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Traffic Control: Subversion of Plant Membrane Trafficking by Pathogens

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Abstract

Membrane trafficking pathways play a prominent role in plant immunity. The endomembrane transport system coordinates membrane-bound cellular organelles to ensure that immunological components are utilized effectively during pathogen resistance. Adapted pathogens and pests have evolved to interfere with aspects of membrane transport systems to subvert plant immunity. To do this, they secrete virulence factors known as effectors, many of which converge on host membrane trafficking routes. The emerging paradigm is that effectors redundantly target every step of membrane trafficking from vesicle budding to trafficking and membrane fusion. In this review, we focus on the mechanisms adopted by plant pathogens to reprogram host plant vesicle trafficking, providing examples of effector-targeted transport pathways and highlighting key questions for the field to answer moving forward.

1. INTRODUCTION

Plants engage in countless microscopic battles with a multitude of diverse pathogens both above- and belowground. To detect and eliminate intruders, plants employ a multilayered innate immune system that fundamentally relies on membrane trafficking to provide effective resistance. Specifically, the endomembrane transport system coordinates the activities of membrane-bound cellular organelles to ensure the precise and timely deposition of immune components in the correct location and quantity. Consistent with this notion, a growing number of studies have revealed pathogen manipulation of plant intracellular transport systems as a crucial infection strategy (14, 28, 85, 89).

To establish a safe replicative niche, pathogens must prevail through a series of critical phases during host invasion. First, they must endure or neutralize toxic compounds and hydrolytic enzymes deployed by the invaded plant cells. Those that survive must then establish secure contacts with their host cells. While doing so, pathogens must also avoid excessive local immune activation, which could lead to host cell suicide and failed infection. To fully govern their hosts and facilitate efficient nutrient uptake, pathogens must actively suppress immune recognition and subvert host trafficking pathways for their own benefit. Therefore, it is not surprising that the endomembrane trafficking system is a major cellular hub commonly targeted by plant-parasitic organisms (63, 93). The emerging paradigm is that adapted pathogens secrete virulence factors known as effectors, some of which target host membrane trafficking pathways, to gain control of their host cells. This review focuses on the mechanisms adopted by pathogens to reprogram host plant vesicle trafficking, providing examples of effector-targeted transport pathways. We also briefly introduce plant membrane trafficking pathways and how they contribute to immunity.

2. PLANT MEMBRANE TRAFFICKING PATHWAYS

The membrane trafficking pathways cooperate to keep cells healthy and operational by ensuring the proper functioning of vital cellular processes, such as cell metabolism, immune response, and cell differentiation. Plants transport cargoes—such as extracellular, membrane, and lysosomal proteins—via membrane-bound vesicles. These trafficking pathways are tightly regulated by a series of vesicle transport regulators and vesicle fusion proteins such as small GTPases and tethering factors (24, 99). Although trafficking mainly takes place through the default secretory and endocytic pathways, there are also alternative pathways that we discuss in this section (**Figure 1**).

2.1. Default Secretory Pathway: Basic Principles and Key Components

Conventional secretion is initiated by the cotranslational transport of newly produced secretory proteins in the endoplasmic reticulum (ER) and their subsequent delivery from the ER to the Golgi apparatus for further post-translational modifications. The exchange of secretory cargoes between different membrane interfaces is facilitated by membrane-bound transport vesicles that bud from a donor membrane and fuse with an acceptor membrane (48). At the *trans*-Golgi network (TGN), mature cargoes are subsequently loaded into secretory vesicles that navigate through the cytoplasm and eventually fuse with the plasma membrane or the vacuole. The movement of vesicles is facilitated by actin or microtubule tracks under the guidance of molecular motor proteins (119).

2.1.1. Small GTPases. Conventional secretion has three key steps: vesicle budding, transport, and fusion. The Ras superfamily of small guanosine triphosphatase (small GTPase)-related proteins, including ADP-ribosylation factor GTPases (Arfs) and Rab GTPases (Rabs), are key regulators of endomembrane trafficking. Vesicle budding is primarily regulated by the Arf1/Sar1

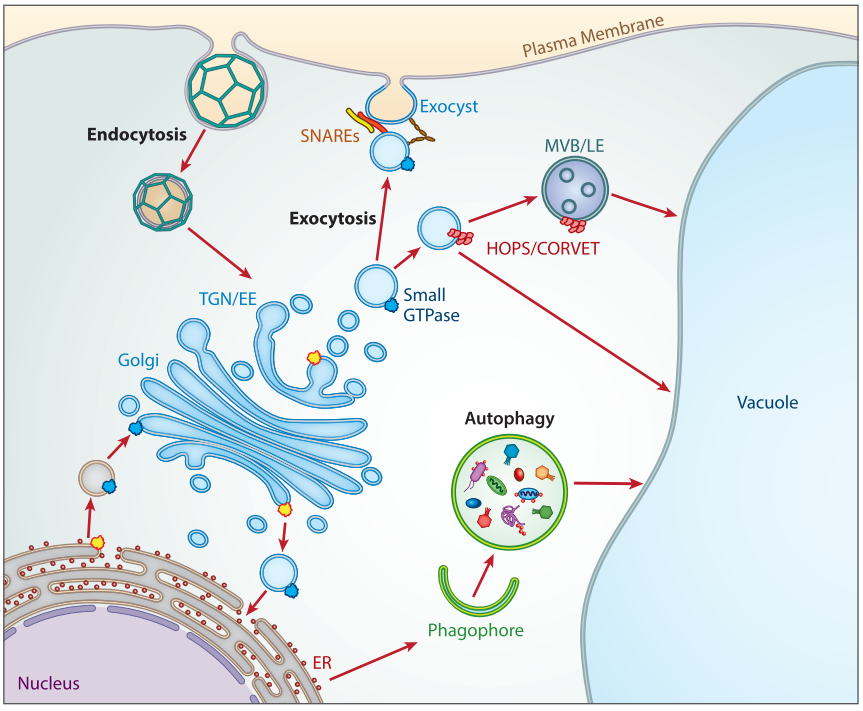


Figure 1

Overview of membrane trafficking pathways in plants. Secretory proteins are cotranslationally translocated in the endoplasmic reticulum (ER), where they are modified and folded into their final three-dimensional structure. They are then transported to the Golgi apparatus and the *trans*-Golgi network/early endosome (TGN/EE), where they are sorted and packaged into vesicles for transport to their final destinations, such as plasma membranes or lysosomes. Small GTPases such as ADP-ribosylation factor (Arf) and Rab GTPases are key regulators of membrane trafficking, which mediates vesicle formation, budding, tethering, and fusion events. Vesicle fusion to the plasma membrane is conducted through the coordination of small GTPases, soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), and the exocyst complex. Surface-localized proteins are internalized by the formation of clathrin-coated vesicles across the plasma membrane. These vesicles are then sorted within the TGN/EE, bringing the molecules into the cell. Endocytosed proteins can then be recycled back to the plasma membrane or delivered to multivesicular bodies/late endosomes (MVBs/LEs), which eventually fuse with the vacuole for degradation. Another pathway to sort proteins to the vacuole is through the catabolic process of autophagy, which facilitates the destruction of cargoes in the vacuole through double-membrane vesicles called autophagosomes. Abbreviations: CORVET, class C core vacuole/endosome tethering complex; HOPS, homotypic fusion and vacuole protein sorting complex.

members, whereas vesicle transport, tethering, and fusion events are governed by Rabs (24, 83). Activated Rabs coat vesicle surfaces to facilitate the movement and fusion of vesicles with target membranes. Both Rab and Arf1/Sar1 members act as molecular switches, converting between GTP-bound (on) and GDP-bound (off) states. These conversions are mediated by GTPase-activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs). GEFs act to replace GDP with GTP, activating their small GTPase partners, whereas GAPs deactivate their cognate GTPases by stimulating their GTP hydrolysis function (108).

2.1.2. Tethering complexes. The final stage of secretion, vesicle fusion, is executed through the concerted actions of Rabs, tethering factors/complexes, and soluble N-ethylmaleimide-sensitive

factor attachment protein receptors (SNAREs) (99, 104). Several multisubunit tethering complexes (MTCs) operate in vesicle trafficking to facilitate vesicle fusion to different membranes. Fusion to the tonoplast during endosomal and vacuolar sorting is governed by two distinct MTCs known as class C core vacuole/endosome tethering complex and homotypic fusion and vacuole protein sorting complex, whereas fusion to the plasma membrane is facilitated by an MTC called the exocyst (103). The exocyst is a eukaryotic protein complex involved in tethering post-Golgi secretory vesicles to the plasma membrane prior to their final SNARE-mediated membrane fusion (77). It was originally identified in yeast and has since been shown to be formed of the same eight conserved subunits across eukaryotes: Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84 (97). Interestingly, unlike other eukaryotes, the plant exocyst has multiple paralogs encoding several of the subunits (135).

2.1.3. SNAREs. SNAREs are transmembrane proteins that mediate vesicle fusion. SNAREs can be classified as Q-SNAREs or R-SNAREs. Q-SNAREs have a central glutamine residue and, as they are usually located on the target membrane, are also called t-SNAREs. R-SNAREs have a central arginine residue and, as they are usually located on the transport vesicle, are also called v-SNAREs. v-SNAREs interact with their cognate t-SNAREs on the target membrane to initiate membrane fusion. After membrane fusion, the SNARE complex is dissociated and recycled by the N-ethylmaleimide-sensitive factor (NSF) and the soluble NSF attachment protein (α-SNAP) (99, 104).

2.2. Endocytic System: Recycling or Destruction of Membrane Proteins

Much like other eukaryotes, plants recycle and remove membrane proteins from the cell surface through the endocytic system. The endocytic system consists of a network of membrane-bound organelles—including early endosomes (EEs), recycling endosomes, late endosomes (LEs), and the vacuole—that coordinate intracellular trafficking and protein homeostasis. In plants, cargoes freshly endocytosed from the plasma membrane are sorted through the TGN, or a TGN-derived compartment, which serves as an EE (TGN/EE hereafter) (33, 61, 116). Internalized proteins are then either recycled back to the plasma membrane or delivered to LEs, also known as multivesicular bodies (MVBs), which eventually fuse with the vacuole. Prior to vacuolar sorting, some membrane cargoes can still be retrieved from the MVBs/LEs and recycled back to the plasma membrane (40).

2.3. Alternative Secretion in Plants

Typically, protein cargoes of the conventional secretion system contain an N-terminal signal peptide, also known as the secretion signal, that mediates cotranslational protein translocation into the ER. However, previous studies identified many plant proteins that are secreted despite lacking signal peptide sequences (20, 95, 123, 134). The ways these proteins are secreted appear to vary, but collectively they are referred to as the unconventional protein secretion (UPS) pathways. The mechanisms of UPS remain cryptic, but the emerging view is that UPS occurs via transmembrane transport, MVBs/LEs, and autophagy (75).

2.4. Autophagy

Autophagy is a conserved eukaryotic pathway that facilitates the destruction of cytoplasmic components in the vacuole. Autophagy involves the formation and transport of double-membrane vesicles called autophagosomes, which engulf cytoplasmic cargoes to mediate their vacuolar degradation or unconventional secretion (81). In yeast (*Saccharomyces cerevisiae*), autophagy is controlled

by more than 30 core proteins called ATG (autophagy-related) proteins, and most of these proteins, including ATG8 members, are conserved in plants. Autophagy can act selectively to capture target substrates through specific interactions of autophagy cargo receptors/adaptors and ATG8 proteins (64). To do this, autophagy receptors carry a short sequence called the ATG8-interaction motif (AIM) to bind ATG8 proteins anchored on autophagosomal membranes (55). In different species, selective forms of autophagy mediate specific trafficking pathways, including alternative secretion in humans and vacuolar sorting in yeast (90). That said, whether secretory autophagy exists in plants remains to be determined.

3. THE ROLE OF ENDOMEMBRANE TRAFFICKING IN PLANT INNATE IMMUNITY

Membrane trafficking pathways play a variety of prominent roles in eliminating infectious diseases. Specifically, membrane trafficking pathways direct immune receptors, signaling molecules, antimicrobial compounds, hydrolytic enzymes, and other immune components toward intruders (14, 29, 59, 64, 68, 72, 76). Additionally, defunct immune complexes are either repaired and recycled or eliminated by the endomembrane trafficking system to make room for the newly generated signaling complexes (9).

3.1. Positioning Pattern Recognition Receptors and Induced Defense Components by the Default Secretory Pathway

The plant innate immune system relies on timely recognition of pathogens/pests through surface-localized pattern recognition receptors (PRRs) and intracellular nucleotide-binding leucine-rich repeat immune receptors (NLRs). PRRs are transmembrane immune receptors that sense extracellular modified-self cues or nonself molecules such as pathogen-associated molecular patterns (PAMPs) (31). PRR activation stimulates intracellular signaling cascades that result in a variety of downstream immune outputs, including the induction of defense-related genes; the secretion of pathogenesis-related proteins and defense hydrolases; the generation of reactive oxygen species (ROS); production of defense hormone; and the deposition of cell wall components such as callose (β-1,3-glucan) to fortify the cell wall at the plant–pathogen interface (1, 31, 107). Successful immune responses rely on the presence of sufficient levels of PRRs and PRR-triggered immune components at the cell surface. The quantity and localization of PRRs are typically ensured by the endomembrane trafficking system. Accordingly, mutations that perturb any key steps of membrane trafficking lead to the depletion of immune PRRs and other immune components at the cell surface, enhancing plant susceptibility to pathogens (4, 5, 22, 46, 51, 58, 59, 87, 88).

3.2. Impact of Endocytosis and Recycling of Pattern Recognition Receptors on Plant Immunity

PRRs are subject to constitutive endocytic recycling between the plasma membrane and endosomal compartments (9). This mediates PRR homeostasis at the cell surface, thereby preserving the integrity of the pathogen surveillance system. This view is supported by the fact that pathogen virulence can be enhanced using genetic and pharmaceutical approaches that impair constitutive endocytic recycling (85). Moreover, some PRRs undergo ligand-induced endocytosis moving through the TGN/EE and MVB/LE compartments (76). The functional ramifications of ligand-induced internalization are not fully understood. Despite this, the internalization of PRRs and their signaling partners, such as BIK1, is implicated in improving immune signal propagation (72).

3.3. Interplay Between Nucleotide-Binding Leucine-Rich Repeat Immune Receptors and Membrane Trafficking

NLRs are intracellular immune sensors that recognize pathogen effectors either directly by binding the effector itself or indirectly by guarding pathogen-targeted host proteins. Interestingly, several NLRs, including TN2 and Pii-2, were found to guard Exo70 family members (43, 137), providing a direct link between NLR-mediated immunity and membrane trafficking.

In uninfected plant cells, NLRs localize to various cellular compartments and membrane interfaces (11, 37, 98, 110, 125). Once activated, NLRs trigger a form of programmed cell death known as the hypersensitive response (HR) that restricts pathogen invasion. Recent breakthroughs have shown that activated NLRs oligomerize into multimeric structures called resistosomes that insert into the plasma membrane and form calcium-permeable pores, resulting in HR (10, 118). Owing to HR, live cell imaging of NLRs during infection with the relevant pathogens has not been feasible until recently. Analysis of resistosome structures revealed critical residues in the N-terminal coiled-coil (CC) domains of NLRs that can be mutated to avoid triggering HR without perturbing other NLR functions (2, 52, 118). This has enabled imaging of activated/unactivated NLRs, which has revealed that NLRs are highly dynamic immune receptors that target different membrane interfaces upon activation (25, 36). Furthermore, genetic and pharmaceutical approaches inhibiting membrane trafficking have been shown to impair functions of the NLR protein R3a (38) and resistance to powdery mildew 8.2 (RPW8.2)—an atypical resistance protein that carries CC domains found in other NLRs but lacks the nucleotide-binding and leucine-rich repeat domains (58). However, the extent to which NLRs rely on membrane trafficking for their proper localization is currently not fully understood.

3.4. Focal Immunity: Diversion of Plant Defenses to the Sites of Pathogen Invasion

Pathogens and pests intimately interact with the host cells by forming specialized infection structures to deliver effectors and uptake nutrients. These structures vary from species to species: Bacteria form the type 3 (T3) injection apparatus to penetrate host cells; nematodes and insects deploy stylets; and most fungal and oomycete pathogens (herein referred to as filamentous pathogens) form specialized hyphae that invade the plant cell. Processes taking place at these host–pathogen interfaces are thought to have a major impact on the outcome of plant–pathogen interactions.

Plants can sense contacts made by the pathogens at the cell surface. This activates reprogramming of the plant cytoskeleton to eliminate pathogen penetration attempts through a spatially confined defense response termed focal immunity. For example, the focal deployment of the plant cell wall material callose has been shown to enhance penetration resistance to fungal pathogens (114). This involves a major reorganization of the cytoskeleton to position cellular defenses and secretory pathways toward pathogen contact sites (13). Several key plant components that contribute to penetration resistance have been discovered (23, 71, 106). One such component, PEN1, associates with v-SNAREs and Arf-GAPs/GEFs to facilitate the secretion of exosomes at penetration sites to form cell wall appositions termed papillae (84). Apart from the endomembrane system, other organelles such as peroxisomes and mitochondria also accumulate at pathogen penetration sites and facilitate focal deployment of defense components (42).

The haustorium is a specialized infection structure produced by filamentous pathogens that penetrates host cells. During host penetration, the haustorium becomes surrounded by an enigmatic plant-derived extrahaustorial membrane (EHM). The biochemical content of the EHM contrasts with that of the plasma membrane. Indeed, the majority of plasma membrane

proteins, including PRRs, are excluded from the EHM (13). In contrast, a helper NLR, termed NLR required for cell death-4 (NRC4), shifts its localization from the cytosol to the EHM during infection by the oomycete pathogen *Phytophthora infestans*. Despite these advances, how and why plant immune receptors are differentially recruited at the EHM remain unknown.

Macromolecule transport mechanisms across the EHM remain largely uncharacterized. In theory, the extracellular vesicles detected at the extrahaustorial matrix during fungal invasion of plant cells could serve as a shuttling system (79). Supporting this notion, an MVB/LE pathway was discovered to target the EHM during infection by *P. infestans*. That said, whether these transport pathways contribute to immunity is currently unknown (12). Notably, haustoria are also subject to targeted defense responses. For instance, accumulation of the cell wall material callose provides protection against hyphal penetration (15). Furthermore, the atypical resistance protein RPW8.2 accumulates at the EHM engulfing fungal and oomycete haustoria and contributes to disease resistance, but how it achieves this remains elusive (58). In addition, a selective form of plant autophagy is diverted to the haustorium interface and contributes to focal immunity (29). Lastly, chloroplasts also navigate toward the haustorium of the oomycete pathogen *P. infestans*, but functional aspects of this process are currently unknown (100). Despite these discoveries, how endomembrane trafficking mediates focal deployment of defense-related components and organelles at the plant–pathogen interface is still poorly understood.

3.5. Autophagy Contributes to Plant Immunity

It is becoming increasingly clear that vesicle trafficking pathways are required for autophagy and immunity (133). There is evidence that during pathogen attack the autophagy cargo receptor Joka2/NBR1, which normally depletes plant protein aggregates, is engaged in a form of defense-related autophagy. For example, Joka2/NBR1-containing autophagy complexes are diverted to the EHM to restrict pathogen infection (28, 29). Furthermore, Joka2/NBR1 mediates antiviral immunity by eliminating viral components (49, 50) and contributes to immunity against bacteria (67, 115). Despite these advances, the regulation and functions of defense-related autophagy remain largely unknown.

4. REPROGRAMMING OF MEMBRANE TRAFFICKING BY PATHOGEN EFFECTORS

Advances in genome sequencing have helped identify a plethora of effectors from various plant parasites. This paved the way for effector biology, which improved our understanding of the plant immune system by revealing major host defense components and susceptibility factors. Bacterial and oomycete effectors have been of particular interest because their unique amino acid sequence signatures make them relatively easy to identify through genomics-based approaches. Although host-translocated bacterial effectors carry an N-terminal T3 secretion signal (17), most oomycete effectors that are delivered inside the plant cells carry a conserved RXLR motif downstream of the N-terminal secretion signal (127). The RXLR motif serves as a host-translocation signal and is cleaved off from the mature effector protein before entering the host cells (124).

The emerging paradigm based on a vast number of effectoromics studies is that effectors from a given pathogen tend to target the same cellular processes in their host plants. Typically, multiple steps of a specific host pathway are targeted redundantly by multiple effectors. It is also possible that effectors from different pathogens with distinct evolutionary origins converge on the same host targets (129). Given the immune-related roles of various transport pathways, it is no surprise that pathogens deploy effectors specifically targeting major host transport pathways to promote virulence (**Figure 2**; **Table 1**).

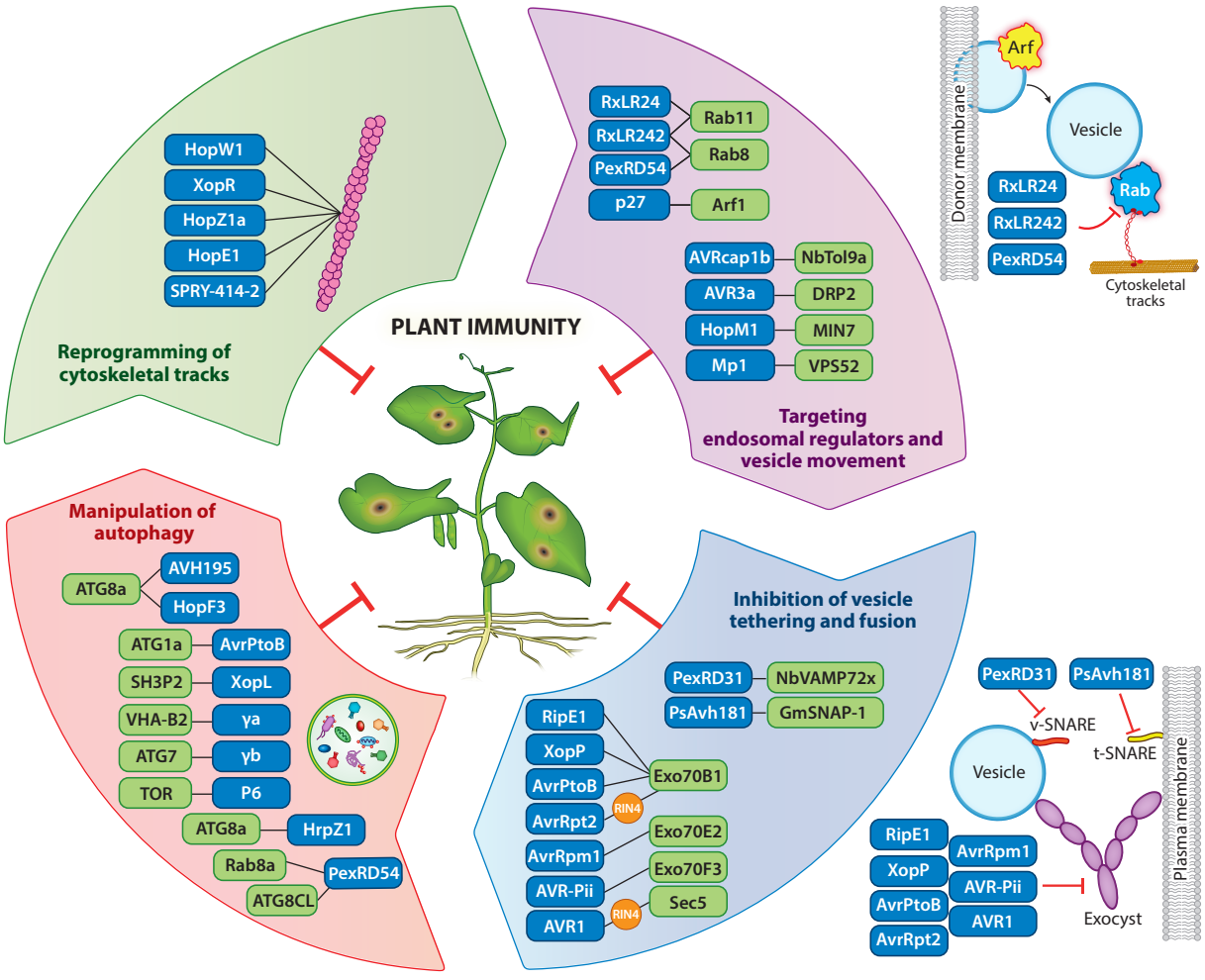


Figure 2

An overview of pathogen effectors and their plant targets implicated in host membrane trafficking. The mechanisms employed by pathogens to manipulate plant membrane trafficking can be broadly sorted into four main categories: targeting endosomal regulators and vesicle movement; inhibition of vesicle tethering and fusion; manipulation of autophagy; and reprogramming of cytoskeletal tracks. Effectors that manipulate endosomal regulators and trafficking can suppress vesicle movement by targeting Rab GTPases (e.g., RXLR242 and RXLR24 from oomycete pathogens) or hijack key transport regulators, such as Arf GTPases (e.g., viral protein p27). Some effectors converge on inhibiting vesicle tethering and fusion by directly targeting tethering components (e.g., RipE1 from *Ralstonia solanacearum*) or indirectly targeting them through modulating their regulators, such as RIN4 (e.g., AvrRpm1 from *Pseudomonas syringae*). Some effectors promote autophagy to facilitate pathogenesis. For example, *Phytophthora infestans* effector PexRD54 interacts with at least two host proteins—Rab8a (Rab GTPase) and ATG8CL (core autophagy protein)—to stimulate autophagosome biogenesis to increase pathogen virulence, whereas other effectors suppress autophagy to increase their virulence (e.g., XopL from *Xanthomonas campestris*). Effectors that target the actin and microtubule cytoskeleton (e.g., HopZ1a from *P. syringae* and XopR from *X. campestris*) could affect overall membrane trafficking events in plants. Abbreviations: t-SNARE, target-soluble N-ethylmaleimide-sensitive factor attachment protein receptor; v-SNARE, vesicle-soluble N-ethylmaleimide-sensitive factor attachment protein receptor.

Table 1 Summary of pathogen effectors that target plant membrane trafficking pathways and their means of increasing virulence

Effector	Pathogen(s)	Host process(es) targeted	Specific host target(s)	Means of increasing virulence	Reference(s)
AvrPto	*Pseudomonas syringae*	Vesicle trafficking via Rab GTPase	Rab8	In planta interaction not detected; could interfere with flagellin receptor FLS2 and BAK1	30
RxLR24	*Phytophthora brassicae/infestans*	Vesicle trafficking via Rab GTPase	Rab11	Inhibits secretion of antimicrobial PR1 and PDF1.2	33
RxLR242	*Phytophthora capsici*	Vesicle trafficking via Rab GTPase	Rab8, Rab11	Inhibits PR1 secretion; interferes with FLS2 trafficking to the PM	19
PexRD31	*P. infestans*	Vesicle trafficking via Rab GTPase and SNARE	RabC1, NbVAMP72x	Increases number of FYVE-labeled endosomes, which regulates MVB/LE activities	25
p27	Red clover necrotic mosaic virus	ER remodeling via Arf GTPase	Arf1	Requires Arf1 for ER remodeling and viral replicase complex assembly	13, 14
BEC4	*Blumeria graminis*	Vesicle trafficking via Arf-GAP	HvARF-GAP	In planta interaction not detected; possibly interferes with HvARF-GAP-dependent resistance to pathogens	29
AvrPtoB	*P. syringae*	Vesicle fusion via exocyst; autophagy	Exo70B1, ATG8a, ATG1a	Causes Exo70B1 to be degraded by proteasome; promotes ATG1 kinase phosphorylation to suppress autophagy	16, 37
RipE1	*Ralstonia solanacearum*	Vesicle fusion via exocyst	Exo70B1	Binds and cleaves Exo70B1 using its cysteine protease activity, inhibiting exocytosis	34
XopP	*Xanthomonas campestris*	Vesicle fusion via exocyst	Exo70B1	Downregulates Exo70B1 to block exocytosis of defense molecules like callose and FLS2	21
AvrRpm1	*P. syringae*	Vesicle fusion via exocyst	Exo70E2 via RIN4	Promotes the inhibitory association of RIN4 with Exo70E2 to inhibit secretion of callose	26
AvrRpt2	*P. syringae*	Vesicle fusion via exocyst	Exo70B1 via RIN4	Prevents RIN4 from recruiting Exo70B1 to the PM to inhibit exocytosis	28
AVR-Pii	*Magnaporthe oryzae*	Vesicle fusion via exocyst	Exo70F2/F3	Exo70F3 appears to act as a decoy target for Avr-Pii to activate Pii-triggered immunity	10
AVR1	*P. infestans*	Vesicle fusion via exocyst	Sec5	Stabilizes SEC5 to inhibit exocyst function in order to prevent focal secretion of callose	8
PsAvh181	*Phytophthora sojae*	Vesicle fusion via SNARE	GmSNAP-1	Interferes with GmSNAP-1 and GmNSF interaction to suppress antimicrobial GmGIP1, P68B, and PR1 secretion	36

(*Continued*)

Table 1 (*Continued*)

Effector	Pathogen(s)	Host process(es) targeted	Specific host target(s)	Means of increasing virulence	Reference(s)
AVR3a	*P. infestans*	Endocytosis via GTPase	DRP2	Reduces internalization of activated FLS2 receptor	1
AVRcap1b	*P. infestans*	Endocytosis via ESCRT	NbTol9a	Suppresses the immune activity of the helper NLRs NRC2 and NRC3	7
HopM1	*P. syringae*	Autophagy; vesicle trafficking via Arf-GEF	MIN7	Promotes degradation of MIN7; induces autophagic removal of proteasome	22, 23, 35
Mp1	*Myzus persicae*	Endosome retrograde trafficking via GARP	VPS52	Reduces protein levels of VPS52 during infection	27
VPg	Turnip mosaic virus	Autophagy	SGS3, RDR6, NBR1	Uses autophagy to degrade key components of host RNA silencing machinery; inhibits NBR1 and HCpro degradation by autophagy	3, 12
6K2	Turnip mosaic virus	Autophagy	NBR1	Inhibits NBR1 and HCpro degradation by autophagy	12
P0	Polerovirus	Autophagy	AGO1	Induces degradation of AGO1, a core component of host RNA silencing system, through autophagy	4
NSvc4	Rice stripe virus	Autophagy	Rem1.3, Rem1.4	Initiates degradation of remorins by autophagy	9
P6	Cauliflower mosaic virus	Autophagy	TOR kinase	Promotes TOR activation to suppress autophagy	40
γa	Barley stripe mosaic virus	Autophagy	VHA-B2	Interrupts vacuolar acidification, thus suppressing autophagic degradation	38
γb	Barley stripe mosaic virus	Autophagy	ATG7	Interferes with ATG7-ATG8 interaction to inhibit autophagy	39
PexRD54	*P. infestans*	Autophagy	ATG8CL, Rab8a	Outcompetes Joka2 binding to ATG8CL and subverts Rab8a-mediated vesicle trafficking to promote autophagy	5, 6, 24
AVH195	*Phytophthora parasitica*	Autophagy	ATG8	Slows down autophagic flux	32
NMAS1	*Globodera rostochiensis*	Autophagy	ATG8	Interacts with ATG8, leading to ROS suppression	2
HrpZ1	*P. syringae*	Autophagy	ATG8a	Induces ATG4b-mediated cleavage of ATG8 to enhance autophagy	16
HopF3	*P. syringae*	Autophagy	ATG8a	Suppresses autophagy	16
XopL	*X. campestris*	Autophagy	SH3P2	Degrades SH3P2 to interfere with autophagosome biogenesis	18

(*Continued*)

Table 1 (*Continued*)

Effector	Pathogen(s)	Host process(es) targeted	Specific host target(s)	Means of increasing virulence	Reference(s)
HopW1	*P. syringae*	Actin cytoskeleton	Actin	Inhibits actin-dependent endocytosis and trafficking of proteins to vacuole	15
XopR	*X. campestris*	Actin cytoskeleton	Actin	Cross-links F-actin; modulates actin biogenesis; antagonizes actin-depolymerization factors	31
HopZ1a	*P. syringae*	Microtubule cytoskeleton	Tubulin	Acetylates tubulin and deconstructs microtubule network to inhibit protein secretion and cell wall–based defense	17
HopE1	*P. syringae*	Microtubule cytoskeleton	MAP65	Dissociates MAP65 from microtubule network to inhibit PR1 secretion and cell wall–based defense	11
SPRY-414-2	*Globodera pallida*	Microtubule cytoskeleton	CLASP	Targets CLASP that is involved in microtubule stability and growth	20

Abbreviations: CLASP, cytoplasmic linker protein-associated protein; ER, endoplasmic reticulum; ESCRT, endosomal sorting complex required for transport; FYVE, Fab1p, YOTB, Vac1p, and EEA1; GAP, GTPase-activating protein; GARP, Golgi-associated retrograde protein; GEF, guanine nucleotide exchange factor; GTPase, guanosine triphosphatase; LE, late endosome; MVB, multivesicular body; NLRs, nucleotide-binding leucine-rich repeat immune receptors; PM, plasma membrane; ROS, reactive oxygen species.

4.1. Effector-Mediated Manipulation of Defense-Related Secretion

Functional screens based on heterologous expression of pathogen effectors inside the plant cells have uncovered an increasing number of effectors that can modify defense-related secretion. Many studies employed cell biology techniques to detect the pathogenesis-related protein 1 (PR1) secretory signal fused to a fluorescent protein marker, whereas biochemical assays have also been used successfully to measure the extracellular accumulation of defense-related proteins. These studies have discovered a plethora of effectors that seem to target practically every stage of membrane trafficking that we discuss here.

4.1.1. Effectors take control of small GTPases to regulate membrane trafficking. Small GTPases contribute to immunity by facilitating proper positioning of PRRs and other immune components at plant–pathogen interfaces (22, 82, 105). For instance, Rab8 and Rab11 members are required for PR1 secretion and proper plasma membrane localization of Flagellin Sensing 2 (FLS2), the PRR that recognizes bacterial PAMP flagellin (22). Given their key roles in membrane trafficking and immunity, an increasing number of effectors are being discovered that target Arf/Sar1 and Rab GTPase members. For example, the bacterial T3 effector (T3E) AvrPto was the first to be reported to interact with a Rab GTPase, namely Rab8, in yeast two-hybrid assays. However, efforts to validate this interaction using in vitro/in vivo binding assays were unsuccessful (105).

Recent studies showed several RXLR effectors target Rab8 and Rab11 members. When transiently expressed in plant cells, RXLR24, a conserved effector deployed by several *Phytophthora* species, interacts with Rab11 and prevents PR1 secretion. Mechanistically, how RXLR24 perturbs Rab11 function is still unknown (112). Intriguingly, a different effector from *Phytophthora capsici*, RXLR242, interacts with multiple host Rabs, including Rab11 (also known as RabA4-3), RabD2-1,

RabG1-1, RabH1-3, and Rab8 (also known as RabE1-7) (70). It is unclear whether RXLR242 interferes with the function of all these Rabs, but it does block PR1 secretion and plasma membrane localization of FLS2. The current model is that RXLR242 may inhibit defense-related secretion by disrupting the interactions between Rab GTPases and their regulators (70). Indeed, multiple oomycete effectors seem to converge on Rab8 members to promote pathogen virulence. For example, a *P. infestans* RXLR effector termed PexRD54 recruits a subset of the Rab8a pool to stimulate a selective form of autophagy (87), presumably subverting the defense-related roles of Rab8a.

Another *P. infestans* effector, PexRD31, enhances the number of membrane-bound vesicles in plant cells (89). PexRD31's mode of action is currently undetermined, but it specifically localizes to RabC1-positive mobile endosomal bodies. A potential link to lipid droplets could be investigated, as RabC1 has been implicated in lipid droplet regulation (45).

There is evidence that Arf GTPases are also targeted by pathogen effectors. For instance, Arf1, which mediates the assembly and formation of the coat protein complex I (COP1) vesicles on the Golgi membrane, is hijacked by the red clover necrotic mosaic virus replication protein P27 (53, 54). How P27 manipulates Arf1 remains unknown, but both genetic and pharmacological disruption of Arf1 function impaired the assembly of the viral replicase complex. Furthermore, genetic interference of Sar1, an Arf GTPase that facilitates packing of vesicles at the ER, also led to reduced viral replication (126). These experiments show that viral pathogens can usurp host GTPases responsible for vesicle formation to facilitate their virulence and replication (53, 54).

Small GTPase functions are controlled through their GAP and GEF regulators. An Arf-GAP in barley (HvArf-GAP) was identified as a potential interactor of the *Blumeria graminis* effector BEC4 in a yeast two-hybrid screen. However, different variants of tagged HvArf-GAP and BEC4 did not interact in protein–protein interaction assays in plant cells. Thus, further research is needed to determine whether HvArf-GAP is a genuine effector target as well as the biological significance of this interaction (101). Taken in concert, these studies have revealed that transport pathways regulated by small GTPases, such as Arf and Rab members, are commonly targeted by pathogen effectors.

4.1.2. Effectors that disrupt vesicle fusion: rendering tethering complexes inactive. Cell surface trafficking pathways play crucial roles in plant protection. For instance, the exocyst complex, which facilitates vesicle tethering to the plasma membrane, is linked to several key processes related to PRR-mediated immunity, including callose deposition, the polarized exocytosis of defense molecules, and the proper localization and functioning of immune receptors (34, 80, 91, 121). The immune functions of the Exo70 subunit have been of particular interest, as several Exo70 paralogs have been linked to PRR-mediated immunity to bacterial, fungal, and oomycete plant pathogens (35). Consistent with this notion, several exocyst complex subunits are targeted by various pathogen effectors. Exo70B1 in particular is directly targeted by three bacterial effectors: AvrPtoB, RipE1, and XopP. AvrPtoB is an effector from *Pseudomonas syringae* that ubiquitinates Exo70B1, stimulating its degradation by the host proteasome. This modification is sensed by the NLR protein TN2, triggering HR cell death (122). RipE1 is a *Ralstonia solanacearum* T3E that directly binds and cleaves Exo70B1 via its cysteine protease activity. Once again, this results in TN2-mediated HR cell death (113). Finally, XopP, an effector from *Xanthomonas campestris*, directly interacts with and downregulates Exo70B1. Thus, XopP prevents the surface deployment of defense molecules such as callose while avoiding the activation of TN2-mediated immunity (80).

There are several bacterial effectors that indirectly target Exo70 paralogs (91, 96). For example, the *P. syringae* effector protein AvrRpm1 perturbs the proper functioning of Exo70B1, Exo70E1,

Exo70E2, and Exo70F1 by directly modifying their interacting partner RPM1-interacting protein 4 (RIN4). RIN4 is a plant plasma membrane resident protein that negatively regulates PRR-mediated immune responses by interacting with Exo70 members and preventing vesicle fusion (91). PTI activation triggers phosphorylation of RIN4 at its S141 position, which releases interacting Exo70 paralogs, leading to defense-related secretion (91). AvrRpm1 functions as an ADP-ribosyltransferase to ADP-ribosylate RIN4's C-terminal nitrate-induced (NOI) domain. This subsequently leads to RIN4 phosphorylation at a different site, T166, which enhances RIN4-Exo70E2 interaction. This, in turn, disrupts exocyst function and defense-related secretion. The *Arabidopsis* NLR protein RPM1 detects the AvrRpm1-induced modifications of RIN4, resulting in HR cell death and resistance to *P. syringae* (91). Exo70B1 also appears to be indirectly targeted by the bacterial effector AvrRpt2 (96). AvrRpt2 uses its cysteine protease activity to cleave RIN4, preventing RIN4-mediated recruitment of Exo70B1 to the plasma membrane and possibly impairing defense-related secretion (96). Consistent with this view, RIN4 fragments, released by AvrRpt2, suppress defense-related secretion and enhance growth of a *P. syringae* mutant impaired in effector delivery (3). Once again, the manipulation of RIN4 by AvrRpt2 is monitored by the NLR protein RPS2, which triggers HR cell death and mediates *P. syringae* resistance (74). Also, there is evidence suggesting that the *Xanthomonas* effectors XopB and XopJ contribute to bacterial infection by interfering with protein secretion; however, their plant targets responsible for vesicle trafficking have not yet been identified (7, 102).

Filamentous plant pathogens, such as the rice blast pathogen *Magnaporthe oryzae*, have also been found to deploy effectors that directly target the exocyst complex. In particular, two Exo70 paralogs, Exo70F2 and Exo70F3, have been identified as targets of the *M. oryzae* effector AVR-Pii, which is recognized by the rice NLR protein Pii (43). Depletion of Exo70F3 has been shown to reduce Pii-mediated resistance to *M. oryzae* in rice, suggesting that Pii might be guarding Exo70F3 similarly to how TN2 guards Exo70B1 in *Arabidopsis* (43). Indeed, although the recognition of AVR-Pii by Pii is indirect, it relies on direct physical interaction between Ex70F2/F3 and an integrated NOI domain found in Pii. As mentioned earlier, Exo70 members are linked to NOI domain–containing proteins, such as RIN4, which are targeted by multiple bacterial effectors that interfere with exocyst function (96). However, RIN4 is guarded by several NLRs in *Arabidopsis*. These findings reveal a highly intricate relationship between pathogen effectors, Exo70 members, RIN4, and NLR proteins, with each playing a role in exocyst function and resistance to bacterial pathogens.

Lastly, the RXLR effector AVR1 from *P. infestans* has been reported to target the Sec5 subunit of the exocyst (34). AVR1 is proposed to interrupt exocyst function and prevent focal secretion of PR1 and callose at the infection sites, enhancing plant susceptibility. AVR1 activity is sensed by the NLR protein R1 that triggers HR cell death, leading to disease resistance (34). These findings further highlight that effectors from different pathogens converge on key components of membrane trafficking, some of which are guarded by NLR immune receptors.

4.1.3. Effectors that disrupt vesicle fusion: dismantling SNARE complexes. There is a plethora of evidence that SNAREs are key components of basal plant immunity—from cell wall fortification at pathogen penetration sites to defense-related secretion (6, 16, 23, 94, 128, 136). In addition, several lines of evidence implicate SNARE proteins in NLR-mediated immunity (44, 58) as well as in the induction of HR cell death leading to nematode resistance (8).

Despite the prominent impact of SNAREs on plant immunity, the extent to which SNAREs are manipulated by pathogen effectors remained obscure until relatively recently. A large-scale interactome screen of *P. infestans* effectors revealed that the PexRD12/31 effector family, which

converges on vesicle-mediated trafficking, associate with several host SNAREs (89). PexRD31 interaction with the v-SNARE protein NbVAMP72x was further validated by coimmunoprecipitation assays in plant cells. How PexRD12/31 perturbs membrane trafficking is unknown, but the findings that it associates with v-SNAREs and enhances vesicle accumulation in host cells have prompted the view that it prevents vesicle fusion by interrupting SNARE functions (89).

Another effector that targets the SNARE complexes is PsAvh181 from *Phytophthora sojae*, which localizes to the host plasma membrane. PsAvh181 binds to a soluble NSF attachment protein (GmSNAP-1), which typically helps with the recycling of SNARE proteins by guiding NSF to dismantle SNARE complexes formed during vesicle fusion. PsAvh181 prevents GmSNAP-1 from binding to GmNSF, suppressing defense-related secretion (117). This likely leads to a depletion of functional SNAREs, but more research is needed to fully understand the relationship between PsAVh181 and SNARE complexes.

4.2. Effectors Targeting the Endosomal Sorting Pathway

The endosomal sorting pathway is essential for the recycling and destruction of transmembrane proteins such as plant surface immune receptors as well as susceptibility factors that are exploited by pathogens. Consistent with this notion, various pathogens have been discovered to encode effectors that perturb various steps of the endosomal sorting pathway. Yet the molecular mechanisms by which many of these effectors function and how they contribute to pathogen virulence remain poorly understood.

4.2.1. Pathogen efforts to interfere with immune receptor endocytosis. PRRs such as FLS2 undergo constitutive endocytosis and recycle between the plasma membrane (PM) and the TGN/EE to maintain their steady-state levels at the cell surface (9). Typically, ligand-free (inactive) PRRs are recycled through constitutive endocytosis, whereas ligand-bound PRRs are internalized from the PM through clathrin-mediated endocytosis (76). The *P. infestans* RXLR effector AVR3a was the first effector reported to impair PRR internalization. AVR3a reduces the internalization of the activated FLS2 and suppresses FLS2-mediated immune signaling. Consistent with these findings, AVR3a was found to associate with the plant Dynamin Related Protein 2a (DRP2a)—a GTPase involved in membrane scission during endocytosis—which is required for FLS2 endocytosis (18). However, whether the suppression of PRR endocytosis by AVR3a directly impacts immune signaling remains unknown.

The internalization of membrane proteins into the TGN/EE and their subsequent sorting inside the MVBs/LEs require the endosomal sorting complex required for transport (ESCRT) complex (56). In plants, ESCRT-0, the ESCRT component responsible for initial recognition and sorting of ubiquitinated cargo, has not yet been identified. Instead, TOM-1-like (Tol) proteins have emerged as key components of the ESCRT machinery in plants. Tol proteins play a role in the degradation of ubiquitinated membrane cargo. They act as ubiquitin adaptors, binding to ubiquitinated cargo and interacting with the ESCRT machinery to facilitate endosomal sorting and degradation (56). Tol9a, in particular, has been shown to negatively regulate HR cell death triggered by the NLR proteins NRC2 and NRC3; however, the mechanism of this phenomenon is currently unclear (32). This hints at the presence of an endocytic route that negatively regulates activated NLRs, a mechanism that could be hijacked by pathogens. Indeed, the *P. infestans* RXLR effector AVRcap1b was discovered to target NbTol9a to indirectly suppress the immune activity of the helper NLRs NRC2 and NRC3 (32).

4.2.2. Reprogramming of trans-Golgi network/early endosomes: perturbation of secretion or the endocytic pathway? TGN/EE vesicles are central to both secretory and endocytic

pathways (116). Therefore, effectors targeting this compartment could potentially interrupt either the secretory vesicles, the endocytic pathways, or even both simultaneously. The fact that MVBs/LEs can also discharge their contents as extracellular vesicles during pathogen attack provides another layer of complexity (95). For instance, MIN7, a TGN/EE-localized Arf-GEF protein, contributes to immunity against a wide range of pathogens, as it was suggested to facilitate protein secretion and callose deposition (73, 86). However, MIN7 is targeted by the *P. syringae* effector HopM1. HopM1 suppresses basal plant immunity by promoting the depletion of MIN7 at the TGN/EE via host 26S proteasome (85, 86). Due to its association with TGN/EE, MIN7 is thought to control vesicle trafficking of immunity-related cargoes. Further research is needed to clarify the intricate dynamics of MIN7-mediated immune trafficking and how these pathways are manipulated by HopM1.

4.2.3. Reprogramming of multivesicular bodies/late endosomes. Following their endocytic sorting inside TGN/EE, PRR complexes that are not recycled back to the plasma membrane are packaged into MVBs/LEs and targeted to the central vacuole for degradation. The lumen of MVBs/LEs is typically more acidic than that of TGN/EEs. This helps dismantle PRR-ligand complexes so that they can be recycled back to the plasma membrane through TGN/EEs (40). Vacuolar protein sorting associated protein 52 (VPS52) is a component of the Golgi-associated retrograde protein (GARP) complex that mediates transport of cargo from endosomes to the TGN. Recent work has shown that plant VPS52 is targeted by Mp1, an effector found in the salivary secretions of the aphid pest species *Myzus persicae* (92). VPS52 contributes to immunity against aphids through an unknown mechanism, and aphids can reduce VPS52 protein levels to promote infestation. However, the extent to which VPS52-regulated trafficking pathways are manipulated by Mp1 and how this promotes aphid virulence are still unknown (92). Considering the implications of GARP in retrograde trafficking from MVBs/LEs to TGN/EEs, Mp1 could act to perturb PRR recycling, encouraging vacuolar degradation of VPS52 to suppress PRR-triggered immunity.

4.3. Subversion of Plant Autophagy Machinery by Pathogen Effectors

Autophagy is an intracellular degradation pathway that heavily relies on membrane trafficking. Each step of autophagy is executed through the coordinated functions of core ATG proteins. Vesicle transport systems channel proteins and lipids required for autophagosome formation and facilitate the subsequent delivery of autophagosomes to their target compartments (87). A growing number of studies show that selective forms of autophagy contribute to plant immunity (29, 64, 66). Although the mechanisms of defense-related autophagy are poorly understood, the discovery of effectors from various plant pathogens helps our understanding of the mechanisms governing autophagy-related immune responses.

4.3.1. Viral strategies to manipulate autophagy. Viruses are intracellular pathogens that specialize in avoiding host cell autonomous immune responses. In particular, viruses have to escape autophagic clearance to enable plant colonization. An increasing number of viral strategies are being discovered that can counteract, avoid, or co-opt autophagy machinery to undermine host antiviral defenses (64). For example, turnip mosaic virus (TuMV) encodes a protein termed VPg that co-opts autophagy to deplete key components of the host RNA silencing machinery (21). Additionally, along with another TuMV effector 6K2, VPg inhibits NBR1/Joka2-mediated defense-related autophagy (50). A similar mechanism is used by the polerovirus P0 protein to destroy a core component of the host RNA silencing system, although the mechanism behind this process is still not well understood (26). Rice stripe virus (RSV) has also been shown to co-opt host

autophagy machinery. RSV encodes a movement protein called NSvc4 that promotes autophagic depletion of host membrane proteins, which restricts viral spread (41).

Some viruses employ an alternative strategy and have specialized to suppress host autophagy. For instance, cauliflower mosaic virus (CaMV) encodes a protein termed P6 to counteract defense-related autophagy by promoting the activity of the autophagy suppressor target of rapamycin (TOR) kinase (139). Another example is the barley stripe mosaic virus (BSMV), which produces two proteins that are known to interfere with autophagy: γa and γb. The γa protein undermines the acidification of the vacuolar lumen and thus indirectly suppresses autophagic degradation (130). In contrast, the γb protein inhibits autophagy by targeting ATG7, a protein that is central to autophagy initiation (131). These studies highlight the vast number of mechanisms that viruses have evolved to avoid depletion by the host autophagy machinery. That said, this is likely just the tip of the iceberg, given the vast number of viruses that can infect the plants.

4.3.2. Reprogramming of autophagy by filamentous plant pathogens. Autophagy is also implicated in defense against filamentous plant pathogens. For example, a defense-related autophagy pathway mediated by the autophagy cargo receptor Joka2/NBR1 targets the EHM during *P. infestans* infection (28). *P. infestans* has evolved an RXLR effector called PexRD54 that counteracts Joka2/NBR1. PexRD54 carries an AIM that binds to the host ATG8 variant ATG8CL with a higher affinity than Joka2/NBR1, depleting Joka2/NBR1 from the autophagy machinery. PexRD54 stimulates the formation of autophagosomes, reminiscent of those that are found during starvation-induced autophagy, that are then diverted to the pathogen haustorium (29). Currently, the content of the perihaustorial PexRD54 autophagosomes remains unknown. Plausibly, PexRD54 could activate autophagy to selectively eliminate plant defense molecules and/or uptake host nutrients (28, 29, 87). PexRD54-induced autophagy relies on host vesicle transport machinery, as PexRD54 recruits Rab8a to stimulate autophagosome formation. By subverting Rab8a-mediated vesicle trafficking, PexRD54 is proposed to hijack lipid droplets to facilitate the biogenesis of autophagosomes diverted to pathogen penetration sites. These findings show extensive remodeling of endomembrane trafficking to selectively support autophagosome formation at the host–pathogen interface (87). A different *Phytophthora* species, *Phytophthora parasitica*, deploys another RXLR effector called AVH195 that is proposed to target autophagy to promote virulence. AVH195 interacts with ATG8 proteins from *Arabidopsis* in a yeast two-hybrid system and promotes *P. parasitica* virulence. It also has the activity to slow down autophagic flux in the green alga *Chlamydomonas reinhardtii* (111). These two different examples highlight host autophagy manipulation as a strategy that is favored by *Phytophthora* species during infection.

4.3.3. Bacterial tricks to subvert autophagy. Most bacterial pathogens employ T3Es to modulate host autophagy in various ways (27, 115). *P. syringae* relies on a functional T3 secretion system that dampens basal plant resistance to promote autophagic activity. The previously mentioned *P. syringae* effector HopM1 appears to have autophagy-inducing features during bacterial infection. HopM1 promotes the removal of proteasomes through a selective autophagy process called proteaphagy, which in turn promotes bacterial virulence (115). As HopM1 was originally discovered to deplete MIN7 through the plant proteasome, its function of inducing proteaphagy directly challenges the notion of utilizing proteasomes (85, 115). Nonetheless, defense-related autophagy mediated by NBR1/Joka2 counteracts bacterial pathogenicity and limits the formation of the HopM1-induced water-soaking phenotype (115), highlighting the intricate interplay between autophagy-related plant defenses and bacterial effectors.

There are several other effectors employed by *P. syringae* to co-opt the plant autophagy machinery. For instance, the core autophagy protein ATG8, which is typically produced as an inactive

precursor, is activated by the T3E HrpZ1. HrpZ1 achieves this by enhancing the ATG4-mediated cleavage of negative regulatory residues present at the C-terminus of the ATG8 precursor (60). In contrast, a different *P. syringae* effector, HopF3, targets ATG8 to suppress autophagy, although its molecular mechanism remains obscure. Intriguingly, both HrpZ1 and HopF3 are found to promote bacterial infection despite their opposite impact on host autophagy (60). Another *P. syringae* effector, AvrPtoB, suppresses host autophagy by promoting ATG1 phosphorylation (60).

Other bacterial pathogens also target plant autophagy. The T3E XopL from *X. campestris* promotes proteasomal degradation of SH3P2, a plant protein that positively regulates autophagy. XopL ubiquitinates SH3P2 to promote its depletion. This enhances bacterial infection by preventing the SH3P2-ATG8 interaction required for autophagosome biogenesis (138). As a counter-response, NBR1/Joka2-mediated defense-related autophagy can capture and deplete XopL, revealing a highly dynamic and complex antagonistic interplay between XopL and the host autophagy machinery (67). Collectively, the emerging view is that autophagy is either promoted or suppressed by bacterial effectors to facilitate pathogenesis (60).

4.4. Remodeling of Actin and Microtubule Dynamics by Pathogen Effectors

Almost all types of membrane trafficking, including intracellular vesicle motility, require remodeling of the actin and microtubule cytoskeleton (119). Inevitably, actin and microtubule cytoskeleton rearrangement is essential for polarized immune responses that are diverted to pathogen penetration sites (30, 69).

How plant pathogens remodel the host cytoskeleton remains relatively less understood (62). Nevertheless, several strategies employed by pathogens to evade host defenses by modifying the cytoskeleton have been identified. For instance, the *Verticillium dahliae* toxin can induce changes in the organization of actin microfilaments (132). *P. syringae* T3E HopW1 promotes bacterial virulence by inhibiting actin-dependent endocytosis and other membrane-trafficking processes by reducing filamentous actin (F-actin) (57). Another bacterial T3E from *X. campestris*, termed XopR, carries potential actin-binding motifs (39). XopR is implicated in cross-linking of F-actin and antagonizing actin-depolymerizing factors by competing for F-actin binding. Thus, XopR can hijack and subvert the host actin cytoskeleton, possibly to divert vesicle trafficking and support bacterial virulence (109).

Other T3Es, such as HopZ1 and HopE1 from *P. syringae*, were found to manipulate microtubules to promote infection. HopZ1a is an acetyltransferase that can acetylate tubulin and lead to deconstruction of the host microtubule network. On the other hand, HopE1 purges the host microtubule-associated protein 65 (MAP65) from microtubules. Both effectors can inhibit protein secretion and compromise cell wall–based defenses (47, 65). In addition, nematode effectors also target plant microtubules to promote infection. An effector called SPRY-414-2 from the white potato cyst nematode *Globodera pallida* was identified to target a potato microtubule-associated protein named cytoplasmic linker protein-associated protein (CLASP) that is involved in microtubule stability and growth (78).

These studies highlight extensive strategies used by plant pathogens to reprogram the host cellular transport mechanisms that rely on actin and microtubule dynamics. Nevertheless, how these processes specifically alter membrane trafficking events remain undetermined.

4.5. Diversion of Trafficking Pathways Targeted to the Haustorium

Cell biology studies in infected cells have revealed that effectors operate at various host cell compartments. An interesting group of oomycete effectors, termed perihaustorial effectors,

specifically target the EHM. Perihaustorial effectors have been of particular interest because they have the potential to probe focal immune responses targeted at the pathogen interface. Indeed, proteomics screens revealed that two perihaustorial effectors, AVRblb2 and AVR1, interfere with defense-related secretion, although the exact mechanisms involved remain unknown (14, 120). Other perihaustorial effectors include PexRD54, which counteracts defense-related autophagy, and PexRD31, which associates with a host v-SNARE (29, 89). The studies on perihaustorial effectors have begun to shed light on plant focal immune responses. Surely, developing a better understanding of these cellular trafficking pathways would enable novel strategies to tackle filamentous plant pathogens.

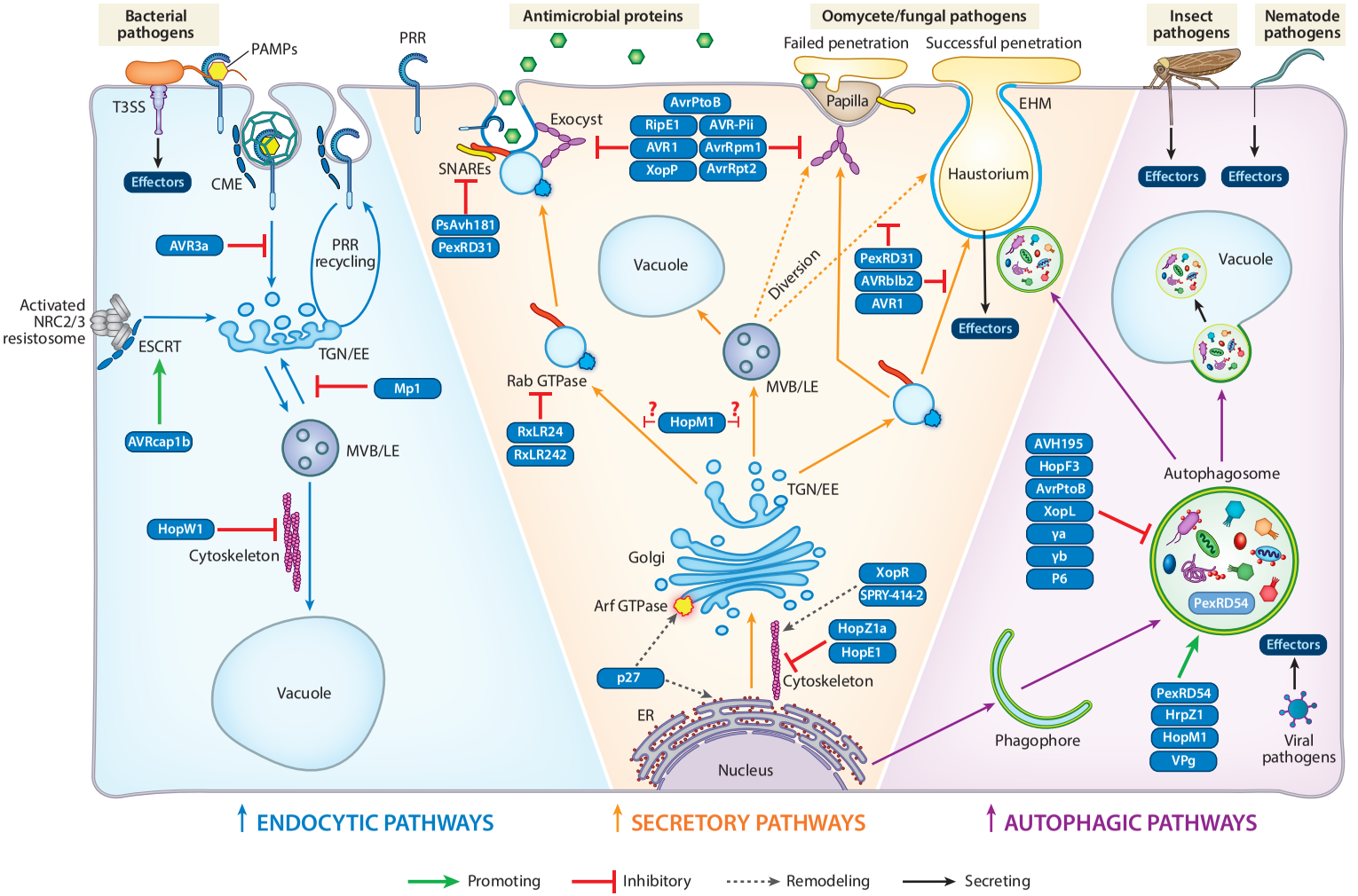
5. OUTLOOK

Many studies have revealed that plant transport pathways are extensively manipulated by the pathogens and pests that threaten our food supply (**Figure 3**). It is often difficult to determine the specific immune-related roles of trafficking components via standard genetic approaches, as many transport regulators show redundancy and are implicated in multiple trafficking routes. Another issue is that secretory systems required for growth are often co-opted by immune responses. That said, effectors present an alternative tool to study the cell biology of plant–pathogen interactions by guiding us toward the most critical components of defense-related trafficking in plants. A deeper understanding of these processes is essential to fully dissect the mechanisms employed by pathogens to subvert the plant immune system. This knowledge is crucial for the targeted breeding of crops that are more resilient to pests and pathogens, an ever-growing threat to food security in this era of climate change.

6. OUTSTANDING QUESTIONS

The recent surge of research in effector biology has only begun to uncover the intricate interplay between diverse membrane trafficking events and plant immunity. Indeed, many crucial questions remain about the specific roles of membrane trafficking in plant–pathogen interactions:

- Do endomembrane trafficking processes promote plant immune signaling? If so, how do pathogens manipulate it?
- Do pathogens deploy effectors to divert host membrane trafficking pathways to uptake host nutrients?
- To what extent does the targeting of activated NLR resistosomes rely on membrane trafficking? Is this targeted by pathogens?
- Do pathogens encode effectors to perturb defense-related trafficking of organelles such as chloroplasts, mitochondria, and peroxisomes?
- To what extent do pathogens remodel the host–pathogen interfaces, such as the EHM, for their own benefit?
- Do effectors target membrane contact sites between organelles and pathogen interface membranes?
- What is the fate of plasma membrane–targeted vesicles that are inhibited by effectors?
- To what extent do filamentous pathogens rely on host endomembrane trafficking for translocating effector proteins?
- What are the origins and contents of the vesicles observed at extracellular space and the extrahaustorial matrix? Do pathogens alter the content of plant-derived exosomes?



(Caption appears on following page)

Figure 3 (*Figure appears on preceding page*)

A summary of plant immune-related membrane trafficking pathways targeted by pathogen effectors. Immune-related membrane trafficking pathways in plants can be broadly classified into endocytic, secretory, and autophagic pathways. In the endocytic pathway, ligand-free plasma membrane (PM)-localized pattern recognition receptors (PRRs) go through constitutive endocytosis and recycling to maintain PRR homeostasis at the PM. Ligand-bound PRRs undergo clathrin-mediated endocytosis (CME), during which the cargoes pass through the *trans*-Golgi network/early endosome (TGN/EE) and multivesicular bodies/late endosomes (MVBs/LEs) before entering the vacuole for degradation. Pathogens are known to secrete effectors that inhibit endocytosis of PRRs and their shuttling between TGN/EE and MVBs/LEs. It has also been shown that the effector AVRcap1b influences the endosomal sorting complex required for transport (ESCRT) machinery to suppress the activity of activated immune receptors. In the secretory pathway, antimicrobial cargoes and PRRs are produced in the endoplasmic reticulum (ER) and transported through the Golgi and TGN/EE route, eventually reaching their destination via secretory vesicles to fuse with the plasma membrane/pathogen interface, e.g., the extrahaustorial membrane (EHM). Although there are effectors that target almost every step of the secretory pathway, many effectors appear to converge on the exocyst complex, which mediates vesicle tethering at the cell surface. There are also other effectors that appear to interfere with vesicle fusion by targeting soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs). By targeting these secretory pathways, these effectors prevent focal deployment of antimicrobial components and callose, inhibiting the formation of cell wall appositions called papillae. Vacuole-targeted MVBs/LEs can be diverted to the plasma membrane/pathogen interface during some infections. Finally, in the autophagic pathway, double-membraned autophagosomes are believed to have originated from the ER through an intermediate membrane called the phagophore. Normally, during autophagy, double-membrane autophagosomes fuse with the vacuole for cargo degradation. However, effectors can activate, suppress, or even change the cargo content of the autophagosomes during infection. These are then diverted to the EHM instead of the default vacuolar pathway. Effectors that do not have evidence of in vivo interaction with their potential host targets are not shown in this figure. Abbreviations: PAMPs, pathogen-associated molecular patterns; T3SS, type III secretion system.

DISCLOSURE STATEMENT

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