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Functional characterization of *Arabidopsis thaliana* Synaptotagmin1 domains using Tricalbin3 chimeras in *Saccharomyces cerevisiae*

Francisco Benitez-Fuente[1], Javier Collado[2], Vitor Amorim-Silva[1], Ruben Fernandez-Busnadiego[2], Miguel A. Botella[1*]

[1]*IHSM-UMA-CSIC, Malaga, Spain.*
[2]*Institute of Neuropathology, University Medical Center Göttingen, Germany.*
[*]*Corresponding author: Miguel A. Botella (mabotella@uma.es)*

f.benitez@uma.es

Summary

- Synaptotagmin1 (SYT1) is an *Arabidopsis thaliana* endoplasmic reticulum (ER)-plasma membrane contact site tether involved in biotic and abiotic stress resistance. These resistance roles have been related to SYT1 tethering and lipid-transport functions. However, the specific contributions of SYT1 domains to these functions and their relevance in stress resistance remain unknown.
- To efficiently investigate each SYT1 domain *in vivo*, we carried out domain interchanges in the model organism *Saccharomyces cerevisiae* (yeast). Tricalbin3 (Tcb3) is a SYT1 homolog in yeast, and it is essential for heat-shock tolerance. Tcb3 also mediates the formation of high-curvature peaks at the ER, which is promotes during heat, and would facilitate lipid homeostasis between the PM and the ER.
- We generated constructs expressing SYT1/Tcb3 chimeras tagged to fluorescent proteins, transformed them into *tcb3Δ* yeast cells and studied their subcellular localization and ability to complement the heat-shock hypersensitivity of *tcb3Δ.* We are further analyzing the ER-peak formation ability of these chimeras by cryo-electron tomography.
- Our work revealed that SYT1 did not show the localization pattern of Tcb3, nor could complement the heat-shock hypersensitivity of *tcb3Δ*. However, Tcb3 chimeras containing either SYT1 SMP or C2 domains showed a Tcb3 subcellular pattern and a heat-shock tolerance similar to that of *tcb3Δ*/Tcb3-GFP complemented strains. Further, Tcb3 N-terminal region was required, although not sufficient, for heat-shock tolerance and localization. Preliminary data suggests that chimeras complementing *tcb3Δ* heat-shock hypersensitivity also mediate ER peak formation.

Results

SYT1 SMP and C2 domains, but not its N-terminal region, drive Tcb3 ER-PM localization

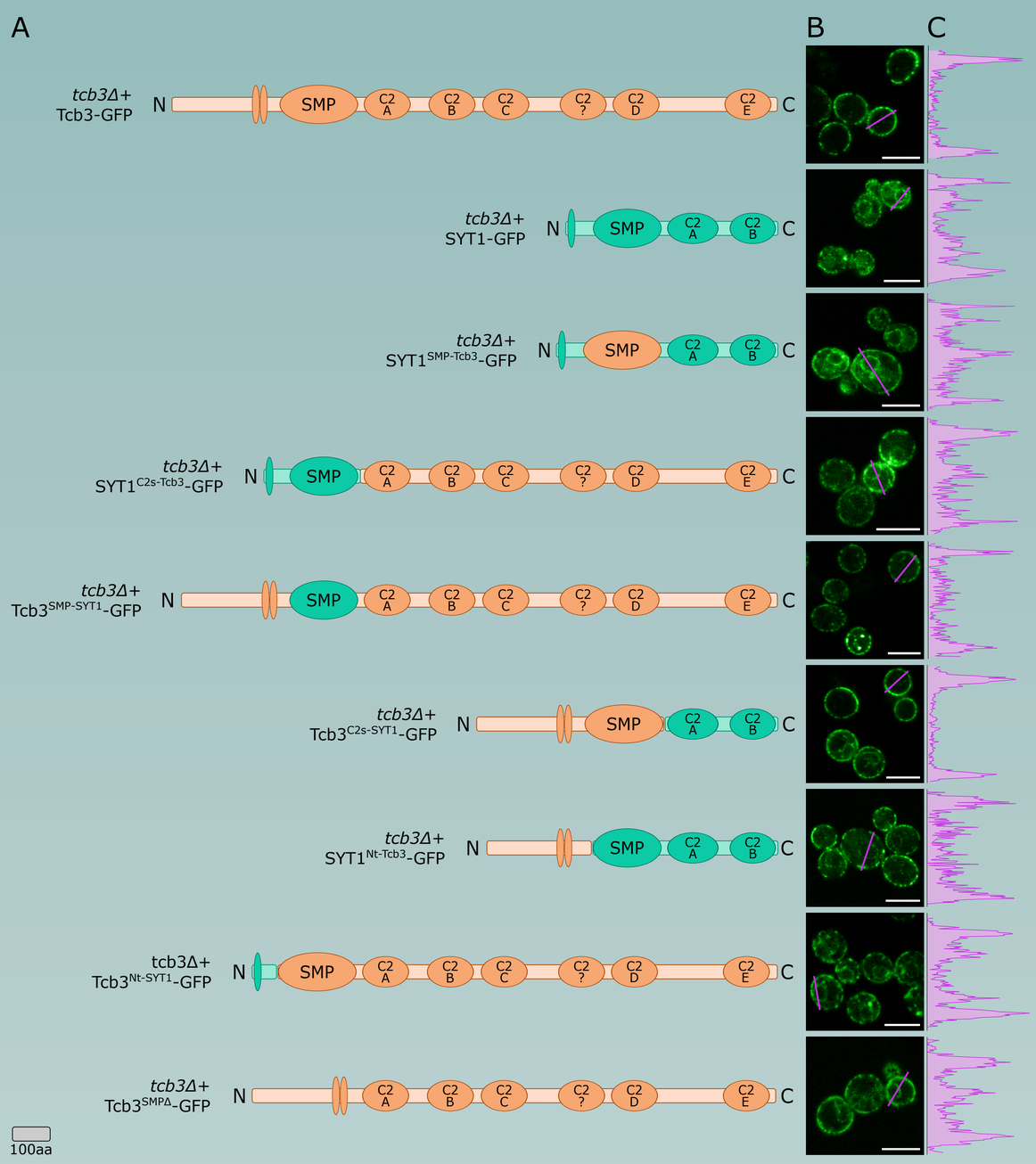


Figure 1: Subcellular localization pattern of SYT1/Tcb3 chimeras tagged to GFP. A, schematic representation of the chimeric proteins used in this study. Tcb3 domains are shown in orange; SYT1 domains are shown in teal. B, chimeras subcellular localization in *tcb3Δ* yeast cells. White bars represent scale bars. Magenta bars show the region analyzed by the intensity plot. C, Intensity plots of along the magenta lines labelled in B. Scale bars, 5µm.

SYT1 SMP and C2 domains complement *tcb3Δ* heat-shock hypersensitivity

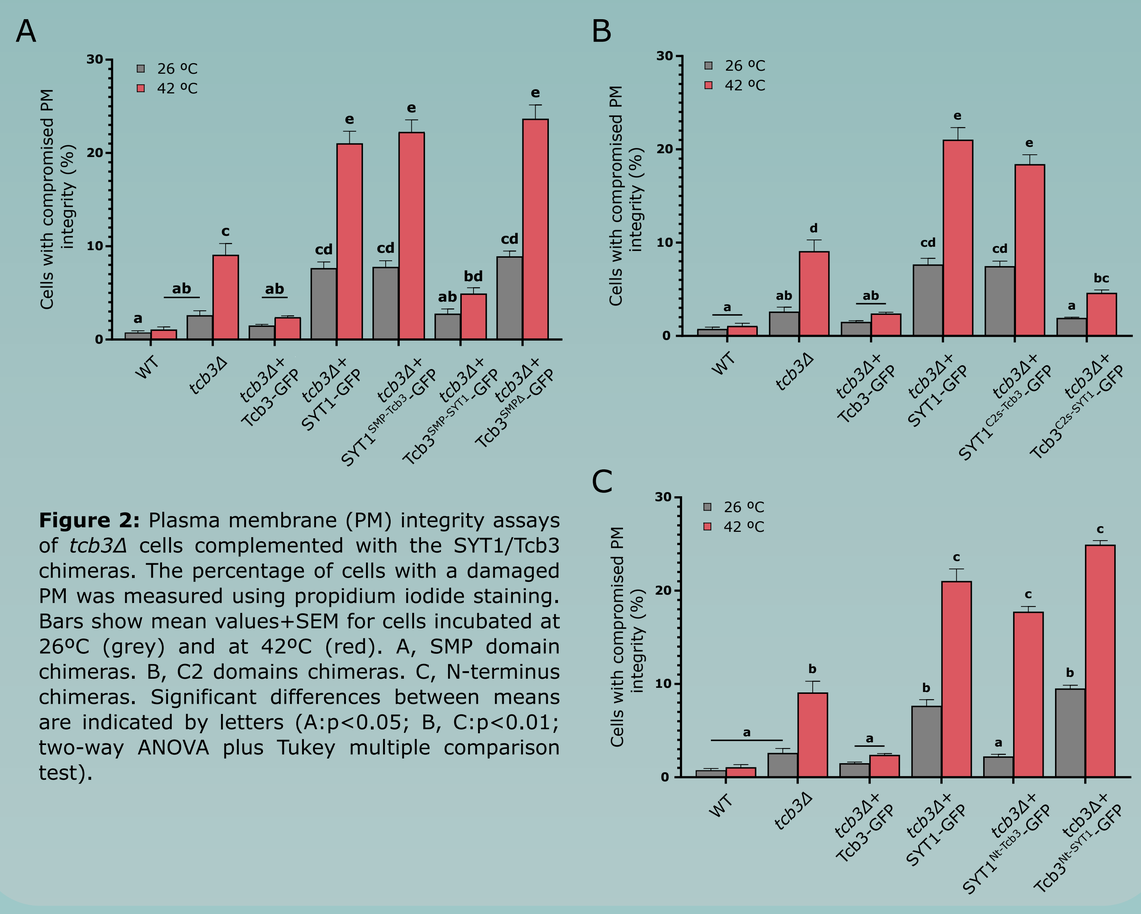


Figure 2: Plasma membrane (PM) integrity assays of *tcb3Δ* cells complemented with the SYT1/Tcb3 chimeras. The percentage of cells with a damaged PM was measured using propidium iodide staining. Bars show mean values+SEM for cells incubated at 26ºC (grey) and at 42ºC (red). A, SMP domain chimeras. B, C2 domains chimeras. C, N-terminus chimeras. Significant differences between means are indicated by letters (A:p<0.05; B, C:p<0.01; two-way ANOVA plus Tukey multiple comparison test).

Tcb3$^{C2s\text{-}SYT1}$-GFP forms ER peaks at ER-PM contact sites

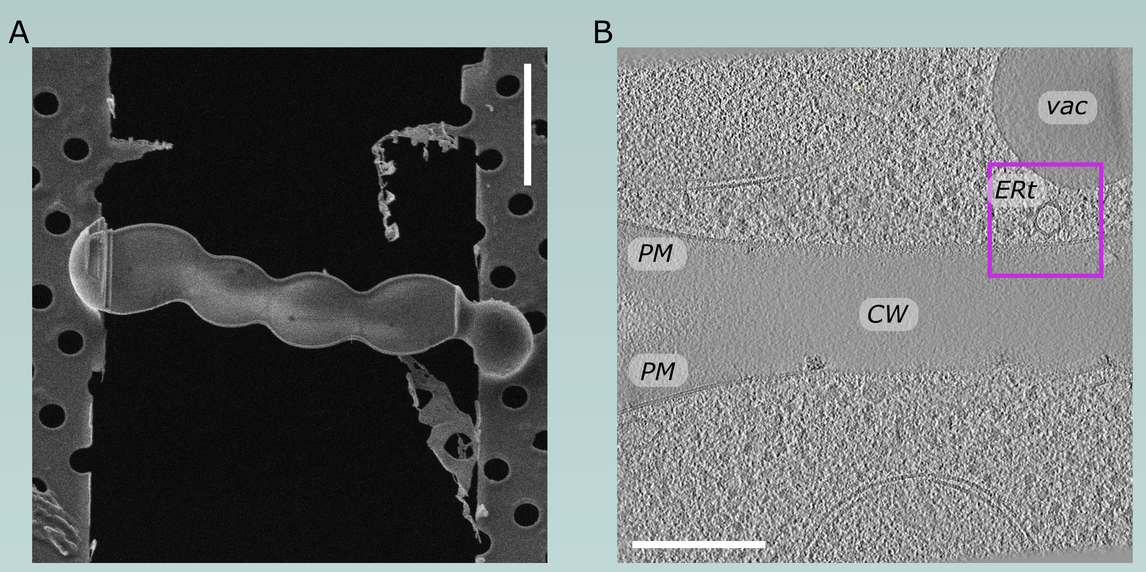
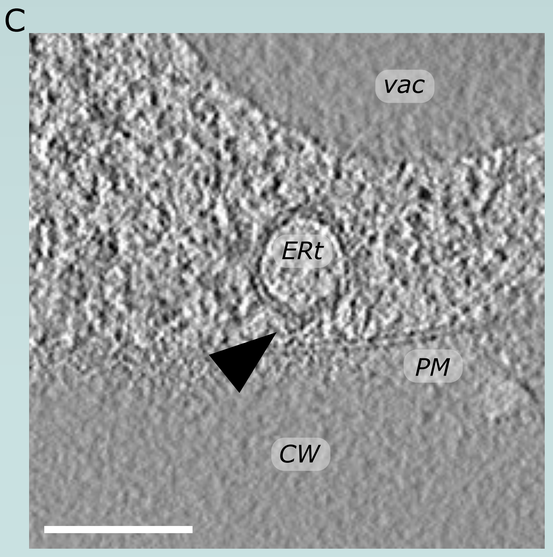


Figure 3: Cryo-electron tomography of *tcb3Δ*+Tcb3$^{C2s\text{-}SYT1}$-GFP cells. A, yeast cells lamella visualized by cryo-SEM after cryo-focused ion beam milling. B, tomogram slice of the periphery of two cells. CW: cell wall; vac: vacuole; PM: plasma membrane; ERt: ER tubule. Magenta square indicates the region enlarged in C. C, magnified section of the tomogram slice showed in B. Black arrowhead points to a ER peak within the ER tubule. Scale bars: A, 5µm; B, 200nm; C, 50nm.



Conclusions

- The use of SYT1/Tcb3 chimeras in yeast is a useful strategy for revealing domain contribution *in vivo.*
- SYT1 SMP and C2 domains function as those of Tcb3, conferring heat-shock tolerance and an ER-PM localization.
- Tcb3 N-terminus is essential, yet not sufficient, for ER-PM localization and heat-shock tolerance.
- Our results highlight the functional relevance of the N-terminal region of these tethers, which usually is not employed on *in vitro* studies due to technical difficulties.