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Toward a better understanding of signaling networks in plants: yeast has the power!

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In response to abiotic stresses, plants produce the hormone abscisic acid (ABA). The ABA signaling pathway is highly complex and relies on a large number of gene copies encoding homologous signaling components, theoretically enabling numerous permutations. In this issue, Ruschhaupt *et al* (2019) used yeast as a reconstitution system to examine the functionality, plasticity, and efficiency of this complex and highly multiplexed core signaling pathway.

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See also: **M Ruschhaupt *et al*** (2019)

As sessile organisms, plants need to tightly integrate cues from their environment in order to survive. To respond to their changing surroundings, plants produce hormones and transduce the associated message through complex signaling pathways. Abscisic acid (ABA) is a major phytohormone implicated in many developmental processes and in mounting resilience to environmental stresses (Cutler *et al*, 2010). A major breakthrough in the ABA scientific community happened a decade ago with the discovery of the ABA receptors, named "PYRABACTIN RESISTANCE/REGULATORY COMPONENTS OF ABA RECEPTOR" (PYR/RCARs; Ma *et al*, 2009; Park *et al*, 2009). In the presence of ABA, the PYR/RCAR receptors bind to type 2C protein phosphatases (PP2Cs), in a gate–latch–lock mechanism (Melcher *et al*, 2009). The signal is then transduced through SNF1-related protein kinases (SnRK2s) that are activated by phosphorylation. SnRK2 kinases, in turn, phosphorylate many downstream ABA signaling components in response to abscisic acid (Kline *et al*, 2010). In the absence of ABA, SnRK2s are dephosphorylated by PP2Cs and cannot transduce the signal (Fig 1).

Adding complexity and difficulty to understanding this ABA signaling core, the *Arabidopsis* genome encodes 9 PP2C co-receptors and 14 different PYL/RCAR receptors, the latter being divided into three subfamilies (Tischer *et al*, 2017). Such redundancy translates into numerous possible combinatorial permutations, and closely related receptor complexes differ in their apparent ABA affinity and sensitivity (Dupeux *et al*, 2011; Hao *et al*, 2011). Previous to their findings published in this issue, the same group studied the ABA response regulation by expressing different combinations of ABA receptors and PP2Cs in plant protoplasts (Tischer *et al*, 2017). While the interactions between putative PYR/PYL/RCAR-PP2C pairings were uncovered, previous research had not focused on the SnRK2 protein kinases, the other central component of the core ABA signaling pathway, a gene family consisting of 10 members (Cutler *et al*, 2010; Raghavendra *et al*, 2010). Three of the 10 SnRK2s are known to function in ABA signaling: OST1 (SnRK2.6), SnRK2.2, and SnRK2.3 (for review: Raghavendra *et al*, 2010).

How to study the functionality and efficiency of signaling components when the number of possible permutations is so large? Five years ago, Pierre-Jerome and colleagues reconstituted the early auxin receptor signaling pathway in *Saccharomyces cerevisiae* (Pierre-Jerome *et al*, 2014). This first article paved the way toward a better understanding of complex molecular mechanisms, using this heterologous system. In this issue, Ruschhaupt *et al* shed light on early ABA signaling events in yeast. The authors used synthetic biology for studying key aspects of the ABA-dependent signaling components. They were first able to reconstruct the ABA signaling pathway using the receptor RCAR11 (PYR1), the ABI1 PP2C phosphatase, and the OST1 (SnRK2.6) protein kinase. In the presence of ABA, OST1 was able to phospho-activate the downstream transcription factor ABF2, resulting in expression of a luciferase reporter in yeast. The authors were also able to recapitulate in yeast the earlier observations on the responsiveness of PYR/RCAR receptor subfamilies to ABA in protoplasts (Tischer *et al*, 2017). They notably identified additional SnRK2 kinases as putative candidates that can function in the reconstituted ABA response pathway: SnRK2.1, 2.4, 2.5, and 2.10 (part of the subgroup I of the *Arabidopsis* SnRK2 protein family) (Fig 1). In yeast, these SnRK2 candidates appeared to have a different ABA sensitivity compared to OST1. For instance, SnRK2.4 triggered a stronger ABA response with a lower dose of the phytohormone compared to OST1. But are these properties characterized in yeast also found *in planta*? The authors looked at the effect of ABA receptors and ABA on the interaction between

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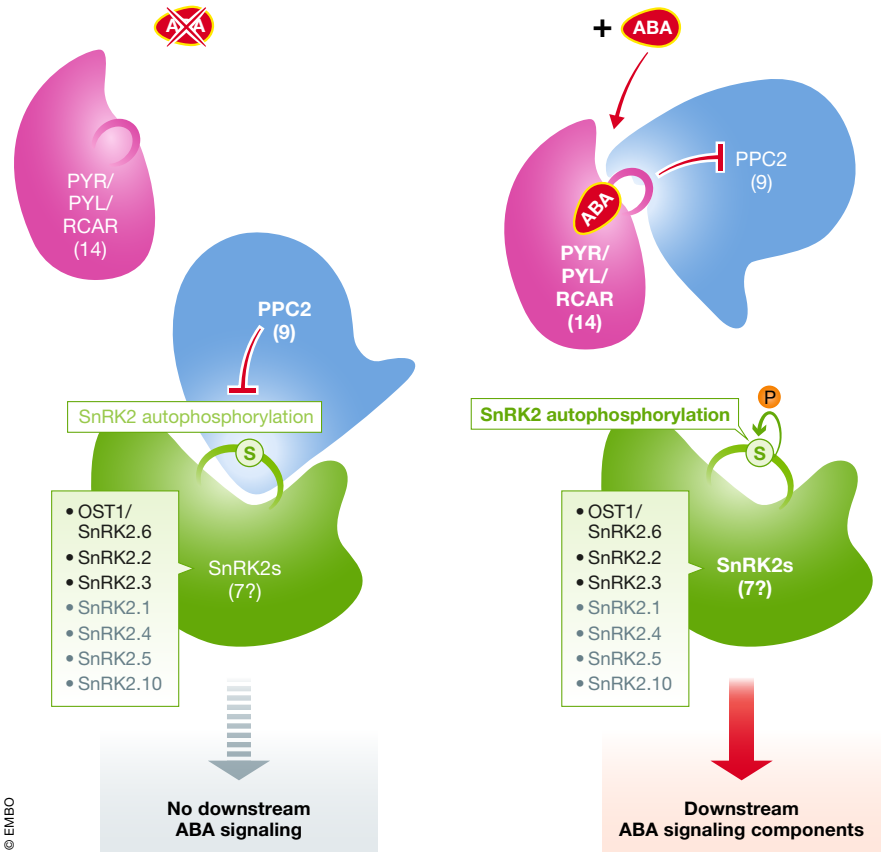

Figure 1. The ABA receptor signal transduction core is complex and includes large numbers of homologous protein components.

ABA binds to the intracellular PYR/PYL/RCAR receptors (14 genes in *Arabidopsis*). In the presence of ABA, the PYR/RCAR receptors bind to and inhibit type 2C protein phosphatases (PP2Cs, 9 genes) via a gate–latch–lock mechanism. As a result, SnRK2 kinases (~3 to up to 10 genes) become activated by phosphorylation and thus transduce the message by phosphorylating many downstream ABA signaling components. Without ABA, PP2Cs are active and dephosphorylate SnRK2s, thus deactivating these protein kinases and rendering them unable to transduce the signal. SnRK2.1, 2.4, 2.5, and 2.10 were uncovered by Ruschhaupt *et al* in this issue as putative new actors in the direct ABA signaling pathway using reconstitution in yeast, with evidence in plant cells for a function of SnRK2.4 in the ABA signaling core. The number of genes encoding the presented core signaling components is indicated in parentheses.

SnRK2s and the ABI1 co-receptor using FRET/FLIM in *Nicotiana benthamiana* leaves. Upon ABA binding to RCAR1 (PYL9), SnRK2.4 was displaced from ABI1 more readily than OST1. Similarly, less ABA was required for RCAR14 (PYL2) to be able to antagonize the interaction between SnRK2.4 and ABI1 in comparison with the ABA levels required to disrupt the OST1-ABI1 heterodimer. These data point to how the yeast system can uncover new mechanisms in plants.

The present article reports on a system that enables analyses of large numbers of signaling component permutations and will undoubtedly trigger new studies in plants, for example, on the direct ABA signal transduction roles of the newly identified SnRK2 kinase candidates found in this study (Ruschhaupt *et al*, 2019). Further work will be needed to investigate which new models derived from reconstitution of the ABA signal transduction pathway in yeast reflect signaling mechanisms present in plants.

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