

Characterizing the NTMC2T5 proteins at ER-plastid contact sites

Oliver Cuevas¹, Carolina Huercano¹, Miriam Moya-Barrientos¹ & Noemí Ruiz-López¹

¹Instituto de Hortofruticultura Subtropical y Mediterránea “La Mayora” (Facultad de Ciencias, Universidad de Málaga/CSIC, Málaga)

Membrane contact sites (MCS) are specialized regions where the membranes of two distinct organelles come into close proximity (10-30 nm) without fusing. These regions facilitate lipid exchange between chloroplasts and the endoplasmic reticulum (ER), playing a key role in plant glycerolipid synthesis. Our research focuses on MCS-associated proteins containing an SMP domain, a conserved lipid-binding module implicated in lipid transfer. We have identified NTMC2T5 proteins, which putatively localize to the outer envelope membrane of chloroplasts and establish contacts with the ER, although their precise molecular function remains largely unknown.

To investigate NTMC2T5 role, we generated *A. thaliana ntmc2t5.1/ntmc2t5.2* double mutants via CRISPR-Cas9. These lines exhibit a distinct post-germination cotyledon yellowing, mirroring the phenotype previously observed in *N. benthamiana ntmc2t5* mutants, and confirming the protein's role in plastid development.

To further characterize NTMC2T5, we expressed truncated versions of the *N. benthamiana* protein NbNTMC2T5 in *E. coli*, comprising its functional domains (SMP and/or C2) and the hydrophobic C-terminal region (HR), which will be used for lipid transfer assays. Furthermore, we have conducted BiFC assays, which support that NTMC2T5 proteins localize to the outer chloroplast envelope, although the precise positioning of their HR remains unclear. Together, these approaches provide a foundation for dissecting the molecular function of NTMC2T5 at ER-plastid MCS.

References

Huercano, C., Moya-Barrientos, M., Cuevas, O., Sanchez-Vera, V. & Ruiz-Lopez, N. ER-plastid contact sites as molecular crossroads for plastid lipid biosynthesis. *BMC Biol.* **23**, (2025).

Funding/Acknowledgements

This work has been funded by grant PID2024-159647NB-I00 (by MICIU/AEI/10.13039/501100011033/FEDER, UE) and an FPU fellowship (FPU23/00493) by the Spanish Ministry for Science, Innovation & Universities.