WOX3 in the scene: intimacy with hormones

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How do WOX genes function to regulate key developmental programs in plants? We’re aware of their love affair with hormones, but lack good evidence of direct cause-and-effect relationships linking transcriptional activity and hormonal changes. In this issue of *Journal of Experimental Botany* (pages 1677–1687), Cho et al. provide that evidence for rice OsWOX3A and gibberellic acid.

The name WOX stands for *WUSCHEL*-related homeobox, named after the founding member of the group, Arabidopsis *WUSCHEL* (*WUS*) (*Mayer* et al., 1998). WOX transcription factors are plant-specific, homeodomain-containing transcriptional regulators known to function in several key plant developmental programs including embryo development, root and shoot apical meristem maintenance, lateral root and crown root development, tillering and vegetative growth, leaf blade development, vascular patterning, inflorescence development, floral organ development and seed development. A major outstanding question is how WOX genes function to regulate these important processes.

WOX genes do appear to have a love affair with hormones since auxin, cytokinin, abscisic acid (ABA) and gibberellic acid (GA) have been implicated in their actions. However, most of these associations were inferred based on gene expression changes and fluorescent markers signaling changes in the activity of the corresponding hormones. Direct cause-and-effect relationships that demonstrate transcriptional activity of WOX genes linked to measured steady-state level hormonal changes, with classical reversal of effects with hormone applications, have not been well established.

Now, Cho et al. (2016) have demonstrated that rice OsWOX3A is induced by GA and directly binds to the promoter of *ent*-kaurenoic acid oxidase (KAO), an enzyme involved in GA biosynthesis, repressing its activity. Transgenic rice plants overexpressing OsWOX3A became dwarf (Fig. 1), suggesting a defect in GA biosynthesis or signaling. By quantifying endogenous GA intermediates, Cho and colleagues were able to show that GA₉₀ and GA₃ levels decrease in these plants. Although in vivo data were not provided for the binding of OsWOX3A to the KAO promoter and other potential OsWOX3A binding sites in the GA pathway are still unclear, the induction of OsWOX3A by GA₃ treatment, reversion of the dwarf phenotype in OsWOX3A transgenic plants by GA application, the measurement of GA intermediates and the interaction of OsWOX3A with the KAO promoter using yeast one-hybrid and EMSA assays are powerful pieces of evidence that together speak loudly for the involvement of OsWOX3A in gibberellin negative-feedback regulation. But then what is the contribution of GA to the Oswox3a (nal2/3) mutant phenotypes? What other hormones are involved in generating the pleiotropic defects?
Leaf blade development

Despite the conservation of most Arabidopsis WOX genes in eudicots and monocots, WOX1 and WOX3 show an interesting detachment. Phylogenetic analyses revealed that Arabidopsis and most eudicots have at least one copy of the WOX1 and WOX3 homologs, but grasses have no copy of the WOX1 homolog at all (Nardmann et al., 2007; Vandenbussche et al., 2009; Tadege et al., 2011; Zhang et al., 2014).

Functionally, Arabidopsis WOX3 – also called PRESSED FLOWER (PRS) – has been proposed to regulate lateral axis-dependent development of flowers (Matsumoto and Okada, 2001), in which the prs mutant shows defects or loss in lateral sepals, lateral stamens, and stipules (Nardmann et al., 2004). We now know that WOX1 and PRS function redundantly to regulate leaf blade outgrowth in Arabidopsis (Vandenbussche et al., 2009; Nakata et al., 2012). The woxt1 mutant alone has no obvious leaf phenotype under standard growth conditions but the woxt1 prs double mutant displays narrow leaf blades. However, mutants in WOX1 homologs in other dicot species show strong leaf blade and petal fusion phenotypes as single mutants. These mutants were variously named based on their mutant phenotypes, including maucest (maw) in petunia (Vandenbussche et al., 2009), stenofolia (stl) in Medicago truncatula (Tadege et al., 2011), bladeless lam1 in Nicotiana sylvestris (McHale, 1992; Tadege et al., 2011), lathyroides (lath) in pea and narrow organs1 (naol1) in Lotus (Zhuang et al., 2012). Although some variations exist in the strength of the mutant phenotypes, they all have narrow leaf blades and narrow petals in common. In stf and lam1 mutants where phenotypes are strongest, blades are drastically reduced to less than a third of the wild-type width and carpels are unfused leading to protrusion of ovules and female sterility.

Promotion of cell proliferation with transcriptional repression

In Medicago, STF is specifically expressed in leaf primordia in a narrow range of cells in the leaf margin and middle mesophyll at the adaxial–abaxial juxtaposition (Tadege et al., 2011). This type of expression pattern is thought to represent a third (middle) leaf domain (Nakata et al., 2012) between the well-characterized adaxial and abaxial domains. STF/LAM1/WOX1 promotes cell proliferation at the leaf margin, which is mediated by a transcriptional repression activity that involves the transcriptional co-repressor TOPLESS (TPL) (Lin et al., 2013; Zhang et al., 2014). It is, thus, likely that STF/WOX1 maintains a cell proliferation zone at the leaf margin by repressing polarity factors. According to this model, STF at the adaxial–abaxial junction provides new cells that ultimately differentiate into adaxial and abaxial domains. This highlights a novel mechanism for blade outgrowth, notwithstanding the well-defined functions of polarity factors (Kerstetter et al., 2001; McConnell et al., 2001). The genetic data are consistent with WOX1 being a repressor of polarity factors (Nakata et al., 2012) and a recent report demonstrated that STF can directly repress the adaxial factor ASYMMETRIC LEAVES2 (AS2) at the margin (Zhang et al., 2014). Because WOX1 is redundant with PRS in Arabidopsis leaf blade outgrowth, but WOX1 homologs in other dicots independently regulate blade outgrowth, the role of PRS/WOX3 has at least been investigated in Medicago. Loss-of-function mutation in Mtwox3 causes a loose flower (lf) phenotype affecting petal fusion (Niu et al., 2015) but no effect on leaf blade outgrowth. This suggests that STF is independently recruited for leaf blade outgrowth functions in eudicots, despite the WOX1 and PRS redundancy in Arabidopsis.

In maize, there is no STF/WOX1 homolog but two WOX3 homologs, NARROW SHEATH1 (NS1) and NARROW SHEATH2 (NS2), redundantly regulate blade outgrowth (Nardmann et al., 2004). Similarly in rice, two WOX3 duplicate genes, NARROW LEAF2 (NAL2) and NARROW LEAF3 (NAL3), encoding the same protein OsWOX3A, regulate leaf blade outgrowth (Cho et al., 2013; Ishiwata et al., 2013). The n2l3 double mutant displays a pleiotropic phenotype including narrow curved leaves, more tillers, fewer lateral roots, opened spikelets and narrow, thin grains (Cho et al., 2013), indicating a wider effect on overall plant growth and development. Thus, NS/OsWOX3A is the monocot equivalent of STF/WOX1 required for leaf blade outgrowth. Whether OsWOX3A mainly functions as a repressor and recruits OsTPL for this purpose is not known but the results of Cho et al. (2016) demonstrate that OsWOX3A represses KAO to maintain GA homeostasis during rice growth and development.

A sizzling affair with hormones

STF/LAM1 has been proposed to modulate multiple hormone homeostasis and sugar metabolism as part of a mechanism in its regulation of leaf blade outgrowth. Apart from changes in hormone-related gene expression, significantly lower levels of free IAA and ABA have been reported and a similarly low level of free cytokinin has been proposed in stf and lam1 mutants (Tadege et al., 2011), suggesting that STF directly or indirectly affects at least auxin, cytokinin and ABA homeostasis and/or signaling. However, these effects of STF appear to increase hormone levels probably by repressing hormone-conjugating enzymes and/or repressing hormone biosynthesis repressors, but OsWOX3A appeared to directly repress GA biosynthesis. Does this mean that STF and OsWOX3A oppositely affect hormones and the mechanisms for leaf blade development are drastically different in dicots and grasses? Probably not. I imagine that we are just scratching the surface. STF and OsWOX3A are likely to coordinate the effects of several hormones in multiple pathways, but more data are required to pinpoint the similarities and differences.

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Insight
The tonoplast – where sweetness is dispensable

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The proteolytic machinery of certain cellular organelles is potentially harmful to resident proteins. In mammals, the large luminal domains of integral lysosomal proteins are heavily glycosylated to protect them from degradation. In this issue of Journal of Experimental Botany (pages 1769–1781) Pedrazzini and co-workers reveal that plants are very different.

Glycosylation is one of the most common protein modifications in all eukaryotes. Resident proteins of different intracellular organelles including the endoplasmic reticulum (ER), Golgi apparatus and hydrolysing compartments are often glycosylated at multiple sites with oligosaccharide chains of varying length and composition. The attached carbohydrates modulate protein stability, activity and trafficking and mediate protein–protein interactions. N-glycosylation, the most prevalent form of protein glycosylation in eukaryotes, is defined by the covalent linkage of an oligosaccharide (N-glycan) to selected asparagine residues of newly synthesized secretory proteins as they are translocated into the ER. Once attached to the polypeptide chain, the N-glycans play crucial roles in protein folding and quality control processes (Helenius and Aebi, 2001) – for example, they regulate a specific cell death event in Arabidopsis (de Oliveira et al., 2016).

Immediately after the transfer to the protein, N-glycans are subjected to step-wise processing reactions that alter the composition of the N-glycan and by doing so control the fate of glycoproteins. As a consequence, the exposure of a defined

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