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Brassinosteroids *en route*

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Brassinosteroids (BR) hormones promote root growth controlling meristem size and cell elongation. Unlike other plant hormones that have shown to move across plant cells, BR transport remain elusive. A new study shows that BR precursors move via intercellular pores named plasmodesmata to modulate BR cellular levels and their signaling function.

Brassinosteroids (BRs) are essential for plant growth and development across the entire life cycle [1]. Together with BRs, other phytohormones, e.g., auxins, and abscisic acid, are part of intervening signaling networks to determine plant organogenesis and adaptation. Hormone signaling is spatially regulated by the relative activity of biosynthetic and catabolic enzymes and by short- and long– distance transport mechanisms that facilitate their distribution across the plant [2]. Despite the current advances in understanding the spatiotemporal control of BRs in root growth and development [3], the mechanisms that determine BR levels and differential distribution across neighboring tissues remain unknown. In this issue of *Nature Chemical Biology*, Wang *et al* [4] report that intercellular transport of BR precursors at short distances occur via plasmodesmata (pores in cell walls connecting the cytoplasm of neighboring cells [5]), and that a feedback mechanism is triggered in response to BR endogenous levels to control plasmodesmata flow rate thus BR biosynthesis and function (Fig. 1)

Plasmodesmata are membranous pores on the cell wall delimiting a cytoplasmic sleeve for the cell-to-cell diffusion of small and large molecules such as metabolites, RNAs and proteins [5, 6]. The transport of molecules via plasmodesmata (symplasmic transport) is restricted by the deposition of the cell wall glucan callose [6] (Fig. 1). Callose turnover (synthesis/degradation) at plasmodesmata is controlled by members of the families of

CALLOSE SYNTHASES (CALS, also known as glucan synthase-like or GSL) and ß- 1,3 GLUCANASES (BGs) and is also regulated by PLASMODESMATA LOCATED PROTEINS (PDLPs) and PLASMODESMATA CALLOSE BINDING proteins (PDCBs) [6]. Mutations in the plasmodesmata-located CALS3 (cals3m) and double mutant in the plasmodesmata-located BGs, PdBG1 and PdBG2 (*pdbg1pdbg2*) affect root growth and lateral root development [7].

Wang *et al* [4] found that increasing callose reduces the amount of dephosphorylated and nuclear-localized BES1 (the active version of a transcription factor downstream BR signaling). Conversely, ectopic expression of the callose-degrading enzyme PdBG1 leads to the accumulation of dephosphorylated BES1. To further investigate callose- BR interaction, the authors treated Arabidopsis roots with the BR inactive precursor 22-hydroxycampesterol (22-OHCR). Blocking plasmodesmata by induction of cals3m in the endodermis, restricted 22-OHCR transport and its capacity to complement the BR biosynthesis mutant *dwf4*. A newly-developed chemical probe (castasterone-alkyne, CSA) enabled fluorescent detection of BR biological activity in Arabidopsis roots. CSA accumulation was restricted to the cortical or epidermal tissues in roots expressing cals3m offering compelling evidence that callose deposition affects the transport of BRs across tissues (Fig. 1). High BR concentration, on the other hand, enhances callose deposition and reduces symplasmic (plasmodesmata-mediated) transport. The authors concluded that BR levels alter callose and regulate intercellular transport through a feedback mechanism controlling BR signaling.

This study adds to our understanding of BR signaling by revealing the contribution of plasmodesmata in the BR-mediated response (Fig. 1). Two negative feedback loops regulate BR homeostasis by controlling i) cellular expression of BR-induced transcription factors and BR biosynthetic enzymes; and ii) the intercellular transport of BR precursors via regulation of callose at plasmodesmata. It remains to be determined how BRs control callose metabolism, but transcriptomic analysis identified CALSs and PDLPs upregulation after BR treatment [8]. Future work is required to reveal the nature of the endogenous mobile BR signal. It is possible that a mobile upstream component (e.g., a transcription factor) could regulate BR distribution. The requirement for BR biosynthesis to occur at the plasmodesmata vicinity for effective intercellular transport also needs further investigation.

To summarize, the work reveals that symplasmic intercellular transport is important for BR signaling (and likely for other hormonal signaling networks), while opening significant questions about the routes for hormonal movement in plants. These findings strengthen the

view of plasmodesmata as signaling hubs integrating hormonal, environmental and developmental cues, to define plant development. The precise molecular mechanism allowing plasmodesmata to remain open to certain signals while blocking others is unknown exposing the inherent complexity of plant cells. Research in these mechanisms is required to identify strategies that modify plasmodesmata for crop improvement.

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Figure 1. Symplasmic intercellular movement of Brassinosteroid compounds in Arabidopsis primary root. The model represents the passive molecular transport via Plasmodesmata (PD, pores found in callose-enriched cell walls) of castasterone (CS, a precursor converted to brassinolide in the endoplasmic reticulum, ER), as a mechanism regulating root growth in *Arabidopsis thaliana*.

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