

# NTMC2T5 proteins as lipid bridges between plastids and ER during cotyledon development.

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The conversion of proplastids to etioplasts is a critical process during seed germination, being essential for the development of cotyledons and the plant itself. Etioplasts play a crucial role in these initial stages as they serve as precursors to chloroplasts [1]. The shift from one type of plastid to another involves massive structural changes. Among these, a key step is the formation of all chloroplastic membranes, which requires the synthesis and delivery of lipids to the plastids [1,2]. Galactolipids are the most prevalent lipids found in chloroplasts. Notably, during the initial phases of photomorphogenesis, there is a noticeable build up of galactolipids within the plastid membranes, originated from the endoplasmic reticulum (ER) [2]. Since vesicular transport between these organelles has not been documented, lipid transport may be mediated by lipid transport proteins (LTP) at membrane contact sites (MCS). LTPs are characterized by unique lipid transport domains, such as the synaptotagmin-like mitochondrial lipid-binding (SMP) domain [3]. However, no specific LTP has been identified as responsible for lipid exchange between the endoplasmic reticulum (ER) and chloroplasts.

In our studies, we have identified the NTMC2T5 proteins, as putative lipid chloroplast transfer proteins, as they contain a SMP domain. Our confocal microscopy experiments have demonstrated that these proteins are anchored to the outer envelope membrane of the chloroplast, and BiFC analysis we shown that they interact with the ER, forming ER-Chloroplast MCS [3]. QRT-PCR analysis have revealed that both *Arabidopsis* and *N. benthamiana* NTMC2T5 genes are mainly expressed in early stages of seedling development. Further analysis in *Nicotiana benthamiana* CRISPR/Cas9 knock-out mutants showed (1) delayed greening in cotyledons when germinated in light, (2) an albino-like phenotype when germinated in darkness, (3) clear defect in plastid morphogenesis by transmission electron microscopy (TEM) and (4) lower content of galactolipids.

Overall, our studies have identified the NTMC2T5 proteins, containing a SMP domain and localized at ER-Chloroplast MCS. Our results suggest NTMC2T5 proteins are involved in various aspects of chloroplast development, serving as shuttles for lipids between ER and chloroplast.

## References

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