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Cycling with brakes: ABA-INSENSITIVE4 controls cell cycle arrest in the root meristem

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Roots play critical roles in anchoring plants to the soil and taking up nutrients and water required for plant growth. Under favorable conditions, roots grow by a combination of cell division in the root apical meristem and cell elongation in the more proximal part of the root.

Root growth is tightly regulated in response to environmental changes, and stress signals affect root growth dynamics. For example, under moderate drought stress, root growth is maintained to seek water in deeper layers, while further elongation of the root is blocked when drought stress becomes severe and plants enter survival mode. Dynamic processes, such as plant organ growth, are often regulated by phytohormones (Pacifici et al., 2015). Amongst them, the hormone abscisic acid (ABA) plays key roles in regulating root growth according to water availability. Previous studies have shown that ABA can stimulate root growth when its concentration in the root is slightly increased by mild drought (Zhang et al., 2010). In contrast, high ABA concentrations trigger root growth arrest (Rowe et al., 2016), but the precise molecular mechanisms remain unknown, particularly with respect to how ABA inhibits cell division in the root meristem.

In the current issue of the *Plant Physiology*, Luo et al. (2022) identify a regulatory module connecting the ABA signaling pathway to cell division arrest in the root. By combining genetic, molecular, and cellular approaches, Luo et al. demonstrated that the transcription factor ABA-INSENSITIVE4 (ABI4) represses the cell cycle in root meristems of Arabidopsis (*Arabidopsis thaliana*; Figure 1). They measured the length of primary roots of 3-day-old seedlings exposed to ABA treatments or control conditions and observed that ABA negatively influenced root growth in wild-type (WT) plants, while *abi4* mutants were significantly less affected. In contrast, seedlings overexpressing *ABI4* were hypersensitive to ABA treatments, with roots even shorter than WT. At the cellular level, the number of cells forming the division zone in the root meristem decreased in the WT with ABA treatments (Figure 1), a negative effect on cell division that was not observed in the absence of ABI4. These phenotypic measurements indicated that ABA and the downstream factor ABI4 have the potential to inhibit cell division in the root meristem.

To further explore the effect of ABI4 on root cell division, the authors performed gene expression analysis in root meristems by specifically microdissecting the root tips of WT plants and *abi4* mutants. Most of the cell cycle genes were expressed at a significantly higher level in root tips of the *abi4* mutant than in WT, suggesting that *abi4* undergoes more cell divisions. Cell cycle is intimately orchestrated by core proteins that allow progression through the different cell cycle phases. Typically, the plant cell cycle is divided into four major phases: the DNA-synthesis phase (S), the mitotic division phase (M), and gap phases preceding each of these phases (G1 and G2; Figure 1). The cell cycle is driven mainly by CYCLIN-DEPENDENT KINASES (CDKs) that phosphorylate downstream genes acting in the different cell cycle phases. As their name suggests, CDK activity depends on their association with CYCLIN proteins (CYCs), which also determine the specificity of the CDK target proteins. For example, CYCB1;1 is a typical mitotic CYC that stimulates M-phase progression when associated with the plant-specific B-type CDKs, CDKB1;1 or CDKB2;2 (Van Leene et al., 2011). Luo et al. (2022) found that the *abi4* mutant had higher expression of cell cycle genes associated with multiple cell cycle phases, indicating stimulation of the whole cell cycle rather than an enrichment of one specific phase.

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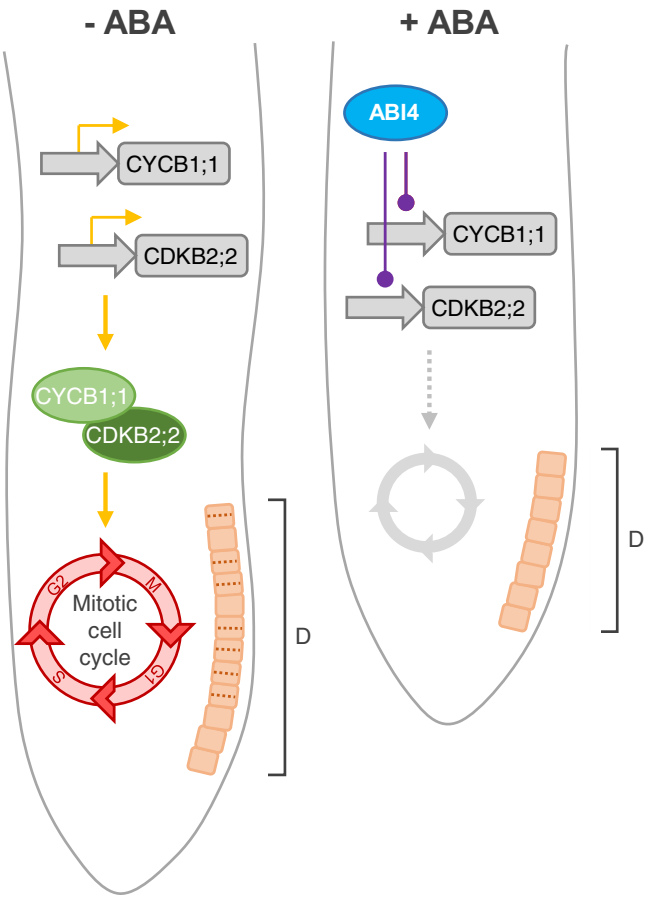

Figure 1 Schematic overview of how ABA blocks the cell cycle in root meristems. In the absence of ABA (left), the progression of the mitotic cell cycle is triggered by the expression of cell cycle-stimulating proteins, such as *CYCLIN B1;1* (*CYCB1;1*) and *CYCLIN-DEPENDENT KINASE B2;2* (*CDKB2;2*). An active mitotic cell cycle ultimately results in new cell divisions (red dashed lines) in different root cell layers (here, orange cells represent one cell layer). When roots are treated with ABA, the expression of the transcription factor *ABA-INSENSITIVE4* (*ABI4*) is induced and ABI4 directly inhibits the expression of *CYCB1;1* and *CDKB2;2* (Luo et al., 2022). As a result, the production of these core cell cycle proteins is reduced and cell cycle activity decreases. Consequently, cell division is inhibited, and the size of the division zone (D) is reduced compared to control conditions. Gold and purple connections represent positive and negative actions, respectively. G1, first gap phase; S, DNA-synthesis phase; G2, second gap phase; M, mitotic division phase.

ABI4 is a well-known transcriptional repressor downstream of the ABA signaling pathway (Shu et al., 2016, 2018); however, ABI4 target genes in the root meristem were previously unknown. To identify ABI4 targets at the genome-wide level, Luo et al. (2022) performed a Chromatin Immuno Precipitation (ChIP) experiment pulling down a GFP-tagged ABI4 followed by sequencing the DNA that interacted with ABI4 (ChIP-sequencing). Thousands of putative ABI4 target genes were identified and, notably, the ABI4-binding sites were located in the promoter region (3,000-bp upstream of the start codon) in ~90% of these putative targets, consistent with what we can expect from a transcription factor–target interaction (Luo et al., 2022). Amongst the identified target genes, dozens of genes were related to cell cycle, and the authors further focused on two of them, *CYCB1;1* and *CDKB2;2*, which encode core proteins acting during mitosis. Luo et al. (2022) confirmed the direct interaction between ABI4 and the promoters of *CYCB1;1* and *CDKB2;2* using molecular assays in Arabidopsis, *Nicotiana benthamiana*, and yeast (*Saccharomyces cerevisiae*). Together with the increased expression of those genes in *abi4* mutants, these data strongly suggest that ABI4 inhibits the expression of *CYCB1;1* and *CDKB2;2*. To further validate this at phenotypic level, the authors generated transgenic lines overexpressing both *ABI4* and one of the two target genes (*CYCB1;1* or *CDKB2;2*). They observed that the short root phenotype of *ABI4*-overexpressing plants was rescued when one of the two downstream cell cycle genes was co-overexpressed. Similarly, mutating *CYCB1;1* or *CDKB2;2* by CRISPR–Cas9 in the *abi4* mutant background suppressed the longer root phenotype of the *abi4* mutant during ABA treatment.

Altogether, the work presented by Luo et al. (2022) uncovered a regulatory module in which the ABA-responsive transcription factor ABI4 directly inhibits the promoter activity of at least two core cell cycle genes, *CYCB1;1* and *CDKB2;2*. These findings contribute to our knowledge on the regulation of the plant cell cycle, a mechanism at the basis of primary plant growth, but also of multiple specific developmental processes. Despite the long history of cell cycle research in Arabidopsis (Gutierrez, 2016) and the importance of understanding how the cell cycle is regulated, surprisingly little is known about how environmental signals act on the core cell cycle (Qi and Zhang, 2020). Acquiring insights into how stress or stress-responsive hormones, like ABA, can control cell cycle activity in meristems is a major step towards engineering plants that could circumvent the stress-induced cell cycle arrest. The engineered plants could potentially maintain better proliferative growth under adverse conditions, a trait that will become increasingly important in the challenge of securing food production in a changing climate.

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References

Gutierrez C (2016) 25 years of cell cycle research: what's ahead? Trends Plant Sci **21**: 823–833

Luo X, Xu J, Zheng C, Yang Y, Wang L, Zhang R, Ren X, Wei S, Aziz U, Du J, et al. (2022) Abscisic acid inhibits primary root growth by impairing ABI4-mediated cell cycle and auxin biosynthesis. Plant Physiol **191**: 265-279

Pacifici E, Polverari L, Sabatini S (2015) Plant hormone cross-talk: the pivot of root growth. J Exp Bot **66**: 1113–1121

Qi F, Zhang F (2020) Cell cycle regulation in the plant response to stress. Front Plant Sci **30**: 1765

Rowe JH, Topping JF, Liu J, Lindsey K (2016) Abscisic acid regulates root growth under osmotic stress conditions via an interacting

hormonal network with cytokinin, ethylene and auxin. New Phytol **211**: 225–239

Shu K, Chen Q, Wu Y, Liu R, Zhang H, Wang P, Li Y, Wang S, Tang S, Liu C, et al. (2016) ABI4 mediates antagonistic effects of abscisic acid and gibberellins at transcript and protein levels. Plant J **85**: 348–361

Shu K, Zhou WG, Yang WY (2018) APETALA 2-domain-containing transcription factors: focusing on abscisic acid and gibberellins antagonism. New Phytol **217**: 977–983

Van Leene J, Boruc J, De Jaeger G, Russinova E, De Veylder L (2011) A kaleidoscopic view of the Arabidopsis core cell cycle interactome. Trends Plant Sci **16**: 141–150

Zhang H, Han W, De Smet I, Talboys P, Loya R, Hassan A, Rong H, Jurgens G, Paul Knox J, Wang MH (2010) ABA promotes quiescence of the quiescent centre and suppresses stem cell differentiation in the Arabidopsis primary root meristem. Plant J **64**: 764–774