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# Oxylipin Signaling in Plant Stress Responses

Oxidized fatty acids, termed oxylipins, are an important class of signaling molecule in plants, especially related to plant stress responses and innate immunity. The best-characterized oxylipins are jasmonic acid (JA) and its immediate precursor 12-oxo-phytodienoic acid (OPDA), which are formed enzymatically and accumulate in response to various stresses, in particular wounding and pathogen infection (Block et al., 2005). In addition, a number of biologically active oxylipins are formed nonenzymatically via the action of reactive oxygen species (ROS), which also accumulate in response to pathogen infection, heavy metal uptake, and other stresses. There is growing evidence that nonenzymatically formed oxylipins, including hydroxy fatty acids and phytoprostanes, play important signaling roles in plant stress responses (Sattler et al., 2006).

Recent work with JA and OPDA has shown that both are active as signaling molecules and induce the expression of overlapping but distinct sets of genes. JA signaling leads to the interaction of the F-box ubiquitin ligase CORONATINE-INSENSITIVE1 (COI1) with JAZ transcriptional repressors, mediating degradation of these repressors of downstream JA-induced genes, many of which are dependent on the key transcription factor MYC2/JIN1 (Chini et al., 2007; reviewed in Santner and Estelle, 2007). Whereas JA induces a set of COI1-dependent genes, OPDA has been found to induce a set of largely COI1-independent genes (Stintzi et al., 2001; Taki et al., 2005). Phytoprostanes have also been shown to activate the expression of stress response genes, leading to enhanced protection from subsequent oxidative stress (Thoma et al., 2003; Loeffler et al., 2005), but little is known about phytoprostanane signal transduction.

Phytoprostanes are grouped into a number of classes, depending on the ring and side chain structures, and plants are known

to produce a number of different types (Thoma et al., 2004). The A<sub>1</sub>-type (PPA<sub>1</sub>) and the deoxy-J<sub>1</sub>-type phytoprostanes, as well as OPDA, belong to a subgroup of oxylipins known as reactive electrophile species (RES) because they contain a reactive  $\alpha,\beta$ -unsaturated carbonyl structure that may contribute to their biological activity. RES, for example, have the ability to modify proteins directly by binding to free thiol groups, a property that is not shared by weak electrophilic oxylipins, including JA and the B<sub>1</sub>-type phytoprostanes (PPB<sub>1</sub>). It has been suggested that RES, including those produced nonenzymatically, induce a common set of defense genes (Almeras et al., 2003; Weber et al., 2004; Farmer and Davoine, 2007).

In this issue of *The Plant Cell*, **Mueller et al. (pages 768–785)** examine the effects of OPDA and phytoprostanes on gene expression and plant growth and development in *Arabidopsis* and show that these oxylipins share similar biological activity that appears to differ considerably from that of JA. In addition, phytoprostanes might have unique activities and targets not shared by OPDA or JA. The authors employ whole-genome microarray analysis, liquid chromatography–tandem mass spectrometry analysis, and enzymatic assays to investigate the biological activity of phytoprostanes in relation to OPDA and JA. Differences in gene induction between JA and OPDA/phytoprostanes suggest the existence of multiple oxylipin signal transduction pathways. Interestingly, many of the genes induced by phytoprostanes and OPDA were found to be dependent on the TGA transcription factors TGA2, TGA5, and TGA6, demonstrating that these factors play an important role in oxylipin signaling (see figure). In addition, it was shown that some of the genes induced by OPDA and phytoprostanes encode enzymes involved in their metabolism (conjugation and/or detoxification). This work shows that nonenzymatically produced oxylipins likely constitute an important component of oxylipin signaling in plant stress responses.

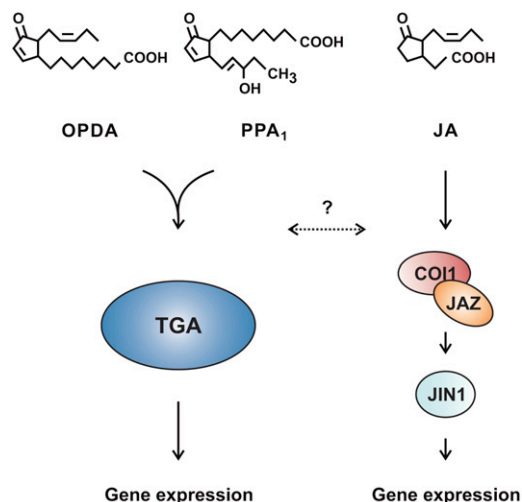
## PHYTOPROSTANES AND OPDA HAVE OVERLAPPING AND INDEPENDENT FUNCTIONS IN *ARABIDOPSIS*

The authors first conducted microarray analysis of gene expression in *Arabidopsis* cell cultures after treatment with A<sub>1</sub>-type phytoprostanes (PPA<sub>1</sub>). In response to PPA<sub>1</sub>, 926 genes showed statistically significant up- or downregulation greater than twofold, and many of the induced genes were classified as putatively involved in stress responses, such as cytochrome P450 enzymes, UDP-glucuronyl-/glycosyltransferases, glutathione S-transferases (GSTs), ABC transporters, and heat shock proteins. Repressed genes included those involved in cell cycle regulation and cell growth.

Further RNA gel blot analysis of the expression of 13 of the genes induced by PPA<sub>1</sub> showed that most also were strongly induced by OPDA, as well as dJ<sub>1</sub>-type and B<sub>1</sub>-type phytoprostanes, but exhibited more moderate or no induction in response to JA. These results showed that biological activities of JA, OPDA, and various phytoprostanes partially overlap, and PPA<sub>1</sub> appears to have activity very similar to that of OPDA, which differs considerably from that of JA. However, the activities of all species did not correlate strictly with electrophilicity (e.g., the weak electrophile PPB<sub>1</sub> appeared to show more similarity to the RES than to JA for the genes tested).

Further experiments conducted in plants offered confirmation that PPA<sub>1</sub> and OPDA share similar biological activities. Microarray analysis using whole plants and comparisons with publicly available microarray data through Genevestigator (www.genevestigator.ethz.ch; Zimmermann et al., 2004) showed that 42% of the PPA<sub>1</sub>-induced genes were upregulated more than threefold by both PPA<sub>1</sub> and OPDA, whereas only 7% of PPA<sub>1</sub>-induced genes were also induced by JA. The set of genes upregulated by PPA<sub>1</sub> also showed considerable overlap with genes induced by pathogens, such as *Pseudomonas syringae*.

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## Oxylipin Signaling Pathways.

JA signaling is to some extent dependent on the key transcriptional activator MYC2/JIN1, which is regulated by COI1-dependent degradation of JAZ repressors (Chini et al., 2007). The work of Mueller et al. (2008) suggests a model wherein TGA transcription factors contribute to phytoprostane and OPDA signaling. Although not shown here, phytoprostanes and OPDA also appear to have their own unique responses.

These data support the notion that pathogen infection leads to enhanced production of ROS that can trigger the formation of nonenzymatic oxylipins, as also shown by Grun et al. (2007). It is also important to note that, although there was considerable overlap between PPA<sub>1</sub> and OPDA, PPA<sub>1</sub> appeared to have a unique signature. The authors speculate that phytoprostanes may have a particular function in detoxification of xenobiotic compounds (various chemicals and pathogen infection situations that elicit the accumulation of ROS/RES).

### TGA TRANSCRIPTION FACTORS PLAY A ROLE IN OXYLIPIN SIGNALING

Mueller et al. next searched the promoters of genes induced by PPA<sub>1</sub> for a high abundance of specific binding sites to identify transcription factors potentially involved in the response to phytoprostanes. They found that approximately half of the PPA<sub>1</sub>-induced genes in both cell culture and whole-plant experiments contain a TGA-motif (TGACG) in the first 500 bp upstream of the start codon. They conducted microarray gene expression analysis using a *tga2*

*tga5 tga6* triple mutant of *Arabidopsis* and found that 60% of the genes upregulated by PPA<sub>1</sub> in the wild type (247 of 411) were not induced in the triple mutant, providing confirmation that these TGA transcription factors are involved in mediating gene regulation by PPA<sub>1</sub>.

These TGA transcription factors were also found to be important in OPDA-mediated regulation of gene expression. In response to OPDA, 30% of the genes induced in the wild type (225 of 760) were not induced in the *tga2 tga5 tga6* triple mutant. Although similar in number, the sets of genes induced in a TGA-dependent manner by PPA<sub>1</sub> (247) and by OPDA (225) contained just 48 genes in common (i.e., expression of 48 genes was increased by PPA<sub>1</sub> and OPDA in a TGA-dependent manner). This could reflect some real differences in the response to PPA<sub>1</sub> versus OPDA but could partly be due to limitations of the microarray analysis. The take-home message is that a large percentage of genes regulated by OPDA and PPA<sub>1</sub> (30 to 60%) is dependent on TGA transcription factors. The microarray data further showed that a relatively large number of genes (>200)

were upregulated by PPA<sub>1</sub> and OPDA in the *tga2 tga5 tga6* triple mutant but were not induced by these oxylipins in the wild type, suggesting that TGA transcription factors might negatively regulate the expression of some genes.

These results build upon previous information on the role of TGA factors in stress responses. For example, TGA2, TGA5, and TGA6 are known to interact with NPR1, a key factor required for salicylic acid-dependent induction of systemic acquired resistance in *Arabidopsis* (Zhang et al., 1999). Previous analysis of the *tga2 tga5 tga6* triple mutant has also shown that these factors play overlapping and essential roles in this process in vivo (Zhang et al., 2003). Ndamukong et al. (2007) found that these TGA factors, together with salicylic acid-responsive glutaredoxin, also mediate suppression of the JA-responsive gene *PDF1.2*. Future work will focus on identifying other TGA-interacting partners that may be involved in the response to oxylipins and to what extent the JA response differs from that of OPDA and phytoprostanes.

### METABOLISM OF REACTIVE OXYLIPINS

Because of the highly reactive nature of OPDA and PPA<sub>1</sub> oxylipins, Mueller et al. investigated whether they induce genes encoding enzymes capable of detoxifying them or metabolizing them into less reactive compounds. Two types of enzymes that might reduce the reactive cyclopentenone ring to an unreactive ring are GSTs and OPDA-reductases (OPRs). The gene expression analyses showed that OPDA and PPA<sub>1</sub> induce several genes encoding OPR and GST enzymes. Using enzymatic assays conducted with purified heterologous proteins produced in *Escherichia coli*, the authors further demonstrate that OPDA and PPA<sub>1</sub> are in vitro substrates of two strongly induced enzymes, OPR1 and GST6. In addition, the authors were able to measure an accumulation of PPA<sub>1</sub>- and OPDA-glutathione conjugates in *Arabidopsis* leaves following infection with *P. syringae*.

The work of Mueller et al. presents convincing data that nonenzymatically formed oxylipins, such as phytoprostanes,

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contribute significantly to plant response to stress and impact signaling pathways that regulate gene expression. In addition, they demonstrate that phytoprostanes are metabolized and converted to less active compounds by induced OPR and GST enzymes. Although there appears to be significant overlap between phytoprostane and OPDA activity, the responses were not identical, suggesting that phytoprostanes might also have a unique set of responses. Phytoprostanes therefore constitute another class of oxylipin in plants (in addition to JA and OPDA) that must be taken into account in considering oxylipin signaling.

**Nancy A. Eckardt**  
News and Reviews Editor  
neckardt@aspb.org

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