

Research Article

Methylation of two-component response regulator MtrA in mycobacteria negatively modulates its DNA binding and transcriptional activation

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Post-translational modifications such as phosphorylation, nitrosylation, and pupylation modulate multiple cellular processes in *Mycobacterium tuberculosis*. While protein methylation at lysine and arginine residues is widespread in eukaryotes, to date only two methylated proteins in *Mtb* have been identified. Here, we report the identification of methylation at lysine and/or arginine residues in nine mycobacterial proteins. Among the proteins identified, we chose MtrA, an essential response regulator of a two-component signaling system, which gets methylated on multiple lysine and arginine residues to examine the functional consequences of methylation. While methylation of K207 confers a marginal decrease in the DNA-binding ability of MtrA, methylation of R122 or K204 significantly reduces the interaction with the DNA. Overexpression of S-adenosyl homocysteine hydrolase (SahH), an enzyme that modulates the levels of S-adenosyl methionine in mycobacteria decreases the extent of MtrA methylation. Most importantly, we show that decreased MtrA methylation results in transcriptional activation of *mtrA* and *sahH* promoters. Collectively, we identify novel methylated proteins, expand the list of modifications in mycobacteria by adding arginine methylation, and show that methylation regulates MtrA activity. We propose that protein methylation could be a more prevalent modification in mycobacterial proteins.

Introduction

Mycobacterium tuberculosis (*Mtb*), the causative agent of tuberculosis, is responsible for nearly one million deaths annually around the globe [1]. It resides dormant in the host for decades without detection and when the immune system wanes, it proliferates and causes active disease. The adeptness of mycobacteria to hijack the host cell can be attributed to the fine-tuning of signaling pathways. Post-translational modifications (PTMs) including serine/threonine phosphorylation, nitrosylation, and pupylation (addition of prokaryotic ubiquitin-like protein) play an important role in regulating mycobacterial physiology and virulence [2–6]. While there are few specific examples of how these modifications affect the function of a protein, more mechanistic insight is required to delineate their regulatory roles. In addition to these modifications, proteins can be post-translationally modified by the addition of methyl groups, catalyzed by S-adenosyl methionine (SAM) dependent methyltransferases [7], at the ε-amino group of lysine, guanidino group of arginine, or oxygen in the carboxylate side chain of glutamate [8–10]. Glutamate methylation of methyl-accepting chemotaxis proteins play a biologically conserved role in chemotaxis and provide rotational directionality to bacteria [11].

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