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The endodermis, a tightly controlled barrier for nutrients

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Plant roots acquire nutrients from the soil and transport them upwards to the aerial parts. To reach the central vasculature of the root, water and nutrients radially cross all external cell layers. The endodermis surrounds the vascular tissues and forms diffusion barriers. It thereby compartmentalizes the root and allows control of nutrient transport from the soil to the vasculature, as well as preventing backflow of nutrients from the stele. To achieve this role, endodermal cells undergo two specialized differentiations states consisting of deposition of two impermeable polymers in the cell wall: lignin, forming the Casparian strips, and suberin lamellae. Recent publications showed that endodermal barrier formation is not a hard-wired, irreversible process. Synthesis and degradation of suberin lamellae is highly regulated by plant hormones in response to nutrient stresses. Moreover, Casparian strip continuity seems to be constantly checked by two small peptides produced in the vasculature that diffuse into the apoplastic space in order to test endodermal barrier integrity. This review discusses the recent understanding of endodermal barrier surveillance and plasticity and its role in plant nutrition.

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Introduction

Plants cannot escape the fluctuations in nutrient availability and/or toxicity in their environment. The plant root is the organ in charge of nutrient acquisition, but at the same time, it must provide an efficient boundary against external stresses in order to maintain plant fitness. The endodermis, the innermost cortical layer surrounding the vasculature, forms barriers controlling root waterproofing by undergoing two differentiation states, impregnating cell walls with lignin (giving rise to Casparian strips) and adding suberin lamellae, respectively [1]. These endodermal barriers have long been considered to be acting as passive barriers in the soil-vasculature pathway [2,3]. Recent studies with Arabidopsis have highlighted that the endodermis forms a bidirectional barrier controlling the access to the root vasculature but also to prevent solutes from leaking out [4,5••,6,7•,8•,9••]. More surprisingly, the endodermal barrier formation is tightly modulated in response to external and internal signals thereby fine-tuning nutrient acquisition and endodermal integrity [5••,10••,11••]. This review focuses on the recent advances with the model plant Arabidopsis on endodermal barrier establishment, integrity, function and plasticity.

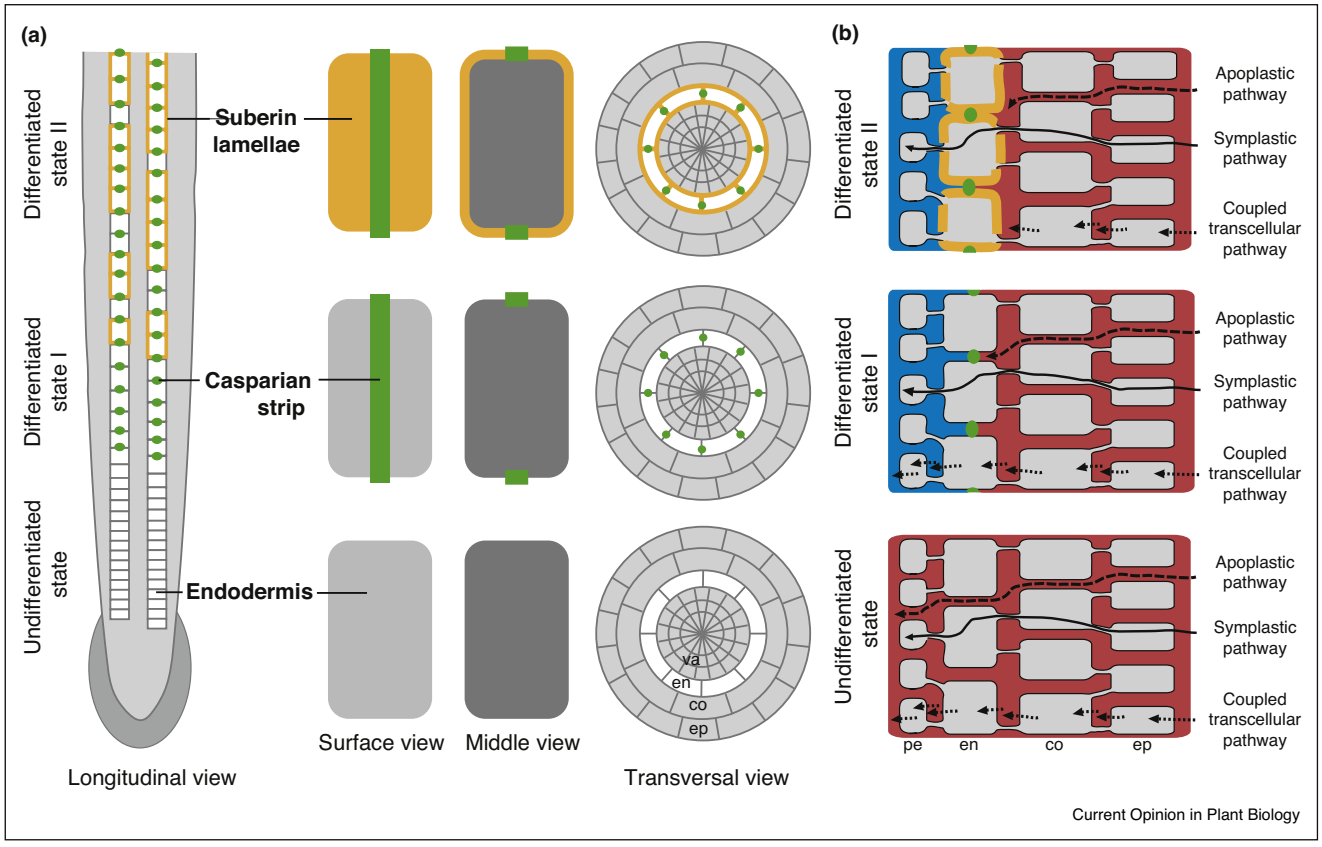
Endodermal barrier formation

Endodermal cells are specified close to the quiescent center of root meristems, associated with a periclinal division of the cortex-endodermis initial cells [12]. After this specification, the endodermis undergoes two distinct levels of differentiation characterized by formation of Casparian strips and suberin lamellae (Figure 1a) [13,14].

The Casparian strips correspond to localized lignin depositions at the junction between adjacent endodermal cells that fuse to form a ring, sealing the apoplastic space (Figure 1a) [15,16,17•]. The transcription factor MYB DOMAIN PROTEIN36 (MYB36) is the major transcriptional regulator of the transition to the first state of endodermal differentiation and controls the expression of the main genes involved in Casparian strip establishment [8•,18•]. The Casparian strip deposition is initiated by the localization of CASPARIAN STRIP DOMAIN PROTEINs (CASP1-CASP5) at the Casparian strip membrane domain (CSD) [19]. CASPs recruit secreted proteins to the CSD such as PEROXIDASE64, and the dirigent domain-containing protein ENHANCED SUBERIN1 (ESB1) which together with the NADPH oxidase F (RbohF) form localized lignin depositions [7•,20]. The leucine-rich repeat receptor-like kinase (LRR-RLK) SCHENGEN3 (SGN3, also known as GSO1) and the receptor-like cytoplasmic kinase SGN1, which localize around the forming CSD and to the outer plasma membrane site of endodermal cells respectively [9••,21•], have been shown to control the fusion of the Casparian strips into a continuous ring and to control Casparian strip integrity [9••,21•].

The state II of endodermal differentiation is marked by suberin lamellae, a hydrophobic polymer forming a secondary cell wall deposition at the inner surface of primary cell walls covering the entire surface of endodermal cells (Figure 1a) [13,22,23]. Transition from state I to state II starts in a patch-like manner, until the whole endodermis is suberized with the exception of passage cells. While the

Figure 1



Endodermal differentiation and barriers. **(a)** Schematic representation of endodermal differentiation, presented as longitudinal (left panel), single cells (middle panel) and transversal views (right panel) for undifferentiated and differentiated states I and II of the endodermis (not at scale). **(b)** Radial transport of nutrients across root cell layers. Three pathways coexist in the undifferentiated endodermis: apoplastic, coupled transcellular and symplastic pathways. In state I endodermal differentiation, the apoplastic pathway is blocked by the Casparian strip and in state II suberin lamellae blocks the coupled transcellular pathway. Red and blue colors show the continuity or block in the apoplastic space, respectively. va, vascular tissue; pe, pericycle; en, endodermis; co, cortex; ep, epidermis.

biosynthetic pathway for suberin is well documented, the regulatory components controlling suberization remain to be elucidated. Recently, endodermal abscisic acid (ABA) signaling was shown to control suberization under non-stressed conditions [5••]. The transcription factor MYB41, whose ectopic expression triggers ectopic suberization and whose expression in the endodermis is induced by ABA, represents a good candidate to control state I to state II transition but its function in the endodermis remains to be clarified [24•].

The endodermis as a bidirectional barrier for nutrients

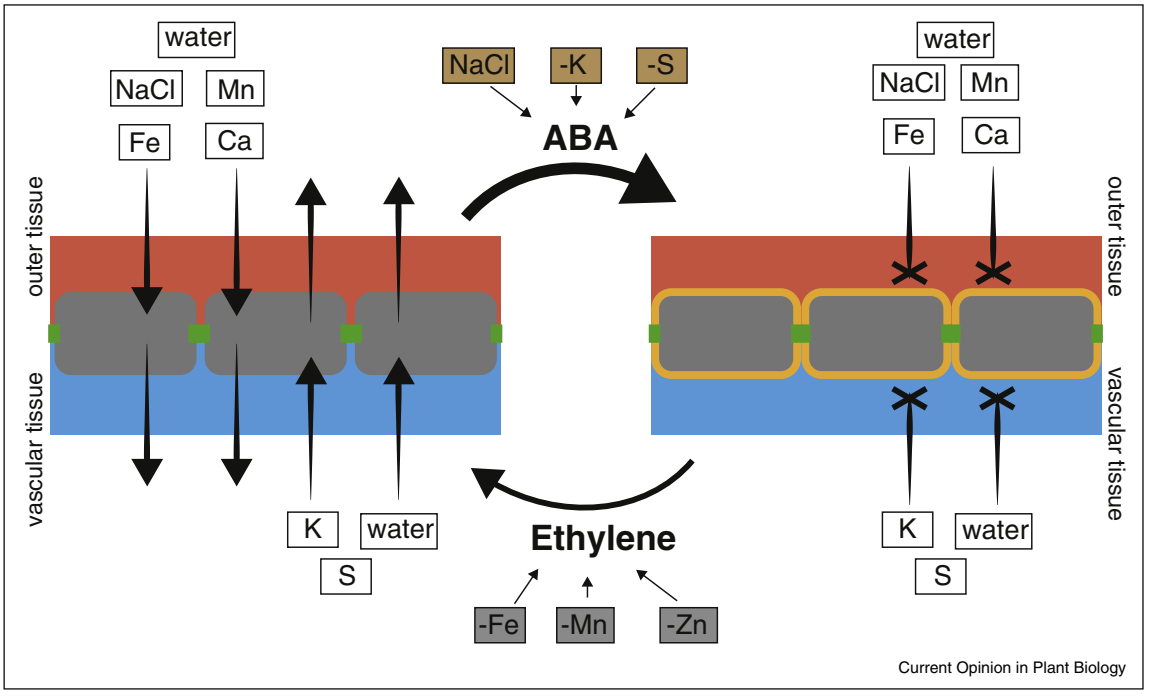
On their way from the soil to the central vasculature water and nutrients can follow three different paths: (i) apoplastic, via diffusion in the extracellular space; (ii) symplastic, via cytosolic connections called plasmodesmata; (iii) transcellular, via polarized influx and efflux carriers and/or diffusion gradients through the plasma membrane (Figure 1b).

By sealing the apoplastic space between adjacent endodermal cells the Casparian strips form an apoplastic barrier blocking the free diffusion of water and nutrients entering the inner part of the root (Figure 1b) [2,3]. In Arabidopsis, this well-known property can be easily observed with the fluorescent apoplastic tracer propidium iodide (PI) that penetrates extracellular spaces of every cell layer until being blocked by Casparian strips [15,17•]. Therefore, in the mutant *sgn3* displaying interrupted Casparian strips, PI can reach the central vasculature all along the root demonstrating loss of an apoplastic barrier in this mutant [9••]. However, it is important to acknowledge that most Casparian strip mutants such as *esb1*, *casp1 casp3*, *myb36* and *lotr1* (*lord of the rings 1*) are only delayed in the establishment of a functional apoplastic barrier. This is due to an ectopic lignification together with a precocious suberization close to the root meristem [6,7•,8•,25•]. These mutants, displaying very similar endodermal barrier defects, were shown to also have comparable changes in mineral content with higher

potassium (K) and sulfur (S) content and a lower calcium (Ca), manganese (Mn) and iron (Fe) content [7•,8•,25•]. However, these differences were difficult to assign to either CS defects or enhanced suberization, as both occurred concomitantly in these mutants. Therefore, the *sgn3* mutant, displaying interrupted Casparian strips with no ectopic lignin or suberin compensations, represented the best opportunity to characterize the function of Casparian strips in plant nutrition. Ionomic analysis in *sgn3* revealed that the lack of an apoplastic barrier does not result in a massive accumulation of minerals but to subtle changes, the main consequences being a higher magnesium (Mg) content and a reduced zinc (Zn) and K content associated with K deficiency [4,9••].

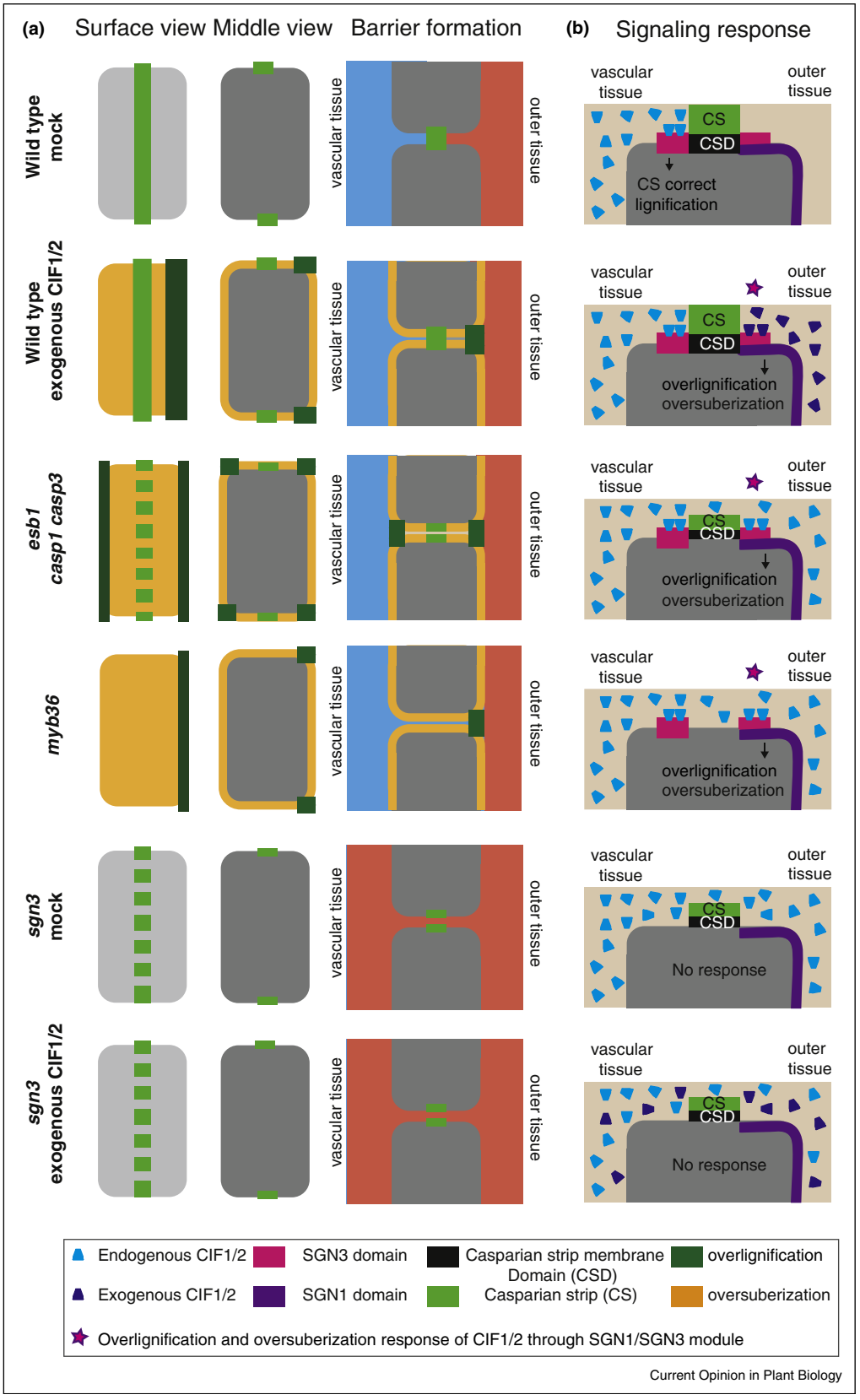
In primary roots, suberin lamellae are not forming an apoplastic barrier [5••,7•,8•,9••,17•]. This can be demonstrated with Casparian strip mutants where PI block and suberin deposition are not correlated [7•,8•,9••,17•]. Interestingly, a recent study shows that at the site of lateral root emergence, (i.e. when Casparian strips are disrupted) [26], the ability to deposit suberin can be important to reseal the apoplastic barrier [25•]. This could explain the appearance of apoplastic barrier discontinuities observed in the older parts of the root of suberin mutants [25•,27]. In addition there is clear evidence that in primary roots suberin controls the uptake or passive diffusion from the apoplast into endodermal cells (transcellular barrier). This function has been demonstrated in Arabidopsis with the fluorescent cellular tracer fluorescein diacetate (FDA), whose uptake in endodermal cells is delayed in presence of suberin [5••]. Illustrating the function of suberin as a bidirectional barrier, carboxyl-FDA unloading from the phloem was shown to be block at the level of endodermal suberization [28]. The function of suberin in plant nutrition has been extensively studied in various plant species by comparing transport, accumulation and fitness of cultivars displaying variations in suberin [29–37]. In Arabidopsis, the suberin biosynthesis mutants, *gpat5* (*glycerol-3-phosphate sn2-acyltransferase 5*), *horst* (*hydrolase of root suberized tissues*) and the triple mutant *abcg2 abcg6 abcg20* (*ATP-binding cassette transporters clade g 2, 6 and 20*), displaying reduced suberization were shown to exhibit higher root hydraulic and osmotic conductivities and higher permeability to solutes, in particular to sodium (Na) [27,38,39]. Physiological and ionomic analysis of suberin-deficient lines (CDEF1 lines, expressing the CUTICULE DESTRUCTING FACTOR 1 in the endodermis), revealed a moderate but specific effect of suberization on mineral accumulation with an increase in lithium (Li), arsenic (As) and Na content and a decrease in K content associated with salt hypersensitivity

Figure 2



Nutrient-induced plasticity of suberin. Schematic representation of antagonistic effects of nutrient availability on suberization of endodermal cells through modulation of abscisic acid (ABA) and ethylene. NaCl excess and K and S deficiencies increased suberization mediated by ABA while Fe, Mn and Zn deficiencies reduced suberization mediated by ethylene. Water, NaCl, Fe, Mn, Zn and Ca pass through the endodermis to reach the stele as long as endodermal cells are not suberized. K, S and water can flow out from the stele across non-suberized endodermal cells.

Figure 3


(a)
Surface view
Middle view
Barrier formation
(b)
Signaling response
Wild type mock
Wild type exogenous CIF1/2
esb1 casp1 casp3
myb36
sgn3 mock
sgn3 exogenous CIF1/2
vascular tissue
outer tissue
CS
CSD
CS correct lignification
overlignification oversuberization
No response
Endogenous CIF1/2
Exogenous CIF1/2
SGN3 domain
SGN1 domain
Casparian strip membrane Domain (CSD)
Casparian strip (CS)
overlignification
oversuberization
Overlignification and oversuberization response of CIF1/2 through SGN1/SGN3 module
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and K deficiency [5••,17•]. Recently, the analysis of CDEF1 lines in the *lotr1* and *esb1* mutant backgrounds allowed dissecting the contribution of endodermal suberin versus lignin in the ionomic phenotypes of these mutants [25•]. This elegant approach allowed to demonstrate that the reduced Ca content observed in *lotr1* and *esb1*, associated with low Ca sensitivity was caused primarily by enhanced suberization, suberin forming a barrier for the uptake of Ca [25•].

Altogether these analyses highlight a fundamental but often overlooked property of the endodermis as a bidirectional barrier, controlling not only the path from the soil to the vasculature but also preventing backflow from the central vasculature [4,9••]. This can be nicely illustrated with the examples of Ca and K whose accumulation in endodermal mutants vary in opposite ways, suberin controlling the entrance of Ca and preventing the leakage of K from the vasculature [5••,7•,8•,25•,40]. In agreement with this model, suberization was shown to interrupt Ca^{2+} influx [40] and K to accumulate principally in the root vasculature [41].

Nutrient-induced plasticity of endodermal differentiation

Endodermal barriers are modified in response to a multitude of abiotic stresses. Salt stress was particularly well studied and shown to affect the width of Casparian strips and to induce suberization in many plant species [5••,29,30,32,34,42,43]. Induced suberization in toxic environments seems to be a general feature in plants suggesting a strategy to block the entry of toxic elements [36,44–46]. In the same way, waterlogging and drought stress conditions induce suberization suggesting a strategy to prevent water and oxygen loss [31,34,45,47,48]. In Arabidopsis, nutritional deficiencies were also shown to affect suberization with Fe, Mn and Zn limitations leading to a reduced suberization and K and S deficiencies leading to an increased suberization (Figure 2) [5••]. These opposite effects could reflect an adaptation of endodermal suberization to modulate the uptake of Fe, Mn and Zn and retain K and S under limiting conditions, reflecting the role of the endodermis as a bidirectional barrier. In agreement with this hypothesis, the CDEF1 lines (with reduced suberization) were shown to have a reduced K content and the mutants with ectopic suberin (*esb1*, *casp1 casp3*, *myb36* and *lotr1*) were shown to have a reduced Mn and Fe content and an increased K and S content (Figure 2) [4,5••,7•,8•,25•]. Extending this model further, reduced suberization restores growth and development of the iron-limited mutant *irt1* (*iron regulated transporter 1*) and exacerbates the growth defect of the sulfate-limited double mutant *sultr1;1 sultr1;2* (*sulfate transporter*) [5••]. This nutrient-induced plasticity of suberization was shown to be control by the antagonistic action of ABA and ethylene leading to increase and reduction of suberin respectively [5••]. However, the signaling cascade controlling suberization is still poorly understood in particular concerning ethylene that has a moderate effect on suberin compared to ABA [5••]. ABA seems to be a general regulator of suberization, its endodermal signaling being required for suberization in non-stressed conditions and most genes involved in suberization being regulated by this hormone [5••,24•,27].

Endodermal barrier surveillance by a small peptide

As mentioned above, most mutants with impaired Casparian strips display a compensatory response with ectopic lignin and suberin, in turn counteracting the initial apoplastic barrier loss [7•,8•,25•]. Hence, the *myb36* mutant impaired in transition to the state I of endodermal differentiation lacks Casparian strips, but forms an ectopic deposition of lignin in the outer corners of endodermal cells and ectopic suberin close to the root tip (Figure 3a) [8•,18•]. In the same way, the mutants *esb1*, *casp1 casp3* and *lotr1* display interrupted Casparian strips later compensated with ectopic lignification at both sides of the endodermal cells and ectopic suberization in early stages of endodermal differentiation (Figure 3a) [7•,19,25•]. Interestingly, this compensatory response to Casparian strip defects was shown to be SGN3-dependent, ectopic lignification and suberization not occurring in a *sgn3* mutant and *sgn3* being epistatic to mutants such as *esb1* and *casp1 casp3* [9••]. Likewise, *sgn1* mutant also did not present ectopic lignification, and strong ectopic suberization indicating that also SGN1 is required for this compensatory mechanism [21•].

Recently, two publications shed light on the molecular mechanism underlying this compensation. In both publications two small secreted peptides were identified as ligands of SGN3 by two independent approaches: (i) by identifying TyrosylProtein SulfoTransferase (TPST) as the causal gene of the barrier-defective *sgn2* mutant and subsequent search for the sulfated peptide substrate of TPST (ii) by bioinformatic analysis based on conserved C-terminal domain of peptides, and demonstration of

(Figure 3 Legend) Model for endodermal barrier integrity surveillance by CIF1/2. **(a)** Schematic representation of Casparian strip and suberin deposition in single endodermal cells, at differentiation state I (left panel) and apoplastic barrier formation between two cells (right panel) in WT mock, WT with externally applied CIF1/2 peptides, *esb1, casp1 casp3* and *myb36* mutants without CIF treatment, and *sgn3* mock and with externally applied CIF1/2 peptides. Red and blue colors show the continuity or separation in the apoplastic space, respectively. **(b)** Hypothetical model (not at scale) of CIF1/2-SGN3 signaling in the respective genotypes and conditions described in A. Note that SGN3 and SGN1 localization in *esb1*, *casp1 casp3* and *myb36* mutants are predicted based on localization in WT.

their binding-activity to heterologously-expressed LRR-receptors [10••,11••]. The two peptides, named Casparian strip Integrity Factor (CIF1/2), are produced in the vascular tissue, are activated by sulfation as post-translational modification carried out by TPST [49] and were shown to bind with very high affinity to the LRR-RLK receptor SGN3 [10••,11••]. It was noted that exogenous application of these peptides complements *sgn2*, and in high concentration leads to ectopic lignin deposition at the outer side of the CSD and oversuberization in wild-type, in a fashion very comparable to the compensation observed in Casparian strip defective mutants (Figure 3) [10••]. Moreover, CIF1/2 overstimulation effects are totally absent in the *sgn3* mutant (Figure 3), and attenuated in *sgn1* mutant. This led us to hypothesize that when the endodermal layer is properly sealed, CIF1/2 are retained in the stele and cannot diffuse through the apoplast to the outer side of the CSD, where a complex of SGN3 and SGN1 could transduce the signal. However, in the absence of an intact Casparian strip, the peptides could leak out between the endodermal cells physically reaching the SGN3/SGN1 module and activating a signaling cascade producing extra-lignification and extra-suberization to seal the barriers (Figure 3). This mechanism would represent an exquisite model for how the root can sense whether its endodermal barrier is intact or not.

Conclusions

Altogether the recent works presented here highlight that endodermal differentiation is more plastic than previously expected, with external signals such as nutrient availability and internal signals with the CIFs peptides controlling and remodeling endodermal barriers. It will be essential in the future to establish whether these two signaling cascades are interconnected and if other signals such as biotic or mechanical stresses are also affecting endodermal differentiation. We can expect that the molecular knowledge gained from Arabidopsis could now be transposed in plants with agronomical interests. This would have a tremendous impact on our understanding of the mechanisms of plant nutrition, most root systems being more complex than Arabidopsis, and could open perspectives in plant breeding. In this context, the discovery of the CIFs peptides controlling Casparian strip integrity is particularly interesting. This endodermal barrier surveillance mechanism by CIF/SGN3 appears to be evolutionarily conserved, CIF peptides being widespread among all seed plants [50•]. Therefore, a conserved mechanism of action could allow for a direct manipulation of root waterproofing capacities, after application of CIF analogues, reinforcing barriers in turn modulating water and nutrient transport.

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