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Endosomal trafficking and signaling in plant defense responses

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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.

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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