Strapline: Root barriers

How to establish a GAPLESS Casparian strip

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To control the movement of water and nutrients, vascular plants seal the paracellular space between adjacent endodermal cells with a tight junction-like complex comprised of the Casparian strip and Casparian strip membrane domain. In rice, GAPLESS proteins mediate the attachment of these two components enabling nutrient homeostasis.

Most plants rely on their roots to forage the soil for water and nutrients, while simultaneously preventing microorganisms from invading and causing disease. To achieve this dual role in uptake and protection, endodermal cells surrounding the vasculature deposit lignin at their junctions to seal the apoplastic space, thereby creating a diffusion barrier. This belt of lignin deposition around endodermal cells is called the Casparian strip (CS) and is tightly attached to the Casparian strip membrane domain (CSD) embedded in the cells' plasma membrane. Although this attachment has been known for almost 90 years, its importance and molecular architecture have remained unknown [1]. In this issue of *Nature Plants*, Song *et al.* report that GAPLESS proteins mediate tethering of the CS to the CSD in rice, and loss-of-function of both GAPLESS1 and -2 results in a disabled cell-cell junction and disrupted nutrient homeostasis [2].

The first identified proteins required for the construction of the CS were the Arabidopsis CASPARIAN STRIP DOMAIN PROTEINS, (CASPs). CASPs localize in the CSD and have functionally conserved orthologues in different plant species [3,4]. In Arabidopsis, no obvious growth defects were observed in *casp1* mutants, but knockout of *OsCASP1* in rice resulted in a significant growth reduction [4]. These differences could stem from a variance in ectopic lignin or suberin compensations that exist between rice and Arabidopsis, or possible differences in functional roles of CASP

proteins and their mechanisms, although this remains unclear. Song *et al.* analyzed genes co-expressed with *OsCASP1* to identify candidates for tethering the CS and CSD and identified three proteins, containing a **G**lycine/**A**lanine/**P**roline-rich domain, a **LE**ctin domain, and a **S**ecretory **S**ignal peptide, thus naming them GAPLESS1-3. These genes are specifically expressed in the endodermis, as revealed by β-glucuronidase (GUS)-reporter lines in rice [2].

To study their function, Song *et al.* created knockout lines for each of the three *GAPLESS* genes. While *gapless2* and *gapless3* showed no significant growth differences from wild-type plants, *gapless1* displayed a slight but significant decrease in height, tiller numbers and yield. To test for functional redundancy, *gapless1/2* and *gapless2/3* double mutants were generated. While the phenotype of gapless2/3 mutants were relatively mild, *gapless1/2* mutants showed more severe growth phenotypes, confirming that there is functional redundancy of *GAPLESS* genes. Moreover, efforts to create a *gapless1/2/3* mutant failed beyond the heterozygous state, suggesting that a loss-of-function of all three genes may not be viable.

The observed symptoms of potassium deficiency and results of the analysis of the ion content in leaves indicated that the observed growth phenotypes probably resulted from a perturbation of nutrient homeostasis in *gapless1, -1/2* and -2/3 mutants, especially for potassium and calcium. Such disorders in elemental homeostasis resemble rice and Arabidopsis mutants with impaired CS function. Therefore Song *et al.* tested the apoplastic permeability of mutant roots using a fluorescent tracer and found that the CS barrier in the endodermis appeared to be dysfunctional in *gapless1*, *gapless2/3*, and even more so in *gapless1/2*. This defect seemed not to be caused by altered CS formation and pattern since, if anything, the CS in *gapless1/2* mutants appeared enhanced rather than impaired compared to the wild type. In the *gapless1/2* mutants the CS appears to no longer be properly tethered to the CSD as the CS zone was no longer devoid of suberin; *gapless1/2* showed a continuous suberin deposition around the whole endodermal cell, whereas this was interrupted at the site of the CS/CSD complex in the wild type. Also, endodermal cells in the roots of *gapless1/2* mutant plants displayed enhanced suberization, a known symptom of plants with an malfunctioning CS, such as in the Arabidopsis ENHANCED SUBERIN1 (*esb1-1*) mutant [5].

To further explore *the gapless1/2* phenotype, Song *et al.* observed the CS zone in endodermal cells using transmission electron microscopy. In *gapless1/2*, the CSD was indeed completely detached from the CS in many endodermal cells. In cells fixed after plasmolysis they saw that whereas the CSD in wild type endodermal cells remained attached to the CS, the CSD was detached from the CS of endodermal cells in *gapless1/2* mutants. Likewise, in endodermal cells that still displayed attachment of the CS to the CSD, the length of the CSD-CS junction was significantly reduced in *gapless1*, *gapless2/3* and most noteworthily in *gapless1/2*. On the other hand, OsCASP1 was still localized to the CSD of *gapless1/2*, showing that localization of OsCASP1 does not require GAPLESS1 and 2.

Immunolocalization of GAPLESS1 in wild-type plants revealed its specific localization in the CS, complementary to OsCASP1 in the CSD. GAPLESS1 localization was not altered in loss-of-function *oscasp1* despite a significant decrease in CSD-CS junctional length and a disordered ion profile like that of *gapless1/2*. Hence, Song *et al*. tested whether GAPLESS1 and CASP1 could interact to tether the CS to the CSD. A physical connection between these two proteins was demonstrated using bimolecular fluorescence complementation assays in which YFP fluorescence was reconstituted in tobacco leaves expressing both YFPN-OsCASP1 and GAPLESS1-YFPC. Moreover, while both N- and C-terminus of AtCASP1 are exposed to the cytoplasm, Song *et al.* showed that OsCASP1 has a long N-terminus which is turned over to the outside of the cell by an unknown mechanism. Finally, pull-down and microscale thermophoresis assays provided further evidence of a strong interaction between OsCASP1 and GAPLESS1.

By studying how the tight junction-like complex of the CS and CSD is connected in rice, Song *et al.* revealed that the novel class of GAPLESS proteins mediate the attachment of the CS to the CSD through a direct interaction with OsCASP1 embedded in the CSD. That the N-terminus of OsCASP1 is able to cross the plasma membrane despite the lack of any signal peptide might help to better predict the topology of the N-terminus of other transmembrane proteins.

GAPLESS proteins are widely distributed within gymnosperms and angiosperms, although some dicots such as Arabidopsis do not have GAPLESS orthologues. This study nicely demonstrates the importance of conducting research

on different plant species, especially important crops, while also suggesting that species without orthologs of GAPLESS proteins must have evolved different ways to tether the CS to the CSD.

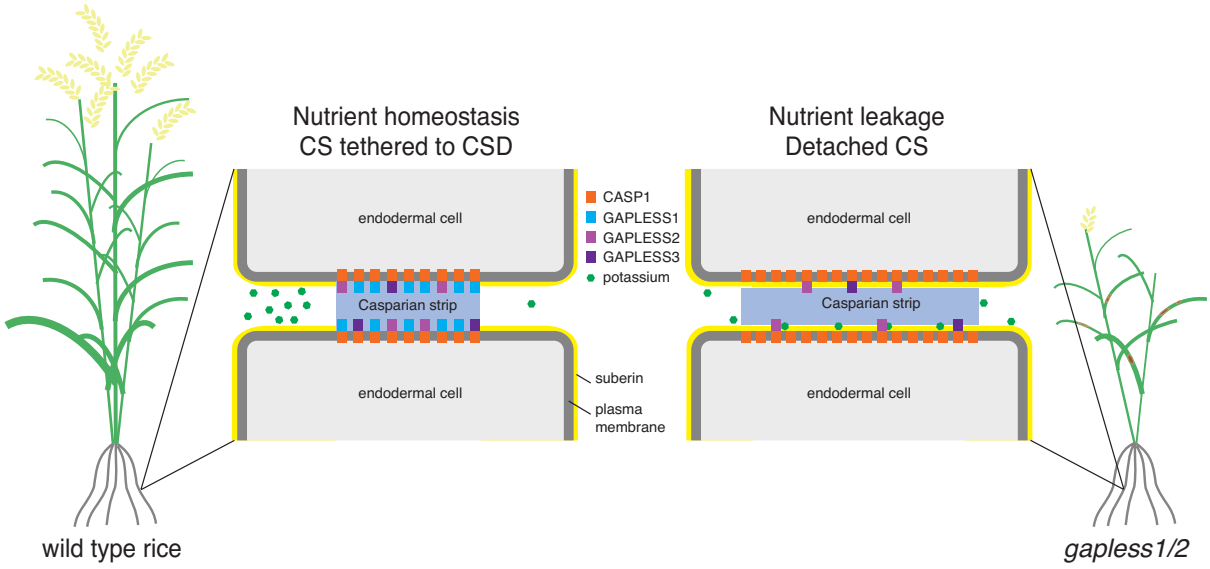

Figure 1. How to secure a Casparian strip.

In rice, GAPLESS and CASP proteins form a complex to tightly attach the cell wall localized Casparian strip (CS) to the CS domain (CSD) in the plasma membrane, thus sealing the paracellular space between adjacent endodermal cells. This tight junction-like complex blocks the apoplastic movement of water and nutrients between cells and enables nutrient homeostasis, which is important for normal plant growth. Besides the CS, plants have another barrier in the form of suberin deposition in the cell wall of endodermal cells. In wild-type plants, the CS is tightly tethered to the CSD, rendering the cell wall:plasma membrane junction inaccessible for suberin deposition. However, in the *gapless1/2* mutant, due to disrupted tethering of CS to CSD, suberin can occupy the CSD domain area.

gapless1/2 double mutants display decreased height, tiller numbers and yield, brown leaf margins and necrotic spots in leaves, symptomatic of potassium deficiency. Although not shown in the present study, we speculate that the other GAPLESS proteins also interact with OsCASP proteins.

References

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