Ancient regulatory role of lysine acetylation in central metabolism

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Introduction

- Post-translational modifications (PTMs) can regulate the activity and localization of proteins inside the cell.
- Whether enzyme function regulation by PTM is evolutionary conserved is poorly understood.
- We investigated evolutionary and functional aspects of PTMs by searching them in a phyloproteomics dataset from 48 distant bacteria.
- Our observations suggest that lysine acetylation is an evolutionary conserved mechanism of controlling central metabolic activity.

Methods

Proteomic workflow

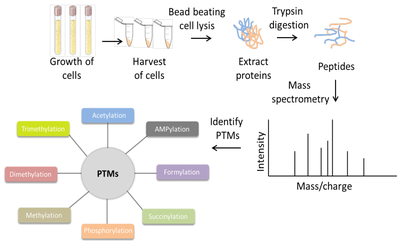

Fig. 1. Proteomic Workflow. Each organism was grown in pure cell culture, harvested, digested with trypsin and analyzed by liquid chromatography tandem mass spectrometry. MS/MS data was analyzed using MSGF+ for the identification of peptides, proteins and PTMs.

Results

- A comprehensive phyloproteomics library was built containing data from 48 diverse bacteria.
- PTMs were detected on evolutionary conserved amino acid residues.
- Conserved lysine acetylation is frequently observed in catalytic sites of enzymes.
- Acetylation of these lysine residues leads to inhibition of enzymatic activity.

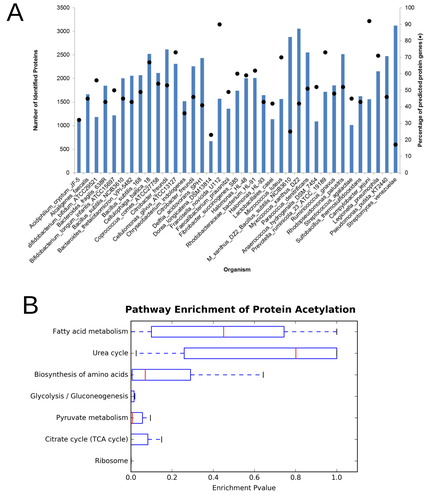

Figure 2. Proteome Coverage of Biodiversity Library. (A) Identified proteins for each organisms. (B) Pathways enriched in lysine-acetylated proteins. The graph represents the average of enrichment p-values across the 48 different bacterial strains.

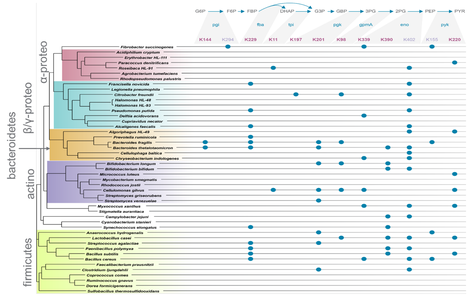

Fig. 3. Acetylation of substrate/cofactor binding lysine residues from glycolysis across taxa. Enzymes are listed along with universally conserved lysine residues. Observed acetylations are indicated with blue dots.

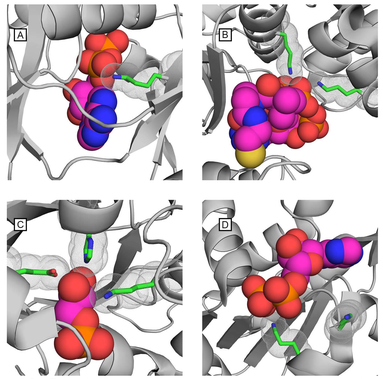

Fig. 4. Substrate binding lysine residues in metabolic enzymes. (A) Succinyl-CoA synthetase with the ADP cofactor. (B) Phosphotransacetylase with coenzyme A. (C) Triosephosphate isomerase glycerol-3-phosphate. Catalytically essential histidine and glutamic acid are also shown. (D) Phosphoglycerate kinase with the ADP cofactor.

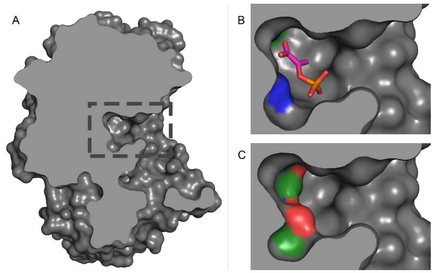

Fig. 5. Functional consequence of enolase acetylation. (A) The catalytic binding site of *S. elongatus* enolase. The boxed region is zoomed for panels B and C. (B) The binding pocket utilizes two lysine residues (shown in blue) to bind and stabilize phosphoenolpyruvate (shown as stick diagram). (C) Acetylating either active site lysine disrupts the electrostatic binding potential and the geometry of the binding site, precluding substrate binding.

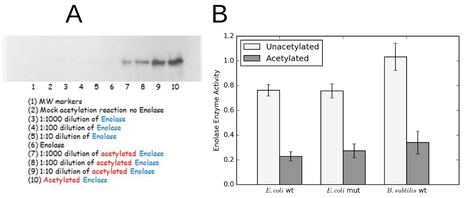

Fig. 6. – Effects of lysine acetylation on enolase activity. (A) Western blot showing lysine acetylation on samples treated with acetyl phosphate. (B) Enzyme activity assay showing that acetylation inhibits enolase catalytic activity, assay performed in triplicate. The *E. coli* mutant strain replaced seven lysine residues with glutamine (K->Q) that are not conserved or at the active site.

Conclusions

- We discovered a widespread lysine acetylation on evolutionarily conserved amino acid residues that are involved in catalysis.
- Our investigation shows that 2/3 of glycolytic and TCA cycle enzymes can be impacted by acetylation of highly conserved lysine residues located within their catalytic pockets.
- Lysine acetylation of catalytic sites represents an evolutionary conserved mechanism of enzymatic regulation.

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Career Opportunities: For potential openings in Integrative Omics Group at PNNL please visit http://omics.pnl.gov/careers

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