COMMENTARY

Caspary's conductor

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Cell walls are essential features of plant cells and modifications of this apoplastic space implement a number of structural and physiological functions. The Casparian strip (CS), named after its 1865 discoverer Robert Caspary (1), is a remarkable cell wall modification that all biology students become acquainted with in undergraduate plant anatomy courses. Typically, CSs occur in the endodermis of roots, the organ performing the uptake of water and nutrients. The CS can be envisioned as a belt-like structure, specifically localized in the middle of the anticlinal wall all around an endodermal cell (Fig. 1). CSs, chemically dominated by lignin (2, 3), bridge the cell wall of adjacent endodermal cells and thus seal the extracellular space. Consequently, apoplastic passage of ions is restricted and transporter and channel proteins in stele and soil-directed plasma membranes selectively permit the radial transport of ions from the soil to the stele (4). Conversely, CSs in the endodermis also prevent the apoplastic backflow of ions from the stele to the cortex. The resulting ion build-up in the stele and subsequent osmotic water movements generate the root pressure, enabling vertical water and nutrient transport when transpiration is limited. When roots develop further, suberin, a cell wall modification of aromatic and aliphatic materials, is deposited as lamella all around the endodermal cell (5). This additionally prevents transcellular paths across membranes and thus complicates the evaluation of the CS's impact on water relations and nutrient homeostasis. However, although a consistent root structure and physiologically essential, little is known about the molecular components that regulate CS localization and biogenesis. In PNAS, Kamiya et al. (6) present a great step forward in the understanding of how and what controls CS formation.

Reports on the molecular factors governing CS development started only recently. Roppolo et al. (7) aligned endodermal enriched transcripts with membrane protein predictions. This strategy identified a family of proteins that localize to the plasma membrane domain at the site of CS formation, the Casparian strip membrane domain (CSD). Furthermore, they demonstrated that the Casparian strip membrane domain proteins (CASPs) CASP1 and CASP3 are required for correct CS formation. Subsequent genetic screens for impeded CS barrier function identified a NADPH oxidase called respiratory burst oxidase homolog F (RBOHF) as part of the biosynthetic machinery for the CS lignin (8). RBOHF generates reactive oxygen species that further downstream feed H_2O_2 to Peroxidase 64 (PER64) to initiate radicals for monolignol polymerization (Fig. 1C). Colocalization experiments indicated that CASPs interact with and precisely position PER64 and RBOHF at the site where CSs develop. Enhanced suberin 1 (ESB1), a dirigent-like
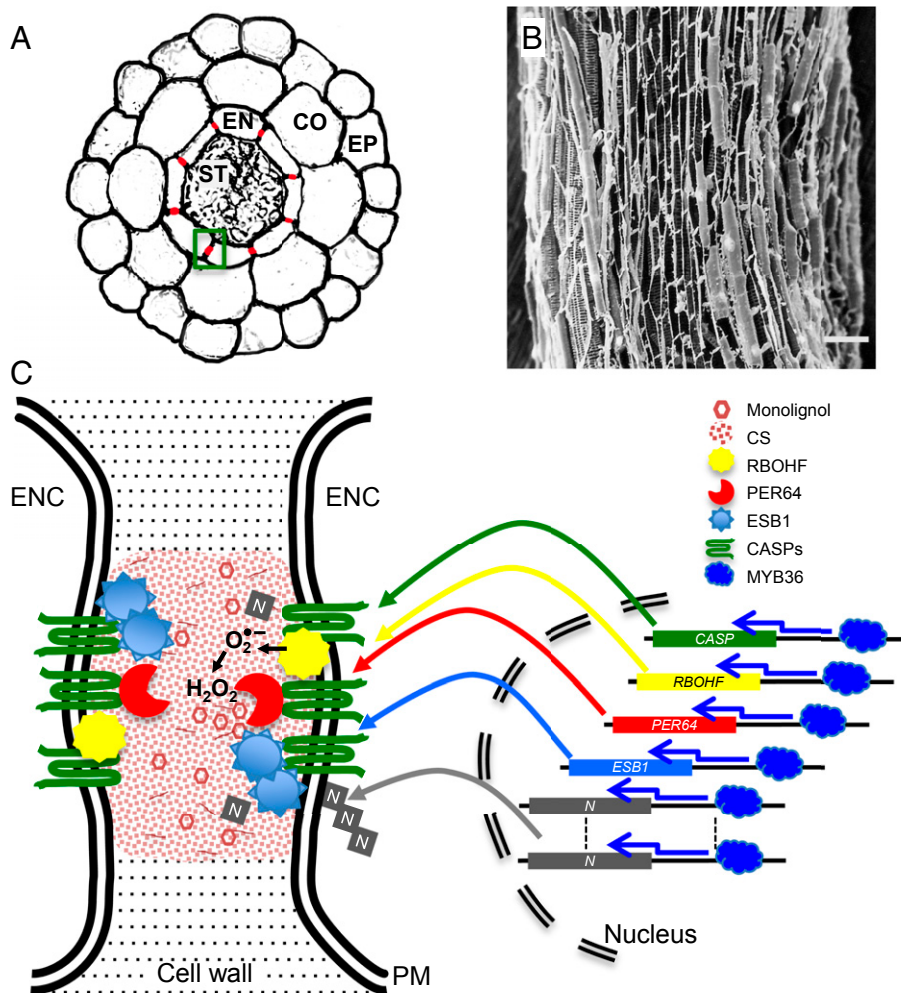

Fig. 1. The CS in the root endodermis. (A) Schematized micrograph of a cross-section through a primary root of *Arabidopsis*. The green box indicates position of the CS (red). CO, cortex; EN, endodermis; EP, epidermis; ST, stele. (B) Scanning electron micrograph of an enzymatically isolated Casparian strip from *Clivia miniata* roots. Longitudinal view. The lignified anticlinal cell walls restrict enzyme digestion and remain as net. (Scale bar, 100 µm.) Adapted from Schreiber et al. (17). (C) Schematic diagram of Casparian strip formation between endodermal cells. Kamiya et al. (6) show that MYB36 (blue) positively regulates the known genes for Casparian strip biosynthesis, such as RBOHF (yellow), PER64 (red), and ESB1 (bright blue), and genes for the assembly and localization of this scaffold, such as CASPs (green). Furthermore, MYB36 controls novel genes (N; gray) for additional factors presumably involved CS formation. ENC, endodermis cell; PM, plasma membrane.

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protein proposed to function in stereo selective linkage of monolignols (9), is also part of this assembly. Mutations in *ESB1* result in a defective and disorganized CS similar to the *casp1 casp3* double-mutant (10). In addition, ESB1 stabilizes the CASP protein scaffold and, vice versa, requires the CASP complex for its CS localization. Despite these breakthroughs, many questions remained: What other proteins contribute? What brings the CS-forming protein scaffold in its precise position and holds it in place? And most interestingly, how are the different processes, including monomer biosynthesis and export, protein export, and localized complex assembly coordinately regulated?

Answers to these questions evolved from large-scale ionomic profiling (11) of mutants in genes encoding above proteins. It revealed that leaf ionomics represents a powerful tool to uncover root barrier mutants. Kamiya et al. (6) demonstrate that 11250, a previously isolated *Arabidopsis* mutant (12), has elevated levels of sodium, magnesium, and zinc, and reduced calcium, manganese, and iron in leaves. This ion profile is very similar to that of *esb1* and *casp1 casp3*. Unfortunately, chromosomal rearrangements in 11250 delayed the identification of *MYB36* as the causal defective gene. To see whether the CS is affected in the *myb36* mutant, Kamiya et al. (6) inspected the distribution of lignin-like materials in the endodermis. No CS lignin was present in the middle of the endodermal cell wall of *myb36*. Remarkably, the CS is misplaced and lignin-like material appears in the corners of endodermal cells. This finding indicates that lignin biosynthesis is not affected but demonstrates that *MYB36* is required for the directed deposition of lignin.

Kamiya et al. (6) then tested the CS functionality using the apoplastic tracer propidium iodide (PI). They found that in regions of *myb36* roots where a CS is expected and lignin-like material is present in the corners, PI easily penetrated the endodermis. Thus, this displaced lignin-like material is not sufficient to form an effective barrier. The authors speculate that, depending on the concentration gradient for a certain ion over the endodermis, the defective CS barrier could result in the bidirectional ionomic changes observed in *myb36*. The chemistry of the mis-localized CS is also interesting to know. Compositional and structural differences might cause an insufficient sealing or affect the CSD anchoring. However, in more mature root zones, where suberin lamellae are normally formed in the endodermis, a PI barrier is found. Surprisingly, Kamiya et al.'s histochemical suberin survey revealed that, similar to *esb1*, ectopic suberin is deposited earlier in the *myb36* endodermis. This finding supports the previous proposition of that research group, that a system perceiving CS integrity may trigger a compensatory

suberin response (10). This suberin could also contribute to the ionome by affecting the movement of ions that cross endodermal membranes. It is particularly interesting that ectopic suberin in *myb36* already appears in regions that are permeable to PI, which raises the question about whether ectopic suberin is chemically or structurally different from normal suberin, and thus PI permeable.

The *MYB36* gene encodes a putative transcription factor. To test the hypothesis that MYB36 controls the expression of instruments involved in CS formation, Kamiya et al. (6) conducted chromatin immunoprecipitation quantitative PCR. This experiment showed that MYB36 regulates *CASP1*, *PER64*, and *ESB1*, presumably by directly connecting with their promoters. Up to this stage it was unclear whether MYB36 also controls other factors or even all processes in CS formation. To address these questions the authors investigated plants that ectopically express *MYB36*. Overwhelmingly, they detected CSs in the root cortex and epidermis, tissues that normally do not produce a CS. This finding demonstrates that the CS apparatus is latently present in other cells. Moreover, MYB36 alone is sufficient to initiate and run the CS formation program in other tissues. Textbooks only rarely mention that most angiosperms, excluding *Arabidopsis*, also form a CS in the soil-facing hypodermal tissue, the exodermis (13). Future experiments will show whether MYB36 orthologous also control the genesis of exodermal CSs, which are evenly important and widely present in crops such as corn and rice. Potentially, MYB36 can also control CSs in nonroot dermal tissues, such as the endodermis of conifer needles (14, 15).

Clearly, MYB36 regulates the biosynthetic apparatus for CSs. But does MYB36 also control the precise localization of CSs? Kamiya et al. (6) generated answers by very elegantly

visualizing and localizing the CSD-defining CASP1 protein, because of fusions with fluorescent proteins. In the *myb36* mutant, CASP1-mCherry does not localize in a central band as in wild-type. Instead, it remains localized throughout the plasma membrane, similar to CASP1-GFP when ectopically expressed in nonendodermal cells (7). However, in plants ectopically expressing *MYB36*, ectopic CASP1-GFP in the cortex and endodermis localizes in a CSD-like domain that precisely overlaps with the CS-like structure. Taken together, these data demonstrate that MYB36 also controls the expression of factors required for the guidance and accurate localization of proteins that mark the plasma membrane region where the CS is laid down.

With the *MYB36* gene in hand, it should now become possible to uncover additional—if not all—molecular components of the CS building complex. Therefore, Kamiya et al. (6) conducted genome-wide transcriptomics in roots of *myb36* mutants. From the initial set of 77 differentially expressed genes, they selected endodermis-expressed genes and deselected *esb1*-effected genes, because they potentially respond to ectopic suberin and lignin; this manifested a shortlist of genes directly regulated by MYB36. The authors confirmed that 23 genes are positively regulated by MYB36, including *PER64*, all *CASP's*, and six *ESB1*-like dirigent protein genes. Thus, upcoming studies on MYB36 might also shed some light on the function of dirigent proteins, which was controversially discussed in the past (16). Most excitingly, this putative core set of genes required for CS biogenesis included uncharacterized receptors, protein kinases, and cell wall-targeted and membrane-targeted proteins that might include the proteins that guide and hold the CS in place. We can look forward to the characterization of these unknown proteins as it unveils the details to conduct CS formation.

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