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About the cover:
Each plant cell is encapsulated in a semi-rigid extracellular matrix—the cell wall—composed of polysaccharides, proteins, ions, and water. The modification of the conformation and arrangement of the cell wall polysaccharides allows plant cells to grow into their functional size and shapes. The cover image is produced based on a micrograph obtained from plant epidermal cell walls using a confocal laser scanning microscope (Figure 1C, page 16). The sample was labeled using Calcofluor White, a fluorophore with affinity to β-glucans with proven usefulness for visualization of the orientation of cellulose microfibrils in plant cell walls. In the image, the meandering borders of a pavement cell and a pair of bean-shaped guard cells forming a stomatal pore can be observed. The lines radiating from the indentation side of wavy borders represent the arrangement of bundles of cellulose microfibrils in cell walls of these interlocking cells.
Growing plant cells are encapsulated in a polysaccharide-rich envelope referred to as the primary plant cell wall. The arrangement of the polysaccharide macromolecules in the cell wall is linked to cell growth and morphogenesis. In vivo localization of cell wall polysaccharides is, therefore, a crucial means towards understanding plant cell development. We discuss live fluorescence labeling of cellulose and pectin in selected primary plant tissues using available fluorescent probes.

The primary cell wall enveloping the plant cell is synthesized at the surface of the plasma membrane and comprises several polysaccharides, ions, proteins, glycoproteins, and water. The chemical composition of the primary cell wall confers it with mechanical properties that control the expansive growth of plant cells [1]. Cellulose is considered a major cell wall component with the volume density, crystallinity, and orientation of the microfibrils determining the orientation of cell expansion [1,2]. Homogalacturonan (HG) pectin is abundant in the primary cell wall and its chemistry is linked with cell morphogenesis and organogenesis [3-6]. Visualization of the spatiotemporal arrangement of the cell wall components is, therefore, instrumental to the study of cell and plant development and confocal fluorescence microscopy is a method of choice. In this project, we developed protocols for some of the available fluorophores to localize HG pectin and cellulose microfibrils in the wall of epidermal cells of Arabidopsis thaliana seedlings and Camellia japonica pollen tubes.

Cellulose arrangement was visualized using Calcofluor White (CFW) and Pontamine Fast Scarlet 4B (PFS). To evaluate their binding, Carbohydrate Binding Module 3a (CBM3a) specific for crystalline cellulose was used to label fixed pollen tubes. Fluorescent labeling using CBM3a was performed with mouse antipolyhistidine and goat anti-mouse IgG Alexa Fluor 488. We used Propidium iodide (PI) and COS488 suggested to preferentially bind weakly esterified pectin [7,8] to study the distribution of HG pectin in epidermal pavement cells and pollen tubes. To evaluate the binding of COS488 and PI, we labeled pollen tubes with LM19 and JIM5 monoclonal antibodies (mAbs) specific to weakly esterified pectin and LM20 and JIM7 specific to highly esterified pectin. Details on sample preparation, fixation, and reagent concentrations are available in [9]. The secondary antibodies for JIM and LM mAbs were goat anti-rat IgG Alexa Fluor 594 and goat anti-rat IgM Alexa Fluor 488, respectively. For visualization, we used Zeiss LSM 510 META or 710 confocal laser scanning microscopes. For live imaging, we used Yokogawa CSU X1 mounted on a Zeiss Axio Observer Z1 spinning disk confocal microscope.
Staining with CFW and PFS was successful in visualizing cellulose microfibril orientation in epidermal cells. The organization of cellulose microfibrils can be observed in guard cells and wavy pavement cells of cotyledons (Figure 1A-D). For other epidermal cell types refer to [9]. Using CFW and PFS, the majority of pollen tubes demonstrated a gradient in signal intensity that decreased toward the apical region (Figure 2A), while some displayed strong signal in the apical region as well. These results were corroborated by the labeling of pollen tubes with CBM5a, which revealed a heterogeneous behavior among pollen tubes in terms of the distribution of cellulose along the length of the cell. CFW also stained pollen tube plugs and plasmodesmata in walls of pavement cells; both are structures that are also enriched in callose.

Labeling cotyledon pavement cells for pectin using PI and COS488 demonstrated differential signal along the meandering anticlinal cell walls with an elevated signal in the periclinal walls adjacent to the indentation sides of undulations (Figure 1E, F). These observations suggest a differential distribution of de-esterified pectin in pavement cell walls (see also [5]). Labeling pollen tubes using COS488 showed a decreasing signal towards the tip (Figure 2B), matching labeling with LM19 and JIM5. PI staining in pollen tubes, however, unlike COS488 labeling, showed signal in all regions of the pollen tube including the tip (Figure 2C). PI also stained callose plugs.

Importantly, labeling live pollen tubes with CFW, COS488 and PI, we observed that when used at proper concentration [9], these probes do not seem to hamper cell growth and allow time-lapse visualization of polysaccharide dynamics in living cells.

Immunohistochemical labeling is the standard procedure for analyzing the spatial organization of plant cell wall components. However, this method typically requires sample fixation or extensive sample preparation that precludes live cell studies. Our results indicate that the cell wall composition, especially in rapidly growing cells, is highly dynamic, and hence live cell imaging is preferable. The available probes allowing imaging of the chemical composition of the plant cell walls are promising, but additional studies are required to further establish their specificity. Novel live cell fluorescent probes, such as those allowing discrimination of subtle changes in pectin esterification, will prove highly valuable.

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REFERENCES
**Figure 1:** Fluorescence confocal micrographs of epidermal cells of cotyledons of living *Arabidopsis thaliana* seedlings. **A)** Staining for cellulose with Pontamine Fast Scarlet 4B (PFS, 10 mg/mL in PBS) shows a nonuniform distribution of fluorescence signal in cotyledon epidermal cells. Specifically, fine bundles of cellulose microfibrils are localized at the indentation (neck) sides of the wavy borders of pavement cells. **B-D)** Epidermal cells stained for cellulose with calcofluor white (CFW, 10 mg/mL in ddH$_2$O). **B)** Similar to (A), staining with CFW shows a nonuniform signal distribution in pavement cells. Bundles of cellulose microfibrils can be discerned using CFW. **C)** Close up of a pair of guard cells stained with CFW forming an open stomatal pore. CFW staining demonstrates bundles of cellulose microfibrils with a radial/transverse orientation in each guard cell. **E)** Epidermal cells of cotyledon stained with Propidium iodide (PI, 0.5 mg/mL in ddH$_2$O) show a nonuniform distribution of signal between lobes and necks of interlocking pavement cells with higher signal observed at indentation sides (necks). **F)** Epidermal cells of cotyledon labeled with COS$_{488}$ (1:1000 in MES) for de-esterified pectin. The left micrograph shows a maximum projection xy view of a z-stack while the right micrograph shows the corresponding orthogonal yz view at the marked location. The arrow and arrowhead in both views point to periclinal cell walls of two neighboring cells interlocked at a neck and a lobe, respectively. The indentation side of waves (neck, shown by arrow) demonstrates a higher signal. All micrographs are maximum projections of a z-stack, except for the yz view of F (on the right) which is a single optical slice. Scale bars = 10 µm, and 5 µm (D, yz view of F).

**Figure 2:** Fluorescence confocal micrographs of growing *Camellia japonica* pollen tubes labeled with **A)** Calcofluor white (CFW, 1.3 µg/ml in pollen tube growth medium). The staining signal decreases in the apical region. **B)** COS$_{488}$ (1:300 in pollen tube growth medium). The signal appears dimmer in the apical region and **C)** Propidium iodide (PI, 0.1 mg/ml in pollen tube growth medium). All regions of the pollen tube demonstrated staining and the signal was often prominent in the apical region. All micrographs are maximum projections of z-stacks. Scale bars = 10 µm.