

Beyond the Barrier: Communication in the Root through the Endodermis¹

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The root endodermis is characterized by the Casparian strip and by the suberin lamellae, two hydrophobic barriers that restrict the free diffusion of molecules between the inner cell layers of the root and the outer environment. The presence of these barriers and the position of the endodermis between the inner and outer parts of the root require that communication between these two domains acts through the endodermis. Recent work on hormone signaling, propagation of calcium waves, and plant-fungal symbiosis has provided evidence in support of the hypothesis that the endodermis acts as a signaling center. The endodermis is also a unique mechanical barrier to organogenesis, which must be overcome through chemical and mechanical cross talk between cell layers to allow for development of new lateral organs while maintaining its barrier functions. In this review, we discuss recent findings regarding these two important aspects of the endodermis.

Soil contains water and dissolved nutrients needed for plant growth, but also holds pathogens and toxic compounds that can be detrimental to the plant. The root system, which is directly in contact with soil particles, can integrate environmental cues to adjust its development in order to optimize nutrient (Péret et al., 2011; Lynch, 2013) and water uptake (Cassab et al., 2013; Lynch, 2013; Bao et al., 2014) or avoid regions of high salinity (Galvan-Ampudia et al., 2013). Once anchored in the soil, roots must deal with the constraints of their local environment and develop specific barriers to balance uptake of nutrients, water, and interactions with symbionts with protection against detrimental biotic and abiotic factors.

In young roots, these barriers are mainly formed by the deposition of hydrophobic polymers such as lignin and suberin within the primary cell wall of the endodermis, which separates the pericycle from the cortex (Fig. 1), and of the exodermis, which lies between the cortex and the epidermis (Nawrath et al., 2013). Although formation of an exodermis is species dependent, the endodermis is a distinguishing figure of extant vascular plants (Raven and Edwards, 2001). Within this layer, two barriers (i.e. the Casparian strip and the suberin lamellae) are sequentially deposited and regulate water and nutrient movements between the inner and outer parts of the root. In this review, we discuss how the presence of these two major endodermal barriers affects communication between the different cell layers of the root. We focus on recent articles highlighting

the importance of the endodermis in this communication during various biological and developmental processes.

ENDODERMAL BARRIERS AS REGULATORS OF WATER AND NUTRIENT UPTAKE

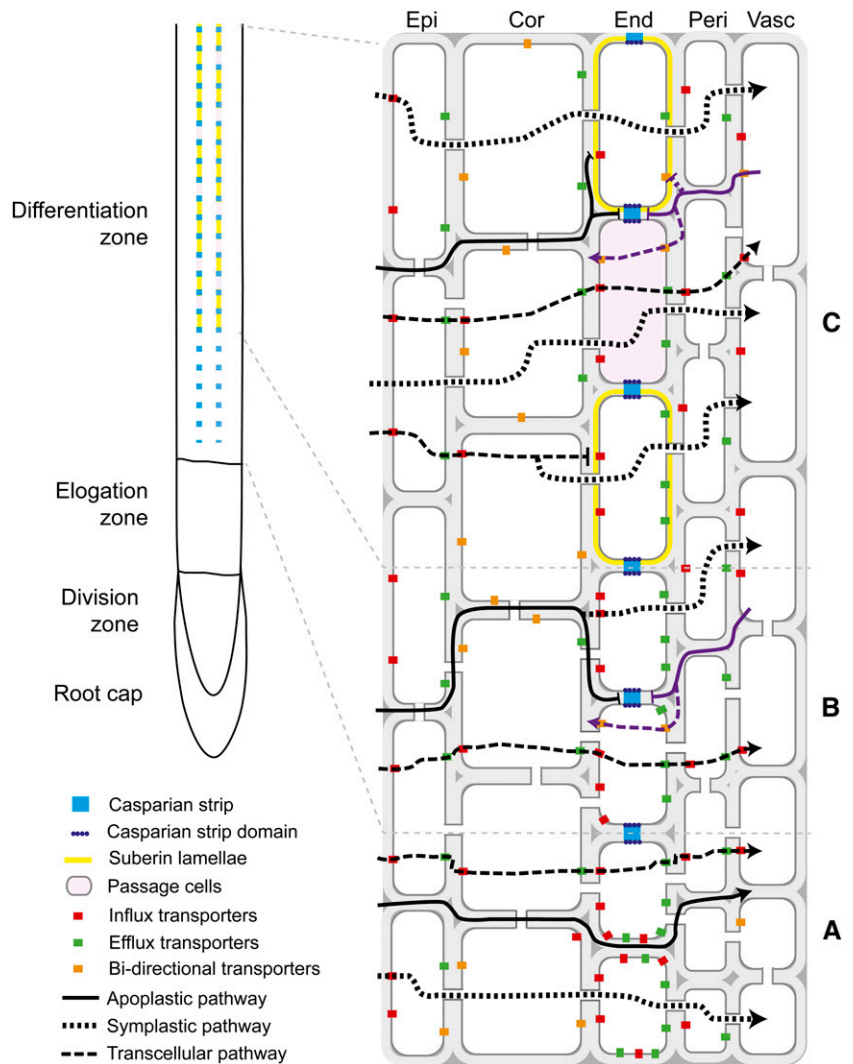
Water and solutes move between the cell layers of the root using either the apoplast or the symplast (Fig. 1). The apoplast consists of the cell walls, extracellular spaces, and the lumen of tracheary elements, whereas the symplast is the continuum of cytoplasm between cells united through plasmodesmata (Steudle and Peterson, 1998). Solutes and water can also repeatedly cross plasma membranes and tonoplasts going from cell to cell to reach the stele, a transport route usually referred to as the directional transcellular pathway (Steudle and Peterson, 1998; Geldner, 2013). The symplastic and transcellular modes are difficult to distinguish experimentally and are usually referred to as the cell-to-cell pathway. Because molecules have to cross the plasma membrane at least once in this pathway, their flow is largely controlled by the presence, number, and gating of transporters localized at the plasma membranes. For example, the expression levels, localization, and gating of aquaporin water channels have been shown to play a major role in controlling water flow within the root (Chaumont and Tyerman, 2014). However, the plasma membrane is not a perfect barrier against entry of undesirable compounds into the symplast; passive diffusion of various molecules through phospholipid bilayers can occur and movement through membrane transporters is not always specific to a single molecular species. If harmful compounds enter the symplast through these pathways, they can later be transported out of the cells or sequestered in vacuoles, preventing them from being further transported to the stele (Schaaf et al., 2006; Miwa et al., 2007). Such control does not exist in the apoplast, where molecules likely diffuse freely depending

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Figure 1. Endodermal barriers affect radial movement of water and solutes through the root. A, At the root tip, to move from the soil to the outer tissues of the root and then into the stele, water and solute molecules can use either the apoplastic (black lines), symplastic (dotted lines), or transcellular (dashed lines) pathways. B, The deposition of the Casparian strip in the endodermis prevents the free apoplastic diffusion of molecules between the outer part and the inner part of the root forcing molecules to pass through the symplast of endodermal cells. C, The deposition of suberin lamellae prevents the uptake of molecules from the apoplast directly into the endodermis forcing molecules to enter the symplast from more outer tissue layers. Suberin deposition is also likely to prevent the backflow of water and ions out of the stele. Passage cells are unsuberized and may facilitate the uptake of water and nutrients in older parts of the root. Cor, Cortex; End, endodermis; Epi, epidermis; Peri, pericycle; Vasc, vasculature. Figure redrawn and modified from Geldner et al. (2013).



on concentration gradients or are carried by bulk water flow driven by transpiration (Enstone et al., 2002). The mobility of some ions, such as calcium, can be affected by the composition of the extracellular pectocellulosic matrix (Gilliham et al., 2011).

As the root differentiates, the free apoplastic diffusion of solutes between the outer cell layers and the vasculature is prevented by the formation of an apoplastic barrier in the extracellular matrix of the endodermis called the Casparian strip. The Casparian strip is a highly localized lignin impregnation (Naseer et al., 2012) of the primary cell wall that forms a longitudinal belt-like structure in the middle of the anticlinal walls of endodermal cells (Fig. 1). This deposition is coordinated within the whole layer to form a supracellular network that completely isolates the apoplast of the stele from the apoplast of the outer tissue layers. The role of the Casparian strip as a barrier for solutes and ions has been suggested by the absence of diffusion of fluorescent dyes beyond the Casparian strip into the stele (Alassimone et al., 2010), by the accumulation of

salts at the cortical side of the Casparian strip (Nagahashi et al., 1974; Alassimone et al., 2012), and by the drop in root pressure observed after puncturing the endodermis (Peterson et al., 1993; Steudle and Peterson, 1998). However, it is still unclear how well water and small dissolved solutes can move through the Casparian strip (Steudle and Peterson, 1998; Steudle, 2000; Geldner, 2013). In absence of apoplastic water flow directly through the Casparian strip, aquaporins could provide a route for water uptake, and their transcriptional and posttranslational regulation could allow for rapid control of water flow across the endodermis into the stele (Bramley et al., 2007). This model is supported by the high expression levels of some aquaporin isoforms in the endodermis (Chaumont and Tyerman, 2014).

The molecular actors involved in the local deposition of the Casparian strip in endodermal cells have just begun to be discovered (Roppolo et al., 2011; Hosmani et al., 2013; Lee et al., 2013). The Casparian strip membrane domain proteins accumulate in the sub-domain of the plasma membrane where the Casparian

strip will form and likely guide the formation of a protein-rich membrane domain, termed the Casparian strip domain (CSD; Roppolo et al., 2011). The CSD anchors the plasma membrane to the cell wall (Alassimone et al., 2010) and recruits the enzymes necessary for lignin biosynthesis (Hosmani et al., 2013; Lee et al., 2013). Interestingly, it has been shown that membranous proteins cannot diffuse freely within this region (Alassimone et al., 2010). As a result, the CSD creates two distinct and exclusive plasma membrane domains, possibly reinforcing the polarized localization of several ion transporters observed at earlier stages of endodermal differentiation (Ma et al., 2006, 2007; Alassimone et al., 2010). The asymmetric distribution of influx and efflux carriers at endodermal plasma membranes associated with the apoplastic barrier provided by the Casparian strip ensures control of solute movement from the outer tissue layers to the vasculature and prevents the backflow of ions from the stele to the apoplast of the cortex when transpiration is low (Steudle and Peterson, 1998). However, because of the bidirectionality of water transport through aquaporins, backflow of water to the outer part of the root could still occur under unfavorable conditions such as in dry soils (Bramley et al., 2007). Tight regulation of aquaporin expression levels, localization, and gating is likely essential to balance uptake and loss of water in such conditions (Bramley et al., 2007).

After the formation and maturation of the Casparian strip, the endodermis can undergo a second stage of differentiation, which consists of the deposition of a hydrophobic secondary cell wall lying between the primary cell wall and the plasma membrane that completely covers the cell surface (Fig. 1). These lamellae are made of suberin, an aliphatic polyester highly resistant to degradation, but they likely do not function as efficient apoplastic barriers (Geldner, 2013). Indeed, in the *enhanced suberin1* (*esb1*) mutant, which is defective in Casparian strip formation, the free diffusion of propidium iodide into the stele is not blocked as early as in wild-type plants. However, this defect cannot be compensated by the strong ectopic deposition of suberin observed in the endodermis relatively close to the root tip in these genetic backgrounds (Hosmani et al., 2013). This suggests that the Casparian strip and suberin lamellae do not have interchangeable functions. Instead, suberin lamellae might prevent the uptake of solutes and water from the apoplast directly into endodermal cells, forcing solutes to use the symplastic pathway from more outer tissue layers. Geldner (2013) proposed that the outer tissues essentially increase the absorptive surface area of the root and funnel solutes to the endodermis through the symplast. Thus, restricting the uptake of solutes from the apoplast by the endodermis may have a low impact on nutrient uptake if entry into the symplast initially occurs at outer tissue layers. Conversely, water movement should be strongly affected by the deposition of suberin lamellae, provided that entry of water into the symplast occurs primarily at the endodermis, and that flow through plasmodesmata is slower than

flow through endodermal plasma membranes. Suberin deposition may also be important for preventing bulk movement of water out of the stele. This role is consistent with observations that suberin deposition can be enhanced in more mature regions of the root (Naseer et al., 2012) and in response to environmental stress (Chen et al., 2011). However, experimental results regarding the importance of the suberin lamellae in controlling the radial hydraulic conductivity of roots have been variable, with both positive and negative effects observed (Steudle and Peterson, 1998; Baxter et al., 2009; Ranathunge and Schreiber, 2011). Overall, the effect of the suberin lamellae on solutes and water movement is likely dependent on relative transport capabilities of the endodermis compared with more outer tissue layers.

It has been suggested that the participation of the endodermis in nutrient uptake can be particularly important in the regions of the root where Casparian bands have formed but suberin lamellae are not yet established (Geldner, 2013). However, it is worth noting that secondary cell walls are not deposited in every endodermal cell (Naseer et al., 2012). Patchy patterns observed with fluorol yellow staining or with expression of GUS reporters of suberin biosynthesis genes point to the existence of passage cells that lack secondary cell wall deposition in the proximity of xylem poles (Wu et al., 2011; Naseer et al., 2012). These passage cells may be hubs of endodermal nutrient uptake (Geldner, 2013). For example, PHOSPHATE1 (*PHO1*) phosphate transporters are expressed in individual endodermal cells that might correspond to passage cells adjacent to protoxylem (Hamburger et al., 2002). The concomitant use of *PHO1:GUS* and suberin staining would be necessary to clarify the importance of passage cells in phosphate transport to the stele. The expression of aquaporins in passage cells has not yet been analyzed to our knowledge, so it is still unclear whether passage cells are important contributors to water uptake in the more mature region of the root.

Once the Casparian strip and suberin lamellae are deposited, molecules such as nutrients, ions, and hormones have to pass through the endodermis cytoplasm to move between the inner and outer parts of the root, making this cell layer a potential hub for receiving and transferring signals between cell layers. This function of the endodermis was recently reviewed (Dinneny, 2014) and is particularly important during biotic and abiotic stresses.

ENDODERMIS AS A COMMUNICATION CENTER DURING ROOT RESPONSES TO THE EXTERNAL ENVIRONMENT

The function of diffusion barriers in the endodermis is especially important when considering the uptake of toxic solutes into the root. In high-saline environments, the endodermis limits free apoplastic diffusion of sodium ions into the vascular stream. This leads to the accumulation of sodium ions in tissues peripheral to the endodermis (Møller et al., 2009). Under drought

conditions, suberization in the endodermis can be accelerated and may prevent the desiccation of inner tissue layers (Enstone et al., 2002; Henry et al., 2012). Thus, the Casparian strip and suberin lamellae not only prevent uptake of toxic compounds but may also prevent the loss of water under unfavorable environments (Soukup et al., 2007; Baxter et al., 2009; Chen et al., 2011).

Because of its role in solute and water transport, the endodermis may act as a signaling center during the perception of stressful environmental conditions and subsequent regulation of growth and development. Recent studies have highlighted the importance of the endodermis in regulating root growth under salt stress through abscisic acid (ABA) signaling. Duan et al. (2013) reveal that the postemergence growth of lateral roots is strongly suppressed during salt stress in *Arabidopsis thaliana*. ABA signaling is specifically induced in these quiescent lateral roots and is necessary for growth inhibition. A GALACTOSE4/Virion Protein 16-Upstream Activation Sequence transactivation system (Haseloff and Hodge, 2001) was used to express the mutant protein *abi1-1* in different tissue types to interrupt ABA signaling in a spatially restricted manner. Endodermal ABA signaling was found to be most important to inhibit lateral root growth during salt stress. Surprisingly, although suppression of ABA signaling allowed lateral roots to bypass growth quiescence, growth was not prolonged indefinitely and eventually these roots senesced, suggesting that other aspects of salinity tolerance were not properly regulated (Duan et al., 2013). Primary roots enter a similar, but more short-lived quiescent phase when seedlings are treated with high concentrations of salt; however, in this case, endodermal ABA signaling promotes growth recovery, perhaps as a result of interactions with the ethylene-signaling pathway (Sharp, 2002; Geng et al., 2013). ABA-responsive genes are up-regulated in all tissue layers of the root under salt stress (Dinnyen et al., 2008); thus, the downstream targets of ABA must somehow be unique in the endodermis in order to regulate growth.

Calcium is a versatile signaling component involved in regulating multiple physiological and developmental responses such as stomatal opening (Allen et al., 2001) and root hair elongation (Ehrhardt et al., 1996; Wymer et al., 1997). Through the induction of rapid changes in cytosolic calcium concentration, cells use calcium to signal environmental stresses such as cold (Knight et al., 1996; Carpaneto et al., 2007), drought, salt stress (Knight et al., 1997), and biotic stress (Blume et al., 2000; Navazio et al., 2007). To quantify changes in cytosolic calcium concentration, calcium-binding proteins have been identified and used to develop Förster resonance energy transfer-based sensors (Horikawa et al., 2010). Choi et al. (2014) recently utilized a high-affinity calcium sensor in *Arabidopsis* to characterize a calcium-signaling wave, which propagates rapidly from a point of local salt treatment to the rest of the root system. Intriguingly, upon localized salt treatment at a lateral root tip, the signal travels to the rest of the root system through the

cortex and endodermis at speeds of up to 400 $\mu\text{m/s}$, eventually reaching the shoot (Choi et al., 2014). Activation of stress-responsive genes was detected within 1 to 2 min in the tissue directly treated with salt, and was detected within 10 min in the aerial part of the plant. The transcriptional regulation of some of these genes can be affected by applying lanthanum (III) chloride, which inhibits calcium channels. This suggests that the calcium wave plays an important role in systemic responses under salt stress. Among these stress-induced genes, a vacuole channel protein, TWO-PORE CHANNEL1 (TPC1), shows very rapid and transient induction in the shoot within 1 min of treatment and is also involved in determining the speed with which the calcium wave propagates (Choi et al., 2014). This study provides an interesting mechanism for rapid and long-distance signaling after localized salt exposure. With the rapid propagation of calcium signals from a localized stressed region to the rest of the root system and further to the shoot, plants can trigger systemic stress-responsive programs as a preventive mechanism against further damage from stressful environments.

These observations highlight a potentially important role of the endodermis in calcium signaling and downstream responses under salt stress, which is supported by the work done by Kiegle et al. (2000). By expressing a calcium reporter, aequorin, in specific cell types, Kiegle et al. (2000) discovered that prolonged cytosolic calcium oscillation occurs in the endodermis and pericycle upon osmotic or salt-stress treatment. It will be interesting to examine tissue-specific functions of TPC1 to determine whether the roles of cortical and endodermal calcium signaling can be differentiated. Whether suberin deposition in the endodermis affects the rate of signal propagation by affecting the flow of calcium from the apoplast to endodermal cells would also be intriguing to determine. In addition, calcium signaling in guard cells has been shown to enhance ABA regulation of stomatal movements (Allen et al., 1999; Webb et al., 2001). Experiments to determine whether similar cross talk occurs between these pathways during regulation of root growth may be fruitful.

Aside from abiotic stresses, the plant root system also faces challenges and opportunities from microorganisms in soil. The Casparian strip and suberin lamellae can function as physical barriers to exclude pathogenic microbes (Bernards, 2002; Schreiber and Franke, 2011), but the root is also capable of forging symbioses with beneficial microbes to assist in nutrient acquisition. One of the most common of such symbioses occurs between plants and arbuscular mycorrhizae (AM). Penetrating fungal hyphae differentiate and form a branch-like structure with a large membrane interface in the root cortex (Genre et al., 2008). This network of hyphae indirectly increases the contact area between the root system and the soil, which facilitates the uptake of water and nutrients by the plant. Reciprocally, the fungi obtain carbon resources from the phloem of the root (Parniske, 2008). Here, the endodermis may function to monitor selective nutrient exchange processes between the cortex

and vascular tissue; therefore, signaling events in the endodermis may be important to facilitate communication between the root and fungal symbionts.

AM development is greatly dependent on plant-derived signals. For example, an endogenous plant hormone, strigolactone, has been identified as an important branching factor for fungal hyphae (Akiyama et al., 2005; Besserer et al., 2006). In *petunia* (*Petunia hybrida*), the identification of a strigolactone transporter, PLEIOTROPIC DISEASE RESISTANCE-LIKE1, provides a mechanism to guide the entry of arbuscular hyphae through hypodermal passage cells (Kretzschmar et al., 2012). Recent work suggested that strigolactone signaling may have endodermis-specific functions during the regulation of root branching (Koren et al., 2013). It will be interesting for future studies to determine whether such endodermal-specific strigolactone signaling is also necessary for AM infection.

Gibberellins (GAs), another class of plant hormones, may also have endodermis-specific roles in controlling root development and AM colonization. In *Arabidopsis*, fluorescently labeled GA derivatives predominantly accumulate in elongating endodermal cells in the root (Shani et al., 2013) and DELLA proteins, which antagonize GA signaling, function in the endodermis to suppress primary root elongation and meristem size (Ubeda-Tomás et al., 2008, 2009). DELLA proteins may also interact with ABA signaling in the endodermis to suppress the post-emergence growth of lateral roots under salt stress (Duan et al., 2013). GA was recently found to inhibit AM colonization, and DELLA proteins are important in this process (Foo et al., 2013; Yu et al., 2014). Indeed, Floss et al. (2013) recently reported that AM formation in the root cortex is strongly suppressed in the *Medicago truncatula* double mutant *della1/della2*. Surprisingly, fungal hyphae are still able to absorb nutrients from the mutant plant, but fail to produce spores and complete their life cycle (Floss et al., 2013). By expressing the dominant mutant protein *della1-Δ18* under both the native promoter of *MtDELLA1* and *Mt PHOSPHATE TRANSPORTER9*, a phosphate transporter, the AM defect in the *della1/della2* double mutant can be rescued. In both situations, the mutant protein is mainly localized in the nuclei of cells of the endodermis and stele, indicating a possible role of the endodermis in regulating AM formation in the cortex (Floss et al., 2013).

The above examples illustrate the importance of the endodermis in coordinating signals to regulate root growth and responses to biotic and abiotic stimuli. This tissue layer also plays a central role in mediating cross talk between different cell layers during development of new lateral organs.

ENDODERMIS AS A BARRIER AND FACILITATOR OF LATERAL ORGAN MORPHOGENESIS

The root system of higher plants is a highly branched network, and new lateral branches are initiated deep within the inner tissue layers of the parent root. In many dicot species, lateral root primordia are derived

from the pericycle, a layer of cells that lies immediately internal to the endodermis. As primordia develop and mature, they must pass through overlying tissue layers in order to emerge from the parent root and enter the soil environment. This process must be highly regulated in order to allow the primordium to emerge without damaging the parent root and significantly compromising barriers provided by the endodermis.

During lateral root emergence in *Arabidopsis*, enzymatic wall loosening takes place in the epidermis and cortex to allow developing primordia to push aside overlying cells as they expand and elongate. However, recent work suggests that the endodermis behaves differently during this process. Simple separation of endodermal cells would likely compromise the diffusion barrier provided by this layer, so it is likely that alternative mechanisms are employed to allow lateral root primordia to emerge through it. A common theme for all overlying tissue layers is a role of auxin signaling in setting cellular modifications in motion. The auxin influx carrier *LIKE AUX13* (*LAX3*) was shown to be critical for proper emergence of lateral root primordia based on the decreased number of emerged lateral roots in *lax3*-null mutants (Swarup et al., 2008). This transporter is expressed in cortical and epidermal cells immediately overlying developing primordia and is required for expression of wall-remodeling enzymes by these cells (Fig. 2).

Auxin signaling is also activated in endodermal cells overlying developing primordia, and has been linked to induction of wall-remodeling enzymes such as PECTIN LYASE2 and GLYCOSYL HYDROLASE17 (Swarup et al., 2008). However, these enzymes lack the ability to degrade the lignified Casparian strip. Vermeer et al. (2014) show that cells of the endodermis instead undergo dramatic morphological changes to accommodate growth of new lateral roots (Fig. 2). The cells become thinner and flatter in regions in contact with developing primordia, accompanied by small, localized breaks in the Casparian strip. These modifications cause the endodermal cells to press tightly around the base of the developing primordium, allowing for emergence without creating significant gaps in the diffusion barrier that would occur if the cells completely separated from one another.

Auxin signaling in the endodermis is necessary for the morphological changes that occur to accommodate developing primordia. This was demonstrated through endodermis-specific expression of *short hypocotyl2-2* (*shy2-2*), which encodes a dominant-negative allele of an AUXIN RESISTANT/INDOLE-3-ACETIC ACID INDUCIBLE (Aux/IAA) protein normally expressed in the endodermis, hypocotyl, and other tissues (Vermeer et al., 2014). Lateral root primordia and emerged lateral roots are completely absent from plants expressing this transgene, implying that the block occurs very early in development. Although exogenous auxin treatment partially rescues this phenotype, developing primordia appear to be flatter and more elongated, and emerge at a much lower frequency than those of wild-type plants. These morphological defects could be attributable to an

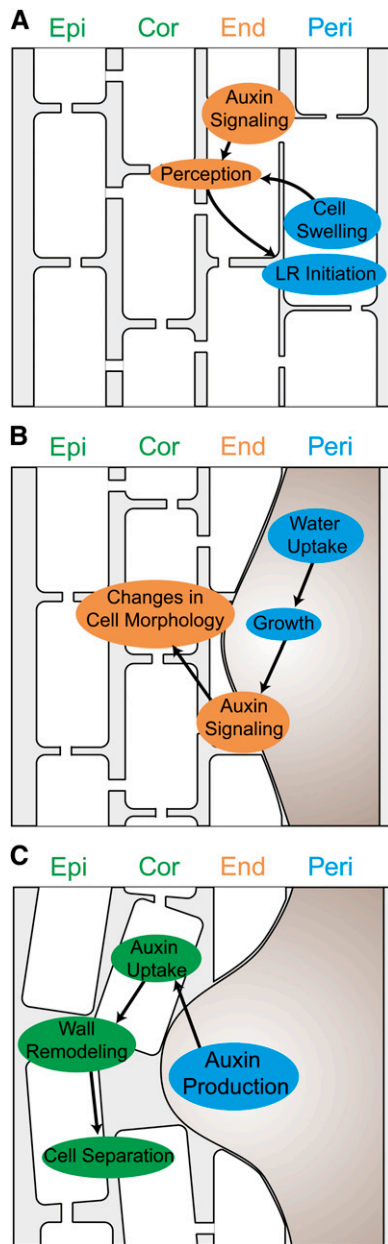


Figure 2. Mechanical and auxin signals mediate cell-to-cell communication during lateral root development. A, Cell swelling in the pericycle sends a mechanical signal to the endodermis. The endodermis responds in an auxin-dependent manner to allow the pericycle cell to undergo the first asymmetric division marking lateral root initiation. B, The endodermis responds to turgor-driven growth of the developing lateral root primordium by undergoing auxin-dependent cell morphological changes. These changes allow the primordium to pass through the endodermis without creating significant apoplastic gaps between the primordium and neighboring endodermal cells. C, Auxin produced by the developing primordium is taken up by overlying cells in the cortex and endodermis through the transporter LAX3. Auxin signaling in these cells then activates expression of cell wall-remodeling enzymes that allow the cells to separate from one another as the primordium pushes past them. Events are color coded according to the tissue layer they occur in. Cor, Cortex; End, endodermis; Epi, epidermis; LR, lateral root; Peri, pericycle.

imbalance of mechanical forces between the primordium and overlying tissue layers during development.

The primordium must generate a force to push past the cells of the outer tissue layers, and these layers must make modifications that allow them to yield to this force (Fig. 2). Accordingly, altering either side of this coordinated interaction leads to developmental defects. The force that allows primordia to push past overlying cells arises as a result of water uptake by the cells of the primordia, which causes them to expand and maintain turgor pressure. Thus, it was proposed that water movement would be a critical factor in mediating the outgrowth of new lateral roots. Péret et al. (2012) tested this hypothesis through a combination of mathematical modeling and reverse genetic approaches. Predictions made using the model were tested empirically through misexpression of the aquaporin *PLASMA MEMBRANE INTRINSIC PROTEIN2;1*. Both overexpression and null mutations of this gene lead to delays in lateral root emergence and flattening of developing primordia. Similar defects are observed when the ability of overlying tissues to yield to the force from primordia is disrupted. This was demonstrated by blocking auxin perception in tissues outside of developing primordia, which prevents these cells from undergoing auxin-dependent changes to their morphology and/or cell wall structure (Lucas et al., 2013). An inducible enhancer-trap system was used to express *auxin resistant3-1* in all root cells outside of developing primordia. This gene encodes a dominant-negative Aux/IAA protein that leads to constitutive suppression of the auxin-signaling pathway. Expression of this transgene leads to delayed emergence coupled with flattening of primordia, similar to what is observed with misexpression of aquaporins during lateral root development. Interestingly, the expression of aquaporin genes is tightly regulated by auxin during lateral root emergence (Péret et al., 2012), implicating a role for this hormone both in the generation of force by the primordium and the yielding to this force by outer tissue layers.

How can signaling from one hormone lead to different outcomes in different tissue layers? In the way that ABA plays tissue-specific roles during salt stress (Duan et al., 2013), auxin apparently acts in multiple tissue layers during lateral root development, serving to generate a mechanical force in some layers while decreasing resistance to mechanical force elsewhere. It is likely that other conditions or factors that are unique to each cell type give rise to these tissue-specific responses. For instance, cell wall loosening is activated by auxin to allow the cells in the outer tissue layers to separate as lateral root primordia grow. Cell wall loosening may also play a role in primordia themselves, since wall relaxation is limiting in turgor-mediated cellular expansion (Cosgrove, 1993; Schopfer, 2006). Perhaps it is the water status of these cells and the rate they can take up water that distinguish developing primordia from overlying tissues during lateral root development.

It appears that mechanical interactions between lateral root primordia and surrounding tissue layers are

important for proper morphogenesis, and the endodermis may play a central role in this mechanical cross talk. Blocking auxin signaling in the endodermis through misexpression of *shy2-2* has effects on development preceding lateral root initiation (Vermeer et al., 2014). Cells of the pericycle increase in volume immediately preceding the first asymmetric cell division that marks lateral root initiation, a process that requires endodermal auxin signaling (Vermeer et al., 2014). It was proposed that resistance of the endodermis to pericycle cell swelling might be perceived by the pericycle cell and used to gauge the level of mechanical resistance provided by the endodermis. The endodermis would then respond to this force by undergoing the morphological adjustments necessary to accommodate the developing primordium (Fig. 2). The molecular mechanisms by which plant cells sense such mechanical forces remain to be elucidated, although numerous efforts have been made to address this issue (Hamant, 2013).

Although considerable progress has been made on the role of the endodermis in lateral root emergence in Arabidopsis, relatively few studies have been done beyond this species. The endodermis plays a more active role in the generation of new lateral roots in many species. In maize (*Zea mays*) and other cereal crops, the lateral root cap and epidermis of new lateral roots are of endodermal origin (Orman-Ligeza et al., 2013). Histology of developing lateral root primordia in maize also suggests that the lignin in the walls of the endodermal cells that contribute to the primordium is degraded (Karas and McCully, 1973). However, it is unclear how this delignification occurs and whether there are any mechanisms employed by the plant to compensate for this localized breakdown of the apoplastic barrier. Suberin deposition has been observed in endodermal cells surrounding lateral root primordia in Arabidopsis (Martinka et al., 2012); such a modification could serve to provide some level of protection in species in which endodermal delignification takes place. Many species also employ programmed cell death in outer tissue layers to clear a path for emergence of developing primordia. The effects of such processes on maintaining endodermal diffusion barriers, if any, remain unexplored.

CONCLUSION

It is generally accepted that the endodermis protects the inner part of the root from the external environment by developing both an apoplastic diffusion barrier (the Casparian strip) and later a barrier to the movement of molecules directly from the apoplast into the endodermis (the suberin lamellae). Nevertheless, many mysteries remain concerning the precise effects that these barriers have on the flow of water and solutes. Despite the fact that these barriers differentially affect the movement of molecules through the endodermis, the Casparian strip and the suberin lamellae are not always well distinguished when studies have analyzed specific biological functions of the endodermis. A more rigorous and

systematic characterization of the developmental progression of the endodermis should be made for this purpose. Mutants specifically affecting one barrier would be useful, but as highlighted with the *esb1* mutant (Hosmani et al., 2013), cross talk between lignin and suberin deposition might make this kind of approach difficult. Regarding solute movement, it is likely that different molecules will preferentially use different paths and their movement is also likely dependent on the age of the root, the species under study, and environmental conditions. All of these parameters should be brought together to synthesize a more holistic understanding of solute flow in roots.

The endodermis is also a useful signaling center, mediating interactions between the root and its environment as well as between different cell types. An important question in this regard is how the endodermis perceives various inputs that must be transduced to affect biological processes. Regarding high salinity, the activation and rapid propagation of calcium signaling could serve as a mechanism. Hormone intermediates, such as auxin and ABA, also provide a means of communication during development and environmental stress. In addition, recent work points to an important role for perception of mechanical cues in mediating cross talk between neighboring cell layers. The responses of the endodermis to this wide array of signals appear to be central to a number of biological processes, including root-microbe interactions and the morphogenesis of new lateral organs. However, many of these signals are activated and perceived in multiple tissue layers. What molecular properties of the endodermis allow it to generate unique responses compared with other cell types? In addition, many of these signaling processes, such as long-distance propagation of calcium waves and stress-induced calcium oscillations, appear to involve the endodermis as well as tissue layers that neighbor it. Is this simply a consequence of symplastic connections between these cells and the endodermis, or do the actions of these cells have functional importance as well? The utilization of tissue-specific genetic tools will be of great utility in addressing these questions.

The role of the endodermis as a center for signaling during development and environmental stress has become increasingly apparent. Future work into the basic biology of this protective barrier layer and its functions in signaling will provide a greater understanding of the mechanisms underlying how plants perceive and respond to their external environment to regulate internal physiology and development.

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