95. Functional overexpressions and characterizations of lipogenesis-related genes in oleaginous yeast Yarrowia lipolytica

Kangjian Qiao[1,*] (kjqiao@mit.edu), Andrew Silverman[1,*](asilver@mit.edu), Peng Xu[1], and **Gregory Stephanopoulos[1]**

[1]Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA

Project Goals: We focused on achieving a fundamental understanding of the metabolic pathways of the oleaginous yeast *Yarrowia lipolytica* and developing tools to characterize and engineer it. More specifically, we aimed to improve its fermentation characteristics towards the development of a cost-effective process which converts renewable resources to lipids for biodiesel production. The conversion yield and volumetric productivity on various carbon sources are the key metrics for optimization.

As an oleaginous yeast, *Yarrowia lipolytica* can naturally accumulate more than 20% of its biomass as triacylglycerols. Recently, due to the availablilty of its sequenced genome and a limited set of genetic tools, it was extensively investigated as a model organism for *de novo* lipid biosynthesis and accumulation. In spite of previous efforts, many gene functions of *Y. lipolytica* are still unknown. Therefore, in our study, we applied a systematic approach to identify and investigate a diverse group of lipogenesis-related genes of *Y. lipolytica*. Many aspects to lipid synthesis are represented in our set of gene targets, including glycerolipid synthesis pathways, the fatty acid synthesis pathway, NADPH generation, transcriptional and protein-level regulators, lipid transporters, and central carbon metabolism. So far, 35 of the selected genes were cloned into a previously established expression platform, featuring the strong constitutive TEF (Transcription Initiation Factor-1α) promoter, and transformed into *Y. lipolytica* po1g; elevated transcription levels for each construct was demonstrated by RT-qPCR. Strains were evaluated in fermentations using either glucose or acetate as sole carbon source and the total lipid titer and content was quantified via GC-FID.

Preliminary results are encouraging, as many of our single-overexpression constructs demonstrated an increase in lipid titer and dry cell weight fraction over wild-type in our fermentations. In particular, overexpression of DGA2 (YALI0E07986g) was demonstrated to significantly boost the lipid content (~55%) as its counterpart DGA1 (YALI0E32769g). On the other hand, overexpression of two other diacylglyceride acyltransferases DGA (YALI0F06578p) and PDAT (YALI0E16797g) elevate the lipid content by 1.4 fold. Together, this evidence supports the hypothesis that integrating intracellular free fatty acid into neutral lipid (located at lipid droplet) is rate limiting step and overexpression of appropriate enzymatic steps can efficiently activate de novo lipogenesis and storage. Moreover, moderate increase (1.2 – 2.1 fold) of lipid titer and content were observed by overexpressing most genes in the Kennedy pathway using both glucose and acetate as the carbon sources, indicating that an increase in the concentration of all Kennedy pathway intermediates could facilitate the biosynthesis of triacylglycerides. Aside from just Kennedy pathway intermediates, overexpressing glycerol-3-phosphate dehydrogenase to produce more of the glycerol backbone significantly increases lipid titer and content in glucose fermentations. Additionally, overexpressing the delta-12 desaturase didn't seem to facilitate lipogenesis but to a considerable extent changed the lipid distributions: the content of C18:2 is increased by 9- fold.

Our results strongly suggest that the oxidative pentose phosphate pathway (oxPPP) is the major NADPH generation pathway for lipid synthesis in *Y. lipolytica*. Of the several gene products that convert $NADP^+$ to NADPH, only those involved in oxPPP conferred increases in lipid titer and content to

strains that overexpressed them, and every oxPPP gene (ZWF1, SOL3, and GND) overexpression led to increased lipogenesis. These increases in performance were only present with glucose as the carbon source. One surprising result was that the most effective of these was SOL3, the intermediate step (and the one that does not by itself produce NADPH), with increases in end-point fermentation lipid content over wild-type ranging from 13% in high carbon-nitrogen ratio media to 177% in lower C:N media. The dependence of comparative results of these strains on the carbon-nitrogen ratio in the media possibly signifies that in *Y. lipolytica*, oxPPP flux is increased in response to nitrogen exhaustion during fermentation.

Lastly, although only a few of our regulators have been tested so far, we saw significant improvements in glucose fermentation performance when overexpressing one particular transcription factor (YALI0C02387g, annotated as "YAS1"). YAS1 is a helix-loop-helix TF demonstrated to be involved in up-regulating genes needed to utilize alkanes as a carbon source. However, *Y. lipolytica* YAS1 also has high homology to the INO4 transcription factor in *Saccharomyces cerevisiae*, which is involved in expressing genes needed for glycerolipid synthesis, so the exact mechanism of YAS1 influencing lipid synthesis is unclear at this time. Moving forward, we aim to test more potentially lipogenic genes as well as conduct further experiments on our most effective targets to elucidate their mechanisms of action as they relate to lipid synthesis.