Commentary

OPDA interaction with CYP20-3 is a benchmark for understanding retrograde signalling in plants

alternative title:

Sulfur metabolism in the centre of plant retrograde signalling

short title:

OPDA signalling revealed

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Despite their different life styles animals and plants share the dependence on small molecules, hormones, for systemic regulation of development and other cellular processes. The hormones act through specific receptors either through triggering secondary signalling cascades or through direct effect of the nuclear receptor complexes on gene transcription (1). Plant hormones, however, use different mechanisms of action, their nuclear receptors are not transcription factors but act through protein-protein interactions, resulting usually in degradation of their interacting partners (2). In PNAS, Park et al. (3) describe a very different hormone receptor with an alternative localization and a mode of action. Not only is the receptor, cyclophilin 20-3 (CYP20-3), found in the chloroplast, its hormone complex binds a metabolic enzyme and results in increased production of cysteine. The newly synthesised cysteine alters the redox state of the cell, resulting in activation of TGA transcription factors (3). The action of this receptor thus connects retrograde signalling, sulfur metabolism, and redox regulation.

In plants, jasmonic acid (JA) is the best known member of the oxylipin phytohormone family with main function in regulation of defence (4). JA is conjugated to isoleucine by JASMONATE RESISTANT1 (JAR1) (5) and the conjugate is perceived by the receptor CORONATINE INSENSITIVE1 (COI1) (6). The F-Box COI1 is part of ubiquitine E3 ligase complex which upon binding of JA-Ile targets a number of JASMONATE-ZIM DOMAIN (JAZ) repressor proteins for degradation (7-9). This relieves inhibition of a specific set of transcription factors and results in rapid activation of a large number of genes (10). The mechanism of JA signalling thus seems well understood, except for a small problem: not all JA regulated genes are COI1-dependent (11). In their search for alternative JA receptors, Park et al. (3) discovered a new JA-binding protein, CYP20-3. Instead of being a straightforward alternative to COI1, however, this receptor presents a few surprises and provides interesting links from oxylipin signalling to other areas of plant metabolism.

Why is the CYP20-3 so interesting? Firstly, although found in a screen for proteins interacting with JA, its physiological ligand is actually the intermediate in JA synthesis, 12-oxo-phytodieonic acid (OPDA) (3). Indeed, a set of genes was shown previously to be specifically regulated by OPDA and not JA (12), and these genes were also independent from COI1. The results of Park et al. (3) thus provide the mechanistic explanation for this observation and place OPDA onto the growing list of phytohormones for which the receptor has been identified (2).

Importantly, CYP20-3 is the first hormone receptor localised not at the plasma membrane or nucleus, but in the plastids. While this seems to make sense for a receptor of a hormone synthesised in chloroplast (13), it involves the inconvenience of transmitting the signal across the plastid envelope to the nucleus, the retrograde signalling (14, 15). Retrograde signalling is essential to enable the cell to react to signals perceived in the plastids, such as high light, drought, or reactive oxygen species and re-adjust homeostasis (15). A number of such signals acting through diverse pathways have been proposed, such as Mg protoporphyrin IX, haem, singlet oxygen, 3’-phosphoadenosine 5’-phosphate (PAP), methylerythritol cyclodiphosphate, or plant homeodomain type transcription factors with transmembrane domain, which are normally present in plastid envelopes and upon stress migrate to the nucleus (reviewed in (15)). So why is addition of OPDA on this list remarkable? The answer is in the detailed dissection of the mechanism of action of CYP20-3 revealed in Park et al. (3). The authors were able to find the interaction partner of the OPDA receptor, to measure the redox changes triggered by the signal, to identify the transcription

factors controlled by these redox changes and to record the resulting changes in gene expression. In contrast, for the other proposed signals the molecular mechanisms of their action and the interacting proteins and/or molecules are largely unknown. The detailed dissection of OPDA signalling by Park et al. (3) thus forms a benchmark for characterisations of other retrograde signalling pathways.

The OPDA receptor, CYP20-3, has previously been shown to bind serine acetyltransferase (SAT), an enzyme essential for sulfate assimilation and a component of cysteine synthase complex (16, 17). The binding increased SAT activity leading to higher production of cysteine, since SAT is limiting for its synthesis (18). The interaction of CYP20-3 with SAT seemed to be important for abiotic stress signalling, e.g., the *cyp20-3* mutants were sensitive to salt stress (16). The results of Park et al. (3) add another layer to these observations, showing that the interaction of CYP20-3 and SAT is facilitated by OPDA and is a part of OPDA signalling mechanism. The OPDA-CYP20-3 promotes the formation of cysteine synthase complex, which is necessary for SAT activity. But for the resulting cysteine to work as a signal or second messenger for regulation of gene expression, it has to move to the nucleus. The mediator of such OPDA retrograde signalling, however, seems to be not the cysteine per se, but a change in redox potential caused by increased concentration of thiol groups. Increased cysteine synthesis leads to synthesis of glutathione that can convey the redox signal to the TGA transcription factors, which have been shown to be redox sensitive before (19). Thus, sulfur metabolites are necessary for the OPDA triggered retrograde signalling.

The involvement of sulfur merits emphasis as this is not the first case in the retrograde signalling field. A typical example of such signalling is the induction of *APX2* gene for ascorbate peroxidase expression by high light. This process obviously requires the transmission of a signal generated in chloroplast to the nucleus. Interestingly, in a genetic screen for mutants impaired in this signalling only two mutants with the same lesion in γ-glutamylcysteine synthetase (γECS), the first enzyme in glutathione synthesis from cysteine, were identified (20). It is tempting to speculate that γECS is involved in the transmission of the redox signal generated by ODPA as well, but to prove it will require some additional work. A different link between retrograde signalling and sulfur metabolism is one of the newest signals, PAP. PAP is a by-product of biological sulfations, which uses an activated sulfate in the form of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) (17). PAP is dephosphorylated in the plastids, as its accumulation inhibits the sulfation reactions and ribonucleases and triggers large changes in gene transcription (21, 22). The role of PAP as a retrograde signal was inferred from the observation that PAP accumulates in both plastids and nucleus of plants lacking the enzyme degrading PAP and also in plants subjected to drought stress resulting in similar expression alterations (21). Interestingly, both branches of sulfate assimilation, the reduction of sulfate to sulfide for cysteine synthesis as well as formation of PAPS are under control of JA and are also redox regulated on a post-translational level (17, 23, 24), indicating a crosstalk of the signals. The identification of mechanisms of OPDA signalling (3) can form a starting point for the dissection of the interactions between the multiple retrograde signalling pathways. This will help to unravel the complex way in which plant cells react to signals localised in organelles and understand the fundamental basis of control of plant homeostasis.

References

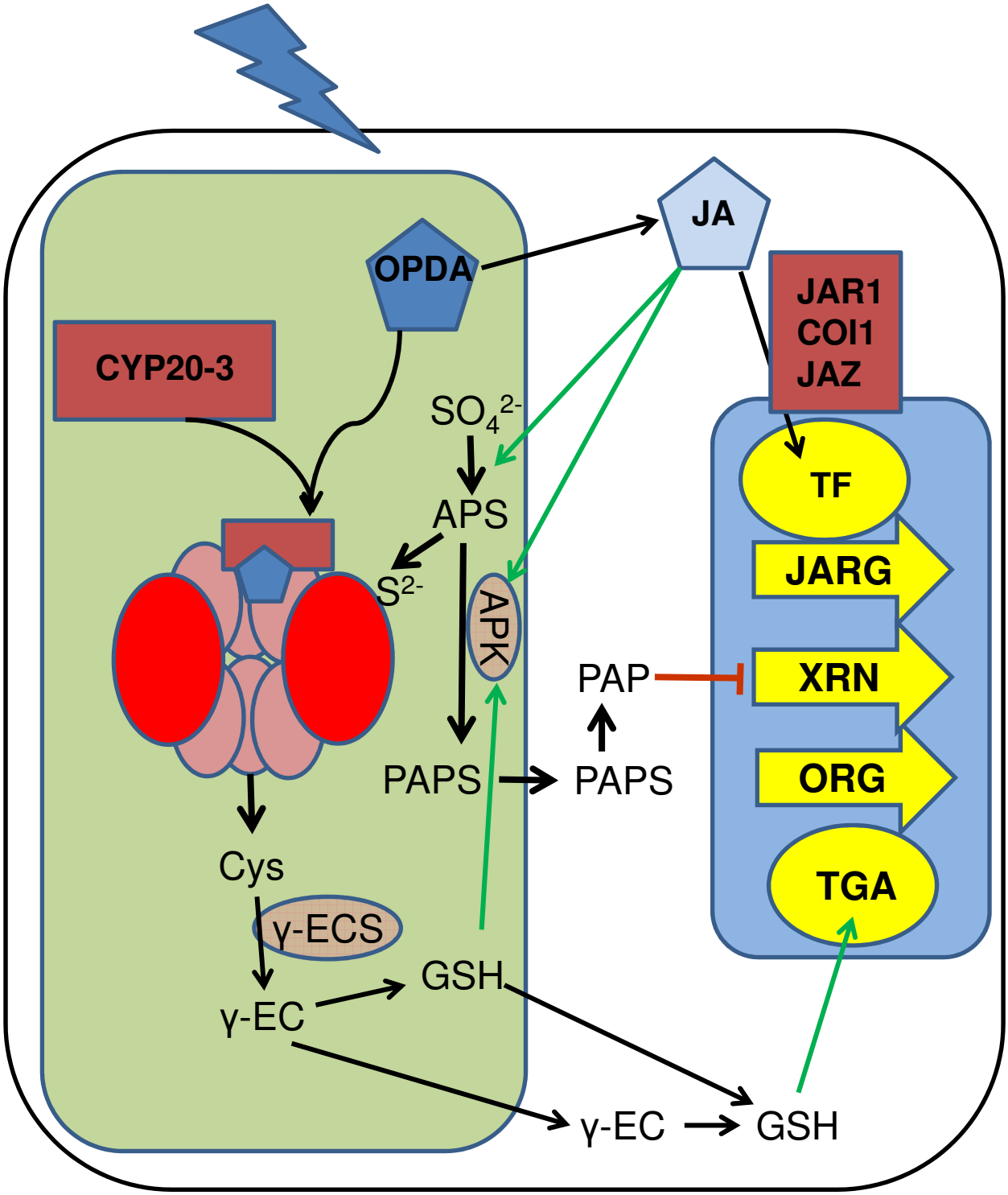
1. Evans RM (2005) The nuclear receptor superfamily: a rosetta stone for physiology. *Mol Endocrinol* 19(6):1429-1438.
2. Lumba S, Cutler S, & McCourt P (2010) Plant nuclear hormone receptors: a role for small molecules in protein-protein interactions. *Annual review of cell and developmental biology* 26:445-469.
3. Park S-W*, et al.* (2013) Cyclophilin 20-3 relays a 12-oxo-phytodienoic acid signal during stress responsive regulation of cellular redox homeostasis. *Proc Natl Acad Sci U S A*.
4. Ballare CL (2011) Jasmonate-induced defenses: a tale of intelligence, collaborators and rascals. *Trends Plant Sci* 16(5):249-257.
5. Staswick PE & Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in Arabidopsis. *Plant Cell* 16(8):2117-2127.
6. Yan J*, et al.* (2009) The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. *Plant Cell* 21(8):2220-2236.
7. Chini A*, et al.* (2007) The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* 448(7154):666-671.
8. Thines B*, et al.* (2007) JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature* 448(7154):661-665.
9. Yan Y*, et al.* (2007) A downstream mediator in the growth repression limb of the jasmonate pathway. *Plant Cell* 19(8):2470-2483.
10. Pauwels L & Goossens A (2011) The JAZ proteins: a crucial interface in the jasmonate signaling cascade. *Plant Cell* 23(9):3089-3100.
11. Devoto A*, et al.* (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 Mol Biol* 58(4):497-513.
12. Taki N*, 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 Physiol* 139(3):1268-1283.
13. Bell E, Creelman RA, & Mullet JE (1995) A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in Arabidopsis. *Proc Natl Acad Sci U S A* 92(19):8675-8679.
14. Chan KX, Crisp PA, Estavillo GM, & Pogson BJ (2010) Chloroplast-to-nucleus communication: current knowledge, experimental strategies and relationship to drought stress signaling. *Plant Signal Behav* 5(12):1575-1582.
15. Estavillo GM, Chan KX, Phua SY, & Pogson BJ (2012) Reconsidering the nature and mode of action of metabolite retrograde signals from the chloroplast. *Front Plant Sci* 3:300.
16. Dominguez-Solis JR*, et al.* (2008) A cyclophilin links redox and light signals to cysteine biosynthesis and stress responses in chloroplasts. *Proc Natl Acad Sci U S A* 105(42):16386-16391.
17. Takahashi H, Kopriva S, Giordano M, Saito K, & Hell R (2011) Sulfur Assimilation in Photosynthetic Organisms: Molecular Functions and Regulations of Transporters and Assimilatory Enzymes. *Annu Rev Plant Biol* 62:157-184.
18. Blaszczyk A, Brodzik R, & Sirko A (1999) Increased resistance to oxidative stress in transgenic tobacco plants overexpressing bacterial serine acetyltransferase. *Plant J* 20(2):237-243.
19. Bottcher C & Pollmann S (2009) Plant oxylipins: plant responses to 12-oxo-phytodienoic acid are governed by its specific structural and functional properties. *Febs J* 276(17):4693-4704.
20. Ball L*, et al.* (2004) Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. *Plant Cell* 16(9):2448-2462.
21. Estavillo GM*, et al.* (2011) Evidence for a SAL1-PAP chloroplast retrograde pathway that functions in drought and high light signaling in Arabidopsis. *Plant Cell* 23(11):3992-4012.

22. Lee BR*, et al.* (2012) Effects of fou8/fry1 mutation on sulfur metabolism: is decreased internal sulfate the trigger of sulfate starvation response? *PLoS One* 7(6):e39425.
23. Ravilious GE, Nguyen A, Francois JA, & Jez JM (2012) Structural basis and evolution of redox regulation in plant adenosine-5'-phosphosulfate kinase. *Proc Natl Acad Sci U S A* 109(1):309-314.
24. Jost R*, et al.* (2005) Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulfur-related pathways in Arabidopsis thaliana. *Photosynth Res* 86(3):491-508.

Figure Legends

Fig. 1. Scheme of the interaction between OPDA signalling and sulfur metabolism.

Park et al. (3) showed that the OPDA synthesised in the chloroplast in reaction to stress binds to cyclophilin CYP20-3, and the hormone-receptor complex interacts with SAT to stabilise formation of cysteine synthase and increase cysteine synthesis. Cysteine is metabolised to glutathione (GSH) resulting in changes of redox homeostasis in plastid as well as in the cytosol. The cytosolic GSH migrates to nucleus to activate TGA transcription factors and induce transcription of OPDA-responsive genes (ORG). In parallel, OPDA is metabolised to JA which up-regulates set of genes responsive to JA (JARG). Among JARGs are genes for components of sulfate assimilation, providing the reduced sulfur needed for increased cysteine production. The redox signalling may be coupled with PAP retrograde signalling: reduced GSH in plastids activates APS kinase synthesising PAPS, which is converted in cytosol to PAP. Increased PAP blocks XRN ribonucleases and triggers changes in transcript levels of another subset of genes.


JA
OPDA
CYP20-3
JAR1
COI1
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SO_4^{2-}
APS
TF
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PAP
XRN
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PAPS
ORG
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TGA
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