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Systematic Analysis of Mycobacterial Acylation Reveals First Example of Acylation-mediated Regulation of Enzyme Activity of a Bacterial Phosphatase*[S]

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Background: Post-translational modifications regulate *Mycobacterium tuberculosis* physiology and pathogenesis.
Results: Several novel signal transduction proteins are regulated by lysine acylation, including the virulence-associated protein-tyrosine phosphatase PtpB.
Conclusion: *M. tuberculosis* lysine acylation-regulated pathways affect other signal transduction modules.
Significance: This is the first report of acylation-dependent regulation of enzyme activity of a bacterial phosphatase.

Protein lysine acetylation is known to regulate multiple aspects of bacterial metabolism. However, its presence in mycobacterial signal transduction and virulence-associated proteins has not been studied. In this study, analysis of mycobacterial proteins from different cellular fractions indicated dynamic and widespread occurrence of lysine acetylation. *Mycobacterium tuberculosis* proteins regulating diverse physiological processes were then selected and expressed in the surrogate host *Mycobacterium smegmatis*. The purified proteins were analyzed for the presence of lysine acetylation, leading to the identification of 24 acetylated proteins. In addition, novel lysine succinylation and propionylation events were found to co-occur with acetylation on several proteins. Protein-tyrosine phosphatase B (PtpB), a secretory phosphatase that regulates phosphorylation of host proteins and plays a critical role in *Mycobacterium* infection, is modified by acetylation and succinylation at Lys-224. This residue is situated in a lid region that covers the enzyme's active site. Consequently, acetylation and succinylation negatively regulate the activity of PtpB.

Regulatory mechanisms in prokaryotic cells confer complexity to their relatively simpler genomes and proteomes. Post-translational modifications (PTMs)[7] add diversity to the proteome and regulate a myriad of cellular processes. The two extensively studied and arguably the most important PTMs are phosphorylation and acetylation. *Mycobacterium tuberculosis*, the causative agent of tuberculosis, employs several mechanisms to evade the host immune system; and PTMs play a significant role in this process. So far, the most widely known PTM in *Mycobacterium* is serine/threonine (Ser/Thr) phosphorylation, which is shown to be important for its survival and virulence (1–4). In addition, *Mycobacterium* encodes two tyrosine phosphatases (PtpA and PtpB) that are secreted in the host phagosome during infection and are critical for pathogenesis (5, 6).

Lysine acetylation is a ubiquitous modification that is conserved from eukaryotes to prokaryotes (7–10). The initial evidence of protein lysine acetylation in mycobacteria came with the characterization of a NAD^+-dependent deacetylase (11) and the identification of the first mycobacterial acetyltransferase (12). The acetyltransferases in *Mycobacterium smegmatis* (MSMEG_5458) and *M. tuberculosis* (Rv0998) contain a cyclic AMP (cAMP)-binding domain that is fused to a Gcn5-related *N*-acetyltransferase (GNAT)-like protein acetyltransferase domain (12). The mycobacterial acetyltransferase and deacetylase enzymes regulate the fatty acid metabolism via reversible acetylation of acetyl-CoA synthetase and fatty acyl-CoA synthetases (13–15). However, acetylation substrates in other biological pathways have yet to be revealed. The presence of extensive protein acetylation in several pathogenic and commercially utilized bacteria (10, 16–21) and the prediction of multiple acetyltransferases in *Mycobacterium* (Ref. 22 and the

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[7] The abbreviations used are: PTM, post-translational modification; pNPP, *p*-nitrophenol phosphate; Ni^{2+}-NTA, Ni^{2+}-nitrilotriacetic acid; PDB, Protein Data Bank.