

**Put on a diet: Lipid accumulation inhibits TOR signaling in *Saccharomyces cerevisiae***

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**Project Goals: 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 (TAG) function as energy storage and source of membrane building blocks. TAG are of particular interest since they can serve as a feedstock for production of oleochemicals and biodiesel. We are interested in studying the regulatory mechanisms behind TAG formation.**

We have engineered *Saccharomyces cerevisiae* to accumulate increased levels of TAG. Accumulation was achieved by introducing a push and pull on TAG biosynthesis. A push was introduced by overexpression of acetyl-CoA carboxylase double mutant *ACCI*<sup>S659A S1157A</sup> (*ACCI*\*\*). The gene product of *ACCI*\*\* is constitutively active because the protein kinase *Snf1p* cannot inactivate the mutated carboxylase by phosphorylation. A pull was introduced by overexpression of phosphatidate phosphatase *PAH1* and diacylglycerol acyltransferase *DGAI*. The resulting strain was analyzed for transcriptional changes in fermentative and respiratory growth phase compared to a reference strain.

Despite a strongly increased flux towards fatty acids and TAG in respiratory phase, we were unable to observe major transcriptional changes for genes involved in fatty acid and TAG biosynthesis. However, we observed an upregulation of  $\beta$ -oxidation, and a downregulation of phospholipid metabolism. Gene set analysis (GSA) revealed changes in biological processes that are not directly linked to lipid metabolism. We observed a downregulation of translational initiation, ribosome biogenesis, cellular amino acid biosynthesis, and rRNA processing, as well as an upregulation of the cell wall integrity pathway, and autophagy. These biological processes are (partially) regulated by the TOR complex. TOR is a protein complex that responds to energy and amino acid levels. It regulates many aspects that are related to cell growth. These data indicate that lipid accumulation impinges on TOR signaling.

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