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Spotlight

Endoreplication controls cell size via mechanochemical signaling

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During hypocotyl development, an asymmetric auxin gradient causes differential cell elongation, leading to tissue bending and apical hook formation. Recently, Ma *et al.* identified a molecular pathway that links auxin with endoreplication and cell size through cell wall integrity sensing, cell wall remodeling, and regulation of cell wall stiffness.

After seed germination in the soil, the hypocotyl forms an apical hook to protect the cotyledons and the shoot apical meristem while being pushed through the surrounding soil, preventing damage to these important tissues. This apical hook is maintained until the seedling emerges from the soil. The formation and maintenance of this apical hook results from asymmetrical cell elongation between outer and inner epidermal cell layers, which is accompanied by asymmetries in the distribution of the hormone auxin and of mechanical properties of the cell walls throughout the bend. Rapidly elongating cells at the outer side of the hook have a low auxin content and more flexible walls, while cells at the inner side of the hook contain more auxin and stiffer walls, inhibiting cell elongation and, thus, resulting in smaller cells [1,2]. After the hook is formed, this structure needs to be maintained until the seedling emerges from the soil, after which the hook is opened and cotyledons are exposed to sunlight. The prevention of premature hook opening during growth in the soil requires tight control of cell size in the different cell layers that build up the hook.

The contribution of endoreplication to the control of cell and organ size is a matter of debate, because changes in endoreplication do not always clearly explain the observed cell size phenotypes [3,4]. For instance, mutants with severe inhibition of endoreplication can continue to grow [5], contradicting the ploidy-dependent growth hypothesis. Additionally, correlation between cell size and endoreplication is not universal because it can be tissue type specific and/or depend on cell position within tissues (e.g., [6]). Recent studies also indicate that cell size can even differ in actively dividing mitotic cells [3], and endoreplication may drive cell wall modifications to support changes associated with cell growth instead of ploidy-dependent growth [7].

Thus, to study the biochemical and mechanical bases of cell size control by endoreplication, Ma *et al.* [8] used apical hook formation in the dark-grown hypocotyl as an elegant model system. In *ccs52a2*, a mutant in CELL CYCLE SWITCH PROTEIN 52A2 with strongly reduced endoreplication, the hook maintenance phase is 32 h shorter than normal. In wild-type hooks, endoreplication levels, measured by DAPI and DRAQ5 staining of nuclear DNA and by nuclear volume measurements, go hand in hand with epidermal cell size and display a gradient from the outer to the inner side of the bend. Parallel to this, fluorescently tagged CCSA52 protein abundance was higher at the outer side of the hook, and lower in cells at the inner side. As expected, this endoreplication asymmetry was absent in *ccs52a2*. All these data point to the importance of CCS52A2-mediated endoreplication asymmetry, with high endoreplication levels in cells at the outer side and lower at the inner side, for hook maintenance in the etiolated hypocotyl.

Ma *et al.* then studied whether the asymmetry in auxin response, which is high in cells at the inner side of the hook and low at the opposite side [1,2], is responsible for the observed endoreplication asymmetry. They used the *pin3pin4pin7*-triple mutant, which shows impaired formation of the auxin gradient and the hook [9,10], and treated wild-type plants with the auxin-transport inhibitor NPA. This approach showed that PIN-mediated auxin transport is required for the creation of CCS52A2 abundance asymmetry and the resulting endoreplication gradient in the hook.

Comparison of cellular elongation rates at opposite sides of the hook in wild-type and *ccs52a2* plants revealed that a strong reduction in the elongation rate in the cells at the outer hook side is responsible for the observed hook maintenance defects in *ccs52a2*. The authors then showed that this absence of growth asymmetry in *ccs51a2* is caused by increased stiffness of the walls at the outer side of the hook, which causes slower cell growth and, thus, affects the formation of the cell growth asymmetry. In normal conditions, rapid elongation of cells at the outer side is due to less rigid cell walls with a low content of methylesterified homogalacturonan (HG) pectin. It has been shown before that interference with pectin demethylesterification, by overexpression of *PECTIN METHYLESTERASE INHIBITOR 5* (*PMEI5*) or by exogenous application of epigallocatechin gallate (EGCG), leads to hook defects because of increased wall stiffness and slower elongation of the outer cells [1]. Treatment of wild-type and *ccs52a2* with low amounts of EGCG had no effects on wild-type hook formation, but made the defects worse in *ccs52a2*. Quantification of the labeling by antibodies specific for methyl- or demethylesterified HG confirmed higher demethylesterification levels in the walls at the outer side versus the inner side in the wild-type. This is consistent with the increased cell wall stiffening

and consequently slower cell elongation on the outer side of the hook. The authors also confirmed that the hook defects are caused by enhanced HG methylesterification in *ccs52a2*.

Having established the importance of differential HG methylesterification in the growth asymmetry, Ma *et al.* used a genetic approach to further unravel the link between endoreplication, HG methylesterification levels, and hook defects. They crossed a loss-of-function mutant in *RECEPTOR-LIKE PROTEIN 44* (*RLP44*), a plasma membrane receptor that responds to pectin modification [11], in *ccs52a2* and noticed that this additional mutation suppressed hook maintenance defects seen in *ccs52a2,* which suggests that sensing of apoplastic pectin status is important in the regulation of differential cell elongation. Furthermore, high upregulation of transcription factor ETHYLENE RESPONSE FACTOR 115 (ERF115) and its close homologs *ERF108*, *ERF113*, and *ERF114* in *ccs52a2* relative to wild-type, and especially *ccs52a2*-like hook maintenance defects in the overexpression line of ERF115, led the authors to verify whether the *ccs52a2*-hook defects could be caused by misexpression of ERF155. Crosses between *erf115* and ERF115-overexpressing lines with the *ccs52a2* mutant showed partially rescued and aggravated hook maintenance defects, respectively, confirming the idea that increased ERF115 activity contributes to the hook defect of the *ccs52a2* mutant. This idea was further strengthened by the observation that introducing a dominant-negative ERF115^{SRDX} fusion into the *ccs52a2* background almost completely suppressed the *ccs52a2*-hook defects, by reverting the cell growth rates of the outer side cells to wild-type levels and, thus, also by restoring the growth gradient throughout the hook. These experiments identified ERF115 family transcription factors as negative regulators of hook development, downstream of CCS52A2-mediated endoreplication.

Next to the identification of this molecular pathway, the authors investigated the potential contribution of mechanochemical feedback during hook development. Given previous observation that members of the *Catharanthus roseus* RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1Ls) superfamily monitor cell wall integrity in different organs and mediate changes in HG methylesterification (e.g. [12]), they crossed the *theseus1* (*the1*) mutant in the *ccsa52a2* background. Whereas *the1-1* did not show any hook defects, the introduction of *the1-1* in the *ccs52a2* background largely suppressed the hook maintenance defect, by restoring cell growth rates in the outer side cells to normal, which reintroduced growth asymmetry throughout the hook cells. Furthermore, the hook defect phenotype seen in the ERF155-overexpressing line was greatly rescued by introducing *the1-1*. This suggests that THE1 has a role in the perception of, and signaling pathway induced by, the increased wall stiffness resulting from elevated HG methylesterification in *ccs52a2*.

These data collectively led the authors to propose a working mechanism (Figure 1) in which PIN-dependent polar auxin transport controls cellular auxin abundance in cells of the apical hook. Auxin-level asymmetries then regulate spatial differences in CCS52A2-dependent endoreplication levels, which, in turn, control the degree of ERF115-mediated HG methylesterification, which affects cell wall properties. Cell elongation is then controlled by mechanical properties of the cell wall and signaling via cell wall integrity receptor-like kinase THE1, a proposed mediator of HG methylesterification levels. This model links endoreplication with control of cell size through modulation of cell wall stiffness via a mechanochemical feedback that involves cell wall integrity sensing.

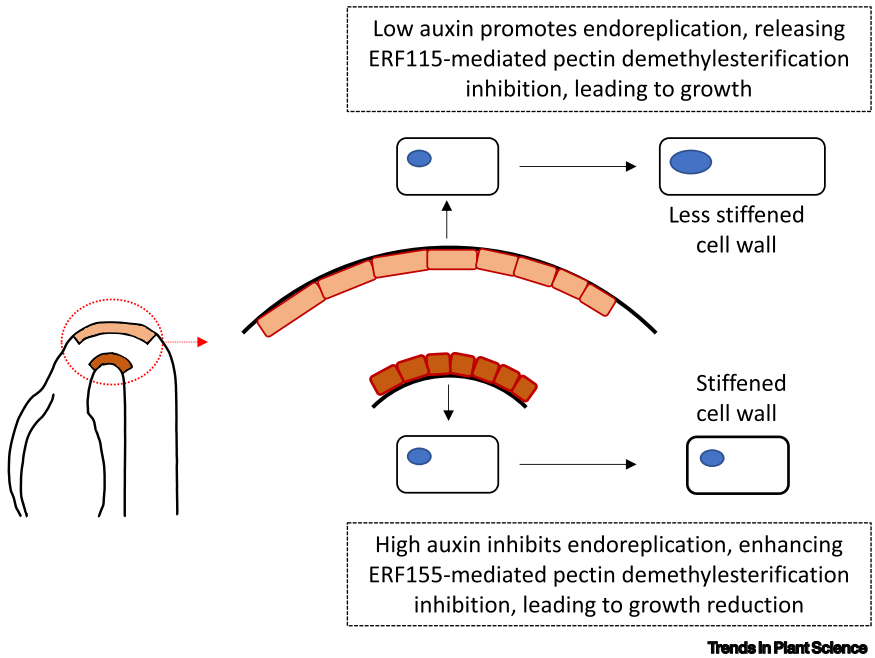

Figure 1. Working mechanism proposed by Ma *et al.* linking endoreplication with control of cell size during apical hook formation [8]. An asymmetric auxin gradient controls differential endoreplication on the outer (cells colored light orange) and inner (cells colored dark orange) side of the hook. This negatively regulates ETHYLENE RESPONSE FACTOR 115 (ERF115) and related members to modulate homogalacturonan (HG) methylesterification levels and, consequently, cell stiffness to control differential cell size.

Overall, this model appears consistent with the notion that endoreplication drives cell wall changes that support cell growth instead of ploidy-dependent growth. Future research could verify whether this model is conserved between organs and plant species in the contexts of development and environmental stress adaptation.

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Declaration of interests

No interests are declared.

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