Localization and characterization of SMP-containing proteins in Membrane Contact Sites

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Abstract: Membrane contact sites (MCS) are discrete regions within the cell where two membranes from different organelles are closely apposed (10-30 nm). In those regions, non-vesicular transfer of lipids takes place to ensure proper organelle functioning. The formation of MCS depends on proteins which promote the apposition of the membranes and mediate the lipid transport between the membranes.

Arabidopsis SYT1 is one of the most characterized MCS protein, and it plays a relevant role in tolerance to abiotic stresses, such as wounding, cold or high salinity, by glycerolipid transport and MCS formation thanks to its synaptotagmin-like mitochondrial-lipid-binding (SMP) domain. However, little is known about other SMP-containing proteins in plants, as their localization or their role in abiotic stress.

We have studied the SMP-containing proteins in *Arabidopsis thaliana* and *Solanum lycopersicum*. To identify them, human E-Syt1 sequence was used to find the remote orthologues in plants. An interesting highlight of those results was that some SMP-containing proteins are exclusive from plants, there are no orthologues in human nor yeast. The subsequent analysis of the subcellular location of these proteins was carried out in *Nicotiana benthamiana* by transient expression of the SMP-containing proteins of *Arabidopsis* and *Solanum*, and confocal microscopy imaging. We have found that those proteins locate in different MCS across the cell: SYT6, NTMC2T6 and Tex2 localized in ER-Golgi contact sites, NTMC2T5 in ER-Chloroplast contact sites, and we have also confirmed that *Solanum* CLB1 and SYT5 behave the same way as their *Arabidopsis* counterpart in the ER-PM contact sites. Additionally, we have analysed the root growth, seed germination rates and fully expanded cotyledons of *Arabidopsis* mutants for these genes in media supplemented with salt or ABA, and our results suggest that some of these proteins might be implicated in abiotic stress signalling through an ABA-dependent pathway.