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Review article

Asymmetry and cell polarity in root development

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ABSTRACT

Within living systems, striking juxtapositions in symmetry and asymmetry can be observed and the superficial appearance of symmetric organization often gives way to cellular asymmetries at higher resolution. It is frequently asymmetry and polarity that fascinate and challenge developmental biologists. In multicellular eukaryotes, cell polarity and asymmetry are essential for diverse cellular, tissue, and organismal level function and physiology and are particularly crucial for developmental processes. In plants, where cells are surrounded by rigid cell walls, asymmetric cell divisions are the foundation of pattern formation and differential cell fate specification. Thus, cellular asymmetry is a key feature of plant biology and in the plant root the consequences of these asymmetries are elegantly displayed. Yet despite the frequency of asymmetric (formative) cell divisions, cell/tissue polarity and the proposed roles for directional signaling in these processes, polarly localized proteins, beyond those involved in auxin or nutrient transport, are exceedingly rare. Indeed, although half of the asymmetric cell divisions in root patterning are oriented parallel to the axis of growth, laterally localized proteins directly involved in patterning are largely missing in action. Here, various asymmetric cell divisions and cellular and structural polarities observed in roots are highlighted and discussed in the context of the proposed roles for positional and/or directional signaling in these processes. The importance of directional signaling and the weight given to polarity in the root-shoot axis is contrasted with how little we currently understand about laterally oriented asymmetry and polarity in the root.

Contents

1. Introduction

The organization of living systems often displays fascinating dichotomies between symmetry and asymmetry within structures. With increased resolution one can often find asymmetry underlying cells and tissues with apparent symmetrical organization. At the cellular level, asymmetry can occur in cell shape, function and distribution of cellular constituents including organelles and proteins, all of which contribute to a cell's polarity. Polarity in its broadest definition is any asymmetry between two

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or more axes. The consequences of polarity can be observed in many aspects of growth and development and viewed at many scales in biology. A cell can be described as having polarity when aspects of its development or function occur preferentially at one axis or in one direction. Additionally, polarity can also describe a process that occurs in along one axis of a tissue or organism, but in either direction (Sachs, 1991).

In multicellular eukaryotes, cell polarity and asymmetry are essential for diverse cellular, tissue, and organismal level function and physiology, including response to the external environment. These attributes are particularly crucial for developmental processes, such as body plan establishment and tissue system formation (Abrash and Bergmann, 2009; Scheres and Benfey, 1999; Menke and Scheres, 2009). Asymmetric cell divisions provide a foundation for differential cell fate specification in tissue and organ patterning and regeneration and in subsequent physiological processes. In development, asymmetric cell divisions often generate two daughter cells of distinct fates. These fates may arise due to distinct intrinsic or extrinsic properties of the daughter cells (Petricka et al., 2009; Horvitz and Herskowitz, 1992). How cells proliferate, grow, and differentiate in a coordinated manner to form highly reproducible tissues and organs is a key question in developmental biology.

These fundamental questions inspire biologists characterizing development in diverse species. Despite vast leaps in imaging technology and molecular and genomic tools, questions posed more than 25 years ago continue to drive research in developmental systems. In 1991, Sachs (1991) discussed cell polarity and asymmetry, raising the following fundamental question:

> *"Cell polarization is the specialization of developmental events along one orientation or one direction. Such polarization must be an early, essential stage of tissue patterning. The specification of orientation could not occur only at the level of the genetic system and it must express a coordination of events in many cells".*
>
> *"From the point of view of pattern formation a central question raised by polarity is how the orientation and direction of the cells is specified, and specified in ways which integrate the cells of entire tissues".*

In plants, where development is primarily postembryonic and continuous, developmental cues are integrated with environmental stimuli. Because plant cells are immobile, surrounded by rigid cell walls, the amazing variety of form and structure observed in plant organs is driven by precisely controlled, oriented cell division and cell expansion. In plant systems, asymmetric cell divisions are the foundation of pattern formation and differential cell fate specification (De Smet and Beeckman, 2011). Thus, cellular asymmetry is a key feature of plant biology and arguably nowhere in the plant are the consequences of asymmetries in cell divisions, structure, and function more elegantly displayed than in the root.

2. Asymmetric cell divisions in root development

2.1. Formative divisions in the root meristem and stem cell niche

Roots have an overall cylindrical appearance with their outermost tissues, the epidermis and ground tissues, organized as concentric cylinders stacked within each other (Fig. 1A, B). These tissues surround the stele, which includes the vascular tissues, xylem and phloem, surrounded by the pericycle cell layer (Dolan et al., 1993). The radial symmetry of the root's overall external appearance and its outermost tissue layers are juxtaposed with the bilateral symmetry of the tissues in the stele (Fig. 1B) (Dolan et al., 1993; Parizot et al., 2008). The continuous formation of root cell types occurs through asymmetric (formative) divisions within the stem cell niche. Within the root stem cell niche, there is an organizing center comprised of a small number of cells that divide infrequently and are thus termed the quiescent center (QC) (Fig. 1C). Immediately surrounding the QC are initial (or stem) cells that give rise to all root cell/tissue types through asymmetric cell divisions (Fig. 1A) (Dolan et al., 1993; van den Berg et al., 1997). These asymmetric cell divisions give rise to daughter cells that will undergo amplifying divisions within the meristematic zone (Fig. 1A). These cells will then undergo rapid cell elongation and, finally, in the differentiation zone, acquire the specialized cellular features and functions unique to each cell type (Dolan et al., 1993).

In *Arabidopsis*, the four major cell types of the root, the vascular, ground, columella and dermal tissues have dedicated initial cells. The initial cells are immediately adjacent to and in physical contact with the QC (Fig. 1). They undergo asymmetric cell divisions to maintain the stem cell population and produce daughter cells that will differentiate into each of the root cell types (Scheres and Benfey, 1999). These cell divisions are specifically oriented in order to produce cells at precise positions in the root (Fig. 1D–F) (Dolan et al., 1993; Scheres and Benfey, 1999). Oriented cell divisions are classified by their position with respect to the root's surface, with periclinal divisions occurring parallel to roots surface and anticlinal divisions occurring perpendicular to the root's surface. Cells of the vasculature develop through asymmetric divisions of the vascular cambium where periclinal cell divisions towards the interior or exterior (not shown) produces a daughter cell that will differentiate into xylem or phloem cell types, respectively (De Rybel et al., 2016; Scheres et al., 1994). The columella initials conduct anticlinal divisions, such that daughter cells are positioned more rootward of the QC (Fig. 1F). The ground tissue executes a series of oriented divisions, first, anticlinally, to produce a daughter cell more shootward of the QC. Then the daughter cell will re-orient its division plane and divide periclinally to produce two cell layers, endodermis towards the inside and cortex towards the outside. The endodermis will maintain division potential and divide periclinally again to produce the secondary (or middle) cortex layer (Fig. 1D) (Baum et al., 2002). The cell division resulting in middle cortex formation occurs in the meristematic zone shootward of the stem cell niche. The initial cell that gives rise to the lateral root cap and the epidermal tissues also undergoes a series of asymmetric divisions to produce each of these cell types in the correct position. These alternating divisions will produce epidermal cells in a more shootward direction and cells of the root cap more laterally (Fig. 1E) (Dolan et al., 1993; Scheres et al., 1994; Duckett et al., 1994).

In the *Arabidopsis* root, the orientation of division planes in initial cells and their daughters is exquisitely ordered and precise; the regularity of cell division orientation lends predictability and relative ease of phenotyping cellular defects in the root. A further advantage of the root as a developmental model organ is its accessibility and continuous development over time. Despite this and extensive work by an ever increasing group of researchers interested in root development, the mechanisms modulating how these formative cell divisions achieve this level of order and precision in remain largely unclear. While some cell autonomous factors (e.g. transcription factor activity within specific initial cells) have been identified (Moreno-Risueno et al., 2012; Petricka et al., 2012), cell ablation experiments revealed a critical role for intercellular signaling in stem cell maintenance and for daughter cell differentiation, yet the molecular mechanisms underlying these observations remain largely obscure (see below).

2.2. Asymmetric cell divisions in lateral root development

As root systems develop and grow they form branched networks through the iterative formation of lateral roots (or

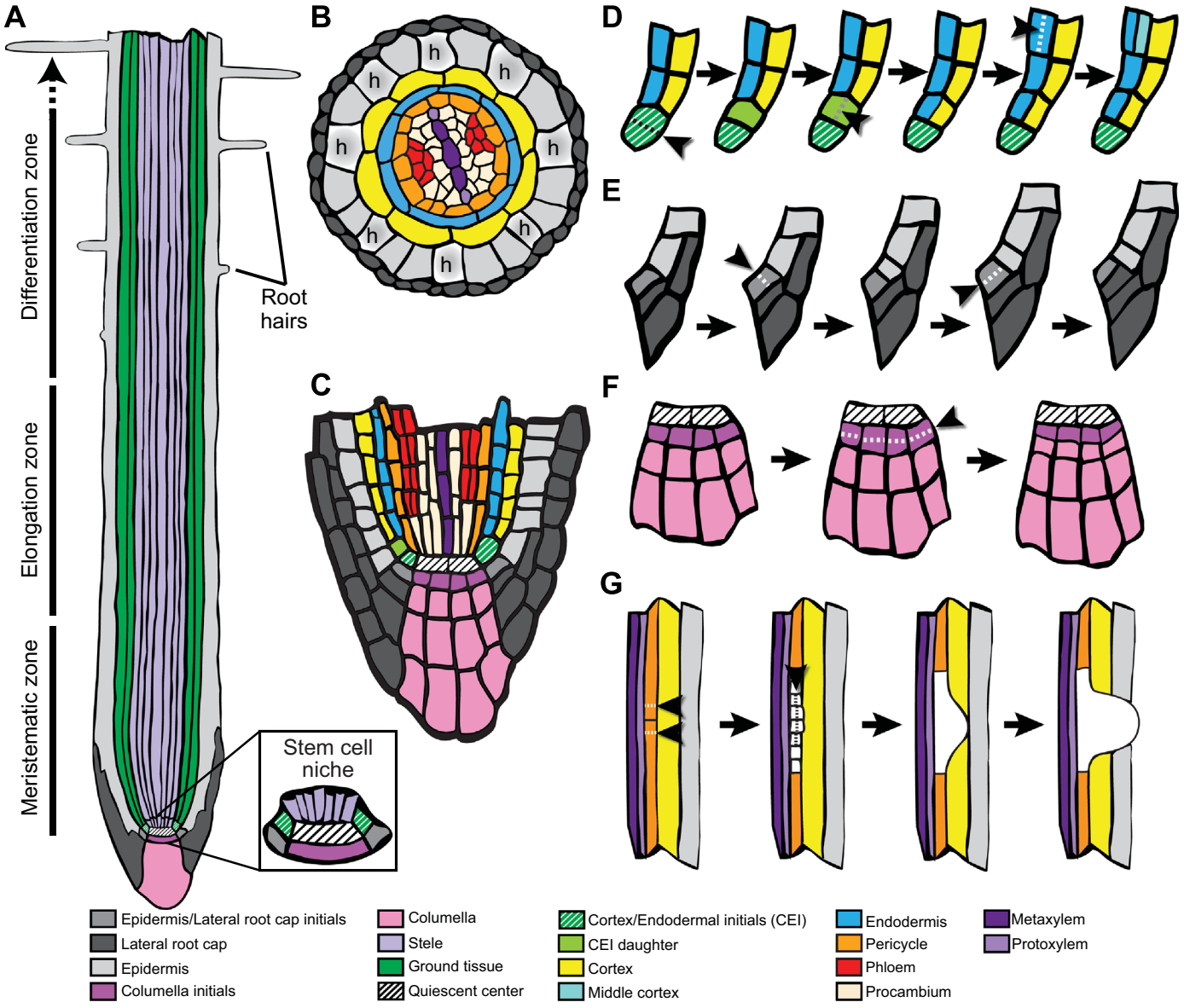

Fig. 1. Schematics showing root organization and asymmetric cell divisions in the stem cell niche in *Arabidopsis*. (A) Median longitudinal cross section showing the major tissue groups, developmental zones and the stem cell niche. In the meristematic zone, cells are actively undergoing formative and amplifying divisions; in the elongation zone, cells stop dividing and begin rapid elongation; finally, in the differentiation zone, cell stop elongating and acquire specialized cellular features, such as root hairs. (B) Transverse cross section through the meristematic zone of a root showing the radial symmetry of the three outer cell layers and the bilateral symmetry of the stele tissues; (h) indicates hair cells in the epidermal layer. (C) Median longitudinal cross section of the root apex. (D) Formation of the cortex and endodermal cell layers from a shared initial cell. First the cortex/endodermal initial cell undergoes an asymmetric cell division in the anticlinal orientation, then the daughter cell undergoes an asymmetric cell division in the periclinal orientation to form endodermis towards the inside and cortex towards the outside. Later in development, the endodermis will undergo a further asymmetric cell division to produce the middle cortex. (E) Formation of the epidermal and lateral root cap cell layers from a shared initial cell. The initial will undergo an asymmetric division in the anticlinal direction to form epidermal cells shootward of the QC and then reorient the division plan and divide perclinally forming lateral root cap cells towards the lateral periphery of the root tip. (F) Asymmetric division of the columella initial cells occurs in the anticlinal direction generating cells rootward of the QC. (G) Formation of a lateral root primordium. Lateral root founder cells are specified within the pericycle layer in cells located at the xylem axis. These founder cells will undergo anticlinal, asymmetric cell divisions and then the centermost cells, will undergo a division that is oriented in the periclinal direction generating a primordium with two cell layers. The primordium will become ellipsoid-shaped through further divisions and cellular morphogenesis and finally, emerge from the parent root tissues. Arrows (D–G) indicate the developmental progression of each tissue type and arrowheads (D–G) indicate asymmetric cell divisions (also indicated by dotted lines).

branches) along a root's longitudinal axis. Asymmetric (formative) cell divisions have a foundational role in the formation of lateral root primordia. The first asymmetric cell division in this process occurs in a pair of lateral root founder cells, which are specified from cells of an internal cell layer called the pericycle that surrounds the vasculature. These founder cells are specifically located at positions adjacent to the xylem poles (Fig. 1B) (Dubrovsky et al., 2000; De Smet et al., 2007). Founder cells undergo an asymmetric cell division to produce a single layered primordium comprised of small cells (Fig. 1G). Subsequently, the centermost cells of the primordium re-orient their division plane, dividing periclinally to generate a primordium with two cell layers (Malamy and Benfey, 1997). Through successive rounds of cell division and oriented cell expansion, an ellipsoid-shaped primordium is formed within and eventually emerges from the parent root tissues (Péret et al., 2009; von Wangenheim et al., 2016). The series of asymmetric cell division in the early stages of lateral root development are key to the *de novo* formation of the stem cell niche within the new

primordium.

The regular pattern of cell divisions in the root meristem lead to early predictions that clonal relationships were principal in cell fate specification, instead, it was shown that positional information is a key cell fate determinant (see below). Observations suggest that positional, often directional, signaling is key to the division itself or differentiation of the resulting daughter cells, yet our understanding of how this positional information is generated, perceived and/or transduced remains mysterious. Additionally, a role for directional or positional information in cell division and fate specification implies that cells generating and/or perceiving these signals display polarity in their cellular features or functions.

3. Cell polarity in the root

Cell polarity typically refers to differences between regions or "sides" of a cell in structural and/or functional aspects of a given cell. Examples of various types of cell polarity can be found within the root tissues. Because of the ordered structure of the root, a cell's axes can be clearly delineated. In the radial axis, the cellular domain nearest the vasculature is described as the inner (or central) polar domain and the domain nearer to the soil interface is defined as the outer (or peripheral) domain (Fig. 2A, B) (Alassimone et al., 2010; Langowski et al., 2010). While in the longitudinal axis, the root and shoot tips (apices) are used for orientation, with the surface of the cell nearer to the root tip being the rootward (or basal) domain and the surface nearer to the shoot being the shootward (or apical) domain (Baskin et al., 2010).

3.1. Polarity in the longitudinal axis: auxin transporter localization

Study of the rootward-shootward (basal-apical) polar domains of root cells is dominated by the examination of proteins involved in the polar transport of the plant hormone auxin (Friml et al., 2003; Baster and Friml, 2014). A role for auxin has been found for nearly every plant process that can be encompassed by the terms growth and development (Vanneste and Friml, 2009; Kazan, 2013); yet, how this ubiquitous signaling molecule achieves specificity in signaling is frequently unclear and is likely a consequence of the existing cellular environment. Localized changes in a cell's or tissue's transcriptional response to auxin is often used as a proxy for changes in auxin levels and these changes are further linked with basic processes, such as cell proliferation and/or morphogenesis (De Smet et al., 2007; Laskowski et al., 2008). When the levels of auxin itself change within a cell or tissue this change can be due to altered auxin synthesis or catabolism (Ljung, 2013), but is largely attributed to changes in the directional, transcellular transport of auxin (Teale et al., 2006; Petrásek and Friml, 2009). It should be noted that when discussing the polarity of auxin transport, polarity does not necessarily indicate a preferential direction of transport, and instead often refers to transport occurring preferentially along one axis, without regard for direction (Sachs, 1991).

Polar auxin transport and therefore, the variable distribution of auxin, is mediated by a family of efflux carriers, called PIN-FORMED (PIN) proteins, which, in roots, most frequently show polar localization to the rootward or shootward polar domains (Wisniewska et al., 2006). However, PINs can also be found in lateral polar domains or with nonpolar localization depending on cell type and developmental context. For example, PIN2 is localized to the shootward plasma membrane domain in epidermal cells, but in the adjacent layer, the cortex, PIN2 is localized to the rootward domain (Müller et al., 1998). Upon ectopic expression in the QC and endodermis, PIN2 is nonpolar in the QC and in the endodermis is localized to the rootward polar domain in the

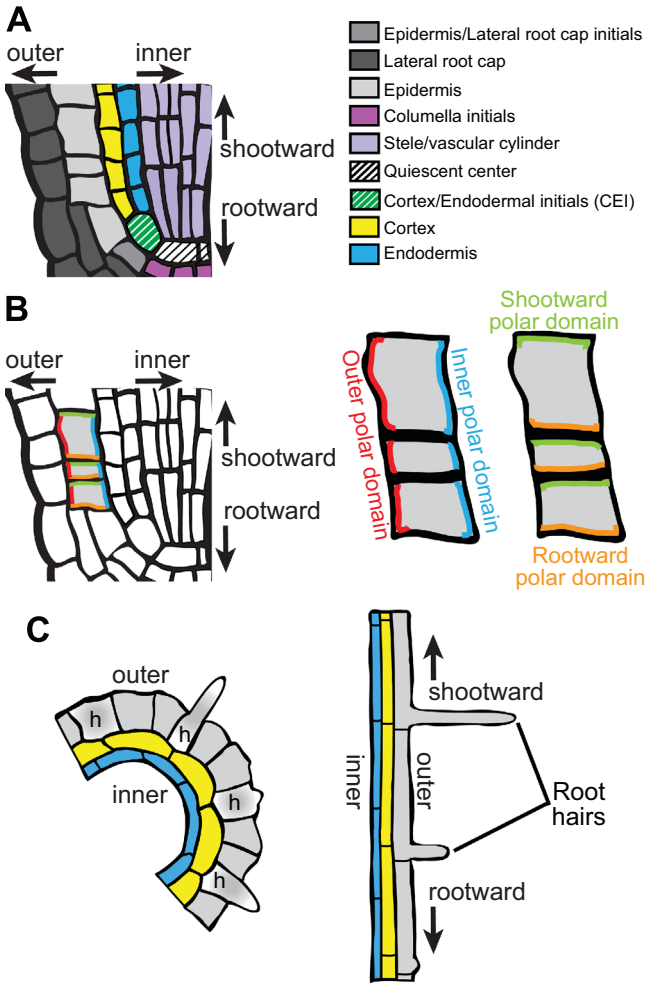

Fig. 2. Schematics showing organ axes, cellular polar domains and epidermal patterning. (A) Portion of a median longitudinal cross section of the root apex showing the axes of the root, the inner and outer and rootward and shootward polar domains. (B) Details of the cellular polar domains in the root using epidermal cells for illustration. Polar domains in the context of the organ (left) and isolated, enlargement the polar domains, inner (blue) and outer (red) at center and rootward (orange) and shootward (green) at right. (C) Portions of cross sections of outermost cell types showing epidermal patterning and root hair outgrowth. (left) Transverse cross section, (h) indicates hair cells. Note that hair cells are positioned over two adjacent cortex cells. (right) Longitudinal cross section; note that root hairs form at the rootward edge of hair cells.

meristematic zone, the shootward polar domain in the elongation zone and finally, to the inner polar domain in the differentiation zone (Alassimone et al., 2010). PIN3 and PIN7 are nonpolar in the columella, but upon gravistimulation, polarize in the direction of the gravity vector (Friml et al., 2002; Kleine-Vehn et al., 2010). Thus, PIN protein localization involves mechanisms influenced by cellular context, features of individuals PINs, and/or intrinsic or extrinsic cues. The cellular mechanisms regulating PIN localization have been extensively studied and reviewed (Löfke et al., 2013; Luschnig and Vert, 2014; Pan et al., 2015; Baster and Friml 2014; Habets and Offringa 2014), and thus are only briefly touched upon here.

A primary mechanism of trafficking PINs to the plasma membrane is mediated by an ADP-ribosylation factor (ARF) GTPase guanine-nucleotide exchange factor (ARF-GEF) called GNOM, which mediates exocytic protein trafficking (Richter et al., 2010; Geldner et al., 2004, 2003). Although *gnom* mutants have severe phenotypes that reflect defects in polar auxin transport, PIN proteins are still localized to the plasma membrane in these mutants (Steinmann et al., 1999). This suggests that localization of PINs to

the plasma membrane occurs via multiple routes. In support of this, a role for the exocyst complex in PIN trafficking has been uncovered (Drdová et al. 2013). This complex is highly conserved throughout eukaryotes, and functions in vesicle fusion at the plasma membrane. In PIN trafficking specifically, the exocyst complex appears to function in moving PINs between endocytic compartments and plasma membrane domains. PIN localization can also be modified by phosphorylation status, which is mediated by the PINOID (PID) protein kinase and its family members, where PID activity can alter PIN polarity within a given cell (Friml et al., 2004). However, PIN polar localization based on phosphorylation status is not entirely dependent on PID activity, as *PIN* alleles that are phosphomimetic or dephosphomimetic are still able to accumulate at the shootward or rootward domains, respectively, regardless of PID activity (Zhang et al., 2010; Huang et al., 2010).

Once polar localization of PINs is established in a cell or tissue, it is maintained by restricting PIN mobility in the plasma membrane and through endocytosis specifically at the edges of the polar domain (Kleine-Vehn et al., 2011). Components of the retromer complex, an evolutionarily conserved complex that functions in recycling and retrograde transport of cargo, are important for balancing PIN recycling back to the plasma membrane and to the lysosome for degradation (Jaillais et al., 2007; Kleine-Vehn et al., 2008). In addition to all of the intracellular factors, the plant cell wall has also been shown to function in maintaining polar localization of PIN and other transmembrane proteins (Feraru et al., 2011; Martinière et al., 2012; Martinière and Runions, 2013). Finally, a positive feedback system is proposed between polar auxin transport and PIN localization, whereby PIN polarization directs polar auxin transport, which re-enforces the polar localization of PINs (Paciorek et al., 2005; Vieten et al., 2005). The presence of a positive feedback system necessitates that the mechanisms needed to disrupt or reorient the direction of auxin transport be decisive, but also flexible, particularly as it is often the change in auxin distribution or auxin response that is linked to key developmental events. The interplay between different PIN localization patterns based on changes in phosphorylation status implies that an as yet undefined component is required mediate these changes. It is clear that the directional (polar) transport of auxin is a highly sensitive, rapid response system by which plants can signal changes in the endogenous or exogenous conditions throughout a tissue or organ.

3.2. Polarity in the radial axis: nutrient transporter localization

An essential function of roots is the uptake of water and nutrients from the environment for transport throughout the plant body. Water and nutrients must move inward through the outer tissue layers to the vasculature for transport. In roots, there are three paths for radial transport, the passive, apoplastic, path through the spaces in and between the cell walls. Then two active paths, the first through direct cell-to-cell connections (plasmodesmata) and the other, a transcellular path, comprised of protein channels and transporters at the plasma membrane. As the root differentiates, a structure called the Casparian strip forms at the transverse surfaces of endodermal cells (reviewed in Barberon and Geldner (2014)). The Casparian strip is a lignified feature in the cell wall of differentiated endodermal cells that blocks the apoplastic path, thereby forcing symplastic or transcellular transport at this point (Naseer et al., 2012; Alassimone et al., 2010; Robbins et al., 2014). Recent work has revealed exciting insights into lateral cell polarity as a key mechanism for the radial, transcellular transport of nutrients.

In the regulation of nutrient movement, transport protein abundance at the plasma membrane is important, however, the intracellular positioning or polar localization of some transporters has emerged as an important mechanism in controlling both the level and directionality of nutrient movement. Nutrient transporters that are polarly localized within a cell are specifically positioned in the inner (central) or outer (peripheral) plasma membrane domains and an inverted localization pattern between pairs of transporters reflects their functions either as influx or efflux carriers. Excellent reviews dedicated to the polarity of nutrient transporters have recently been published (Barberon and Geldner, 2014; Zelazny and Vert 2014), therefore examples are only highlighted here.

In rice root cell types, the silicon influx channel, LOW SILCON RICE 1 (LSI1) is localized to the outer polar domain, while the silicon efflux channel, LOW SILICON RICE 2 (LSI2) is localized to the inner polar domain (Ma et al., 2006, 2007). In *Arabidopsis* roots, the acquisition and transport of boron is mediated through uptake channel, NODULIN26-LIKE INTERNSIC PROTEIN5;1 (NIP5;1), which is localized to the outer polar domain in epidermal cells (at the soil interface) and its counterpart, a borate exporter (BOR1) is localized to the inner polar domain (Alassimone et al., 2010; Takano et al., 2010). Additionally, IRON-REGULATED TRANPORTER 1 (IRT1), which is involved in the uptake of iron and other metals from soil, is found at the outer polar domain in epidermal cells (Barberon et al., 2014). How iron is then transported across the other root tissues to the vascular is unclear; as a counterpart efflux carrier positioned at the inner plasma membrane is predicted (Dubeaux et al., 2015), but has not yet been identified.

Despite the necessity for transcellular movement imposed by the Casparian strip, polar localization of these transporters is observed in diverse cell types; additionally, these transporters are consistently localized to their respective lateral polar domains upon ectopic expression and prior to or in the absence of Casparian strip formation (Alassimone et al., 2010; Pfister et al., 2014). This suggests that transcellular transport is not merely employed at the Casparian strip, but is used more globally across root tissues. Additionally, these observations indicate that the barrier function of the Casparian strip itself doesn't impart lateral polarity or create its appearance due to partitioning of the plasma membrane. The polarity of nutrient transporters exposes not only mechanisms of nutrient transport, but essential roles for cell polarity in function and physiology at the cell, tissue, and organ level. Unlike the rootward-shootward polarity of the PIN proteins, the mechanisms involved in the establishment and maintenance of laterally polar proteins do not appear to involve the known components of root-shootward targeting and, thus, remain somewhat unclear (Alassimone et al., 2010; Takano et al., 2010). Additionally, several proteins localized to lateral polar domains appear undergo targeted secretion and are not repositioned to a polar domain by vesicle recycling at the plasma membrane (Langowski et al., 2010). Understanding the similarities and differences between how cells establish and maintain proteins at each polar domain will provide insight into fundamental questions about cellular asymmetry and provide firm links between these asymmetries and cellular function.

3.3. Structural polarity of root hairs

Roots also exhibit a prime example of polarized cell growth and cell structure in plants. In the longitudinal axis of *Arabidopsis* roots, epidermal cells are clearly delineated into files of hair (trichoblast) and nonhair (atrichoblast) cells (Dolan et al., 1993). Epidermal cells that have hair cell fate will initiate the formation of a root hair as they differentiate (Fig. 1A). In *Arabidopsis*, root hairs can be about 1 mm in length and are thought to increase the root's surface area and function uptake of water and nutrients. These hairs are the result of polarized (tip) growth of a single cell (Grierson et al., 2014), which are initiated at the rootward-most region of the hair cell (Fig. 2C) (Carol and

Dolan, 2002). How this rootward site of root hair initiation is determined is not entirely clear, but it may involve the directional transport of the plant hormone auxin and differential accumulation of auxin in hair and nonhair cells (reviewed in Balcerowicz et al. (2015)). The initiation of a root hair on the rootward outer surface of a hair cell initially appears as a bulge due to localized loosening of the plant cell wall. Subsequently, tip growth begins and through rapid, local cell expansion a long single-celled projection is extended outward from the root's surface (Carol and Dolan, 2002; Grierson et al., 2014). In addition to the polar growth and structure of root hairs, the patterning of hair and nonhair cells is spatially regulated by positional information from the underlying cortex layers (Grierson et al., 2014) (Fig. 2C, discussed below). Thus in this one cell type, a complex interplay between polarity in the lateral and root-shootward axes appears to be crucial to tissue patterning and cellular differentiation.

4. Positional information and directional signaling in root development

The regular orientation of cell division planes in the root appeared to suggest that cell types were defined by rigid clonal relationships and that a prepattern was established in the plant embryo, which was then maintained during postembryonic root development (Dolan et al., 1993; Scheres et al., 1994). In other words, it was predicted that cell fate was defined by intrinsic cues propagated from a specific initial cell to its daughters. Yet, experiments examining the consequences of cell ablation in and around the stem cell niche indicate that positional or extrinsic cues are critical for root stem cell maintenance and in cell fate specification (van den Berg et al., 1995', 1997).

Following ablation of either the QC cells or various initial cells, adjacent internal cells invade the gap left by the dead cell and divide, thereby physically replacing the missing cell. The 'invader' then adopts the fate of and functionally replaces the missing QC or initial cell as shown both by reporter gene expression and the production of daughter cells with the expected differentiation markers (van den Berg et al., 1995). It was further demonstrated that differentiation of daughter cells failed to occur if the three contacting shootward cells were ablated. This result suggests that signaling from more differentiated cells in the rootward direction, rather than cell lineage, drives cell fate in the root. Furthermore, it was found that the QC actively represses differentiation of the initial cells it is in contact with (van den Berg et al., 1997). Ablation of individual QC cells leads to expansion and differentiation of immediately adjacent initials, suggesting cell-cell communication via cytoplasmic channels (plasmodesmata) or short range signaling molecule(s). Thus, directional signaling both outward from the QC and rootward from more differentiated cells have roles in modulating root development.

The formation of lateral roots and passage cells in alignment with the xylem axis has implied roles for additional positional or directional cues in root development. As mentioned previously lateral root founder cells are specified from the pericycle cell layer, but these founder cells are specified only from pericycle cells located at the xylem pole (*Arabidopsis*) or the phloem pole (maize) (Dubrovsky et al., 2000; Hochholdinger and Zimmermann, 2008). In *Arabidopsis*, it has been proposed that an, as yet unknown, signal from the underlying xylem cells is directed outward to the adjacent pericycle cells prior to founder cell specification (De Smet et al., 2007). Additionally, two recent publications suggest that competence to form a LR also depends on local, intercellular signaling pathways. First, carotenoid biosynthesis was shown to be necessary for the formation of prebranch (competent) sites, yet carotenoid production flanks the region were competence is acquired suggesting a non-cell-autonomous carotenoid-derivative participates in this process (Van Norman et al., 2014). It was also shown that production of auxin in cell types external to and not in contact with the pericycle cell layer is also important for competence to form a lateral root primordia (Xuan et al., 2015). Yet how these signaling events might inform or permit the cellular asymmetries that give rise to the first asymmetric cell division of lateral root founder cells is unclear.

Passage cells are present in the differentiated endodermal cell layer and are aligned with the xylem pole (Geldner, 2013). Recall that the endodermis forms the casparian strip, and is then suberinized to create an apoplastic transport barrier; passage cells remain unsuberinized and serve to facilitate symplastic transport from the soil interface to the vasculature. In the cases of lateral root and passage cells, the mechanisms that serve to align these structures with the axes of the vascular tissues are unknown.

In the epidermis of Arabidopsis roots, not only do root hairs develop specifically at the rootward end of a hair cell, but hair cells are also specified at a specific position. Epidermal cells surround the cortex and the specification of hair cells occurs at positions overlaying two adjacent cortex cells, which produces an alternating pattern of hair and nonhair cells (Figs. 1B and 2C). This pattern is specified during embryogenesis through the activity of a well-defined transcription factor network, which creates cellular dimorphisms that are detectable well before any root hairs are formed (Grierson et al., 2014; Costa and Dolan, 2000). However during postembryonic growth and as epidermal cells differentiate refinement of the expression of *SCRAMBLED*, a transmembrane receptor kinase, to the hair cells is essential for the correct pattern to be maintained (Kwak et al., 2005; Kwak and Schiefelbein, 2008). It is predicted that SCRAMBLED serves as a receptor for an unknown, cortex-secreted ligand (Schiefelbein et al., 2009). This ligand may be present at higher concentrations at the junctions between two adjacent cortex cells and thereby refining *SCRAMBLED* expression to epidermal cells at this position and promoting or maintaining hair cell identity at that position.

Despite the frequency at which positional cues are implicated in developmental processes in the root, there are relatively few instances in which the cue has been concretely identified. The outward signaling mechanisms from the QC that maintain stem cells in an undifferentiated state (van den Berg et al., 1997) remain largely unknown, although a peptide ligand-receptor kinase pair has been identified in maintenance of the columella initial cells (below). The identity of the rootward moving signal(s) within a given cell type that promotes the specific cell fates of newly formed daughter cells as they exit the niche remains unclear. As are the positional and apparently directional cues that are predicted to be involved in aligning the formation of lateral roots, passage cells, and root hairs at each of their specific positions in the radial axis. While concrete evidence for the existence of these signals and their specific identities are being actively sought, the identities of several key cell-to-cell communication systems in root development have been elucidated.

The number of columella initial cells is jointly regulated by a rootward signal from the QC (Sarkar et al., 2007) and a peptide-ligand pair signaling from the differentiated columella cells. The signal from the QC is unknown, but the peptide-ligand pair is comprised of CLE40, a small CLAVATA3/ENDOSPERM SURROUNDING-RELATED peptide, and ACR4 (ARABIDOPSIS CRINKLY 4), a transmembrane receptor kinase, respectively, which promote initial cell differentiation (De Smet et al., 2008; Stahl et al., 2009). CLE40 and ACR4 are both expressed in differentiated cells of the columella where they repress expression of a transcription factor that promotes stem cell proliferation. The balance between these signals limits the columella initials to a single layer of cells.

Mobile transcription factors and microRNAs are used in plants for cell-cell communication and the root provides a fascinating example of their sequential use as positional cues that signal from inside-out and then outside-in to pattern various tissues (Van Norman et al., 2011). The SHORT ROOT (SHR) transcription factor is required for normal development of the xylem cell types and the formation of the ground tissue layers and, yet, *SHR* mRNA is expressed exclusively in the stele (Carlsbecker et al., 2010; Helariutta et al., 2000). The formation of the ground tissue layers requires the SHR protein to move outward one cell layer into the QC, cortex-endodermal initial cells and endodermis (Nakajima et al., 2001). Specifically, SHR is required for asymmetric division of the cortex/endodermal initial cell, for specification of endodermal cell fate and later, for the asymmetric division that gives rise to middle cortex (Fig. 1D) (Paquette and Benfey, 2005; Cui et al., 2007; Nakajima et al., 2001; Koizumi et al., 2012). The movement of SHR is highly regulated both in terms of its movement out of the stele and the prevention of its further movement (Gallagher et al., 2004; Gallagher and Benfey, 2009; Cui et al., 2007). Furthermore, although *SHR* mRNA and protein are found in the stele this was, unexpectedly, not sufficient for the normal development of the xylem (Carlsbecker et al., 2010). Instead, it was observed that normal xylem development required SHR activity specifically in the endodermis. In an elegant set of experiments by Carlsbecker et al. (2010) it was found that endodermal SHR activates expression of a pair of microRNAs (miR165/166), which then move inward to the stele where they repress expression of their target gene (Carlsbecker et al., 2010). The resulting gradient of target gene expression in the radial axis allows for the development of two distinct xylem cell types, metaxylem towards the inside and protoxylem towards the outside of the stele (Fig. 1B). Thus the outward movement of SHR followed by the inward movement of miR165/166 is necessary to non-cell-autonomously direct the formation of ground tissue and xylem cell types.

4.1. Summary and perspectives

The importance of positional and/or directional signaling in plant and root development is predicted by many observations, yet relatively few signaling pathways and their components have been concretely described. The particular orientations of asymmetric cell divisions in the root stem cell niche and in lateral root formation evoke the involvement of directional signaling and cell polarity. Directional signaling is typically proposed to be achieved through the directional movement of a molecule, with two predominant examples being the variable direction of auxin movement and the outward movement of SHR protein. However, directional signaling may also be achieved through the polarized perception of a signal. In particular, one might predict that proteins with polar localization would be involved in signal secretion or perception, partitioning the plasma membrane, and/or orienting division planes. Yet, outside of those proteins involved in auxin and nutrient transport, there are very few reports of polarly localized proteins in the root. Nevertheless, proteins of this type do occur in plant systems and several have been identified in stomata development in *Arabidopsis* and maize. Stomata are pores in the epidermal layer of aerial plant organs and are formed through one of the best-characterized developmental processes in plants, which integrates asymmetric cell division, cell fate specification and cell polarity factors. This topic is reviewed in this issue of Developmental Biology by Shao and Dong (2016).

The study of tissue and cell polarity in the root has long been focused on the rootward-shootward axis by the study of PIN localization and polar auxin transport, and PIN localization is often regarded as a hallmark of cell polarity (Baster and Friml, 2014; Pan et al., 2015). While the rootward-shootward polarity of the PIN proteins is important for growth of the overall root and maintenance of the niche as a whole, it is not clear that these proteins are specifically important for orienting the asymmetric or formative cell divisions of the niche. It is particularly difficult to match the rootward-shootward polarity of PINs and auxin transport with the laterally oriented cell division planes. There is a link between increased auxin levels in the endodermis and cortex/endodermal initial cells and the occurrence of asymmetric cell divisions regulated by the SHR network (Cruz-Ramírez et al., 2012), which forges a path for the further coupling of the longitudinal auxin gradient and radial patterning factors in root development. Additionally, there are examples, such as early in embryo development, where polar auxin transport appears to drive polarity of the embryo (Vieten et al., 2005; Costa and Dolan, 2000; Friml et al., 2003); yet, how such a responsive pathway, one that shows rapid switches in the direction of polar transport even in the embryo, contributes to a stable feature like cell polarity is not entirely clear. In other words, how can a highly dynamic signal, such as auxin, act as a cue in the stable establishment and/or maintenance of cell polarity? This question is important not only in terms of a single cell type, but also in the context of coordinated polarity during organ patterning? If, as suggested by some, auxin acts as an instructive cue to direct cell polarization and/or polar cell growth (Luschnig and Vert, 2014; Pan et al., 2015), how does it achieve this in different cell types and under variable conditions of flux and directions of transport?

Reflecting on a broader question in root development, it remains unclear whether the signals involved in early embryo polarity and patterning of the root are the same as those that maintain this pattern postembryonically. A recent study found that upon root tip excision, various cell types in the stump contribute to regeneration of the stem cell niche and this regeneration occurs in a sequence matching that observed in embryonic root patterning (Efroni et al., 2016). However, establishment of tissue patterning in the embryo or following injury and maintenance of a pattern during postembryonic growth may not utilize entirely overlapping molecular mechanisms. For example, SHR is required in the embryo and root for formation of the ground tissue layers, yet in the *shr* mutant a single layer of ground tissue is formed. This indicates that the key tangential divisions in the embryo that sequentially separate the inner and outer (dermal) layers and then delineate the ground and vasculature layers persist in *shr* (ten Hove and Heidstra, 2008; ten Hove et al., 2015). Thus, SHR is not required for the specification of the primary ground tissue layer in the radial axis of the embryo, but is specifically required for the elaboration of multiple ground tissue layers. Further, the expression of *SCRAMBLED* is uniform across epidermal cells in the embryo and then its expression becomes refined to the hair cells after the seed germinates (Kwak and Schiefelbein, 2008). This refinement is predicted to depend on local differences in ligand concentration and is necessary to maintain the hair and nonhair cell pattern during postembryonic growth. These examples suggest there are distinctions between cellular and molecular mechanisms that operate in establishment and maintenance of polarity and tissue patterning in the embryo and the root, respectively.

Perhaps in the root, the polarity of PIN protein localization doesn't reflect the state or changing state of a cell's polarity and instead purely indicates the changing state of auxin transport? Given the significance of positional information and intercellular signaling in cell fate determination in plants, could cell polarity be described more as a manifestation of a cell's position and identity? Under this hypothesis, the overall, intrinsic polarity of a cell, shootward vs. rootward and inner vs. outer, remains stable regardless of the direction of auxin flow. Indeed, a cell's spatial relationship to the vasculature and soil interface does not change in the face of altered auxin transport. Given the complexity and

variety of cell-to-cell signaling mechanisms employed by plants, the potential for orienting cues beyond polar auxin transport and in addition to the root-shoot axis is considerable.

Indeed, the role of lateral polarity as a key feature of root development and function is being propelled forward by lateral movement of proteins and miRNAs and in the lateral polarity of nutrient transporters (Van Norman et al., 2011; Zelazny and Vert, 2014; Dubeaux et al., 2015). Nutrient transporter polarity and the resulting directionality of transport are important for root physiology and function, indicating that lateral polar domains are essential for plant survival. Additionally, the importance of distinct cellular morphologies between tissues at the soil interface and those at the root's interior is intuitive. And yet despite the prevalence and intuitiveness of laterally oriented asymmetric cell divisions and cellular features, it is striking to consider how little we understand about the proteins and signals that are directly involved in orienting these essential processes.

Interestingly, lateral polarity of nutrient transporters is observed not just in the root, but also in the embryo, which is prior to the functional necessity for nutrient uptake (Alassimone et al., 2010). This suggests that polar localization of nutrient transporters is driven not by the physiological necessity for nutrients, but instead may serve as a readout of existing lateral polarity or asymmetry in cells. It has been proposed that an orienting cue produced in the stele serves to orient laterally localized nutrient transporters and that this cue becomes active once the vascular tissues have been formed (Alassimone et al., 2010). With proposed mechanisms to establish and maintain the laterally polarity of nutrient transporters, it is straightforward to predict how proteins involved in directional signaling and cellular polarity or asymmetry would be polarly localized in the lateral domains. Perhaps the orienting cues for these cell divisions and cellular asymmetries are also aligned with the proposed transporter orienting cue in the stele. An orienting cue originating in the stele would succinctly allow for coordinated organization of tissues and cellular polarity around the central axis. Yet, it is unclear how different proteins would orient themselves to the inner or outer polar domains in response to the same cue. Furthermore, this model seems to predict that the stele-derived cue would be present as a gradient, with the cue being more diffuse in the outer tissues. Could such a gradient offer sufficient spatial resolution to account for the opposing polarity of influx and efflux transporters within a single cell type or would another mechanism have to be evoked? These and other questions regarding lateral polarity are currently being investigated. Although laterally localized signaling molecules in the root are largely missing in action, their discovery would begin to satisfy many long-standing predictions in the field and further our understanding of fundamental questions about the role of cell polarity in root development.

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