

Plasma membrane lipid remodeling during cold acclimation is mediated by the ER-PM contact sites-localized synaptotagmins 1 and 3

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Cold acclimation is the capacity of certain plants to increase their freezing tolerance in response to a period of low non-freezing temperatures. Cold acclimation involves a series of biochemical and physiological adaptations, including a deep transcriptional reprogramming and drastic changes in the lipid composition of cellular membranes in order to prevent the freeze-induced damage (1). While a profound knowledge has been acquired on the regulation of gene expression triggered by cold-acclimation, very little is known about the mechanisms governing the cold-induced changes in membranes' lipid composition. In this study we report that in *Arabidopsis*, the constitutively expressed Synaptotagmin 1 (SYT1) and the cold-induced homolog Synaptotagmin 3 (SYT3) are essential for cold-acclimated freezing tolerance and for the lipid remodelling of the plasma membrane during cold-acclimation. SYT1 and SYT3 are phospholipid-binding proteins located in Endoplasmic Reticulum-Plasma Membrane contact sites (ER-PMcs), conserved structures defined as regions of the cortical ER in close apposition to the PM (2). ER-PMcs facilitate the non-vesicular lipid transport between ER and PM in yeast and mammals, and are essential for lipid homeostasis (3). In contrast to the high and ubiquitous SYT1 expression, SYT3 expression is low and mainly restricted to meristemoids, young stomata, and old primary root. TIRF microscopy analyses show that during cold acclimation there is an increase of SYT1::SYT1:GFP and SYT3::SYT3:GFP signals as spots at the PM. High-resolution lipidome analyses show the over-accumulation of phosphatidylinositols phosphate (PIPs) and glycerolipids *in vivo* in *syt1* and specially *syt1/syt3* mutant plants compared to WT in one-week cold-acclimated plants. Interestingly, protein-lipid overlay assays (membrane-strips and PIP-strips) reveal PIPs and glycerolipids as major interactors for both, SYT1 and SYT3. Here we show that 1) *Arabidopsis SYT1* and *SYT3* are induced by cold, 2) SYT1 and SYT3 localize to ER-PMcs, 3) the specific lipids that directly interact with SYT1 and SYT3 accumulate in *syt1/syt3* mutant after cold acclimation, and 4) *syt1/syt3* show reduced cold acclimated freezing tolerance. We propose that SYT1 and SYT3 have essential roles in ER-PMcs mediated lipid remodelling during cold acclimation, which in turn leads to freezing tolerance.

References:

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- (3) Prinz, W.A. (2014). *J. Cell Biol*. 205: 759–769.