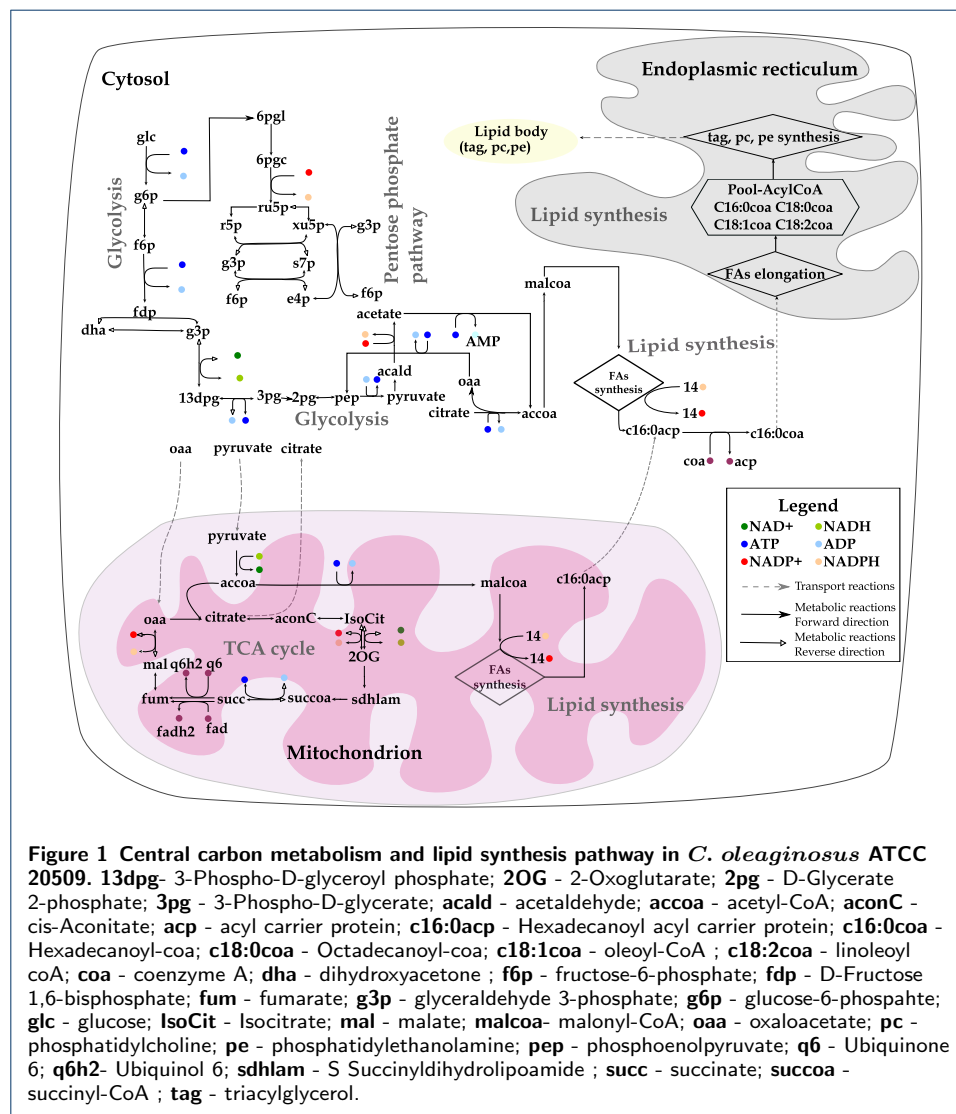


Lipid synthesis pathways

C. oleaginosus ATCC 20509 metabolizes sugars by using standard central metabolic pathways including glycolysis, pentose phosphate pathway and the citric acid (TCA) cycle (Figure 1). These pathways provide the precursors and energy required for lipid biosynthesis. Lipid biosynthesis can be divided into three steps: formation of fatty acids, synthesis of triacylglyceride (TAG), and synthesis of phospholipids.



Formation of fatty acids

In yeasts, fatty acids can derive from either a *de novo* synthesis pathway, from hydrolysis of complex lipids and delipidation of proteins or from hydrolysis of external fatty acids sources [1]. *De novo* fatty acid synthesis mainly occurs in the cytosol [2], and in some cases, in the mitochondrion [3]. The process is catalyzed by the multi-enzyme fatty acid synthetase complex (FAS) [2]. We found multiple genes, g2870.t1, g5734.t1, g570.t1 and g5733.t1, that together encode this enzyme com-

plex in *C. oleaginosus* ATCC 20509. The overall process of lipid synthesis can be simplified as follow:

- **ATP- citrate lyase (ACL)**. Citrate + ATP \rightarrow oxaloacetate + acetyl-CoA + ADP + P_i
- **Acetyl-CoA Carboxylase (ACC)**. acetyl-coA + CO₂ + ATP \rightarrow malonyl-CoA + ADP + P_i
- **Fatty acid synthetase (FAS)**. acetyl-CoA + 7 malonyl-CoA + 14 NADPH + 14 H⁺ \rightarrow palmityl-CoA + 14 NADP⁺ + 7 CoA + 7 CO₂

To sustain lipid synthesis, a constant supply of fatty acids is required [4]. This process in turn requires a continuous supply of acetyl-CoA [4]. For this oleaginous yeast species [5] typically have a cytosolic ATP :Citrate Lyase [6], in *C. oleaginosus* ATCC 20509, encoded by g3238.t1, that catalyzes the cleavage of citrate to yield acetyl CoA and oxaloacetate.

The next step in *de novo* fatty acid synthesis is the condensation of an acetyl-CoA and a bicarbonate anion to generate malonyl-CoA [2] and gene g570.t1 was predicted to encode the required acetyl-CoA carboxylase.

Malonyl-CoA is then converted to malonyl-ACP by malonyl-CoA ACP transacylase (g5734.t1 and g5733.t1) [7], followed by a series of elongation reactions. For each step of the carbon chain elongation reaction, two molecules of NADPH are required for FAS. NADPH can come from the malic enzyme, which is found in the mitochondrion of most of oleaginous microorganism in the malate dehydrogenase carboxylase reaction [4]: S-Malate + NADP⁺ \rightarrow pyruvate + NADPH + CO₂ . In *C. oleaginosus* ATCC 20509 two genes encoding this enzyme g1447.t1 or g6387.t1 are present.

Synthesis of triacylglyceride

The process of triacylglyceride (TAG) synthesis can be split into 3 steps: starting with the formation of phosphatidic acid (PtdOH) from glycerol-3-phosphate either through the glycerol-3-phosphate or the dihydroxyacetone phosphate pathway [3, 8, 9] .

- Formation of PtdOH catalyzed by two different acyltransferase encoded by g1820.t1 and g6923.t1 respectively.
acyl-CoA + glycerol 3-phosphate \Leftrightarrow 1-acyl-sn-glycerol 3-phosphate + coenzyme A + 2 H⁺
1-acyl-sn-glycerol 3-phosphate + acyl-CoA \Leftrightarrow coenzyme A + phosphatidate
- Formation of diacylglyceride (DAG) reaction catalyzed by phosphohydrolase enzyme (g920.t1)
Phosphatidate + H₂O \Leftrightarrow diglyceride + phosphate + 2 H⁺
- Formation of TAG catalyzed by acyltransferase enzyme (g561.t1)
Acyl-CoA + diglyceride \Leftrightarrow coenzyme A + triglyceride

Phospholipid synthesis

CDP-DAG pathway starts with the synthesis of liponucleotide CDP-DAG from phosphatidic acid and cytidine triphosphate (CTP) in the Endoplasmic reticulum (ER) [4, 10]. This reaction is catalyzed by CDP-diacylglycerol synthase enzyme (g6796.t1). CDP-DAG is then converted to PS by CHO1-encoded phosphatidylserine synthase (g3238.t1). PS is then transported to the mitochondrion, where it is

further decarboxylated to produce PE [10, 11, 12] by the enzyme encoded from g6020.t1. Transport of lipids from the ER and mitochondria is often considered via contact sites without long-distance through the cytosol [13, 10, 14]. However, a recent study showed that PS is transported to the mitochondrion via a cytosolic factor [11]. PE is transported to other cellular locations including ER to continue the synthesis of phospholipid [4, 10]. PC, the final product of the pathway is synthesized from PE through a series of methylation reactions. PC and PE can also form in the Kennedy pathway via two branches CDP-choline and CDP-ethanolamine, respectively [10]. The two branches start with external Ethanolamine or Choline, which need to be supplied in the culture medium [10]. Ethanolamine and Choline are phosphorylated in the cytosol with ATP to produce ethanolamine-P and choline-P, respectively. They are then further phosphorylated with CTP and incorporate DAG to produce PE or PC, respectively [15]. The later two steps are associated with enzymes in the nuclear/ER membrane [16]. In the absence of ethanolamine and/or choline in the growth medium, CDP-DAG is the main route to synthesize PE and/or PC [16].

Author details

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