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Auxin in Root Development

Suruchi Roychoudhry and Stefan Kepinski

Centre for Plant Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom

Correspondence: s.roychoudhry@leeds.ac.uk; s.kepinski@leeds.ac.uk

Root system architecture is an important determinant of below-ground resource capture and hence overall plant fitness. The plant hormone auxin plays a central role in almost every facet of root development from the cellular to the whole-root-system level. Here, using *Arabidopsis* as a model, we review the multiple gene signaling networks regulated by auxin biosynthesis, conjugation, and transport that underpin primary and lateral root development. We describe the role of auxin in establishing the root apical meristem and discuss how the tight spatio-temporal regulation of auxin distribution controls transitions between cell division, cell growth, and differentiation. This includes the localized reestablishment of mitotic activity required to elaborate the root system via the production of lateral roots. We also summarize recent discoveries on the effects of auxin and auxin signaling and transport on the control of lateral root gravitropic setpoint angle (GSA), a critical determinant of the overall shape of the root system. Finally, we discuss how environmental conditions influence root developmental plasticity by modulation of auxin biosynthesis, transport, and the canonical auxin signaling pathway.

In vascular plants, root systems form the interface between the plant body and the soil. They play a crucial role in basic functions such as anchorage and nutrient and water uptake, as well as in species-specific functions such as vegetative reproduction, minimizing underground competition with adjacent individuals, and maintaining abiotic and biotic interactions within the rhizosphere (Morris et al. 2017). Plants encounter myriad subterranean macro- and micro-environmental stresses during their lifetimes, including biotic stresses, such as pathogen attack, and abiotic stresses such as nutrient and water scarcity. Root systems must therefore retain the ability to perceive and respond to these stresses at multiple levels, ranging from individual cells to tissues and eventually whole organs. The ability of root systems to maintain a high degree of plasticity is therefore key to ensuring the survival, fitness, and adaptation of individual plants (Khan et al. 2016).

Roots can have one of two distinct origins: the embryo (embryonic, i.e., primary or seminal roots) or other roots and/or non-root tissues (postembryonic). Roots that arise from pre-existing roots are usually secondary or lateral roots (LRs), while those that arise from non-root tissues are adventitious or basal roots (for review, see Du and Scheres 2018). In dicot species, including *Arabidopsis*, the primary embryonic root is dominant and produces a number of secondary LRs. Over time, these LRs reiterate the formation of new LRs, which leads to the production of a highly ordered and spatially dis-

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tinct root system termed as the "taproot" or allorhizic root system. In contrast, monocots form "homorhizic" root systems comprised of numerous adventitious roots that in turn produce additional secondary or LRs (Osmont et al. 2007; Bellini et al. 2014). An important distinction between the two types of root systems lies in the life span of the primary embryonic root: in taproot systems, the primary root remains dominant during the entire life span of the plant, while in homorhizic systems, embryonic roots are often short-lived and play a role in the establishment of the root systems at the seedling stage, before the development of adventitious roots, which dominate the root system (Bellini et al. 2014).

Root system architecture (RSA) describes the spatial distribution of the root system, capturing the specific deployment of root axes in the soil matrix or substrate upon which the plant grows (Smith and De Smet 2012; Khan et al. 2016). Macro RSA, the large-scale overall form of the root system, comprises three major parameters: length, degree of branching, and angle of growth. At a smaller scale, the "micro" parameters of RSA that influence the extent of root surface area include root diameter and the formation of root hairs. These macro- and micro-scale components of RSA are a critical determinant of the plant's ability to capture the water and nutrients that are typically distributed heterogeneously within the soil (Smith and De Smet 2012; Morris et al. 2017; for review, see Rellán-Álvarez et al. 2016). RSA parameters can be modulated by multiple genetic, physiological, and environmental factors, allowing for an efficient capture of resources from the rhizosphere (for review, see Rellán-Álvarez et al. 2016). Understanding the molecular mechanisms that regulate RSA and its plasticity is therefore key to enhancing both the productivity and sustainability of agriculture through developing crop varieties that are better able to take up water and nutrients, reducing the need for supplementary fertilizer application and irrigation (Lynch 2013).

The plant hormone auxin is the master regulator of plant architecture (for review, see Benjamins and Scheres 2008; Overoode et al. 2010). Auxin is a small molecule that is dynamically distributed via a system of polar auxin transport. The resulting gradients, minima and maxima of auxin concentration that are formed to act to pattern, trigger developmental transitions, and control growth. Auxin can enter cells passively, but is also actively imported into cells by the influx carriers AUX1 and LIKE AUX1 (LAX) 1-3 (Swarup et al. 2005; Péret et al. 2012). Auxin must be exported from cells by the PIN-FORMED family of efflux transporter proteins as well as the ABCB and TWISTED DWARF1 (TWD1) proteins (for review, see Geisler et al. 2017; Zwiewka et al. 2019; Hammes et al. 2021). It is this requirement for active auxin efflux from cells that forms the basis of the directional control of auxin movement. *Arabidopsis* contains eight PIN proteins, of which PINs 1–4 and 7 are canonical "long PINs" targeted to the plasma membrane, while PINs 5 and 6 are short PINs targeted to the endoplasmic reticulum (ER). Although each of the long PINs have specific expression patterns and play distinct roles in root and shoot development, recent work has shown that all five canonical PINs share equivalent capacity to generate auxin maxima and regulate different aspects of root (and shoot) development (Zhang et al. 2020). Thus, coordinated regulation of the subcellular distribution of PIN proteins within the field of cells allows flows of auxin to be generated (Blilou et al. 2005). Spatiotemporal variation in auxin concentration is then translated into developmental control principally via the TIR1/AFB-Aux/IAA-ARF system of auxin-dependent transcriptional regulation (see Morffy and Strader 2021). Briefly, in this system auxin acts to regulate the abundance of Aux/IAA transcriptional corepressor proteins, which are targeted to auxin-regulated genes via their interaction with DNA-binding transcription factors called auxin response factors (ARFs) (for review, see Leyser 2018). Auxin promotes the formation of an auxin coreceptor complex comprising an F-box protein of the TIR1/AFB family and an Aux/IAA protein. This auxin-enhanced interaction catalyzes to ubiquitin-mediated proteolysis of the Aux/IAA, thereby de-repressing the ARF-bound loci previously transcriptionally silenced by the presence of the Aux/IAA (Gray et al. 2001; Dharmasiri et al. 2005; Kepinski and Leyser

2005). Here, we review the complex role of auxin in root development and its impact on RSA.

AUXIN IN PRIMARY ROOT DEVELOPMENT

In the *Arabidopsis* root, tissues originate through the activity of the root meristem. Just above the columella, which constitutes the root tip, is the stem cell niche, which is composed of a group of slow-dividing cells that constitute the organizing center, also called quiescent center (QC), and the surrounding long-term stem cells (for review, see García-Gómez et al. 2021). These stem cells divide asymmetrically to short-term stem cells, which undergo multiple cell divisions with little expansion, in the proximal meristem (PM). The PM is enveloped by the lateral root cap (LRC), an important tissue for auxin transport and homeostasis in the root meristem. Cells are displaced away from the stem cell niche by subsequent cell divisions, until they reach the transition zone (TZ), where they stop dividing and start to differentiate. Cells then enter the elongation zone (EZ) within which cells undergo vast increases in length (up to 300% in 3 h) with virtually no division. The EZ is followed by the differentiation zone (DZ), where cells stop elongating and complete their differentiation gaining cell-type-specific features and functions (Verbelen et al. 2006; Markakis et al. 2012). Research has shown that some of the signaling processes that mediate differentiation begin while cells are still elongating (see below) (Datta et al. 2015). For this reason, the EZ and DZ have sometimes been referred to collectively as the elongation–differentiation zone (EDZ), a name that better captures the overlap in the processes of cell elongation and cell differentiation (Takatsuka and Umeda 2014).

The patterning and coordination of the processes that maintain the constant self-renewal and growth of the root is complex. At first sight, the extent of auxin's involvement in so many of them seems quite remarkable. First, a localized, auxin maximum in the root apex acts to position and maintain the QC and stem cell niche (Sabatini et al. 1999; Grieneisen et al. 2007). This auxin maximum is specified during embryogenesis in the hypophyseal cell at the base of the embryo, where it first establishes the population of root stem cells. In both the embryo and postembryonic root, the formation of these auxin maxima is principally dependent on the expression and subcellular localization of members of the PIN family of auxin efflux transporters (see Fig. 1 and legend for details; Blilou et al. 2005; Michniewicz et al. 2007; Zourelidou et al. 2014). Auxin maintains stem cell organization by regulating the differentiation of distal stem cells (the cells that give rise to the columella) through a signaling pathway involving the transcription factors PLETHORA (PLTs) and the homeodomain transcription factor WUSCHEL RELATED HOMEOBOX 5 (WOX5) (Sabatini et al. 2003; for review, see Drisch and Stahl 2015). Through an ARF10/16-IAA17/AXR3 signaling pathway, local auxin levels are tightly regulated in the root tip by biosynthesis and transport, in turn repressing the expression of WOX5 and restricting it to the QC where it is required for the expression of PLTs (Ding and Friml 2010).

Next, a complex network of hormone signaling pathways specify the boundaries of mitotic activity in the primary root apex, positioning the TZ and maintaining a meristem of sufficient size to sustain continuous root growth. At the TZ, auxin and cytokinin act antagonistically to regulate the expression of the PIN efflux transporters through modulation of the expression of the auxin signaling corepressor SHY2/IAA3. This, in turn, promotes auxin redistribution leading to cell differentiation. Conversely, auxin promotes SHY2 degradation thereby promoting cell division (Dello Ioio et al. 2008). Further insight into auxin-dependent TZ maintenance came from a recent study that combined computational and genetic approaches (Di Mambro et al. 2017). This work showed that cytokinin-dependent degradation of auxin, via conjugation by the enzymes of the GRETCHEN HAGEN (GH) family, in the LRC is required for the specification of an auxin minimum, which coincides with the TZ. The authors predicted, using mathematical modeling, that perturbation of this auxin minimum would lead to changes in TZ positioning and root meristem size and, conversely, showed that manipulation of GH expression lev-

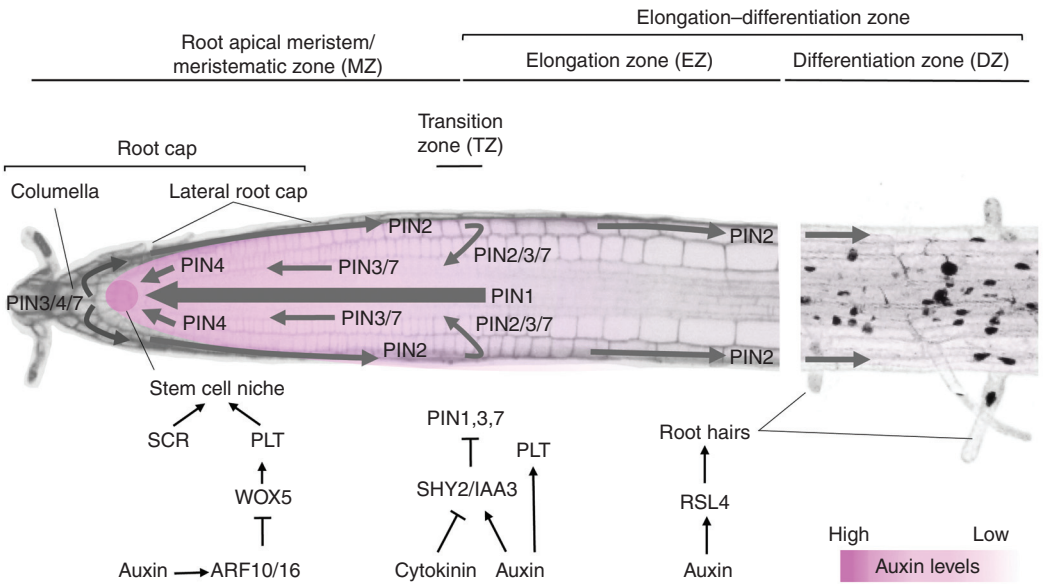

Figure 1. Organization of the primary root in *Arabidopsis*. In the root apex, individual PINs are localized to specific cell membranes to generate a pattern of auxin distribution in the root tip. In the stele, several PINs but principally PIN1 are localized to rootward cell membranes leading to a strong tipward flux of auxin. In the columella cells of the root cap, PIN3, PIN4, and PIN7 distribute auxin laterally toward the lateral root cap, from where it is transported shootward through the epidermis by AUX/LAX proteins and PIN2 (Hu et al. 2021). At the transition zone, some auxin is also refluxed back into the rootward stream in the stele via lateral, inward transport by PIN2, PIN3, and PIN7 (Blilou et al. 2005). This topology of PINs proteins in the root apex creates both the maximum of auxin that positions the stem cell niche and through the gravity-responsive, asymmetric lateral redistribution of auxin from the columella, a means to steer the growth of the root with reference to gravity (for review, see García-Gómez et al. 2021). The identity of the stem cell niche is also dependent on the high levels of *PLT* expression, tightly regulated by complex coordinated hormone signaling pathways. Further shootward, antagonistic interaction between auxin and cytokinin maintain the identity of the meristematic and transition zones, partially through the regulation of levels of the Aux/IAA, SHY2/IAA3. At the transition zone *PLT* levels are intermediate and become further reduced in the elongation–differentiation zone, where cells begin to stop elongating and differentiate to acquire specific functions, such as root hairs.

el resulted in variations in root meristem size (Di Mambro et al. 2017). In addition, auxin also controls TZ positioning by regulating the graded distribution of PLTs (Aida et al. 2004; Mähönen et al. 2014; Durgaprasad et al. 2019). PLTs are transcribed in a narrower domain around the stem cell niche, and PLT proteins form a gradient through a mechanism that involves coordinated cell divisions and cell-to-cell transport. Auxin regulates PLT levels and distribution by both inducing PLT expression around the stem cell niche and by regulating cell division in the PM. PLT controls root zonation in a dose-dependent manner: high levels of PLT in the QC are necessary to maintain slow mitotic stem cell identity, intermediate PLT levels in the meristem induce rapid cell divisions, and low PLT levels at the TZ allow for the onset of cell expansion and differentiation (Galinha et al. 2007; Mähönen et al. 2014). A recent systems biology approach further revealed the complex interplay between auxin-cytokinin-PLT signaling pathways in root development. This study showed that PLT2 levels drop in the root TZ, leading to ARR12 activation. The resulting PLT2-ARR12 antagonism controls the growth of the root meristem in the initial phases of primary root growth. Subsequent activation of ARR1 further reduces PLT2

levels through induction of the cell-cycle repressor KRP2, thus setting final meristem size (Salvi et al. 2020).

Proximal to the meristem, auxin concentrations decrease as daughter cells repeatedly divide before they differentiate. Crucially, auxin concentrations rise again after the TZ, where it is needed for appropriate cell differentiation. In the DZ, auxin is an essential driver of developmental events in both proximodistal and radial axes (Marhava et al. 2018). For example, root protophloem sieve element (PPSE) cells accumulate higher levels of auxin and begin the process of differentiation while their neighbouring cells are still meristematic. A recent study has demonstrated the novel role of two plasma membrane proteins, BRX (BREVIS RADIX) and PAX (a D6PK-like protein kinase, member of the AGC protein family) in regulating this pathway. This work showed that BRX and PAX coordinately act at the rootward membrane of PPSE cells to inhibit PIN1-dependent auxin efflux. This causes a rise in auxin levels, which triggers PPSE differentiation. Following the localized increase in auxin levels, BRX is displaced from the membrane, which in turn activates PAX-dependent auxin efflux and leads to localized reduction in auxin levels. The authors propose that BRX and PAX act as a "molecular rheostat" to control the timing of PPSE differentiation in a multicellular context (Marhava et al. 2018).

Surprisingly, the role of auxin homeostasis and signaling networks in regulating root elongation still remains poorly understood. However, a large number of studies have demonstrated that the exogenous application of both natural and synthetic auxins inhibits cellular elongation and, therefore, root growth. Interestingly, recent work also has demonstrated that tissue-specific biosynthesis of auxin effects root growth. In this study, Hu et al. (2021) expressed the YUCCA and TAA1 auxin biosynthesis genes under the control of promoters that restricted their expression to specific root tissues: *pWER*—epidermis, *pSCR*—endodermis, *pSHR*—stele, *pAPL*—phloem (protophloem, companion cells, and metaphloem sieve elements), and *pWOX5*—QC. The authors showed that the ectopic expression of YUCCA-TAA1 in the epidermis, endodermis, and stele caused very short roots, while YUCCA-TAA1 expression in the phloem and QC had relatively mild effects (Hu et al. 2021).

The inhibition of root growth by auxin has been linked at least partially to auxin-dependent disruption of the auxin cytoskeleton (Rahman et al. 2007; for review, see Zhu and Geisler 2015). Auxin homeostasis during overall root development was also recently found to be dependent on the interplay between the dynamic regulation of the auxin oxidase AtDAO1 (DIOXYGENASE FOR AUXIN OXIDATION 1) and the conjugation enzyme GH3. Mellor et al. (2016) used a combination of mathematical modeling, biochemical, and physiological approaches to show that at lower concentrations of auxin, *AtDAO1* was expressed at higher levels to oxidize endogenous auxin, while higher levels of auxin led to expression of the conjugating enzyme *GH3*. Further work by Zhang et al. (2016) showed that *Arabidopsis* contains two distinct DAO genes, *AtDAO1* and *AtDAO2*, and loss-of-function *dao* mutants had elongated primary roots, increased LR density, and longer root hairs (Porco et al. 2016a; Zhang et al. 2016) compared to wild-type (WT) seedlings. All of these phenotypes are attributed to elevated IAA levels in *dao* mutants.

Auxin also regulates epidermal cell patterning. The *Arabidopsis* root epidermis is alternately spaced with files consisting of smaller trichoblast or hair cells, which generate root hair, and longer atrichoblast or non-hair cells (Dolan 2001; Löfke et al. 2013). Interestingly, at least two auxin transporters are known to be differentially expressed within these distinct cell files. First, the activity of the auxin influx carrier AUX1 mediates auxin transport through atrichoblast cells and is necessary to maintain the identity of root hair cells, and promote root hair elongation. Surprisingly, AUX1 itself is not present in root hair cells, suggesting that auxin is transported longitudinally through canals in epidermal tissue (Jones et al. 2009). Second, PIN2 is also targeted to the plasma membrane of non-hair cells at higher levels than the hair cell files, likely by differential rates of trafficking to lytic vacuoles within these files (Löfke et al. 2015). Taken together, these data suggest

that distinctly different downstream auxin-dependent processes occur in these two cell types.

Downstream of PIN/AUX1 auxin transport-dependent patterning, auxin regulates hair cell growth by modulating the expression of a basic helix-loop-helix transcription factor RSL4 (ROOT HAIR DEFECTIVE 6 LIKE 4). RSL4 is sufficient to promote postmitotic hair cell elongation and, accordingly, *rsl4* mutants have very short root hairs (Yi et al. 2010). *RSL4* is an auxin-inducible gene (Yi et al. 2010) and more recently it has been shown that root hair elongation is directly proportional to the amplitude of a pulse of *RSL4* expression that precedes formation of the root hairs in the DZ (Datta et al. 2015). The intensity of this transient increase in *RSL4* expression can be modulated by external environmental signals such as low phosphate, providing another mechanism for the integration of environmental cues to modulate plant development (Datta et al. 2015).

AUXIN IN LATERAL ROOT DEVELOPMENT

In *Arabidopsis*, LRs arise from single-layered pericycle cells. Radially, the pericycle is surrounded by the endodermal, cortical, and epidermal cell layers, and itself surrounds the central root vasculature. The pericycle is composed of two distinct cell types: phloem pole pericycle (PPP) and xylem pole pericycle (XPP). LRs arise exclusively from XPP cells, also known as pericycle founder cells. Crucially, these cells are thought to retain semi-meristematic properties based on their ultrastructure (small vacuoles, dense cytoplasm, and ribosomes) and maintain the ability to undergo cell divisions upon activation by remaining in the G2 phase of the cell cycle for long periods.

LR development occurs in distinct steps, including (1) pericycle priming and specification of founder cells; (2) initiation, defined as the first round of anticlinal divisions of the founder cells; (3) primordium formation, defined as the sum of divisions leading to the formation of a dome-shaped primordium; and (4) emergence, defined as the protrusion of the newly formed LR through overlying ground and epidermal tissue (Fig. 2; covered in detail in Cavallari et al. 2021).

Priming

Early patterning events that determine the regular spacing of LRs coincide with the oscillation of auxin-dependent transcriptional mechanism. In *Arabidopsis*, LRs emerge from the DZ of the primary root at regularly spaced intervals. When roots are observed in a 2D growth system, such as a petri dish, LRs emerge specifically at the outside edges of waves or curves. An auxin-dependent root clock transcriptional signal is one of the earliest drivers of early LR priming and, therefore, patterning as confirmed by several studies (De Smet et al. 2007; Laskowski et al. 2008; Moreno-Risueno et al. 2010; Xuan et al. 2015, 2016). Crucially, these studies first demonstrated the oscillatory expression of the auxin-responsive promoter DR5 driving the expression of two different markers, β-glucuronidase (GUS) and luciferase (LUC). The oscillations have an amplitude of ∼6 h and occur within the basal meristem and elongation zones, together termed the oscillation zone (OZ). However, the periodicity of the root clock can be modified by environmental signals or auxin treatment (Moreno-Risueno et al. 2010; Kircher and Schopfer 2016). In standard conditions, however, over time, a portion of cells within the OZ maintained stable high expressions of DR5:LUC signal. These cells then began to initiate LRs as they reached the DZ in the elongating root and were defined as LR pre-branch sites. Interestingly, the authors found large numbers of genes that oscillate both in phase (2000) and in antiphase (1500) of DR5:LUC. Also, the application of exogenous auxin was not sufficient to induce LR priming. Taken together, these data suggest that priming is a result of fluctuations not only in auxin levels, but in the expression of a significant number of genes.

Studies using of loss-of-function mutants have reiterated the importance of auxin perception and signaling in LR priming and pre-branch site formation. The *arf7*, *tir1*, and *afb2* mutants all display aberrant DR5 oscillatory patterns and have markedly lower numbers of LR pre-branch sites (for review, see Du and Scheres 2018). Recently, ARF7 and its auxin-sensitive inhibitor POTENT/IAA18 were found to regulate gene

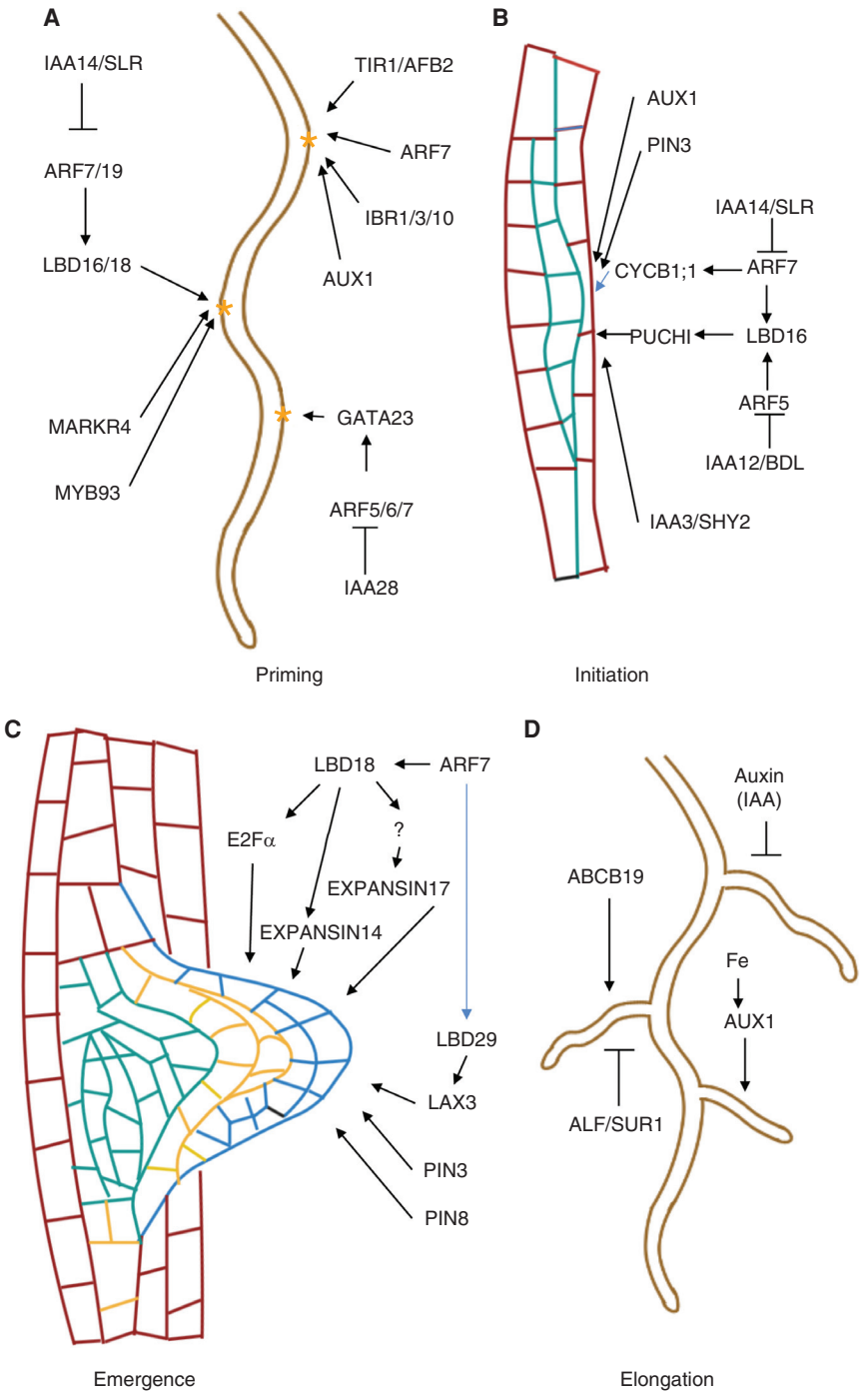

Figure 2. Auxin signaling networks in lateral root development. Visual summary of genes and networks acting both upstream and downstream of the canonical TIR1/AFB-Aux/IAA-ARF auxin signaling pathway during the multiple stages (*A*) priming, (*B*) initiation, (*C*) emergence, and (*D*) elongation of lateral root development in *Arabidopsis*. Yellow asterisks in *A* denote priming sites. Arrowheads denote up-regulation of expression of target genes, while bars denote target down-regulation.

oscillation in the OZ required for the formation of LR pre-branch sites by developing a negative regulatory loop circuit that was predicted to be influenced by environmental cues (Perianez-Rodriguez et al. 2021). Interestingly, an indole-3-butyric acid (IBA) to indole acetic acid ([IAA], the most common form of active auxin) conversion pathway acting specifically within the LRC seems to play a significant role in the generation of stable auxin fluxes required for pre-branch site establishment. Mutations in three INDOLE-3-BUTYRIC ACID RESPONSE genes (IBR1, IBR3, and IBR10) lead to reduced frequency of pre-branch sites (Fig. 2A; Xuan et al. 2015). In a follow-up study, the authors demonstrated the significance of local fluctuations in auxin levels in pre-branch site establishment (Xuan et al. 2016). LRC cells reaching the TZ displayed a gradual reduction in DR5:VENUS signal just prior to their apoptosis and act as a source of auxin for neighboring stele cells, consistent with DR5 activity oscillation. This work also showed that AUX1 expression in the LRC was required for correct DR5 oscillation amplitude and LR patterning.

Interestingly, several studies have demonstrated that not all pre-branch sites showing static DR5 signal go on to become lateral root founder cells (LRFCs) or, indeed, form LRs. One of the earliest molecular markers for LRFC formation is the auxin regulatory GATA23 transcription factor. GATA23 is activated at specific pre-branch sites through an IAA28-ARF 6/7/8/19-dependent pathway in the basal meristem (Fig. 2A). XPP cells leaving the basal meristem displays broad peaks in GATA23 expression and both *gata23* gain- and loss-of-function mutants show altered numbers and spacing of LRs (De Rybel et al. 2010). Moreover, LBD16/ASL18 and other redundantly expressed LBDs, expressed in the adjacent cells of the XPPs undergoing asymmetric cell division downstream of an SLR14-ARF7/19 signaling module, are required for founder cell specification (Fig. 2A; Goh et al. 2012). Additional molecular genetic studies also identified *MARKR4* as another candidate gene in LRFC specification. *markr4* mutants show fewer LRs, but have unaltered numbers of pre-branch sites, suggesting that *MARKR4* is required for the conversion of pre-branch sites into LRFCs (Fig. 2A). *MARKR4* was identified as a downstream molecular component of the IBA to IAA conversion pathway (Xuan et al. 2016). Another potential candidate regulator is *MYB93*, a member of the R2R3 MYB transcription factor family, which is strongly induced by auxin in the basal meristem (Gibbs et al. 2014).

A known positional cue that tightly regulates LR initiation is curvature, either induced mechanically or by gravity. Several studies have convincingly demonstrated that LRs preferentially originate from the convex side of a curved root. Several in silico and mechanotransduction studies have attempted to explain this phenomenon by considering factors such as the differences in geometry of XPP cells on convex and concave sides of the bend, and heterogenous changes in of Ca^{2+} levels in stretched XPP cells. However, experimental work recently showed that curvature alone is not sufficient to specify positioning of the LR, as even if curvature frequency increases, the frequency of LR initiation remains the same (Kircher and Shopfer 2016). Thus, while further work is required to fully understand the signaling pathways that regulate unilateral LR initiation after LRFC specification (Laskowski et al. 2008; Richter et al. 2009), it is evident that auxin transport from the LRC, via the influx transporter AUX1, also plays a role in specifying LR initiation sites. Roots of the *Arabidopsis aux1* mutant display a coiled growth phenotype, with LRs emerging mainly from the convex side of the bend, in complete contrast to the normal wavy growth patterns of WT roots (Marchant et al. 2002; De Smet et al. 2007). Further, *aux1* mutants also have fewer LRs and display lower levels of DR5 oscillatory activity in the basal meristem. However, targeted expression of *AUX1* to the LRC and epidermal cells restores both the agravitropic root and aberrant LR branching patterns in the *aux1* mutant (Swarup et al. 2005).

Studies using a heat-shock-inducible system to generate clonal sectors of XPP cells with local high IAA levels (iaaM) were sufficient to enhance LR production in pericycle cells (Dubrovsky et al. 2008). In silico approaches proposed that existing LRFCs were able to inhibit new

LRFCs in the distal region of the root, by depleting auxin levels in the surrounding cells (Laskowski et al. 2008) These local auxin gradients are formed by the action of influx and efflux carriers. Conversely, molecular genetic studies showed that roots of polar auxin transport mutants (e.g., *pin2pin3pin7* and weak alleles of *gnom*) and loss- (e.g., *mp*/*arf5*) or gain-of-function (e.g., *shy2*, *bdl*) signaling mutants show altered branching patterns, with closely grouped, fused, or fewer LRP/LRs (Laskowski et al. 2008; Geldner 2009; De Smet et al. 2010; Goh et al. 2012; Okumura et al. 2013).

Other regulatory genes/modules also interact with auxin to induce and regulate LRFC patterning. For example, the *PLT* triple loss-of-function *plt357* mutant has several clustered LR primordia (Hofhuis et al. 2013). Finally, auxin-responsive small terminal peptide RALF34, which is expressed around the flanks of the pericycle cells undergoing divisions to form LRPs, seems to be required for spatial distribution of LRs. Indeed, two loss-of-function insertion mutants of *RAFLF4* showed a threefold increase in clustered LRPs (Murphy et al. 2016). Another auxin-repressed peptide signaling module CEP5/CEPR has a similar phenotype in loss-of-function background (Roberts et al. 2016).

LR Initiation and Primordium Formation

The first step in LR initiation involves the formation of an auxin maximum in specified LRFCs (for review, see Torres-Martínez et al. 2019). This maximum is dependent on the influx carrier AUX1 and the activation of *YUCCA4*-mediated auxin biosynthesis by plant-specific B3 transcription factors *FUSCA3* and *LEC2* (Tang et al. 2017). During primordium formation, the first cell division occurs anticlinally to form a series of cells that undergo further periclinal division (for review, see Du and Scheres 2018). This step is preceded by nuclear migration within the XPP cells toward the center of the LR-initiation site. This is followed by asymmetric cell division, which is again regulated by *BDL-ARF5*- and *SLR14-ARF7*-dependent *LBD16* expression in the XPP cells (Fig. 2B; Goh et al. 2012; Lee et al. 2015). *LBD16* is required for LR initiation and subsequent cell fate re-specification after the first round of asymmetric divisions (Goh et al. 2012). LBD16 in turn up-regulates *PUCHI*, an auxin-inducible transcription factor expressed within newly developed LRPs following the first round of asymmetric divisions (Fig. 2A; Goh et al. 2019). Premature induction of *PUCHI* during the preinitiation phase disrupts LR primordium formation.

The divisions necessary to initiate LR formation require pericycle cells to reenter the cell cycle and start dividing actively. This first series of XPP divisions depends on mechanical feedback from the overlying endodermal cells. A *SHY2/IAA3*-dependent signal triggers loss of endodermal cell volume and turgor pressure by relinquishing their tight junction-like diffusion barriers (Vermeer et al. 2014). These biophysical changes trigger *CYCB1;1* expression in the XPP cells, which regulates G2-M transition. Again, the *SLR-ARF7*/*19* signaling module seems to be required for this pathway and *slr* and *arf7*/*19* mutants have almost no LRs and show reduced expression of *CYCB1;1*, likely through an *LBD16*/*LBD18* signaling module (Fig. 2B; Vanneste et al. 2005; Okushima et al. 2007). In addition to triggering changes in endodermal cell shape, auxin signaling in pericyle cells is also required to specify the plane of division. *tir1*/*afb* mutants show periclinal XPP cell divisions that are not competent to form primordia. This auxin-dependent input into cell plane division specification relies on a dynamic microtubule cytoskeleton (for review, see Van Norman et al. 2013).

PIN3 is transiently expressed in the endodermal cells overlying the LRFC, until just after the first round of anticlinal divisions. Interestingly, *pin3* mutants have increased numbers of founder cells but reduced numbers of LRPs, suggesting that transient endodermal *PIN3* expression is required for the transition from founder cell to primordium stage. PIN3 is initially distributed in a nonpolar manner in the endodermal cells in the DZ. However, during the course of LRP formation, PIN3 becomes polarized to the inner cell wall (inner lateralization) (Marhavý et al. 2013, 2016) facilitating auxin efflux into the LRFCs for the first round of anticlinal divisions.

LR Emergence

LRs originate from pericycle cells deep within the parental root tissue and must emerge through three distinct cell layers (endodermis, cortex, and epidermis). These cell layers undergo cell wall loosening and hydraulic separation via multiple auxin-dependent signaling modules. Particularly within the endodermis, the dynamic opening and resealing of lignified and suberized barriers are tightly regulated for LR emergence, and to prevent leakage of nutrients and pathogen entry from the soil. This endodermal remodeling is also critical for root system plasticity (Barberon et al. 2016). A new transcriptomic study identified specific members of GDSL-type esterases/lipases (GELPs), proteins that are expressed sequentially downstream of SHY2 and regulate auxin-dependent desuberization during LR emergence, and consequently resuberization of the endodermal barrier post-LR emergence (Ursache et al. 2021). A study from a few years ago, which used a combination of mathematical modeling and cutting-edge light sheet fluorescence microscopy, showed that while the first division is tightly regulated, subsequent divisions do not occur in a rigid sequence as the primordium center grows, but instead their orientations (anticlinal or periclinal) depend on cell geometry and follow the "shortest wall" rule. The orientations of these division planes, the apical growth of the primordium due to cell proliferation, and accommodation from the overlying cell layers are all key factors that determine the emergence of the characteristic layers that make up the dome-shaped primordium (von Wangenheim et al. 2016).

Interestingly, recent data has suggested that the *Arabidopsis* noncanonical ARF *ETTIN/ARF3* (*ETT*), which lacks the PB1 DNA-binding domain, may play an important role in LR emergence (Stoeckle et al. 2018). Loss-of-function and auxin-insensitive mutants have increased numbers of emerged LRs, suggesting that auxin can act directly through ETT to negatively regulate LR emergence (Simonini et al. 2017).

Downstream of an *ARF7/19* module, LBD transcription factors play multiple roles in LR initiation and emergence. An LBD18/33 dimer are other direct downstream targets of this module and themselves regulate the expression of E2Fα, which encodes a transcriptional activator of cell-cycle genes (Berckmans et al. 2011). Further, LBD18 also directly up-regulates *EXPANSIN14*, and indirectly regulates *EXPANSIN17*, required for loosening of the cell wall during LR emergence (Fig. 2C).

The auxin influx transporters, *AUX1* and *LAX3*, play a crucial role in regulating spatial separation of adjacent overlying cell files as the newly formed LRP emerges. Initially, AUX1 facilitates the loading of shoot-derived IAA into the vascular system to ensure that the auxin required for cell wall loosening is channeled to the overlying tissue via the LRPs (Marchant et al. 2002; Swarup et al. 2005; Péret et al. 2013). At later developmental stages, the primordium itself becomes the source of auxin required in the overlying tissue layers to trigger the expression of cell wall remodeling enzymes required for emergence. Several in silico and in vivo studies have also demonstrated that sequential expression of *PIN3* and *LAX3* in turn in the two adjacent cell files overlying the LRP is crucial to ensure that the auxin-dependent separation of cells occurs solely along the shared walls. *LAX3* is a downstream target of *LBD29*, which is a direct target of *ARF7* (Fig. 2C; Porco et al. 2016a,b).

The intracellular auxin homeostasis required during LR emergence is maintained by the short PIN, *PIN8*. *PIN8* is expressed in the phloem cells adjected to the primordium at a later stage of development. Although the mechanisms by which PIN8 promotes LR emergence is not fully understood, *pin8* mutants show significantly reduced numbers of emerged LRs that are arrested at the primordium stage and have reduced expression levels of *GATA23* and *LBD* genes (Lee et al. 2020).

LR Elongation

Surprisingly, there is little information available on the effect of auxin and the role of auxin signaling networks on LR elongation. In *Arabidopsis*, at least, several studies have reported that auxin inhibits the growth of LRs in young seedlings. Interestingly, however, in tomato, Muday and Haworth (1994) reported that auxin pro-

moted LR growth, suggesting that the growth responses of LRs to auxin are likely to be species specific (for review, see Waidmann et al. 2020).

Gain- and loss-of-function studies using *Arabidopsis* mutants have identified a small number of additional genes that regulate LR elongation. Celenza et al. (1995) reported that the constitutively active allele of *alf1*/*sur* displays hyperproliferation of LRs (Fig. 2D). Whereas Boerjan et al. (1995) established that this mutation leads to an overproduction of endogenous IAA, the *ALF* gene is yet to be mapped. Moreover, Wu et al. (2007) demonstrated that the loss-of-function mutants of the *ABCB14*/*PGP19*/*MDR1* gene show arrested and/or impaired LR growth rates and a 40% reduction of acropetal auxin transport within the LRs. In contrast, no differences in LR growth rates have been reported between WT and *aux1* mutants in normal conditions (Linkohr et al. 2002; Giehl et al. 2012). However, localized iron supply was found to promote LR elongation via altering AUX1-mediated auxin distribution, suggesting that environmental triggers are able to influence LR growth rates via the modulation of auxin transport and/or signaling components (Fig. 2D; see the "Environmental Regulation" section).

GRAVITROPIC SETPOINT ANGLE (GSA)

Perhaps the most significant parameter that determines overall root architecture is root angle. As described previously, secondary roots in most plant species grow at oblique angles in the soil. These angles, and indeed all angles that plant organs may grow at are known as gravitropic setpoint angles (GSAs). GSAs are defined as the angle at which a plant organ grows to maintain equilibrium with gravity (Digby and Firn 1995). Spatiotemporal developmental and environmental cues regulate the setting and maintenance of these root angles within the soil, and can therefore shape root architectures that can contrastingly range from shallow, widely distributed roots, to narrow deeper rooting systems. The distribution of the root system within the soil is key to maximizing the capture of heterogenously distributed soil minerals and water. For example, phosphate and nitrate are often found while water is found in the deeper layers.

In *Arabidopsis*, as described above, LRs emerge from the pericycle layer and emerge through overlying tissues at a near horizontal orientation (stage I). Following emergence, LRs undergo a brief period of downward growth, characterized by asymmetric distribution of auxin (as visualized by the reporter DR5v2) as well as the efflux transporter PIN3 (stage II) (Rosquete et al. 2013). Subsequently, LRs undergo stable angled growth, progressively transitioning through increasingly vertical states to adopt near vertical GSAs (stages III–V). Importantly, LRs actively maintain GSAs from stage II onward (i.e., they retain the capacity to reorient themselves both downward and upward) when moved from the GSA.

Several studies have recently sought to explain the molecular mechanisms that underpin the active setting and maintenance of these GSAs, most often using *Arabidopsis* roots as a model system. One set of studies proposes that LRs grow nonvertically due to the onset of sequential expressions of *PIN3*, *PIN7*, and *PIN4* in the root columella cells of stage II, III, and IV LRs, respectively. This is in direct contrast to primary root tips where PIN3, 4, and 7 are highly expressed within the columella initials (Guyomarc'h et al. 2012; Rosquete et al. 2013; Ruiz Rosquete et al. 2018; Ogura et al. 2019). In this model, LRs grow at nonvertical angles because they lack the molecular machinery required for full gravitropic competence. However, both these studies do not explain how LRs not only grow obliquely, but also maintain the capacity to bend upward to return to their original GSAs upon reorientation. Other work proposes that nonvertical GSAs are a result of balanced auxin fluxes across the upper and lower sides of the root that act antagonistically. In this model, the authors define a gravitropic auxin flux that increases in magnitude when an LR is reoriented above its GSA, to promote downward bending. This increase in downward auxin flux occurs in an angle-dependent manner: the larger the displacement above GSA, the greater the increase in the magnitude of downward auxin flux. However, when the LR is reoriented below its GSA,

the magnitude of this gravitropic auxin flux decreases. In this scenario, the relative magnitude of the antagonistic "antigravitropic offset" flux increases, and the root bends upward toward its original GSA (for review, see Roychoudhry and Kepinski 2015). Interestingly, another recent study discovered that the cytokinin reporter TCSn:GFP is expressed asymmetrically in higher levels toward the upper flank of stage II LRs. The authors proposed that asymmetric cytokinin signaling inhibits downward bending at the upper flank of LRs, and cytokinin therefore functions as an "antigravitropic" signal (Waidmann et al. 2019).

In the last few years, several studies have described the role of multiple members of the *LAZY* gene family in regulating gravitropism and root (and indeed shoot) angles in multiple plant species. Uga et al. first described *DEEPER ROOTING 1* (*DRO1*), a QTL that controlled root angle in rice. Rice accessions that contained a truncated version of the *DRO1* allele, termed DRO1-NIL, had shallow rooting angles and demonstrated impaired root gravitropism in young seedlings. *DRO1* was found to be expressed in the root EZ and was negatively regulated by auxin (Uga et al. 2011). Subsequently, *DRO1* was found to be orthologous to *LAZY4* in *Arabidopsis* (Hollender and Dardick 2015). Subsequent studies showed that *LAZY4* was part of a larger gene family in *Arabidopsis*, termed as the IGT family, which also contains *TAC1* (Guseman et al. 2017). LAZY proteins are expressed in the root tip and EZ, and share a conserved 14 amino acid carboxyl terminus (termed as CCL domain), which seems to be important for directional graviresponse in primary roots (Taniguchi et al. 2017). Several single and multiple loss-of-function mutants of *LAZYs* have multiple phenotypes including shallow LR growth angles and delayed primary root gravitropism. Interestingly, a recent study showed that primary roots of a *lazy234* multiple mutant are negatively gravitropic and bend upward (Ge and Chen 2016). Whether this phenotype is the result of an actively maintained GSA, or loss of the capacity to respond to gravity, remains to be investigated.

While the precise roles of *LAZYs* in regulating root angle are not yet fully understood, what is evident is that *PIN3* polarity is altered in LR columella cells of *lazy* mutants. Several lines of evidence suggest that the shallow LR GSA phenotype in *lazy* loss-of-function mutants is due to increased localization of PIN3 to the upper membrane of LR columella cells (Taniguchi et al. 2017; Furutani et al. 2020). Another recent study showed that LAZY proteins may themselves be polarized in LR columella cells and likely recruit PIN3 to the plasma membrane by interacting with the BRX domain of Regulator of Chromosome Condensation 1 (RCC1)-Like proteins (RLD proteins), identified as novel regulators of PIN polarity (Furutani et al. 2020).

ENVIRONMENTAL REGULATION

Vascular plants have evolved complex 3D root architectures to maximize the volume of soil they can explore and interact with. This is to acquire numerous highly heterogeneously distributed resources within multiple soil strata. The ability of the root system to adapt its genetically determined developmental parameters to multiple environmental signals, such as nutrient and water availability, has been a key factor determining the success of land plants (for review, see Morris et al. 2017). This adaptive behavior is known as developmental plasticity.

Phosphate Availability

Diverse environmental signals can have dramatic and often diametrically opposite effects on RSA. Phosphates and nitrates are both examples of plant macronutrients that are often limiting in soil (Morgan and Connolly 2013). Phosphorus, specifically, is crucial in the building of nucleic acids, ATP, and other molecules, and is acquired by the plant in its inorganic forms of phosphates. Phosphates are highly immobile in soil and often form complexes with soil components or can be converted to organic phosphate, leading to reductions in the availability of soil Pi for the plants (Brady and Weil 2008). In *Arabidopsis*, phosphate deficiency leads to PR length inhibition, increases in LR length, and deeper root angle (Fig. 3). These effects are due to a signaling pathway that enhances sensitivity to

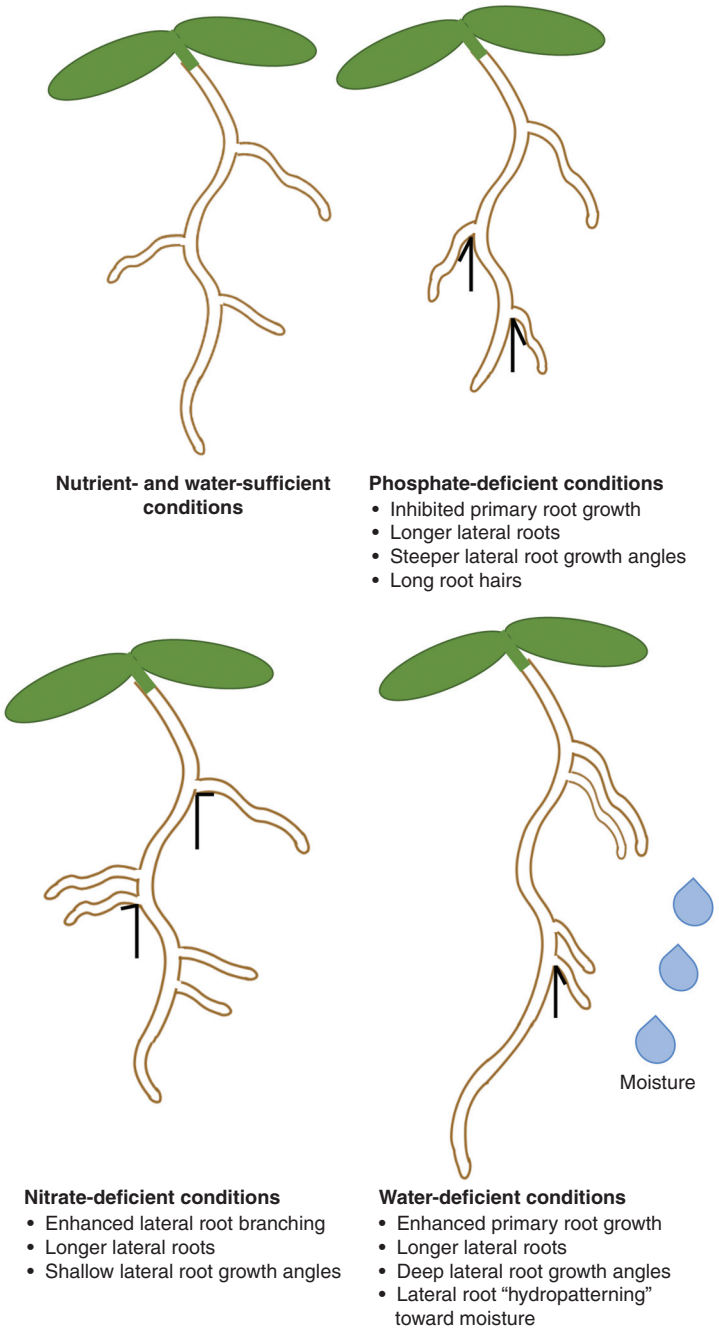

Figure 3. Environmental regulation of root system architecture (RSA) plasticity. Effect of phosphate, nitrate, and water deficiency on different parameters of RSA. Phosphate deficiency leads to inhibition of primary root growth, with contrasting increases in lateral root and root hair length along with steeper lateral root growth angles in *Arabidopsis*. In contrast, nitrate deficiency has little to no effect on primary root length, but leads to increases in lateral root length, and shallower growth angles. Water deficiency broadly enhances primary and lateral root growth, causes steeper lateral root growth angles and stimulates "hydropatterning" of lateral roots (i.e., induces enhanced lateral root branching toward the side of the primary root that is in contact with higher moisture levels).

auxin via up-regulation of the *TIR1* auxin receptor (López-Bucio et al. 2002; Ma et al. 2003; Pérez-Torres et al. 2008; Roychoudhry et al. 2017). Interestingly, these effects of low phosphate appear to be root class specific. In bean, growth under phosphorus deficiency had been demonstrated to induce shallower root angles in basal (adventitious) roots in contrast to the effect of low phosphorus on *Arabidopsis* LR growth angle (Bonser et al. 1996). Further examination of the response to low phosphate in bean showed that, as in *Arabidopsis*, bean LRs become more vertical while, as previously shown, basal roots adopt a shallower growth habit (Roychoudhry et al. 2017). The physiological basis of this root class-specific response is not yet understood.

Nitrate Availability

Nitrogen, a crucial macronutrient required for the production of amino acids and proteins, is primarily acquired by the root system in its inorganic form from nitrates and ammonia, supplied as fertilizers. In smaller quantities, nitrogen may also be taken up in its organic forms (urea, amino acids, and peptides). Depending on its source, nitrogen availability may have different effects on RSA (Fig. 3). For example, ammonium application limits PR growth (Liu et al. 2013). However, local application of ammonium enhances LR length and branching, likely due to increased expression of the ammonium transporter *AMT (AMMONIUM TRANSPORTER) 1;3* in LRs, which enhances nitrogen uptake (Lima et al. 2010). In contrast to these findings, several studies have demonstrated that localized nitrogen supply either in the form of nitrate or L-glutamate inhibits LR elongation, while nitrate deficiency enhances LR elongation and branching (Zhang and Forde 1998; Zhang et al. 1999; Linkohr et al. 2002; Tian et al. 2009). Nitrogen availability or deprivation seems to have a relatively small effect on PR length (for review, see Waidmann et al. 2020). Thus, depending on the source of nitrogen, the root system has distinct responses in terms of LR branching and elongation, suggesting that there are several additional mechanisms/hormone-signaling pathways that fine-tune the uptake of N. A recent study has also demonstrated that nitrate deficiency resulted in shallow LR growth angles in *Arabidopsis* (Roychoudhry et al. 2017). However, the effect of localized nitrate supply on LR growth angle has not yet been explored in detail.

Light

The effect of light on RSA has remained largely unexplored, as the root systems of most terrestrial plants remain in darkness, underground. Interestingly, however, several studies have demonstrated that multiple photoreceptors are expressed in root tissues, enabling them to detect ambient light and launch appropriate tropic and developmental regimes (for review, see van Gelderen et al. 2018). Broadly, total darkness inhibits root growth because of the reduction in basipetal auxin transport, due to depletion of PIN proteins from the plasma membrane (Laxmi et al. 2008). However, when only root systems are placed in darkness, root growth is largely enhanced in *Arabidopsis* (Zhang et al. 2019). In contrast, light exposure limits root growth and promotes negative phototropism, via a signaling pathway that depends on the photoreceptors *PHOT1* and *PHOT2*, with receptors *CRY1*/*CRY2* and *PHYA* playing minor roles. Downstream of the direction of light perception, polar auxin transport is required for directional root growth. The coordinated rootward polarities of PIN1 in the stele and PIN2 in root epidermal cells as well as the lateral relocalization of PIN3 in the columella cells are required for the formation of the auxin gradient (Wang et al. 2012; Zhang et al. 2013). During gradient formation, PIN1 polarity in the stele is also influenced by flavonols, which were found to accumulate on the side of the root exposed to light and promote cell elongation at this side (Buer and Muday 2004; Silva-Navas et al. 2015). Another recent study demonstrated that UV-B light inhibits LR growth through a signaling pathway that involved the UV-B receptor *UVR8*, which antagonistically regulates auxin-dependent gene expression. Upon UV-B irradiation, UVR8 interacts with the MYB73 transcription factor within the nucleus, thereby

inhibiting its DNA-binding activity and resulting in direct repression of target auxin-responsive genes (Yang et al. 2020).

Water

The effect of water on overall RSA has not been extensively studied to date (for review, see Dietrich 2018). Root systems with deeper, soil-penetrating architectures have enhanced resistance to drought, because of their ability to capture available water from deep within the soil. In this light, it is not surprising perhaps that a recent study showed that water deficit promoted vertical growth in *Arabidopsis* LRs (Fig. 3; Rellán-Álvarez et al. 2016). Interestingly, it was found that in addition to regulating growth angles, water availability around the circumferential axis of the root also acts as a positional cue to specify the patterning of LRs. This phenomenon was shown to occur before the initiation step and was defined as "hydropatterning." It seems that high water levels promote patterning through a pathway that creates a local LR inductive signal through the biosynthesis (dependent on *TRYPTOPHAN AMINOTRANSFERASE1*) and transport (*PIN3*-dependent) of auxin (Bao et al. 2014). In addition, it has been shown that ARF7 promotes LR initiation by triggering asymmetric transcription of *LBD16* at the circumference of the root that is in contact with water. This asymmetric induction of *LBD16* was regulated by the preferential posttranslational modification of ARF7 by the small ubiquitin-like modifier (SUMO) protein. SUMOylation of ARF7 leads to an increased association with the repressor IAA3/SHY2, presumably at the "dry" circumference of the root, and consequent inhibition of *LBD16* transcription (Orosa-Puente et al. 2018).

Similar to LRs, primary roots in several species also develop hydrotropic curvatures in response to water availability. Initially, several lines of evidence suggested that this hydrotropic response was antagonistic to gravitropic bending (for review, see Takahashi et al. 2009), but this response appears to be species-specific. For instance, in cucumber, the root cap is required for normal hydrotropism, suggesting that the interactions between the two tropisms may be more complex. In *Arabidopsis*, while auxin transport is not required for the hydrotropic response, a functional auxin response seems to be needed. For example, studies showed that treatment with auxin response inhibitors led to contrasting results. Whereas treatment with *p*-chloro-phenoxy-isobutyl acetic acid (PCIB), an inhibitor of Aux/IAA protein degradation (Oono et al. 2003), produced a decrease in hydrotropic response, addition of auxinole or α-(phenylethyl-2-oxo)-indole acetic acid (PEO-IAA), a TIR1 antagonist (Hayashi et al. 2012), accelerated the response (Kaneyasu et al. 2007; Shkolnik et al. 2016). These contrasting results could be due to different modes of action and target specificities of inhibitors. Thus, currently, additional experiments with multiple auxin-responsive mutants are required to confirm the effect of auxin signaling on the hydrotropic response.

CONCLUSIONS

The number of processes governed by auxin during root, and indeed most aspects of plant development seems extraordinary. This versatility rests in part on the fact that auxin can be moved within tissues to create and maintain gradients of concentration. Equally important is capacity of the auxin signaling system to generate specific outputs relevant to distinct and diverse developmental phenomena. These context-specific responses to auxin are a property of the existence of different TIR1/AFB-Aux/IAA-ARF signaling modules, which can then be wired to downstream genes to effect appropriate modes of developmental control. Nevertheless, even with this rationalization of how so much of development can, often simultaneously, depend on one simple molecule, the sheer robustness of auxin-regulated development is remarkable. A conspicuous example of this robustness is seen in the control of LR formation. This is a process with multiple, distinct, auxin-regulated steps and yet, in the face of high concentrations of auxin, the result is simply an increase in the number of LR primordia, each initiating into perfectly formed LRs. Here, the fact that the

development of individual LRs is so well buffered against large changes in auxin concentrations may well stem from the fact that successive Aux/IAA-ARF signaling modules are activated consecutively in time and space. Understanding this molecular genetic basis of auxin's capacity for self-organization is one of the frontiers of auxin research and one that is at the interface of genetics, biophysics, and mathematical modeling.

This review has been very focused on the dicot model *Arabidopsis* and within that system, principally on auxin's role in primary and LR growth. This leaves out the unsurprisingly central role of auxin in adventitious roots and also in the regulation of root development in monocot species. Nevertheless, many of the paradigms established in *Arabidopsis* have been found to be translatable to cereal species such as maize, rice, and wheat in particular, albeit with interesting differences (for review, see McSteen 2010; Balzan et al. 2014; Steffens and Rasmussen 2016; Waidmann et al. 2020). Understanding these differences and maximizing the potential application of our understanding of auxin's control of root biology will be essential if we are to make rapid progress in developing crop varieties that perform better in more sustainable farming systems in which inputs such as water, nitrogen, and phosphorous must be reduced. Indeed, there was an extent to which root performance as a trait became a casualty of post-Green Revolution breeding programs based on high levels of agricultural inputs. While this was an appropriate technological response to alleviating food insecurity at that point in history, we are now way beyond the stage at which food production needs to be radically decarbonized. Reducing the use of nitrogen fertilizer derived from the environmentally damaging Haber–Bosch process and conserving the world's finite supply of phosphorous are high on the list of targets for crop improvement. It is here that our deep understanding of the networks of auxin response controlling root growth and system architecture can be brought to bear in a way that can accelerate the development of new varieties in time to meet our commitments to limiting global warming and feeding a global population predicted to peak at 9–10 billion people just 30 years from now.

Whereas overall tremendous progress has been made in understanding the role of auxin and its signaling networks in regulating multiple facets of root and, indeed, overall plant development, many questions still remain unanswered. Whereas a large number of gene networks that regulate different steps of both primary and LR development have now been studied, at least partially, many still remain to be discovered. Specific examples of these include exceptionally rapid auxin-dependent signaling pathways that regulate root elongation (Fendrych et al. 2016, 2018) and those that underpin the mechanisms of LR growth angle setting and maintenance. Following on from this, how multiple environmental signals can fine tune these myriad auxin-dependent developmental responses remains to be fully understood. Perhaps the most basic question of all relates to the evolution of such complex systems of auxin response and how the proliferation and subfunctionalization of the three gene families in auxin signaling, including the development of specific preferential interactions between pairs and multimers of different Aux/IAAs, ARFs, and TIR1/AFBs, may have underpinned the evolution of increasingly sophisticated developmental phenomena. Interdisciplinary advances in the multiple fields of biology, physics, and mathematical modeling will enable researchers to answer these and other fundamental, yet exciting, questions in the coming decade.

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