Plant Receptors Go Endosomal: A Moving View on Signal Transduction[W]

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Signaling through cell surface receptors is pivotal for cells to communicate with each other and to interact with the environment. Although overlooked in the past, current knowledge supports the idea of receptor signaling, not only from the surface but also from endosomes. In plants, pioneer studies on receptors that show ligand-induced as well as constitutive endocytosis provide evidence for the accumulation of active receptors in endosomes and uncover complex trafficking routes leading to recycling and degradation. Receptor-mediated endocytosis might have developed as a logical consequence of higher organism complexity. As such, translocation of plasma membrane (PM) resident receptors into endosomes can be seen as a means to extend limited signaling surface, adding plasticity and modularity to the PM and ensuring a robust and efficient cellular signaling system.

Cell surface receptors in plants mediate a plethora of responses according to developmental as well as environmental inputs. During their lifetime, receptors undergo a complex suite of subcellular trafficking events: Synthesis and maturation take place in the endoplasmic reticulum and delivery to the PM requires passage through the Golgi apparatus. Receptors eventually insert into the PM to fulfill their function as sensors. During this time, retrieval from the PM involves the endocytic pathway and subsequent sorting, either for recycling back to the PM or for targeting to late endosomes and eventual degradation in the vacuole.

Classical models of signal transduction cascades are based on the assumption that only the cell surface-localized receptor pool is functionally relevant and downstream signaling components are freely accessible by diffusion. In the last decade, however, it has become evident that subcellular trafficking of cell surface receptors has to be considered as an integral part of signal transduction cascades (von Zastrow and Sorkin, 2007). Many of the initial observations that pointed to a requirement for endosomal localization in signaling have been made with the mammalian epidermal growth factor receptor (EGFR). EGFR exhibits strong ligand-dependent endocytosis that leads to a decrease of receptors at the PM and increased rates of degradation. While this process is important for eventual signal termination, it is also a required step for an efficient assembly of downstream signaling components, such as mitogen-activated protein (MAP) kinases. Some MAP kinase scaffold proteins are exclusively localized to late endosomal compartments, which makes translocation of activated EGFR into endosomes essential for signaling (Teis et al., 2002). In mouse, assembly of an endosomal signaling complex is crucial for EGF-dependent developmental processes (Teis et al., 2006). Such a requirement for endosomal signaling is not specific to EGFR or its family because it has also been demonstrated for other receptor classes, such as the transforming growth factor-β receptor or seven-transmembrane receptors (von Zastrow and Sorkin, 2007). In addition, Toll-like cell surface receptors, which recognize exogenous ligands from potentially infectious agents and share some characteristics with plant receptors, were also shown to involve endocytosis for cellular responses (Latz et al., 2004; Johnsen et al., 2006).

While endosomal signaling in animals is now beyond doubt, its existence irresistibly raises the question of its mechanistic and evolutionary necessity. Until recently, no data were available that would indicate whether endosomal signaling is restricted to the animal lineage or represents a more widespread phenomenon in eukaryotes. Although subject to endocytosis, the archetypal yeast pheromone receptor model does not appear to have a strong positive requirement for signaling (Hicke et al., 1998; Slessareva et al., 2006). However, comparing signaling pathways in unicellular yeast with those of multicellular organisms suggests that cells of complex organisms had to specialize at the cellular level and/or increase complexity at the subcellular level. Mammals, for example, comprise a large number of highly specialized cells, including a circulatory system. Plants, by contrast, are sessile organisms that develop without cell migration and display a lower degree of tissue and organ specialization and centralization. This must leave a much higher charge of signal perception and integration to the individual cell. To integrate the complex signaling cues from neighboring cells as well as the environment,

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plant cells were probably forced to develop highly flexible systems at the subcellular level to allow both parallel signaling and controlled cross talk. Given the enormous numbers of plant cell surface receptors, the PM requires a high degree of plasticity to form multiple, timely, accurate, and highly specific receptor signaling platforms. Receptor endocytosis and endosomal trafficking could allow them to establish such platforms for parallel, yet specific, perception of a multitude of different ligands. In plants, endocytosis via clathrin-mediated processes has been established for the PM-resident proteinase inhibitor (PIN) proteins, auxin efflux carriers that are required for polar transport of the growth hormone auxin (Dhonukshe et al., 2007).

Although some indications for receptor-mediated endocytosis (RME) have existed for a long time, only recently has it become a focus in plant cell biology. In this *Update*, we will review the latest data on receptor endocytosis and trafficking in plants and then discuss how a requirement for endosomal signaling could be explained as necessary adaptations of cells in complex multicellular organisms, putting endosomal signaling into a more general evolutionary context.

CONSTITUTIVE RECEPTOR ENDOCYTOSIS

Only a few endogenous ligand-receptor pairs are known in plants, and one of the prime examples is the Leu-rich repeat (LRR) receptor-line kinase (RLK) BRASSINOSTEROID INSENSITIVE1 (BRI1), which is responsible for the perception of brassinosteroid (BR) in Arabidopsis (*Arabidopsis thaliana*; Kinoshita et al., 2005). Transgenic lines expressing a functional BRI1-GFP fusion protein at endogenous levels revealed fluorescent signals at the PM and intracellular mobile vesicles in root meristem cells (Geldner et al., 2007), confirming earlier studies (Russinova et al., 2004). BRI1-GFP colocalizes with the endocytic tracer FM4-64 and the trans-Golgi network/early endosome marker VHA-a1-RFP, and its localization is sensitive to brefeldin A (BFA), an inhibitor of endosomal trafficking. This sensitivity toward BFA could reflect continuous recycling, as demonstrated for the PIN auxin efflux carriers (Geldner et al., 2001). Because BRI1 is the receptor for endogenously produced BR, it is possible that the observed endosomal localization of BRI1-GFP was BR dependent, indicative of activated RME. However, exogenous application of BR, depletion of endogenous BR levels, and reapplication of BR to previously depleted plants did not cause any changes in the BRI1-GFP endosomal pool (Geldner et al., 2007). Thus, BRI1-GFP endocytic trafficking appears to be constitutive, and BRI1 can be considered a receptor that is resident to both PM and endosomes.

Interestingly, a similar localization was also reported for the Arabidopsis homolog of the receptor-like kinase CRINKLY4 (ACR4; Gifford et al., 2005), a non-LRR-RLK acting in epidermal development. A functional fusion of ACR4 to GFP was expressed in transgenic lines under the control of its native promoter. In roots, ACR4-GFP fluorescence was observed at the PM and in endosomal vesicles, based on the partial colabeling with FM4-64 and their sensitivity to BFA. Thus, ACR4 might represent another example of a receptor that localizes to endosomes in a constitutive fashion. However, because the endogenous ligand has not been identified, it could also be that endosomal localization of ACR4 is a reflection of continuous ligand-dependent stimulation.

Taken together, plant cell surface receptor kinases can enter the endocytic trafficking route constitutively and can accumulate in endosomes. The relative stability and BFA sensitivity of BRI1 suggests receptor recycling with only a fraction of endocytosed receptors being subject to degradation. Both BRI1 and ACR4 are developmental receptors, recognizing endogenous ligands. It is possible that a constant ratio of membrane resident/endosomal receptors in these cases is used as a durable pool of activated receptors for continuous, long-term signaling.

LIGAND-REGULATED RECEPTOR ENDOCYTOSIS

The first compelling case for ligand-induced endocytosis in plants did not involve a receptor for some endogenous growth regulator, but for an exogenous peptide, as part of the self/non-self-discrimination system, which, in many cases, requires acute receptor signaling. Ligand-induced RME could ensure the transient nature of such a signal and, in addition, could serve to clear plant tissues/cells of foreign molecules. Perception of so-called microbe-associated molecular patterns (MAMPs) plays a key role in plant immunity. The receptor recognizing bacterial flagellin (flg22) is encoded by the Arabidopsis LRR-RLK FLAGELLIN SENSING2 (FLS2). Transgenic lines that express a functional FLS2-GFP fusion driven by its native promoter revealed localization of the nonactivated receptor at the PM (Robatzek et al., 2006). Upon activation with flg22, FLS2-GFP was found to translocate to endocytic compartments. This induced uptake of FLS2 was specific to its ligand and required receptor activation. Continuous flg22 stimulation led to a loss of FLS2 fluorescence, which suggests that activated FLS2 is targeted for degradation. Wortmannin, an inhibitor of the formation of prevacuolar endocytic compartments, abrogated flg22-induced FLS2-GFP internalization. In contrast to BRI1, BFA did not have an effect on FLS2 localization. Thus, it appears that BRI1 and FLS2 subcellular trafficking indicates that the routes taken by these receptors could differ in their composition/destination and may require distinct regulatory components (Fig. 1).

According to the current model of receptor internalization, formation of oligomer complexes and lipid mircrodomains of increased density at the receptor site are thought to precede membrane invagination and vesicle budding. However, in protoplasts, fluorescently labeled FLS2 was not found to form homodimers, regardless of the presence or absence of flg22. None-

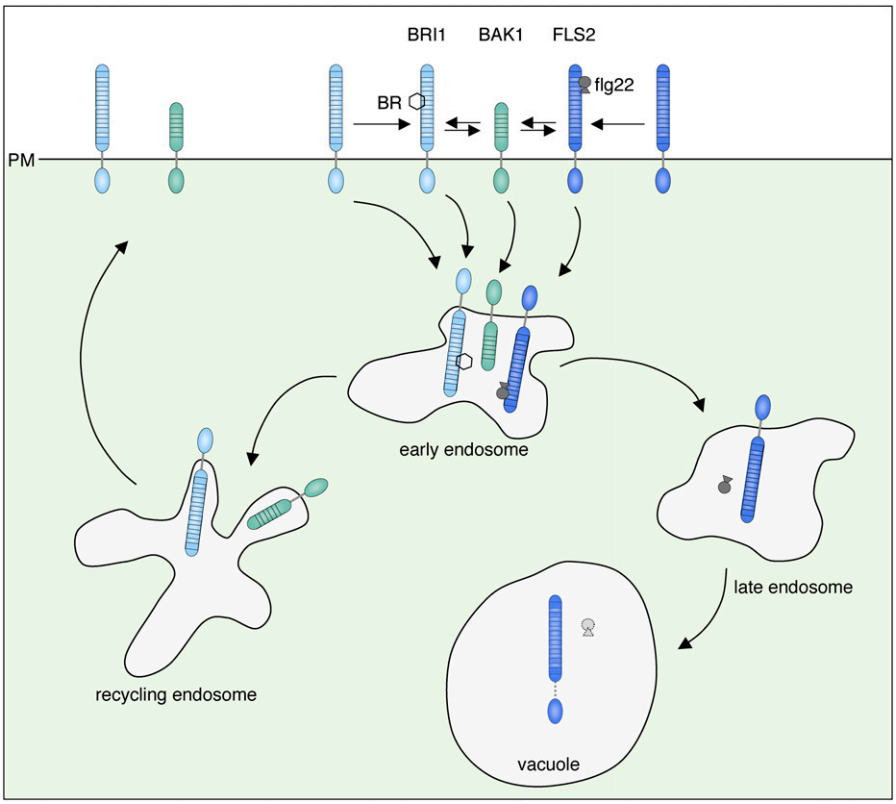

Figure 1. Model of RME subcellular trafficking in plants according to the prime examples of BRI1, BAK1, and FLS2. Both BRI1 and FLS2 are ligand-perceiving, cell surface-localized receptor kinases that recognize BR and bacterial flagellin (flg22), respectively, and form a ligand-dependent complex with the coreceptor BAK1 or likely other members of the SERK family. BRI and BAK1 translocate to endosomal compartments and are recycled back to the PM. BRI1 relocalization is independent of ligand binding, but seems to accumulate at endosomes at its active state. Upon ligand activation, FLS2 internalizes into endosomes and is further sorted for lytic degradation. There is evidence in both cases that BRI1 and FLS2 endocytosis contributes to BR and flg22 signaling pathways.

theless, a reduction in the fluidity of FLS2 upon stimulation was observed, indicative of the formation of lipid microdomains or larger complexes (Ali et al., 2007). Notably, no FLS2-YFP-labeled vesicles were reported using this protoplast system and it is unclear whether transient overexpression data in protoplasts can be compared with stably transformed plants.

LIGAND ENDOCYTOSIS

A hallmark of receptors is their high ligand affinity that often results in irreversible ligand binding (Bauer et al., 2001). Therefore, RME cannot only be observed at the receptor level, but also be visualized using soluble ligand-binding proteins or the ligands themselves. In soybean (*Glycine max*), a β-glucan binding protein (GBP) was identified (Fliegmann et al., 2004). Although devoid of any transmembrane domain, electron microscopy unraveled localization of GBP at the cytoplasmic face of the cell wall and to vesicles at the PM. It is possible that GBP interacts with a receptor-like protein (RLP) or RLK that is targeted for RME. Although there is no receptor or binding protein known for the bacterial MAMP lipopolysaccharide (LPS), there is evidence for endocytosis. Fluorescently labeled LPS was found to bind to the PM and to become internalized into vesicles in tobacco (*Nicotiana tabacum*) suspension cells (Gross et al., 2005). LPS uptake was abolished by amantadine, an inhibitor of RME, and LPS-labeled vesicles exhibited colocalization with the endosomal marker Ara6. Internalization was also observed for bacterial exopolysaccharides (Romanenko et al., 2002). In the case of oomycete-derived cryptogein, it could be demonstrated that the MAMP specifically enhanced uptake of FM4-64 in BY-2 cells (Leborgne-Castel et al., 2008). Also, the number of clathrin-coated pits was increased by cryptogein. This stimulatory endocytic effect of cryptogein was inhibited by tyrphostin A23, which targets RME.

Most exogenous ligands, such as microbial patterns, provoke plant responses that are rapid, but transient, to ensure proper defense while preventing harm for the host cell. Possibly, ligand-induced RME will turn out to be a widespread phenomenon for non-self-recognition receptors because it is a suitable mechanism to achieve high accuracy and short duration in receptor signaling, as well as clearing the host cell of exogenous ligand. However, the lack of knowledge of the cognate receptors in the above cases makes it difficult to determine whether their endocytosis is the result of a constitutive or ligand-induced trafficking.

COCEPTORS INVOLVED IN ENDOCYTOSIS

BRI1-ASSOCIATED KINASE1 (BAK1; also called SERK3 for SOMATIC EMBRYO RECEPTOR KINASE3) was originally characterized as a BRI1 coreceptor and shown to be a member of a small group of RLKs known as the SERK family (Vert et al., 2005). BRI1 was found to interact in a ligand-dependent fashion with BAK1, but

also with other SERK family members (Li et al., 2002; Wang et al., 2005; Karlova et al., 2006; He et al., 2007). Signaling of BRI1 does not depend solely on BAK1 and, in the last years, SERK family members were shown to act in various signal transduction pathways, often with partial redundancy (Albrecht et al., 2005; Colcombet et al., 2005; Chinchilla et al., 2007; He et al., 2007; Kemmerling et al., 2007). Accordingly, multiple SERK mutants display severe pleiotropic phenotypes and seedling lethality (He et al., 2007). Such a situation strongly suggests a more general role for this coreceptor family in RLK signaling and regulation of receptor endocytosis represents an attractive possibility. Coexpression of BRI1 and BAK1 CFP/YFP pairs to high levels in cowpea (*Vigna unguiculata*) protoplasts led to enhanced endosomal localization of BRI1, suggesting a regulatory role for BAK1 in BRI1 translocation (Russinova et al., 2004). In this system, BRI1/BAK1 complex formation could even be investigated at subcellular resolution. At the PM, BRI1, but not BAK1, associated in homodimers, whereas in endosomes both formed a heterodimer complex with each other. When both receptors were expressed, three distinct endosomal compartments, either carrying BRI1 or BAK1 alone or containing both together, could be distinguished, all of which showed labeling with FM4-64. This points to a complex pool of different endosome populations possibly delivering receptors to different trafficking routes.

In planta, however, BRI1-GFP localization was unaltered in a *bak1* mutant background (Russinova et al., 2004). This discrepancy could be due to the different systems used or a result of redundancy within the SERK family. For example, localization of SERK1 in transgenic Arabidopsis lines was detected at the PM and also in endosomes in some cells (Kwaaitaal et al., 2005). Internal accumulation of SERK1 was increased by BFA, suggesting that it follows a similar endocytic route than BRI1. Yet, it remains that BRI1 localization and turnover was reported to be completely ligand independent, whereas interaction with at least some SERKs required ligand binding. Therefore, interaction of SERKs should not influence BRI1 endocytosis. By contrast, FLS2 does require BAK1 for its endocytosis (Fig. 1). Recently, it was demonstrated that BAK1 forms a complex with FLS2 in a ligand-dependent fashion and is necessary for FLS2 signaling (Chinchilla et al., 2007; Heese et al., 2007). Importantly, it was shown that, in *bak1* mutants, flg22-induced internalization of FLS2 does not occur anymore. Considering that SERK family members probably interact with numerous ligand-binding receptors, functional redundancy, increased expression levels, and rapid recycling might be important not to become limiting factors in any of the signaling pathways.

REGULATION OF RECEPTOR ENDOCYTOSIS

Although a heterologous system, the human transferrin receptor (hTfR) and its ligand transferrin (Tfn) were shown to localize to the PM and endosomes in Arabidopsis protoplasts (Ortiz-Zapater et al., 2006). This pattern resembles that of hTfR subcellular localization in human cells and hTfR internal accumulation was increased by BFA, which suggests that endocytic trafficking routes and components required for endocytosis are at least partially conserved between plants and animals. Consistent with this, endocytosis of hTfR was blocked by the inhibitor tyrphostin A23, which could be due to interference with an adapter protein (AP) complex, a conserved class of endocytic regulators targeting the endocytic motif Yxxϕ present in hTfR. This tetrapeptide Yxxϕ (Y = Tyr, x = any amino acid, ϕ = hydrophobic residue) is known to play a role in clathrin-dependent endocytosis (Kurten, 2003) and is also conserved in the cytoplasmic parts of plant cell surface receptors (Table I). The tomato (*Lycopersicon esculentum*) disease resistance receptors Ve and LeEix carry this Yxxϕ motif in their cytoplasmic domains (Kawchuk et al., 2001; Ron and Avni, 2004). LeEix2, which mediates perception of the fungal ethylene-inducing xylanase (EIX), requires a functional Yxxϕ motif for triggering immunity (Ron and Avni, 2004). Sequence alignments of the cytoplasmic domains of selected RLKs (as in Table I) revealed, in addition to several randomly scattered Yxxϕ motifs, a clustering of this motif in the juxta membrane region, within the ATP binding site, and a remarkably conserved Yxxϕ cluster between the ATP-binding site and the kinase active site (Supplemental Fig. S1). None of them has been functionally studied, but the presence of the conserved Yxxϕ cluster in 14 of 17 randomly chosen RLKs, including BRI1 and BAK1, suggests that endocytosis of RLKs could be a more general phenomenon (Table I). Moreover, sequence alignments of the cytoplasmic domains of RLPs showed a conservation of the Yxxϕ motif between hTfR and tomato Ve2. However, inspection of subcellular dynamics of most RLPs and RLKs is forthcoming, and functional analysis of sorting/trafficking motifs within plant receptors remains to be addressed.

Additionally, the di-Leu (D,E)xxxL(I,L) endocytosis motif was identified in animal receptors. While it is also present in some RLPs and RLKs (Table I), there is currently no evidence for a function of this motif in plant endocytic processes. However, even receptors that lack any obvious endocytic signals, like FLS2, do nevertheless enter the endocytic pathway. In animals and yeast, ubiquitination has been identified as a targeting signal for receptor internalization and subsequent degradation (Holler and Dikic, 2004). Post-translational modifications, such as ubiquitination, could act as an additional label for sorting of ligand-activated receptors into the late endosomal pathway. A role for ubiquitination in plant receptor uptake and trafficking was observed with FLS2. Proteasome inhibitors, such as MG132, which could deplete the cell's pool of freely available ubiquitin moieties (Melikova et al., 2006), prevented flg22-induced internalization of FLS2 (Robatzek et al., 2006). FLS2 carries a PEST-like

Table I. *Overview of selected plant cell surface RLKs and RLPs with known ligands and/or subcellular localizations*

The extracellular domains of the receptors are indicated by CRINKLY4-like repeat domain (CR4L), LRR, Lys motif (LysM), or SRK (S) types. Receptor localization is shown as PM and endosomal according to biochemical and microscopic studies. The presence of a putative endocytic motif (the tetrapeptide Yxxϕ, the di-Leu [D,E]xxxL[L,I], or the PEST motif) within the cytoplasmic domains of receptors is provided. Ligands and subcellular localizations labeled with question marks await biochemical and cell biological confirmation, respectively.

Receptor	Origin	Type	Ligand	Function	Localization	Endocytic Motif	Ref.
Role in development							
BRI1	Arabidopsis	LRR-RLK	BR	Plant growth	PM + endosomes	Yxxϕ	Geldner et al. (2007)
CLV1	Arabidopsis	LRR-RLK	CLV3/CLE	Meristem proliferation	PM	Yxxϕ, di-Leu	Ogawa et al. (2008)
ACR4	Arabidopsis	CR4L-RLK	?	L1 cell layer organization	PM + endosomes	Yxxϕ, di-Leu	Gifford et al. (2005)
PEPR1	Arabidopsis	LRR-RLK	Pep1	Wound signaling	PM?	Yxxϕ, di-Leu	Yamaguchi et al. (2006)
PSKR1	Carrot	LRR-RLK	PSK	Cell differentiation/ proliferation	PM	Yxxϕ	Matsubayashi et al. (2002)
WAK1	Arabidopsis	LRR-RLK	GRP3	Cell wall	PM + endomembranes?	Yxxϕ, di-Leu	Kohorn et al. (2006)
Role in self/non-self-discrimination							
EFR	Arabidopsis	LRR-RLK	elf18	Defense to bacteria	PM?	Yxxϕ	Zipfel et al. (2006)
CEBiP	Soybean	LysM-RLP	Chitin	Defense to fungi/ oomycetes	PM?	–	Kaku et al. (2006)
CERK	Arabidopsis	LysM-RLK	Chitin?	Chitin signaling	PM?	Yxxϕ	Miya et al. (2007)
LeEix1/2	Tomato	LRR-RLP	EIX	Fungal xylanase signaling	PM + endosomes?	Yxxϕ	Ron and Avni (2004)
FER	Arabidopsis	RLK	?	Pollen compatibility	PM	Yxxϕ	Escobar-Restrepo et al. (2007)
FLS2	Arabidopsis	LRR-RLK	flg22	Defense to bacteria	PM + endosomes	PEST	Robatzek et al. (2006)
NFR1/5	Lotus	LysM-RLK	Nod factor	Symbiosis with bacteria	PM?	Yxxϕ, di-Leu	Radutoiu et al. (2007)
LePRK1/2	Tomato	LRR-RLK	LAT52?	Pollen compatibility	PM	Yxxϕ, di-Leu	Wengier et al. (2003)
SYMRK	Lotus	LRR-RLK	?	Symbiosis with bacteria/fungi	PM?	Yxxϕ	Stracke et al. (2002)
SRK	Brassica	S-type RLK	SCR	Pollen self-incompatibility	PM?	Yxxϕ	Naithani et al. (2007)
Ve1/2	Tomato	LRR-RLP	?	Resistance to *Verticillium*	?	Yxxϕ, di-Leu	Kawchuk et al. (2001)
Xa21	Rice	LRR-RLK	AvrXa21?	Resistance to *Xanthomonas oryzea*	?	Yxxϕ, di-Leu	Lee et al. (2006)
Role as coreceptors							
BAK1	Arabidopsis	LRR-RLK		BR signaling + immunity	PM + endosomes	Yxxϕ, di-Leu	Russinova et al. (2004)
SERK1	Arabidopsis	LRR-RLK		Somatic embryogenesis	PM + endosomes	Yxxϕ, di-Leu	Shah et al. (2002)

motif in the C-terminal part of its kinase domain. A mutation within this region abolished FLS2 endocytosis. The attachment of ubiquitins to a target protein involves the function of ubiquitin E3 ligases at the last step. Thus, E3 ligases could function as a switch in receptor subcellular trafficking, for example, sorting from early to late endosomes (Duan et al., 2003). In rice (*Oryza sativa*), the RING finger-type E3 ligase Xb3 was identified as interacting partner for the LRR-RLK Xa21 (Wang et al., 2006). Although data of subcellular localization are lacking, Xb3 was found to influence Xa21 protein levels and to be required for Xa21-mediated immunity. The Arabidopsis RING domain ligase 2 was found to associate with the PM, indicative of an involvement in membrane trafficking (Yin et al., 2007), but functions for most E3 ligases to specific plant signaling and trafficking processes remain to be assigned.

Most receptor kinases are capable of autophosphorylation and the phosphorylation status of specific residues is important for overall receptor function, which includes protein levels, oligomerization, receptor activation, and signal transduction. Furthermore, phosphorylation impacts the subcellular localization of RLKs. For ligand-induced RME, only those flg22 peptides that could activate the FLS2 receptor were able to target FLS2 for endocytosis (Robatzek et al., 2006). Addition of the kinase inhibitor K252a and mutation of a conserved potentially phosphorylated residue within the juxta membrane region abolished FLS2 internalization. Furthermore, the protein phosphatase 2A inhibitor cantharidin affected FLS2 subcellular trafficking (Serrano et al., 2007). FLS2 was shown to interact with KAPP, a kinase interacting domain containing protein phosphatase 2C, as was also found for BAK1, BRI1, CLV1, SERK1, and SRK (Trotochaud et al., 1999; Gomez-Gomez et al., 2001; Shah et al., 2002; Vanoosthuyse et al., 2003; Ding et al., 2007). This indicates that KAPP keeps multiple RLKs under control by dephosphorylation. Coexpression of KAPP with fluorescently tagged SERK1 revealed an accelerated rate of SERK1 endocytosis in protoplasts (Shah et al., 2002). SERK1 mutant variants, in which potentially phosphorylated residues important for interaction with KAPP were substituted, also displayed increased internalization. The overall phosphorylation status of cell surface receptors regulated by KAPP is a key component of RME. This is further supported by physical association of SERK1 and KAPP in vesicles, which was not detected at the PM. These results point

to strong mechanistic coupling between receptor signaling and trafficking.

A number of RLK interacting proteins have been identified, among which some are related to endocytic processes. SRK (S locus receptor kinase), for example, was found to interact with a sorting nexin (Vanoosthuyse et al., 2003). Sorting nexins are localized at prevacuolar compartments of the endocytic route in Arabidopsis (Jaillais et al., 2007). Three RLKs of unknown function were identified to interact with Rop GTPases, which could be detected at the PM and in endosomes (Molendijk et al., 2008). GTPases of the Rab type are well-known key players in endocytic processes and are commonly used as markers for early and late endosomes in plants (Ueda et al., 2004). Endocytic trafficking in animals involves phospholipase D, a protein implicated in vesicle formation through regulation of the GTPase cycle of dynamin (Lee et al., 2006). A tomato phospholipase D accumulated in intracellular vesicles when cells were stimulated with the EIX elicitor (Bargmann et al., 2006). On the other hand, EIX-induced responses appeared to be influenced by phospholipase D activity. A general difference between membranes of the PM and endosomes is their lipid composition (Gruenberg, 2001). Triclosan, an inhibitor of the fatty acid synthase type II complex, impaired induced endocytosis of FLS2 possibly by affecting signaling lipids (Serrano et al., 2007). Recently, the responsible gene mutated in Arabidopsis *gravitropism defective2* (*grv2*) was isolated (Silady et al., 2008). It encodes a homolog of *Caenorhabditis elegans* RME-8 with known functions in receptor endocytosis. Transgenic expression of a fluorescently labeled fusion protein exhibited endosomal localization of Arabidopsis GRV2, which appeared to be wortmannin sensitive and BFA resistant. GRV2 is a potential regulator of RME, but its receptor clients are unknown. Current data concerning downstream components of RME are limited and further studies, also by genetic means, should be employed to identify and functionally characterize key molecules of plant endocytosis.

ROLE OF RECEPTOR ENDOCYTOSIS

It is generally accepted that translocation of activated cell surface receptors is associated with an attenuation of ligand-stimulated responses and also contributes to activate downstream signaling cascades (von Zastrow and Sorkin, 2007). Most results have been obtained from animal systems, but what do we know about the significance of receptor endocytosis in plants? Ligand-dependent endocytosis and degradation of FLS2 will evidently contribute to the termination of the MAMP-induced defense signal. When FLS2 internalization was abrogated by cantharidin, flg22-induced accumulation of reactive oxygen species was enhanced and sustained (Serrano et al., 2007). Although only indirectly shown, this would support a role for FLS2 endocytosis in signal termination. Yet, it is also reasonable that FLS2 does not merely pass through endosomes en route to its degradation. Instead, the activated receptor at least transiently accumulates to considerable levels at endosomes before it enters lysosomes, analogous to the situation of EGFR in animals. In these compartments, receptors are perfectly able to signal and could encounter efficient access and higher local concentrations of interacting signaling partners. A variant of FLS2 mutated in a potentially phosphorylated residue within the juxta membrane region was found to be defective in endocytosis and in flg22 responses, such as the oxidative burst (Robatzek et al., 2006). Also, some chemical compounds were identified to impair FLS2 trafficking, together with the oxidative burst and flg22-induced gene expression (Serrano et al., 2007). In particular, wortmannin diminishes the activation of MAP kinases by flg22, while not affecting the oxidative burst (Chinchilla et al., 2007). Therefore, it is important to consider endosomes as possible sites of FLS2 signaling, in addition to the PM, and to keep in mind that the two signaling populations do not necessarily have to be equivalent in their outputs.

In contrast to FLS2, BRI1 always partitions into endosomes and PM, independent of the presence or absence of ligand (Fig. 1). This finding on its own already suggests an endosomal function of BRI1 because such constitutive partitioning is not normal behavior for PM-localized proteins, most of which accumulate exclusively at the PM even when passage through endosomes can be evidenced (Geldner et al., 2001; Takano et al., 2005; Abas et al., 2006). Interestingly, BFA treatment enhanced accumulation of BRI1 in structurally altered endosomal compartments and caused an increase in BRI1-dependent signaling (Geldner et al., 2007). This suggests that BRI1 is able to signal from these compartments, perhaps even in a preferential fashion. In support of this, it was found that the endosomal BRI1 population is devoid of BKI1, an interactor and negative regulator of the receptor, which localizes exclusively to the PM. In the future, it will be important to substantiate these findings by directly monitoring the activity status of BRI1 at subcellular resolution and by localizing, as yet unidentified, direct downstream targets of BRI1.

ENDOSOMAL SIGNALING—A NECESSARY ADAPTATION?

It appears that plants not only display intracellular trafficking machinery that matches or even surpasses that of animals in complexity (Jurgens, 2004)—they also appear to regulate endosomal trafficking of their receptors in similar ways. This was not to be expected if one considers that the hundreds of receptor kinases in plants share no common precursor RLK with animals, but independently evolved and expanded (Shiu and Bleecker, 2001). With BRI1 and FLS2, we now have plant receptor models with different trafficking behaviors in spite of them sharing similar structures and signaling partners (Fig. 1). It will be interesting to see

how this can be explained mechanistically and how it relates to their very different biological function. Both cases, however, suggest that endosomes can carry active receptor complexes and that they can be used as signaling platforms. In general, endocytosis is a fundamental process important for nutrient uptake in unicellular species, which might have been further developed for specific receptor-mediated internalization of cargo and then being used as robust mechanisms in complex receptor signaling pathways. Therefore, it is intriguing to ask whether endosomal signaling reflects an ancient occurrence in the common ancestor of higher organisms or whether it is the result of convergent evolution. Because of the ancient split of the animal and plant lineage and the sparse evidence for signaling endosomes in unicellular organisms like yeast, we currently favor the latter hypothesis.

In our view, some basic, but fundamental, constraints that are common to cells in a multicellular context could have independently driven the development of signaling endosomes. The first driving force could have been the large increase in the number of different receptors that have to be accommodated at the PM of a cell in a higher organism. Simply putting the number of RLKs in relation to the surface area of a representative meristematic cell impressively illustrates this point. Such a rough estimate shows more than a 100-fold increase in the number of RLKs that would have to be accommodated in the PM of an Arabidopsis cell as compared to *Chlamydomonas* (Fig. 2A). Therefore, we can consider the PM of higher organisms as a crowded place where available signaling surface has become limiting. If this is the case, signaling endosomes could have arisen as additional, less restricted, inner membrane surfaces allowing the accommodation of activated receptors in sufficient local concentrations and access to downstream signaling components over the necessary time spans. This additional level of membrane plasticity might have been necessary to sustain complex multicellular life.

Another constraint that could have driven the development of signaling endosomes might be associated with an increase in cell volume of differentiated cells, which could easily render diffusion-based mechanisms of signal transduction insufficient in range (Howe, 2005). This is especially obvious in neurons, where activated receptors at the axon terminal have to elicit responses in the nucleus based at the cell body. For example, directional, microtubule-based trafficking of the activated receptor TrkA in endosomes is necessary for signal transduction to the nucleus (Miaczynska et al., 2004). It was also proposed that, in cells with a less extreme morphology, the directional movement of endosomes from the periphery to the center could facilitate signal transduction by bringing activated receptors into closer proximity to the nucleus (Howe, 2005). However, both models are valid only in animal systems. In plants, endosome motility is actin-based and does not display any observable directionality toward the nucleus. Considering an elongated

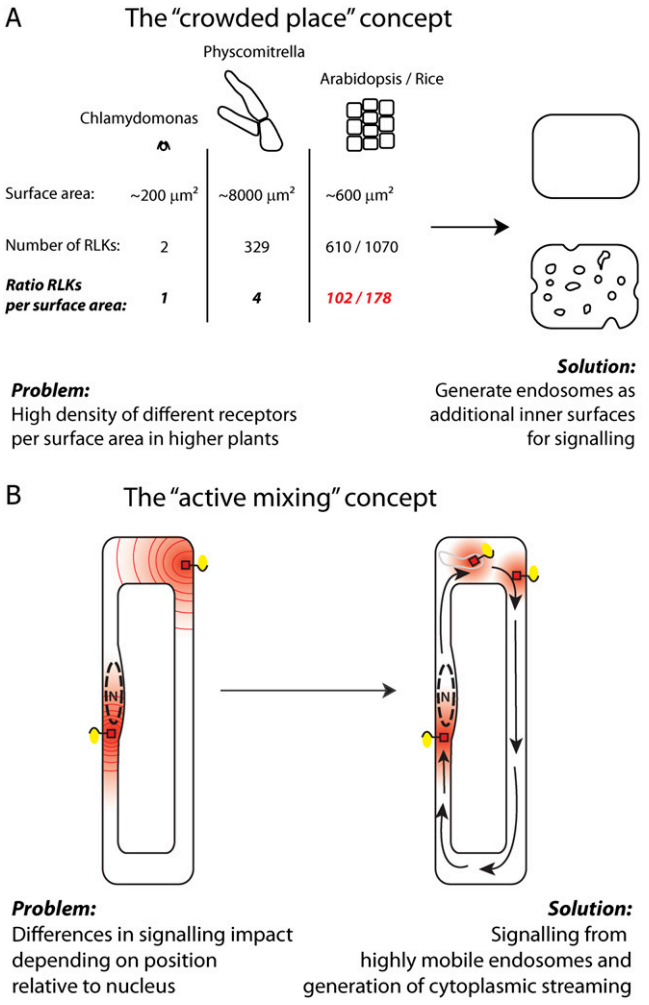

Figure 2. Explanations for the evolution of endosomal signaling in plants. A, Larger increases in receptor number than in cell surface area could have rendered cell surface area limiting for signal transduction. Endosomal signaling would be a solution to this because it increases the available surface area for signaling. We put into relation the number of RLKs encoded in genomes of the unicellular algae *Chlamydomonas*, the simple, multicellular moss *Physcomitrella*, and higher plants. Cell surface area was arbitrarily estimated for small, actively dividing cells (chloronema cell in *Physcomitrella*, root meristem epidermal cell in Arabidopsis/rice). RLK numbers are a personal communication from M. Lehti-Shiu. The actual increase of receptor numbers per surface area will be lower because not all RLKs will be expressed in a given cell type. B, In differentiated cells with a large central vacuole, the nucleus is normally apposed closely to some small patch of PM, whereas considerable distances exist in other parts of the PM. Considering this, it is evident that the same ligand-induced receptor complex should elicit responses of very different strength in the nucleus depending on where it is placed in the PM—if transduction relies solely on diffusion. Placing activated receptors in highly mobile endosomes would equilibrate such position-dependent bias by active mixing, especially in the case of cytoplasmic streaming that differentiated plant cells display.

plant cell nonetheless highlights the same problems in different ways. The large central vacuole leads to the close apposition of the nucleus to a small region of the PM, whereas other parts of the PM reside at a consid-

erable distance. Within a strictly diffusion-based model of signal transduction, this could cause a strong bias in signaling impact on the nucleus, depending on the position of the activated receptor. Translocating activated receptors into highly motile endosomes could largely alleviate this bias simply by active mixing of the receptor pool more than a strictly directional transport (Fig. 2B). This would even be reinforced by the phenomenon of actin-based cytoplasmic streaming that is occurring in differentiated plant cells.

PERSPECTIVES

Despite recent progress in plant receptor endocytosis, a lot remains to be done until this area of research can unfold its full explanatory potential. First of all, we need to identify and localize immediate downstream signaling components of activated receptors to assess the degree of compartmentalization of signaling complexes in plants. Moreover, we need to better understand the factors that regulate endocytosis and their interplay with the signal transduction cascades. In view of the conserved but multiplied, as well as novel molecules regulating RME in plants, both homology-based reverse genetic and unbiased forward genetic or proteomic approaches will be necessary for a mechanistic understanding of RME. The concept of receptors interacting with different, localized signaling scaffolds, depending on their position in the cell, is fascinating, but also very challenging to dissect experimentally. It emphasizes the need for more sophisticated tools to monitor signaling, ideally in single cells and possibly with subcellular resolution, and to follow dynamic changes at quantitative levels. The next few years will provide us with better insights of the plant's endosomal system and its use for robustness and specificity in signaling of the awe-inspiring array of cell surface receptors.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Conservation of endocytic sorting motifs in kinase domains of plant RLKs.

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