

The endocytic network in plants

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Endocytosis and vesicle recycling via secretory endosomes are essential for many processes in multicellular organisms. Recently, higher plants have provided useful experimental model systems to study these processes. Endocytosis and secretory endosomes in plants play crucial roles in polar tip growth, a process in which secretory and endocytic pathways are integrated closely. Plant endocytosis and endosomes are important for auxin-mediated cell–cell communication, gravitropic responses, stomatal movements, cytokinesis and cell wall morphogenesis. There is also evidence that F-actin is essential for endocytosis and that plant-specific myosin VIII is an endocytic motor in plants. Last, recent results indicate that the *trans* Golgi network in plants should be considered an integral part of the endocytic network.

Endocytosis is an essential process in all eukaryotic cells. It is involved in the internalization of molecules from the plasma membrane and extracellular environment, plasma membrane recycling, including uptake and the degradation of signal molecules [1]. The recent increase of interest in endocytosis has focused, to a large extent, on its extensive roles in signaling. This includes the identification of endosomes as motile signaling platforms [2], Ca²⁺-regulated secretion and cycling of synaptic vesicles, as well as of other exocytic and endocytic vesicles [3]. Moreover, endocytosis and endocytic proteins are linked closely with human cancer [4,5]. Recent research has questioned the text-book view of the structural and functional segregation of exocytic and endocytic pathways, and indicated that secretory pathways in both animal and plant cells are integrated closely with their endocytic networks [3,6,7]. This is true not only for early endosomes and recycling endosomes. Surprisingly, even lysosomes and vacuoles, which are end-stations of the degradative endocytic pathway, can serve as secretory organelles in some situations [8–10].

The study of endocytosis in higher plants remained relatively dormant for a long period because of the notion that endocytosis would not work against the high turgor pressure in plant cells. However, recent evidence argues against this [6,11–17], and plants provide useful model systems for studying signaling endosomes and cell–cell communication based on vesicle recycling [6,11,12].

Moreover, plant cells can internalize components of their cell wall [10,11,13,15], which might provide an important paradigm of an effective mechanism for remodelling extracellular matrices in other organisms.

Compartments, molecules and markers

The endocytic machinery, which encompasses both molecules and membranous compartments, is well conserved in higher plant species (Box 1; Figure I). In plants, animals and yeast, some plasma membrane proteins together with extracellular cargo are delivered to endosomes via clathrin-coated vesicles, whereas other proteins are internalized from plasma membrane domains that are enriched with structural sterols (Box 1; Figure I).

In plants, several proteins that are involved in clathrin-dependent endocytosis have been identified. These include clathrin, adaptor proteins such as AP180, and two adaptins [16,17]. Clathrin-coated vesicles in plant cells are smaller (70–90 nm) [16] than in animal cells (120 nm), which might facilitate endocytic uptake against the high turgor pressure of plant cells [14]. Other components that interact with the clathrin coat and its adaptor proteins (e.g. dynamins and associated proteins that contain an SH3 domain) occur in plants and are involved in endocytosis, vesicular trafficking and cytokinesis [18,19]. These data demonstrate that clathrin-dependent endocytosis and its molecular machinery are conserved in plants. Structural sterols and lipid-raft domains also occur in plants but have not been characterized in detail [20]. Nevertheless, plant structural sterols are reported to be internalized into endosomes [21] and distributed within endocytic networks (Box 1; Figure I) [11,12]. They have been proposed to play a role in a constitutive endocytic cycling of some plasma membrane transport proteins [6].

Three Rab GTPases, Ara6, Ara7 and Rha1, are specific molecular markers of plant endosomes (Box 1; Figure I). However, there is controversy about using Rab markers to identify early and late endosomes in transient over-expression systems. For example, Ara7 and Rha1 have been localized to both early [22] and late endosomes [23]. This might be explained by a model in which there is domain-specific maturation of early endosomes into late endosomes when early endosomes, which are enriched with Ara7 and Rha1, progressively recruit Ara6 and, presumably, other late endocytic molecules, in a domain-specific fashion [22,23]. Because the plant endomembrane system is highly dynamic [24], with extensive interactions

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Box 1. Endosomal compartments in plants

Early endosomes are the first station and branch point in the endocytic pathway in plants. They accumulate endocytic tracers within 2–5 min and serve as sorting compartments for the newly internalized cargo. They are believed to participate in the rapid cycling of plasma membrane molecules but are not well defined structurally (Figure 1). **Recycling endosomes** are loosely defined compartments that are involved in the recycling and secretion of cargo to the plasma membrane. **Late endosomes** are identical to multivesicular bodies and/or prevacuolar compartments where endocytic and biosynthetic pathways converge. They play an important role in the sorting of new synthesized proteins to vacuoles. Late endosomes are most likely to represent a branch-point in the endocytic pathway that leads to the secretion of cargo to either the vacuole or the cell surface. Late endosomes have been defined structurally and molecularly (Figure 1). They contain small internal vesicles in their lumen, and are labelled 10–20 min after exposure to endocytic marker dyes (Figure 1). **Secretory endosomes** participate in exocytosis by either generating secretory vesicles or fusing completely with the plasma membrane. **Lytic vacuoles** are compartments that are specialized for the degradation and turnover of cargo, and are equivalent to mammalian lysosomes and yeast vacuoles (Figure 1).

The identity of endocytic compartments that correspond to mammalian early/recycling and late endosomes, lysosomes and TGN, are defined operationally using either the time-course of internalization or appearance of endocytic tracers such as FM dyes and filipin-labelled sterols, and by the functional accumulation of molecular markers such as Rab GTPases, SNAREs, ARF, ARF-GEF, vacuolar-sorting receptors and PtdIns(3)P-binding FYVE-domain (Figure 1).

Plant-specific problems with endosomal terminology

Plant-cell biologists have tended to develop their own terms such as 'partially-coated reticulum' (PCR) and 'pre-vacuolar compartment' (PVC) for some endocytic organelles [6,11,12,29]. PCR might correspond to either the early endosome or the TGN, but its identity has not been confirmed *in vivo* [6,12]. By contrast, the PVC and MVB are thought to represent the same compartment, which is most likely to belong to the late endosomal system in plants [29]. Therefore, we use the term 'late endosome' to refer to the PVC and MVB in this review. We use the term 'lytic vacuole' for the plant compartment that is equivalent functionally to the mammalian lysosome.

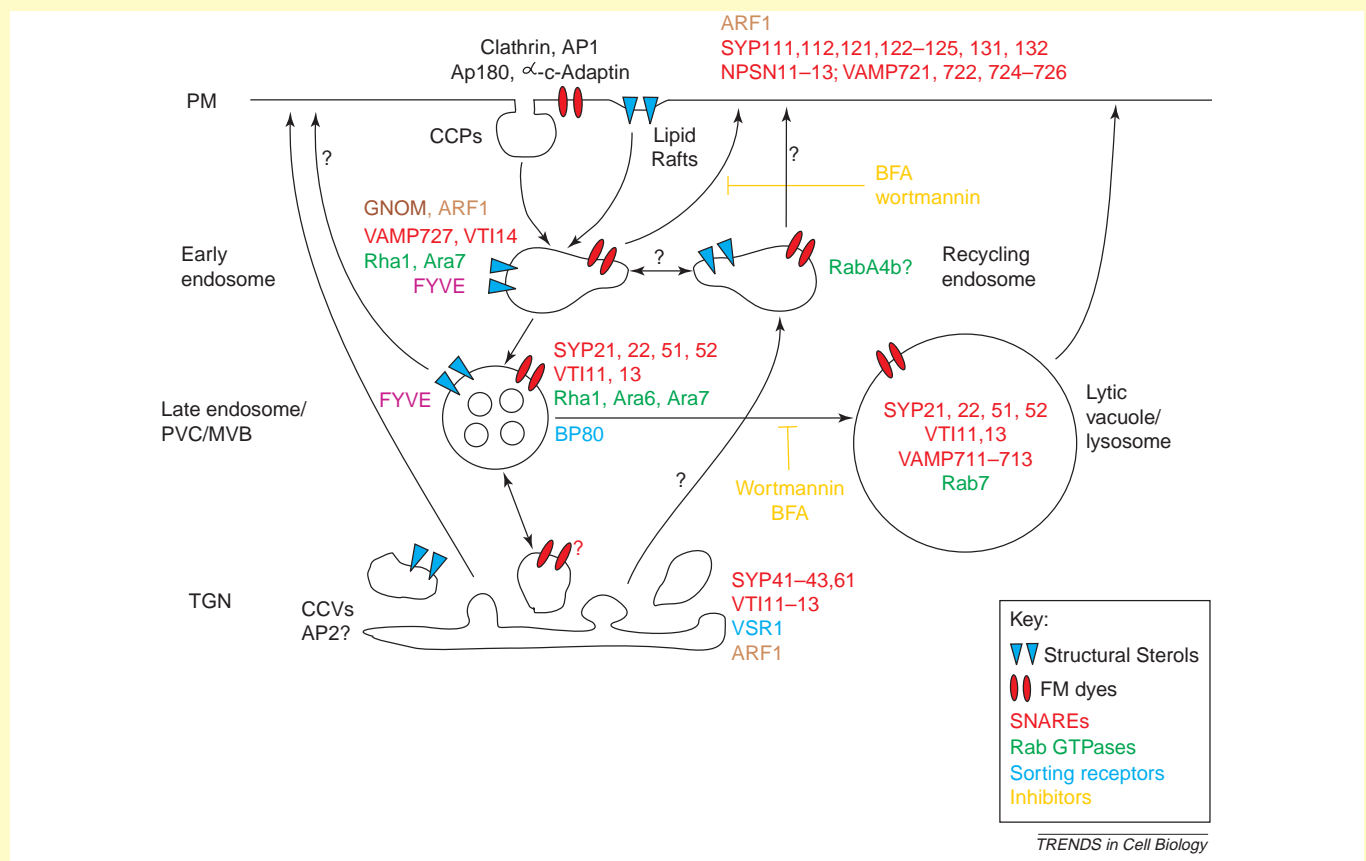


Figure 1. Endocytic network in plant cells. Clathrin-coated pits (CCPs) and lipid-raft domains that are enriched with structural sterols (double blue triangles) on the plasma membrane (PM) serve as internalization platforms for plasma membrane-associated molecules and cargo. These internalized molecules are sorted by two populations of plant endosomes: (1) by early/recycling endosomes; and (2) late endosomes/prevacuolar compartment (PVC)/multivesicular body (MVB); and by the TGN. On sorting, these molecules are either recycled and secreted back to the PM or delivered to lytic vacuoles for degradation and turnover. Internalization of membrane-bound, fluorescent, styryl FM dyes is indicated by double red ellipses. Corresponding molecular markers are depicted in different colours: SNAREs are in red; Rab-GTPases are in green; and vacuolar sorting receptors are in blue. Inhibitory effects of BFA and wortmannin are indicated in yellow. Abbreviations: AP, adaptor protein; ARF, ADP ribosylation factor (small GTPase); BP, binding protein; CCV, clathrin-coated vesicle; FYVE, PtdIns(3)P-binding domain; GNOM, ARF-GEF (guanine nucleotide exchange factor for ARF); VSR, vacuolar-sorting receptor. The terminology of plant Rab GTPases is not yet settled. Ara6 corresponds to AtRabF1, Ara7 to AtRabF2b, and Rha1 to AtRabF2a.

between diverse endocytic compartments [22], caution should be taken when interpreting data obtained in transient overexpression systems. This is particularly significant if molecular markers are overexpressed in

heterologous species because this might result in ectopic expression rather than the true localization pattern. Recent data from our laboratory [24] indicate that strong overexpression of a FYVE-domain construct results in its

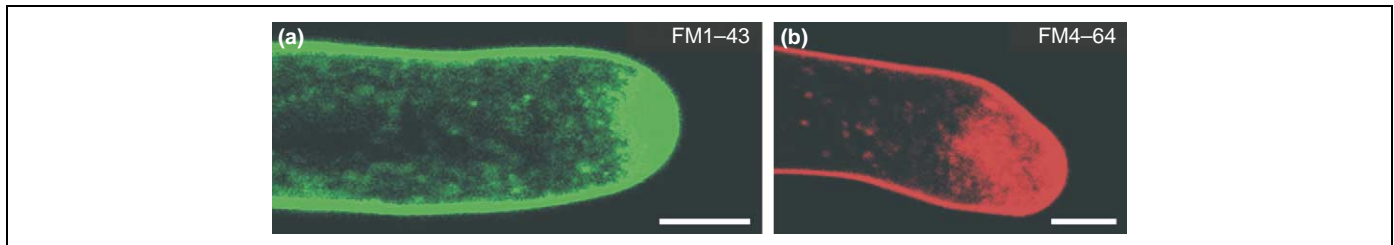


Figure 1. Integrated endocytic and secretory networks in tip-growing root hairs of plants. (a,b) Actively growing root hairs, like pollen tubes [35,71], internalize endocytic tracers FM1–43 (a) and FM4–64 (b) within minutes and accumulate them throughout their growing tips. Bars, 4 μ m. Pictures courtesy of Miroslav Ovecka (University of Vienna, Austria).

mislocalization to a vacuolar compartment [24,25], whereas moderate and low expression levels assure proper localization to endosomes. To avoid such artefacts, results from transient overexpression systems should be supported by data from stably transformed, homologous plant lines that, ideally, carry marker constructs under the control of their own promoters, and/or by localization of corresponding endocytic proteins *in situ*.

In *Arabidopsis*, Ara7 localization is sensitive to ARF-GEF GNOM [26]. In addition, Ara7 colocalizes with the soluble N-ethyl-maleimide-sensitive factor attachment protein receptor (SNARE) VAMP727 [22]. By contrast, structural sterols that are internalized from the plasma membrane and prevacuolar SNAREs, such as SYP21 and SYP22, colocalize with Ara6 in late endosomes [21,22]. Systematic analysis of the expression and localization of SNAREs in *Arabidopsis* revealed that 17 SNAREs, including SYP111/KNOLLE and AtVAMP721, localize to both the plasma membrane and endosomes [27]. Two SNAREs, VAMP727 and AtVTI14, are specific for early/recycling endosomes, and six SNAREs, SYP21, SYP22, SYP51, SYP52, AtVTI11 and AtVTI13, regulate transport between late endosomes and the lytic vacuole [27]. Vacuolar transport also needs specific vacuolar-sorting receptors such as BP80 [28], which has been identified recently as a reliable marker for prevacuolar compartments that represent late endosomes in plants (Box 1; Figure I) [29]. It is possible that Rab GTPases and SNAREs are specialized for either early or late endosomal compartments, but their precise functions, mechanisms of actions and interactions are unknown in plants.

FM-dyes are water-soluble, amphiphilic, styryl compounds that are non-fluorescent in aqueous media but fluoresce intensely on insertion into the outer leaflet of the plasma membrane [30]. They have been used extensively as membrane-selective markers to monitor endocytosis and exocytosis. In neurons that are actively releasing neurotransmitters, these dyes are first internalized and then secreted via recycling synaptic vesicles, and whole nerve terminals become brightly stained [31]. FM4–64 is an invaluable tool to study endocytosis and endosomes in plants [14,22,24,26,27,29,30,32]. Thus, it is generally accepted that FM4–64 is an endocytic tracer and vacuolar marker, depending on the exposure times (Box 1; Figure I). In tobacco BY-2 cells in suspension, FM4–64 partially colocalizes with sialyltransferase, a molecular marker of the trans-Golgi cisternae and the trans-Golgi network (TGN) [30,33], but not with the GDP-mannose transporter [29,34], a true Golgi marker. The physico-chemical

properties of FM1–43, an alternative endocytic marker, differ only slightly from that of FM4–64 [14,36]. However, the reliability of FM1–43 as an endocytic tracer in plants is not unequivocally settled yet [14,35–37]. Although FM1–43 sometimes labels mitochondria in turgid guard cells [14], it is, apparently, a reliable endocytic marker in other cell types such as BY-2 suspension cells and root hairs in which the labelling pattern seems indistinguishable from that of FM4–64 (Figure 1) [36,37].

Exposure of plant cells to FM-dyes for up to 10 min results in colocalization of the dyes with Ara7, Rha1, GNOM, the KAT1 K⁺-channel, SYP111/KNOLLE, AtVAMP721, AtVAMP727, AtVTI14, and the FYVE-domain marker for phosphatidylinositol 3-phosphate [PtdIns(3)P] in early and recycling endosomes (Box 1; Figure I) [14,22,24,26,27]. With longer exposures (10 min–2 h), FM4–64 labels also late endosomes and colocalizes with Ara6, BP80, prevacuolar SNAREs and vacuoles [21,22,24,27,29].

Endocytosis of receptors

Biotinylated proteins such as bovine serum albumin (BSA) and horseradish peroxidase (HRP) have been used as endocytic markers that are internalized into plant cells by a process with the characteristics of receptor-mediated endocytosis [38]. Non-biotinylated BSA and HRP do not enter plant cells, which indicates that the uptake of these markers depends on a specific receptor that recognizes the biotin moiety. Additionally, bacterial elicitors such as lipopolysaccharides are internalized into plant cells by endocytosis [39]. However, the receptors that mediate endocytic uptake of biotin and lipopolysaccharides are still to be discovered and characterized.

Receptor endocytosis in plant cells has been proved for the brassinosteroid receptor BRI1, which regulate diverse aspects of plant growth and development. BRI1, the transmembrane leucine-rich repeat (LRR) protein kinase, interacts with another LRR kinase BAK1 (also known as SERK3). Recently, heterodimerization, endocytic internalization and constitutive cycling of BRI1 and SERK3 has been reported *in vivo* [40]. This constitutive cycling is independent of the brassinosteroid signal but co-expression of BRI1 and SERK3 results in accelerated endocytosis. The same group reported previously that the similar receptor-like kinase (RLK) SERK1 must interact with kinase-associated protein phosphatase (KAPP) to accomplish endocytosis [41]. Another plant RLK, CRINKLY4, undergoes rapid endocytic internalization and both signaling and turnover of this RLK depend on its extracellular domain, which forms a β -propeller [42]. Additionally, endocytic trafficking of

RLKs such as CLV1 (CLAVATA 1) and other RLKs that belong to the SERK subfamily is expected because their kinase domains interact with the endosomal sorting nexin [43]. Another example is the receptor for the fungal elicitor ethylene-inducing xylanase (LeEix2) which is a cell-surface glycoprotein that possesses a signal for receptor-mediated endocytosis. A point mutation (Tyr993 to Ala) in the endocytosis signal of LeEix2 abolishes its ability to induce a hypersensitive response [44].

Fluid-phase endocytosis and phagocytosis-like internalization of bacteria

Lucifer yellow (LY) is a membrane-impermeable fluorescent dye that is used widely as a marker of fluid-phase endocytosis [45], but its use in plants cells should be treated with caution because it is transported actively across membranes in some cell types [46]. Plants possess the plant-specific class VIII myosins that act as an endocytic motor for the fluid-phase endocytosis pathway that is visualized with LY [45]. Plant cells internalize sucrose via fluid-phase endocytosis, and sucrose even acts as a signal for the induction of this endocytic pathway [47]. Furthermore, the combination of LY and FM4-64 has been used recently to identify autolysosomes in plant cells in culture [48]. Plant cells also undergo phagocytosis-like internalization of symbiotic bacteria of the genus *Rhizobium*, a process that depends on the action of Rab GTPases and the generation of PtdIns(3)P [49,50].

Endocytosis, endosomes and the TGN

It is known that endosomes interact extensively with the TGN during protein sorting. Generally, the TGN is considered to be an integral part of the Golgi apparatus, but several observations indicate that the TGN is an independent organelle in plant cells [27,28]. Moreover, some data from plant and animal cells are at variance with the TGN as part of the Golgi apparatus and indicate that the TGN is part of the endocytic network. For example, endosomes and TGN share endocytic molecules such as dynamin, clathrin, endocytic SNAREs, Rabs, ARFs, and ARF-GEFs such as GNOM and BIG2 [11,27,28,51–53], and they are characterized by abundant structural sterols in their membranes [21] and by the formation of tubules [54]. Finally, plant endosomes and TGN elements aggregate in brefeldin A (BFA)-treated cells and form BFA-induced compartments that show typical perinuclear localization [11–13,21,26,55]. BFA is a fungal toxin that targets specifically ARF-GEF proteins that are involved in vesicle trafficking and regulated secretion from either the TGN or endosomes. In this way BFA blocks secretory pathways, but not the first internalization steps in endocytosis [11]. Similarly, in BFA-treated animal cells the endomembrane system divides at the TGN–Golgi interface and endosomes aggregate with TGN elements to form a perinuclear TGN–endosomal hybrid organelle [56,57] that resembles the BFA-induced compartments of plant cells [11–13,21,26]. By contrast, the Golgi apparatus of BFA-treated animal and plant cells in suspension cultures merge with the endoplasmic reticulum (ER) to form a Golgi apparatus–ER hybrid organelle [55–57].

In plant cells analyzed by electron microscopy, the TGN does not colocalize with the Golgi apparatus [28], and several reports show that this is also the case in animal cells. In one of the most dramatic examples in animal cells, the TGN accumulates at the synapses of neuronal cells [58] whereas the Golgi apparatus is localized deep within these cells near the nuclear surface, as in all animal cells. Moreover, the characteristic split of the endomembrane system after treatment with BFA, when TGN elements accumulate with endosomes to form the BFA-induced compartments of plant cells [11] and the TGN–endosomal hybrid organelle of animal cells [56,57], provides strong support for a concept in which the TGN is an inherent part of the endocytic network in plants. Finally, the most convincing argument for the independent nature of the TGN is provided by the analysis of plant SNAREs [27].

These studies indicate that the TGN is obviously an inappropriate term for this organelle, especially because many authors use the terms TGN and Golgi apparatus interchangeably. Thus, changing the term TGN to ‘post-Golgi network’, as proposed in [27], would be more consistent with its characteristics as part of the dynamic system of the endocytic network.

Endocytosis, endosomes and the actin cytoskeleton

In plant cells, pharmacological studies using the actin-disrupting drugs latrunculin B and cytochalasin D reveal that the intact F-actin cytoskeleton is important for the endocytic internalization of plasma membrane proteins, structural sterols, cell wall pectins and extracellular fluids [11–13,21,26,45]. Furthermore, endocytosis and cycling of structural sterols is compromised in actin mutants [21]. These data are consistent with a crucial role of the actin cytoskeleton in endocytosis in other eukaryotic systems such as yeast and mammals [59,60].

In addition to its involvement in endocytic internalization, the actin cytoskeleton is also required for short-range endosomal movements in mammalian and yeast cells [59,60], and for short-range and long-range movements in plant cells [24]. Further studies reveal that uptake of the fluid-phase marker LY occurs preferentially at plasmodesmata domains, which are enriched with F-actin and the plant-specific myosin VIII [45,61,62]. Inhibition of myosin ATPase activity with 2,3 butanedione monoxime slows endocytic uptake of LY, which indicates an active role for myosin(s) in this process [45]. Plants contain two classes of myosin; class VIII and XI. Cell-periphery-associated myosins of class VIII are the only candidates for an endocytic motor because class XI myosins do not localize to the cell periphery and are important for intracellular trafficking [63]. In animal cells, myosin VI has been implicated in endocytosis, vesicular trafficking and cellular processes such as cell migration and mitosis [64]. Interestingly, plant myosin VIII localizes to plant synapses [61,62,65], while myosin VI is localized to neuronal synapses, where it is involved in the endocytosis of glutamate receptors [66]. The particular functions of class VIII myosins in endocytosis in plants remain to be established.

Integrated endocytic and secretory networks regulate cell polarity and tip growth

Endocytosis plays an important role in polarity and tip growth. For example, the endocytic protein Sla2p/End4, which links the endocytic machinery with the actin cytoskeleton [67], is crucial in establishing zones of polarized growth in yeast [68]. Tip-growing plant cells, such as root hairs and pollen tubes, have persistent polarized growth that depends on both secretory and endocytic pathways. It is believed that tip-growing cells need balanced exocytosis and endocytosis to regulate the amount of plasma membrane at the apices of these cells, and to maintain their highly polarized pattern of growth [24,35,37,69,70]. Importantly, endocytosis is concentrated in the growing tips of these highly polarized cells [24,35,37,70].

The endocytic tracers, FM4-64 and FM1-43, are internalized rapidly by pollen tubes and root hairs, preferentially at the growing apices [24,35,37,71]. In growing root-hair tips, highly motile plant endosomes have been visualized with independent molecular markers including a double FYVE-domain peptide and endosomal Rab proteins such as Rha1 and Ara6 [24]. FM dyes label the whole apex, the so-called clear zone, of pollen tubes and root hairs after 10–15 min (Figure 1a,b) [37,71]. These findings indicate that the endocytic and exocytic pathways are integrated closely in polarized, tip-growing plant cells.

Clathrin-coated pits and vesicles are also concentrated at the apices of pollen tubes and root hairs (Figure 2) [69,70]. Actin patches, which are assumed to be sites of endocytosis, are tip-focused in growing root hairs [24], which correspond with important roles of the actin cytoskeleton in endocytosis and polarized-tip growth. Moreover, actin polymerization and intact actin filaments, but not microtubules (for animal cells see [72]), are essential for the movement of early and late endosomes in root hairs [24].

Polar auxin transport, synaptic cell–cell communication and gravisensing

Transcellular transport of auxin, which is a typical feature of plant tissues and organs [73], depends on endocytosis

and endocytic networks [6,26,74]. In addition to aberrant cell walls (see below), *gnom/emb30* mutants have defects in the polar transport of auxin because of failure in endocytic recycling of putative auxin transporters of the PIN family [26,51]. In addition, classical inhibitors of the polar transport of auxin also inhibit endocytosis [74]. Further close links between auxin and endocytosis in plants have been reported recently: first, auxin treatment increases endosomal PtdIns(3)P [75]; and second, exogenous auxin inhibits endocytosis in plant cells [76]. Studies of plant endocytosis reveal that ARF-GEF GNOM is an endosomal protein that maintains the integrity of endosomes and is essential for cycling of the putative auxin-efflux cofactor PIN1 and polar auxin transport dependent on vesicle recycling [26,51]. This link between ARF-GEF and endosomes is surprising [51]. Recently, however, a BFA-sensitive ARF-GEF in animal cells, BIG2, is also reported to localize to endosomes and the TGN [52], and to be essential for their integrity [53]. These findings demonstrate that data obtained using plant cells might also be relevant to animal cells.

BFA-mediated inhibition of all secretory pathways stops cell–cell transport of auxin almost immediately [77]. Thus, it is possible that auxin is secreted in a neurotransmitter-like fashion [65,76,78]. Auxin is transported across F-actin- and myosin VIII-enriched end-poles, which represent plant synapses [65]. Its transcellular transport is extremely sensitive to gravity, and most auxin is transported along the gravity vector [65]. Recent studies reveal that the auxin-dependent graviresponse of *Arabidopsis* shoots depends on endocytic-like proteins that have similarities to the J-domain protein RME8 of *Caenorhabditis elegans* [79]. The significance of this finding is far-reaching because the endocytic network might participate in both plant gravisensing and graviresponses. For example, gateable cell–cell channels called plasmodesmata [62], which interconnect plant cells into a syncytium-like ‘supercell’, might be relevant for these processes. In this respect, the RME8-like protein is also implicated in the endocytic recycling of viral-movement proteins [80] that target plasmodesmata and regulate

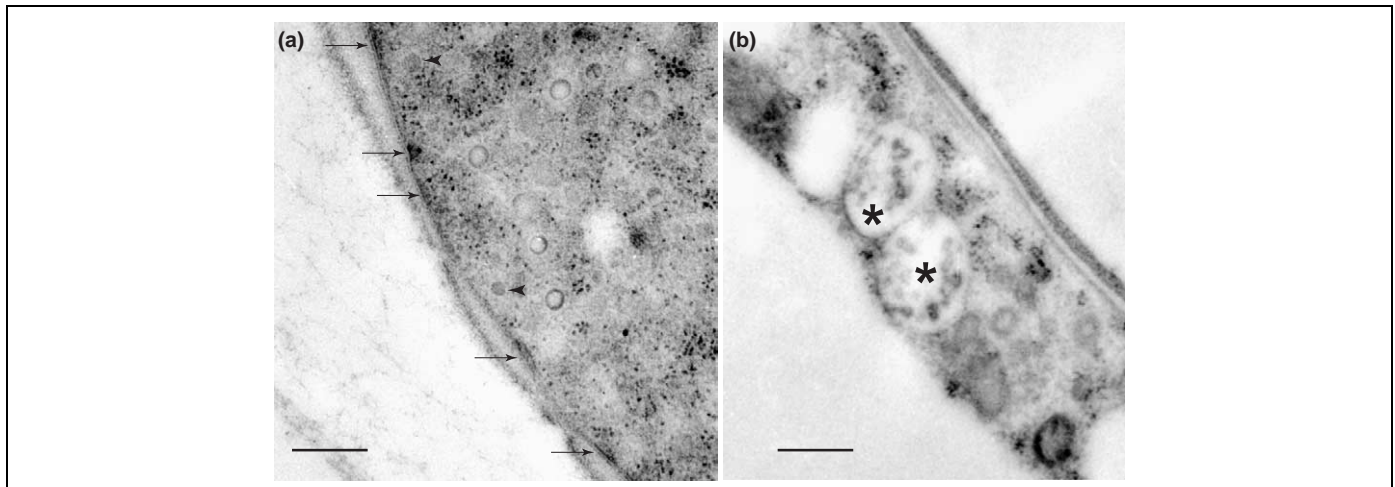


Figure 2. Clathrin-coated pits and vesicles in pollen tubes. (a) Clathrin-coated pits (arrows) and clathrin-coated vesicles (arrowheads) localize to the apex, and (b) multivesicular bodies (asterisks) localize to the sub-apical region of cryofixed/freeze-substituted pollen tubes of *Arabidopsis*. Bars, 350 nm. Pictures courtesy of Jan Derksen (University of Nijmegen, Netherlands).

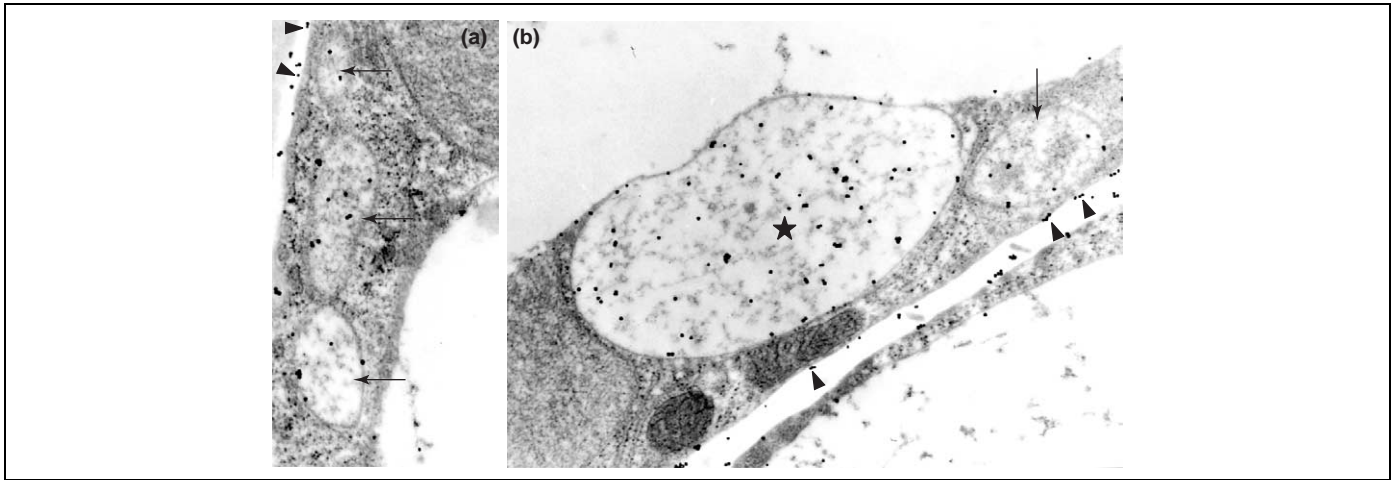


Figure 3. Endocytosis of cell wall cargo represented by GPI-anchored arabinogalactan-proteins (AGPs). (a,b) Immunogold electron-microscopic localization of AGPs with the monoclonal antibody JIM13 shows presumptive cell wall signaling molecules at the plasma membrane, and in late endosomes and lytic vacuoles. AGPs are thought to be internalized endocytically from the plasma membrane (indicated by arrowheads) and delivered to late endosomes resembling MVB and/or prevacuolar compartments (a,b arrows), and then to lytic vacuoles (b, star) for degradation and turnover. For details, see [15,83].

cell-cell transport of virus particles and endogenous proteins [62,80]. Mutations that affect other proteins, including VAM3 and VTI11 that are involved in the vesicular traffic to lytic vacuoles, also result in defects in shoot gravitropism [81,82]. Importantly, the VTI11 SNARE is invoked in vacuolar organization and gravitropism and in the transcellular transport of auxin [82].

Cytokinesis and guard cells: the role of endocytosis and endosomes in cell wall morphogenesis

Dividing and growing plant cells internalize massive amounts of cross-linked cell wall pectins, and also cell wall-associated arabinogalactan proteins anchored with glycosylphosphatidylinositol to the plasma membrane (Figure 3) [10,13,15,83]. Internalized pectins accumulate within BFA-induced compartments alongside several recycling proteins, which indicates that pectins might also be recycled [11,13]. An attractive possibility is that pectins which are cross-linked by either Ca^{2+} or boron are released from their cross-linkages and recycled back to cell walls. This would maintain cell walls in a loosened state that is essential for their growth. However, another possible reason for the accumulation of cross-linked pectins in endosomes of dividing plant cells is that plant cells undergoing cytokinesis need to generate a new cell wall very quickly (in a few minutes). Plant endosomes, the integrity of which is dependent on ARF1-related GNOM/EMB30 activity, are filled with cross-linked cell wall pectins and xyloglucan in dividing root cells [10]. Endosomal pectins serve as 'ready-to-use' building blocks for the formation of new cell walls. In support of these data, ADP ribosylation factor 1 (ARF1), which associates with the plasma membrane and endocytic BFA-induced compartments in maize [13], also localizes to the plasma membrane, endosomes and TGN (Box 1; Figure I) [84] and is essential for cytokinesis in root cells of *Arabidopsis* [84]. These data fit well with recent studies showing that both endocytosis and endosomes are crucial in animal cytokinesis [85]. Intriguingly, endosomes are involved in the execution of cytokinesis [10] and in setting the division plane in plant cells (Figure 4) [86].

Another plant system that requires the rapid degradation and formation of cell walls are guard cells of stomatal complexes. These drive repetitive opening and closing of stomata via rapid increases and decreases in their size. The resulting dramatic change in cell-surface area is accomplished by active endocytosis [32], and endosomal PtdIns(3)P is essential for these stomatal movements [87]. Pectins are abundant in guard-cell walls, and those involved in endocytic internalization [10] are also essential for guard-cell movements [88].

Moreover, the importance of endocytosis for cell wall morphogenesis is apparent from the development of aberrant cell walls in mutants of endocytic proteins such as dynamin [19] and the sec7-like protein GNOM with ARF-GEF activity [26], which is also known as EMB30 [89]. Interestingly, pectins are mislocalized in cells of the *emb30* mutant [89], which links the GNOM/EMB30 protein to cell wall biogenesis. This is perhaps not surprising, considering that GNOM/EMB30 regulates endosomal recycling of PIN1 in early endosomes [26]. Cell wall pectins undergo the same route of vesicular recycling as PIN1 [11,13,76], which might also depend on GNOM/EMB30.

Outlook and perspectives

There is a resurgence of interest in plant endocytosis [6,11,12]. Despite all the negative predictions, highly turgid plant cells accomplish endocytosis [11,12] and rapid recycling via secretory endosomes [6,14]. This is a strong argument for the immense, ancient importance of endocytosis in eukaryotic cells, which can be traced back to the early evolution of eukaryotes. Many of the old dogmas of plant biology might be on 'shaky ground' in the face of new data on endocytosis. For example, new data reveal that cell walls undergo rapid remodelling via endocytosis [10,11,13,15,83] and that the TGN is probably part of the endocytic network. Furthermore, intriguing similarities between the roles of endocytosis in plants and animals are emerging. Recent results [6,65,78] indicate that plants, like animals, undergo a form of synaptic cell-cell communication that involves rapid endocytosis

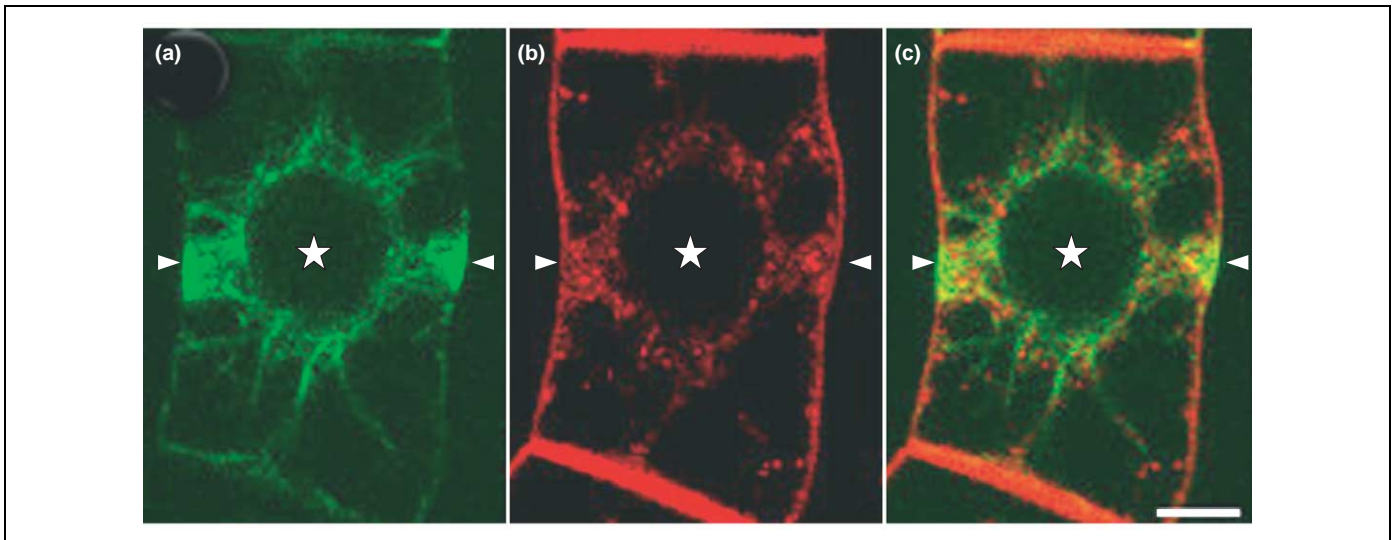


Figure 4. An endocytic belt predicts the division plane in premitotic plant cells. (a) The preprophase band (PPB) of microtubules predicts the cell-division plane. (b,c) Dynamic PPB microtubules induce local endocytosis as visualized with FM4-64 (B and C). Green color represents microtubules visualized with a GFP-MAP4 construct, and red color depicts the FM4-64-labelled plasma membrane, endosomes and endocytic vesicles. For further details, see [86]. Images were taken from [86] with the agreement of the corresponding authors, Pankaj Dhonukshe and Theodor Gadella. Bar, 10 μm .

and vesicle recycling. Moreover, the K^+ -channel TWIK1, which is involved in neuronal excitation, has been identified as a modulator of the endocytic/recycling machinery in animals [90]. Interestingly in this respect, the plant K^+ -channel TRH1 [91] is required for the transcellular transport of auxin, which is driven by endocytosis and vesicular recycling [26,65,73,74,78]. The endocytic network, particularly secretory endosomes, is now in the spotlight of diverse fields including neurobiology, immunobiology, pathology, virology and plant biology. Higher plants will surely be at the forefront of this fascinating field of cell biology.

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Note added in proof

Two important papers have been published recently that advance the issues covered in our review. First, out of 590 human kinases screened, 210 are involved in endocytosis [92]. Second, recycling chemokine CX3CL1 (fractalkine) accumulates in mildly acidic juxtannuclear endosomes and TGN elements, whereas it becomes trapped in BFA-induced compartments near nuclei [93]. These perinuclear, BFA-induced compartments resemble those reported for BFA-treated plant cells.

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