1

Aim and outline of the thesis.

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Outline of this thesis

Vascular plant genomes have an higher number of SNAREs encoding genes compared to the unicellular plants and this might be associated with novel trafficking functions such as polarized secretion (Sanderfoot, 2007) which might require new specialized SNAREs (Lukowitz et al., 1996; Collins et al., 2003; Leucci et al., 2007). Next to the increase in gene numbers, also the capability of SNAREs to interact with different regulatory factors is proposed to have generated the diversification of SNARE complexes. In Chapter III we present a new experimental approach that relies on the “in vivo” measurement of an enzymatic reporter of secretion in protoplasts (Di Sansebastiano et al., 2007). With this experimental set up we could assess the functional difference between two plasma membrane syntaxins, SYP121 and SYP122 through the use of a dominant negative mutant for Rab11, a small GTP binding. Co expression of such Rab11 mutant with an SYP121 dominant negative mutant decreased secretion of the reporter secRGUS further compared with the expression of the Rab11 mutant alone, whereas co-expression of the same Rab11 mutant with a mutant version of SYP122 had no synergistic effect. The results suggest that Rab11 regulates anterograde transport from the TGN to the plasma membrane in combination with SYP122, rather than SYP121.

The pathway of traffic to the vacuole in the late secretory pathway (Rojo et al., 2001) has shown a high cell type dependent variability. In Arabidopsis many SNAREs have been showed to be involved in membrane trafficking to the tonoplast, these include the Qa-SNAREs SYP21 and SYP22, the Qb-SNAREs VTI11 and VTI12, and the Qc-SNAREs SYP51 and SYP52. So far there are no reports describing the function of SNAREs in cell-type specific processes so not much is known about their role in e.g. the sorting to vacuoles with different functions. Chapter IV describes a simple protocol for the isolation and transformation of petal protoplast from Petunia hybrida. We report that protoplasts isolated from different tissues maintain tissue specificity of gene expression and endomembrane organization within the time required for transient expression studies. This findings provides a formidable tool for the analysis of endomembrane traffic in different cell types.

The steady state pH in the lumen of organelles and vesicles is determined by the activity of proton pumps that generate a proton gradient across the membrane. The central vacuole of P. hybrida epidermal petal cells is specialized for the accumulation of anthocyanin, which are one of the major plant pigments and serve a variety of eco-physiological
functions (Koes et al., 2005). Because the color displayed by these pigments is dependent on pH, they act as natural pH indicators of the vacuolar lumen, where the anthocyanin accumulate, and thus provide a unique tool to identify mutants in which pH homeostasis is impaired (Quattrocchio et al., 2006). By studying such ph mutants we discovered a new mechanism by which vacuoles are acidified and a novel pathway by which proteins and vesicles are delivered to the vacuole. In Chapter V, we describe a novel acidification machinery, involving two P-ATPases encoded by the PH1 and PH5 genes, both needed for the acidification of vacuoles in epidermal petal cells. The combination of these two pumps, can drive vacuolar acidification also in other cell types where they are normally not expressed, suggesting they form an efficient acidification unit that can translocate protons across the tonoplast independently from other cell-specific factors.

In Chapter VI we describe vacuolar proteins sorted to the vacuole of anthocyanin accumulating cells. PH1 and PH5 localize, in first instance, on small vacuolar compartments, vacuolinos, that later probably fuse to the main tonoplast. To better characterize the nature of these vacuolar compartments, which are specific for epidermal petal cells, we isolated two petunia SNARE gene (PhSYP22, encoding a Qa-SNARE and PhSYP51, encoding a Qc-SNARE domain) by homology with the vacuolar SNAREs SYP22 and SYP51 from Arabidopsis. GFP-PhSYP22 and GFP-PhSYP51, and several other vacuolar proteins, follow the same protein sorting pathway by which PH5-GFP and GFP-PH1 reach the tonoplast. In addition we identified several mutations that disrupt the “vacuolino pathway”. Mutations in the regulators PH3 and PH4, (transcription factors required for the expression of PH1, PH5 and some 10 other petal specific genes), disrupt the formation of vacuolino, while mutations in PH1, knockdown of PhSYP51 or over expression of either PhSYP22 or PhSYP51, impair the fusion of vacuolino with the central vacuole. These observations strongly suggest that PhSYP51 and PH1 together mediate membrane docking and/or fusion and are supported by preliminary data showing the physical interaction of PH1 and PhSYP51 (as well as PhSYP22).

Conclusions

It has been proposed that different intracellular compartments of the eukaryotic cell emerged by events of duplication and diversification of a simpler molecular machinery
possibly consisting of about 20 basic SNARE types (Cavalier-Smith, 2002; Kloepper et al., 2007). Similarly, the duplication and divergence of other important factors involved in intracellular membrane trafficking have been demonstrated, for example for the organelle-specific Rab GTPases (Rutherford & Moore, 2002). The fine-tuning of the membrane trafficking processes is far from being fully understood, partially due to a large number of proteins involved. As it became apparent, the full characterization of SNARE functions is largely dependent on relevant in vivo assays.

In our opinion the exhaustive comparison of SNAREs in different organisms has scientific but also biotechnological implications. It will shed more light onto the organization and origins of the eukaryotic endomembrane system but will also provide new possibility to engineer new, up to now unthinkable, functions into transgenic eukaryotic cells. No question that membrane traffic is mediated by far more components than just SNAREs, some well-known like the Rab GTPases (Rutherford & Moore 2002) and other less studied like the long-range tethering factors known as golgins (Short et al., 2005), but the specificity of SNARE can be now investigated both in vivo and in vitro thanks to a large collection of new approaches.

A new aspect of SNARE function is their interaction with membrane proteins which is required for the activity of such protein (Grefen et al., 2010) and for the fusion of membranes (this thesis).

These exciting new findings will lead in the near future to a better understanding of how cells control the sorting of proteins and vesicles to specific compartments and how different combinations of SNAREs and membrane proteins possibly shapes the cell-type specific biogenesis and architecture of endomembrane compartments.
REFERENCES.


Sanderfoot, A. (2007) Increases in the number of SNARE genes parallels the rise of multicellularity among the green plants. Plant Physiol, 144, 6-17.
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