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Review

Beyond gene expression: The impact of protein post-translational modifications in bacteria☆

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ABSTRACT

The post-translational modification (PTM) of proteins plays a critical role in the regulation of a broad range of cellular processes in eukaryotes. Yet their role in governing similar systems in the conventionally presumed 'simpler' forms of life has been largely neglected and, until recently, was thought to occur only rarely, with some modifications assumed to be limited to higher organisms alone. Recent developments in mass spectrometry-based proteomics have provided an unparalleled power to enrich, identify and quantify peptides with PTMs. Additional modifications to biological molecules such as lipids and carbohydrates that are essential for bacterial pathophysiology have only recently been detected on proteins. Here we review bacterial protein PTMs, focusing on phosphorylation, acetylation, proteolytic degradation, methylation and lipidation and the roles they play in bacterial adaptation — thus highlighting the importance of proteomic techniques in a field that is only just in its infancy.
This article is part of a Special Issue entitled: Trends in Microbial Proteomics.

Contents

Abbreviations: AcK, lysine acetylation; AcS, acetyl-coA synthetase; ART, ADP-ribosyltransferase; CID, collision-induced dissociation; GNAT, GCN5-like protein *N*-acetyltransferase; HCD, higher collisional dissociation; LMW, low molecular weight; Lnt, apolipoprotein transacylase; Lol, localisation of lipoproteins; MCP, methyl-accepting chemotaxis protein; NAT, *N*-acetyltransferase; pCh, phosphocholine; pEtN, phosphoethanolamine; Pup, protein ubiquitin-like protein; PTM, post-translational modification; TCR, two-component regulation.

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1. Introduction

The lower than anticipated number of coding regions contained within the human genome has led to the conundrum of how our complexity is derived. We are now aware of several levels of regulation at both the transcript and protein levels. The diversity of protein functions is generated by post-translational modifications (PTM) that alter the structure/function relationship and impact protein complex formation, enzyme catalysis and other biomolecule interactions (Table 1). The sheer number of potential PTM and the diverse range of protein targets has challenged the central dogma of molecular biology that postulates one gene giving rise to one gene-product (protein) with a single function — a concept originally derived from the study of bacterial systems. The advent of mass spectrometry (MS)-based proteomics has shown that prokaryotes are capable of modifying proteins with an extensive array of PTM and that these have a profound influence on bacterial physiology and virulence [1]. As an example, recent work has demonstrated that prokaryotes are capable of modifying proteins by phosphorylation at previously assumed 'eukaryotic-type' serine/threonine and tyrosine kinase sites, in addition to the well-characterized two-component regulatory systems that sense changes in the environment and relay this to effector proteins *via* phospho-relay from a sensor histidine kinase to aspartic acid in a 'response regulator' that binds to the promoter regions of target genes. Such 'fast switching' events allow microorganisms to adjust to external stimuli in a fashion that cannot be met as quickly with gene expression. One specific example of this is the acetyltransferase AcuA from *Bacillus subtilis* that has a k_{cat} of 0.3 s^{-1} and is energy efficient, and most importantly — reversible [2].

Methods for high-throughput PTM identification have vastly improved in efficacy with the application of MS. Modified proteins are typically in abundances that cannot be observed by regular shotgun proteomics without prior enrichment. Enrichment strategies and the increased power in sensitivity, scan rate, resolution and fragmentation in emerging instrumentation has provided an unprecedented potential for detecting PTMs. The presence of a PTM can be inferred through differences in the predicted mass compared to the experimentally obtained mass of a peptide (or protein), while isobaric PTMs are typically distinguished based upon unique differences in fragmentation patterns [3]. New bacterial PTM are constantly being discovered (e.g. hydroxymethylation — Waridel and colleagues recently demonstrated the presence of hydroxymethylated Asn and Gln on surface-exposed proteins from *Staphylococcus aureus* [4]), as well as new targets of known PTM. Consequently, characterisation of PTM, particularly within bacterial systems, is a burgeoning field. The aim of this review is to provide an overview of the state of current research into the identification and biological significance of bacterial protein PTM.

2. Phosphorylation

Protein phosphorylation is the attachment of phosphate onto the functional groups of amino acid side chains. This is observed commonly in eukaryotes on the hydroxyl groups of Ser and Thr, and to a lesser extent, the phenol-based side chain of Tyr. Attachment of phosphate to Lys, Arg, His, Asp, Glu and Cys has also been observed to varying degrees *in vivo* [5,6], with His and Asp (two-component regulation) thought to be the most common in bacteria. Classically, phosphorylation has been studied