Insight

Endogenous auxin biosynthesis and de novo root organogenesis

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Induction of adventitious roots is essential for vegetative propagation of plants, and auxin has long been used as an exogenous root-inducing agent. In this issue of Journal of Experimental Botany, Chen et al. (pages 4273–4284) demonstrate that different members of the YUCCA family orchestrate the endogenous auxin biosynthesis that is required for the induction of adventitious roots.

Sun Wukong, also known as the Monkey King, is the main character in the classical Chinese novel Journey to the West. As a fabled deity, he was endowed with magical properties allowing each of his hairs to be transformed into clones of himself as needed. Plants also possess the remarkable ability of multiplication, with detached pieces of adult tissues capable of forming an entire plant body (Gordon et al., 2007; Birnbaum and Sánchez Alvarado, 2008; Sugimoto et al., 2010). This unique ability is mainly based on de novo organogenesis, in which adventitious shoots or roots are generated from isolated tissues or organs (Duclercq et al., 2011; Cheng et al., 2013; Xu and Huang, 2014).

De novo organogenesis can be induced under both natural and tissue culture conditions (Chen et al., 2014). Plant organs, such as stems and leaves, give rise to adventitious roots under natural growth conditions and this property has long been used for vegetative propagation of elite genotypes in agriculture, forestry and horticulture (De Klerk et al., 1999). Six decades ago, Skoog and Miller demonstrated that the entire plant could be regenerated by tissue culture (Skoog and Miller, 1957). They showed that culturing explants in medium containing high levels of cytokinin induced the formation of adventitious shoots, whereas medium with high levels of auxin triggered initiation of adventitious roots. This classic system laid the foundations for plant micropropagation and genetic transformation (Duclercq et al., 2011; Li et al., 2011). In both cases, a key step ensuring the success of plant regeneration is de novo root organogenesis.
which guarantees the water and nutrient supply for regeneration and survival of the new organism (Chen et al., 2014).

**Cell fate transition**

The first cellular event of de novo organogenesis is cell fate transition (Duclercq et al., 2011; Chatfield et al., 2013). Evidence from different species has indicated that adventitious roots initiate from the procambium or cambium regions (Liu et al., 2014; Xu and Huang, 2014). To study the underlying mechanisms, Chen et al. have developed a simple system to mimic the formation of adventitious roots under natural conditions (Chen et al., 2014). By culturing Arabidopsis leaves on B5 medium without exogenous hormones for six to eight days, adventitious roots can be generated from the mid-vein near the wound (Liu et al., 2014). Using this system, Liu et al. revealed that the cell fate transition during the initiation of rooting contains two steps. In the first step, WUSCHEL-RELATED HOMEobox 11 (WOX11) and WOX12 act redundantly to regulate the transition from procambium cells to root founder cells. In the second step, root founder cells are further switched into root primordium cells, marked by WOX5 expression.

Notably, endogenous auxin plays essential roles in the cell fate reprogramming process. Inhibiting polar auxin transport using naphthylphthalamic acid (NPA) abolishes rooting, an effect which can be rescued by exogenous indole-3-acetic acid. During adventitious root formation, the distribution of auxin response signals overlaps with the expression regions of WOX11. Mutations of the auxin response elements within the WOX11 promoter or NPA treatment disrupt the expression pattern of WOX11 (Liu et al., 2014). Moreover, the auxin response signals are progressively enhanced and distributed in the region of root initiation, suggesting that the wounding induces auxin accumulation in this area. However, the molecular events between explant detachment and adventitious root initiation remain to be elucidated.

**YUCCA enzymes**

The research reported in this issue by Chen et al. (2016) describes the involvement of different members of the YUCCA (YUC) family, which encode enzymes catalysing the rate-limiting step of auxin biosynthesis in de novo root organogenesis. Under natural growth conditions, some plant species can generate adventitious roots from detached organs spontaneously, but in most cases application of exogenous auxin is required (De Klerk et al., 1999), suggesting that the endogenous auxin biosynthesis varies among the different species. However, using culture methods to induce rooting using exogenous auxin could bypass the function of endogenous hormones. In the system used by Chen et al. (2016), no exogenous hormones were added. Thus, adventitious root generation depended on endogenous hormones, providing an opportunity to investigate the roles of endogenous auxin in de novo root organogenesis.

Together with the previous findings from this lab (Chen et al., 2014; Liu et al., 2014), the results support a working model for de novo root organogenesis (see Chen et al., 2016, Fig. 9). The detachment of leaf explants leads to significant increases in the level of auxin. The elevated auxin content results from the function of YUC genes, which respond to wounding and act upstream of cell fate transition from competent cells (procambium and vascular parenchyma cells) to root founder cells. The YUC genes show division of labour and orchestrate auxin biosynthesis required for the formation of adventitious roots. Of them, YUC1, 2, 4 and 6 play major roles under both light and dark conditions. YUC1 and 4 function in a wounding-induced way, whereas YUC2 and 6 contribute to the basal auxin level (Box 1). In addition, YUC5, 8 and 9 mainly produce auxin in leaf margin and mesophyll cells in response to darkness.

**More questions**

A critical open question is how wounding signals trigger the spatial expression of YUC1 and 4. The authors suggest that wounding-response factors could regulate YUC1 and 4 in cooperation with epigenetic factors. This is supported by the fact that up-regulation of YUC1 and 4 expression is accompanied by a reduced level of histone H3 lysine 27 trimethylation, a marker of transcriptional repression (Schatlowski et al., 2008; He et al., 2013). It would be interesting to investigate the relationship between wounding signals and epigenetic factors, as well as their regulatory role in de novo root organogenesis.
Despite its importance to plant industries worldwide, adventitious root induction is still difficult in many species, hampering the development of forestry and horticulture (Rasmussen et al., 2012). The issue is mainly limited knowledge about the mechanisms controlling adventitious root formation, and therefore the findings presented here provide valuable new information. Genetic engineering approaches allowing the modification of endogenous auxin biosynthesis would now be powerful in enhancing our abilities.

Key words: Adventitious root, auxin biogenesis, de novo organogenesis, plant regeneration, YUCCA family.

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