Abstract 2069

ATP-citrate lyase regulates lipid biosynthesis in *Yarrowia lipolytica*

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ATP citrate lyase (ACL) catalyzes the ATP-dependent conversion of cytosolic citrate to the fatty acid precursor, acetyl-CoA. ACL presence in yeasts has been associated with their ability to accumulate lipids (i.e., oleaginous phenotype), but little is known about the regulation of this enzyme in oleaginous yeasts. In the model oleaginous yeast *Yarrowia lipolytica*, ACL is a heterodimer composed of a catalytic and a regulatory subunit encoded by the *ACL1* and *ACL2* genes, respectively. To better understand the contribution of each of the subunits of ACL in lipid biosynthesis, we constructed strains that lack (i.e., *acl1Δ; acl2Δ; acl1Δacl2Δ*) or overexpress (OE) (i.e., *ACL*1OE; *ACL*2OE; *ACL*1/2OE) Acl1 and Acl2, either alone or in combination. The expression of ACL in the strains was confirmed by immunoblot analysis using antibodies directed against its two subunits (i.e., Acl1 and Acl2). We examined the time-dependent expressions of Acl1 and Acl2 and found that their levels decline over time. The expression profiles of Acl1 and Acl2 showed that both subunits were expressed at 24 h and 48 h, but their expression declined at 72 h and 96 h of growth. The decline in expression could indicate decreased expression of the *ACL*1 and *ACL*2 genes or degradation of the proteins. Overexpression of Acl1 increased the protein levels of Acl2. In addition, Acl2 was more expressed than Acl1 in the strain overexpressing Acl1 and Acl2. We also examined the effects of the ACL mutations on growth and lipid biosynthesis on high glucose media that induce lipid accumulation. The loss of Acl1 or Acl2 resulted in slower growth compared to the wild type, while ACL overexpression did not significantly affect growth. Lipid analysis showed that triacylglycerol levels decreased by 74% in the *acl*1Δ mutant and increased by 35% in the *ACL*1/2 OE strains compared to the wild type. The phospholipid levels decreased by 19% in the *acl*1Δ mutant and 28% in the *ACL*1/2 OE strains. The ACL mutations also affected the fatty acid profiles of the triacylglycerols and phospholipids. These effects are being further examined using an integrated omics approach.

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Abstract 2117

A short-chain acyl-CoA synthetase that supports branched-chain fatty acid synthesis in *Staphylococcus aureus*

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Staphylococcus aureus produces a combination of branched and straight chain fatty acids to maintain membrane homeostasis. Branched-chain ketoacid dehydrogenase (Bkd) is the enzyme responsible for the formation of branched-chain acyl-CoA primers from α-keto acids. 3-ketoacyl-ACP synthase III (FabH) then catalyzes the initial condensation step in fatty acid biosynthesis drawing on the available short-chain acyl-CoA primer pool. Most experiments with *S. aureus* are carried out in rich media that contains an abundant supply of branched-chain amino acids. Under these laboratory conditions isoleucine is the favored substrate for the IlvE transaminase and Bkd produces 2-methylbutyryl-CoA for anteiso fatty acid synthesis. In bkd knockout strains, however, branched-chain fatty acids are still present and the enzyme(s) responsible for this alternate biosynthetic pathway is unknown. Gas chromatography analysis of the fatty acid composition of bkd knockout strains grown in rich media shows an increase in iso even fatty acid chains and a decrease in iso odd chains. The anteiso branched-chain fatty acid composition is elevated by supplementation with 2-methylbutyrate suggesting a pathway that activates short-chain acids to their acyl-CoAs. The bkd knockout strains are auxotrophs for either 2-methylbutyric acid or isobutyric acid in defined media supporting the activation pathway hypothesis. A screen of candidate genes identified the mbcS gene (methylbutyryl-CoA synthetase; SAUSA300_2542) as the first required step in the incorporation of 2-methylbutyric and isobutyric acids into phosphatidylglycerol. MbcS is an ATP/Mg2 +-dependent acyl-CoA synthetase that selectively catalyzes the conversion of isobutyrate and 2-methylbutyrate to their respective acyl-CoA. Butyrate and isovalerate are poor MbcS substrates and activity was not detected using acetate or short-chain dicarboxylic acids as substrates. Thus, MbcS functions as a salvage pathway to convert extracellular 2-methylbutyric and isobutyric acids to their respective acyl-CoAs that are used by FabH to initiate branched-chain fatty acid biosynthesis for membrane formation independent of Bkd function.

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