2001). Among them, ORA47 is a COI1-dependent positive regulator of JA biosynthesis, whereas ORA59, ERF1, ORA37, MYC2 and WRKY70 act positively or negatively on different groups of defence genes (Fig. 6). The antagonistic action of MYC2 and ERF1 may cause the independence between wound signalling and pathogen-defence signaling (Lorenzo *et al.*, 2004; Lorenzo and Solano, 2005).
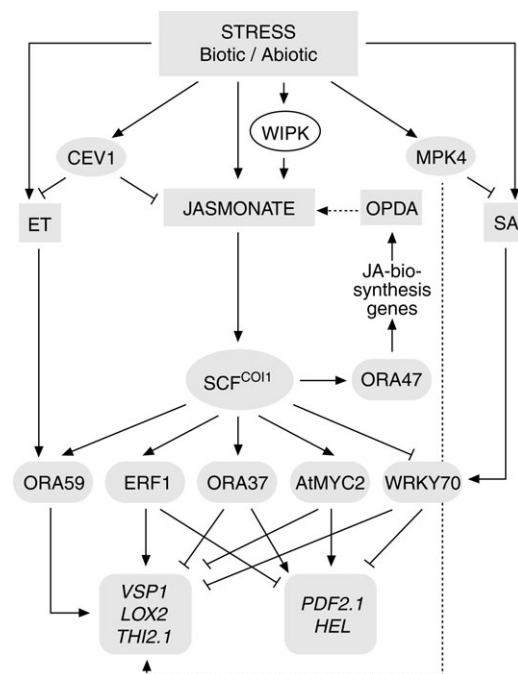

FIG. 6. Transcription factors involved in signalling pathways of JA and cross-talk to ethylene and SA (adapted from Pre, 2006, and Delker, 2007).

Jasmonates in development

Some aspects on role of jasmonates in developmental processes are summarized in Table 1.

Root growth inhibition. This was one of the first physiological effects detected for JA (Dathe *et al.*, 1981; Staswick *et al*, 1992). JA or its methyl ester supplemented to the

TABLE 1. *Jasmonates in development*

Developmental process	Putative signal	Alteration/species	Reference for initial observation
Root growth	JA, JA-Ile	Inhibition	Dathe *et al.* (1981); Staswick *et al.* (1992)
Seed germination	JA	Inhibition	Corbineau *et al.* (1988)
Tuber formation	12-OH-JA	Induction/potato	Yoshihara *et al.* (1989)
Tendril coiling	OPDA	Stimulation/*Bryonia*	Falkenstein *et al.* (1991)
Nyctinasty	12-OH-JA Glu	Stimulation/*Albizzia*	Nakamura *et al.* (2006)
Trichome formation	JA	Induction/tomato	Li *et al.* (2004)
Senescence	JA	Stimulation	Ueda and Kato (1980)
Flower development			
Anther development + dehiscence	JA	Induction/*Arabidopsis*	McConn and Browse (1996)
Female organ development	JA	Induction/tomato	Li *et al.* (2004)
Filament elongation	JA	Induction/*Arabidopsis*	Stintzi and Browse (2000)

growth medium are active in a picomolar range. Consequently, JA-insensitive mutants such as *coi1*, *jin1*, or *jar1* are similar in root length upon JAME treatment compared with the untreated wild type (Fig. 7). In contrast, mutants such as *cev1*, *cet1*, *joe2* or *cex1* that are characterized by constitutive over-expression of JA-responsive genes due to constitutively elevated JA levels have reduced root length and a stunted growth phenotype similar to JA-treated plants (Ellis *et al.*, 2002). Most mutants that are affected in components of JA- and COI-dependent signal transduction (including transcription factors such as MYC2, the SCF proteasom and its upstream-acting proteins such as AXR1, JAI4/SGT1b or MPK4) exhibit a reduction in root-growth inhibition (Fig. 6; cf. Browse, 2005; Wasternack, 2006).

Tuber formation. This has been known for a long time to be induced by 12-OH-JA (tuberonic acid; Yoshihara *et al.*, 1989), the glucoside of which has been suggested to be the transport form. Initially, 12-OH-JA was found mainly in Solanaceaen species. More recently, it has been detected in *A. thaliana* and identified as the substrate of a sulfotransferase (*AtST2a*), one of the 18 sulfotransferases occurring in the *Arabidopsis* genome (Gidda *et al.*, 2003). A recent screening revealed abundant occurrence of 12-OH-JA in different tissues and plant species, for example seeds of *Glycine max* (O. Miersch and C. Wasternack, unpubl. res.).

Tendril coiling and touch. Touch-mediated processes in plants are initiated by specific organs, such as tactiles. In the case of tendril coiling in *Bryonia*, a highly sensitive JA-dependent mechanism has been found (Falkenstein *et al.*, 1991; Weiler, 1997). Inspection of the response kinetics revealed that OPDA was more active than JA, and this was substantiated by the endogenous rise of OPDA upon touch (Stelmach *et al.*, 1998; Blechert *et al.*, 1999). More
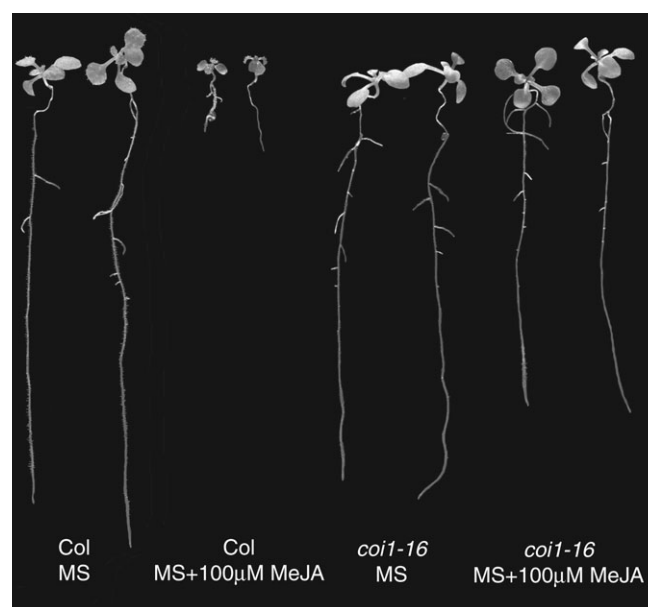

F<small>IG</small>. 7. Root growth inhibition by 100 μM methyl jasmonate in wild-type (Col) and the JA-insensitive mutant *coi1-16* (photograph: C. Delker).

recently, an excellent approach combining cell biology, chemistry and biochemistry has given indications as to how nyctinasty takes place in *Albizzia* plants. The leaf movement of *Albizzia* was shown to be dependent on a specific enantiomer of 12-OH-JA-*O*-glucoside, which binds with cell-type-specificity to the motor cells of leaves of *Albizzia julibrissica* (Nakamura *et al.*, 2006).

Flower development. This is essentially linked to intact JA biosynthesis and JA signalling. Clear evidence for a JA-dependent phenotype in flower development has come from *Arabidospis* mutants affected in JA biosynthesis, such as *dad1*, *fad3-2fad7-2fad8*, *dde2-2*, *dde1*, *opr3* and *aim1* as well as mutants in JA-signalling, such as *coi1*. All these mutants are male-sterile due to delayed anther development and incomplete anther dehiscence, or are impaired in filament elongation (cf. Turner *et al.*, 2002; Delker *et al.*, 2006). Surprisingly, in *Arabidopsis* an impaired F-box protein COI1 leads to male sterility, whereas its tomato homolog is essential for proper female fertility (Xie *et al.*, 1998; Li *et al.*, 2004).

The reduced filament elongation in JA-deficient mutant such as *dad1* and *opr3* corresponds to reduced JA levels in filaments of the double mutant *arf6/arf8*. These mutants are impaired in two auxin response factors responsible for proper filament elongation, and they indicate auxin signalling upstream of JA (Nagpal *et al.*, 2005). Recently, a large-scale analysis of gene expression in *opr3* stamen development following JA treatment has led to an excellent overview on JA-dependent processes in stamen development (Mandaokar *et al*, 2006). Among 13 transcription factors identified to function in stamen maturation, two of them, MYB21 and MYB24, are JA-inducible and mediate the jasmonate response during stamen development (Mandaokar *et al*, 2006).

Specific roles for JA and other oxylipins are also suggested by the abundant occurrence of AOC protein in ovules of tomato flowers and by the distinct oxylipin signature in different tomato-flower organs (Hause *et al.*, 2000) (Fig. 8). The total amount and the ratio between OPDA and JA can be increased by constitutive over-expression of *AOC* (Miersch *et al.*, 2004). It will be interesting to see whether the oxylipin signature is responsible for distinct steps in flower development, flower abscission, embryo development and seed formation.

Senescence. One of the first biological activities observed for jasmonates was the senescence-promoting effect (Ueda and Kato, 1980; Parthier, 1990). Although it occurs ubiquitously following JA treatment, monocotyledonous plants are more sensitive. Senescence is the last stage in plant development. Consequently, genes expressed during leaf senescence (senescence-associated genes, SAGs) code for proteins with functions in sink/source relationships, photosynthesis, intermediary metabolism, proteolysis and plant defence (cf. Wasternack and Hause, 2002; Wasternack, 2004). The role of JA in senescence is linked to (1) downregulation of housekeeping proteins encoded by photosynthetic genes and (2) upregulation of genes active in defence reactions against biotic and abiotic stresses (Wasternack, 2004, 2006). Several microarray analyses have led to more detailed
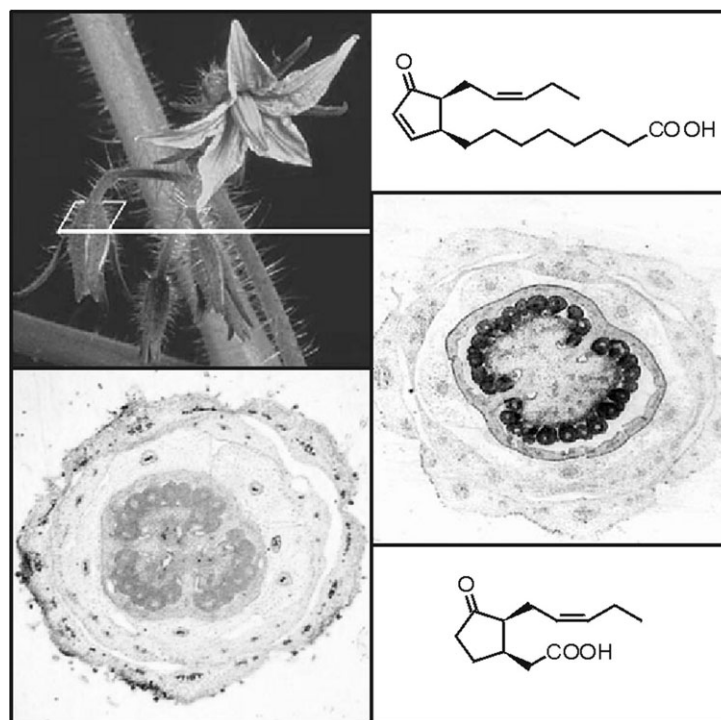
F<small>IG</small>. 8. The allene oxide cyclase (AOC) that establishes the correct enantiomeric structure of the cyclopentanone ring of jasmonate is encoded by a single copy gene in tomato, which is specifically expressed in ovules of young flower buds as revealed by *AOC* promoter activity tests (staining, left bottom) and immunocytological detection of AOC protein (black staining, right). The structure of jasmonic acid (bottom) and its precursor 12-oxophytodienoic acid (top) is shown.

information, including cross-talk between plant hormones (Lin and Wu, 2004; Buchanan-Wollaston *et al.*, 2005; van der Graaff *et al.*, 2006). A direct role of JA in leaf senescence of *A. thaliana* was shown by expression-analysis of JA biosynthetic genes (He *et al.*, 2002). Another molecular explanation for the role of JA in leaf senescence was given by identification of a novel nuclear-localized CCCH-type zinc finger protein in rice (Kong *et al.*, 2006). This protein acts as a negative regulator of the JA pathway and leads to a delay in the onset of leaf senescence. Another negative regulator of senescence is ORE9, an F-box protein (Woo *et al.*, 2001). However, there are also JA-independent processes in leaf senescence, as shown by identification of NAC family transcription factors that have an important role in leaf senescence (Guo and Gan, 2006).

CONCLUSIONS

In the last few years interesting new data on JA biosynthesis and action have appeared. Crystal structures for several JA biosynthetic enzymes now allow molecular explanations for substrate specificity. Fatty acid β-oxidation enzymes and 4-Cl-like CoA ligases have been identified to function in JA biosynthesis. New esterified oxylipin derivatives such as the arabidopsides suggest new functions in defence reactions. Occurrence and specific functions of JA metabolites have been detected. Transcription factors acting in JA signalling have shed new light on cross-talk in signalling pathways that are active following biotic and abiotic stresses, as well as in developmental processes. Finally, it is possible

that a putative role of jasmonates in cancer formation may be substantiated.

Future work will address questions on specific actions of JA metabolites, on JA and oxylipin perception, on MAP kinases active in JA signalling, on the diversity of action of JA in plant–plant and plant–microbe interactions; and, finally, cell- and organ-specific functions of jasmonates in plant development will be analysed.

LITERATURE CITED

Andersson MX, Hamberg M, Kourtchenko O, Brunnström Å, McPhail KL, Gerwick WH, *et al.* 2006. Oxylipin profiling of the hypersensitive response in *Arabidopsis thaliana*: formation of a novel oxophytodienoic acid containing galactolipid, arabidopside E. *Journal of Biological Chemistry* **281**: 31528–31537.

Bakan B, Hamberg M, Perrocheau L, Maume D, Rogniaux H, Tranquet O, *et al.* 2006. Specific adduction of plant lipid transfer protein by an allene oxide generated by 9-lipoxygenase and allene oxide synthase. *Journal of Biological Chemistry* **281**: 38981–38988.

Baker A, Graham IA, Holdsworth M, Smith SM, Theodoulou FL. 2006. Chewing the fat: β-oxidation in signalling and development. *Trends in Plant Science* **11**: 124–132.

Bargmann BO, Munnik T. 2006. The role of phospholipase D in plant stress responses. *Current Opinion in Plant Biology* **9**: 515–522.

Birkett MA, Campbell CAM, Chamberlain K, Guerrieri E, Hick AJ, Martin JL, *et al*. 2000. New roles for *cis*-jasmone as an insect semiochemical and in plant defense. *Proceedings of the National Academy of Sciences of the USA.* **97**: 9329–9334.

Blechert S, Brodschelm W, Hölder S, Kammerer L, Kutchan TM, Mueller MJ, Xia Z-Q, Zenk MH. 1995. The octadecanoic pathway: signal molecules for the regulation of secondary pathways. *Proceedings of the National Academy of Sciences of the USA* **92**: 4099–4105.

Blechert S, Bockelmann C, Füßlein M, von Schrader T, Stelmach BA, Niesel U, Weiler EW. 1999. Structure activity analyses reveal the existence of two separate groups of active octadecanoids in elicitation of the tendril-coiling response of *Bryonia dioica* Jacq. *Planta* **207**: 470–479.

Blée E. 2002. Impact of phyto-oxylipins in plant defense. *Trends in Plant Sciences* **7**: 315–321.

Breithaupt C, Strasser J, Breitinger U, Huber R, Macheroux P, Schaller A. 2001. X-Ray structure of 12-oxophytodienoate reductase 1 provides structural insight into substrate binding and specificity within the family of OYE. *Structure* **9**: 419–429.

Breithaupt C, Kurbauer R, Lilie H, Schaller A, Strassner J, Huber R, Macheroux P, Clausen T. 2006. Crystal structure of 12-oxophytodienoate reductase 3 from tomato: self-inhibition by dimerization. *Proceedings of the National Academy of Sciences of the USA* **103**: 14337–14342.

Browse J. 2005. Jasmonate: an oxylipin signal with many roles in plants. *Vitamines and Hormones* **72**: 431–456.

Bruce TJ, Martin JL, Pickett JA, Pye BJ, Smart LE, Wadhams LJ. 2003. Cis-Jasmone treatment induces resistance in wheat plants against the grain aphi, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). *Pest Managment Sciences* **59**: 1031–1036.

Buchanan-Wollaston V, Page T, Harrison E, Breeze E, Lim PO, Nam HG, *et al*. 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. *The Plant Journal* **42**: 567–585.

Bücking H, Förster H, Stenzel I, Miersch O, Hause B. 2004. Applied jasmonates accumulate extracellularly in tomato, but intracellularly in barley. *FEBS Letters* **562**: 45–50.

Buhot N, Gomès E, Milat M-L, Ponchet M, Marion D, Lequeu J, *et al*. 2004. Modulation of the biological activity of a tobacco LTP1 by lipid complexation. *Molecular Biology of the Cell* **15**: 5047–5052.

Buseman CM, Tamura P, Sparks AA, Baughman EJ, Maatta S, Zhao J, *et al*. 2006. Wounding stimulates the accumulation of glycerolipids containing oxophytodienoic acid and dinor-oxophytodienoic acid in *Arabidopsis* leaves. *Plant Physiology* **142**: 28–39.

Charron J-BF, Quellet F, Pelletier M, Danyluk J, Chauve C, Sarhan F. 2005. Identification, expression, and evolutionary analyses of plant lipocalins. *Plant Physiology* **139**: 2017–2028.

Chen H, McCaig BC, Melotto M, He SY, Howe GA. 2004. Regulation of plant arginase by wounding, jasmonate, and the phytotoxin coronatine. *Journal of Biological Chemistry* **279**: 45998–46007.

Chen H, Wilkerson CG, Kuchar JA, Phinney BS, Howe GA. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proceedings of the National Academy of Sciences of the USA* **102**: 19237–19242.

Corbineau F, Rudnicki RM, Come D. 1988. The effects of methyl jasmonate on sunflower (*Helianthus annuus* L.) seed germination and seedling development. *Plant Growth Regulation* **7**: 157–169.

Cruz Castillo M, Martínez C, Buchala A, Métraux JP, León J. 2005. Gene-specific involvement of β-oxidation in wound-activated responses in *Arabidopsis*. *Plant Physiology* **135**: 85–94.

Dathe W, Rönsch H, Preiss A, Schade W, Sembdner G, Schreiber K. 1981. Endogenous plant hormones of the broad bean, *Vicia faba* L. (−)-Jasmonic acid, a plant growth inhibitor in pericarp. *Planta* **155**: 530–535.

Delker C. 2007. *Jasmonatbiosynthese in* Arabidopsis thaliana — *Charakterisierung der Allenoxidcyclase-Genfamilie und von Mutanten der Fettsäure-β-Oxidation*. Ph.D. thesis, Martin-Luther-Universität Halle-Wittenberg, Germany.

Delker C, Stenzel I, Hause B, Miersch O, Feussner I. Wasternack C. 2006. Jasmonate biosynthesis in *Arabidopsis thaliana* — enzymes, products, regulation. *Plant Biology* **8**: 297–306.

Demole E, Lederer E, Mercier D. 1962. Isolement et détermination de la structure du jasmonate de méthyle, constituant odorant caractéristique de lèssence de jasmin. *Helvetica Chimica Acta* **45**: 675–685.

De Vos M, Van Zaanen W, Koornneef A, Korzelius JP, Dicke M, Van Loon LC, Pieterse CMJ. 2006. Herbivore-induced resistance against microbial pathogens in Arabidopsis. *Plant Physiology* **142**: 352–363.

Devoto A, Ellis C, Magusin A, Chang H-S, Chilcott C, Zhu T, Turner JG. 2005. Expression profiling reveals *COI1* to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions. *Plant Molecular Biology* **58**: 497–513.

Dharmasiri N, Dharmasiri S, Estelle M. 2005. The F-box protein TIR1 is an auxin receptor. *Nature* **435**: 441–445.

Dörmann P. 2005. Membrane lipids. In: Murphy DJ, ed. *Plant lipids: biology, utilization and manipulation*. Oxford: Blackwell Publishing Ltd, 123–161.

Ellis C, Karafyllidis I, Wasternack C, Turner JG. 2002. The *Arabidopsis* mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. *The Plant Cell* **14**: 1557–1566.

Falkenstein E, Groth B, Mithöfer A, Weiler EW. 1991. Methyljasmonate and α-linolenic acid are potent inducer of tendril coiling. *Planta* **185**: 316–322.

Farmer EE, Ryan CA. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the USA* **87**: 7713–7716.

Farmer EE, Alméras E, Krishnamurthy V. 2003. Jasmonates and related oxylipins in plant responses to pathogenesis and herbivory. *Current Opinion in Plant Biology* **6**: 372–378.

Feussner I, Wasternack C. 2002. The lipoxygenase pathway. *Annual Review of Plant Biology* **53**: 275–297.

Feys BJF, Benedetti CE, Penfold CN, Turner JG. 1994. *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate and resistant to a bacterial pathogen. *The Plant Cell* **6**: 751–759.

Flescher E. 2005. Jasmonates — a new family of anti-cancer agents. *Anticancer Drugs* **16**: 911–916.

Froehlich JE, Itoh A, Howe GA. 2001. Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450 involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. *Plant Physiology* **125**: 306–317.

Gidda KS, Miersch O, Schmidt J, Wasternack C, Varin L. 2003. Biochemical and molecular characterization of a hydroxy-jasmonate sulfotransferase from *Arabidopsis thaliana*. *Journal of Biological Chemistry* **278**: 17895–17900.

Grun C, Berger S, Matthes D, Mueller M. 2007. Early accumulation of non-enzymatically synthesized oxylipins in *Arabidopsis thaliana* after infection with *Pseudomonas syringae*. *Functional Plant Biology* **34**: 65–71.

Grzyb J, Latowski D, Strzałka K. 2006. Lipocalins — a family portrait. *Journal of Plant Physiology* **163**: 895–915.

Gundlach H, Müller MJ, Kutchan TM, Zenk MH. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proceedings of the National Academy of Sciences of the USA* **89**: 2389–2393.

Guo Y, Gan S. 2006. AtNAP, a NAC family transcription factor, has an important role in leaf senescence. *The Plant Journal* **46**: 601–612.

Halitschke R, Baldwin IT. 2004. Jasmonates and related compounds in plant–insect interactions. *Journal of Plant Growth Regulation* **23**: 238–245.

Hause B, Stenzel I, Miersch O, Maucher H, Kramell R, Ziegler J, Wasternack C. 2000. Tissue-specific oxylipin signature of tomato flowers — allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. *The Plant Journal* **24**: 113–126.

Hause B, Maier W, Miersch O, Kramell R, Strack D. 2002. Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. *Plant Physiology* **130**: 1213–1220.

Hause B, Hause G, Kutter C, Miersch O, Wasternack C. 2003. Enzymes of jasmonate biosynthesis occur in tomato sieve elements. *Plant & Cell Physiology* **44**: 643–648.

Hause B, Mrosk C, Isayenkov S, Strack D. 2007. Jasmonates in arbuscular mycorrhizal interactions. *Phytochemistry* **68**: 101–110.

He Y, Fukushige H, Hildebrand DF, Gan S. 2002. Evidence supporting a role of jasmonic acid in *Arabidopsis* leaf senescence. *Plant Physiology* **128**: 876–884.

Hisamatsu Y, Goto N, Hasegawa K, Shigemori H. 2003. Arabidopsides A and B, two new oxylipins from *Arabidopsis thaliana*. *Tetrahedron Letters* **44**: 5553–5556.

Hisamatsu Y, Goto N, Sekiguchi M, Hasegawa K, Shigemori H. 2005. Oxylipins arabidopsides C and D from *Arabidospis thaliana*. *Journal of Natural Products* **68**: 600–603.

Hisamatsu Y, Goto N, Hasegawa K, Shigemori H. 2006. Senescence-promoting effect of arabidopside A. *Zeitschrift für Naturforschung* **61**: 363–366.

Hofmann E, Zerbe P, Schaller F. 2006. The crystal structure of *Arabidospis thaliana* allene oxide cyclase: insights into the oxylipin cyclization reaction. *The Plant Cell* **18**: 3201–3217.

Hornung E, Walter M, Kühn H, Feussner I. 1999. Conversion of cucumber linoleate 13 − lipoxygenase to a 9-lipoxygenating species by site-directed mutagenesis. *Proceedings of the National Academy of Sciences of the USA* **96**: 4192–4197.

Howe GA. 2004. Jasmonates as signals in the wound response. *Journal of Plant Growth Regulation* **23**: 223–237.

Howe GA, Schilmiller AL. 2002. Oxylipin metabolism in response to stress. *Current Opinion in Plant Biology* **5**: 230–236.

Howe GA, Lee GI, Itoh A, Li L, DeRocher AE. 2000. Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. *Plant Physiology* **123**: 711–724.

Huffaker A, Pearce G, Ryan CA. 2006. An endogenous peptide signal in *Arabidopsis* activates components of the innate immune response. *Proceedings of the National Academy of Sciences of the USA* **103**: 10098–10103.

Hughes KH, Belfield EJ, Ashton R, Fairhurst SA, Göbel C, Stumpe M, Feussner I, Casey R. 2006. Allene oxide synthase from *Arabidopsis thaliana* (CYP74A1) exhibits dual specificity that is regulated by monomer-micelle association. *FEBS Letters* **580**: 4188–4194.

Ishi Y, Kiyota H, Sakai S, Honma Y. 2004. Induction of differentiation of human myeloid leukemia cells by jasmonates, plant hormones. *Leukemia* **18**: 1413–1419.

Ishiguro S, Kwai-Oda A, Ueda J, Nishida I, Okada K. 2001. The *DEFECTIVE IN ANTHER DEHISCENCE1* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation. *The Plant Cell* **13**: 2191–2209.

Kang JH, Wang L, Giri A, Baldwin IT. 2006. Silencing threonine deaminase and *JAR4* in *Nicotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta*. *The Plant Cell* **18**: 3303–3320.

Kepinski S, Leyser O. 2005. The *Arabidopsis* F-box protein TIR1 is an auxin receptor. *Nature* **26**: 446–451.

Kim JH, Lee SY, Oh SY, Han SI, Park HG, Yoo MA, Kang HS. 2004. Methyl jasmonate induces apoptosis through induction of Bax/Bcl-XS and activation of caspase-3 via ROS production in A549 cells. *Oncology Reports* **12**: 1233–1238.

Koch T, Bandemer K, Boland W. 1997. Biosynthesis of *cis*-jasmone: a pathway for the inactivation and the disposal of the plant stress hormone jasmonic acid to the gas phase? *Helvetica Chimica Acta* **80**: 838–850.

Koda Y. 1992. The role of jasmonic acid and related compounds in the regulation of plant development. *International Reviews in Cytology* **135**: 155–199.

Koljak R, Boutaud O, Shieh B-H, Samel N, Brash AR. 1997. Identification of a naturally occurring peroxidase-lipoxygenase fusion protein. *Science* **277**: 1994–1996.

Kong Z, Li M, Yang W, Xu W, Xue Y. 2006. A novel nuclear-localized CCCH-type zinc finger protein, OsDOS, is involved in delaying leaf senescence in rice. *Plant Physiology* **141**: 1376–1388.

Koo AJK, Chung HS, Kobayashi Y, Howe GA. 2006. Identification of a peroxisomal acyl-activating enzyme involved in the biosynthesis of jasmonic acid in *Arabidopsis*. *Journal of Biological Chemistry* **281**: 33511–33520.

Kramell R, Atzorn R, Schneider G, Miersch O, Brückner C, Schmidt J, Sembdner G, Parthier B. 1995. Occurrence and identification of jasmonic acid and its amino acid conjugates induced by osmotic stress in barley leaf tissue. *Journal of Plant Growth Regulation* **14**: 29–36.

Kramell R, Miersch O, Hause B, Ortel B, Parthier B, Wasternack C. 1997. Amino acid conjugates of jasmonic acid induce jasmonate-responsive gene expression in barley (*Hordeum vulgare* L.). *FEBS Letters* **414**: 197–202.

Kramell R, Miersch O, Atzorn R, Parthier B, Wasternack C. 2000. Octadecanoid-derived alteration of gene expression and the 'oxylipin signature' in stressed barley leaves – implications for different signalling pathways. *Plant Physiology* **123**: 177–186.

Kubigsteltig I, Laudert D, Weiler EW. 1999. Structure and regulation of the *Arabidopsis thaliana* allene oxide synthase gene. *Planta* **208**: 463–471.

Laudert D, Schaller F, Weiler EW. 2000. Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. *Planta* **211**: 163–165.

Li C, Schilmiller AL, Liu GL, Lee GI, Jayanty S, Sageman C, et al. 2005. Role of β-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. *The Plant Cell* **17**: 987–999.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell* **16**: 126–143.

Liavonchanka A, Feussner I. 2006. Lipoxygenases: occurrence, functions and catalysis. *Journal of Plant Physiology* **163**: 348–357.

Lin J-F, Wu S-H. 2004. Molecular events in senescing *Arabidopsis* leaves. *The Plant Journal* **39**: 612–628.

Liu J, Xia K-F, Zhu J-C, Deng Y-G, Huang X-L, Hu B-L, Xu X, Xu Z-F. 2006. The nightshade proteinase inhibitor IIb gene is constitutively expressed in glandular trichomes. *Plant & Cell Physiology* **47**: 1274–1284.

Lorenzo O, Solano R. 2005. Molecular players regulating the jasmonate signalling network. *Current Opinion in Plant Biology* **8**: 532–540.

Lorenzo O, Chico JM, Sanchez-Serrano JJ, Solano R. 2004. *JASMONATE-INSENSITIVE1* encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. *The Plant Cell* **16**: 1938–1950.

Mandaokar A, Thines B, Shin B, Lange BM, Choi G, Koo YJ, Yoo YJ, et al. 2006. Transcriptional regulators of stamen development in *Arabidopsis* identified by transcriptional profiling. *The Plant Journal* **46**: 984–1008.

Maucher H, Hause B, Feussner I, Ziegler J, Wasternack C. 2000. Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome) – tissue-specific regulation in seedling development. *The Plant Journal* **21**: 199–213.

McConn M, Browse J. 1996. The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. *The Plant Cell* **8**: 403–416.

Memelink J, Verpoorte R, Kijne JW. 2001. ORCAnization of jasmonate-responsive gene expression in alkaloid metabolism. *Trends in Plant Sciences* **6**: 212–9.

Meyer A, Miersch O, Büttner C, Dathe W, Sembdner G. 1984. Occurrence of the plant growth regulator jasmonic acid in plants. *Journal of Plant Growth Regulation* **3**: 1–8.

Miersch O, Wasternack C. 2000. Octadecanoid and jasmonate signaling in tomato (*Lycopersicon esculentum* Mill.) leaves: endogenous jasmonates do not induce jasmonate biosynthesis. *Biological Chemistry* **381**: 715–722.

Miersch O, Sembdner G, Schreiber K. 1989. Occurrence of jasmonic acid analogues in *Vicia faba*. *Phytochemistry* **28**: 339–340.

Miersch O, Bohlmann H, Wasternack C. 1999a. Jasmonates and related compounds form *Fusarium oxysporum*. *Phytochemistry* **50**: 517–523.

Miersch O, Kramell R, Parthier B, Wasternack C. 1999b. Structure-activity relations of substituted, deleted or stereospecifically altered jasmonic acid in gene expression of barley leaves. *Phytochemistry* **50**: 353–361.

Miersch O, Porzel A, Wasternack C. 1999c. Microbial conversion of jasmonates – hydroxylations by *Aspergillus niger*. *Phytochemistry* **50**: 1147–1152.

Miersch O, Weichert H, Stenzel I, Hause B, Maucher H, Feussner I, Wasternack C. 2004. Constitutive overexpression of allene oxide cyclase in tomato (*Lycopersicon esculentum* cv. Lukullus) elevates levels of jasmonates and octadecanoids in flower organs but not in leaves. *Phytochemistry* **65**: 847–856.

Mithöfer A, Maitrejean M, Boland W. 2004. Structural and biological diversity of cyclic octadecanoids, jasmonates, and mimetics. *Journal of Plant Growth Regulation* **23**: 170–178.

Mithöfer A, Wanner G, Boland W. 2005. Effects of feeding *Spodoptera littoralis* on lima bean leaves. II. Continuous mechanical wounding resembling insect feeding is sufficient to elicit herbivory-related volatile emission. *Plant Physiology* **137**: 1160–1168.

Mueller MJ. 2004. Archetype signals in plants: the phytoprostanes. *Current Opinion in Plant Biology* **7**: 441–448.

Nagpal P, Ellis CM, Weber H, Ploenase SE, Barkawi LS, Guilfoyle TJ, et al. 2005. Auxin response factors ARF6 and ARF8 promote jasmonic acid production and flower maturation. *Development* **132**: 4107–4118.

Nakamura Y, Matsubara A, Miyatake R, Okada M, Ueda M. 2006. Bioactive substances to control nyctinasty of *Albizzia* plants and its biochemistry. *Regulation of Plant Growth & Development* **41**(Supplement): 44.

Narváez-Vásquez J, Ryan CA. 2004. The cellular localization of prosystemin: a functional role for phloem parenchyma in systemic wound signaling. *Planta* **218**: 360–369.

Narváez-Vásquez J, Florin-Christensen J, Ryan CA. 1999. Positional specificity of a phospholipase A2 activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *The Plant Cell* **11**: 2249–2260.

Oh K, Asami T, Matsui K, Howe GA, Murofushi N. 2006. Characterization of novel imidazole derivative, JM-8686, a potent inhibitor of allene oxide synthase. *FEBS Letters* **580**: 5791–5796.

Oldham ML, Brash AR, Newcomer ME. 2005. The structure of coral allene oxide synthase reveals a catalase adapted for metabolism of a fatty acid hydroperoxide. *Proceedings of the National Academy of Sciences of the USA* **102**: 297–302.

Pan Z, Durst F, Werck-Reichhart D, Gardner HW, Camara B, Cornish K, Backhaus RA. 1995. The major protein of guayule rubber particles is a cytochrome P450. *Journal of Biological Chemistry* **70**: 8487–8494.

Parthier B. 1990. Hormonal regulators or stress factors in leaf senescence? *Journal of Plant Growth Regulation* **9**: 1–7.

Pauw B, Memelink J. 2004. Jasmonate-responsive gene expression. *Journal of Plant Growth Regulation* **23**: 200–210.

Pearce G, Ryan CA. 2003. Systemic signaling in tomato plants for defense against herbivores. Isolation and characterization of three novel defense signaling glycopeptide hormones coded in a single precursor gene. *Journal of Biological Chemistry* **278**: 30044–30050.

Pearce G, Moura DS, Stratmann J, Ryan CA. 2001. Production of multiple plant hormones from a single polyprotein precursor. *Nature* **411**: 817–820.

Pieterse CMJ, Schaller A, Mauch-Mani B, Conrath U. 2006. Signaling in plant resistance responses: divergence and cross-talk of defense pathways. In: Tuzun S, Bent E, eds. *Multigenic and induced systemic resistance in plants*. New York: Springer, 166–196.

Powers RA, Rife CL, Schilmiller AL, Howe GA, Garavito RM. 2006. Structure determination and analysis of acyl-CoA oxidase (ACX1) from tomato. *Acta Crystallographica, SectionD: Biological Crystallography.* **62**: 683–6.

Pozo MJ, Van Loon LC, Pieterse CMJ. 2004. Jasmonates – signals in plant microbe interactions. *Journal of Plant Growth Regulation* **23**: 211–222.

Pre MR. 2006. *Functional analysis of jasmonate-responsive AP2/ ERF-domain transcription factors in* Arabidopsis thaliana. Ph.D. thesis, University of Leiden, Germany.

Prost I, Dhondt S, Rothe G, Vicente J, Rodriguez MJ, Kift N, et al. 2005. Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiology* **139**: 1902–1913.

Reymond P, Weber H, Diamond M, Farmer EE. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *The Plant Cell* **12**: 707–719.

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M, Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *The Plant Cell* **16**: 3132–3147.

Rosahl S, Feussner I. 2005. Oxylipins. In: Murphy DJ, ed. *Plant lipids: biology, utilization and manipulation*. Oxford, UK and Boca Raton, FL: Blackwell Publishing Ltd/CRC Press, 329–354.

Rotem R, Heyfets A, Fingrut O, Blickstein D, Shaklai M, Flescher E. 2005. Jasmonates: novel anticancer agents acting directly and selectively on human cancer cell mitochondria. *Cancer Research* **65**: 1984–1993.

Rusterucci C, Montillet JL, Agnel JP, Battesti C, Alonso B, Knoll A, et al. 1999. Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogein on tobacco leaves. *Journal of Biological Chemistry* **274**: 36446–36455.

Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB. 2000. The *Arabidopsis DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell* **12**: 1041–1061.

Schaller F, Hennig P, Weiler EW. 1998. 12-Oxophytodienoate-19,11-reductase: occurrence of two isoenzymes of different specificity against stereoisomers of 12-oxophytodienoic acid. *Plant Physiology* **188**: 1345–1351.

Schaller F, Biesgen C, Müssig C, Altmann T, Weiler EW. 2000. 12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. *Planta* **210**: 979–984.

Schilmiller AL, Howe GA. 2005. Systemic signaling in the wound response. *Current Opinion in Plant Biology* **8**: 369–377.

Schilmiller AL, Koo AJK, Howe GA. 2007. Functional diversification of acyl-coenzyme A oxidases in jasmonic acid biosynthesis and action. *Plant Physiology* **143**: 812–824.

Schmidt S, Baldwin IT. 2006. Systemin in *Solanum nigrum*. The tomato-homologous polypeptide does not mediate direct defense responses. *Plant Physiology* **142**: 1751–1758.

Schneider K, Kienow L, Schmelzer E, Colby T, Bartsch M, Miersch O, et al. 2005. A new type of peroxisomal acyl-coenzyme A synthetase from *Arabidopsis thaliana* has the catalytic capacity of activate biosynthetic precursors of jasmonic acid. *Journal of Biological Chemistry* **280**: 13962–13972.

Schüler G, Mithöfer A, Baldwin IT, Berger S, Ebel S, Santos JG, et al. 2004. Coronalon: a powerful tool in plant stress physiology. *FEBS Letters* **563**: 17–22.

Sembdner G, Parthier B. 1993. The biochemistry and the physiological and molecular actions of jasmonates. *Annual Review of Plant Physiology and Plant Molecular Biology* **44**: 569–589.

Seo HS, Song JT, Cheong J-J, Lee Y-H, Lee Y-W, Hwang I, Lee JS, Choi YD. 2001. Jasmonic acid carboxyl methyl transferase: a key enzyme for jasmonate-regulated plant responses. *Proceedings of the National Academy of Sciences of the USA* **98**: 4788–4793.

Shiojiri K, Kishimoto K, Ozawa R, Kugimiya S, Urashimo S, Arimura G, et al. 2006. Changing green leaf volatile biosynthesis in plants: an approach for improving plant resistance against both herbivores and pathogens. *Proceedings of the National Academy of Sciences of the USA* **103**: 16672–16676.

Song WC, Funk CD, Brash KAR. 1993. Molecular cloning of an allene oxide synthase. A cytochrome P-450 specialized for metabolism of fatty acid hydroperoxides. *Proceedings of the National Academy of Sciences of the USA* **90**: 8519–8523.

Staswick PE, Tiryaki I. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *The Plant Cell* **16**: 2117–2127.

Staswick PE, Su W, Howell SH. 1992. Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proceedings of the National Academy of Sciences of the USA* **89**: 6837–6840.

Staswick PE, Tiryaki I, Rowe M. 2002. Jasmonate response locus *JAR1* and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and

indole-3-acetic acids in an assay for adenylation. *The Plant Cell* **14**: 1405–1415.

Staswick PE, Serban B, Rowe M, Tiryaki I, Maldonado MT, Maldonado MC, Suza W. 2005. Characterization of an *Arabidopsis* enzyme family that conjugates amino acids to indole-3-acetic acid. *The Plant Cell* **17**: 616–627.

Stelmach BA, Müller A, Hennig P, Laudert D, Andert L, Weiler EW. 1998. Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signalling compound in plant mechanotransduction. *Phytochemistry* **47**: 539–546.

Stelmach BA, Müller A, Hennig P, Gebhardt S, Schubert-Zsilavecz M, Weiler EW. 2001. A novel class of oxylipins, sn1-O-(12-Oxophytodienoyl)-sn2-O-(hexadecatrienoyl)-monogalactosyl diglycer ide, from *Arabidopsis thaliana*. *Journal of Biological Chemistry* **276**: 12832–12838.

Stenzel I, Hause B, Maucher H, Pitzschke A, Miersch O, Ziegler J, Ryan C, Wasternack C. 2003a. Allene oxide cyclase dependence of the wound response and vascular bundle specific generation of jasmonates in tomato – amplification in wound-signalling. *The Plant Journal* **33**: 577–589.

Stenzel I, Hause B, Miersch O, Kurz T, Maucher H, Weichert H, Ziegler J, Feussner I, Wasternack C. 2003b. Jasmonate biosynthesis and the allene oxide cyclase family of *Arabidopsis thaliana*. *Plant Molecular Biology* **51**: 895–911.

Stintzi A, Browse J. 2000. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences of the USA* **97**: 10625–10630.

Stintzi A, Weber H, Reymond P, Browse J, Farmer EE. 2001. Plant defense in the absence of jasmonic acid: the role of cyclopentenones. *Proceedings of the National Academy of Sciences of the USA* **98**: 12837–12842.

Strassner J, Schaller F, Frick UB, Howe GA, Weiler EW, Amrhein N, Macheroux P, Schaller A. 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. *The Plant Journal* **32**: 585–601.

Stumpe M, Feussner I. 2006. Formation of oxylipins by CYP74 enzymes. *Phytochemical Reviews* **5**: 347–357.

Stumpe M, Göbel C, Demchenko K, Pawlowski K, Feussner I. 2006. Identification of an allene oxide synthase (CYP74C) that leads to formation of α-ketols from 9-hydroperoxides of linoleic and linolenic acid in below ground organs of potato. *The Plant Journal* **47**: 883–896.

Swiatek A, Van Dongen W, Esmans EL, Van Onckelen H. 2004. Metabolic fate of jasmonates in tobacco bright yellow-2 cells. *Plant Physiology* **135**: 161–172.

Taki N, Sasaki-Sekimoto Y, Obayashi T, Kikuta A, Kobayashi K, Ainai T, et al. 2005. 12-Oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiology* **139**: 1268–1283.

Tani T, Chujo T, Jikumaru Y, Okada K, Nojiri H, Yamane H. 2006. Biological functions of 12-oxophytodienoic acid reductase homologs in *Arabodopsis thaliana*. *Regulation of Plant Growth & Development* **41**(Supplement): 47.

Theodoulou FL, Job K, Slocombe SP, Footitt S, Holdsworth M, Baker A, Larson TR, Graham IA. 2005. Jasmonic acid levels are reduced in COMATOSE ATP-binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. *Plant Physiology* **137**: 835–840.

Turner JG, Ellis C, Devoto A. 2002. The jasmonate signal pathway. *The Plant Cell* **Supplement**: S153–S164.

Ueda J, Kato J. 1980. Isolation and identification of a senescence-promoting substance from wormwood (*Artemisia absinthium* L.). *Plant Physiology* **66**: 246–249.

Uppalapati SR, Ayoubi P, Weng H, Palmer DA, Mitchell RE, Jones W, Bender CL. 2005. The phytotoxin coronatine and methyl jasmonate impact multiple phytohormone pathways in tomato. *The Plant Journal* **42**: 201–217.

Van der Fits L, Memelink J. 2001. The jasmonate-inducible AP2/ERF-domain transcription factor ORCA3 activates gene expression *via* interaction with a jasmonate-responsive promoter element. *The Plant Journal* **25**: 43–53.

Van der Graaff E, Schwacke R, Schneider A, Deesimone M, Flügge U-I, Kunze R. 2006. Transcription analysis of arabidopsis membrane transporters and hormone pathways during developmental and induced leaf senescence. *Plant Physiology* **141**: 776–92.

Vick BA, Zimmerman DC. 1984. Biosynthesis of jasmonic acid by several plant species. *Plant Physiology* **75**: 458–461.

Vick BA, Zimmerman DC. 1987. Pathways of fatty acid hydroperoxide metabolism in spinach leaf chloroplasts. *Plant Physiology* **85**: 1073–1078.

Walling LL. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation* **19**: 195–216.

Wasternack C. 2004. Jasmonates – biosynthesis and role in stress responses and developmental processes. In: Nooden LD, ed. *Plant cell death processes*. New York: Elsevier/Academic Press, 143–155.

Wasternack C. 2006. Oxilipins: biosynthesis, signal transduction and action. In: Hedden P, Thomas S. eds. *Plant hormone signaling. Annual Plant Reviews*. Oxford: Blackwell Publishing Ltd, 185–228.

Wasternack C, Hause B. 2002. Jasmonates and octadecanoids: signals in plant stress responses and plant development. *Progress in Nucleic Acid Research and Molecular Biology* **72**: 165–221.

Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, et al. 2006. The wound response in tomato – role of jasmonic acid. *Journal of Plant Physiology* **163**: 297–306.

Weidhase RA, Lehmann J, Kramell H, Sembdner G, Parthier B. 1987a. Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methyl ester, and counteraction by cytokinin. *Physiologia Plantarum* **69**: 161–166.

Weidhase RA, Kramell R, Lehmann J, Liebisch HW, Lerbs W, Parthier B. 1987b. Methyl jasmonate-induced changes in the poly-peptide pattern of senescing barley leaf segments. *Plant Sciences* **51**: 177–186.

Weiler E. 1997. Octadecanoid-mediated signal transduction in higher plants. *Naturwissenschaften* **84**: 340–349.

Woo HR, Chung KM, Park JH, Oh SA, Ahn T, Hong SH, Jang SK, Nam HG. 2001. ORE9, an F-box protein that regulates leaf senescence in *Arabidopsis*. *The Plant Cell* **13**: 1779–1790.

Xie DX, Feys BF James S, Nieto-Rostro M, Turner JG. 1998. *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* **280**: 1091–1094.

Yamaguchi Y, Pearce G, Ryan CA. 2006. The cell surface leucine-rich repeat receptor for A*t*Pep1, an endogenous peptide elicitor in *Arabidospis*, is functional in transgenic tobacco cells. *Proceedings of the National Academy of Sciences of the USA* **103**: 10104–10109.

Yoshihara T, Omer E-LA, Koshino H, Sakamura S, Kikuta Y, Koda Y. 1989. Structure of a tuber-inducing stimulus from potato leaves (*Solanum tuberosum* L.). *Agriculture & Biological Chemistry* **53**: 2835–2837.

Zerbe P, Weiler EW, Schaller F. 2007. Preparative enzymatic solid phase synthesis of *cis*(+)-12-oxo-phytodienoic acid – physical inter-action of AOS and AOC is not necessary. *Phytochemistry* **68**: 229–236.

Ziegler J, Stenzel I, Hause B, Maucher H, Miersch O, Hamberg M, et al. 2000. Molecular cloning of allene oxide cyclase: the enzyme establishing the stereochemistry of octadecanoids and jasmonates. *Journal of Biological Chemistry* **275**: 19132–19138.

APPENDIX

Abbreviations used in the text

ACX	Acyl-CoA oxidase
α-LeA	α-linolenic acid
AOC	Allene oxide cyclase
AOS	Allene oxide synthase
ERF1	Ethylene response factor 1
HPL	Hydroperoxide lyase
HPOD	Hydroperoxyoctadecadienoic acid
HPOT	Hydroperoxyoctadecatrienoic acid
IP$_3$	Inositol triphosphate
JA	Jasmonic acid
JAME	Jasmonic acid methyl ester
JAR1	JA conjugate synthase
JIP	Jasmonate-induced protein
LOX	Lipoxygenase
LTP	Lipid transfer protein
MFP	Multifunctional protein
OPDA	*cis*(+)-12-oxophytodienoic acid
OPR	OPDA reductase
OYE	Warburg's old yellow enzyme
PA	Phosphatidic acid
PIN	Proteinase inhibitor
PUFA	Polyunsaturated fatty acid
SAG	Senescence-associated gene
TD	Threonine deaminase