J. Plant Biol. (2009) 52:88–101
DOI 10.1007/s12374-009-9014-1

REVIEW

Tail-Anchored Proteins in Plants

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Received: 23 January 2009 / Accepted: 25 January 2009 / Published online: 10 March 2009

Abstract Tail-anchored (TA) proteins are a class of polypeptides integrated into the membrane by a C-terminally located hydrophobic sequence which are present in all three domains of life. Proteins of this class lack an N-terminal signal peptide and reach their destination within the cell by posttranslational mechanisms. TA proteins perform a variety of essential functions on the cytosolic face of cellular membranes and, in several cases, determine the organelle identity. Some TA proteins insert directly into the lipid bilayer without the help of molecular machinery, suggesting that they may be ancestral proteins able to recruit lipids, contributing to the formation of intracellular compartments during cell evolution. Relevant progress has been made in recent years on the identification of TA protein sorting and the posttranslational translocation machineries. Interestingly, membrane lipid components were also found to be involved in the insertion mechanism. A bioinformatic approach is used to produce a catalogue of putative TA proteins encoded by the *Arabidopsis thaliana* genome, and intracellular localization is predicted based on features of well-characterized TA proteins. A recent strategy aimed at improving the accumulation of recombinant proteins expressed in transgenic plants is also discussed.

Keywords Tail-anchored · Posttranslational Targeting · Membrane protein · Membrane lipids · Intracellular membranes

The term "tail-anchored" (TA) proteins was introduced by the group of Tom Rapoport more than 10 years ago (Kutay et al. 1995) in a study on mammalian synaptobrevin, a protein of the soluble *N*-ethylmaleimide (NEM)-sensitive factor attachment protein receptors class (SNAREs). This term well describes the topology of proteins that are anchored to the membrane via a C-terminally located hydrophobic domain, followed by a short (or null) polar sequence. In the resulting topology, the bulk of the protein (functional, catalytic domain) faces entirely the cytosol, and the short C-terminal region is translocated into the lumen of the organelle of residence. Proteins with this topology are present in all eukaryotes and in bacteria (Kalbfleisch et al. 2007; Borgese et al. 2009), on the cytosolic face of any cellular membrane, and carry out a variety of functions, some of which are fundamental for cell metabolism and survival. Moreover, the TA protein class also includes several viral proteins (Brideau et al. 1998; da Fonseca et al. 2000; Schmidt-Mende et al. 2001; Koshizuka et al. 2002; Koshizuka et al. 2008).

The position of the TMD, which is located near to the C terminus, is responsible for the peculiar mechanism of insertion into the lipid bilayer. Indeed, the hydrophobic domain emerges from the ribosome only after translation has been completed. The polypeptide is therefore released in the cytosol, and necessarily, its insertion into the target membrane occurs posttranslationally (Fig. 1). This holds true also for TA proteins inserted into the endoplasmic reticulum (ER) membrane, whereas ER insertion of most integral membrane proteins is a co-translational process.

Electronic supplementary material The online version of this article (doi:10.1007/s12374-009-9014-1) contains supplementary material, which is available to authorized users.

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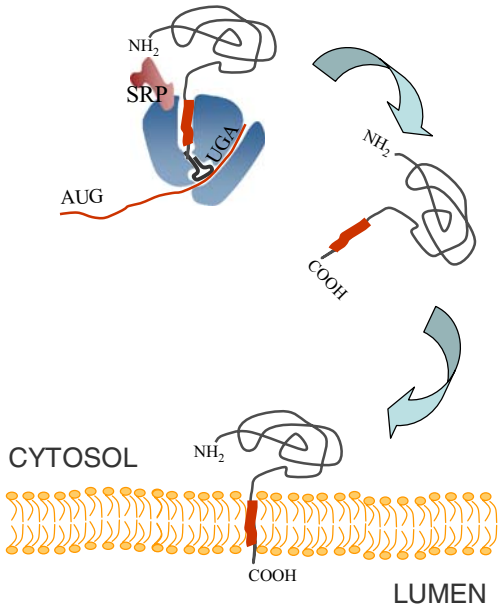


Fig. 1 Posttranslational insertion of tail-anchored proteins. Tail-anchored proteins are translated on free ribosomes in the cytosol and cannot interact co-translationally with canonical translocation systems, such as SRP, because the C-terminal hydrophobic domain emerges from the ribosome only when the translation was completed. Once the protein is released in the cytosol, it can follow different pathway (see Fig. 3) to interact with membrane and to translocate the short polar sequence in the lumen of the target organelle

Not all bilayers are able to support the posttranslational insertion of tail anchors (see below): TA proteins can be targeted to peroxisomes, chloroplasts, mitochondria, and the ER. Those resident of the Golgi complex, plasma membrane, vacuoles, and endosomes are first inserted into the ER bilayer and then travel along the secretory pathway until they reach their membrane of residence (Fig. 2).

There are three essential questions regarding TA proteins: (1) How do they choose their target membrane? (2) Which is the machinery involved in their post-translational translocation? (3) How do TA proteins maintain their localization in spite of membrane flow along the secretory pathway? In the first part of this review, these questions will be discussed, focusing on TA proteins in plants. In the second part, we will report an "in silico" analysis of *Arabidopsis thaliana* proteome, with the aim of creating a catalogue of putative plant TA proteins. These proteins will be classified both by function and localization. On the basis of our knowledge on TA protein biogenesis, in particular in plants, for each membrane, common features will be tentatively identified of its resident members of this protein class, with the intent of predicting the localization (and function) of uncharacterized TA proteins.

Results obtained to date on the machinery involved TA protein membrane insertion in mammalian and yeast cells will be discussed and summarized.

Finally, a recent work will be presented that propose the use of tail anchors in biotechnology as a strategy to increase the accumulation of recombinant antigens in transgenic plants.

How Do TA Proteins Choose Their Target Membrane?

Once a TA protein is released from free ribosomes, it must reach the correct target membrane. There is general consensus among scientists that the sorting and targeting seem to be governed by physical–chemical features of TA polypeptides rather than by defined sequence motifs (see reviews Borgese et al. 2003, 2007).

The fact that only a subset of intracellular membranes is able to accept TA protein insertion mainly depends on lipid composition, which determines fluidity and plasticity of the bilayer (Brambillasca et al. 2005). Non-acceptor membranes contain high levels of certain lipids, such as sterols,

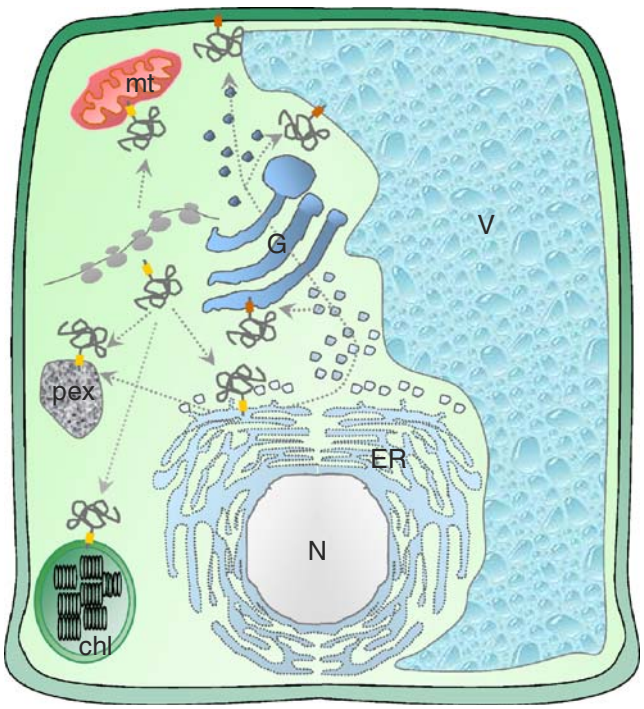


Fig. 2 How do tail-anchored proteins choose the correct target membrane? TA proteins translated on free ribosomes are released in the cytosol and can be addressed to a limited number of intracellular membrane: endoplasmic reticulum, chloroplasts outer envelope, mitochondrial outer membrane, peroxisomal membrane. TA proteins resident in the endomembrane system, downstream the ER are first inserted into the ER membrane and travel along the secretory pathway until reach their destination. Peroxisomal TA proteins can be address to these organelles directly from the cytosol or by passing through a specialized region of the ER (peroxisomal ER). *ER* endoplasmic reticulum, *N* nucleus, *mt* mitochondria, *G* Golgi complex, *V* vacuole, *pex* peroxisome

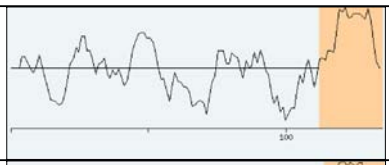
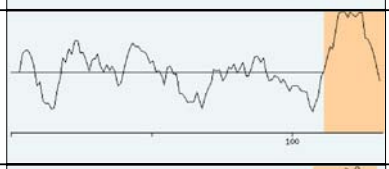
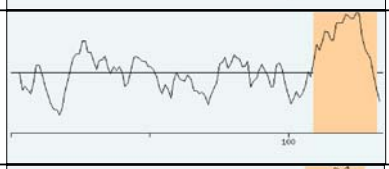
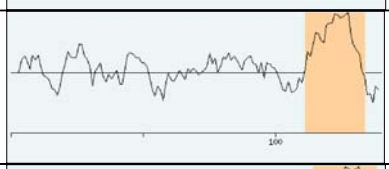
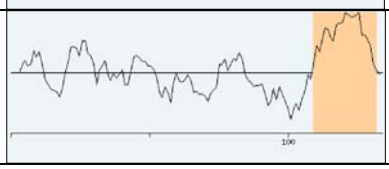
that increase bilayer rigidity and thus impair C terminus translocation (Brambillasca et al. 2005).

A useful model to study sorting and targeting of TA proteins is the electron carrier cytochrome b5 (cyt b5; D'Arrigo et al. 1993). Most of the studies have been performed on animal cells where cyt b5 is present in two isoforms, ER-b5 and MOM-b5, that reside in the ER and in the outer mitochondrial membrane (MOM), respectively (D'Arrigo et al. 1993). It has been demonstrated that the short C-terminal polar region determines targeting to the ER or MOM. This region carries a net negative charge in ER-b5, and reversal of this charge results in mistargeting of the mutated proteins to the MOM (Borgese et al. 2001). In contrast to animal cyt b5s, no plant isoform with a negative C terminus has been identified (see Table 2 in Borgese et al. 2001), suggesting that other mechanisms, instead of charge-based sorting, determine the localization in plants.

The targeting of four tung (*Aleurites fordii*) cyt b5 isoforms (Cb5-A, -B, -C, and -D) has been studied (Hwang et al. 2004). Mitochondrial targeting of Cb5-D is mediated by a combination of hydrophylic amino acids along one side of the TMD, an enrichment of branched β-carbon-containing residues in the medial portion of the TMD, and a dibasic -R-R/K/H-x motif in the C-terminal tail. By contrast, targeting to the ER depended primarily upon the overall length and hydrophobicity of the TMD, although an -R/H-x-Y/F- motif in the tail was also a targeting determinant (Hwang et al. 2004).

The Arabidopsis genome contains five putative TA cyt b5 isoforms, all with a positive luminal C terminus (see Fig 5a in Maggio et al. 2007). One of these isoforms is sorted to the ER and another to the chloroplast outer envelope (At5g48810/AtCb5-3 and At1g26340/AtCb5-6, respectively, Table 1; Maggio et al. 2007). In cells lacking chloroplasts, AtCb5-6 is targeted to mitochondria, indicating that there is a competition between the two organelles in capturing this cyt b5 isoform and that chloroplasts have the stronger affinity (Maggio et al. 2007). In search of differences between ER and COE Arabidopsis isoforms, it can be noticed that the hydrophobicity profiles of their TA are slightly dissimilar. There is a gradual increase of hydrophobicity in the first half of AtCb5-3 TMD (Table 1, At5g48810). Conversely, AtCb5-6 TMD starts with a sharp increase in hydrophobicity, which slightly decreases in the

Table 1 Classification of different cytochrome b5 isoforms from *A. thaliana*

Entry	Tail-anchor	Hydrophobicity profile	TMD hydrophobicity index
At1g26340 (AtCb5-6)	YKKDQPQDSVQKLFDLTK ***QYWVVPVSIITISVAVSVLFS*** RKT		0.554
At2g46650 (AtCb5-1)	WEKESTAAETTKEESGKK ***LLIYLIPLLILGVAFAL*** RFYNNK		0.539
At5g53560 (AtCb5-2)	APQQPAYNQDKTPEFIIK ***ILQFLVPILILGLALVV*** RHYTKKD		0.63
At5g48810 (AtCb5-3)	TSTKAVATQDKSSDFVIK ***LLQFLVPLLILGLAFGI*** RYYTKTKAPSS		0.574
At2g37720 (AtCb5-4)	PPKQPHYNQDKTSEFIIK ***LLQFLVPLAILGLAVGI*** RIYTKSG		0.57

For each cytochrome b5, the tail-anchor is shown. The TMDs are in bold and italicized. The hydrophobicity profiles of the molecules are reported. The TMD is highlighted in orange and its hydrophobicity index is listed

middle region (Table 1, compare At1g26340 and At5g48810). This could reflect differences in lipid composition of ER and COE. With regard to this point, Toc34, a TA component of the COE translocon, is able to insert itself in the lipid bilayer of the chloroplast envelope in the absence of helper proteins (Schleiff et al. 2001; Qbadou et al. 2003). The authors established that two positive charges in close proximity to the cytosolic end of the TMD dictate the topology of Toc34 (Qbadou et al. 2003). Importantly, the insertion of Toc34 is dependent on the lipid asymmetry present in the outer envelope and the presence of the non-bilayer lipids monogalactosyldiacylglyceride (MGDG) and phosphatidylethanolamine (PE) (Epand 1998; Qbadou et al. 2003). MGDG and PE, like cardiolipin in mitochondria, are a non-bilayer-forming lipid because they have a small polar headgroup relative to the diameter occupied by the two acyl chains. This small headgroup gives the lipid the shape of a cone when rotated along its long axes. Such cone-shaped lipids form inverted hexagonal phases characterized by high local curvature rather than bilayers. Non-bilayer property of membranes is essential for the function of the translocases (Rietveld et al. 1995; Epand 1998).

Comparing the hydrophobicity profiles of the five *Arabidopsis* cyt b5s, it can be observed that AtCb5-2 and AtCb5-4 are similar to AtCb5-3, and therefore, we can hypothesize that they are ER isoforms. The profile of AtCb5-1 resembles that of AtCb5-6, but the amino acid composition of the C terminus is more similar to that of ER isoform. Notice that the ER and putative ER isoforms contain a tyrosine in the C-terminal polar region which is absent in the COE cyt b5. The analysis of putative phosphorylation site using Net-Phos 2.0 server (http://www.cbs. dtu.dk/services/NetPhos/), predicted that the C-terminal tyrosine of AtCb5-3 could be phosphorylated. The phosphorylation in the extreme C terminus could have regulatory implications in the mechanisms of targeting/insertion (Maggio et al. 2007).

The implementation of knowledge both on tail anchor structures and membrane lipid composition will contribute to elucidate the biogenesis of TA proteins in respect to intracellular membranes.

Posttranslational Insertion into the ER: More Than One Pathway

The first evidence of posttranslational insertion of proteins into ER membrane came from a study on rat liver microsomal ER-b5, which is considered the archetype of TA proteins. The results showed a preferential association of cyt b5 polyA+ messenger RNA (mRNA) with free ribosomes, despite the ER localization of the protein, and indicated that it is synthesized in a soluble form and only subsequently is inserted into the ER (Rachubinski et al. 1980). A few years later, it was shown that ER-b5 synthesized in vitro in a wheat germ cell-free translation system was able to tightly bind posttranslationally added dog pancreas microsomal membranes (Anderson et al. 1983). In the following years, several studies have contributed to acquire new information on TA protein biogenesis. In particular, the setup of increasingly sophisticated translocation assays has partially clarified the mechanism of posttranslational insertion (at least in mammalian and yeast cells).

According to current models, TA protein insertion into the ER membrane can follow three different pathways depending mainly on the physical–chemical features of the TMD; in certain cases, these pathways can also partially overlap.

The first pathway was described in the laboratory of Nica Borgese (Brambillasca et al. 2006) where the assay for TA protein translocation was also developed (Pedrazzini et al. 2000; Brambillasca et al. 2005). The authors used ER-b5 as a model and then extended their studies on other TA proteins. ER-b5 insertion does not depend on Sec61 channel and/or translocon accessory proteins (Yabal et al. 2003; Brambillasca et al. 2006) and can occur spontaneously, without protein assistance, across pure lipid vesicles (Fig. 3a; Brambillasca et al. 2006). At least another protein, protein tyrosine phosphatase 1B (PTP-1B), is able to follow this spontaneous pathway, indicating that the feature is not unique to cyt b5 (Brambillasca et al. 2006). What is the common characteristic between these two proteins? As we will illustrate below, other TA proteins are unable to insert spontaneously into the bilayer. The mild hydrophobicity of cyt b5 and PTP-1B TMDs can provide an explanation: TMDs with higher hydrophobicity could cause irreversible aggregation of the polypeptide immediately after its release from the ribosome and thus need interactions with molecular chaperones to avoid this.

The lipid composition of membranes is also important for an effective translocation of ER-b5 C terminus; indeed, cholesterol-loaded artificial vesicles impaired ER-b5 insertion completely, even if low concentration of sterol was used (Brambillasca et al. 2005). This is probably due to the increased order and thickness of the lipid bilayer caused by the sterols and can reflect the in vivo inability of TA proteins to insert into sterol-enriched membranes.

Our experimental evidence (unpublished) supports the hypothesis that the ability of spontaneous membrane integration is maintained also by plant ER-b5. As suggested by Maggio et al. (2007), the ability to insert directly in the lipid bilayer, without the help of translocons and protein machinery, could be a feature of ancestral proteins which were able to recruit lipids, contributing to the biogenesis of cellular membrane.

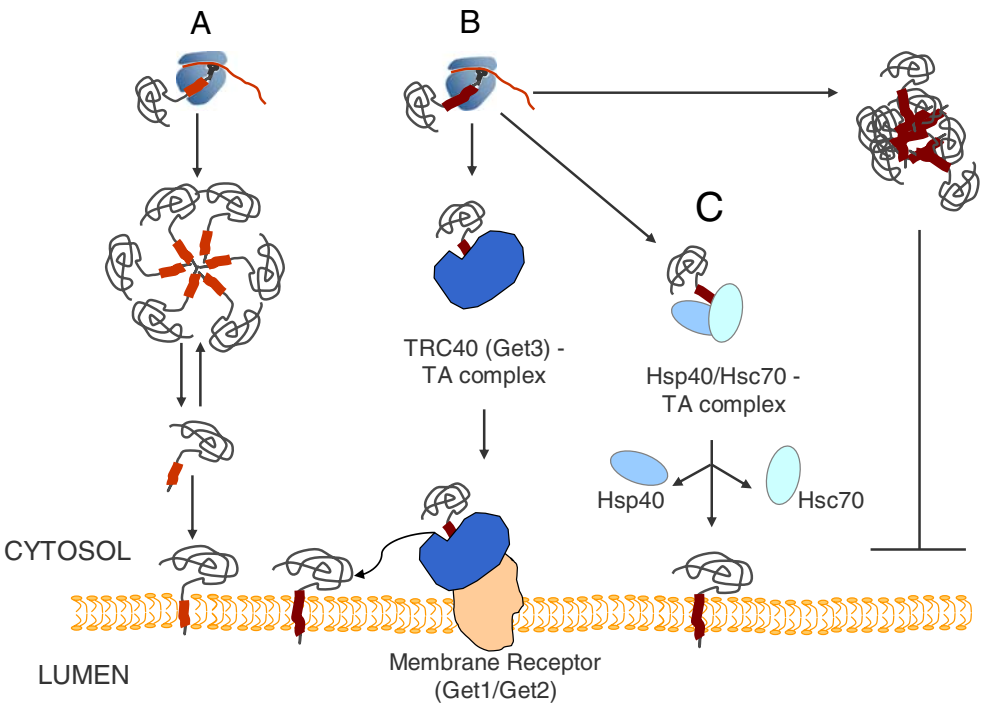


Fig. 3 Different pathways for TA proteins sorting. TA protein targeting to the ER membrane can follow three different pathways depending on the protein physical–chemical features; in certain cases, these pathways can also partially overlap. TA proteins which have TMDs with moderate hydrophobicity can remain in solution, forming micellae, and are therefore inserted into the membrane without assistance of molecular chaperones (**a**). TA proteins with a longer or more hydrophobic TMD need molecular chaperones, such as TRC40/Get3 or Hsp40/Hsc70, to avoid irreversible aggregation. A membrane receptor complex (Get1/Get2) has been identified in yeast, which recognized Get3-TA protein complex (**b**, **c**)

The second pathway involves a TMD recognition complex (TRC) and is ATP-dependent. This pathway is followed by a subset of TA proteins, such as the mammalian Sec61β and synaptobrevin, which have more hydrophobic TMDs, rendering them reliant on an incompletely characterized, ATP-dependent mechanism (High and Abell 2004; Abell et al. 2007; Stefanovic and Hegde 2007; Favaloro et al. 2008). The major player in the TRC pathway is the 40-kDa cytosolic factor TRC40/Asna1, the homologue of bacterial ArsA and yeast GET3 ATPases (Bhattacharjee et al. 2001; Shen et al. 2003). Mammalian Asna-1 has 27% homology to the bacterial ArsA, which is involved in arsenite transport (Kurdi-Haidar et al. 1996). However, the mammalian protein has little or no arsenite-stimulated ATPase activity and plays a different role from its distant bacterial homolog (Kurdi-Haidar et al. 1998). TRC40 binds directly TMDs with high hydrophobicity. The energy used to insert the protein into the bilayer comes from ATP hydrolysis (Fig. 3b). Moreover, the membrane insertion mediated by Asna1 is sensitive to NEM and oxidants, indicating that cytosolic redox conditions can influence the binding of Asna1 substrates (Favaloro et al. 2008). A recent study by Schuldiner et al. (2008) has shown that cytosolic GET3 recognition represents the key decision step for the insertion of TA proteins into yeast ER; loss of this factor can lead to mistargeting to mitochondria. Moreover, Get3–TA protein complexes are recruited by the Get1/Get2 receptor that resides on the ER membrane. The absence of Get1/Get2 causes cytosolic aggregation of Get3–TA complexes.

The third pathway of TA membrane insertion is stimulated by the chaperone Hsc70 in conjunction with Hsp40: this complex binds the TMD to avoid cytosolic aggregation (Fig. 3c; Abell et al. 2007; Rabu et al. 2008).

The mechanism of plant TA protein integration has not been investigated in detail yet, but the knowledge acquired on mammals and yeast combined with the large availability of proteomic data and tools that plant biologists can access (see http://www.arabidopsis.org; http://aramemnon.botanik.uni-koeln.de/) might quickly narrow the gap. Moreover, the analysis of *A. thaliana* knockout mutants can provide important information on the role played by individual TA proteins and by the components of the machineries that take care of their biogenesis in plant development and reproduction.

In the following part of this review, we will describe bioinformatic data on *A. thaliana* TA proteome that could be useful to elucidate the biogenesis of TA proteins in plants.

Putative TA Proteins in the Arabidopsis Proteome

An overall picture of the TA proteome in *A. thaliana* can cast light on the variety of functions of these proteins, reveal new functions, and help in identifying the target membranes. To this purpose, a first investigation of *A. thaliana* proteome has been performed using the tools provided by the site http://www.arabidopsis.org.

As a first step, the *bulk protein search* tool (http://www.arabidopsis.org/tools/bulk/protein/index.jsp) was used to

extract putative proteins with a single TMD. It has been also fixed, as *restricted by predicted protein characteristic*, the intracellular location as *other* and *undefined*, to exclude proteins with predicted signal peptide (for co-translational translocation into the ER), chloroplast, or mitochondrial targeting signals. The option *entire range* was set for values of both isoelectric point (p*I* 0.00 to 14.00) and molecular weight (M_W 0.00 to 1,000,000 Da) of the protein. Two lists of loci were obtained in this first step: one containing 877 loci coding for putative proteins with a single TMD and *undefined localization* (Electronic Supplementary Materials Table S1) and the second containing 338 loci coding for putative proteins with a single transmembrane domain and *other localization* (*cytoplasm* or *not identified*; Electronic Supplementary Materials Table S2). It should be noted that this first search did not provide any information about the position of the TMD. To restrict the two lists to TA proteins (which have the TMD near to the C terminus), each putative sequence was further analyzed one by one using the plant membrane protein database ARAMEMNON (http://aramemnon.botanik.uni-koeln.de/index.ep); sequences having no other putative TMD that might have escaped the first step selection, no farther than 60 residues from the C terminus and with average hydrophobicity more than 0.3 (calculation of the average hydrophobicity is based on the hydrophobicity scale published by Eisenberg et al. 1984 and is directly provided by ARAMEMNON—transmembrane detail window), were validated as TA protein. The *60 residues* criterion was more restrictive than the limits of in vitro unassisted translocation of ER-b5 (85 residues) reported by Brambillasca et al. (2006). TA proteins with a C-terminal polar region longer than 35 residues could in theory be recognized by SRP and inserted co-translationally in vivo because in this case, the TMD would emerge from the ribosome before the end of translation (Borgese et al. 2003). Experimental evidence will be necessary to assess the posttranslational insertion of these proteins in vivo. Moreover, sequences that had a value of consensus prediction for localization in chloroplast, mitochondria, or secretory pathway higher than 8 were excluded as well. In the end, the list summarized in Table 2 was obtained, which contains 164 putative TA proteins.

These proteins were classified by putative function, once more using ARAMEMNON database. Table 2 shows nine different functional groups and confirms the variety of TA protein roles. Putative TA proteins with a C terminus longer than 35 residues are marked by an asterisk.

The most abundant group is constituted by SNARE polypeptides, which are involved in vesicle fusion to target compartments along the secretory pathway (Sanderfoot et al. 2000): from this analysis, 59 SNAREs (out of a total of 64 SNAREs entries) have a C-terminal putative TMD. The comparison of the data with the list of *A. thaliana* SNARE proteins published in a recent review (Lipka et al. 2007) indicates that 15 unclassified TA-SNARES encoded by the *A. thaliana* genome are revealed by the present analysis.

As mentioned above, TA proteins resident of the different compartments of the secretory pathway are first inserted into the ER and then traffic along the pathway until they reach their residence (Kutay et al. 1995). The maintenance of their localization, in spite of continuous vesicle traffic, is due predominantly to the length and hydrophobicity of their TMD (Pedrazzini et al. 1996; Bulbarelli et al. 2002). TA SNAREs were ordered based on the increasing average hydrophobicity value of their C terminus, and the results are listed in Table 3. When the established subcellular localization of known SNAREs is taken into account, it is rather clear that the more an Arabidopsis SNARE is distal from the ER along the secretory pathway, the more the hydrophobicity of its TMD increases. With the only exception of SYP132, all known SNAREs of the plasma membrane, tonoplast, and cell plate are in the second half of the list.

Another interesting group of putative TA proteins that has been identified is constituted by "transcription factor/DNA binding proteins" (Table 2). Membrane-bound transcription factors (MTFs) have been found in prokaryotes, yeast, animals, and plants (Brown et al. 1997; Hoppe et al. 2000; Kim et al. 2007; Seo et al. 2008). In plants, several MTFs were previously characterized (Kim et al. 2007; Chen et al. 2008). For example, the NAC MTF named NTM1 resides on the ER membrane: when the tail anchor of NTM1 is removed by proteolysis, the cytosolic portion, containing the NAC domain, is able to enter the nucleus and activates genes involved in cellular division (Kim et al. 2006). The transcription factor AtbZIP60 regulates ER stress response by shuttling from the ER membrane, where the stress signal is sensed, and the nucleus: its detachment from the ER seems to be mediated by proteolysis (Iwata et al. 2008). Therefore, signal transduction across intracellular bilayers, such as the ER membrane, seems to require less intracellular mediators in plants than animals because of the high availability of TFs which are directly bound to the membrane.

A number of components of translocation complexes of the ER and outer mitochondrial membrane (TOM) are TA proteins (Kalbfleisch et al. 2007). The present analysis indicates that only one component of the chloroplast outer envelope translocon (Toc34) has a TA topology, while seven TOM components are predicted to be TA proteins (Table 2). This observation could reflect differences in the biogenesis of chloroplasts and mitochondria. TA proteins of the ER translocon are Sec61β and Sec61γ (Table 2).

The TA proteome identified by this approach also includes putative TA enzymes with a variety of functions: the already reported five cytochrome b5 isoforms (Maggio

Table 2 Putative and known tail-anchored proteins in *A. thaliana*

Locus	Description
Vesicle traffic	
AT1G04750.1	R-SNARE domain protein, synaptobrevin (AtVAMP721)
AT1G04760.1	R-SNARE domain protein, synaptobrevin (AtVAMP726)
AT1G08560.1	vesicle transport syntaxin-type t-SNARE protein (AtSYP111/AtKNOLLE)
AT1G08820.1	SNARE-like VAP33 family-related protein (AtVAP27-2)
AT1G11250.1	vesicle transport syntaxin-type t-SNARE protein (AtSYP125)
AT1G11890.1	R-SNARE domain protein, synaptobrevin (AtSEC22)
AT1G15880.1	Vesicle transport v-SNARE protein (AtGOS11) (ER/Golgi)
AT1G16225.1	Putative syntaxin-type t-SNARE protein
AT1G16240.1	Syntaxin-type t-SNARE protein (AtSYP51)
AT1G26670.1	Vesicle transport v-SNARE protein (AtVTI12/AtVTI1b)
AT1G27700.1	Putative vesicle transport syntaxin-type t-SNARE protein
AT1G28490.1	Putative vesicle transport syntaxin-type t-SNARE protein (AtSYP61/AtOSM1)
AT1G29060.1	Putative vesicle transport t-SNARE protein
AT1G33475.1	Identical to Probable VAMP-like protein At1g33485
AT1G48240.1	Vesicle transport np-SNARE protein (AtNPSN12)
AT1G51740.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP81/AtUFE1)
AT1G54110.1	Cation exchanger, putative (CAX10) (USE11)
AT1G61290.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP124)
AT1G79590.1	Syntaxin-type t-SNARE protein (AtSYP52)
AT2G01470.1	SEC12p protein / St12p protein
AT2G18260.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP112)
AT2G18860.2	Putative vesicle transport syntaxin-type t-SNARE protein
AT2G19950.1	Putative golgin, involved in tethering of vesicles to Golgi membrane (AtGC1)
AT2G33110.1	R-SNARE domain protein, synaptobrevin (AtVAMP723)
AT2G33120.1	R-SNARE domain protein, synaptobrevin (AtVAMP722)
AT2G35190.1	vvesicle transport np-SNARE protein, interacts with syntaxin KNOLLE (AtNPSN11)
AT2G36900.1	Vesicle transport v-SNARE protein (AtMEMB11) (ER/Golgi)
AT2G45140.1	VAMP (vesicle-associated membrane protein)-associated protein, putative
AT2G45200.1	Vesicle transport v-SNARE protein (AtGOS12)
AT3G03800.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP131)
AT3G05710.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP43)
AT3G09740.1	Syntaxin-type t-SNARE protein (AtSYP71)
AT3G11820.1*	Vesicle transport syntaxin-type t-SNARE protein (AtPEN1/AtSYP121/AtSYR1)
AT3G17440.1	Vesicle transport np-SNARE protein (AtNPSN13)
AT3G24315.1*	sec20 protein
AT3G24350.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP32)
AT3G45280.1	Syntaxin-type t-SNARE protein (AtSYP72)
AT3G52400.1*	Vesicle transport syntaxin-type t-SNARE protein (AtSYP122)
AT3G54300.1	R-SNARE domain protein, synaptobrevin (AtVAMP727)
AT3G58170.1	Syntaxin-type t-SNARE protein (AtBET11/AtBS14a) (ER/Golgi)
AT3G58890.1	Putative syntaxin-type t-SNARE protein
AT3G60600.1	Putative vesicle-associated membrane protein (VAMP) (AtVAP27-1)
AT3G61450.1	Syntaxin-type t-SNARE protein (AtSYP73)
AT4G00170.1	VAMP (vesicle-associated membrane protein)-associated protein
AT4G02195.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP42)
AT4G03330.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP123)
AT4G10170.1	Synaptobrevin-related protein
AT4G14455.1	Syntaxin-type t-SNARE protein (AtBS14)

Table 2 (continued)

Locus	Description
AT4G14600.1	Putative vesicle transport t-SNARE protein
AT4G30240.1	Putative vesicle transport syntaxin-type t-SNARE protein
AT5G05760.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP31/AtSed5) (ER/Golgi)
AT5G08080.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP132)
AT5G16830.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP21/AtPEP12) (PVC)
AT5G26980.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP41)
AT5G39510.1	Vesicle transport v-SNARE protein (AtZIG/AtSGR4/AtVTI11/AtVTI1a) (tonoplast)
AT5G46860.1	Vesicle transport syntaxin-type t-SNARE protein (AtSYP22/AtSGR3/AtVAM3) (Tonoplast)
AT5G47180.2	VAMP (vesicle-associated membrane protein)-associated protein-like
AT5G50440.1	Vesicle transport v-SNARE protein (AtMEMB12)
AT5G50550.1	St12p protein
Transcription factors/DNA binding	
AT1G01010.1	Putatively membrane-associated NAC-type transcription factor (AtNTL10/AtANAC001))
AT1G32870.1	Putatively membrane-associated NAC-type transcription factor (AtNTL1/AtANAC013)
AT1G33060.1	Putatively membrane-associated NAC-type transcription factor (AtNTL2/AtANAC014)
AT1G34180.1	Putatively membrane-associated NAC-type transcription factor (AtNTL3/AtANAC016)
AT1G34190.1	Putatively membrane-associated NAC-type transcription factor (AtNTL7/AtANAC017)
AT1G42990.1*	bZIP-Type transcription factor, mediates ER stress response (AtbZIP60) Cleaved by SP1 protease
AT2G27300.1	Putatively membrane-associated NAC-type transcription factor (AtNTL8/AtANAC040)
AT2G47070.1	Squamosa promoter binding protein-like 1 (spl1)
AT3G10500.1	Putatively membrane-associated NAC-type transcription factor (AtNTL4/AtANAC053)
AT3G11580.2	DNA-binding protein, putative
AT3G44290.1	Putatively membrane-associated NAC-type transcription factor (AtNTL5/AtANAC060)
AT3G49530.1	Putatively membrane-associated NAC-type transcription factor (AtNTL6/AtANAC062)
AT3G60030.1	Squamosa promoter binding protein-like 12
AT4G01540.1	Membrane-bound NAC-type transcription factor, controls cell division (AtNTM1/AtNTL12/AtANAC068)
AT4G01550.1	Membrane-bound NAC-type transcription factor (AtNTM2/AtNTL13/AtANAC069)
AT4G35580.1	Putatively membrane-associated NAC-type transcription factor (AtNTL9)
AT5G04410.1	Putatively membrane-associated NAC-type transcription factor (AtNTL11/AtANAC078)
AT5G06710.2	Homeobox-leucine zipper protein 14 (HAT14) / HD-ZIP protein 14
AT5G18830.1	Squamosa promoter binding protein-like 7 (spl7)
Components of translocation complexes	
AT1G04070.1	Mitochondrial outer membrane translocase component (AtTOM9-1/AtTOM22-1)
AT1G27390.1	Putative mitochondrial outer membrane translocase component (AtTOM20-2)
AT1G64220.1	Mitochondrial outer membrane translocase component (AtTOM7-2)
AT3G27070.1	Putative mitochondrial outer membrane translocase component (AtTOM20-1)
AT3G27080.1	Putative mitochondrial outer membrane translocase component (AtTOM20-3)
AT3G48570.1	Protein transport protein SEC61 gamma subunit, putative
AT4G24920.1	Protein transport protein sec61 gamma subunit-like
AT5G40930.1	Putative mitochondrial outer membrane translocase component (AtTOM20-4)
AT5G43970.1	Mitochondrial outer membrane translocase component (AtTOM9-2/AtTOM22-2)
AT5G50460.1	Protein translocation complex Sec61 gamma chain
AT5G05000.1	Chloroplast Outer membrane tranclocase component TOC34
Enzymatical activity	
AT1G17280.1	Ubiquitin-conjugating enzyme, putative
AT1G26340.1	Chloroplast-targeted tail-anchored membrane protein cytochrome b5 (AtCb5-6)
AT1G30040.2	Gibberellin 2-oxidase / GA2-oxidase (GA2OX2)
AT1G33480	Putative ubiquitin ligase, ATL subfamily (AtATL58)
AT1G53110.1	Proton pump interactor, putative

Table 2 (continued)

Locus	Description
AT2G32720.1	Tail-anchored membrane protein cytochrome b5 (AtCb5-4)
AT2G41910.1	Putative kinase, NtNPK1L subfamily
AT2G44490.1	Peroxisomal beta-glycosyl hydrolase (AtPEN2)
AT2G46650.1	Tail-anchored membrane protein cytochrome b5 (AtCb5-1)
AT3G21640.1	Membrane-bound peptidyl-prolyl isomerase, TWisted Dwarf (AtFKBP42/AtTWD1)
AT3G22920.1	Cyclophilin-type peptidyl-prolyl isomerase (AtCYP26-1)
AT3G52190[a]	Phosphate transporter traffic facilitator (AtPHF1)
AT3G54010.1	FKBP-type peptidyl-prolyl isomerase (AtFKBP72/AtPAS1) (PASTICCINO 1)
AT3G56300.1	Putative class-I tRNA synthetase
AT4G09760.3	Choline kinase, putative
AT4G24730.1	Calcineurin-like phosphoesterase protein
AT4G27500.1	Putative proton pump interactor (AtPPI2)
AT4G35000.1	Putative peroxisomal ascorbate peroxidase (AtAPX3)
AT4G35970.1	Putative microsomal ascorbate peroxidase (AtAPX5)
AT5G40510.1	Sucrose cleavage protein-like
AT5G48810.1	ER-targeted tail-anchored membrane protein cytochrome b5 (AtCb5-3)
AT5G53560.1	Tail-anchored membrane protein cytochrome b5 (AtCb5-2)
Heat shock	
AT1G54400.1	Heat shock protein
AT1G72416.3	Similar to DNAJ heat shock N-terminal domain-containing protein
AT1G76770.1	Putative Hsp20-type stress-responsive protein
AT2G27140.1	Putative Hsp20-type stress-responsive protein
AT1G76780.1	Putative heat shock protein
AT3G10680.1	Putative Hsp20-type stress-responsive protein
AT5G04890.1	Putative Hsp20-type stress-responsive protein (AtRTM2)
Containing zinc finger/RING finger domains	
AT1G09920.1	TRAF-type zinc-finger-related
AT2G26130.1	RING domain protein with so-called in between RING fingers domain
AT2G26135.1	Zinc finger (C3HC4-type RING finger) protein
AT2G34200.1	RING domain protein with zinc-binding domain
AT2G37950.1	Putative RING domain protein
AT3G45480.1	RING domain protein with so-called in between RING fingers domain
AT3G45540.1	RING domain protein with so-called in between RING fingers domain
AT5G01980.1	Putative RING domain protein
AT5G05830.1	Putative RING domain protein
AT5G59000.1	Putative RING domain protein
Miscellaneous	
AT1G05320.1	Myosin-related
AT1G06530.1	Myosin heavy chain-related
AT1G72090.1	Radical SAM domain-containing protein/TRAM domain-containing protein
AT1G77880.1	F-box protein
AT2G27310.1	F-box protein
AT2G32240.1	Putative myosin heavy chain
AT2G32340.1	TraB protein
AT2G43270.1	F-box protein
AT3G12140.3	emsy N terminus domain-containing protein/ENT domain-containing protein
AT3G49430.2	Pre-mRNA splicing factor, putative
AT4G19910.1	Toll-interleukin resistance (TIR) domain-containing protein
AT4G23515.1	Toll-interleukin resistance (TIR)

Table 2 (continued)

Locus	Description
AT4G28640.2	Auxin-responsive protein / indoleacetic acid-induced protein 11 (IAA11)
AT5G48630.1*	Cyclin family
Unknown	
AT1G10657.1	Similar to unknown protein
AT1G14688.1	Unknown protein
AT1G54385.1	Protein of unknown function
AT2G25120.1	Protein of unknown function
AT3G03970.1	Protein of unknown function
AT3G26580.1	Protein of unknown function (contains protein-protein interaction mediating site)
AT3G57090.1	Protein of unknown function, required for peroxisome fission (AtFis1a)
AT4G08590.1	Protein of unknown function (contains putative histone-interacting domain)
AT5G61490.1	Protein of unknown function
Hypothetical	
AT1G19400.1	Hypothetical protein
AT1G27330.1	Hypothetical protein
AT1G27350.1	Hypothetical protein
AT1G75180.1	Hypothetical protein
AT3G01311.1	Hypothetical protein
AT3G45460.1	Hypothetical protein
AT3G50170.1	Hypothetical protein
AT3G55600.1	Hypothetical protein
AT3G58840.1	Hypothetical protein
AT3G60470.1	Hypothetical protein
AT5G06560.1	Hypothetical protein
AT5G13190.1*	Hypothetical protein
AT5G15880.1	Hypothetical protein
AT5G46850.1	Hypothetical protein

Encoded proteins are classified into functional groups

*Encoded proteins with a C-terminal polar region longer than 35 residues

et al. 2007), proteins involved in proteasomal degradation (AT1G17280 and AT1G33480), as well as two FKBP-like and one cyclophilin-like peptidyl-prolyl isomerases. Among these, PASTICCINO1 plays an important role in the control of plant development (Vittorioso et al. 1998).

A number of polypeptides having similarity of the N-terminal region with small heat shock proteins (sHsps) are also classified as TA proteins by the current bioinformatic analysis. One of them, RTM2, is involved in resistance of Arabidopsis to tobacco etch potyvirus (TEV) by blocking long-distance movement of the virus (Whitham et al. 2000). Several lines of evidence suggest that although the RTM2 N-terminal domain is related to sHSPs, RTM2 is unlikely to possess typical chaperone functions because it is not heat-inducible under conditions that stimulate the heat shock response (Whitham et al. 2000). The substrate of RTM2 is unknown, and several hypotheses on the mechanism of its action were postulated (Whitham et al. 2000). Certainly, the localization of membrane-bound plant sHSPs could contribute in the identification of real substrates, in understanding the role of these TA proteins in stress tolerance and development, and in unraveling their impressive multiplicity. Knowing sHSP substrates may also help understand whether the same sHSP plays the same role upon different conditions as well as recognize differences between plant species.

Protein containing zinc finger/RING finger domains are also putative TA and could have a role in protein–protein interactions on membrane surfaces. Finally, the screen identified TA proteins with various other functions, a number of TA proteins with unknown function, and some hypothetical TA proteins (Table 2—miscellaneous, unknown and hypothetical, respectively).

On the basis of the TMD properties and hydrophobicity profiles, it should be possible to predict the intracellular localization of TA proteins with as not yet identified functions.

Table 3 Classification of TA SNARES by increasing hydrophobicity index of their C-terminal region, which include the TMD

SNARE	Entry	Localization	C-terminal region	Hydrophobicity
BET11 (Qc)	AT3G58170	Golgi	mldrmgndmdssrgflsgtmdrfktvfetkSSRMLTLVASFVGLFLVIYYLTr	0.411
v-SNARE/AtMEMB11 (Qb)	AT2G36900	ER/Golgi	lksaqrkaldvlntvglsnsvlrlierrnrVDTWIKYAGM IATLVILYLFIRWtr	0.475
v-SNARE/AtMEMB12 (Qb)	AT5G50440	ER/Golgi	lksaqrkaldvlntvglsnsvlrlierrnrVDTWIKYAGMIATLVILYLFIRWtr	0.475
SYP72 (Qc)	AT3G45280	ER	eemetkvdgatsdlkntnvrlkkqlvqmrsSRNFCIDIILLCVILGIVSYIYNaln	0.480
Putative SYP	AT4G30240	?	akpmfwlqrcrdynqlfdrvkvyqrrfrvpLSRPIKLILSLTLILILLLFILRt	0.515
Putative Golgin AtGC1	AT2G19950	Golgi	hrhmatastqlqnavklldsgavratrflwRYPIARMFLLFYLVFVHLFLMYLihrlqeqaeaqevaamtnnvfrl	0.522
SYP71 (Qc)	AT3G09740	ER	eidtkvdratsdlkntnvrlkdtvnqlrssRNFCIDIVLLCIVLGIAAYLYNVlk	0.523
SYP81 (Qa)	AT1G51740	ER	qieflydqaveatknvelgnkelsqaiqrnSSSRFLLLFFFVLTFSVLFLDWys	0.531
SYP73 (Qc)	AT3G61450	ER	deidtkidkaatdlkstnvrlkdtvtklrsSRNFCIDIILLCILLGIAAFIYNsvk	0.534
Putative SYP	AT4G14455	?	vgnkmdsargimsgtinrfklvfekksnrkSCKLIAYFVLLFLIMYYLIRLLNyikg	0.537
v-SNARE/AtGOS12 (Qb)	AT2G45200	Golgi	lfsdvqgkvknlgdkfpvirgllgsikrkrSRDTLILSAVIAACTLFLIIYWLsk	0.540
Putative SYP	AT3G58890	?	kgtmressmiwhfmtsfpclkflvytpmdkKWIFGVAFSNVRVFIIDIVMIiifls	0.545
AtSEC22	AT1G11890	ER	ekldqvsemssrltsesriyadkakdlnrqALIRKWAPVAIVFGVVFLLFWVKnklw	0.585
Putative SYP	AT1G16225	?	elglqtrlidrldhhvdvsasdveelqrsiDTGVCMTLLLSVVVVVFGLSEFVkfgrrrilg	0.607
VAMP-associated Protein	AT4G00170	?	klteektsatqqsqklrlelemlrketskkQSGGHSLLLMLLVGLLGCVIGYLlnri	0.609
VTI12 (Qb)	AT1G26670	TGN/PVC	lhahnklhgvddaidkskkvltamsrrmtrNKWIITSVIVALVLAIILIISYKl sh	0.617
putative t-SNARE	AT1G29060	?	lqmtliraqagvknnirklnsiirSGNNHIMHVVLFALLLFFILYMWskmfkr	0.639
AtSEC12p/St12p	AT2G01470	ER	ltfspdsrglvsvsfdsrarltmieqkgdkPGVRWWLLVLLIVLLYVVAYYYMkakg iip	0.658
Putative t-SNARE	AT4G14600	?	ldelqmtliraqagvknnirklnmsiirsGNNHIMHVVLFALLVFFVLYI WSkmfkr	0.659
VAMP-like	AT1G33475	?	nassesatyvprrgrsggsqsierkwrrqvKIVLA IDIAICLTLLGVWLAICHgi ectrs	0.663
Putative SYP	AT2G18860	?	ksvlwmqrlpdhnqlfdktgcfqnpirlpfNHPIKFIVSLLLMVFLLLPFVVYls	0.665
R.SNAREAtVAMP727	AT3G54300	Endosome	lvdktenlqfqadsfqrqgrqlrrkmwlqslQMKLMVAGA VFSFILIVWVVACGgfkcss	0.667
AtVAP27-2	AT1G08820	?	lmeersissqhrqslqhelaelrtkkivkeVHNGFPLLYVCVVAFIAYVIGHFlrt	0.670
VAMP-associated protein-like	AT5G47180	?	erdaavkqtqqlqheletvrrrrnqrnsgnGLSLKLAAMVGLIGLIIGFILKLtlaspt	0.688
SYP132 (Qa)	AT5G08080	Plasma membrane	dniesqvssavdhvqsgntalqrakslqknSRKWMCIAIIILLIVVAVIVVGVlkpwknksa	0.701
VAMP-associated Protein, putative	AT2G45140	?	klteeknsavqlnnrlqqeldqlrreskrsKSGGIPFMYVLLVGLIGLILGYImkrt	0.703
SYP32 (Qa)	AT3G24350	Golgi	iridqnmedtlanvegaqsqlarylnsissNRWLMMKIFFVLIAFLMIFLFFVa	0.705
St12p	AT5G50550	ER	tfspdsrclvsvsfdsrarltvikqkgekrRVYLWVAALLFVLVYVVLYYLMVamgiih	0.709
Sec20 (Qb)	AT3G24315*	ER/Golgi	vlkkaeseykghrsllsrtrnllstmqrqdVIDRIILIVGFSLFVFAVVYVVSkrigilklqrmataaikaqlagkaang vgddvmplgqqfdgntvptvniplqqrmhdel	0.710
Putative SYP	AT1G27700	?	ceedcyekqlhgwygalqrqlqrsqyrmrySKSVHAAIWIILLVFLIVVVAVHsm	0.712
AtGOS11 Qb)	AT1G15880	Golgi	tfgginsklsnvasrlptvntilaaikrkkSMDTIILSLVAAVCTFLIFIYWItk	0.717
SYP51 (Qc)	AT1G16240	Tonoplast	dldyhvdvtdsrlrrvqkslavmnknmrsgCSCMSMLLSVLGIVGLAVVIWMLvkym	0.727
USE11 (Qc)	AT1G54110	ER?	dsteeaieqslastghatvratkiysesskTSCFQWLLILAMTCVFIMVVMLIrvt	0.730

Table 3 (continued)

SNARE	Entry	Localization	C-terminal region	Hydrophobicity
AtVAP27-1	AT3G60600	?	lteekqsaiqlnnrlqreldqlrreskksQSGGIPFMYVLLVGLIGLILGYImkrt	0.731
SYP31 (Qa)	AT5G05760	Golgi	iriddnmdeslvnvegarsallqhltrissNRWLMMKIFAVIILFLIVFLFFVa	0.733
SYP112 (Qa)	AT2G18260	Plasma membrane	dieanvanagsfvsggtnslyyanqmkkktKSWVLWVSILGVLILLVCVISMLasr	0.734
SYP131 (Qa)	AT3G03800	Plasma membrane	nienmvssavdhvqsgnnqltkavksqkssRKWMCIAILILLIIIIITVISVLkpwtqknga	0.736
SYP124 (Qa)	AT1G61290	Plasma membrane	ieshvskassfvrrgtdqlqdareyqkssrKWTCYAILLFIVVFALLLIPALPhimlmlk	0.737
SYP52 (Qc)	AT1G79590	Tonoplast	dldydvditdsrlrrvqkslalmnksmksgCSCMSMLLSVLGIVGLALVIWLLvkyl	0.745
AtVAMP721 (R)	AT1G04750	Plasma membrane	vdktenlrsqaqdfrttgtqmrrkmwlqnmKIKLIVLAIIIALILIIVLSVCHgfkc	0.762
R-SNARE AtVAMP726	AT1G04760	Plasma membrane	vdktenlrsqaqdfrtqgtkmkrklwfenmKIKLIVFGIIVALILIIILSVCHgfkct	0.762
AtZIG/AtSGR4/AtVTI11/ AtVTI1a (Qb)	AT5G39510	Tonoplast	rahetlhgvddnigkskkiltdmtrrmnknKWTIGAIIIALIAAIFIILYFKLt k	0.764
SYP41 (Qa)	AT5G26980	TGN	dridynienvattvedglkqlqkaertqrhGGMVKCASVLVILCFIMLLLLILkeifl	0.772
Synaptobrevin-related	AT4G10170	?	sagagtslekecvsssgrsvtqsfewkwrrLVQIVLAIDAAICLTLFGIWLAIcrgiectrs	0.774
AtNPSN13 (Qb)	AT3G17440	Plasma Membrane	rvvnhldtiqfsikkasqlvkeigrqvatdKCIMGFLFLIVCGVVAIIIVKIVnpnnkdirdipglappaqsrkllylrnqdym	0.782
SYP43 (Qa)	AT3G05710	TGN	vdridyniqnvastvddglkqlqkaertqrQGGMVMCASVLVILCFIMLVLLIlkeill	0.783
SYP42 (Qa)	AT4G02195	TGN	dridynvqnvstsveegykqlqkaertqreGAMVKCATILLVLCLIMIVLLILknilf	0.792
SYP61 (Qc)	AT1G28490	TGN	ideldtemdstknrlefvqkkvgmvmkkaAKGQMMMICFLLVLFIILFVLVFlt	0.800
AtNPSN12 (Qb)	AT1G48240	Plasma Membrane	rvvndldtiqfslkkasqlvkeigrqvatdKCIMAFLFLIVCGVIAIIIVKIVnpnnkdirdipglappaqsrkllyfre	0.801
SYP125 (Qa)	AT1G11250	Plasma membrane	ieshvakassfvrrgtdqlqdareyqkssrKWTCYAIILFIVIFILLLIPLLPhimlmlk	0.816
SYP122 (Qa)	AT3G52400*	Plasma membrane	iegnvkranslvrsgadrlvkarfyqkntrKWTCFAILLLLIIVVLIVVFTvkpwesngggggggaprqatpvqaqpppppavnrrllr	0.840
SYP22 (Qa)	AT5G46860	Tonoplast	ddigthidnsraatsqgksqlvqaaktqksNSSLTCLLLVIFGIVLLIVIIVLaa	0.844
AtNPSN11 (Qb)	AT2G35190	Cell plate	rvvneldsihfslkkasklvkeigrqvatdKCIMAFLFLIVIGVIAIIIVKIVnpnnkdirdipgvglappamnrrllwnhy	0.848
SYP123 (Qa)	AT4G03330	Plasma membrane	iesnvskassfvmrgtdqlhgakvlqrnnrKWACIATILAIVVVIVILFPILFntllrp	0.856
SYP121 (Qa)	AT3G11820*	Plasma membrane	ieshvgrassfirggtdqlqtarvyqkntrKWTCIAIIILIIIITVVVLAVLkpwnnssgggggggggttggsqpnsgtppnppqarrllr	0.876
SYP111 (Qa)	AT1G08560	Cell Plate	iehhvinashyvadganelktakshqrnsrKWMCIGIIVLLLIILIVVIPIITsfsss	0.900
R-SNARE domain/ AtVAMP723	AT2G33110	?	mlgssesqpqafyikrtqmkrkkwfqnmkIKLIVLAIIIALILIIILSVCGGfncgk	0.900
R-SNARE domain/ AtVAMP722	AT2G33120	?	dktenlrsqaqdfrtqgtqmrrkmwfqnmkIKLIVLAII IALILIIILSICGGfncgk	0.923
SYP21 (Qa)	AT5G16830	PVC	dissnldnshaattqatvqlrkaaktqrsnSSLTCLLILIFGIVLLIVIIVVLv	0.938

The classes of known and putative TA SNARES are indicated in the first column. When established, the localization is indicated. For each protein, the sequence used to calculate hydrophobicity is in uppercase

*Encoded proteins with a C-terminal polar region longer than 35 residues

Tail Anchors and Biotechnology

It has been recently shown that addition of a tail anchor improves the accumulation of HIV-negative factor (Nef), a promising target for vaccine development against HIV infection expressed in transgenic tobacco plants (Barbante et al. 2008). Nef is a cytosolic protein, but its accumulation levels in the cytosol or as a secretory protein (produced by adding a signal peptide) has been unsatisfactory (Marusic et al. 2007). In an effort to improve its accumulation, Nef was anchored to the cytosolic face of the ER membrane via the addition of the tail anchor of mammalian ER-b5. The chimeric protein (termed Nef-TA) has the expected TA topology. Nef-TA has a longer half-life and accumulates to higher levels (more than threefold) than its cytosolic counterpart. The half-lives of TA proteins are highly protein-specific and the turnover mechanism is, up to date, unknown. For example, the yeast TA protein Ubc6p is a very short-lived protein, but when the transmembrane domain (TMD) is removed, its half-life increases (Walter et al. 2001). On the contrary, when the TMD of the yeast TA long-lived protein Ubc4p was replaced with the TMD of Ubc6p, the half-life of the former decreased in spite of the fact that its localization on the ER membrane was maintained (Walter et al. 2001). Therefore, the half-life of TA proteins is strongly influenced by the structure of their TMDs. ER-b5 is a very long-lived protein, both in mammalian and plant cells (Borgese et al. 2001; Maggio et al. 2007; Pedrazzini et al. 2000). This is probably the reason why the cyt b5 tail anchor stabilizes Nef in the cytosolic environment. Other pharmaceutical proteins were fused to the TA domain of ER-b5, and also in those cases, accumulation of the chimeric proteins was improved (Alessandra Barbante and Emanuela Pedrazzini, unpublished data). Several GFP fusions to TA proteins have been expressed in plant cells (Lisenbee et al. 2003; Uemura et al. 2004); however, the different stabilities have not been compared. It will be interesting to produce GFP fusions to the tail anchors of different TA proteins located in the same compartment and compare stabilities. This would provide clues on the structural features that determine turnover. Besides the biotechnological implications, these results could cast light on the mechanisms of membrane protein degradation in plant cells.

Acknowledgments I am very grateful to Marco Marazza for technical assistance in computing TA proteome data. I thank The Arabidopsis Information Resource (TAIR) Center for providing bioinformatic tools. I am indebted to Alessandro Vitale and Nica Borgese for helpful discussions and critical reading of the manuscript. This work was supported by the project 'Recombinant Pharmaceuticals from Plants for Human Health—Pharma-Planta', VI European FW Programme Priority [LSH-2002-1.2.5-2] and by the project PRIN 20073YHRLE of the Ministero dell'Istruzione, dell'Università e della Ricerca, Italy.

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