Routes to and from the plasma membrane: bulk flow versus signal mediated endocytosis

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Transport of proteins via the secretory pathway is controlled by a combination of signal dependent cargo selection as well as unspecific bulk flow of membranes and aqueous lumen. Using the plant vacuolar sorting receptor as model for membrane spanning proteins, we have distinguished bulk flow from signal mediated protein targeting in biosynthetic and endocytic transport routes and investigated the influence of transmembrane domain length. More specifically, long transmembrane domains seem to prevent ER retention, either by stimulating export or preventing recycling from post ER compartments. Long transmembrane domains also seem to prevent endocytic bulk flow from the plasma membrane, but the presence of specific endocytosis signals overrules this in a dominant manner.

When soluble proteins are transported to the apoplast or the vacuole, they effectively leave the secretory pathway. In contrast membrane proteins remain within the pathway, unless they segregate into the intraluminal vesicles of post Golgi organelles to reach either the vacuole lumen for degradation[1] or to be secreted as "exosomes."[2-4] While in the pathway, membrane proteins visit and recycle between many compartments, and they thus need a multitude of signals to regulate their steady state levels.

The plant vacuolar sorting receptor (VSR) is a valuable type I membrane spanning protein that completes many transport cycles before it is degraded.[5] VSR export from the ER occurs in a COPII dependent manner by bulk flow, without specific signals.[6] However, the conserved peptide sequence YMPL in its short cytosolic tail mediates segregation from biosynthetic secretory bulk flow.[7] When VSRs reach the prevacuolar compartment (PVC), the YMPL motif also facilitates VSR recycling,[8] a rate-limiting transport step that explains why VSRs are best detected at the PVC.[9]

Interestingly, the VSR tail contains additional contingency signals to deal with mis-sorting. The conserved IM motif prevents plasma membrane accumulation of VSRs when YMPL-mediated sorting to the PVC is impaired.[10] To test if the IM motif mediates increased endocytosis or reduced exocytosis, we have used the ligand-binding and release properties of full-length VSRs. By monitoring VSR-mediated vacuolar cargo release at the cell surface, we could show that the IM motif increases endocytic recycling of VSRs rather than preventing plasma membrane arrival (Gershlick et al. 2014, **Fig. 9**[6]). In contrast, the YMPL motif prevents VSRs arrival at the plasma membrane most probably via interactions with AP1 and/or AP4 complexes at the level of TGN or the Golgi apparatus.[6,11]

These recent results prompted us to ask if endocytosis also has a bulk flow component, similar to biosynthetic bulk flow[12] Although it was shown before that long transmembrane domains (TMDs) promote plasma membrane accumulation of membrane proteins,[13] it is unknown if this is due to increased anterograde transport or reduced endocytosis. For this reason, we modified the series of C-terminal truncation mutants of VSR (**Fig. 1A**) by imposing a long TMD. This would allow us to test the role of the TMD within the context of the presence or absence of biosynthetic and endocytic transport signals in the VSR tail.

Figure 1B shows that the longest deletion (ΔCT) was mostly ER retained, similar to the standard ER marker GFP-HDEL, except for bright punctae earlier shown to be Golgi and post-Golgi compartments.[6] However, introduction of a long TMD led to efficient accumulation at the plasma membrane with only weak punctae detectable (**Fig. 1B**, ΔCTLoTM). This suggests that long TMDs either enhance ER export or inhibit recycling back to the ER. The following deletion Δ23 was detected at the plasma membrane and bright punctate, but showed a significant redistribution to the plasma membrane when a long TMD was introduced (compare Δ23 with Δ23LoTM). While ΔCT and Δ23 with the wild type TMD were targeted very differently, ΔCTLoTM and Δ23LoTM could hardly be distinguished. In sharp contrast, the shorter deletions (Δ19 and Δ15) were hardly affected by the lengthening of the TMD. The Δ19 construct (containing the IM motif) continues to accumulate in bright punctate with only a very weak plasma membrane signal apparent (compare Δ19 with Δ19LoTM). These results suggest that a long TMD may reduce endocytic bulk flow but it cannot prevent signal-mediated endocytosis. Finally, when both IM and YMPL motifs are present (Δ15), PM localization is completely abolished
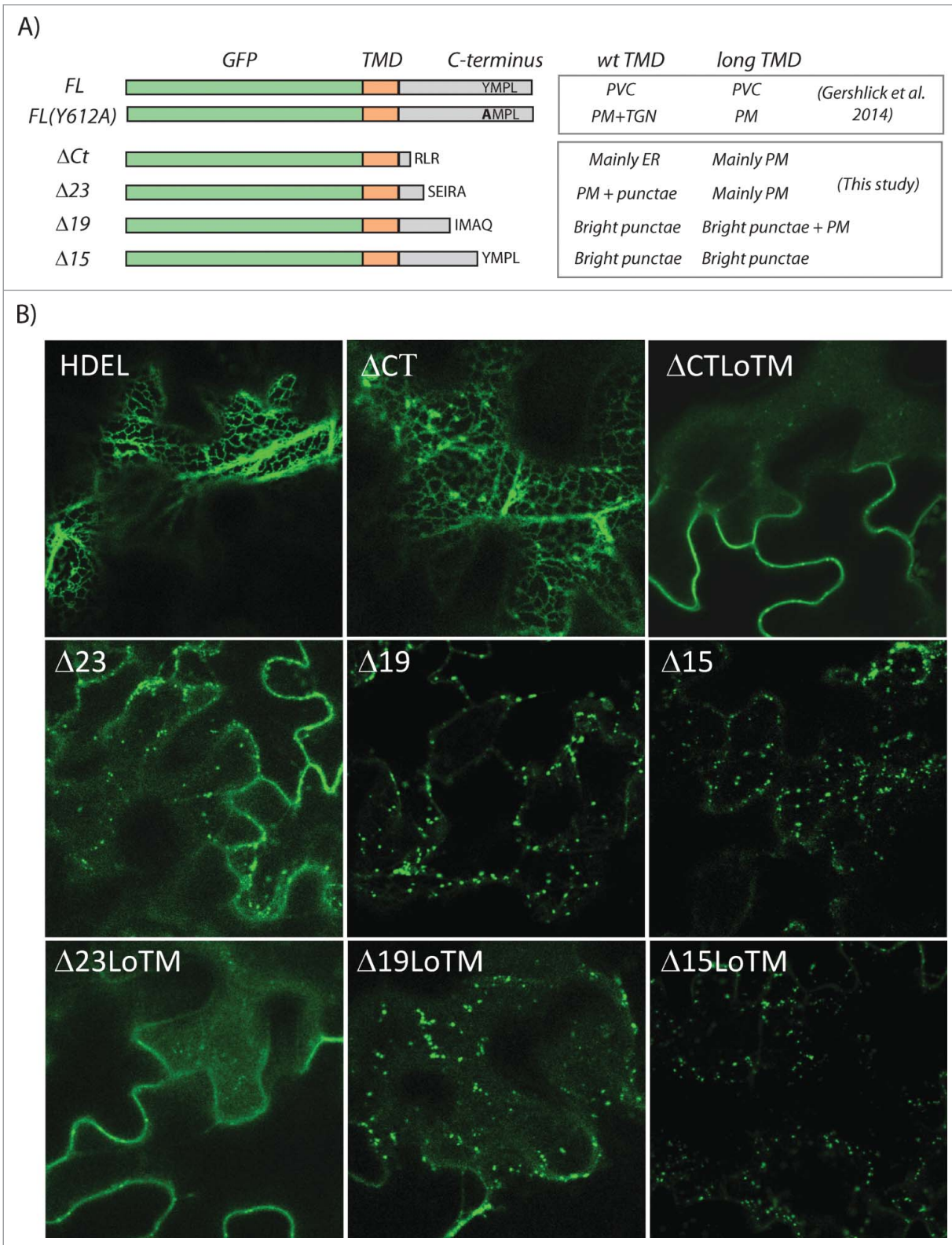
Figure 1. Summary of localization of various VSR mutants. (**A**) A schematic of the various tail deletion mutants described previously[6] and in this study, together with the corresponding subcellular locations in the presence of a wild type transmembrane domain (wt TMD) or a longer version (Long TMD). (**B**) Representative confocal laser scanning micrographs of Agrobacterium-infiltrated tobacco leaf epidermis cells expressing either a soluble ER marker (GFP-HDEL) or various mutants of the fluorescent receptor model membrane cargo GFP-VSR2. All VSR variants in this study have been cloned under the control of the weak TR2 promoter.

regardless of the presence of a long TMD due to the action of two complementary signals, one to minimize plasma membrane arrival (YMPL-motif) and another to retrieve the few molecules that escaped the YMPL pathway (IM motif).

The results obtained are consistent with the following model for membrane protein transport. Short or medium length TMDs are compatible with biosynthetic bulk flow of membrane proteins to the plasma membrane, but they can also undergo endocytic bulk flow, leading to degradation in the vacuole. This explains why the complete deletion of the VSR tail does not totally prevent vacuolar arrival of the truncated receptor.[7] Our model also explains why deletion of the di-lysine motif of p24 proteins causes the truncated protein to be mis-targeted to the plasma membrane, multivesicular bodies (PVCs and LPVCs) and the vacuole.[14] When active sorting signals are lacking, long TMDs promote plasma membrane accumulation by inhibiting endocytic bulk flow, as suggested for mammalian cells.[15] However, active endocytosis signals such as the IM motif are dominant signals that take precedence (**Fig. 1B**, Δ19, Δ19LoTM, Δ15 and Δ15LoTM). Other dominant internalization signals may be constituted by AP2-interacting tyrosine motifs[16,17] or ubiquitination.[18,19]

Besides its influence during endocytosis, long TMDs may also affect the early secretory pathway. Even though the shortest deletion (ΔCT) was shown to cycle via the plasma membrane[6] the dramatic difference between ΔCT and ΔCTLoTM cannot be explained by an inhibition of endocytosis alone as it would not explain the redistribution of the ER-retained portion. Long TMDs could either promote incorporation into COPII coated ER export carriers or be poor cargo for COPI-mediated recycling. Further work will be required to distinguish between these two possibilities.

We can now ask if plasma membrane transport of different types of membrane proteins occurs via the same pathway as constitutive secretion of soluble cargo. The established VSR constructs with specific deletions and point mutations provides an ideal toolbox to test single parameters that influence membrane protein sorting and with the help of Rab6 and Rab11 mutants we may be able to dissect the role of the Golgi and TGN in biosynthetic as well as endocytic transport to and from the plasma membrane.[20-24]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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