

Oxidative Pentose Phosphate Pathway is the Major Cytosolic NADPH Source in *Rhodospiridium toruloides*

Tianxia Xiao^{1,2*} (tianxiax@princeton.edu), Christopher Rao,^{2,3} and Joshua Rabinowitz^{1,2}

¹Princeton University, Princeton, NJ; ²Carl R. Woese Institute For Genomic Biology, Urbana, IL; ³University of Illinois, Urbana-Champaign, Urbana, IL

<https://www.igb.illinois.edu/DOEcenter>

Project Goals: Understand the metabolic pathways in *R.toruloides*.

Understanding the native metabolism of microbes is important for facilitating metabolic engineering efforts. *Rhodospiridium toruloides* is a promising yeast for fatty-acids production, but relatively understudied. Nitrogen limitation elevates *R. toruloides*' already high native production of fatty acids. Fatty acid production causes a high demand for cytosolic NADPH. Here we investigated the source of cytosolic NADPH in *R. toruloides*. Specifically, we employed a ²H-glucose strategy to trace directly the source of the redox-active hydride of NADPH, an approach that had not previously been applied to any yeast. This strategy requires complementary deuterated water tracing experiments to determine the extent of ¹H-²H exchange in NADPH, which otherwise leads to underestimation of pathway contributions. By this approach, we find that the oxidative pentose phosphate pathway (oxPPP) contributes most of *R. toruloides*' cytosolic NADPH. The oxidative pentose phosphate pathway contribution is equivalent in both batch growth and N-limitation. 1,2-¹³C-glucose tracing data indicate that the ratio of oxidative pentose phosphate pathway flux to glycolysis is also similar in these two conditions. These data suggest a shift during nitrogen limitation in NADPH utilization from reductive nutrient assimilation to fat synthesis. These observations lay foundation for future efforts to enhance fatty acid production in *Rhodospiridium* species via metabolic engineering.

References

1. Zhang, Z., Chen, L. *et al.* Chemical basis for deuterium labeling of fat and NADPH. *JACS*, **139**, 14368-14371 (2017).