



Plant Exocytic Secretion of Toxic Compounds for Defense

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In contrast to animals, plants do not have a circulatory system as well as mobile immune cells that allow them to protect themselves against pathogens. Instead, plants exclusively depend on the innate immune system to defend against pathogens. As typically observed in the animal innate immunity, plant immune responses are composed of pathogen detection, defense signaling which includes transcriptional reprogramming, and secretion of antimicrobial compounds. Although knowledge on recognition and subsequent signaling of pathogen-derived molecules called elicitors is now expanding, the mechanisms of how these immune molecules are excreted are yet poorly understood. Therefore, current understandings of how plants secrete defense products especially via exocytosis will be discussed in this review.

Key words: Plant innate immunity, Secretory pathway, Exocytosis, SNARE

INTRODUCTION

Due to a lack of the circulatory system and mobile immune cells such as B and T cells, plants depend on the innate immunity to potential pathogens. In order to activate immune responses, similarly to animals plants utilize the pattern-recognition receptors (PRRs) to recognize pathogen-associated molecular patterns (PAMPs) (1-3). PRRs such as FLS2, EFR and LYM1/3 are known to recognize bacterial flagellin, elongation factor Tu and peptidoglycan, respectively (4-6). Other PRRs include CERK1, Eix1/2 and Ve1 which are used for detecting fungal chitin, xylanase and Ave1, respectively (7-9). These PAMP-PRR interactions result in transcriptional re-programming in host plants by intracellular

signaling via MAPK cascade and WRKY/TGA transcription factors (10). In addition to the above mentioned surface PRRs, plants also possess intracellular immune receptors called Resistance (R) proteins that can recognize effectors which are transported from the pathogens into host plant cells to interfere with plant immune responses (1,2). Compared to the PAMP-triggered immunity (PTI), the effector-triggered immunity (ETI) is more heightened because the latter is often accompanied by host plant cell death to restrict the pathogen spread (1,2). Some R proteins have been reported to translocate into the nucleus depending on the presence of effectors, which is critical for their immune activity (11-13). This may explain why the ETI response is faster than PTI likely by bypassing the cytoplasmic signaling processes.

As the result of immune signaling, plants produce antimicrobial molecules in the form of peptides and/or secondary metabolites. Although many of these antimicrobial molecules are detected in the extracellular space in plants (14,15), how they are secreted to repel the invading pathogens is not clear yet. Best studied are pathogenesis-related (PR) proteins that are known to be secreted to function in plant immunity. However, over-producing a single PR failed to enhance plant defense against pathogens, strongly suggesting that the effective plant immunity requires the collaboration of various PR proteins possibly working together with pathogen-toxic compounds. It is so far known that two distinct secretory pathways are involved in plant immune responses. One pathway is mediated by the atypical PEN2 myrosinase and the plasma membrane (PM)-residing PEN3

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Abbreviation: ARF-GEF, ADP ribosylation factor-guanine nucleotide exchange factor; BFA, brefeldin A; ETI, effector-triggered immunity; PAMP, pathogen-associated molecular pattern; PM, plasma membrane; PR, pathogenesis-related; PRR, pattern-recognition receptors; PTI, PAMP-triggered immunity; R, resistance; SM, Sec1/Munc-18; SNARE, soluble N-ethylmaleimide sensitive factor attachment protein receptor; SYP, syntaxin of plant; VAMP, vesicle-associated membrane protein

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ATP-binding cassette transporter (16,17). PEN2 has been suggested to generate glucosinolate derivatives that are toxic to fungi (18). The overlapping immune activity between PEN2 and PEN3 to fungal pathogens revealed by genetic studies suggests PEN3 as a transporter of PEN2-generated fungal toxin(s). The other pathway is the soluble *N*-ethylmaleimide sensitive factor attachment protein receptor (SNARE) protein-associated exocytosis. Although the cargo transported by this pathway is yet to be revealed, biochemical, cell biological and genetic studies indicate that the PM-localized PEN1 syntaxin, SNAP33 adaptor and vesicle-associated membrane protein (VAMP) 721/722 drive the immune exocytosis by forming the SDS-resistant ternary SNARE complex (19,20). Since the exocytic pathway can deliver diverse molecules via secretory vesicles compared to the PEN2-PEN3 pathway, the SNARE-mediated secretory pathway will be focused in this review.

SNAREs in plant exocytosis-associated immunity. Due to the subcellular compartmentalization, eukaryotic cells utilize vesicles for transporting required materials between organelles. For this delivery process, SNARE proteins are used because they can drive membrane fusion events (21,22). SNARE proteins are grouped into target membrane (t)- and vesicle (v)- SNAREs depending on their localization or into glutamine (Q)- and arginine (R)-SNAREs by their conserved central amino acid (23,24). Q-SNAREs can be further classified into Qa-, Qb-, Qc- and Qb+c-SNARE by their sequence similarities (25). During exocytosis (vesicle fusion with the PM), Qa-SNARE (also called syntaxin) and SNAP-25 (Qb+c-SNARE containing two SNARE motifs) at the PM form the SNARE complex containing a four antiparallel helical bundle with R-SNARE (also called v-SNARE or VAMP) anchored in vesicular membrane (21,22).

The key role of exocytic activities in plant immune responses has been understood based on indirect evidence. Analysis of transcriptome induced by the plant defense hormone, salicylic acid (SA), revealed that genes involved in ER chaperoning and protein secretion were up-regulated by the SA receptor NPR1 (26). Mutational approaches on those genes suggested that protein secretory pathway is critically important for plant immune responses, because mutant plants showed reduced export of PR1 protein and impaired resistance to the phytopathogenic *Pseudomonas syringae* bacterium (26). *P. syringae* is known to contain about 20 effectors to disturb the host plant immunity (27). Among them, HopM1 was reported to interact with the ADP ribosylation factor-guanine nucleotide exchange factor (ARF-GEF) MIN7 protein which is regarded to function in vesicle trafficking (28). Degradation of MIN7 protein by HopM1 and the compromised defense in the *min7* mutant plants against *P. syringae* (28) also indicate the importance of protein secretion in plant

immune responses.

The first SNARE protein required for plant immunity is the PEN1 PM syntaxin which was identified by screening *Arabidopsis* mutants that showed impaired resistance to powdery mildew fungi (19). Subsequent biochemical approaches revealed that PEN1 is engaged in plant extracellular immunity via exocytosis with SNAP33 and VAMP721/722 by forming the SNARE complex (20). The contribution of same SNARE proteins in barley to defense against powdery mildew fungi (20) indicates that this exocytic pathway is an ancient secretory system in both mono- and dicotyledonous plants. In tobacco, syntaxin of plant (SYP) 132 that is another PM syntaxin was reported to play a role in defense against bacterial pathogens (29). Interestingly, both PEN1 and SYP132 preferentially interact with VAMP721/722 (30). However, the compromised resistance to bacteria in tobacco plants lacking of SYP132 but not PEN1 (29) suggests that plants utilize distinct PM syntaxins for immunity to different pathogen types.

A default or immune secretory pathway. Dispensability of a PEN1 homolog, SYP122 in defense against fungal pathogens (31,32) indicates that PEN1 syntaxin is specialized for immunity to those pathogens. However, the *pen1 syp122* double mutant plants exhibit retarded growth phenotype (31,33), suggesting that both syntaxins are involved in plant growth. It was reported that SYP132 is also required for plant growth because deletion or silencing of *SYP132* gene resulted in lethality or dwarfism, respectively (29). All these results suggest that PEN1, SYP122 and SYP132 are involved in a default secretory pathway to govern plant growth as t-SNARE proteins in the PM to receive secretory vesicles. The PM-localized SNAP33 (Qb+c-SNARE) was originally identified as an interacting partner of the KN (SYP111) syntaxin that is localized in the phragmoplast (a scaffold for cell plate formation) and required for cell division in plants (34). However, its co-expression and co-localization with PEN1 in leaves, and *in vitro* and *in planta* specific interactions with PEN1 (20) indicate that SNAP33 is also engaged in the PEN1-associated secretory immunity. Interestingly, deletion of *SNAP33* gene leads to seedling lethality (34). This suggests that SNAP33 also plays an important function in plant growth as a t-SNARE protein. VAMP721/722 that are localized in vesicles specifically interact and form the SNARE complex with PEN1 and SNAP33 *in vitro* and *in planta* (20). Although VAMP721/722 together with PEN1 and SNAP33 comprise an immune exocytic pathway, they are also essential for plant growth because VAMP721/722-depleted plants exhibit dwarfism or even lethality (20,35).

Since all PEN1/SYP122/SYP132 PM syntaxins, SNAP33 and VAMP721/722 are required for plant growth, they most likely form the default secretory pathway in plants. Although the exact nature of cargo transported by this pathway is not

known, their growth-related mutant phenotypes implicate that vesicles-containing growth-associated proteins and/or lipids might fuse with the PM by the PEN1/SYP122/SYP132-SNAP33-VAMP721/722 SNARE complexes. The randomly scattered fluorescent signals in healthy transgenic plants expressing functional GFP-VAMP722 (20) suggest that VAMP721/722 vesicles non-directionally deliver growth molecules, although it is unknown whether there is a biased interaction of VAMP721/722 with any of the above mentioned PM syntaxins. Upon infection, the GFP-VAMP722 vesicles in transgenic plants are however redistributed to directionally move towards pathogen attacking sites (20). It was reported that when challenged by pathogens many sub-cellular organelles in plant cells are accumulated beneath pathogen contacting sites, accompanied by cytoskeletal remodeling (36,37). Therefore, it is likely that the rearranged movement of GFP-VAMP722 vesicles in infected plant cells might be also caused by cytoskeletal reorganization. This targeted redistribution of organelles including vesicles to pathogen attempting sites suggests that plants put efforts in trying to defend against pathogens by concentrating intracellular compartments possibly to reduce the time and distance for producing and transporting immune molecules.

Regulation of the plant exocytic immunity. Although SNARE proteins can promiscuously form the SNARE complex *in vitro* (38,39), their *in vivo* interactions are tightly and specifically regulated. Transient expression assays of fluorescently tagged SNARE proteins revealed that they are distinctly localized in different subcellular compartments in Arabidopsis (40). This suggests that the fusogenic specificity of SNARE proteins in vesicle trafficking can be determined by their localization patterns. It was found that SYP122 fails to replace the PEN1 function in plant immunity (31,33). Recently, it was also reported that SYP132 but not PEN1 can substitute for the KN activity in cell division (41). Since PEN1 forms the SNARE complex with members belonging to the VAMP72 but not VAMP71 group (20), this further suggests that the specificity of some plant SNARE proteins resides in themselves.

The protein-interacting activity of syntaxin is known to be self-regulated. Three alpha helices in the N-terminal region of syntaxin can interact intramolecularly with its SNARE domain, which hinders its complex formation with other SNARE proteins and therefore is called a closed conformation (21,22). Sec1/Munc-18 (SM) protein by binding to a syntaxin with this closed conformation can control the SNARE complex formation, and eventually regulate vesicle fusion (21,22). A PEN1 variant that cannot form the closed conformation was reported not to be able to completely complement the *pen1* mutant phenotype (32), which strongly suggests a similar function of SM protein in plant immune exocytosis. The KEULE, which was originally

identified as an Arabidopsis SM protein that controls the KN activity in cell division (42,43), was recently found to control the PEN1 interaction with SANP33 and VAMP721 (44). This suggests that KEULE may have a dual function in both cytokinesis and immunity in plants, although its immune activity remains to be tested.

Rab small GTPase is regarded as a specificity controller of vesicle trafficking, which functions by tethering vesicles to specific target membranes (45). In plant exocytic immunity, the PEN1 accumulation at pathogen attacking sites is critically important for focal discharge of immune molecules. Indeed, treatment of brefeldin A (BFA) which is an inhibitor of vesicle trafficking results in compromised resistance to powdery mildew fungus accompanied by lack of PEN1 deposition at fungal attempting sites (46). Since PEN1 was reported to continuously cycle between the PM and endocytosed vesicles (41), it is suggested that upon infection the PM-retrieved PEN1 is relocalized focally to pathogen contacting sites. Interestingly, this phenomenon found in the BFA-treated plants was also observed in plants lacking the plant specific ARFA1b/1c Rab GTPases or the plant unique GNOM ARF-GEF (46,47). Therefore, it is suggested that the ARFA1b/1c aided by GNOM helps PEN1 to be focally accumulated underneath pathogen attacking sites likely for effective and undiluted secretion of defense molecules. The requirement of GNOM for plant growth (48) further suggests that it may control the destination of PEN1 either for plant growth or immunity likely by regulating the PEN1 location.

CONCLUSION

The exocytic pathway mediated by the PEN1/SYP122/SYP132-SNAP33-VAMP721/722 SNARE complexes plays a fundamental role in both plant growth and immunity (Fig. 1). The key question is how the same secretory pathway can be involved in at least two different physiological processes. KEULE and GNOM that were originally identified as plant growth regulators (42,48) are now known to regulate the complex-forming activity and membrane localization of PEN1 (44,46), which hints how plants co-opt the default secretory pathway for immunity. Likewise, isolation of the above mentioned SNAREs-interacting proteins and subsequent understanding of their biological functions can broaden our knowledge on how to regulate this growth/immune exocytic pathway. Delayed deposition of callose, beta-1,3-glucan, at fungal attacking sites in PEN1- or VAMP721/722-depleted plants (20,31) suggests that a cell wall constituent or modifier might be delivered within the VAMP721/722 vesicles, although the exact immune activity of callose is still unclear. Therefore, to identify the cargo transported via VAMP721/722 vesicles would be an alternative way to understand how the same exocytic pathway can engage in both plant growth and immunity.

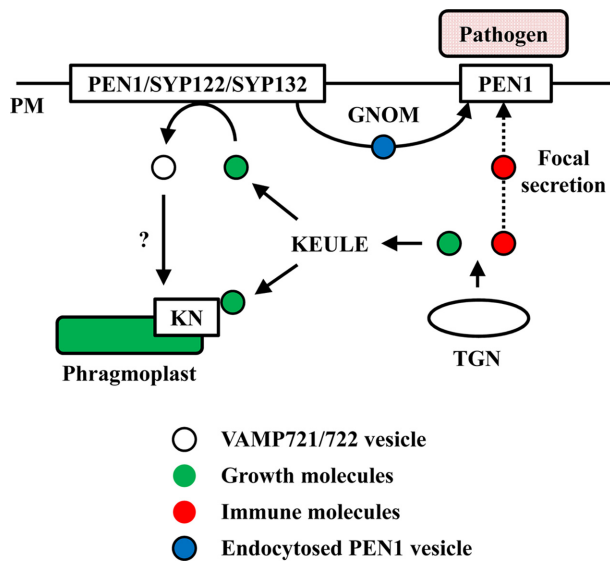


Fig. 1. Versatile physiological activities of the PEN1/SYP122/SYP132-SNAP33-VAMP721/722 secretory pathway. VAMP721/722 vesicles containing growth-associated molecules (green) move either non-directionally to the PM or directionally to phragmoplast. The KEULE SM protein by controlling the SNARE complex-forming activity may determine the vesicle destinations. However, immune molecules (red) are targeted to pathogen attacking sites within VAMP721/722 vesicles along the rearranged cytoskeleton. For focal secretion of immune molecules, PEN1 proteins are relocalized to pathogen attempting sites via endocytosed vesicles (blue), which is mediated by GNOM. PM, plasma membrane; TGN, trans-Golgi network.

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