CHAPTER FOURTEEN

Just passing through: The auxin gradient of the root meristem

Bruno Guillotin, Kenneth D. Birnbaum*
New York University, The Department of Biology, The Center for Genomics and Systems Biology, New York, NY, United States
*Corresponding author: e-mail address: kdb5@nyu.edu

Contents

1. Introduction 434
   1.1 Proximo-distal maturation gradient of the root 434
2. Signals across cell walls 436
   2.1 Plasmodesmata 436
   2.2 Auxin 437
3. The auxin gradient of the root 438
   3.1 Setting up the gradient 438
   3.2 Positional inputs to the auxin gradient 438
   3.3 Local auxin synthesis influences the root maximum 439
4. PLETHORAs mediate auxin but form their own gradient 440
   4.1 Downstream auxin responses 440
   4.2 Gene level regulation of PLTs 440
   4.3 Protein level regulation of PLTs 441
   4.4 PLTs show dose dependent effects 442
5. Direct outputs of the auxin gradient 444
   5.1 Auxin has effects independent of PLTs 444
   5.2 Prepatterned responses 444
   5.3 Combinatorial inputs 445
   5.4 Repressive lockdown states 446
   5.5 Prospects 446
References 448
Further reading 454

Abstract

The root meristem—one of the plant's centers of continuous growth—is a conveyer belt in which cells of different identities are pushed through gradients along the root's longitudinal axis. An auxin gradient has long been implicated in controlling the progression of cell states in the root meristem. Recent work has shown that a PLETHORA (PLT) protein transcription factor gradient, which is under a delayed auxin response, has a dose-dependent effect on the differentiation state of cells. The direct effect of auxin

Current Topics in Developmental Biology, Volume 137
ISSN 0070-2153
https://doi.org/10.1016/bs.ctdb.2019.12.001

concentration on differential transcriptional outputs remains unclear. Genomic and other analyses of regulatory sequences show that auxin responses are likely controlled by combinatorial inputs from transcription factors outside the core auxin signaling pathway. The passage through the meristem exposes cells to varying positional signals that could help them interpret auxin inputs independent of gradient effects. One open question is whether cells process information from the changes in the gradient over time as they move through the auxin gradient.

1. Introduction

1.1 Proximo-distal maturation gradient of the root

The growing tissue of the Root Apical Meristem (RAM) continuously produces cells on a homeostatic anatomy, like a road crew adding new sections to a multilane highway (Fig. 1). In the RAM, cells are born around a structure known as the quiescent center, QC (Dolan et al., 1993; Haecker, 2004; van den Berg, Willemsen, Hendriks, Weisbeek, & Scheres, 1997). While plant cells do not migrate, the cylinder-like root contains files of cells that are displaced from the tip by the birth, expansion, and division of new cells behind them. Meanwhile, the gradients that form in the meristem are stationary with respect to the growing tip (Motte, Vanneste, & Beeckman, 2019), analogous to a highly organized road crew moving along as the highway gets built. Thus, behind the root cap, cells in the meristem progress in an assembly-line fashion, such that, in theory, a morphogen gradient along this length-wise (proximo-distal) axis of the root could stage each step of cellular maturation for a cell in a given file (Fig. 1). We consider the prospects of such a maturation-instructive morphogen here—a substance whose concentration instructs the progressive differentiation states of cells.

Below the QC, a separate set of divisions gives rise to the columella cells of the cap. The QC has been shown to be the source of signals that maintain columella stem cells (Pi et al., 2015; Sarkar et al., 2007), but the maturation gradient in that axis is extremely short so here we focus on the gradients above. This means that the root really has two conveyer belts of cell production, with the very slowly dividing QC remaining stationary in the middle (Cruz-Ramirez et al., 2013; Rahni & Birnbaum, 2018). Thus, as will be discussed below, the QC (and surrounding cells) appear to serve as a focal point of signals that help polarize the gradients above the cap.

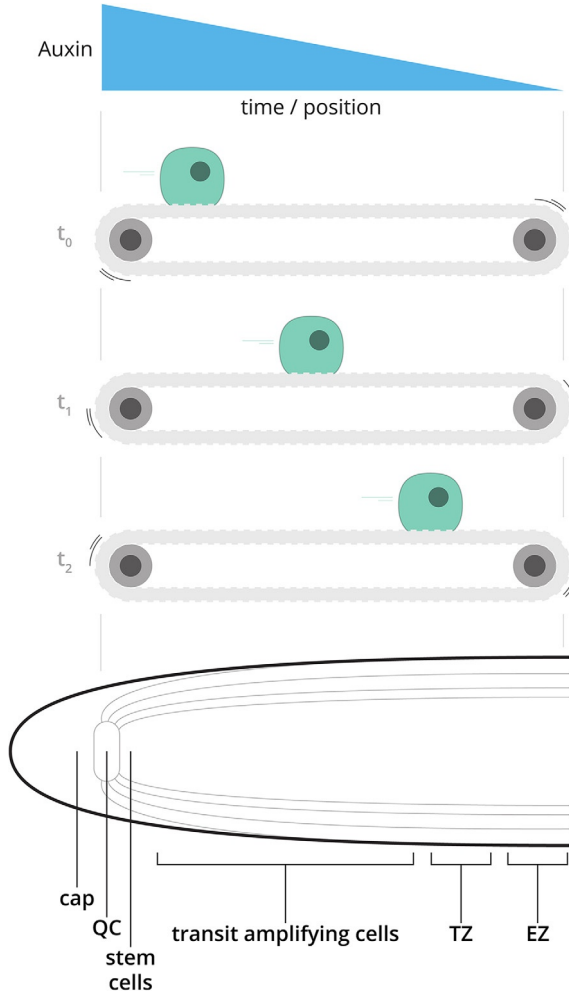

Fig. 1 Cells in the plant meristem pass through the auxin gradient of the root. At top, for most of the radial files of the root, there is a decreasing gradient of auxin within the division zone as cells mature. At middle, the division and expansion of cells create a conveyer-belt like mechanism whereby cells are pushed through the meristem over time, passing through each level of the gradient. At bottom, the key landmarks in the root meristem on the maturation axis are indicated, with the QC surrounded by stem cells, followed by a zone of transit amplification, a transition zone (TZ), and then an elongation zone (EZ) at the start of differentiation.

2. Signals across cell walls

2.1 Plasmodesmata

To understand the gradients that form in the roots, it's useful to consider the plant's common modes of signaling—some of which are unique to plants. One such feature of plant signaling is the gap-junction-like structure known as plasmodesmata, which forms channels through cell walls connecting the cytoplasm of neighboring cells (Sager & Lee, 2018). Remarkably, transcription factors use these conduits to move from cell to cell, acting as direct signaling agents. For example, the transcription factor SHORTROOT (SHR) moves at the protein level from the vascular and surrounding pericycle cells into the adjacent ground tissue to mediate a tissue-forming division and help specify the endodermal cell identity (Nakajima, Sena, Nawy, & Benfey, 2001). Constructs that block plasmodesmata prevented the movement of SHR (Vatén et al., 2011). Many other plant transcription factors and microRNAs have now been shown to move from cell to cell (Daum, Medzihradszky, Suzaki, & Lohmann, 2014; Mähönen et al., 2014; Miyashima et al., 2019; Pi et al., 2015; Skopelitis et al., 2018; Yadav et al., 2011), presumably mediated by passage through the plasmodesmata.

Transport through plasmodesmata—known as symplastic signaling—appears to have some size exclusion limit, although there may also be active mechanisms that control movement (Sager & Lee, 2018). For example, small RNA diffusion was also shown to be directional—that is, polarized in one direction (Skopelitis et al., 2018). There is also some evidence that metabolites travel through plasmodesmata (Benitez-Alfonso, Faulkner, Ritzenthaler, & Maule, 2010; Sivaguru et al., 2000; Vatén et al., 2011). Thus, the plasmodesmata appear to mediate symplastic gradients that allow a certain level of diffusion for transcription factors, much like the syncytium of the Drosophila embryo (Daniels, Rikhy, Renz, Dobrowsky, & Lippincott-Schwartz, 2012).

Plants, like animals, also signal through peptides and cognate membrane bound receptor-like kinases (Olsson et al., 2019). Although it's feasible that peptides could diffuse through cell walls, there are several reports that receptors and their ligands interact at the site of plasmodesmata (Faulkner, 2013; Stahl et al., 2013; Vaddepalli et al., 2014), although more research is needed to determine how plasmodesmata mediate peptide signaling.

2.2 Auxin

Auxin is not the only plant hormone that affects root development (Kamiya, 2010), but auxin takes center stage, not only for its ubiquity in plant development, but also because of both its necessity and sufficiency for root development. Seminal experiments showed that treating callus—a blastula-like mass of regenerative cells—with high auxin and low cytokinin leads to the regeneration of a fully functional root (Skoog & Miller, 1957). Mutations in *Auxin Response Factor 5* (*ARF5* or *MONOPTEROS, MP*)—in a family of transcription factors that mediate direct auxin responses—led to rootless embryos (Hardtke & Berleth, 1998), while blocking auxin signaling in roots leads to severe defects in the root meristem (Overvoorde, Fukaki, & Beeckman, 2010; Sato & Yamamoto, 2008).

Auxin is a small metabolite derived from tryptophan in just a few enzymatic steps (Zhao, 2012). The bulk of auxin synthesis is in the shoot and its long-distance transport to the root relies, in part, on the transport system of the phloem (Robert & Friml, 2009; Weijers, Nemhauser, & Yang, 2018). However, auxin's localization within organs is precisely fluxed and positioned by what amounts to its own private circulatory system. Auxin appears to be able to enter cells by passive mechanisms, although auxin influx carriers also have essential roles in development (van Berkel, de Boer, Scheres, & ten Tusscher, 2013). Once inside the cell, auxin is unable to passively diffuse through the membrane. Thus, much of auxin flux is mediated by efflux carriers, known as PINs, that are polarly localized in the cell, such that auxin is shuttled along in one cell and out the other in specific directions (Adamowski & Friml, 2015). This planar polarity among cells shifts at strategic points fluxing auxin through organs like traffic patterns through city streets (Benkova et al., 2003; Friml et al., 2003). In the root, distinct PINs flux auxin down the vasculature toward the tip, then outwards toward the outer cap and epidermis, then upwards in the epidermis toward the shoot, and finally back into the vascular flux in what has been likened to a reverse fountain (Grieneisen, Xu, Marée, Hogeweg, & Scheres, 2007; Swarup & Bennett, 2003).

Downstream, auxin signaling is mediated by an E3-ligase receptor complex that leads to the degradation of a set of repressors called Aux/IAAs (Dharmasiri, Dharmasiri, & Estelle, 2005; Kepinski & Leyser, 2005), the core of the widely used auxin degron (Pierre-Jerome, Jang, Havens, Nemhauser, & Klavins, 2014). The repressors bind to a subset of ARF family members (Ulmasov, Hagen, & Guilfoyle, 1997;

Ulmasov, Murfett, Hagen, & Guilfoyle, 1997), which mediate a large set of transcriptional responses (Bargmann et al., 2013; Lewis et al., 2013). There are more than 20 members each in both ARF and Aux/IAA gene families in Arabidopsis with similarly high gene family expansion in other flowering plants, allowing for combinations of the core machinery with different responses to auxin (Leyser, 2018; Roosjen, Paque, & Weijers, 2018). Some of the direct targets of positively regulating ARFs are, in fact, their own repressors, the Aux/IAAs (Leyser, 2018). Thus, there is a strong feedback built into core response, with auxin leading to the degradation of Aux/IAAs but also inducing their expression. In addition, while genes in the ARF5 clade function as activators, genes in another ARF clade appear to act as repressors and may compete for the ARF5 clade binding sites (Roosjen et al., 2018; Wang & Estelle, 2014). The negative feedback and repressive modes of auxin signaling are increasingly at the forefront of auxin signaling research, as we will cover below.

3. The auxin gradient of the root

3.1 Setting up the gradient

Several lines of evidence have established the existence of an auxin gradient within the root meristem with a high concentration at the stem cell niche that gradually decreases in the differentiation zone of the root (Petersson et al., 2009; Sabatini et al., 1999; Santuari et al., 2011). In the last several years, new auxin sensors, which are based on the Aux/IAA receptor rather than transcriptional responses mediated by ARF binding sites, have painted a more complex picture of the auxin gradient. In addition to a diminishing gradient, auxin appears to have a second peak after the transition from cell division as cells elongate and differentiate (Brunoud et al., 2012). The epidermal layers appear to have lower auxin concentration near the stem cell niche, with the auxin peak building up in cells positioned several cell lengths toward the shoot. Thus, the auxin gradient is bimodal over the entire root, with most cells experiencing a decreasing gradient within the meristem before the onset of differentiation with cell elongation.

3.2 Positional inputs to the auxin gradient

Computational models suggest that the polar auxin transport system described above could generate a local maximum at the stem cell niche (Grieneisen et al., 2007). A recent extension of this model includes the

opposing effects of the plant hormone cytokinin on auxin at the end of the meristem (Di Mambro et al., 2017). The model shows that cytokinin determines the position of the auxin minimum and the transition zone by both downregulating PIN transporters and inactivating auxin (Di Mambro et al., 2017). In a validation of the model, manipulation of a *GRETCHEN HAGEN3* gene, which deactivates auxin and was shown to be downstream of cytokinin, moved the position of the transition zone and extended the meristem (Di Mambro et al., 2017). This mechanism sheds light on how the familiar antagonism between auxin and cytokinin helps shape the root auxin gradient.

Furthermore, recent work shows how specific cell files can modulate the auxin gradient locally to "customize" their own developmental timing. The work showed that two phloem-specific plasma membrane-localized proteins interact with PINs to mediate auxin flux, resulting in a steeper gradient within this vascular cell type (Marhava et al., 2018). When the cell-specific gradient was perturbed, cellular maturation was altered (Marhava et al., 2018). The phloem work showed that cell-type specific mechanisms could alter the auxin gradient and speed the maturation of one cell file—analogous to a lane of the highway being constructed early. It is plausible there is a need to speed the maturation of a cell type that delivers sucrose to an organ that is an energy sink.

Both of these studies draw attention to the way local signals reshape the auxin gradient to influence maturation, either across many cell types, as in the case of cytokinin inputs, or within a specific cell type, as in phloem.

3.3 Local auxin synthesis influences the root maximum

While auxin flux via PINs is of central importance to auxin localization, a body of work now shows that local auxin biosynthesis also has a key role in the establishment of the RAM auxin gradient (Brumos et al., 2018; He et al., 2011; Stepanova et al., 2008; Yang et al., 2014). For example, mutants in the auxin biosynthesis gene *WEAK ETHYLENE INSENSITIVE 8/Tryptophan aminotransferase-related protein 2*, which is localized to the QC, columella and surrounding cells, fail to maintain an auxin maximum centered around the QC (Brumos et al., 2018; Stepanova et al., 2008). This work firmly established the need to account for local auxin synthesis in the root when considering auxin's ability to self-organize its own flux and localization. That is, again, positional signals outside the core auxin signaling machinery may help the auxin transport system establish or maintain fluxes.

4. PLETHORAs mediate auxin but form their own gradient

4.1 Downstream auxin responses

To date, auxin's clearest role in regulating progressive stages of differentiation in the root comes from its rather indirect effects on members of the *PLETHORA* (*PLT*) transcription factor family—*PLT1*, *PLT2*, *PLT3* and *BABY BOOM* (*BBM*, also known as *PLT4*). Several lines of evidence put the PLTs downstream of auxin. First, several *PLT*s are no longer expressed in the embryo in *ARF5* mutants (Aida et al., 2004). Still, in the primary root, it was shown that auxin reporters respond almost immediately to auxin treatment, while *PLT* reporters required 72 h of treatment to show dramatic up regulation (Mähönen et al., 2014). Thus, the *PLT*s appear to be a slow response to auxin, which led to a model where PLTs regulated auxin's role in long-term developmental processes while rapid auxin responses mediated faster processes like auxin-mediated gravitropism (Mähönen et al., 2014).

4.2 Gene level regulation of PLTs

Interestingly, a menagerie of signaling mechanisms that are not directly tied to auxin appear to shape the PLT protein gradient. At the transcriptional level, *PLT*s are induced in the zone of highest auxin concentration within the stem cell niche and some surrounding daughter cells (Mähönen et al., 2014). However, above the niche, the *PLT*s are repressed by *Growth Regulating Factors* (*GRF*s; Kim, Choi, & Kende, 2003). In Arabidopsis roots, *GRF1*, *2,* and *3* are expressed above the QC (Fig. 2; Rodriguez et al., 2015) and are co-activated by *GRF Interacting Factors, GIF*s (Fig. 2; Debernardi et al., 2014; Ercoli et al., 2018; Horiguchi, Kim, & Tsukaya, 2005; Kim & Kende, 2004; Nelissen et al., 2015). Thus, the *GRF*s keep *PLT* mRNA expression within a narrow zone near the QC and stem cells.

Meanwhile, *PLT* expression appears to be shielded from its negative regulators in the niche by microRNA396 family, which directly targets 7 out of 9 *GRF*s for transcriptional degradation (Rodriguez et al., 2015). For example, it was shown that miRNA resistant *GRF*s are ectopically expressed in the QC resulting in a smaller meristem size and development defects (Ercoli et al., 2018; Rodriguez et al., 2015). Finally, this local expression of *PLT* mRNAs appears to be on a feedforward loop, as high levels of PLT proteins directly promote miR396s expression

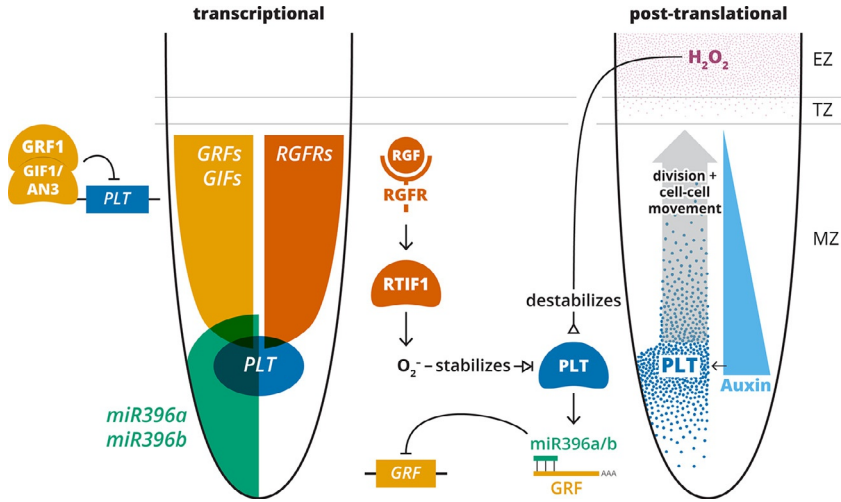

Fig. 2 The PLETHORA gradient is shaped at both the transcriptional and posttranscriptional level starting with an auxin gradient. On the right, the PLT gradient is induced transcriptionally in the region of the auxin maximum near the stem cell niche. The GRFs and GIFs mediate PLT transcriptional down regulation in the transit amplifying zone, while miR396 protects PLT expression levels around the stem cell niche. PLTs form a gradient through both cell–cell movement and through protein carry over and dilution from cell division. In addition, an RGF peptide gradient recognized by RGF receptors (RGFRs) helps stabilize the PLT transcription factor gradient, through RTIF1 and reactive oxygen species.

and protect *PLT* transcription in the stem cell niche from *GRF* activity (Rodriguez et al., 2015). Thus, rather than a gradient at this point, the transcriptional regulatory circuit appears to concentrate the *PLT* expression in a narrow zone of high concentration near the QC (Fig. 2).

4.3 Protein level regulation of PLTs

In addition to the mechanisms that restrict PLT mRNA expression, protein-level regulation shapes the PLT gradient. First, PLT proteins appear quite stable, allowing them to a "ride" a wave of cell divisions in the transit amplifying zone of the meristem, where dilution helps form a decreasing gradient (Mähönen et al., 2014). Second, in a very plant-centric signaling mode, the PLTs also appear to be able to move from cell to cell, most likely through the plasmodesmata, to spread out toward the transition zone. For example, one litmus test for plasmodesmata-mediated mobility is attaching a triple GFP

protein to the candidate transcription factor (Pi et al., 2015). These bulky fusions appear to exceed the plasmodesmatal size exclusion limit. Aiding in phenotypic interpretation, they can typically rescue cell-autonomous but not cell nonautonomous defects in their own mutant backgrounds (Pi et al., 2015; Schlereth et al., 2010). In the PLT longitudinal gradient, a nonmobile PLT2-3xYFP exhibited a shorter gradient than a mobile version when cell division was halted with a chemical block, showing that protein movement as well as protein persistence and division dilution were important in generating the gradient (Mähönen et al., 2014).

Critically, the PLT protein gradient is strongly shaped by members of the *CLAVATA-LIKE* (*CLE*) peptide family, known as *Root meristem Growth Factors* (*RGF*s). The small peptide subfamily has nine members, more than half of which are expressed around the QC, stem cell niche, and cap area (Matsuzaki, Ogawa-Ohnishi, Mori, & Matsubayashi, 2010). The proteins diffuse locally out of the QC region with the effect of stabilizing PLT proteins (Matsuzaki et al., 2010). The gradient of RGFs is critical for PLT function and meristem maintenance, as a triple mutant in the family, *rgf123*, causes a short-root phenotype characterized by a decrease in meristematic cell number. Exogenous treatment with the RGF peptides drastically increases the PLT gradient length and meristem size (Shinohara, Mori, Yasue, Sumida, & Matsubayashi, 2016; Yamada, Han, & Benfey, 2019). Furthermore, the increase in PLT protein is visible within only a few hours, implying a fairly direct action on PLTs (Shinohara et al., 2016; Yamada et al., 2019).

Recently, several key components of the pathway downstream of RGFs were discovered, including the *RGF* receptors, *RGFR*s (Fig. 2, Shinohara et al., 2016) and a downstream mediator *RGF1 INDUCIBLE TRANSCRIPTION FACTOR 1* (*RITF1*), which then signals to the PLTs via reactive oxygen species (Yamada et al., 2019). This entire circuit appears to be independent of auxin since auxin treatment does not affect the *RGF*s (Matsuzaki et al., 2010).

4.4 PLTs show dose dependent effects

The information conveyed by the PLT gradient has largely been assessed by loss- and gain-of-function experiments. For example, overexpression of PLT2 increases the potency of distal cells to regenerate after wounding, associating high levels of PLTs with cellular potency and youth

(Marhava et al., 2019). At the next level, moderate PLT concentrations appear to maintain cell division in the transit amplifying zone of the meristem, as increasing *PLT* expression with 35S:PLT2 increases the root meristem size (Galinha et al., 2007). Finally, low concentrations of PLTs correlate with the shift from cell division to elongation—the transition zone that marks meristem size. Indeed, decreasing the numbers of functional PLTs in higher level mutants led to a decrease in meristem size (Galinha et al., 2007). Thus, PLT levels correlate with maturation landmarks in the root both in stereotypical development and upon perturbations.

How the PLT gradient is perceived by cells remains an open question. An in-depth study of downstream PLT regulation showed that many PLT targets were expressed in discrete zones within the meristem (Santuari et al., 2016). This could be the result of threshold effects stemming from PLT dosage. However, it was not clear if PLT dosage or combinatorial regulation with other spatially discrete factors generated the zoned expression (Santuari et al., 2016). A PLT binding site has been identified and it was enriched under PLT ChIP-seq peaks in the root studies (Santuari et al., 2016). However, no analysis so far has uncovered a relationship between binding site affinity or arrangement and spatial expression along the PLT gradient. This may be a prospect for future work with more information on PLT binding affinities.

In addition, another level of feedback complexity is evident from the list of PLT target genes. One of the direct targets of PLT proteins was the auxin biosynthetic gene, *YUCCA3* (Santuari et al., 2016), providing evidence, along with other studies, that the PLTs provide feedback to influence auxin levels and transport in the root (Aida et al., 2004; Santuari et al., 2016).

Thus, the PLTs offer one of the clearest examples of a gradient in plants that can instruct cellular maturation. The variety of positional signals that go into shaping the gradient raises an important question on how the system assembles. A hallmark of plant development is the capacity for *de-novo* meristem assembly both in regeneration and postembryonic development like lateral root formation. For example, the entire meristem and its maturation gradient can reform over a few days after severe injury (Sena, Wang, Liu, Hofhuis, & Birnbaum, 2009). Is there an order to the assembly of the positional signals that shape meristem gradients? For example, is there is a signaling center that emerges first to set up positional signals, or, could physical properties like distal tip vs internal proximal positions provide cues that provide a proximo-distal coordinate system at once?

5. Direct outputs of the auxin gradient

5.1 Auxin has effects independent of PLTs

Downstream analyses of the auxin and PLT pathways showed a significant overlap in their targets (Santuari et al., 2016). Still, global studies of auxin responsive genes suggest a much wider set of genes affected by auxin than are regulated by the PLTs (Bargmann et al., 2013; Lewis et al., 2013; Santuari et al., 2016). In addition, perturbations to the auxin gradient can alter the position of the transition zone relatively rapidly, suggesting a role for auxin signaling independent of the slowly induced PLTs (Dello Ioio et al., 2008; Di Mambro et al., 2017). Is there a role for the auxin gradient in cellular maturation aside from its effect on the PLTs?

The question goes to the heart of one of the central issues in auxin biology. How does this single hormone, which has a role in virtually every developmental process, exert so many different effects?

In terms of a dose response to ARFs, there is also evidence that ARF-binding motif variants, copy number, and arrangement in regulatory regions can determine the sensitivity to auxin signaling (Boer et al., 2014; Liao et al., 2015; Pierre-Jerome et al., 2014; Pierre-Jerome, Moss, Lanctot, Hageman, & Nemhauser, 2016). However, evidence has yet to emerge of binding motif patterns that could correlate with local expression along the gradient of the root.

5.2 Prepatterned responses

Overall, auxin appears to be more of a trigger than a signal whose level conveys specific information to cells (Benkova, Ivanchenko, Friml, Shishkova, & Dubrovsky, 2009; Leyser, 2018; Stewart & Nemhauser, 2010). Auxin has been likened to an orchestra conductor instructing cells to "carry out" their preprogrammed response (Leyser, 2006). It has also been compared to currency, informing cells of the "wealth" of auxin concentration in their vicinity (Stewart & Nemhauser, 2010), implying a role for concentration.

The differential competence of cells to respond to auxin is illustrated in an experiment in which roots were treated with auxin, and cell types differentially labeled with fluorescent reporters were separated by cell sorting to measure their global response (Bargmann et al., 2013). The analysis showed that hundreds of genes respond differently to the same treatment in different cell types (Bargmann et al., 2013). This showed the extent to which intrinsic cell state could determine a cell's response to auxin.

Mechanistically, generating differential auxin responses along the gradient might be controlled by differences in the auxin response machinery itself, and there is evidence that different ARFs and/or AUX/IAA repressors allow cells to respond differently to auxin inputs (Bargmann & Birnbaum, 2009; O'Malley et al., 2016; Pierre-Jerome et al., 2014; Vernoux et al., 2011; Weijers et al., 2018; Zemlyanskaya, Wiebe, Omelyanchuk, Levitsky, & Mironova, 2016). For example, in the embryo, ARF9 is expressed in specific root-precursor cells, mediating a response that cannot be rescued by other ARFs (Rademacher et al., 2012). Such a change in the auxin response machinery along the meristem gradient could explain the differentiation effects of auxin along the gradient. However, it is not clear that such differences in the core auxin machinery could explain the differential responses to auxin along the maturation axis of the root meristem. For example, in a recent report in the yeast system, activating ARFs did not show specific preferences in binding motifs, suggesting their first-order interactions with regulatory sequences cannot determine differences in a cell's intrinsic response to auxin (Lanctot, Taylor-Teeples, Oki, & Nemhauser, 2019).

5.3 Combinatorial inputs

Transcription factors outside of the core auxin machinery that are expressed in zones along the meristem could help auxin regulate downstream genes in discrete zones. For example, ARFs have been shown to interact with other transcription factors (Roosjen et al., 2018; Shin et al., 2007; Varaud et al., 2011). In global analyses, genes responding at different times to auxin treatments showed enrichments of both ARF-responsive motifs and binding sites for other transcription factors such as basic helix-loop-helix proteins (Cherenkov et al., 2017; Mironova, Omelyanchuk, Wiebe, & Levitsky, 2014). These studies strongly suggest that differences in auxin responses are likely the result of combinatorial regulation that includes transcription factors outside the ARF gene family.

Combinatorial control of auxin responses could explain differential responses along the auxin gradient without the need for auxin concentration to carry any information other than off or on signaling. It's not necessarily an argument against gradient control, as the current efforts in the field may be progressing more quickly on the combinatorial aspects of auxin's transcriptional regulation. Nonetheless, concentration effects for auxin do not appear obvious. For example, we treated cells with varying levels of auxin in culture and then cell sorted specific cell types to measure their global response to auxin dose. The cells showed only quantitative responses of largely the same

target genes rather than qualitatively different responses at different auxin levels that are indicative of different maturation states or cell identities (unpublished results). Of course, many caveats in such an experiment exist, including the need for tissue context in regulating a cell's primary response to auxin.

5.4 Repressive lockdown states

Some of the information contained in the auxin gradient may be encoded in its disappearance. Low auxin states are known to carry information for plant cells (Dubrovsky et al., 2011; Sorefan et al., 2009; Wang, Kohlen, Rossmann, Vernoux, & Theres, 2014), such as when low auxin marks the site where valve margin separation will form in the seed-bearing organ, the silique (Sorefan et al., 2009). Mechanistically, ARF-Aux/IAA complexes were shown to recruit *TOPLESS*, a Groucho-like homolog, that interacts with histone deacetylases in low auxin states to form a repressive complex (Szemenyei, Hannon, & Long, 2008)—a flip switch-type lockdown of auxin targets. In turn, *ARF5* can recruit SWI/SNF chromatin remodelers to make chromatin accessible upon auxin treatment (Wu et al., 2015). In this way, different auxin environments can regulate differential chromatin accessibility and, potentially, a cell's competence to respond to other signals. Going from a high to a low auxin environment could conceivably prime a specific response. Still, it's still not clear if such a progression of chromatin states occurs along the root axis.

Alternative to combinatorial or repression "flip switch" models, auxin levels may be read out directly by a mechanism yet to be identified. Finally, it remains possible that auxin levels may not have a primary role in staging the maturation of cells outside of their effects on PLTs.

5.5 Prospects

Most of our understanding of the role of auxin in staging root maturation has emerged through a more detailed understanding of the PLTs. Extensive work has shown how the PLT gradient is set up by an auxin maximum at the root tip. The strong foundational work on the PLTs has made it perhaps one of the best characterized morphogen candidates in plants. Perturbations strongly suggest that a gradient of PLTs carries information at different levels. This comes largely through the genetic manipulation of PLT levels that show corresponding shifts in the meristem size.

A key question remains whether PLT levels directly affect downstream gene regulation. It is possible that the multiple effects of PLTs arise from

combinatorial regulation with other transcription factors that are localized to different parts of the meristem. The eventual dissipation of the gradient could then serve as a switch to allow other genetic programs to take over. With direct targets now in hand (Santuari et al., 2016), there will be new opportunities to gain a mechanistic understanding of how PLT levels are interpreted at the promoter/regulatory sequence level. Thus, the PLTs provide an important tool in understanding how mobile transcription factors—a primary signaling mode in plants—can act as morphogens.

The role of an auxin gradient remains a more difficult question. Two important properties of auxin will likely be important considerations in analyzing a gradient role in the root. The so-called "auxin code" represents the concept that a prepatterned, intrinsic cellular state dictates a cell's response to an auxin signal. Progress has been made to show that differences in the core auxin machinery can prime a specific response to auxin. Increasingly, evidence for intrinsic cellular mechanisms points to combinatorial regulation with transcription factors outside the auxin machinery. In the root meristem, cells at different stages of maturity respond differently to the same auxin input (Bargmann et al., 2013), at least in part because of inputs from cytokinin (Di Mambro et al., 2017). Positional signals appear to set up differences in the auxin response code that allow cells near the stem cell niche to respond differently than those near the transition zone. Could auxin levels provide another level of fine tuning to a combinatorial code? A strong reliance on combinatorial inputs along the gradient does not leave a clear role for different levels of the gradient to carry specific information for cells.

Still, auxin is notoriously self-organizing, for example, influencing its own transport polarity (Leyser, 2018). The conveyer belt-like nature of the meristem means that, cells not only experience each level of the auxin gradient, but they do so in a particular order. Others have raised the possibility that a prior auxin state could influence a future auxin state (Pierre-Jerome, Moss, & Nemhauser, 2013). Extending that idea to the root meristem, cells may interpret the sequence of auxin inputs rather than the immediate level of auxin. It would be a tangled web: auxin would set up the prepattern that shapes its response to the next auxin level. Still, such a scenario brings the auxin gradient back into a focus as a primary organizing feature of the meristem.

Auxin's feedback and feedforward behaviors have been difficult to disentangle. The yeast system has been powerful in assessing the function of the core response machinery (Pierre-Jerome et al., 2014). However, the role of the gradient in the root will require more context from the

meristematic environment. One potential tool lies in the plant's high capacity to self-organize its meristems. The root regeneration protocols mentioned above provide "organoid" systems in which the order of meristem assembly can be tracked (Skoog & Miller, 1957).

In addition, advances in fine-scale analyses at the molecular and imaging level have the potential to dissect cellular responses at progressive stages of the gradient. For example, single-cell RNA-seq studies have ordered cells of the root by both cell type and developmental trajectories represented in pseudo-time (Denyer et al., 2019; Jean-Baptiste et al., 2019; Mironova & Xu, 2019; Ryu, Huang, Kang, & Schiefelbein, 2019; Wang, Ryu, Barron, & Schiefelbein, 2019; Zhang, Xu, Shang, & Wang, 2019). This tool offers a comparative analysis of different cell types moving through a similar, if not a uniform, gradient for each cellular position in the meristem. In addition, the root is highly amenable to live imaging, which should allow analyses of auxin levels and responses that could be mapped back to high-content RNA-seq profiles of single cells. Such fine-scale temporal analysis of meristematic cells could provide models of how cells transition from one state to another during maturation.

For testing the function of auxin feedback mechanisms, loss- and gain-of-function mutants have been useful, but auxin's involvement in every step of plant development make perturbations highly pleiotropic, complicating interpretations of phenotype. We anticipate gene-editing techniques that allow localized, conditional perturbations will help parse out auxin's potential sequential roles in meristem maturation. These tools hold promise to provide a perspective on auxin signaling that has been difficult to assess: how do cells respond to dynamic auxin environments over time?

References

Adamowski, M., & Friml, J. (2015). PIN-dependent auxin transport: Action, regulation, and evolution. *The Plant Cell*, *27*, 20–32.

Aida, M., Beis, D., Heidstra, R., Willemsen, V., Blilou, I., Galinha, C., et al. (2004). The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. *Cell*, *119*, 109–120.

Bargmann, B. O., & Birnbaum, K. D. (2009). Positive fluorescent selection permits precise, rapid, and in-depth overexpression analysis in plant protoplasts. *Plant Physiology*, *149*, 1231–1239.

Bargmann, B. O., Vanneste, S., Krouk, G., Nawy, T., Efroni, I., Shani, E., et al. (2013). A map of cell type-specific auxin responses. *Molecular Systems Biology*, *9*, 688.

Benitez-Alfonso, Y., Faulkner, C., Ritzenthaler, C., & Maule, A. J. (2010). Plasmodesmata: Gateways to local and systemic virus infection. *Molecular Plant-Microbe Interactions*, *23*, 1403–1412.

Benkova, E., Ivanchenko, M. G., Friml, J., Shishkova, S., & Dubrovsky, J. G. (2009). A morphogenetic trigger: Is there an emerging concept in plant developmental biology? *Trends in Plant Science*, *14*, 189–193.

Benkova, E., Michniewicz, M., Sauer, M., Teichmann, T., Seifertova, D., Jurgens, G., et al. (2003). Local, efflux-dependent auxin gradients as a common module for plant organ formation. *Cell*, *115*, 591–602.

Boer, D. R., Freire-Rios, A., van den Berg, W. A., Saaki, T., Manfield, I. W., Kepinski, S., et al. (2014). Structural basis for DNA binding specificity by the auxin-dependent ARF transcription factors. *Cell*, *156*, 577–589.

Brumos, J., Robles, L. M., Yun, J., Vu, T. C., Jackson, S., Alonso, J. M., et al. (2018). Local auxin biosynthesis is a key regulator of plant development. *Developmental Cell*, *47*, 306–318.e305.

Brunoud, G., Wells, D. M., Oliva, M., Larrieu, A., Mirabet, V., Burrow, A. H., et al. (2012). A novel sensor to map auxin response and distribution at high spatio-temporal resolution. *Nature*, *482*, 103–106.

Cherenkov, P., Novikova, D., Omelyanchuk, N., Levitsky, V., Grosse, I., Weijers, D., et al. (2017). Diversity of cis-regulatory elements associated with auxin response in *Arabidopsis thaliana*. *Journal of Experimental Botany*, *69*, 329–339.

Cruz-Ramirez, A., Diaz-Trivino, S., Wachsman, G., Du, Y., Arteaga-Vazquez, M., Zhang, H., et al. (2013). A SCARECROW-RETINOBLASTOMA protein network controls protective quiescence in the Arabidopsis root stem cell organizer. *PLoS Biology*, *11,* e1001724.

Daniels, B. R., Rikhy, R., Renz, M., Dobrowsky, T. M., & Lippincott-Schwartz, J. (2012). Multiscale diffusion in the mitotic *Drosophila melanogaster* syncytial blastoderm. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 8588–8593.

Daum, G., Medzihradszky, A., Suzaki, T., & Lohmann, J. U. (2014). A mechanistic framework for noncell autonomous stem cell induction in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 14619–14624.

Debernardi, J. M., Mecchia, M. A., Vercruyssen, L., Smaczniak, C., Kaufmann, K., Inze, D., et al. (2014). Post-transcriptional control of GRF transcription factors by microRNA miR396 and GIF co-activator affects leaf size and longevity. *The Plant Journal*, *79*, 413–426.

Dello Ioio, R., Nakamura, K., Moubayidin, L., Perilli, S., Taniguchi, M., Morita, M. T., et al. (2008). A genetic framework for genetic control of cell division and differentiation in the root meristem. *Science*, *322*, 1543–1547.

Denyer, T., Ma, X., Klesen, S., Scacchi, E., Nieselt, K., & Timmermans, M. C. P. (2019). Spatiotemporal developmental trajectories in the Arabidopsis root revealed using high-throughput single-cell RNA sequencing. *Developmental Cell*, *48*, 840–852 e845.

Dharmasiri, N., Dharmasiri, S., & Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. *Nature*, *435*, 441–445.

Di Mambro, R., De Ruvo, M., Pacifici, E., Salvi, E., Sozzani, R., Benfey, P. N., et al. (2017). Auxin minimum triggers the developmental switch from cell division to cell differentiation in the Arabidopsis root. *Proceedings of the National Academy of Sciences of the United States of America*, *114*, E7641–E7649.

Dolan, L., Janmaat, K., Willemsen, V., Linstead, P., Poethig, S., Roberts, K., et al. (1993). Cellular organisation of the *Arabidopsis thaliana* root. *Development*, *119*, 71–84.

Dubrovsky, J. G., Napsucialy-Mendivil, S., Duclercq, J., Cheng, Y., Shishkova, S., Ivanchenko, M. G., et al. (2011). Auxin minimum defines a developmental window for lateral root initiation. *The New Phytologist*, *191*, 970–983.

Ercoli, M. F., Ferela, A., Debernardi, J. M., Perrone, A. P., Rodriguez, R. E., & Palatnik, J. F. (2018). GIF transcriptional coregulators control root meristem homeostasis. *The Plant Cell*, *30*, 347–359.

Faulkner, C. (2013). Receptor-mediated signaling at plasmodesmata. *Frontiers in Plant Science*, *4*, 1–6.

Friml, J., Vieten, A., Sauer, M., Weijers, D., Schwarz, H., Hamann, T., et al. (2003). Efflux-dependent auxin gradients establish the apical-basal axis of Arabidopsis. *Nature*, *426*, 147–153.

Galinha, C., Hofhuis, H., Luijten, M., Willemsen, V., Blilou, I., Heidstra, R., et al. (2007). PLETHORA proteins as dose-dependent master regulators of Arabidopsis root development. *Nature*, *449*, 1053–1057.

Grieneisen, V. A., Xu, J., Marée, A. F. M., Hogeweg, P., & Scheres, B. (2007). Auxin transport is sufficient to generate a maximum and gradient guiding root growth. *Nature*, *449*, 1008–1013.

Haecker, A. (2004). Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in *Arabidopsis thaliana*. *Development*, *131*, 657–668.

Hardtke, C. S., & Berleth, T. (1998). The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *The EMBO Journal*, *17*, 1405–1411.

He, W., Brumos, J., Li, H., Ji, Y., Ke, M., Gong, X., et al. (2011). A small-molecule screen identifies l-kynurenine as a competitive inhibitor of TAA1/TAR activity in ethylene-directed auxin biosynthesis and root growth in Arabidopsis. *The Plant Cell*, *23*, 3944–3960.

Horiguchi, G., Kim, G.-T., & Tsukaya, H. (2005). The transcription factor AtGRF5 and the transcription coactivator AN3 regulate cell proliferation in leaf primordia of *Arabidopsis thaliana*. *The Plant Journal*, *43*, 68–78.

Jean-Baptiste, K., McFaline-Figueroa, J. L., Alexandre, C. M., Dorrity, M. W., Saunders, L., Bubb, K. L., et al. (2019). Dynamics of gene expression in single root cells of *Arabidopsis thaliana*. *Plant Cell*, *31*, 993–1011.

Kamiya, Y. (2010). Plant hormones: Versatile regulators of plant growth and development. *Annual Review of Plant Biology*, *61*, 301–307.

Kepinski, S., & Leyser, O. (2005). The Arabidopsis F-box protein TIR1 is an auxin receptor. *Nature*, *435*, 446–451.

Kim, J. H., Choi, D., & Kende, H. (2003). The AtGRF family of putative transcription factors is involved in leaf and cotyledon growth in Arabidopsis. *The Plant Journal: For Cell and Molecular Biology*, *36*, 94–104.

Kim, J. H., & Kende, H. (2004). A transcriptional coactivator, AtGIF1, is involved in regulating leaf growth and morphology in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 13374–13379.

Lanctot, A., Taylor-Teeples, M., Oki, E. A., & Nemhauser, J. L. (2019). *Specificity in auxin responses is not explained by the promoter preferences of activator ARFs* (p. 843391). bioRxiv.

Lewis, D. R., Olex, A. L., Lundy, S. R., Turkett, W. H., Fetrow, J. S., & Muday, G. K. (2013). A kinetic analysis of the auxin transcriptome reveals cell wall remodeling proteins that modulate lateral root development in Arabidopsis. *The Plant Cell*, *25*, 3329–3346.

Leyser, O. (2006). Dynamic integration of auxin transport and signalling. *Current Biology*, *16*, 424–433.

Leyser, O. (2018). Auxin signaling. *Plant Physiology*, *176*, 465–479.

Liao, C. Y., Smet, W., Brunoud, G., Yoshida, S., Vernoux, T., & Weijers, D. (2015). Reporters for sensitive and quantitative measurement of auxin response. *Nature Methods*, *12*, 207–210. 202 p following 210.

Mähönen, A. P., Tusscher, K. T., Siligato, R., Smetana, O., Díaz-Triviño, S., Salojärvi, J., et al. (2014). PLETHORA gradient formation mechanism separates auxin responses. *Nature*, *515*, 125–129.

Marhava, P., Bassukas, L., Zourelidou, M., Kolb, M., Moret, B., Fastner, A., et al. (2018). A molecular rheostat adjusts auxin flux to promote root protophloem differentiation. *Nature*, *558*, 297–300.

Marhava, P., Hoermayer, L., Yoshida, S., Marhavý, P., Benková, E., & Friml, J. (2019). Re-activation of stem cell pathways for pattern restoration in plant wound healing. *Cell*, *177*, 957–969.

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*, 1065–1067.

Mironova, V. V., Omelyanchuk, N. A., Wiebe, D. S., & Levitsky, V. G. (2014). Computational analysis of auxin responsive elements in the *Arabidopsis thaliana* L. genome. *BMC Genomics*, *15*(Suppl. 12), S4.

Mironova, V., & Xu, J. (2019). A single-cell view of tissue regeneration in plants. *Current Opinion in Plant Biology*, *52*, 149–154.

Miyashima, S., Roszak, P., Sevilem, I., Toyokura, K., Blob, B., Heo, J. O., et al. (2019). Mobile PEAR transcription factors integrate positional cues to prime cambial growth. *Nature*, *565*, 490–494.

Motte, H., Vanneste, S., & Beeckman, T. (2019). Molecular and environmental regulation of root development. *Annual Review of Plant Biology*, *70*, 465–488.

Nakajima, K., Sena, G., Nawy, T., & Benfey, P. N. (2001). Intercellular movement of the putative transcription factor SHR in root patterning. *Nature*, *413*, 307–311.

Nelissen, H., Eeckhout, D., Demuynck, K., Persiau, G., Walton, A., van Bel, M., et al. (2015). Dynamic changes in ANGUSTIFOLIA3 complex composition reveal a growth regulatory mechanism in the maize leaf. *The Plant Cell*, *27*, 1605–1619.

Olsson, V., Joos, L., Zhu, S., Gevaert, K., Butenko, M. A., & De Smet, I. (2019). Look closely, the beautiful may be small: Precursor-derived peptides in plants. *Annual Review of Plant Biology*, *70*, 153–186.

O'Malley, R. C., Huang, S. C., Song, L., Lewsey, M. G., Bartlett, A., Nery, J. R., et al. (2016). Cistrome and epicistrome features shape the regulatory DNA landscape. *Cell*, *166*, 1598.

Overvoorde, P., Fukaki, H., & Beeckman, T. (2010). Auxin control of root development. *Cold Spring Harbor Perspectives in Biology*, *2*, a001537.

Petersson, S. V., Johansson, A. I., Kowalczyk, M., Makoveychuk, A., Wang, J. Y., Moritz, T., et al. (2009). An auxin gradient and maximum in the Arabidopsis root apex shown by high-resolution cell-specific analysis of IAA distribution and synthesis. *The Plant Cell*, *21*, 1659–1668.

Pi, L., Aichinger, E., van der Graaff, E., Llavata-Peris, C. I., Weijers, D., Hennig, L., et al. (2015). Organizer-derived WOX5 signal maintains root columella stem cells through chromatin-mediated repression of CDF4 expression. *Developmental Cell*, *33*, 576–588.

Pierre-Jerome, E., Jang, S. S., Havens, K. A., Nemhauser, J. L., & Klavins, E. (2014). Recapitulation of the forward nuclear auxin response pathway in yeast. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 9407–9412.

Pierre-Jerome, E., Moss, B. L., Lanctot, A., Hageman, A., & Nemhauser, J. L. (2016). Functional analysis of molecular interactions in synthetic auxin response circuits. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 11354–11359.

Pierre-Jerome, E., Moss, B. L., & Nemhauser, J. L. (2013). Tuning the auxin transcriptional response. *Journal of Experimental Botany*, *64*, 2557–2563.

Rademacher, E. H., Lokerse, A. S., Schlereth, A., Llavata-Peris, C. I., Bayer, M., Kientz, M., et al. (2012). Different auxin response machineries control distinct cell fates in the early plant embryo. *Developmental Cell*, *22*, 211–222.

Rahni, R., & Birnbaum, K. D. (2018). *Week-long imaging of cell divisions in the Arabidopsis root meristem* (p. 268102). bioRxiv. Provisionally accepted at Plant Methods.

Robert, H. S., & Friml, J. (2009). Auxin and other signals on the move in plants. *Nature Chemical Biology*, *5*, 325–332.

Rodriguez, R. E., Ercoli, M. F., Debernardi, J. M., Breakfield, N. W., Mecchia, M. A., Sabatini, M., et al. (2015). MicroRNA miR396 regulates the switch between stem cells and transit-amplifying cells in Arabidopsis roots. *The Plant Cell*, *27*, 3354–3366.

Roosjen, M., Paque, S., & Weijers, D. (2018). Auxin response factors: Output control in auxin biology. *Journal of Experimental Botany*, *69*, 179–188.

Ryu, K. H., Huang, L., Kang, H. M., & Schiefelbein, J. (2019). Single-cell RNA sequencing resolves molecular relationships among individual plant cells. *Plant Physiology*, *179*, 1444–1456.

Sabatini, S., Beis, D., Wolkenfelt, H., Murfett, J., Guilfoyle, T., Malamy, J., et al. (1999). An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. *Cell*, *99*, 463–472.

Sager, R. E., & Lee, J.-Y. (2018). Plasmodesmata at a glance. *Journal of Cell Science*, *131*, jcs209346-jcs209346.

Santuari, L., Sanchez-Perez, G. F., Luijten, M., Rutjens, B., Terpstra, I., Berke, L., et al. (2016). The PLETHORA gene regulatory network guides growth and cell differentiation in Arabidopsis roots. *The Plant Cell*, *28*, 2937–2951.

Santuari, L., Scacchi, E., Rodriguez-Villalon, A., Salinas, P., Dohmann, E. M. N., Brunoud, G., et al. (2011). Positional information by differential endocytosis splits auxin response to drive Arabidopsis root meristem growth. *Current Biology*, *21*, 1918–1923.

Sarkar, A. K., Luijten, M., Miyashima, S., Lenhard, M., Hashimoto, T., Nakajima, K., et al. (2007). Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers. *Nature*, *446*, 811–814.

Sato, A., & Yamamoto, K. T. (2008). Overexpression of the non-canonical Aux/IAA genes causes auxin-related aberrant phenotypes in Arabidopsis. *Physiologia Plantarum*, *133*, 397–405.

Schlereth, A., Moller, B., Liu, W., Kientz, M., Flipse, J., Rademacher, E. H., et al. (2010). MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature*, *464*, 913–916.

Sena, G., Wang, X., Liu, H. Y., Hofhuis, H., & Birnbaum, K. D. (2009). Organ regeneration does not require a functional stem cell niche in plants. *Nature*, *457*, 1150–1153.

Shin, R., Burch, A. Y., Huppert, K. A., Tiwari, S. B., Murphy, A. S., Guilfoyle, T. J., et al. (2007). The Arabidopsis transcription factor MYB77 modulates auxin signal transduction. *Plant Cell*, *19*, 2440–2453.

Shinohara, H., Mori, A., Yasue, N., Sumida, K., & Matsubayashi, Y. (2016). Identification of three LRR-RKs involved in perception of root meristem growth factor in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*, 3897–3902.

Sivaguru, M., Fujiwara, T., Šamaj, J., Baluška, F., Yang, Z., Osawa, H., et al. (2000). Aluminum-induced 1→3-β-d-glucan inhibits cell-to-cell trafficking of molecules through plasmodesmata. A new mechanism of aluminum toxicity in plants. *Plant Physiology*, *124*, 991–1006.

Skoog, F., & Miller, C. O. (1957). Chemical regulation of growth and organ formation in plant tissues cultured in vitro. *Symposia of the Society for Experimental Biology*, *54*, 118–130.

Skopelitis, D. S., Hill, K., Klesen, S., Marco, C. F., von Born, P., Chitwood, D. H., et al. (2018). Gating of miRNA movement at defined cell-cell interfaces governs their impact as positional signals. *Nature Communications*, *9*, 1–14.

Sorefan, K., Girin, T., Liljegren, S. J., Ljung, K., Robles, P., Galvan-Ampudia, C. S., et al. (2009). A regulated auxin minimum is required for seed dispersal in Arabidopsis. *Nature*, *459*, 583–586.

Stahl, Y., Grabowski, S., Bleckmann, A., Kühnemuth, R., Weidtkamp-Peters, S., Pinto, K. G., et al. (2013). Moderation of Arabidopsis root stemness by CLAVATA1 and ARABIDOPSIS CRINKLY4 receptor kinase complexes. *Current Biology*, *23*, 362–371.

Stepanova, A. N., Robertson-Hoyt, J., Yun, J., Benavente, L. M., Xie, D.-Y., Doležal, K., et al. (2008). TAA1-mediated auxin biosynthesis is essential for hormone crosstalk and plant development. *Cell*, *133*, 177–191.

Stewart, J. L., & Nemhauser, J. L. (2010). Do trees grow on money? Auxin as the currency of the cellular economy. *Cold Spring Harbor Perspectives in Biology*, *2*, 1–14.

Swarup, R., & Bennett, M. (2003). Auxin transport: The fountain of life in plants? *Developmental Cell*, *5*, 824–826.

Szemenyei, H., Hannon, M., & Long, J. A. (2008). TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science*, *319*, 1384–1386.

Ulmasov, T., Hagen, G., & Guilfoyle, T. J. (1997). ARF1, a transcription factor that binds to auxin response elements. *Science*, *276*, 1865–1868.

Ulmasov, T., Murfett, J., Hagen, G., & Guilfoyle, T. J. (1997). Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell*, *9*, 1963–1971.

Vaddepalli, P., Herrmann, A., Fulton, L., Oelschner, M., Hillmer, S., Stratil, T. F., et al. (2014). The C2-domain protein QUIRKY and the receptor-like kinase STRUBBELIG localize to plasmodesmata and mediate tissue morphogenesis in *Arabidopsis thaliana*. *Development*, *141*, 4139–4148.

van Berkel, K., de Boer, R. J., Scheres, B., & ten Tusscher, K. (2013). Polar auxin transport: Models and mechanisms. *Development*, *140*, 2253–2268.

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*, 287–289.

Varaud, E., Brioudes, F., Szecsi, J., Leroux, J., Brown, S., Perrot-Rechenmann, C., et al. (2011). AUXIN RESPONSE FACTOR8 regulates Arabidopsis petal growth by interacting with the bHLH transcription factor BIGPETALp. *Plant Cell*, *23*, 973–983.

Vatén, A., Dettmer, J., Wu, S., Stierhof, Y. D., Miyashima, S., Yadav, S. R., et al. (2011). Callose biosynthesis regulates symplastic trafficking during root development. *Developmental Cell*, *21*, 1144–1155.

Vernoux, T., Brunoud, G., Farcot, E., Morin, V., Van den Daele, H., Legrand, J., et al. (2011). The auxin signalling network translates dynamic input into robust patterning at the shoot apex. *Molecular Systems Biology*, *7*, 508.

Wang, R., & Estelle, M. (2014). Diversity and specificity: Auxin perception and signaling through the TIR1/AFB pathway. *Current Opinion in Plant Biology*, *21*, 51–58.

Wang, Q., Kohlen, W., Rossmann, S., Vernoux, T., & Theres, K. (2014). Auxin depletion from the leaf axil conditions competence for axillary meristem formation in Arabidopsis and tomato. *Plant Cell*, *26*, 2068–2079.

Wang, W., Ryu, K. H., Barron, C., & Schiefelbein, J. (2019). Root epidermal cell patterning is modulated by a critical residue in the WEREWOLF transcription factor. *Plant Physiology*, *181*, 1239–1256.

Weijers, D., Nemhauser, J., & Yang, Z. (2018). Auxin: Small molecule, big impact. *Journal of Experimental Botany*, *69*, 133–136.

Wu, M. F., Yamaguchi, N., Xiao, J., Bargmann, B., Estelle, M., Sang, Y., et al. (2015). Auxin-regulated chromatin switch directs acquisition of flower primordium founder fate. *eLife*, *4,* e09269.

Yadav, R. K., Perales, M., Gruel, J., Girke, T., Jonsson, H., & Reddy, G. V. (2011). WUSCHEL protein movement mediates stem cell homeostasis in the Arabidopsis shoot apex. *Genes & Development*, *25*, 2025–2030.

Yamada, M., Han, X., & Benfey, P. N. (2019). RGF1 controls root meristem size through ROS signalling. *Nature*. https://www.nature.com/articles/s41586-019-1819-6.pdf.

Yang, Z.-B., Geng, X., He, C., Zhang, F., Wang, R., Horst, W. J., et al. (2014). TAA1-regulated local auxin biosynthesis in the root-apex transition zone mediates the aluminum-induced inhibition of root growth in Arabidopsis. *The Plant Cell*, *26*, 2889–2904.

Zemlyanskaya, E. V., Wiebe, D. S., Omelyanchuk, N. A., Levitsky, V. G., & Mironova, V. V. (2016). Meta-analysis of transcriptome data identified TGTCNN motif variants associated with the response to plant hormone auxin in *Arabidopsis thaliana* L. *Journal of Bioinformatics and Computational Biology*, *14*, 1641009.

Zhang, T. Q., Xu, Z. G., Shang, G. D., & Wang, J. W. (2019). A single-cell RNA sequencing profiles the developmental landscape of Arabidopsis root. *Molecular Plant*, *12*, 648–660.

Zhao, Y. (2012). Auxin biosynthesis: A simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Molecular Plant*, *5*, 334–338.

Further reading

Brady, S. M., Orlando, D. A., Lee, J. Y., Wang, J. Y., Koch, J., Dinneny, J. R., et al. (2007). A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science*, *318*, 801–806.