Identification of NTMC2T5, a new lipid transfer protein family at ER-chloroplast contact sites involved in stress response

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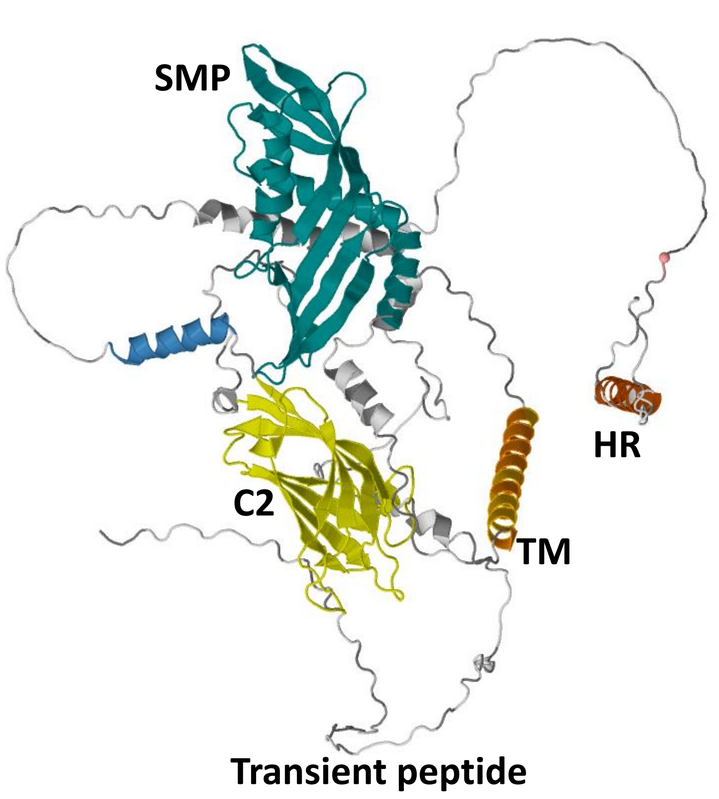
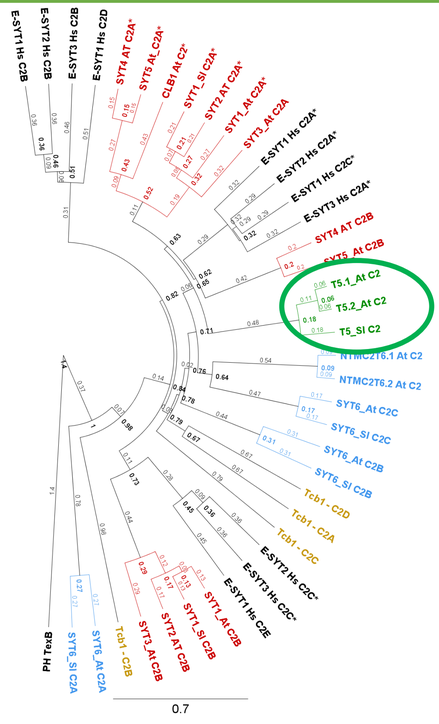
Plants are sessile organisms and therefore they have perfected a complex molecular signalling network to detect and respond to the different environmental stresses. In plants, fatty acid synthesis takes place at chloroplasts, and they are assembled into glycerolipids and sphingolipids at the endoplasmic reticulum (ER). Then, the newly synthetized lipids in the ER are delivered to chloroplast 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 MCS. They are involved in tethering of these MCS and in glycerolipid transferring 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) and we have identified NTMC2T5 proteins.

Our results show the first identified LTPs localized in ER-Chloroplast MCS which conceivably would have a role in lipid transfer between these two membranes. Specifically they are attached to chloroplast and they are transiently interacting with the ER that form the nuclear envelope. Our results suggest that NTMC2T5 proteins are involved in stress signalling after pathogen perception by promoting the clustering around the nucleus. Also, they may be involved in abiotic stress signalling (cold and salt) through ABA.



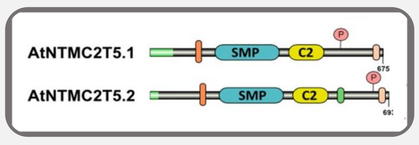


Analysis of NTMC2T5 C2 domains revealed they are a new type of C2 Ca^{2+} independent domains.

Two genes codify to NTMC2T5 proteins in *A. thaliana:* NTMC2T5.1 *(At1g50260)* and NTMC2T5.2 *(At3g19830).*

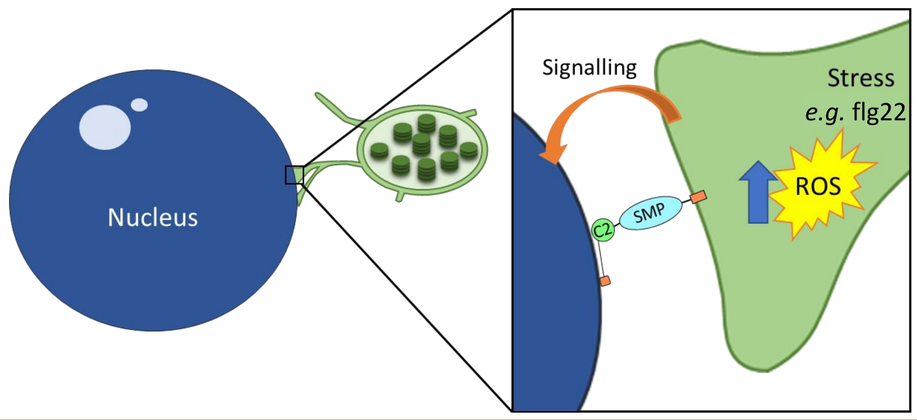
NTMC2T5 proteins share a common structure

- **Transit peptide** - presence of a large aminoacidic sequence before the transmembrane (**TM)** domain to guide the protein to the chloroplasts.
- **SMP domain** – involved in lipid transferring.
- **C2 domain** – likely responsible of the transient interaction with the ER.
- **Hydrophobic region (HR)** at the C terminal – of unknown function.



Scheme of our hypothesis

After stress perception *(e.g.,* pathogen *via* flg22), ROS are produced in the chloroplast. This signal could be delivered from the chloroplast stromules to the nucleus through proteins such as NTMC2T5 proteins.



1. NTMCT5 proteins are localized in ER-chloroplast contact sites.

NTM2T5 proteins were transient expressed in *N. benthamiana* leaves and visualized using confocal microscopy.

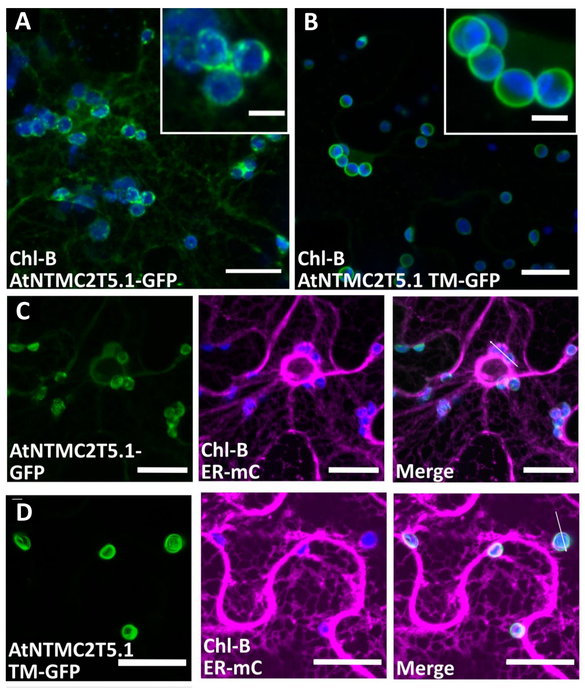


Image A

NTMC2T5.1-GFP is localized around chloroplasts, but the GFP signal is concentrated in some areas of chloroplast outer envelope membrane (OEM). We also noticed that GFP signal spread out of the chloroplast in a signal resembling the ER.

Image B

The transmembrane domain of NTMC2T5.1 is only localized around chloroplast. Additionally, in this experiment the GFP signal is evenly distributed at chloroplasts.

Image C

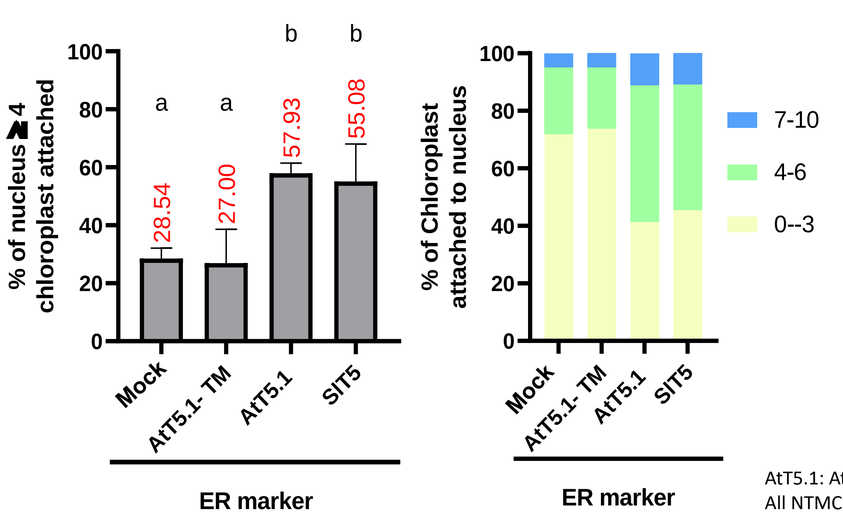
The overexpression of the NTMC2T5.1-GFP protein with the ER marker shows a clearly association of this protein with the nucleus membrane.

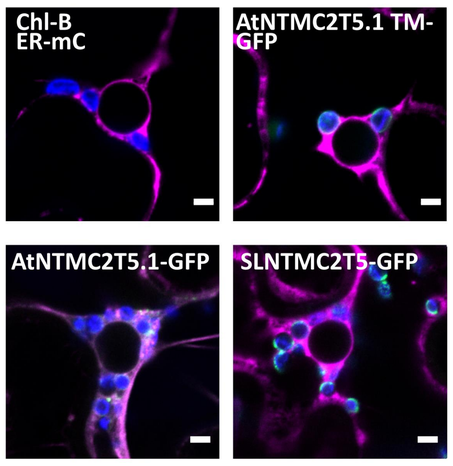
Image D

When we overexpressed the TM of NTMC2T5.1-GFP with ER marker, the association with the ER seems to disappear, in contrast with the overexpression of the full version of the protein.

2. NTMC2T5 proteins are involved in clustering around nucleus after pathogen perception.

***2A) NTMC2T5 overexpression induces clustering of chloroplast around the nucleus*.**

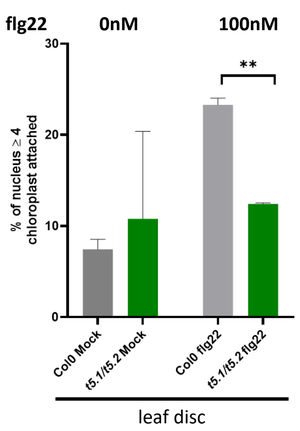




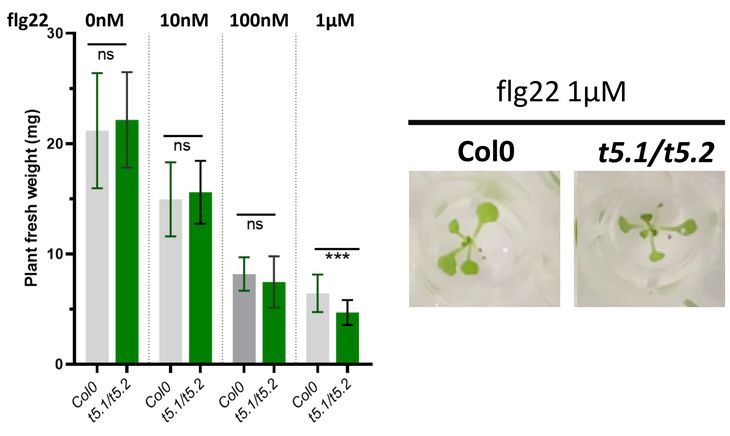
AtT5.1: AtNTMC2T5.1; SLT5: SlNTMC2T5
All NTMC2T5 proteins are fused to GFP

Scale bar: 5 um

2B) Arabidopsis double knock-out mutant showed less chloroplasts attached to the nucleus in presence of flg22.

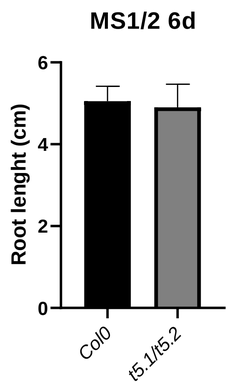


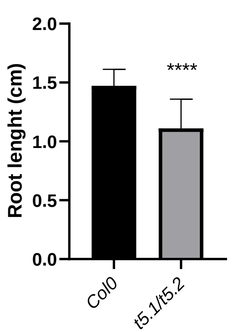
2C) Arabidopsis ntmc2t5.1/ntmc2t5.2 showed a significant reduced growth when treated with flg22.



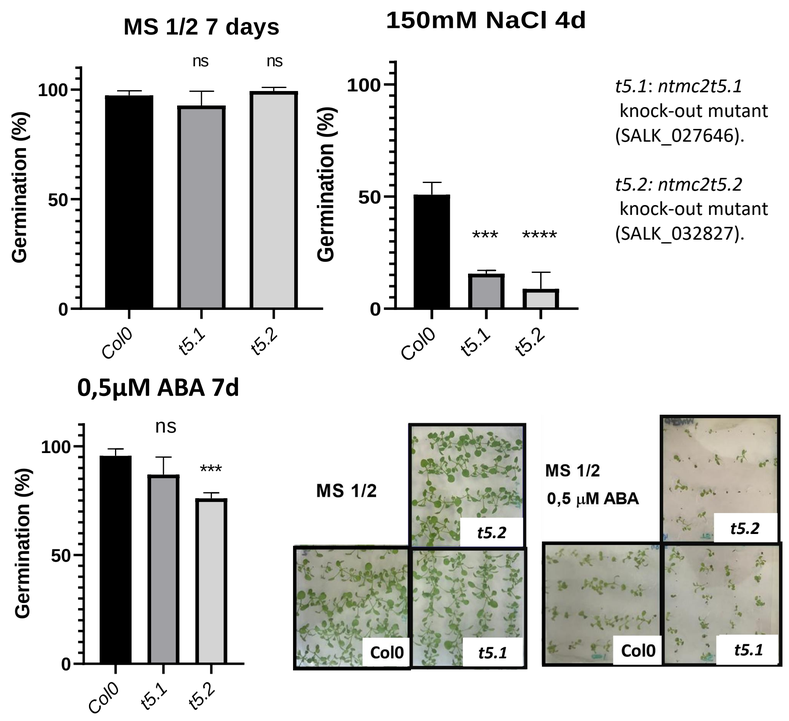
3. Mutant *ntmc2t5* plants showed reduced growth under some abiotic stress conditions.

3A) ntmc2t5 double mutant showed shorter root lenght in cold conditions (10ºC)





***3B) ntmc2t5* plants showed reduced germination in NaCl and ABA presence.**



t5.1: ntmc2t5.1 knock-out mutant (SALK_027646).

t5.2: ntmc2t5.2 knock-out mutant (SALK_032827).

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