ROLE OF ARABIDOPSIS DGK1 AND DGK2 IN COLD STRESS

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There are regions, present in all eukaryotic cells, where the membranes of two different organelles are very close (10-30nm), but without fusion, due to proteins which act as tether. These regions are named Membrane Contact Sites (MCS), as for example those formed by endoplasmic reticulum (ER) and plasma membrane (PM). ER-PM CS play important roles in communication between membranes, lipid homeostasis and Ca^{2+} influx.

When a plant is challenge with a stress, phospholipase C is activated at PM producing diacylglycerol (DAG) and inositol phosphates from the hydrolysis of PIP(4,5)P2 or PI4P. DAG is then phosphorylated by diacylglycerol kinases (DGKs) producing phosphatidic acid (PA). Both, DAG and PA, are molecules involved in signalling (Arisz *et al*., 2009). The genome of *Arabidopsis thaliana* contain 7 genes encoding DGKs. Most of them are predicted to be cytosolic, with only DGK1 and DGK2 are anchored to the ER due a transmembrane domain. Using different approaches (Co-Immunoprecipitation, confocal microscopy, FRET, Tap-tag…), our group has uncover, that AtDGK1 and AtDGK2 (Diacylglycerol kinase 1, AT5G07920 and Diacylglycerol kinase 2, At5g63770) form a complex with the lipid transport protein located at ER-PM CS known as Synaptotagmin1 (SYT1, At2g20990) (Pérez-Sancho *et al*., 2015). SYT1 is able to bind preferentially DAG (Ruiz-Lopez *et al*., 2021), which support the idea that SYT1, DGK1, and DGK2 function coordinated to regulate the levels of DAG at the PM.

DGK1 and *DGK2* are induced upon low temperatures and found that *dgk2* mutants show reduced root growth in low temperature and have reduced freezing tolerance. Our studies suggest that DGK1 and DGK2 act in concert with SYT1 to regulate the production of DAG and PA at ER-PM CS and highlight the importance of these proteins for the correct response to stress.

References:

Arisz, S. A., Testerink, C., & Munnik, T. (2009). Plant PA signaling via diacylglycerol kinase. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids, 1791(9), 869–875. https://doi.org/10.1016/j.bbalip.2009.04.006

Pérez-Sancho, J., Vanneste, S., Lee, E., McFarlane, H. E., del Valle, A. E., Valpuesta, V., Friml, J., Botella, M. A., & Rosado, A. (2015). The arabidopsis synaptotagmin1 is enriched in endoplasmic reticulum-plasma membrane contact sites and confers cellular resistance to mechanical stresses. Plant Physiology, 168(1), 132–143. https://doi.org/10.1104/pp.15.00260

Ruiz-Lopez, N., Pérez-Sancho, J., del Valle, A. E., Haslam, R. P., Vanneste, S., Catalá, R., Perea-Resa, C., van Damme, D., García-Hernández, S., Albert, A., Vallarino, J., Lin, J., Friml, J., Macho, A. P., Salinas, J., Rosado, A., Napier, J. A., Amorim-Silva, V., & Botella, M. A. (2021). Synaptotagmins at the endoplasmic reticulum–plasma membrane contact sites maintain diacylglycerol homeostasis during abiotic stress. Plant Cell, 33(7), 2431–2453. https://doi.org/10.1093/plcell/koab122

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