NTMC2T5: newly identified lipid transfer proteins at ER-chloroplast contact sites.

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In plants, fatty acid synthesis takes place at chloroplasts, and they are assembled into glycerolipids and sphingolipids at the endoplasmic reticulum (ER). Then, the newly synthetized lipids in the ER are delivered to chloroplast (mostly) via a non-vesicular pathway, likely through lipid transport proteins (LTP). These LTP would be localized in ER-chloroplast membrane contact sites (MCS), which are microdomains where membranes of these two different organelles are closely apposed but not fussing.

Synaptotagmin-like mitochondrial-lipid-binding (SMP) domain proteins are evolutionarily conserved LTP in eukaryotes that localize at membrane CS. They are involved in tethering of these MCS through interaction with other proteins/membrane lipids and in transferring of glycerolipids between these two membranes. We have studied the occurrence of SMP proteins in *A. thaliana* and *S. lycopersicum* by searching remote orthologs of human E-Syt1 (SMP protein). By using transient expression in *N. benthamiana* leaves and confocal microscopy, we have identified the NTMC2T5 family with two homologs in *A. thaliana* and only one in *S. lycopersicum* that are anchored to the chloroplast outer membrane and are interacting with the ER (at ER-chloroplast CS).

Our preliminary data have unequivocally demonstrated that NTMC2T5 proteins are anchored to the outer envelope membrane in chloroplast, and they bind in *trans* the ER. Additionally, it is predicted that these proteins contain a SMP domain which is a lipid-transfer domain, indicating that these proteins could be responsible for some of the lipid transferring events at ER-chloroplast CS that are still unknown. Our preliminary phenotypic analyses have shown that these proteins are involved in hypocotyl length in darkness and plant growth under Nitrogen deficiency. Interestingly, we have observed that clustering of chloroplasts around the nucleus occurred when we overexpressed these proteins in *Nicotiana benthamiana* leaves and *Arabidopsis* double *knock-out* mutant for these proteins showed less chloroplasts attached to the nucleus in epidermal cells. Finally, we will also show the results of our biotinylation-based proximity labelling proteomics experiments that have been performed to identify interactors of these proteins. And we will also show the results of the lipidomic analysis we have performed in order to understand the role of these proteins.