Cell

A Mechanism for Localized Lignin Deposition in the Endodermis

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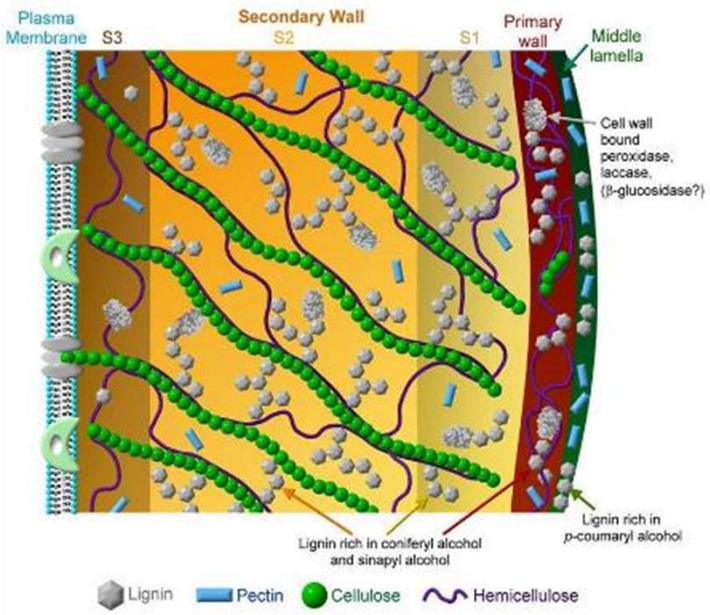
INTRODUCTION

Lignin

- A complex chemical compound most commonly derived from wood, and an integral part of the secondary cell walls of plants
- One of the most abundant organic polymers on Earth
- Constituting from 1/4 to 1/3 of the dry mass of wood.
- The support through strengthening of wood (xylem cells) in trees.
- Lignin deposition is a major cell wall modification that plants employ in many different cell types.

Cell wall

- Primary: cellulose, hemicellulose, pectin in epidermis, cortex, pith cells
- Secondary: cellulose, hemicellulose, ligni in vessel elements, fiber cells



Konandoor et al., 2010, Molecules

<Wikipedia and Lee et al., 2013, Cell>

INTRODUCTION

The endodermis separates outer (peripheral) from inner (central) cell layers by virtue of its Casparian strips (Alassinone et al., 2010)

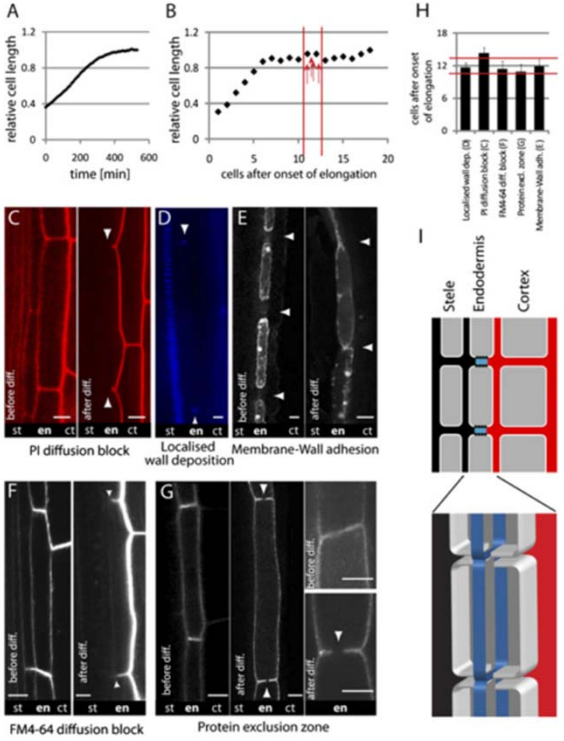
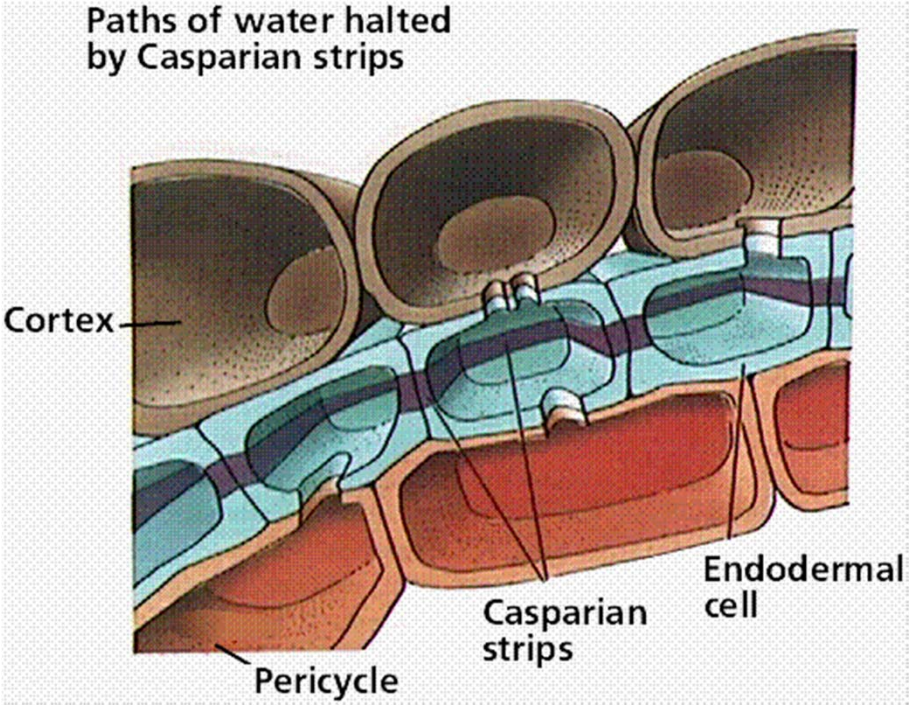


Fig. 1. Molecular and quantitative analysis of endodermal differentiation. (*A*) Average rate and duration of endodermal cell elongation in 9-h time-series/10-min intervals (13 cells/6 roots). (*B*) Average cell length vs. position in the cell file ($n = 25$). Red arrows mark the position of the differentiation events depicted in *D*–*G*. (*C*) Penetration of propidium iodide (PI) into the stele (*Left*) is blocked in differentiated roots (*Right*). Block at 14.4 cells ($n = 30$) is shown. (*D*)



http://www.emc.maricopa.edu/faculty/farabee/biobk/biobookplanthorm.html

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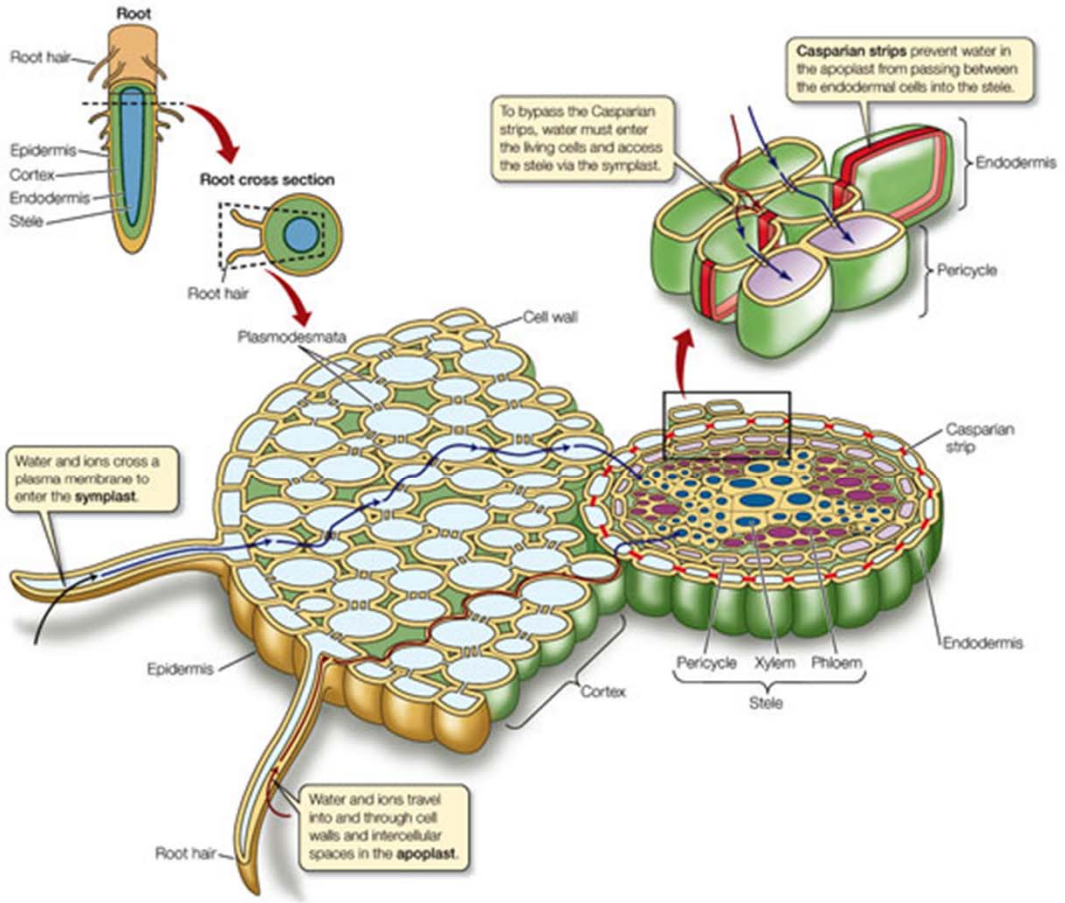

***LIFE 8e*, Figure 35.4**

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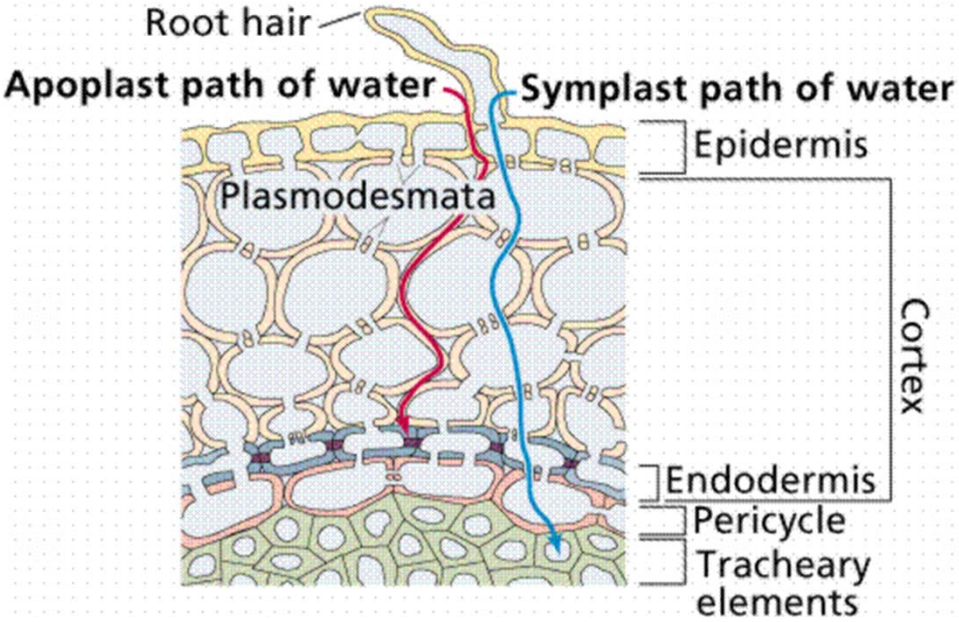
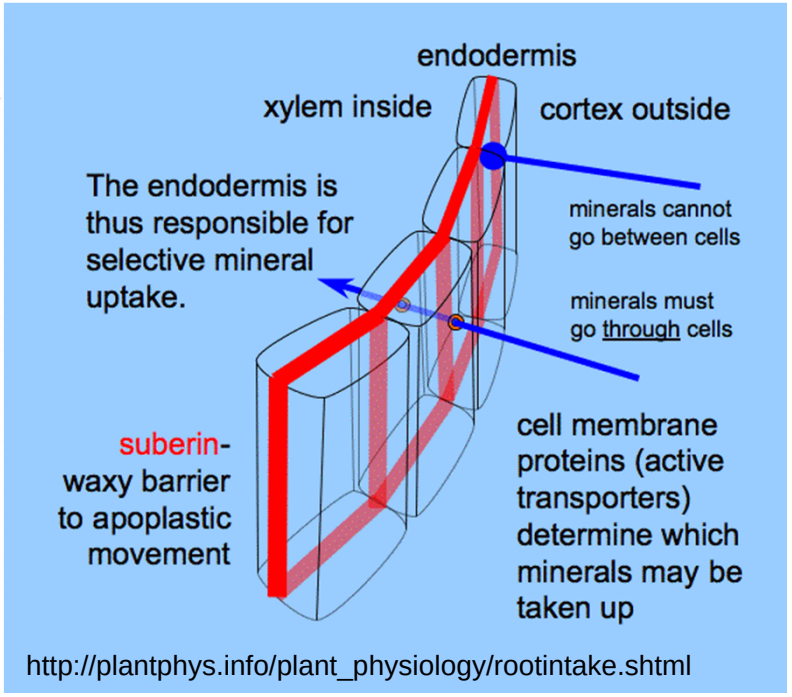
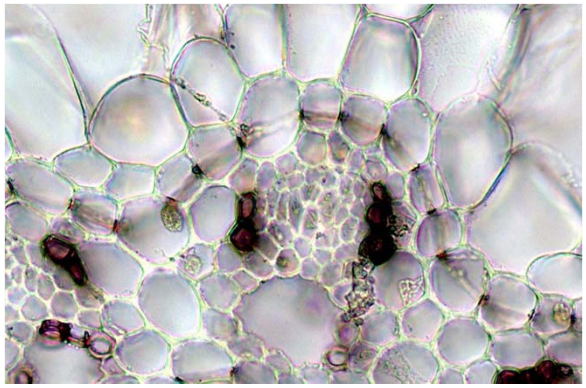


http://www.emc.maricopa.edu/faculty/farabee/biobk/biobookplanthorm.html



http://plantphys.info/plant_physiology/rootintake.shtml

INTRODUCTION



Casparian strip (CS)

- A band of cell wall material deposited on the radial and transverse walls of the endodermis
- It is used to block the passive flow of materials, such as water and solutes into the stele of a plant.
- In Arabidopsis, CS in endodermal cells are lignin-based structures.
- Casparian strop domain proteins(CASPs), a family of small transmembrane proteins has been identified, which predict the localization formation of CS and are necessary for its correct formation. (Ropplo et al., 2011)

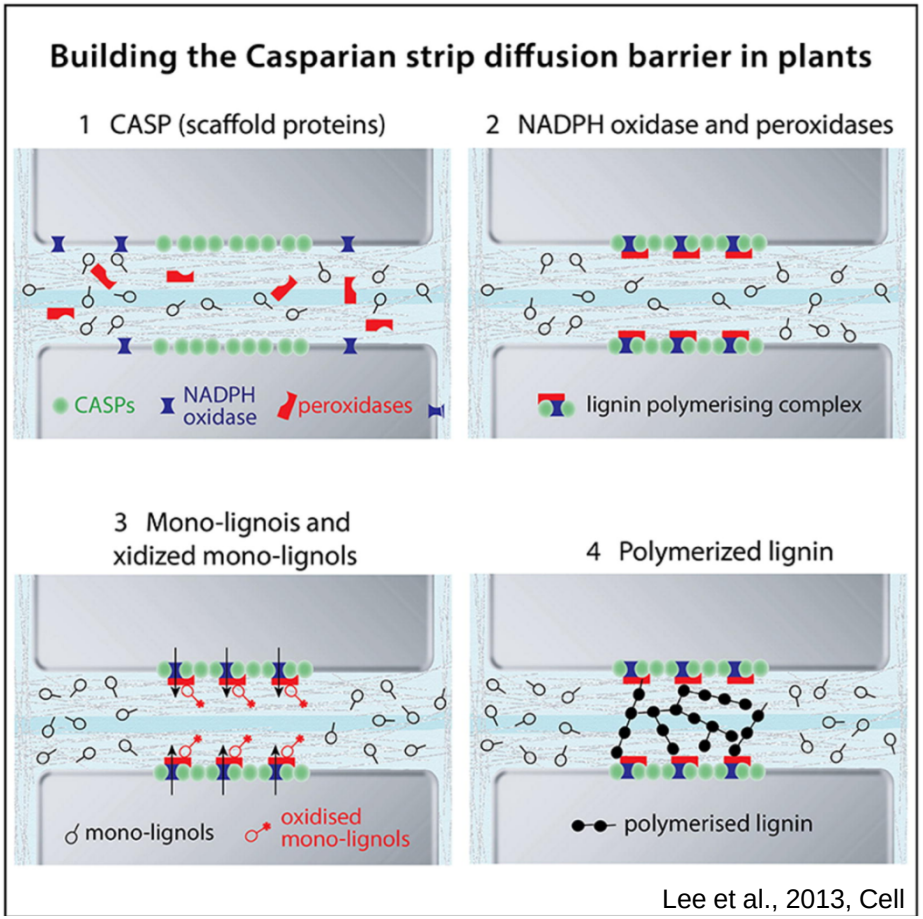
This paper demonstrate..

- CASP1 is a determinant for the subcellular localization of a specific endodermal peroxidase.
- Mutant in one of the NADPH oxidase strongly delays formation of CS.
- They draw the model..

<Wikipedia and Lee et al., 2013, Cell >

INTRODUCTION

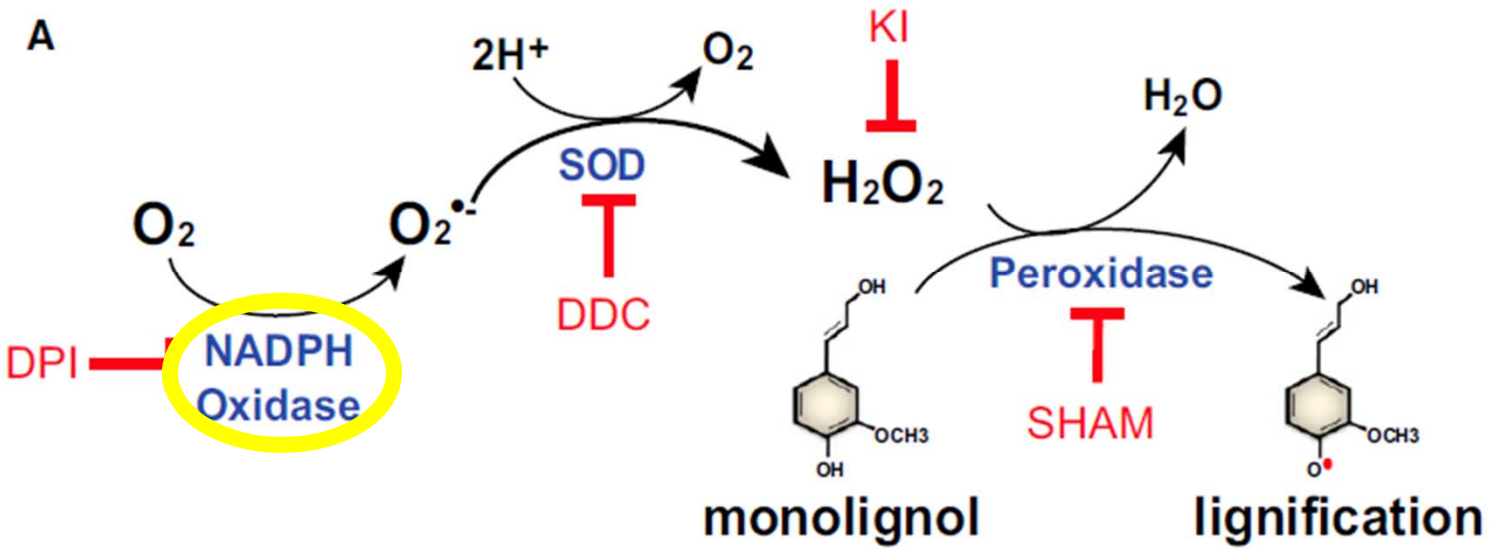
The Casparian strip, a lignin based, paracellular diffusion barrier in plants, forms as a precise, median ring by the concerted action of a specific, localized NADPH oxidase, brought into proximity of localized peroxidases through the action of CASPs.



Lee et al., 2013, Cell

INTRODUCTION

In Arabidopsis, They provide a simple mechanistic model of how plant cells regulate lignin formation with subcellular precision.



INTRODUCTION

RBOH

- Plant NADPH oxidases, also known as respiratory burst oxidase homologues (RBOHs)
- Their roles as ROS producers during cell growth, plant development and plant response to abiotic environmental constraints and biotic interactions, both pathogenic and symbiotic.
- This broad range of functions suggests that **RBOHs may serve as important molecular 'hubs' during ROS-mediated signalling in plants**.

AtRbohB is expressed principally in germinating seeds and a mutant of this gene has been shown to result in altered seed germination

AtRBOHD may be involved in the production of a ROS wave that propagates through the entire plant.

atrbohD/atrbohF double mutant displays impaired ABA-induced stomatal closure.

<Marino et al,. 2012, Cell>

INTRODUCTION

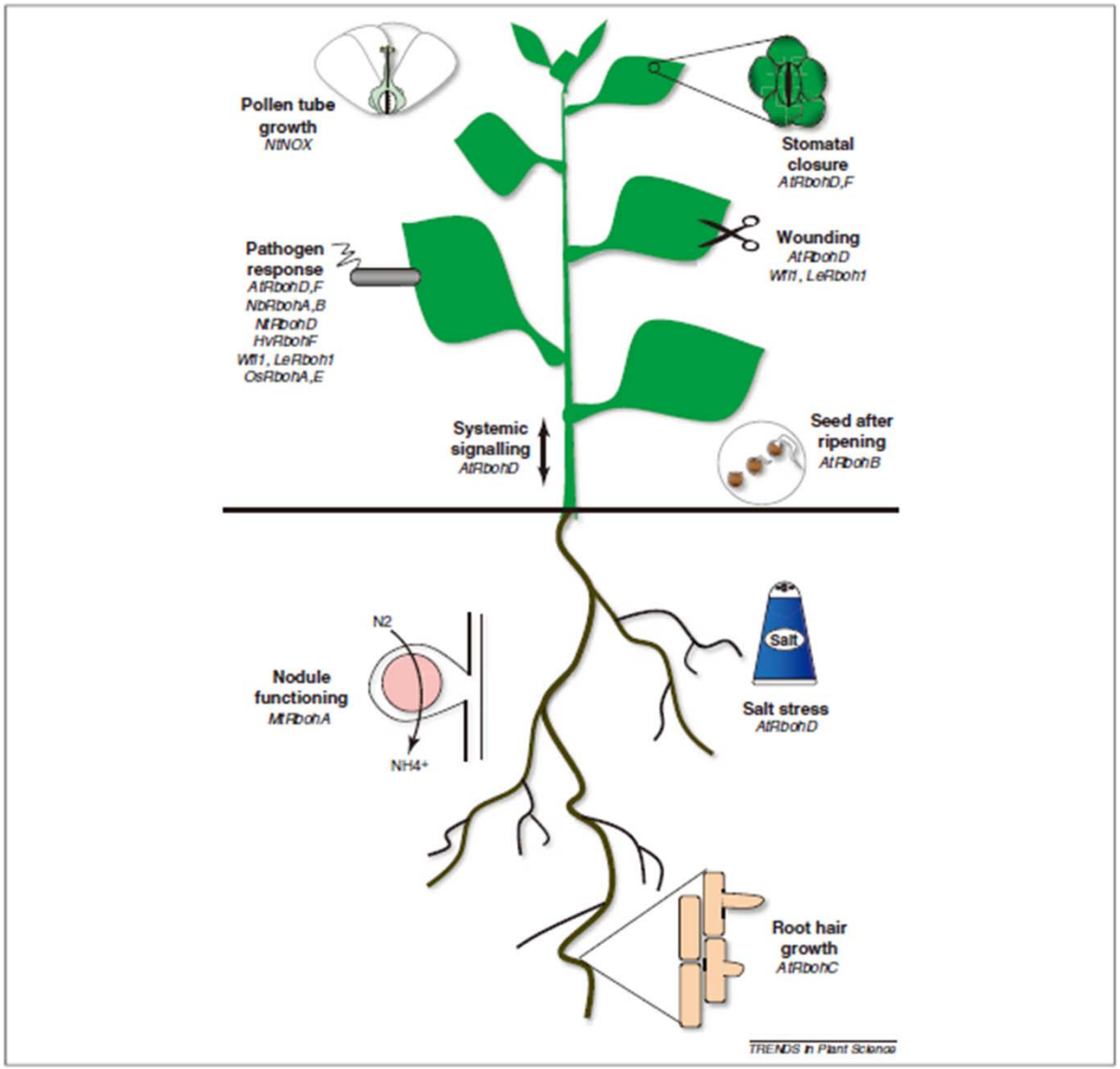


Figure 1. Schematic diagram of the plant processes for which experimental genetic evidence demonstrating a role for RBOHs has been obtained. The genes implicated in each process are indicated. References corresponding to each case are provided in the main text.

HIGHLIGHTS

- **A specific NADPH oxidase is crucial for formation of lignified Casparian strips**
- **NADPH oxidase specificity depends on subcellular localization and regulatory domain**
- **CASPs recruit secreted peroxidases to the Casparian strip domain**
- **The assembly of NADPH oxidase and peroxidases drives localized lignin formation**

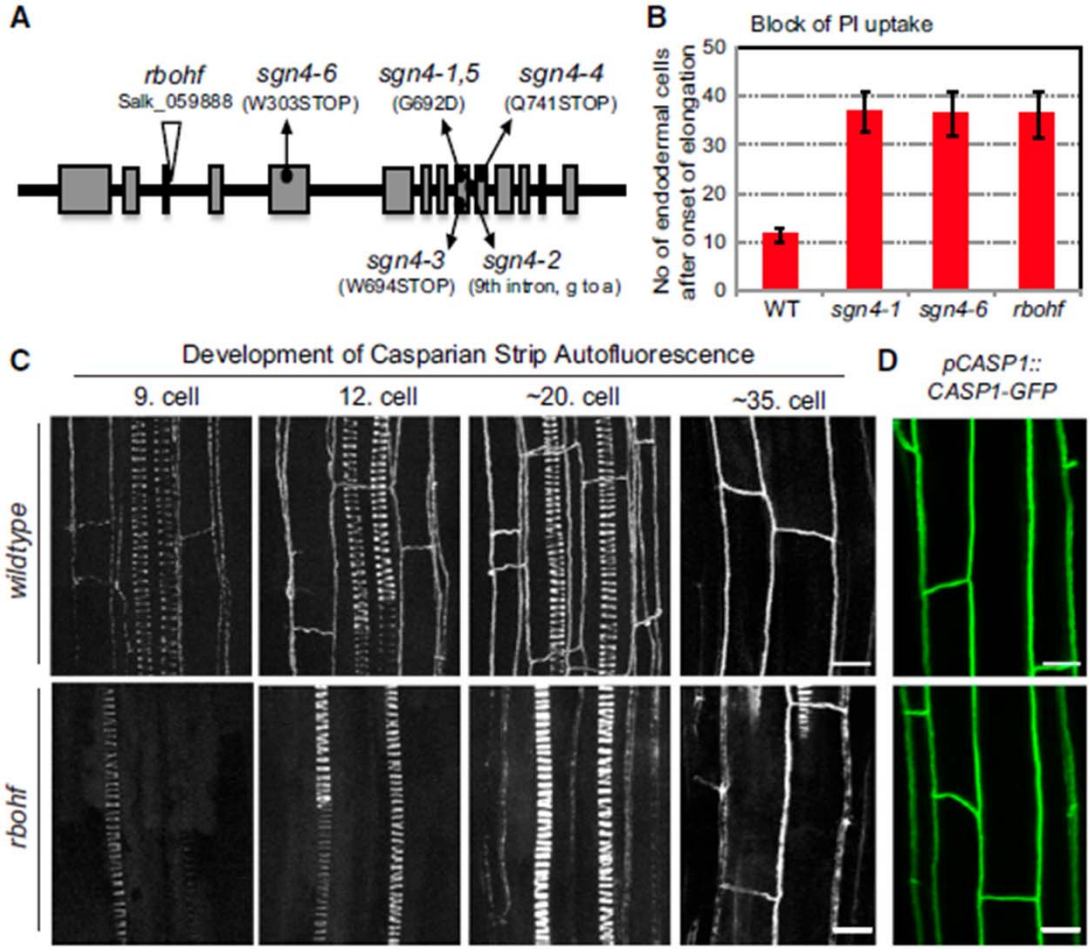


Figure 1. Casparian Strip Formation Is Delayed in *sgn4/rbohf* Mutants

(A) A diagram of RBOHF genomic region shows insertion site of the T-DNA and the positions of the different stop- and missense mutations identified in the six *sgn4* alleles (see also Tables S1 and S2).

(B) Apoplastic diffusion barrier was visualized by block of penetration of externally applied PI (15 μM) into the stele. Quantification was done by counting endodermal cells after "onset of elongation," defined when an endodermal cell in a median optical section was at least three times its width. From this point, cells in the file were counted until the PI signal was blocked at endodermal cells (5-day-old seedlings, mean ± SD, $21 < n < 31$).

(C) Casparian strip networks are visualized as autofluorescence after clearing in different developmental stages which are represented by counts of endodermal cells after "onset of elongation" (see above). Pictures are maximum projections of longitudinal, surface-to-median confocal image stacks, allowing visualization of the net-like nature of the Casparian strips. Note that xylem vessels are also observed as brightly fluorescent spiral structures.

(D) pCASP1::CASP1-GFP localizes at the net-like Casparian strip membrane domain (CSD) in wild-type and *rbohf*. Projections as in (C).

See also Figure S1. Scale bars, 10 μm.

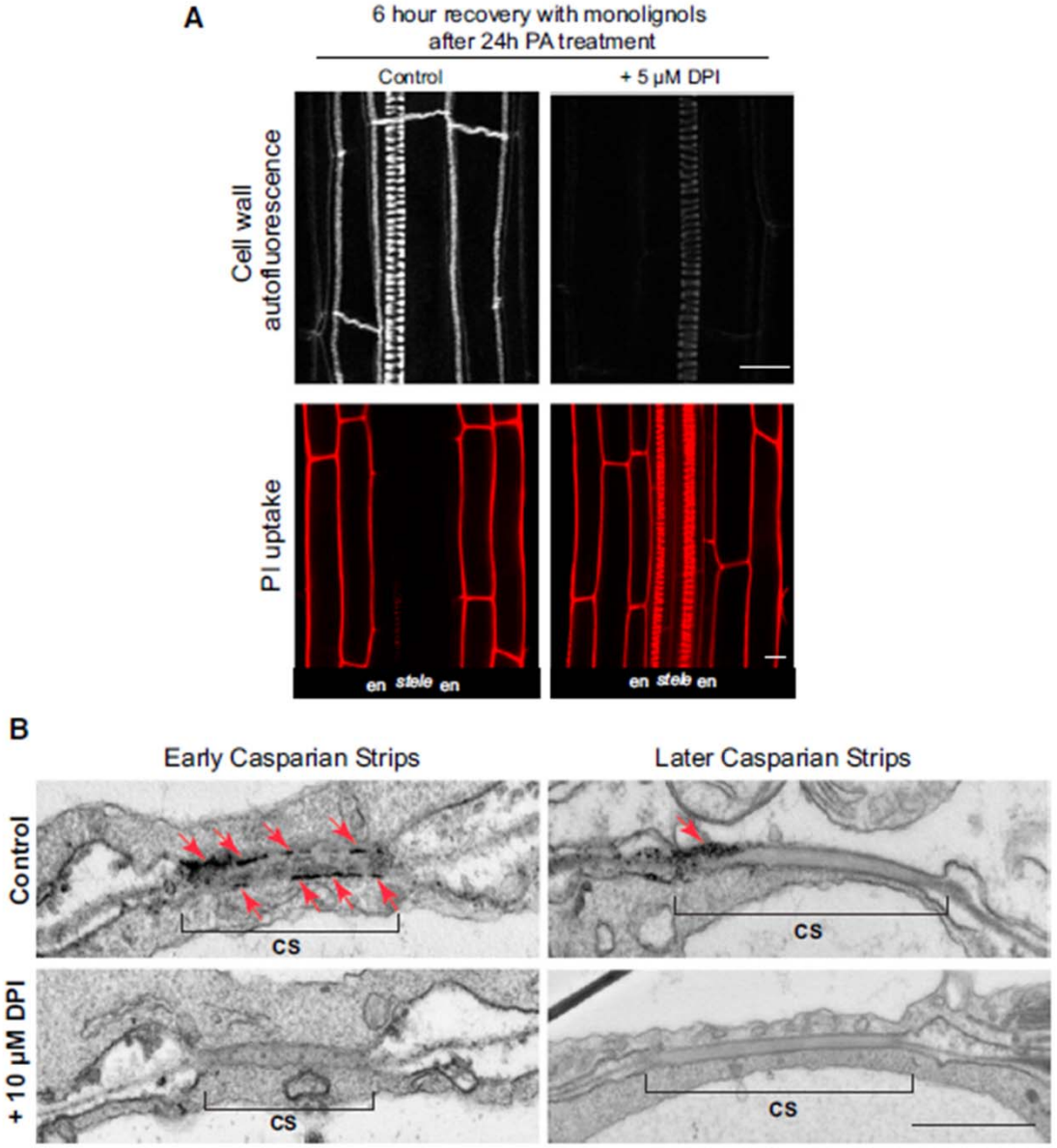


Figure 2. NADPH Oxidase Is Essential for Casparian Strip Formation and Localized ROS Production

(A) The NADPH oxidase inhibitor DPI abolishes complementation observed by monolignol addition in the newly grown root zone of 6-day-old seedlings. Roots were treated for 24 hr with lignin biosynthesis inhibitor PA. After this, seedling roots were incubated for 6 hr on plates with monolignols (20 µM coniferyl and sinapyl alcohol) with or without 5 µM DPI. Casparian strip networks (cell wall autofluorescence, upper panel) and diffusion barrier formation (PI tracer uptake, lower panel) were assessed (see also Figures S2 and S3). en, endodermis; stele, cell layers enclosed by endodermis.

(B) Localized H_2O_2 is detected in electron micrographs by its reaction with 10 mM cerium chloride ($CeCl_3$) to produce electron-dense deposits. DPI (10 µM) was pretreated for 1 hr. Arrows point to electron-dense deposits. Note the deposits are formed throughout CS region in developing strips, but are confined to outer edge of more established strips, probably reflecting block of cerium chloride uptake by the strip itself (see also Table S3).

Scale bars, 10 µm (A); 0.5 µm (B).

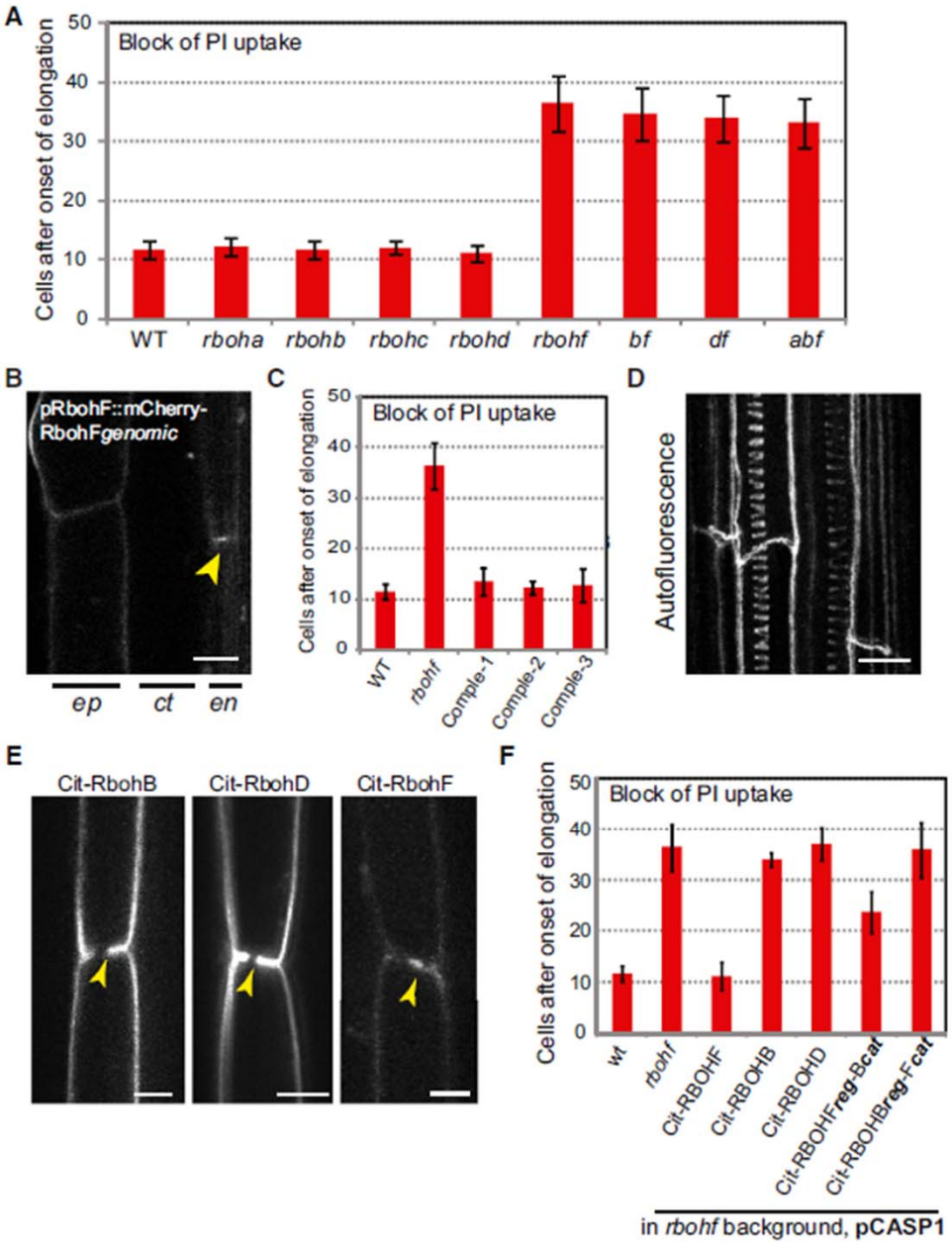


Figure 3. CSD Localization and Regulatory Input Determine Specificity of RBOHF Action in Casparian Strip Formation

(A) Establishment of a functional diffusion barrier is visualized by block of PI diffusion (mean ± SD, 13 < n < 31) (see also Figure S4 and Table S2).

(B) Line of mCherry-RBOHF genomic construct, driven by own promoter shows localization at the plasma membrane and expresses in every cell type of 5-day-old seedling roots, including endodermis where it accumulates at the CSD. Arrowhead points to CSD accumulation (see also Figure S5).

(C) Delayed PI block phenotype of *rbohf* is complemented in three independent complementation lines expressing pRBOHF::mCherry-RBOHF genomic construct (comple-1-3) (mean ± SD, 20 < n < 31).

(D) Rescued Casparian strip autofluorescence in a complementation line.

(E) Endodermal localization of mCitrine fused to RBOHB, D, and F under the CASP1 promoter. Only mCitrine-RBOHF localizes at the CSD. Arrowheads point to CSD (see also Table S5).

(F) Delayed PI block phenotype of *rbohf* is complemented by introduction of Cit-RBOHF CDS under the CASP1 promoter. When N-terminal regulatory domain of RBOHF is replaced by that of RbohB (Cit-RBOHB*reg*-F*cat*), no complementation is observed. By contrast, regulatory domain of RBOHF combined with catalytic domain of RbohB (Cit-RBOHF*reg*-B*cat*) shows partial complementation ($p < 0.01$, mean ± SD, 19 < n < 31) (see also Figure S5C).

Scale bars, 10 μm (B and D); 5 μm (E). ep, epidermis; ct, cortex; en, endodermis; st, stele.

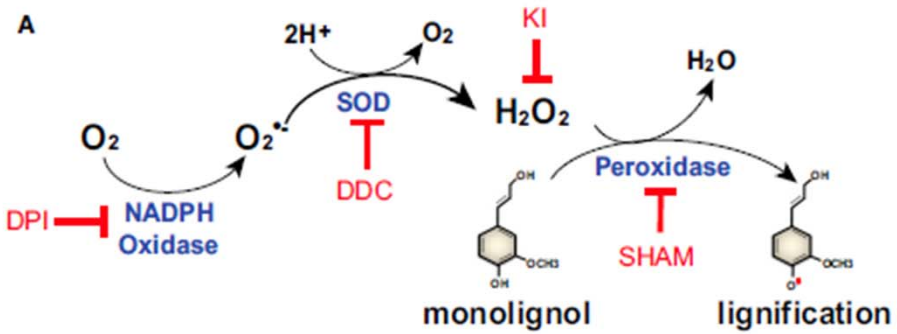


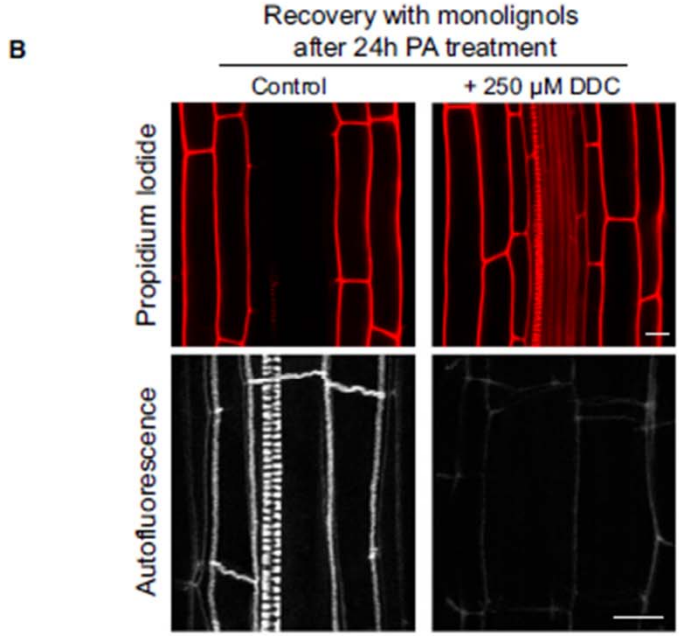
Figure 4. NADPH Oxidase Drives H_2O_2 Production for Peroxidase-Mediated Lignin Polymerization in Casparian Strips

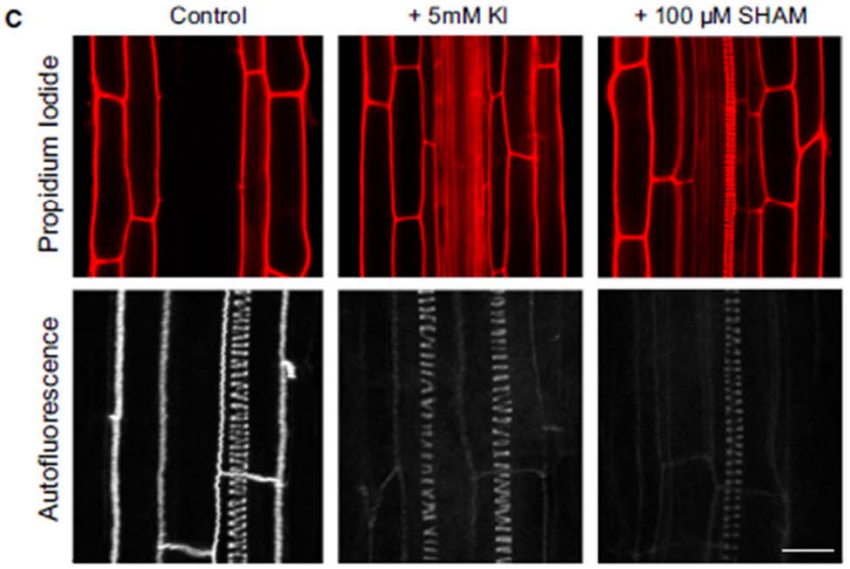
(A) Schematic representation of the required steps between NADPH oxidase activity and lignin polymerization. Diphenyleneiodonium (DPI, NADPH oxidase inhibitor), diethyldithiocarbamic acid (DDC, superoxide dismutase inhibitor), potassium iodide (KI, H_2O_2 scavenger), salicylhydroxamic acid (SHAM, peroxidase inhibitor).

(B) When 250 μM DDC is applied together with monolignols after 24 hr of PA treatment, as in Figure 2A, Casparian strip reconstitution does not occur (see also Figure S2 and Table S6).

(C) When 5 mM KI and 100 μM SHAM is applied for 24 hr, root growth continues, but Casparian strip formation is inhibited in the newly grown root zone (see also Figure S2 and Table S6).

Scale bars, 10 μm.





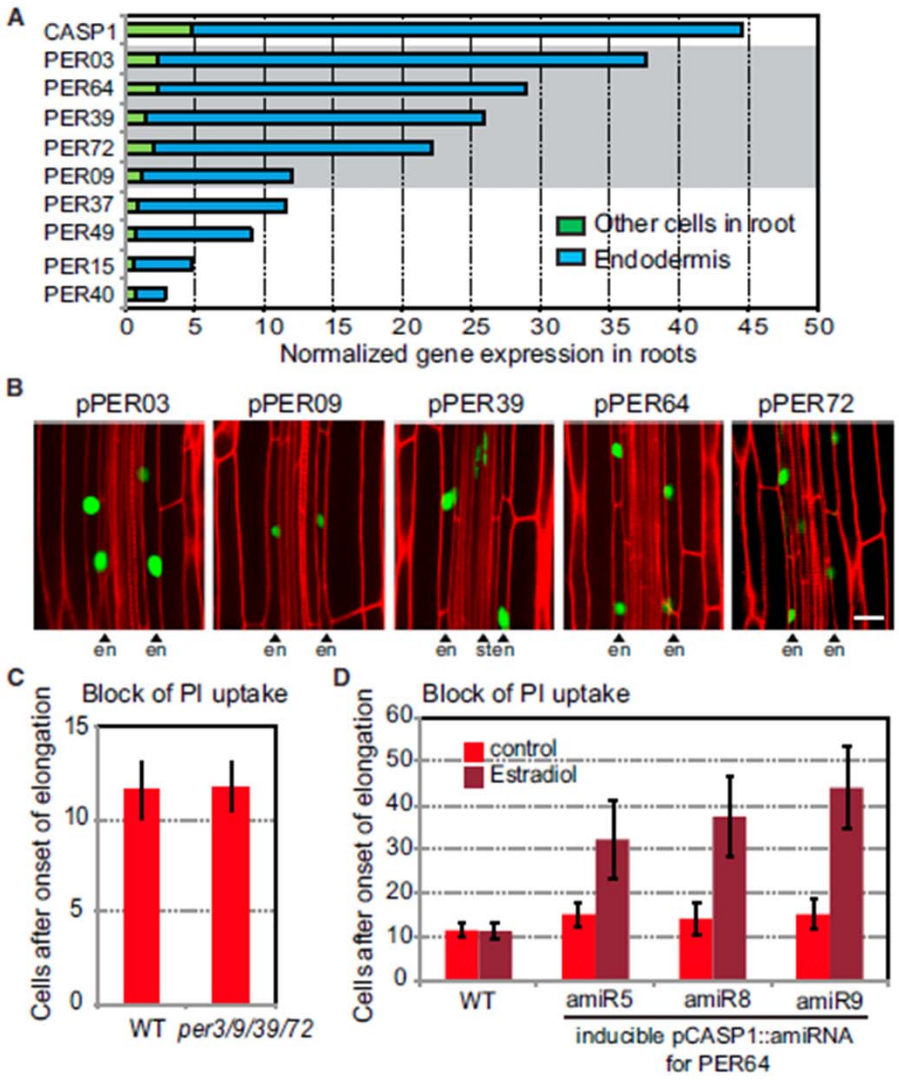


Figure 5. Peroxidases Are Involved in the Casparian Strip Formation

(A) Microarray data showing endodermis-enriched gene expression of CASP1 and peroxidases in roots (data from Birnbaum et al., 2003; Brady et al., 2007).

(B) Promoter gene expression analysis of peroxidases with nuclear-localized GFP-GUS as reporter confirms endodermis-specific/enriched gene expression in the endodermis. Arrowheads point to cell layers showing expression. en, endodermis; st, stele (see also Table S4). Scale bar, 20 μm.

(C) Establishment of a functional diffusion barrier, visualized by PI, is not affected in the quadruple mutant of *per3 per9 per39 per72* ($p > 0.5$, mean ± SD, $25 < n < 29$) (see also Table S2).

(D) Establishment of a functional diffusion barrier is delayed in mutants expressing inducible artificial microRNA for PER64 germinated in 1/2 MS plates including 10 μM estradiol ($p < 0.001$, mean ± SD, $16 < n < 27$) (see also Table S5).

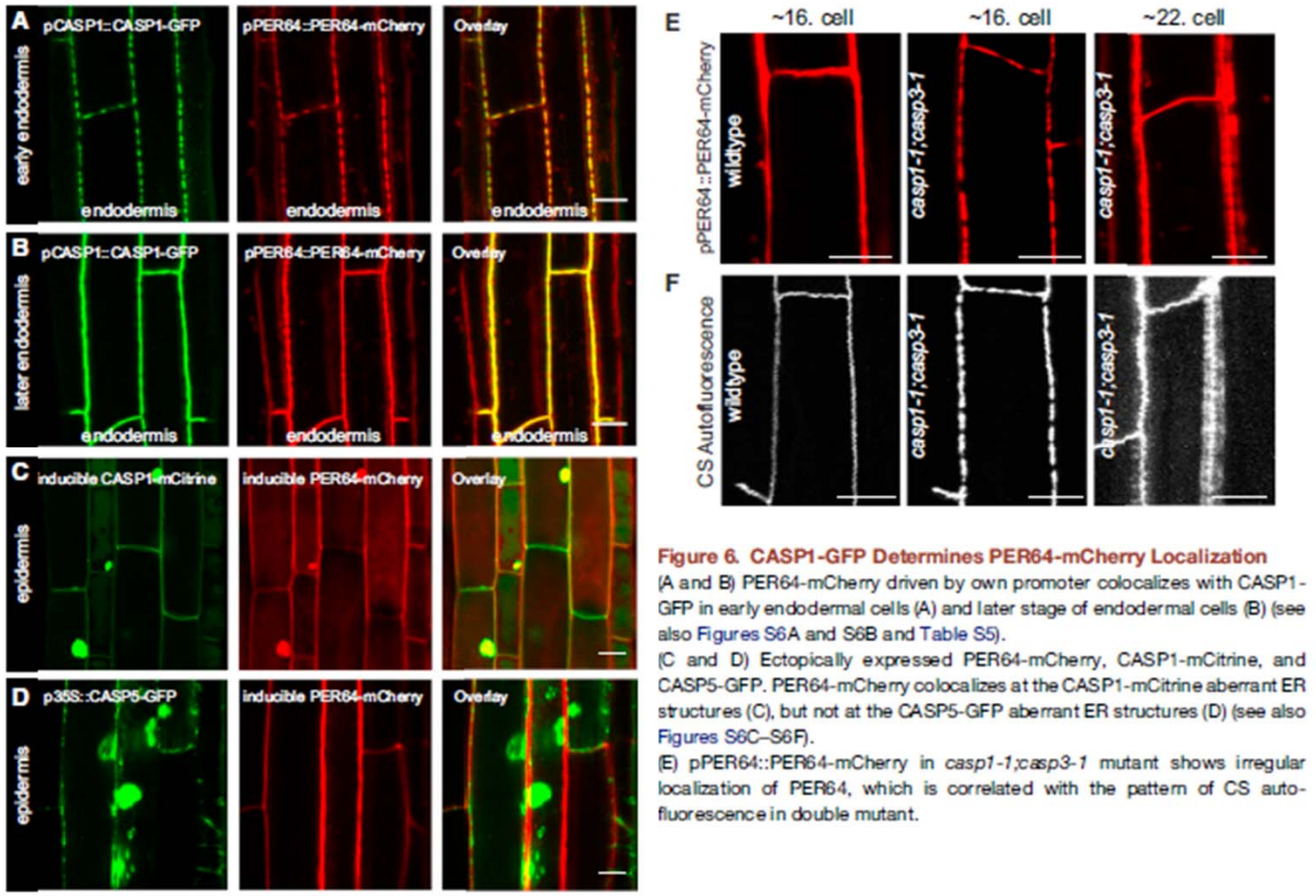


Figure 6. CASP1-GFP Determines PER64-mCherry Localization

(A and B) PER64-mCherry driven by own promoter colocalizes with CASP1-GFP in early endodermal cells (A) and later stage of endodermal cells (B) (see also Figures S6A and S6B and Table S5).

(C and D) Ectopically expressed PER64-mCherry, CASP1-mCitrine, and CASP5-GFP. PER64-mCherry colocalizes at the CASP1-mCitrine aberrant ER structures (C), but not at the CASP5-GFP aberrant ER structures (D) (see also Figures S6C–S6F).

(E) pPER64::PER64-mCherry in *casp1-1;casp3-1* mutant shows irregular localization of PER64, which is correlated with the pattern of CS auto-fluorescence in double mutant.