FRIDAY - 1036

Quantitative Profiling of Post-Translational Modifications in *E. coli*

Charles L. Farnsworth[1], Xiaoying Jia[1], Jian Min Ren[1], Kimberly Lee[1], Justin Topp[2], Wayne Gatlin[2], Zach Norring[3]

[1]Cell Signaling Technology, Inc., Danvers MA 01923
[2]Endicott College, Beverly, MA 01915,
[3]U. Massachusettes, Amherst, MA

INTRODUCTION

Protein post-translational modification (PTM) serves to regulate nearly every function of cellular biology including growth, development and disease. Antibody-based enrichment coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS) has long been used to study PTM changes and associated cellular signaling in mammalian cells and tissues. Application of these methods to study prokaryotic systems may be critical to understanding the underlying the regulation of protein function that cause phenotypic changes. Advances in technology and bioinformatics allow the identification and quantification of 10's of thousands of peptides in one experiment. Moreover, the same antibodies which can immunoprecipitate PTM containing peptides can be used to visualize changes in PTM abundance by western blotting. This ability to prescreen experimental samples for changes in PTM's prior to mass spectrometry can optimize the experimenters workflow to focus on the conditions demonstrating the most significant changes.

METHODS

In this study we first examined changes in the abundance of post-translational modifications or PTM's in *E. coli* strain MG1655 grown in minimal M9 media with differing sources of carbon by Western blotting. Here we compared glucose to mannitol, glycerol or succinate. We also examined the lysine acylation's acetylation and succinylation, and tyrosine phosphorylation when the cells were grown in M9 media with lower nitrogen or phosphate.
From a 5ml overnight culture cells were grown in 200mls of M9 media, M9 with low nitrogen or phosphate. The cells were washed in PBS, harvested in 9M urea buffer, and subjected to western blotting with a panel of PTM-specific antibodies to determine by band pattern/intensity changes which were good candidates for the mass spectrometry-based analysis. Samples were then reduced, alkylated, digested with trypsin, and subjected to immuno-affinity enrichment with the antibodies that showed changes in the western blotting screen (Phospho-S/T/Y, Acetyl-K, Succinyl-K). Enriched peptides were run on an Orbitrap Q Exactive mass spectrometer in data-dependent mode. MS/MS data was matched to peptide sequences, score filtered, and the relative amount of each peptide was quantified across samples.

CONCLUSIONS

This analysis identified over 200 phosphorylated peptides, over 7,500 acetylated peptides, and 7,800 succinylated peptides across the samples. These PTM peptides were from proteins representing all aspects of cellular biology. Among these, thousands of peptides were identified that changed between complete media and low nitrogen or low phosphate conditions. Many proteins in cellular metabolic pathways changed between samples, providing potential insights into how *E. coli* cellular signaling is regulated in response to low nutrient conditions. A perhaps surprising result is the number of proteins that show little change at many sites of acylation, however, they do show larger fold changes at specific lysine residues. This method can also be applied to other prokaryotic systems to study aspects of bacterial cell signaling, disease biology, or microbiome research.

REFERENCES

Rush J, Moritz A, Lee KA, Guo A, Goss VL, Spek EJ, Zhang H, Zha XM, Polakiewicz RD, Comb MJ (2005). "Immunoaffinity profiling of tyrosine phosphorylation in cancer cells". Nat. Biotechnol. 23(1), 94-101.

Jimmy K. Eng, Ashley L. McCormack, and John R. Yates, III (1994). "An Approach to Correlate Tandem Ma
Spectral Data of Peptides with Amino Acid Sequences in a Protein Database". J Am Soc Mass
Spectrom. 5 (11): 976–989

Biasini, M., Bienert, S., Waterhouse, A., Arnold, K., Studer, G., Schmidt, T., Kiefer, F., Cassarino, T.G.,
Bertoni, M., Bordoli, L., Schwede, "T. SWISS-MODEL: modelling protein tertiary and quaternary structure
using evolutionary information". Nucleic Acids Res. 42, W252-W258 (2014)

Quantitative Profiling of the Acetylome in Mouse Tissue using Lysine Acetylation Reagents in
Immunoaffinity LC-MS/MS. 0.9 MB. Hongbo Gu, Matthew P. Stokes, Allan Guo, Kimberly Lee, Jian Min Re
Xiaoying Jia, Vipin Suri, Jeffrey Silva. In 61st Annual American Society for Mass Spectrometry, ASMS (201
Minneapolis, MN

PhosphoSitePlus®,www.phosphosite.org

PTMScan Discovery

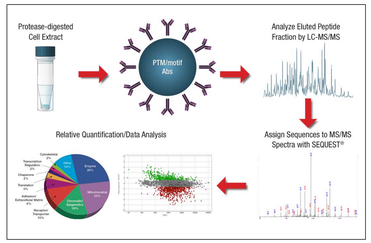

Figure 1. PTMScan® Discovery: PTMScan allows identification and quantification of hundreds to thousands of novel sites of post-translational modification (PTM) in a single LC-MS/MS analysis. This method can be used to profile protein phosphorylation, ubiquitination, acetylation, methylation, and many other critical PTMs.

PTMScan Antibodies for Lysine Acylation

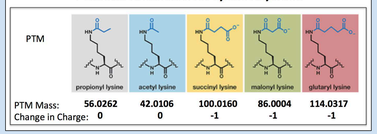

Figure 2. Acyl Lysine Modifications: Antibodies designed to enrich for peptides with the above acyl-lysine modifications: propionyl, acetyl, succinyl, malonyl, and glutaryl lysine. The change in mass and charge for each PTM is shown.

Experimental Details

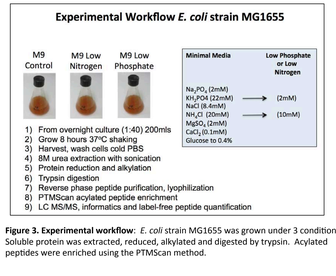

Figure 3. Experimental workflow: *E. coli* strain MG1655 was grown under 3 conditions. Soluble protein was extracted, reduced, alkylated and digested by trypsin. Acylated peptides were enriched using the PTMScan method.

Western Blotting

PTMScan Antibodies for Lysine Acylation

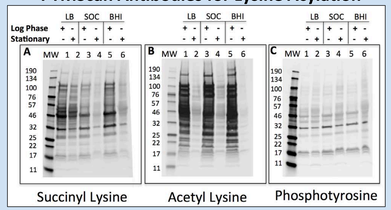

Figure 4. Western blotting results: The same antibodies used for peptide capture were used for western blotting. 20ug of protein extracted from MG1655 cells were run on 4-20% SDS-PAGE gradient gels and probed with antibodies (1:1,000) to succinyl lysine, **Panel A;** acetyl lysine, **Panel B** or phosphotyrosine, **Panel C**.

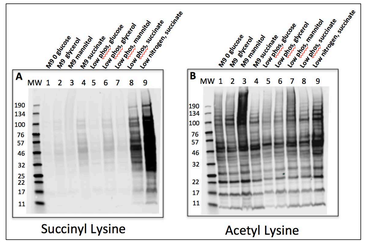

Figure 5. Western blotting results: The same antibodies used for peptide capture were used for western blotting. 20ug of protein extracted from MG1655 cells were run on 4-20% SDS-PAGE gradient gels and probed with antibodies (1:1,000) to succinyl lysine, **Panel A;** or acetyl lysine, **Panel B**.

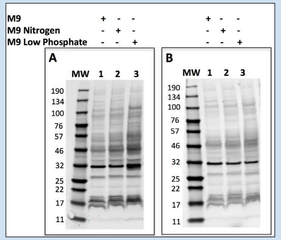

Figure 6. Western blotting results: 20ug of protein extract from MG1655 cells were loaded per lane and run on 4-20% SDS-PAGE gradient gels before transfer to nitrocellulose and probed with antibodies (1:1,000) to succinyl lysine, **Panel A;** acetyl lysine, **Panel B.**

Qualitative PTMScan Results

Peptides Identified

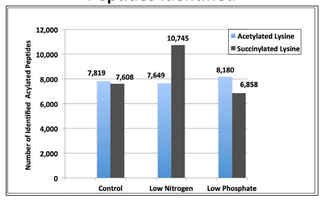

Figure 7. Qualitative Summary of PTMScan results: Shown is the number of unique tryptic peptides derived from 10mg of soluble protein. Acetylated and succinylated peptides were enriched for using the PTMScan method. Cells were grown in minimal media; minimal media with low nitrogen or low phosphate. MS/MS spectra were evaluated using SEQUEST 3G. Results for the average of technical replicates are shown for each condition.

Proteins identified

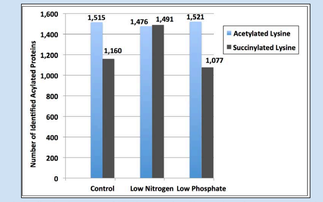

Figure 8. Qualitative Summary of PTMScan results: The number of parent proteins identified from PTMScan enrichment of acetylated or succinylated peptides derived from 10mg of soluble protein using *E. coli* strain MG1655. Cells were grown in minimal M9 media media; minimal media with low nitrogen or low phosphate.

Acylation Sequence Logos

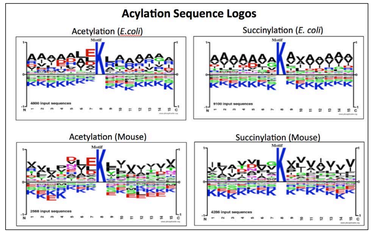

Figure 9. Motif Analysis of Acetylated and Succinylated peptides: Non-redundant peptide sequences were searched for motifs surrounding the modified lysine residue using Site Logo Generator from PhosphoSitePlus®. Peptides identified from the M9 minimal media condition were used for the *E. coli* samples; the acetylated mouse peptides were derived from Balb-C mouse liver.

Quantitative PTMScan Results

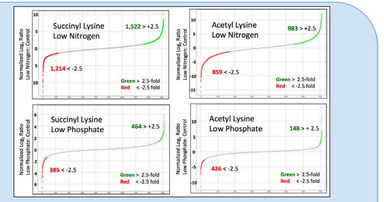

Figure 10. Sigmoidal Plots: Log_2 ratios were calculated from peptide intensity measurements (Skyline) between control and treated conditions. Peptides with ratios showing a fold change greater than 2.5-fold are in **green**, between 2.5 and -2.5 are **grey**, ratios lower than -2.5 are **red**. Control, M9 media; low nitrogen M9 media [10mM NH_4Cl]; low phosphate M9 media [2mM KH_2PO_4].

Changes In Succinylation cAMP-activated global transcriptional regulator

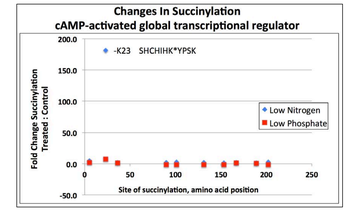

Figure 11. Plot of changes in succinylation: Log_2 median corrected fold-change ratios were determined by label-free quantitation (Skyline) of peptide intensity in the MS1 channel. Ratios were plotted for low nitrogen condition, **blue diamonds** and low phosphate **red squares**. Results show little change except for K23 which increases 181.3-fold under low nitrogen conditions.

Peptide identification and Quantification

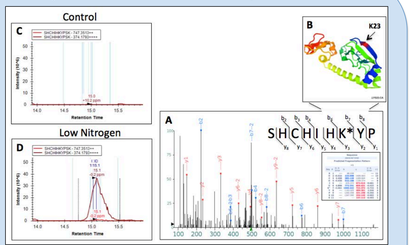

Figure 12. Peptide identification and quantification: The identification of the peptide succinylated at K23 of crp is based on the assignment of fragment ions, **Panel A.** SwissModel localizes the site of succinylation K23 in crp, **Panel B**. Peptide quantification based on area under the curve measurements for control condition, **Panel C,** as compared to low nitrogen condition, **Panel D**.

Quantitative PTMScan Results

Changes in Acylation of aceA Isocitrate lyase

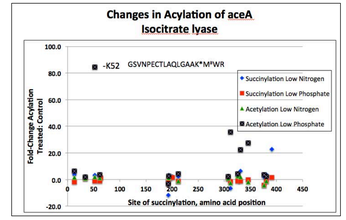

Figure 13., Plot of changes in acylation: For aceA, isocitrate lyase, the log_2 median corrected fold-change ratios were determined by label-free quantitation of peptide intensity in the MS1 channel (Skyline). Ratios were plotted for succinylation under low nitrogen condition, **blue diamonds** and low phosphate **red squares**; changes in acetylation under low nitrogen **green** triangles, low phosphate **black** squares.

Peptide identification and Quantification

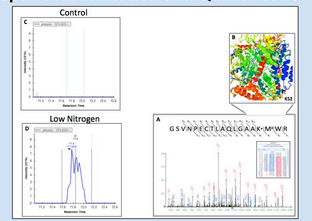

Figure 14. Peptide identification and quantification. The identification of the peptide acetylated at K52 of aceA is based on the assignment of fragment ions, **Panel A.** SwissModel shows the site of succinylation K52 in AceA, **Panel B**. Peptide quantification based on area under the curve measurements for control condition, **Panel C**, as compared to low nitrogen condition, **Panel D**.

Qualitative Overlap

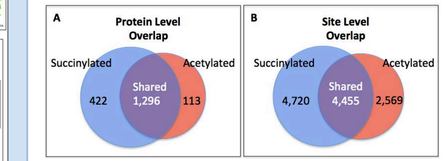

Figure 15. Venn diagrams: Summary of overlap between protein acetylation and succinylation. The total number of proteins identified with acetylated or succinylated lysine residues from all three conditions are shown, **Panel A**. The number of sites of acylation shared between the three experiments, **Panel B**.

For Research Use Only. Not For Use in Diagnostic Procedures

Charles L. Farnsworth, Ph.D.
email: cfarnsworth@cellsignal.com
978-867-2376
www.cellsignal.com/posters