ANNUAL REVIEWS

Annual Review of Plant Biology

Molecular and Environmental Regulation of Root Development

Hans Motte,[1,2] Steffen Vanneste,[1,2,3] and Tom Beeckman[1,2]

[1]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium

[2]Center for Plant Systems Biology, VIB, 9052 Ghent, Belgium; email: tom.beeckman@ugent.vib.be

[3]Lab of Plant Growth Analysis, Ghent University Global Campus, Incheon 21985, Republic of Korea

Annu. Rev. Plant Biol. 2019. 70:465–88

First published as a Review in Advance on March 1, 2019

The *Annual Review of Plant Biology* is online at plant.annualreviews.org

https://doi.org/10.1146/annurev-arplant-050718-100423

Keywords

root development, root architecture, lateral root, gravitropism, root meristem

Abstract

In order to optimally establish their root systems, plants are endowed with several mechanisms to use at distinct steps during their development. In this review, we zoom in on the major processes involved in root development and detail important new insights that have been generated in recent studies, mainly using the *Arabidopsis* root as a model. First, we discuss new insights in primary root development with the characterization of tissue-specific transcription factor complexes and the identification of non-cell-autonomous control mechanisms in the root apical meristem. Next, root branching is discussed by focusing on the earliest steps in the development of a new lateral root and control of its postemergence growth. Finally, we discuss the impact of phosphate, nitrogen, and water availability on root development and summarize current knowledge about the major molecular mechanisms involved.

ترجمه
به منظور استقرار بهینه سیستم ریشه خود، گیاهان دارای وقف
هستند
.چندین مکانیسم برای استفاده در مراحل متمایز در طول توسعه آنها

Contents

1. INTRODUCTION

In plants, root systems represent the below-ground organs that develop from the root apical meristem initiated during embryogenesis and possibly from multiple not-root-borne adventitious root apical meristems. After germination, root systems continue to expand in size to become as extensive as—or even to considerably exceed—their above-ground parts. Depending on the species, root systems show a high level of morphological diversity varying from deeply penetrating to shallow and horizontally extending roots, with all possible combinations in between. This evolutionarily acquired diversity provides plants with a means to adapt to local climatological and/or edaphic conditions. In plant communities, such morphological diversification allows for a stratification of root systems from different plant species thereby evading too much competition for water and nutrients. It is unsurprising that, due to their inaccessibility, the study of root systems has been neglected for a long time. This has resulted in a serious lack of knowledge about root biology as compared with the shoot. However, over the last couple of years, the root research community has grown rapidly, and it has become nearly impossible to keep up with the recent appearance of new methods, approaches, and protocols to study roots. Advanced digital photography, X-ray computed tomography, transparent soils, automated rhizotron and aeroponic installations, high-throughput 3D reconstructions, and fluorescence-based and luminescence-based imaging systems have all recently been developed and improved (156) and will contribute to a better understanding of root system architecture in the near future. At the same time, the well-established *Arabidopsis* reference organism, which ushered in a new era in root developmental studies now 25 years ago (32), has continuously been used and is still valid to use in identifying genes involved in root developmental processes. The achievements of the *Arabidopsis* root community have led to a broader understanding of fundamental aspects of root biology, such as meristem organization, gravitropic response, root branching, and, more recently, the response of roots toward their environment. This review zooms in on these recent developments obtained primarily in the *Arabidopsis* reference organism with some exceptions in Section 4 on nutrient and water availability, where recent data has also been obtained on other species.

Meristem: a discrete group of undifferentiated cells with high division capacity responsible for producing new tissues and organs

Rhizotron: a laboratory set up to observe and study roots in soil conditions, usually by growing roots on a transparent surface

2. REGULATION OF ROOT GROWTH BY THE ROOT APICAL MERISTEM

Generally, young growing roots have a relatively simple radial organization in which the vascular cylinder or stele is surrounded by concentric ground tissue layers (cortex, endodermis) and delineated from the environment by a root epidermis or rhizodermis that can produce root hairs. The root tip is surrounded by a root cap, a protective tissue, which, according to fossil records, was already present in lycophytes of the Devonian period (58, 90). Besides its radial structure, the root consists of roughly three longitudinal zones: the meristematic, elongation, and differentiation zones (**Figure 1**). In the meristematic zone (meristem), all cell types derive from one or more precursors or stem cells located at the very tip (stem cell niche).

As stem cells divide, they generate tissue-specific transit-amplifying cells, which in turn divide anticlinally to produce the bulk of cells constituting the meristem. Cells that leave the meristem start to anisotropically expand (in the elongation zone) and later mature into their final shape and function (in the differentiation zone). The rate of cell proliferation and subsequent elongation propels the root stem cell niche forward through the soil and defines the rate of root growth.

Transit-amplifying cells: cells in the meristem that arise from stem cells and divide a finite number of times until they become differentiated

Quiescent center: a region in the root apical meristem where cell division proceeds very slowly or not at all

Columella: a tissue layer in the central part of the root cap below the quiescent center where cells are arranged in columns

2.1. Transcriptional Control of the Stem Cell Niche and the Proximal Meristem

Most of our knowledge about meristem functioning comes from the *Arabidopsis* meristem, where stem cells are situated around a four-celled quiescent center. Genetic or physical ablation of the quiescent center cells causes collapse of the meristem, a loss of proliferative activity, and the onset of differentiation; this indicates the role of the quiescent center in preventing differentiation of the stem cells (139, 151). This key function of the quiescent center in root meristem organization depends largely on the activity of the WUSCHEL-LIKE HOMEOBOX5 (WOX5) transcription factor (121). *WOX5* is expressed specifically in the quiescent center, where it prevents cell proliferation via suppression of *CYCD3;3* expression (40). From the quiescent center, WOX5 can move into the abutting cells, where it represses differentiation. In the columella stem cells, a WOX5–TOPLESS-HISTONE DEACETYLASE19 complex is formed to prevent differentiation (109). Homologs of WOX5 identified in both other dicots and more anciently diverged lineages have been found to functionally complement the *wox5* mutant (163), suggesting that the role of WOX5 in the stem cell niche is widely conserved.

Members of the PLETHORA (PLT) transcription factor family are master regulators of root organogenesis. Mutants in multiple PLTs have profound effects on root meristem organization, including a defective stem cell niche (1). No strong reduction in WOX5 expression was observed in *plt1 plt2* double mutants (121), but ectopic activation of PLT1 causes expansion of the WOX5 expression domain into the cortex-endodermis initial daughter cells (124). Very strong PLT overexpression during embryogenesis results in ectopic root development, whereas intermediate PLT doses keep transit-amplifying cells proliferative and inhibit elongation (1, 43, 88), illustrating a dose-dependent readout of PLT levels. This is consistent with a central role for PLTs in root meristem zonation. Analysis of the PLT-responsive transcriptional network corroborates PLTs as master regulators of root organogenesis via the activation of proliferation and repression of differentiation (120).

The key to the coordinative function of PLT in root meristem zonation lies in the PLT protein gradients that exist across the meristem (43, 88). In the root stem cells, where PLT levels are highest, PLT activates MIR396 to degrade mRNA of the proliferation-inducing GROWTH-REGULATING FACTOR (GRF), which in turn represses *PLT* expression in transit-amplifying cells (112). This feedback mechanism contributes to confining the *PLT* expression domain more

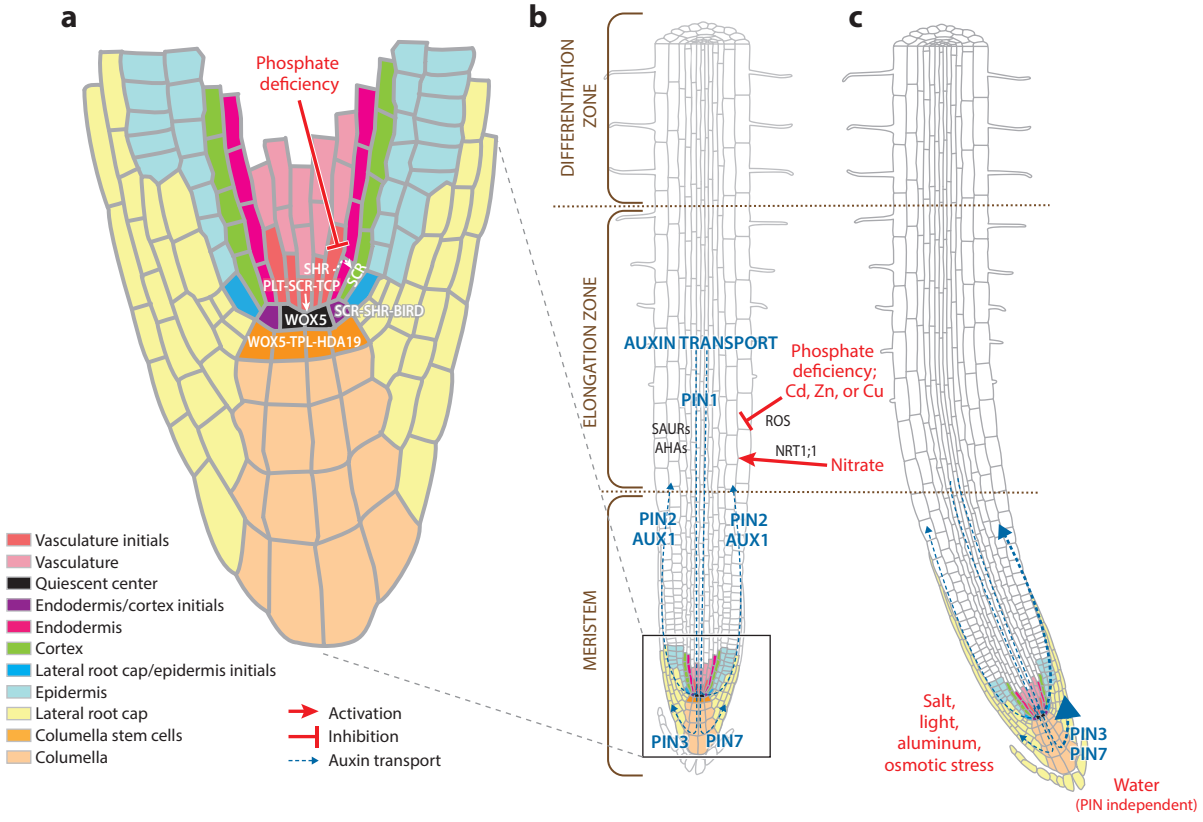


Figure 1

Anatomy of the primary root and the pathways that contribute to root growth by regulating cell divisions in the meristematic zone or elongation of the cells in the elongation zone and environmental cues affecting these pathways (*red arrows*). (*a*) Detailed view of the root meristem and the molecular players contributing to the cell divisions in the meristem and meristem organization. (*b*) Growing root. Both the cell divisions via the molecular players indicated in panel *a* as the cell elongation depends on the auxin level, and hence auxin transport (indicated by *dashed blue arrows*) as well. In the elongation zone, auxin induces cell expansion via the concerted activity of SAURs, AHAs, and cell wall–modifying proteins. NRT1.1 activates auxin signaling. ROS signaling impedes cell expansion by stiffening the cell wall. (*c*) A root subjected to a directional stimulus. Differential auxin transport caused by relocalization of PIN3 and PIN7 auxin transporters in the columella results in a shift in auxin transport (indicated by different arrow sizes) and thus in auxin distribution. High auxin levels inhibit cell elongation. As such, the root bends toward the high auxin level side. Environmental cues that have an impact on one of the pathways presented in the figure are indicated in red. Abbreviations: AHA, *Arabidopsis* H^+-ATPase; AUX1, Auxin transporter protein 1; Cd, cadmium; Cu, copper; HDA19, HISTONE DEACETYLASE19; NRT1.1, NITRATE TRANSPORTER 1.1; PIN, PIN-FORMED; PLT, PLETHORA; ROS, reactive oxygen species; SAURs, Small Auxin Up RNAs; SCR, SCARECROW; SHR, SHORT-ROOT; TCP, PLANT-SPECIFIC TEOSINTE-BRANCHED CYCLOIDEA PCNA; TPL, TOPLESS; WOX5, WUSHEL-LIKE HOMEOBOX5; Zn, zinc.

or less to the stem cell niche. Yet, the PLT proteins can be found throughout the meristem (43). Based on cell-to-cell movement and dilution via cell division, gradients of these stable proteins are established across the meristem and translate via a dose-dependent readout into its longitudinal meristem zonation (88). PLT protein stability depends heavily on sulfated ROOT GROWTH FACTOR (RGF)/GOLVEN (GLV) peptide signaling. Exogenous application of RGF/GLV causes expansion of the root apical meristem (148), similar to plants overexpressing PLT, whereas higher-order mutants defective in RGF/GLV and RGF receptors have severe defects in meristem organization, which are associated with reduced PLT protein levels (91, 104, 165).

The GRAS transcription factor SCARECROW (SCR) has a cell-autonomous function that partially defines quiescent center identity (119), regulates cortex-endodermis initial daughter cell division (24, 126), and stimulates endodermis differentiation (75). In addition, SCR activates MIR165 in the endodermis, which diffuses into the surrounding tissues to control nonradially symmetric vascular bundle patterning (19) and transit-amplifying cell proliferation (122).

To be able to affect these distinct processes, SCR forms tissue-specific higher-order transcription factor complexes. One of the main interaction partners of SCR is the stele-expressed GRAS transcription factor, SHORT ROOT (SHR), which moves symplastically through plasmodesmata into the SCR expression domain (81, 141). In these cell types, SHR activates the expression of not only SCR but also BIRD zinc fingers [e.g., JACKDAW (JKD), NUTCRACKER, BLUE JAY, and MAGPIE], which are additional regulatory components of the SCR-SHR transcription factor complex (82, 93). The formation of such higher-order transcription factor complexes provides a means for tissue-specific activation of *WOX5*, *CYCD6;1*, or *CASP1* (82). In the cortex-endodermis initial daughter cells, SCR-SHR-BIRD complexes activate *CYCD6;1* expression to promote the asymmetric cell division that leads to the formation of the ground tissue defined by the endodermis and cortex (26, 57, 126), an activity that can be enhanced by auxin (24). In addition, the BIRDs JKD and BALD IBIS (BIB), together with SCR, constrain SHR movement via nuclear retention, while JKD and BIB repress the *CYCD6;1*-inducing capacity of the SCR-SHR complex (82). The SCR-SHR complex activates cell division, but its transcriptional activity is also coupled to the cell cycle through direct interaction of SCR with the canonical cell cycle repressor RETINOBLASTOMA RELATED (RBR) and proteasomal degradation of SCR and RBR during cell division (24, 25). The recent identification of the PLANT-SPECIFIC TEOSINTE-BRANCHED CYCLOIDEA PCNA (TCP) transcription factors as a bridging factor between PLT and SCR complexes for direct activation of *WOX5* expression (124) further supports the emerging theme that higher-order complexes in overlapping expression domains of different key regulators delineate tissue-specific developmental programs in the root meristem.

Plasmodesmata: microscopic channels that traverse the cell walls of plant cells and allow for symplastic movement of signaling molecules

Asymmetric cell division: involves polarization of the mother cell to produce two daughter cells with different cell fates; typical of stem cells

2.2. Auxin Distribution Tunes Root Apical Meristem Organization and Root Elongation

Root development results from the concerted action of multiple plant hormones (21). Among these, auxin stands out as a key instructive signal on which many, if not all, other plant hormones converge to regulate root development. Already during embryogenesis, auxin distribution patterns determine the position around which the embryonic roots starts developing. Later in development, auxin distribution patterns in and around the meristem instruct root meristem organization, regeneration after damage, tropic root growth, and lateral root spacing.

Analysis of the spatial dynamics of auxin-responsive genes, the readout of synthetic auxin-signaling reporters, and simulations of auxin transport consistently predict the existence of a physical auxin gradient that is centered on the very tip of the meristem and that gradually lowers toward the end (8, 11, 17, 49, 74).

Indeed, a steep auxin-signaling gradient with its peak on the quiescent center is required to maintain columella stem cell identity (134), cell division, and columella shedding (36). Moreover, gradients of auxin and PLT proteins across the root meristem are tightly interconnected (15, 43, 49, 146). Auxin can induce *PLT* expression, and PLT directly controls PIN-mediated auxin transport and auxin biosynthesis (1, 120). However, the PLT gradient in the root meristem is not a direct readout of the auxin gradient. Instead, the effects of auxin on meristem zonation can be separated in two cooperative responses (88): (*a*) a slow response that is connected to the PLT expression domain and that subsequently translates into a gradient via diffusion and cell

division–based protein dilution and (*b*) a superimposed, fast response controlling proliferation, elongation, and differentiation.

TRANSPORT INHIBITOR RESPONSE 1 (TIR1)-based signaling: nuclear auxin signaling based on auxin binding to the F-box TRANSPORT INHIBITOR RESPONSE 1 auxin receptor

Reactive oxygen species (ROS) signaling: in addition to generating oxidative stress in cells, byproducts of aerobic metabolism can induce specific signaling to regulate biological and physiological processes

The fast auxin response probably allows for the integration of environmental challenges in root growth while maintaining normal root meristem organization. This is particularly relevant during the root's navigation through the soil in search of water and nutrients while avoiding obstacles and stresses. Very low auxin concentrations stimulate root elongation in *Arabidopsis* (10). Conversely, minute auxin increases potently inhibit root elongation via a nontranscriptional branch of TRANSPORT INHIBITOR RESPONSE 1 (TIR1)-based signaling (38). Therefore, many regulating stimuli of root elongation increase auxin levels in the elongation zone to modulate root growth.

The redirection of auxin flows within the meristem, as seen during tropisms, allows for asymmetric auxin distribution in the root tip, differential inhibition of elongation, and, as a consequence, reorientation of the root growth direction (reviewed in 56). PIN3 and PIN7 are key to redirecting the auxin as they relocalize in the columella upon directional stimuli (41, 70). Subsequently, PIN2 and AUX1 transport auxin shootward and are thus essential in most tropisms but are not responsible for changed auxin distribution (14, 22, 83, 86, 96, 138). Of important note is that not all tropisms act through modulation of auxin homeostasis and signaling (56). Hydrotropism, which is the bending of the root toward a higher water potential, is such an example and is actually dependent on abscisic acid (ABA) (2, 132).

The mechanism by which low auxin levels stimulate elongation is poorly understood but is probably similar to the one that acts in the shoot (10, 62). In hypocotyls, auxin sensing via a TIR1/AUXIN-SIGNALING F-BOX (AFB)-based mechanism activates the transcription of Small Auxin Up RNAs (SAURs). These SAURs activate plasma membrane–localized proton ATPases (AHAs) to lower apoplastic pH, thereby promoting cell expansion due to the activation of expansins and other cell wall–modifying proteins (127, 131). However, the rapid and reversible inhibition of root elongation also depends on TIR1-based auxin signaling via an unknown nontranscriptional mechanism (38). Interestingly, inhibition of auxin signaling via expression of a stabilized AXR3/INDOLE-3-ACETIC ACID17 (IAA17) isoform in the expanding epidermal cells renders root growth partially resistant to auxin (129), suggesting that more downstream components of canonical transcriptional auxin signaling are involved in this nontranscriptional pathway. However, the best characterized interaction partners of Aux/IAAs, such as AXR3/IAA17, are auxin-response transcription factors (ARFs), raising the possibility of still unidentified intracellular targets of the auxin-dependent TIR-Aux/IAA interaction.

Notably, reactive oxygen species (ROS) signaling affects root growth independently from auxin. More specifically, ROS signaling modulates the balance between cell proliferation and elongation (136), controls PLT expression in the root meristem (155), and is even involved in quiescent center identity by controlling *SCR* and *SHR* expression (160).

3. ROOT BRANCHING

3.1. Lateral Roots

In angiosperm roots, branching occurs through the formation of lateral roots, which, strictly speaking, are roots that form off of an existing root. This is opposed to adventitious roots, which are produced from nonroot tissues. During evolution, the first roots appeared in the Early Devonian in the lycophytes, but they did not produce lateral roots. In these first land plants, roots colonized the soil by repeatedly cleaving their apices thereby producing twin root meristems. This bifurcation process can still be observed in the extant representatives of the lycophytes, such as *Selaginella* (42). In the euphyllophytic lineage extending toward the seed plants, roots evolved a

second time (58, 59). In this evolution, roots acquired the capacity to expand laterally into the soil by producing lateral roots, which represents a much more efficient root branching strategy that not only increases branching capacity but also provides developmental plasticity to changing soil conditions (95). The contribution of these different root types to the mature root system varies among the different taxa. In general, monocotyledons, such as the grasses, predominantly use adventitious roots that bear lateral roots to build their fibrous root systems, whereas dicotyledons, such as *Arabidopsis*, predominantly produce a taproot system with a prevailing primary root that is the source of all lateral roots and their branches. In the following sections, we zoom in on the early developmental steps of the latter, which has been extensively studied in *Arabidopsis*.

Protoxylem: the first-formed xylem cell type developing from the procambium, confined between, centrally, the metaxylem and, peripherally, the pericycle

Anticlinal division: cell division occurring at right angles to the tissue or organ surface

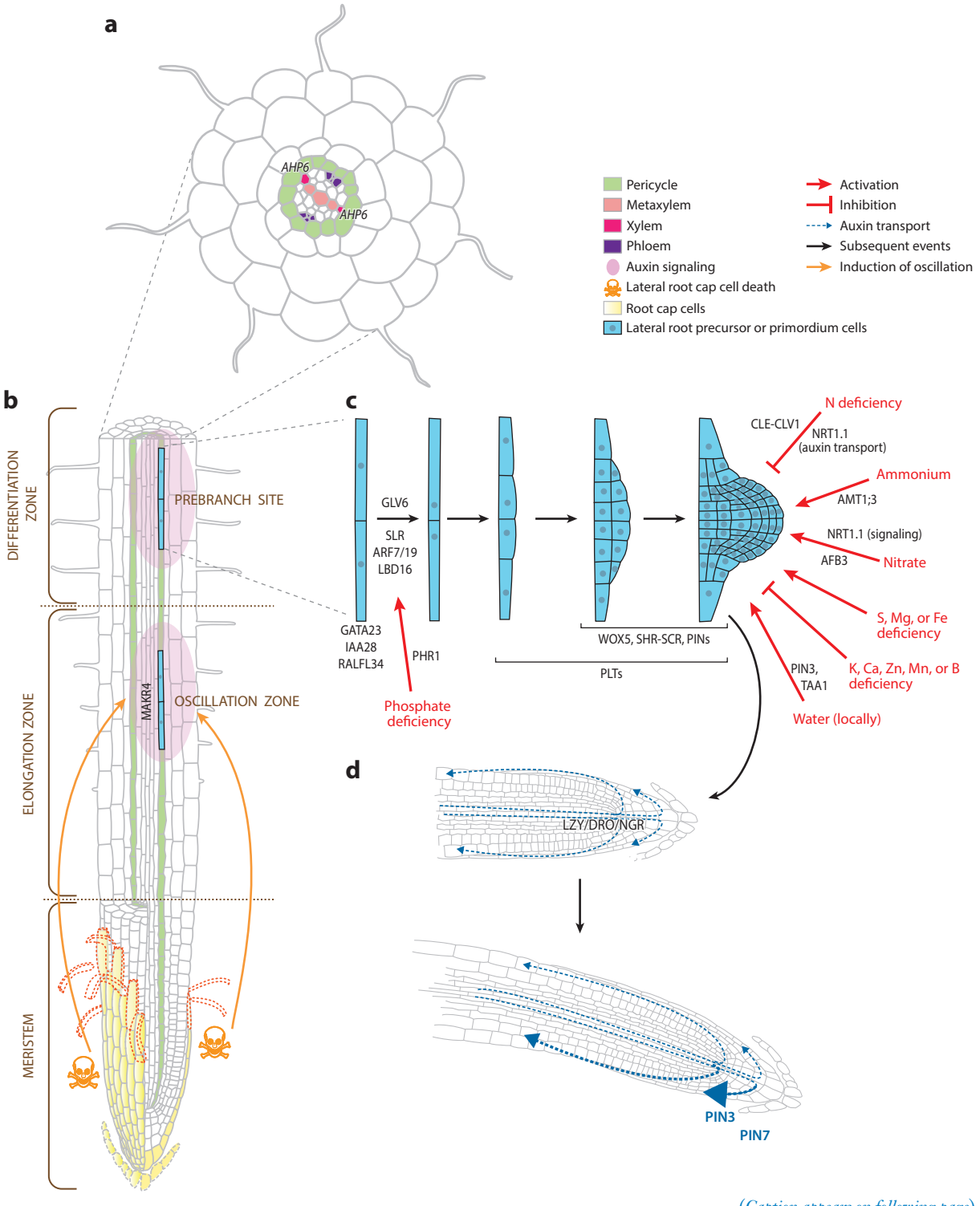
DR5 auxin-response marker: a synthetic promoter construct activated upon auxin perception that can be fused to reporter genes, such as luciferase

Transition zone: a region in the root tip located between the apical meristem and the basal elongation region

Prebranch site: a region in the young root with strong DR5-luciferase activity correlated with early stages of lateral root formation

3.1.1. Xylem pole pericycle cells. In *Arabidopsis*, as in the majority of the angiosperms, new lateral roots initiate in the pericycle at the xylem poles, termed the xylem pole pericycle (XPP) cells. The pericycle is a single-layered tissue that surrounds the central vascular tissue of the root (**Figure 2**). Because it is confined within the endodermis, the pericycle is commonly regarded as being part of the stele. Moreover, there is accumulating evidence that pericycle cells at the phloem versus xylem poles differ from each other in postembryonic roots and instead have features in common with their respective neighboring vascular tissues. Support for this hypothesis comes from a study of marker lines for pericycle identity, analysis of mutants defective in vascular development, and use of ultrastructural analysis, which together have argued for the existence of at least two different pericycle cell identities—each of them closely associated with the adjacent vascular elements (106). AHP6, a negative regulator of cytokinin signaling, is required for normal protoxylem formation (87). *AHP6* is expressed in the developing protoxylem and in the associated pericycle cell files. This suggests that the specification of XPP cells as competent to form lateral roots is correlated with vascular patterning. Based on the *AHP6* expression pattern, starting from the stem cell area near the quiescent center, specification of XPP cell files seems to already happen in the pericycle initials. However, once specified, several developmental steps are required to produce lateral roots from a subset of the XPP cells. Especially for this part of lateral root development there is still a lot to discover. Yet, while taking into account the latest insights, and to our best knowledge, the following chronological steps can be discriminated: (*a*) specification of lateral root founders in a subset of XPP cells, (*b*) lateral root initiation (the first visible event marked by an asymmetric cell division pattern), (*c*) lateral root primordium formation (during which a new meristem is established), and (*d*) lateral root emergence (passage of the primordium through the overlying cells) (**Figure 2**).

3.1.2. Specification of lateral root founder cells: the root cap and oscillations in auxin response. Due to continued anticlinal division and cell elongation, XPP cells leave the meristem and become exposed to an auxin-signaling maximum, as can be visualized by the DR5 auxin-response marker (28). Because this maximum is not constitutive but rather shows recurrent peaks that are interrupted by periods of low auxin response, the idea of an oscillatory behavior in gene expression was proposed and the region of the root where this occurs was termed the oscillation zone. The oscillation zone describes a region in the root tip roughly stretching from the transition zone into the elongation zone (94). The dynamics of the auxin-signaling peaks can be studied using a *DR5*::*luciferase* (*DR5*::*LUC*) reporter, and the occurrence of the peaks can be correlated with the sites of later lateral root initiation. This correlation holds true as long as the amplitude of the peak in auxin response is strong enough and as long as it is maintained into the elongation zone (154). These strong *DR5*::*LUC* maxima are even maintained into the differentiation zone and are regarded as prebranch sites because they represent the sites of founder cell specification and



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Pathways that contribute to lateral root initiation, formation, and outgrowth, as well as environmental cues affecting these pathways (*red arrows*). (*a*) Cross-section of an *Arabidopsis* root showing the vascular bundle and xylem pole pericycle cells, in *Arabidopsis* possible lateral root founder cells, marked by increased *AHP6* expression. (*b*) Lateral root initiation is preceded by a recurrent cell death of lateral root cap cells that induce an auxin signaling oscillation in the elongation zone. From these cells marked by a persistent auxin signal, a prebranch site, which has the competence to form a lateral root primordium, may originate. (*c*) Lateral root primordium formation. The onset of primordium formation is hallmarked by coordinated nuclear migration in two or more adjacent xylem pole pericycle cells and followed by a series of well-organized cell divisions. The different steps before or during lateral root formation require the contribution of specific molecular players as indicated. Red arrows indicate an effect during lateral root initiation or formation. (*d*) ~~An emerged lateral root. At first, the gravitropic response is suppressed, allowing an initial horizontal outgrowth of the lateral root. Later in development, gravitropic growth is partially restored (differential auxin transport indicated by *blue dashed arrows*)~~. Abbreviations: AFB3; AUXIN SIGNALING F-BOX3; *AHP6*, *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6*; AMT1;3, AMMONIUM TRANSPORTER1;3; ARF, AUXIN RESPONSE FACTOR; B, boron; Ca, calcium; CLE, CLAVATA3-LIKE; CLV1, CLAVATA1; Fe, iron; GATA23, GATA TRANSCRIPTION FACTOR 23; GLV6, GOLVEN6; IAA28, INDOLE-3-ACETIC ACID INDUCIBLE 28; K, potassium; LBD16, LOB DOMAIN-CONTAINING PROTEIN 16; LZY/DRO/NGR, LAZY/DEEPER ROOTING/NEGATIVE GRAVITROPIC RESPONSE OF ROOTS; MAKR4, MEMBRANE-ASSOCIATED KINASE REGULATOR4; Mg, magnesium; Mn, manganese; NRT1.1, NITRATE TRANSPORTER 1.1; PIN, PIN-FORMED; PLT, PLETHORA; RALFL34, RALF-LIKE 34; S, sulfur; SLR, SOLITARY-ROOT; SCR, SCARECROW; SHR, SHORT-ROOT; TAA1, TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1; WOX5, WUSHEL-LIKE HOMEOBOX5; Zn, zinc.

lateral root initiation (94, 140). Following the appearance of the auxin maximum, new root tissue is produced by the continuous activity of the root meristem, and, for some time (it takes 4–6 h for a new peak to be observed), cells leave the meristem without being exposed to an auxin-response maximum; the process is then repeated (94, 153, 154). This periodicity correlates well with the recurrent programmed cell death of the most distal lateral root cap cells and the temporary release of auxin from these cells into the root meristem (154). The periodicity of programmed cell death–derived auxin further correlates with the oscillation and the formation of prebranch sites (154). In other words, the dynamics of programmed cell death in the root cap appear to be involved in steering the oscillation pattern to result in the recurrent formation of prebranch sites. The question of how the programmed cell death dynamics in the root cap are regulated remains, however, unanswered. The transcriptional regulator SOMBRERO (SMB) controls the persistence of lateral root cap cells. Using an inducible overexpression construct of *SMB*, it was possible to induce precocious programmed cell death in the root cap, resulting in roots that were temporarily devoid of a root cap. During this period of growth in the absence of a lateral root cap, no oscillation in *DR5* expression could be observed and no prebranch sites were formed. In *smb* mutants, however, programmed cell death was delayed and lateral root spacing was strongly impeded (154). The link between the rate of root cap formation and root branching might represent a mechanism by which plants control the spacing of lateral roots or at least a way for them to adjust the number of lateral roots in order to keep up with the elongation rate of the primary root.

3.1.3. Prebranch sites: specification of lateral root founder cells and initiation. The prebranch sites, as visualized using the *DR5*::*LUC* reporter, encompass a broad region of the root that starts at the end of the elongation zone and spreads into the differentiation zone. The low cellular resolution of the *DR5*::*LUC* marker does not allow one to determine exactly when and how many XPP cells become specified as lateral root founder cells in each prebranch site. However, by comparative analysis with light and confocal microscopy studies using the *DR5*::*GFP* or *DR5*::*GUS* marker lines, it can be deduced that, within the prebranch sites, lateral root founder cells become specified by a new, strongly focused auxin-response maximum in one or two adjacent XPP cells (37). The outcome of the specification event is that two adjacent XPP cells undergo radial swelling, which is accommodated by the relaxation of the overlying endodermal cells (142), and dramatically

repolarize, which is reflected in the migration of their nuclei toward the common anticlinal cell wall (27). This process is initiated in one cell file and reiterated in the neighboring founder cells. As such, founder cell specification can start in one single XPP cell, and gradually more cells are recruited to create a group of about 11 founder cells (72). After repolarization and radial swelling, founder cells divide asymmetrically, and a central cluster of small cells flanked by larger cells is generated. In a comprehensive cell lineage study using light sheet-based fluorescence microscopy allowing for in vivo recording of the complete process of lateral root formation, it became clear that not all founder cells contributed equally to the lateral root primordium (147). XPP cells in the median cell file contribute to the majority (76%) of the cells in the primordium.

3.1.4. Specification of lateral root founder cells: candidate regulators. Few candidate regulators for lateral root founder cell specification have been proposed so far, and the lack of data on their interdependence hinders us from proposing a unifying model for founder cell specification. In the absence of further insight into their mode of action, we are therefore forced to merely list these components. The GATA23 transcription factor is transcriptionally induced specifically in XPP cells before nuclear migration (27) and provides the best approximation of a founder cell–specification factor. After lateral root initiation, GATA23 expression decreases and soon disappears from young lateral root primordia. Genetic data underline its potential role as a founder cell–specification factor with altered numbers of lateral roots and disturbed spacing of primordia. Its expression is controlled upstream by an auxin-signaling cascade involving IAA28 and its interacting ARF binding factors (ARF5, ARF6, ARF7, ARF8, and ARF19) in the transition zone of the root (27); however, the GATA23 downstream signaling remains enigmatic. Furthermore, the rather weak lateral root phenotypes of loss-of-function and gain-of-function mutants, together with its outsider position within the GATA family of transcription factors having a Brassicaceae-specific degenerate LLM domain at the C terminus (12), do not support its central role as a conserved regulator of lateral root founder cell specification.

The F-box protein S-Phase Kinase-Associated Protein2B (SKP2B) is expressed in founder cells, lateral root primordia, and the root apical meristem. SKP2B negatively regulates the cell cycle and lateral root formation because it represses meristematic and founder cell divisions (89). This suggests that, during lateral root formation, the over-proliferation of pericycle cells is enough of a risk to require a mechanism for deaccelerating the cell cycle.

Recently, the identification of the TARGET OF LBD SIXTEEN 2–RECEPTOR-LIKE KINASE7 (TOLS2-RLK7) pathway, a peptide hormone-receptor signaling cascade that prevents lateral root primordia from forming in close proximity, led to additional evidence for the existence of cell-division-restraining mechanisms in founder cells (135).

MEMBRANE-ASSOCIATED KINASE REGULATOR4 (MAKR4), a downstream molecular component of the auxin pulses coming from the root cap (153), accumulates at the plasma membrane of XPP cells before nuclear migration. Loss-of-function mutants show fewer lateral root primordia and fewer lateral roots but unaltered lateral root prebranch sites, suggesting that MAKR4 helps to convert lateral root prebranch sites into lateral root primordia.

Like the above-mentioned genes, *LATERAL ORGAN BOUNDARIES DOMAIN16* (*LBD16*)/*ASYMMETRIC LEAVES2-LIKE18* (*ASL18*) is also expressed in lateral root founder cells and gradually increases its transcription during nuclear migration and asymmetric cell division. When *LBD16* is dominantly repressed using an *LBD16-SRDX* construct, lateral root founder cell specification still occurs normally; however, nuclear migration and subsequent asymmetric cell division is blocked, prohibiting lateral root initiation to take place (47). Therefore, LBD16, whose expression in founder cells is controlled by the SOLITARY ROOT (SLR)/IAA14-ARF7-ARF19

auxin-signaling module, might be required to break the symmetry of the founder cells, allowing repolarization and asymmetric cell division.

GLV6, which encodes a small signaling peptide, is transcribed in the founder cells prior to nuclear migration and asymmetric cell division (39). An excess of GLV6 peptide seems to impede the polarization of founder cells required to set up the first asymmetric cell divisions. The result is that cells tend to divide more symmetrically. In addition, divisions are not restricted to a limited number of founder cells; instead excessive anticlinal divisions are induced.

Periclinal cell division: cell division occurring parallel to the tissue or organ surface

Another gene encoding a small signaling peptide, RALF-LIKE 34 (RALFL34), is expressed in lateral root founder cells before asymmetric cell division. Based on the reduced GATA23 expression in *ralfl34* mutants, it might be a component of an upstream regulatory mechanism (98). However, *ralfl34* mutants show phenotypic resemblance with GATA23 gain-of-function plants, which have more stage I primordia clustered together, precluding a straightforward hypothesis for its role in the early steps of lateral root formation.

The *PLT* transcription factor genes, *PLT3*, *PLT5*, and *PLT7*, are expressed during the early developmental stages of lateral root formation, and their earliest expression has been described as taking place in a subset of pericycle cells prior to the first founder cell division (60). All three transcription factors, indeed, show early accumulation in two migrated nuclei, as demonstrated by the use of PLT-YFP protein fusions. There is, however, no demonstrated expression of these genes prior to nuclear migration, suggesting that the transcription factors might become expressed after the repolarization of pericycle founder cells.

3.1.5. Postinitiation steps of lateral root formation. Recent work has indicated that the early activation of *PLT* genes in incipient stage I primordia is essential to regulate the postinitiation steps of lateral root formation and is required to regulate the asymmetric periclinal cell divisions that generate a two-layered primordium (stage II) (35, 48). The occurrence of these periclinal divisions can be regarded as the first sign of a 90° turn in the polarity of the cells involved and of the generation of proximal (inner) and distal (outer) domains in the primordium. After stage II, genes have been found whose expression pattern differs between the inner and outer layers of the primordium, such as the family members *PLT1* and *PLT2* and the GRAS transcription factors *SHR* and *SCR* (35). The latter were described previously for their role in regulating the cellular pattern of the root (see Section 2.1). The cellular pattern contributing to the root's dome shape is essential for the successful outgrowth and emergence of the primordium. For example, *plt3 plt5 plt7* triple mutants show abnormal cell division patterns and irregular cell shapes whose variable morphology makes it difficult for the primordium to emerge from the parent root. On its way to the surface, the newly formed organ needs to grow through three overlying cell layers: the endodermis, cortex, and epidermis. In recent years, it has become increasingly clear that in addition to its own organization, a tight integration of molecular signaling and of mechanical aspects between the lateral root and the surrounding tissue is essential for the outgrowth and emergence of the primordium. For a recent review of this complex but fascinating facet of lateral root formation, we refer the reader to Stoeckle et al. (128).

3.2. Lateral Root Emergence and Root Branch Angle

After emergence, lateral roots grow, at least initially, away from the primary axis of the plant at nonvertical orientations. This contributes to the radial expansion of the root system, increases access to important resources, and enhances anchoring in the soil. Early emerging lateral roots still lack an elongation zone and grow perpendicular to the primary root (54, 114). As the roots grow out, usually gravity will affect differential cell elongation, and the roots will start to bend at

the gravitropic set-point angle (31). Interestingly, after making this angle, the lateral roots continue to grow during a so-called plateau phase, maintaining the same gravitropic set-point angle and not showing a purely gravitropic response (114). This plateau phase is variable, and early lateral roots generally show a longer plateau phase compared with later lateral roots (115). The plateau phase ends after another gravitropic response, which leads to a second bending and stronger vertical growth, usually—at least in the *Arabidopsis* accession Col-0—resulting in an angle between 0° and 30° (114, 115). As such, during the different phases of lateral root emergence and growth, the gravitropism signaling is weakened or counteracted to generate the nonvertical growth of lateral roots.

Gravitropic set-point angle: the angle at which a lateral root is maintained by gravitropism; characteristic of the root developmental state and environmental conditions

Acropetal sequence: sequence with more advanced stages at the root base and gradually younger lateral root stages toward the root tip

Although the lateral root meristem resembles many aspects of the primary root meristem, they clearly have distinct molecular programs in response to gravity, and this distinction is useful to optimize root system architecture. One partial cause of this difference might be due to the *LAZY* (*LZY*)*/DEEPER ROOTING* (*DRO*)*/NEGATIVE GRAVITROPIC RESPONSE OF ROOTS* (*NGR*) genes, which affect auxin flow. The triple *lzy1 lzy2 lzy3* mutant, for example, leads to an inverse auxin redistribution and asymmetry of PIN3 expression in the columella of the lateral root but not in the primary root (133). As a consequence, *lzy1 lzy2 lzy3*—just like the single *lzy2* or *lzy3/dro1* mutants—has lateral roots with a more horizontal or even an upward growth direction but normally growing primary roots (52, 133, 158). The difference with the primary root might be the presence of a fourth member of this family, *LZY4/DRO2/NGR3*. Independent of the direction of initial root growth, *ngr1 ngr2 ngr3* (which is similar to *lzy2 lzy3 lzy4*) triple mutants show almost entirely negative gravitropic growth of the primary root (44). This observation further supports the presence of a mechanism that is dependent on sensing the gravity vector, but which causes the root to grow in the opposite direction of the gravity vector (116). Again, a different response is present in the lateral roots of *ngr1 ngr2 ngr3* triple mutants: Lateral roots grow in a horizontal position independent of gravity—even in seedlings manually reoriented perpendicular to the gravity vector (158).

Hence, outgrowing lateral roots are subjected to (gravi)tropic mechanisms that are similar to those of the primary root, but the different regulation of these and other mechanisms counteracts total downward growth and contributes to the radial expansion of the root system.

3.3. Lateral Roots Versus Adventitious Roots

Lateral and adventitious roots share key elements of genetic and hormonal regulatory networks but are subject to different regulatory mechanisms, and we refer to excellent recent reviews comparing lateral and adventitious roots (13, 78). Such comparisons are not so straightforward, however: The recent identification of lateral roots in *Arabidopsis* that can be produced on primary roots outside the normal acropetal sequence in soil conditions or upon wounding in medium (123) challenges the sharp distinction between lateral and adventitious roots, according to their original definitions. Additional support to classify these nonacropetal roots as adventitious can be found in the molecular regulation of their initiation. Whereas ARF7 and ARF19 transcription factors are essential during lateral root initiation in activating the downstream LBD16 transcriptional regulator, in adventitious root formation, ARF7 and ARF19 function is taken over by another transcription factor, WOX11 (77). Interestingly, although they are also produced from the primary root pericycle, the nonacropetal roots in *Arabidopsis* seem to require WOX11 rather than ARF7 or ARF19 activity for their initiation (123). Therefore, it should be logical that the molecular discrimination between WOX11-mediated roots and ARF7/ARF19-mediated roots coincide with the lateral-versus-adventitious-root distinction, but this is not the case. ARF7 and ARF19 have been shown to control adventitious root formation from *Arabidopsis* hypocotyls (149),

precluding a molecular basis for the longstanding morphological discrimination between lateral and adventitious roots. Taken together, these data clearly indicate the existence of different types of lateral roots, whether they can be considered as adventitious or not. The presence of alternative mechanisms to induce lateral roots surely enhances root branching capacity, e.g., in the case of unusual situations, such as wounding, or exceptional environmental conditions as is the case for WOX11-mediated roots in *Arabidopsis*. In persistent roots of other dicot plants, it was reported that roots can even be produced from secondary tissues (105), which would again argue for the idea that roots can indeed produce real adventitious roots in addition to lateral roots. In these perennial species, the molecular mechanisms responsible for generating roots are still to be determined. Recently, it was reported that mechanical stress can induce the growth of adventitious roots from older parts of the root, for example, on the *Arabidopsis* taproot, which had already undergone secondary thickening (5). The authors of this study demonstrated that this happened by converting the initials of the cambium, which normally produced xylem and phloem mother cells, into founder cells for new lateral roots. This transition seems to be mediated by WOX11 together with the PRE3/ATBS1/bHLH135/TMO7 transcription factor and suggests that the vascular cambium might replace pericycle competences during the emergence of new lateral and adventitious roots from a secondary structure.

Secondary tissues: tissues formed in vascular plants by the activity of lateral meristems in more mature parts

Cambium: the layer of dividing cells between xylem and phloem tissues, which is responsible for the secondary growth of stems and roots

4. ROOT RESPONSES TO ENVIRONMENTAL CUES

4.1. Nutrients and Nutrient Starvation

Mineral nutrients are essential in plants for structural, signaling, or metabolic components and, hence, are of major importance for plant growth. As these nutrients are almost exclusively acquired from the soil, the root system is key in retrieving them and is highly responsive to changes in nutritional conditions. Especially as nutrients show a generally heterogeneous distribution in the soil, a plastic root system is important. More specifically, roots may respond to nutrient starvation or nutrient-rich patches by developing a root system that allows greater exploration or greater nutrient acquisition, respectively. In addition, certain nutrients—particularly mobile nutrients, such as nitrate—tend to be more abundant in deeper soil layers, whereas immobile nutrients—for example, phosphate—are more abundant in the upper soil (46). Mobile and immobile nutrients require different root systems for optimal acquisition. A high density in lateral roots, for example, is favorable for the acquisition of immobile nutrients but inefficient for the acquisition of mobile nutrients (157). Similarly, a deep and steep root system is more favorable for the acquisition of mobile nutrients but is a wasted investment for the plant if immobile nutrients are the limiting factor (85). During evolution, plants acquired ways for their roots to respond to changing nutrients and optimized their root systems accordingly. In the following subsections, we give for the most important nutrients an overview of how plants react to nutrient availability with respect to their root system architecture. In terms of the underlying molecular pathways, we focus specifically on *Arabidopsis*.

4.1.1. Phosphate. Phosphate represents the roots' sole source of phosphorus, which is a major element in several important cellular components (e.g., nucleic acids, phospholipids, and ATP) and, hence, is crucial for plant fitness. As a consequence, plant roots are highly responsive to phosphate starvation. Phosphate deficiency generally induces a shallow root system in order to increase topsoil exploration or foraging by inhibiting primary root growth, stimulating axial branching, and favoring a more horizontal growth angle of adventitious roots (84, 107). In *Arabidopsis*, phosphate-dependent root growth inhibition is partially caused by an enhanced iron uptake. Indeed,

Callose: a plant polysaccharide composed of glucose residues produced in various places, such as the plasmodesmata, where it regulates permeability

phosphate starvation typically induces the secretion of organic acids that help to release anionic phosphate from bound cations, but these acids also release iron at the same time (7, 33, 92, 97). Iron subsequently leads to callose deposition at the plasmodesmata, which prevents correct SHR movement; affects the expression of *SHR*, *SCR*, and *WOX5*; and results in advanced differentiation of root meristem cells (53, 97). Additionally, the increase in iron results in ROS signaling in the elongation zone, thereby stimulating cell wall stiffening and further reinforcing root growth inhibition (7). The enhanced branching results in more root tips, which represent the predominant sites of phosphate uptake (66). Both the higher lateral root density and the decreased gravitropic set-point angle of lateral roots (6, 108, 117) are parameters strongly affected by auxin and auxin signaling. *tir1 afb3* auxin receptor mutants are resistant to the phosphate starvation–induced decrease in the gravitropic set-point angle (117), whereas *tir1 afb2 afb3* triple mutants show no lateral root induction at low phosphate levels (108). Furthermore, phosphate starvation was shown to induce auxin-signaling maxima and *TIR1* expression in lateral root primordia (99, 108), suggesting that it might locally enhance auxin sensitivity.

Recent progress in phosphate research has led to a relatively good understanding of phosphate starvation signaling (reviewed in 4, 23, 55, 64, 110) with a central role for the transcription factor PHOSPHATE STARVATION RESPONSE1 (PHR1) to target a broad range of phosphate starvation–responsive genes (18, 20). Among the possible PHR1 targets are a number of prominent auxin-signaling components, which might point to a relatively direct link between phosphate starvation signaling and auxin-affected lateral root formation and gravitropic set-point angle. Indeed, in addition to the major auxin receptor *TIR1*, the lateral root–related *ARF7* and *ARF19*, and the PIN2-affecting phosphatase *PP2A-2*, other presumptive PHR1 targets are the phospholipase Dζ2 (*PLDζ2*) and the kinase *WAG2* (20, 23, 102). Remarkably, *ARF7* and *ARF19* are possible targets of PHR1 at the same time that they control *PHR1* expression. *PHR1* overexpression partially rescues the lateral root phenotype of the *arf7/arf19* mutant; hence, additional PHR1 targets seem to be important for phosphate starvation–induced lateral root formation (61).

4.1.2. Nitrogen. As the central element in amino acids and proteins, nitrogen is obviously of the utmost importance to plant functioning. Nitrogen is taken up by the roots mainly in the form of ammonium or nitrate. These two forms have different, sometimes opposing properties and affect root system architecture in particular ways (69). Ammonium is described as inhibiting root elongation and gravitropism, whereas nitrate stimulates root elongation. If locally present, both nitrogen forms stimulate root branching in a broad range of plant species (34, 76, 80, 111), thereby enabling root systems to forage for nitrogen in heterogeneous soil environments (118). In nitrogen-poor regions of the soil, root growth is probably suppressed through the activation of the auxin transport function of NITRATE TRANSPORTER 1.1 (NRT1.1), impeding auxin accumulation in lateral root primordia and, hence, inhibiting their further development (16, 71). Alternatively, nitrogen deficiency might suppress the outgrowth of lateral root primordia by inducing CLE peptides that activate CLAVATA1 signaling (3). Another peptide family, the C-terminally encoded peptides (CEP), are also induced in the root upon nitrogen deficiency and are transported to the shoot to induce *CEP DOWNSTREAM1* (*CEPD1*) and *CEPD2* (130). CEPDs are transported back to the root (100) to induce several genes in nitrate-rich, growing root parts, including *NRT2.1* and *NRT1.1* (100, 130). Here, the receptor function of NRT1.1 becomes important and induces a signaling pathway controlling lateral root initiation, lateral root elongation, and primary root elongation (143). This pathway includes the TCP20 transcription factor (51), which was recently shown to interact with PLTs and SCR to control *WOX5* expression (124). Activation of NRT1.1 also leads to the induction of *AFB3*, which encodes an auxin receptor important for both primary root growth and lateral root density (144, 145). In contrast, overall mild

nitrate starvation can have positive effects on root development. Low nitrogen levels induce, via *AGAMOUS-LIKE21* (*AGL21*), auxin-biosynthesis genes leading to increased auxin levels in lateral root primordia and lateral roots to stimulate initiation and elongation (159). For ammonium, the situation is less clear. Ammonium-induced lateral root initiation occurs in an AMMONIUM TRANSPORTER1;3 (AMT1;3)-dependent way but does not seem to be dependent on ammonium uptake. Moreover, mutation in *AMT1;1*, having similar ammonium transport properties as AMT1;3, does not seem to affect root branching, and this argues for a signaling function for AMT1;3 (76). In addition to its local, positive effect on lateral root development, ammonium inhibits primary and lateral root elongation (79). As in phosphate starvation, ROS plays a central role in this (80, 150). In *Lotus japonicus*, AMT1;3 contributes to the inhibition of root elongation, again, independent of ammonium uptake levels, supporting a role for AMT1;3 in ammonium signaling (113).

Hence, both nitrogen forms, especially if locally present, have a positive effect on lateral root formation, and, conversely, nitrogen deficiency generally reduces lateral root formation. This strategy allows plants to minimize the cost of their metabolic requirements for root branching and to invest in root elongation for nutrient foraging. Indeed, maize lines producing fewer lateral roots were shown to perform better upon nitrogen deficiency by developing a deeper root system that acquired more nitrogen (161).

4.1.3. Other nutrients. In addition to nitrogen and phosphate, other nutrients may also have a clear effect on the root system, but very little is known about the controlling mechanisms. In *Arabidopsis*, sulfur, magnesium, and iron deficiencies lead to a reduction in lateral root density, whereas deficiencies in potassium, calcium, zinc, manganese, and boron result in an increased lateral root density (50, 67, 68). With the exception of zinc-deficient roots, the increase in lateral root density is accompanied by an inhibition of primary root growth; therefore, it is not always clear to what extent lateral roots are effectively induced by the nutrient deficiency. Manganese excess is shown to have a negative effect on auxin biosynthesis and *PIN4* and *PIN7* expression, which is probably associated with the reduction in lateral root density (164). Similarly, an excess of iron represses *PIN2* and negatively affects lateral root formation (73) while it induces *AUX1* to trigger root elongation (45). Multiple other genes involved in root development might, however, be affected by iron deficiency as indicated by an in silico analysis showing iron-deficiency-specific differential expression profiles for *PIN4*, *TIR1*, *LBD16*, and *LBD29* (45).

4.2. Water and Drought

Water is obviously of major importance for plants, and roots have developed different strategies to forage specifically for water. Drought, if not too severe, will enhance root growth, allowing plants to explore deeper layers of the soil for water (152). Similarly, induction of *DRO1* results in a steeper root system and an increase in drought tolerance (137, 158). This, however, does not involve a specific drought response but instead is based on an enhanced gravitropism. The *DRO1* example nicely illustrates the benefits of a steep and deep ideotype to cope with drought (85). Drought sensing and the downstream signaling leading to growth responses involve a complex and not yet fully understood network, with a key role for ABA signaling. ABA is indeed essential for the drought response and rapidly increases upon drought stress (63, 152). ABA is shown to induce the PIN2 and AUX1 auxin transporter encoding genes, resulting in increased auxin redistribution toward the epidermal and cortical cells to stimulate cell elongation (152). Hydrotropism is also regulated by ABA (2, 30, 132) but does not require auxin transport or auxin redistribution (65, 125). Low water potential is sensed at the elongation zone and not at the root

tip as is the case for other tropisms (29, 30). The lower water potential affects ABA signaling specifically in the cortical cells of the elongation zone triggering cell expansion and, hence, enabling the root to bend toward the higher water potential. In contrast to water stress–induced and auxin-dependent root elongation, ABA itself is central for cell elongation during hydrotropism (30).

Unequal water distribution in the soil also affects lateral root formation. As such, lateral roots will be preferentially positioned toward available water in a process known as hydropatterning (9). In contrast, the complete absence of water, for instance in an air gap, will arrest lateral root formation. This was recently termed xerobranching (101). The hydropatterning and xerobranching mechanisms are both adaptive toward water availability to allow roots to efficiently use internal resources. The general arrest of lateral root initiation in locally dry microenvironments may point to a similar control mechanism for the two responses. Xerobranching coincides with increased ABA signaling, which is, similar to other water or drought responses, probably key in this mechanism (101). In contrast, hydropatterning is unaffected in ABA-signaling mutants, indicating that ABA is not required for this response (9). Although hydropatterning would still be regulated by a drought response, it would involve the auxin machinery. It is suggested that posttranslational modification of the important lateral root factor ARF7 (see Section 3.1.4), specific at the air side of the root, triggers its binding to the IAA3/SHORT HYPOCOTYL 2 (SHY2) to form a transcriptional repressor complex and block auxin-responsive gene expression required for lateral root formation. Nonmodified ARF7 proteins at the water side do not bind to the IAA3/SHY2 repressor and induce the lateral root genetic program (103).

Like nitrogen starvation, maize lines with fewer but longer lateral roots are also more tolerant to drought. This suggests that reduced lateral root formation in dry microenvironments can be favorable for plants to cope with general drought stress conditions (162).

5. PERSPECTIVES

Thanks to the efforts of a rapidly growing root community, we have finally reached the point where it is not a matter of course anymore to call the below-ground parts of plants the hidden half. Progress in our understanding of root biology has come from different angles. Depending on the aspect of root development that is the subject of study, the level of resolution, ranging from signaling molecules to root system architecture, can be very different as is exemplified in the present review. It is obvious that the longstanding in-depth work on root development mainly performed using the *Arabidopsis* reference organism has generated considerable insights into the development of the primary root. The primary root with its meristem and stem cell niche has revealed a lot of its secrets. We are not dealing anymore with one single transcription factor; transcriptional networks have been uncovered and higher-order transcription factor complexes have been identified. Great methodological advances in the discovery and validation of protein–protein interactions have recently heralded a new era in root developmental studies, and we anticipate that this will generate important novel contributions in investigations of lateral root formation and responses to environmental cues. At present, very little information is available about the protein networks that control these developments. A second important new trend is the increased effort to observe and study root developmental processes in vivo. The generation of specific reporter lines combined with improved microscopic installations allows for a dynamic analysis of developmental processes that were hitherto invisible and were not even thought to exist. The use of the *DR5*::*LUC* marker is exemplary for this trend and has introduced new developmental stages prior to lateral root initiation with concepts such as prebranch sites and the oscillation zone. Other markers have contributed to the recognition of the involvement of the root cap in root branching. More in vivo

studies, eventually making simultaneous use of multiple reporter constructs, promise to generate spectacular new findings when combined with the induction of environmental stress conditions. We strongly believe that the coming years will be as exciting as what we have experienced during the last decade and will shed light on the fine-tuned regulation of root developmental programs in normal and suboptimal conditions.

SUMMARY POINTS

1. Decades of root research using the *Arabidopsis* model system has led to deep knowledge of the molecular mechanisms regulating root development. Our review illustrates that present-day root research is still steadily improving and deepening our understanding of root developmental processes.
2. Three examples of how the root developmental field has progressed recently are the discoveries of nontranscriptional but TIR-dependent root growth regulation, a WOX11-dependent alternative lateral root pathway, and posttranslational modifications affecting the action of ARF7 on lateral root formation.
3. In contrast to the level of insight acquired into the molecular regulation of root development, the understanding of how environmental cues interact with the pathways controlling root growth and lateral root formation remains limited, despite its importance to future applied research strategies.
4. Based on current progress and the widening scope of an increasing root research community, more and more pathways at the intersection of environment–root development have the potential to become demystified in the near future. This was recently exemplified by the elucidation of the hydropatterning pathway.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Maria Njo for assistance with artwork in making the figures. We apologize to our colleagues whose work on root development is not cited in this review owing to space limitations. This work was supported by grants G002817N [Bilateral Research Cooperation with the Chinese Ministry of Science and Technology (MOST) 2016YFE0109900], G022516N, G020918N, and G024118N from the Research Foundation-Flanders (FWO).

LITERATURE CITED

1. Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, et al. 2004. The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. *Cell* 119:109–20
2. Antoni R, Gonzalez-Guzman M, Rodriguez L, Peirats-Llobet M, Pizzio GA, et al. 2013. PYRABACTIN RESISTANCE1-LIKE8 plays an important role for the regulation of abscisic acid signaling in root. *Plant Physiol.* 161:931–41

3. Araya T, Miyamoto M, Wibowo J, Suzuki A, Kojima S, et al. 2014. CLE-CLAVATA1 peptide-receptor signaling module regulates the expansion of plant root systems in a nitrogen-dependent manner. *PNAS* 111:2029–34
4. Azevedo C, Saiardi A. 2017. Eukaryotic phosphate homeostasis: the inositol pyrophosphate perspective. *Trends Biochem. Sci.* 42:219–31
5. Baesso B, Chiatante D, Terzaghi M, Zenga D, Nieminen K, et al. 2018. Transcription factors PRE 3 and WOX 11 are involved in the formation of new lateral roots from secondary growth taproot in *A. thaliana*. *Plant Biol*. 20:426–32
6. Bai HW, Murali B, Barber K, Wolverton C. 2013. Low phosphate alters lateral root setpoint angle and gravitropism. *Am. J. Bot.* 100:175–82
7. Balzergue C, Dartevelle T, Godon C, Laugier E, Meisrimler C, et al. 2017. Low phosphate activates STOP1-ALMT1 to rapidly inhibit root cell elongation. *Nat. Commun.* 8:15300
8. Band LR, Wells DM, Fozard JA, Ghetiu T, French AP, et al. 2014. Systems analysis of auxin transport in the *Arabidopsis* root apex. *Plant Cell* 26:862–75
9. Bao Y, Aggarwal P, Robbins NE, Sturrock CJ, Thompson MC, et al. 2014. Plant roots use a patterning mechanism to position lateral root branches toward available water. *PNAS* 111:9319–24

10. Elucidates the link between auxin and root cell expansion and hence (directional) root growth.

10. Barbez E, Dunser K, Gaidora A, Lendl T, Busch W. 2017. Auxin steers root cell expansion via apoplastic pH regulation in *Arabidopsis thaliana*. *PNAS* 114:E4884–93

11. Bargmann BO, Vanneste S, Krouk G, Nawy T, Efroni I, et al. 2013. A map of cell type-specific auxin responses. *Mol. Syst. Biol.* 9:688
12. Behringer C, Schwechheimer C. 2015. B-GATA transcription factors—insights into their structure, regulation, and role in plant development. *Front. Plant Sci.* 6:90
13. Bellini C, Pacurar DI, Perrone I. 2014. Adventitious roots and lateral roots: similarities and differences. *Annu. Rev. Plant Biol.* 65:639–66
14. Bennett MJ, Marchant A, Green HG, May ST, Ward SP, et al. 1996. *Arabidopsis AUX1* gene: a permease-like regulator of root gravitropism. *Science* 273:948–50
15. Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, et al. 2005. The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. *Nature* 433:39–44
16. Bouguyon E, Perrine-Walker F, Pervent M, Rochette J, Cuesta C, et al. 2016. Nitrate controls root development through posttranscriptional regulation of the NRT1.1/NPF6.3 transporter/sensor. *Plant Physiol*. 172:1237–48
17. Brunoud G, Wells DM, Oliva M, Larrieu A, Mirabet V, et al. 2012. A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature* 482:103–6
18. Bustos R, Castrillo G, Linhares F, Puga MI, Rubio V, et al. 2010. A central regulatory system largely controls transcriptional activation and repression responses to phosphate starvation in *Arabidopsis*. *PLOS Genet*. 6:e1001102
19. Carlsbecker A, Lee J-Y, Roberts CJ, Dettmer J, Lehesranta S, et al. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465:316–21
20. Castrillo G, Teixeira PJPL, Paredes SH, Law TF, de Lorenzo L, et al. 2017. Root microbiota drive direct integration of phosphate stress and immunity. *Nature* 543:513–18
21. Chaiwanon J, Wang W, Zhu J-Y, Oh E, Wang Z-Y. 2016. Information integration and communication in plant growth regulation. *Cell* 164:1257–68
22. Chen R, Hilson P, Sedbrook J, Rosen E, Caspar T, Masson PH. 1998. The *Arabidopsis thaliana AGRAVITROPIC 1* gene encodes a component of the polar-auxin-transport efflux carrier. *PNAS* 95:15112–17
23. Crombez H, Motte H, Beeckman T. 2019. Tackling plant phosphate starvation by the roots. *Dev. Cell.* In press
24. Cruz-Ramírez A, Díaz-Triviño S, Blilou I, Grieneisen VA, Sozzani R, et al. 2012. A bistable circuit involving SCARECROW-RETINOBLASTOMA integrates cues to inform asymmetric stem cell division. *Cell* 150:1002–15
25. Cruz-Ramírez A, Díaz-Triviño S, Wachsman G, Du Y, Arteága-Vázquez M, et al. 2013. A SCARECROW-RETINOBLASTOMA protein network controls protective quiescence in the Arabidopsis root stem cell organizer. *PLOS Biol*. 11:e1001724

26. Cui H, Levesque MP, Vernoux T, Jung JW, Paquette AJ, et al. 2007. An evolutionarily conserved mechanism delimiting SHR movement defines a single layer of endodermis in plants. *Science* 316:421–25
27. De Rybel B, Vassileva V, Parizot B, Demeulenaere M, Grunewald W, et al. 2010. A novel aux/IAA28 signaling cascade activates GATA23-dependent specification of lateral root founder cell identity. *Curr. Biol.* 20:1697–706
28. De Smet I, Tetsumura T, De Rybel B, Frey NFD, Laplaze L, et al. 2007. Auxin-dependent regulation of lateral root positioning in the basal meristem of *Arabidopsis*. *Development* 134:681–90
29. Dietrich D. 2018. Hydrotropism: how roots search for water. *J. Exp. Bot.* 69:2759–71
30. Dietrich D, Pang L, Kobayashi A, Fozard JA, Boudolf V, et al. 2017. Root hydrotropism is controlled via a cortex-specific growth mechanism. *Nat. Plants* 3:17057
31. Digby J, Firn RD. 1995. The gravitropic set-point angle (GSA): the identification of an important developmentally controlled variable governing plant architecture. *Plant Cell Environ*. 18:1434–40
32. Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, et al. 1993. Cellular organisation of the *Arabidopsis thaliana* root. *Development* 119:71–84
33. Dong JS, Piñeros MA, Li X, Yang H, Liu Y, et al. 2017. An *Arabidopsis* ABC transporter mediates phosphate deficiency-induced remodeling of root architecture by modulating iron homeostasis in roots. *Mol. Plant* 10:244–59
34. Drew MC, Saker LR, Ashley TW. 1973. Nutrient supply and the growth of the seminal root system in barley I. The effect of nitrate concentration on the growth of axes and laterals. *J. Exp. Bot.* 24:1189–202
35. Du YJ, Scheres B. 2017. PLETHORA transcription factors orchestrate de novo organ patterning during *Arabidopsis* lateral root outgrowth. *PNAS* 114:11709–14
36. Dubreuil C, Jin X, Grönlund A, Fischer U. 2018. A local auxin gradient regulates root cap self-renewal and size homeostasis. *Curr. Biol.* 28:2581–87
37. Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Friml J, et al. 2008. Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. *PNAS* 105:8790–94
38. Fendrych M, Akhmanova M, Merrin J, Glanc M, Hagihara S, et al. 2018. Rapid and reversible root growth inhibition by TIR1 auxin signalling. *Nat. Plants* 4:453–59
39. Fernandez A, Drozdzecki A, Hoogewijs K, Vassileva V, Madder A, et al. 2015. The GLV6/RGF8/CLEL2 peptide regulates early pericycle divisions during lateral root initiation. *J. Exp. Bot.* 66:5245–56
40. Forzani C, Aichinger E, Sornay E, Willemsen V, Laux T, et al. 2014. WOX5 suppresses *CYCLIN D* activity to establish quiescence at the center of the root stem cell niche. *Curr. Biol.* 24:1939–44
41. Friml J, Wiśniewska J, Benková E, Mendgen K, Palme K. 2002. Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis*. *Nature* 415:806–9
42. Fujinami R, Yamada T, Nakajima A, Takagi S, Idogawa A, et al. 2017. Root apical meristem diversity in extant lycophytes and implications for root origins. *New Phytol.* 215:1210–20
43. Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, et al. 2007. PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. *Nature* 449:1053–57
44. Ge L, Chen R. 2016. Negative gravitropism in plant roots. *Nat. Plants* 2:16155
45. Giehl RFH, Lima JE, von Wirén N. 2012. Localized iron supply triggers lateral root elongation in *Arabidopsis* by altering the AUX1-mediated auxin distribution. *Plant Cell* 24:33–49
46. Giehl RFH, von Wirén N. 2014. Root nutrient foraging. *Plant Physiol.* 166:509–17
47. Goh T, Joi S, Mimura T, Fukaki H. 2012. The establishment of asymmetry in *Arabidopsis* lateral root founder cells is regulated by LBD16/ASL18 and related LBD/ASL proteins. *Development* 139:883–93
48. Goh T, Toyokura K, Wells DM, Swarup K, Yamamoto M, et al. 2016. Quiescent center initiation in the *Arabidopsis* lateral root primordia is dependent on the SCARECROW transcription factor. *Development* 143:3363–71
49. Grieneisen VA, Xu J, Marée AFM, Hogeweg P, Scheres B. 2007. Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature* 449:1008–13
50. Gruber BD, Giehl RFH, Friedel S, von Wirén N. 2013. Plasticity of the Arabidopsis root system under nutrient deficiencies. *Plant Physiol.* 163:161–79

30. Shows that the root elongation zone during hydrotropism, unlike other tropisms, both senses and responds in an abscisic acid–dependent manner.

35. Comprehensive study of the role of the PLETHORAs in lateral root formation and their relation towards key root meristem factors.

38. Pinpoints novel TIR1-dependent but nontranscriptional regulation of cell expansion and hence root growth by auxin.

51. Guan P, Wang R, Nacry P, Breton G, Kay SA, et al. 2014. Nitrate foraging by *Arabidopsis* roots is mediated by the transcription factor TCP20 through the systemic signaling pathway. *PNAS* 111:15267–72
52. Guseman JM, Webb K, Srinivasan C, Dardick C. 2017. *DRO1* influences root system architecture in Arabidopsis and Prunus species. *Plant J.* 89:1093–105
53. Gutiérrez-Alanís D, Yong-Villalobos L, Jiménez-Sandoval P, Alatorre-Cobos F, Oropeza-Aburto A, et al. 2017. Phosphate starvation-dependent iron mobilization induces CLE14 expression to trigger root meristem differentiation through CLV2/PEPR2 signaling. *Dev. Cell* 41:555–70
54. Guyomarc'h S, Léran S, Auzon-Cape M, Perrine-Walker F, Lucas M, Laplaze L. 2012. Early development and gravitropic response of lateral roots in *Arabidopsis thaliana*. *Philos. Trans. R. Soc. B* 367:1509–16
55. Ham B-K, Chen J, Yan Y, Lucas WJ. 2018. Insights into plant phosphate sensing and signaling. *Curr. Opin. Biotechnol.* 49:1–9
56. Harmer SL, Brooks CJ. 2018. Growth-mediated plant movements: hidden in plain sight. *Curr. Opin. Plant Biol.* 41:89–94
57. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, et al. 2000. The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* 101:555–67
58. Hetherington AJ, Dolan L. 2018. Stepwise and independent origins of roots among land plants. *Nature* 561:235–38
59. Hetherington AJ, Dolan L. 2019. Rhynie chert fossils demonstrate the independent origin and gradual evolution of lycophyte roots. *Curr. Opin. Plant Biol.* 47:119–26
60. Hofhuis H, Laskowski M, Du Y, Prasad K, Grigg S, et al. 2013. Phyllotaxis and rhizotaxis in *Arabidopsis* are modified by three PLETHORA transcription factors. *Curr. Biol.* 23:956–62
61. Huang K-L, Ma G-J, Zhang M-L, Xiong H, Wu H, et al. 2018. The ARF7 and ARF19 transcription factors positively regulate *PHOSPHATE STARVATION RESPONSE1* in *Arabidopsis* roots. *Plant Physiol.* 178:413–27
62. Inoue S-i, Takahashi K, Okumura-Noda H, Kinoshita T. 2016. Auxin influx carrier AUX1 confers acid resistance for *Arabidopsis* root elongation through the regulation of plasma membrane H^+-ATPase. *Plant Cell Physiol.* 57:2194–201
63. Janiak A, Kwaśniewski M, Szarejko I. 2016. Gene expression regulation in roots under drought. *J. Exp. Bot.* 67:1003–14
64. Jung J-Y, Ried MK, Hothorn M, Poirier Y. 2018. Control of plant phosphate homeostasis by inositol pyrophosphates and the SPX domain. *Curr. Opin. Biotechnol.* 49:156–62
65. Kaneyasu T, Kobayashi A, Nakayama M, Fujii N, Takahashi H, Miyazawa Y. 2007. Auxin response, but not its polar transport, plays a role in hydrotropism of *Arabidopsis* roots. *J. Exp. Bot.* 58:1143–50
66. Kanno S, Arrighi J-F, Chiarenza S, Bayle V, Berthomé R, et al. 2016. A novel role for the root cap in phosphate uptake and homeostasis. *eLife* 5:e14577
67. Kellermeier F, Armengaud P, Seditas TJ, Danku J, Salt DE, Amtmann A. 2014. Analysis of the root system architecture of *Arabidopsis* provides a quantitative readout of crosstalk between nutritional signals. *Plant Cell* 26:1480–96
68. Kellermeier F, Chardon F, Amtmann A. 2013. Natural variation of Arabidopsis root architecture reveals complementing adaptive strategies to potassium starvation. *Plant Physiol.* 161:1421–32
69. Kiba T, Krapp A. 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. *Plant Cell Physiol.* 57:707–14
70. Kleine-Vehn J, Ding Z, Jones AR, Tasaka M, Morita MT, Friml J. 2010. Gravity-induced PIN transcytosis for polarization of auxin fluxes in gravity-sensing root cells. *PNAS* 107:22344–49
71. Krouk G, Lacombe B, Bielach A, Perrine-Walker F, Malinska K, et al. 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. *Dev. Cell* 18:927–37
72. Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM. 1995. Formation of lateral root meristems is a two-stage process. *Development* 121:3303–10
73. Li G, Song H, Li B, Kronzucker HJ, Shi W. 2015. Auxin Resistant1 and PIN-FORMED2 protect lateral root formation in Arabidopsis under iron stress. *Plant Physiol.* 169:2608–23
74. Liao C-Y, Smet W, Brunoud G, Yoshida S, Vernoux T, Weijers D. 2015. Reporters for sensitive and quantitative measurement of auxin response. *Nat. Methods* 12:207–10

75. Liberman LM, Sparks EE, Moreno-Risueno MA, Petricka JJ, Benfey PN. 2015. MYB36 regulates the transition from proliferation to differentiation in the *Arabidopsis* root. *PNAS* 112:12099–104
76. Lima JE, Kojima S, Takahashi H, von Wirén N. 2010. Ammonium triggers lateral root branching in *Arabidopsis* in an AMMONIUM TRANSPORTER1;3-dependent manner. *Plant Cell* 22:3621–33
77. Liu J, Sheng L, Xu Y, Li J, Yang Z, et al. 2014. *WOX11* and *12* are involved in the first-step cell fate transition during de novo root organogenesis in *Arabidopsis*. *Plant Cell* 26:1081–93
78. Liu W, Yu J, Ge Y, Qin P, Xu L. 2018. Pivotal role of *LBD16* in root and root-like organ initiation. *Cell. Mol. Life Sci.* 75:3329–38
79. Liu Y, Lai N, Gao K, Chen F, Yuan L, Mi G. 2013. Ammonium inhibits primary root growth by reducing the length of meristem and elongation zone and decreasing elemental expansion rate in the root apex in *Arabidopsis thaliana*. *PLOS ONE* 8:e61031
80. Liu Y, von Wirén N. 2017. Ammonium as a signal for physiological and morphological responses in plants. *J. Exp. Bot.* 68:2581–92
81. Liu Y, Xu M, Liang N, Zheng Y, Yu Q, Wu S. 2017. Symplastic communication spatially directs local auxin biosynthesis to maintain root stem cell niche in *Arabidopsis*. *PNAS* 114:4005–10
82. Long Y, Smet W, Cruz-Ramírez A, Castelijns B, de Jonge W, et al. 2015. *Arabidopsis* BIRD zinc finger proteins jointly stabilize tissue boundaries by confining the cell fate regulator SHORT-ROOT and contributing to fate specification. *Plant Cell* 27:1185–99
83. Luschnig C, Gaxiola RA, Grisafi P, Fink GR. 1998. EIR1, a root-specific protein involved in auxin transport, is required for gravitropism in *Arabidopsis thaliana*. *Genes Dev.* 12:2175–87
84. Lynch JP. 2011. Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. *Plant Physiol.* 156:1041–49
85. Lynch JP. 2013. Steep, cheap and deep: an ideotype to optimize water and N acquisition by maize root systems. *Ann. Bot.* 112:347–57
86. Maher EP, Martindale SJB. 1980. Mutants of *Arabidopsis thaliana* with altered responses to auxins and gravity. *Biochem. Genet.* 18:1041–53
87. Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, et al. 2006. Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* 311:94–98
88. Mähönen AP, ten Tusscher K, Siligato R, Smetana O, Díaz-Triviño S, et al. 2014. PLETHORA gradient formation mechanism separates auxin responses. *Nature* 515:125–29
89. Manzano C, Ramirez-Parra E, Casimiro I, Otero S, Desvoyes B, et al. 2012. Auxin and epigenetic regulation of *SKP2B*, an F-box that represses lateral root formation. *Plant Physiol.* 160:749–62
90. Matsunaga KK, Tomescu AM. 2016. Root evolution at the base of the lycophyte clade: insights from an Early Devonian lycophyte. *Ann. Bot.* 117:585–98
91. Matsuzaki Y, Ogawa-Ohnishi M, Mori A, Matsubayashi Y. 2010. Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*. *Science* 329:1065–67
92. Mora-Macías J, Ojeda-Rivera JO, Gutiérrez-Alanís D, Yong-Villalobos L, Oropeza-Aburto A, et al. 2017. Malate-dependent Fe accumulation is a critical checkpoint in the root developmental response to low phosphate. *PNAS* 114:E3563–72
93. Moreno-Risueno MA, Sozzani R, Yardımcı GG, Petricka JJ, Vernoux T, et al. 2015. Transcriptional control of tissue formation throughout root development. *Science* 350:426–30
94. Moreno-Risueno MA, Van Norman JM, Moreno A, Zhang J, Ahnert SE, Benfey PN. 2010. Oscillating gene expression determines competence for periodic *Arabidopsis* root branching. *Science* 329:1306–11
95. Motte H, Beeckman T. 2019. The evolution of root branching: increasing the level of plasticity. *J. Exp. Bot.* 70:785–93
96. Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, et al. 1998. *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. *EMBO J.* 17:6903–11
97. Müller J, Toev T, Heisters M, Teller J, Moore KL, et al. 2015. Iron-dependent callose deposition adjusts root meristem maintenance to phosphate availability. *Dev. Cell* 33:216–30
98. Murphy E, Vu LD, Van den Broeck L, Lin ZF, Ramakrishna P, et al. 2016. RALFL34 regulates formative cell divisions in Arabidopsis pericycle during lateral root initiation. *J. Exp. Bot.* 67:4863–75

82. Unravels how both SHORT ROOT (SHR) movement and activity are regulated by BIRD proteins to specify cell fates in the root meristem.

99. Nacry P, Canivenc G, Muller B, Azmi A, Van Onckelen H, et al. 2005. A role for auxin redistribution in the responses of the root system architecture to phosphate starvation in Arabidopsis. *Plant Physiol.* 138:2061–74
100. Ohkubo Y, Tanaka M, Tabata R, Ogawa-Ohnishi M, Matsubayashi Y. 2017. Shoot-to-root mobile polypeptides involved in systemic regulation of nitrogen acquisition. *Nat. Plants* 3:17029
101. Orman-Ligeza B, Morris EC, Parizot B, Lavigne T, Babé A, et al. 2018. The xerobranching response represses lateral root formation when roots are not in contact with water. *Curr. Biol.* 28:3165–73
102. Oropeza-Aburto A, Cruz-Ramírez A, Acevedo-Hernández GJ, Pérez-Torres C-A, Caballero-Pérez J, Herrera-Estrella L. 2012. Functional analysis of the *Arabidopsis PLDZ2* promoter reveals an evolutionarily conserved low-Pi-responsive transcriptional enhancer element. *J. Exp. Bot.* 63:2189–202
103. Orosa-Puente B, Leftley N, von Wangenheim D, Banda J, Srivastava AK, et al. 2018. Root branching toward water involves posttranslational modification of transcription factor ARF7. *Science* 362:1407–10
104. Ou Y, Lu X, Zi Q, Xun Q, Zhang J, et al. 2016. RGF1 INSENSITIVE 1 to 5, a group of LRR receptor-like kinases, are essential for the perception of root meristem growth factor 1 in *Arabidopsis thaliana*. *Cell Res.* 26:686–98
105. Paolillo DJ Jr., Zobel RW. 2002. The formation of adventitious roots on root axes is a widespread occurrence in field-grown dicotyledonous plants. *Am. J. Bot.* 89:1361–72
106. Parizot B, Laplaze L, Ricaud L, Boucheron-Dubuisson E, Bayle V, et al. 2008. Diarch symmetry of the vascular bundle in Arabidopsis root encompasses the pericycle and is reflected in distich lateral root initiation. *Plant Physiol.* 146:140–48
107. Péret B, Desnos T, Jost R, Kanno S, Berkowitz O, Nussaume L. 2014. Root architecture responses: in search of phosphate. *Plant Physiol.* 166:1713–23
108. Pérez-Torres C-A, López-Bucio J, Cruz-Ramírez A, Ibarra-Laclette E, Dharmasiri S, et al. 2008. Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. *Plant Cell* 20:3258–72
109. Pi L, Aichinger E, van der Graaff E, Llavata-Peris CI, Weijers D, et al. 2015. Organizer-derived WOX5 signal maintains root columella stem cells through chromatin-mediated repression of *CDF4* expression. *Dev. Cell* 33:576–88
110. Puga MI, Rojas-Triana M, de Lorenzo L, Leyva A, Rubio V, Paz-Ares J. 2017. Novel signals in the regulation of Pi starvation responses in plants: facts and promises. *Curr. Opin. Plant Biol.* 39:40–49
111. Robinson D. 1994. The responses of plants to non-uniform supplies of nutrients. *New Phytol.* 127:635–74
112. Rodriguez RE, Ercoli MF, Debernardi JM, Breakfield NW, Mecchia MA, et al. 2015. MicroRNA miR396 regulates the switch between stem cells and transit-amplifying cells in Arabidopsis roots. *Plant Cell* 27:3354–66
113. Rogato A, D'Apuzzo E, Barbulova A, Omrane S, Parlati A, et al. 2010. Characterization of a developmental root response caused by external ammonium supply in *Lotus japonicus*. *Plant Physiol.* 154:784–95
114. Rosquete MR, von Wangenheim D, Marhavý P, Barbez E, Stelzer EH, et al. 2013. An auxin transport mechanism restricts positive orthogravitropism in lateral roots. *Curr. Biol.* 23:817–22
115. Ruiz Rosquete M, Waidmann S, Kleine-Vehn J. 2018. PIN7 auxin carrier has a preferential role in terminating radial root expansion in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* 19:1238
116. Roychoudhry S, Del Bianco M, Kieffer M, Kepinski S. 2013. Auxin controls gravitropic setpoint angle in higher plant lateral branches. *Curr. Biol.* 23:1497–504
117. Roychoudhry S, Kieffer M, Del Bianco M, Liao CY, Weijers D, Kepinski S. 2017. The developmental and environmental regulation of gravitropic setpoint angle in Arabidopsis and bean. *Sci. Rep.* 7:42664
118. Ruffel S, Krouk G, Ristova D, Shasha D, Birnbaum KD, Coruzzi GM. 2011. Nitrogen economics of root foraging: transitive closure of the nitrate–cytokinin relay and distinct systemic signaling for N supply versus demand. *PNAS* 108:18524–29
119. Sabatini S, Heidstra R, Wildwater M, Scheres B. 2003. SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. *Genes Dev.* 17:354–58
120. Santuari L, Sanchez-Perez GF, Luijten M, Rutjens B, Terpstra I, et al. 2016. The PLETHORA gene regulatory network guides growth and cell differentiation in Arabidopsis roots. *Plant Cell* 28:2937–51

103. Elucidation of the molecular mechanism of hydropatterning that involves posttranslational modification of a specific auxin response factor.

121. Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, et al. 2007. Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature* 446:811–14
122. Sebastian J, Ryu KH, Zhou J, Tarkowská D, Tarkowski P, et al. 2015. PHABULOSA controls the quiescent center-independent root meristem activities in *Arabidopsis thaliana*. *PLOS Genet*. 11:e1004973
123. Sheng LH, Hu XM, Du YJ, Zhang GF, Huang H, et al. 2017. Non-canonical *WOX11*-mediated root branching contributes to plasticity in *Arabidopsis* root system architecture. *Development* 144:3126–33
124. Shimotohno A, Heidstra R, Blilou I, Scheres B. 2018. Root stem cell niche organizer specification by molecular convergence of PLETHORA and SCARECROW transcription factor modules. *Genes Dev*. 32:1085–100
125. Shkolnik D, Krieger G, Nuriel R, Fromm H. 2016. Hydrotropism: Root bending does not require auxin redistribution. *Mol. Plant* 9:757–59
126. Sozzani R, Cui H, Moreno-Risueno MA, Busch W, Van Norman JM, et al. 2010. Spatiotemporal regulation of cell-cycle genes by SHORTROOT links patterning and growth. *Nature* 466:128–32
127. Spartz AK, Ren H, Park MY, Grandt KN, Lee SH, et al. 2014. SAUR inhibition of PP2C-D phosphatases activates plasma membrane H^+-ATPases to promote cell expansion in *Arabidopsis*. *Plant Cell* 26:2129–42
128. Stoeckle D, Thellmann M, Vermeer JE. 2018. Breakout—lateral root emergence in *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 41:67–72
129. Swarup R, Kramer EM, Perry P, Knox K, Leyser HMO, et al. 2005. Root gravitropism requires lateral root cap and epidermal cells for transport and response to a mobile auxin signal. *Nat. Cell Biol.* 7:1057–65
130. Tabata R, Sumida K, Yoshii T, Ohyama K, Shinohara H, Matsubayashi Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. *Science* 346:343–46
131. Takahashi K, Hayashi K-i, Kinoshita T. 2012. Auxin activates the plasma membrane H^+-ATPase by phosphorylation during hypocotyl elongation in Arabidopsis. *Plant Physiol.* 159:632–41
132. Takahashi N, Goto N, Okada K, Takahashi H. 2002. Hydrotropism in abscisic acid, wavy, and gravitropic mutants of *Arabidopsis thaliana*. *Planta* 216:203–11
133. Taniguchi M, Furutani M, Nishimura T, Nakamura M, Fushita T, et al. 2017. The Arabidopsis LAZY1 family plays a key role in gravity signaling within statocytes and in branch angle control of roots and shoots. *Plant Cell* 29:1984–99
134. Tian H, Wabnik K, Niu T, Li H, Yu Q, et al. 2014. WOX5–IAA17 feedback circuit-mediated cellular auxin response is crucial for the patterning of root stem cell niches in *Arabidopsis*. *Mol. Plant* 7:277–89
135. Toyokura K, Goh T, Shinohara H, Shinoda A, Kondo Y, et al. 2019. Lateral inhibition by a peptide hormone-receptor cascade during *Arabidopsis* lateral root founder cell formation. *Dev. Cell* 48:64–75.e5
136. Tsukagoshi H, Busch W, Benfey PN. 2010. Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* 143:606–16
137. Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, et al. 2013. Control of root system architecture by *DEEPER ROOTING 1* increases rice yield under drought conditions. *Nat. Genet.* 45:1097–102
138. Utsuno K, Shikanai T, Yamada Y, Hashimoto T. 1998. *AGR*, an *Agravitropic* locus of *Arabidopsis thaliana*, encodes a novel membrane-protein family member. *Plant Cell Physiol.* 39:1111–18
139. van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B. 1997. Short-range control of cell differentiation in the *Arabidopsis* root meristem. *Nature* 390:287–89
140. Van Norman JM, Xuan W, Beeckman T, Benfey PN. 2013. To branch or not to branch: the role of pre-patterning in lateral root formation. *Development* 140:4301–10
141. Vatén A, Dettmer J, Wu S, Stierhof Y-D, Miyashima S, et al. 2011. Callose biosynthesis regulates symplastic trafficking during root development. *Dev. Cell* 21:1144–55
142. Vermeer JEM, von Wangenheim D, Barberon M, Lee Y, Stelzer EHK, et al. 2014. A spatial accommodation by neighboring cells is required for organ initiation in *Arabidopsis*. *Science* 343:178–83
143. Vidal EA, Álvarez JM, Moyano TC, Gutiérrez RA. 2015. Transcriptional networks in the nitrate response of *Arabidopsis thaliana*. *Curr. Opin. Plant Biol.* 27:125–32
144. Vidal EA, Araus V, Lu C, Parry G, Green PJ, et al. 2010. Nitrate-responsive miR393/*AFB3* regulatory module controls root system architecture in *Arabidopsis thaliana*. *PNAS* 107:4477–82

123. Describes an alternative, ARF7/ARF19-independent lateral root formation mechanism that is partially overlapping with the adventitious root formation pathway.

145. Vidal EA, Moyano TC, Riveras E, Contreras-López O, Gutiérrez RA. 2013. Systems approaches map regulatory networks downstream of the auxin receptor AFB3 in the nitrate response of *Arabidopsis thaliana* roots. *PNAS* 110:12840–45
146. Vieten A, Sauer M, Brewer PB, Friml J. 2007. Molecular and cellular aspects of auxin-transport-mediated development. *Trends Plant Sci*. 12:160–68
147. von Wangenheim D, Fangerau J, Schmitz A, Smith RS, Leitte H, et al. 2016. Rules and self-organizing properties of post-embryonic plant organ cell division patterns. *Curr. Biol.* 26:439–49
148. Whitford R, Fernandez A, Tejos R, Cuéllar Pérez A, Kleine-Vehn J, et al. 2012. GOLVEN secretory peptides regulate auxin carrier turnover during plant gravitropic responses. *Dev. Cell* 22:678–85
149. Wilmoth JC, Wang S, Tiwari SB, Joshi AD, Hagen G, et al. 2005. NPH4/ARF7 and ARF19 promote leaf expansion and auxin-induced lateral root formation. *Plant J.* 43:118–30
150. Xie Y, Mao Y, Xu S, Zhou H, Duan X, et al. 2015. Heme-heme oxygenase 1 system is involved in ammonium tolerance by regulating antioxidant defence in *Oryza sativa*. *Plant Cell Environ*. 38:129–43
151. Xu J, Hofhuis H, Heidstra R, Sauer M, Friml J, Scheres B. 2006. A molecular framework for plant regeneration. *Science* 311:385–88
152. Xu W, Jia L, Shi W, Liang J, Zhou F, et al. 2013. Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytol*. 197:139–50
153. Xuan W, Audenaert D, Parizot B, Möller B, Njo M, et al. 2015. Root cap-derived auxin pre-patterns the longitudinal axis of the *Arabidopsis* root. *Curr. Biol.* 25:1381–88

154. Associates lateral root cap cell death with lateral roots and pinpoints an early lateral root formation mechanism.

154. Xuan W, Band LR, Kumpf RP, Van Damme D, Parizot B, et al. 2016. Cyclic programmed cell death stimulates hormone signaling and root development in *Arabidopsis*. *Science* 351:384–87

155. Yang L, Zhang J, He J, Qin Y, Hua D, et al. 2014. ABA-mediated ROS in mitochondria regulate root meristem activity by controlling *PLETHORA* expression in *Arabidopsis*. *PLOS Genet*. 10:e1004791
156. York LM, Lobet G. 2017. Phenomics of root system architecture: measuring and analyzing root phenes. *Plant Cell* 29:tpc.117.tt0917
157. York LM, Nord EA, Lynch JP. 2013. Integration of root phenes for soil resource acquisition. *Front. Plant Sci.* 4:355
158. Yoshihara T, Spalding EP. 2017. LAZY genes mediate the effects of gravity on auxin gradients and plant architecture. *Plant Physiol*. 175:959–69
159. Yu L-H, Miao Z-Q, Qi G-F, Wu J, Cai X-T, et al. 2014. MADS-box transcription factor AGL21 regulates lateral root development and responds to multiple external and physiological signals. *Mol. Plant* 7:1653–69
160. Yu Q, Tian H, Yue K, Liu J, Zhang B, et al. 2016. A P-loop NTPase regulates quiescent center cell division and distal stem cell identity through the regulation of ROS homeostasis in *Arabidopsis* root. *PLOS Genet*. 12:e1006175
161. Zhan A, Lynch JP. 2015. Reduced frequency of lateral root branching improves N capture from low-N soils in maize. *J. Exp. Bot.* 66:2055–65
162. Zhan A, Schneider H, Lynch JP. 2015. Reduced lateral root branching density improves drought tolerance in maize. *Plant Physiol*. 168:1603–15
163. Zhang Y, Jiao Y, Jiao H, Zhao H, Zhu Y-X. 2017. Two-step functional innovation of the stem-cell factors WUS/WOX5 during plant evolution. *Mol. Biol. Evol.* 34:640–53
164. Zhao J, Wang W, Zhou H, Wang R, Zhang P, et al. 2017. Manganese toxicity inhibited root growth by disrupting auxin biosynthesis and transport in *Arabidopsis*. *Front. Plant Sci*. 8:272
165. Zhou W, Wei L, Xu J, Zhai Q, Jiang H, et al. 2010. *Arabidopsis* tyrosylprotein sulfotransferase acts in the auxin/PLETHORA pathway in regulating postembryonic maintenance of the root stem cell niche. *Plant Cell* 22:3692–709