A transferase interactome that may facilitate channeling of polyunsaturated fatty acid moieties from phosphatidylcholine to triacylglycerol

Received for publication, August 12, 2019, and in revised form, August 25, 2019 Published, Papers in Press, September 3, 2019, DOI 10.1074/jbc.AC119.010601

© 2019 Xu et al. Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.

ACCELERATED COMMUNICATION

Polyunsaturated fatty acids (PUFAs) such as α-linolenic acid (ALA, 18:3\[^{9, cis, 12, cis, 15}\] have high nutritional and industrial values. In oilseed crops, PUFAs are synthesized on phosphatidylcholine (PC) and accumulated in triacylglycerol (TAG). Therefore, exploring the mechanisms that route PC-derived PUFA to TAG is essential for understanding and improving PUFA production. The seed oil of flax (Linum usitatissimum) is enriched in ALA, and this plant has many lipid biosynthetic enzymes that prefer ALA-containing substrates. In this study, using membrane yeast two-hybrid and bimolecular fluorescence complementation assays, we probed recombinant flax transferase enzymes, previously shown to contribute to PUFA enrichment of TAG, for physical interactions with each other under in vivo conditions. We found that diacylglycerol acyltransferases, which catalyze the final reaction in acyl-CoA-dependent TAG biosynthesis, interact with the acyl-editing enzymes phosphatidylcholine:diacylglycerol cholinephosphotransferase, and lysophosphatidylcholine acyltransferase. Physical interactions among the acyl-editing enzymes were also identified. These findings reveal the presence of an assembly of interacting transferases that may facilitate the channeling of PUFA from PC to TAG in flax and possibly also in other oleaginous plants that produce seeds enriched in PC-modified fatty acids.

Seed oils of flax (Linum usitatissimum), which are predominantly in the form of triacylglycerol (TAG),\(^2\) contain 45 to 65% α-linolenic acid (ALA; 18:3\[^{9, cis, 12, cis, 15}\] , a polyunsaturated fatty acid (PUFA) with great nutritional and industrial value (1–4). Similar to the process of PUFA biosynthesis in many other plant species, flax ALA enrichment involves the coordinate action of multiple enzymes in various subcellular compartments.

In developing seeds of oleaginous plants, oleic acid (18:1\[^{9, cis}\] , hereafter 18:1), which is de novo-synthesized in the plastid, is exported and undergoes further desaturation on phosphatidylcholine (PC) to form linoleic acid (18:2\[^{9, cis, 12, cis}\] ) and ALA by the sequential catalytic actions of fatty acid desaturase 2 and 3, respectively (5, 6). PC is also the major site for the synthesis of unusual fatty acids, such as hydroxy, epoxy, and conjugated fatty acids (7–10). Subsequently, PUFA-s or other modified fatty acids at the sn-2 position of PC are routed into TAG via several enzyme reactions involving in acyl-editing and TAG assembly (8, 11, 12). PUFA on PC can enter the acyl-CoA pool through the reverse reaction catalyzed by lysophosphatidylcholine acyltransferase (LPCAT) or through the combined catalytic actions of phospholipase A\(_2\) and long-chain acyl-CoA synthetase. The resulting PUFA-CoAs can then be used as acyl donors for the acyl-CoA-dependent acyltransferases of the Kennedy pathway. The acylation of sn-1,2-diacylglycerol (DAG) to form TAG in this pathway is catalyzed by diacylglycerol acyltransferase (DGAT) (8). Alternatively, PUFA-enriched DAG can be supplied to DGAT as a result of the symmetrical phosphocholine head group exchange between PUFA-enriched PC and 18:1-enriched DAG catalyzed by phosphatidylcholine diacylglycerol cholinephosphotransferase (PDCT) (13, 14). The resulting 18:1-enriched PC can then undergo further desaturation. In addition, the catalytic actions of phospholipase C and D can potentially generate PUFA-enriched DAG and phosphatidic acid, respectively, for utilization by the Kennedy pathway (15, 16). Furthermore, PUFA-enriched PC can also serve as an acyl-donor in the acyl-CoA-independent synthesis of TAG catalyzed by phospholipid:diacylglycerol acyltransferase (PDAT) (17, 18).

Physical interactions between lipid biosynthetic enzymes have been identified in mammals (19–22) and a dynamic protein interactionome to facilitate these processes has been recently proposed (23). Considering that TAG assembly and acyl-editing enzymes for plant PUFA enrichment either share the same substrate/product or catalyze consecutive reactions, in which the product of the reaction catalyzed by the first enzyme is the
substrate for the second enzyme, it is reasonable to hypothesize that these enzymes might form assemblies to effectively route the PUFA moieties from PC to TAG. In flax, DGAT (24, 25), PDAT (26), PDCT (14), and LPCAT (25) have been shown to display preference toward ALA-containing substrates and contribute to ALA-enriching processes. The aim of this study was to probe for possible physical interactions between flax transferases involved in routing PUFA from PC to TAG. Using a split-ubiquitin membrane yeast two-hybrid system (MYTH) and a bimolecular fluorescence complementation (BiFC) assay using *Nicotiana benthamiana* leaves, several interactions among the flax transferases were identified. It is proposed that these physical interactions contribute to channeling of ALA from PC into TAG.

**Results and discussion**

Protein-protein interactions between LuDGAT1, LuDGAT2, and other transferases contributing to flax ALA enrichment, including LuPDAT1, LuLPCAT2, and LuPDCT1 were tested using MYTH and BiFC systems. The principle of the split-ubiquitin MYTH is similar to the traditional yeast two-hybrid assay but it is adapted to test the protein-protein interactions in cellular membranes (27). In a MYTH system, two membrane proteins being tested for possible interactions (bait and prey) are fused to the C- and N-terminal subfragments of ubiquitin (Cub-Lex reporter and NubG), respectively, and only if the two proteins interact, their tight contact brings Cub-Lex reporter and NubG into close proximity and thus leads to re-association of ubiquitin, which then releases the transcription factor and activates the expression of the downstream reporter genes, including HIS3, ADE2, and LacZ. As a result, the yeast cells co-expressing bait and prey grow on synthetic drop-out (SD) agar plates lacking Ade, His, Leu, and Trp (SD-A-H-L-T) and produce blue colonies at the presence of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). As shown in Fig. 1A, an interaction between LuDGAT1 and itself or LuPDCT1 was observed in yeast, when LuDGAT1 was used as a bait. An interaction between LuLPCAT2 and LuDGAT1 was also observed in yeast by using LuLPCAT2 as a bait and LuDGAT1 as a prey. To confirm the MYTH results, BiFC experiments were carried out. BiFC is based on the complementation of the N- and C-terminal subfragments of a fluorescent protein (28). In our case, two proteins of interest are fused to the N-terminal fragment of the yellow fluorescent protein Venus (Venus<sup>YF</sup>) and the C-terminal fragment of the super cyan fluorescent protein SCFP3A<sup>C</sup> (SCFP3A<sup>C</sup>), respectively, and only if the two proteins interact, the reconstitution of the fluorescing protein is triggered, resulting in unique fluorescence emission (BiFC signal is shown in green and grey scale; Fig. 1B). Consistently, the BiFC experiments showed that LuDGAT1 interacted with itself, LuLPCAT2 or LuPDCT1 in tobacco leaves when fused with either the Venus N-terminal fragment or the SCFP3A C-terminal fragment (Fig. 1B). As expected, these flux transferases appear to reside in the endoplasmic reticulum (ER), because the observed BiFC signal showed a similar pattern to the fluorescent signal of the SCFP3A fused *Arabidopsis thaliana* glycero-3-phosphate acyltransferase (Fig. 1C), which is a known ER-localized protein (29). Further evidence is still required to confirm the subcellular pattern of the interaction of flux transferases, although this reticular appearance of the ER of tobacco leaf epidermal cells (Fig. 1C) has been previously shown in other reports (30, 31). The demonstrated interaction between LuLPCAT2 and LuDGAT1 is consistent with the recent observation of the interaction between LPCAT2 and DGAT1 from *Arabidopsis* (*A. thaliana*) using MYTH (32) and further supports the previous evidence for biochemical coupling of LuDGAT1- and LuLPCAT-catalyzed reactions for incorporating ALA into TAG (25).

LuDGAT2 interacted with LuLPCAT2 or LuPDCT1 in yeast when using LuDGAT2 as a prey (Fig. 2A), which was only partially supported by the BiFC results (Fig. 2B). The interaction of LuDGAT2 with LuLPCAT2 was observed in tobacco leaves using BiFC, in which the Venus N-terminal fragment or the SCFP3A C-terminal fragment was fused with LuDGAT2 or LuLPCAT2, whereas the absence of or a very weak BiFC signal was observed for a possible interaction between LuDGAT2 and LuPDCT1 (Fig. 2B).

LuLPCAT2 interacted with itself, LuPDAT1, or LuPDCT1 in yeast (Fig. 3A). Only the self-interaction of LuLPCAT2 and the interaction between LuLPCAT2 (fused with the SCFP3A C-terminal fragment) and LuPDAT1 (fused with the Venus N-terminal fragment) were observed using the BiFC system (Fig. 3B). LuPDCT1 also self-interacted in yeast (Fig. 3A), but only a very weak BiFC signal was seen in tobacco leaves in support of an LuPDCT1 self-interaction (Fig. 3B).

It should also be noted that in the MYTH experiments, different bait and prey combinations of the same protein pairs led to different results (Figs. 1A, 2A, and 3A). This discrepancy has also been reported when testing the interactions between *Arabidopsis* DGAT and LPCAT (32). One possible explanation may be that the membrane-bound feature of these proteins would restrict the orientation of both termini of proteins and thus affect the re-association process of ubiquitin.

The observed self-interactions of LuDGAT1, LuLPCAT2, and LuPDCT1 suggested that these enzymes may have a quaternary structure. Previously, self-association of DGAT1 mediated by the hydrophilic N-terminal domain was demonstrated for the recombinant enzyme from *Brassica napus* (33), mouse (*Mus musculus*) (34), and human (*Homo sapiens*) (35). The hydrophilic N-terminal domain of *B. napus* DGAT1 has also been shown to possess a noncatalytic allosteric binding site for acyl-CoA or CoA, which regulates the enzyme’s activity based on surrounding concentrations of acyl-CoA/CoA (33, 36). Allosteric enzymes often exhibit a quaternary structure that can involve intersubunit communication. As for the other transferases examined in the current study, it remains to be determined whether these enzymes and their animal homologues exhibit self-interactions and allosteric properties. It should be noted, however, that a conserved member of the animal sphingomyelin synthase enzyme family, which is phylogenetically close to plant PDCT (13), was recently shown to undergo self-association (37).

The identified protein-protein interactions reveal a possible network of TAG assembly and acyl-editing transferases in flax (Fig. 4A), in which the enzymes from the acyl-editing processes and DGAT, which catalyzes the final reaction in acyl-CoA–de-
dependent TAG biosynthesis, might form multienzyme complexes to facilitate effective channeling of ALA moieties from PC to TAG. This network of interacting enzymes may lead to enhanced pathway efficiency wherein the substrates and products of these enzyme-catalyzed reactions are not equilibrated with bulk phases such as cytosol or nearby phospholipids, but instead, the stream of intermediates is channeled through the active sites of the various transferases. More specifically, the protein-protein interaction between LuPDCT1 and LuDGAT1 suggests that DAG enriched in ALA through PDCT action could be an effective acyl acceptor for LuDGAT1 selectively utilizing \( \alpha \)-linolenoyl-CoA to form TAG (Fig. 4B). In addition, LuLPCAT2, an acyl-editing enzyme involved in PC and lyso-PC interconversion, may operate at a strategic intersection between the acyl-editing process and the TAG assembly for enriching the ALA content of TAG by direct interactions with
LuDGATs (Fig. 4B). Considering the high α-linolenoyl-CoA selectivity of LuDGAT2 (24), the LuDGAT2-catalyzed forward reaction may exhibit even more effective coupling with LuLP-CAT-catalyzed reverse reaction than the LuDGAT1-catalyzed reaction. Furthermore, the interaction between LuLP-CAT2 and LuPDAT1 suggests the presence of a specific complex for PC/lyso-PC recycling and editing in developing flax seeds (Fig. 4B), in which PDAT and the reverse reaction of LPCAT could efficiently channel the ALA moieties from PC into TAG directly or in the form of DAG or α-linolenoyl-CoA. In turn, the resulting lyso-PC could be recycled to PC by the forward reaction of LPCAT to catalyze incorporation of 18:1 at the sn-2 position. 18:1-enriched PC could then undergo further desaturation to produce PUFA-enriched PC.

Similar physical interactions between transferases involved in routing PC-modified fatty acids into TAG may also be operative in other oleaginous plants. Direct interactions among a few plant lipid metabolic enzymes and proteins have been observed and/or proposed in tung tree (Vernicia fordii), including the interactions between sn-glycerol-3-phosphate acyltransferase (GPAT) 8 and DGAT2 or GPAT9 (MYTH) (38), and in Arabidopsis, including the interactions between acyl-CoA-binding protein 2 and lysophospholipase 2 (yeast two-hybrid assay, co-immunoprecipitation, and thermodynamic analysis) (39, 40), among trigalactosyldiacylglycerol proteins (co-immunoprecipitation) (41), among fatty acid desaturases (MYTH and cross-linking) (42), between GPAT9 and lysophosphatidic acid acyltransferase (LPAAT) or LPCAT, and between DGAT1 and LPCAT or lysophosphatidic acid acyltransferase (MYTH) (32). Taken together, these studies indicate that plant lipid metabolic enzymes may also participate in a dynamic protein interactome for lipid channeling, similar to what has been proposed in mammals (23).

In the future, it would be interesting to extend this interaction analysis to other plant lipid biosynthetic enzymes (and their possible isoforms). It will also be important to determine the interacting regions of these enzymes. The usage of the overexpression constructs in both MYTH and BiFC systems might lead to excess accumulation of encoded proteins in vivo and possibly false-positive results, although various negative controls were included in the current study to avoid this. In this regard, future experiments may include a protein partner with a mutated interaction domain as a negative control or test the interaction using native promoters to drive the expression. In addition, it is worthwhile to quantify the strength of interaction because transient interaction may occur; this information would, in turn, expand our understanding in the possible dynamic transferase interactome. These studies would obviously benefit from the availability of highly purified enzymes, which can be a challenging aspect when dealing with mem-
brane-bound enzymes such as the transferases discussed in this study. Thus far, only recombinant *B. napus* DGAT1 has been highly purified from yeast over-expressing *BnaDGAT1* (43). In addition, studies with plant systems producing lipid biosynthetic enzymes with modified interaction interfaces would further shed light on the importance of these physical interactions in potential substrate-product channeling leading to PUFA enrichment of TAG. Furthermore, it will be interesting to determine whether a particular transferase can in fact interact with more than one other transferase (excluding itself). More specifically, if LuDGAT1 interacts with LuPDCT1 or LuLPCAT2, can it accommodate both LuPDCT1 and LuLPCAT2 at the same time? Studies of this nature will assist in revealing the complexity and overall functionality of this transferase interactome.

**Experimental procedures**

The MYTH system was kindly provided by Dr. Igor Stagljar (University of Toronto). The MYTH assay was performed as described by Snider et al. (27). In brief, cDNAs encoding LuDGAT1, LuDGAT2, LuPDAT1, LuLPCAT2, or LuPDCT1 were amplified by PCR and cloned into the pBT3N or pAMBV bait vector or pPR3N prey vector. After confirming the integrity of each sequence, the resulting pAMBV:bait or pBT3N:bait and pPR3N:prey or control prey (Ost-NubI “positive” control prey or Ost-NubG “negative” control prey) were co-transformed into yeast strain NMY51 (*MATa, his3/H9004200, trp1–901, leu2–3,112, ade2, LYS2::(lexAop)4-HIS3,ura3::(lexAop)8-lacZ,ade2::(lexAop)8-ADE2, GAL4*). Yeast cells expressing each bait/prey combination were selected on SD agar plates lacking Leu and Trp (SD-L-T) to ensure the presence of both bait and prey vector, and the interaction was assayed on SD-A-H-L-T plates by 1:10 dilution of X-Gal.
serial dilution of cell cultures starting from an $A_{600}$ value of 0.4. Activation of the $LacZ$ gene in the yeast strain was visualized by spotting yeast cells (with an $A_{600}$ value of 0.4) onto SD-A-H-L-T plates containing 80 mg/liter of X-Gal. Various baits constructed by fusing the Lex A–C-terminal fragment of ubiquitin ($C_{ub}$) or $C_{ub}$-Lex A to the N (pB7T3N) or C terminus (pAMBV) of each enzyme were subsequently validated via the NubGI control test. With exception for LuLPAT2, only fusing the Lex A–$C_{ub}$ to the N terminus of the enzymes showed positive reporter gene activation when paired with the NubI positive control prey, whereas fusion proteins containing the $C_{ub}$-Lex A linked at the C terminus of the enzymes failed to activate the reporter gene when paired with NubI. In terms of LuLPAT2, both C and N termini fusion proteins activated the reporter gene.

For the BiFC assay, flax cDNAs encoding LuDGAT1, LuDGAT2, LuLPAT2, LuPDCT1, and LuPDAT1 were PCR amplified and subcloned into binary vectors pDEST-VYNE(R)$^{GW}$ and pDEST-SCYCE(R)$^{GW}$ (kindly provided by Dr. Jörg Kudla, University of Münster) (28), respectively. After verifying sequence integrity, individual constructs were transformed to Agrobacterium tumefaciens GV3101::mp90 cells using electroporation. The transformed A. tumefaciens cells containing different BiFC constructs and the p19 vector encoding a viral suppressor protein were mixed in a transformation medium containing 50 mM MES, 2 mM Na$_3$PO$_4$, 0.5% (w/v) glucose, and 0.1 mM acetosyringone and diluted to yield a final $A_{600}$ of each culture equal to 0.25. The leaves of 4–5-week-old N. benthamiana, which were grown in a growth chamber at 25 °C, 50% humidity, and 16/8 h day/night cycle, were used for infiltration. The fluorescence of the lower epidermis of leaves after 2 days of infiltration was visualized using a fluorescent microscope (Axio Imager M1m microscope; Carl Zeiss Inc., Germany). BiFC and chlorophyll autofluorescence were excited at 470/40 and 575–625 nm, respectively, and emissions were recorded at 525/50 and 660–710 nm, respectively.

The sequences used in this study can be accessed in the PhytotechnologyTM databases under the following accession numbers: LuDGAT1, KC485337; LuDGAT2, KC437084; LuPDAT1, KC437085; LuLPAT2, Lus10006325; LuPDCT1, KC669705.

Acknowledgments—We are grateful to Dr. Igor Staggljar (University of Toronto) for providing the membrane yeast two-hybrid system and Dr. Jörg Kudla (University of Münster) for providing the BiFC system. We also thank Dr. Stacy Singer (Agriculture and Agri-Food Canada) for sharing her experience on tobacco leaf infiltration, Dr. Michael Gänzle, Dr. Yuan Fang, and Kosala Waduthanthri (University of Alberta) for their assistance in fluorescence microscopy, and Dr. Shanjida Khan (University of Alberta) for providing the N. benthamiana seeds.

References

1. Das, U. N. (2006) Essential fatty acids: a review. *Curr. Pharm. Biotechnol.* 7, 467–482 CrossRef Medline
2. Lorente-Cebrián, S., Costa, A. G., Navas-Carretero, S., Zabala, M., Martínez, J. A., and Moreno-Aliaxi, M. J. (2013) Role of ω-3 fatty acids in obesity, metabolic syndrome, and cardiovascular diseases: a review of the evidence. *J. Physiol. Biochem.* 69, 633–651 CrossRef Medline
3. Ihala, A. J., and Hall, L. M. (2010) Flax (*Linum usitatissimum* L.): current uses and future applications. *Aust. J. Basic Appl. Sci.* 4, 4304–4312
4. Hall, L. M., Booker, H., Siloto, R. M. P., Ihala, A. J., and Weselake, R. J. (2016) Flax (*Linum usitatissimum* L.), in Industrial Oil Crops (McKeon, T., Hildebrand, D., Hayes, D., and Weselake, R., eds) Elsevier/AOCS Press, Urbana, IL.
5. Radovanovic, N., Thambugala, D., Duguig, S., Loewen, E., and Cloutier, S. (2014) Functional characterization of flax fatty acid desaturase FAD2 and FAD3 isoforms expressed in yeast reveals a broad diversity in activity. *Mol. Biotechnol.* 56, 609–620 CrossRef Medline
6. Vrinten, P., Hu, Z., Munchinsky, M. A., Rowland, G., and Qiu, X. (2005) Two FAD3 desaturase genes control the level of linoleic acid in flax seed. *Plant Physiol.* 139, 79–87 CrossRef Medline
7. van de Loo, F. J., Broun, P., Turner, S., and Somerville, C. (1995) An oleate 12-hydroxylase from *Ricinus communis* L is a fatty acyl desaturation homolog. *Proc. Natl. Acad. Sci.* 92, 6743–6747 CrossRef Medline
8. Xu, Y., Caldo, K. M. P., Pal-Nath, D., Ozga, J., Lemieux, M. J., Weselake, R. J., and Chen, G. (2018) Properties and biotechnological applications of acyl-CoA diacylglycerol acyltransferase and phospholipidacylglycerol acyltransferase from terrestrial plants and microalgae. *Lipids* 53, 663–688 CrossRef Medline
9. Cahoon, E. B., Carlson, T. J., Ripp, K. G., Schweiger, B. J., Cook, G. A., Hall, S. E., and Kinney, A. J. (1999) Biosynthetic origin of conjugated double bonds: production of fatty acid components of high-value drying oils in transgenic soybean embryos. *Proc. Natl. Acad. Sci. U.S.A.* 96, 12935–12940 CrossRef Medline
10. Lee, M., Lenman, M., Bana, A., Bafor, M., Singh, S., Schweizer, M., Nilsson, R., Liljenberg, C., Dahlqvist, A., Gummesson, P. O., Sjödahl, S., Green, A., and Stymne, S. (1998) Identification of non-heme diiron proteins that catalyze triple bond and epoxy group formation. *Science* 280, 915–918 CrossRef Medline
11. Bates, P. D., Fatihii, A., Snapp, A. R., Carlsson, A. S., Browse, J., and Lu, C. (2012) Acyl editing and headgroup exchange are the major mechanisms that direct polysaturated fatty acid flux into triacylglycerols. *Plant Physiol.* 160, 1530–1539 CrossRef Medline
12. Lager, I., Glab, B., Eriksson, L., Chen, G., Bana, A., and Stymne, S. (2015) Novel reactions in acyl editing of phosphatidylcholine by lysophosphatidylcholine transacylase (LPCT) and acyl-CoA:lyso phosphatidylcholine acyltransferase (GPCAT) activities in microsomal preparations of plant tissues. *Planta* 241, 347–358 CrossRef Medline
13. Lu, C., Xin, Z., Ren, Z., Miquel, M., and Browse, J. (2009) An enzyme regulating triacylglycerol composition is encoded by the *ROD1* gene of *Arabidopsis*. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18837–18842 CrossRef Medline
14. Wickramarathna, A. D., Siloto, R. M., Mietkiewska, E., Singer, S. D., Pan, X., and Weselake, R. J. (2014) Functional characterization of flax fatty acid desaturase FAD2 and FAD3 isoforms expressed in yeast reveals a broad diversity in activity. *Mol. Biotechnol.* 56, 609–620 CrossRef Medline
15. Meli, C. E., Cussac, M., Haslam, R. P., Beaudoin, F., Wong, Y. S., Maréchal, E., and Rébéllé, F. (2017) CI metabolism inhibition and nitrogen deprivation trigger triacylglycerol accumulation in *Arabidopsis thaliana* cell cultures and highlight a role of NPC in phosphatidylcholine-to-triacylglycerol pathway. *Front. Plant Sci.* 7, 2014 Medline
16. Yang, W., Wang, G., Li, J., Bates, P. D., Wang, X., and Allen, D. K. (2017) Phospholipase D replenishes diacylglycerol flux into triacylglycerol. *Plant Physiol.* 174, 110–123 CrossRef Medline
17. Dahlqvist, A., Ståhl, U., Lenman, M., Bana, A., Lee, M., Sandager, L., Ronne, H., and Stymne, S. (2000) Phospholipid:diacylglycerol acyltransferase: an enzyme that catalyzes the acyl-CoA-independent formation of
triaclylglycerol in yeast and plants. Proc. Natl. Acad. Sci. U.S.A. 97, 6487–6492 CrossRef Medline

28. Gehl, C., Waadt, R., Kudla, J., Mendel, R. R., and Hänsch, R. (2009) New dynamic insights into an interaction between ACYL-COA-BINDING PROTEIN2 and LYSOPHOSPHOLIPASE2 in Arabidopsis. J. Biol. Chem. 285, 6214–6226 Medline

29. Bobba, S., Farese, R. V., Jr., and Mak, H. Y. (2012) The FATP1-DGAT2 complex facilitates lipid droplet expansion at the ER-lipid droplet interface. J. Cell Biol. 198, 895–911 CrossRef Medline

30. Crooks, E., P. J., Li, D., Kudla, J., Mendel, R. R., and Hänsch, R. (2010) Topological orientation of acyl-CoA:diacylglycerol acyltransferase-1 (DGAT1) and identification of a putative active site histidine and the role of the N-terminal in dimer/tetramer formation. J. Biol. Chem. 285, 37377–37387 CrossRef Medline

31. Cheng, D., Meequa, R. L., He, B., Cromley, D. A., Bilheimer, J. T., and Young, P. R. (2001) Human acyl-CoA:diacylglycerol acyltransferase is a tetrameric protein. Biochem. J. 359, 707–714 CrossRef Medline

32. Caldo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

33. Cabukosta, B., Kol, M., Kneller, L., Hilderink, A., Bickert, A., Mina, I. G. M., Korneev, S., and Holthuis, J. C. (2017) ER residency of the ceramide phosphoethanolamine synthase SMSr relies on homotypic oligomerization mediated by its SAM domain. Sci. Rep. 7, 41290 CrossRef Medline

34. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

35. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

36. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

37. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

38. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

39. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

40. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

41. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

42. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

43. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

44. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

45. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

46. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

47. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

48. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

49. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

50. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

51. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

52. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

53. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

54. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline

55. Calo, K. M. P., Acedo, J. Z., Panigrahi, R., Vederas, J. C., Weselake, R. J., and Lemieux, M. J. (2017) Diacylglycerol acyltransferase 1 is regulated by its N-terminal domain in response to allosteric effectors. Plant Physiol. 175, 667–680 Medline