DISPATCH

Plant Biology: Building Barriers…In Roots (au: I changed the title a bit to make it more within our house style. Is this ok with you?)

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The Casparian strip is an important barrier regulating water and nutrient uptake into root tissues. New research reveals two pepide signals and their co-receptors play critical roles patterning and maintaining barrier integrity.

Plant roots are required to explore highly heterogeneous soil environments to secure essential resources such as water and nutrients [1]. Roots have developed barrier-like structures to regulate the flow of these resources between the external soil environment and xylem tissue that transport them to the shoot (Figure 1A). These root barriers include the Casparian strip that forms a watertight structure in the endodermal layer [2] and blocks the free passage of chemicals between cortex and xylem (Figure 1A). How such a precisely positioned barrier forms has recently attracted significant research interest. New papers published in *Current Biology* [3] and *Science* [4,5] provide novel molecular insights, reporting new components and an elegant patterning mechanism that controls Casparian strip formation and integrity.

The Casparian strip is initially composed of lignin [6]. The later deposition of a glycerolipid termed suberin into the intercellular space surrounding endodermal cells creates a barrier that is essentially impermeable and prevents water and nutrients from seeping through (Figure 1A). This creates a continuous barrier stretching from the root differentiation zone (near the tip) to the root-shoot junction. Whilst water, minerals and nutrients can freely flow between cells in root tip tissues (younger tissue), these resources can only enter vascular tissues through endodermal cells in older root tissues once the Casparian strip forms in the root differentiation zone (Figure 1A).

Mutants that cause the Casparian strip to form discontinuously exhibit changes in elemental accumulation in shoot tissues. For example, Li *et al.* [3] reported the isolation of a new *A. thaliana* mutant (termed *lord of the rings 1; lotr1*) which, like almost every other Casparian strip mutant characterised to date, exhibits significantly lower levels of elements like calcium in their shoot tissues. All of these Casparian strip mutants share an inability to form a continuous barrier. However, this common defect is coupled with enhanced suberin levels, presumably to compensate for the compromised integrity of the lignin barrier. Li *et al.* [3] elegantly tested this hypothesis by crossing *lotr1* with the *CDEF1* transgenic line (that degrades suberin), which reversed the mutant's shoot calcium accumulation defect, proving that the enhanced suberin is causing the reduced Ca concentration in the shoot.

The *schengen3* (*sgn3*) mutant also features a discontinuous Casparian strip [7]. However, *sgn3* mutant roots fail to trigger compensatory changes in suberin levels, causing even larger effects on shoot elemental accumulation [3]. The absence of

compensatory suberin feedback regulation suggests that SGN3 functions as part of the signalling machinery which monitors the integrity of the Casparian strip. Consistent with such a signalling function, SGN3 encodes a leucine-rich repeat (LRR) receptor-like kinase localised either side of the Casparian strip (Figure 1A) [7]. However, until now the identity of the ligand binding SGN3 remained a mystery.

Employing distinct genetic and biochemical based ligand discovery approaches, Doblas *et al.* [4] and Nakayama *et al.* [5] report in *Science* that SGN3 and its co-receptor SGN1 [7,8] bind the closely related peptide signals CASPARIAN STRIP INTEGRITY FACTOR 1 & 2 (CIF1 & CIF2). Together they are able to trigger Casparian strip synthesis. Consistent with CIF1 and CIF2 being SGN3 ligands, the *cif1 cif2* mutant phenocopied the *sgn3* Casparian strip defect, while addition of the CIF1/2 peptide could rescue the former, but not the latter, mutant phenotype [4,5]. SGN3 controls Casparian strip formation by promoting the formation of a continuous band of membrane proteins termed CASPs that function as a scaffold upon which the lignin-based barrier forms [7,9]. Addition of the CIF1/2 peptide to the *cif1 cif2* mutant was able to rescue formation of the CASP band, which was visualised using a CASP1–GFP reporter [5]. Hence, the CIF1/2–SNG3–SNG1 signalling module is critical for correctly patterning the formation of a contiguous CASP band and Casparian strip.

So how do these components function to monitor the integrity of the Casparian strip? The key appears to lie in their relative sub-cellular and cellular distributions (Figure 1A) [5]. The plasma membrane localised receptor-like kinase SGN1 is targeted to the outer face of endodermal cells (i.e. facing cortical cells) [8], whereas SGN3 flanks the Casparian strip [7], while CIF1/2 are expressed in root vascular tissues [4,5]. When the Casparian strip is intact, CIF2 cannot diffuse to the outer face of endodermal cells (Figure 1A), meaning that SGN3 and SGN1 do not interact to promote Casparian strip formation. If this barrier is disrupted, CIF2 can diffuse between endodermal cells, where it binds to SGN3 and forms a co-receptor complex with SGN1, triggering the formation of a band of CASP proteins, leading to Casparian strip synthesis.

The novel LOTR1 protein also appears to be essential for CASP protein localisation [3]. Mutant roots lacking LOTR1 exhibit CASP1–GFP mis-localisation to the vascular face of endodermal cells (in addition to a correctly localised strip that forms as normal). Exactly how LOTR1 regulates CASP targeting remains to be elucidated. LOTR1 is predicted to be targeted to the endodermal apoplastic space. However, based on the invaluable mechanistic insights gained from the detailed knowledge of SGN1/SGN2/CIF1/CIF2 localisation, elucidating LOTR1 sub-cellular position will be necessary to better understand its role in Casparian strip formation and maintenance.

The integrity of the Casparian strip is frequently compromised when lateral root primordia (LRP) emerge (Figure 1B). LRP originate from pericycle cells, which have to push through overlying endodermal, cortical and epidermal tissues before emerging into the soil [10,11] (Figure 1A,B). So how does a root compensate for the disruption to the Casparian strip? Intriguingly, Nakayama *et al.* [5] observed that CIF2 was not expressed at sites of lateral root initiation, suggesting that the Casparian strip repair mechanism is supressed at the site of organ emergence.

Instead, Li *et al*. [3] reports that a layer of suberin is deposited at lateral root emergence sites, covering both endodermal cells and new primordia (Figure 1B). This suberin layer acts as an apoplastic diffusion barrier, providing a seal around the new primordia and ensuring that continuity with the mature Casparian strip in the main root is maintained. As a result, the apoplastic tracer, propidium iodide is only able to enter vascular tissues at lateral root emergence sites when suberin is degraded in the *CDEF1* transgenic line [3]. Surprisingly, ectopic deposition of suberin in *lotr1* caused emergence of new root primordia to be delayed [4]. However, this defect can be reversed by expressing the suberin-degrading CDEF1 enzyme in this mutant background [3]. This observation highlights the importance of regulating the spatio-temporal distribution of suberin in root tissues, since ectopic deposition will create apoplastic barriers that could disrupt aquaporin-mediated water transport fluxes which promote lateral root emergence [12].

Is the Casparian strip simply a passive barrier or can its properties change depending on environmental conditions? Barberon *et al*. [13] elegantly demonstrated that suberin could be added or removed depending on external nutrient conditions. Nakayama *et al*. [5] also noted that *CIF1* and *CIF2* mRNAs are upregulated by excess external iron [5]. Consistent with this observation, *cif1 cif2* is more sensitive to excess iron than wildtype, most likely as a result of this element leaking into the xylem of the mutant, causing elevated levels to be transported to the shoot.

We conclude from these recent studies that the Casparian strip provides a highly effective barrier, and its permeability is far more dynamically regulated than originally imagined. This enables the root to rapidly adapt to dynamic changes in the highly heterogeneous environment that soil represents. Building impermeable, rigid barriers makes little sense for plants. We humans have much to learn…

Figure 1. The Casparian strip provides a root barrier regulating water and nutrient uptake whose integrity needs to be maintained where new lateral roots are formed.
(A) Schematic illustrating how the Casparian strip is patterned and functions as a root barrier. The Casparian strip forms a ring-like structure between endodermal cells, sealing the intercellular (apoplastic) space. If disrupted, CIF1/2 peptides diffuse from inner root tissues and promote co-receptors SGN1 and SGN3 to interact and trigger Casparian strip synthesis. When intact, CIF1/2 peptides remain within inner root tissues and SGN1 and SGN3 don't interact in outer facing endodermal plasma membranes. If taken up into the cytoplasm of epidermal cells, solutes can bypass the Casparian strip by moving through intercellular pores called plasmodesmata from cell to cell into inner root tissues before being loaded into xylem cells and transported to the shoot (symplastic transport). Solutes can also move in the apoplast between epidermal and cortical cells (apoplastic transport) until reaching the Casparian strip, where they must move into endodermal cells using membrane carriers before reaching xylem cells and shoot tissues. (B) Casparian strip integrity is disrupted when new lateral roots initiate in the pericycle and emerge through overlying tissues. This triggers the root to deposit the glycerolipid suberin (denoted in yellow) onto outer endodermal (red) and LRP (grey) surfaces in order to seal holes and maintain the root barrier integrity.

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in Brief

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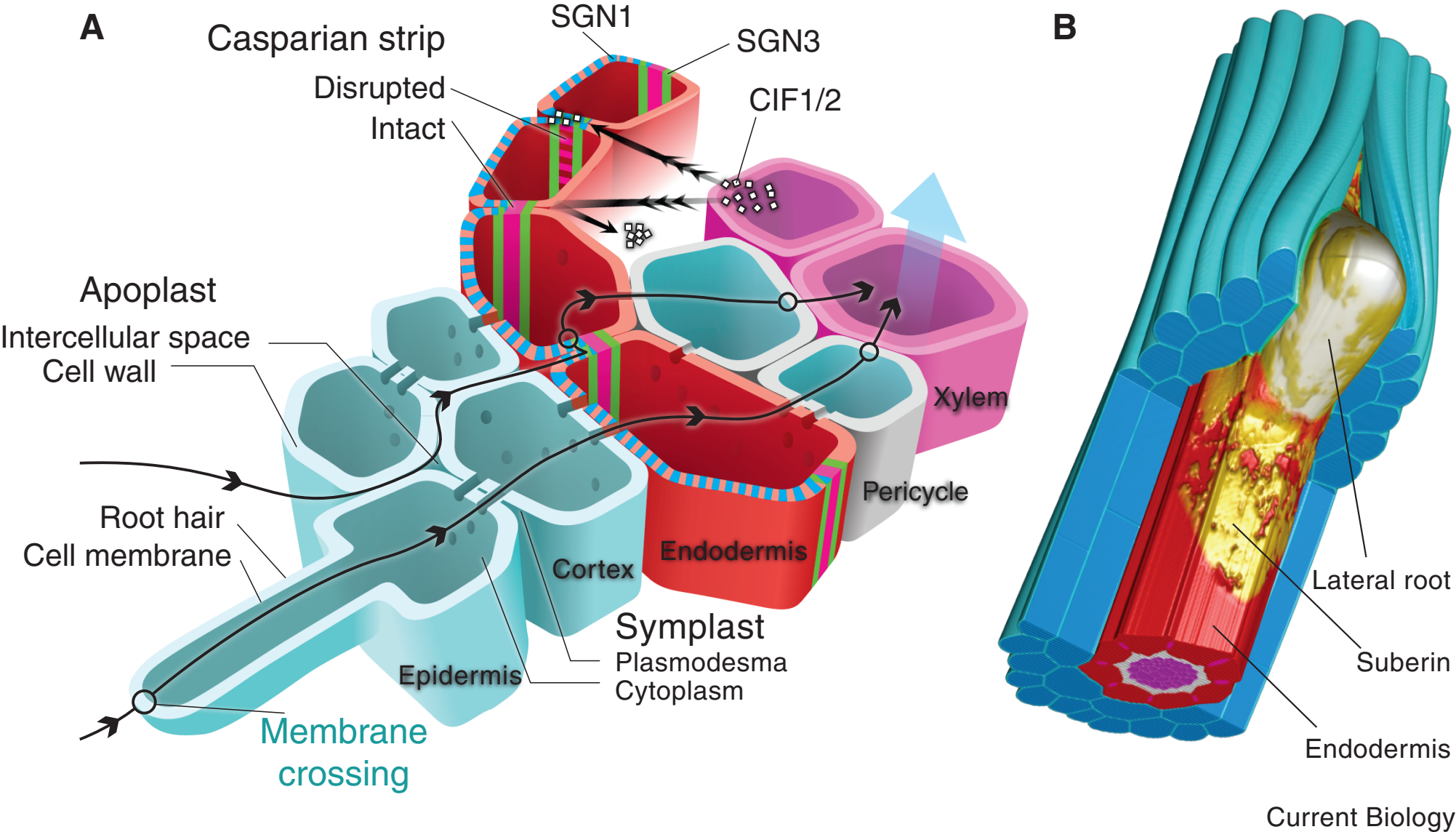
A
Casparian strip
SGN1
SGN3
Disrupted
Intact
CIF1/2
Apoplast
Intercellular space
Cell wall
Xylem
Pericycle
Root hair
Cell membrane
Cortex
Endodermis
Symplast
Plasmodesma
Cytoplasm
Epidermis
Membrane crossing
B
Lateral root
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