Protein Acylomes in Fuel-Producing *E. coli* – Changes in Posttranslational Modifications with Different Carbon Sources

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Project Goals: The goal of this project is to gain deeper insights into posttranslational remodeling of engineered microorganisms, and specifically their protein acylomes under different growth conditions. Lysine acetylation, for example is a common post-translational modification that eukaryotes, archaea, and bacteria employ to regulate protein activity. We and others have recently shown that lysine acetylation predominantly targets metabolic enzymes – in fact, most metabolic enzymes are subject to lysine acetylation. Due to the dynamic nature of protein acetylation and deacetylation mechanisms in the cell, lysine acetylation can likely be considered a global mechanism to regulate metabolism in response to their energy and redox status. Here, we are comparing acetylation status under different growth conditions, using different sugar supplementation strategies and different *E. coli* strains. 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.

In our recent study, we reported that glucose-regulated lysine acetylation (Kac) was predominant in central metabolic pathways and overlapped with acetyl phosphate-regulated acetylation sites (1). We proposed that acetyl phosphate-dependent acetylation across hundreds of proteins is a response to carbon flux that can regulate central metabolism. Since then, we have extended our investigations to examine the effect on both protein expression levels and the alterations in acetylation status across the *E. coli* proteome when it is provided with alternative carbon sources beyond glucose.

Typically, for mass spectrometric studies protein fractions are either analyzed on the protein level to assess protein expression changes, or protein fractions are subjected to antibody-based affinity-enrichment of acetyllysine-containing peptides and are then measured by quantitative mass spectrometry. To monitor changes in protein acetylation and expression, we used a novel label-free and data-independent acquisition (DIA or SWATH) approach on a SCIEX TripleTOF 6600 LC/MS system that we have modified for this purpose (2). In any of our acetylation studies, we typically identify >3,000 unique acetylation sites. Subsequent quantification then reveals sites that show statistically significant relative changes in acetylation abundance between different growth conditions.

In this study, we grew wild-type *E. coli* cells in minimal medium supplemented either xylose (Xyl) or glucose (Glc) as the sole carbon source at either 0.4% or 4% sugar. Using SWATH, we identified hundreds of acetylation sites in more than 150 proteins that showed robust and statistically significant increases in acetylation when the cells were grown with the larger amount of sugar, independent of the supplemented sugar source (Glc or Xyl). These sites overlap substantially with those that are acetyl-phosphate-sensitive. Together, these results support our hypothesis that acetyl-phosphate-dependent protein acetylation is a response to carbon flux and not exposure to a specific carbon source.

The 'regulated' acetylated proteins included many involved in multiple central metabolic processes, e.g., glycolysis/gluconeogenesis, pyruvate metabolism, and the TCA cycle. A functional annotation analysis for regulated acetylated proteins showed a statistically relevant enrichment for the ontology term "generation of precursor and metabolites and energy." To visualize acetylation changes in the context of these protein pathways, we developed novel visual display algorithms. These results are supportive of the hypothesis that protein acetylation could regulate central metabolism in response to carbon flux.

We have been particularly interested in two acetylation sites of XylA, the first enzyme in xylose catabolism, as we determined regulation both on the protein and acetylation site levels. Most intriguingly, acetylation of two sites (K17 and K381) behaved oppositely when growth on 4% glucose was compared to growth on 4% xylose. Differential regulation is supportive of the hypothesis that acetylation may dictate enzyme function.

References

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