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Diacylglycerol acyltransferases (DGATs) of type 1 and type 2 are well-known ER membrane enzymes playing a major role in TAG synthesis in Eukaryotes. A third type of protein, DGAT3, has been purified from the cytosolic fraction of peanut (*Arachis hypogea*) cotyledon in 2006 (Saha et al.).

Since then, the DGAT3 gene family appears to be widely and exclusively distributed among plants and (micro)algae (Turchetto-zolet 2016). Deduced amino acid sequences share a lack of predicted transmembrane domains ('soluble' proteins) and the presence of a conserved thioredoxin-ferredoxin-like domain, which may carry Fe-S cluster. Indeed, DGAT3 of *Arabidopsis thaliana* has been identified as a 2Fe-2S protein when expressed in *E. coli* (Ayme *et al.* 2018).

Although the capacity of diverse DGAT3s to synthesize TAG *in vitro* (Saha et al. 2006, Ayme et al. 2018) or *in vivo* has been observed, their physiological role remains unclear (or diverse), as well as their subcellular location. DGAT3 has been proposed to be involved in lipid remodeling in germinating *A. thaliana* (Hernandez *et al.* 2012) or to take part in a chloroplastic TAG synthesis pathway in *Chlamydomonas reinhardtii*.

In order to investigate activities, substrate specificities or potential lipid ligand, we developed an *in vitro* approach based on recombinant his-tagged proteins purified from *E. coli*. Using protein-lipid overlay assays and/or microscale thermophoresis, we observed that two distantly related land plant DGAT3 (from *A. thaliana* and *Amborella trichopoda*) bind to a subset of membrane lipids including phosphatidic acid and PI4P.

We thank E. Hornung and I. Feussner (Göttingen Univ., Germany) for the gift of expression plasmid for *A. thaliana DGAT3*.

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