

# Cyclopentenone signals for plant defense: Remodeling the jasmonic acid response

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Compounds derived from the oxidative metabolism of polyunsaturated fatty acids are synthesized in response to external stimuli and serve important roles in controlling diverse processes in both plants and animals. Members of the eicosanoid family of lipid mediators have been studied extensively with respect to their biosynthesis from C<sub>20</sub> fatty acids and their function in the regulation of cell differentiation, immune responses, and homeostasis in animal systems (1). In plants, oxygenated derivatives of C<sub>18</sub> and C<sub>16</sub> fatty acids participate in the regulation of many defense-related and developmental processes. Research on fatty acid-based signaling systems in plants has focused mainly on the hormonally active compound, jasmonic acid (JA). A rapidly growing body of literature indicates that plant defense responses against insect herbivores (Fig. 1) and some microbial pathogens are orchestrated by signaling pathways involving the biosynthesis and subsequent action of JA. In this issue of PNAS, Stintzi *et al.* (2) used an elegant biochemical genetic approach to determine whether the cyclopentenone precursor of JA, 12-oxo-phytodienoic acid (OPDA), is also a physiological signal for defense. Their demonstration that OPDA confers broad-spectrum resistance in the absence of JA marks a major advance in our understanding of jasmonate-signaled responses.

JA is a terminal product of the octadecanoid pathway (ref. 3; Fig. 2). This series of reactions is initiated by lipoxygenase, which adds molecular oxygen to linolenic acid. The resulting 13-hydroperoxide is converted in the chloroplast to a specific stereoisomer of OPDA (9*S*,13*S*-OPDA) by the sequential action of allene oxide synthase and allene oxide cyclase. The next step in the pathway involves reduction of the cyclopentenone ring of OPDA by OPDA reductase (OPR). OPR likely catalyzes this reaction in peroxisomes (4), where  $\beta$ -oxidation enzymes complete the synthesis of JA. The jasmonate family of compounds includes biologically active cyclopentenones (e.g., OPDA) and cyclo-



Fig. 1. Jasmonic acid and related compounds regulate plant defense responses against hostile invaders. This photograph shows a tobacco hornworm (*Manduca sexta*) attacking a tomato plant.

pentanones (e.g., JA) of related structure and biosynthetic origin.

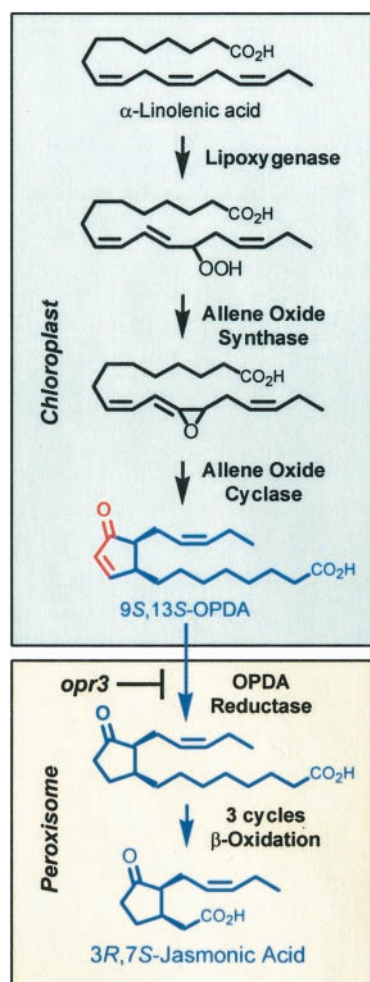
Several lines of evidence support an essential role for the octadecanoid pathway in various aspects of defense. First, treatment of plants with exogenous JA (or methyl-JA) results in major reprogramming of gene expression, including defense-related genes that are activated by wounding and pest attack (5–8). Second, endogenous levels of JA increase rapidly in response to wounding and other biotic stress (3). Finally, mutants defective in either the biosynthesis or perception of JA are dramatically compromised in resistance to numerous plant invaders (9–14). These findings have led to the general assumption that JA is the physiological signal for several wound- and pathogen-induced responses. A few studies, however, indicate that OPDA is also active as a signal without prior metabolism to JA. Some of the best evidence for this comes from studies of the tendrils of *Bryonia* to mechanical stimulation. Dose-response studies with intermediates of the octadecanoid pathway provide strong evidence that OPDA is the

physiologically relevant signal for this response (15, 16).

To investigate the potential role of OPDA in defense, Stintzi *et al.* took advantage of the Arabidopsis *opr3* mutant (also known as *dde1*), which fails to metabolize OPDA to JA. The *opr3/dde1* mutant (hereafter referred to as *opr3*) was identified on the basis of a defect in male gametophyte development (4, 17). A T-DNA insertion in the mutant disrupts the *OPR3* locus encoding one of three known OPR isozymes. Biochemical and genetic studies have established that OPR3 catalyzes the reduction of 9*S*,13*S*-OPDA, the physiologically relevant precursor of JA (4, 17, 18). The possibility that other OPR isozymes can substitute for OPR3 in the biosynthesis of JA was ruled out by two observations. First, recombinant forms of OPR1 and OPR2 do not use 9*S*,13*S*-OPDA as a substrate (18). Second, wounded leaves of *opr3* plants accumulate 9*S*,13*S*-OPDA

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**Fig. 2.** The octadecanoid pathway for biosynthesis of jasmonic acid. Members of the jasmonate family of oxylipins are shown in blue. The reactive  $\alpha,\beta$ -unsaturated carbonyl group in the cyclopentenone ring of OPDA is highlighted in red. The biosynthetic step blocked by the *opr3* mutation is also indicated.

but lack detectable levels of 3*R*,7*S*-JA (2). Although further metabolism (e.g.,  $\beta$ -oxidation) of OPDA in the absence of OPR3 remains a formal possibility, these findings demonstrate that *opr3* plants are completely blocked in the conversion of OPDA to JA (Fig. 2). Accordingly, this mutant provides a useful genetic tool to disentangle the physiological effects of endogenous OPDA from those of JA.

Mutants of *Arabidopsis* have been instrumental in establishing a role for JA in defense against a broad spectrum of pests. Of particular importance have been the *fad3 fad7 fad8* "triple" mutant, which fails to synthesize the linolenate precursor of JA

(19), and the *coil* mutant, which is deficient in the JA signaling cascade (20). Both mutants are highly compromised in resistance to the dipteran insect *Bradysia impatiens* and the necrotrophic fungus *Alternaria brassicicola*. Based on these results and the JA-deficient phenotype of *opr3*, one might have predicted that *opr3* plants would also be compromised in resistance. When this was put to the test, however, *opr3* plants exhibited a wild-type level of resistance to both *Bradysia* and *Alternaria*. This striking result indicates that resistance in *opr3* plants is mediated by a signal other than JA, the most likely candidate being OPDA.

Two independent signaling pathways have been shown to regulate the expression of wound-responsive genes in *Arabidopsis* (6, 21). One pathway relies on wound-induced JA biosynthesis and subsequent signaling through COI1. A second pathway regulates the expression of a distinct set of target genes independently of JA and COI1. To further investigate the molecular basis of resistance in *opr3* plants, cDNA microarray analysis was used to examine the expression of numerous defense-related genes in response to wounding and applied OPDA. Results of these experiments point to two important conclusions. First, the COI1 pathway can receive input from both JA and OPDA. Second, full activation of wound responsive genes involves the combined action of JA and OPDA.

Although both JA and OPDA work through COI1 to control expression of many of the same genes, these two signals also effect different responses. For example, not all COI1-dependent genes are expressed in *opr3* plants in response to wounding or exogenous OPDA. The most striking example of this phenomenon is the *VSP* gene, which is activated by JA but not OPDA. Prior insight into the differential action of JA and OPDA has come from studies showing that the strict requirement for JA in anther and pollen maturation cannot be fulfilled by OPDA (4), and that JA and OPDA induce different patterns of volatiles in leaves of lima beans (22). These and other studies (15, 16) point to the existence of cellular mechanisms that can distinguish JA from OPDA.

Interestingly, exogenous OPDA and wounding activate several COI1-indepen-

dent genes whose expression is not affected by JA. Stintzi and coworkers suggest that this effect is mediated by the defining structural feature of cyclopentenones, namely the  $\alpha,\beta$ -unsaturated carbonyl group located in the cyclopentenone ring (Fig. 2). Of relevance to this hypothesis is the fact that many biological actions of cyclopentenone prostaglandins, including effects on gene expression, require the  $\alpha,\beta$ -unsaturated carbonyl moiety (23). The electrophilic properties of this reactive center render cyclopentenones susceptible to conjugate addition reactions (Michael addition) with various intracellular targets. Structure-activity studies will be necessary to determine the extent to which such a mechanism accounts for the unique biological activity of OPDA, or other cyclopentenones produced from related biosynthetic pathways (24, 25).

The function of JA, OPDA, and other oxylipins (26, 27) as signals for defense supports the concept that host responses to hostile invaders are fine-tuned by a complex mix of signals, termed the oxylipin signature (24). A recent study by Hause *et al.* (28) highlights the fact that oxylipin profiles are shaped not only by external stimuli, but also by developmental cues that exert control on the octadecanoid pathway in specific tissues and cell types. Future advances in our understanding of the jasmonate signaling pathway will likely come from research aimed at filling several important knowledge gaps. First, what specific gene products and metabolites account for jasmonate-mediated resistance to various pests? The JA-independent resistance phenotype of *opr3* plants may provide a useful starting point for such studies. Second, what are the control points that govern the synthesis and accumulation of jasmonates? Of potential relevance to this question is the recent observation that the major fraction of OPDA occurs esterified in chloroplast glycerolipids (29). Finally, what are the mechanisms by which jasmonate signals are perceived and transduced into diverse functional responses? Answers to these questions promise to shed new light on the intriguing similarities and evolutionary origins of fatty acid-based signaling processes that are essential for the well being of both plants and animals.

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