

The Arabidopsis Genome. An Abundance of Soluble *N*-Ethylmaleimide-Sensitive Factor Adaptor Protein Receptors¹

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Many factors have been characterized as essential for vesicle trafficking, including a number of proteins commonly referred to as soluble *N*-ethylmaleimide-sensitive factor adaptor protein receptor (SNARE) components. The Arabidopsis genome contains a remarkable number of SNAREs. In general, the vesicle fusion machinery appears highly conserved. However, whereas some classes of yeast and mammalian genes appear to be lacking in Arabidopsis, this small plant genome has gene families not found in other eukaryotes. Very little is known about the precise function of plant SNAREs. By contrast, the intracellular localization of and interactions between a large number of plant SNAREs have been determined, and these data are discussed in light of the phylogenetic analysis.

An essential function for all eukaryotic cells is to control the traffic of proteins and lipids through the endomembrane system (for review, see Sanderfoot and Raikhel, 1999). Most protein cargo first enters the endomembrane system at the endoplasmic reticulum (ER) before moving on to the Golgi apparatus. After sequential passage through the stacks of the Golgi, proteins arrive at the trans-Golgi network (TGN). At the TGN, cargo is sorted into vesicles destined for various endosomal organelles including the prevacuolar compartment (PVC) or vacuole, or is targeted to the plasma membrane (PM) for secretion. Each of these compartments must maintain some independence and a unique protein content, while accommodating a vast amount of cargo in transit to another destination.

Many factors including proteins and lipids are thought to be involved in packaging cargo into vesicles and delivering them to different organelles. A large diverse group of proteins called soluble *N*-ethylmaleimide-sensitive factor (NSF) adaptor proteins (SNAPs) receptors (SNAREs) and their associated proteins are vital for this process. The term SNARE describes the activity of these proteins as binding partners of two proteins, NSF and SNAPs. It is now clear that the role of SNAREs is much deeper than simply as receptors for SNAPs. As diagrammed in Figure 1, the role of the SNAREs appears to be in

the assembly of a four-helix SNARE bundle (often called a trans-SNARE complex or a SNAREpin), which probably drives the fusion of transport vesicles with target membranes. Three of the helices form a "t-SNARE complex" on the target membrane. One of the helices is always derived from a member of the syntaxin family of SNAREs, whereas the other two helices come from other types of SNAREs. A fourth helix (the v-SNARE) is derived from the vesicle membrane, and assembly of this helix into the t-SNARE complex is very energetically favorable and is sufficient to drive fusion the membranes *in vitro* (Weber et al., 1998; McNew et al., 2000). Following vesicle fusion the four-helix bundle exists as a cis-SNARE complex where all four proteins are found on the same membrane. It is at this point that NSF and SNAPs bind onto the cis-SNARE complex and the ATPase activity of NSF is used to disassemble the complex, freeing the individual members for additional rounds of vesicle fusion.

The mixing of synthetic liposomes containing just SNAREs can result in fusion of membranes *in vitro* (Weber et al., 1998; McNew et al., 2000); however, this process must be highly regulated *in vivo*. This regulation is the role of proteins of the Rab and Sec1 families of proteins. Sec1 proteins directly bind to syntaxins and regulate their activity and conformation (for review, see Hanson, 2000). The Rab proteins do not interact directly with the SNAREs, and instead function through various effectors, as well as interacting with the Sec1 proteins (Gonzalez and Scheller, 1999). As they do not interact directly with the SNAREs, the Rab family of proteins will be covered in a separate analysis.

The availability of the complete sequence of several model eukaryotes has provided useful information

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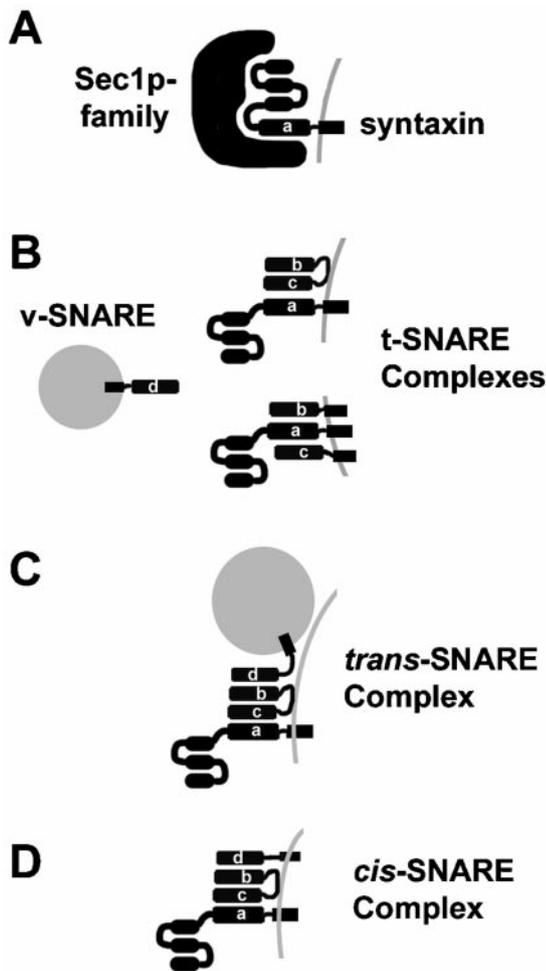


Figure 1. SNAREs in vesicle fusion. A, Syntaxins are usually found in an inactive state associated with a member of the Sec1p family. In some manner the Sec1 protein and other effectors activate the syntaxin, allowing it to associate with other SNAREs to form a t-SNARE complex. B, Four SNARE helices are required for vesicle fusion to occur (labeled the a-, b-, c-, and d- helices as described in Scales et al., 2000). The a-helix is always contributed by a syntaxin residing on a target membrane. At certain targeting steps (e.g. at the PM) the syntaxin associates with a member of the SNAP25 class of SNAREs, which contributes a b- and c- helix to the t-SNARE complex (B, top right). Other steps, including at most intracellular membranes, the syntaxin associates with two other SNAREs who each contribute a b- or a c-helix to the t-SNARE complex (B, bottom right). C, Vesicle fusion likely occurs when the v-SNARE (or d-helix) from the vesicle assembles into a trans-SNARE complex (or SNAREpin) with the t-SNARE complex (here just showing the SNAP25-containing complex). For simplicity a single trans-SNARE complex is drawn; it is possible that the concerted action of many trans-SNARE complexes forming simultaneously may be required for a vesicle to fuse. D, The four-helix cis-SNARE complex results, where all four proteins are on the same membrane. The cis-SNARE complex is a binding site for SNAPs and NSF, proteins that disassociate the SNARE complex, freeing the individual SNAREs for future fusion events.

on the overall conservation of the vesicle fusion machinery. Many important distinctions are also found, notably the absence of certain proteins in some eukaryotes, an increase in the number of SNAREs in

multicellular eukaryotes, and the presence of novel proteins found only in particular eukaryotes. The genome sequence of the model plant *Arabidopsis* has provided the first look at the complete complement of SNARE components in plants.

Because it is now clear that a single SNARE may function at multiple targeting events and may be required on the vesicle (as a v-SNARE) in one case and on the target membrane (as a t-SNARE) in another (McNew et al., 2000), definition of a particular SNARE as a v- or t-SNARE is now inaccurate. It is fortunate that the particular types of SNAREs appear to be conserved across the eukaryotes and we can therefore discuss these SNAREs as orthologous groups rather than describing a particular protein as a t- or v-SNARE. Here we will first describe the syntaxin family, a group of SNAREs that are always found as part of the t-SNARE complex. We will then describe the other SNAREs groups, proteins that are in some cases part of the t-SNARE complex or that in other cases function as v-SNAREs. In addition, we will also discuss the Sec1p family of proteins and those proteins involved in breaking up the cis-SNARE complex (SNAPs, NSF, and its homologs).

SYNTAXINS

Syntaxins can be recognized by sequence homology and by common structural features. For example, all are membrane proteins most commonly anchored to membranes via their C-terminal transmembrane domains, although some are anchored by post-translational addition of lipids to a C-terminal Cys residue. Adjacent to the membrane anchor is a coiled-coil domain centered on a Glu residue. At the N terminus of the protein are three other α -helical domains, which form a bundle in the crystal structure of mammalian syntaxin 1 (see Fig. 1A; Fernandez et al., 1998; Misura et al., 2000). Other syntaxins are predicted to contain these N-terminal helices and this has been confirmed by structural studies in some cases (Fiebig et al., 1999).

Syntaxins have been well studied in a few eukaryotes, and several isoforms are known to localize to distinct compartments where they function in vesicle fusion (for review, see Sanderfoot and Raikhel, 1999). All syntaxins can be recognized by the high degree of sequence homology in their coiled-coil domain, however, certain syntaxins form groups where the sequence homology is extended throughout the entire protein (i.e. groups of orthologs). It is interesting that when the functions of the members of a particular orthologous group are examined they each seem to have a common function or at least intracellular localization. Although sophisticated algorithms have been used in the past to identify SNAREs in sequence databases (Weimbs et al., 1998), BLAST searches (Altschul et al., 1997) can be used to distinguish syntaxins from other proteins, including other

SNAREs (Adavani et al., 1998; Holthuis et al., 1998; Simonsen et al., 1998; Steegmaier et al., 1998; Sanderfoot et al., 1999).

Yeast (*Saccharomyces cerevisiae*) is known to contain eight syntaxins (for review, see Pelham, 1999), two of which share high sequence identity and are known to be functionally redundant (Aalto et al., 1993). These syntaxins minimally define the known compartments of the yeast secretory pathway and can serve as a basis for classification of syntaxins from other eukaryotes. Although some syntaxins have a broad localization, and in fact, often cycle between target organelles, a steady-state localization for the yeast syntaxins can be defined. Ufe1p resides on the ER, Sed5p on the Golgi, Tlg2p on the late Golgi, Pep12p on the PVC, Vam3p on the vacuole, Tlg1p on the endosome, and the redundant pair of Sso1p and Sso2p (hereafter Sso1/2p) on the PM (see Pelham, 1999 and refs. therein).

Based upon our analysis of the sequence databases for several eukaryotes with complete or near-complete genome sequences, *Caenorhabditis elegans* (The *C. elegans* Sequencing Consortium, 1998) contains nine syntaxins, the fruit fly (Adams et al., 2000) contains 10, and humans contain at least 15. Similar analyses carried out by others (Pelham, 1999; Koushika and Nonet, 2000; Littleton, 2000) have yielded comparable results. The completed genome of Arabidopsis has revealed 24 syntaxins (The Arabidopsis Genome Initiative, 2000). In a general sense, animals and plants have syntaxins that are orthologous to one of the yeast syntaxins; and where it has been examined, they have a similar localization and/or function (see below). Often, the multicellular eukaryotes have multiple paralogs of the single yeast syntaxins (i.e. genes having higher sequence identity to a gene within the same genome than to the ortholog from a different organism). In a converse manner, none of the multicellular eukaryotes contain an ortholog of the yeast vacuolar syntaxin Vam3p (Pelham, 1999; Littleton, 2000; Fig. 3). On the other hand, multicellular eukaryotes have evolved novel groups that yeast appears to lack. For example, plants, fruit fly, and mammals have elaborated the Tlg1p group to create the Syntaxin 6/10 subgroup and the Syntaxin 8 subgroup; plants appear to have a third novel group of Tlg1p-like syntaxins.

Some of the 24 syntaxins of Arabidopsis have been previously examined and have been named according to various (often conflicting and potentially confusing) criteria. To synchronize the nomenclature we have developed a system for naming the plant syntaxins using the name syntaxin of plants (SYP), and here we apply this system to the syntaxins of Arabidopsis. The syntaxins of Arabidopsis (and of other plants, see below) cluster into eight groups based upon sequence homology—groups that we named SYP1 to SYP8 (Fig. 2). As can be seen in Figure 3, each of these groups is paralogous, having a higher degree

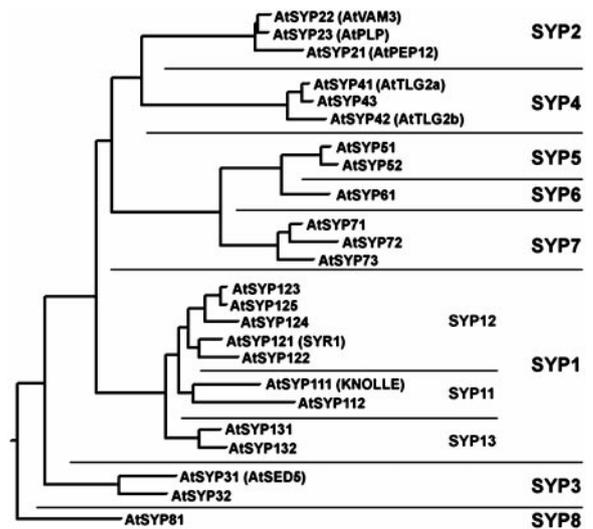


Figure 2. Arabidopsis syntaxin groups. The protein sequences of Arabidopsis syntaxins were aligned using default parameters of the CLUSTAL algorithm of the MEGALIGN program in the DNASTAR package. The resulting phylogenetic tree revealed the presence of eight groups (SYP1–8), which were numbered according to various criteria (see text). For those Arabidopsis syntaxins that have been previously published, the prior name is given in parenthesis. The accession numbers for all the Arabidopsis SNAREs can be acquired from <http://www.msu.edu/~sanderfo/atsnare.htm>

of homology with members of their own group than with orthologous syntaxins from fungi or animals.

Within most of the groups the individual members share between 50% and 80% protein sequence identity. In the SYP3 family only approximately 44% identity is found, consistent with the members of this group being more highly diverged. The members of the SYP1 group were found to have a large range of sequence identities (between 34% and 63% protein sequence identity among each other), and the members seemed to cluster into three subgroups. To further examine this finding we examined the structure of each syntaxin gene locus to determine the location of introns within each gene (Fig. 4). We found that the SYP1 group consisted of three gene families, each of which corresponded to the subgroups suggested by sequence identity. A gene family is defined here as a paralogous group of genes that share a common splicing pattern (and presumably, arose by way of gene duplication). We, therefore, subdivided the SYP 1 group into three subgroups (1, 2, and 3; or SYP11, SYP12, and SYP13), and named the individual members sequentially within the subgroups (i.e. SYP111, SYP112. . .) in the order of prior publication or of deposition into sequence databases. With the exception of the SYP6 and SYP8 groups, which contained only a single member, we found that each of the other groups of Arabidopsis syntaxins was represented by a gene family (Fig. 4). The two members of the SYP3 group share a somewhat similar splicing pattern, especially near the 3' end of the gene (Fig. 4) and are likely a gene family whose members have

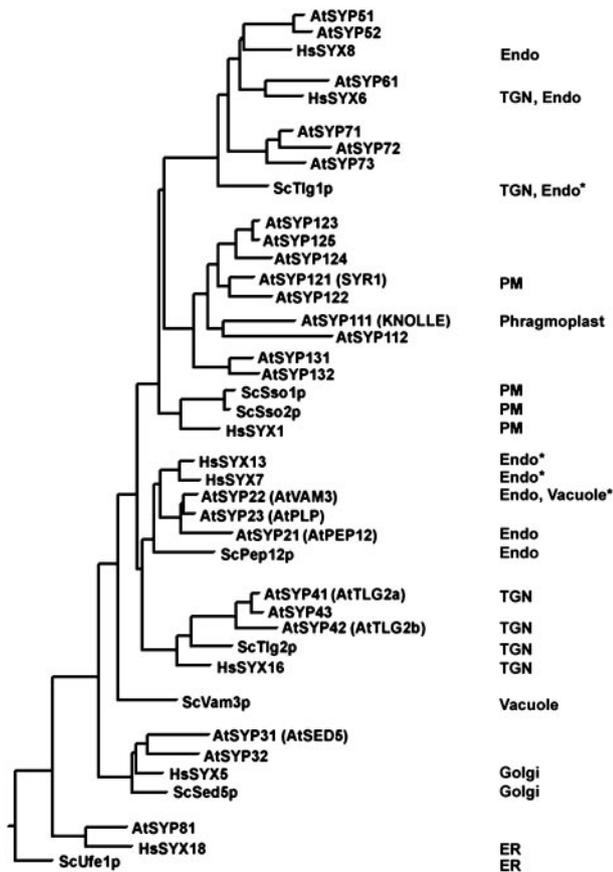


Figure 3. Phylogenetic analysis of eukaryotic syntaxins. Full-length sequences of the Arabidopsis (At), yeast (Sc), and selected human (Hs) syntaxins were acquired from GenBank and aligned as described in Figure 2. Where it is known, the intracellular localization of the indicated syntaxins is given (see text for references). Because the nature of the endosomal compartments (i.e. early, late, prevacuolar, etc.) can vary considerably depending on the cell type we use the general term endosome (Endo) for those syntaxins localized to these types of endomembranes. Often, different researchers have found conflicting results with the same syntaxin and these are noted by an asterisk. More information on these sequences, as well as further alignments with other eukaryotic syntaxins, can be acquired from <http://www.msu.edu/~sanderfo/atsnare.htm>.

diverged significantly. As with the SYP1 subgroups, we named each member of the various groups sequentially (i.e. SYP21, SYP22. . .) in the order of prior publication or of deposition into sequence databases. It should be noted that 20 of the 24 Arabidopsis syntaxins are known to be expressed, as judged by the existence of a cloned cDNA or a cognate expressed sequence tag (EST; see Table I). This suggests that this large number is not due to multiple pseudogenes. The complete complement of Arabidopsis syntaxins is listed in Table I.

When compared with other eukaryotic syntaxins the members of the SYP1 group show highest homology with yeast Sso1/2p or mammalian Syntaxin 1, which reside on the PM (Aalto et al., 1993; Bennett et al., 1993). However, compared with the other syn-

taxin groups (see below), this association of SYP1 with the PM syntaxins of other organisms is relatively weak. On the other hand, one member of the SYP1 group is known to localize to the PM (Leyman et al., 1999) and the others seem to have roles somewhat analogous to those of the PM syntaxins, suggesting this association may be real. AtSYP111 or KNOLLE was first characterized as a seedling lethal gene (Lukowitz et al., 1996; and see Lethality below). KNOLLE is expressed only during cell division and is found at the phragmoplast (Lauber et al., 1997). AtSYP121 (AtSYR1) is found on the PM of guard cells and may play a role in abscisic acid signaling (Leyman et al., 1999). Of the other four members of this gene family (AtSYP122–125), two are known to be expressed, but their function or localization remains to be determined. The AtSYP131/132 group has not been examined.

The AtSYP2 family has three members orthologous to Pep12p and to mammalian syntaxins 7 and 13. Yeast Pep12p is found on the late endosome/PVC (Becherer et al., 1996), whereas syntaxins 7 and 13 are found on distinct endosomal structures and likely

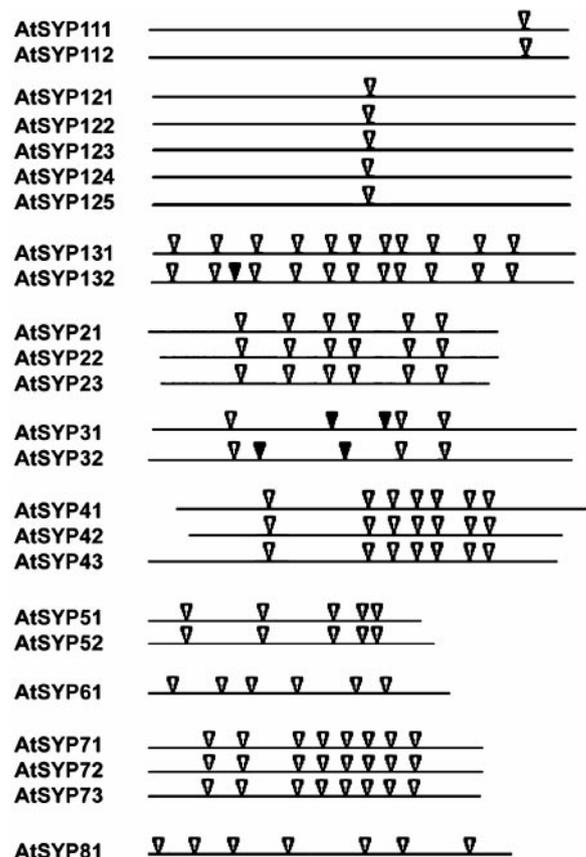


Figure 4. Gene structure of the Arabidopsis syntaxins. The cDNA sequence of each Arabidopsis syntaxin is represented schematically as a line with the position of the introns indicated by the triangles. White triangles within each group indicate an identical intron position within a particular gene family and black triangles indicate introns in a position not found in other members of the gene family.

Table I. *Syntaxins of Arabidopsis*

Name	Previous Name ^a	Genomic Locus ^b	AGI ID ^c	Intracellular Localization ^d	Expressed Gene? ^e	Note ^f
AtSYP111	KNOLLE	F22O13.4	At1g08350	Phragmoplast	Yes	1
AtSYP112		T30D6.23	At2g18260		?	
AtSYP121	AtSYR1	F26K24.11	At3g11820	PM	Yes	2
AtSYP122		F22O6.220	At3g52400		Yes	3
AtSYP123		F4C21.26	At4g03330		Yes	
AtSYP124		T1F9.22	At1g55410		?	
AtSYP125		T28P6.10	At1g10980		?	
AtSYP131		F20H23.28	At3g03800		?	
AtSYP132		T22D6.20	At5g08080		?	
AtSYP21	AtPEP12	F5E19.180	At5g16830	PVC	Yes	4
AtSYP22	AtVAM3	MSD23.4	At5g46860	PVC or vac	Yes	5
AtSYP23	AtPLP	dl14901c	At4g17730		Yes	6
AtSYP31	AtSED5	MJJ3.17	At5g05760		Yes	
AtSYP32		K7M2.10	At3g24350		Yes	
AtSYP41	AtTLG2a	F2P16.240	At5g26980	TGN	Yes	7
AtSYP42	AtTLG2b	T10M13.19	At4g**	TGN	Yes	7
AtSYP43		F18C1.4	At3g05710		Yes	8/9
AtSYP51		F3O9.4	At1g15930		Yes	9
AtSYP52		F20B17.2	At1g73260		Yes	9
AtSYP61		F3M18.7	At1g27550		Yes	9
AtSYP71		F11F8.33	At3g09740		Yes	9
AtSYP72		F18N11.40	At3g45280		Yes	9
AtSYP73		F2A19.50	At3g61450		Yes	9
AtSYP81		F19C24.5	At1g47920		Yes	

^a Name given to previously published Arabidopsis syntaxins, see notes for references. See Tables I and II for accession nos. and associated protein sequences. ^b Designation of gene locus on annotated bacterial artificial chromosome. ^c Systematic designation given to gene by Arabidopsis Genome Initiative, At4g03330 indicates the 333rd annotated gene (counting from top) on chromosome 4. Genes marked by a double asterisk were not assigned nos. during annotation. ^d Intracellular localization, if known; see note for reference. ^e Indicates whether gene is expressed, based on cloned cDNA (see note for reference) or presence of cognate EST. ^f 1, Lukowitz et al., 1996; 2, Leyman et al., 1999; 3, X. Gansel and L. Sticher, GenBank accession no. AJ245407; 4, Bassham et al., 1995; 5, Sato et al., 1997; Sanderfoot et al., 1999; 6, Zheng et al., 1999a; 7, Bassham et al., 2000; 8, X. Gansel and L. Sticher, GenBank accession no. AJ245408; 9, A.A. Sanderfoot and N.V. Raikhel, unpublished data.

have distinct functions (Prekeris et al., 1999). AtSYP21 (AtPEP12) is found on the PVC in Arabidopsis (Conceição et al., 1997; Sanderfoot et al., 1998). AtSYP22 (AtVAM3) has been reported to localize to the vacuole in the shoot apical meristem (Sato et al., 1997). However, we have shown that it is found only on the PVC, colocalized with AtSYP21 in vegetative cells (Sanderfoot et al., 1999). Whether this syntaxin is found on different membranes in different cell types is currently being investigated. AtSYP23 is known to be expressed (Zheng et al., 1999a), though its localization has not been examined.

The AtSYP3 family contains two members orthologous to Sed5p and mammalian syntaxin 5, which localize to the Golgi (Hardwick and Pelham, 1993; Dascher et al., 1994). Both members are known to be expressed, although the localization of either has yet to be reported.

The AtSYP4 family has three members that are orthologs of Tlg2p and mammalian syntaxin 16, syntaxins of the late Golgi/TGN (Abeliovich et al., 1998; Holthuis et al., 1998; Séron et al., 1998; Simonsen et al., 1998). Two members of this group, AtSYP41

(AtTLG2a) and AtSYP42 (AtTLG2b), have been examined, both localizing to the TGN; however, they do not colocalize, but instead are found on distinct domains of the TGN (Bassham et al., 2000).

Like fruit flies and mammals, plants have elaborated the Tlg1p-group. The two members of the SYP5 family are orthologous to syntaxin 8, a protein found preferentially on the late endosomes in mammalian cells (Prekeris et al., 1999). The SYP6 family (one member) is related to syntaxins 6 and 10. Syntaxin 6 is a protein involved in TGN to late endosome trafficking in mammalian cells that is found preferentially on the TGN (Bock et al., 1997). Syntaxin 10 is paralogous to Syntaxin 6 and is also found in the TGN, though its functions have not been examined (Tang et al., 1998). We have recently begun to characterize the members of these groups (SYP5 and SYP6), and preliminary results suggest that these syntaxins have locations similar to their eukaryotic orthologs (A.A. Sanderfoot and N.V. Raikhel, unpublished data), though their functions remain unclear. The SYP7 family (three members) does not appear to

have an ortholog among the yeast or animal syntaxins, and this group may be unique to plants.

The SYP8 family has a single member and is related to ER-localized syntaxins such as Ufe1p and syntaxin 18 (Lewis et al., 1997; Hatsuzawa et al., 2000). An EST is found for AtSYP81, but its localization has not been examined.

That Arabidopsis syntaxins are encoded in gene families appears not to be indicative of redundancy. In fact, research has indicated that the individual members of the families may have distinct localizations (Bassham et al., 2000) and expression patterns (Zheng et al., 1999a) and are likely to possess distinct functions as well. Gene disruption experiments of individual Arabidopsis syntaxins (i.e. AtSYP21, AtSYP22, AtSYP41, and AtSYP42) indicate that loss of a single member of a gene family is lethal (A.A. Sanderfoot and N.V. Raikhel, unpublished data). These results suggest that members of these gene families have diverged such that each has a unique essential function. Further work is needed to define the exact functions of each Arabidopsis syntaxin, yet these studies should provide much useful information about the functioning of the plant endomembrane system.

OTHER SNAREs

Other SNAREs besides syntaxins are required for vesicle fusion. The members of these groups contribute helices to the four-helix bundle at various trafficking steps throughout the endomembrane system (for review, see Scales et al., 2000). Often, the members of these groups may have roles in more than a single trafficking event and may interact with many different syntaxins (i.e. be a part of several different t-SNARE complexes). Even more confusing, the same SNARE that is part of a t-SNARE complex at one trafficking step can act as a v-SNARE in another. Thus classification as to function in these groups is difficult. It is fortunate that many of these groups appear to be conserved across eukaryotes and they can be classified by sequence homology. Just as was seen for the syntaxins above, these SNAREs are often encoded by small gene families made of two to seven members in Arabidopsis. As with the syntaxins, these gene families generally share identical splicing patterns (data not shown). A complete list of these SNAREs can be found in Table II.

SNAP25-Like SNAREs

The SNAP25 class of SNAREs was first described in the mammalian neuron. SNAP25 contributes two helices (one N-terminal and one C-terminal) to the t-SNARE complex of the mammalian neuronal synaptic membrane (McNew et al., 2000). SNAP25 is bound to the membrane by post-translational lipid addition; however, other members of the SNAP25

class are found to associate with membranes without addition of lipid anchors (Steegmaier et al., 1998). Mammalian cells have at least three members of this group: at the PM, there is one neuron-specific and one ubiquitous SNAP25; a third member functions at other endomembranes (Oyler et al., 1989; Ravichandran et al., 1996; Steegmaier et al., 1998). Yeast have two members of the SNAP25 class. Sec9p interacts with Sso1/2p at the PM as part of general secretion (Brennwald et al., 1994), whereas Spo20p is a sporulation-specific protein (Neiman et al., 2000). Arabidopsis has three genes that encode proteins of the SNAP25 group, one of which (AtSNAP33) has been found to localize to the PM and likely functions in vesicle secretion (X. Gansel and L. Sticher, personal communication).

Vti1p-, Gos1p-, and Membrin-Like SNAREs

In yeast, Vti1p was first described as a v-SNARE involved in TGN-to-PVC trafficking (Fischer von Mollard et al., 1997). Further work has indicated that this SNARE functions in many additional pathways such as intra-Golgi trafficking (Fischer von Mollard et al., 1997; Lupashin et al., 1997), as well as all three vesicle trafficking steps to the yeast vacuole where it is part of a vacuolar t-SNARE complex (Fischer von Mollard and Stevens, 1999; Fukuda et al., 2000). Mammals contain two Vti1p orthologs (Fischer von Mollard and Stevens, 1998; Xu et al., 1998), whereas *C. elegans* and fruit fly contain one (Littleton, 2000). Arabidopsis contains three orthologs, two of which probably have distinct functions in vesicle trafficking (Zheng et al., 1999b; Bassham et al., 2000).

Yeast Gos1p is believed to have a role in intra-Golgi trafficking, probably as part of a t-SNARE complex with the Golgi syntaxin Sed5p (McNew et al., 1998), though the precise role of this SNARE remains unclear. Mammals have a Gos1p-ortholog called GS28 (Subramaniam et al., 1997), whereas Arabidopsis has two. Trafficking between the ER and Golgi in yeast requires the action of the two SNAREs: Bet1p is the v-SNARE for this step, whereas Bos1p is part of a t-SNARE complex that includes Sec22p (see VAMPs) and the Golgi syntaxin Sed5p (Newman et al., 1990; Parlati et al., 2000). Arabidopsis has two orthologs of Bet1p, however, no homologs of Bos1p are found. In mammals it is believed that the SNARE membrin has taken on the role of Bos1p (Hay et al., 1998). This is likely to be true in plants as well, since Arabidopsis has two membrin orthologs. In addition, Arabidopsis has a novel SNARE gene family of three members that has no counterparts in other eukaryotes.

VAMPs

VAMPs (also called synaptobrevins) are anchored to membranes by a C-terminal transmembrane domain or by post-translational addition of lipids. Ad-

adjacent to the membrane anchor is a coiled-coil domain centered on an Arg residue. Mammals contain many VAMP isoforms that play roles in many different trafficking steps, usually as v-SNAREs. Yeast encodes for only five, including a redundant pair. Sec22p is required for ER-to-Golgi (where it is part of the t-SNARE complex; Parlati et al., 2000) and Golgi-to-ER trafficking (probably as a v-SNARE; Newman et al., 1990; Sacher et al., 1997), Ykt6p is involved in intra-Golgi and perhaps vacuolar trafficking (McNew et al., 1997; Ungermann et al., 1999; Fukada et al., 2000), Nyv1p is required for homotypic vacuole fusion as a v-SNARE (Nichols et al., 1997; Fukada et al., 2000), and the redundant pair of Snc1/2p are the v-SNAREs involved in secretion (Protopopov et al., 1993; McNew et al., 2000). Arabidopsis encodes 14 VAMP isoforms (see Table II).

Arabidopsis seems to lack a VAMP of the Snc1/2p-class (orthologous with VAMP 1/synaptobrevin 1 in mammals). VAMPs of this class are known to be required for secretion (Protopopov et al., 1993; McMahon and Sudhof, 1995). Whether another Arabidopsis VAMP-homolog has replaced the Snc1/2p-type protein in this capacity should be examined.

Equally unique is the observation that the only ortholog of Sec22p in Arabidopsis actually encodes a protein with a C-terminal coiled-coil centered on a Val residue instead of an Arg. This gene is expressed in Arabidopsis; however, whether it can function in the role of a Sec22p-type protein remains to be determined. If not, it seems that Arabidopsis lacks two vital classes of VAMP isoforms.

Arabidopsis does have a gene family of two members encoding Ykt6p-orthologs. In a similar manner,

Table II. Other SNAREs of Arabidopsis^a

Name	Previous Name	Genomic Locus	AGI ID	Intracellular Localization	Expressed Gene?	Note ^b
SNAP25 Group						
AtSNAP33	AtSNAP33	MAF19.6	At5g61210	PM	Yes	1
AtSNAP29	AtSNAP29	MXM12.4	At5g07880		Yes	2
AtSNAP30	–	F16A14.10	At1g13530		?	
Vti1p Group						
AtVTI11	AtVTI1a	MUL8.190	At5g38510	TGN, PVC	Yes	3
AtVTI12	AtVTI1b	T24P13.5	At1g25740	TGN, PVC	Yes	3
AtVTI13	–	MXE2.6	At3g29100		Yes	
Gos1p Group						
AtGOS11	–	F7H2.21	At1g15590		Yes	
AtGOS12	–	F4L23.29	At2g45200		Yes	
Bet1p Group						
AtBET11	–	F9D24.80	At3g58170		Yes	
AtBET12	–	dl3265w	At4g14450		?	
Membrin Group						
AtMEMB11	–	T1J8.8	At2g36900		Yes	
AtMEMB12	–	MXI22.9	At5g**		?	
Novel plant Group						
AtNPSN11	–	T4C15.14	At2g35190		Yes	
AtNPSN12	–	F11A17.20	At1g44640		Yes	
AtNPSN13	–	MTO12.3	At3g17440		?	
VAMPS						
AtSEC22	–	F12F1.27	At1g11610		Yes	
AtYKT61	–	K21L19.5	At5g58060		Yes	
AtYKT62	–	MCK7.5	At5g58180		?	
AtVAMP711	VAMP7C	F10N7.4	At4g32150		Yes	4
AtVAMP712	–	T22F11.7	At2g25340		Yes	
AtVAMP713	–	F2I11.40	At5g11150		?	
AtVAMP714	–	MWD9.16	At5g22360		?	
AtVAMP721	SAR1	T1G11.1	At1g04630		Yes	5
AtVAMP722	VAMP7B	F25I18.14	At2g33120		Yes	6
AtVAMP723	–	F25I18.15	At2g33110		Yes	
AtVAMP724	–	dl3930c	At4g15780		Yes	
AtVAMP725	–	F24L7.19	At2g32670		?	
AtVAMP726	–	F13M7.25	At1g04650		?	
AtVAMP727	–	F24B22.260	At3g54300		?	

^a Headings and definitions as given in Table I. ^b 1, X. Gansel and L. Sticher, GenBank accession no. X92419; 2, C. Dickey and S. Bednarek, personal communication; 3, Zheng et al., 1999b; 4, D.M. Nikoloff and C. Somerville, GenBank accession no. AF025333; 5, M. Schena and R.W. Davis, GenBank accession no. M90418; 6, D.M. Nikoloff and C. Somerville, GenBank accession no. AF025332.

a gene family of four members encodes VAMP7 orthologs, a mammalian VAMP isoform probably involved in endosomal/lysosomal trafficking (Advani et al., 1999). It is interesting that Arabidopsis encodes a distinct gene family of seven members that also show homology to VAMP7, including some genes that encode proteins lacking membrane anchors. The roles of this large number of potential VAMP7 homologs remains unstudied.

NSF AND SNAPs

The SNARE complex is remarkably stable once formed and requires a great deal of energy to disassemble (Fasshauer et al., 1998). It is believed that this is the role of two soluble proteins, NSF and SNAP. The SNARE complex serves as a binding site for SNAP, which then recruits NSF. The ATPase activity of NSF then serves to disassemble the SNARE complex, freeing the components for subsequent pairing and fusion events. As in other eukaryotes NSF is a single-copy gene in Arabidopsis (see Table III). The yeast genome encodes a single SNAP, called Sec17p, whereas the mammalian genome codes for three types of SNAPs: α -, β -, and γ -SNAP. Arabidopsis encodes two isoforms of the α -SNAP type and one of the γ -SNAP type (see Table III).

A second large ATPase, CDC48 (p97 in mammals), is known to interact with certain SNAREs as part of specialized membrane fusion events where vesicles derived from the same organelle fuse (called homotypic fusion; Patel et al., 1998; Rabouille et al., 1998). Three orthologs are encoded in Arabidopsis and at least two are expressed (see Table III). Although the precise functions of these factors have not yet been determined, AtCDC48 has been localized to the phragmoplast in dividing cells, suggesting some role in cytokinesis (C. Dickey and S. Bednarek, personal communication).

SEC1p Family

The Sec1p-family is another well-conserved group of large peripheral membrane proteins. In neuronal cells Sec1 proteins positively regulate vesicle fusion by inducing conformational changes in syntaxins (for review, see Hanson, 2000); however, the precise role of Sec1 proteins remains unclear. Yeast contains four members of this family. Sec1p functions at the PM, forming complexes with Sso1/2p and somehow controlling the activity of these syntaxins in secretion (Aalto et al., 1991, 1993). A second member, Sly1p, functions in traffic in both directions between the ER and Golgi (Ossig et al., 1991). Vps45p plays roles in anterograde transport to the PVC through interactions with the prevacuolar syntaxin Pep12p (Peterson et al., 1999) and in an alternate pathway to the vacuole in concert with the TGN syntaxin Tlg2p (Abeliovich et al., 1999). Vps33p is found on the vacuolar membrane and functions in several trafficking steps to that organelle through interactions with the vacuolar syntaxin Vam3p (Darsow et al., 1997).

Arabidopsis contains six members of the Sec1p family (see Table III and Fig. 4). Three are orthologous to Sec1p. Among these is KEULE, which is required for cytokinesis rather than secretion (Assaad et al., 2001) Perhaps the other Sec1 orthologues function in secretion. A single ortholog of Sly1p is found in Arabidopsis, perhaps functioning in the early secretory pathway. Arabidopsis also has a single gene encoding a Vps33p-ortholog. This is interesting considering that no Vam3p-type syntaxin is found in Arabidopsis. It is likely that this protein has adopted a new function in plants, though this has yet to be investigated. What has been investigated is the function of AtVPS45, a Vps45p-homolog from Arabidopsis. Expression of this gene in yeast is able to complement all the defects associated with a *vps45* Δ mutation (Bassham and Raikhel, 1998), suggesting

Table III. SNARE interacting proteins^a

Groups	Gene Names	Genomic Locus	AGI ID	Intracellular Localization	Expressed Gene?	Note ^b
α -SNAP	α -SNAP1	T5P19.100	At3g56450		Yes	
	α -SNAP2	F18O21.150	At3g56190		Yes	
γ -SNAP	γ -SNAP	F9F13.60	At4g20410		Yes	
	NSF	T1J1.4	At4g04910		Yes	
CDC48	AtCDC48a	F8A24.11	At3g09840		Yes	1
	AtCDC48b	F19B11.22	At2g03670		Yes	
	AtCDC48c	F4P13.15	At3g01610		?	
Sec1p-Family						
Sec1p-Group	KEULE	F5O11.8	At1g12080	Phragmoplast	Yes	2
	AtSEC1a	F22M8.14	At1g01980		Yes	2
	AtSEC1b	F16J13.190	At4g12120		Yes	2
Vps33p-Group	AtVPS33	F28P10.160	At3g54860		Yes	
Vps45p-Group	AtVPS45	T14N5.4	At1g70890	TGN	Yes	3
Sly1p-Group	AtSLY1	T27K22.15	At2d17980		Yes	

^a Headings and definitions as given in Table I.

^b 1, Feiler et al., 1995; 2, Assaad et al., 2001; 3, Bassham and Raikhel, 1998; Bassham et al., 2000.

that this *Arabidopsis* protein can interact with the yeast TGN-to-PVC trafficking machinery (including the PVC syntaxin Pep12p). However, in *Arabidopsis* we have found that this protein is found only at the TGN and does not interact with any of the *Arabidopsis* PVC syntaxins (AtSYP21 or AtSYP22); instead, AtVPS45 was found to only interact with the TGN syntaxins of the SYP4 gene family (Bassham et al., 2000). It is clear that the function of AtVPS45 is different in plants and is the subject of ongoing research. These results clearly indicate that simple homology and even complementation of yeast mutants is not proof of conserved function, a finding that has been noted for other plant genes as well (Bassham and Raikhel, 2000).

There is a total of six Sec1 proteins compared with 24 syntaxins. It is clear that there are not enough Sec1 proteins to individually bind to so many syntaxins. This is similar to the situation found in mammalian cells, and to a lesser extent in yeast. Though this has not been studied in great detail, it is believed that a single Sec1 protein can interact specifically with a number of syntaxins (Abeliovich et al., 1999; Peterson et al., 1999; Bassham et al., 2000).

THE LETHALITY OF VESICLE TRAFFICKING MUTANTS

Although analyses have been carried out on the intracellular localization of *Arabidopsis* SNAREs and related proteins, little is known about the functions of the corresponding genes. In fact, until recently the only vesicle trafficking mutants of *Arabidopsis* were, surprisingly, ones defective in cytokinesis. Building a structure as complex as a cell wall in the brief period of time between anaphase and telophase requires a rapid and efficient mobilization of resources. This is achieved by the targeting and fusion of Golgi-derived vesicles at the equator of a dividing cell: the vesicle contents build the cell wall and the vesicle membranes form the flanking PMs. A number of *Arabidopsis* mutants affect the execution of cytokinesis (F.F. Assaad, U. Mayer, and G. Jürgens, unpublished data). These mutants all have similar phenotypes, characterized by multinucleate cells with gapped or incomplete cross walls. Two cytokinesis-defective genes of *Arabidopsis* have been cloned: *KNOLLE* (AtSYP111) encodes a cytokinesis-specific syntaxin (Lukowitz et al., 1996) and *KEULE* encodes a cytokinesis-related Sec1p family member (Assaad et al., 2001). *KNOLLE* and *KEULE* interact genetically (Waizenegger et al., 2000) and have been shown to bind to each other *in vitro* (Assaad et al., 2001). *KEULE* and *KNOLLE* show some similarity to groups of Sec1 or syntaxin genes involved in exocytosis. Although this might appear surprising for proteins involved in cytokinesis, it is consistent with the idea that cytokinesis in plants is a specialized form of exocytosis. Further analysis of these and related

genes will shed light on the as yet elusive biological function of the Sec1-syntaxin complex in the novel context of cell cycle progression.

It is to be noted in this context, however, that lethality in plants comes in three different forms: seedling lethality, embryo lethality, and gametophytic lethality. Thus, although *keule* and *knolle* mutants complete embryogenesis, germinate, and die as seedlings, *keule-knolle* double mutants are embryo lethal and die during embryogenesis as bags of nuclei with no cross walls (Waizenegger et al., 2000). The striking phenotype of the double mutant compared with the attenuated phenotypes of the single mutants suggests that other *Arabidopsis* genes are partially redundant to *KEULE* and *KNOLLE*, allowing the corresponding cytokinesis-defective mutants to muddle through life until the seedling stage. In a consistent manner, phylogenetic analysis places *KEULE* and *KNOLLE* in subfamilies with several members: *KEULE* has two close paralogues, and *KNOLLE* has eight (see Figs. 1 and 5).

Gene disruptions in syntaxins other than *KNOLLE* are also lethal (A.A. Sanderfoot and N.V. Raikhel, unpublished data). In general, the severe lethality of vesicle trafficking mutants renders their genetic characterization difficult. The isolation and characterization of conditional mutants would greatly further our understanding of the plant SNAREs.

HOW GOOD A MODEL IS ARABIDOPSIS FOR OTHER PLANTS?

Limited genomic sequencing as well as several extensive EST projects have been carried out in other plants. Examination of these databases reveals that the patterns outlined above for *Arabidopsis* also hold in other plants such as soybean, tomato, rice, and maize; namely, the presence of small gene families encoding SNARE components and the presence of

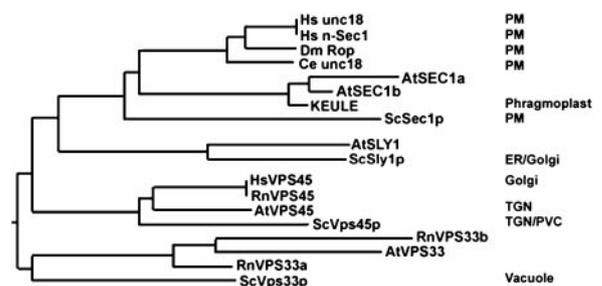


Figure 5. Phylogenetic analysis of Sec1 proteins from several eukaryotes. Protein sequences from representative Sec1p family members from yeast (*Sc*), *Arabidopsis* (*At*), Human (*Hs*), Rat (*Rattus norvegicus*; *Rn*), fruit fly (*Dm*), and *C. elegans* (*Ce*) were acquired from GenBank and aligned as described in Figure 2. Where it is known the intracellular localization of Sec1 proteins is indicated. Note that although the closest homologs of AtSec1a, AtSec1b, and *KEULE* are the animal exocytic Sec1 proteins, the three *Arabidopsis* Sec1 proteins cluster as a somewhat separate group and may have evolved to play novel roles (such as cytokinesis in the case of *KEULE*).

syntaxins and other SNARE groups not found in animals or yeast. Investigation of the functions of these proteins in other plants, especially in the monocots such as rice and maize, may provide useful information that will help clarify the functions of these essential genes.

WHY SO MANY SNAREs IN SUCH A COMPACT GENOME?

The large number of SNAREs in the Arabidopsis genome is unprecedented: Arabidopsis has at least two times more syntaxins than worms or flies, although the genomes are of similar complexity. In a similar manner, Arabidopsis will likely have more syntaxins than humans, which have greater than 10-fold more DNA. Although the majority of these “extra” SNAREs come in gene families, several lines of evidence suggest that they are not functionally redundant. In cases where it has been studied, gene disruptions of syntaxins are lethal (Lukowitz et al., 1996; A.A. Sanderfoot and N.V. Raikhel, unpublished data), indicating that each SNARE fulfills a unique essential function. Furthermore, although the two highly conserved syntaxins, AtSYP41 and AtSYP42, localize to the TGN, they are in fact on distinct domains of this network. Of the 24 syntaxin genes, at least 20 are expressed and therefore unlikely to be pseudogenes.

But why so many? It appears that some of these proteins have specialized in the plant-specific method of cytokinesis (Lauber et al., 1997; Assaad et al., 2001), whereas others appear to have taken on roles in hormone signaling (SYP121/SYR1; Leyman et al., 1999). Since it is known that protein targeting in plant cells can be polarized to particular domains of the PM (Steinmann et al., 1999), it is possible that other “extra” SNAREs have specialized into these roles. These speculations require further analysis of the Arabidopsis SNAREs before one can explain why this modest plant has evolved such a sophisticated and differentiated vesicle trafficking machinery.

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