Citric Acid Metabolites Regulate Phosphatidate Phosphatase Activity from the Oleaginous Yeast *Yarrowia lipolytica*

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Abstract

Phosphatidate phosphatase (PAP) catalyzes the conversion of phosphatidate (PA) to diacylglycerol (DAG) in a reaction that depends on Mg^{2+}. This reaction is critical for *de novo* lipid accumulation because it provides the DAG needed for the biosynthesis of triacylglycerol (TAG). Lipid accumulation is triggered by the downregulation of the citric acid cycle, which results in the exit of citrate from the mitochondria to the cytosol. In the cytosol, citrate is converted to oxaloacetate and acetyl-CoA which is channeled to fatty acid biosynthesis. Oxaloacetate is converted to malate by malate dehydrogenase and malate to pyruvate by malic enzyme. Also, citrate stimulates the activity of acetyl-CoA carboxylase, which catalyzes the rate-limiting step in fatty acid biosynthesis. In this work, we examined the effect of citric acid cycle metabolites on PAP activity in cell extracts prepared from wild type cells and cells that lack *PAH1* (i.e., *pah1*Δ). The cells were grown for 96 h on high glycerol media that induce lipid accumulation, and cell extracts were prepared. Control PAP assays done in the presence of Mg^{2+} showed that the *pah1*Δ mutation resulted in a 95% decrease in PAP activity, indicating that *PAH1* encoded for almost all PAP activity. The effects of citrate, malate, and pyruvate were examined at concentrations ranging from 0.1 mM to 5 mM. In wild type cell extracts, citrate (1 mM) and malate (0.1 mM) caused a 220%, 217% increase in PAP activity, respectively. In contrast, the addition of these acids to cell extracts prepared from *pah1*Δ cells did not affect PAP activity. The stimulatory effect of these organic acids on PAP activity could provide a direct link between fatty acid biosynthesis and DAG synthesis by Pah1. The buildup of citrate and its metabolites in the cytosol could induce PAP activity to direct the lipid biosynthetic pathway towards the synthesis of DAG. This regulation, combined with the stimulation of acetyl-CoA carboxylase by citrate, could contribute to the induction in TAG synthesis typically observed during lipogenesis. On the other hand, loss of *PAH1* resulted in diminished TAG levels while the PLs and FFAs levels increased by 85% and 60%, respectively.

Introduction

- *PAH1*-encoded PAP regulates lipid biosynthesis by catalyzing the reaction converting phosphatidic acid (PA) to diacylglycerol (DAG).
- Deletion of the *PAH1* gene resulted in 30% decrease of total PAP activity, and lower TAG levels (up to 90%) in high glucose media. (Ukey et al. 2020, Carmon et al., under preparation).

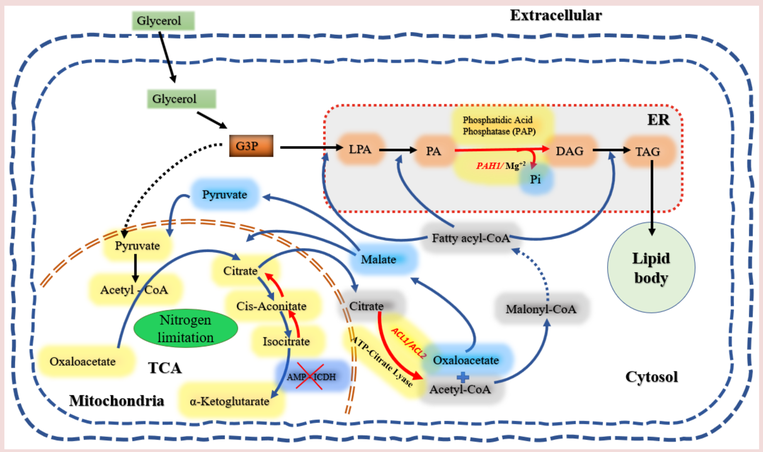


Figure 1: Diagram of *Y. lipolytica* metabolic pathways relevant to this study. This diagram shows the utilization of glycerol and the generation of cytosolic citrate under nitrogen-limited conditions. Citrate converts to acetyl-CoA, which is used for the synthesis of fatty acyl-CoAs and triacylglycerols. Magnesium (Mg^{+2})-dependent PAP enzymes encoded by *PAH1* catalyzes the conversion of PA to DAG.

Materials & Methods

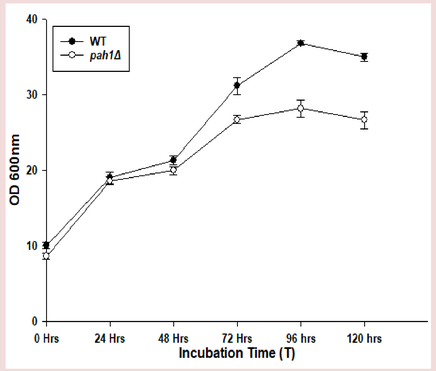
High Glycerol Media

PAP Assay

Gas Chromatography

Thin-Layer Chromatography

Results



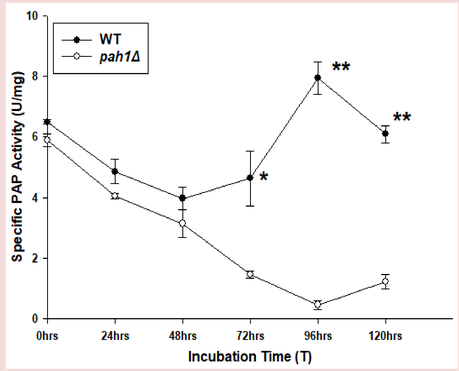
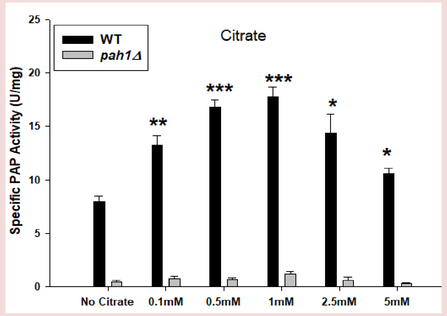
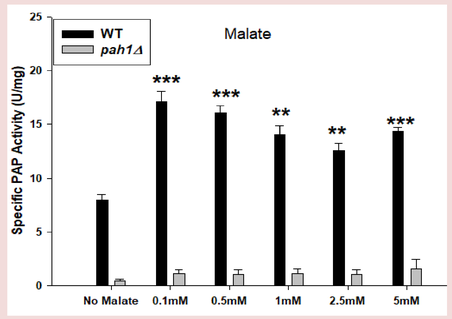
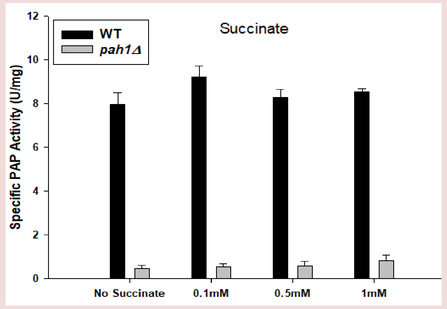


Figure 2. Effect of *pah1*Δ mutation on growth and PAP activity. A. Cells were inoculated in fresh media and their $A_{600\ nm}$ was measured. Each data point represents the average of three biological replicates ± SD (error bars). **B.** Cell extacts were prepared and the PAP activity was measured. Each data point represents the average of three biological replicates ± SE (error bars). ***** indicates the level of significant difference of mean values (P < 0.05) between WT and *pah1*Δ strains during lipogenesis.







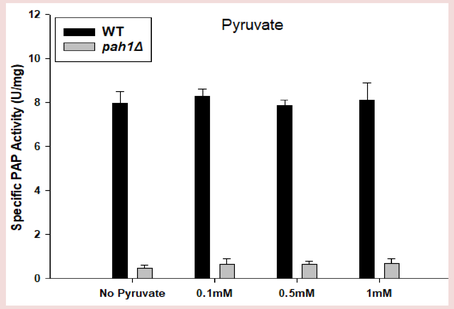


Figure 2: Effect of potassium citrate, malate, succinate and pyruvate on PAP activity. Wild type and *pah1*Δ cells were grown for 96h, cell extacts were prepared and the PAP activity was measured. Each bar represents the average of three biological replicates ± SE (error bars). ***** indicates the level of significant difference of mean values (P < 0.05) between the control (no TCA metabolites) and 0.1mM, 0.5mM, 1mM, 2.5mM and 5mM of metabolites.

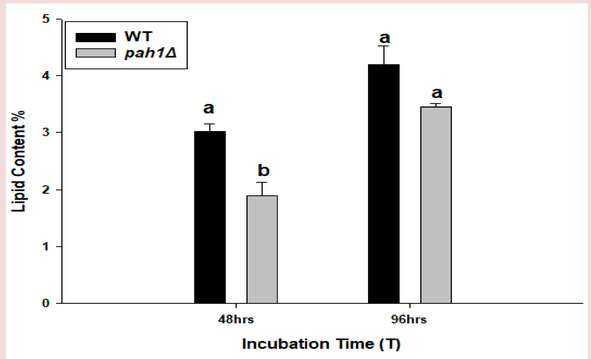


Figure 3. Effect of *pah1*Δ mutation on lipid content. Lipids were extracted from cells and quantified by gas chromatography. Each data point represents the average of three biological replicates ± SE (error bars). Different letters indicate the significant difference of mean values (P < 0.05) between WT and *pah1*Δ strains at specific time point.

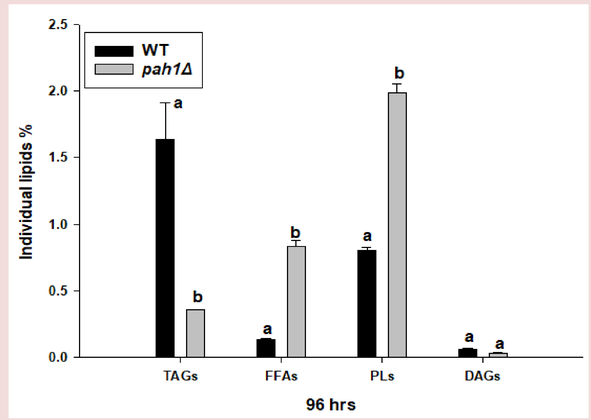


Figure 4. Effect of *pah1*Δ mutation on lipid profiles. Each data point represents the average of three biological replicates +- SE (error bars). Different letters indicate the significant difference of mean values (P < 0.05) between WT and *pah1*Δ strains of individual lipids

Conclusions

- ❑ *PAH1* encodes for the major PAP activity in *Y. lipolytica*
- ❑ Loss of *PAH1* resulted in lower TAG levels and an increase in PLs and FFAs
- ❑ Citrate and malate significantly increased the Pah1 PAP activity

References

Ukey, R., Carmon, T., Hardman, D., Hill, N., & Fakas, S. (2020). The *Yarrowia lipolytica PAH1* homologue contributes but is not required for triacylglycerol biosynthesis during growth on glucose. *Yeast*, *37*(1), 93–102. https://doi.org/10.1002/yea.3447

Acknowledgements

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