

NTMC2T5: a newly identified lipid transfer proteins at ER-chloroplast contact sites involved in development and stress response.

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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 ER synthesized lipids are sent back to the chloroplast to form part of their membranes. Since, no vesicular transport has been described between these two organelles, lipid transport might be mediated by lipid transport proteins (LTP) via a non-vesicular pathway.

Many of these LTP are localized in membrane contact sites (MCS). LTP at MCS contain specific lipid transport domains, as the synaptotagmin-like mitochondrial lipid-binding (SMP) domain. To our knowledge, no protein has been identified to be directly involved in transfer of lipids between ER and chloroplast.

Our analysis has identified the NTMC2T5 family proteins, SMP proteins, localized at chloroplast. They are anchored to the outer membrane of the chloroplast and interact with the nuclear ER (ER-Chloroplast MCS). Additionally, when we overexpress them, clustering of chloroplasts around the nucleus occurs, which have been associated with pathogen perception and retrograde signalling.

Furthermore, *Nicotiana benthamiana* CRISPR/Cas9 knock-out mutants showed delayed greening of cotyledons, which could be due to impaired lipid transport to the chloroplast and, therefore, chloroplast biogenesis. Arabidopsis knock-down mutant plants have shown reduced root growth under nitrogen and carbon deprivation conditions.

Overall, these results show the first identified proteins localized in ER-Chloroplast MCS which might have a role in lipid transfer. Our results suggest they might be involved in various aspects of plant development and stress signalling through changes in membrane lipids between nucleus and chloroplast.