Discovery and characterization of regulatory mechanisms affecting the heteromeric acetyl-coenzyme A carboxylase in Arabidopsis

Matthew J. Salie, Advisor: Jay J. Thelen

ABSTRACT

Vegetable oil is produced by the fatty acid biosynthesis (FAS) pathway. Acetyl-CoA carboxylase (ACC) catalyzes the committed step of this pathway by converting acetyl-CoA to malonyl-CoA. The heteromeric form of this enzyme requires four different subunits for activity: biotin carboxylase, biotin carboxyl carrier protein (BCCP), and α- and β-carboxyltransferases (CT). Heteromeric ACC is present in prokaryotes and the plastids of most plants, and has been a focus of biotechnology research due to its prominent role in FAS. Many different regulatory mechanisms have been identified in both plants and *E. coli*. However, it is still unknown how most of these regulatory mechanisms are mediated.

This work focused on clarifying two unknowns in ACC regulation: 1) Identify suspected regulatory factors associated with ACC, and 2) characterize a role for phosphorylation of α-CT. First, using *in vivo* co-immunoprecipitation coupled with quantitative mass spectrometry, yeast two-hybrid, and co-expression in *E. coli*, two unknown proteins annotated as 'biotin/lipoyl attachment domain containing' (BADC) proteins were identified to interact with ACC. These proteins were found to resemble the BCCP subunit, but are not biotinylated. BADC gene orthologs were found only in plant and green algae species that contain a heteromeric ACCase suggesting BADC genes co-evolved with this form of ACCase. Furthermore, BADC was shown to directly reduce ACCase activity in both *E. coli* and *A. thaliana*. Seed specific RNAi-silencing of BADC expression in *A. thaliana* increased total seed oil content. The BADCs are proposed to function as competitive inhibitors of ACC and negatively regulate the complex. Second, expression of phosphomimic α-CT protein in *A. thaliana* showed no obvious phenotype. However, overexpression of α-CT protein was observed to have a positive effect on ACC activity, suggesting that α-CT is the limiting subunit of the ACC complex.