Transcriptional regulation and lipid accumulation in *Yarrowia lipolytica*

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Project Goals: Our goal is to elucidate the regulation of lipid metabolism in *Y. lipolytica* to identify new targets to improve the TAG yield.

Oleaginous yeasts such as *Yarrowia lipolytica* are capable of accumulating lipids up to 70% of their biomass, predominantly in the form of triacylglycerols (TAGs), and this has fuelled interest in exploiting these fungi for the production of biodiesel. To optimise yields, we are studying the metabolic fluxes and their regulation in *Y. lipolytica* in different growth conditions on a genomic scale.

A multi-factorial chemostat experiment was designed, including two growth conditions (nitrogen and carbon limitation) and two strains (a wild-type and a DGA1 overexpressor). These cultures were sampled for various omics technologies (RNAseq, proteomics [performed under the Pan-omics program at PNNL], metabolomics, lipidomics) and integrative data analysis was performed with this comprehensive dataset and the aid of a genome-scale model of *Y. lipolytica* metabolism.

Lipid accumulation is substantially increased upon nitrogen limitation and DGA1 overexpression, however, both transcriptomics and proteomics indicate the lack of regulation of expression levels of lipid biosynthetic enzymes. The genes that were differentially expressed showed a strong correlation between transcript and protein levels, indicating that many of these are under transcriptional regulation.

The strongly correlated genes were enriched for involvement in amino acid biosynthesis. More precisely, specifically leucine biosynthesis was transcriptionally downregulated upon high lipid accumulation in the DGA1 strain during lipid accumulation. This was further supported by metabolomics data, which indicated the intermediate of leucine biosynthesis 2-isopropylmalate as overflow metabolite during nitrogen limitation in the WT strain, while absent at high lipid accumulation during nitrogen limitation in the DGA1 strain.

While the lipid biosynthetic pathway is not transcriptionally regulated at high lipid accumulation, leucine biosynthesis is specifically down-regulation, having the effect that carbon flux is redirected from amino acid biosynthesis towards lipid accumulation. This suggests new and unexpected targets for further metabolic engineering.