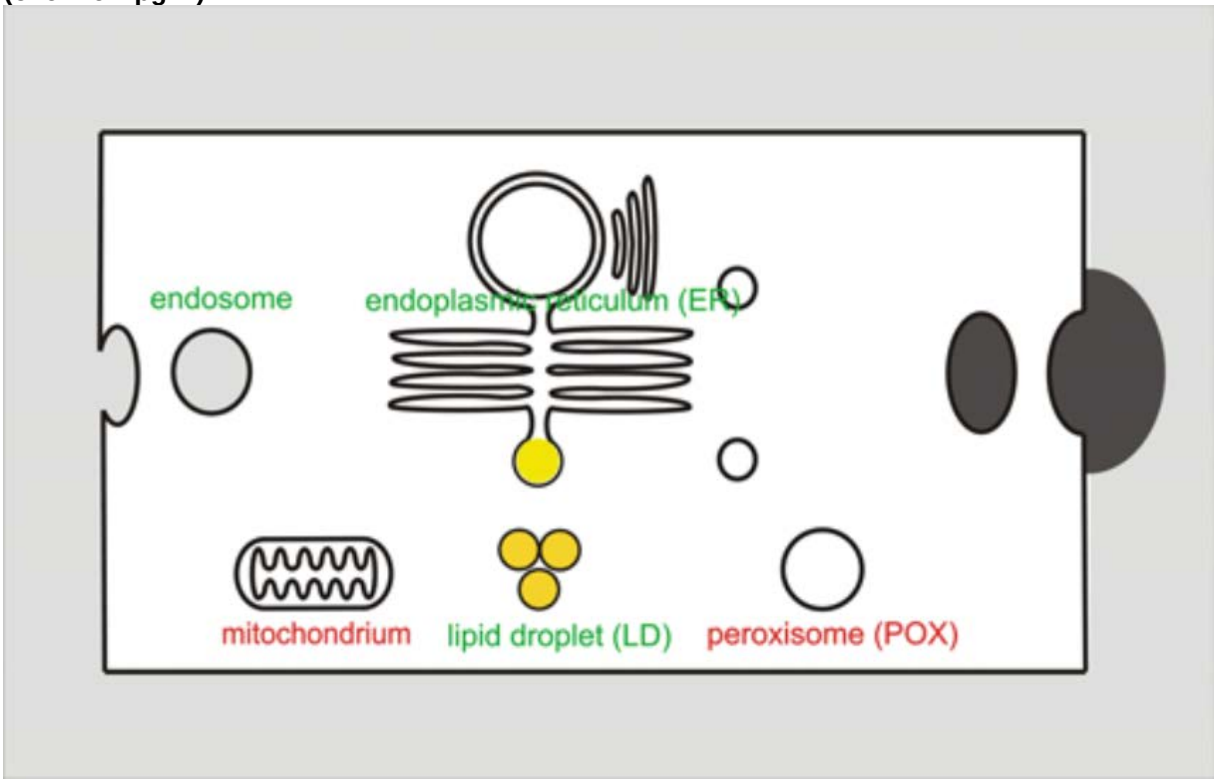
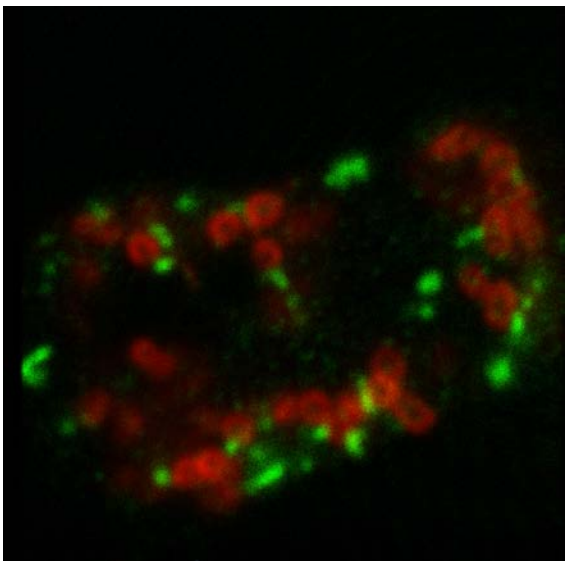
Proteins involved in lipid metabolism
(overview pg. 1)



Top: Representation of a *Dictyostelium* cell (white box) in liquid medium (gray). Organelles that contribute to lipid synthesis (green) or lipid degradation (red) are indicated.

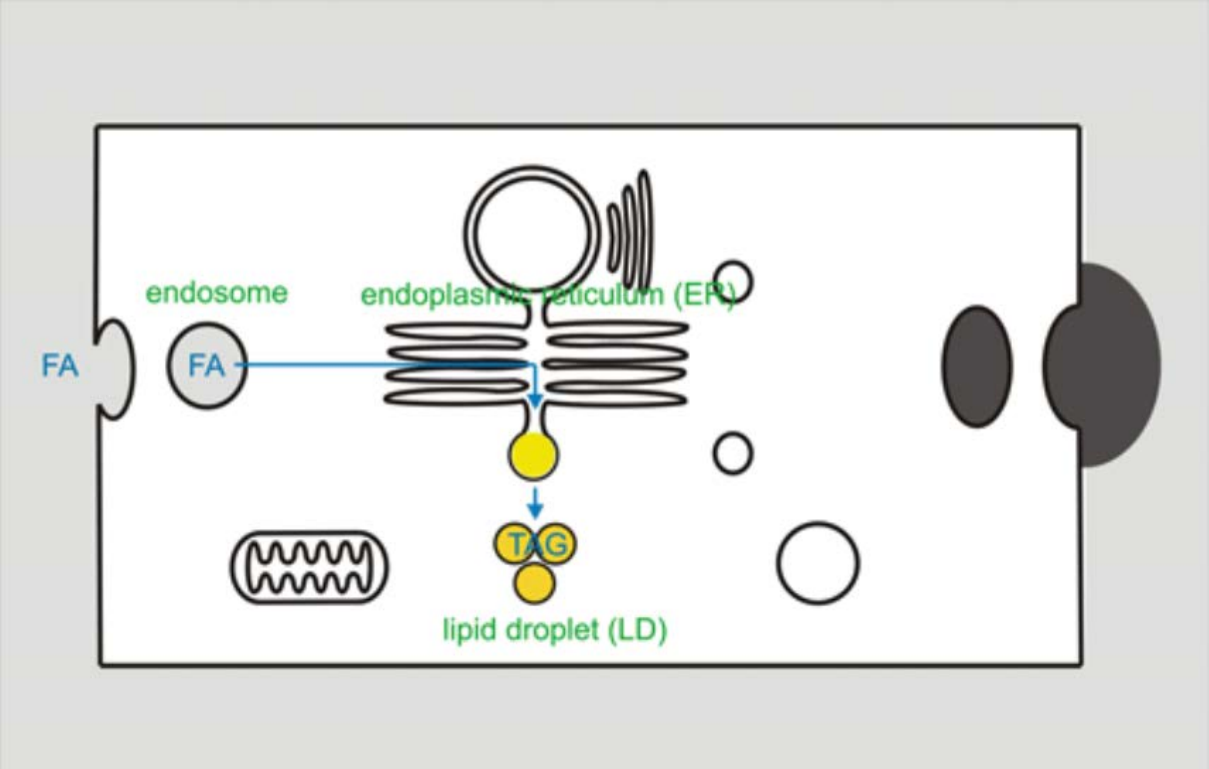
Right: Single optical section through a cell showing the outer membrane of mitochondria in red and the variable shapes of peroxisomes in green.



References: We have not published a review on Dictyostelium fat metabolism yet, but we definitively should do so. Until then, please look at these pages summarizing key findings of our research.

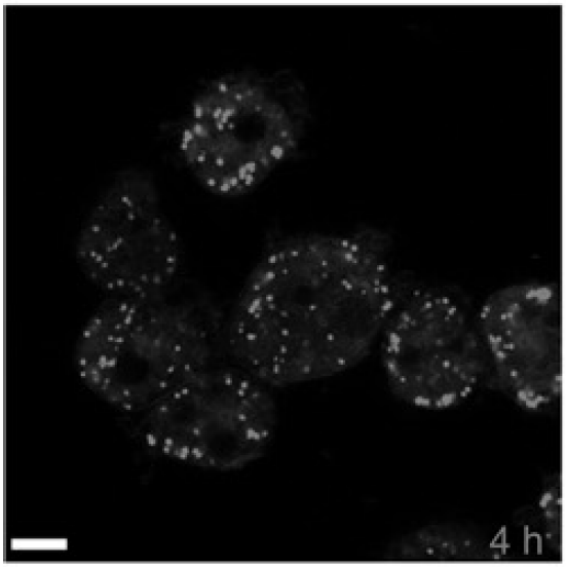
Functional details on various other proteins are obtained following the links below (jump to):
(overview pg. 1) (overview pg. 2) (overview pg. 3) (FcsA) (GPAT) (Lipin) (DGAT1) (DGAT2) (FARAT) (Seipin) (Plin pg. 1) (Plin pg. 2) (Smt1, Ldp, Net4) (FcsB pg. 1) (FcsB pg. 2)

Proteins involved in lipid metabolism
(overview pg. 2)



Top: Fatty acids (FA) added to the medium are internalized and converted to neutral lipids in the endoplasmic reticulum. These hydrophobic molecules coalesce between the leaflets of one ER membrane and bulge out. The final storage form is a lens of neutral lipids (yellow) surrounded by a phospholipid monolayer (thin black line), a so-called lipid droplet. Other than triacylglycerol (TAG) it contains monoalkyldiacylglycerol (MDG) and steryl esters (SE). Their different roles are investigated.

Right: Accumulation of lipid droplets over several hours after the addition of fatty acid.



Function: Lipid droplets are an economic storage form of energy used in virtually all organisms.

Reference: Du, X., Barisch, C., Paschke, P., Herrfurth, C., Bertinetti, O., Pawolleck, N., Otto, H., Rühling, H., Feussner, I., Herberg, F.W., Maniak, M.: Dictyostelium lipid droplets host novel proteins. Eukaryotic Cell 12, 1517-1529, 2013

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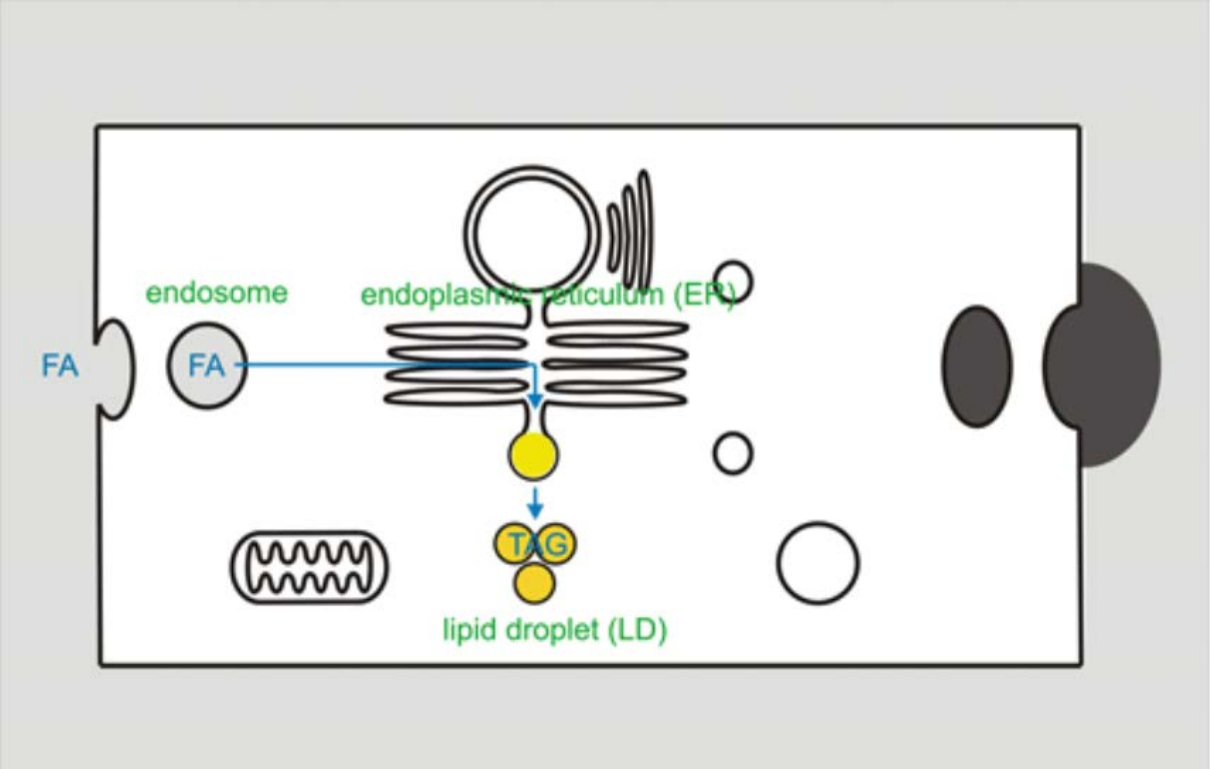
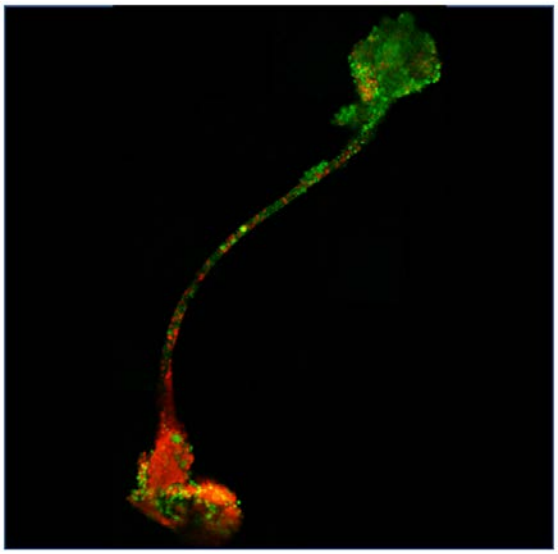
Proteins involved in lipid metabolism
(overview pg. 3)



Top: We call cells that have converted fatty acids (FA) into TAG-containing lipid droplets during their growth phase “fat cells”, whereas cells that did not receive fatty acids in their medium are designated “lean”. In Dictyostelium development, that is initiated by starvation, fat cells have a strong selective disadvantage.

Right: GFP-expressing lean cells (green) predominate among the viable spores in the head of the fruiting body, whereas fat cells (red) form the dead stalk and basal plate.



Function: Mutant strains that do not accumulate lipid droplets even in fatty acid containing medium, because they lack FcsA, GPAT or DGAT enzymes are saved.

Reference: Kornke, J. M. & Maniak, M.: Fat-containing cells are eliminated during *Dictyostelium* development. Biology Open 6: 1294-1304, 2017.

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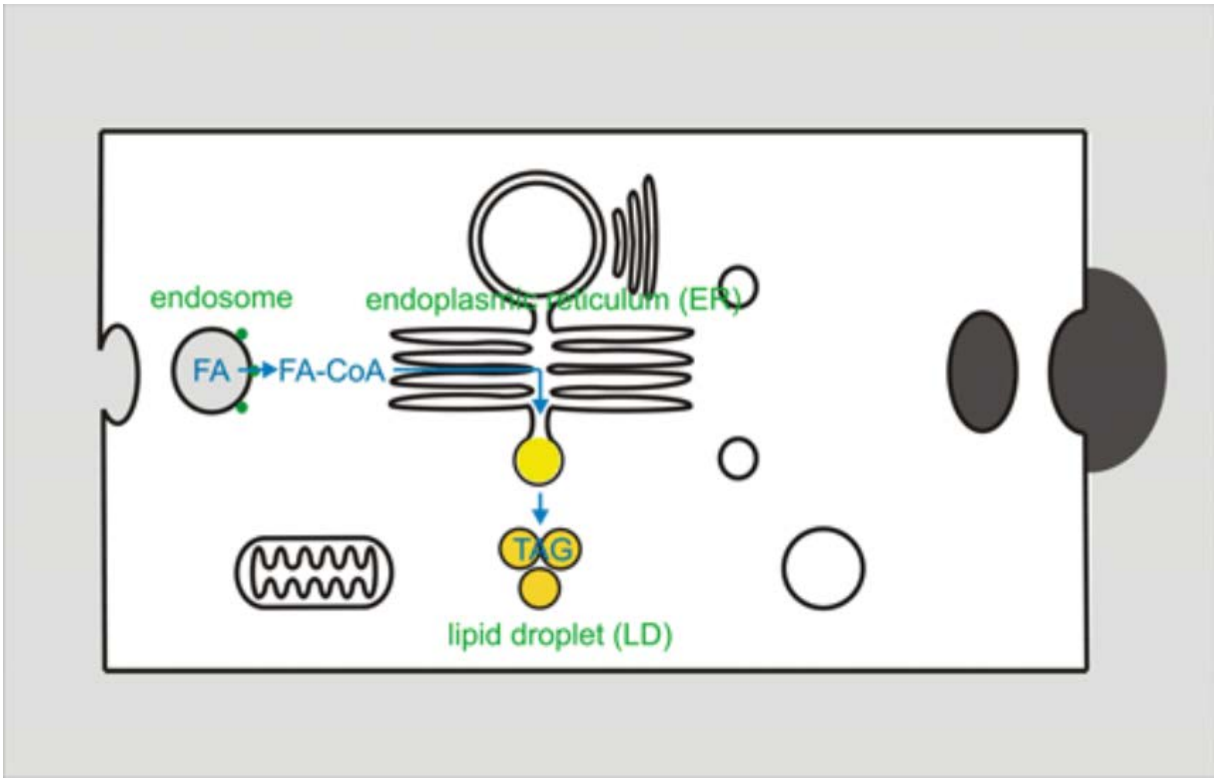
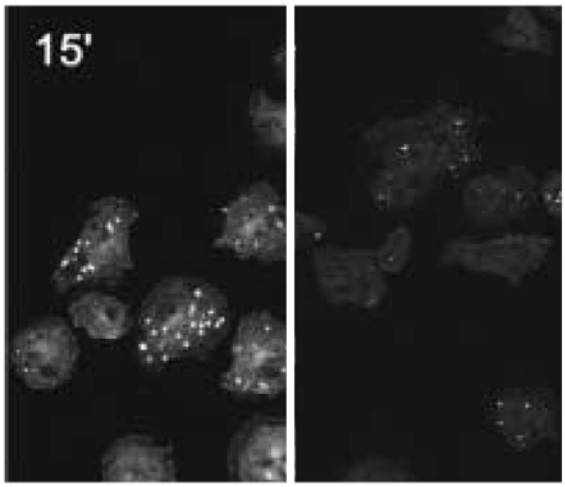
Proteins involved in lipid metabolism
(FcsA)



Top: Fatty acids (FA) taken up into endosomes are activated by linkage to coenzyme a (CoA). This occurs through the action of the FcsA protein (green dots) bound to the endosomal membrane. Only FA-CoA molecules can be converted into TAG, filling lipid droplets.

Right: Images of wildtype Dictyostelium cells (left) and a fcsA mutant (right) in the presence of fluorescently labelled fatty acids.



Function: The fcsA-mutant is strongly impaired in the formation of lipid droplets.

Reference: von Löhneysen, K., Pawolleck, N., Rühling, H., and Maniak, M.: A Dictyostelium Long Chain Fatty Acyl Coenzyme A Synthetase mediates fatty acid retrieval from endosomes. Eur.J. Cell Biol., 82, 505 - 514, 2003

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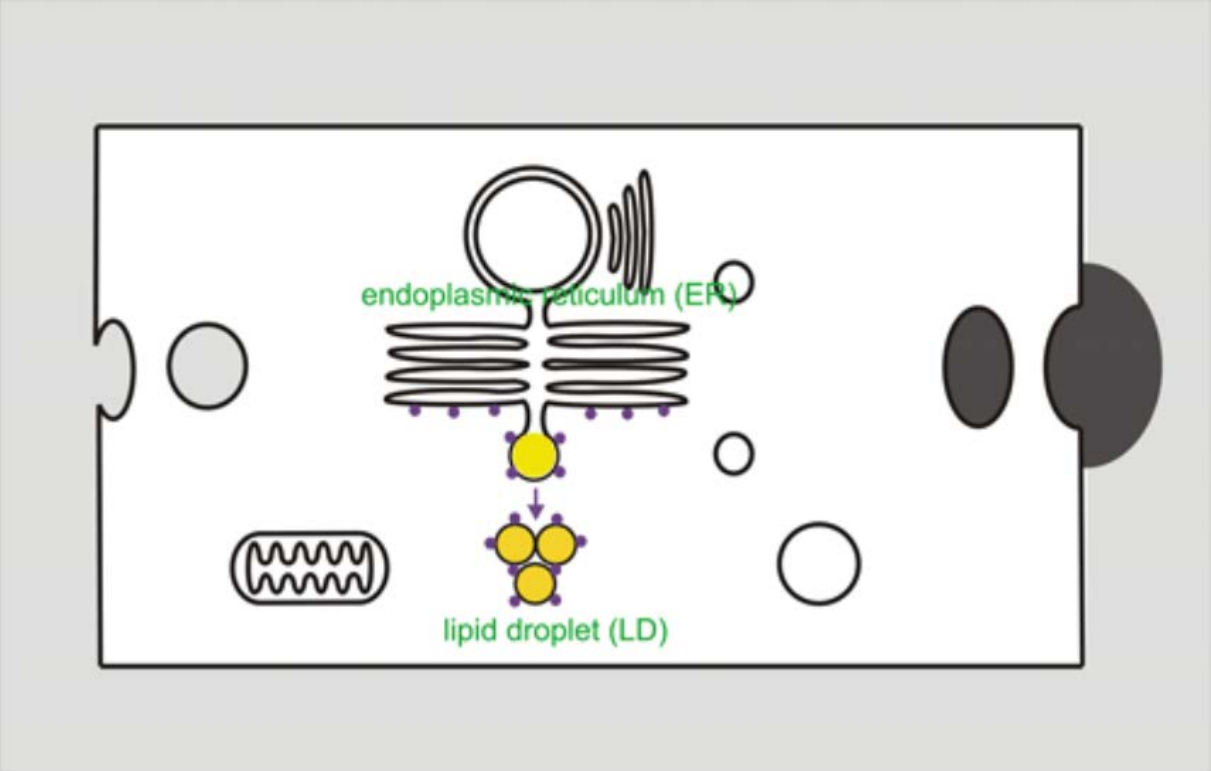
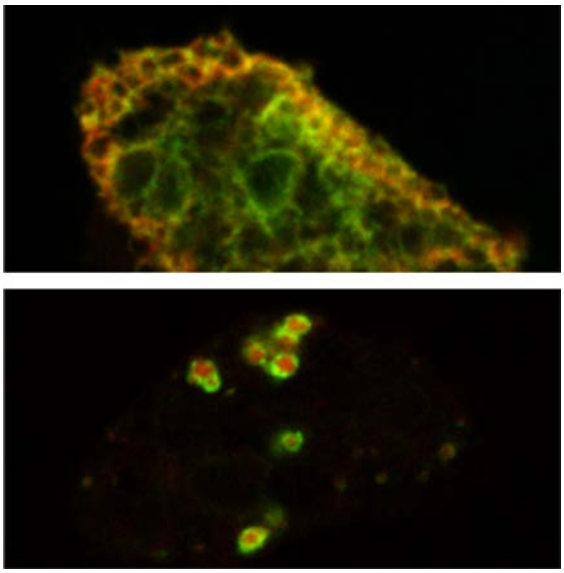
Proteins involved in lipid metabolism
(GPAT)



Top: The first fatty acid is added to glycerol-3-phosphate by the action of GPAT, a glycerol-3P-acyl-transferase. In the cell it distributes like the purple dots. Our knowledge on this enzyme is summarized below. Thereafter, a second fatty acid is added by one or likely more enzymes that we could not yet identify. From this product, phosphatidic acid, the phosphate needs to be removed by an enzyme called lipin.

Right: GFP-tagged GPAT (green) is present in a reticular pattern (ER) in the absence of fatty acid (top). If fatty acids are added to the medium GPAT associates with small round organelles, the lipid droplets (bottom).



Function: Deletion of GPAT form the genome strongly reduces the production of TAG, but not MDG (for explanation see overview pg2).

Reference: Kappelt, F., Du Ma, X., Abou Hasna, B. Kornke, J.M. & Maniak, M.: Phospholipids containing ether-bound hydrocarbon-chains are essential for efficient phagocytosis and neutral lipids of the ester-type perturb development in Dictyostelium. Biology Open 9:7, 2020

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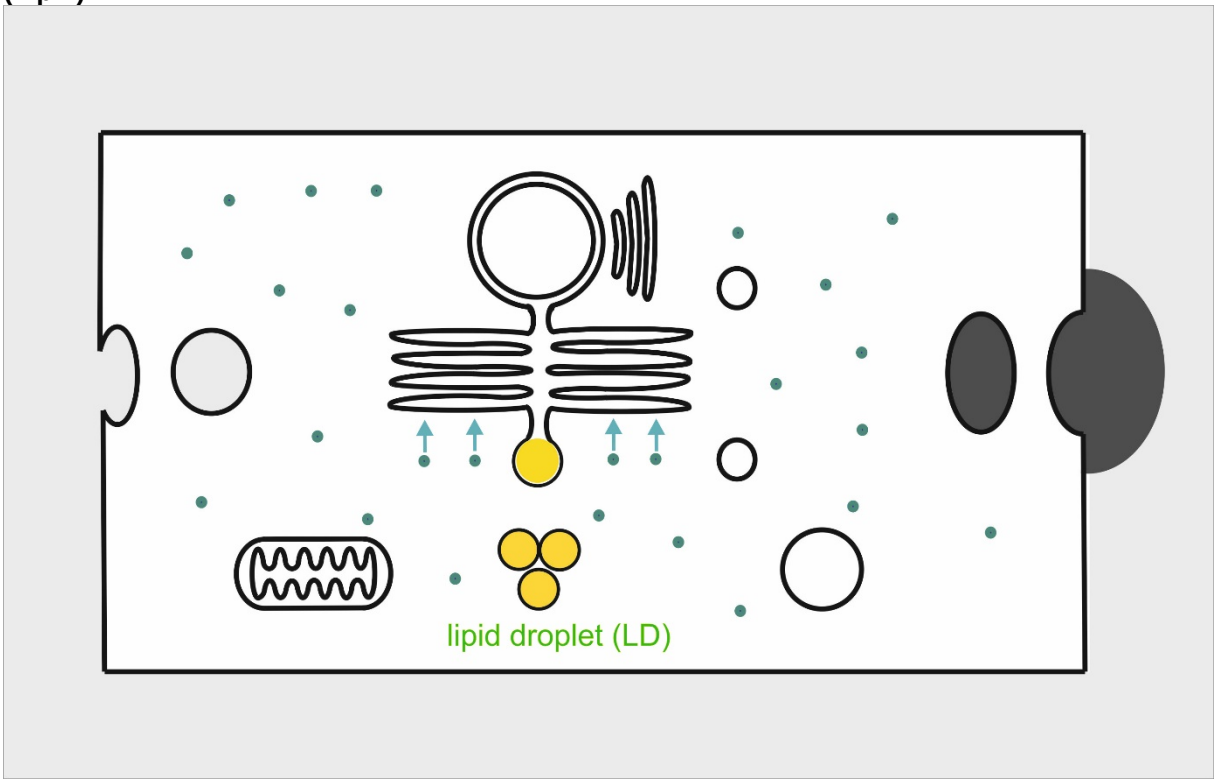
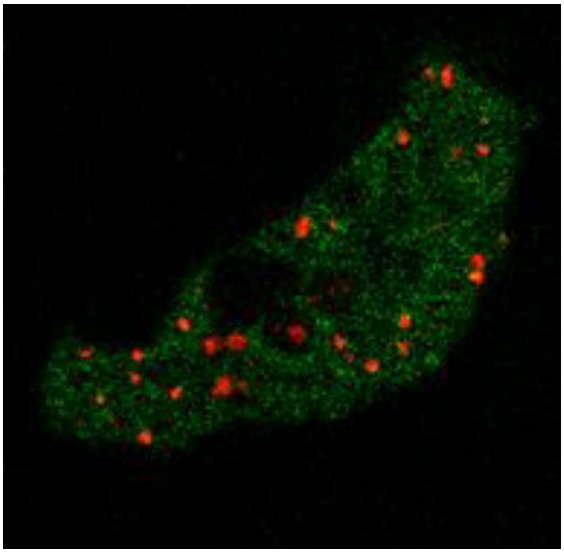
Proteins involved in lipid metabolism
(Lipin)



Top: The lipid phosphatase Lipin (light blue dots) is a cytoplasmic enzyme that, however, needs to undergo at least transient association with the ER surface (arrows) to find its substrate, phosphatidic acid, in order to convert it into diacylglycerol.

Right: GFP-Lipin (green) is homogeneously distributed throughout the cytoplasm. It does not visibly associate with the ER or lipid droplets (red).



Function: A lipin-mutant neither forms significant amounts of TAG nor any MDG and thus is devoid of lipid droplets in the vegetative cell cycle.

Reference: Kappelt, F. PhD-Thesis, 2021

Functional details on various other proteins are obtained following the links below (jump to):
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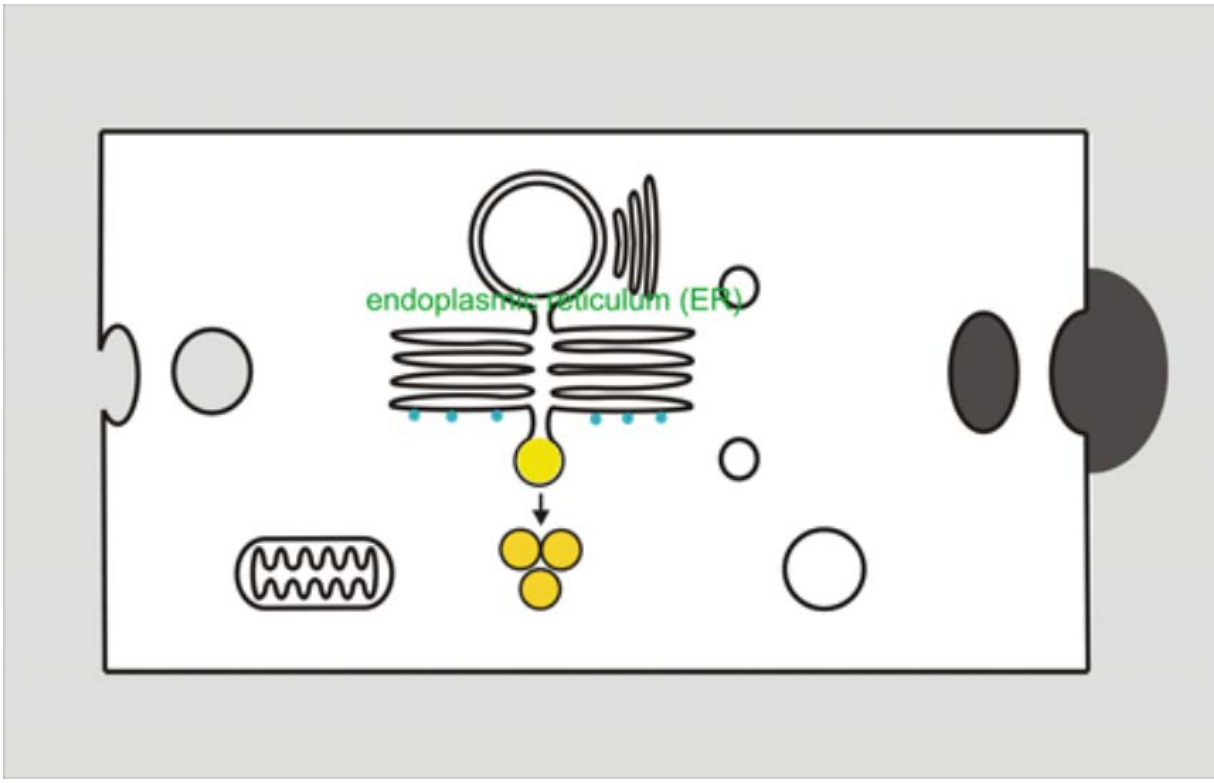
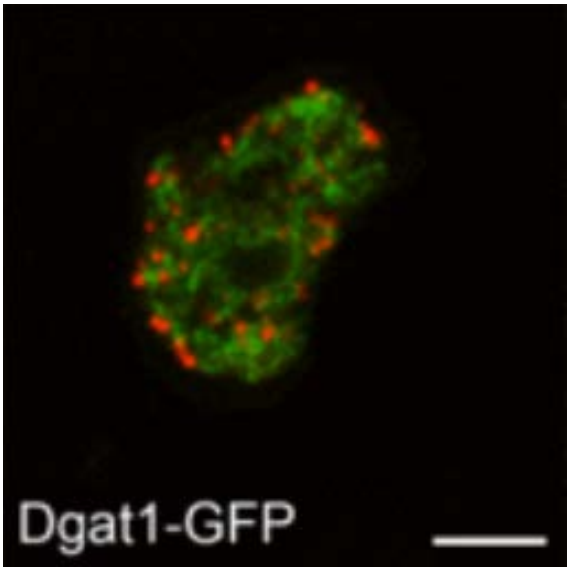
Proteins involved in lipid metabolism
(DGAT1)



Top: The addition of the third fatty acid to the amphiphilic diacylglycerol molecule is the step that produces storage fat (TAG) with only hydrophobic properties. This reaction is carried out by acyltransferases abbreviated DGAT. DGAT1 (blue dots) is an enzyme that permanently resides on the endoplasmic reticulum, whereas DGAT2 is exclusively found on lipid droplets.

Right: GFP-tagged DGAT1 (green) highlights the network of the ER and does not coincide with lipid droplets (red).



Function: Under normal conditions DGAT1 is the major enzyme producing TAG in Dictyostelium cells. It is also able to synthesize a substance called mono-alkyl-di-acyl-glycerol (MDG), that differs from TAG only in that one chain is connected by an ether-bond while the other two are ester-linked.

References: Du, X., et al.: The essential function of *Dictyostelium* Dgat1 in triglyceride production, but not in ether lipid synthesis, can be substituted by Dgat2. Eukaryotic Cell, 13, 517-526, 2014

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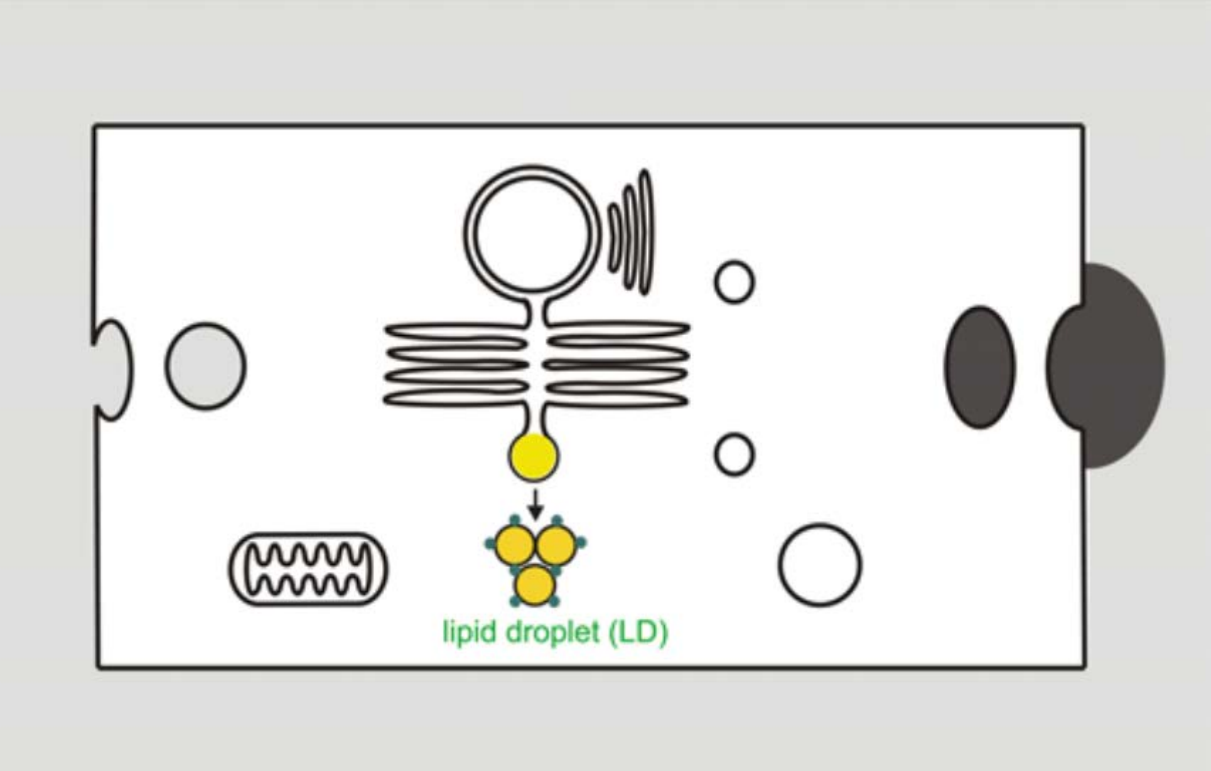
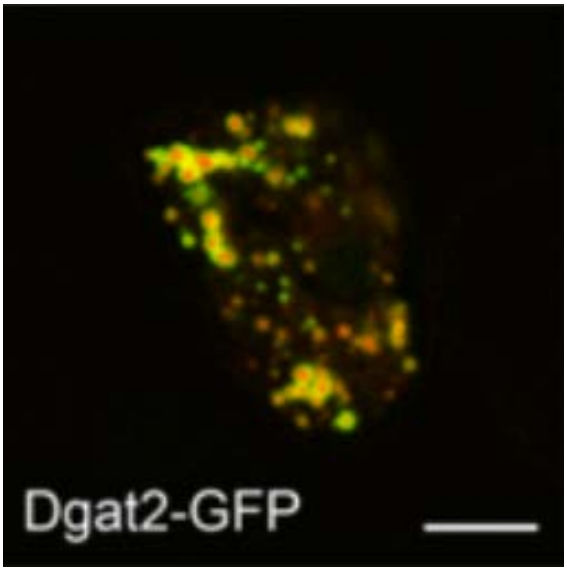
Proteins involved in lipid metabolism
(DGAT2)



Top: The addition of the third fatty acid to the amphiphilic diacylglycerol molecule is the step that produces storage fat (TAG) with only hydrophobic properties. This reaction is carried out by acyltransferases abbreviated DGAT. DGAT2 (blue dots) is an enzyme that only associates with lipid droplets whereas DGAT1 is only active on the endoplasmic reticulum.

Right: A cell showing GFP-DGAT2 (green) surrounding the TAG core of lipid droplets stained by a fluorescent dye called LD540 (red).



Function: In a mutant lacking DGAT1, overexpressed DGAT2 can fully compensate the defect in TAG synthesis. DGAT2, however, is unable to produce MDG.

References: Du, X., Herrfurth, C., Gottlieb, T. Kawelke, S., Feussner, K., Rühling, H., Feussner, I., Maniak, M.: The essential function of *Dictyostelium* Dgat1 in triglyceride production, but not in ether lipid synthesis, can be substituted by Dgat2. Eukaryotic Cell, 13, 517-526, 2014

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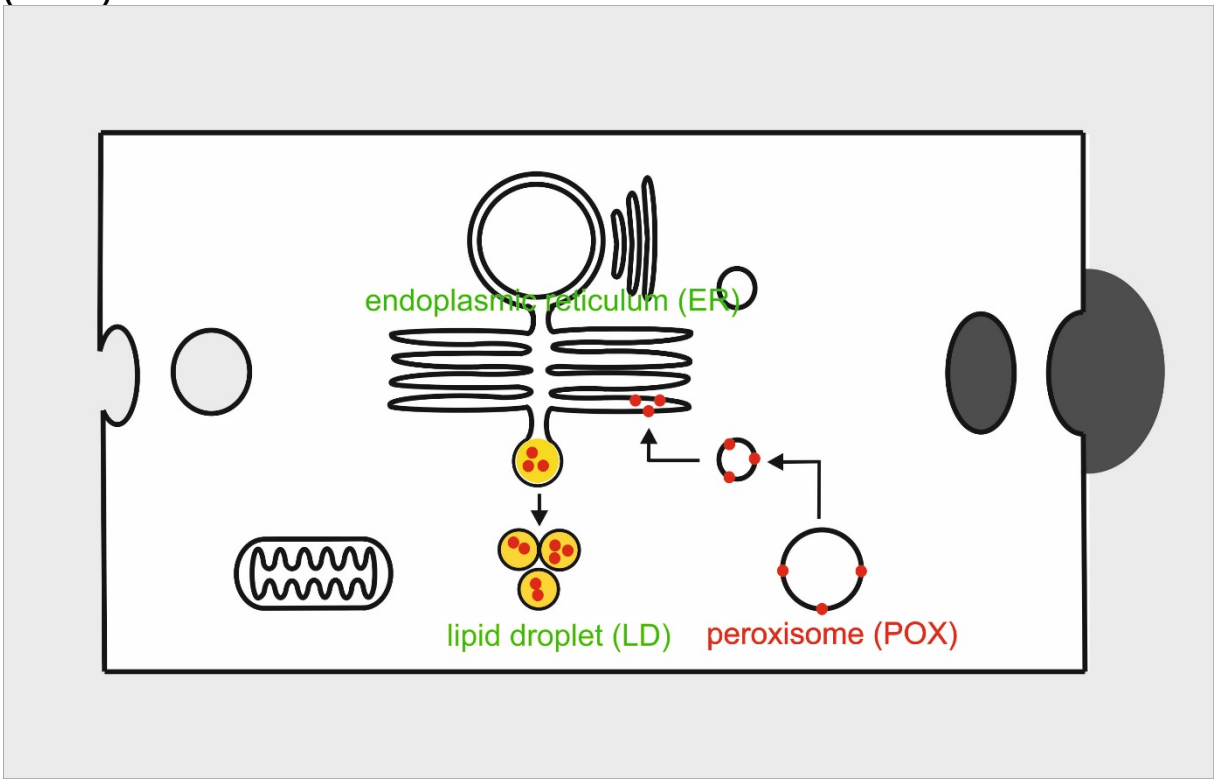
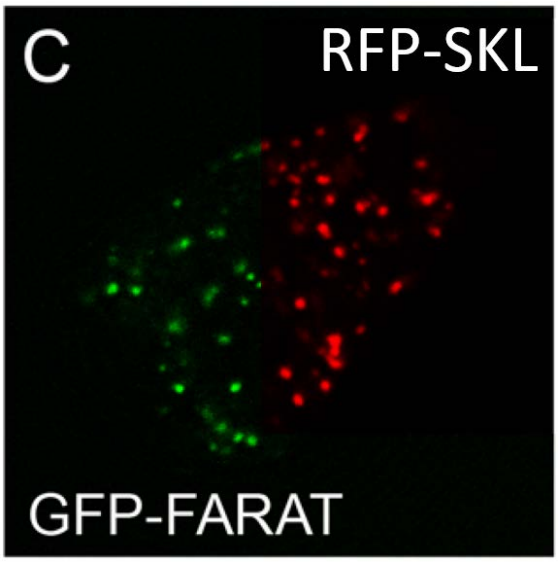
Proteins involved in lipid metabolism
(FARAT)



Top: FARAT is a peroxisomal protein generating a lipid precursor (red dots) which is tranferred to the endoplasmic reticulum for further anabolic reactions. One of these products (MDG, also red dots) is stored in lipid droplets (for explanation see overview pg2).

Right: Adding the GFP-tag to the N-terminus of FARAT (green) demonstrates its presence in peroxisomes together with the marker RFP-SKL (mounted halves of the same cell).



Function: FARAT initiates the synthesis of ether lipids that promote phagocytosis as phospholipid species or become the precursor of the neutral storage lipid MDG.

Reference: Kappelt, F., Du Ma, X., Abou Hasna, B. Kornke, J.M. & Maniak, M.: Phospholipids containing ether-bound hydrocarbon-chains are essential for efficient phagocytosis and neutral lipids of the ester-type perturb development in Dictyostelium. Biology Open 9:7, 2020

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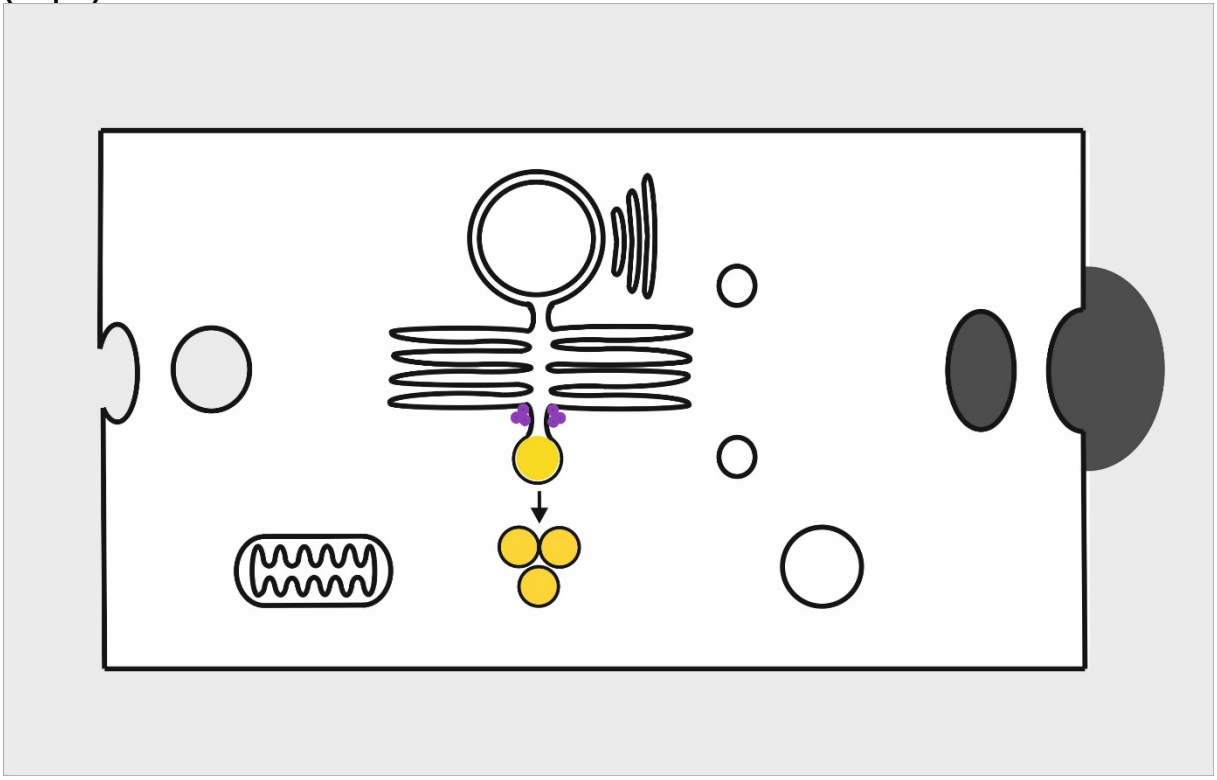
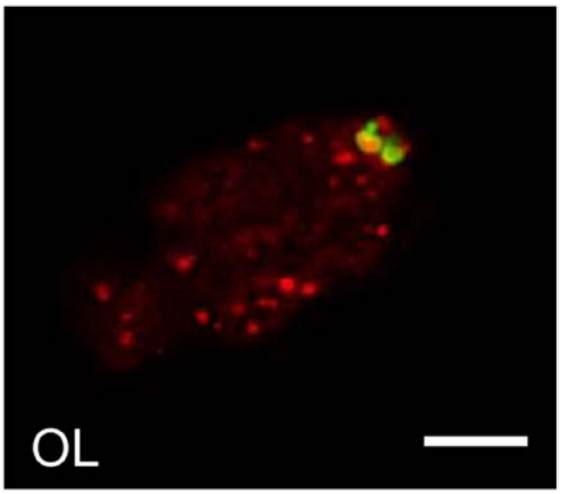
Proteins involved in lipid metabolism
(Seipin)



Top: Seipin (purple dots), a protein named after an MD who described a hereditary lipodystrophy, assembles in many copies at the ER membrane to regulate the access of specific proteins to the lipid droplet.

Right: GFP-tagged seipin (green) associated only with a subset of lipid droplets (red) i.e. the ones that are about to bud off from the ER.



Function: In a seipin knockout-mutant lipid droplets are about threefold bigger by volume, but their number is reduced to one third of wildtype.

Reference: Kornke, J. M. & Maniak, M.: Fat-containing cells are eliminated during *Dictyostelium* development. Biology Open 6: 1294-1304, 2017.

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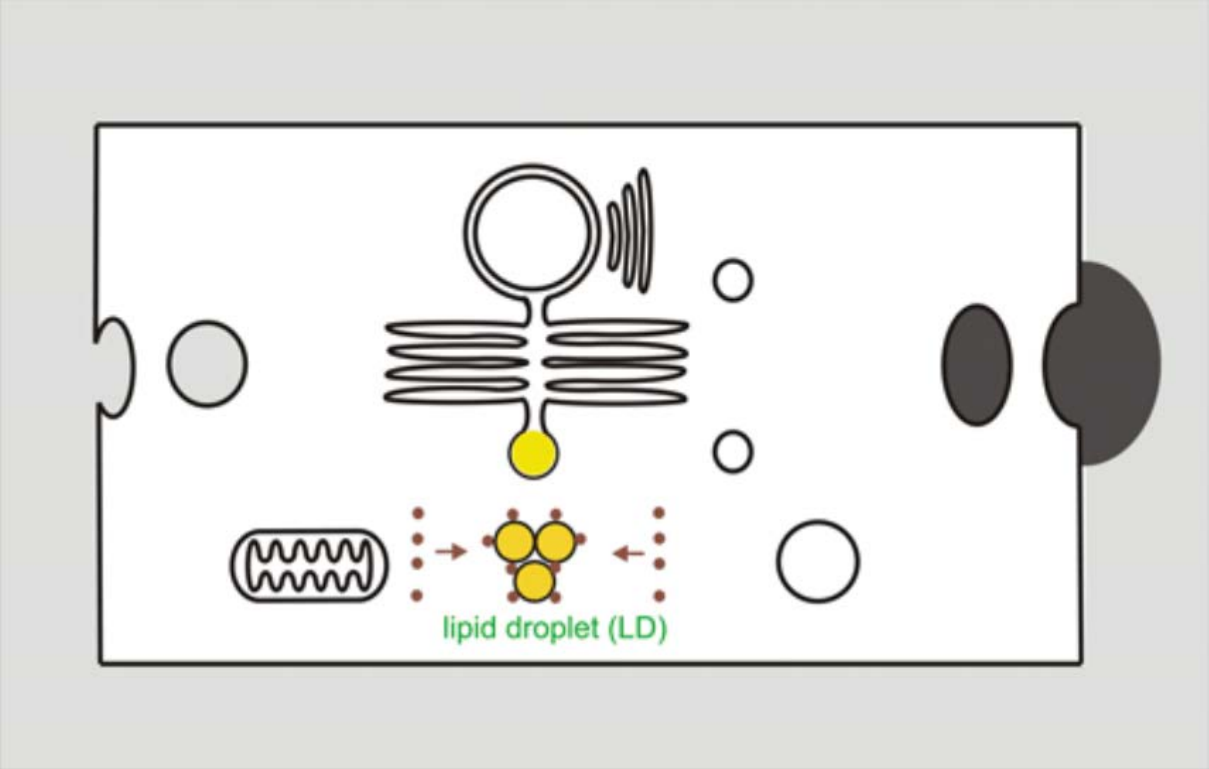
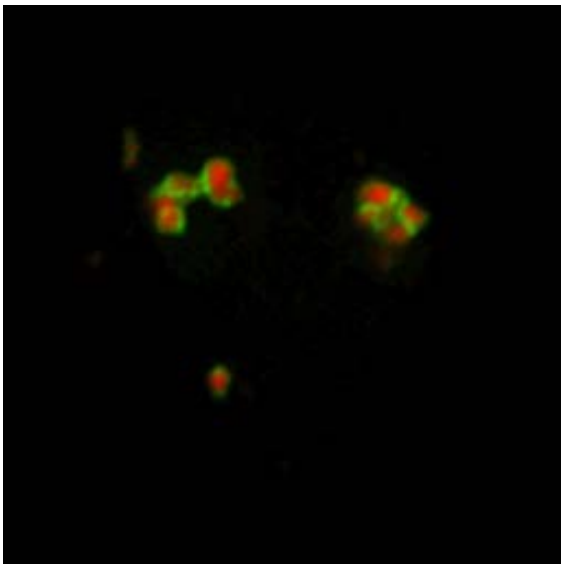
Proteins involved in lipid metabolism
(Plin pg. 1)



Top: The perilipin (Plin) protein is present in almost all organisms. It is synthesized in the cytoplasm (brown dots) and subsequently coats the surface of lipid droplets.

Right: A cell showing GFP-Plin (green) surrounding the TAG core of lipid droplets stained by a fluorescent dye called LD540 (red).



Function: We have used GFP-tagged perilipin to aid the biochemical purification of lipid droplets from Dictyostelium, allowing us to fully characterize their protein composition and lipid contents.

Reference: Du, X., Barisch, C., Paschke, P., Herrfurth, C., Bertinetti, O., Pawolleck, N., Otto, H., Rühling, H., Feussner, I., Herberg, F.W., Maniak, M.: Dictyostelium lipid droplets host novel proteins. Eukaryotic Cell 12, 1517-1529, 2013

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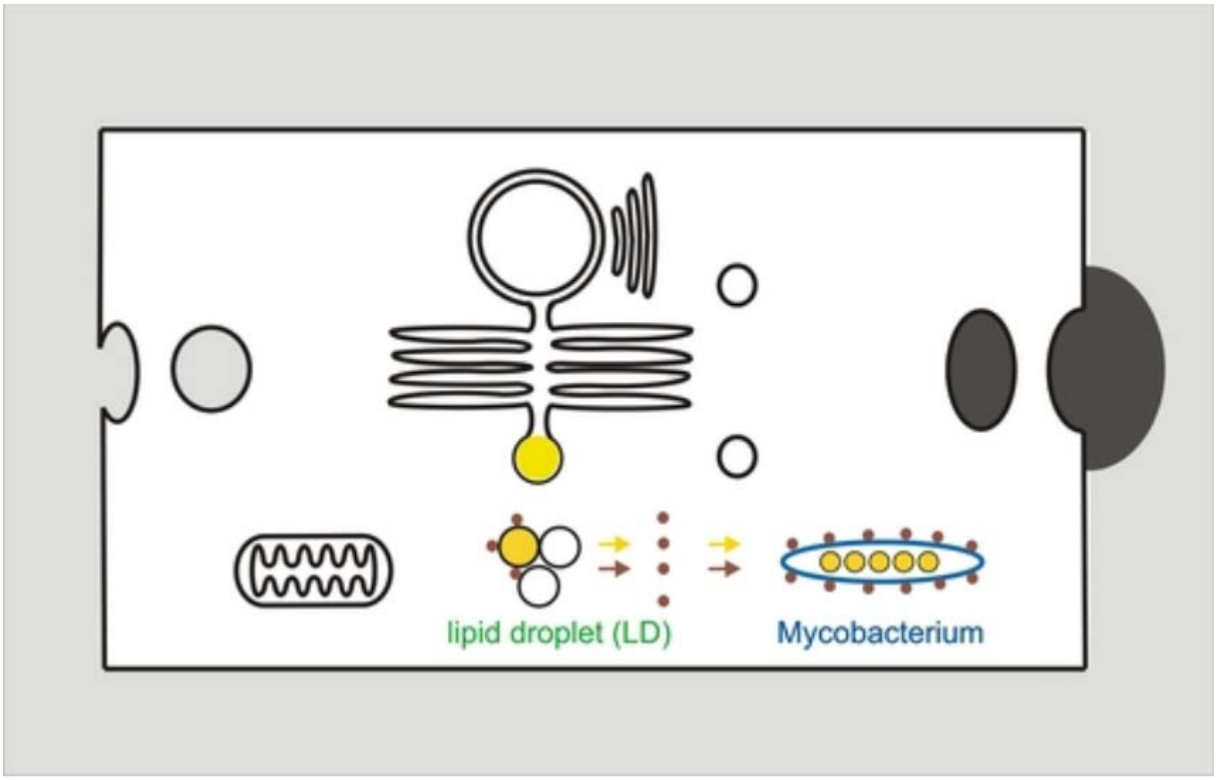
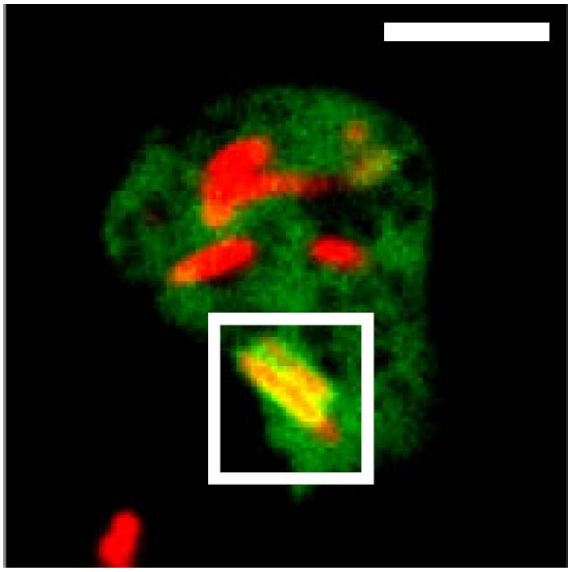
Proteins involved in lipid metabolism
(Plin pg. 2)



Top: In a *Dictyostelium* cell infected with the intracellular pathogen *Mycobacterium marinum*, fat (yellow) is transferred from cellular lipid droplets to the invader (blue). Surprisingly, the surface of *Mycobacteria* is then coated with the perilipin protein of the host (brown dots).

Right: An infected *Dictyostelium* cell showing GFP-Plin (green) surrounding *Mycobacteria* expressing a red fluorescent protein (red).



Function: In a perilipin knockout strain, the growth of *Mycobacteria* in the host cytoplasm is reduced, indicating that the perilipin coat is protective against host defence mechanisms.

Reference: Barisch, C., Paschke, P., Hagedorn, M., Maniak, M., Soldati, T.: Lipid droplet dynamics at early stage of Mycobacterium marinum infection in Dictyostelium. Cell. Microbiol. 17, 1332-1349 , 2015

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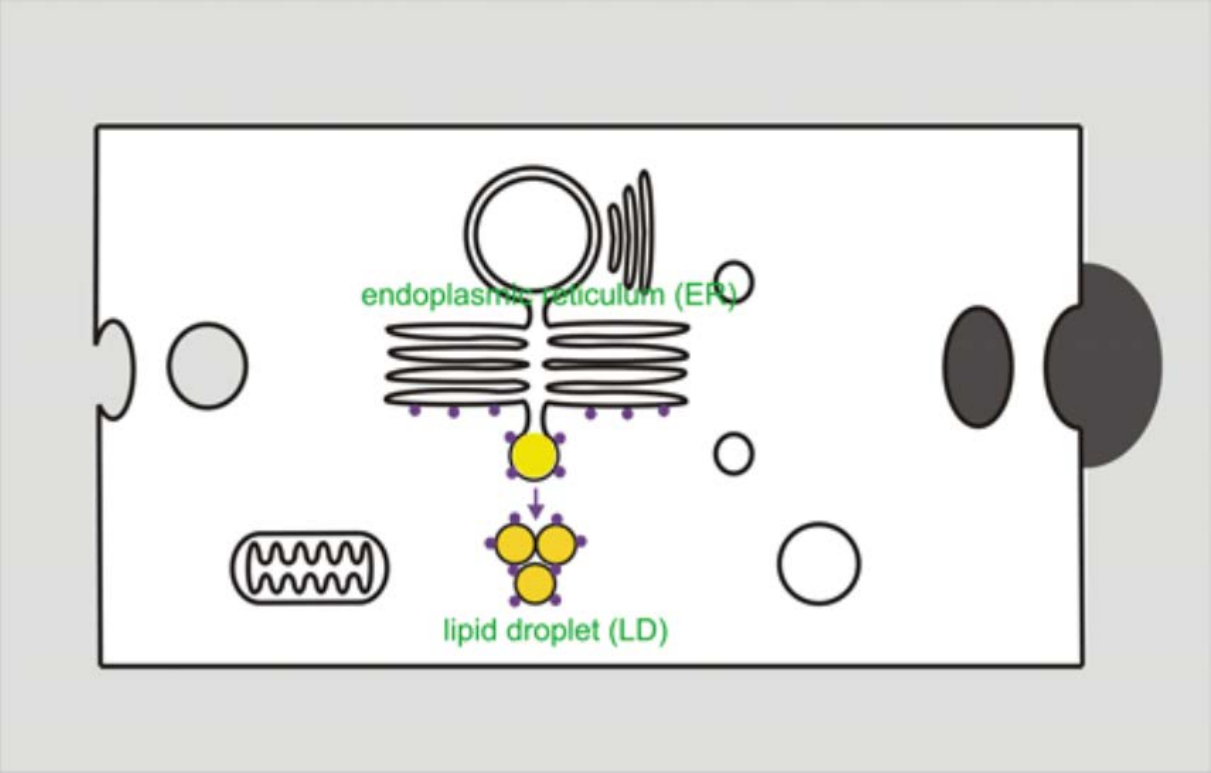
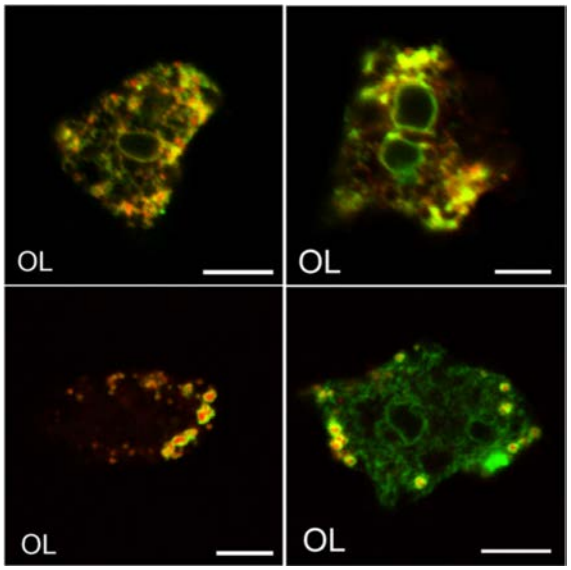
Proteins involved in lipid metabolism
(Smt1, Ldp, Net4)



Top: Surprisingly many proteins normally reside on the endoplasmic reticulum and are translocated to lipid droplets only after their formation. We have established this behaviour for Smt1, Ldp, and Net4, shown as purple dots. Thus, perilipin is rather an exception, being made in the cytoplasm.

Right: GFP-tagged proteins Ldp, and Net4 (green) are present in a reticular pattern (ER) in the absence of fatty acid (top row). If fatty acid is added to the medium the green proteins associate with small round organelles, the lipid droplets (bottom row).



Function: The human homologue of Net4 was previously known as a component of the nuclear envelope. We now could prove that it also surrounds lipid droplets in mammalian cells.

Reference: Du, X., et al.: Dictyostelium lipid droplets host novel proteins. Eukaryotic Cell 12, 1517-1529, 2013

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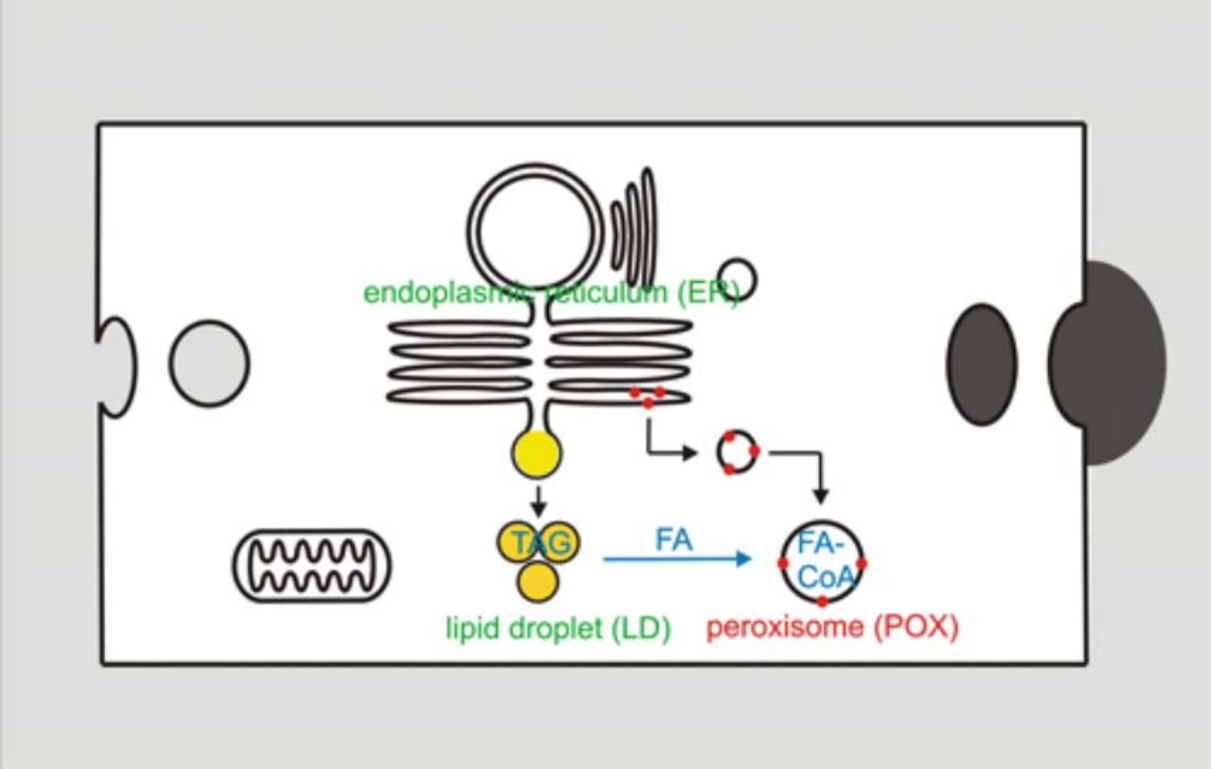
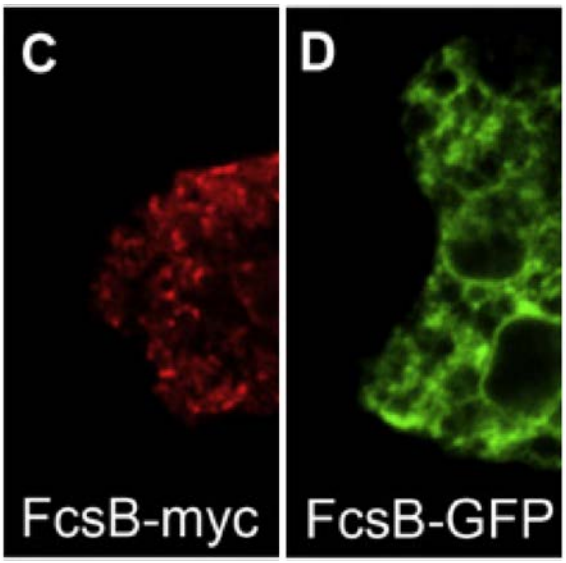
Proteins involved in lipid metabolism
(FcsB pg. 1)



Top: The FcsB Protein (red dots) is translated into the endoplasmic reticulum and then further transported to peroxisomes, which is a very rare and unusual pathway.

Right: Insertion of a myc epitope into the middle of the protein (red) allows the passage through the entire pathway, yielding a peroxisomal pattern. In contrast, adding the GFP-tag to the C-terminus of FcsB (green) blocks its egression from the ER.

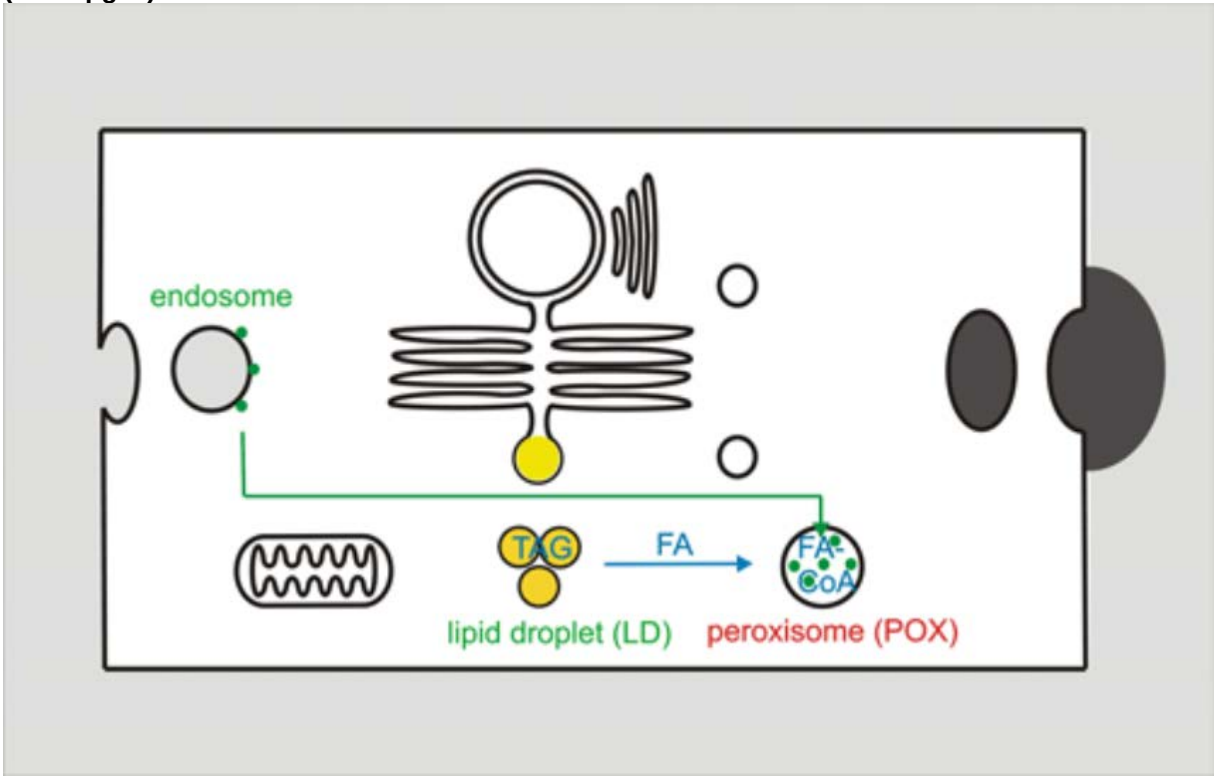


Function: FcsB may activate fatty acids (FA) by addition of coezyme A (CoA) in the lumen of the peroxisome

Reference: Paschke, et al.: The isoform B of the Dictyostelium long-chain fatty-acyl-coenzyme A synthetase is initially inserted into the ER and subsequently provides peroxisomes with an activity important for efficient phagocytosis. Eur. J. Cell Biol. 91, 717-727, 2012

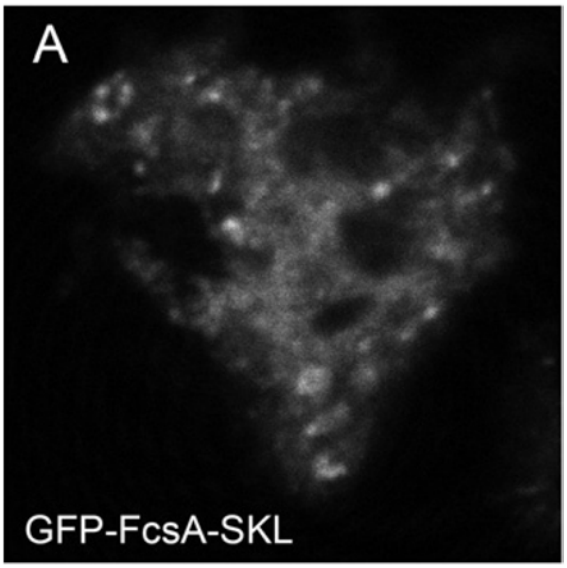
Functional details on various other proteins are obtained following the links below (jump to):
(overview pg. 1) (overview pg. 2) (overview pg. 3) (FcsA) (GPAT) (Lipin) (DGAT1) (DGAT2) (FARAT) (Seipin) (Plin pg. 1) (Plin pg. 2) (Smt1, Ldp, Net4) (FcsB pg. 1) (FcsB pg. 2)

Proteins involved in lipid metabolism
(FcsB pg. 2)



Top: The endosomal enzyme FcsA (green dots) can be forced to enter peroxisomes solely by the addition of a three amino acid signal (Ser-Lys-Leu) to its C-terminal end.

Right: GFP-FcsA-SKL (bright spots) enters peroxisomes.



Function: FcsA artificially targeted to peroxisomes can cure the phagocytic defect seen in a FcsB mutant, proving that the peroxisomal acyl-CoA synthetase activity is required for this process.

Reference: Paschke, P., Pawolleck, N., Haenel, F., Otto, H., Rühling, H., Maniak, M.: The isoform B of the Dictyostelium long-chain fatty-acyl-coenzyme A synthetase is initially inserted into the ER and subsequently provides peroxisomes with an activity important for efficient phagocytosis. Eur. J. Cell Biol. 91, 717-727, 2012.

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