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Plant signaling: Interplay of brassinosteroids and auxin in root meristems

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Summary (no more than 40-word): Plant hormones brassinosteroids and auxin mutually interact to regulate the meristem size of roots. A new study revealed dual and opposing roles for brassinosteroids in auxin biosynthesis and signaling output in the root epidermis, and demonstrates that epidermis-derived BR signaling is required for root meristem maintenance through its effect on auxin signaling.

Plant root growth relies on a balanced control on cell division and elongation. High cell division activity in meristems allows for its self-renewal and provides cells for continuous growth. Root growth has, however, to be correlated with the overall growth of the plant and with the environment but many questions remain unanswered on how plants get this done. Plant hormones such as auxin were shown to play a key role, either through the activation of their signaling components, or through interaction with other hormones.[1,2] As several hormones have been shown to impact root growth in various ways, one of the big challenges for future research will be to find out how these pathways interconnect in order to control root growth and development. In this issue of Current Biology, Ackerman-Lavert et al., investigate the influence of steroidal phytohormone brassinosteroids (BRs) on root meristem maintenance, and unveil dual BR effects on auxin synthesis and signaling that rely on BR signaling in outer root tissues.

Over the past decades, BR has been found to cause pleiotropic effects in plant development and responses to stresses,[3,4] while BR biosynthesis and signaling mutants display a short-root phenotype along with a strong shoot defect.[5-7] Although it is suggested that the core BR receptor kinase BRI1-ativated signaling in the epidermis is critical for BR effects on root meristem activity,[8] the molecular mechanism has not yet been fully determined.

A recent study revealed that the role of BR in root development involves spatiotemporal regulation of its metabolism in root tissues.[6] BR biosynthetic pathway genes were found to be expressed in different tissue layers in the root meristem and elongation zone (EZ), and a short-distance movement of BR and its intermediates in the radial direction is required to complete BR biosynthesis (Fig.1). BR biosynthesis is more active in the EZ than in the meristem mirroring a BR signaling gradient along the longitudinal axis of the root. This spatial variation in BR concentration and signaling leads to distinguished cellular behaviors: expansion in the EZ and division in the meristem (Fig.1). Thus, increasing BR levels in the EZ contributes to the decision of meristematic cells to transit into an elongation/differentiation state.

Besides sustaining low BR levels, root meristems are hypersensitive to brassinolide (BL) (an active BR) treatment causing a reduction of the root meristem and shorter roots.[9] This is suggested to result from antagonistic action of BR versus auxin on gene expression and root meristem activity.[10,11] Auxin concentration and signaling in the root tip contrast the expression of BR biosynthesis and signaling genes, and a mutually regulation between BR and auxin biosynthesis has been reported.[12] However, it remains undetermined whether this BR-auxin interaction contributes to the control on meristematic activity and how this interaction is integrated in the spatiotemporal regulation of BR signaling.

In this new study, Ackerman-Lavert et al, revealed dual, seemingly opposing, effects of BR in, on the one hand, promoting auxin biosynthesis and on the other hand inhibiting the output of auxin signaling. They further demonstrate that both activities co-exist in the outer tissues (i.e. epidermis and lateral root cap) of the root meristem and are critical for the meristematic state of the root (Fig.1).

To probe a role for auxin in the BR regulated meristematic state, the authors took off by evaluating the spatial changes in the endogenous auxin content and signaling output in the root meristem under treatments of BL and a BR biosynthesis inhibitor BRZ. The authors found that increasing BR elevates the expression of the auxin biosynthesis gene *YUC9* and auxin levels in the root meristem. However, high BR levels attenuated auxin signaling in the root meristem visualized by reduced expression of high auxin-sensitivity markers, while BRZ treatment on the opposite elevated auxin sensitivity. This opposite impact of BR on auxin biosynthesis and signaling synchronously occurs in the epidermis, indicative for a dual role of BR in auxin metabolism in a tissue-specific manner. These results further corroborate previous studies showing that the outer layer tissues of roots are highly sensitive to BR signaling.[6,8]

To assess the influence of this dual effect of BR on the meristematic state of the root, the authors examined the response of the root meristem to BR in auxin biosynthesis mutants. Either high BR or low auxin levels reduced root meristem size, while high BR/low auxin levels led to a complete loss of the root meristem. Exogenous auxin supply or a specific induction of auxin production in epidermis could recover the root meristem, demonstrating epidermis-derived auxin is required to buffer the BR effect on meristem maintenance. These effects are also reflected at the transcriptional level, with high BR or low auxin levels only causing a transient change in the expression of a small number of genes whereas a synergistic reprogramming of massive gene expression was observed upon the high BR/low auxin level. These findings thus indicate a feed-back regulation of BR-induced auxin biosynthesis on transcriptional responses in line with the meristematic state.

The authors also determined the impact of reduced BR levels and signaling on auxin homeostasis and the meristematic state of the root, and showed that reduced BR biosynthesis and signaling can override the meristem loss by the perturbation of auxin biosynthesis. Root meristems are lost when auxin levels are extreme low, but is maintained upon the perturbations of BR biosynthesis and signaling. Noticeably, perturbation of BR biosynthesis cannot alleviate auxin biosynthesis, but recovers the auxin signaling in the stele and root meristem, further confirming the dual function of BR on auxin biosynthesis and signaling. Furthermore, the authors showed that this regulatory mechanism also functions during the root meristem regeneration after wounding.

Given auxin levels and signaling output are affected by BR signaling in the epidermis, the question arises whether the role of BR in regulating meristematic state is restricted to this tissue. To address this, the authors applied an elegant "BR toolkit" to explore the requirement of BR-regulated signaling in distinct root tissues for meristem maintenance. Through tissue-specific activation of *bin2-1*, a dominant mutant of the BR signaling inhibitor kinase, the authors showed that ectopically blocking BR signaling in the outer tissues of the root, rather than other tissues, resulted in a root meristem phenotype reminiscence of BR signaling mutants. These results highlight a vital role of epidermis-derived BR signaling on the meristematic state and root growth.

In contrast, blocking BR signaling in the stele resulted in longer cells and a thinner meristems, reminiscent of enhanced BR effects. The authors further provided evidence that in these plants transcripts of BR biosynthesis genes (i.e. *DWF4* and *CDP*) were induced in the stele thereby resulting in higher levels of BR precursors and leading to a hypersensitive meristematic state and root waviness. By developing a ratiometric BES1/H2B based BR signaling reporter, the authors further demonstrated that the increased BR levels are perceived in the epidermis. This is accompanied with increased *YUC9* expression and enhanced meristem sensitivity to low auxin, mimicking high BR effects. These data collectively suggest inhibition of BR signaling in the stele shows a positive feedback on BR biosynthesis, in turn promoting epidermal BR signaling and meristem sensitivity to low auxin (Fig.1).

Moreover, in plants with interrupted epidermis-derived BR signaling, *YUC9* expression was reduced under high BL treatment, while an enhanced auxin signaling output in epidermis was observed. Correspondingly, these plants also maintained intact root meristems under the combination of BL and L-Kyn, again confirming a dual role of BR on auxin action, while relying on BR signaling in the epidermis.

Hormonal interactions are multilayered in nature and the majority of studies on root growth and development only focused on one hormone at the time making it impossible to get a global picture how the different hormones act together to maintain meristematic activity. Ackerman-Lavert et al. were brave enough to undertake this challenge and succeeded in providing a novel mechanistic understanding of the antagonistic function of BR and auxin in root meristem maintenance (Fig.1), whereby a balance of these hormones rather than their overall levels determine meristematic activity. Furthermore, this study highlights the importance of the outer tissues, mainly the epidermis, for BR perception and signaling output. The epidermis-derived BR responses are unevenly distributed along the longitudinal axis, thereby ensuring the transition of meristematic into the cell elongation phase.[6]

<u>Legend to Figure 1</u>

Figure 1. BR exerts dual effects on auxin biosynthesis and signaling in the root epidermis to mediate meristem maintenance.

In the elongation zone of the Arabidopsis root, BR biosynthesis genes, CPD and DWF4, are highly expressed in the stele and epidermis leading to elevated BR levels promoting cell elongation through BR signaling. On the opposite, in the meristem BR levels and signaling are kept low to ensure cell division activity thereby maintaining the root meristem. In the meristem, BR biosynthesis is negatively regulated by stele-derived BR signaling. In the case of reduced BR signaling in the stele as shown by Ackerman-Lavert et al., BR biosynthesis will be induced and BR levels will be elevated as the result of the known feedback regulation on BR biosynthesis. In such a case, elevated BR levels will subsequently activate BR signaling in the epidermis, which in turn enhances local auxin biosynthesis but represses auxin signaling output, finally determining meristem shape and maintenance and nicely demonstrating how the root meristem is buffered towards hormonal fluctuations

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