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Functional characterization of NTMC2T5 proteins in the development of chloroplasts in *Nicotiana benthamiana*

Oliver Cuevas[1], Carolina Huercano[1], Victoria Sánchez-Vera[1] & Noemí Ruiz-López[1]

[1]Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora" (IHSM-UMA-CSIC) 29010, Departamento de Biología Molecular y Bioquímica, Málaga, Spain.

Membrane contact sites (MCS) are specialized regions where the membranes of two different organelles come into close proximity, typically within 10–30 nm. These regions are enriched in specific lipids and proteins and play a crucial role in plant lipid metabolism by facilitating lipid transport between chloroplasts and the endoplasmic reticulum (ER) during the eukaryotic pathway of glycerolipid synthesis.

Our research focuses on proteins that localize to MCS and contain an SMP domain, which mediates lipid transfer between organelle membranes. Our recent studies have identified NTMC2T5 proteins, which are found in the outer envelope membrane of the chloroplast and associates the ER, although their specific functions remain unclear.

To explore their functional roles, we generated *Nbntmc2t5* mutant lines of *Nicotiana benthamiana*. These mutants displayed a characteristic yellowing of cotyledons post-germination, prompting us to analyse plastid development under both light and etiolation conditions using confocal and transmission electron microscopy.

Our results indicate that the absence of NbNTMC2T5 protein significantly impacts plastid biogenesis, disrupting the transition from proplastids to mature chloroplasts in seedlings germinated in both light and darkness. These findings suggest that these alterations originate during early plastid development, specifically at the proplastid stage. Furthermore, the disruption of ER-chloroplast MCS in the *Nbntmc2t5* mutants highlights the critical role of MCS-mediated lipid transfer in proper plastid differentiation and function.

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