Pinstatic acid as a dissection tool-kit for transcriptional and non-transcriptional auxin responses.

Auxin is one of the most studied plant hormones and it comes in various forms. Indole 3-acetic acid (IAA) is the most biologically active form of auxin among the endogenous auxins. Interestingly, phenylacetic acid (PAA), is more abundant auxin form across plant species and organs(Sugawara et al., 2015). While IAA and PAA affect the expression of the same set of auxin-responsive genes(Sugawara et al., 2015), PAA is not transported through the PIN proteins, which are responsible for IAA relocation. Interestingly, PAA does influence the rate of IAA transport(Johnson and Morris, 1987; Simon and Petrášek, 2011), possibly by affecting PIN trafficking in a more direct way. Due to the low mobility of PAA, local control of regulation of PAA metabolism might be the sole regulatory mechanism of PAA levels(Cook, 2019). While we know that PAA is the only phenyl derivative of from endogenous auxins, the metabolic pathway for making and breaking of PAA remains elusive(Cook et al., 2016).

In this issue of *Plant Physiology* Oochi (et al., (2019) reports on a screen of PAA derivates that affect the PIN localization without inducing the transcriptional responses through $SCF^{TIR/AFB}$ complex. They identified 4-ethoxyphenylacetic acid, dubbed by Oochi (et al., 2019) pinstatic acid (PISA), which enhanced hypocotyl elongation without activating the IAA-responsive DR5::GUS reporter. PISA treatment enhances auxin flow by blocking PIN endocytosis and reorientation. When the gravity vector is changed by turning a root sideways, PISA blocks IAA redistribution, which in turn prevents the root bending towards the gravity axis. While PISA treatment led to reduced polar PIN localization, the consequences for severe defects in plant development were visible only at high concentrations.

Interestingly, this loss of polar PIN localization was enough to rescues the effect of PIN misalignment in the PINOID (PID) overexpression lines. PID encodes a proteinkinase which is responsible for regulating PIN1 localization. In plants carrying the 35S::PID construct, PIN1 transporters are mislocalized on the shoot-ward facing side of the plasma membrane, leading to auxin depletion in the root meristem and meristem collapse,as PIN1 transporters are mislocalized on the shoot-ward facing side of the plasma membrane. PISA treatment rescued the collapsed root meristem of 35S::PID lines, restoring the auxin maximum by inducing the apolar PIN1 pattern and restoring auxin accumulation in the meristem.

Ochi (et al., 2019) observed that treatment with PISA results in an increased rate of auxin transport both root- and shoot shoot-ward. The PISA-increased rate of auxin transport resulted in lower auxin levels across the root length, leading to reduced root hair and lateral root development. Supplementation of PISA with IAA led to the increased lateral root development, suggesting that increased auxin transport might deplete IAA from developing lateral root primordia. The auxin-dependent effect of PISA was

additionally demonstrated as because the auxin synthesis and signaling mutants are insensitive to PISA (Ochi et al., 2019). These results illustrate the importance of auxin transport dynamics, resulting in depletion of local auxin maxima, which are essential for lateral organ formation.

Oochi (et al., 2019) presents an exciting new tool, PISA, that will allow the dissection between the physiological processes dependent on auxin transport and those dependent on transcriptional activation. Although the PISA's *modus operandi* remains to be decoded in the future studies, it will be exciting to examine its effect in other plant developmental processes relying on a change in auxin transport rate in response to the developmental signals or the changing environment.

Cook SD (2019) An Historical Review of Phenylacetic Acid. Plant Cell Physiol. doi: 10.1093/pcp/pcz004

Cook SD, Nichols DS, Smith J, Chourey PS, McAdam EL, Quittenden L, Ross JJ (2016) Auxin Biosynthesis: Are the Indole-3-Acetic Acid and Phenylacetic Acid Biosynthesis Pathways Mirror Images? Plant Physiol **171**: 1230–1241

Johnson CF, Morris DA (1987) Regulation of auxin transport in pea (Pisum sativum L.) by phenylacetic acid: effects on the components of transmembrane transport of indol-3yl-acetic acid. Planta **172**: 400–407

Oochi A, Hajny J, Fukui K, Nakao Y, Gallei M, Quareshy M, Takahashi K, Kinoshita T, Harborough SR, Kepinski S, Kasahare H, Napier R, Friml J, Hatashia K (2019) Pinstatic acid is a modulator of PIN trafficking. Plant Phys. XXX

Simon S, Petrášek J (2011) Why plants need more than one type of auxin. Plant Sci **180**: 454–460

Sugawara S, Mashiguchi K, Tanaka K, Hishiyama S, Sakai T, Hanada K, Kinoshita-Tsujimura K, Yu H, Dai X, Takebayashi Y, et al (2015) Distinct Characteristics of Indole-3-Acetic Acid and Phenylacetic Acid, Two Common Auxins in Plants. Plant Cell Physiol **56**: 1641–1654