SNARE Proteins as Signaling Elements

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Abstract

SNAREs (*N-ethylmaleimide-sensitive* factor adaptor protein receptors) are small polypeptides (~200–400 amino acid) which are characterized by a particular domain, the SNARE motif that can form a coiled-coil structure via hetero-oligomeric interactions. These protein interactions are highly stable leading to the formation of the so-called SNARE complex which allows the membrane fusion. SNAREs also interact with several proteins acting as regulators of SNARE complex formation. By regulating vesicle traffic, SNAREs have a clear influence on several signaling pathways. SNAREs take part to receptors turnover through endocytosis and exocytosis, but they can also directly gate channels and interact with membrane proteins potentially involved in signaling processes. Phosphorylation of SNAREs upon elicitation is known, and hormonal control confirms that SNAREs have a role in signaling processes.

Keywords

SNAREs (*N-ethylmaleimide-sensitive* factor adaptor protein receptors) • Endocytosis • Exocytosis • Endomembrane system • Synaptobrevins

2.1 Introduction

Eukaryotes have evolved a sophisticated system within the endomembrane system for extracellular transport of cargo molecules from the endoplasmic reticulum (ER) via the Golgi apparatus. The small membrane-coated transport vesicles shuttle between the compartments (including ER, Golgi apparatus, plasma membrane, and vacuoles) of the secretory pathway. The plant vacuoles are complex and multifunctional organelles that serve diverse functions such as storage, digestion,

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and recycling, and unlike in yeast the functional vacuoles present are essential for plant cells (Rojo et al. 2001). The vesicles in addition to extracellular transport, i.e., anterograde transport (exocytosis), are also required for uptake of material from the extracellular space, i.e., endocytosis and other retrograde transport processes. There are several classes of polypeptides that contribute in these shuttling activities, and the fusion process between a coated vesicle and a target membrane is an unfavorable process in terms of energy. The eukaryotic systems have evolved a specialized class of proteins that drives the membrane fusion and these are named as soluble *N*-ethylmaleimide-sensitive factor adaptor protein receptors (SNAREs). The SNAREs provide the force to bridge membrane lipid bilayers together and provide the specific matching specificity between vesicles and targeted compartments. In doing so they contribute to targeting and delivery of membranes and soluble proteins in all eukaryotic cells (Lipka et al. 2007).

The role of SNAREs in relation to membrane trafficking is not only limited to general homeostatic and housekeeping functions, but it also represents important signaling and response elements associated with growth, osmotic stress, gravitropism, and defense. The first evidences arrived with the discovery of the tobacco SNARE NtSYP121 (=NtSYR1) and its homologue in *Arabidopsis*, and this protein was found involved in abiotic stress signaling (Leyman et al. 1999) and, later, in pathogen resistance (Collins et al. 2003).

The canonical model of SNAREs function describes them as complementary sets of interacting proteins which target vesicles to the specific destination membrane. This model has to be enriched by the evidences of their contribution in scaffolding and anchoring of other membrane proteins that play their roles in response to environment, in growth as well as development. In comparison to animals and fungi, the plants have a larger number of SNAREs. In contrast to the members of the former kingdoms, the plants are lacking some of the particular SNARE protein subfamilies but have additionally evolved some novel types of SNAREs. After the description of the canonical model, this chapter will review the latest evidences about the relation of SNAREs with signaling in the plant cell.

2.2 SNARE Structure and Function

2.2.1 The Canonical Model

The SNAREs comprise of a large superfamily of relatively small polypeptides (~200–400 amino acids) which are characterized by the presence of a particular domain, the SNARE motif (Jahn and Scheller 2006). This domain has a stretch of 60–70 amino acids which consists the heptad repeat forming a coiled-coil structure. The SNAREs form highly stable protein-protein interactions via the hetero-oligomeric interactions that facilitate in overcoming the energy barrier required for membrane fusion. The SNAREs are required for mediating the fusion events between membranes in vesicle-associated traffic, and for performing this function they are thus distributed on vesicles and the organelles of the endomembrane system, including the plasma membrane.

The SNAREs possess *C*-terminal transmembrane (TM) domains that usually help in its association with membrane bilayer (Fig. 2.1a). The SNAREs that lack the TM domains are associated or attached with the membranes via the lipid anchors. There is an exception to possession of one SNARE motif, viz., SNAP-25-like SNARE protein that contains two SNARE domains that are separated by a flexible linker (Fig. 2.1a). In addition to the SNARE domain and the *C*-terminal TM domain, many SNAREs have an *N*-terminal region with regulatory function that generally controls the activity of SNARE protein and coordinates its activity with several accessory polypeptides (Fig. 2.1a).

The membrane fusion is mediated by interactions among the complimentary SNAREs associated with the vesicles and the target membrane. Once the SNAREs are matched, they form a highly stable association known as the "SNARE complex." This complex comprises of three

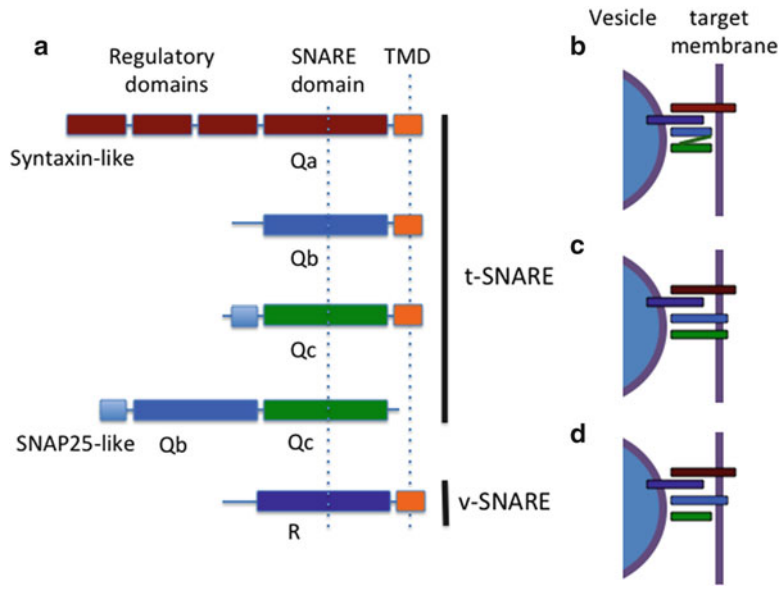

Fig. 2.1 The figure proposes a simple representation of SNARE structure and assembly. (**a**) Organization of Q- and R-SNARE domains; (**b**) SNARE complex composed by three proteins, typically observed in synaptic transport (Syn1/Vamp/SNAP25) and other transport event on the PM; (**c**) SNARE complex composed by four proteins, typically observed on yeast ER and endosomes (Syn7/Vamp/Syn8/VTi1); (**d**) SNARE complex composed by four proteins, typically observed on yeast vacuoles (Vam3/Vamp/Vam7/VTi1)

or four types of distinctive SNARE proteins which contribute to form a four-helix bundle of intertwined SNARE domains (Brunger 2005; Jahn and Scheller 2006).

The classification of SNAREs is based on either their localization (functional classification) or on the basis of the presence of specific amino acids in the center of the SNARE motif (structural classification). On the basis of functional classification, SNAREs are divided into vesicle-associated (v-SNAREs) and target membrane-associated SNAREs (t-SNAREs) (Lipka et al. 2007). This classification does not take into account the role of SNAREs in the context of homotypic fusion events or progressive anterograde traffic. The structural classification of SNAREs has been indicated as Q- and R-SNAREs based on the presence of either a conserved glutamine or an arginine residue in the middle of the SNARE domain (Fasshauer et al. 1998). Further, the functionally classified t-SNAREs correspond to the structurally classified Q-SNAREs, and similarly functionally classified v-SNAREs correspond to the structurally classified R-SNAREs. The target membrane-localized Q-SNAREs are of three types which are further subdivided into Qa-, Qb-, and Qc-SNAREs. The SNAP-25-like proteins of Q-SNAREs constitute a special class with both Qb- and Qc-SNARE motif. Historically the SNAREs are often designated and described on the basis of their role in synaptic exocytosis, and thus the Qa-SNAREs are frequently called as syntaxins (Bennett et al. 1992) and vesicle-resident R-SNAREs are called as VAMPs (vesicle-associated membrane proteins). The R-SNAREs can either have a short or a long *N*-terminal regulatory region, gaining the designation of brevins (lat. brevis, short) and longins (lat. longus, long). The brevins for their role in synaptic exocytosis have been frequently called as synaptobrevins; however, this evolutionary class of R-SNAREs is absent in plants, and thus all R-SNAREs present in the plants are only longins (Uemura et al. 2005).

2.2.2 SNARE Genomics in Plants

Several SNAREs have been located in the genomes of all higher plant species as such that there are 60 SNAREs in dicotyledonous model species *Arabidopsis thaliana* (The Arabidopsis Genome Initiative 2000), 57 SNAREs in monocotyledonous *Oryza sativa* (International Rice Genome Sequencing Project 2005), and 69 SNAREs in the *Populus trichocarpa* (Tree black cottonwood: Tuskan et al. 2006). In contrast the yeast *Saccharomyces cerevisiae* encodes for 21–25 SNAREs, and the humans (*Homo sapiens*) are thought to encode 35–36 SNAREs (Jahn and Scheller 2006; Sutter et al. 2006).

There are comparable numbers of SNAREs in the same subfamily in various plant genomes that suggest that the enlarged SNARE number, compared to other eukaryotes, is not related to a particular plant lifestyle or habitat but related to an essential aspect of plant biology. The higher number of SNAREs found in plant species as compared to fungi and animals is predominantly thought to be due to the expansion of number of members in conserved SNARE subfamilies and not due to the evolution of new isoforms. There are only two subfamilies which appear to be plant specific: the novel plant-specific SNARE (NPSN) Qb- and the SYP7 Qc-SNAREs (Sanderfoot et al. 2000; Lipka et al. 2007). The presence of most of these SNARE genes in green algae *Chlamydomonas reinhardtii* and moss *Physcomitrella patens* also indicates that these essential characteristics evolved early in plants to satisfy the necessity which arose for plant-specific biological processes. There are many plant-specific processes such as a specific type of cytokinesis, gravitropic responses, and phytohormones transport, and several of these processes are related to signaling. As well as in other eukaryotes, many of these functions are also involved in establishing and maintaining cellular processes polarization (Surpin and Raikhel 2004).

2.2.3 Q-SNAREs

Plant genomes encode multiple syntaxin-like isoforms called SYP (syntaxin of plants) subfamilies. These SNAREs were originally intended and indicated with an *N*-terminal autoregulatory domain, a linker, the SNARE domain, and a TM region (Fig. 2.1). This description has to be considered as a general indication with several exceptions in terms of autoregulatory and transmembrane domain. The examples being SYP5, 6, and 7, which were initially grouped as syntaxins but are now being regarded as Qc-SNAREs even if they are still designated as syntaxins (Pratelli et al. 2004; Sutter et al. 2006). The SNAREs that better correspond to the original description of syntaxins are all the Qa-SNAREs, but they also possess a natural genetic polymorphism. For example, AtSYP23 a Qa-SNARE lacks the *C*-terminal TM domain in the *Arabidopsis* ecotype Col-0 (Ohtomo et al. 2005). The *N*-terminal auto-inhibitory domain in AtSYP23 is composed of three helices which are also called the Habc motif, and this domain is folded into three helical bundles which mimics the parallel four-helix bundle of the SNARE complex. Furthermore, the folded domain interacting with the Q-domain in the so-called close conformation prevents undesired interaction of the protein with the partners before activation (Munson et al. 2000). The plant Qa- and Qb-SNAREs have been reported to have roles in several biological processes such as shoot gravitropism, cytokinesis, and autophagy. However up until now, no phenotype was revealed upon genetic screens for a Qc-SNARE. The Qb- and Qc-SNAREs are also known to possess an extended *N*-terminal domain occasionally, and this domain may also adopt (in animals) a coiled-coil structure similar to a Habc motif (Hong 2005). For example, the SNAP-25 comprises of 2 SNARE motifs and thus is classified as a Qb+Qc-SNARE. The Qb-domain of SNAP-25 is equivalent to *N*-terminal domain, and the Qc-domain is equivalent to a *C*-terminal domain. Moreover, the *Arabidopsis* SNAP-25-like proteins also lack a TM domain (Lipka et al. 2007). The mammalian SNAP-25 is attached to the PM by palmitoylation (Veit et al. 1996); however, the *Arabidopsis* SNAP-25s do not have an appropriate conserved palmitoylation sites (Lipka et al. 2007). Nevertheless, the AtSNAP33 has been reported to localize to the PM (Wick et al. 2003) more like the animal homologue (Hong 2005).

2.2.4 R-SNAREs

The R-SNAREs are grouped in three subfamilies, the SEC22s, VAMPs, and YKT6s. These are located mostly on trafficking vesicles and are anchored to them by the *C*-terminal TM domain. In animals and yeast the R-SNAREs, AtYKT61, and AtYKT62 are attached to the vesicular membranes by lipid anchors which are added to these posttranslationally (McNew et al. 1997). The plant R-SNAREs can be classified as longins because of the presence of longin domain (an extended *N*-terminal stretch). This longin domain is responsible for subcellular localization as well as regulation of SNARE complex assembly in other eukaryotes (Lipka et al. 2007). When compared to Q-SNAREs the information about the biological roles of plant R-SNAREs is scarce. However, there is only one exception of a recently discovered salt resistance phenotype (Leshem et al. 2006).

2.2.5 Expression and Subcellular Localization

The availability of microarray data (https://www.genevestigator.ethz.ch) allows the expression profiling of SNARE isoforms in various plant cell types, tissue, and organs. The expression of many SNAREs is ubiquitous, in particular SYP22 and SYP32 (Qa-SNAREs); VTI11 and GOS12 (Qb-SNAREs); BET11, SYP71, SFT11, and USE11 (Qc-SNAREs); SNAP33 (Qb+Qc-SNARE); and VAMP713, VAMP714, VAMP721, and VAMP722 (R-SNAREs) (Lipka et al. 2007).

Interestingly many SNAREs have apparently a relatively maximum transcript accumulation in pollen. This fact supports the idea that for the pollen development and function, a comprehensive vesicle-associated transport processes are required which are essential to pollen tube: cell polarity and tip growth (Hepler et al. 2001). Mostly the SNAREs are associated with specific membranous compartments, but some of them are localized to two or more distinct organelles which are possibly due to their shuttling between various subcellular compartments. The overexpression studies utilizing the full-size SNAREs or the cytoplasmic SNARE domains have provided fresh insights into the potential role of these isoforms in vesicle trafficking.

2.2.6 Regulators and Associated Proteins

The SNARE proteins are able to drive vesicle fusions in vitro; however, in vivo these SNAREs are not the only determinants of vesicle fusion and targeting specificity. For the formation of SNARE complex, the SNAREs are known to interact with many proteins which act as the regulators (Lobingier and Merz 2012; Schafer et al. 2012). The regulatory factors Sec1/Munc18 (SM) proteins are also important determinants interacting with non-conserved SNARE domains which control the conformational changes of syntaxins *N*-terminal Habc domains. The genome of *Arabidopsis* is reported to contain six members of the Sec1 family, and out of these one of the member which is called as KEULE (KEU) was shown to be involved in cytokinesis (Assaad et al. 2001). Another important class of regulatory proteins is the Ras-related GTPases belonging to the Rab GTPase family, and these are involved with SNAREs in controlling the multiple steps of vesicle transport. The *Arabidopsis* has 57 Rab GTPases classified in eight subfamilies (RabA to RabH) (Rutherford and Moore 2002). The regulatory role of Rabs interactions with SNAREs or the multi subunit protein complex called exocyst complex will be treated in a separate chapter. For the specificity of membrane fusion, the SNAREs may also fine-tune their function by partly acting as inhibitory SNAREs (i-SNAREs) (Sansebastiano 2013). In this action it may either substitute or bind to the fusogenic SNARE protein and thus forming a non-fusogenic complex (Varlamov et al. 2004). These later possibilities remind the importance of in vivo SNAREs stoichiometry in membrane fusion events (Fig. 2.2).

Further, for the process of SNARE recycling, the soluble accessory proteins, viz., *N*-ethylmaleimide-sensitive fusion protein NSF (an ATPase) and alpha-SNAP, are required. The interaction of an NSF with the SNARE complex

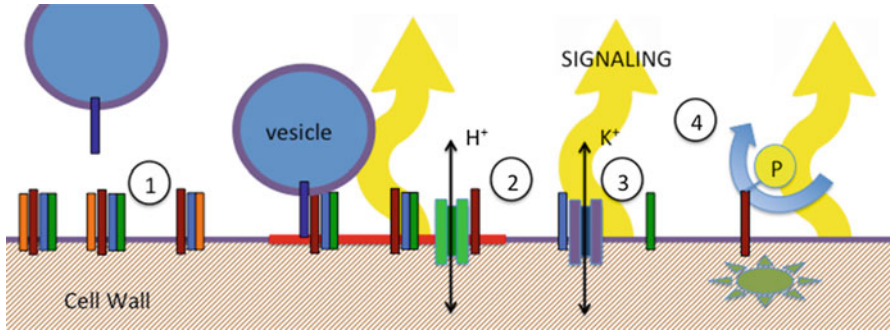

Fig. 2.2 SNAREs are involved in signaling in different ways. SNARE stoichiometry tells us they are more abundant than required for membrane traffic; they assemble also to form non-fusogenic complexes (1) and to interact with proton pumps to define membrane microdomains and potential (2) and other unknown partners; SNARE influence turnover of channels, but it is known that gating of Ca^{2+} and K^+ depends on SNAREs through direct protein-protein interactions (3); PM SNAREs can be phosphorylated as part of the signaling cascade elicited by interaction with microorganisms or hormonal stimulation (4)

takes place via the alpha-SNAP resulting in hydrolyzing ATP and dissociation of the complex (Barnard et al. 1997). *Arabidopsis* has three genes for alpha-SNAP and one for NSF, but nothing is known about their specific biological role (Sanderfoot et al. 2000).

2.3 SNAREs and Signaling

2.3.1 Development

The gravity direction is sensed by the plants which affect their growth orientation. The gravitropic responses are controlled by complex molecular mechanisms which involve signaling (perception and transduction) and growth adjustments. Initially during the gravity perception, the starch-filled amyloplasts undergo the changes in sedimentation, and these organelles are present in the endodermis (statocytes) of shoots and columella cells (statocytes) of root cap. They have an influence over the signal transduction cascades that are involved in auxin transporters relocalization resulting in altering of auxin flux. This alteration causes the compensatory asymmetric growth responses that are largely reliant on the vacuoles (Lipka et al. 2007; Bassham and Blatt 2008). Many gravitropic responsive genes have been isolated, and in *Arabidopsis* plant the Qa-SNARE AtVAM3/SYP22 (*SGR3*) and the Qb-SNARE AtVTI11 (*ZIG*/*SGR4*) of the SNARE complex play an important role in shoot gravitropism (Yano et al. 2003). Further the v-SNAREs comprising of VTI1 group are the best studied among the SNARE subfamily. The single mutations in VTI1 were shown to be viable while as the double mutations were embryo lethal (Surpin et al. 2003). The mutants with single mutation were having minor defects which placed them into two distinct phenotypes, viz., *vti11* and *vti12*. The knockout mutant's *vti11* and *vti12* showed developmental and growth-related defects. The *vti11* showed defects such as vascular patterning, auxin transport, and shoot gravitropism (Kato et al. 2002; Surpin et al. 2003) while as the *vti12* mutants were hypersensitive to starvation, and these showed premature senescence (Surpin et al. 2003) similarly to autophagy mutants. The studies on VTI11 and VTI12 revealed that the vacuolar trafficking is affected as such that in *vti11* mutant showed defects in protein trafficking to lytic vacuoles (LV), while as the *vti12* mutant showed defects in storage protein transport to protein storage vacuoles (PSV). The specificity of function in the single mutants, viz., *vti11* and *vti12* means that there can be partial substitution of proteins in SNARE complexes or in other words the redundancy of function (Sanmartín et al. 2007). The mechanism that allows SNAREs

to respond to gravity is unknown; however, there is a possibility that the mutant phenotype arises because of an indirect effect on the structure and composition of the vacuolar membranes rather than the direct effect of vesicle trafficking on gravitropism. There is an abnormal vacuolar structure in these mutants with an absence of transvacuolar strands and accumulated presence of vesicle-like structures which may be possibly impeding the amyloplast movement and may also be interacting with the cytoskeleton.

Membrane traffic affects growth most probably by altering the correct sorting of membranes and cell wall components. Tip growth in pollen tubes can be taken as a good example of growth processes because there exists a continuous vesicle secretion as well as the delivery of new wall material. It was recently shown that SNAREs have probably a determinant role in the pollen growth processes. The localization of pollen-specific syntaxin SYP125 (*AtSYP125*) was shown to be associated with the plasma membrane (PM) and apical vesicles in growing pollen tubes. The AtSYP125 was asymmetrically localized behind the apex at the plasma membrane, while as AtSYP124, another pollen-specific syntaxin was distributed differently (Rehman et al. 2011; Silva et al. 2010). Thus, the syntaxins asymmetric distribution in pollen tubes helps to define the exocytic sub-domains; however, there is also the requirement and role of other signaling and functional mechanisms such as phosphoinositides and small GTPases. Membrane traffic also regulates the transport capacity of selected ion and solute transporters (2, 3 in Fig. 2.2). Among these the most characterized in mammalian cells is the trafficking of GLUT4 (Na^+-coupled Glc transporter). It has been shown in the intestinal epithelial cells that GLUT4 cycles between apical membrane and cytosolic vesicle pool. The SNARE complexes involved in fusion of GLUT4 vesicles include mammalian syntaxin 4, SNAP-23, and VAMP2 within the lipid rafts of the plasma membrane. The recovery of these GLUT4 transporters form the apical plasma membrane takes place by endocytosis, and the sequestering takes place in specialized GLUT4 vesicles before recycling occurs (Grefen and Blatt 2008). This example shows how the traffic is characterized by changes in the integral membrane proteins turnover. Unlike GLUT4, many other proteins follow the path leading to the vacuole for final degradation after endocytosis. There is no information about the molecular mechanics of this trafficking; however, SNAREs do certainly play a role. Another interesting example is the traffic of the KAT1 (Kv-like K^+ channel) of the epidermal cells whose turnover at the plasma membrane is tightly controlled through a mechanism evoked by ABA that leads to recycling in an endomembrane pool that is distinctive from the degradation pathways leading to the vacuole. The studies using the dominant negative fragments of syntaxin SYP121 have revealed that the KAT1 transport to the plasma membrane depends on SYP121 function (Grefen and Blatt 2008). The lateral mobility of KAT1 also increased greatly (100-fold) in the presence of dominant negative fragments, and this is an indicative of an additional role of SNARE helping to anchor the KAT1 protein present within the lipid microdomains (2 in Fig. 2.2). Recent work directly evidences that SYP121 is the key structural element which determines the gating of another K^+ channel (Grefen et al. 2010) which makes it clear that in fact SNARE is part of a scaffold of proteins that is associated with membrane transport of K^+. Thus, the SNARE may be essentially required for channel-mediated K^+ nutrition that is a wholly distinct function from other roles in membrane traffic. In fact a few SNARE proteins such as in mammalian nerve cells, the syntaxin 1A is known to interact with ion channels binding several Ca^{2+} and K^+ channels.

2.3.2 Hormones

In the plants the vesicle trafficking has been implicated in a variety of responses pertaining to hormonal and environmental stimuli. This paragraph can only make limited examples about the existing cases of study. For example, ABA signaling was shown to be related to evoked endocytosis at the plasma membrane which ably selected among the integral membrane proteins and also

regulated their recycling back to the plasma membrane. The phytohormone ABA is pivotal in regulating cellular responses to abiotic stresses, and it acts as a signal that rapidly triggers changes in three K^+ and Cl^- channels such as in the control of ion transport of stomatal guard cells resulting in transpiration suppression from the leaf tissues. Concurrently, ABA is known to initiate endocytosis of KAT1 K^+ ion channel which is otherwise normally active during the K^+ uptake for stomatal opening. Furthermore, a study on a cDNA screen in frog (*Xenopus laevis*) oocytes for ABA-related genes ultimately led to the identification of a *Nicotiana tabacum* syntaxin NtSYR1 (which is an ortholog of *Arabidopsis thaliana* AtSYP121/AtSYP122). The SYP121 or the close homologue SYP122 contributes in early changes during ion channel gating in ABA response. It has been shown that the SYP121 and SYP122 dominant negative fragments were blocking changes in K^+ and Cl^- channel gating in response to ABA (Leyman et al. 1999; Geelen et al. 2002). The changes due to action of the dominant negative fragments can lead to the rise in intercellular calcium $[Ca^{2+}]_i$ due to suppression of the Ca^{2+} channel gating and its entry across the plasma membrane. The fact that in the guard cells K^+ and Cl^- channel currents are controlled mainly by the $[Ca^{2+}]_i$, it underscores the importance of the mechanisms regulating the integration of SNARE with ion channel regulation during the process of stomatal closure. Further, it also raises similar questions about integration and role of Ca^{2+} signaling with other physiological responses such as the responses to pathogens (see following paragraph) (Grefen et al. 2010, 2011). In one such response studies, the genetic screening in *Arabidopsis* for altered salt tolerance identified a knockout line [*osm1* (osmotic stress-sensitive mutant 1)] in which the T-DNA insertion was found to be similar to SYP61, and it co-segregated closely with osm1 phenotype and was found to be only a functional mutant (Zhu et al. 2002). In the root bending assay, the mutant showed sensitivity towards osmotic stresses (both ionic and nonionic). It also showed the hypersensitivity towards the drought stress, and the stomata showed insensitivity towards the ABA-induced opening and closing. However, it was surprising to note that upon the expression of antisense *AtVAMP711*, there was an improved salt tolerance in the above mutant lines. Similarly, the individual T-DNA insertional lines of *AtVAMP711*, *AtVAMP713*, and *AtVAMP714* also exhibited the improved salt tolerance. This phenotype coincided with a failure of osmotic stress-induced, reactive oxygen species-containing endosomal vesicles to fuse with the central vacuole. Thus the SNAREs can then have an antagonistic function in abiotic stress responses.

About the contributions of SNAREs to trafficking associated with auxin, some intriguing ideas emerged from the study of the PIN (for PIN-formed) and AUX1 (for AUXIN1) proteins. The SNARE proteins in the mammalian epithelial cells are responsible for the differential targeting of solute transporters (e.g., Na^+/K^+-ATPase, gastric H^+-ATPase) and the coupled transporters (e.g., GLUT4:Glc transporter) to apical and basal cell membranes. Considering the analogy to mammalian epithelial polarity to be true, it was expected that the AUX1 (an auxin uptake carrier) and PIN (an auxin efflux carrier) traffic will be dependent on the different subsets of Q- and R-SNARES because the AUX1 is localized on the apical ends of the cells (in *Arabidopsis* epidermis and cortex of stem and root), while as the PIN1 protein is present on the opposite ends of the same cells. However, these results are still controversial and need further clarification (Grefen and Blatt 2008).

2.4 SNAREs in Plant Interaction with Microorganisms

The SNAREs have been reported to have several roles in plant defense responses against the pathogen attacks (Reichardt et al. 2011). The resistance of *Arabidopsis* to powdery mildew of barley (*Hordeum vulgare*) is the best characterized example. On the *Arabidopsis* (a nonhost plant) the spores of the powdery mildew are able to germinate but are not able to penetrate the cells and thus are unsuccessful in establishing an infection. This is an example of nonhost resistance which is

an active mechanism where the cell wall provides a physical barrier at the site of the pathogen attempting to penetrate by secreting deposits known as papillae (Kwon et al. 2008). The genetic screening of *Arabidopsis* identified a defective nonhost resistance mutants called as pen mutants (having increased penetration of pathogen), and among these pen1 mutant was discovered with mutation in SYP121 gene. To find again SYP121 may look surprising, but biotic and abiotic stress have many common points. However, the precise function of SYP121 in pathogen resistance is not yet clear. In barley it has been seen that the cells below the site of infection have hydrogen peroxide filled in their vesicles, but these vesicles were decreased in *ROR2* mutant (*SYP121*-homologue). Due to the decrease in vesicles in *ROR2* mutants, they were more susceptible to fungal penetration, because of the fact that vesicles potentially function in cross-linking of the cell wall components in response to the attempts of infection. It was also found that the *Arabidopsis SYP121* was more susceptible to infection due to the delay in fungus-induced formation of papillae. These mutants also had an increase in the expression of PR-1 (pathogen response gene) and SA (salicylic acid). The increase in SA (a signal molecule in defense pathway) in these mutants suggests that SYP121 acts as a regulator of SA-mediated defense pathway. These findings indicate that SYP121 may have distinct and opposite roles in modulating several different pathogen-responsive pathways.

Another SNARE SYP122 is regulated in a different manner in spite of being very similar to SYP121 (Grefen and Blatt 2008). The expression of SYP122 is induced by viral, bacterial, and fungal infections. In response to flg22 (a bacterial elicitor) it showed rapid phosphorylation that may suggest its role in pathogen defense like its compatriot SYP121. However, the SYP121 phosphorylation takes place in response to Avr9 (race-specific elicitor) and not flg22 (4 in Fig. 2.2). Furthermore, the SYP121 mutants did not show any detectable defects in disease resistance like the SYP121. The double-mutant SYP121/122 indicated overlapping of individual function of SYP121 and SYP122. Further, unlike either of the mutants, the double mutant SYP121/122 was dwarfed, developed necrosis in patches, and had higher levels of SA and PR-1. These studies suggest that SYP121 and SYP122 are functionally distinct (Rehman et al. 2008) and they can partially substitute for one another.

A third plasma membrane syntaxin that was the object of deeper studies, SYP132, has also been implicated in defense response against the bacterial infection. It is also phosphorylated upon elicitor treatment and thus suggesting that phosphorylation may be a general phenomenon required for regulation of defense-related SNAREs. Probably many of the secretory SNAREs are multifunctional proteins which under the normal conditions are having a role in the general secretory pathway. But during the pathogen attack, they are recruited to defend the cells and are required for delivery of cell wall material and defense proteins during the infection. In fact the expression of *SNAP33* a Qa+Qb partner of both SYP121 and SYP122 is also induced by pathogen attack, and its knockout mutants are dwarfed and develop necrotic lesions.

2.4.1 Plant-Symbiont Interactions

During the endosymbiotic interactions the host forms the specialized membrane compartments. There are two well-studied situations which are of agricultural as well as ecological relevance: firstly the arbuscular mycorrhizal symbiosis and the second rhizobium-legume symbiosis. In both of these cases the host produces the specialized membrane surrounding the microbes to form a symbiotic interface which facilitates the exchange of nutrients. Despite their importance, the mechanisms for the formation of these specialized membrane interfaces are largely unknown (Lipka et al. 2007). There is little information about the role of SNAREs in these symbiotic relationships. In the legume species (*Lotus japonicus*) it has been reported that one of the *LjSYP32* isogenes (*LjSYP32-1*) which encodes orthologs of the AtSYP32 syntaxin appears to function in root nodule development. The *AtSYP32* and

LjSYP32-1 are expressed ubiquitously but having a preference for roots. The transgenic antisense *Lotus* lines proved that this protein has in fact a role in plant development as well as root nodule organogenesis.

The *Medicago truncatula* MtSYP132, an ortholog of AtSYP132, was shown localized in root nodules in the specialized symbiosome membranes of the so-called infection threads and infection droplets. Finally, the R-SNARE exocytotic vesicle-associated membrane proteins (VAMPs) are also required for the formation of the membrane at the symbiotic interface in both interactions.

2.5 Conclusion

We have seen SNAREs taking part to receptor turnover through endocytosis and exocytosis, we have seen them gating channels (e.g., KAT1), and recent experiments also suggest the regulation of proton pumps activity. These mechanisms have a clear effect on signaling. Moreover SNAREs phosphorylation upon elicitation and hormonal control confirm that future studies on signaling will find more and more evidences about the role of these proteins in all signaling processes.

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