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Functions of the Plant Qbc SNARE SNAP25 in Cytokinesis and Biotic and Abiotic Stress Responses

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Eukaryotes transport biomolecules between intracellular organelles and between cells and the environment via vesicle trafficking. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE proteins) play pivotal roles in vesicle and membrane trafficking. These proteins are categorized as Qa, Qb, Qc, and R SNAREs and form a complex that induces vesicle fusion for targeting of vesicle cargos. As the core components of the SNARE complex, the SNAP25 Qbc SNAREs perform various functions related to cellular homeostasis. The *Arabidopsis thaliana* SNAP25 homolog AtSNAP33 interacts with Qa and R SNAREs and plays a key role in cytokinesis and in triggering innate immune responses. However, other *Arabidopsis* SNAP25 homologs, such as AtSNAP29 and AtSNAP30, are not well studied; this includes their localization, interactions, structures, and functions. Here, we discuss three biological functions of plant SNAP25 orthologs in the context of AtSNAP33 and highlight recent findings on SNAP25 orthologs in various plants. We propose future directions for determining the roles of the less well-characterized AtSNAP29 and AtSNAP30 proteins.

Keywords: abiotic stress responses, cytokinesis, innate immune response, Qbc SNARE, SNAP25

INTRODUCTION

Vesicle trafficking is a fundamental mechanism for maintaining cellular homeostasis in eukaryotes. Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins play an essential role in vesicle trafficking by participating in vesicle fusion at the membrane. Distinct combinations of SNARE proteins allow for many different types of cargo to travel to specific subcellular locations. SNARE proteins are classified as Q SNAREs or R SNAREs depending on the contributing residue (glutamine or arginine, respectively) in the structurally assembled core SNARE complex. Q SNAREs are further categorized as Qa, Qb, and Qc, depending on their location in the four-helix bundle (Bock et al., 2001), which forms the core SNARE complex. The first identified SNARE complex, which is involved in synaptic vesicle exocytosis, forms a parallel four-stranded coiled-coil structure composed of a helix of syntaxin 1A, two helical domains of SNAP25, and a helix of vesicle-associated membrane protein 2 (VAMP2) (Hayashi et al., 1994; Niemann et al., 1994).

The first cDNA encoding SNAP25 was identified in mouse brain (Oyler et al., 1989). Five years later, human *SNAP25* was cloned and sequenced (Zhao et al., 1994), and other *SNAP25* homologs, *SNAP23*, *SNAP29*, and *SNAP47*, were subsequently identified (Ravichandran et al., 1996; Steegmaier et al., 1998; Holt et al., 2006). SNAP25 functions in synaptic vesicle trafficking by interacting with syntaxin1A and

VAMP2 (Hayashi et al., 1994; Niemann et al., 1994). Moreover, SNAP25 functions in cell division as a subunit of the trafficking machinery along with syntaxin 2 and VAMP8, a component that facilitates abscission of the midbody (Gromley et al., 2005). Enrichment of SNAP25 at the midbody was also observed in a study on zebrafish embryos (Li et al., 2006). These reports indicated that SNAP25 proteins are essential for the final stages of cytokinesis during cell division. SNAP23 proteins play a pivotal role in immune cells by mediating the secretion of cytokines, antibodies, and granules (Pagan et al., 2003; Reales et al., 2005; Mollinedo et al., 2006). SNAP29 interacts with syntaxin17 (STX17) and VAMP8 to form a SNARE complex, which mediates the fusion of autophagosomes and lysosomes (Itakura et al., 2012; Takats et al., 2013). The characterized animal SNAP25 homologs are essential for overall cellular homeostasis.

The *Arabidopsis thaliana* genome encodes 65 putative SNARE proteins, considerably more than in other eukaryotes (35 in humans, 21 in *Saccharomyces cerevisiae*, and 20 in *Drosophila*) (Saito and Ueda, 2009). Similar to *Arabidopsis*, plants encode multiple SNARE proteins (70 in *Oryza sativa*, 129 in *Brassica rapa*, and 151 in *Glycine max* [Phytozome, Plant Genome Resource]). *Arabidopsis* SNARE proteins localize in distinct subcellular organelles and are involved in various cellular functions, including development, gravitropism, pathogen responses, and abiotic stress responses (Yano et al., 2003; Heese et al., 2001; Kwon et al., 2008; Pajonk et al., 2008; Zhu et al., 2002). Several *Arabidopsis* SNARE proteins show functional redundancy but form distinct complexes depending on the cellular environment. For example, VACUOLAR PROTEIN SORTING 10-INTERACTING 11 (VTI11) and VTI12 are involved in trafficking of the marker VAC2 (composed of CLAVATA3 fused to a vacuolar sorting signal from a barley [*Hordeum vulgare*] lectin); however, VTI11 and VTI12 mediate trafficking to lytic and storage vacuoles, respectively, through their SNARE partners (Surpin et al., 2003; Sanmartin et al., 2007). Therefore, determining the interactions and locations of SNARE proteins is crucial to identifying their functional mechanisms.

Here, to examine plant SNAP25 proteins, we first generated a phylogenetic tree of plant SNAP25 proteins based on the Qbc SNARE motifs in *A. thaliana*, *B. rapa*, *Capsicum annuum*, *Solanum lycopersicum*, *Solanum tuberosum*, *G. max*, *Medicago truncatula*, *O. sativa*, and *Marchantia polymorpha*. These proteins were analyzed with BLAST using the full-length amino acid sequence of AtSNAP29/30/33 as a query. SNAP25 proteins from *Glycine soja*, *H. vulgare*, *Triticum aestivum*, *Gossypium hirsutum*, and *Cynanchum komarovii* were only listed when functional reports were available in the literature (Fig. 1, Table 1). Using this framework, we discuss recent findings regarding SNAP25 homologs in various plants and their molecular roles in protein–protein or environmental interactions. We also propose future directions for studies on less well-characterized SNAP25 homologs in plants.

STRUCTURE OF THE HUMAN SNAP25 PROTEIN

In general, SNARE proteins have a transmembrane domain and an α-helical coiled-coil domain termed the "SNARE domain", which forms part of the SNARE complex (Weimbs et al., 1997). The coiled-coil domains in SNARE proteins twist together to induce the fusion of vesicles and membranes. Unlike general SNARE proteins, SNAP25 proteins consist of two SNARE domains, namely Qb and Qc, and a linker (Weimbs et al., 1997). The N-terminal Qb SNARE domain is connected via a linker region to the C-terminal Qc SNARE domain of the SNAP25 protein (Fig. 2).

SNAP25 homologs have no transmembrane domains; therefore, it is unclear how SNAP25 homologs localize to the cellular membranes. One possibility is that lipid modifications allow the SNAP25 proteins to associate with membranes. For example, PtSNAP25 from *Paramecium tetraurelia* contains a myristoylation site for membrane attachment (Schilde et al., 2008). Moreover, mammalian membrane-targeted SNAP25 is palmitoylated at a cysteine residue in the linker region (Gonzalo and Linder, 1998; Gonzalo et al., 1999). A *snap25* mutant lacking the cysteine site showed a slower rate of synaptic vesicle fusion in mouse cells than the wild-type protein (Nagy et al., 2008). Recovery of the cysteine residue in the linker region of SNAP23 complemented the *snap25* mutant with respect to the rate of fusion in synaptic vesicles (Nagy et al., 2008). However, the mammalian SNAP25 homologs SNAP29 and SNAP47 localize to subcellular membranes even without the cysteine residue; thus, these homologs cannot complement the function of SNAP25 in *snap25* mutants (Arora et al., 2017).

Similar to SNAP29 and SNAP47, plant SNAP25 homologs have a linker region lacking the cysteine residue. This indicates that plant SNAP25 homologs use another mechanism, possibly fatty acid modification, for cell membrane attachment. However, to our knowledge, no structural studies on plant SNAP25 homologs have been reported and further studies are needed to determine how plant SNAP25 localizes to the cellular membrane, and how it forms complex structures with other SNARE proteins.

MOLECULAR FUNCTIONS OF SNAP25 FAMILY PROTEINS IN PLANTS

Cytokinesis

The mechanism of cytokinesis in plants differs from that in animals. An animal cell is divided by cytoplasmic abscission through the formation of a cleavage furrow (Cao and Wang, 1990; Mierzwa and Gerlich, 2014), whereas a plant cell is divided by the formation of a cell plate through vesicle fusion and concomitant formation of vesicular-tubular structures (Ahn et al., 2017). Plants contain a Qa SNARE, a cytokinesis-specific syntaxin termed SYP111 (SYNTAXIN OF PLANTS 111)/KNOLLE (meaning tuber-shaped in German), which localizes to the Golgi stacks and plasma membranes, and is involved in the fusion of vesicles from the Golgi to the center of the dividing cell (Lauber et al., 1997; Volker et al., 2001). The delivered vesicles and *de novo*-synthesized tubular structures fuse and expand toward the parental plasma membrane to form the cell plate. The *Arabidopsis* SNAP25 homolog AtSNAP33 is the principal interacting partner of KNOLLE/SYP111 together with AtVAMP721 (Heese et al., 2001). *Arabidopsis snap33* mutants show severe necrotic cotyledons
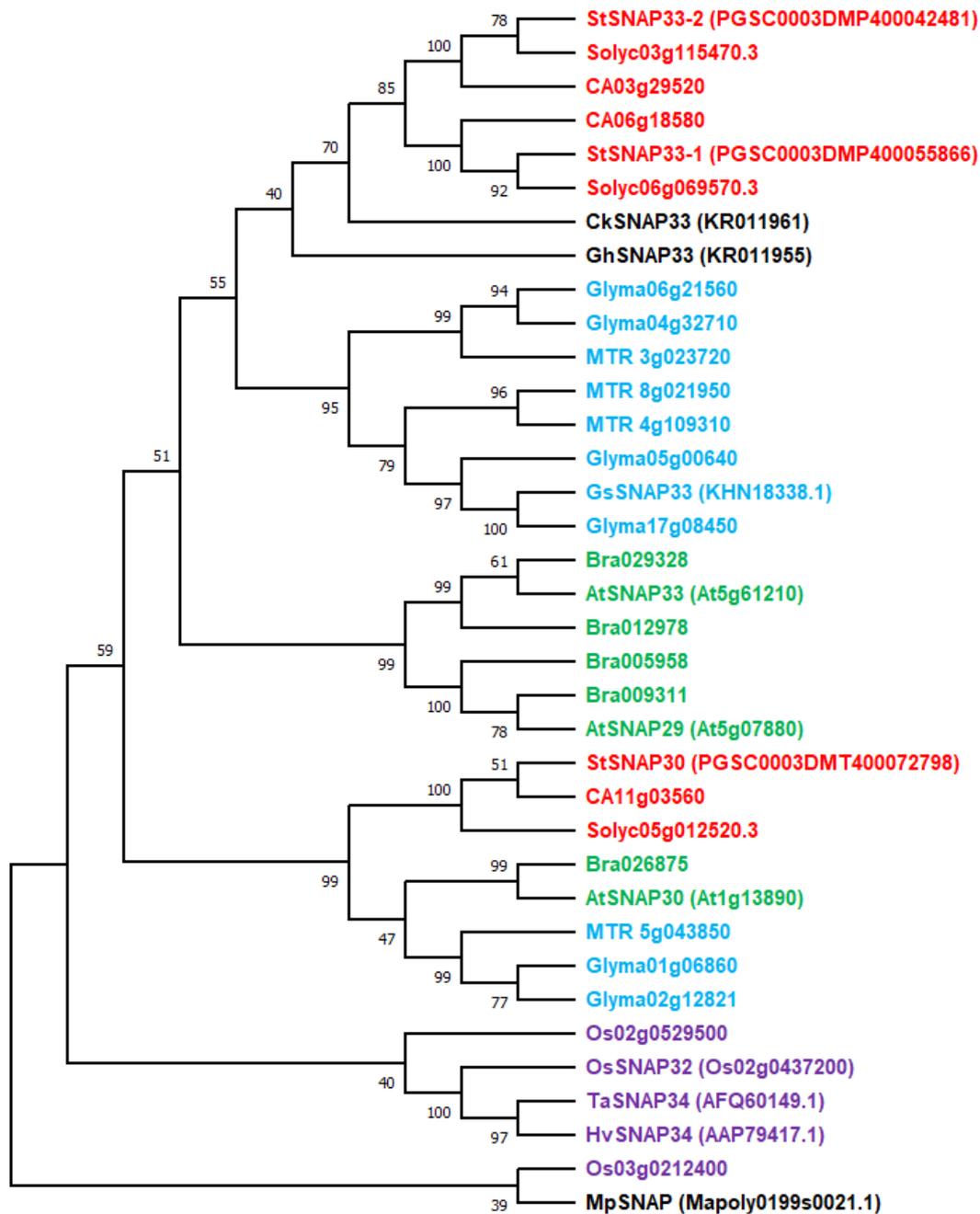
Fig. 1. Phylogenetic tree of SNAP25 proteins in plants. Phylogenetic analysis was performed using MEGAX software (Kumar et al., 2018) with full amino acid sequences of the retrieved SNAP25 proteins from public databases; UniProt, GenBank, Sol Genomics, and Phytozome, and additionally only functionally studied SNAP25 proteins from *G. soja*, *H. vulgare*, *T. aestivum*, *G. hirsutum*, and *C. komarovii*. The bootstrap values from 1000 replicates are depicted at the nodes. Different colors indicate the following: green, *Brassicaceae*; red, *Solanaceae*; blue, *Fabaceae*; violet, *Poaceae*; black, other functionally reported SNAP25 proteins. All information in the tree is described in Table 1.

and a seedling-lethal phenotype (Heese et al., 2001). A genetic study on *snap33 npsn11* (NPSN11, Novel Plant SNARE 11) and *snap33 syp71* double mutants, along with mutants of other cytokinesis-specific SNARE proteins, revealed a major cytokinetic defect with dividing cells showing abnormal morphologies (El Kasmi et al., 2013). Seminal studies showed

that AtSNAP33 plays a critical role in cytokinesis. Heterologously expressed *CkSNAP33* from *C. komarovii* in *Arabidopsis* transgenic plants promotes growth thereby increasing root length and leaf area (Wang et al., 2017). Direct evidence of increased cell numbers of the root and leaf in *CkSNAP33*-expressing lines suggests that heterologous expression of *Ck*-

Table 1. Plant SNAP25 proteins used to construct the phylogenetic tree

Species	Name (in tree)	Accession No.	Gene ID	ID source
Arabidopsis thaliana	AtSNAP29	Q9SD96	At5g07880	UniProt
	AtSNAP30	Q9LMG8	At1g13890	
	AtSNAP33	Q9S7P9	At5g61210	
Brassica rapa	Bra012978	M4D918	Bra012978	UniProt
	Bra029328	M4EKL1	Bra029328	
	Bra005958	M4CP20	Bra005958	
	Bra009311	M4CYL4	Bra009311	
	Bra026875	M4EDL5	Bra026875	
Glycine max	Glyma04g32710	C6T803	Glyma04g32710	UniProt
	Glyma06g21560	I1KCY1	Glyma06g21560	
	Glyma05g00640	I1JZK8	Glyma05g00640	
	Glyma17g08450	C6TJG5	Glyma17g08450	
	Glyma02g12821	K7K7S4	Glyma02g12821	
	Glyma01g06860	I1J5Y3	Glyma01g06860	
Glycine soja	GsSNAP33	KHN18338.1	KHN18338	GenBank
Medicargo trancatula	MTR_3g023720	KEH33106	MTR_3g023720	GenBank
	MTR_4g109310	KEH31992	MTR_4g109310	
	MTR_5g043850	AES96982	MTR_5g043850	
	MTR_8g021950	AET01821	MTR_8g021950	
Oriza sativa	OsSNAP32	Q5EEP3	AAW82752	UniProt
	OsSNAP29	Q10Q25	Os03g0212400	
Hordeum vulgare	HvSNAP34	AAP79417.1	AAP79417	GenBank
Triticum aestivum	TaSNAP34	AFQ60149.1	AFQ60149	GenBank
Zea mays	Zm00001d019505_P001	A0A1D6HXY8	Zm00001d019505_P001	UniProt
	Zm00001d016686_P002	A0A1D6H9U2	Zm00001d016686_P002	
Capsicum annuum	CA03g29520	CA03g29520	CA03g29520	Sol Genomics
	CA06g18580	CA06g18580	CA06g18580	
	CA11g03560	CA11g03560	CA11g03560	
Solanum lycopersicum	Solyc06g069570.3	Solyc06g069570.3	Solyc06g069570.3	Sol Genomics
	Solyc03g115470.3	Solyc03g115470.3	Solyc03g115470.3	
	Solyc05g012520.3	Solyc05g012520.3	Solyc05g012520.3	
Solanum tuberosum	PGSC0003DMP400055866	PGSC0003DMP400055866	PGSC0003DMP400055866 (StSNAP33-1)	Sol Genomics
	PGSC0003DMP400042481	PGSC0003DMP400042481	PGSC0003DMP400042481 (StSNAP33-2)	
	PGSC0003DMP400049245	PGSC0003DMP400049245	PGSC0003DMP400049245	
Gossypium hirsutum	GhSNAP33	ALD83640.1	KR011955	GenBank
Cynanchum komarovii	CkSNAP33	ALH22085.1	KR011961	GenBank
Marchantia polymorpha	MpSNAP	Mapoly0199s0021.1	Mapoly0199s0021.1	Marchantia

All information regarding SNAP25 protein species, name, accession No., and gene ID was retrieved from publicly available databases; UniProt, GenBank (MpSNAP sequence obtained from Marchandia), Sol Genomics, and Phytozyome. SNAP25 proteins in *A. thaliana*, *B. rapa*, *C. annuum*, *S. lycopersicum*, *S. tuberosum*, *G. max*, *M. truncatula*, *O. sativa*, and *M. polymorpha* were found by BLAST using AtSNAP29/30/33 as a query. The other SNAP25 proteins from *G. soja*, *H. vulgare*, *T. aestivum*, *G. hirsutum*, and *C. komarovii* were only listed when a functional report was available in the literature.

SNAP33 is associated with cytokinesis.

Other SNAP25 homologs, such as the Qbc SNAREs AtSNAP29 and AtSNAP30, also interact with KNOLLE/ SYP111 *in vitro* (Heese et al., 2001). *AtSNAP33* is expressed throughout the plant, whereas *AtSNAP29* and *AtSNAP30* are rather highly expressed in the roots and flowers, respectively (Lipka et al., 2007). However, the functions of AtSNAP29 and AtSNAP30 have not been characterized. AtSYP132 is a Qa SNARE, which interacts with VAMP721/722/724 to form a SNARE complex in growing root hairs, and is involved in cytokinesis in flowering plants (Ichikawa et al., 2014; Park et al., 2018). AtSNAP29 and AtSNAP30, may be involved in the development of specific tissues by interacting with other At-SYPs.

Members of *Fabaceae*, such as *G. max*, *M. truncatula*, and *Lotus japonicus*, contain SYP132 orthologs and various VAMP72 family members, which are involved in symbiosis as well as in delivering cargos to the cell plate in dividing cells (Gavrin et al., 2016; Ivanov et al., 2012; Sogawa et al., 2018). *G. max* has six putative SNAP25 homologs, which are
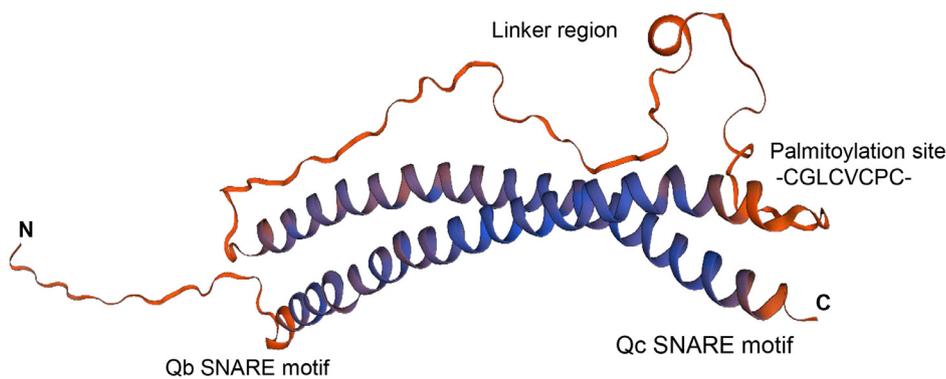
Fig. 2. Structure of SNAP25 in humans. SNAP25 proteins comprise a coiled-coil of the Qb domain at the N-terminus, a linker region, and a coiled-coil of the Qc domain at the C-terminus. Images were prepared by SWISS-MODEL, an automated protein structure homology-modeling server, using SNAP25 protein from humans (UniProtKB - P60880) (Waterhouse et al., 2018).

likely components of the SNARE complex and are involved in symbiosis and cytokinesis (Sharma et al., 2016). Analysis of the *G. max* genome suggests that six members of the GlymaSNAP25 protein family have evolved to have novel functions in symbiosis with environmental microbes and cellular trafficking, in addition to their roles in cytokinesis.

Innate immune responses

Unlike animals, plants do not have an adaptive immune system, but depend on their innate immunity against various pathogens. Secreted defense molecules are crucial for defense against non-host-adapted pathogens and plants primarily secrete compounds associated with defense, such as pathogenesis-related (PR) proteins and secondary metabolites, via SNARE complex-mediated vesicle trafficking. PENETRATION RESISTANCE1 (PEN1)/AtSYP121 is the main syntaxin involved in mediating resistance responses (Collins et al., 2003). Additionally, the PEN2/PEN3 pathway transports phytoalexins to the outside of the cell via a membrane transporter (Lipka et al., 2005; Stein et al., 2006). In response to pathogen infection, *Arabidopsis* increases the expression of *AtSNAP33*, which is regulated by the salicylic acid (SA) pathway (Wick et al., 2003). AtSNAP33 forms SNARE complexes with PEN1/AtSYP121 and VAMP721/722, which deliver undetermined defense cargos to the penetration sites of fungal pathogens (Kim et al., 2014; Kwon et al., 2008; Pajonk et al., 2008). *Arabidopsis pen1* mutants and *VAMP721/722*-silenced mutants were vulnerable to infection by the plant pathogenic fungus *Blumeria graminis* (Kwon et al., 2008; Pajonk et al., 2008).

SNAP25 orthologs play a role in the innate immune response against fungal pathogens in *Arabidopsis* and in monocots, such as *H. vulgare*, *O. sativa*, and *T. aestivum* (Collins et al., 2003; Bao et al., 2008; Luo et al., 2016; Chandra et al., 2017). HvSNAP34 is required for the development of resistance against *B. graminis* penetration by forming ternary SNARE complexes with ROR2 (REQUIRED FOR mlo SPECIFIED RESISTANCE 2), a PEN1 ortholog, and HvVAMP721, an AtVAMP721 ortholog (Collins et al., 2003; Kwon et al., 2008). The expression of *OsSNAP32*, an *O. sativa* SNAP25 ortholog, increases upon infection with the blast fungus *Magnaporthe oryzae*. The *O. sativa* cultivar Suyunuo (a glutinous rice variety) lacks resistance to *M. oryzae*. Overexpression of *OsSNAP32* in blast-susceptible Suyunuo plants increased

resistance to the blast fungus (Luo et al., 2016). Reciprocally, RNA interference (RNAi) lines of the blast-resistant *O. sativa* landrace Heikezijing, with reduced expression of *OsSNAP32* produced more lesions, as observed for the susceptible plants (Luo et al., 2016). *T. aestivum* SNAP25 homologs are also involved in the defense against leaf rust (Chandra et al., 2017). The expression levels of *GhSNAP33* in *G. hirsutum* and *CkSNAP33* in *C. komarovii* increased upon inoculation with the fungal pathogen *Verticillium dahlia*, which shows a broad host range (Wang et al., 2017; 2018). Ectopic expression of *CkSNAP33* and *GhSNAP33* in transgenic *Arabidopsis* plants resulted in increased resistance to *V. dahlia* compared to that observed in wild-type *Arabidopsis* (Wang et al., 2017; 2018).

SNAP25 orthologs also participate in defense against bacterial and oomycete pathogens. For example, the pathogens *Pseudomonas syringae* pv. *maculicola* and *Phytophthora infestans* increase the expression of *StSNAP33* in *S. tuberosum* (Eschen-Lippold et al., 2012). *StSNAP33*-silenced plants exhibited a chlorotic phenotype, enhanced SA concentrations, increased *StPR1* gene expression, and enhanced callose deposition. However, *StSNAP33*-silenced plants did not show greater resistance when inoculated with *P. infestans* and the necrotrophic pathogen *Botrytis cinerea* compared to *StSYP1*-RNAi plants. In contrast, *StSNAP33*-silenced plants showed hypersensitive responses to *Agrobacterium tumefaciens* and *Escherichia coli* (Eschen-Lippold et al., 2012). These results indicate that StSNAP33 uses different modes of action for modulating innate immune responses based on the interacting microbes in the environment.

SNAP25 homologs in *G. max* may have specific roles in the defense against nematodes in roots. Overexpression of *Glyma17g08450* (*SNAP25-3*) in *G. max* increases the resistance to nematodes, whereas RNA interference of *Glyma17g08450* in plants allowed for greater nematode invasion (Sharma et al., 2016).

The AtSNAP33 orthologs in dicots as well as in monocots have conserved functions as a component of a ternary SNARE complex in innate immune responses against environmental pathogens. However, plants have other SNAP25 homologs, such as AtSNAP29 and AtSNAP30, which are differentially expressed throughout the plant and may have evolved to counter distinct pathogens or to trigger immune responses via different SNARE interactions to enable plant survival in varied environments.

Abiotic stress responses

Plants have evolved high-order trafficking mechanisms via numerous SNAREs to maintain cellular homeostasis against environmental stresses. Plant vacuoles and the *trans*-Golgi network function in plant responses to stressful environments. Various endosomal-specific SNARE proteins such as VTI11, SYP22, and SYP51 interact with multiple members in the VAMP71 family, which are involved in directing the vesicle transport to the vacuole and plasma membrane (Ebine et al., 2008; Leshem et al., 2010). These SNARE proteins, such as Qa SNAREs and R SNAREs, are widely involved in endosomal trafficking by carrying cargos containing storage proteins, reactive oxygen species (ROS), and unknown materials to respond to abiotic stresses (Ebine et al., 2008; Leshem et al., 2010). A study on the extreme halophyte *Salicornia brachiate* suggested that a novel salt-inducible gene *SbSLSP* (*S. brachiata* SNARE-like superfamily protein), confers salt and drought tolerance by maintaining membrane stability, and reduces the accumulation of Na$^+$ ion and ROS (Singh et al., 2016).

How SNARE and SNAP25 proteins mechanistically function in abiotic stress responses remains unclear; however, several studies have suggested that they have important roles in mediating these responses. For example, AtSYP61 and AtSYP121 are involved in aquaporin distribution, and *Arabidopsis* SNAP25 proteins may interact with these SNARE proteins during abiotic stress responses (Hachez et al., 2014). *AtVAMP71* suppression in *Arabidopsis* increased water loss and altered the control of stomatal opening/closing via inappropriate ROS localization under drought stress (Leshem et al., 2010). Therefore, SNARE proteins in endosomal compartments may function in mediating resistance to abiotic stresses (Leshem et al., 2010). Exposure to mechanical stresses such as wind and wounding increased the expression of *AtSNAP33* (Wick et al., 2003). Moreover, the expression of *OsSNAP32* increased under drought and cold stress in *O. sativa* (Bao et al., 2008). *Arabidopsis* plants heterologously overexpressing *GhSNAP33* or *GsSNAP33* showed increased drought tolerance (Nisa et al., 2017; Wang et al., 2018). Moreover, the SNARE proteins interacting with SNAP25 homologs in plants such as AtVAMP721/722 and NtSYP121 are regulated by abscisic acid (ABA) for abiotic stress responses. AtVAMP721/722-deficient plants are sensitive to ABA, and the *PEN1/AtSYP121* ortholog in *Nicotiana tabacum*, *NtSYP121*, is regulated by ABA (Kargul et al., 2001; Yi et al., 2013).

How SNAP25 homologs are maintained at appropriate levels in various organelles, such as the *trans*-Golgi network, vacuole, or plasma membrane, during abiotic stress responses is unclear. Additional studies will provide insight into how plants adapt to varied environments with the involvement of their highly expanded families of SNARE proteins.

UNDETERMINED ASPECTS OF PLANT SNAP25

Interacting partners

Work on animal SNAP25s has identified interactions with proteins lacking SNARE motifs; however, whether plant SNAP25s interact with other non-SNARE proteins remains to be determined. For example, during mitosis, the C-terminal SNARE domain of SNAP29 directly recruits kinetochores and supports tissue development in *Drosophila* (Morelli et al., 2016). The eye disks in the *Drosophila snap29* mutant showed defects in epithelial architecture and increased apoptosis (Morelli et al., 2014; 2016).

Similarities in domain structure can help identify candidate interactors. Unlike other SNAP25 homologs in mammals, human SNAP29 harbors an NPF (Asn-Pro-Phe) motif, which interacts with the Eps15-Homology (EH) domain-containing protein1, EHD1 (Rotem-Yehudar et al., 2001; Rapaport et al., 2010). EHD1 is required for the redistribution of the endocytic recycling compartment (Lin et al., 2001). EHD1 and SNAP29 directly interact and form a complex with insulin-like growth factor 1 receptor, IGF-1R (Rotem-Yehudar et al., 2001; Rapaport et al., 2010). Based on these interactions in animals, we predicted that AtSNAP29 and AtSNAP33, which harbor the NPF motif, can interact with AtEHD1, a regulator of endocytosis. It has been shown that down-regulation of AtEHD1 delays the internalization of the styryl dye FM4-64, an endocytosis marker (Bar et al., 2008).

Studies on SNAP29 and SNAP47 in animals suggested that plant SNAP25 homologs could interact with other proteins in autophagy or endocytosis, or other functions, in addition to their role in regulating SNARE complexes. As an autophagy-regulating Qbc SNARE in animals, SNAP29 was identified together with STX17 on autophagosomes (Diao et al., 2015). Autophagy Related 14 (ATG14), an essential autophagy-specific regulator, directly binds to the STX17–SNAP29 binary SNARE complex on autophagosomes, thus priming the SNAP29 complex to interact with VAMP8 and stimulating autophagosome–endolysosome fusion (Diao et al., 2015). Additionally, animal SNAP47 interacts with STX16 and VAMP7, which localize in ATG9a-resident vesicles from recycling endosomes (Aoyagi et al., 2018). The autophagic trafficking of SNAP29 and SNAP47 proteins was hijacked by coxsackievirus B3 (CVB3) and enterovirus D68 (EV-D68), respectively, to enhance viral replication (Mohamud et al., 2018; Corona et al., 2018).

A recent report identified QUIRKY, a member of the family of multiple C2 domain and transmembrane region proteins, as interacting with an *Arabidopsis* Qa SNARE, and suggested that PEN1/AtSYP121 is engaged in the export of florigen from phloem companion cells to sieve elements through its interaction with QUIRKY in the induction of flowering (Liu et al., 2019). Based on the study of QUIRKY, a Qbc SNARE partner of PEN1/AtSYP121 may be involved in regulating developmental phases by exporting florigen.

Post-translational modifications

Animal SNAP25 proteins undergo several post-translational modifications to modulate their functions. In animals, *O*-GlcNAc-modification of SNAP29 exacerbates the dysfunction of autophagy induced by arsenic and diabetes (Dodson et al., 2018; Huang et al., 2018). NEK3 (NIMA-never in mitosis gene A-related kinase 3)-mediated serine 105 (S105) phosphorylation of SNAP29 is important for its membrane association (Rapaport et al., 2018). A serine 105 to alanine (S105A) mutant of SNAP29 could not localize to the Golgi or rescue the CEDNIK (cerebral dysgenesis, neuropathy, ichthyosis, and

palmoplantar keratoderma) syndrome that occurs because of an early stop codon in *SNAP29* (Rapaport et al., 2018). These reports indicate that post-translational modifications of mammalian SNAP25 proteins are important for their cellular functions.

The NetOGlyc 4.0 Server (http://www.cbs.dtu.dk/services/NetOGlyc/) predicts distinct *O*-GlcNAc-modification sites on *Arabidopsis* SNAP25 proteins, with 17 residues in AtSNAP29, 18 residues in AtSANP30, and 27 residues in AtSNAP33. However, there is no clear evidence of the function of *O*-GlcNAc-modification in plants. Phosphoproteome profiling of *Arabidopsis* seedlings revealed that AtSNAP33 in the plasma membrane has one phosphoserine, although AtSNAP29 and AtSNAP30 were not identified (Reiland et al., 2009). The role of phosphoserine in AtSNAP33 has not been characterized.

Numerous tools for predicting post-translational modifications based on large-scale proteome profiling are available, but the predictions require additional experimental validation. For example, regarding the phosphorylation site in AtSNAP33, one prediction tool (Functional Analysis Tools for Post-Translational Modifications, FAT-PTM; https://bioinformatics.cse.unr.edu/fat-ptm/proteins/) identified 12 residues and another prediction tool (a database of phosphorylation sites in Arabidopsis thaliana and a plant-specific phosphorylation site predictor, PhosPhAt 4.0; http://phosphat.uni-hohenheim.de/) identified 20 residues. However, only 9 residues were identified by both prediction tools and whether the predicted residues are functionally meaningful, such as S105 of SNAP25 in CEDNIK syndrome, requires further validation.

AtSNAP29 proteins contain K119 as a potential ubiquitination modification site without a phosphorylation site from FAT-PTM tool and 13 phosphorylation sites from PhosPhAt4.0 tool. AtSNAP30 proteins contain S183 as a matched phosphorylation site from both 1 residue in FAT-PTM and 14 residues in PhosPhAt4.0. Interestingly, phosphorylated

AtSNAP30 proteins were enriched in pollen (Mayank et al., 2012), where AtSNAP30 proteins are specifically and highly expressed. However, which residues contribute to post-translational modifications in specific cellular functions require further analysis.

CONCLUSION AND PERSPECTIVES

Based on genetic, biochemical, and cell biological studies in *Arabidopsis*, the unique Qbc SNAREs, which are SNAP25 homologs, function in several vesicle-trafficking processes, such as cytokinesis, innate immune responses, and abiotic stress responses. The results of various studies of plant SNAP25 proteins support the idea that the characterized biological roles of SNAP25 proteins are fairly conserved in monocots and dicots (Table 2). The recently identified interactors of SNAP25 homologs have novel functions, such as the regulation of development of specific tissues (Ichikawa et al., 2014; Liu et al., 2019; Park et al., 2018). Additionally, in the *Fabaceae*, potential SNAP25 interactors, such as *G. max* VAMP721a and VAMP721d, are required for symbiotic interactions. GlymaSNAP25 homologs are involved in suppressing nematodes, which are deadly parasites in the roots of legumes and *L. japonicus* LjVAMP72a and LjVAMP72b are required for root symbiosis and root hair formation (Gavrin et al., 2016; Ivanov et al., 2012; Sharma et al., 2016; Sogawa et al., 2018). These SNAP25 homologs may function as targets for interacting with other SNARE proteins in different organisms to affect survival.

AtSNAP29 and AtSNAP30 have distinct expression profiles throughout development of *Arabidopsis* and in different tissues. They also respond differently to environmental stresses; therefore, their roles likely reflect their spatiotemporal effects. AtSNAP29 or AtSNAP30 may interact independently with Qa SNAREs and R SNAREs, whose expression is synchronized.

Table 2. Reported functions of plant SNAP25 proteins

Name	Expression	Interaction	Reference	Function
AtSNAP33	Whole plant	KNOLLE, VAMP721/722	(Heese et al., 2001; El Kasmi et al., 2013)	Cell division
		SYP123, VAMP721/722/724	(Ichikawa et al., 2014)	
		SYP132, VAMP721/722	(Park et al., 2018)	
		PEN1, VAMP721/722	(Kwon et al., 2008; Pajonk et al., 2008)	Biotic stress
AtSNAP29	Root, whole plant	KNOLLE/SYP111	(Heese et al., 2001)	ND
AtSNAP30	Flower	KNOLLE/SYP111	(Heese et al., 2001)	ND
Glyma17g08450	Root	ND	(Sharma et al., 2016)	Biotic stress
GsSNAP33	Pod, root, seed, stem	ND	(Nisa et al., 2017)	Abiotic stress
OsSNAP32	Leaf, root, flowering panicle	ND	(Luo et al., 2016)	Biotic stress
			(Bao et al., 2008; Luo et al., 2016)	Abiotic stress
HvSNAP34	ND	ROR2, HvVAMP721	(Collins et al., 2003)	Biotic stress
GhSNAP33	Leaf, root, stem	ND	(Wang et al., 2018)	Biotic stress Abiotic stress
StSNAP33-1	ND	StSYP1	(Eschen-Lippold et al., 2012)	Biotic stress
CkSNAP33	Root, stem, leaf	ND	(Wang et al., 2017)	Biotic stress

ND, not determined.

Studies on the subcellular localization, post-translational modification, identification of interacting Qa SNAREs or R SNAREs for the SNAP25 proteins, and structural analysis of new SNARE complexes at the cellular membrane are essential for exploring the mechanisms and roles of Qbc SNARE SNAP25 proteins in plants. *snap29* and *snap30* mutants of *Arabidopsis* and *snap25* mutants of other crops can be generated using clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) genome editing tools to study the developmental phenotypes and tissue-specific biological roles of SNAP25 proteins in plants.

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K.H.W. and H.K. wrote and approved the manuscript.

CONFLICT OF INTEREST
The authors have no potential conflicts of interest to disclose.

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