SNARE-Ware: The Role of SNARE-Domain Proteins in Plant Biology

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Abstract

In yeast and animal cells, members of the superfamily of N-ethylmaleimide-sensitive factor adaptor protein receptor (SNARE)-domain-containing proteins are key players in vesicle-associated membrane fusion events during transport processes between individual compartments of the endomembrane system, including exocytosis and endocytosis. Compared with genomes of other eukaryotes, genomes of monocotyledonous and dicotyledonous plants encode a surprisingly high number of SNARE proteins, suggesting vital roles for this protein class in higher plant species. Although to date it remains elusive whether plant SNARE proteins function like their yeast and animal counterparts, genetic screens have recently begun to unravel the variety of biological tasks in which plant SNAREs are involved. These duties involve fundamental processes such as cytokinesis, shoot gravitropism, pathogen defense, symbiosis, and abiotic stress responses, suggesting that SNAREs contribute essentially to many facets of plant biology.
INTRODUCTION

Cellular compartmentalization is a hallmark of eukaryotic cells. Compartmentalization allows functional and spatial partitioning into self-contained reaction chambers, each with its own characteristic ambiance, such as organelle-specific biomolecule (e.g., polypeptide, carbohydrate, lipid, ion, etc.) composition. The establishment and maintenance of complex eukaryotic compartmentalization require comprehensive transport activities between the various locations within the endomembrane system. Eukaryotes have evolved a sophisticated system to ensure the shipping of cargo within the secretory pathway, a major transport route within the endomembrane system, leading from the endoplasmic reticulum via the Golgi apparatus to the extracellular space (Hanton & Brandizzi 2006, Sanderfoot & Raikhel 2003). Small, spherical, membrane-coated transport units called vesicles shuttle and carry loads (e.g., polypeptides and small compounds) between the compartments of the secretory pathway. These include the above-mentioned organelles, the plasma membrane (PM), and the vacuole, another membrane-enclosed organelle. Plant vacuoles are particularly elaborate and can serve diverse purposes, including storage, digestion, and recycling (Bassham & Raikhel 2000). Unlike in yeast, the presence of a functional vacuole is essential for plant cells (Rojo et al. 2001). In addition to facilitating transport toward the outside of the cell (anterograde transport) and extrusion of cargo (exocytosis), vesicles also contribute to the uptake of loading from the extracellular space (endocytosis) and backward (retrograde) transport processes. Several classes of polypeptides contribute to the targeting and fusion of acceptor and target membranes during these shuttling activities.

In terms of energy, the fusion of a membrane-coated vesicle with a target lipid bilayer is a rather unfavorable process. Eukaryotes have evolved a specialized class of proteins, the soluble N-ethylmaleimide-sensitive factor (SNAREs), that function as mediators of fusion between vesicular and target membranes. These adaptor polypeptides spontaneously form highly stable protein-protein interactions that help to overcome the energy barrier required for membrane fusion. Members of the superfamily of SNARE proteins are found in a variety of contexts, including the endoplasmic reticulum, Golgi apparatus, and plasma membrane.
in all eukaryotes, including plants. Notably, plants have a somewhat distinct and considerably expanded repertoire of SNAREs compared with that of animals and fungi: In contrast to members of the latter kingdoms, plants lack particular SNARE protein subfamilies but have also evolved few novel types of SNAREs (see below). During the past decade, comprehensive cell biological and biochemical studies as well as both forward and reverse genetic screens have begun to shed light on the roles of SNARE proteins in plant biology. This review focuses on the determined functions of SNAREs in the context of plant biological processes and discusses essential future questions in this research area.

**SNARE PROTEINS**

SNARE-domain proteins comprise a superfamily of comparatively small (∼200–400 amino-acid) polypeptides that are characterized by the presence of a particular peptide domain, the SNARE motif (Jahn & Scheller 2006). The SNARE domain is a stretch of 60–70 amino acids consisting of heptad repeats that can form a coiled-coil structure. Via hetero-oligomeric interactions, SNAREs mediate fusion events between membranes in the course of intracellular vesicle-associated traffic and therefore occur on vesicles, organelles of the endomembrane system, and the PM. The association of SNAREs with lipid bilayers is usually conferred by C-terminal transmembrane (TM) domains (Figure 1a). Some SNAREs, however, are attached to membranes via lipid anchors. With the exception of SNAP-25-like SNARE proteins, which harbor two SNARE domains separated by a flexible linker, the vast majority of SNAREs possess one SNARE motif (Figure 1a). In addition to the SNARE domain and the C-terminal TM domain, many SNAREs contain N-terminal regulatory sequence motifs that control in vivo SNARE protein activity in concert with a range of accessory polypeptides (Figure 1a). SNAREs mediate membrane fusion by intermolecular interactions among complementary vesicle- and target membrane–associated SNAREs. Upon contact, matching types of SNAREs form a highly stable protein association called the SNARE complex. A typical SNARE complex involves three distinct types of SNARE proteins residing on the target membrane and one SNARE polypeptide located on the vesicle that together contribute to a four-helix bundle of intertwined SNARE domains (for recent reviews, see Brunger 2006, Hong 2005, Jahn & Scheller 2006). Complex formation is associated with conformational and free-energy changes that are commonly believed to drive the membrane fusion process (Figure 1b). On the basis of (a) sequence conservation between animal and plant SNAREs, (b) complementation of yeast mutants by plant SNAREs (Bassham & Raikhel 1998, Bassham et al. 1995, Sato et al. 1997, Tai & Banfield 2001, Zheng et al. 1999b), (c) accumulating protein-protein interaction data (Bassham & Raikhel 1999, Chen et al. 2005, Heese et al. 2001, Rancour et al. 2002, Sanderfoot et al. 2001a, Zheng et al. 2002), and (d) evidence from in vitro liposome fusion studies (Chen et al. 2005), the in planta formation of ternary SNARE complexes and their contribution to membrane fusion events appear very likely. However, direct experimental proof of this is still lacking to date.

SNAREs can be classified either on the basis of their subcellular localization (functional classification) or according to the occurrence of invariant amino acid residues in the center of the SNARE motif (structural classification). Functional classification divides SNAREs into vesicle-associated and target membrane–associated SNAREs (v- and t-SNAREs, respectively) (Sollner et al. 1993), a categorization that can become problematic in the context of homotypic vesicle fusion events and anterograde trafficking routes. Alternatively, under the structural classification, SNAREs can be grouped as Q- and
Disassembly and component recycling

Fusion, cis complex, and cargo release

**Disassembly and component recycling**

**Fusion, cis complex, and cargo release**
R-SNAREs owing to the occurrence of either a conserved glutamine or arginine residue in the center of the SNARE domain (Fasshauer et al. 1998). Generally, t-SNAREs correspond to Q-SNAREs, and v-SNAREs correspond to R-SNAREs. The three types of target membrane–localized Q-SNAREs that contribute to the formation of a SNARE complex can be further subdivided into Qa-, Qb-, and Qc-SNAREs (Bock et al. 2001). SNAP-25-like proteins constitute a special case because they comprise a Qb- and a Qc-SNARE motif within a single polypeptide chain, possibly as a result of an ancient gene fusion event. For historical reasons, on the basis of their role in synaptic exocytosis, Qa-SNAREs are also frequently referred to as syntaxins (Bennett et al. 1992). Likewise, vesicle-resident R-SNAREs are often designated as VAMPs (vesicle-associated membrane proteins). R-SNAREs can have either a short or long N-terminal regulatory region, further subdividing them into brevins (lat. brevis, short) and longins (lat. longus, long). Again on the basis of their role in synaptic exocytosis, R-SNAREs with a short N-terminal domain are also frequently referred to as synaptobrevins (Baumert et al. 1989). In plants, however, this evolutionary young class of R-SNAREs is absent; all known plant R-SNAREs belong to the longin category (Üemura et al. 2005).

**PLANT SNARE GENOMICS**

The genomes of all higher plant species investigated so far code for a large repertoire of SNAREs: 60 in the dicotyledonous model species *Arabidopsis thaliana* (a weed growing in temperate zones) (The Arabidopsis Genome Initiative 2000), 57 in monocotyledonous rice (*Oryza sativa*, a grass species of subtropical and tropical regions) (International Rice Genome Sequencing Project 2005), and 69 in black cottonwood (*Populus trichocarpa*, a deciduous dicotyledonous tree endemic to moist woods, ravines, shores, and prairies of western North America) (Tuskan et al. 2006; Table 1). Likewise, comparable numbers of subfamily members are encoded by the various plant genomes (Table 1), suggesting that the enlarged SNARE complement is not related to a particular plant lifestyle and/or biogeographic habitat. Baker’s yeast (*Saccharomyces cerevisiae*) and humans (*Homo sapiens*) are thought to encode a total of 21–25 and 35–36 members, respectively (Jahn & Scheller 2006, Sutter et al. 2006a). The higher number of SNAREs in plant species compared with yeast and humans is due predominantly to the enormous expansion of member quantities in conserved SNARE subfamilies and not the evolution of novel SNARE isoforms. In fact, only two subfamilies appear to be plant specific: the NPSN (novel plant-specific SNARE) Qb- and the SYP7 Qc-SNAREs (Sanderfoot et al. 2005).

**Figure 1**

Domain architecture of major plant SNARE subfamilies and molecular mechanics of SNARE complex formation and vesicle fusion. (a) Scheme of the general domain organization of plant SNARE proteins. Shown are Qa-, Qb-, and Qc-SNARE motifs, the R-SNARE domain (R), regulatory N-terminal regions of Q-SNAREs, the longin domain (LD) of R-SNAREs, and the C-terminal TM helices (T) present in most SNARE proteins. N and C denote the N- and C-terminal ends of the polypeptides, respectively. (b) Scheme of the principle of binary and ternary SNARE complex formation. Vesicle fusion is initiated by regulatory protein–mediated opening of the closed conformation of a target membrane–anchored Qa-SNARE. The resulting exposition of the Qa helix allows association with helices provided by a membrane-associated Qb+Qc-SNARE and an R-SNARE. The latter resides in a vesicle, which buds from a donor compartment after cargo loading and is transported to the acceptor compartment. Association in a trans complex is accompanied by an increase in core α-helical structure density that drives transition to the cis complex, membrane fusion, and the release of vesicle cargo into the acceptor compartment. After energy-dependent disassembly and recycling of the individual complex units, the cycle can restart (for more details, see Jahn & Scheller 2006). For simplicity, the N-terminal longin domain of the R-SNARE is omitted in this scheme.
Table 1 Number of SNARE type and SNARE subfamily members encoded by the genomes of the dicotyledonous weed *Arabidopsis thaliana*, the monocot *Oryza sativa* (rice), and the dicotyledonous tree species *Populus trichocarpa* (black cottonwood)

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Cytokinesis: the division of the cytoplasm to form two separate daughter cells immediately after mitosis

2000). The presence of respective genes in the moss *Physcomitrella patens* and even green algae such as *Chlamydomonas reinhardtii* suggests that they evolved early in plant evolution. The greater diversification of SNARE isoforms in plants presumably reflects the necessity for some SNAREs to be devoted to plant-specific biological processes such as the plant-specific type of cytokinesis, gravitropic responses, and the transport of phytohormones [e.g., abscisic acid (ABA) and auxin; see below]. Consistent with the role of SNAREs in other eukaryotes, many of these functions appear to be related to the establishment and/or maintenance of polarity at the (sub)cellular level (Surpin & Raikhel 2004).

**PLANT Q-SNARES**

Plant genomes encode multiple syntaxin-like Qa isoforms belonging to five distinct SYP (syntaxin of plants) subfamilies (Supplemental Figure 1; for all supplemental items follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org). SYP5, 6, and 7, which were initially grouped as syntaxins, are now regarded as Qc-SNAREs (Pratelli et al. 2004, Sutter et al. 2006a; http://www.cbs.umn.edu/~sande099/atsnare.htm); however, they have retained their original designation. As in yeast and mammals, syntaxins are also the best-studied SNARE proteins in plants.
The overall structure of the syntaxins consists of an N-terminal autoregulatory domain, a linker, the SNARE domain, and a TM region (Figure 1a). Interestingly, owing to a natural genetic polymorphism, AtSYP23 lacks the C-terminal TM domain in the Arabidopsis reference ecotype Col-0 (Ohtomo et al. 2005, Zheng et al. 1999a). The N-terminal autoinhibitory domain is composed of three helices that are also called the Habc motif. The Habc domain of neuronal syntaxins is folded into three helical bundles that can interact with the SNARE domain, mimicking the parallel four-α-helix bundle of the SNARE complex. The intramolecular interaction of the Habc stretch with the SNARE domain, which is also called a closed conformation (Figure 1b), prevents interaction of the syntaxin with other SNAREs (Fernandez et al. 1998, Lerman et al. 2000, Munson et al. 2000). Genetic screens have revealed the contribution of individual syntaxin isoforms to various biological processes such as cytokinesis, pathogen defense, and ABA signaling (see below).

In association with syntaxin-like Qa-SNAREs, individual Qb- and Qc- (or Qb+Qc-) SNAREs are thought to complement the SNARE complex at the target membrane (Figure 1b). Like Qa-SNAREs, Arabidopsis Qb-SNAREs have been implicated in a range of biological processes, including cytokinesis (AtNPSN11), shoot gravitropism (AtVti11), and autophagy (AtVti12) (see below). Remarkably, genetic screens have so far revealed no phenotype for a Qc-SNARE, suggesting at least partial functional redundancy. Qb- and Qc-SNAREs generally also possess an extended N-terminal domain, which, in the case of some animal isoforms, may adopt a coiled-coil structure related to the syntaxin Habc motif (Hong 2005).

Unlike other SNAREs, SNAP-25s contain two SNARE motifs and are classified as a Qb+Qc-SNARE because the N-terminal half is equivalent to Qb-SNAREs and the C-terminal half is similar to Qc-SNAREs (Bock et al. 2001, Fasshauer et al. 1998). Like their mammalian counterpart, SNAP-25, Arabidopsis SNAP-25-like proteins also lack a TM domain. Mammalian SNAP-25 is attached to the PM by palmitoylation of conserved cysteines located in the linker region (Gonzalo et al. 1999, Koticha et al. 1999, Veit et al. 1996). Arabidopsis SNAP-25s, however, have no conserved cysteine(s). Nevertheless, AtSNAP33 localizes to the PM (Heese et al. 2001), suggesting that different post-translational lipid addition(s) may target it to the PM.

**PLANT R-SNARES**

The R-SNAREs encoded by plant genomes can be grouped into three major subfamilies, the VAMPs, YKT6s, and SEC22s (Supplemental Figure 1). Most R-SNAREs are located on trafficking vesicles, anchored by a C-terminal TM domain. A few members, such as AtYKT61 and AtYKT62, are thought to be attached to the vesicular membrane by posttranslational addition of lipid anchors, as in the case of their yeast and mammalian counterparts (McNew et al. 1997). All plant R-SNAREs are so-called longins, comprising an extended N-terminal stretch (the longin domain; see Figure 1a) that, on the basis of data from human R-SNAREs, may be involved in subcellular localization and SNARE complex formation, e.g., by interaction with regulatory polypeptides (Martinez-Arca et al. 2003, Uemura et al. 2005). Compared with Q-SNAREs, little is known about the biological roles of plant R-SNAREs. AtVAMP711 suppresses Bax-triggered programmed cell death in yeast, suggesting that intracellular vesicle traffic can regulate the execution of apoptosis (Levine et al. 2001). With the exception of a recently discovered salt resistance phenotype (Leshem et al. 2006, see below), no further phenotype has been found in any Arabidopsis R-SNARE mutant, suggesting that most R-SNAREs act at least partially redundantly, rendering it difficult to infer their function in plants.
IN SILICO EXPRESSION AND SUBCELLULAR LOCALIZATION ANALYSES OF ARABIDOPSIS SNARE GENES AND GENE PRODUCTS

The availability of comprehensive microarray data (Zimmermann et al. 2004; https://www.genevestigator.ethz.ch) enables a synopsis of the expression profile of SNARE isoforms in various organs, tissue, and cell types of Arabidopsis. Many SNARE genes appear to be expressed in a range of plant tissues. The Qa-SNAREs—in particular SYP22 and SYP32; VTI11 and GOS12 (Qb-SNAREs); BET11, SYP71, SFT11, and USE11 (Qc-SNAREs); SNAP33 (Qb+Qc-SNARE); and VAMP713, VAMP714, VAMP721, and VAMP722 (R-SNAREs)—all exhibit a ubiquitous expression pattern (Figure 2). Interestingly, many SNARE-encoding genes reveal an apparent relative maximum of transcript accumulation in pollen (Figure 2), consistent with the notion that pollen development and function require comprehensive vesicle-associated transport processes, e.g., during the establishment of cell polarity and tip growth of the pollen tube (Hepler et al. 2001).

Recently, Uemura et al. (2004) systematically examined the localization of fluorophore-tagged versions of all Arabidopsis SNARE proteins by transient overexpression in protoplasts. These studies revealed that most SNAREs are associated with specific intracellular compartments, whereas some localize to two or more distinct organelles, possibly owing to shuttling between subcellular compartments. Despite the inherent caveats typically associated with cauliflower mosaic virus 35S promoter–driven mis-/overexpression, these and similar experiments by other groups (e.g., Chatre et al. 2005, Latijnhouwers et al. 2005, Takeuchi et al. 2002) suggest at least preferential association of individual SNARE proteins with particular organelles. Likewise, overexpression of full-size SNAREs or cytoplasmic SNARE domains has provided insights into the potential role of these isoforms in subcellular vesicle trafficking (e.g., Chatre et al. 2005, Foresti et al. 2006, Geelen et al. 2002, Sutter et al. 2006b). Together with the above-mentioned gene expression metadata and combined with the results obtained from immunolocalization (e.g., Bassham et al. 2000, Conceicao et al. 1997, Müller et al. 2003, Rancour et al. 2002, Sanderfoot et al. 2001a, Sato et al. 1997) and proteomic studies (e.g., Carter et al. 2004, Marmagne et al. 2004, Mongrand et al. 2004, Morel et al. 2006, Nühse et al. 2003), these experiments provide a preliminary localization and activity atlas for the Arabidopsis SNARE inventory. Supplemental Table 1 summarizes these results.

SNARE REGULATORS AND SNARE COMPLEX-ASSOCIATED PROTEINS

Although SNARE proteins themselves are able to drive vesicle fusions in vitro, they are not the sole determinants of vesicle fusion and targeting specificity. Beyond in vivo SNARE gene expression, posttranslational modifications, protein turnover and localization patterns, regulatory factors such as...
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Sec1/Munc18 (SM) proteins are also important (Gerst 2003). SM proteins interact with nonconserved SNARE residues and regulate conformational changes of syntaxins (from closed to open), thereby controlling SNARE complex formation capacity and vesicle fusion (Dulubova et al. 1999, Misura et al. 2000, Yang et al. 2000). The Arabidopsis genome contains six members of the Sec1 family (Sanderfoot & Raikhel 2003), of which one, KEULE (KEU), has been reported to be involved in cytokinesis (Assaad et al. 2001; see below).

In addition to SM proteins, Ras-related GTPases of the Rab family control multiple steps of eukaryotic vesicle transport such as budding, cargo selection, movement, tethering, and docking (Deneka et al. 2003). Arabidopsis is equipped with 57 potential Rab GTPases that are categorized into eight subfamilies (RabA to RabH) (Sanderfoot & Raikhel 2003). Although the exact biological functions of most plant Rabs are still elusive, investigators have recently shown that Rha1 and Rab-E1 play crucial roles in vacuolar trafficking and exocytosis, respectively (Sohn et al. 2003, Zheng et al. 2005). In yeast and mammal cells, both Rab and Rho GTPases potentially interact with a multisubunit protein assembly, the so-called exocyst (Sec6/8) complex. This complex may be critical for site specification of vesicle docking and fusion by its actions as a tethering complex before SNAREs and associated proteins mediate the actual fusion event (Elias et al. 2003 and references therein). Bioinformatic analyses of the Arabidopsis genome have identified plant homologs of all known exocyst subunits, suggesting a possibly conserved function in plants (Elias et al. 2003).

Intriguingly, SNARE proteins may fine-tune membrane fusion specificity by acting in part as inhibitory SNAREs (i-SNAREs) via substituting for or binding to a subunit of a fusogenic SNARE protein to form a nonfusogenic complex (Varlamov et al. 2004). These findings highlight the importance of in vivo SNARE stoichiometry and call for a careful interpretation of transgenic (over)expression data.

To maintain a stable cellular architecture and continuous vesicle trafficking, it is important for eukaryotic cells to recycle proteins and lipids used for vesicle fusion. This recycling process is usually performed by the cooperation of soluble accessory proteins termed α-SNAP and NSF (an ATPase). NSF interacts with the SNARE complex via α-SNAP and, by ATP hydrolysis, dissociates the complex into compartments (May et al. 2001). Although Arabidopsis has three genes encoding α-SNAP and one gene encoding NSF (Sanderfoot et al. 2000), no evidence on the biological role of these genes has been reported.

THE ROLE OF SNARE PROTEINS IN CYTOKINESIS

Cytokinesis partitions the cytosol and organelles of a dividing cell following separation of the daughter chromosomes (Mayer & Jürgens 2004, Seguí-Simarro et al. 2004). In somatic plant cells this process is initiated in the center of the division plane by the formation of a transient membrane compartment called the cell plate. Electron microscopic analyses, in vivo imaging, and electron tomographic studies indicate that cell plate formation is the result of Golgi-derived vesicle fusions that give rise to a tubular membrane network at the cell equator (Nebenführ et al. 2000, Samuels et al. 1995, Seguí-Simarro et al. 2004). Recent data suggest that distinct membrane fusion pathways, including endocytotic transport of cell-surface material (Dhonukshe et al. 2006), may also be involved (Rancour et al. 2002).

A genetic screen for Arabidopsis mutants with aberrant seedling body organization allowed the isolation of seedling-lethal knolle (kn) mutants (Lukowitz et al. 1996). kn embryos are characterized by incomplete and disoriented cell divisions resulting in multinucleate and enlarged cells, which defines the mutant as cytokinesis defective and suggests
an important function for the KN protein in cell plate formation. Moreover, membrane vesicles accumulate at the plane of cell division but apparently do not fuse normally (Lauber et al. 1997). The mutated gene was identified as the Qa-SNARE AtSYP111 (Lukowitz et al. 1996), which led to the conclusion that KN may be involved in homotypic fusions of Golgi-derived vesicles. Consistent with a role as a cytokinesis-specific Qa-SNARE, the KN gene is tightly cell-cycle regulated. Its mRNA accumulates transiently in actively dividing tissues of developing flowers and siliques (Lukowitz et al. 1996), and meta-analysis of publicly available microarray data shows high expression levels in the shoot apex and the root tip (Figure 2). Immunofluorescence microscopy revealed that the KN protein accumulates during M phase, relocates to the cell division plane during telophase, and disappears at the end of cytokinesis (Lauber et al. 1997). Most importantly, Lauber et al. (1997) noticed that KN is tightly associated with membranes and postulated a specific association with post-Golgi vesicles destined to the cell plate. Recently, however, a series of colocalization experiments showed that KN is at least partially localized in endosomes (Dhonukshe et al. 2006). Dhonukshe et al. (2006) suggest that both Golgi-derived and endocytotic trafficking pathways are involved in cell plate formation and that KN may be required for homotypic fusion of endosomes.

Interestingly, ectopic expression of KN by the strong constitutive cauliflower mosaic virus 35S promoter leads to PM mislocalization in nonproliferating cells but has no phenotypic effect, whereas comparatively low 35S promoter–driven expression in proliferating cells is insufficient to rescue cytokinesis-defective kn mutant embryos (Völker et al. 2001). Thus, high transcription rates during M phase appear essential for KN function in cytokinesis. Conversely, KN cis-regulatory sequence–driven expression of Arabidopsis Qa-SNAREs is not generally sufficient to complement kn-mutant cytokinesis defects (Müller et al. 2003). Only one out of three tested Qa-SNAREs with no cytokinesis-related function, AtSYP112, was targeted to the cell plate and sufficiently related to KN to perform its function. Intrinsic protein sequence information determining target membrane identity or functional specificity was considered responsible for complementation failure of two other tested Qa-SNAREs, the pathogen defense–associated and PM-localized AtSYP121 (see below) and the male gametophyte–related and prevacuolar compartment–localized AtSYP21. These intrinsic features may determine interaction capabilities with regulatory proteins and/or cognate SNARE complex partners.

The mutated gene in another cytokinesis-defective Arabidopsis mutant encodes the Sec1 protein KEU (Assaad et al. 2001; Figure 3a). Sec1 proteins are known regulators of SNARE-mediated vesicle tethering and fusion (see above; Gerst 2003). In vitro protein binding studies and gene interaction analyses suggest that KN and KEU interact in vivo to promote vesicle fusion in the cell division plane (Assaad et al. 2001, Waizenegger et al. 2000). Because there are only 6 Sec1 protein family members but 18 Qa-SNAREs encoded by the Arabidopsis genome (see above), it is likely that KEU also regulates other Qa-SNAREs; that keu, but not kn, mutants exhibit aberrant root hair morphogenesis corroborates this idea (Söllner et al. 2002).

The Arabidopsis genome harbors three genes encoding putative KN Qb+Qc-SNARE complex partners, AtSNAP29, 30, and 33 (see above). SNAP30 appears to be expressed exclusively in pollen. In contrast, SNAP29 and 33 are both expressed in a variety of different tissues; the latter has significantly higher expression levels (Figure 2). The derived proteins can all interact with KN in yeast two-hybrid experiments, suggesting functional redundancy (Heese et al. 2001). In addition to its KN-binding capacity, the ubiquitously expressed SNAP33 is localized predominantly to the PM and colocalizes with KN at the cell plate of dividing cells (Heese et al. 2001). Consistent with a role in cell plate
vesicle fusion processes, *map33*-knockout mutants show cytokinetic defects such as incomplete cell walls. Remarkably, they also develop aberrant callose deposits and large necrotic lesions on cotyledons and rosette leaves that are reminiscent of pathogen-induced responses (Figure 3a). Thus, AtSNAP33 appears to be functionally involved not only in cytokinesis but also in other processes (Heese et al. 2001). Indeed, recent findings suggest an interaction of a tobacco homolog of AtSNAP33 with the tobacco Qa-SNARE NtSYP121 (Kargul et al. ...
2001) as well as recruitment of AtSNAP33 and its presumptive barley ortholog HvSNAP34 for defense mechanisms executed at the cell periphery (see below).

With KN and SNAP33 as likely partners of a cytokinesis-specific SNARE complex, the only component missing for an authentic SNARE complex until recently was a specific R-SNARE contributing the required fourth helix. That one of the three NPSN genes in the Arabidopsis genome, NPSN11, is highly expressed in actively dividing cells prompted Zheng et al. (2002) to study its gene product in more detail. Immunofluorescence microscopy showed that KN and NPSN11 colocalize at the cell plate and in punctate subcellular organelles, which do not correspond to Golgi stacks. The latter finding reinforces the notion that non-Golgi-derived membrane fusions may contribute to cell plate formation. Coimmunoprecipitation experiments demonstrated that KN specifically interacts with NPSN11, suggesting that the latter may contribute the fourth helix to a cytokinesis-specific SNARE complex driving homotypic fusions of a membrane compartment that still remains to be unequivocally identified. Intriguingly, however, npsn11-knockout mutants did not show any obvious phenotype, suggesting that other members of the NPSN family can functionally substitute for NPSN11.

Experiments with AtCDC48, an AAA-type ATPase whose yeast and mammal homologs regulate SNARE complex assembly and integrity during cell division, further support the idea that cell plate formation depends on several different membrane fusion pathways that may involve distinct SNARE complexes (Rancour et al. 2002). In Arabidopsis, CDC48 colocalizes at the cell division plane with KN and another Qa-SNARE, SYP31 (Rancour et al. 2002). In vitro, CDC48 appears to interact specifically in an ATP-dependent manner with SYP31 but not with KN, which assembles into a large 20S complex with the adaptor proteins Sec18/NSF and α-SNAP. Interestingly, immunolocalization analyses showed that SYP31 is present throughout the cell cycle, associates with both large and small punctate membrane structures of unknown identity during interphase, and marks the division plane during cytokinesis (Rancour et al. 2002). Thus, potentially distinct SNARE complexes seem to mediate a variety of different (homotypic and heterotypic?) subcellular membrane compartment fusions at the forming cell plate during cytokinesis.

Figure 3
Selected phenotypes of Arabidopsis mutants defective in SNARE protein–mediated biological processes. (a) Seedling phenotypes of five-day-old wild-type (WT) Arabidopsis (upper panel, left); cytokinesis-defective, dwarfish ken mutant (upper panel, right); and spontaneous brownish lesion development on a map33-mutant plant (lower panel). Adapted from Heese et al. (2001). Copyright 2001 by The Rockefeller University Press. (b) WT (upper panel, left) and abnormal inflorescence stem morphology of zig-1 mutant (upper panel, right). WT plants (lower panel, left) show a normal gravitropic response after 90 min horizontal gravity stimulation, whereas zig-1 mutants (lower panel, right) show little or no response. Reprinted with permission from Niihama et al. (2005), pp. 555–60. Copyright 2005, Elsevier. (c) Five days postinoculation, low-magnification fluorescence microscopy reveals an enhanced incidence of autofluorescent areas on barley powdery mildew–inoculated pen1-1-mutant leaves (upper panel, right) when compared with WT (upper panel, left). Fixation of leaf tissue, callose staining, and subsequent fluorescence microscopy at higher magnification demonstrate efficient deposition of callosic plugs at sites of attempted fungal penetration (red arrowheads) on wild-type plants (lower panel, left). In marked contrast, pen1-1 mutants (lower panel, right) allow fungal invasion (encased and fluorescent fungal feeding structure; red arrowhead), which coincides with local plant-cell-death execution accompanied by whole-cell fluorescence. (d) Phenotypes of one-month-old WT (upper panel) or salt-sensitive osmi1-mutant plants (lower panel) watered with different concentrations of NaCl (from left to right: 0 mM, 200 mM, 300 mM, 400 mM) every three days for three additional weeks. Reproduced with permission from Zhu et al. (2002). Copyright by the American Society of Plant Biologists.
**THE ROLE OF SNARE PROTEINS IN GRAVITROPISM, TISSUE IDENTITY, AND AUTOPHAGY**

Most higher plants are sessile organisms that show a bidirectional growth orientation: Shoots grow upward to maximize light-harvesting capabilities, and roots grow downward to anchor the plant in the ground and to ensure sufficient uptake of water and nutrients. The term gravitropism refers to a plant’s ability to control organ growth at a specified angle from the gravity vector. The molecular mechanisms that govern gravitropic responses are complex and involve signal perception, transduction, and subsequent adaptive growth adjustments (Blancaflor & Masson 2003).

Changes in sedimentation of starch-filled organelles, called amyloplasts, are believed to constitute one of the initial events in gravity perception (Sack 1991). In shoots these specialized organelles occur in the endodermis (statocytes), and in roots they occur in the columella cells of the root cap (statocytes). Ultimately, signal transduction cascades that mediate the relocalization of auxin transporters and thus alter auxin flux cause compensatory asymmetric growth responses.

Several agravitropic mutants that are affected in various aspects of this complex process have been isolated (Masson et al. 2002). These include *Arabidopsis* plants with mutations in two different SNARE-encoding genes. In the corresponding mutants, sgr3 and sgr4 (the latter is also known as zig or zigzag) (Figure 3b), shoot endodermal statocytes do not sediment according to the gravity vector, whereas root cap statocytes behave normally (Kato et al. 2002, Morita et al. 2005, Yano et al. 2003). Consistent with these results, both mutants show abnormal inflorescence stem gravitropic responses (Figure 3b). Interestingly, however, hypocotyl gravitropism is abnormal in sgr4, but not in sgr3, mutants, documenting tissue-specific differences (Fukaki et al. 1996, Yamauchi et al. 1997). Pronounced morphological defects such as zigzag growth of the inflorescence stem, small size, and wrinkled leaves are additional phenotypic characteristics of sgr4 mutants, whereas sgr3-1 mutant plants exhibit no general growth defects (Yano et al. 2003). SGR3 encodes the Qa-SNARE AtVAM3/SYP22 (Yano et al. 2003), and ZIG/SGR4 codes for the Qb-SNARE AtVTI11 (Kato et al. 2002). Of the three described sgr4 mutant alleles, one (zig-2) completely lacks the coding region for AtVAM3, whereas zig-3, whose gravitropic response is the most weakly affected, shows a substitution in a conserved amino acid of the coiled-coil domain (Kato et al. 2002). In the original sgr3-1 mutant, a single amino acid substitution adjacent to the SNARE motif appears to reduce the protein’s affinity for putative gravitropism-specific SNARE complex partners (Yano et al. 2003). However, this particular mutation may not be deleterious enough to cause other morphological phenotypes. Indeed, a novel semidwarf mutant with reduced elongation of inflorescence stems and leaves has been recently identified. This mutant harbors a 34-bp deletion in the sixth intron of *AtVAM3/SYP22*, probably preventing proper mRNA splicing and protein synthesis (Ohtomo et al. 2005). Remarkably, this mutant also exhibits abnormal development of idioblasts, specialized leaf cells accumulating thioglucoside glucohydrolases (myrosinases) that are involved in the release of toxic compounds to repel phytophagous insects or phytopathogenic microorganisms (Ueda et al. 2006). These findings reinforce the notion that AtVAM3/SYP22 is required for various aspects of plant development.

Both AtVTI11 and AtVAM3/SYP22 are localized to prevacuolar or vacuolar compartments (Sato et al. 1997, Zheng et al. 1999b), and together with SYP51 they may form a SNARE complex that contributes to vesicular trafficking to these organelles (Sanderfoot et al. 2001a, Yano et al. 2003). On the subcellular level, sgr3 and sgr4 mutants have abnormally structured vacuoles that do not flexibly surround the statocytes, as do wild-type plants.
In Arabidopsis, the VTI11 family is composed of four closely related members, VTI11, 12, 13, and 14, the first two of which are expressed at reasonable levels in a variety of organs and tissues (Figure 2; Surpin et al. 2003). VTI12 forms SNARE complexes at the trans-Golgi network with the Sec1 family protein VIP45 and members of the SYP4 and SYP6 t-SNARE families (Bassham et al. 2000, Sanderfoot et al. 2001a), reflecting marked differences with VTI11 with respect to localization and putative SNARE complex partners. Recent analyses suggest, however, that the gene products of VTI11 and VTI12 are at least partially functionally redundant. Surpin et al. (2003) found that homozygous double mutants are embryo lethal, suggesting vital functional overlap between the two genes. Moreover, vti11/vti11 vti12/vti12 plants exhibit an enhanced zigzag stem morphology when compared with vti11 (sgr4) single mutants, suggesting that VTI12 can at least partially substitute for VTI11. Plants with an enhanced zig phenotype are characterized by a loss of discernible tissue types. This demonstrates a significant role for VTI11 in the establishment and maintenance of tissue organization and identity in addition to its role in plant gravitropism. Conversely, VTI11/vti11 vti12/vti12–mutant plants are indistinguishable from their vti12 parents and exhibit no abnormal developmental phenotype under normal growth conditions. However, under nutrient-poor conditions and in assays with detached leaves, vti12 mutants exhibit accelerated senescence phenotypes similar to previously characterized Arabidopsis autophagy mutants (Surpin et al. 2003). This suggests that, analogous to yeast autophagy, VTI12 may be involved in autophagosome traffic to the vacuole, where cytosolic contents or organelles are degraded and reallocated for essential processes.

A recent screen for suppressors of the zig1 (vti11; sgr4-1) mutation yielded one dominant mutant, zip1 (zig suppressor 1), which fully restored wild-type morphology and gravitropism (Niihama et al. 2005; Figure 3b). A map-based cloning strategy revealed a point mutation in the closest homolog of VTI11, VTI12, which at the protein level converts a glutamate to a lysine. Surprisingly, this particular amino acid exchange appears to convert SNARE complex formation specificity and subcellular localization, allowing ZIP1 to function as VTI11.

In summary, plant SNAREs appear to play essential roles in plant development, a statement already expressed by Sanderfoot et al. (2001b), who found that disruption of individual members of the SYP2 and SYP4 families is embryonic lethal. However, growing evidence suggests the potential for at least partial functional redundancy, whereas the above-mentioned ZIP1/VTI11 example of specificity conversion (Niihama et al. 2005) demonstrates how easily subtle mutations can affect functional specialization. Evolutionary selection for such modifications may have had (and possibly still has) a significant impact on functional diversification, resulting in the recruitment of SNARE variants for different biological processes. Notably, Qa-SNAREs SYP121 and 122 are closely related to the cytokinesis-specific Qa-SNARE families.
Symbiosis: the relation between two different species of organisms (e.g., plant and microbe) that are interdependent; each gains benefits from the other

Paramural bodies: membranous vesicle-like structures located between the cell wall and the plasma membrane

Multivesicular bodies: lipid-bilayer-covered vesicle-like cytoplasmic structures that contain a number of small vesicles

SNARE proteins in plant-microbe interactions may relate to the uptake of microbial effectors, to vesicle-associated defense responses, and to the accommodation of microbial infection structures.

**Plant-Pathogen Interactions**

Researchers have thought for a long time that secretory processes are likely to contribute to defense responses in plant-microbe interactions. For example, site-specific, localized accumulation of antimicrobial secondary metabolites (so-called phytoalexins) was observed in the apoplast of *Sorghum bicolor* cells attacked by the fungal pathogen *Colletotrichum graminicola* (Snyder & Nicholson 1990). In this study, the deposition of the phytoalexin initiated with the occurrence of spherical cytoplasmic inclusions of approximately 1 μm diameter that subsequently coalesced to larger entities of ~20 μm diameter. Likewise, in barley powdery mildew interactions, the accumulation of H₂O₂-containing vesicular structures of 2–3 μm diameter has been reported (Collins et al. 2003, Hückelhoven et al. 1999). Ultrastructural analysis revealed that these vesicle-like bodies consist of a range of cellular structures, including miniature cell wall appositions, paramural bodies, and multivesicular bodies. This situation possibly reflects the specialized transport of different antimicrobial proteins, regulatory polypeptides, cell wall components, and other defense-related cargo (An et al. 2006). Besides the extrusion of phytoalexins, the exocytosis of a bouquet of defense-associated polypeptides, so-called pathogenesis-related proteins, into the apoplastic space is a common response to microbial attack that has been observed in numerous pathosystems (for example, see Carr et al. 1987).

Despite the longstanding, well-acknowledged role of secreted antimicrobial compounds and polypeptides at the cell periphery, transport routes from the site of synthesis to the site of action have remained largely unknown. Recently, Collins et al. (2003) demonstrated a fundamental role for a SNARE-domain protein in plant defense. Loss-of-function alleles of *AtSYP121* (*PEN1*; encoding a Qa-SNARE) and its barley ortholog, *HvROR2*, enable elevated levels of host cell entry either by nonadapted fungal species (on *Arabidopsis*; Figure 3e) or in highly resistant barley *mlo* mutant alleles. Lack of *AtSYP121* results in a delay in the formation of localized cell wall appositions (so-called...
papillae) at attack sites of nonadapted powdery mildews, suggesting that AtSYP121 contributes to the timely establishment of pathogen-triggered cell wall reinforcements (Assaad et al. 2004). Recent findings, however, indicate that the roles of AtSYP121 and the closely-sequence-related AtSYP122 extend beyond limiting fungal penetration and possibly also include the regulation of postinvasive defense layers (Zhang et al. 2007). Genetic studies involving triple mutants defective in AtSYP121 and AtSYP122 and the salicylic acid signaling pathway revealed that the two t-SNAREs may represent negative regulators of salicylic acid–, jasmonic acid–, and ethylene-dependent defense pathways (Zhang et al. 2007).

Silencing of the barley Qa+Qb–SNARE-encoding gene HvSNAP34, the ortholog of AtSNAP33, revealed increased fungal entry rates in the otherwise fully resistant mlo genotype, similar to the loss of HvROR2 Qa–SNARE function (Collins et al. 2003). In addition to mlo-mediated resistance, HvSNAP34 appears to be required for nonhost immunity and basal defense but not for isolate-specific resistance (Douchkov et al. 2005). In yeast two-hybrid assays, HvSNAP34 interacted with the PM-resident HvROR2 syntaxin, suggesting that HvROR2 and HvSNAP34 may represent cognate SNARE partners that form binary SNARE complexes in planta. Taken together, these results suggest an essential and evolutionary conserved role for a PM-localized SNARE complex during defense against fungal pathogens (Collins et al. 2003, Schulze-Lefert 2004). On the basis of the pathogen-responsive gene expression (Wick et al. 2003), a contribution of the Arabidopsis ortholog AtSNAP33 in plant defense also appears likely. Early seedling lethality of the respective Atsnap33-knockout mutant (Figure 3a) unfortunately has precluded experimental assessment of this prediction.

Following challenge by powdery mildew fungi, fluorophore-tagged variants of barley and Arabidopsis syntaxins HvROR2 and AtSYP121 became concentrated at attempted fungal entry sites (Assaad et al. 2004, Bhat et al. 2005). In addition to the HvROR2 syntaxin, a subset of further PM-resident barley proteins also accumulates at locations of fungal challenge (Bhat et al. 2005, Eichmann et al. 2006), suggesting the formation of a pathogen-triggered PM microdomain. Intriguingly, focal accumulation of fluorophore-tagged AtSYP121 syntaxin was not seen beneath attempted entry sites of adapted or nonadapted Colletotrichum species, fungal pathogens that cause the widespread anthracnose disease (Shimada et al. 2006). However, as in the case of nonadapted powdery mildew species (Assaad et al. 2004), Atsyp121 mutants exhibited a delay in the deposition of callose in papillae, reinforcing the notion that AtSYP121 function is associated with the timely formation of cell wall appositions in response to attack by various fungal pathogen species. Interestingly, despite delayed papilla formation there was no increase in the entry rate of nonadapted anthracnose fungi, suggesting that other defense reactions suffice to prevent ingress by these fungal pathogens. These assumed defense reactions possibly involve distinct vesicle populations tagged with R-SNAREs that interact with cognate Qa–SNAREs other than AtSYP121.

In a proteomic study aimed at identifying PM-resident polypeptides that become phosphorylated upon treatment with the general bacterial elicitor flg22, Nühse et al. (2003) discovered that the syntaxin AtSYP122, a close relative of the above-mentioned AtSYP121, is rapidly phosphorylated. In vitro phosphorylation of AtSYP122 occurs at two serine residues at the N terminus of the protein in a calcium-dependent manner, providing a potential link between defense-associated calcium signaling and protein phosphorylation. Likewise, Heese et al. (2005) have reported rapid phosphorylation of a tobacco syntaxin upon triggering a race-specific resistance response. These findings suggest that the phosphorylation of Qa–SNAREs may be a conserved early response in various types of plant defense.
Although most of the above-mentioned findings corroborate a role for SNARE proteins in defense-related exocytosis, a recent report also highlights the crucial role of host endocytosis in plant-microbe interactions. The Arabidopsis PM-resident receptor kinase FLS2 serves as a pattern recognition receptor for flagellin, the main building subunit of the bacterial motility organ (reviewed in Gómez-Gómez & Boller 2002). Flagellin represents a widespread pathogen-associated molecular pattern (PAMP), and its perception triggers innate immune responses that ultimately restrict bacterial invasion (Zipfel et al. 2004). Work by Robatzek et al. (2006) now shows that a functional GFP-FLS2 fusion protein is internalized into intracellular mobile vesicles upon activation by the flagellin epitope flg22. It is, however, currently unclear whether this ligand-triggered receptor endocytosis plays a role in receptor desensitization and/or defense signaling.

Finally, in the context of plant-pathogen interactions, it is not only defense reactions on the host side for which SNARE proteins are crucial. A recent study provides evidence that the t-SNARE Yup1 is essential for endocytosis during the initial steps of development of the plant-pathogenic corn smut fungus Ustilago maydis, highlighting the importance of SNAREs for both partners of plant-microbe interactions (Fuchs et al. 2006).

**Plant-Symbiont Interactions**

In comparison with the continuously growing knowledge about the function of SNARE proteins in the context of pathogen defense, little is known about the role of these polypeptides in symbiotic relationships. In the legume species Lotus japonicus, one of two LjSYP32 isogenes (LjSYP32-1) encoding orthologs of the AtSYP32 syntaxin appears to function in the development of root nodules, specialized organelle-like structures that form the interaction zone between symbiotic bacteria (rhizobia) and the plant’s root tissue (Mai et al. 2006). Like AtSYP32 (Figure 2), LjSYP32-1 is ubiquitously expressed, with a preference for roots. Transgenic Lotus lines expressing LjSYP32-1 antisense constructs showed a general growth defect and exhibited smaller roots and in part irregularly shaped nodules, suggesting that LjSYP32-1 plays a role in plant development and root nodule organogenesis (Mai et al. 2006). Reminiscent of Atsyp121/Atsyp122 double-mutant studies (Assaad et al. 2004), these findings in Lotus reinforce the notion that particular SNAREs have at least dual roles in developmental programs and interactions with microorganisms (Lipka & Panstruga 2005; see above). Similar to LjSYP32-1, in Medicago truncatula, MtSYP132, the ortholog of AtSYP132, was localized to root nodules (Catalano et al. 2004, 2007). Western blot analysis and immunolocalization revealed the presence of MtSYP132 in the specialized symbiosome membranes of so-called infection threads and infection droplets (Catalano et al. 2007). Taken together, these studies provide the first evidence for a role for plant SNARE isoforms in the establishment of plant-symbiont interactions, possibly by their contributions to the delivery of specialized cargo toward the symbiosome membrane. However, unequivocal genetic evidence for a function for any SNARE protein–encoding gene during symbiosis is still lacking. Pleiotropic effects (Mai et al. 2006) or even lethality associated with respective loss-of-function mutants may be preventing the identification of plant SNARE mutants in forward genetic screens for symbiosis-deficient mutants (Mao et al. 2005; NodMut database, http://nodmutdb.vbi.vt.edu/).

**THE ROLE OF SNARE PROTEINS IN ABSICIC ACID SIGNALING AND ABIOTIC STRESS RESPONSES**

Along with biotic stresses, plants also have to cope with a range of abiotic stresses, e.g., drought, heat, cold, and salinity. Stomatal closure is a common response in reply to various abiotic stress cues, including
heat, drought, and salinity. The phytohormone ABA is thought to play a pivotal role in the regulation of cellular responses upon abiotic stresses. A cDNA screen in frog (*Xenopus laevis*) oocytes led to the identification of a tobacco syntaxin, NtSYR1 (the ortholog of *Arabidopsis AtSYP121/AtSYP122*), which interferes with ABA-triggered potassium and chloride ion fluxes in both *Xenopus* oocytes and *Nicotiana tabacum* guard cell protoplasts (Leyman et al. 1999). Likewise, transient expression of a truncated version of NtSYR1, expected to exert a dominant negative effect, led to a complete loss of ABA-induced changes in ion fluxes. Because some mammalian syntaxin isoforms physically associate with ion channels (Naren et al. 1997), these findings have been interpreted as evidence for a potential additional role for syntaxins in the regulation of plant ion channel activities (Pratelli et al. 2004, Sutter et al. 2006a). NtSYR1 appears to interact with a tobacco homolog of the Qb+Qc-SNARE AtSNAP33 (Kargul et al. 2001). Consistent with a potential role in abiotic stress responses, NtSYR1 expression is responsive to ABA, salt stress, and wounding in the leaves but not roots of tobacco (Leyman et al. 2000). Recent data, however, revealed that the truncated dominant-negatively-acting variant of the AtSYP121 syntaxin contributes to the proper trafficking and localization of the KAT1 potassium channel rather than to the regulation of its activity (Sutter et al. 2006b). This suggests that the observed differential ion channel responses may be related to altered channel turnover upon syntaxin misexpression.

In a genetic screen for altered salt tolerance in *Arabidopsis*, a knockout line [osm1 (osmotic stress-sensitive mutant 1)] of the gene encoding the AtSYP61 syntaxin was identified in a population of T-DNA-tagged mutants (Zhu et al. 2002). The mutant exhibited increased sensitivity to both ionic and non-ionic osmotic stresses in a root bending assay. Additionally, the knockout line was hypersensitive to drought stress (*Figure 3d*), and its stomata were insensitive to ABA-induced closing and inhibition of opening. Surprisingly, transgenic *Arabidopsis* lines expressing an AtVAMP711 antisense fragment as well as individual AtVAMP711, AtVAMP713, or AtVAMP714 T-DNA insertion lines exhibited the opposite phenotype, namely improved salt tolerance (Leshem et al. 2006). This phenotype coincided with a failure of osmotic stress–induced, reactive oxygen species–containing endosomal vesicles to fuse with the central vacuole. Collectively, these data suggest antagonistic functions for individual SNAREs in abiotic stress responses.

ASSOCIATION OF SNARES WITH LIPID RAFTS

Lipid rafts represent cholesterol- and sphingolipid-enriched PM microdomains that can selectively include or exclude proteins and that supposedly serve as cellular signaling platforms (Pike 2006). Individual rafts are thought to be small (10–200 nm) but able to coalesce into larger entities in a stimulus-dependent manner (Pike 2006). In various types of animal cells, several syntaxins have been reported to reside in lipid raft–like PM microdomains (e.g., Chamberlain et al. 2001, Lang et al. 2001; reviewed in Salaün et al. 2004). There is increasing evidence for the existence of lipid rafts in plants (Mongrand et al. 2004; reviewed in Bhat & Panstruga 2005), and proteomic studies in tobacco recently revealed the potential presence of several syntaxin isoforms in PM microdomains (Mongrand et al. 2004, Morel et al. 2006). Reminiscent of the reported stimulus-triggered clustering of lipid rafts, fluorophore-tagged *Arabidopsis* and barley syntaxins AtSYT121 and HvROR2 exhibit focal accumulation beneath attempted fungal attack sites (Assaad et al. 2004, Bhat et al. 2005). Moreover, staining with the sterol-specific dye filipin revealed the local accumulation of sterols around sites of fungal challenge (Bhat et al. 2005). Studies in our laboratory have indeed confirmed
a partial sterol-dependent association of the cellular AtSYP121 syntaxin pool with detergent-resistant membranes, a characteristic feature of raft-associated proteins (N. Zappel & R. Panstruga, unpublished data). Taken together, the findings in plants seem to corroborate the data obtained in animal cells, pointing toward a concentration of certain SNAREs in PM microdomains, possibly to govern (polarized) sites of exocytosis temporally and spatially (Salaün et al. 2004). Recent experiments employing a fluorophore-tagged variant of the tobacco KAT1 potassium channel and dominantly acting NtSYR1 syntaxin fragments revealed a potential role for SNARE proteins in the traffic and distribution of KAT1 in distinct PM microdomains (Sutter et al. 2006b). These findings suggest that syntaxins may not only reside in lipid rafts but also, at least in part, contribute to the establishment of these PM microdomains.

### SUMMARY POINTS

1. All studied higher plant genomes encode a similar comprehensive set of SNAREs, including two plant-specific Qb subfamilies (NPSN and SYP7).
2. Many plant SNARE genes exhibit tissue-specific expression patterns as well as a distinct subcellular localization of the respective gene products.
3. Plant SNAREs contribute to essential developmental processes (cytokinesis, autophagy, idioblast morphogenesis) and physiological responses (gravitropism, abiotic stress, pathogen defense, symbiosis), many of which at the (sub)cellular level involve the establishment and/or maintenance of polarity.
4. Some SNAREs appear to fulfill dual roles in development as well as in physiological response pathways.
5. Like their animal counterparts, some SNARE isoforms seem to be associated with lipid raft–like plasma membrane microdomains.

### FUTURE ISSUES

1. The capability of plant SNAREs to form ternary SNARE complexes and their actual contribution to in planta vesicle trafficking should be verified.
2. The full set of biologically meaningful binary and ternary SNARE complexes occurring in plants needs to be established.
3. Methods for the isolation of vesicle subpopulations to determine the cargo transported by individual vesicle types characterized by the presence of distinct SNARE isoforms need development.
4. The (plant-specific) regulatory proteins of binary/ternary SNARE complex assembly/disassembly should be uncovered.
5. Future research should determine whether the dual function of some SNARE proteins in development and physiological responses is due to (a) changes in expression pattern and/or subcellular localization, (b) the presence/absence of particular regulatory proteins, or (c) changes in vesicle cargo.
6. Whether the localization of particular SNAREs in lipid raft–like plasma membrane microdomains is related to the sites of exocytosis/endocytosis needs resolution.
7. Potential functions of SNAREs distinct from vesicle trafficking (e.g., regulation of ion channels?) should be clarified.
8. Whether—and if so, which—SNAREs contribute to further plant-specific and cell polarity–related biological processes such as pollen tube expansion, root hair growth, and trichome development needs to be unraveled.

DISCLOSURE STATEMENT
The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Provides the first firm genetic evidence (based on a loss-of-function mutant allele) for the contribution of a SNARE in plant abiotic stress responses.
RELATED RESOURCES

Plant SNAREs. Genomic analysis of *Arabidopsis*, rice, and *Chlamydomonas* SNAREs:
http://www.cbs.umn.edu/~sande099/pln_snare.htm