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Review

Enzymes in jasmonate biosynthesis – Structure, function, regulation

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ABSTRACT

Jasmonates are a growing class of lipid-derived signaling molecules with diverse functions ranging from the initiation of biotic and abiotic stress responses to the regulation of plant growth and development. Jasmonate biosynthesis originates from polyunsaturated fatty acids in chloroplast membranes. In a first lipoxygenase-catalyzed reaction molecular oxygen is introduced to yield their 13-hydroperoxy derivatives. These fatty acid hydroperoxides are converted by allene oxide synthase and allene oxide cyclase to 12-oxophytodieneoic acid (OPDA) and dinor-OPDA, i.e. the first cyclic intermediates of the pathway. In the subsequent step, the characteristic cyclopentanone ring structure of jasmonates is established by OPDA reductase. Until recently, jasmonic acid has been viewed as the end product of the pathway and as the bioactive hormone. It becomes increasingly clear, however, that biological activity extends to and may even differ between the various jasmonic acid metabolites and conjugates as well as its biosynthetic precursors. It has also become clear that oxygenated fatty acids give rise to a vast variety of bioactive compounds including but not limited to jasmonates. Recent insights into the structure, function, and regulation of the enzymes involved in jasmonate biosynthesis help to explain how this variety is generated while specificity is maintained.

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1. Introduction to jasmonate biosynthesis

Polyunsaturated fatty acids (PUFAs) including linoleic acid (18:2), linolenic acid (18:3) and hexadecatrienoic acid (16:3) are abundant in chloroplast membranes and are readily oxidized to yield the corresponding fatty acid hydroperoxides. Under conditions of oxidative stress, fatty acid hydroperoxides are formed by free-radical-catalyzed oxidation of PUFAs and may be further oxidized non-enzymatically to generate phytoprostanes, which are considered to be archetypal mediators of oxidative stress responses (Mueller, 2004). Alternatively, fatty acid hydroperoxides are synthesized enzymatically involving lipoxygenase (LOX) or α -dioxygenase (DOX) activities. While numerous positional

isomers are generated as racemic mixtures during chemical lipid peroxidation, the LOX-catalyzed regio- and stereo-specific dioxygenation of PUFAs at C9 or C13 results in the specific formation of 9(S) and 13(S) hydroperoxy fatty acids, respectively. These hydroperoxides are substrates for at least six different families of enzymes, resulting in the formation of different classes of oxylipins including jasmonates (JAs) (Blee, 2002; Feussner and Wasternack, 2002; Mosblech et al., 2009; Wasternack, 2007).

The committed step of JA biosynthesis (Fig. 1) is catalyzed by allene oxide synthase (AOS), an unusual cytochrome P450 which does not bind molecular oxygen but uses already oxygenated fatty acid hydroperoxide substrates as oxygen donor and as source for reducing equivalents (Howe and Schillmiller, 2002; Werck-Reichhart et al., 2002). The dehydration of 13(S)-hydroperoxy-octadecatrienoic acid (13-HPOT) by AOS results in the formation of an unstable allylic epoxide (allene oxide), 12,13(S)-epoxy-octadecatrienoic acid

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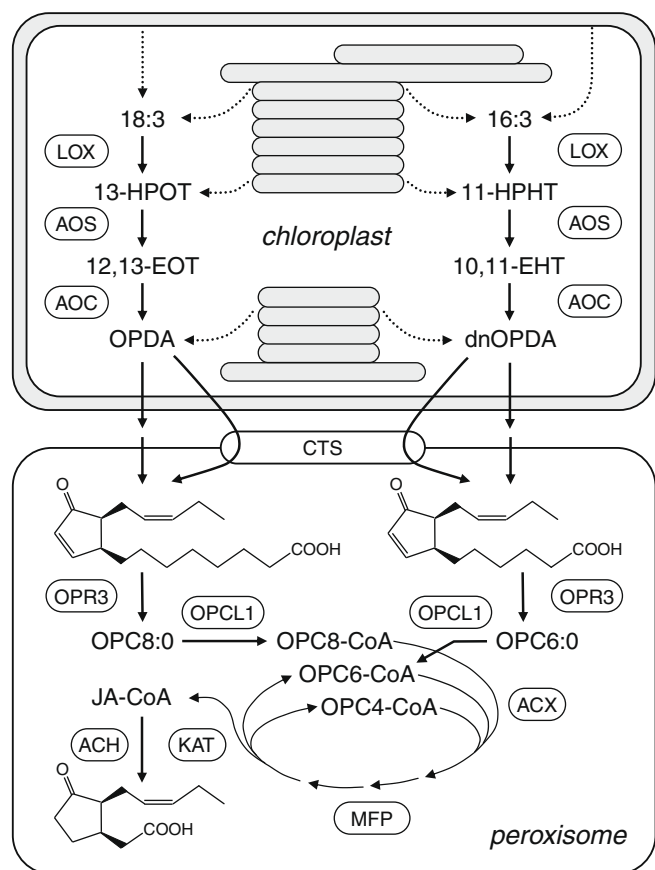


Fig. 1. Jasmonate biosynthesis. Polyunsaturated fatty acids (18:3 and 16:3) are converted to OPDA and dnOPDA by the consecutive action of plastid-localized lipoxygenase (LOX), allene oxide synthase (AOS), and allene oxide cyclase (AOC). Within peroxisomes, jasmonic acid is formed by oxophytodieneoate reductase 3 (OPR3) followed by three cycles of β -oxidation (see text for further details and abbreviations). Broken arrows represent the lipase-mediated release of pathway substrates and intermediates from chloroplast membranes, which is still partly hypothetical (modified from Schaller and Stintzi (2008)).

(12,13-EOT). In aqueous media, 12,13-EOT is hydrolyzed spontaneously into α - and γ -ketols, or undergoes cyclization to form 12-oxo-phytyldienoic acid (OPDA) (Brash et al., 1988). As opposed to spontaneous cyclization which yields a racemate of OPDA enantiomers, optically pure (9*S*,13*S*)-OPDA is formed as the predominant product in the presence of allene oxide cyclase (AOC) (Hamberg and Fahlstadius, 1990). In a parallel pathway, dinor-OPDA (dnOPDA) is generated by AOS and AOC from 11(*S*)-hydroperoxy-hexadecatrienoic acid (11-HPHT; Fig. 1) (Weber et al., 1997). The short half-life of allene oxides in water (20 s at 0 °C and pH 7.4) (Brash et al., 1988) and the optical purity of endogenous OPDA (Laudert et al., 1997) suggest tight coupling of the AOS and AOC reactions *in vivo*. While coupling is in fact required to establish the absolute configuration of the substituted cyclopentanone ring of JAs, physical contact of AOS and AOC in an enzyme complex does not seem to be required for stereochemical control of the cyclization reaction (Zerbe et al., 2007).

The formation of (9*S*,13*S*)-OPDA and (7*S*, 11*S*)-dnOPDA as the first cyclic intermediates concludes the plastid-localized part of the JA biosynthetic pathway. The remaining steps of JA biosynthesis are located in peroxisomes raising the question as to how OPDA (and/or dnOPDA) is released from chloroplasts and taken up by peroxisomes. While specific transporters for OPDA have not been identified, there is evidence that the peroxisomal ATP-binding cassette (ABC) transporter COMATOSE (CTS) (Footitt et al., 2002), also known as PXA1 (Zolman et al., 2001) or PED3 (Hayashi et al.,

2002)) mediates import of OPDA, and thus contributes to the biosynthesis of JAs (Theodoulou et al., 2005). CTS catalyzes the ATP-dependent uptake of multiple β -oxidation substrates into peroxisomes. Reduced levels of JAs, impaired wound-induced JA accumulation, and reduced expression of the JA-dependent *VSP1* gene in the *cts* mutant suggest that (dn)OPDA or the corresponding CoA esters are among the CTS substrates (Theodoulou et al., 2005). However, as indicated by residual JA levels in the *cts* mutant, additional pathways for (dn)OPDA import must exist. CTS-independent uptake of (dn)OPDA into peroxisomes may in part be explained by anion trapping as a result of the pH difference between peroxisomes and the cytoplasm (Theodoulou et al., 2005).

A peroxisomal OPDA reductase (OPR) catalyzes the subsequent step in JA biosynthesis, i.e. the reduction of the cyclopentenone ring of (9*S*,13*S*)-OPDA and dnOPDA to 3-oxo-2-(2'(Z)-pentenyl)-cyclopentane-1-octanoic (OPC-8:0) and hexanoic (OPC-6:0) acids, respectively (Fig. 1). The JA precursors OPDA and dnOPDA (i.e. cyclopentenones) and JAs (i.e. cyclopentanones) differ in bioactivity (Blechert et al., 1999; Stintzi et al., 2001; Taki et al., 2005). The reduction of the cyclopentenone ring may therefore be particularly important, as it controls the relative levels of these two classes of signaling molecules. The reaction is catalyzed by OPR3, which is the only member of a small family of related enone reductases accepting the (9*S*,13*S*)-enantiomer of OPDA as a substrate (Schaller et al., 2000; Stintzi and Browse, 2000; Strassner et al., 2002).

The shortening of the hexanoic and octanoic acid side chains of OPC-6:0 and OPC-8:0 yields jasmonic acid and involves two or three rounds of β -oxidation, respectively. Prior to entry into the β -oxidation cycle, the carboxylic moiety needs to be activated as CoA ester. In *Arabidopsis thaliana*, there is a large family of 63 ATP-dependent acyl-activating enzymes potentially involved in this process (Shockey et al., 2003). Within this family, a subgroup of fatty acyl-CoA synthetases was shown to activate JA precursors *in vitro*, including OPDA, dnOPDA, OPC-8:0, and OPC-6:0 (Kienow et al., 2008; Koo et al., 2006; Schneider et al., 2005). However, a physiological role in JA biosynthesis was confirmed for only one of them, OPCL1 (OPC-8:CoA ligase 1, locus At1g20510) (Koo et al., 2006). Loss-of-function mutants for OPCL1 hyper-accumulate OPC-8:0, OPC-6:0, and OPC-4:0 suggesting a partial metabolic block in OPC-CoA ester formation (Kienow et al., 2008). The mutants are also compromised in wound-induced JA accumulation which is consistent with a role of OPCL1 in JA biosynthesis. However, about 50% of wild-type levels remain in the mutants indicating that OPCL1 is responsible for only part of the wound-induced JA production, and that additional acyl-CoA synthetases may be involved (Kienow et al., 2008; Koo et al., 2006). The available data are consistent with the view that OPCs are activated as CoA esters within peroxisomes and subsequently channeled into β -oxidation. The formation of OPDA-CoA and dnOPDA-CoA either in the cytosol or within peroxisomes prior to the reduction by OPR3 remains an alternative possibility.

Beta-oxidation itself involves three core enzymes, acyl-CoA oxidase (ACX), multifunctional protein (MFP; comprising enoyl-CoA hydratase and β -hydroxy-acyl-CoA dehydrogenase activities), and 3-ketoacyl-CoA thiolase (Fig. 1). Despite early findings implicating β -oxidation in JA biosynthesis (Vick and Zimmerman, 1984), direct evidence for the contribution of these enzymes is very recent. ACX1A was shown to catalyze the first step in the β -oxidation of OPC-8:0-CoA, and was found to be responsible for the bulk of wound-induced JA production in tomato (Li et al., 2005). Consistent with its essential role in JA biosynthesis, the *acx1* tomato mutant is impaired in wound-induced defense gene activation and insect resistance (Li et al., 2005). In *Arabidopsis*, ACX1 is responsible for about 80% of JA production after wounding (Cruz Castillo et al., 2004; Schilmiller et al., 2007), and only the *acx1/5* double mutant showed severe JA deficiency symptoms (Schilmiller et al., 2007).

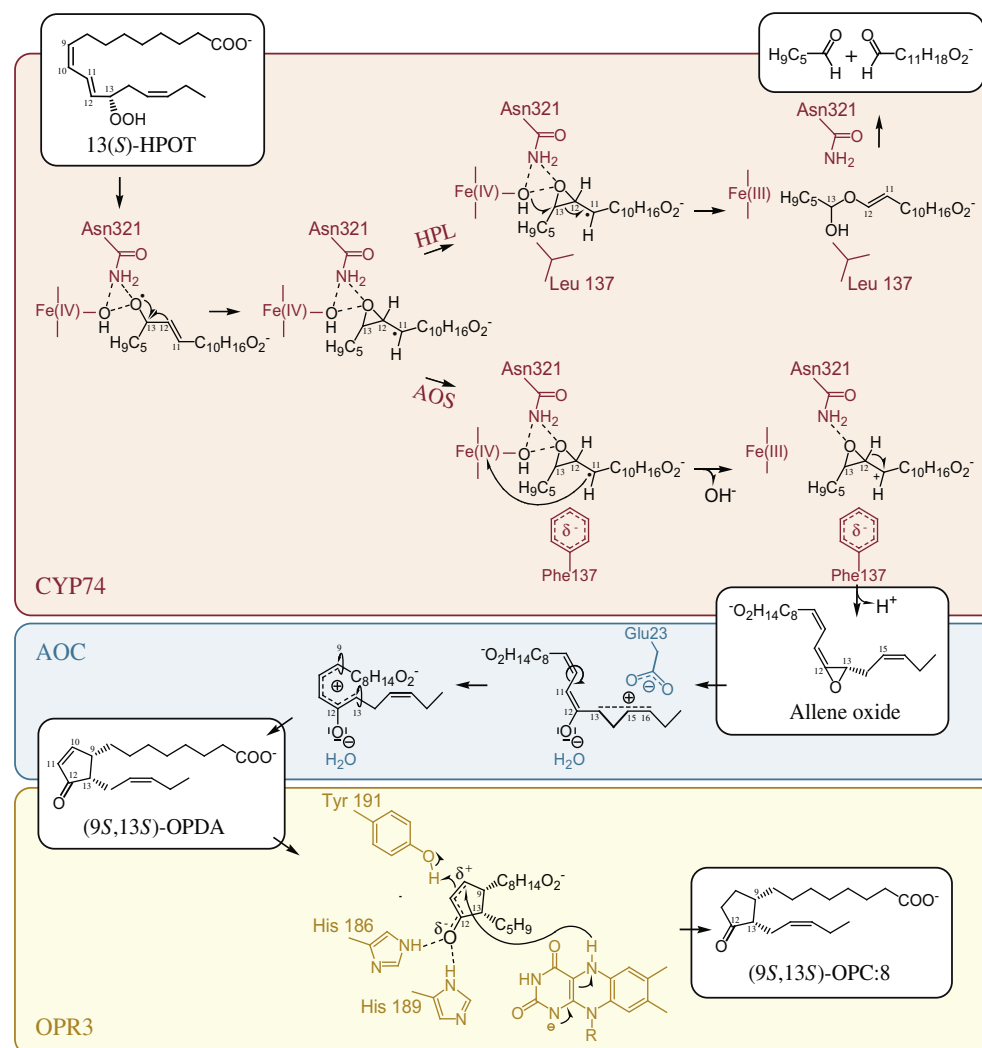


Fig. 2. Reaction mechanisms proposed for CYP74s, AOC, and OPR3. In **CYP74s** (top panel), the terminal oxygen of the hydroperoxide substrate coordinates the heme iron. Asn321 assists in the homolytic cleavage of the O–O bond. The resulting alkoxy radical adds to the C11=C12 double bond leading to the formation of an epoxide with an unpaired electron at C11 as the common intermediate of both, the AOS and the HPL reactions. One-electron-oxidation by the ferryl center and the formation of a carbocation at C11 are favored by Phe137 in the AOS as opposed to the HPL-catalyzed reaction. Finally, a C=C double bond is generated adjacent to the epoxide upon proton abstraction resulting in the formation of 12,13-EOT (allene oxide). Upon binding to **AOC** (middle panel), the epoxide oxygen of 12,13-EOT is coordinated by a bound water molecule. Delocalization of the C15=C16 double bond by Glu23 assists in epoxide opening and the resulting oxanion is stabilized by the bound water molecule. Critical for stereoselectivity is the *trans-cis* isomerization around the C11–C10 bond enforced by hydrophobic parts of the binding pocket, followed by ring closure as the final step in the formation of (9S,13S)-OPDA. OPDA is then bound above the *si* face of the flavin cofactor of **OPR3** (bottom panel). Its carbonyl oxygen forms hydrogen bonds with His186 and His189 leading to a polarization of the α,β -double bond. Consequently, hydride transfer from the reduced flavin cofactor to the substrate C β is facilitated, followed by a protonation of the C α by Tyr191. Substrates, reaction intermediates, and products are shown in black; enzyme-bound cofactors and amino-acid residues involved in catalysis are indicated in red, blue, and gold for CYP74s, AOC2, and OPR3, respectively. (Modified from Lee et al. (2008), Hofmann and Pollmann (2008), and Breithaupt et al. (2001); residues numbered according to the *Arabidopsis* sequences.)

The *Arabidopsis aim1* mutant disrupted in one of two MFP genes is similarly impaired in wound-induced accumulation of JA and the expression of JA-dependent genes (Delker et al., 2007). Among the five 3-ketoacyl-CoA thiolase genes in *Arabidopsis*, KAT2 appears to be the one most relevant for JA biosynthesis. In transgenic plants silenced for KAT2 expression, a reduction in wound-induced JA accumulation and JA-dependent marker gene (*JR2*) expression was observed (Cruz Castillo et al., 2004). As the final step in jasmonic acid biosynthesis, jasmonoyl-CoA is supposedly hydrolyzed to release the free acid. Candidate acyl-thioesterases have been identified in *Arabidopsis*, two of which appear to be peroxisomal (*AtACH1* and *AtACH2*) (Tilton et al., 2000, 2004), but a direct involvement in JA biosynthesis remains to be shown.

It becomes apparent from the above discussion that of all the steps involved, only those catalyzed by AOS, AOC, and OPR3 are truly committed to the JA biosynthetic pathway. Crystal structures

of these enzymes have recently been solved and in the remainder of this article, we will focus on the findings of these studies. For other aspects of JA biosynthesis, JA conjugation and metabolism, as well as JA activity and signaling the reader is referred to articles published elsewhere (Browse, 2005; Delker et al., 2006; Schaller and Stintzi, 2008; Schaller et al., 2005; Schilmiller and Howe, 2005; Wasternack, 2007) and also in this issue of *Phytochemistry*.

2. Allene oxide synthase (AOS)

Allene oxide synthases differ in specificity for 9(S) and/or 13(S) hydroperoxy fatty acids which are generated by cytosolic 9-LOX and plastidial 13-LOX activities. 13-AOS was first cloned from flaxseed (Song et al., 1993). It is encoded by a single gene in *Arabidopsis* as compared to two genes in tomato (Howe et al., 2000; Laudert

et al., 1996; Sivasankar et al., 2000). Consistent with plastid-localized 13-LOX activity, all known 13-AOSs – with the single exception of AOS from guayule (Pan et al., 1995) – are targeted to plastids, where they associate with plastid membranes (Farmaki et al., 2007; Froehlich et al., 2001) and plastoglobules (Vidi et al., 2006). Membrane binding is not mediated by transmembrane helices nor lipid anchors, but rather by a large non-polar patch of 2400 Å² at the enzymes surface. Substrates appear to access the active site from within this region which is consistent with their localization in plastid membranes (Lee et al., 2008).

AOSs belong to an atypical cytochrome P450 subfamily, the CYP74 enzymes. Unlike other P450s, they do not require molecular oxygen nor NAD(P)H-dependent cytochrome P450 reductases as cofactors, but use their hydroperoxide substrates as source for reducing equivalents and as oxygen donor (Howe and Schillmiller, 2002; Werck-Reichhart et al., 2002). While most P450s function as monooxygenases, CYP74 enzymes rearrange their fatty acid hydroperoxide substrates into various structurally different products: 13-AOSs catalyze the formation of 12,13-(*S*)-epoxy-octadecanoic acid and 10,11-(*S*)-epoxy hexadecatrienoic acid (10,11-EHT) as the first committed step of JA biosynthesis, whereas divinyl ether synthases (DESs) convert hydroperoxides to divinyl ethers, and hydroperoxide lyases (HPLs) produce short-lived hemiacetals that decompose to aldehydes and ω-fatty acids (Howe and Schillmiller, 2002; Stumpe and Feussner, 2006) (Fig. 2, top panel). The crystal structures recently solved for 13-AOSs from *Arabidopsis* and guayule (*Parthenium argentatum*) reveal that these plant CYP74s share a common fold with other P450s and provide a rationale for their unique reaction specificity (Lee et al., 2008; Li et al., 2008).

In both the guayule and the *Arabidopsis* AOS structures and similar to classical P450s, the heme group is inserted between two conserved α-helices, the I- and the L-helix. The fifth ligand for the heme iron is provided by a conserved cysteine which is located in the loop between helices K' and L. Within this loop, however, a nine amino-acid insertion is one of the most prominent distinctive features of CYP74s as compared to other P450s (Lee et al., 2008). As a result of this insertion, the Fe–S bond is significantly longer in CYP74s which affects the redox properties of the heme iron and has important consequences for reactivity and product specificity (see below).

CYP74s do not bind molecular oxygen and this is reflected in the absence of a generally conserved (A/G)GxxT oxygen-binding motif within the I-helix. In conventional P450s, the conserved Gly of the I-helix motif is positioned above the heme iron. This position is occupied by an Asn residue which is conserved in AOSs (N276 and N321 in guayule and *Arabidopsis* AOS, respectively) and other CYP74s. The carboxamide group of the Asn side chain is catalytically important as it assists in the homolytic cleavage of the O–O bond of the hydroperoxide substrate (Fig. 2, top panel). The resulting alkoxyl radical (RO•) adds to the substrate C11=C12 double bond to generate an epoxide between C12 and C13 and a carbon-centered radical (C•) at C11. Stabilization of this radical and the incipient carbocation at C11 (C⁺) is important in AOS to favor oxidation (transfer of the electron to the ferryl center), whereas C–C scission in the HPL and DES catalyzed reactions results in the production of hemiacetals and divinyl ethers, respectively. Lee et al. (2008) hypothesized that the aromatic π-system of a nearby Phe residue (F137) may be important for the stabilization of the radical, and may thus be essential for product specificity of CYP74s (Fig. 2, top panel). F137 was in fact found to be strictly conserved in all known AOS sequences, and replaced by Leu in HPL and DES. The critical role of F137 in AOSs was further confirmed by site-directed mutagenesis. Substitution of F137 in *Arabidopsis* and rice AOS with Leu resulted in altered product specificity: 12-oxododecenoic acid rather than allene oxide was identified as the reaction product indicating robust HPL activity for the site-directed mutants (Lee et al., 2008). A similar change in reaction specificity

was observed in a 9-AOS from tomato (*SIAOS3*), when F295 was changed to isoleucine by site-directed mutagenesis. The F295I mutant showed HPL activity producing 9-oxononanoic acid from 9-HPOD. F295 is located next to the catalytically important Asn (N296) in the I-helix of *LeAOS3* (Toporkova et al., 2008). Whether or not F295 of *SIAOS3* plays a role similar to F137 in 13-AOS from *Arabidopsis* and stabilizes a radical intermediate of the 9-AOS reaction remains to be seen.

It is evident that CYP74s evolved by precluding the monooxygenation chemistry of typical P450s, and the crystal structures provide fascinating insights into how the reactivity of reaction intermediates is controlled in order to achieve different product specificities in individual members of the CYP74 subfamily.

3. Allene oxide cyclase (AOC)

The allylic epoxides produced by AOS (12,13-EOT from 18:3 and 10,11-EHT from 16:3; Fig. 1) are highly unstable in water. Rapid hydrolysis results in the formation of α- and γ-ketols, and spontaneous cyclization yields (dn)OPDA in a racemic mixture of *cis*(+) and *cis*(–) enantiomers (i.e. the (9*S*,13*S*) and (9*R*,13*R*) enantiomers of OPDA, respectively) (Brash et al., 1988). In presence of AOC, the *cis*(+)–isomers of OPDA and dnOPDA are formed exclusively as precursors of bioactive JAs. AOC is thus crucially important to establish the enantiomeric structure of the cyclopentenone ring.

The enzyme was first isolated from corn seeds (Ziegler et al., 1997) and a few years later cloned from tomato (Ziegler et al., 2000). AOC is encoded by a single gene in tomato and a small gene family in *Arabidopsis* (Stenzel et al., 2003b). Despite comprehensive analyses of substrate specificity (Ziegler et al., 1999), the mechanism by which stereochemical control is exerted on the cyclization reaction remained enigmatic until the recent elucidation of several crystal structures for *Arabidopsis* AOCs (Hofmann and Pollmann, 2008; Hofmann et al., 2006; Levin et al., 2007).

Considering the fact that cyclization occurs spontaneously in aqueous solution, AOC does not need to be much of a catalyst in terms of lowering the activation energy barrier. Rather, the protein environment imposes steric restrictions on the substrate's hydrocarbon tail in the AOC-mediated as opposed to the spontaneous cyclization reaction, and these restrictions are sufficient to enforce the necessary conformational changes resulting in stereoselectivity (Hofmann et al., 2006). Consistent with this mechanism of action, the substrate binding site is located within a rigid barrel and binding of the substrate or the transition state does not involve any induced fit mechanism (Hofmann and Pollmann, 2008). Binding and correct positioning of the substrate 12,13-EOT is facilitated by the hydrophobic protein environment and very few ionic interactions, involving a tightly bound water molecule and Glu23 (*Arabidopsis* AOC2 numbering; Fig. 2, middle panel), which was shown to be essential for AOC activity (Schaller et al., 2008).

Interestingly, AOCs appear to be distantly related to dirigent proteins (Pleiss and Schaller, unpublished observation) and the somewhat passive role of AOCs in the stereoselective synthesis of (9*S*,13*S*)-OPDA is reminiscent of the way in which dirigent proteins promote stereoselective formation of lignans. Lignan biosynthesis involves the regio- and stereochemically controlled oxidative coupling of two phenols, e.g. the formation of (+)-pinoreosinol from two molecules of coniferylalcohol (Davin et al., 1997). In a first oxidative step, resonance-stabilized phenoxy radicals are formed which, in the absence of dirigent proteins, couple randomly to result in a mixture of racemic lignans. Dirigent proteins lack a catalytic center and were shown to promote stereoselective coupling by binding and orienting the free radical intermediates (Davin and Lewis, 2005; Davin et al., 1997).

First and foremost, dirigent proteins and AOCs appear to be binding proteins for unstable lipophilic substrates (phenoxy radicals and allene oxides, respectively). Their protein scaffold precludes all but one of the possible reaction channels of the protein-bound substrates resulting in stereochemical control in the synthesis of lignans and OPDA, respectively. Such a mechanism of action is reminiscent of the way in which molecular chaperones assist in protein folding. Chaperones do not themselves increase the rate of individual folding steps, but rather increase the efficiency of the overall process by reducing the probability of competing reactions (Dobson, 2003). Considering the importance of the hydrophobic protein environment and the mere binding of the substrates for dirigent protein and AOC activity, one might suspect that these proteins and their specific functions have evolved from more general binding proteins of hydrophobic ligands, e.g. lipocalins (Flower et al., 2000). Lipocalins, like AOCs, are β -barrel proteins comprising a central hydrophobic cavity for binding of small lipophilic molecules with functions in e.g. olfaction, pheromone transport, retinol transport, and invertebrate cryptic coloration (Charron et al., 2005). However, there is only very limited sequence similarity between lipocalins on the one, and AOC and dirigent proteins on the other hand. Therefore, the topological similarity observed between AOCs and lipocalins (Hofmann and Pollmann, 2008; Hofmann et al., 2006) may well be the result of convergent evolution.

4. 12-Oxophytodienoate reductase 3 (OPR3)

OPR3 belongs to a small family of related flavin-dependent oxidoreductases comprising at least three members in tomato, six in *Arabidopsis*, six in pea, eight in maize, and 10 in rice. The JA-deficient phenotype of OPR3 loss-of-function mutants in *Arabidopsis* (Sanders et al., 2000; Stintzi and Browse, 2000) and tomato (Stintzi and Bosch, unpublished) indicates that other OPR isoforms cannot substitute for OPR3 in JA biosynthesis. This is somewhat surprising since all OPRs catalyze the reduction of α,β -unsaturated carbonyls (conjugated enones) and their broad substrate spectrum may even include (9*R*,13*R*)-OPDA. However, the *in vitro* reduction of (9*R*,13*R*)-OPDA is physiologically irrelevant, since only the (9*S*,13*S*)-OPDA enantiomer is a precursor of bioactive jasmonic acid. The unique function of OPR3 in jasmonate biosynthesis is a result of its relaxed stereoselectivity with respect to OPDA stereoisomers. The physiologically relevant (9*S*,13*S*)-enantiomer of OPDA is not a substrate for the majority of OPRs, but is reduced exclusively by OPR3 from tomato and *Arabidopsis*, and orthologs in other species (OsOPR7) (Breithaupt et al., 2001, 2006; Jung et al., 2007; Schaller et al., 2000; Schaller and Weiler, 1997; Sobajima et al., 2003; Strassner et al., 1999, 2002).

- Brash, A.R., Baertschi, S.W., Ingram, C.D., Harris, T.M., 1988. Isolation and characterization of natural allene oxides: unstable intermediates in the metabolism of lipid hydroperoxides. *Proc. Natl. Acad. Sci. USA* 85, 3382–3386.
- Breithaupt, C., Strassner, J., Breitingner, U., Huber, R., Macheroux, P., Schaller, A., Clausen, T., 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., Kurzbauer, R., Lillie, 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. *Proc. Natl. Acad. Sci. USA* 103, 14337–14342.
- Breithaupt, C., Kurzbauer, R., Schaller, F., Stintzi, A., Schaller, A., Huber, R., Macheroux, P., Clausen, T., 2009. Shaping the oxylipin signature: control of stereospecificity in plant 12-oxophytodienoate reductases. *J. Mol. Biol.* doi:10.1016/j.jmb.2009.07.0870.
- Browse, J., 2005. Jasmonate: an oxylipin signal with many roles in plants. *Vitam. Horm.* 72, 431–456.
- Charron, J.B.F., Ouellet, F., Pelletier, M., Danyluk, J., Chauve, C., Sarhan, F., 2005. Identification, expression, and evolutionary analyses of plant lipocalins. *Plant Physiol.* 139, 2017–2028.
- Chehab, E.W., Perea, J.V., Gopalan, B., Theg, S., Dehesh, K., 2007. Oxylipin pathway in rice and *Arabidopsis*. *J. Int. Plant Biol.* 49, 43–51.
- Chung, H.S., Koo, A.J.K., Gao, X., Jayanty, S., Thines, B., Jones, A.D., Howe, G.A., 2008. Regulation and function of *Arabidopsis* JASMONATE ZIM-Domain genes in response to wounding and herbivory. *Plant Physiol.* 146, 952–964.
- Cruz Castillo, M., Martinez, C., Buchala, A., Mettraux, J.P., Leon, J., 2004. Gene-specific involvement of beta-oxidation in wound-activated responses in *Arabidopsis*. *Plant Physiol.* 135, 85–94.
- Davin, L.B., Lewis, N.G., 2005. Dirigent phenoxyl radical coupling: advances and challenges. *Curr. Opin. Biotech.* 16, 398–406.
- Davin, L.B., Wang, H.B., Crowell, A.L., Bedgar, D.L., Martin, D.M., Sarkanen, S., Lewis, N.G., 1997. Stereoselective bimolecular phenoxyl radical coupling by an auxiliary (dirigent) protein without an active center. *Science* 275, 362–366.
- Delker, C., Stenzel, I., Hause, B., Miersch, O., Feussner, I., Wasternack, C., 2006. Jasmonate biosynthesis in *Arabidopsis thaliana* – enzymes, products, regulation. *Plant Biol. (Stuttg.)* 8, 297–306.
- Delker, C., Zolman, B.K., Miersch, O., Wasternack, C., 2007. Jasmonate biosynthesis in *Arabidopsis thaliana* requires peroxisomal beta-oxidation enzymes – additional proof by properties of *pex6* and *aim1*. *Phytochemistry* 68, 1642–1650.
- Dobson, C.M., 2003. Protein folding and misfolding. *Nature* 426, 884–890.
- Farmaki, T., Sanmartin, M., Jimenez, P., Paneque, M., Sanz, C., Vancanneyt, G., Leon, J., Sanchez-Serrano, J.J., 2007. Differential distribution of the lipoxygenase pathway enzymes within potato chloroplasts. *J. Exp. Bot.* 58, 555–568.
- Feussner, I., Wasternack, C., 2002. The lipoxygenase pathway. *Annu. Rev. Plant Biol.* 53, 275–297.
- Flower, D.R., North, A.C., Sansom, C.E., 2000. The lipocalin protein family: structural and sequence overview. *Biochim. Biophys. Acta* 1482, 9–24.
- Footitt, S., Slocumbe, S.P., Larner, V., Kurup, S., Wu, Y., Larson, T., Graham, I., Baker, A., Holdsworth, M., 2002. Control of germination and lipid mobilization by COMATOSE, the *Arabidopsis* homologue of human ALDP. *EMBO J.* 21, 2912–2922.
- Fox, B.G., Malone, T.E., Johnson, K.A., Madson, S.E., Aceti, M., Bingman, C.A., Blommel, P.G., Buchan, B., Burns, B., Cao, J., Cornilescu, C., Doreleijers, J., Ellefson, J., Frederick, R., Geetha, H., Hruby, D., Jeon, W.B., Kimball, T., Kunert, J., Markley, J.L., Newman, C., Olson, A., Peterson, F.C., Phillips, G.N., Primm, J., Ramirez, B., Rosenberg, N.S., Runnels, M., Seder, K., Shaw, J., Smith, D.W., Sreenath, H., Song, J., Sussman, M.R., Thao, S., Troestler, D., Tyler, E., Tyler, R., Ulrich, E., Vinarov, D., Vojtki, F., Volkman, B.F., Wesenberg, G., Wrobel, R.L., Zhang, J., Zhao, Q., Zolnai, Z., 2005. X-ray structure of *Arabidopsis* Atlg77680, 12-oxophytodienoate reductase isoform 1. *Proteins* 61, 206–208.
- Froehlich, J.E., Itoh, A., Howe, G.A., 2001. Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450s involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. *Plant Physiol.* 125, 306–317.
- Glauser, G., Grata, E., Dubugnon, L., Rudaz, S., Farmer, E.E., Wolfender, J.L., 2008. Spatial and temporal dynamics of jasmonate synthesis and accumulation in *Arabidopsis* in response to wounding. *J. Biol. Chem.* 283, 16400–16407.
- Hamberg, M., Fahlstadius, P., 1990. Allene oxide cyclase: a new enzyme in plant lipid metabolism. *Arch. Biochem. Biophys.* 276, 518–526.
- Hause, B., Stenzel, I., Miersch, O., Wasternack, C., 2003. Occurrence of the allene oxide cyclase in different organs and tissues of *Arabidopsis thaliana*. *Phytochemistry* 64, 971–980.
- Hayashi, M., Nito, K., Takei-Hoshi, R., Yagi, M., Kondo, M., Suenaga, A., Yamaya, T., Nishimura, M., 2002. Ped3p is a peroxisomal ATP-binding cassette transporter that might supply substrates for fatty acid beta-oxidation. *Plant Cell Physiol.* 43, 1–11.
- Hofmann, E., Pollmann, S., 2008. Molecular mechanism of enzymatic allene oxide cyclization in plants. *Plant Physiol. Biochem.* 46, 302–308.
- Hofmann, E., Zerbe, P., Schaller, F., 2006. The crystal structure of *Arabidopsis thaliana* allene oxide cyclase: insights into the oxylipin cyclization reaction. *Plant Cell* 18, 3201–3217.
- Howe, G.A., Schillmiller, A.L., 2002. Oxylipin metabolism in response to stress. *Curr. Opin. Plant Biol.* 5, 230–236.
- Howe, G.A., Lee, G.L., Itoh, A., Li, L., DeRocher, A.E., 2000. Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. *Plant Physiol.* 123, 711–724.
- Jung, C., Yeu, S.Y., Koo, Y.J., Kim, M., Do Choi, Y., Cheong, J.J., 2007. Transcript profile of transgenic *Arabidopsis* constitutively producing methyl jasmonate. *J. Plant Biol.* 50, 12–17.
- Kienow, L., Schneider, K., Bartsch, M., Stuible, H.-P., Weng, H., Miersch, O., Wasternack, C., Kombrink, E., 2008. Jasmonates meet fatty acids: functional analysis of a new acyl-coenzyme A synthetase family from *Arabidopsis thaliana*. *J. Exp. Bot.* 59, 403–419.
- Koo, A.J.K., Chung, H.S., Kobayashi, Y., Howe, G.A., 2006. Identification of a peroxisomal acyl-activating enzyme involved in the biosynthesis of jasmonic acid in *Arabidopsis*. *J. Biol. Chem.* 281, 33511–33520.
- Laudert, D., Pfannschmidt, U., Lottspeich, F., Holländer-Czytko, H., Weiler, E.W., 1996. Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase (CYP74), the first enzyme of the octadecanoid pathway to jasmonates. *Plant Mol. Biol.* 31, 323–335.
- Laudert, D., Hennig, P., Stelmach, B.A., Müller, A., Andert, L., Weiler, E.W., 1997. Analysis of 12-oxo-phytyldienoic acid enantiomers in biological samples by capillary gas chromatography-mass spectrometry using cyclodextrin stationary phases. *Anal. Biochem.* 246, 211–217.
- Laudert, D., Schaller, F., Weiler, E.W., 2000. Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. *Planta* 211, 163–165.
- Lee, D.S., Nioche, P., Hamberg, M., Raman, C.S., 2008. Structural insights into the evolutionary paths of oxylipin biosynthetic enzymes. *Nature* 455, 363–368.
- Levin, E.J., Kondrashov, D.A., Wesenberg, G.E., Phillips, G.N., 2007. Ensemble refinement of protein crystal structures: validation and application. *Structure* 15, 1040–1052.
- Li, L., Zhao, Y., McCaig, B.C., Wingerd, B.A., Wang, J., Whalon, M.E., Pichersky, E., Howe, G.A., 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell* 16, 126–143.
- Li, C.Y., Schillmiller, A.L., Liu, G.H., Lee, G.L., Jayanty, S., Sageman, C., Vrebalov, J., Giovannoni, J.J., Yagi, K., Kobayashi, Y., Howe, G.A., 2005. Role of beta-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. *Plant Cell* 17, 971–986.
- Li, L., Chang, Z., Pan, Z., Fu, Z.Q., Wang, X., 2008. Modes of heme binding and substrate access for cytochrome P450 CYP74A revealed by crystal structures of allene oxide synthase. *Proc. Natl. Acad. Sci. USA* 105, 13883–13888.
- Malone, T.E., Madson, S.E., Wrobel, R.L., Jeon, W.B., Rosenberg, N.S., Johnson, K.A., Bingman, C.A., Smith, D.W., Phillips, G.N., Markley, J.L., Fox, B.G., 2005. X-ray structure of *Arabidopsis* At2g06050, 12-oxophytodienoate reductase isoform 3. *Proteins* 58, 243–245.
- Mosblech, A., Feussner, I., Heilmann, I., 2009. Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiol. Biochem.* 47, 511–517.
- Mueller, M.J., 2004. Archetype signals in plants: the phytoprostanes. *Curr. Opin. Plant Biol.* 7, 441–448.
- Pan, Z., Durst, F., Werck-Reichhart, D., Gardner, H.W., Camara, B., Cornish, K., Backhaus, R.A., 1995. The major protein of guayule rubber particles is a cytochrome P450. *J. Biol. Chem.* 270, 8487–8494.
- Park, J.H., Halitschke, R., Kim, H.B., Baldwin, I.T., Feldmann, K.A., Feyereisen, R., 2002. A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. *Plant J.* 31, 1–12.
- Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W., Goldberg, R.B., 2000. The *Arabidopsis* DELAYED DEHISCENCE1 gene encodes an enzyme in the jasmonic acid synthesis pathway. *Plant Cell* 12, 1042–1061.
- Schaller, A., Stintzi, A., 2008. Jasmonate biosynthesis and signaling for induced plant defense against herbivory. In: Schaller, A. (Ed.), *Induced Plant Resistance Against Herbivory*. Springer, Heidelberg, pp. 349–365.
- Schaller, F., Weiler, E.W., 1997. Molecular cloning and characterization of 12-oxophytodienoate reductase, an enzyme of the octadecanoid signaling pathway from *Arabidopsis thaliana*. Structural and functional relationship to yeast old yellow enzyme. *J. Biol. Chem.* 272, 28066–28072.
- Schaller, F., Biesgen, C., Müssig, C., Altmann, T., Weiler, E.W., 2000. 12-Oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. *Planta* 210, 979–984.
- Schaller, F., Schaller, A., Stintzi, A., 2005. Biosynthesis and metabolism of jasmonates. *J. Plant Growth Regul.* 23, 179–199.
- Schaller, F., Zerbe, P., Reinbothe, S., Reinbothe, C., Hofmann, E., Pollmann, S., 2008. The allene oxide cyclase family of *Arabidopsis thaliana* – localization and cyclization. *FEBS J.* 275, 2428–2441.
- Schillmiller, A.L., Howe, G.A., 2005. Systemic signaling in the wound response. *Curr. Opin. Plant Biol.* 8, 369–377.
- Schillmiller, A.L., Koo, A.J.K., Howe, G.A., 2007. Functional diversification of acyl-coenzyme A oxidases in jasmonic acid biosynthesis and action. *Plant Physiol.* 143, 812–824.
- Schneider, K., Kienow, L., Schmelzer, E., Colby, T., Bartsch, M., Miersch, O., Wasternack, C., Kombrink, E., Stuible, H.P., 2005. A new type of peroxisomal acyl-coenzyme A synthetase from *Arabidopsis thaliana* has the catalytic capacity to activate biosynthetic precursors of jasmonic acid. *J. Biol. Chem.* 280, 13962–13972.
- Shockey, J.M., Fulda, M.S., Browse, J., 2003. *Arabidopsis* contains a large superfamily of acyl-activating enzymes. Phylogenetic and biochemical analysis reveals a new class of acyl-coenzyme A synthetases. *Plant Physiol.* 132, 1065–1076.
- Sivasankar, S., Sheldrick, B., Rothstein, S.J., 2000. Expression of allene oxide synthase determines defense gene activation in tomato. *Plant Physiol.* 122, 1335–1342.

- Sobajima, H., Takeda, M., Sugimori, M., Kobashi, N., Kiribuchi, K., Cho, E.M., Akimoto, C., Yamaguchi, T., Minami, E., Shibuya, N., Schaller, F., Weiler, E.W., Yoshihara, T., Nishida, H., Nojiri, H., Omori, T., Nishiyama, M., Yamane, H., 2003. Cloning and characterization of a jasmonic acid-responsive gene encoding 12-oxophytodiene reductase in suspension-cultured rice cells. *Planta* 216, 692–698.
- Song, W.C., Funk, C.D., Brash, A.R., 1993. Molecular cloning of an allene oxide synthase: a cytochrome P450 specialized for the metabolism of fatty acid hydroperoxides. *Proc. Natl. Acad. Sci. USA* 90, 8519–8523.
- Stenzel, I., Hause, B., Maucher, H., Pitzschke, A., Miersch, O., Ziegler, J., Ryan, C.A., Wasternack, C., 2003a. Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato – amplification in wound signalling. *Plant J.* 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 Mol. Biol.* 51, 895–911.
- Stintzi, A., Browse, J., 2000. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodiene reductase required for jasmonate synthesis. *Proc. Natl. Acad. Sci. USA* 97, 10625–10630.
- Stintzi, A., Weber, H., Reymond, P., Browse, J., Farmer, E.E., 2001. Plant defense in the absence of jasmonic acid: the role of cyclopentenones. *Proc. Natl. Acad. Sci. USA* 98, 12837–12842.
- Straßner, J., Fürholz, A., Macheroux, P., Amrhein, N., Schaller, A., 1999. A homolog of old yellow enzyme in tomato. Spectral properties and substrate specificity of the recombinant protein. *J. Biol. Chem.* 274, 35067–35073.
- Strassner, J., Schaller, F., Frick, U.B., Howe, G.A., Weiler, E.W., Amrhein, N.A., Macheroux, P., Schaller, A., 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodiene reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. *Plant J.* 32, 585–601.
- Stumpe, M., Feussner, I., 2006. Formation of oxylipins by CYP74 enzymes. *Phytochem. Rev.* 5, 347–357.
- Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Aina, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K., Shibata, D., Kobayashi, Y., Ohta, H., 2005. 12-Oxo-phytodiene acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiol.* 139, 1268–1283.
- Theodoulou, F.L., Job, K., Slocombe, S.P., Footitt, S., Holdsworth, M., Baker, A., Larson, T.R., Graham, I.A., 2005. Jasmonic acid levels are reduced in COMATOSE ATP-binding cassette transporter mutants. Implications for transport of jasmonate precursors into peroxisomes. *Plant Physiol.* 137, 835–840.
- Tilton, G., Shockey, J., Browse, J., 2000. Two families of acyl-CoA thioesterases in *Arabidopsis*. *Biochem. Soc. Trans.* 28, 946–947.
- Tilton, G.B., Shockey, J.M., Browse, J., 2004. Biochemical and molecular characterization of ACH2, an acyl-CoA thioesterase from *Arabidopsis thaliana*. *J. Biol. Chem.* 279, 7487–7494.
- Toporkova, Y.Y., Gogolev, Y.V., Mukhtarova, L.S., Grechkin, A.N., 2008. Determinants governing the CYP74 catalysis: conversion of allene oxide synthase into hydroperoxide lyase by site-directed mutagenesis. *FEBS Lett.* 582, 3423–3428.
- Vick, B.A., Zimmerman, D.C., 1984. Biosynthesis of jasmonic acid by several plant species. *Plant Physiol.* 75, 458–461.
- Vidi, P.A., Kanwischer, M., Baginsky, S., Austin, J.R., Csucs, G., Dormann, P., Kessler, F., Brehelin, C., 2006. Tocopherol cyclase (VTE1) localization and vitamin E accumulation in chloroplast plastoglobule lipoprotein particles. *J. Biol. Chem.* 281, 11225–11234.
- Wang, C., Avdiushko, S., Hildebrand, D.F., 1999. Overexpression of a cytoplasm-localized allene oxide synthase promotes the wound-induced accumulation of jasmonic acid in transgenic tobacco. *Plant Mol. Biol.* 40, 783–793.
- Wasternack, C., 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Ann. Bot.* 100, 681–697.
- Weber, H., Vick, B.A., Farmer, E.E., 1997. Dinor-oxo-phytodiene acid: a new hexadecanoid signal in the jasmonate family. *Proc. Natl. Acad. Sci. USA* 94, 10473–10478.
- Werck-Reichhart, D., Bak, S., Paquette, S., 2002. Cytochromes P450. In: *The Arabidopsis Book*. American Society of Plant Biologists, Rockville, MD. doi:10.1199/tab.002.
- Zerbe, P., Weiler, E.W., Schaller, F., 2007. Preparative enzymatic solid phase synthesis of *cis*(+)-12-oxo-phytodiene acid – physical interaction of AOS and AOC is not necessary. *Phytochemistry* 68, 229–236.
- Ziegler, J., Hamberg, M., Miersch, O., Parthier, B., 1997. Purification and characterization of allene oxide cyclase from dry corn seeds. *Plant Physiol.* 114, 565–573.
- Ziegler, J., Wasternack, C., Hamberg, M., 1999. On the specificity of allene oxide cyclase. *Lipids* 34, 1005–1015.
- Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Hamberg, M., Grimm, R., Ganai, M., Wasternack, C., 2000. Molecular cloning of allene oxide cyclase. The enzyme establishing the stereochemistry of octadecanoids and jasmonates. *J. Biol. Chem.* 275, 19132–19138.
- Zolman, B.K., Silva, I.D., Bartel, B., 2001. The *Arabidopsis pxa1* mutant is defective in an ATP-binding cassette transporter-like protein required for peroxisomal fatty acid β -oxidation. *Plant Physiol.* 127, 1266–1278.



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