Cell Reports

CellPress
OPEN ACCESS

Review

Hormonal regulation of primary root development

Michela De Nittis,[1,2] Mirko De Vivo,[1,2] Raffaele Dello Ioio,[1,*] and Sabrina Sabatini[1,*]
[1]Department of Biology and Biotechnology "Charles Darwin", University of Rome "Sapienza", via dei Sardi, 70, 00185 Rome, Italy
[2]These authors contributed equally
*Correspondence: raffaele.delloioio@uniroma1.it (R.D.I.), sabrina.sabatini@uniroma1.it (S.S.)
https://doi.org/10.1016/j.celrep.2025.115751

SUMMARY

Plant post-embryonic development is characterized by the unceasing generation of new tissues and organs that allow plants to adapt their growth and architecture to environmental conditions. Primary root indeterminate growth is sustained by the activity of the root apical meristem (RAM), an organized structure containing stem cells that continuously divide to generate new tissues. RAM, thanks to its simple and symmetric structure, is an excellent system for studying how hormone distributions and interactions regulate growth. Plant hormones are pivotal actors in establishing and maintaining RAM patterning, and their developmental outputs are dependent on their integrated activities. In this review, we discuss recent findings on hormonal crosstalk that shapes the root meristem, focusing our attention on both the root radial and longitudinal axes.

INTRODUCTION

Plant hormones are endogenous signals that play a pivotal role in coordinating plant indeterminate growth in response to internal and external inputs, contributing to a robust but also plastic development, necessary to adapt the plant architecture to variable environmental conditions.[1] Post-embryonically, the generation of new structures and organs is due to the activity of meristems, organized structures containing stem cells that continuously self-renew and produce a population of transit-amplifying cells that eventually differentiate and give rise to new tissues and organs.[2] In this review, we focus on the role of hormones in coordinating *Arabidopsis thaliana* primary root growth, which is sustained by the activity of the root apical meristem (RAM). The RAM is located at the root tip and has a simple structure, and cells of different lineages and developmental stages can be easily followed during development, based on their cell position, shape, and dimension[3,4] (Figure 1). Different tissues are distributed in concentric layers with the most external tissue, the epidermis, followed by the cortex, endodermis, pericycle, and, most internal, the vascular tissues (Figure 1B). Apically, the RAM is surrounded by the root cap, composed by the columella at the very root tip and the lateral root cap (LRC). RAM presents two main developmental axes: the longitudinal axis, extending from the root tip through the root-shoot junction, and the radial axis, across which different tissues are distributed in concentric layers.[3,5,6] All root tissues are generated by the activity of stem cells contained in the stem cell niche (SCN) present at the tip. The SCN is composed of an organizer, the quiescent center (QC), surrounded by five sets of cell-lineage-specific stem cells: the epidermis and LRC initials, cortex and endodermis initials (CEIs), pericycle initials, vasculature initials, and columella initials[3,5,7](Figure 1C). The QC is enveloped by these groups of stem cells, and it functions to preserve their stemness by cell-to-cell contact.[7] Asymmetric and anticlinal divisions of stem cells in the RAM generate daughter cells that give rise to both the proximodistal and radial axes, through stereotypical divisions. Tissue organization in concentric layers on the radial axis of the root is achieved through periclinal divisions of stem cell daughter cells, which leads to the development of tissues with distinct identities. Additionally, radial expansion of the root meristem is achieved through periclinal divisions, which increase the number of procambium cells in the stele, and through tangential divisions, which add new cell files to specific tissues.[6]

Among the longitudinal axes (Figure 1A), cells of different tissues show a coordinated developmental stage, generating progressive developmental zones: in the SCN, stem cells divide asymmetrically producing a population of transit-amplifying cells that undergo a fixed number of divisions along the longitudinal axis, forming the meristematic zone (MZ). The MZ is followed by the elongation zone (EZ), where cells stop dividing and start to elongate longitudinally. In the EZ, the differentiation processes begin and are eventually completed in the differentiation zone (DZ), where cells develop the lineage-specific characteristics of the different root tissues. The boundary between the MZ and EZ is the transition zone (TZ), where cells coordinately change their cellular state from division to differentiation.[3,4] Indeed, along the longitudinal axis, the TZ is a fundamental developmental boundary, and its positioning is finely tuned to guarantee the correct balance between division and differentiation necessary to sustain root growth.[4,8,9]

Hormones are fundamental actors in root patterning, and their activity is integrated into a complex network of interactions that is dependent on their spatiotemporal distribution.[1,10–12] The peculiar distribution and site of activity of different hormones are achieved through hormone-specific biosynthesis, degradation, transduction, and transport pathways.[10,13–15]

Different hormones can establish synergistic or antagonistic interactions in a context-specific manner. These outputs can be achieved through different mechanisms such as, for example,

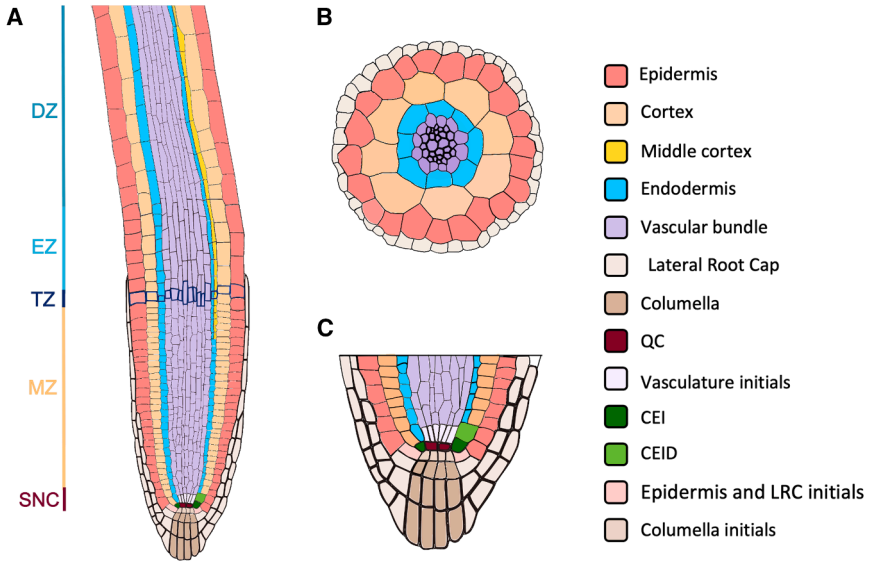

Figure 1. Representation of the *Arabidopsis* root tip structure

(A) Representation of a longitudinal section of the RAM. The color-coded bar on the left represents root zonation among the longitudinal axes: SCN, stem cell niche; MZ, meristematic zone; TZ, transition zone; EZ, elongation zone; DZ, differentiation zone. Tissues of the root are represented in different colors.

(B) Representation of the radial section in the RAM where root tissues, highlighted by different colors, are distributed in concentric layers.

(C) Representation of the SCN of RAM showing the QC and the 5 sets of initials that give rise to all the root tissues.

the direct regulation of specific proteins or a common set of genes or, more indirectly, by modulating another hormone's metabolism, signal transduction, and transport.[1]

In this review, we focus on the well-characterized molecular pathways that are controlled by auxin, cytokinin (CK), gibberellins (GAs), brassinosteroids (BRs), abscisic acid (ABA), and ethylene and their role in RAM development in both the longitudinal and radial axes.

Hormonal crosstalk among the longitudinal axes

To sustain indeterminate root growth, proliferation, elongation, and differentiation processes occurring along the RAM longitudinal axis are finely coordinated to maintain the correct balance between cell division and differentiation rates.[3,16,17] At 5 days post-germination, a steady state between division and differentiation processes is established, resulting in TZ positioning.[17,18] Hormones and their interactions are fundamental for instructing and maintaining the molecular networks necessary to establish cell activity and root zonation.[4,9,18–20]

Auxin plays a major role in root patterning and development: this hormone shows morphogenetic activities and is shaped in a gradient along the longitudinal axis of the RAM. This gradient peaks at the SCN, exhibits intermediate levels in the MZ, reaches its minimum at the TZ, and rises again in the EZs and DZs[8,21,22] (Figure 2B).

It has been shown that the auxin maximum is necessary to position and maintain the activities of the SCN,[21,23,24] while the auxin minimum is necessary to position and maintain the TZ.[8] Auxin distribution depends on PIN-FORMED (PIN) transporters, which direct polar auxin flux through the meristem, depending on their expression pattern and position in the cell plasma membrane. PIN1, PIN3, and PIN7 in the vascular tissues and PIN2 in the cortex direct auxin through the root tip, whereas PIN2 from the epidermis cells redirects auxin through the internal tissues.[22,23] Interestingly, a recent study demonstrated that different PIN proteins not only have a specific membrane distribution but also show specific transport rates that contribute to the regulation of auxin directional flux. For example, PIN2 shows a slower rate compared to PIN1 and PIN3. This difference in auxin transport rate may be crucial to regulate proper auxin distribution through the apex. For instance, the authors showed that PIN2 slower transport rate is important for root gravitropism, probably as it allows auxin perdurance in epidermis cells to direct root bending.[25]

In shaping the auxin gradient, and thus root zonation, the antagonistic interaction of auxin with another hormone, CK, is fundamental.[9] The auxin-CK antagonistic interaction is well characterized (Figure 2B1): CK contributes to the auxin minimum formation in the uppermost meristematic cells of the meristem, inducing cell differentiation and positioning the TZ.[8,9] CK at the TZ regulates both auxin transport and degradation by activating a two-component signaling pathway that involves the ARABIDOPSIS HISTIDINE KINASE 3 (AHK3) CK receptor and the primary CK-responsive transcription factor (TF) ARABIDOPSIS RESPONSE REGULATOR 1 (ARR1). CK, binding to AHK3, prompts a series of phosphorylation events that lead to ARR1 activation in the nucleus. ARR1 then directly induces the expression of the auxin signaling repressor *SHORT HYPOCOTYL2/INDOLE-3-ACETIC ACID INDUCIBLE 3* (*SHY2/IAA3*) in the vasculature of the TZ and the *GRETCHEN HAGEN 3.17* (*GH3.17*) auxin-conjugating enzyme in the epidermis and LRC. SHY2 downregulates *PIN1*, *3*, and *7* in the vasculature, thus inhibiting auxin flux in this domain, whereas GH3.17, acting specifically from the LRC at the TZ, directs auxin to degradation, catalyzing its irreversible conjugation to glutamate.[8,9,26] In addition, ARR1, specifically in the LRC, activates the expression of PIN5, an auxin intracellular transporter that pumps auxin from the cytosol in the endoplasmic reticulum (ER). Thus, the LRC controls auxin levels through both GH3.17-mediated degradation and PIN5-dependent sequestration in the ER. Indeed, the LRC seems to play a prominent role in shaping the auxin gradient, acting as an auxin sink that regulates the entire auxin flux through the meristem.[26] In this way, the ARR1/PINs/GH3.17 networks positions an auxin minimum in the last meristematic cells.

In the TZ, CK promotes the expansion of cells entering the EZ, providing them the competence to differentiate. Specifically, CK

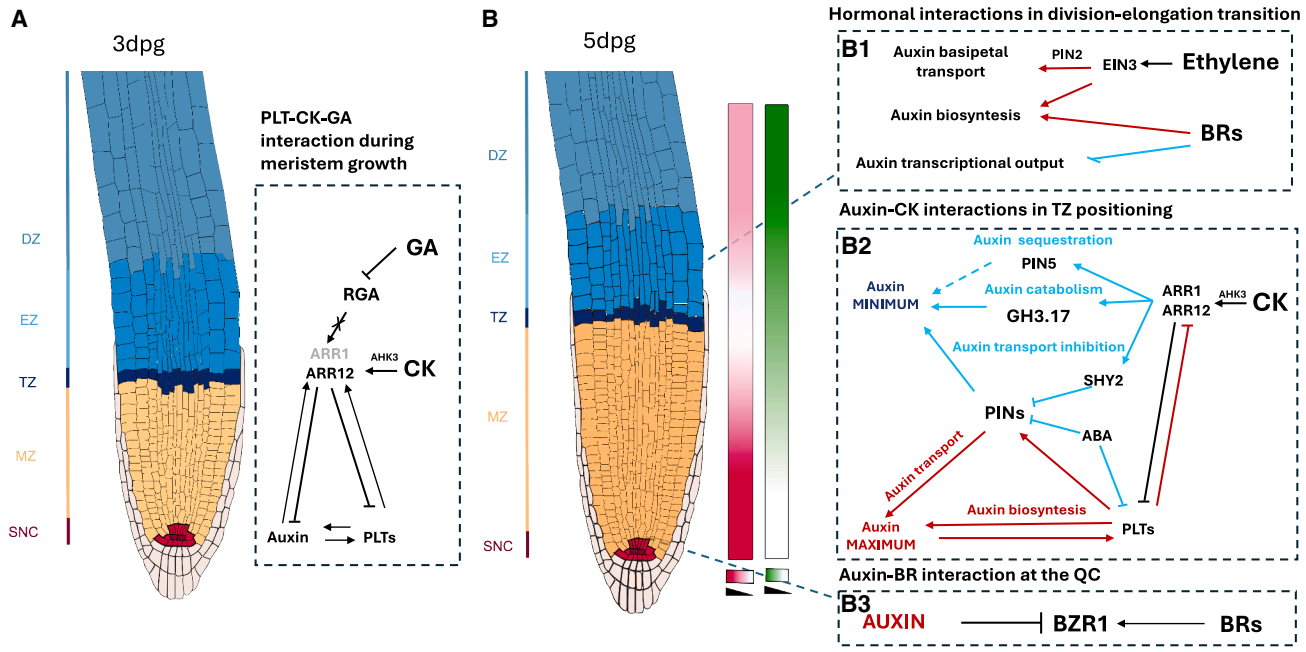

Figure 2. Hormonal crosstalk along the longitudinal axis

Representation of the RAM at 3 dpg (growth phase) (A) and 5 dpg (adult meristem) (B) showing the developmental zones highlighted by different colors (SCN in red, MZ in yellow, TZ in dark blue, EZ in light blue, and DZ in green). (A) Hormonal interactions involved in meristem expansion. CK, via AHK3, activates ARR12 enabling early TZ formation. The reciprocal inhibition between PLTs and ARR12 confines their expression at the SCN and at the TZ, respectively. GA inhibits RGA thus preventing ARR1 expression and TZ stabilization. In this way, PLT, CK, and GA activities enable meristem expansion. (B) Hormonal interactions involved in maintaining meristem activity. In the color-coded boxes are represented the auxin (red/blue to white) and BR (green to white) gradients. (B1) Hormonal interactions in division-elongation transition: ethylene interacts with auxin promoting its basipetal transport and biosynthesis to regulate elongation. BRs activate auxin biosynthetic genes but repress auxin transcriptional output to finely tune division and elongation processes. (B2) Auxin-CK interaction in TZ positioning: CK regulates auxin minimum formation activating ARR1 at the TZ via AHK3. ARR1 activates specifically the TZ, GH3.17, and SHY2 expression, thus inducing auxin catabolism and PIN-mediated transport inhibition. A mutual inhibition between PLT2 and ARR1 is also established, maintaining PLT and ARR1 in separate domains of activity. (B3) Auxin-BR antagonistic interaction at the QC regulates cell renewal. BRs and auxin show opposite effects on BZR1 nuclear accumulation in QC cells: BZR1 enhances its nuclear localization while auxin inhibits it.

induces pH-dependent cell expansion via ARR1, which induces the member of expansin family *EXPANSIN A1* (*EXPA1)* and the proton pumps *AHA1* and *AHA2*. EXPA1 is a pH-dependent cell-wall-remodeling enzyme, which, in the presence of AHA1 and AHA2 activity, leads to apoplast acidification and mediates cell expansion.[27] Furthermore, recent studies have shown that CK plays a role in mediating the transition from elongation to differentiation by inducing growth cessation in upper EZ cells, when cells reach their final size. Interestingly, in this region, CK promotes cell wall stiffening through the auxin influx carrier AUXIN TRANSPORTER PROTEIN 1 (AUX1), suggesting that CK may induce growth cessation by facilitating auxin accumulation in the last elongating cells.[28]

The regulation of CK levels is essential for maintaining the correct balance between division and differentiation in the meristem,[9,17] and its production is therefore finely regulated. CK biosynthesis in the meristem is promoted by PHABULOSA (PHB), a member of the Class III Homeodomain Leucine Zipper TFs (HDZIPIII), which directly induces *ISOPENTENYL TRANSFERASE 1* and *7* (*IPT1* and *IPT7*) biosynthetic enzymes.[29] PHB-mediated CK biosynthesis is sufficient to activate the ARR1/PINs module to position the TZ.[29] On the other hand, ARR1 downregulates both *PHB* and its inhibitors *miR165/6*, creating a negative incoherent feedforward loop, probably as a mechanism to maintain the correct cell differentiation rate.[29] Interestingly, the PHB/mir165-166/ARR1 module is also important for controlling root plastic development in response to external stimuli, such as salt stress. Indeed, when plants are treated with salt, the levels of miR165/166 decrease, leading to an increase in the expression of PHB. The elevated PHB levels, in turn, promote the expression of IPTs, resulting in higher CK levels, promoting the root meristem shortening.[29]

Besides the auxin/CK antagonistic interaction, a drop of the PLETHORA (PLT) TFs is needed at the TZ to induce cell differentiation. PLTs are key determinants of root identity and are expressed from embryogenesis in an auxin-dependent manner. Their distribution across the meristem mirrors the auxin gradient, and they control root zonation in a dose-dependent manner, following the same trend of auxin: high PLT levels in the SCN are necessary to maintain stem cell activity, intermediate levels promote rapid cell proliferation, and a drop at the TZ is necessary to allow cell differentiation.[30–32]

It has been shown that the mutual inhibition between the PLTs and the CK-responsive TFs ARR1 and ARR12 is necessary to establish and position the TZ, contributing to root zonation.[33,34] During the early stages of root development (Figure 2A), an

antagonistic ARR12/PLT2 network regulates meristem growth and is responsible for early TZ formation: cell divisions lead to a PLT drop that enables differentiation processes to start. The PLT drop allows *ARR12* activation at the TZ, and, in turn, ARR12 inhibits PLTs. This reciprocal inhibition regulates meristem expansion. TZ positioning depends on ARR1 activation occurring at 5 days post-seed germination (dpg): at this time, ARR1 inhibits cell-cycle progression by activating the expression of the cell-cycle inhibitor *KIP-RELATED PROTEIN 2* (*KRP2*), and the mutual inhibition between PLT2 and ARR1 is established, maintaining PLT and ARR1 in separate activity domains.[33,34] This ARR1/PLT network (Figure 2B2) enables a stable TZ positioning and contributes to maintain root zonation by separating division and differentiation processes.[33–35] Also, GAs are a class of hormones involved in meristem size setting, acting as positive regulators of meristem longitudinal expansion, especially at early stages of RAM development, when they are present at high levels to rapidly decline between 3 and 5 dpg.[14] GAs promote meristem growth by repressing *ARR1* expression through DELLA PROTEIN REPRESSOR OF GA (RGA) degradation (Figure 2A). Conversely, when GAs decrease at 5 dpg, RGA levels increase, thus enabling *ARR1* expression and meristem size setting.[18] Moreover, RGA stability is also positively regulated by SCARECROW (SCR), a TF expressed in the QC and endodermis, which is involved in stem cell maintenance and ARR1 repression in the SCN. From the endodermis, SCR sustains GA signal directly activating *SNEEZY* (*SNE*), an F box involved in RGA protein degradation.[7,36,37] GAs are also known to be involved in cell proliferation control: in particular, GAs accumulate in the endodermis[38] and control cell proliferation specifically from this tissue by restraining the activity of the cell-cycle inhibitors KRP2 and SIAMESE (SIM).[39,40]

In recent years, increasing interest has been directed toward dissecting and integrating the roles of BRs in regulating root development. Along the longitudinal axis, BRs act in a tissue-specific and dose-dependent manner in coordinating root zonation.[20,41] The spatial distribution of BRs response in the RAM was uncovered by evaluating the nuclear accumulation of fluorescently tagged BRASSINAZOLE RESISTANT 1 (BZR1) and BRI1-EMS- SUPPRESSOR 1 (BES) TFs that are key effectors of BR signaling pathway. BZR1/BES1 nuclear accumulation correlates with BR signaling strength and is distributed along the longitudinal axis exhibiting a maximum in the epidermis of the EZ and lower levels in the MZ[20] (Figure 2A). Concordantly, this BR signaling maximum overlaps with the primary site of BR biosynthesis in the RAM.[42] The graded pattern of BR activity through different developmental zones is required for optimal growth, demonstrating a dose-dependent effect of BRs on RAM development.[20,43] In particular, the presence of high levels and activity of BRs in the EZ regulates the correct transition from division to differentiation: in the EZ, BRs promote cell longitudinal expansion, regulating microtubules orientation and cell wall deposition and remodeling.[13] Recently, single-cell RNA sequencing approaches were used to identify gene regulatory networks controlling BR response in the *Arabidopsis* root with a spatiotemporal resolution. These analyses revealed that BRs are involved in regulating the shift from division to elongation by inducing cell-wall-related genes in the cortex.[44,45] Two TFs, HOMEOBOX FROM ARABIDOPSIS THALIANA 7 (HAT7) and GT-2-LIKE 1 (GTL1), were identified as BR-induced factors along cortex developmental trajectories that regulate cell elongation. Notably, GTL1 was found to regulate cell-wall-related genes, suggesting that BRs control cortex cell wall remodeling during cell elongation via this TF.[45]

In the MZ, BR signaling, arising specifically from the epidermis, promotes cell expansion and mitotic activity, acting as a positive regulator of meristem size.[43] The spatial shaping of BR signaling is complementary to auxin distribution, and it has been shown that these two hormones exert overall opposite activities in RAM development, regulating in opposite directions a common set of genes through the different developmental zones.[20] However, auxin and BRs show both positive and negative interactions (Figure 2B). For example, BRs induce auxin biosynthesis in the epidermis and LRC but repress its transcriptional output, probably as a feedback mechanism to maintain the correct balance between BR-promoted cell elongation processes and auxin-driven division in the meristem[19,20] (Figure 2B1). Conversely, BRs and auxin show an antagonistic interaction in regulating BZR1 nuclear accumulation in the QC, a site where BRs were shown to promote QC cell division[20,43,46] (Figure 1B3). Specifically, BRs are involved in regulating QC cell renewal by inhibiting BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER (BRAVO), a TF factor that represses QC divisions. BRs regulate QC division cell-autonomously by activating the BES1 effector via the membrane BR receptor BRASSINOSTEROID INSENSITIVE 1 (BRI1). Subsequently, BES1 directly represses *BRAVO* expression.[46,47]

Among the plant hormones that regulate growth and physiology, ethylene and ABA have been found to influence root development, particularly through interactions with other hormones. Ethylene is a gaseous hormone that negatively regulates primary root growth by controlling both cell proliferation and cell elongation.[48,49] Specifically, ethylene affects meristem activity by inducing irregular divisions in the QC[50] and inhibiting cell expansion in epidermal tissue, thus reducing cell proliferation in the MZ and affecting cell elongation in the EZ.

In inhibiting cell proliferation and elongation, ethylene acts synergistically with auxin by promoting its biosynthesis and basipetal transport through the EZ, leading to auxin-dependent cell growth inhibition.[49,51] Interestingly, ETHYLENE INSENSITIVE 3 (EIN3), a key TF in the ethylene response, has been found to positively regulate both auxin biosynthesis[52,53] and basipetal auxin transport via PIN2 modulation.[54] ABA affects primary root growth in a concentration-dependent manner: low ABA levels promote root growth, while higher concentrations inhibit it.[55] Auxin and ethylene mediate the biphasic effect of ABA, with auxin primarily driving its promotive effect, while both auxin and ethylene contribute to growth inhibition.[55] At inhibitory concentrations, ABA reduces auxin levels in roots by downregulating transport genes such as AUX1 and PINs and inhibiting auxin signaling components.[55] Additionally, high ABA levels decrease PLT1 and PLT2 expression.[56,57] Ethylene signaling is also required for ABA-induced root growth inhibition. In this context, ABA

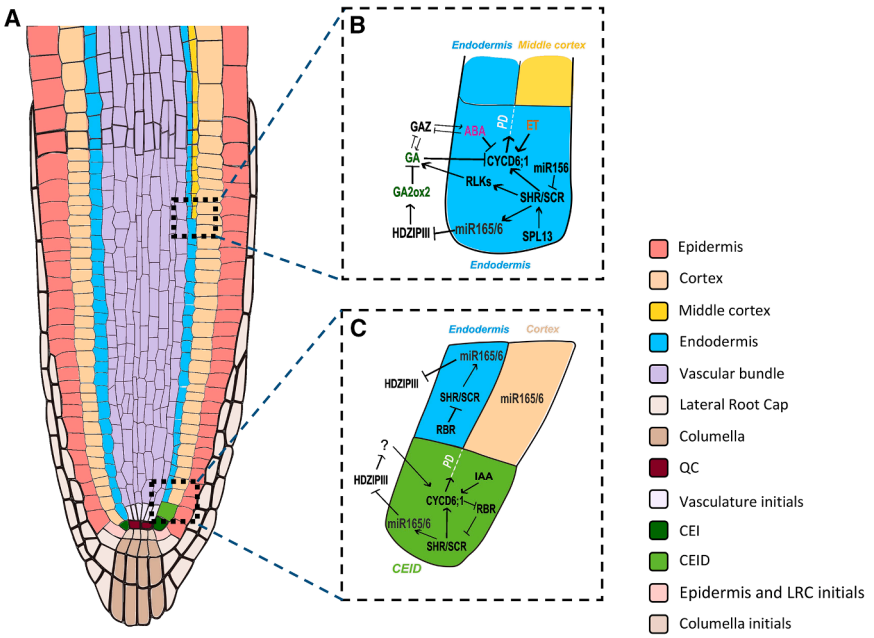

Figure 3. Model depicting the main pathways contributing to asymmetric cell division in *Arabidopsis* root

(A) A schematic representation of RAM longitudinal section. Different cell types are indicated by colors.

(B) Model of middle cortex formation. miR165/6 levels decrease at 8 dpg, resulting in higher HDZIPIII levels in the vasculature. High HDZIPIII levels promote the expression of GA2OX2, which in turn causes GA degradation and consequent stabilization of GAI proteins in the endodermis, essential for the reactivation of CYCD6; 1 and the formation of the MC. GAZ, serving as a convergent point for the interaction between the ABA and GA pathways, plays a role in maintaining a constant flux of ABA and GA, which in turn modulates the timing of MC formation during GT maturation. High levels of GAs at 5 dpg support SHR levels in the endodermis, which at high concentrations inhibit the activity of CYCD6; 1 and, hence, the periclinal division.

(C) Model of cortex-endodermis formation. In the *Arabidopsis* root, CEI (dark green box) divides anticlinally to generate the CEID (light green box); an SHR/SCR complex in CEID promotes PD via activation of CYCD6; 1. High levels of auxin (IAA) in the CEID contribute to CYCD6; 1 activation, whereas CYCD6; 1 inactivates RBR, in turn promoting SCR activity. Besides activating CYCD6; 1, SHR/SCR also induce the transcription of miRNA165/6, restricting HDZIPIII expression in the vasculature. As the expression of PHB in the endodermis results in ectopic CYCD6; 1 activation, some other factor might mediate the PHB-dependent CYCD6; 1 activation. In the endodermis cells (light blue), RBR binds to SCR inhibiting cell division, whereas SHR instructs endodermis fate. In the cortex (yellow), SHR/SCR complex promotes miR165/6 expression restricting PHB to the stele.

promotes ethylene biosynthesis that mediates root growth suppression.[58]

Hormonal crosstalk among the radial axes

Timing and spatial triggering of periclinal divisions are crucial for proper root radial patterning, as they enable formation of tissues with distinct identities in specific positions.[6,59] This regulation also drives radial root expansion by continuously introducing new cell files to specific tissues, such as the procambium in the stele.[60,61] Hence, it is not surprising that the precise crosstalk between TFs and different hormonal pathways is fundamental to fine-tuning asymmetric cell division during development.

One of the best characterized mechanisms involved in radial axis formation is the one generating the cortex and the endodermis root tissues. These tissues arise from the CEI, which divides first anticlinally to produce a daughter cell (cortex and endodermis initial daughter cell, CEID). Then, the CEID divides periclinally to generate two distinct tissues with different identities: the cortex and endodermis[5,62–65] (Figure 3C). The combination of cortex and endodermis is named ground tissue (GT). In *Arabidopsis*, a molecular network conveying on the regulation of SHR and SCR TFs and the cell-cycle regulator CYCLIND6;1 (CYCD6;1) is crucial to pattern GT.[65,66] In this molecular network, hormonal interplays largely contribute to regulate SHR/SCR and CYCD6; 1 module, which is fundamental to trigger CEID periclinal divisions that generate both endodermis and cortex tissues.

In particular, SHR is a mobile intercellular TF that is produced in the vasculature of the root and moves outward into adjacent cells, including the endodermis, and the CEID. In the CEID, SHR induces *SCR* expression, and together they form a complex that induces the expression of CYCD6; 1[62,65–67] (Figure 3C). The interplay between SHR/SCR and CYCD6; 1 constitutes a bistable molecular network that position asymmetric cell division in the GT. High levels of SHR/SCR heterodimers promotes CEID asymmetric cell division inducing high levels of CYCD6; 1, which in turn inhibits retinoblastoma-related (RBR) activity via phosphorylation. On the contrary, low levels of these heterodimers diminish CYCD6; 1 activity, thus promoting RBR activity and hence blocking asymmetric cell divisions.[63] Auxin maximum in the SCN defines this bistable circuit as this hormone promotes CYCD6; 1 expression, generating a flip flop mechanism between CEI, CEID, and CEID daughters.[63]

Notably, recent findings indicate that low levels of SHR and SCR operate at early stages during the cell cycle to alter the orientation of the CEID division plane.[64] In particular a window of sensitivity to these TFs during G1 and early S phases aligns with the activity of D-type cyclins, such as CYCD6; 1, in phosphorylating RBR during the transition from G1 to S phase.[63] The activation of these D-type cyclins initiates the bistable switch prompting an irreversible progression through mitosis.[68] Interestingly, SHR induction cannot initiate formative division outside of the meristem, suggesting that SHR alone is not enough to independently trigger cell division.[64] This might occur because of low levels of auxin and/or of SCR outside of the meristem.

Additional TFs can induce asymmetric cell divisions in the GT by promoting CYCD6; 1 independently of the SHR/SCR pathway. One such example is the HDZIPIII member PHABULOSA (PHB). This TF indirectly supports *CYCD6;1* expression, as gain-of-function mutants of *PHB* lead to ectopic

CYCD6; 1 activation, triggering additional periclinal divisions in the GT[59] (Figure 3C). In *Arabidopsis*, the GT undergoes dynamic changes during post-embryonic development. Specifically, between 7 and 10 dpg, a new cortical layer, the middle cortex (MC), is formed[69] (Figure 3B). This layer originates from sporadic asymmetric cell divisions in endodermal cells.[62] MC formation largely depends on SHR levels; high levels of SHR inhibit MC formation, whereas low levels promote that.[70] SHR levels in the GT are defined by GAs,[71] whose physiological level decreased with the aging of the root.[18,72,73] Several studies have highlighted the central role of GAs in regulating the timing of MC formation, with high levels of GAs acting as repressor.[73–76]

Time-dependent GA levels in the root are regulated by PHB and its homolog PHAVOLUTA (PHV). The expression of these TFs increases in the vasculature at 7 dpg, enhancing the expression of the GA2ox2 gene, which encodes an enzyme involved in GA catabolism. This regulation allows PHB and PHV to promote MC formation cell non-autonomously, by inducing CYCD6; 1 expression in MC progenitors[75,77] (Figure 3B). Interestingly, the time-dependent expression of PHB and PHV is linked to reduced miR165 and miR166 levels in the endodermis, which, in turn, are regulated by GA levels. Recently it has been suggested that regulation of RLK proteins such as ARH1, FEI1, and FEI2 by SHR and SCR is also necessary for maintaining high levels of GAs during early root development, hence repressing MC formation.[78]

Research indicates that ethylene also plays a significant role in regulating radial root patterning, controlling cortical cell proliferation. This gaseous phytohormone, along with GAs and ABA, influences cortex proliferation through complex regulatory mechanisms. Notably, enhanced ethylene signaling, as observed in the *eto1* mutant, leads to an anticipated MC phenotype in *Arabidopsis* roots.[76]

Similarly to GAs, ABA also acts as a suppressor of MC formation in *Arabidopsis.* Loss-of-function ABA biosynthesis mutants, such as *aba2-2*, display anticipated MC formation, whereas exogenous ABA application inhibits periclinal divisions required to form the MC layer. This pattern regulation is mediated through GA- AND ABA-RESPONSIVE ZINC FINGER (GAZ), a zinc-finger TF responsive to both ABA and GAs. GAZ integrates ABA and GA signaling to fine-tune the timing of asymmetric cell divisions in the GT, ensuring proper root development.[74,79]

It is suggested that HDZIPIII TFs may contribute to ABA biosynthesis: PHB has been shown to directly induce the expression of *BETA-1,3-GLUCANASE 1* (*BG1*), which converts ABA-glucose ester into bioactive ABA.[80] While this has not been explicitly demonstrated in roots, it is plausible that a similar regulatory mechanism might exists in the GT, where HDZIPIII factors could modulate both ABA and GAs levels, thereby influencing radial root development. Remarkably, recent studies have revealed that asymmetric cell divisions in the root meristem are also regulated by miR156 and its targets, the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL)* TFs.[81] These TFs are well known for their role in controlling the transition from juvenile to adult phases in plants.[82] Interestingly, increased expression of SPL13—either through misexpression or through the repression of miR156—promotes the formation of additional cortical layers by triggering periclinal divisions in endodermal cells. Conversely, reduced SPL activity, as observed in *spl* mutants or miR156 overexpression lines, suppresses these divisions, resulting in the maintenance of a single cortical layer.[81] These findings open new avenues for understanding the age-dependent regulation of root patterning.

Another important step toward root radial axis establishment is the development of the vascular bundle. In *Arabidopsis*, the root vasculature consists of an inner xylem bundle (metaxylem in the center and protoxylem aside) with two juxtaposed phloematic bundles[5] generated by the procambium meristematic tissue (Figure 4A).

Procambial cells divide periclinally to increase the number of vascular cell files. This developmental process, crucial for generating the correct number of vascular cell files, is regulated by the combined activities of various TFs and phytohormones, including auxin and CK.

Auxin and CK signals occupy distinct and complementary domains: high auxin signaling is necessary for xylem identity cells, while adjacent undifferentiated procambial and phloem cells exhibit high CK signaling.[83,84]

During xylem development, auxin elicits the activity of the MONOPTEROS (MP) TF. MP directly triggers the activation of *TARGET OF MONOPTEROS 5* (*TMO5*) a basic-helix-loop-helix (bHLH) TF. Within xylem cells, TMO5 creates heterodimers with another TF, called LONESOME HIGHWAY (LHW). The resulting TMO5/LHW heterodimers activates transcription of the *LONELY GUY3* and *4* (*LOG3,4*) responsible of catalyzing the final step of CK biosynthesis.[85,86] CK produced locally in the xylem diffuses to nearby procambial cells inducing their periclinal division[87] (Figure 4B).

High CK levels in procambial cells regulate and coordinate the polar localization of the auxin efflux transporter PIN1, PIN3, and PIN7. PIN relocalization directs auxin toward the xylem, ensuring a continuous auxin signal in the xylem.[84,85,88,89] Even though xylem cells serve as a CK source, CK response is precluded in these cells. Indeed, MP, activated by auxin, not only activates TMO5 but also directly induces the expression of *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFERASE6* (*AHP6*), a gene that encodes for a CK signaling inhibitor (Figure 4B). AHP6 acts cell-autonomously to block CK signaling in the protoxylem cells (the outermost cell in the primary xylem).[84] However, it remains unclear whether a similar CK signaling suppressor exists in the metaxylem position (the inner cells in the primary xylem).

Several different negative feedback loops limit TMO5/LHW activity: TMO5/LHW heterodimers, besides activating the *LOG3,4* CK biosynthesis genes, also activates transcription of *SUPPRESSOR OF ACAULIS LIKE* (SACL) genes and *MYB12*, both encoding for unusual bHLH proteins.[90,91] The SACL proteins and MYB12 can form heterodimers with LHW and TMO5, respectively. These heterodimers, possessing a different or no DNA-binding domain, move on different transcriptional network contexts than the TMO5/LHW heterodimers, preventing TMO5/LHW activity.[90,91] In the xylem, TMO5/LHW triggers the expression of *SHR*. SHR moves into adjacent procambial cells to activate transcription of a CK-degrading enzyme, the *CYTOKININ OXIDASE3* (*CKX3*) gene (Figure 4B). In this way, the TMO5/LHW heterodimers control CK level in the procambium thus contributing to its activity.[92]

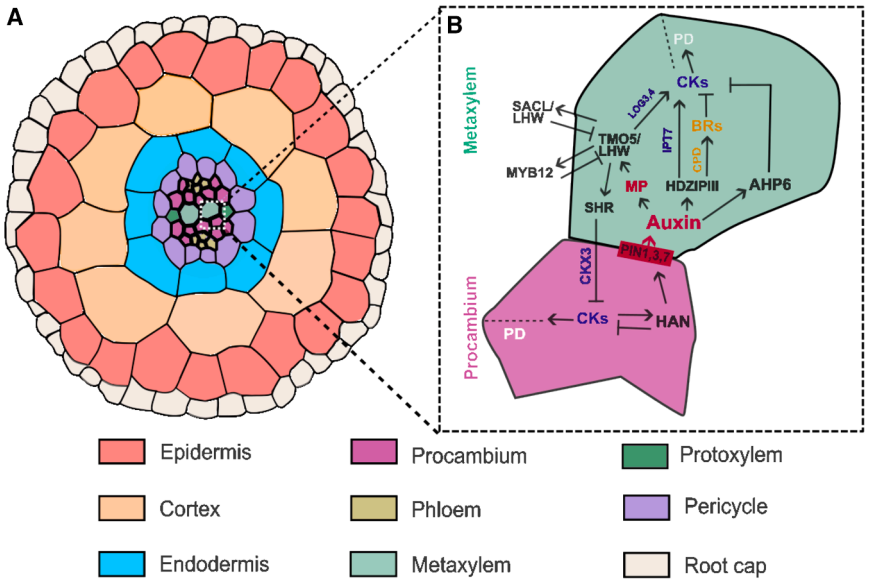

Figure 4. Hormonal crosstalk controlling vascular radial patterning

(A) A schematic representation of a RAM cross-section. Different cell types are indicated by colors. (B) Model and spatial distribution of hormones/TFs/miRNAs that ensure the correct positioning of divisional activity during procambial development. High auxin activity in the metaxylem (light-green box) leads to local production of CK through the TMO5-LHW-LOG4 pathway. In parallel, auxin induces HDZIPIII TF expression and IPT7 that help increase CK levels in the xylem, so promoting periclinal divisions (PDs). Moreover, TMO5/LHW heterodimer also induces SHR expression, a mobile TF that migrates to the surrounding procambial cells (pink box) and promotes the expression of CKX3, to control CK levels in this cell type. TMO5/LHW heterodimer level is also controlled by 2 different negative feedback loops, with one being the induction of SACLs, which sequestrate LHW, mediated by ACL5 and second MYB12 that physically interact with TMO5. Another negative feedback loop that regulates hormone level, and specifically CK in the procambium, involves the HAN protein, which inhibits the type-B ARR response. High CK level in the procambium not only promotes proper cell proliferation by inducing PDs, but also enhances auxin influx into xylem cells by regulating the expression of PIN1 and PIN7. Here, locally produced CKs also induce the PD and xylem cell proliferation, but their effect is strongly downregulated by BRs, produced by CPD, in turn activated by HDZIPIII and AHP6.

In addition, there are several other molecular networks that control vascular development by acting on CK biosynthesis or signaling.

It was recently demonstrated that CK activity in the procambium also depends on the expression of the GATA TF, *HANABA-TARANU* (*HAN*). HAN is induced by CK, but it has a negative role in CK signaling (Figure 4B), potentially by offsetting the CK response type-B ARR.[93]

The radial gradient of HDZIPIIIs is sufficient to pattern xylem fate specification, as high HDZPIII levels promote metaxylem formation, whereas low levels promote protoxylem. miR165/166 plays a crucial role in patterning this gradient.[88] They are produced in the endodermis, and from here they move several cells away toward the stele, to limit the expression domain of the HDZIPIII genes. They, in turn, regulate vascular patterning and ensure its proper development.[94]

To control vascular development, the HDZIPIII-microRNA (miRNA) module controls CK activities.[84] In the stele, PHB controls the biosynthesis and activity of the phytohormone CK directly activating the CK biosynthesis gene *IPT7* by binding to its promoter[29] (Figure 4B).

Interestingly, exogenous BR application suppresses the CK response in xylem cells. The suppression of xylem cell division by BRs is caused by HDZIPIIIs that mediate the transcriptional activation of CONSTITUTIVE PHOTOMORPHOGENIC DWARF (CPD), a BR biosynthesis gene in the RAM vascular cells[95] (Figure 4B). On the other hand, it has been shown that HDZIPIII enhances CK biosynthesis by increasing IPT7 expression in the root TZ.[29] These facts point to the existence of strict location-dependent regulation of BRs and CK by HDZIPIII TFs even in the RAM.[95]

This intricate process secures precise control over CK levels and response in the xylem and procambium, ensuring the establishment and proliferation of those cell types, which serve as the precursors for radial growth.

Hormonal regulation of root development in different plants

Hormonal interactions are increasingly well characterized in *Arabidopsis*, but our understanding of these processes remains fragmented for other species. The evolution of the root system across plant lineages has been shaped by both conserved and independently derived developmental mechanisms. Roots are believed to have evolved independently in lycophytes and euphyllophytes (which include ferns, gymnosperms, and angiosperms), suggesting that distinct genetic programs were recruited to generate similar organ structures.[96,97] Despite these independent origins, modern vascular plants share fundamental regulatory modules, particularly in hormonal control of root patterning.[98]

Generally, in angiosperms, auxin promotes root elongation, while CKs inhibit it.[98,99] Similarly, GAs promote meristematic cell division, whereas ethylene restricts it by reducing bioactive GA levels.[100] BRs show dose-dependent effects in regulating root elongation.[101,102]

Auxin response factors regulating auxin-mediated gene expression and auxin transporters like AUX1 and PINs modulating its distribution have been found in many different angiosperms such as rice, maize, wheat, and *Populus*.[103–112] In monocots, PIN family is more expanded compared to *Arabidopsis*, suggesting functional diversification.[103]

Although most of the discoveries have been made using *Arabidopsis* as a model system, significant insights into the hormonal regulation of root development have emerged from studies on

rice, a key model organism for monocots, showing conserved mechanisms.

In rice, CK signaling shares similar two-component elements with *Arabidopsis*, including histidine kinases and phosphotransferase proteins. However, while both possess the same types of response regulators, rice exhibits significant lineage-specific expansions, contributing to genome plasticity, allowing functional overlap and the potential emergence of new functions.[113]

In rice, auxin modulates CK levels by suppressing *OsIPT7* and inducing *OsCKX4*, while promoting *LOG* expression to activate CKs.[114,115] This highlights a conserved auxin-CK interplay in angiosperm root development. However, in ferns like *Azolla filiculoides*, with a determinate root meristem, CKs promote meristem activity while auxin restricts it,[116] suggesting evolutionary shifts in auxin-CK signaling.

The integration of BR signaling in rice root longitudinal development has also been investigated. As in *Arabidopsis*, experimental evidence has demonstrated that low BR levels promote root elongation, while high concentrations suppress root growth.[43,101,102,117]

Recent studies have elucidated another hormonal interplay conserved between rice and *Arabidopsis*: in rice high ethylene production leads to reduced bioactive GA levels, which in turn inhibit cell proliferation in the root meristem, gradually suppressing primary root elongation. This regulation involves the ethylene signaling TF ETHYLENE INSENSITIVE1-LIKE (OsEIL1), which activates GA catabolic genes, leading to decreased GA activity and subsequent inhibition of meristematic cell division.[100,118]

Root radial axis patterning has been extensively studied in rice, which is characterized by multiple cortical layers. In rice, the SHR/SCR interaction is conserved,.[62,119] Various studies suggest that SHR movement is not restricted to the CEI and endodermis as in *Arabidopsis* but extends to the inner cortical layers, where it promotes the formation of multiple cortical layers by activating CYCD6; 1 expression.[120–122] In maize, SHR protein has been found to exhibit notable mobility, extending several layers deep into the cortex. Higher-order SHR mutants in both maize and *Setaria* display a reduction in cortical layer number formation, highlighting the role of the SHR pathway in promoting cortical patterning.[123] Interestingly, studies in *Selaginella moellendorffii*, a lycophyte, indicate that, while SHR and SCR are present, their interaction and mobility differ from their function in seed plants, suggesting modifications in the deployment of this module.[124] Similarly, spatial transcriptomics in *Isoetes*, a basal lycophyte, have revealed partial conservation of root regulatory networks but with distinct hormonal integration.[125]

Also the regulation of HDZIPIII by miR165/166 is conserved across land plants,[126] yet species-specific spatial modifications generate diverse formative gradients. In *Cardamine hirsuta*, a close relative of *Arabidopsis*, HDZIPIII TFs contribute to the formation of a second cortical layer. Here, the narrower expression domain of miR165/166 leads to an expanded PHB activity, highlighting a key regulatory shift in cortical layer patterning.[59]

Hormonal crosstalk might be key for functional adaptation of roots to the environment. In rice, ethylene is important for radial root expansion under mechanical stress. Its accumulation induces ABA biosynthesis, which, together with auxin, promotes cortical cell expansion and increases root diameter, enabling roots to penetrate compacted soil. Mutants deficient in ABA biosynthesis fail to undergo radial expansion, highlighting the role of ABA-ethylene crosstalk in mechano-adaptive root growth.[127]

CONCLUSIONS

Hormones play a pivotal role in root development, generating a complex network of molecular pathways that act and intersect along the longitudinal and radial axes.

Hormones can interact, in a spatiotemporal regulated manner, at various stages of hormones signaling, by either modulating biosynthesis and transport or regulating key TFs.

For instance, CK shapes the auxin gradient among the longitudinal axes generating the auxin minimum that triggers cell to differentiation by inhibiting auxin transport and promoting its degradation.[8,17] Adding another layer to the regulation of auxin levels, BRs positively regulate its biosynthesis in the root outer tissues to finely tune the transition from mitotic activity to cell expansion.[19] Hormonal signaling output frequently converges on the fine regulation of key TF activity and localization at specific stages of development. For example, during meristem formation, CK-responsive ARRs and auxin-dependent PLTs interact and establish mutual inhibition across the different phases of RAM development[4,34] to regulate the balance from division to differentiation,[17] a process that is further modulated by GA and BR activity.[13,39]

Moreover, as exposed in this review, research has elucidated how hormonal networks regulate developmental zonation along the longitudinal and radial axes, but an integrate view of how these hormonal networks intersect to coordinate the development of the two axes remains elusive. To this aim, computational models offer powerful tools to integrate multi-level data and uncover these intricate processes. Another fundamental aspect is that, althogh extensive research has been performed in *Arabidopsis*, studies in other angiosperms have shown that the core hormonal framework underlying RAM development remains largely conserved. The auxin-CK antagonistic interaction, GA-mediated expansion, and the SHR/SCR regulatory module serve as fundamental mechanisms across angiosperms. However, the mobility of key TFs, hormonal gradients, and environmental responsiveness have been evolutionarily tuned to optimize root architecture in different species probably according to the environment in which they evolved. Understanding these variations will be crucial for future efforts to enhance crop resilience and productivity by exploiting the plasticity of hormonal regulatory networks. This comparative approach could reveal evolutionary adaptations in root development that are functional to improve fitness of crop.

ACKNOWLEDGMENTS

This study was carried out within the Agritech National Research Center and received funding from the European Union NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA [PNRR] – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022), 2022NZ7M3W-PRIN2022 to S.S. and 20229LHY5L-PRIN2022 to R.D.I. This manuscript

reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. Vanstraelen, M., and Benková, E. (2012). Hormonal Interactions in the Regulation of Plant Development. Annu. Rev. Cell Dev. Biol. *28*, 463–487. https://doi.org/10.1146/annurev-cellbio-101011-155741.
2. Heidstra, R., and Sabatini, S. (2014). Plant and animal stem cells: similar yet different. Nat. Rev. Mol. Cell Biol. *15*, 301–312. https://doi.org/10.1038/nrm3790.
3. Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., and Scheres, B. (1993). Cellular organisation of the *Arabidopsis thaliana* root. Development *119*, 71–84. https://doi.org/10.1242/dev.119.1.71.
4. Salvi, E., Di Mambro, R., and Sabatini, S. (2020). Dissecting mechanisms in root growth from the transition zone perspective. J. Exp. Bot. *71*, 2390–2396. https://doi.org/10.1093/jxb/eraa079.
5. Scheres, B., Benfey, P., and Dolan, L. (2002). Root Development. Arab. Book *1*, e0101. https://doi.org/10.1199/tab.0101.
6. Di Mambro, R., Sabatini, S., and Dello Ioio, R. (2018). Patterning the Axes: A Lesson from the Root. Plants *8*, 8. https://doi.org/10.3390/plants8010008.
7. Sabatini, S., Heidstra, R., Wildwater, M., and Scheres, B. (2003). SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes Dev. *17*, 354–358. https://doi.org/10.1101/gad.252503.
8. Di Mambro, R., De Ruvo, M., Pacifici, E., Salvi, E., Sozzani, R., Benfey, P. N., Busch, W., Novak, O., Ljung, K., Di Paola, L., et al. (2017). Auxin minimum triggers the developmental switch from cell division to cell differentiation in the *Arabidopsis* root. Proc. Natl. Acad. Sci. USA *114*, E7641–E7649. https://doi.org/10.1073/pnas.1705833114.
9. Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M.T., Aoyama, T., Costantino, P., and Sabatini, S. (2008). A Genetic Framework for the Control of Cell Division and Differentiation in the Root Meristem. Science *322*, 1380–1384. https://doi.org/10.1126/science.1164147.
10. Pacifici, E., Polverari, L., and Sabatini, S. (2015). Plant hormone cross-talk: the pivot of root growth. J. Exp. Bot. *66*, 1113–1121. https://doi.org/10.1093/jxb/eru534.
11. Yamoune, A., Cuyacot, A.R., Zdarska, M., and Hejatko, J. (2021). Hormonal orchestration of root apical meristem formation and maintenance in Arabidopsis. J. Exp. Bot. *72*, 6768–6788. https://doi.org/10.1093/jxb/erab360.
12. Zluhan-Martínez, E., López-Ruíz, B.A., García-Gómez, M.L., García-Ponce, B., De La Paz Sánchez, M., Álvarez-Buylla, E.R., and Garay-Arroyo, A. (2021). Integrative Roles of Phytohormones on Cell Proliferation, Elongation and Differentiation in the Arabidopsis thaliana Primary Root. Front. Plant Sci. *12*, 659155. https://doi.org/10.3389/fpls.2021.659155.
13. Ackerman-Lavert, M., and Savaldi-Goldstein, S. (2020). Growth models from a brassinosteroid perspective. Curr. Opin. Plant Biol. *53*, 90–97. https://doi.org/10.1016/j.pbi.2019.10.008.
14. Shtin, M., Dello Ioio, R., and Del Bianco, M. (2022). It's Time for a Change: The Role of Gibberellin in Root Meristem Development. Front. Plant Sci. *13*, 882517. https://doi.org/10.3389/fpls.2022.882517.
15. Svolacchia, N., and Sabatini, S. (2023). Cytokinins. Curr. Biol. *33*, R10–R13. https://doi.org/10.1016/j.cub.2022.11.022.
16. Beemster, G.T., and Baskin, T.I. (1998). Analysis of Cell Division and Elongation Underlying the Developmental Acceleration of Root Growth in *Arabidopsis thaliana* 1. Plant Physiol. *116*, 1515–1526. https://doi.org/10.1104/pp.116.4.1515.
17. Dello Ioio, R., Linhares, F.S., Scacchi, E., Casamitjana-Martinez, E., Heidstra, R., Costantino, P., and Sabatini, S. (2007). Cytokinins Determine Arabidopsis Root-Meristem Size by Controlling Cell Differentiation. Curr. Biol. *17*, 678–682. https://doi.org/10.1016/j.cub.2007.02.047.
18. Moubayidin, L., Perilli, S., Dello Ioio, R., Di Mambro, R., Costantino, P., and Sabatini, S. (2010). The Rate of Cell Differentiation Controls the Arabidopsis Root Meristem Growth Phase. Curr. Biol. *20*, 1138–1143. https://doi.org/10.1016/j.cub.2010.05.035.
19. Ackerman-Lavert, M., Fridman, Y., Matosevich, R., Khandal, H., Friedlander-Shani, L., Vragović, K., Ben El, R., Horev, G., Tarkowská, D., Efroni, I., and Savaldi-Goldstein, S. (2021). Auxin requirements for a meristematic state in roots depend on a dual brassinosteroid function. Curr. Biol. *31*, 4462–4472.e6. https://doi.org/10.1016/j.cub.2021.07.075.
20. Chaiwanon, J., and Wang, Z.-Y. (2015). Spatiotemporal Brassinosteroid Signaling and Antagonism with Auxin Pattern Stem Cell Dynamics in Arabidopsis Roots. Curr. Biol. *25*, 1031–1042. https://doi.org/10.1016/j.cub.2015.02.046.
21. Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., Benfey, P., Leyser, O., Bechtold, N., Weisbeek, P., and Scheres, B. (1999). An Auxin-Dependent Distal Organizer of Pattern and Polarity in the Arabidopsis Root. Cell *99*, 463–472. https://doi.org/10.1016/s0092-8674(00)81535-4.
22. Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., Offringa, R., and Jürgens, G. (2003). Efflux-dependent auxin gradients establish the apical–basal axis of Arabidopsis. Nature *426*, 147–153. https://doi.org/10.1038/nature02085.
23. Blilou, I., Xu, J., Wildwater, M., Willemsen, V., Paponov, I., Friml, J., Heidstra, R., Aida, M., Palme, K., and Scheres, B. (2005). The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature *433*, 39–44. https://doi.org/10.1038/nature03184.
24. Petersson, S.V., Johansson, A.I., Kowalczyk, M., Makoveychuk, A., Wang, J.Y., Moritz, T., Grebe, M., Benfey, P.N., Sandberg, G., and Ljung, K. (2009). An Auxin Gradient and Maximum in the *Arabidopsis* Root Apex Shown by High-Resolution Cell-Specific Analysis of IAA Distribution and Synthesis. Plant Cell *21*, 1659–1668. https://doi.org/10.1105/tpc.109.066480.
25. Janacek, D.P., Kolb, M., Schulz, L., Mergner, J., Kuster, B., Glanc, M., Friml, J., Ten Tusscher, K., Schwechheimer, C., and Hammes, U.Z. (2024). Transport properties of canonical PIN-FORMED proteins from Arabidopsis and the role of the loop domain in auxin transport. Dev. Cell *59*, 3259–3271.e4. https://doi.org/10.1016/j.devcel.2024.09.020.
26. Di Mambro, R., Svolacchia, N., Dello Ioio, R., Pierdonati, E., Salvi, E., Pedrazzini, E., Vitale, A., Perilli, S., Sozzani, R., Benfey, P.N., et al. (2019). The Lateral Root Cap Acts as an Auxin Sink that Controls Meristem Size. Curr. Biol. *29*, 1199–1205.e4. https://doi.org/10.1016/j.cub.2019.02.022.
27. Pacifici, E., Di Mambro, R., Dello Ioio, R., Costantino, P., and Sabatini, S. (2018). Acidic cell elongation drives cell differentiation in the *Arabidopsis* root. EMBO J. *37*, e99134. https://doi.org/10.15252/embj.201899134.
28. Liu, S., Strauss, S., Adibi, M., Mosca, G., Yoshida, S., Dello Ioio, R., Runions, A., Andersen, T.G., Grossmann, G., Huijser, P., et al. (2022). Cytokinin promotes growth cessation in the Arabidopsis root. Curr. Biol. *32*, 1974–1985.e3. https://doi.org/10.1016/j.cub.2022.03.019.
29. Dello Ioio, R., Galinha, C., Fletcher, A.G., Grigg, S.P., Molnar, A., Willemsen, V., Scheres, B., Sabatini, S., Baulcombe, D., Maini, P.K., and Tsiantis, M. (2012). A PHABULOSA/Cytokinin Feedback Loop Controls Root Growth in Arabidopsis. Curr. Biol. *22*, 1699–1704. https://doi.org/10.1016/j.cub.2012.07.005.
30. Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., Nussaume, L., Noh, Y.-S., Amasino, R., and Scheres, B. (2004). The

PLETHORA Genes Mediate Patterning of the Arabidopsis Root Stem Cell Niche. Cell *119*, 109–120. https://doi.org/10.1016/j.cell.2004.09.018.

31. Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R., and Scheres, B. (2007). PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. Nature *449*, 1053–1057. https://doi.org/10.1038/nature06206.
32. Mähönen, A.P., Ten Tusscher, K., Siligato, R., Smetana, O., Díaz-Triviño, S., Salojärvi, J., Wachsman, G., Prasad, K., Heidstra, R., and Scheres, B. (2014). PLETHORA gradient formation mechanism separates auxin responses. Nature *515*, 125–129. https://doi.org/10.1038/nature13663.
33. Salvi, E., Rutten, J.P., Di Mambro, R., Polverari, L., Licursi, V., Negri, R., Dello Ioio, R., Sabatini, S., and Ten Tusscher, K. (2020). A Self-Organized PLT/Auxin/ARR-B Network Controls the Dynamics of Root Zonation Development in Arabidopsis thaliana. Dev. Cell *53*, 431–443.e23. https://doi.org/10.1016/j.devcel.2020.04.004.
34. Shtin, M., Polverari, L., Svolacchia, N., Bertolotti, G., Unterholzner, S.J., Di Mambro, R., Costantino, P., Dello Ioio, R., and Sabatini, S. (2023). The Mutual Inhibition between PLETHORAs and ARABIDOPSIS RESPONSE REGULATORs Controls Root Zonation. Plant Cell Physiol. *64*, 317–324. https://doi.org/10.1093/pcp/pcad001.
35. Svolacchia, N., Salvi, E., and Sabatini, S. (2020). Arabidopsis primary root growth: let it grow, can't hold it back anymore. Curr. Opin. Plant Biol. *57*, 133–141. https://doi.org/10.1016/j.pbi.2020.08.005.
36. Moubayidin, L., Di Mambro, R., Sozzani, R., Pacifici, E., Salvi, E., Terpstra, I., Bao, D., van Dijken, A., Dello Ioio, R., Perilli, S., et al. (2013). Spatial Coordination between Stem Cell Activity and Cell Differentiation in the Root Meristem. Dev. Cell *26*, 405–415. https://doi.org/10.1016/j.devcel.2013.06.025.
37. Moubayidin, L., Salvi, E., Giustini, L., Terpstra, I., Heidstra, R., Costantino, P., and Sabatini, S. (2016). A SCARECROW-based regulatory circuit controls Arabidopsis thaliana meristem size from the root endodermis. Planta *243*, 1159–1168. https://doi.org/10.1007/s00425-016-2471-0.
38. Shani, E., Weinstain, R., Zhang, Y., Castillejo, C., Kaiserli, E., Chory, J., Tsien, R.Y., and Estelle, M. (2013). Gibberellins accumulate in the elongating endodermal cells of *Arabidopsis* root. Proc. Natl. Acad. Sci. USA *110*, 4834–4839. https://doi.org/10.1073/pnas.1300436110.
39. Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T.S., and Genschik, P. (2009). Gibberellin Signaling Controls Cell Proliferation Rate in Arabidopsis. Curr. Biol. *19*, 1188–1193. https://doi.org/10.1016/j.cub.2009.05.059.
40. Ubeda-Tomás, S., Federici, F., Casimiro, I., Beemster, G.T.S., Bhalerao, R., Swarup, R., Doerner, P., Haseloff, J., and Bennett, M.J. (2009). Gibberellin Signaling in the Endodermis Controls Arabidopsis Root Meristem Size. Curr. Biol. *19*, 1194–1199. https://doi.org/10.1016/j.cub.2009.06.023.
41. Vragović, K., Sela, A., Friedlander-Shani, L., Fridman, Y., Hacham, Y., Holland, N., Bartom, E., Mockler, T.C., and Savaldi-Goldstein, S. (2015). Translatome analyses capture of opposing tissue-specific brassinosteroid signals orchestrating root meristem differentiation. Proc. Natl. Acad. Sci. USA *112*, 923–928. https://doi.org/10.1073/pnas.1417947112.
42. Vukašinović, N., Wang, Y., Vanhoutte, I., Fendrych, M., Guo, B., Kvasnica, M., Jiroutová, P., Oklestkova, J., Strnad, M., and Russinova, E. (2021). Local brassinosteroid biosynthesis enables optimal root growth. Nat. Plants *7*, 619–632. https://doi.org/10.1038/s41477-021-00917-x.
43. González-García, M.-P., Vilarrasa-Blasi, J., Zhiponova, M., Divol, F., Mora-García, S., Russinova, E., and Caño-Delgado, A.I. (2011). Brassinosteroids control meristem size by promoting cell cycle progression in *Arabidopsis* roots. Development *138*, 849–859. https://doi.org/10.1242/dev.057331.
44. Graeff, M., Rana, S., Wendrich, J.R., Dorier, J., Eekhout, T., Aliaga Fandino, A.C., Guex, N., Bassel, G.W., De Rybel, B., and Hardtke, C.S. (2021). A single-cell morpho-transcriptomic map of brassinosteroid action in the Arabidopsis root. Mol. Plant *14*, 1985–1999. https://doi.org/10.1016/j.molp.2021.07.021.
45. Nolan, T.M., Vukašinović, N., Hsu, C.-W., Zhang, J., Vanhoutte, I., Shahan, R., Taylor, I.W., Greenstreet, L., Heitz, M., Afanassiev, A., et al. (2023). Brassinosteroid gene regulatory networks at cellular resolution in the *Arabidopsis* root. Science *379*, eadf4721. https://doi.org/10.1126/science.adf4721.
46. Vilarrasa-Blasi, J., González-García, M.-P., Frigola, D., Fàbregas, N., Alexiou, K.G., López-Bigas, N., Rivas, S., Jauneau, A., Lohmann, J.U., Benfey, P.N., et al. (2014). Regulation of Plant Stem Cell Quiescence by a Brassinosteroid Signaling Module. Dev. Cell *30*, 36–47. https://doi.org/10.1016/j.devcel.2014.05.020.
47. Lozano-Elena, F., Planas-Riverola, A., Vilarrasa-Blasi, J., Schwab, R., and Caño-Delgado, A.I. (2018). Paracrine brassinosteroid signaling at the stem cell niche controls cellular regeneration. J. Cell Sci. *131*, jcs204065. https://doi.org/10.1242/jcs.204065.
48. Street, I.H., Aman, S., Zubo, Y., Ramzan, A., Wang, X., Shakeel, S.N., Kieber, J.J., and Schaller, G.E. (2015). Ethylene Inhibits Cell Proliferation of the Arabidopsis Root Meristem. Plant Physiol. *169*, 338–350. https://doi.org/10.1104/pp.15.00415.
49. Růžička, K., Ljung, K., Vanneste, S., Podhorská, R., Beeckman, T., Friml, J., and Benková, E. (2007). Ethylene Regulates Root Growth through Effects on Auxin Biosynthesis and Transport-Dependent Auxin Distribution. Plant Cell *19*, 2197–2212. https://doi.org/10.1105/tpc.107.052126.
50. Ortega-Martínez, O., Pernas, M., Carol, R.J., and Dolan, L. (2007). Ethylene Modulates Stem Cell Division in the *Arabidopsis thaliana* Root. Science *317*, 507–510. https://doi.org/10.1126/science.1143409.
51. Stepanova, A.N., and Alonso, J.M. (2005). *Arabidopsis* Ethylene Signaling Pathway. Sci. STKE *2005*, cm4. https://doi.org/10.1126/stke.2762005cm4.
52. Mao, J.-L., Miao, Z.-Q., Wang, Z., Yu, L.-H., Cai, X.-T., and Xiang, C.-B. (2016). Arabidopsis ERF1 Mediates Cross-Talk between Ethylene and Auxin Biosynthesis during Primary Root Elongation by Regulating ASA1 Expression. PLoS Genet. *12*, e1005760. https://doi.org/10.1371/journal.pgen.1005760.
53. Liu, G., Gao, S., Tian, H., Wu, W., Robert, H.S., and Ding, Z. (2016). Local Transcriptional Control of YUCCA Regulates Auxin Promoted Root-Growth Inhibition in Response to Aluminium Stress in Arabidopsis. PLoS Genet. *12*, e1006360. https://doi.org/10.1371/journal.pgen.1006360.
54. Miao, Z.-Q., Zhao, P.-X., Mao, J.-L., Yu, L.-H., Yuan, Y., Tang, H., Liu, Z.-B., and Xiang, C.-B. (2018). HOMEOBOX PROTEIN52 Mediates the Crosstalk between Ethylene and Auxin Signaling during Primary Root Elongation by Modulating Auxin Transport-Related Gene Expression. Plant Cell *30*, 2761–2778. https://doi.org/10.1105/tpc.18.00584.
55. Li, X., Chen, L., Forde, B.G., and Davies, W.J. (2017). The Biphasic Root Growth Response to Abscisic Acid in Arabidopsis Involves Interaction with Ethylene and Auxin Signalling Pathways. Front. Plant Sci. *8*, 1493. https://doi.org/10.3389/fpls.2017.01493.
56. Promchuea, S., Zhu, Y., Chen, Z., Zhang, J., and Gong, Z. (2017). ARF2 coordinates with PLETHORAs and PINs to orchestrate ABA-mediated root meristem activity in *Arabidopsis*. J. Integr. Plant Biol. *59*, 30–43. https://doi.org/10.1111/jipb.12506.
57. Yang, L., Zhang, J., He, J., Qin, Y., Hua, D., Duan, Y., Chen, Z., and Gong, Z. (2014). ABA-Mediated ROS in Mitochondria Regulate Root Meristem Activity by Controlling PLETHORA Expression in Arabidopsis. PLoS Genet. *10*, e1004791. https://doi.org/10.1371/journal.pgen.1004791.
58. Luo, X., Chen, Z., Gao, J., and Gong, Z. (2014). Abscisic acid inhibits root growth in Arabidopsis through ethylene biosynthesis. Plant J. *79*, 44–55. https://doi.org/10.1111/tpj.12534.
59. Di Ruocco, G., Bertolotti, G., Pacifici, E., Polverari, L., Tsiantis, M., Sabatini, S., Costantino, P., and Dello Ioio, R. (2018). Differential spatial

distribution of miR165/6 determines variability in plant root anatomy. Development *145*, dev153858. https://doi.org/10.1242/dev.153858.

60. Tian, Y., Zhao, N., Wang, M., Zhou, W., Guo, J., Han, C., Zhou, C., Wang, W., Wu, S., Tang, W., et al. (2022). Integrated regulation of periclinal cell division by transcriptional module of BZR1-SHR in *Arabidopsis* roots. New Phytol. *233*, 795–808. https://doi.org/10.1111/nph.17824.

61. Moreno-Risueno, M.A., Sozzani, R., Yardımcı, G.G., Petricka, J.J., Vernoux, T., Blilou, I., Alonso, J., Winter, C.M., Ohler, U., Scheres, B., et al. (2015). Transcriptional control of tissue formation throughout root development. Science *350*, 426–430. https://doi.org/10.1126/science.aad1171.

62. Cui, H., Levesque, M.P., Vernoux, T., Jung, J.W., Paquette, A.J., Gallagher, K.L., Wang, J.Y., Blilou, I., Scheres, B., and Benfey, P.N. (2007). An Evolutionarily Conserved Mechanism Delimiting SHR Movement Defines a Single Layer of Endodermis in Plants. Science *316*, 421–425. https://doi.org/10.1126/science.1139531.

63. Cruz-Ramírez, A., Díaz-Triviño, S., Blilou, I., Grieneisen, V.A., Sozzani, R., Zamioudis, C., Miskolczi, P., Nieuwland, J., Benjamins, R., Dhonukshe, P., et al. (2012). A Bistable Circuit Involving SCARECROW-RETINOBLASTOMA Integrates Cues to Inform Asymmetric Stem Cell Division. Cell *150*, 1002–1015. https://doi.org/10.1016/j.cell.2012.07.017.

64. Winter, C.M., Szekely, P., Popov, V., Belcher, H., Carter, R., Jones, M., Fraser, S.E., Truong, T.V., and Benfey, P.N. (2024). SHR and SCR coordinate root patterning and growth early in the cell cycle. Nature *626*, 611–616. https://doi.org/10.1038/s41586-023-06971-z.

65. Benfey, P.N., Linstead, P.J., Roberts, K., Schiefelbein, J.W., Hauser, M. T., and Aeschbacher, R.A. (1993). Root development in Arabidopsis: four mutants with dramatically altered root morphogenesis. Development *119*, 57–70. https://doi.org/10.1242/dev.119.Supplement.57.

66. Van Den Berg, C., Willemsen, V., Hage, W., Weisbeek, P., and Scheres, B. (1995). Cell fate in the Arabidopsis root meristem determined by directional signalling. Nature *378*, 62–65. https://doi.org/10.1038/378062a0.

67. Levesque, M.P., Vernoux, T., Busch, W., Cui, H., Wang, J.Y., Blilou, I., Hassan, H., Nakajima, K., Matsumoto, N., Lohmann, J.U., et al. (2006). Whole-Genome Analysis of the SHORT-ROOT Developmental Pathway in Arabidopsis. PLoS Biol. *4*, e143. https://doi.org/10.1371/journal.pbio.0040143.

68. Stallaert, W., Kedziora, K.M., Chao, H.X., and Purvis, J.E. (2019). Bistable switches as integrators and actuators during cell cycle progression. FEBS Lett. *593*, 2805–2816. https://doi.org/10.1002/1873-3468.13628.

69. Baum, S.F., Dubrovsky, J.G., and Rost, T.L. (2002). Apical organization and maturation of the cortex and vascular cylinder in *Arabidopsis thaliana* (Brassicaceae) roots. Am. J. Bot. *89*, 908–920. https://doi.org/10.3732/ajb.89.6.908.

70. Paquette, A.J., and Benfey, P.N. (2005). Maturation of the Ground Tissue of the Root Is Regulated by Gibberellin and *SCARECROW* and Requires *SHORT-ROOT*. Plant Physiol. *138*, 636–640. https://doi.org/10.1104/pp.104.058362.

71. Koizumi, K., Hayashi, T., Wu, S., and Gallagher, K.L. (2012). The SHORT-ROOT protein acts as a mobile, dose-dependent signal in patterning the ground tissue. Proc. Natl. Acad. Sci. USA *109*, 13010–13015. https://doi.org/10.1073/pnas.1205579109.

72. Heo, J.-O., Chang, K.S., Kim, I.A., Lee, M.-H., Lee, S.A., Song, S.-K., Lee, M.M., and Lim, J. (2011). Funneling of gibberellin signaling by the GRAS transcription regulator SCARECROW-LIKE 3 in the *Arabidopsis* root. Proc. Natl. Acad. Sci. USA *108*, 2166–2171. https://doi.org/10.1073/pnas.1012215108.

73. Gong, X., Flores-Vergara, M.A., Hong, J.H., Chu, H., Lim, J., Franks, R. G., Liu, Z., and Xu, J. (2016). SEUSS Integrates Gibberellin Signaling with Transcriptional Inputs from the SHR-SCR-SCL3 Module to Regulate Middle Cortex Formation in the Arabidopsis Root. Plant Physiol. *170*, 1675–1683. https://doi.org/10.1104/pp.15.01501.

74. Cui, H. (2016). Middle Cortex Formation in the Root: An Emerging Picture of Integrated Regulatory Mechanisms. Mol. Plant *9*, 771–773. https://doi.org/10.1016/j.molp.2016.05.002.

75. Bertolotti, G., Unterholzner, S.J., Scintu, D., Salvi, E., Svolacchia, N., Di Mambro, R., Ruta, V., Linhares Scaglia, F., Vittorioso, P., Sabatini, S., et al. (2021). A PHABULOSA-Controlled Genetic Pathway Regulates Ground Tissue Patterning in the Arabidopsis Root. Curr. Biol. *31*, 420–426.e6. https://doi.org/10.1016/j.cub.2020.10.038.

76. Cui, H., and Benfey, P.N. (2009). Interplay between SCARECROW, GA and LIKE HETEROCHROMATIN PROTEIN 1 in ground tissue patterning in the Arabidopsis root. Plant J. *58*, 1016–1027. https://doi.org/10.1111/j.1365-313X.2009.03839.x.

77. Miyashima, S., Koi, S., Hashimoto, T., and Nakajima, K. (2011). Non-cell-autonomous microRNA165 acts in a dose-dependent manner to regulate multiple differentiation status in the *Arabidopsis* root. Development *138*, 2303–2313. https://doi.org/10.1242/dev.060491.

78. Chang, J., Hu, J., Wu, L., Chen, W., Shen, J., Qi, X., and Li, J. (2024). Three RLKs integrate SHR-SCR and gibberellins to regulate root ground tissue patterning in Arabidopsis thaliana. Curr. Biol. *34*, 5295–5306.e5. https://doi.org/10.1016/j.cub.2024.09.074.

79. Lee, S.A., Jang, S., Yoon, E.K., Heo, J.-O., Chang, K.S., Choi, J.W., Dhar, S., Kim, G., Choe, J.-E., Heo, J.B., et al. (2016). Interplay between ABA and GA Modulates the Timing of Asymmetric Cell Divisions in the Arabidopsis Root Ground Tissue. Mol. Plant *9*, 870–884. https://doi.org/10.1016/j.molp.2016.02.009.

80. Yan, J., Zhao, C., Zhou, J., Yang, Y., Wang, P., Zhu, X., Tang, G., Bressan, R.A., and Zhu, J.-K. (2016). The miR165/166 Mediated Regulatory Module Plays Critical Roles in ABA Homeostasis and Response in Arabidopsis thaliana. PLoS Genet. *12*, e1006416. https://doi.org/10.1371/journal.pgen.1006416.

81. Yang, B., Sun, Y., Minne, M., Ge, Y., Yue, Q., Goossens, V., Mor, E., Callebaut, B., Bevernaege, K., Winne, J.M., et al. (2024). SPL13 controls a root apical meristem phase change by triggering oriented cell divisions. Science *386*, eado4298. https://doi.org/10.1126/science.ado4298.

82. Wu, G., Park, M.Y., Conway, S.R., Wang, J.-W., Weigel, D., and Poethig, R.S. (2009). The Sequential Action of miR156 and miR172 Regulates Developmental Timing in Arabidopsis. Cell *138*, 750–759. https://doi.org/10.1016/j.cell.2009.06.031.

83. Miyashima, S., Roszak, P., Sevilem, I., Toyokura, K., Blob, B., Heo, J.O., Mellor, N., Help-Rinta-Rahko, H., Otero, S., Smet, W., et al. (2019). Mobile PEAR transcription factors integrate positional cues to prime cambial growth. Nature *565*, 490–494. https://doi.org/10.1038/s41586-018-0839-y.

84. Bishopp, A., Help, H., El-Showk, S., Weijers, D., Scheres, B., Friml, J., Benková, E., Mähönen, A.P., and Helariutta, Y. (2011). A Mutually Inhibitory Interaction between Auxin and Cytokinin Specifies Vascular Pattern in Roots. Curr. Biol. *21*, 917–926. https://doi.org/10.1016/j.cub.2011.04.017.

85. De Rybel, B., Adibi, M., Breda, A.S., Wendrich, J.R., Smit, M.E., Novák, O., Yamaguchi, N., Yoshida, S., Van Isterdael, G., Palovaara, J., et al. (2014). Integration of growth and patterning during vascular tissue formation in *Arabidopsis*. Science *345*, 1255215. https://doi.org/10.1126/science.1255215.

86. Ohashi-Ito, K., Saegusa, M., Iwamoto, K., Oda, Y., Katayama, H., Kojima, M., Sakakibara, H., and Fukuda, H. (2014). A bHLH Complex Activates Vascular Cell Division via Cytokinin Action in Root Apical Meristem. Curr. Biol. *24*, 2053–2058. https://doi.org/10.1016/j.cub.2014.07.050.

87. Smet, W., Sevilem, I., De Luis Balaguer, M.A., Wybouw, B., Mor, E., Miyashima, S., Blob, B., Roszak, P., Jacobs, T.B., Boekschoten, M., et al. (2019). DOF2.1 Controls Cytokinin-Dependent Vascular Cell Proliferation Downstream of TMO5/LHW. Curr. Biol. *29*, 520–529.e6. https://doi.org/10.1016/j.cub.2018.12.041.

88. Muraro, D., Mellor, N., Pound, M.P., Help, H., Lucas, M., Chopard, J., Byrne, H.M., Godin, C., Hodgman, T.C., King, J.R., et al. (2014). Integration

of hormonal signaling networks and mobile microRNAs is required for vascular patterning in *Arabidopsis* roots. Proc. Natl. Acad. Sci. USA *111*, 857–862. https://doi.org/10.1073/pnas.1221766111.

89. el-Showk, S., Help-Rinta-Rahko, H., Blomster, T., Siligato, R., Marée, A. F.M., Mähönen, A.P., and Grieneisen, V.A. (2015). Parsimonious Model of Vascular Patterning Links Transverse Hormone Fluxes to Lateral Root Initiation: Auxin Leads the Way, while Cytokinin Levels Out. PLoS Comput. Biol. *11*, e1004450. https://doi.org/10.1371/journal.pcbi.1004450.
90. Vera-Sirera, F., De Rybel, B., Úrbez, C., Kouklas, E., Pesquera, M., Álvarez-Mahecha, J.C., Minguet, E.G., Tuominen, H., Carbonell, J., Borst, J.W., et al. (2015). A bHLH-Based Feedback Loop Restricts Vascular Cell Proliferation in Plants. Dev. Cell *35*, 432–443. https://doi.org/10.1016/j.devcel.2015.10.022.
91. Wybouw, B., Arents, H.E., Yang, B., Nolf, J., Smet, W., Vandorpe, M., Minne, M., Luo, X., De Clercq, I., Van Damme, D., et al. (2023). The transcription factor AtMYB12 is part of a feedback loop regulating cell division orientation in the root meristem vasculature. J. Exp. Bot. *74*, 1940–1956. https://doi.org/10.1093/jxb/erad020.
92. Yang, W., Cortijo, S., Korsbo, N., Roszak, P., Schiessl, K., Gurzadyan, A., Wightman, R., Jönsson, H., and Meyerowitz, E. (2021). Molecular mechanism of cytokinin-activated cell division in *Arabidopsis*. Science *371*, 1350–1355. https://doi.org/10.1126/science.abe2305.
93. Fujiwara, M., Imamura, M., Matsushita, K., Roszak, P., Yamashino, T., Hosokawa, Y., Nakajima, K., Fujimoto, K., and Miyashima, S. (2023). Patterned proliferation orients tissue-wide stress to control root vascular symmetry in Arabidopsis. Curr. Biol. *33*, 886–898.e8. https://doi.org/10.1016/j.cub.2023.01.036.
94. Ramachandran, P., Carlsbecker, A., and Etchells, J.P. (2017). Class III HD-ZIPs govern vascular cell fate: an HD view on patterning and differentiation. J. Exp. Bot. *68*, 55–69. https://doi.org/10.1093/jxb/erw370.
95. Ohashi-Ito, K., Iwamoto, K., Yamagami, A., Nakano, T., and Fukuda, H. (2023). HD-ZIP III-dependent local promotion of brassinosteroid synthesis suppresses vascular cell division in Arabidopsis root apical meristem. Proc. Natl. Acad. Sci. USA *120*, e2216632120. https://doi.org/10.1073/pnas.2216632120.
96. Augstein, F., and Carlsbecker, A. (2018). Getting to the Roots: A Developmental Genetic View of Root Anatomy and Function From Arabidopsis to Lycophytes. Front. Plant Sci. *9*, 1410. https://doi.org/10.3389/fpls.2018.01410.
97. Hetherington, A.J., and Dolan, L. (2018). Stepwise and independent origins of roots among land plants. Nature *561*, 235–238. https://doi.org/10.1038/s41586-018-0445-z.
98. Jan, M., Muhammad, S., Jin, W., Zhong, W., Zhang, S., Lin, Y., Zhou, Y., Liu, J., Liu, H., Munir, R., et al. (2024). Modulating root system architecture: cross-talk between auxin and phytohormones. Front. Plant Sci. *15*, 1343928. https://doi.org/10.3389/fpls.2024.1343928.
99. Rani Debi, B., Taketa, S., and Ichii, M. (2005). Cytokinin inhibits lateral root initiation but stimulates lateral root elongation in rice (Oryza sativa). J. Plant Physiol. *162*, 507–515. https://doi.org/10.1016/j.jplph.2004.08.007.
100. Qin, H., Pandey, B.K., Li, Y., Huang, G., Wang, J., Quan, R., Zhou, J., Zhou, Y., Miao, Y., Zhang, D., et al. (2022). Orchestration of ethylene and gibberellin signals determines primary root elongation in rice. Plant Cell *34*, 1273–1288. https://doi.org/10.1093/plcell/koac008.
101. Qian, W., Wu, C., Fu, Y., Hu, G., He, Z., and Liu, W. (2017). Novel rice mutants overexpressing the brassinosteroid catabolic gene CYP734A4. Plant Mol. Biol. *93*, 197–208. https://doi.org/10.1007/s11103-016-0558-4.
102. Yamamuro, C., Ihara, Y., Wu, X., Noguchi, T., Fujioka, S., Takatsuto, S., Ashikari, M., Kitano, H., and Matsuoka, M. (2000). Loss of function of a rice brassinosteroid insensitive1 homolog prevents internode elongation and bending of the lamina joint. Plant Cell *12*, 1591–1606. https://doi.org/10.1105/tpc.12.9.1591.
103. Forestan, C., Farinati, S., and Varotto, S. (2012). The Maize PIN Gene Family of Auxin Transporters. Front. Plant Sci. *3*, 16. https://doi.org/10.3389/fpls.2012.00016.
104. Li, Y., Han, S., and Qi, Y. (2023). Advances in structure and function of auxin response factor in plants. J. Integr. Plant Biol. *65*, 617–632. https://doi.org/10.1111/jipb.13392.
105. Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Shibata, Y., Gomi, K., Umemura, I., Hasegawa, Y., Ashikari, M., Kitano, H., and Matsuoka, M. (2005). *Crown rootless1* , Which Is Essential for Crown Root Formation in Rice, Is a Target of an AUXIN RESPONSE FACTOR in Auxin Signaling. Plant Cell *17*, 1387–1396. https://doi.org/10.1105/tpc.105.030981.
106. Sims, K., Abedi-Samakush, F., Szulc, N., Macias Honti, M.G., and Mattsson, J. (2021). OsARF11 Promotes Growth, Meristem, Seed, and Vein Formation during Rice Plant Development. Int. J. Mol. Sci. *22*, 4089. https://doi.org/10.3390/ijms22084089.
107. Qiao, L., Zhang, W., Li, X., Zhang, L., Zhang, X., Li, X., Guo, H., Ren, Y., Zheng, J., and Chang, Z. (2018). Characterization and Expression Patterns of Auxin Response Factors in Wheat. Front. Plant Sci. *9*, 1395. https://doi.org/10.3389/fpls.2018.01395.
108. Wang, L., Guo, M., Li, Y., Ruan, W., Mo, X., Wu, Z., Sturrock, C.J., Yu, H., Lu, C., Peng, J., and Mao, C. (2018). LARGE ROOT ANGLE1, encoding OsPIN2, is involved in root system architecture in rice. J. Exp. Bot. *69*, 385–397. https://doi.org/10.1093/jxb/erx427.
109. Li, J., Wu, F., He, Y., He, B., Gong, Y., Yahaya, B.S., Xie, Y., Xie, W., Xu, J., Wang, Q., et al. (2022). Maize Transcription Factor ZmARF4 Confers Phosphorus Tolerance by Promoting Root Morphological Development. Int. J. Mol. Sci. *23*, 2361. https://doi.org/10.3390/ijms23042361.
110. Yu, C., Sun, C., Shen, C., Wang, S., Liu, F., Liu, Y., Chen, Y., Li, C., Qian, Q., Aryal, B., et al. (2015). The auxin transporter, Os AUX 1, is involved in primary root and root hair elongation and in Cd stress responses in rice (*Oryza sativa* L.). Plant J. *83*, 818–830. https://doi.org/10.1111/tpj.12929.
111. Wang, J.-R., Hu, H., Wang, G.-H., Li, J., Chen, J.-Y., and Wu, P. (2009). Expression of PIN Genes in Rice (Oryza sativa L.): Tissue Specificity and Regulation by Hormones. Mol. Plant *2*, 823–831. https://doi.org/10.1093/mp/ssp023.
112. Liu, B., Zhang, J., Wang, L., Li, J., Zheng, H., Chen, J., and Lu, M. (2014). A survey of Populus PIN-FORMED family genes reveals their diversified expression patterns. J. Exp. Bot. *65*, 2437–2448. https://doi.org/10.1093/jxb/eru129.
113. Kim, H.J., Kieber, J.J., and Schaller, G.E. (2012). Overlapping and lineage-specific roles for the type-B response regulators of monocots and dicots. Plant Signal Behav. *7*, 1110–1113. https://doi.org/10.4161/psb.21293.
114. Tsai, Y.C., Weir, N.R., Hill, K., Zhang, W., Kim, H.J., Shiu, S.H., Schaller, G.E., and Kieber, J.J. (2012). Characterization of genes involved in cytokinin signaling and metabolism from rice. Plant Physiol. *158*, 1666–1684. https://doi.org/10.1104/pp.111.192765.
115. Gao, S., Fang, J., Xu, F., Wang, W., Sun, X., Chu, J., Cai, B., Feng, Y., and Chu, C. (2014). CYTOKININ OXIDASE/DEHYDROGENASE4 Integrates Cytokinin and Auxin Signaling to Control Rice Crown Root Formation. Plant Physiol. *165*, 1035–1046. https://doi.org/10.1104/pp.114.238584.
116. De Vries, J., Fischer, A.M., Roettger, M., Rommel, S., Schluepmann, H., Bräutigam, A., Carlsbecker, A., and Gould, S.B. (2016). Cytokinin-induced promotion of root meristem size in the fern *Azolla* supports a shoot-like origin of euphyllophyte roots. New Phytol. *209*, 705–720. https://doi.org/10.1111/nph.13630.
117. Müssig, C., Shin, G.-H., and Altmann, T. (2003). Brassinosteroids Promote Root Growth in Arabidopsis. Plant Physiol. *133*, 1261–1271. https://doi.org/10.1104/pp.103.028662.
118. Achard, P., Vriezen, W.H., Van Der Straeten, D., and Harberd, N.P. (2003). Ethylene Regulates Arabidopsis Development via the Modulation of DELLA Protein Growth Repressor Function. Plant Cell *15*, 2816–2825. https://doi.org/10.1105/tpc.015685.

119. Shaar-Moshe, L., and Brady, S.M. (2023). *SHORT-ROOT* and *SCARECROW* homologs regulate patterning of diverse cell types within and between species. New Phytol. *237*, 1542–1549. https://doi.org/10.1111/nph.18654.

120. Henry, S., Dievart, A., Divol, F., Pauluzzi, G., Meynard, D., Swarup, R., Wu, S., Gallagher, K.L., and Périn, C. (2017). SHR overexpression induces the formation of supernumerary cell layers with cortex cell identity in rice. Dev. Biol. *425*, 1–7. https://doi.org/10.1016/j.ydbio.2017.03.001.

121. Wu, S., Lee, C.-M., Hayashi, T., Price, S., Divol, F., Henry, S., Pauluzzi, G., Perin, C., and Gallagher, K.L. (2014). A plausible mechanism, based upon SHORT-ROOT movement, for regulating the number of cortex cell layers in roots. Proc. Natl. Acad. Sci. USA *111*, 16184–16189. https://doi.org/10.1073/pnas.1407371111.

122. Henry, S., Divol, F., Bettembourg, M., Bureau, C., Guiderdoni, E., Périn, C., and Diévart, A. (2015). Immunoprofiling of Rice Root Cortex Reveals Two Cortical Subdomains. Front. Plant Sci. *6*, 1139. https://doi.org/10.3389/fpls.2015.01139.

123. Ortiz-Ramírez, C., Guillotin, B., Xu, X., Rahni, R., Zhang, S., Yan, Z., Coqueiro Dias Araujo, P., Demesa-Arevalo, E., Lee, L., Van Eck, J., et al. (2021). Ground tissue circuitry regulates organ complexity in maize and *Setaria*. Science *374*, 1247–1252. https://doi.org/10.1126/science.abj2327.

124. Woudenberg, S., Renema, J., Tomescu, A.M.F., De Rybel, B., and Weijers, D. (2022). Deep origin and gradual evolution of transporting tissues: Perspectives from across the land plants. Plant Physiol. *190*, 85–99. https://doi.org/10.1093/plphys/kiac304.

125. Yang, X., Poelmans, W., Grones, C., Lakehal, A., Pevernagie, J., Van Bel, M., Njo, M., Xu, L., Nelissen, H., De Rybel, B., et al. (2023). Spatial transcriptomics of a lycophyte root sheds light on root evolution. Curr. Biol. *33*, 4069–4084.e8. https://doi.org/10.1016/j.cub.2023.08.030.

126. Floyd, S.K., Zalewski, C.S., and Bowman, J.L. (2006). Evolution of Class III Homeodomain–Leucine Zipper Genes in Streptophytes. Genetics *173*, 373–388. https://doi.org/10.1534/genetics.105.054239.

127. Pandey, B.K., Huang, G., Bhosale, R., Hartman, S., Sturrock, C.J., Jose, L., Martin, O.C., Karady, M., Voesenek, L.A.C.J., Ljung, K., et al. (2021). Plant roots sense soil compaction through restricted ethylene diffusion. Science *371*, 276–280. https://doi.org/10.1126/science.abf3013.