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## Regulation of Division and Differentiation of Plant Stem Cells

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### Abstract

A major challenge in developmental biology is unraveling the precise regulation of plant stem cell maintenance and the transition to a fully differentiated cell. In this review, we highlight major themes coordinating the acquisition of cell identity and subsequent differentiation in plants. Plant cells are immobile and establish position-dependent cell lineages that rely heavily on external cues. Central players are the hormones auxin and cytokinin, which balance cell division and differentiation during organogenesis. Transcription factors and miRNAs, many of which are mobile in plants, establish gene regulatory networks that communicate cell position and fate. Small peptide signaling also provides positional cues as new cell types emerge from stem cell division and progress through differentiation. These pathways recruit similar players for patterning different organs, emphasizing the modular nature of gene regulatory networks. Finally, we speculate on the outstanding questions in the field and discuss how they may be addressed by emerging technologies.

### Keywords

plant stem cells; asymmetric divisions; meristem; transcriptional regulation; mobile signals; cell identity

## INTRODUCTION

In multicellular organisms, the choice to divide or differentiate lies at the core of cellular decision making. The advent of multicellularity required the diversification of cell types to carry out specialized functions. While all cell types share the same genetic information encoded in their DNA, an array of regulatory mechanisms specify distinct programs of gene expression. These changes in gene expression modify the complement of proteins within a given cell, resulting in an altered competence to respond to signals and patterning cues.

Central to development are asymmetric divisions during which one cell divides to produce two daughter cells that go on to acquire different fates (Horvitz & Herskowitz 1992). Stem cells by definition undergo asymmetric divisions to self-renew and produce new cell types

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(Morrison et al. 1997). Plants maintain a constant pool of stem cells to drive growth throughout their life spans (Heidstra & Sabatini 2014). These pools of stem cells are found in meristems, which are regions of plant tissue composed of a central stem cell niche and actively dividing stem cell progeny (Scheres 2007). This review focuses on the two primary meristems established during embryogenesis, the shoot apical meristem (SAM) and the root apical meristem (RAM). In addition to these primary meristems, other stem cells arise in the establishment of specialized cell lineages during differentiation. Examples include the outgrowth of lateral organs in the shoot and root and the formation of epidermal pores that mediate gas exchange (Nadeau & Sack 2003, Scheres 2007).

Plants exhibit remarkable developmental plasticity—a fundamental feature of the survival of a plant because it is rooted to the ground. Since plant cells are immobile, intercellular communication is vital for the establishment and maintenance of cell identity (Aida et al. 2004, van den Berg et al. 1997, Yadav et al. 2010). In plants, it is well established that positional cues regulate cell identity (Kidner et al. 2000, van den Berg et al. 1995). While there is evidence for lineage-based information encoded in daughter cells, such information can be overridden by extrinsic positional cues. Ablation and regeneration studies have demonstrated that most cell types in the meristem can adopt a new position-dependent fate (Efroni et al. 2016, van den Berg et al. 1997). Thus, most plant cells have the capacity to assume a new cell identity, although maintenance of identity also requires continuous signaling and regulatory feedback (Helariutta et al. 2000; Sabatini et al. 1999, 2003).

Specific combinations of transcriptional regulators can revert the identity of a differentiated cell into a stem cell in both plants and animals (Gallois et al. 2004, Huangfu et al. 2008, Smith et al. 2016). Cell identity is therefore established and maintained by myriad interactions of molecular regulators (Caggiano et al. 2017, Moreno-Risueno et al. 2015). This includes hormonal fluxes and mobile signals from neighboring cells that converge on transcription factor activity (Drisch & Stahl 2015, Galli & Gallavotti 2016). Moreover, the active interplay of transcription factors and other signaling molecules cues cell identity and ensures robust patterning of the plant body (Rahni et al. 2016). Evolution tinkers and reuses molecular regulators to generate diversity, such as the expansion of gene families that take on similar roles in different tissues (Dolzblasz et al. 2016, Zhou et al. 2015). Biology is thus full of repeating patterns that form the foundation of the nuanced information processing that is executed at the molecular level.

## SPATIAL ORGANIZATION OF PLANT STEM CELLS

Aspects of regulation in the SAM and the RAM are almost mirror images and follow opposing gradients of differentiation along the primary axis of the plant body (Figure 1).

The SAM produces all the aerial organs of the plant, including the stem, leaves, and flowers. A small group of cells functions as the organizing center (OC), which maintains the stem cell population in the overlying layers of the central zone (CZ) at the very tip of the meristem (Figure 1, top left). Division of stem cells in the CZ can result in displacement of a daughter cell to the peripheral zone (PZ) at the edges of the meristem or to the rib meristem (RM) beneath the CZ. Daughter cell displacement leads to the competence to differentiate

into leaf or flower primordia at the PZ or into various cell types of the elongating stem at the RM (Gaillochet & Lohmann 2015).

In the root, an organizing group of mitotically inactive quiescent center (QC) cells marks the position of the stem cell niche (van den Berg et al. 1997). Surrounding the QC are the stem cells that divide asymmetrically to generate all the tissue types in the root (Figure 1, bottom left). Because plant cells do not move in relation to each other, as the proximal stem cells divide, their daughters are displaced further from the QC. Each division displaces a daughter cell apically, resulting in the downward growth of the root (Cederholm et al. 2012). Each stem cell type generates a cell file of displaced daughters, which go through several additional rounds of division in the transit-amplifying region of the meristematic zone (MZ). In the elongation zone (EZ), cells exit the traditional cell cycle and enter a period of endoreduplication: multiple cycles of DNA replication accompanied by cell elongation, without an intervening cell division (Polyn et al. 2015). The border between the MZ and the EZ therefore defines the transition from cell division to differentiation (Dello Ioio et al. 2007). Finally, the differentiation zone (DZ) is where cells acquire the features of their differentiated fate (Figure 1, bottom center). As a result, the primary axis of the root serves as a linear timeline of development from stem cell to differentiated tissue.

## HORMONES AND PATTERNING

Hormones play key roles in patterning both plants and animals. In plants, hormone biosynthesis, trafficking, and signaling establish gradients of accumulation and response that result in distinct cell fates. While several hormones play a role, the hormones auxin and cytokinin are essential for positioning and maintaining meristem activity (Figure 1, right). The balance between division and differentiation relies on a mutual steady state reached by the interactions between these two hormones (Schaller et al. 2015). A cell's competence to respond to the two hormones guides the gradual acquisition of identity. What establishes this competence to respond?

In the root, auxin regulates stem cell divisions, and cytokinin triggers the transition to differentiation (Blilou et al. 2005, Dello Ioio et al. 2007). These roles are reversed in the shoot, where local auxin accumulation drives organogenesis in the PZ and cytokinins are crucial for cell proliferation in the CZ (Werner et al. 2003, Zhao et al. 2010). Each hormone stimulates a signaling pathway that culminates in the activation of transcription factors, which mediate global changes in gene expression. Auxin signaling has been tied to almost every aspect of plant growth and development. Briefly, an auxin signal promotes the destabilization of its signaling repressors, resulting in the activation of downstream transcription factors (Weijers & Wagner 2016). In contrast, cytokinin signaling relies on a two-component phosphorelay to phosphorylate and activate transcriptional response regulators (Hwang et al. 2012). Both hormones directly activate the expression of their own signaling repressors. They are thus regulated by negative feedback that can determine the duration and extent of response in a given cell.

While these frameworks have provided key insights into the molecular basis of hormone response, there remains much to be fleshed out. Genetic dissection of these signaling

pathways is plagued by extensive redundancy among members of large families of transcriptional regulators (Keshishian & Rashotte 2015, Lokerse & Weijers 2009). Transcriptional reporters serve as the principal readout of hormone activity, but sifting through the web of signaling components that relay a specific signal remains a major challenge (Liao et al. 2015, Zürcher et al. 2013). Moreover, how these hormones mediate transcriptional repression remains an open question. Almost every hormone pathway has been connected to TOPLESS (TPL), a transcriptional cofactor related to the Groucho/Tup1 family of corepressors (Causier et al. 2012, Szemenyei et al. 2008). TPL recruitment is associated with a histone deacetylase (HDAC), suggesting that repression involves changes in chromatin state (Long et al. 2006). While several chromatin modifiers have been identified to play a role in hormonal regulation of cell fate (Efroni et al. 2013, Wu et al. 2015, Yang et al. 2015), the path from hormone perception to transcriptional response remains largely a black box.

As early as embryogenesis, complementary patterns of auxin and cytokinin signaling appear to define spatial boundaries, but the precise molecular mechanism has not been determined (Müller & Sheen 2008). Overlapping expression patterns of key cytokinin and auxin regulators have led to the identification of genetic circuits in which auxin signaling converges on cytokinin's regulators of biosynthesis, accumulation, and signaling and vice versa (Chandler & Werr 2015). Through a combination of modeling and experimentation, DiMambro et al. (2017) recently demonstrated how an auxin minimum at the boundary between the MZ and the EZ in the root provides a positional cue for the switch to differentiation. Previous work demonstrated that cytokinin induces repression of auxin signaling in this domain, resulting in the inhibition of the PIN1 auxin transporter (Dello Ioio et al. 2008). New evidence indicates that cytokinin further enforces this minimum by inducing members of the *GRETCHEN HAGEN 3 (GH3)* gene family (Di Mambro et al. 2017). GH3 family members function as auxin conjugation enzymes that convert auxin to an inactive state (Staswick et al. 2005). Cytokinin-induced repression of auxin signaling and auxin inactivation by a GH3 result in an auxin minimum positioned where cells exit the MZ and transition to differentiation (Di Mambro et al. 2017). Di Mambro et al. (2017) argue that auxin thus patterns the root in a self-organizing manner, with cytokinin signaling as one of multiple inputs.

The remarkable ability of plants to regenerate is also dependent on auxin and cytokinin (Su & Zhang 2014). Given the right combination of hormones, organogenesis can be induced in explants prepared from almost any tissue. This chemical regulation has been recognized since the 1950s, shortly after the identification of cytokinins (Skoog & Miller 1957). To induce the formation of a callus, explants are grown on media containing a high ratio of auxin to cytokinin. Root induction requires only auxin, while shoot induction requires a high cytokinin-to-auxin ratio (Feldmann & Marks 1986). Thus, the balance of auxin and cytokinin directs organ formation both in vivo and in vitro.

The plasticity of organ identity in plants was recently investigated using alternate auxin and cytokinin treatments to convert lateral root primordia into shoot meristems, without the formation of a dedifferentiated callus (Rosspopoff et al. 2017). Lateral root development has been extensively studied as a model for de novo organogenesis (Malamy & Benfey 1997).

Cytokinin treatment resulted in direct conversion of lateral root primordia into a growing shoot in as few as 4 days (Figure 2). This conversion was possible only after the establishment of a stem cell niche in the lateral root primordia. During this stage, there is a characteristic auxin maximum at the emerging lateral root tip and no cytokinin signal. In the organ undergoing conversion, cytokinin accumulates in the central vasculature, and auxin is depleted from the root tip. As the shoot meristem develops, new auxin maxima form at the base of leaf primordia, and a broad cytokinin domain is established in the inner layers of the meristem. Notably, the hormone domains shift from that of root primordia into domains characteristic of a SAM prior to the expression of SAM-specific regulators.

In a recent study of root regeneration, a similar relationship was observed between the formation of complementary auxin and cytokinin domains and the establishment of cell identity in the regenerated root (Efroni et al. 2016). Following removal of the root tip, including the stem cell niche, Efroni et al. (2016) employed lineage tracing and single-cell RNA-seq to follow the gene expression of specific cells during the reestablishment of the stem cell niche. They found that cells from multiple tissues acquired stem cell identity to regenerate the excised root tip. Moreover, manipulation of auxin or cytokinin during regeneration could alter patterning. The authors argue that, similar to embryogenesis, auxin and cytokinin form complementary signaling domains during regeneration that provide spatial cues for positioning the stem cell niche.

## REGULATORY FEEDBACK IN THE SHOOT AND ROOT STEM CELL NICHES

Members of the *WUSCHEL-RELATED HOMEODOMAIN* (*WOX*) gene family are required non-cell autonomously to maintain stem cell homeostasis. The best-characterized family members are *WUSCHEL* (*WUS*) in the shoot and *WOX5* in the root. The onset of *WUS* expression in the shoot and the onset of *WOX5* expression in the root are early markers of stem cell niche establishment. Their expression is restricted to only a few cells, which function as organizers at the center of each niche. Loss of either transcription factor results in the collapse of its stem cell niche (Sarkar et al. 2007). To ensure proper patterning and development, restriction of *WUS* and *WOX5* expression to the organizing cells is tightly regulated by layered feedback mechanisms.

In the SAM, *WUS* expression is positioned by a gradient of cytokinin (Chickarmane et al. 2012). An activating enzyme of cytokinin is specifically expressed in the uppermost stem cell layer, permitting the diffusion of cytokinin to the lower layers of the SAM (Kurakawa et al. 2007). The underlying *WUS* domain in the shoot OC contains high levels of a cytokinin receptor, sensitizing the cells to cytokinin and resulting in high levels of cytokinin signaling (Gordon et al. 2009). Cytokinin promotes *WUS* expression and *WUS* promotes cytokinin signaling through repression of negative regulators of cytokinin signaling (Leibfried et al. 2005, Meng et al. 2017). Thus, a positive feedback loop between *WUS* and cytokinin ensures that *WUS* expression leads to more *WUS* and high levels of cytokinin signaling (Chickarmane et al. 2012).

*WUS* expression in the OC is critical for stem cell fate in the overlying cells of the CZ (Laux et al. 1996, Yadav et al. 2010). Only cells in the OC express *WUS*, but the *WUS* protein

migrates to the neighboring cell layers of the CZ through plasmodesmata (see sidebar titled Intercellular Trafficking Through Plasmodesmata) (Daum et al. 2014, Yadav et al. 2011). In CZ stem cells, the mobile WUS transcription factor activates expression of its own negative regulator, a small secreted peptide termed CLAVATA3 (CLV3) (Yadav et al. 2011). Recent analysis of WUS binding to *cis* elements in the regulatory region of *CLV3* indicated that the same *cis* elements mediate repression and activation, depending on the concentration of WUS (Perales et al. 2016). WUS binds to these elements as monomers at low concentrations and as dimers at higher concentrations. These observations provide a plausible explanation for the ability of WUS to activate CLV3 only in the CZ and not in the OC where WUS is expressed. When WUS migrates from its source in the OC to neighboring stem cells, it accumulates at lower levels than at its origin. This lower accumulation results in the activation of CLV3 transcription, limiting the expression domain of WUS (Rodriguez et al. 2016). Conversely, cells transcribing WUS accumulate high levels of WUS protein, leading to repression of CLV3 transcription as well as to the self-sustaining expression of WUS solely in the OC (Figure 3).

Similar to WUS expression in the OC, *WOX5* expression is confined to the QC cells in the center of the root stem cell niche (Fiers et al. 2005, Stahl et al. 2009). Yet, *WOX5* is also required for maintenance of the surrounding stem cells, particularly for the stem cells of the columella (Sarkar et al. 2007). The columella serves as a gravity-sensing and protective tissue at the very tip of the root. Columella cells rapidly differentiate and originate from a single stem cell layer positioned immediately beneath the QC. Expression of *WOX5* driven by the WUS promoter demonstrated that *WOX5* is capable of intercellular movement in the SAM (Daum et al. 2014). Recent evidence indicates endogenous *WOX5* is mobile in the RAM (Pi et al. 2015). There, *WOX5* migrates to the columella stem cells to repress differentiation (Pi et al. 2015).

Similar to the relationship between WUS and cytokinin, *WOX5* also has numerous ties with auxin signaling. The auxin gradient in the root meristem peaks in the QC, where *WOX5* is specifically expressed (Sabatini et al. 1999). This peak of auxin activity is sustained and focused by auxin regulation of the PIN transporters and by local auxin biosynthesis (Blilou et al. 2005, Ding & Friml 2010). The regulatory relationship between *WOX5* and auxin also relies on feedback from each other to maintain robust expression and activity in the QC (Tian et al. 2014). Outside the QC, specific auxin signaling repressors and transcription factors repress *WOX5* expression (Ding & Friml 2010).

Chromatin regulation is also emerging as a mechanism for restricting expression of WUS and *WOX5* (Ikeuchi et al. 2015). SPLAYED (SYD), an SNF2 chromatin remodeling complex, regulates the transcriptional expression of WUS in the SAM (Kwon et al. 2005). REPRESSOR OF WUSCHEL1 (ROW1) was originally identified for its role in confining WUS expression to the OC of the SAM (Han et al. 2008). Recently, ROW1 was also demonstrated to repress *WOX5* expression in the proximal meristem. Loss of ROW1 results in the expansion of *WOX5* expression into the proximal meristem, and misexpression of *ROW1* in the QC extinguishes *WOX5* expression (Zhang et al. 2015). ROW1 represses *WOX5* expression by binding to H3K4me3 chromatin marks in the *WOX5* promoter. The *WOX5* promoter is characterized by these H3K4me3 chromatin marks, which remain



unchanged regardless of promoter activity (Aichinger et al. 2011, Zhang et al. 2015). Moreover, both WUS and WOX5 interact with TPL corepressors to mediate repression of their targets (Kieffer et al. 2006, Pi et al. 2015). Many examples of transcriptional repression implicate chromatin modifiers, but the molecular mechanisms remain elusive.

WUS and WOX5 are mostly interchangeable between the root and shoot, indicating that they play similar roles in stem cell maintenance (Sarkar et al. 2007). A recent study tested how well other WOX family members could rescue the *wus* mutant phenotype (Dolzblasz et al. 2016). Only a few of the closely related family members, including WOX5, were capable of various degrees of rescue. Notably, WUS is capable of complete rescue of the *wox5* mutant (Sarkar et al. 2007). Thus, while WUS and WOX5 may differ in some aspects of their functionality, they overlap in many others. HAIRYMERISTEM (HAM) functions as a WUS cofactor in regulating a number of WUS target genes (Zhou et al. 2015). Both the WOX and HAM families are highly conserved and include multiple members whose expression domains overlap, suggesting that the interaction between different pairs of WOX and HAM family members plays a key role in how WOX family members maintain different stem cell niches.

## FEEDBACK BETWEEN MOBILE TRANSCRIPTION FACTORS AND miRNAS

Small RNAs have an important role in transcriptional feedback. Two miRNAs that together form a hairpin structure, *miR165* and *miR166*, have an important role in regulating dividing and differentiating tissues (Aukerman 2003, Carlsbecker et al. 2010; reviewed in Couzigou & Combier 2016). In the SAM, the RAM, and other meristems, *miR165* and *miR166* promote differentiation by repressing members of the HD-ZIP III family of transcription factors (Bao et al. 2004, Williams 2005). These factors include *PHAVOLUTA*, *PHABULOSA*, and *REVOLUTA*, which have important roles in maintaining stem cells in both the SAM and RAM (Emery et al. 2003, Hawker 2004, McConnell et al. 2001, Prigge 2005). In each of these settings, different argonaute (AGO) proteins act in meristematic cells to sequester *miR165* and *miR166* away from targets or to promote differentiation by relieving repression of HD-ZIP transcription factors (Ji et al. 2011, Kidner & Martienssen 2004, McHale 2004, Zhang & Zhang 2012; reviewed in Zhong & Ye 2007). While the key players in this pathway seem to be recycled in meristematic tissues, the mechanisms by which these pathways intersect with those described above to define stem cells are still largely unknown.

## SMALL PEPTIDES IN MERISTEM DEVELOPMENT

Similar to the case for animal development, small peptides such as those of the CLAVATA3/ESR-related (CLE) family are important for signaling in the development and differentiation of plants (Matsubayashi 2014). While the relationship between WUS and CLV3 is well established, much less is understood about the downstream mechanism of CLV3 signaling (Clark et al. 1995, Fletcher et al. 1999, Kondo et al. 2006). In a wild-type SAM, CLV3 represses *WUS*, allowing cells in the PZ to differentiate (Brand et al. 2000, Laux et al. 1996, Mayer et al. 1998, Schoof et al. 2000). Loss of *CLV3* results in enlarged meristems due to expansion of the *WUS* expression domain (Brand et al. 2000, Schoof et al. 2000). Careful

genetic, ectopic expression, and biochemical experiments have demonstrated that CLV3 peptide diffuses to the peripheral meristem, where it activates a signaling cascade through CLAVATA1 (CLV1), a leucine-rich receptor kinase (Clark et al. 1997, Lenhard & Laux 2003, Nimchuk et al. 2011, Ogawa et al. 2008, Rojo 2002). While *WUS* is a transcriptional target of CLV3 signaling, the molecular events leading to transcriptional repression remain elusive. Multiple receptor complexes are involved in CLV3 signaling, and there is mounting evidence that each complex plays a unique role in stem cell regulation (DeYoung & Clark 2008, DeYoung et al. 2005, Nimchuk 2017).

In the root, a small peptide related to CLV3, *CLE40*, represses *WOX5* expression in stem cells surrounding the QC (Stahl et al. 2009). Expression of *CLE40* in the SAM can rescue loss of CLV3 signaling in the shoot, indicating these two peptides are functionally equivalent and differ mainly in expression pattern (Hobe et al. 2003). Unlike *CLV3*, which is expressed in shoot stem cells, *CLE40* is expressed in differentiated columella cells and binds to receptors that are expressed in the overlying columella stem cells (Stahl et al. 2013). Loss of *CLE40* results in additional layers of columella stem cells (Stahl et al. 2009). In the root, the CLV1 receptor interacts with ARABIDOPSIS CRINKLY 4 (ACR4), another receptor-like kinase, to detect *CLE40* and initiate downstream signaling (De Smet et al. 2008; Stahl et al. 2009, 2013). There is also evidence that other receptors involved in CLV3 sensing in the SAM can detect CLE peptides in the RAM (Kinoshita et al. 2010, Muller et al. 2008, Shimizu et al. 2015). Other CLE family peptides have important roles in root development, such as regulating differentiation in root vascular tissue and in several stages of lateral root development (Depuydt et al. 2013; Fernandez et al. 2013, 2015; Kumpf et al. 2013; Meng et al. 2012).

## SMALL PEPTIDES IN DIFFERENTIATION

Outside of the meristem, divisions of specialized stem cell lineages are also regulated by small peptides. Members of the epidermal patterning factor (EPF) family of peptides play important roles in leaf patterning (reviewed in Katsir et al. 2011). Stomata are pores in the epidermis that mediate gas exchange (Pillitteri & Torii 2012). They arise from a defined series of asymmetric divisions and one symmetric division that form guard cells, the two cells that regulate stomatal opening (Figure 4). The stereotypical division pattern begins with asymmetric division of a cell termed the meristemoid mother cell, which divides to give rise to a meristemoid cell and a sister cell type. The two daughter cells have different sizes, with the sister cell being much larger than the meristemoid cell. Both progeny continue to asymmetrically divide in a series of amplifying divisions, with cells regenerated as meristemoids transitioning into a fate termed guard mother cells. The guard mother cells finally symmetrically divide to make rounded guard cells, which form a pore. Once the asymmetric divisions begin in one cell, the surrounding cells become incapable of forming stomata and instead differentiate into puzzle piece-shaped pavement cells. This process results in a defined pattern whereby each stoma is surrounded by at least one pavement cell. Although auxin distribution between mother and daughter cells has been implicated in the transition to the final symmetric division that forms the guard cells (Le et al. 2014, Pillitteri & Torii 2012), it is largely unknown how hormone signaling is integrated with other factors to regulate stomatal spacing (Saibo et al. 2003).



EPF1 and EPF2 have some overlapping effects in promoting division and restricting differentiation at different stages along the path to stomata or pavement cell development (Hara et al. 2009, Hunt & Gray 2009, Pillitteri & Torii 2012). EPF1 inhibits the transition to guard mother cells. EPF2 inhibits the initial asymmetric cell division of meristemoid mother cells and has an important role in ensuring the correct spacing of guard cells (Hara et al. 2009, Hunt & Gray 2009). Overexpression of either *EPF1* or *EPF2* results in fewer stomatal clusters (Hara et al. 2007, 2009; Hunt & Gray 2009; Pillitteri & Torii 2012). These peptides activate members of the ERECTA (ER) family of receptor kinases and the coreceptor TOO MANY MOUTHS (TMM). ER and TMM can form complexes with the somatic embryogenesis receptor kinase (SERK) family of coreceptors to activate downstream signaling through the mitogen-activated protein kinase (MAPK) pathway (Meng et al. 2015, Pillitteri & Torii 2012). An EPF-like (EPFL) peptide termed STOMAGEN promotes stomatal development by inducing guard cell differentiation. One intriguing question is how such related peptides can exert such different effects. It was recently shown that STOMAGEN also acts through ER family receptors competing with EPF2 for binding to ER (Lee et al. 2015). By contrast, STOMAGEN does not activate MAPK signaling and may actually prevent it (Lee et al. 2015). These findings show how finely tuned this system is, with both inhibiting and activating signals spatially organizing the leaf epidermis. Mechanistically, it is still unknown whether these changes in sensitivity are due to changes in receptor sensitivity or changes in peptide concentration.

EPFL2, another EPFL peptide, has an important role in other aspects of leaf morphogenesis and has an established connection with auxin. *Arabidopsis* leaves have serrations, the sawlike projections or teeth found at the edges of leaves. Serrations are initiated from small primordia along the leaf margin (Tameshige et al. 2016). In *epfl2* mutants, serrations are missing, and there is an expansion of tooth primordia. Auxin is normally restricted to the tips of growing teeth and is important for tooth development. The auxin pathway intersects with EPFL signaling, as auxin has a mutually repressive relationship with EPFL2 and ER (Tameshige et al. 2016). These studies demonstrate the importance of the intersection of small peptide signaling and hormonal regulation in defining organ boundaries.

Small peptides also cue the acquisition of specialized features during differentiation. Two recently discovered peptides are required for the proper formation of the root endodermis (Doblas et al. 2017, Nakayama et al. 2017). One role of the endodermis is to provide for selective uptake of solutes. Water and dissolved compounds can pass between cells of the epidermis and cortex. The endodermis generates a waterproof seal made of lignin termed the Casparian strip, which prevents unrestricted flow to the vascular tissue and allows membrane channels to selectively uptake ions (Geldner 2013). The small signaling peptides CASPARIAN STRIP INTEGRITY FACTOR 1 (CIF1) and CIF2 are required for proper sealing of this structure. Mutations in SCHENGEN2 (SGN2), the enzyme required for CIF1 and CIF2 activity, and mutations in both *cif1* and *cif2* result in defective Casparian strips (Doblas et al. 2017, Nakayama et al. 2017). These peptides act on the endodermis but are derived from the neighboring cells of the vascular tissue. Both genetic and biochemical evidence identified the receptor downstream of CIF1 and CIF2 as SCHENGEN3 (SGN3), a receptor-like kinase with enriched expression in the endodermis (Pfister et al. 2014). The *sgn3* mutant also resembles the *cif1cif2* double mutant (Doblas et al. 2017, Nakayama et al.

2017, Pfister et al. 2014). Both the CIF peptides and SHORTROOT (SHR) protein (described below) are mobile signals derived from the vasculature, demonstrating the importance of signaling from neighboring cell layers for proper differentiation. This mechanism is similar to induction in animal cell development, such as the well-studied examples of mesoderm induction and EPIDERMAL GROWTH FACTOR signaling in *Drosophila* eye development (Kimelman 2006, Schweitzer & Shilo 1997).

## MOBILE TRANSCRIPTION FACTORS REGULATE CELL FATE

The sustained accumulation of auxin induces transcription of PLETHORA (PLT) transcription factors, a family of mobile transcription factors (Mähönen et al. 2014). PLT expression is highest in stem cells and gradually decreases in the shootward direction (Aida et al. 2004, Galinha et al. 2007). PLTs regulate QC and stem cell maintenance and differentiation in what is thought to be a dose-dependent manner (Galinha et al. 2007). Another small family of peptides termed root growth factors (RGFs) maintain the root meristem by regulating the gradient of PLT expression (Matsuzaki et al. 2010, Zhou et al. 2010). The enzyme that modifies the RGFs into their mature form is also regulated by auxin, suggesting convergence of hormonal and small peptide regulation of the *PLT* gradient (Zhou et al. 2010). Only recently has the clade of receptors for this pathway been identified, highlighting again the complexity of studying small peptides (Ou et al. 2016, Shinohara et al. 2016, Song et al. 2016).

Perhaps the best-characterized mobile transcription factor in *Arabidopsis* is *SHR*, which plays multiple roles in root patterning. *SHR* is a member of the GRAS family of transcription factors, a plant-specific family that regulates various aspects of plant growth and development (Hirsch & Oldroyd 2009). *SHR* is expressed and translated in the central vascular tissue and moves into the adjacent cell layer (Figure 3b) (Helariutta et al. 2000, Nakajima et al. 2001). This layer includes the root endodermis, the stem cell progenitors of the endodermis, and the QC. As implied by the name, *shr* mutants have short roots (Benfey et al. 1993), largely due to the QC and stem cell defects that occur in the absence of *SHR* protein (Helariutta et al. 2000). The primary role of *SHR* movement, however, is the specification and differentiation of the endodermis. Without *SHR*, there is no endodermis.

Without movement of *SHR* into the stem cell progenitors, asymmetric division of the cortex and endodermis stem cell is compromised (Helariutta et al. 2000). A decade of research on this division has uncovered the integral factors that contribute to the timing and decision to divide. Transcription factors, including *SHR*, can induce cell cycle regulators and phosphorylation of the plant homolog of RETINOBLASTOMA (RB) protein (Cruz-Ramírez et al. 2012, Di Laurenzio et al. 1996, Helariutta et al. 2000, Sozzani et al. 2010). One major question is how auxin signaling is integrated in this process. In modeling of this division, one plausible explanation is that the concentration of auxin at the niche triggers cells with the right levels of transcription factors and RB to divide (Cruz-Ramírez et al. 2012).

Regulating transcription factor movement is also important for robust patterning. *SHR* expression in the vascular tissue is both nuclear and cytoplasmic, while in the neighboring endodermis *SHR* protein is mostly nuclear. Available evidence indicates that *SHR* moves

through plasmodesmata and, unexpectedly, requires nuclear localization for movement. (Gallagher et al. 2004, Vatén et al. 2011). Once in the endodermis, SHR is sequestered to the nucleus. There, SHR induces several transcriptional coregulators that physically interact with SHR and keep it in the nucleus. These cofactors include SCARECROW (SCR), another member of the GRAS family, and several members of the INDETERMINATE DOMAIN (IDD) family of zinc-finger transcription factors, commonly referred to as the BIRDS (Long et al. 2015). Loss of SCR or members of the BIRD family results in the expansion of SHR mobility (Cui et al. 2007, Long et al. 2015). SHR activity in cells external to the endodermis generates additional layers of cortex, the tissue that separates the inner endodermis from the outer epidermis (Cui et al. 2007, Wu et al. 2014). That additional SHR movement results in cortex rather than endodermis formation suggests that additional stele-derived factors are required for endodermis identity.

The transcription factor SHOOT MERISTEMLESS (STM) is mobile and required for SAM maintenance in *Arabidopsis* (Long et al. 1996). STM's maize homolog, KNOTTED1 (KN1), was the first mobile transcription factor discovered (Jackson 2002, Jackson et al. 1994, Lucas et al. 1995). In contrast to STM, KN1 has distinct domains of expression and protein localization (Jackson 2002). Both maize KN1 and *Arabidopsis* STM require their mobility to regulate SAM maintenance (Balkunde et al. 2017, Xu et al. 2011).

While some mobile transcription factors, such as STM, are mobile across large evolutionary distances, others are not. The transcription factor MUTE, which plays a key role in differentiation of stomata (Pillitteri & Torii 2012, Pillitteri et al. 2007), was recently shown to be mobile in the grass species *Brachypodium* (Raissig et al. 2017). MUTE is a key regulator of cell divisions in leaf patterning, but it is not mobile in *Arabidopsis* (Pillitteri et al. 2007, 2008). The mobility of MUTE in *Brachypodium* plausibly explains the presence of additional supporting cells in stomata, which *Arabidopsis* lacks (Raissig et al. 2017). Modified mobility could explain the patterning differences seen between dicots and monocots.

## DISCUSSION

Developmental patterning in time and space emerges from dynamic interactions at the cellular and molecular levels. In plants, intercellular communication is vital for ensuring robust patterning throughout development. Plants utilize recurring modules of intercellular interactions to communicate position and identity. Hormones, mobile transcription factors, small RNAs, and peptides form complementary positional cues to establish and reinforce cell identity. In many cases, duplication and evolution of transcriptional regulators not only confer robustness through redundancy but also generate diversity and specialization. Spatiotemporal development thus relies on the contribution of individual cells and their competence to respond to specific signaling cues.

Signaling between neighboring cells is dynamic and involves multiple feedback loops. This back and forth between cells is essentially an ongoing call and response to both specify and reinforce cell fate. Non-cell autonomous gene regulation by mobile signals provides an elegant solution for robust patterning. The path from signal to gene regulation, however,

remains largely obscure. Hormones have long been recognized as chemical messengers, but how the same hormone delivers different messages continues to be an active area of research. The molecular underpinnings of small peptide perception and signaling are complicated by peptide promiscuity and by the range of receptor complexes that propagate the signal. Finally, the mobility of miRNAs and transcription factors suggests that they have different effects in their origin and their terminus. How is spatiotemporal specificity achieved by these factors?

Cell type-specific expression maps and single-cell sequencing have revealed key markers of identity (Birnbaum et al. 2005, Brady et al. 2007, Efroni & Birnbaum 2016, Efroni et al. 2016, Li et al. 2016, Slane et al. 2014). As precision in these technologies continues to improve, it will be possible to track the gene expression program of a cell as it transitions to a new state (Efroni et al. 2016). A number of transcription factors in the root meristem have a graded expression, which could establish a dose-dependent progression of differentiation (Wendrich et al. 2017). This graded expression could also be distance dependent, communicating position within a cell file. In the case of mobile transcription factors, protein levels can differ between the cells in which they are actively expressed and the cells into which they move. Recent advances in *in vivo* microscopy have enabled the measurement of diffusion and protein binding dynamics of SHR as it moves into the endodermis stem cell to trigger asymmetric cell division (Clark et al. 2016, Long et al. 2017). As described above, SHR plays numerous roles in root development and is active in several cell types. The big outstanding question is how the same transcription factor differs in form or activity across cell types. As our questions continue to become more specific, we will gain new mechanistic insights into the fundamentals of cell identity.

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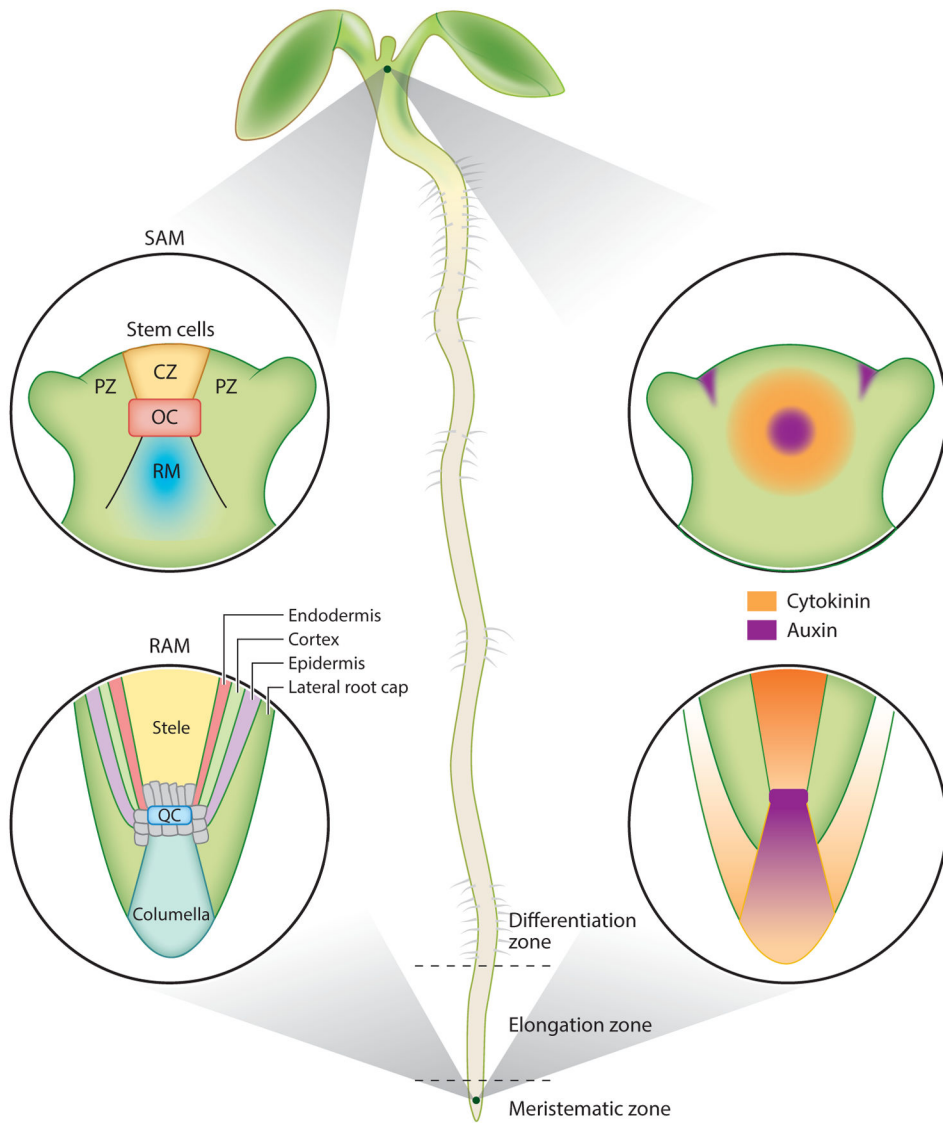


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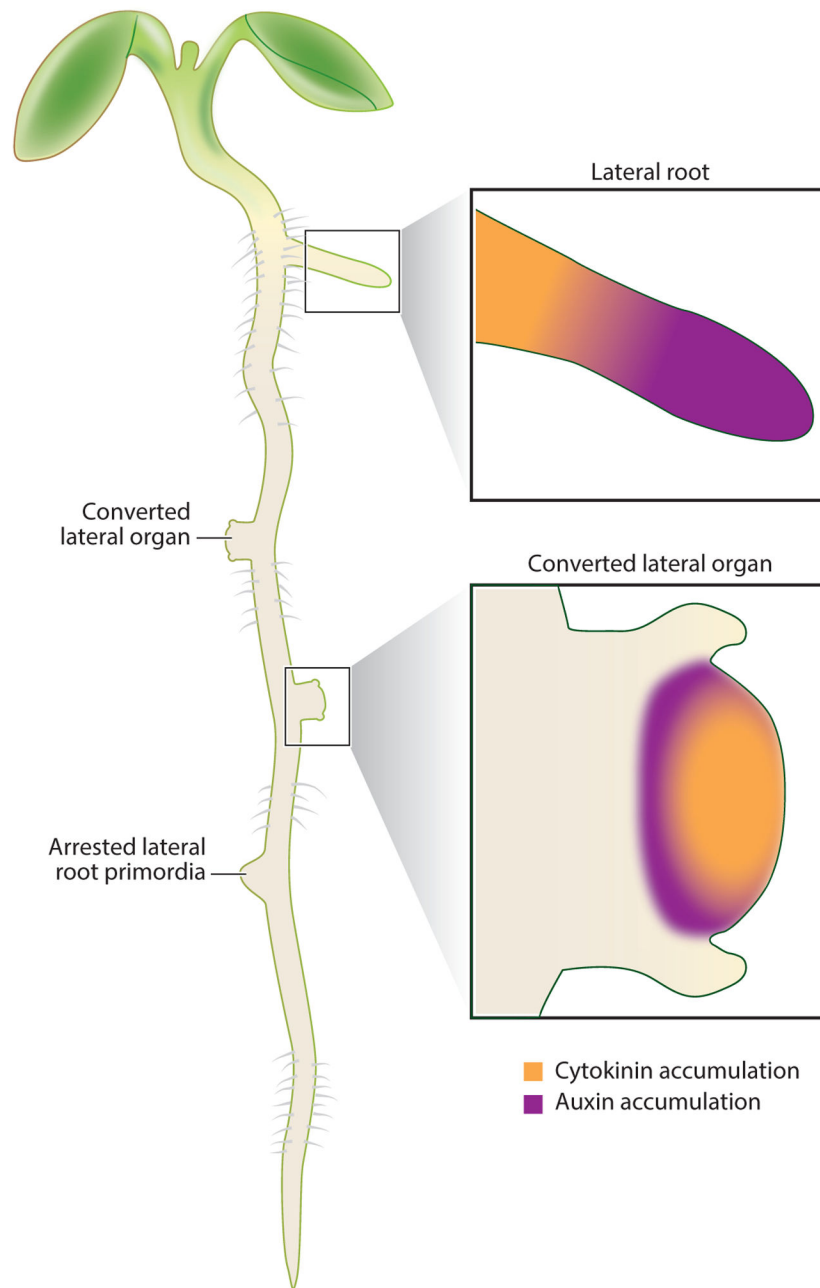


### INTERCELLULAR TRAFFICKING THROUGH PLASMODESMATA

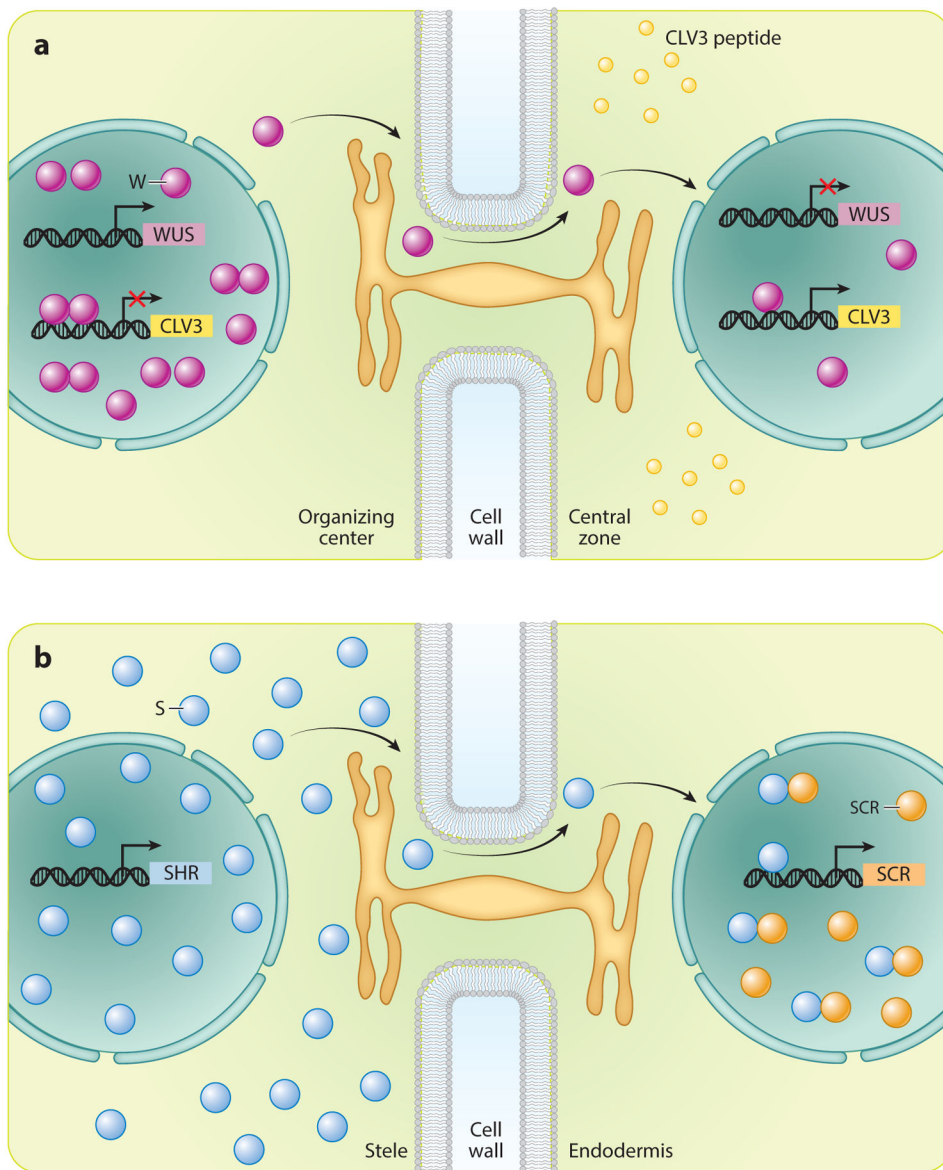
Approximately 30% of transcription factors in *Arabidopsis* are mobile, and there are examples of both transcription factors and small RNAs moving through plasmodesmata to regulate development in plants (Hantke et al. 1995, Lucas et al. 1995, Nakajima et al. 2001, Sessions et al. 2000, Vatén et al. 2011). Passage of signaling molecules can occur through the cell wall matrix or directly through cytoplasmic connections termed plasmodesmata. Plasmodesmata are plasma membrane-lined channels containing a thread of endoplasmic reticulum. Relative to the analogous structure in animals termed gap junctions, plasmodesmata are massive, at a size exclusion limit of 10 kDa and a diameter of 50–60 nm (Kim et al. 2002, Robards & Lucas 1990). There is also evidence that plasmodesmata are selective, allowing passage of some molecules and not others (Hantke et al. 1995, Lucas et al. 1995, Sessions et al. 2000). Recently, a study of how plasmodesmata change during cell maturation showed that plasmodesmata in recently divided cells have almost no space between their plasma membrane and endoplasmic reticulum connections (Nicolas et al. 2017). As cells elongate during maturity, the gap between the plasma membrane and endoplasmic reticulum widens, potentially to accommodate larger molecules (Nicolas et al. 2017).



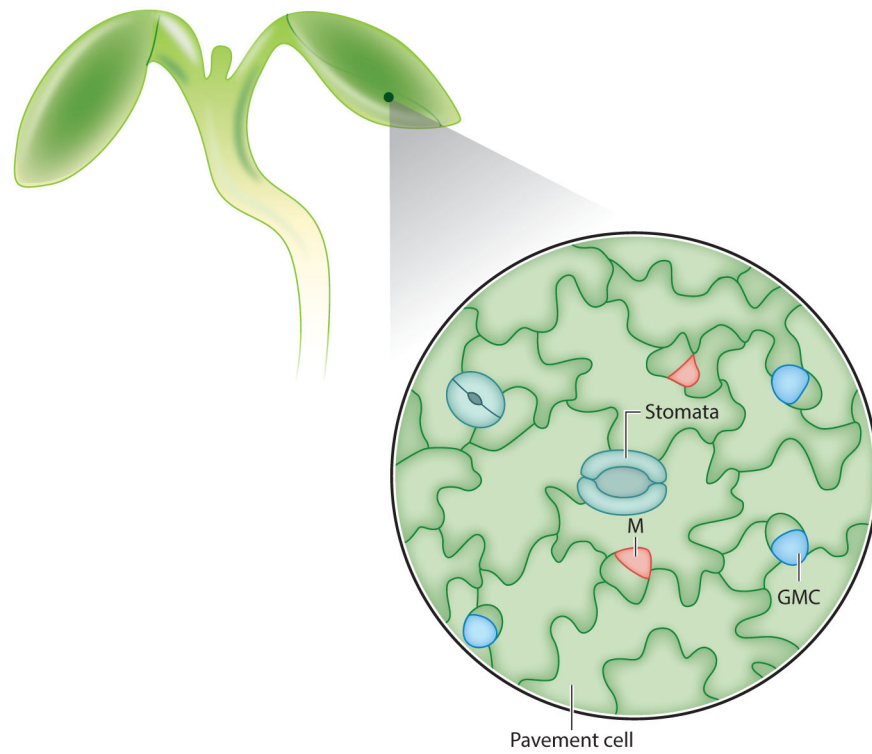
**Figure 1.** An *Arabidopsis* seedling depicting the developmental organization of (*moving counterclockwise from top left*) the shoot apical meristem (SAM), root apical meristem (RAM), root developmental zones, and hormone domains of the RAM and SAM. (*Top left*) The SAM is positioned by the central organizing center (OC), which maintains the stem cells in the overlying central zone (CZ). Cells differentiate into either leaf or shoot cells as they are displaced into the peripheral zone (PZ) or the rib meristem (RM). (*Bottom left*) The quiescent center (QC) maintains the surrounding stem cells (*gray*) that give rise to all the tissues in the root. (*Bottom center*) Actively dividing cells are found in the meristematic zone. The transition to differentiation starts in the elongation zone when cells exit the mitotic cycle and enter a period of endoreduplication. Cells acquire their specialized features, such as root hairs, in the differentiation zone. (*Right*) The hormones auxin and cytokinin establish juxtaposed signaling domains that regulate division and differentiation in the SAM and RAM.



**Figure 2.** Hormonal treatment can convert lateral root primordia into shoot primordia and switch the characteristic auxin-cytokinin gradient of a root within a short developmental window. Presented is a schematic of how lateral root primordia responded to hormonal treatments by Rossopoff et al. (2017) at different developmental stages. Early lateral root primordia are aborted. In contrast, intermediate-stage lateral root primordia transition to accumulate cytokinin in the stem cell niche and grow to resemble a shoot apical meristem. Past a certain stage, lateral root primordia maintain their characteristic auxin-cytokinin domains and emerge as lateral roots.



**Figure 3.** Transcription factor movement through plasmodesmata. (a) *WUSCHEL* (*WUS*) is expressed in the organizing center, and *WUS* protein (W) moves into central zone stem cells. In those cells, W activates the expression of *CLAVATA3* (*CLV3*), a small signaling peptide that prevents *WUS* expression outside the organizing center. New evidence suggests that *WUS* dimers repress *CLV3* expression in the organizing center. (b) *SHORTROOT* (*SHR*) is expressed in the stele, where it localizes to both the nucleus and cytoplasm. *SHR* protein (S) moves into the endodermis, where it activates the expression of *SCARECROW* (*SCR*). Physical interaction with *SCR* sequesters S to the nucleus, preventing further movement.



**Figure 4.** Leaf epidermal cells in various stages of stomatal development. A meristemoid mother cell undergoes the first asymmetric division to produce a meristemoid (M), which continues to divide asymmetrically until it converts into a guard mother cell (GMC). The GMC divides symmetrically to form the two guard cells of the stomatal pore. Each stoma is separated by puzzle piece–shaped pavement cells.