

169. The Systems Biology of Protein Acetylation in Fuel-Producing Microorganisms

Birgit Schilling¹, Kori D. Dunn², David Christensen³, Bozena Zemaitaitis³, Alexandria K. Sahu¹, Bradford W. Gibson¹, Christopher V. Rao^{*2}, and Alan J. Wolfe³

¹ Buck Institute for Research on Aging, Novato, CA; ² University of Illinois at Urbana-Champaign, Urbana IL; ³ Loyola University Chicago, Maywood, IL

* Email: chris@scs.uiuc.edu

Project Goals: The goal of this project is to determine how protein acetylation affects metabolism in engineered microorganisms. Lysine acetylation is a common post-translational modification that eukaryotes, archaea, and bacteria employ to regulate protein activity. Multiple studies have recently shown that lysine acetylation predominantly targets metabolic enzymes – in fact, most metabolic enzymes are subject to lysine acetylation. Still, we know very little about the consequences of lysine acetylation, particularly in the case of bacteria. We hypothesize that bacteria employ lysine acetylation as a global mechanism to regulate metabolism in response to their energy and redox status. Our previous work suggests that lysine acetylation may be an attractive and innovative target for metabolic engineering. We are investigating how lysine acetylation affects fuel production in engineered microorganisms. The significance of this work is that it will address a fundamental gap in our understanding of bacterial metabolism and identify new approaches for overcoming the problems associated with the production of advanced biofuels.

We hypothesized that supplementation of growth media with glucose would significantly increase *E. coli* protein acetylation by elevating the levels of acetyl-phosphate (acP). To test this hypothesis, wild-type *E. coli* cells were grown in buffered tryptone broth (TB7) supplemented with or without 0.4% glucose, and temporal changes in the acetylation status of the bacterial proteome were observed. Anti-acetyllysine Western analysis revealed a proteome that became more acetylated the longer cells were exposed to glucose. To obtain a more precise understanding of glucose-induced acetylation, we affinity-enriched acetyllysine-containing peptide fractions and analyzed by mass spectrometry using an AB SCIEX TripleTOF 6600 and Skyline MS1 Filtering. Across all growth conditions and time points, we confidently identified 2813 unique lysine acetylation sites on 780 unique acetylated *E. coli* proteins. During growth in TB7 with glucose, the number of identified unique acetylated lysines (Kac) and proteins increased steadily over time, with 1068, 1658, 2226 and 2338 Kac sites observed at 2, 5, 8 and 12 h, respectively. To quantify changes at specific lysine acetylation sites over time in glucose, we used Skyline MS1 Filtering analysis. The acetylation status of 1091 lysines on 420 proteins showed statistically significant increases (>2 fold), with median changes of 2.7-fold (5h/2h), 5.7-fold (8h/2h), and 6.6-fold (12h/2h). However, the rate of change in acetylation varied greatly between individual lysines from the same proteins, suggesting a high degree of specificity. Network mapping of these acetylated proteins showed involvement of a diverse set of cellular processes; however, acetylations were particularly prominent in central metabolic pathways, such as the TCA cycle and glycolysis (the Embden-Meyerhoff-Parnas and pentose phosphate pathways). To represent the dynamic nature of acetylation during the investigated glucose time course, we created pictorial representations in which each enzyme of a specific pathway (i.e., TCA cycle) is displayed as a color-coded pie chart that indicates both specific lysine sites and their fold-changes. Such analysis shows that glucose-regulated sites overlap extensively with acP-regulated sites (as previously determined by comparing an *ackA* mutant and its WT parent). We propose that acP-dependent protein acetylation is a response to carbon flux that has the potential to regulate central metabolism.

To explore the significance of this regulation, we measured polyhydroxybutyrate (PHB) production – as a

proxy for acetyl-CoA pools – in engineered strains of *E. coli*. Our data demonstrate that PHB production is significantly delayed and somewhat reduced in mutants that have altered acetylation ability (*yfiQ* and *pta/ack*), suggesting that lysine acetylation plays a key role in regulating acetyl-CoA pools. We are currently assessing these results in light of our MS data.

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