A Tale of Two Domains Pushing Lateral Roots

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ABSTRACT

Successful plant organ development depends on well-coordinated intercellular communication between the cells of the organ itself, as well as with surrounding cells. Intercellular signals often move via the symplasmic pathway using plasmodesmata. Intriguingly, brief periods of symplasmic isolation may also be needed to promote organ differentiation and functionality. Recent findings suggest that symplasmic isolation of a subset of parental root cells and newly forming lateral root primordia (LRPs) plays a vital

role in modulating lateral root development and emergence. Here, we discuss how two symplasmic domains may be established within an LRP and its overlying cells simultaneously, and what the significance of plasmodesmata might be during this process.

Symplasmic Domains Establish New Boundary of Developing Lateral Root

Symplasmic isolation, via a temporary or permanent disconnection of **plasmodesmata** between neighboring plant cells, can occur during the growth, differentiation, and function of certain plant tissues and organs (reviewed in [1-3]). Some **symplasmic domains** form permanently, whereas others may form transiently, and they can occur at the level of a single cell, a cluster of cells, tissues, and even organs (BOX 1). One of the biggest challenges often faced when searching for and studying novel symplasmic domains is the lack of noninvasive, real-time, and in situ methods for measuring cellular connectivity, especially if the domains are small or occur deep within a tissue. It is likely that previously unrecognized symplasmic domains could be more easily detected as new and improved techniques and methods become available for measuring cellular connectivity.

In roots, a period of symplasmic isolation occurs during lateral root development. A lateral root primordium (LRP) initiates inside parental root tissues and grows outward, passing through the overlying tissue layers until it finally emerges into the soil environment (reviewed in [4]). This process, termed lateral root emergence, requires coordination between the growing LRP and the **LRP-overlying root cells**—through hormone signaling, mechanical feedback, and possibly other mechanisms of cell communication—to prevent unwanted LRP abortion or overlying cell death [5-7]. How these two newly forming cellular domains successfully achieve such coordinated actions is not fully understood. However, our recent findings [8] showing that Plasmodesmata-Located Protein (PDLP) 5 may contribute to the production of a symplasmically isolated cellular domain consisting of LRP-overlying cells starting at the earliest stages of LRP development, has begun to reveal some new and unique aspects to the lateral root organ outgrowth process.

In this paper, we discuss how symplasmic isolation in both the LRP and PDLP5-expressing overlying cells could be essential for normal lateral root emergence, and how PDLP5 may have an additional role regulating physiological and defense programs in the lateral root forming zone. We also highlight what we suspect could be a critical, but overlooked, aspect of LRP development: the initial symplasmic separation between the **xylem pole pericycle founder cells** (the tissue in which the LRP originates) and the overlying endodermis. This separation would likely be required for the LRP to create its own identity apart from the overlying tissue, forming the boundary that we are calling the LRP and overlying cell interface (LOI).

Auxin Recruits the Plasmodesmal Regulator PDLP5 to LRP-overlying Cells

PDLP5, a receptor-like protein that localizes to plasmodesmata, is a potent plasmodesmal regulator that restricts molecular movement between cells by stimulating localized **callose** accumulation [9,10]. In the shoot, PDLP5, once induced by the defense hormone salicylic acid (SA)-dependent signaling pathway, not only triggers plasmodesmal closure but also functions as a defense protein amplifying the SA levels via positive feedback regulation. Consequently, loss of PDLP5 results in plants that are impaired in both basal and SA-dependent plasmodesmal closure, as well as susceptible to microbial pathogens.

In the root, auxin released from an LRP induces the spatiotemporal expression of PDLP5 in overlying cells during LRP development [8], usually limited to the 2 to 4 endodermal, cortical, and epidermal cells that successively come in direct contact with the emerging LRP. In contrast to the regulatory relationship between PDLP5 and the SA-dependent signaling pathway, in which SA is amplified by inducing PDLP5 expression, the auxin response in overlying cells is seemingly negatively feedback-regulated by inducing PDLP5. Furthermore, our experimental evidence suggested that ectopic induction of PDLP5 also restricts cell-to-cell movement in roots, while elevating plasmodesmal callose deposition [8]. Importantly, loss of PDLP5 results in faster LRP emergence, while its overexpression suppresses normal root branching. Given PDLP5's role as a plasmodesmal inhibitor, we speculate that its highly-targeted spatiotemporal

expression in overlying cells by auxin would promote their symplasmic isolation, and that this function is critical to modulate lateral root emergence.

LRP and its Overlying Cells Concurrently Undergo Symplasmic Isolation

Over the course of lateral root development in arabidopsis, an LRP undergoes a transient symplastic disconnect from the parental vasculature starting at about stage IV, until soon after emergence when it reestablishes vascular connections (Figure 1). Two reports using different mobile fluorescent molecules have demonstrated the transient nature of LRP symplastic isolation: Oparka et al. [11] using a phloem-loaded fluorescein tracer, and Benitez-Alfonso et al. [12] using a genetically encoded free GFP reporter. During initial development, a nascent LRP (stages I-II) is fully symplastically connected to the parental root, allowing small tracer dye and larger GFP to diffuse into it. However, starting at about late stage III or early stage IV, the size exclusion limit at the LRP-parental root boundary begins to decrease, as deduced from observations that though phloem-loaded tracer dye can still move into the LRP [8,11], phloem-expressed GFP movement is blocked [12]. At mid-to-late LRP stages (VI-VIII), the LRP becomes fully symplastically isolated, until the new vascular system within it reconnects to the parental phloem soon after emergence (Figure 1).

The outermost LRP cells, which will later become the cells of the lateral root cap, physically disconnect from adjacent endodermal cells, allowing the LRP to develop as a new organ separate from the overlying parental tissue. Little is currently known about the timing, signaling molecules, and enzymes involved in this step. Nevertheless, it is conceivable that auxin and auxin-dependent cell wall remodeling enzymes could play a major role as shown in overlying cell separation [5].

Intriguingly, PDLP5 induction occurs in endodermal cells overlying LRP as early as stage I-II [8]. This early timing of induction suggests that PDLP5-controlled symplasmic isolation of the overlying endodermal cells from the dividing xylem pole pericycle founder cells could represent one of the earliest steps allowing founder cells to differentiate (BOX 1 Figure 1). Furthermore, while the interfacial cell walls undergo loosening between the nascent LPR root cap and overlying cells, PDLP5-induced plasmodesmal closure could facilitate two outcomes. First, the two symplasmic domains

could retain certain signals necessary for the autonomous part of their development and physiological changes. Second, they could be protected from any intercellular signals from the other domain that might interfere with those processes. Importantly, **transcellular auxin transport** (reviewed in [13]) should still occur from the LRP into the overlying endodermal and cortical cells at the early stages of LRP development [14] via auxin influx and efflux carriers [5,15,16], even as plasmodesmata begin closing (BOX 1 Figure 1).

Nascent Root Cap Cuticle Fortifies LRP Separation from Overlying Cells

Subsequently from, or perhaps concurrently with, the PDLP5-induced symplasmic uncoupling of the LRP and endodermis, the production of an impermeable **cutin** coating [17] called the root cap cuticle (RCC) may also contribute to the separation of these two domains (BOX 1 Figure 1). Although several cutin biosynthesis genes were previously known to be expressed in mature root caps [18,19], one exciting new study has shown that two cutin biosynthesis enzymes were expressed in the outermost layer of early-stage LRP [20].

Importantly, the RCC study showed that a membrane-permeable dye could not penetrate the newly emerged lateral root when applied externally [20]. This corresponds well with our own results showing that a phloem-loaded symplastic tracer dye is unloaded but retained within the nascent LRP, unable to penetrate into overlying cells [8]. These findings support the possibility that the RCC formation and plasmodesmal disconnection may be key aspects of creating the LRP symplasmic domain. The fate of the plasmodesmata surrounding the RCC layer awaits to be examined in detail; however, the plasmodesmata connecting the RCC cells to the inner LRP cells appear to remain functional, considering how symplastic dye unloads throughout the LRP dome [8]. The outermost surface of the RCC cells are likely plugged and severed prior to, or concurrently with, the onset of cutin deposition.

The formation of the RCC is likely the final step that produces the interface we have dubbed LOI, where the outer cells of the LRP and the overlying cells remain in physical contact yet separated by symplasmic and apoplasmic boundaries (BOX 1 Figure 1). As a nascent LRP develops, its cell walls in contact with the separated walls of the

overlying cells become part of the LOI in each successive overlying tissue layer (Figure 1). Despite the lack of symplasmic communication between them, the cells across the LOI are the likeliest candidates to translate any input between the LRP and overlying cells. Important future questions may address how LRP/overlying cell input, mechanical or otherwise, is translated across the LOI, as well as how auxin may enter the overlying cells to trigger separation of epidermal cells at a late emergence stage, even though by then transcellular transport from the LRP may be blocked by the RCC.

PDLP5-mediated Callose-dependent Plasmodesmal Regulation

The changes in LRP connectivity we introduced earlier seem to rely on a callose-dependent mechanism of plasmodesmal closure. For example, expression of a callose degrading enzyme, PdBG1, can be strongly detected within the LRP at stages I-III, then decreases in later stages [12], coinciding with the increase of a callose-binding protein, PDCB1 [21], which becomes highly expressed within LRP starting around stage IV [22]. Although how exactly those callose-regulating enzymes and proteins in the LRP are activated is not yet known, in overlying cells, PDLP5 and callose deposition appear to be connected in a two-step process, as discussed below.

Since PDLP5 exerts plasmodesmal regulation in shoots via stimulating callose deposition [9,10,23], we propose that a similar mechanism functions in LRP-overlying cells [8]. While it was technically challenging to quantify changes in callose levels directly at plasmodesmata within LOIs, we were able to clearly detect callose in pit fields of overlying cortical cell walls at LRP emergence stage IV-V, and even remnants of pit field callose in endodermal cells (Supplemental Information Figure S1). Based on these results, we hypothesize that auxin-controlled PDLP5 expression stimulates a yet unknown callose synthase(s) to block cell-to-cell movement in and out of the overlying cells via the callose-dependent mechanism of plasmodesmal regulation.

It may seem odd that plants might utilize a two-step system for plasmodesmal callose deposition—first having auxin upregulate PDLP5 expression, followed by PDLP5-dependent activation of a callose synthase—instead of simply having auxin activate the callose synthase enzyme directly. Indeed, we have observed that in certain cases, callose synthases can respond directly to signals. As we have shown recently,

wounding and hydrogen peroxide treatments activate the Callose Synthase (CalS) 8 to quickly deposit plasmodesmal callose independently of PDLP5 [23]. We speculate that this is because fast plasmodesmal closure is the "endpoint" of the wounding/H_2O_2 response, with no feedback signaling required from the changes in plasmodesmal gating status.

However, PDLP5 seems to be upregulated at times when some kind of feedback from the plasmodesmata is required. For example, PDLP5 expression is highly induced in response to SA or bacterial pathogens, and not only does PDLP5 work together with another callose synthase, CalS1, to close plasmodesmata via callose accumulation [23], but this is followed by a PDLP5-dependent positive feedback regulation of the SA biosynthesis pathway to further boost defense [9,10]. This is somewhat similar to what we observe in the LRP-overlying cells, except in this case it is a negative feedback loop, as auxin-upregulated PDLP5 results in repressing auxin accumulation [8]. Taking these points into account, we theorize that PDLP5 acts as a versatile signal transducer which is upregulated not only to facilitate plasmodesmal closure, but then to signal relay this gating status to other parts of the cell. This mechanism could potentially initiate a variety of downstream effects, depending on the intra- or extracellular cues [24].

Potential Functions of the LRP and Overlying Cell Symplasmic Domains

Transient symplasmic isolation of LRP may serve at least two functions. One is to allow turgor pressure to push LRP outgrowth. Studies on mutants of LRP-specific aquaporins found that root emergence rate was altered [25,26], and some speculate that symplasmic isolation may be crucial to retain turgor pressure after aquaporin-mediated water influx [20]. The other could be to control the accumulation and spatial distribution of auxin within the LRP, as suggested in computational modeling showing that plasmodesmata, in conjunction with transporters, are crucial for auxin flow to regulate proper auxin distribution within root tips [27]. We reason that symplasmic isolation of the LRP allows auxin to be retained, reaching a threshold that drives its growth; plausibly, other plant signals would also be impacted by plasmodesmal gating in the LRP.

One may ask if the LRP is already closed off from surrounding tissue, then why would symplasmic isolation in the LRP-overlying cells also be necessary? We postulate that

plasmodesmal closure in overlying cells allows them to undergo physiological changes apart from their neighboring cells, similar to what might occur within the LRP (Figure 2). For example, plasmodesmal closure could help to maintain turgor pressure: LRP-overlying cells often lose their turgidity due to the mechanical pressure of the growing LRP pushing through them [7], and plasmodesmal closure of overlying cells could ensure the water loss occurs in a controlled manner via aquaporins, rather than leak out quickly through the open pores. Furthermore, auxin influx and efflux carriers target the hormone to overlying cells, activating the cell separation program that allows an LRP to emerge [15]. Here, plasmodesmal closure could help prevent symplasmic auxin diffusion, helping it reach the threshold that activates the cell separation [8].

There are also additional possibilities for why symplasmic isolation of the overlying cells may be crucial (Figure 2). Evidence suggests that soil pathogens would have an easy entry point as the LRP emerges from the epidermal layer [28], and sealing plasmodesmata could help prevent them or their harmful signaling molecules from entering (or if they manage to penetrate, from exiting) the LRP-overlying cells. PDLP5 functions as a defense protein in the shoots [9,10], so along with closing plasmodesmata, it is also plausible that PDLP5 could activate defense programs when it is induced in the overlying cells; hypothetically, PDLP5 could even have previously uncharacterized functions that occur only in the LRP-overlying domain (BOX 2).

Furthermore, an interesting new study suggests that the LRP-overlying cells may eventually undergo programmed cell death, to help clear a path for the emerging LRP [29]. During such cell death, various potentially harmful signaling molecules are produced, such as reactive oxygen species which could damage nearby healthy tissues. Symplasmic isolation of the dying LRP-overlying cells would protect surrounding healthy tissues, including the LRP itself, from these harmful molecules [30]. Finally, it is important to note that more than one of these possibilities might occur simultaneously or sequentially in the overlying cells (Figure 2).

Concluding Remarks and Future Perspectives

Spatiotemporal formation of symplasmic domains plays vital roles in many aspects of cell and organ development, and it seems different signaling mechanisms exist to

recruit plasmodesmal regulators into such processes. Creative experimental approaches and novel techniques will be needed to gain more insight into where and how these domains appear during plant growth; for example, recent research cleverly used somatic embryogenesis as a model system for observing how spatiotemporal symplasmic domains alter cell fate [31]. Our own research also illuminates how precise and transient symplasmic domain formation can be, with the LRP-overlying cell domain likely lasting only until soon after the lateral root emerges. Further dissecting the mechanical and proteinaceous elements of the LRP and overlying cells symplasmic domain pathways could provide clues that help us find similar symplasmic events in other plant processes. Eventually, understanding more about those pathways, and the role of PDLP5 and other molecular players, may allow scientists to apply the knowledge to engineer crop plants with desired organ architecture and traits that result from manipulating these complex intercellular communication pathways.

Supplemental information

Supplemental information associated with this article can be found at doi:XXXXXXX'

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FIGURE LEGENDS

Figure 1. Progression of symplasmic domains during lateral root primordium outgrowth. MZ, meristematic zone; EZ, elongation zone; DZ, differentiation zone; XPP, xylem pole pericycle; En, endodermis; Co, cortex; Epi, epidermis; SEL, size exclusion limit; CF, carboxyfluorescein used as a small symplastic tracer dye; GFP, freely diffusible green fluorescent protein used as a large symplastic tracer.

Figure 2. Potential functions of the lateral root primordium-overlying cell symplasmic domain. The microscopic image* is taken from a cross-section of confocal z-stack to show LRP emergence at stage V. Cell wall boundaries are stained red using propidium iodide. False-colored, bright punctate signals represent the location of PDLP5-GFP accumulation at plasmodesmal pit-fields along the cortex cells overlying LRP (marked with dashed arc). Size bar, 25 µm. Panels (A)-(E) illustrate emerging LRP

and overlying cells that are symplasmically isolated by the induction of PDLP5 (red dots) under various scenarios of what the role of symplasmic isolation might be. Blue gradient in (A) and (B) indicates auxin concentration; arrows in (B), turgor pressure; light orange shading in (C) and (E), defense activation and tailed green circles, pathogens; yellow shapes in (D) and (E), programmed cell death signals; dashed arrows in (E), potential movement of signals from LRP-overlying cells. Abbreviations: PD, plasmodesmata; LRP, lateral root primordium; CalS, callose synthase; RCC, root cap cuticle; PCD, programmed cell death. *Image taken from Figure 2A in Sager *et al.*, 2020 with authors' permission.

BOX 1. Examples of Symplasmic Domains

Guard cells: Mature guard cells sever their plasmodesmal connections to surrounding shoot epidermal cells, allowing them to modulate turgor pressure to close or open [32]. Depending on the plant species, guard cells may either be symplasmically isolated from each other too, like in *Allium cepa* and *Arabidopsis thaliana*, or retain plasmodesmal connections between them, as has been shown in *Zea mays* and, recently, *Polypodium vulgare* (ferns) [33-35].

Pollen cells: Initially, pollen mother cells are symplasmically connected to a nurturing tissue called the tapetum. When meiosis begins in the pollen mother cells, callose is heavily deposited between the tapetum and pollen, isolating the pollen grains as they mature, and perhaps protecting them from harmful signals once the tapetum eventually undergoes programmed cell death [36].

Cotton Fibers: Turgor pressure drives the dramatic lengthening of these seed trichomes, facilitated by the symplasmic isolation of the fiber cell. During the elongation phase, callose is deposited at the fiber cell plasmodesmata, sealing them to allow turgor to increase; when the fiber cell reaches a certain length, beta-1,3-glucanases are expressed to degrade this callose, reestablishing intercellular connectivity with the neighboring cells so the fiber can survive [37-39].

Phloem sieve elements: Phloem sieve elements are symplasmically isolated from the surrounding vascular tissues, except in sink zones like the primary and lateral root tips, where their contents become unloaded [40]. Recently it has been shown that this domain is connected to phloem pole pericycle cells via unique funnel-shaped plasmodesmata, to better regulate macromolecular unloading [41].

Root epidermal cells: When root epidermal cells grow out of the elongation zone and into the differentiation zone, epidermal cells primed to differentiate into root hairs become symplasmically isolated [42,43].

LRP-overlying cells? Based on our research, we propose a new symplasmic domain within the LRP-overlying cells during LR emergence. As shown in in BOX 1 Figure 1, we hypothesize that this new domain forms in the overlying endodermal cells during the earliest divisions of the LRP founder cells, facilitating founder cell differentiation into LRP.

Box Figure I. PDLP5 expression may guide early symplasmic domain formation in LRP and overlying cells. (A) LRP stage I: Concurrently with the first divisions of the LRP founder cells, PDLP5 (red dots) is expressed and localizes to plasmodesmata in the overlying endodermis, creating the first level of symplasmic isolation. PDLP5-dependent plasmodesmal closure could prevent LRP differentiation signals (yellow stars) and LRP-overlying cell differentiation signals (green diamonds) from moving out of their functional cellular domains. Prior to RCC formation, auxin (blue dashed arrows) is transcellularly transported from the nascent LRP into overlying cells to initiate the cell wall separation program. (B) LRP late stage III: Cutin biosynthesis in the dome's outer cells creates the RCC apoplasmic barrier (pink arc). The overlying endodermis and LRP are now fully separated; symplasmic and transcellular transport are prevented by PDLP5 and the RCC. The point of physical contact between each cellular boundary is the LOI (thick grey arc). PDLP5 dissipates or is degraded at the LOI; the fate of LOI plasmodesmata is unknown. Dashed outlines represent cells with open plasmodesmata. XPP, xylem pole pericycle; En, endodermis; Co, cortex; Epi, epidermis; LRP, lateral root primordium; RCC, root cap cuticle; LOI, LRP and overlying cell interface.

BOX 2: Speculation on Other Roles for Plasmodesmal Regulator in LRP-overlying Cells

During our imaging of PDLP5 in LRP-overlying cells, we found that it often briefly builds to a high level specifically at the cell walls that will separate, soon before they do [8]. It is possible that PDLP5 may regulate some aspects of recruiting cell wall digesting enzymes to plasmodesmata. Research has suggested that during abscission, cell wall digestion enzymes may accumulate in the central cavities of plasmodesmata within the separating walls [44] (reviewed in [1]). Evidence supporting this possibility includes the recent discovery of a plasmodesmata-localizing expansin, a type of cell wall-degrading enzyme [45]. Alternately, more PDLP5 could collect at cell-cell junctions prior to separation as part of a pathway that will plug the severed pores with cell wall material, also similar to abscission [46]. Post-separation, no PDLP5 is detected at the separated walls, though whether this is because the plasmodesmata there have been degraded would require electron microscopy to find out.

Lateral root emergence rate and branching increased in the *pdlp5-1* loss-of-function mutant, while the primary root length was not significantly affected [8]. If PDLP5 is necessary for proper LRP emergence, why would its loss promote root branching? It could be that PDLP5 locally represses an auxin biosynthesis gene(s), directly or indirectly. It may also be that when plasmodesmata cannot be closed in the LRP-overlying root cells, auxin cannot reach the required threshold for cell separation because it diffuses out. The plant may thus divert more auxin to, or biosynthesize more auxin within, the LR zone in an attempt to reach the threshold, inadvertently stimulating faster LR emergence and longer growth due to more auxin being present. In *pdlp5-1*, *DR5:GUS* staining was increased in LRP regions, supporting these theories [8].

LR architecture can be modified depending on the types and availability of certain nutrients (reviewed in [47]) to be phenotypically similar to *pdlp5-1*; for instance, mild nitrogen deficiency can increase LR length without affecting the primary root [48]. Future research could be performed into how genes involved in the nutrient foraging pathways in roots may alter PDLP5 expression, and vice-versa.

Mycorrhizal fungi in a symbiotic relationship with the roots can also modify LR architecture [49]. Intriguingly, fungi were seen to colonize the lateral root emergence zones in rice [50]. If PDLP5 is required for defense in roots, it may be repressed in host plants to prevent overactive defense during symbiotic interactions with the soil fungi; as a consequence of this repression, open plasmodesmata would decrease the turgor pressure in the LRP-overlying cells, allowing faster LR emergence.

GLOSSARY

Callose: The plant polysaccharide beta-1,3-glucan, which acts as a temporary cell wall material deposited to protect wound or pathogen invasion sites. It also accumulates naturally at the plasmodesmata, and controlling its levels via enzymatic biosynthesis or degradation is a major method of regulating plasmodesmal pore size, and thus intercellular connectivity.

Cutin: A “waxy” (polyester of fatty acids) compound that is the major component of the plant cuticle, a protective hydrophobic barrier on the surface of many outer plant tissues, including root tips.

Founder cells: A pair of xylem pole pericycle cells which, upon stimulation by the phytohormone auxin, will eventually divide and begin undergoing differentiation into a new lateral root organ. Founder cells are often designated in the lower root via pulses of shoot-derived auxin, but exogenous auxin treatments or root bending can trigger new founder cells to form in other root zones.

Plasmodesmata (sg. plasmodesma): Small pores connecting the cytoplasms of adjacent plant cells, lined along the outside with plasma membrane, and often with a strand(s) of appressed endoplasmic reticulum along the inside of the channel.

Symplasm: The basic state of most plant cells is to share cytoplasm with all neighboring cells throughout the plant via plasmodesmata; this plasmodesmata-dependent connectivity is called the symplasm.

Symplasmic domain: A zone, ranging in size from a single cell up to an organ, where cells are symplasmically isolated from surrounding tissue, but may still be connected to each other via plasmodesmata within the bounds of their cellular zone. Symplasmic domains may form temporarily or permanently, usually to enable certain tissues or organs to properly differentiate and function.

Transcellular auxin transport: Active, often directional, transport of auxin across both apoplasmic and symplasmic boundaries. Auxin is exported from the cytoplasm into the apoplasmic space via efflux proteins, where it is converted to a form that could either diffuse through the cell wall, or is taken up by influx proteins, into nearby cells.

Xylem pole pericycle: The root pericycle cells (usually 2-3 in arabidopsis) connected to the major xylem cells on both sides of the root, directly opposite each other. In arabidopsis, the lateral roots differentiate from this tissue, but this may not be the case in all plant species.

Figure 1

Sager et al 2020 TIPS Fig 1

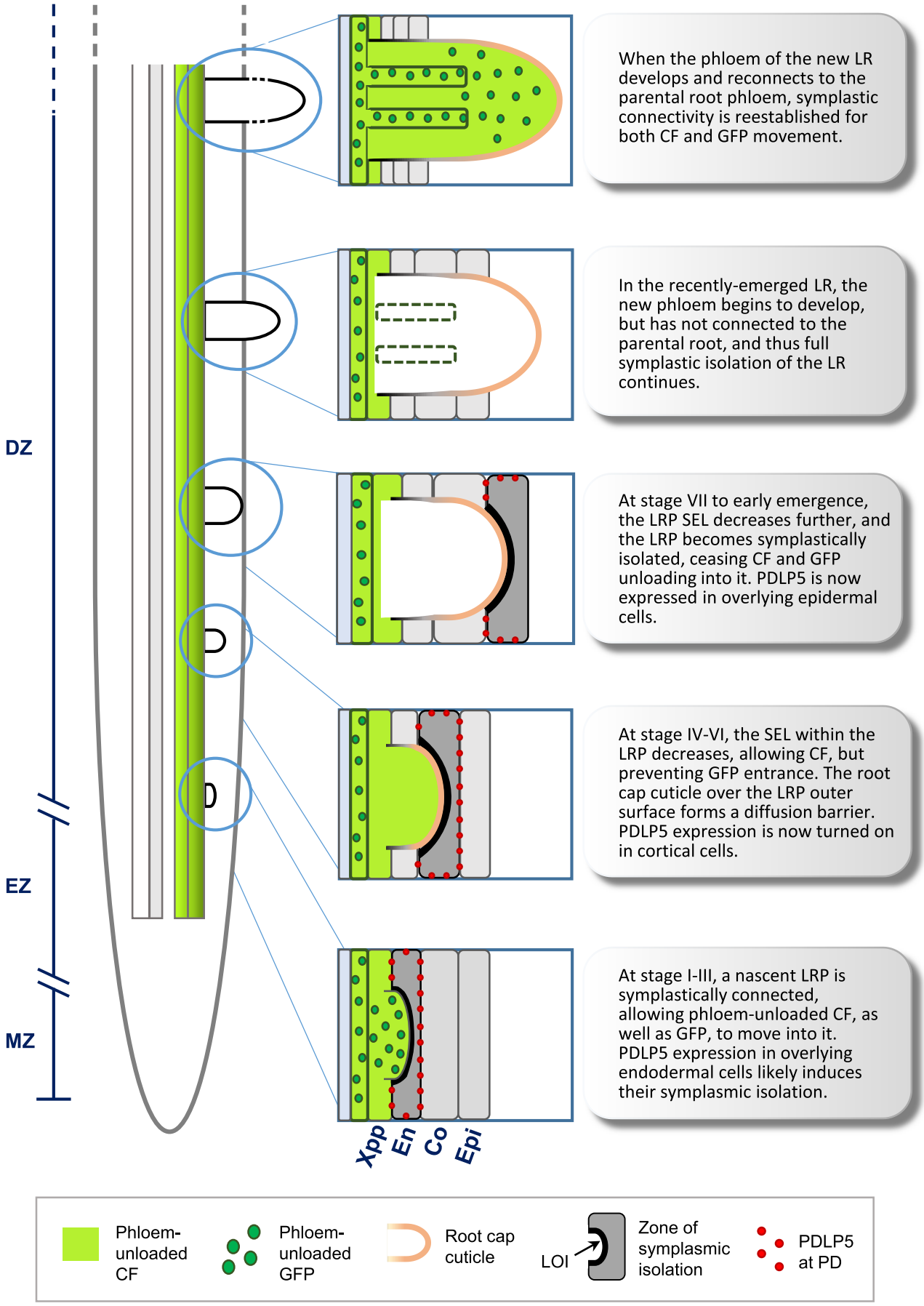
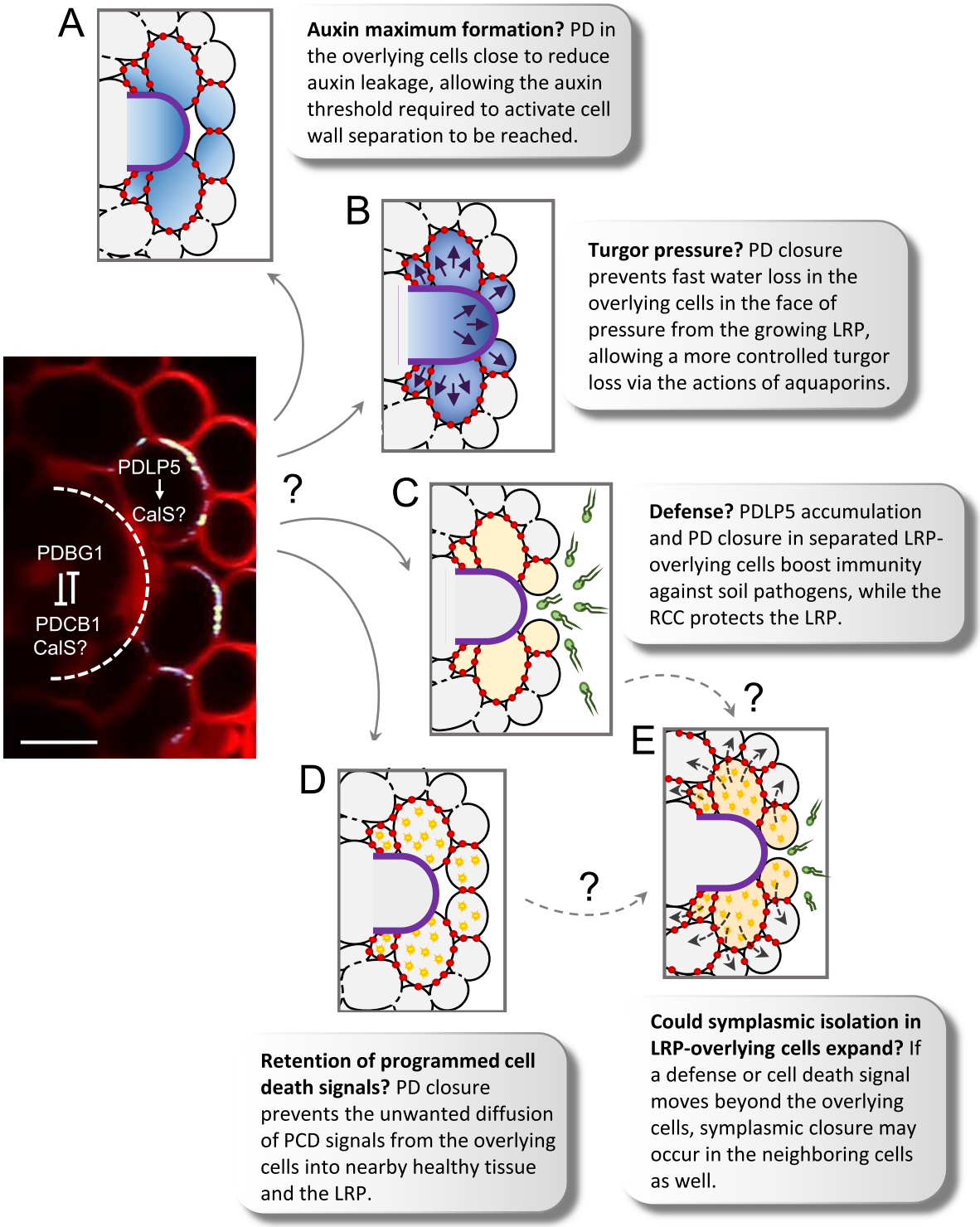
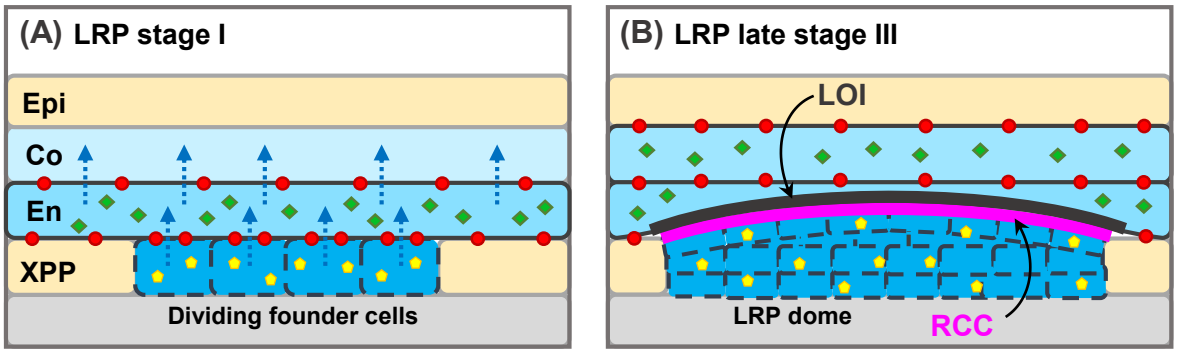


Figure 2

Sager et al 2020 TIPS Fig 2



Sager et al 2020 TIPS BOX 1 Fig 1



A tale of two domains pushing lateral roots

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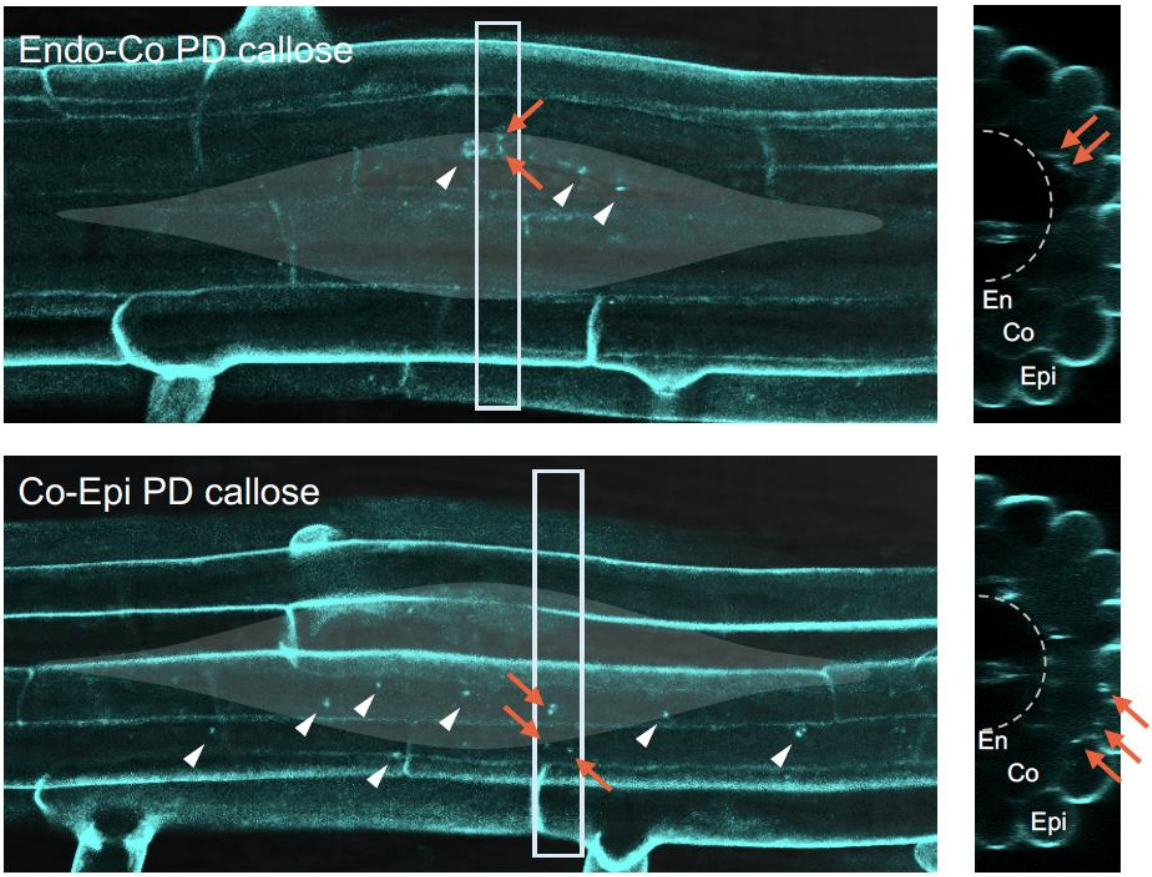

Figure S1. Aniline blue-stained callose in plasmodesmal pit fields of LRP-overlying cells. In longitudinal view, the overlaid grey shape marks the position of the LRP; white arrowheads and orange arrows are callose-stained pit fields; and the white box represents the area of the cross-sectional view. In cross-sectional view, the dashed arc is LRP position; orange arrows are pit fields corresponding to those marked by orange arrows in the longitudinal view. Abbreviations: PD, plasmodesmata; Endo, endodermis; Co, cortex; Epi, epidermis; LRP, lateral root primordium. Images are taken from Supplementary Figure 6b in Sager et al, 2020, with modification and authors' permission.