The ins and outs of plant cell walls
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New findings reveal that many membrane proteins undergo regulated trafficking between intracellular compartments and the plasma membrane. This also appears to be a common regulatory mechanism in the control of cell wall metabolism.

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Introduction

The plant cell wall is made of a highly dynamic composite material, the architecture and composition of which changes dramatically during cellular differentiation. The regulation of cell wall dynamics occurs at multiple levels including regulated membrane trafficking within the secretory pathway. In this review, we first summarize recent evidence indicating that regulated trafficking within the endomembrane system is a much more common phenomenon in plant cells than previously thought. We then discuss how the secretory pathway is involved in regulating the deposition and metabolism of cell wall polysaccharides.

Recycling of plasma membrane proteins

Recent reports on alterations in subcellular compartmentation in response to environmental changes suggest that recycling of plasma membrane proteins is an extremely common regulatory mechanism in plant cells. Two extensive reviews [1,2] on endocytosis in plants have been recently published. We limit our discussion here to the most recent findings.

Arguably the best-studied plasma membrane (PM) protein that undergoes endocytosis is the auxin-efflux carrier PIN-FORMED 1 (PIN1). Polar transport of the phytohormone auxin mediates various processes in plant growth and development. Auxin transport inhibitors block PIN1 cycling along with the transport of other PM proteins such as PM-ATPase and KNOLLE [3]. Recent results show that exogenously applied auxin negatively regulates endocytosis not only of PIN1 but also of other PM proteins and of the steryl dye FM4-64. This process lies at the basis of an autoregulatory loop, in which auxin stimulates PIN1 levels at the PM, and hence its own efflux from cells [4**].

Boron is an essential micronutrient for plants. This metal forms di-ester bonds with rhamnogalacturonan II (RG-II), and hence is essential for the formation of dimers of this highly complex and evolutionary conserved cell wall polysaccharide [5]. RG-II dimers are like press-buttons that cross-link pectins and thus organize the cell wall architecture [6]. Boron deficiency causes a disorganisation of the cell wall and growth inhibition. An excess of boron is toxic for the plant, and boron levels therefore need to be finely tuned. Recent studies show that plants regulate boron levels through regulated intracellular trafficking. BOR1, a boron exporter for the xylem, is essential for efficient boron translocation from roots to shoots when boron is limited. During boron limitation, BOR1–green fluorescent protein (GFP) locates to the PM, and the addition of boron relocates BOR1–GFP into intracellular structures that are targeted by the plant Rab5 homolog ARA7 (RabF2b). BOR1–GFP fluorescence is lost after prolonged boron incubation, indicating that the internalisation and degradation of BOR1 are regulated by the availability of the metal [7]. The boron-dependent regulation of BOR1 allows the plant to protect itself against toxic levels of boron while also protecting the shoot from boron deficiency during boron limitation.

The flagellin receptor, FLAGELLIN SENSTIVE 2 (FLS2), belongs to the large receptor-like kinase family in Arabidopsis. FLS2–GFP locates to the PM but relocates (in a microtubule and actin-dependent manner) into intracellular compartments upon stimulation with the flagellin epitope flg22 [8**]. A complete loss of intracellular and PM-located FLS2–GFP fluorescence was observed upon prolonged incubation with flg22, suggesting that the fusion protein is eventually degraded. In addition, the reoccurrence of PM-located FLS2-GFP is blocked upon removal of flg22 in the presence of cycloheximide, indicating that FLS2 is subjected to degradation by an endosomal/vacuolar pathway rather than being recycled to the PM [8**]. This is (to our knowledge) the first example of ligand-mediated receptor internalisation in plants. Other plant receptor kinases such as SOMATIC EMBRYOGENESIS RECEPTOR KINASE3 (SERK3), the brassinosteroid receptor BRASSINOSTEROID...
INSENSITIVE1 (BRI1), and more recently, the L1-specific receptor kinase *ARABIDOPSIS CRINKLY* (ACR4), have also been shown to internalise into intracellular vesicles [9,10].

These examples illustrate how plant cells can adapt to physiological conditions and environmental needs through a dynamic relocation of key proteins between the PM and intracellular compartments.

**Internalised PM proteins, where do they go?**
The increasing number of plant PM proteins that are known to undergo endocytosis raises the question of their intracellular destinations.

At present, plant endocytic compartments are not well characterised, and the term endosome has often been used for any compartment that is labelled by FM4-64. This dye is incorporated into plant PMs, subsequently endocytosed, and labels a variety of compartments in the cytoplasm in a time-dependent manner before being transported to the vacuole [11–13]. An early study looking at cationised ferritin (CF) uptake in white spruce (WS1) protoplasts demonstrated the presence of CF in Golgi bodies and in partially coated reticulum (PCR) within just 5 min of exposure to CF [14]. The exact identity of the PCR is still unknown, however, the *trans*-Golgi network (TGN) shows a remarkable resemblance to the PCR in that both have been described as a tubulo-vesicular compartment with clathrin buds (reviewed in [1,2,15,16] and references therein).

A recent study of the location of a vacuolar ATPase subunit, VHA-a1, showed that VHA-a1 co-located with the TGN marker Syp41, and immunogold labelling confirmed that VHA-a1 predominantly labelled the TGN [17**]. The VHA-a1-labelled compartment was targeted by FM4-64 in root tip cells within 6 min, indicating that the TGN might indeed act as an early endosome. In root tip cells under the same conditions, the location of ARA7, but not of the plant Rab5 homolog ARA6 (RabF1), showed a partial overlap with that of FM4-64 after 15 min of incubation [17**]. ARA7 and Rha1 (i.e. RabF2a, another plant Rab5 homolog) locate to the prevacuolar compartment (PVC)/multivesicular bodies (MVBs) ([13,18–20]; AM Kotzer, pers. comm.) and are involved in membrane trafficking to the lytic vacuole. Evidence suggests that ARA6 locates to an as-yet-unidentified compartment that is different from the location of ARA7 and Rha1 [13], and which is not involved in vacuolar trafficking [18].

The delayed uptake of FM4-64 (after the TGN) in PVCs/MVBs in root tip cells [17**] correlates well with the electron microscopy evidence of CF uptake in soybean and WS1 protoplast, which showed that the labelling of MVB bodies clearly lagged behind that of the Golgi and PCR [14]. This suggests that the TGN could indeed be a candidate for the early endosome, where the biosynthetic and the endocytic pathways converge. The MVB/PVC might represent a recycling endosome where membrane (protein) traffic is either directed toward the vacuole or recycled back to the TGN (Figure 1).

**Membrane trafficking and the control of cell wall metabolism**
The plant cell wall consists of cellulose microfibrils that are embedded in a complex mixture of matrix polysaccharides and structural proteins. Synthesis of cell wall polysaccharides occurs at two subcellular locations: cellulose microfibrils are synthesised at the PM and matrix polysaccharides in the Golgi apparatus (see article by O Lerouxf et al. in this issue for a more detailed discussion of the structure of cell wall polysaccharides and their synthesis). Numerous observations illustrate the heterogeneity in wall architecture within the same cell, which indicates an important role for targeted secretion to distinct sites within the cell. For instance, outer and inner epidermal cell walls have a distinct architecture and composition. This is also the case for longitudinal and cross walls. Newly formed cross walls have been found to contain three-fold more xylolucan epitopes than older longitudinal walls [21]. Plasmodesmata form in specific cell wall domains [22] and only cross walls are perforated during the differentiation of phloem sieve elements. Root hairs are formed only at the basis of root epidermal cells. This involves the targeted secretion of wall material and wall-modifying enzymes to the site of outgrowth. Antigens raised against specific de-esterified homogalacturonan epitopes (LM7 and PAM1) label only highly specific cell wall domains that face the intercellular spaces in stem parenchyma [23]. This pattern could be the result of the targeted secretion of pectins or pectin-modifying enzymes to cell corners.

**Regulated intracellular trafficking of two essential enzymes for cellulose synthesis**
Cellulose microfibrils are synthesised at the PM from 25 nm hexameric membrane complexes that contain the cellulose synthase catalytic subunits (CESA) [24,25]. These complexes generally contain three non-redundant CESA isoforms [24]. A functional YFP–CESA6 fusion was recently shown to locate not only to the PM but also to the Golgi apparatus and other smaller compartments [26**]. Golgi-located YFP–CESA6 might reflect an intracellular store of cellulose synthase complexes that can be inserted into the PM. Alternatively, the cellulose synthase complexes might constantly cycle between the cells interior and the PM. Our findings suggest that the subcellular location of GFP–CESA6 is highly regulated during cellular differentiation, with a predominant PM location in slowly growing cells at the top of 4-day-old hypocotyls and a more intracellular location in larger cells further down the hypocotyl (S Vernhettes, T Desprez, H Höfte, www.sciencedirect.com
unpublished). Interestingly, treatment with isoxaben, a cellulose synthesis inhibitor that specifically targets a complex containing CESA3 and CESA6, cleared YFP–CESA6 from the PM within 20 min [26]. It is not yet clear whether isoxaben promotes endocytosis or inhibits the secretion of the complexes. If endocytosis is involved, however, a mechanism must exist to remove the complexes from the growing microfibril. Either the complexes disassemble or the glucan chains are cleaved from the complex. KORRIGAN1 (KOR1), an endo-1,4-β-D-glucanase, might play a role in this process [27]. GFP–KOR1 was observed in the Golgi apparatus and at the tonoplast of small epidermis cells within the root meristem [28]. In root tip cells, the styryl dye FM4-64 labels the PM. Upon internalisation of the dye, FM4-64 targets the VHA-a1/Syp41-labelled TGN, and is then taken up into the ARA7/Rha1-labelled PVC/MVB. From the PVC/MVB, FM4-64 is directed to the vacuole [pathway 2] [17]. We do not yet know at which stage FM4-64 targets the ARA6-labelled compartment (before TGN, before or after PVC/MVB?).

In light of recent publications on PM protein recycling, it is tempting to speculate that the PM-located proteins BOR1 and FLS2 might follow pathway 2 upon internalisation for degradation in the vacuole. This might also hold true for the receptor kinase ACR4, whereas SERK3 and BRI1 are recycled to the PM [9], either directly through an ARA6-labelled compartment [pathway 3] or through the TGN before targeting to the ARA6-labelled compartment [pathway 4]. The identity of the ARA6 compartment has yet to be resolved; however, it is distinct from the TGN and the PVC/MVB [13]. It is conceivable that CESA and KOR transit this compartment on their way to the cell surface.

Endocytic cycling in plants. Little is known about endocytosis in plants; however, recent evidence shows that the trans-Golgi network (TGN) could be the ‘early endosome’ where the biosynthetic and the endocytic pathway converge [17]. Newly synthesized proteins and cell wall material are transported from the TGN to the plasma membrane (PM) [pathway 1]. The cellulose synthase subunit CESA6 locates to the Golgi, to the PM and to another unidentified compartment [26]. An endo-1,4-β-glucanase, KOR1, locates to the Golgi apparatus and tonoplast in small epidermis cells within the root meristem [28]. In root tip cells, the styryl dye FM4-64 labels the PM. Upon internalisation of the dye, FM4-64 targets the VHA-a1/Syp41-labelled TGN, and is then taken up into the ARA7/Rha1-labelled PVC/MVB. From the PVC/MVB, FM4-64 is directed to the vacuole [pathway 2] [17]. We do not yet know at which stage FM4-64 targets the ARA6-labelled compartment (before TGN, before or after PVC/MVB?).

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Golgi-located biosynthetic enzymes have been characterised (O Lerouxel et al., in this issue) and it is not known how these enzymes are regulated. Early immuno-labeling studies of clover roots showed that the location of polysaccharides within the Golgi apparatus could change during cellular differentiation [21]. Polygalacturonic acid/RG-I epitopes were found in cis- and medial-Golgi cisternae in cortical cells and in trans-Golgi cisternae and the TGN in epidermal and root cap cells. Given the recent findings showing that the TGN in Arabidopsis behaves as an early endosomal compartment, it is conceivable that a portion of the pectic polysaccharides in this compartment are recycled from the cell surface through endocytosis, and that the degree of pectin endocytosis varies in different cell types. Concerning the endocytosis of cell wall polysaccharides, it was recently suggested that cell wall pectins can be recycled from the surface and inserted into the growing cell plate during cytokinesis [31,32].

Remodelling in situ of cell wall polysaccharides

Cell walls are highly dynamic structures. Upon deposition, cell wall polymers are integrated into the existing structure, and they undergo extensive remodelling during cell expansion. These processes involve a wide array of wall-modifying agents (i.e. expansins, xyloglucan transglycosylases/hydrolases [XET], endo-glucanases, pectin methyl esterases, pectin acetyl esterases, pectate lyases and polygalacturonases, peroxidases and various glycosidases), which are secreted into the apoplasm. We have no evidence of the regulated or polarised secretion of these proteins, except that XET activity has been shown to accumulate specifically at root hair initiation sites [33]. However, the activity of many of these proteins must be tightly controlled in the cell wall. This control might involve the sequestration of the proteins to defined sites in the cell wall; for instance, through specific carbohydrate-binding domains, which are found in many cell wall enzymes [34]. In addition, the activity of many of these enzymes is highly dependent on the extracellular pH and/or the presence of oxygen radicals in the cell wall. Interestingly, auxin also inhibits the endocytosis of H+-ATPases in the PM, which might provide a mechanism for the control of the cell wall pH by auxin [4**]. A similar mechanism can be envisaged for NADPH-oxidase, which produces oxygen radicals.

Polarised secretion induced by non-host pathogens

Another striking example of polarised secretion occurs during non-host infection, with failure of the transition from surface to invasive growth of the pathogen. Genetic analysis in Arabidopsis of its interaction with the non-host pathogen powdery mildew shows that plants have the capacity to target secretory vesicles to the incipient infection site. These vesicles presumably carry defence molecules, cell wall components or enzymes. The targetting of these vesicles to the membrane involves a specific syntaxin, PEN1, which is located at the incipient infection site and which forms a trimolecular complex with SNAP33 (soluble N-Ethylmaleimide-sensitive factor attachment protein33) and a secretory vesicle-associated membrane protein, VAMP722 [35].

Conclusions

Accumulating evidence shows that endocytosis and regulated trafficking are very common processes in plant cells. The tip of the veil has been lifted revealing a highly reactive secretory pathway that underpins the dynamics and the micro-heterogeneity of plant cell walls. Challenges for the future will be to understand the mechanisms that control intracellular cycling and targeted secretion. Another important question is how each cell controls its cell wall composition, including a characteristic cellulose/matrix polysaccharide ratio, given the distinct location of their biosynthetic machinery. This will require the development of quantitative methods for measuring intracellular transport in well-defined systems. This knowledge will pave the way for the rational manipulation of cell wall properties in crop plants.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


The first evidence for ligand-induced internalisation of a plant cell membrane receptor. The authors show that the flagellin receptor is endocytosed upon stimulation with the flagellin epitope flg22 and eventually degraded.


