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Structure and function of endosomes in plant cells

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Summary
Endosomes are a heterogeneous collection of organelles that function in the sorting and delivery of internalized material from the cell surface and the transport of materials from the Golgi to the lysosome or vacuole. Plant endosomes have some unique features, with an organization distinct from that of yeast or animal cells. Two clearly defined endosomal compartments have been studied in plant cells, the trans-Golgi network (equivalent to the early endosome) and the multivesicular body (equivalent to the late endosome), with additional endosome types (recycling endosome, late prevacuolar compartment) also a possibility. A model has been proposed in which the trans-Golgi network matures into a multivesicular body, which then fuses with the vacuole to release its cargo. In addition to basic trafficking functions, endosomes in plant cells are known to function in maintenance of cell polarity by polar localization of hormone transporters and in signaling pathways after internalization of ligand-bound receptors. These signaling functions are exemplified by the BRII brassinosteroid hormone receptor and by receptors for pathogen elicitors that activate defense responses. After endocytosis of these receptors from the plasma membrane, endosomes act as a signaling platform, thus playing an essential role in plant growth, development and defense responses. Here we describe the key features of plant endosomes and their differences from those of other organisms and discuss the role of these organelles in cell polarity and signaling pathways.

Key words: Endosome, Arabidopsis, Endocytosis

Introduction
The eukaryotic endomembrane system functions in the synthesis, sorting, delivery and degradation of macromolecules within the cell. The system is composed of a variety of membrane-bound organelles that are connected either directly or through a series of transport vesicles. The main organelles of the endomembrane system are the endoplasmic reticulum, the Golgi complex and trans-Golgi network (TGN), endosomes, and lysosomes or vacuoles. Endosomes have a core sorting function within the endomembrane system. These organelles are the first point of fusion for endocytic vesicles, which are used to internalize extracellular materials. They are also involved in the transport of materials either from the Golgi to the lysosome or vacuole, or their return from lysosomes to the Golgi. Endosomes provide intermediate compartments where materials can be stored, sorted and then sent to a designated target organelle within the cell (Anitei et al., 2010; Huotari and Helenius, 2011; Otegui and Spitzer, 2008).

In plants, lysosomes are generally absent in favor of a large central vacuole, although there is evidence that lysosome-like organelles can co-exist with the vacuole in some cells (Takatsuka et al., 2011). The vacuole is responsible for degradative functions, in common with lysosomes, and also acts as a large storage compartment for proteins, water, ions and some defense compounds (Marty, 1999). Appropriate sorting of proteins at the TGN is essential for proper maintenance of the central vacuole and cell wall (Saint-Jore-Dupas et al., 2004). In plants, as in other organisms, endosomes serve as an entry point for the endocytosis of external materials and an intermediate compartment in the transport of macromolecules to the vacuole (Otegui and Spitzer, 2008). However, the organization of the endosomal system in plants has some unique features when compared with that in animal cells.

In animal and yeast cells, an array of different types of endosomes have been identified that can be classified according to their function during endocytosis. Early endosomes (EEs) are the first site of deposition of internalized components upon endocytosis. The internalized components might then be transported to a recycling endosome for subsequent recycling to the cell surface, or might enter a multivesicular body (MVB), designated as an intermediate or late endosome (LE), for transport to a lysosome or vacuole, often for degradation (Spang, 2009). The MVB is generally considered to be the point at which endocytic and biosynthetic vacuolar trafficking meet, and is therefore often also termed the prevacuolar compartment (PVC) in both yeast and plants (Gerrard et al., 2000; Tse et al., 2004). A third major endosome type, the recycling endosome (RE), receives internalized material from the EE and directs it back to the cell surface or by a retrograde pathway to the TGN (Hsu and Prekeris, 2010). Although endosomes perform similar functions of cargo sorting and transport in plants, they cannot be classified using the same criteria.

Endosomes serve functions beyond the sorting of cargo from endocytosis and biosynthesis. Plant endosomes are important in the maintenance of the vacuole and for cell growth, including the growth of specialized cells such as pollen tubes (Šamaj et al., 2006; Wang et al., 2010; Richter et al., 2012). The composition and structure of the plasma membrane and cell wall require proper endosomal sorting function (Bonifacino and Jackson, 2003; Chow et al., 2008; Toyoooka et al., 2009). Endosomes have also been linked to activation of several signaling pathways (Bar and Avni, 2009; Geldner et al., 2007; Kang et al., 2010; Robatzek et al., 2006). Here, we describe the classification and structure of...
endosomes in plant cells, and discuss how they differ from those in animal cells. The functions of endosomes in plant cells are then discussed, with examples of the growing number of pathways in which they have been found to participate, including roles in generating cell polarity and in hormonal and defense signaling.

**Endosomal compartments**

In animal cells, endosomes have been classified as early endosomes, which are composed of tubules that are dynamic in nature; late endosomes (also categorized as multivesicular bodies), which are typically spherical and contain internal vesicles; and recycling endosomes, another tubular compartment located close to the microtubule-organizing center (Huotari and Helenius, 2011). These compartments can be distinguished by the presence of specific marker proteins, in particular Rab GTPases (Huotari and Helenius, 2011). For example, early endosomes might be detectable by the presence of RAB4 and RAB5, late endosomes can contain RAB7 or mannose 6-phosphate receptors, and recycling endosomes contain RAB11. Both early and late endosomes can recycle their contents back to the plasma membrane, potentially by a recycling endosome, whereas late endosomes can either recycle components to the TGN or deliver them to the lysosome for degradation (Russell et al., 2006). Transport through the endosomal system in general occurs through a maturation of endosomes, for example, maturation of an EE into a LE by removal of EE-specific components, which is accompanied by morphological changes in the structure of the endosome (Huotari and Helenius, 2011).

Although the general functions of endosomes in plant cells are similar to those in animal cells, the organization of the endosomal trafficking pathways has some distinct features (see Fig. 1). Endocytosis has commonly been traced in plant cells using the fluorescent styryl dye FM4-64 (and related compounds); it labels the plasma membrane upon addition to intact cells, is taken up by endocytosis, transported through endosomal compartments and eventually reaches the vacuolar membrane (Bolte et al., 2004). Organelles on the endocytic route are therefore sequentially labeled with fluorescent dye during a time course of uptake. Various inhibitors that block distinct steps in the endocytic and endosomal trafficking pathways have allowed the dissection of trafficking routes and the analysis of proteins potentially involved in these pathways (see Table 1). To date, there appear to be two clearly defined and studied endosomal compartments in plant cells, the TGN or EE and MVB or LE (Fig. 2). Additional types of endosomes might be present, but their identity awaits further experimental confirmation.

**Early endosomes**

EEs are defined as the first compartments that receive endocytic cargo after internalization from the cell surface, and are labeled very rapidly by FM4-64 (Bolte et al., 2004). There is now strong

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**Fig. 1. The endocytic pathway in plants.** In plants, components of the endocytic pathway are involved in biosynthetic, degradative and recycling transport. The trans-Golgi network and early endosomes act as the point of organization for these three pathways. This diagram displays the organelles involved in endomembane trafficking: nucleus, endoplasmic reticulum (ER), Golgi complex (GC), trans-Golgi network (TGN) or early endosome (EE), late endosome (LE) or multivesicular body (MVB) or prevacuolar compartment (PVC), late prevacuolar compartment (LPVC), vacuole surrounded by the tonoplast, recycling endosome (RE). The colored arrows designate potential traffic between organelles. The green arrows indicate pathway 1, the biosynthetic transport route to the plasma membrane that passes through the TGN and sometimes the MVB. The blue arrow shows a pathway (2) that is taken by plasma membrane components (red ovals) as they are internalized into endocytic vesicles and move through the TGN. Proteins associated with the TGN are listed (Bassham et al., 2000; Chow et al., 2008; Inaba et al., 2002; Lam et al., 2007; Sanderfoot et al., 2001; Ueda et al., 1996). The thick, red arrows represent a recycling pathway (3), by which plasma membrane components might be returned to the plasma membrane through a specialized RE. Proteins associated with the RE are shown (Geldner et al., 2009; Preuss et al., 2006; Rutherford and Moore, 2002). The orange arrows designate the transport pathway (4) for newly synthesized vacuolar components, as well as cellular materials destined for degradation in the vacuole. Proteins associated with MVBs are shown (Bottaneli et al., 2012; da Silva Conceição et al., 1997; Jiang and Rogers, 1998; Lee et al., 2004; Li et al., 2002; Paris et al., 1997; Rutherford and Moore, 2002; Sanderfoot et al., 1998; Sanderfoot et al., 1999; Sohn et al., 2003; Spitzer et al., 2009). Transport from the TGN to MVBs is designated by a black arrow. Retrograde transport from the VAC to the MVB is represented by curved black arrows.
evidence that, rather than existing as a separate organelle as in animal cells, in plants, the TGN takes on the function of an early endosome and is the first site for delivery of endocytosed material. This was initially proposed by Dettmer and colleagues, who, using immunogold electron microscopy and fluorescent labeling, showed that the vacuolar proton–ATPase subunit VHA-a1 localizes to the TGN (Dettmer et al., 2006). Surprisingly, the same marker was also shown to colocalize with FM4-64 very rapidly after addition of the dye, a hallmark of an EE. Inhibition of the vacuolar ATPase with concanamycin A (Table 1), which leads to a block in vesicle trafficking, causes the accumulation of both secretory and endocytic cargo at the TGN (Dettmer et al., 2006). Endocytosed plasma membrane proteins, secretory proteins and polysaccharides can all be found in the same TGN (Viotti et al., 2010), suggesting that these two pathways merged at this site, now often termed the TGN/EE. Similar results were obtained using the secretory carrier membrane protein 1 (SCAMP1) protein from rice, which also localized to the TGN.

Table 1. Examples of inhibitors used to study endosomal trafficking in plants

<table>
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<tr>
<th>Inhibitor</th>
<th>Effect on plant cells</th>
<th>References</th>
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<tr>
<td>Brefeldin A</td>
<td>Blocks trafficking from endosomes to plasma membrane</td>
<td>(Geldner et al., 2003; Nebenführ et al., 2002; Richter et al., 2007; Robinson et al., 2008b; Teh and Moore, 2007; Tse et al., 2006)</td>
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<tr>
<td></td>
<td>Causes formation of endosomal aggregates (BFA compartment)</td>
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<tr>
<td></td>
<td>Causes redistribution of Golgi proteins to ER</td>
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<tr>
<td>Wortmannin</td>
<td>Inhibits PI3K</td>
<td>(Jailais et al., 2006; Kasai et al., 2011; Matsuoka et al., 1995; Miao et al., 2006; Tse et al., 2004; Vermeer et al., 2006)</td>
</tr>
<tr>
<td>Concanamycin A</td>
<td>Inhibits vacuolar ATPase</td>
<td>(Dettmer et al., 2006; Matsuoka et al., 1997)</td>
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<tr>
<td></td>
<td>Blocks transport out of TGN/EE</td>
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<tr>
<td></td>
<td>Prevents vacuolar degradation</td>
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<tr>
<td>Filipin</td>
<td>Binds plasma membrane sterols</td>
<td>(Grebe et al., 2003; Kleine-Vehn et al., 2006)</td>
</tr>
<tr>
<td>Tyrophostin A23</td>
<td>Inhibits clathrin-mediated endocytosis</td>
<td>(Fujimoto et al., 2010; Ortiz-Zapater et al., 2006)</td>
</tr>
<tr>
<td>Endosidin1</td>
<td>Causes formation of endosomal aggregates</td>
<td>(Robert et al., 2008)</td>
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<tr>
<td></td>
<td>Blocks endocytic trafficking</td>
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Fig. 2. Use of three-dimensional electron tomography to distinguish different regions of the endomembrane system. (A) Transmission electron microscopy (TEM) image of a prepared tomographic slice (4.3 mm thick) through a region of the Golgi complex and surrounding cytoplasm. Take note of the division of the Golgi stack from cis-Golgi (cis) to medial-Golgi (med) to trans-Golgi (trans) and the trans-Golgi network (TGN). Specialized labeling techniques can be used to help distinguish different cisternae. A non-coated vesicle (NCV) and a multivesicular body (MVB) are also labeled. (B) Here, cis-, medial- and trans-Golgi cisternae seen in A are visualized as a three-dimensional image, displaying the divisions of the Golgi stack, as well as a distinct TGN with associated non-coated and clathrin-coated vesicles (CCVs). MVBs are also clearly visible as white bodies attached to the TGN. (C) Top-down or face-on views of the Golgi stack. 3D electron tomography allows for the virtual dissection of the Golgi stack, from top to bottom (from top, cisternae are indicated: cis1, cis 2, medial 1, medial 2, medial 3, trans 2, TGN). The colors for each cisterna are as in B. This top-down view also allows visualization of the formation of protein aggregates (arrows) and CCVs (CC). Scale bars: 100 nm. Figure reprinted from Otegui et al. (Otegui et al., 2006) with permission.
and colocalized with FM4-64 at early time points (Lam et al., 2007), confirming that the previous findings are not dependent on the TGN marker used.

Evidence exists that the TGN in plants might contain specialized subdomains with distinct functions. Using immunogold electron microscopy, TGN marker proteins have been shown to localize to separate domains of the same TGN (Bassham et al., 2000). Rab-A2 and Rab-A3 GTPases partially co-localize with a TGN marker at an organelle that also acts as an EE (Chow et al., 2008). However, unlike the TGN marker VHA-a1, these GTPases are found at the cell plate during cytokinesis, suggesting that they label an organelle that contributes to secretory processes (Chow et al., 2008), possibly suggesting functional differentiation of the TGN or EE during cell division. Recently, an Arabidopsis mutant lacking a TGN-localized component was described that is defective in secretory but not endocytic trafficking, indicating a separation of these two functions of the TGN (Gendre et al., 2011). Electron tomography indicates that the TGN is differentiated into early and late compartments (Kang et al., 2011; Staehelin and Kang, 2008); how these relate to functionally specialized regions is not yet entirely clear. This differentiation was further analyzed by live-cell imaging using the secreted protein SCAMP2 as a marker. SCAMP2 could be seen at the TGN, which was associated with the Golgi and also could be labeled with FM4-64, indicating that it was on the endocytic route (Toyooka et al., 2009). It was subsequently found in a cluster of vesicles derived from the TGN that contain secretory cargo, including protein and polysaccharides. This cluster separates from the Golgi, moves to the plasma membrane, where it fuses and releases its contents into the cell wall (Toyooka et al., 2009), in a process that possibly also involves the Rab GTPase ARA6 (Ebine et al., 2011). Tagging of Golgi and TGN with fluorescent markers followed by live-cell imaging has demonstrated that the TGN is not permanently associated with a Golgi stack, but that rather, individual TGNs move rapidly within the cell and can dissociate from their associated Golgi and even reassociate with a different Golgi stack (Viotti et al., 2010). Whether some of these mobile TGN structures correspond to the secretory clusters described by Toyooka and co-workers (Toyooka et al., 2009) is unknown.

**Late endosomes**

Endocytosed material has several possible destinations after arriving at an EE. It might be recycled back to the plasma membrane, maintained in the EE as it matures into a LE, or possibly transported to earlier compartments of the secretory pathway. Unlike EEs, LEs have a multivesicular structure, in which the external limiting membrane of the endosome buds inward to produce internal vesicles. Functionally, this structure allows delivery of membrane to both the lysosomal or vacuolar membrane by fusion of the external endosomal membrane with the lysosomal or vacuolar membrane and to the lumen of the lysosome or vacuole for degradation in the internal vesicles. It has therefore been predicted that membrane proteins that are destined for degradation would be concentrated into the internal vesicles of LEs, whereas those destined for recycling would remain on the limiting outer membrane (Babst, 2011).

Evidence that MVBs, which were identified morphologically by electron microscopy, act as LEs in plants was provided by the demonstration in tobacco cell culture, by FM4-64 labeling, that these organelles are on the endocytic pathway (Tse et al., 2004). MVBs also contain vacuolar sorting receptors and vacuolar proteins that are en route to the vacuole, suggesting that they are also intermediates in biosynthetic trafficking to the vacuole (Bottanelli et al., 2011; Jaillais et al., 2008; Miao et al., 2008; Mo et al., 2006; Tse et al., 2004). Several Rab GTPases (Ebine et al., 2011; Ueda et al., 2004; Ueda et al., 2001) were shown to localize to MVBs and to also colocalize with a PVC marker, but not an EE or TGN marker, confirming that MVBs observed by electron microscopy correspond functionally to both a LE and a PVC (Haas et al., 2007; Miao et al., 2008). Plasma membrane proteins are found in internal vesicles of MVBs and are absent from the limiting membrane, indicating that they are en route to the vacuole for degradation and that recycling to the plasma membrane must occur from an earlier compartment, either the TGN or a separate RE (Viotti et al., 2010).

The lumenal vesicles of MVBs are generated through the endosomal sorting complex required for transport (ESCRT) machinery. This machinery consists of several protein complexes (ESCRT-0, -I, -II, -III) that function in the invagination of the limiting endosomal membrane and release of the vesicles formed, and in targeting of vacuole-destined plasma membrane proteins into these vesicles (Henne et al., 2011). Although most of the ESCRT proteins identified in yeast and animals are conserved in plants, the ESCRT-0 cargo-sorting complex appears to be missing in the majority of eukaryotic lineages, including plants (Leung et al., 2008), and other components might therefore act in cargo recognition in these organisms, with the Tom1 proteins being likely candidates based on their interaction with ESCRT subunits (Richardson et al., 2011). On the basis of protein localization and the phenotypes of mutants in a few of the plant ESCRT proteins, in plants, the ESCRT proteins probably function in LE maturation and sorting of cargo proteins for degradation (see Fig. 3) (Haas et al., 2007; Ibl et al., 2012; Katsiarimpa et al., 2011; Scheuring et al., 2011; Shahriri et al., 2011; Spitzer et al., 2009). An example of regulated transport in which a normally plasma-membrane-localized protein, the BOR1 boron transporter, is internalized and sorted by the ESCRT complex in response to environmental conditions has recently been described (Takano et al., 2005) (see Box 1 for more details), and additional examples probably exist.

A model for endosome formation and maturation has been proposed, in which, instead of vesicle transport moving cargo between endosomes, the TGN matures into a MVB, which then fuses with the vacuole to release its cargo (Scheuring et al., 2011). The authors of this study were able to capture MVBs fusing with the vacuole, suggesting that this transport step does not involve vesicle trafficking. They also imaged MVBs that are connected to tubules, which they hypothesized to correspond to immature MVBs derived from the TGN. In support of their model, ESCRT components are found distributed on the TGN as well as on MVBs, and disruption of endosome maturation leads to increased colocalization of TGN and MVB markers (Scheuring et al., 2011).

An extension of this model is implicated in work by Foresti and colleagues, in which a mutant vacuolar-sorting receptor is shown to accumulate in a compartment that is distinct from the Golgi, TGN and MVB (Foresti et al., 2010). A soluble vacuolar marker also accumulates in these structures, which contains members of the Rab5 family of small GTPases (Bottanelli et al., 2012; Foresti et al., 2010). These structures are therefore suggested to be an intermediate compartment, termed the late...
machinery, back to earlier compartments before fusion of the LPVC with the vacuole. The retromer complex is likely to be a key component in this recycling process, consisting in yeast of two subcomplexes that function in cargo recognition and membrane association and deformation, respectively (Schellmann and Pimpl, 2009). These subcomplexes are conserved in plants (Oliiviousson et al., 2006; Pourcher et al., 2010) and might function in recycling of vacuolar-sorting receptors during endosome maturation (Shimada et al., 2006; Yamazaki et al., 2008; Jaillais et al., 2007). Although the exact site of recycling of vacuolar-sorting receptors is controversial, with different localization of the retromer complex reported by different laboratories (Jaillais et al., 2006; Jaillais et al., 2007; Kleine-Vehn et al., 2008; Niemes et al., 2010a; Niemes et al., 2010b; Oliiviousson et al., 2006; Phan et al., 2008; Pourcher et al., 2010; Yamazaki et al., 2008), the general principles of the endosome maturation model are likely to stand.

### Role of endosomes in plants

The primary role of the endosome in all eukaryotes is in endocytosis. Endocytosis has been studied most extensively in yeast and mammalian systems, partly because of its association with a number of diseases (Durieux et al., 2010; Li and Dificiglia, 2011; Zhang et al., 2009). In plants, endosomes serve these same basic functions of uptake of extracellular materials to the interior of the cell (Meckel et al., 2004), and recycling and maintenance of plasma membrane receptors (Altenbach and Robatzek, 2008).

Plasma membrane maintenance and cell polarity are both linked to the EE or TGN in plants. Plant cell polarity is important in maintaining overall plant structure, organogenesis and tropisms. One family of plant proteins with a role in cell polarity are PIN proteins, plasma-membrane-localized auxin efflux carriers that are involved in the transport of the plant hormone auxin. They are important for determining the direction and extent of growth of individual cells and therefore for the maintenance of plant cell polarity (Grunewald and Friml, 2010). The polar localization and maintenance of PIN family members is controlled by endocytosis (Dhokukshe et al., 2007) and transport by both ESCRT-dependent and ESCRT-independent pathways (Spitzer et al., 2009). Several pathways have been elucidated that control the maintenance of plasma membrane polarity (Fig. 3), at least in part through the use of the inhibitors Brefeldin A, which blocks the cycling of the PIN proteins through endosomes (Jaillais et al., 2006), and wortmannin, which inhibits transport out of endosomes (Jaillais et al., 2006) (see Table 1). For example, localization of PIN1 involves the endosome-localized GNOM protein, a guanine nucleotide exchange factor for an ARF GTPase (Geldner et al., 2003; Tanaka et al., 2009), or an alternative GNOM-like protein GNL2 in tip-growing cells that undergo rapid cell expansion (Richter et al., 2012), whereas a separate pathway is responsible for the internalization of PIN2, which requires GNOM-like 1 (Jaillais et al., 2006; Pan et al., 2009; Teh and Moore, 2007). The auxin influx carrier AUX1 is regulated by yet another distinct pathway, but still requires endosomal trafficking (Du et al., 2011; Kleine-Vehn et al., 2006; Robert et al., 2008). The endosome thus regulates the location of components in different domains of the

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**Box 1. BOR1 borate transporter: an example of regulated endosomal trafficking**

Examples of regulated endocytic trafficking in plants have recently come to light. One of the better studied is the BOR1 borate exporter. Boron is an essential element for plants, required for correct cell wall structure (O’Neill et al., 2004), but an excess of boron is toxic. BOR1 transports borate into the xylem and therefore controls the amount of boron in the plant (Noguchi et al., 1997; Takano et al., 2002). Under boron-limiting conditions, BOR1 is localized to the plasma membrane, but addition of boron leads to uptake of BOR1 into endosomes and further transport to the vacuole (Takano et al., 2005). Study of this protein has provided insight into the signals required for endosomal transport. Upon addition of boron, BOR1 is ubiquitylated on K590, and mutation of this residue blocked MVB sorting for degradation in the vacuole (Viotti et al., 2010). Because BOR1 is present on internal vesicles of MVBs (Viotti et al., 2010), this raises the possibility that ubiquitylation of BOR1 signals its transfer into internal vesicles, presumably through the ESCRT complex.
plasma membrane through endocytosis and recycling, which, in turn, is essential for plant cell growth and polarity.

Endocytosis and signaling are linked through the need for maintenance and internalization of plasma membrane receptors, as well as the role of endosomes as platforms for signaling pathways (Geldner, 2004; Geldner and Robatzek, 2008). In addition to more typical signaling cascades that are initiated at the plasma membrane by membrane-localized receptors, several examples of signaling pathways that involve endosomes are now known in plants (Geldner and Robatzek, 2008). Some leucine-rich repeat receptor kinases can function in signaling mechanisms that involve the endosome. Upon extracellular ligand binding, the ligand–receptor complex is internalized, which in the case of recognition of flagellin by the flagellin-sensitive 2 (FLS2) receptor, initiates the defense response (Gómez-Gómez and Boller, 2000). Mutations in the gene encoding FLS2 that block endocytosis also impair downstream signaling, indicating that there is a relationship between these two processes (Robatzek et al., 2006; Salomon and Robatzek, 2006). Another example is that of the BRI1 brassinosteroid receptor; although its internalization is independent of ligand binding, experimental enhancement of its endosomal localization increases brassinosteroid signaling, suggesting that signaling occurs preferentially from endosomes rather than the plasma membrane (Geldner et al., 2007).

An unusual pathway for initiation of signaling has been described for the extracellular leucine-rich repeat receptor-like protein LeEix2 from tomato (Ron and Avni, 2004). This class of proteins are cell-surface receptors, but lack a functional cytoplasmic domain, which in most receptors functions in signaling to downstream components (Krujút et al., 2005); it is therefore unclear how the signal is transduced in the absence of this domain. LeEix2 induces defense responses by binding to the fungal elicitor ethylene-inducing xylanase (EIX), causing its internalization to endosomes (Bar and Avni, 2009; Bar et al., 2009). Inhibitor studies demonstrated that endosomes and endocytosis are required for LeEix2-mediated initiation of signaling responses (Sharfman et al., 2011). Surprisingly, the addition of EIX leads to changes in endosome mobility, with increased directional movement of endosomes in the presence of the elicitor (Sharfman et al., 2011). Although the significance of this movement is not yet understood, the functional implications of endosome dynamics in the defense of plants against pathogen attack should prove a fascinating topic of future study.

Closing remarks and future challenges

In this Commentary, we have discussed the structure of the endomembrane system in plants, with its two main classes of endosomes, early endosomes (equivalent to the TGN) and late endosomes (equivalent to multivesicular bodies or prevacuolar compartments). The differences between endosome organization in plants compared with animal cells, and in particular the additional role of the TGN as an early endosome (Dettmer et al., 2006), highlight the need to study endosomal structure and function in plant cells to have a complete understanding of these important organelles. Recent research into plant endosomes has focused on the regulation of endosomal trafficking and its relationship to polarized cell growth (Du and Chong, 2011; Ebine et al., 2011; Richter et al., 2012), the structure and maturation pathways of different types of endosomes (Kang et al., 2011; Scheuring et al., 2011; Viotti et al., 2010), and the characterization of endosomal proteins such as ESCRT and retromer components involved in transport and maturation pathways (Pourcher et al., 2010; Shahriri et al., 2011; Wang et al., 2010). Future important avenues of research will include the clarification and further characterization of the pathways and components for recycling from endosomes to the plasma membrane (Jailal et al., 2008; Pan et al., 2009; Richter et al., 2012) and functional analysis of proteins that are required for endosomal trafficking and maturation (Niemes et al., 2010b; Otegui and Spitzer, 2008; Pourcher et al., 2010; Robinson et al., 2008a). The role of endosomes in signaling pathways is still limited to a small number of examples in plants (Geldner and Robatzek, 2008; Gu and Innes, 2011; Kang et al., 2010); it is likely that this number will increase and our understanding of the function of endosomes as signaling platforms will grow over the next few years. As new tools are becoming available for the analysis of endosomal structure, function and trafficking (see Table 1 for examples), new avenues of research might be illuminated, and our understanding of these important organelles will greatly expand.

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