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Endosomal trafficking and signaling in plant defense responses

Maya Bar[1] and Adi Avni[2]

Plant defense responses are initiated by ligand–receptor recognition. The receptor may contain a motif for endocytosis and endocytosis is important for defense signaling in some cases.Recently, endosomal trafficking during defense has begun to be elucidated. In some cases, defense receptors are internalized into early endosomes, recycled back to the plasma membrane (PM) on recycling endosomes, and targeted for degradation via the late endosome pathway in an ESCRT dependent manner.Endosomal signaling has been proposed for several receptors. Defense receptors have been shown to reside on endosomes during the signaling time window. Increasing the endosomal presence of a receptor can cause a concomitant increase in signaling, while abolishing the formation of endosomes after the receptor has already been internalized can cause signaling attenuation.

Addresses
[1] The Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture and The Otto Warburg Minerva Center for Agricultural Biotechnology, Hebrew University, Rehovot 76100, Israel
[2] Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978, Israel

Corresponding author: Avni, Adi (Lpavni@post.tau.ac.il)

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Introduction

Receptor-mediated plant immunity is activated upon the recognition of a microbial associated molecular patterns (MAMPs) by surface-localized immune receptors or the stimulation of cytoplasmic immune receptor by a pathogen effector protein [1]. Leucine-rich-repeat receptor kinases (LRR-RLKs) and Leucine-rich-repeat receptor like proteins (LRR-RLPs) respond to conserved MAMPs by producing a defense response upon detection [2,3] Recognition between the immune receptor and its corresponding MAMP/elicitor activates a signal transduction cascade which can include various defense responses [4].

Many molecules which activate plant defense have been documented, of both pathogen and non-pathogen origin [5]. In several cases, the molecule which activates plant defense is the ligand of a known receptor [6,7].

In several cases where the defense responses are initiated by a ligand–receptor association, the defense receptor contains an endocytic motif, and endocytosis has been shown to be a crucial step in the recognition between the receptor and the ligand [8–10,11••]. This review will focus on recent advances in endocytosis, endosomal trafficking and endosomal signaling during plant defense mediated by LRR receptors.

Requirements for ligand-induced defense

Defense receptors which possess an LRR motif are numerous and have been identified in many plant (as well as mammalian) species [12,13]. The LRR domain is thought to confer specificity to the ligand [14–16], and has been shown to be crucial for effector recognition and signal transduction in the case of *Cf4* and *Cf9* which mediate defense responses elicited by Avr4 or Avr9 from *Cladosporium fulvum* [17], *Ve1* which mediates defense in response to the fungal wilt pathogen *Verticillium* [18,19••], and LeEix2 which mediates defense in response to ethylene inducing xylanase (EIX; Bar and Avni, personal communication). Several defense receptors have also been shown to contain an endocytosis motif. In the case of LeEix2, mutating the clathrin- type endocytic YXXΦ motif abolishes the ability of the receptor to respond to the ligand and mediate defense responses [20]. The tomato Cf LRR-RLP receptors which mediate signaling in response to MAMPs derived from *C. fulvum* also contain a YXXΦ endocytosis motif. The Ve1 receptor also contains two types of endocytic motifs, a C-terminal E/DXXXLΦ motif and a YXXΦ motif [22], though both were recently reported not to be required for Ve1 functionality [19••], although they may still mediate Ve1 endocytosis. FLS2, the LRR-RLK which mediates the response to flagellin, was reported to contain an atypical YXXXΦ motif [23], as well as a PEST-like endocytosis motif which was also reported to be required for FLS2 internalization and possibly signaling [10,24••].

LRR-RLPs in particular have been previously described as 'lacking any particular domain in the short cytoplasmic c-terminal tail', perhaps underscoring bafflement at the mechanism by which a defense signal is transduced from receptors lacking kinase activity. It is

therefore not surprising that co-receptors have recently emerged as important for defense signaling in several of these systems. The suppressor of BAK1-interacting RLK-1 (BIR1), termed SOBIR1, was found to interact *in planta* with Cf4 and Ve1, and to be required for signaling mediated by these receptors. Knock-down of SOBIR1 attenuated Cf4 and Ve1 signaling. SOBIR1 also interacts with LeEix2 and additional RLPs, but did not interact with RLKs such as FLS2, CLV1 or BAK1 [25,26••].

The co-receptor BAK1 was shown to dimerize with FLS2 and EFR, affecting their signaling. The signal transmitted by these receptors is reduced in the absence of BAK1 [27], and cannot be rescued by a BAK1 lacking proper kinase activity [27–29]. BAK1 also binds LeEix1, and was shown to be required for the ability of LeEix1 to attenuate LeEix2 signaling [30]. The kinase activity of BAK1 was also required in this case. Ve1 also requires BAK1 for proper signaling in tomato [31], while Cf4 mediated responses are compromised upon the silencing of tomato SERK1 [32•].

Concomitantly with the documented endocytosis motif of several known defense receptors, internalization itself was also shown to be required for proper defense signaling in some systems [3,20,33], indicating that the endocytic motif present in these defense receptors can mark them for internalization as part of the defense pathway, that is, the endocytosis motif serves to indicate that the internalization of the receptors is related to the defense process itself and not only to a recycling or degradation requirement the receptor may have. Blocking internalization of LeEix2 pharmacologically lead to disruption of the defense response. Blocking internalization of LeEix2, Cf4 and Cf9 by overexpression of the EH-domain protein EHD2 also interfered with signaling of these receptors [33].

Membranal components have also been shown to be required for endocytosis that occurs during plant defense responses. Endocytic processes and vesicular transport in general require participation of membrane components that form transport vesicles with a capability to store and process a number of molecules known to participate in cell signaling [34]. Pharmacological inhibition of phospholipid synthesis has been documented to interfere with plant defense responses [8,35,36]. Inhibition of PI3-kinase using Wortmannin or LY294002 prevents internalization of the LeEix2 receptor [8], and proper EIX induced signaling [11••]. Phospholipase Dβ (PLDβ) mRNA was found to accumulate specifically in response to EIX [36]. In untreated cells, PLDβ localized to the cytosol, while in EIX treated cells, PLDβ localized to vesicles in the cytosol. Further, PLDβ silenced cells exhibited a strong decrease in EIX-induced PLD activity [35]. Tomato cells treated with EIX showed an increase in phosphatidic acid (PA) and a decrease in intracellular PIP, as well as an increase in extracellular phosphatidylinositol 4-phosphate (PI4P). Interestingly, addition of PI4P to tomato cell suspensions triggered the same defense responses as those induced by EIX [37]. Alteration of the phosphatidyl inositol (PI) pathway in plant cells has also been reported to affect plant responses to abiotic stress [38]. We recently demonstrated that tomato cyclopropyl isomerase (SlCPI), a membrane protein involved in sterol biosynthesis, binds directly to LeEix2 and enhances signaling upon overexpression, while knocking down *SlCPI* attenuates defense responses elicited by EIX. Overexpression of *SlCPI* also stimulates the signaling of Cf9, but does not affect the signaling of the cytoplasmic receptor Pto [39•].

In several cases where endocytic internalization is critical for defense response transmission, components of the clathrin pathway have been shown to be required for the endocytic process. The LeEix2 receptor was suggested to interact with the clathrin adaptor complex through Eps15-homology Domain 2 (EHD2) [40], and overexpression of the clathrin HUB domain inhibited LeEix2 mediated signaling [11••]. Overexpression of clathrin HUB was also reported to abolish cryptogein induced endocytosis and expression of defense genes [9,41].

Endosomal trafficking during plant defense

The best characterized plant defense receptors in the context of endosomal trafficking are LeEix2 and FLS2. Using spinning disc confocal microscopy, we previously characterized endosomal movement in the LeEix2 mediated system [11••]. The LeEix2 receptor can be internalized independent of ligand binding, though the percentage of LeEix2 endosomes greatly increases following exposure to EIX [11••]. Following EIX treatment, a subpopulation of endosomes exhibits directional movement. EIX also causes endosomes to move faster and to greater distances. EIX treatment leads to enrichment in endosomes which are directional as well as in tubular endosomes, which may be related to the TGN, and in which sorting functions can possibly occur. The FYVE domain is a conserved protein motif characterized by its ability to bind with high affinity and specificity to phosphatidylinositol 3-phosphate (PI(3)P), a phosphoinositide highly enriched in early endosomes [42]. Interestingly, endosomes which contain a smaller amount of FYVE, exhibit greater displacement in response to EIX than endosomes which contain higher amounts of FYVE, seeming to indicate that there are different endosomal classes (which contain LeEix2 in response to EIX), and not all endosomal classes exhibit similar movement. Directional movement in response to a MAMP/elicitor could stem from targeting to particular cellular organelles, said targeting being a component of the plant defense response or a mechanism originating from the pathogen or

organism from which the elicitor is derived. An intact cytoskeleton is required for EIX induced signaling, possibly indicating that directional movement following EIX treatment occurs on actin filaments. Actin also has documented roles in endocytosis in general [43]. The changes in endosomal content following EIX treatment may also indicate that a particular subpopulation of cellular endosomes is involved in EIX/LeEix2 transport and signaling (Box 1).

Interestingly, reorganization of cytoskeleton and vesicle trafficking was demonstrated in the interaction between plant cells and microorganisms [44]. The cytoskeleton has been known to be linked to fungal pathogenesis for many years [45]. More recently, effects on the actin cytoskeleton during defense responses have also been documented, whereby a rapid increase in actin filament abundance was observed during the immune response to bacteria in tomato [46••], possibly reflecting an increased need for trafficking. Interestingly, the EH domain protein EHD2, which inhibits endocytosis, also induces changes in the actin cytoskeleton upon overexpression [40], and was shown to be transiently overexpressed following EIX treatment [8].

We have also observed the LeEix2 receptor in MVBs (Bar and Avni, personal communication) and believe that part of the activated receptor may be targeted for degradation. It seems probable that LeEix2 trafficking would therefore also depend on ESCRT machinery. Pharmacological studies have also led to the insight that LeEix2 signaling — and therefore possibly also trafficking (or trafficking of downstream components) — is sensitive to Wortmannin, 1-butanol, Dag-Kinase inhibitor, Endosidin 1, and, to a lesser extent, BFA [11••]. These analyses have shed light on the trafficking and endosomal compartments which may be involved in the signal mediated by LeEix2.

In the FLS2 receptor system, Robatzek and colleagues previously characterized the internalization of FLS2 following flg22 treatment [10]. They found that ligand activation of FLS2 is required for its internalization, and suggested that internalization may be required for certain aspects of the defense response against flagellin. However, work published recently by Smith and colleagues [47••] indicates that at least certain aspects of the defense response against flagellin such as MAP kinase activation and ROS production (as also suggested in [10]) are not dependent on receptor internalization. With respect to FLS2 trafficking, following ligand elicitation, FLS2 was found to traffic through EE/TGN vesicles and to subsequently arrive at the LE/MVB, possibly to be targeted for degradation [24••,48••]. Impairing the ESCRT complex by decreasing ESCRT-I causes attenuation of both FLS2 internalization and immunity of Arabidopsis to *pseudomonas* [48••]. Further, FLS2 was found to constitutively re-cycle via BFA-sensitive compartments, independently of ligand activation or protein synthesis. However, upon ligand (flg22) activation, the FLS2 receptor was found to internalize and traffic via ARA7 and ARA6 endosomes which are insensitive to BFA treatment [24••]. The same work analyzed time-frames of FLS2 receptor endocytosis and found a peak of FLS2 residence on endosomes 60–75 min after elicitation.

A model for receptor trafficking during plant defense responses is proposed in Figure 1.

Endosomal signaling during plant defense

In mammalian systems is has been documented that while cell surface signaling is transient in nature (second to minutes), endosomal signaling can sustain for longer

Box 1 Vesicular trafficking in plant cells

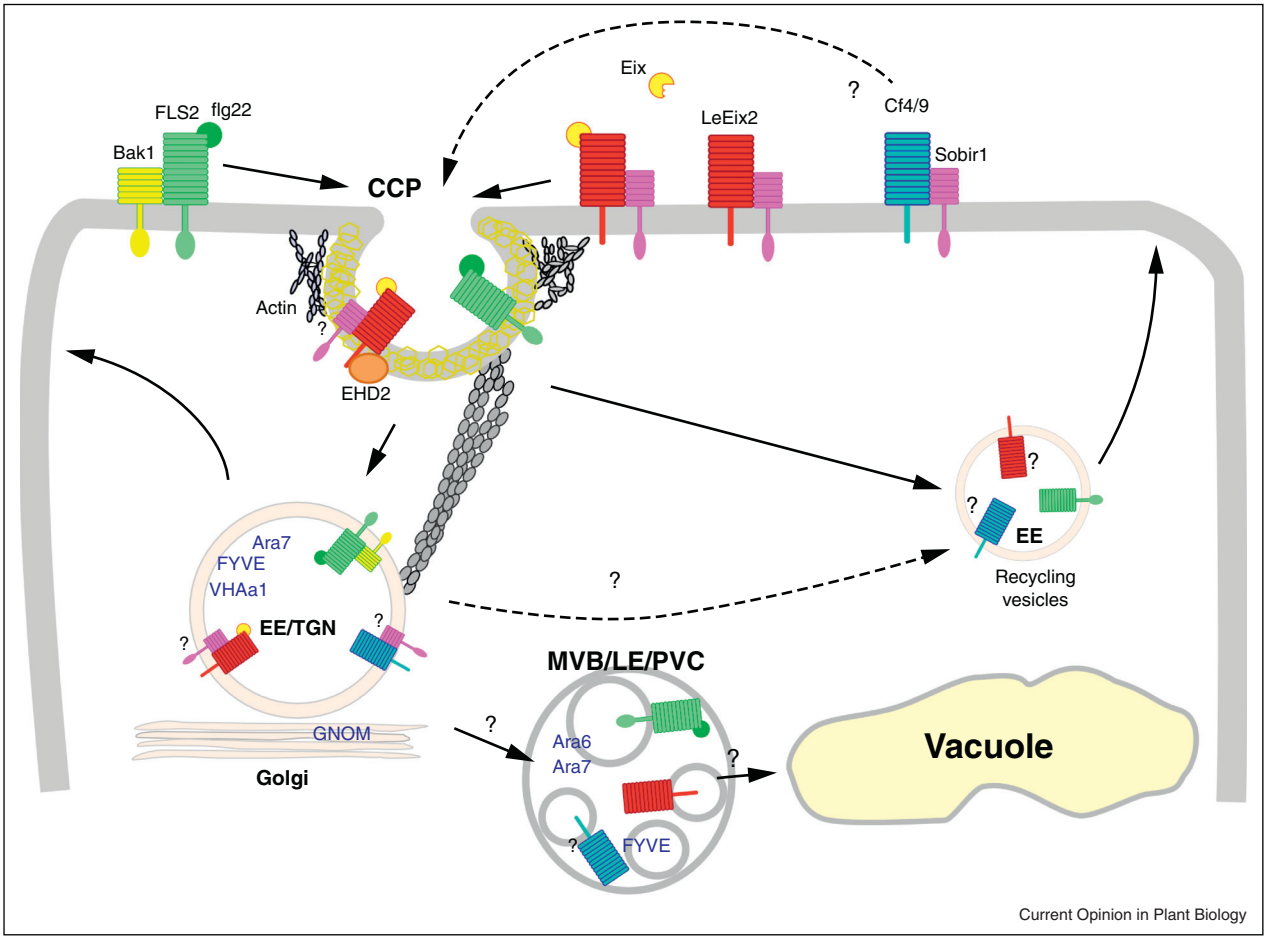
Endosomes in plant cells share functionality with those in animal cells, though the organization of endosomal compartments and trafficking pathways in plants can be different and has some distinctive features [57]. Plant endocytosis has commonly been traced using the fluorescent styryl dye FM4-64 which labels the plasma membrane, is up taken by endocytosis and transported through endosomal compartments to the vacuole [58]. Inhibitors that block distinct steps in endosomal trafficking pathways have been employed to better characterize plant trafficking and attempt to pinpoint the role of various proteins within this system. From these analyses two distinct plant endosomal compartments have emerged, the trans Golgi network (TGN)/early endosome (EE) and the late endosome (LE)/Multi-Vesicular-Body (MBV)/Pre-Vacuolar Compartment (PVC) (see Figure 1). Interestingly, the previously postulated GNOM-positive recycling endosome was in fact found to most probably have been the Golgi apparatus (GA) [59], indicating that the GA may be involved in recycling.

EEs are the first endomembrane compartments that receive endocytic cargo after internalization. It has been suggested that in plants, rather than a separate early endosome, the TGN possesses the early endosome function and is the first site for delivery of endocytosed material [60]. The contents of the early endosome may be recycled back to the plasma membrane, may continue along the endocytic pathway, possibly culminating in degradation, or may also be transported to earlier compartments of the secretory pathway.

MVBs/LEs are also on the endocytic pathway in plants [61], and contain vacuolar sorting receptors and vacuolar proteins that are en route to the vacuole [62–65]. Several Rab GTPases were shown to localize to MVBs and to also co-localize with a PVC marker, but not an EE or TGN marker, confirming that MVBs correspond functionally to both a LE and a PVC [64,66,67]. Plasma membrane proteins are found in internal vesicles of MVBs, indicating that they are en route to the vacuole for degradation and that recycling to the plasma membrane probably occurs from an earlier compartment, either the TGN or a separate RE [60].

The internal vesicles of MVBs are generated through the endosomal sorting complex required for transport (ESCRT) machinery. This machinery consists of several protein complexes 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 [68]. It has been suggested that the ESCRT proteins probably function in LE maturation and sorting of cargo proteins for degradation [69,70].

Figure 1



A model for receptor trafficking during plant defense responses. LeEix2, FLS2 and possibly also the tomato Cf family receptors are internalized in a ligand dependent manner into early endosomes (which are also trans-Golgi-network (TGN) related vesicles). EHD2 can inhibit receptor internalization and attenuate the signaling of LeEix2 and Cf4/9. LeEix and FLS2 can also be internalized ligand-independently, presumably for degradation. Cf4 and Ve1 require SOBIR1 for signaling, while FLS2 requires BAK1 for signaling. LeEix2 was also shown to bind SOBIR1. In the TGN receptors undergo sorting, and can be recycled back to the plasma membrane on early endosomes or specialized recycling vesicles derived from the early endosome. Receptors are also sorted for degradation via the late endosomes/Multi-Vesicular-Body (MBV)/Pre-Vacuolar Compartment (PVC).

times (minutes to hours) by the increased residence of the activated receptor in endosomes [49]. Although signaling was classically considered to occur on the plasma membrane (PM) only, Bergeron, Posner and colleagues were the first to observe that shortly after ligand addition the majority of activated epidermal growth factor receptors (EGFRs) and their downstream signaling factors such as Shc, Grb2 and mSOS were found not on the PM but on early endosomes [50], suggesting that EGFR signaling continues from this compartment [51]. Mammalian endosomes are now known to participate in a variety of different signaling pathways [52]. There seems to be no reason why plant endosomes should not prove to be diverse signaling platforms. In fact, endosomes are an ideal locale for signaling origination, given the accumulation of activated receptors and certain signaling components in a 'simplified' and enriched microenvironment which may in fact prove to be an ad hoc signaling compartment. Evidence to this effect has begun to accumulate.

BRI1 (for Brassinosteroid Insensitive 1, is an LRR-RLK which functions in plant development). In one case, [53] it was reported that treatment with BFA enhanced BRI-dependent signaling by causing accumulation of BRI in endosomal compartments, and the authors suggested that the use of endosomes as signaling compartments may be conserved among eukaryotes. However, a more recent report found BRI1 to transmit its signal primarily from the PM [54] (see below).

Similar to mammalian systems, signaling can occur directly from the PM in plant cells. A recent work demonstrates that BRI1 transmits its signal from the membrane [54]. Interestingly, inhibiting endocytosis caused the signal to intensify as activated receptors were retained at the cell membrane, while increasing endocytosis attenuated the signal. Increasing the pool of endosomal BRI1 did not increase the signal [54]. These results lead to the conclusion that the BRI1 signal is transmitted exclusively from the PM, and that signaling endosomes are not involved in this system. It will be interesting to conduct similar experiments with a fluorescent version of an FLS2 ligand.

In addition to the requirement for internalization in the signaling process of several defense receptors as detailed above, which in itself can allude to the probable existence of signaling originating from plant endosomes, more specific signaling pathways involving endosomes have been documented in plant systems and in plant defense in particular.

In the LeEix/EIX system, preventing the formation of endosomes after internalization has already occurred caused a decrease in EIX-induced signaling, while interfering with vesicle trafficking by ES1 enhanced EIX induced signaling, further supporting the notion of endosomal signaling [11••]. In fact, the temporal windows in which EIX induced signaling occurs (as early as 5–10 min after the addition of EIX) and in which the LeEix2 receptor resides on endosomes (at least 30–45 min after internalization), provide circumstantial evidence indicating a possibility that at least some of the signal originates from endosomes. This will be interesting to investigate further.

Concluding remarks

Recent years have seen substantial advances in the research of plant trafficking and signaling. Trafficking and signaling during plant defense responses are a good model for the study of plant trafficking and signaling in general, due to the swift and transient nature of signals transmitted during plant defense.

Recently accumulated data show that plant defense receptors can be trafficked in several pathways, depending on the desired outcome (e.g., signal propagation via recycling or endosomal signaling, signal attenuation via degradation). These pathways are similar to those reported in trafficking which occurs in mammalian cells, though some of the specialized compartments can have different features.

Perhaps not surprisingly, one of the common features is the signaling from endosomes. Evidence shows that plant endosomes likely serve as a signaling platform. Further experimentation is needed in order to unequivocally confirm the existence of signaling endosomes in plants. LeEIx2, which appears to signal to some extent from endosomes, and FLS2, in which endosomal signaling may have a minimal contribution, can serve as 'compare and contrast' systems. New and exciting techniques which may serve to elucidate this matter include endosomal purification and analysis [55] and endosome visualization techniques [24••,56]. A genetic approach to abolishing particular types of endosomes and examining the signaling of specific plant receptors may also prove to be effective. It will be exciting to observe the upcoming experimental advances in the field, which will no doubt characterize the common and distinct features of trafficking and signaling during plant defense mediated by different receptors.

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References and recommended reading

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

- • of special interest
- •• of outstanding interest

1. Thomma BPHJ, Nurnberger T, Joosten MHAJ: **Of PAMPs and effectors: the blurred PTI-ETI dichotomy**. *Plant Cell* 2011, **23**:4-15.

2. Geldner N, Robatzek S: **Plant receptors go endosomal: a moving view on signal transduction**. *Plant Physiol* 2008, **147**:1565-1574.

3. Robatzek S: **Vesicle trafficking in plant immune responses**. *Cell Microbiol* 2007, **9**:1-8.

4. Elbaz M, Avni A, Weil M: **Constitutive caspase-like machinery executes programmed cell death in plant cells**. *Cell Death Differ* 2002, **9**:726-733.

5. Henry G, Thonart P, Ongena M: **PAMPs, MAMPs, DAMPs and others: an update on the diversity of plant immunity elicitors**. *Biotechnol Agron Soc Environ* 2012, **16**:257-268.

6. Chinchilla D, Boller T: **Innate immunity pattern recognition in plants**. In *Effectors in plant–microbe interactions*. Edited by Martin F, Kamoun S. Wiley-Blackwell; 2011:3-34.

7. Patel HH, Murray F, Insel PA: **Caveolae as organizers of pharmacologically relevant signal transduction molecules**. *Annu Rev Pharmacol Toxicol* 2008, **48**:359-391.

8. Bar M, Avni A: **EHD2 inhibits ligand-induced endocytosis and signaling of the leucine-rich repeat receptor-like protein LeEix2**. *Plant J* 2009, **59**:600-611.

9. Leborgne-Castel N, Lherminier J, Der C, Fromentin J, Houot V, Simon-Plas F: **The plant defense elicitor cryptogein stimulates clathrin-mediated endocytosis correlated with reactive oxygen species production in Bright Yellow-2 tobacco cells**. *Plant Physiol* 2008, **146**:1255-1266.

10. Robatzek S, Chinchilla D, Boller T: **Ligand-induced endocytosis of the pattern recognition receptor FLS2 in Arabidopsis**. *Genes Dev* 2006, **20**:537-542.

11. •• Sharfman M, Bar M, Ehrlich M, Schuster S, Melech-Bonfil S, Ezer R, Sessa G, Avni A: **Endosomal signaling of the tomato leucine-rich repeat receptor-like protein LeEix2**. *Plant J* 2011, **68**:413-423.

This paper documents endosomal dynamics during LeEix2 mediated signaling and demonstrated the importance of signaling derived from endosomes in this system.

12. Bohm H, Albert I, Fan L, Reinhard A, Nurnberger T: **Immune receptor complexes at the plant cell surface**. *Curr Opin Plant Biol* 2014, **20C**:47-54.

13. Lee MS, Min YJ: **Signaling pathways downstream of pattern-recognition receptors and their cross talk**. *Annu Rev Biochem* 2007, **76**:447-480.

14. Kobe B, Kajava AV: **The leucine-rich repeat as a protein recognition motif**. *Curr Opin Struct Biol* 2001, **11**:725-732.

15. Sun Y, Li L, Macho AP, Han Z, Hu Z, Zipfel C, Zhou JM, Chai J: **Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex**. *Science* 2013, **342**:624-628.

16. Wulff BB, Heese A, Tomlinson-Buhot L, Jones DA, de la Pena M, Jones JD: **The major specificity-determining amino acids of the tomato Cf-9 disease resistance protein are at hypervariable solvent-exposed positions in the central leucine-rich repeats**. *Mol Plant Microbe Interact* 2009, **22**:1203-1213.

17. Parniske M, Hammond-Kosack KE, Golstein C, Thomas CM, Jones DA, Harrison K, Wulff BB, Jones JD: **Novel disease resistance specificities result from sequence exchange between tandemly repeated genes at the Cf-4/9 locus of tomato**. *Cell* 1997, **91**:821-832.

18. Fradin EF, Zhang Z, Rovenich H, Song Y, Liebrand TWH, Masini L, van den Berg GCM, Joosten MHAJ, Thomma BPHJ: **Functional analysis of the tomato immune receptor Ve1 through domain swaps with its non-functional homolog Ve2**. *PLOS ONE* 2014:9.

19. •• Zhang Z, Song Y, Liu CM, Thomma BP: **Mutational analysis of the ve1 immune receptor that mediates verticillium resistance in tomato**. *PLOS ONE* 2014, **9**:e99511.
This work employed alanine scanning to functionally characterize motifs that putatively mediate protein–protein interactions and endocytosis in the transmembrane domain and the cytoplasmic tail of the Ve1 receptor. The endocytosis motifs were found not to be crucial for receptor functionality.

20. Ron M, Avni A: **The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato**. *Plant Cell* 2004, **16**:1604-1615.

22. Kozik P, Francis RW, Seaman MN, Robinson MS: **A screen for endocytic motifs**. *Traffic* 2010, **11**:843-855.

23. Beck M, Heard W, Mbengue M, Robatzek S: **The INs and OUTs of pattern recognition receptors at the cell surface**. *Curr Opin Plant Biol* 2012, **15**:367-374.

24. •• Beck M, Zhou J, Faulkner C, MacLean D, Robatzek S: **Spatio-temporal cellular dynamics of the Arabidopsis flagellin receptor reveal activation status-dependent endosomal sorting**. *Plant Cell* 2012, **24**:4205-4219.
The authors examined FLS2 trafficking and found that FLS2 receptors constitutively recycle in a Brefeldin A (BFA)-sensitive manner, while flg22-activated receptors traffic via ARA7/Rab F2b- and ARA6/Rab F1-positive endosomes insensitive to BFA. The authors also employed pharmacological agents to determine that activated FLS2 receptors are targeted to late endosomes and to examine the importance of various components in FLS2 internalization and trafficking.

25. Liebrand TWH, van den Burg HA, Joosten MHAJ: **Two for all: receptor-associated kinases SOBIR1 and BAK1**. *Trends Plant Sci* 2014, **19**:123-132.

26. •• Liebrand TWH, van den Berg GCM, Zhang Z, Smit P, Cordewener JHG, America AHP, Sklenar J, Jones AME, Tameling WIL, Robatzek S *et al.*: **Receptor-like kinase SOBIR1/EVR interacts with receptor-like proteins in plant immunity against fungal infection**. *Proc Natl Acad Sci U S A* 2013, **110**:10010-10015.
The authors show that the tomato ortholog of the *Arabidopsis thaliana* RLK suppressor of BIR1-1 (SOBIR1/EVR) and its close homolog *S. lycopersicum* (Sl) SOBIR1-like interact in planta with both Cf-4 and Ve1 and are required for the Cf-4- and Ve1-mediated signaling and stability. They also show that Tomato SOBIR1/EVR interacts with most of the tested RLPs, including LeEix2, but not with the RLKs FLS2, SERK1, SERK3a, BAK1, and CLV1.

27. Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nurnberger T, Jones JD, Felix G, Boller T: **A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence**. *Nature* 2007, **448**:497-500.

28. Heese A, Hann DR, Gimenez-Ibanez S, Jones AM, He K, Li J, Schroeder JI, Peck SC, Rathjen JP: **The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants**. *Proc Natl Acad Sci U S A* 2007, **104**:12217-12222.

29. Schulze B, Mentzel T, Jehle AK, Mueller K, Beeler S, Boller T, Felix G, Chinchilla D: **Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1**. *J Biol Chem* 2010, **285**:9444-9451.

30. Bar M, Sharfman M, Ron M, Avni A: **BAK1 is required for the attenuation of Eix-induced defense responses by the decoy receptor LeEix1**. *Plant J* 2010, **63**:791-800.

31. Fradin EF, Zhang Z, Juarez Ayala JC, Castroverde CD, Nazar RN, Robb J, Liu CM, Thomma BP: **Genetic dissection of Verticillium wilt resistance mediated by tomato Ve1**. *Plant Physiol* 2009, **150**:320-332.

32. • Fradin EF, Abd-El-Haliem A, Masini L, van den Berg GC, Joosten MH, Thomma BP: **Interfamily transfer of tomato Ve1 mediates Verticillium resistance in Arabidopsis**. *Plant Physiol* 2011, **156**:2255-2265.
The authors show that SERK1 is required in addition to SERK3/BAK1 for Ve1 resistance in Arabidopsis and tomato, and that SERK1 is required for resistance against the fungal pathogen *Cladosporium fulvum* mediated by the RLP Cf-4.

33. Bar M, Avni A: **EHD2 inhibits signaling of leucine rich repeat receptor-like proteins**. *Plant Signal Behav* 2009, **4**:682-684.

34. Hansen CG, Nichols BJ: **Exploring the caves: cavins, caveolins and caveolae**. *Trends Cell Biol* 2010, **20**:177-186.

35. Bargmann BO, Laxalt AM, Riet BT, Schouten E, van Leeuwen W, Dekker HL, de Koster CG, Haring MA, Munnik T: **LePLDbeta1 activation and relocalization in suspension-cultured tomato cells treated with xylanase**. *Plant J* 2006, **45**:358-368.

36. Laxalt AM, ter Riet B, Verdonk JC, Parigi L, Tameling WI, Vossen J, Haring M, Musgrave A, Munnik T: **Characterization of five tomato phospholipase D cDNAs: rapid and specific expression of LePLDbeta1 on elicitation with xylanase**. *Plant J* 2001, **26**:237-247.

37. Gonorazky G, Laxalt AM, Testerink C, Munnik T, de la Canal L: **Phosphatidylinositol 4-phosphate accumulates extracellularly upon xylanase treatment in tomato cell suspensions**. *Plant Cell Environ* 2008, **31**:1051-1062.

38. Golani Y, Kaye Y, Gilhar O, Ercetin M, Gillaspy G, Levine A: **Inositol polyphosphate phosphatidylinositol 5-phosphatase9 (At5ptase9) controls plant salt tolerance by regulating endocytosis**. *Mol Plant* 2013, **6**:1781-1794.

39. • Sharfman M, Bar M, Schuster S, Leibman M, Avni A: **Sterol-dependent induction of plant defense responses by a microbe-associated molecular pattern from *Trichoderma viride***. *Plant Physiol* 2014, **164**:819-827.
This work demonstrates that tomato cyclopropyl isomerase (SlCPI), an enzyme involved in sterol biosynthesis, interacts with the LeEix2 receptor, and regulates the induction of defense responses mediated by LeEix2.

40. Bar M, Sharfman M, Schuster S, Avni A: **The coiled-coil domain of EHD2 mediates inhibition of LeEix2 endocytosis and signaling**. *PLoS ONE* 2009, **4**:e7973.

41. Adam T, Bouhidel K, Der C, Robert F, Najid A, Simon-Plas F, Leborgne-Castel N: **Constitutive expression of clathrin hub hinders elicitor-induced clathrin-mediated endocytosis and defense gene expression in plant cells**. *FEBS Lett* 2012, **586**:3293-3298.

42. Hayakawa A, Hayes SJ, Lawe DC, Sudharshan E, Tuft R, Fogarty K, Lambright D, Corvera S: **Structural basis for endosomal targeting by FYVE domains**. *J Biol Chem* 2004, **279**:5958-5966.

43. Baisa GA, Mayers JR, Bednarek SY: **Budding and braking news about clathrin-mediated endocytosis**. *Curr Opin Plant Biol* 2013, **16**:718-725.

44. Lipka V, Panstruga R: **Dynamic cellular responses in plant–microbe interactions**. *Curr Opin Plant Biol* 2005, **8**:625-631.

45. Schmelzer E: **Cell polarization, a crucial process in fungal defence**. *Trends Plant Sci* 2002, **7**:411-415.

46. •• Henty-Ridilla JL, Shimono M, Li J, Chang JH, Day B, Staiger CJ: **The plant actin cytoskeleton responds to signals from microbe-associated molecular patterns**. *PLOS Pathog* 2013, **9**:e1003290.

The authors show that pathogen triggered immunity involves an immediate increase in actin filament abundance. Treatment of leaves with a MAMP peptide mimic was sufficient to elicit a change in actin organization in epidermal cells, and this actin response required the host–cell MAMP receptor kinase complex, including FLS2, BAK1 and BIK1.

47. •• Smith JM, Salamango DJ, Leslie ME, Collins CA, Heese A: **Sensitivity to Flg22 is modulated by ligand-induced degradation and de novo synthesis of the endogenous flagellin-receptor FLAGELLIN-SENSING2**. *Plant Physiol* 2014, **164**:440-454.

The authors show that within 1 hour after flg22 treatment, Arabidopsis leaf tissue was unable to re-elicit flg22 signaling, suggesting that degradation of FLS2 may serve to desensitize cells to the same stimulus. At later times after the initial flg22 elicitation, recovery of FLS2 protein levels positively correlated with re-sensitization to flg22.

48. •• Spallek T, Beck M, Ben Khaled S, Salomon S, Bourdais G, Schellmann S, Robatzek S: **ESCRT-I mediates FLS2 endosomal sorting and plant immunity**. *PLOS Genet* 2013:9.

This paper shows that ESCRT complex proteins regulate FLS2 endosomal sorting and that ESCRT-I is required for flg22-activated stomatal defenses involved in plant immunity.

49. Rajagopalan S: **Endosomal signaling and a novel pathway defined by the natural killer receptor KIR2DL4 (CD158d)**. *Traffic* 2010, **11**:1381-1390.

50. Diguglielmo GM, Baass PC, Ou WJ, Posner BI, Bergeron JJM: **Compartmentalization of Shc, Grb2 and Msos, and hyperphosphorylation of Raf-1 by Egf but not insulin in liver parenchyma**. *EMBO J* 1994, **13**:4269-4277.

51. Baass PC, Diguglielmo GM, Authier F, Posner BI, Bergeron JJM: **Compartmentalized signal-transduction by receptor tyrosine kinases**. *Trends Cell Biol* 1995, **5**:465-470.

52. Murphy JE, Padilla BE, Hasdemir B, Cottrell GS, Bunnett NW: **Endosomes: a legitimate platform for the signaling train**. *Proc Natl Acad Sci U S A* 2009, **106**:17615-17622.

53. Geldner N, Hyman DL, Wang X, Schumacher K, Chory J: **Endosomal signaling of plant steroid receptor kinase BRI1**. *Genes Dev* 2007, **21**:1598-1602.

54. Irani NG, Di Rubbo S, Mylle E, Van den Begin J, Schneider-Pizon J, Hnilikova J, Sisa M, Buyst D, Vilarrasa-Blasi J, Szatmari AM *et al.*: **Fluorescent castasterone reveals BRI1 signaling from the plasma membrane**. *Nat Chem Biol* 2012, **8**:583-589.

55. Drakakaki G, van de Ven W, Pan S, Miao Y, Wang J, Keinath NF, Weatherly B, Jiang L, Schumacher K, Hicks G *et al.*: **Isolation and proteomic analysis of the SYP61 compartment reveal its role in exocytic trafficking in Arabidopsis**. *Cell Res* 2012, **22**:413-424.

56. Geldner N, Denervaud-Tendon V, Hyman DL, Mayer U, Stierhof YD, Chory J: **Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set**. *Plant J* 2009, **59**:169-178.

57. Contento AL, Bassham DC: **Structure and function of endosomes in plant cells**. *J Cell Sci* 2012, **125**:3511-3518.

58. Bolte S, Talbot C, Boutte Y, Catrice O, Read ND, Satiat-Jeunemaitre B: **FM-dyes as experimental probes for dissecting vesicle trafficking in living plant cells**. *J Microsc* 2004, **214**:159-173.

59. Naramoto S, Otegui MS, Kutsuna N, de Rycke R, Dainobu T, Karampelias M, Fujimoto M, Feraru E, Miki D, Fukuda H *et al.*: **Insights into the localization and function of the membrane trafficking regulator GNOM ARF-GEF at the Golgi apparatus in Arabidopsis**. *Plant Cell* 2014, **26**:3062-3076.

60. Viotti C, Bubeck J, Stierhof YD, Krebs M, Langhans M, van den Berg W, van Dongen W, Richter S, Geldner N, Takano J *et al.*: **Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle**. *Plant Cell* 2010, **22**:1344-1357.

61. Tse YC, Mo B, Hillmer S, Zhao M, Lo SW, Robinson DG, Jiang L: **Identification of multivesicular bodies as prevacuolar compartments in *Nicotiana tabacum* BY-2 cells**. *Plant Cell* 2004, **16**:672-693.

62. Bottanelli F, Foresti O, Hanton S, Denecke J: **Vacuolar transport in tobacco leaf epidermis cells involves a single route for soluble cargo and multiple routes for membrane cargo**. *Plant Cell* 2011, **23**:3007-3025.

63. Jaillais Y, Fobis-Loisy I, Miege C, Gaude T: **Evidence for a sorting endosome in Arabidopsis root cells**. *Plant J* 2008, **53**:237-247.

64. Miao Y, Li KY, Li HY, Yao X, Jiang L: **The vacuolar transport of aleurain-GFP and 2S albumin-GFP fusions is mediated by the same pre-vacuolar compartments in tobacco BY-2 and Arabidopsis suspension cultured cells**. *Plant J* 2008, **56**:824-839.

65. Mo B, Tse YC, Jiang L: **Plant prevacuolar/endosomal compartments**. *Int Rev Cytol* 2006, **253**:95-129.

66. Ebine K, Fujimoto M, Okatani Y, Nishiyama T, Goh T, Ito E, Dainobu T, Nishitani A, Uemura T, Sato MH *et al.*: **A membrane trafficking pathway regulated by the plant-specific RAB GTPase ARA6**. *Nat Cell Biol* 2011, **13**:853-859.

67. Ueda T, Yamaguchi M, Uchimiya H, Nakano A: **Ara6, a plant-unique novel type Rab GTPase, functions in the endocytic pathway of *Arabidopsis thaliana***. *EMBO J* 2001, **20**:4730-4741.

68. Henne WM, Buchkovich NJ, Emr SD: **The ESCRT pathway**. *Dev Cell* 2011, **21**:77-91.

69. Scheuring D, Viotti C, Kruger F, Kunzl F, Sturm S, Bubeck J, Hillmer S, Frigerio L, Robinson DG, Pimpl P *et al.*: **Multivesicular bodies mature from the trans-Golgi network/early endosome in Arabidopsis**. *Plant Cell* 2011, **23**:3463-3481.

70. Shahriari M, Richter K, Keshavaiah C, Sabovljevic A, Huelskamp M, Schellmann S: **The Arabidopsis ESCRT protein–protein interaction network**. *Plant Mol Biol* 2011, **76**:85-96.