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The role of DGK1 and DGK2 in Membrane Contact Sites and Stress Tolerance

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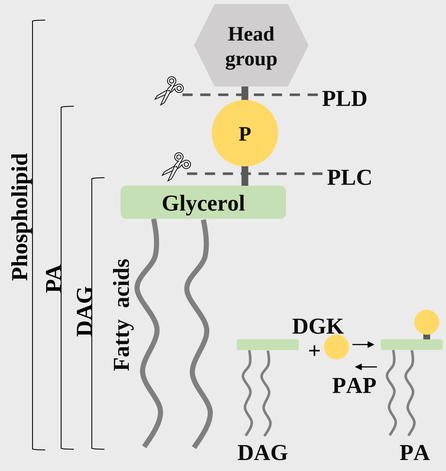
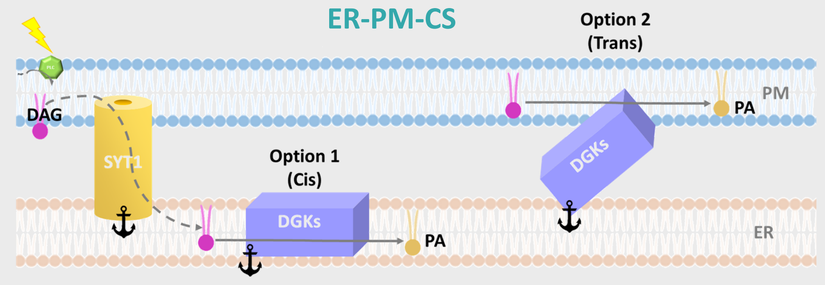
SUMMARY

Substrate (DAG) and product (PA) of DGKs are both signaling lipids, which implicates these proteins in the regulation of the stress response. Here we show the interaction between DGK1, DGK2 and SYT1, all of them ER proteins. SYT1, which preferentially binds DAG, is concentrated at the ER-PM-CS through the binding of the C2 domains to negative phospholipid of the PM. These domains are also responsible for the interaction with the C1 domains of DGK1, which supports the hypothesis that DGK1 and DGK2 may be acting in Trans in ER-PM-CS.

INTRODUCTION:

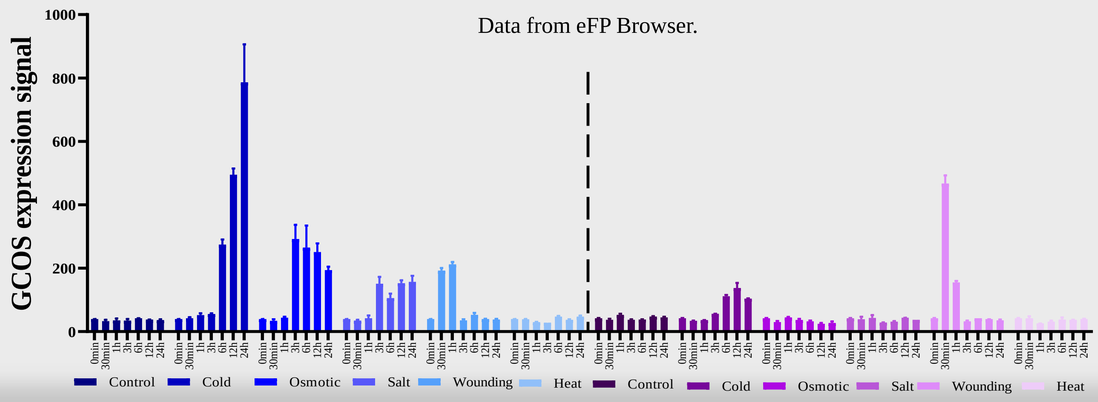
- There are **7 isoforms** of DGKs encoded in *Arabidopsis thaliana* genome. AtDGK1 and AtDGK2 are anchored to ER, the rest are cytoplasmic
- Diacylglycerol kinases (**DGKs**) phosphorylate diacylglycerol (**DAG**), converting it into phosphatidic acid (**PA**), both important signaling molecules
- **Contact Sites** are conserved eukaryotic cellular microdomains where two membranes of different organelles are very close. Contact sites between the endoplasmic reticulum (**ER**) and the plasma membrane (**ER-PM-CS**) have been described to play important roles in cellular signaling. **SYT1** is a well characterized lipid transporter ER-PM-CS protein
- It remains unknown if DGK1 and DGK2 are working in **CIS** or in **TRANS** in ER-PM-CS





DGK1 and DGK2 expression under stresses

Data from eFP Browser.



INTERACTIONS:

1. AtDGK1, AtDGK2 and AtSYT1 interact to each other

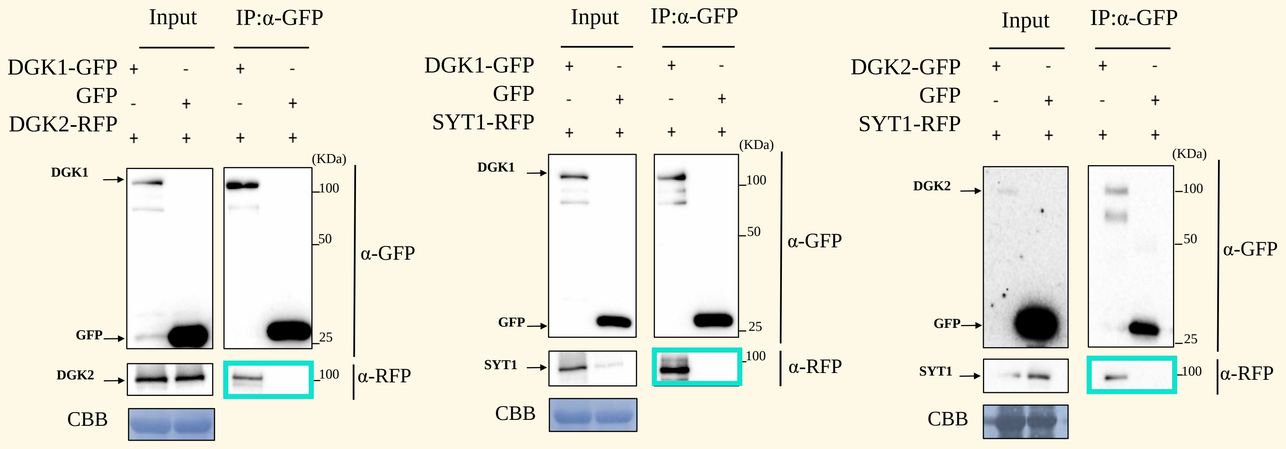


Fig. 1. Co-IP of AtDGK1 with AtDGK2, AtDGK1 with AtSYT1 and AtDGK2 with AtSYT1. Proteins were tagged with GFP or RFP and co-expressed in *N. benthamiana*. 2dpi tissue was collected and analyzed by Co-IP.

2. SYT1 ΔC2 domains loses its location at ER-PM-CS

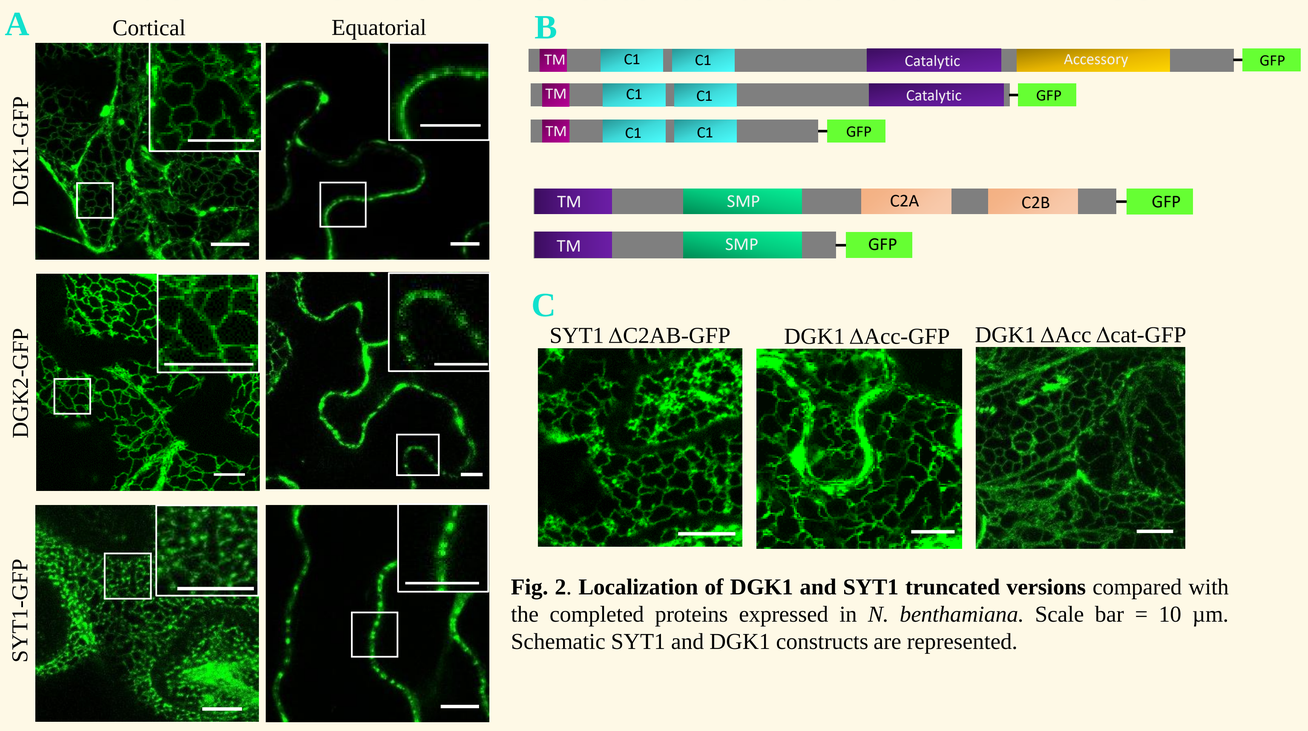


Fig. 2. Localization of DGK1 and SYT1 truncated versions compared with the completed proteins expressed in *N. benthamiana*. Scale bar = 10 µm. Schematic SYT1 and DGK1 constructs are represented.

3. C1s of DGK1 interact with C2s of SYT1. DGK1 and DGK2 interact by the Accessory domain

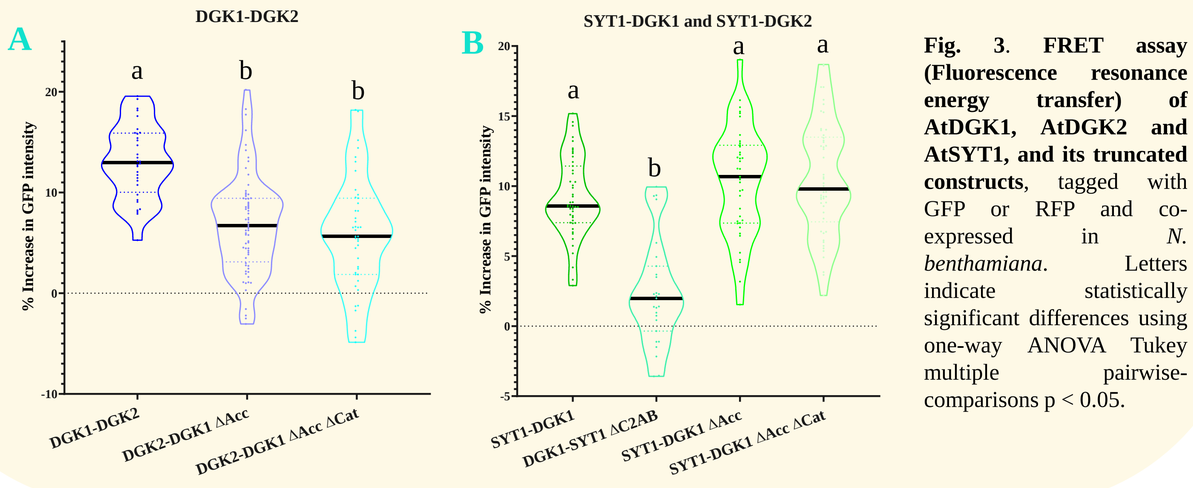


Fig. 3. FRET assay (Fluorescence resonance energy transfer) of AtDGK1, AtDGK2 and AtSYT1, and its truncated constructs, tagged with GFP or RFP and co-expressed in *N. benthamiana*. Letters indicate statistically significant differences using one-way ANOVA Tukey multiple pairwise-comparisons $p < 0.05$.

4. DGK1 and DGK2 protein amount increase under cold treatment

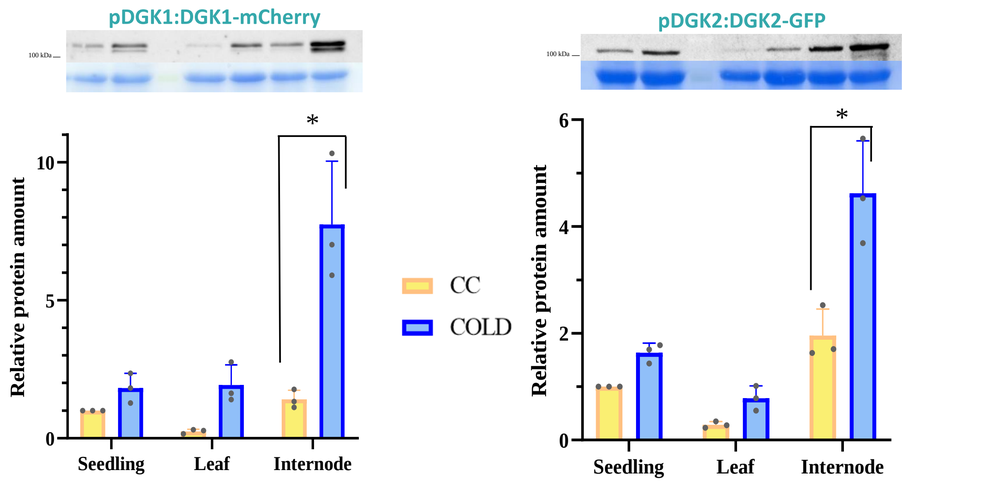
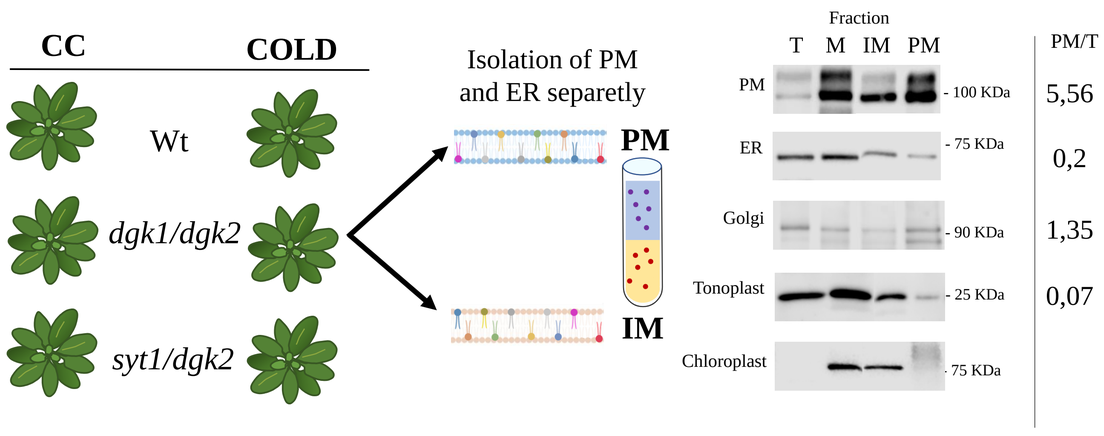


Fig. 4. Relative DGK1 and DGK2 protein amount in seedling, leaf and internode under Control (CC) and Cold Treatment. The amount of protein in each tissue and condition was measured by western blot, and it was relativized to the protein expression of the seedling in CC. Three independent replicates were carried out.

5. On going → DGK1 and DGK2 are acting in Cis or in Trans at the ER-PM CS?



We are going to analyze the lipid composition of both fraction (in CC and COLD condition) to determine whether there is an accumulation of DAG or PA in the PM or ER as a result of the absence of these proteins in the mutant lines

Fig. 5. Membrane lipid analysis. It has been performed using the genotypes Wt, *dgk1/2*, and *syt1/dgk2*, grown under CC and COLD conditions, from which PM and Inner Membranes (IM) fractions were isolated. Western blot analysis confirmed an enrichment of PM compared to the other organelles. (Total extraction, T; Membranes, M)

CONCLUSIONS:

- **SYT1, DGK1 and DGK2 interact with each other**
- **DGK1 and SYT1 interact via the C1 and C2 domains**
- **SYT1 interact with the PM via C2 domains**
- **Under cold condition DGK1 and DGK2 proteins are accumulated**

Acknowledgments:

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