40. Analysis of Lipid Metabolism in Saccharomyces cerevisiae: Elucidating Regulation of Triacylglyceride Synthesis

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The goal of this project is to improve our understanding of regulation of triacylglyceride synthesis in yeast. For this, Saccharomyces cerevisiae is engineered to accumulate increased amounts of triacylglycerides by overexpressing genes involved in their biosynthesis. Lipidome and transcriptome of the engineered strain and a reference strain will be analyzed. Integrated data analysis will reveal associations and correlations between the different components. Obtained knowledge can be transferred to Yarrowia lipolytica, a distantly related oleaginous yeast.

Lipids are a group of highly diverse molecules with a multitude of biological functions such as formation of biological membranes, storage of energy, cell signaling, and apoptosis. Triacylglycerides function as energy storage and source of membrane building blocks. Triacylglycerides are of particular interest since they can serve as a feedstock for microbial production of oleochemicals and biodiesel.

Oleaginous yeasts such as Yarrowia lipolytica, Lipomyces starkeyi or Rhodotorula glutinis are a suitable choice for microbial production, as they can accumulate significant amounts of storage lipids. Lack of genetic tools and incomplete fundamental knowledge about these organisms, however, makes optimization of production difficult. The yeast Saccharomyces cerevisiae, while not an oleaginous yeast, is a well-established model organism for studying the lipid metabolism of eukaryotes. A strong toolbox for genetic modification and extensive knowledge about this model organism makes it a good choice to study the mechanisms behind storage lipid accumulation. While Saccharomyces cerevisiae is only distantly related to the above mentioned oleaginous yeasts, the enzymes involved in biosynthesis and storage of lipids are generally well-conserved across yeast species.

To fully unlock the potential of yeast as a cell factory for products derived from triacylglycerides, an improved understanding about the regulatory mechanisms behind synthesis, storage and breakdown of triacylglycerides is required. To unravel these mechanisms, an engineered yeast strain that produces increased amounts of triacylglyceride was generated. The flux of carbon was redirected into triacylglyceride synthesis by expressing acetyl-CoA carboxylase mutant ACC1S659A S1157A, phosphatidate phosphatase

PAH1 and diacylglycerol acyltransferase DGA1 under the control of strong constitutive promoters. The engineered yeast strain and the reference strain were cultured in batch under different nutritional conditions. Strains were then used to analyze differences in gene expression and lipid composition as a result from changes in metabolic flux.