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Review

Bacterial Phosphoproteomic Analysis Reveals the Correlation Between Protein Phosphorylation and Bacterial Pathogenicity

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Abstract

Increasing evidence shows that protein phosphorylation on serine, threonine and tyrosine residues is a major regulatory post-translational modification in the bacteria. This review focuses on the implications of bacterial phosphoproteome in bacterial pathogenicity and highlights recent development of methods in phosphoproteomics and the connectivity of the phosphorylation networks. Recent technical developments in the high accuracy mass spectrometry have dramatically transformed proteomics and made it possible the characterization of a few exhaustive site-specific bacterial phosphoproteomes. The high abundance of tyrosine phosphorylations in a few bacterial phosphoproteomes suggests their roles in the pathogenicity, especially in the case of pathogen–host interactions; the high abundance of multi-phosphorylation sites in bacterial phosphoprotein is a compensation of the relatively small phosphorylation size and an indicator of the delicate regulation of protein functions.

Key words: protein phosphorylation, bacterium, pathogenicity, phosphoproteomics

Introduction

Post-translational modifications are essential for the rapid and reversible modification of the physiochemical properties of a protein, resulting in the changes of enzyme activity, oligomerization state, protein–protein interaction, subcellular localization or half-life (*1*). Protein phosphorylation is the most abundant and biologically the most important post-translational modification on the tyrosine, serine and threonine residues, and is catalyzed reversibly by specific protein kinases and phosphatases. Bacteria and some plants rely on histidine autophosphorylation of the sensory kinases and aspartate phosphorylation of the response regulators (thus two-component systems) (*2*). Protein phosphorylation is perhaps the best studied due to the close association between dys-regulated phosphorylation and human pathologies (*3*). Therefore, it is extremely important to determine the degree and the site of the *in vivo* protein phosphorylation. However, due to the very low stoichiometry, limited dynamic range, high complexity and quantitative difficulties of protein phosphorylations, highly selective enrichment procedures and sensitive mass spectrometry (MS) are required to decipher the phosphoproteome (*4*). Selective phosphopeptide enrichment has been accomplished in several ways by using anti-phosphotyrosine antibodies, immobilized metal affinity chromatography (IMAC), chemical modifications or strong cation exchange chromatography (*5*). The seamless combination of IMAC and nano-liquid chromatography en-

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ables reproducible separation and identification of phosphopeptides in a low-femtomole range (*6, 7*), and thus it is the most frequently used method in the study of cellular phosphorylation.

For some time, protein phosphorylation was considered to exist exclusively in eukaryotes until the first observation of protein phosphorylation occurring in *Escherichia coli* (*8, 9*). Indeed, protein phosphorylation is fundamental to the regulation of all kinds of physiological processes for the bacteria, especially for several key steps in the infection process, such as adhesion to the host, triggering and regulation of pathogenic functions as well as biochemical warfare, scrambling the host signaling cascades and impairing its defense mechanisms (*10, 11*). The global phosphoproteome has been established in a number of bacteria (**Table 1**), including *Corynebacterium glutamicum* (*12*), *Campylobacter jejuni* (*13*), *Bacillus subtilis* (*14-16*), *E. coli* (*17*), *Latococcus lactis* (*18*), *Streptococcus pneumoniae* (*19*), *Klebsiella pneumoniae* (*20*), *Mycoplasma pneumoniae* (*21*), *Pseudomonas* species (*22*), *Mycobacterium tuberculosis* (*23*), *Streptomyces coelicolor* (*24*) and *Helicobacter pylori* (*13, 25*). Phosphorylation in the bacteria was biased toward threonine compared with serine, while serine phosphorylation may account for 80%-90% of total phosphorylation sites in the eukaryotes (*26*). *L. lactis*, *C. jejuni*, and *S. coelicolor* contain more phosphorylation sites of threonine than serine (Table 1).

The most abundant subset of phosphorylated proteins is the enzymes involved in the central carbon/protein/nucleotide metabolism, and some other phosphorylated housekeeping proteins are helicases, chaperones, ribosomal proteins and amino acyl tRNA-synthetases (*27*). One quantitative phosphoproteomic analysis on the model bacterium *B. subtilis* has been performed with stable isotope labeling by amino acids in cell culture (SILAC) (*28*). The striking distinction between the known metazoan and bacterial phosphoproteomes are the extent: 30%-50% of proteins are phosphorylated in humans (*29, 30*), whereas it is at least one order of magnitude lower in bacteria (*27*). The only notable exception is *M. tuberculosis*, whose yield was significantly high: 516 phosphorylation sites in 301 phosphoproteins, accounting for >7% of *M. tuberculosis* proteins (*23*). Differential roles were proposed for the protein phosphorylations in eukaryotes and prokaryotes: protein phosphorylation in the eukaryotes is extensively used for transduction of signals inter- and intra-cellularly, whereas the function may be less central in the prokaryotes (*16*). Bacterial phosphorylation sites can be conferred by two major search algorithms NetPhosBac (*31*) and Diphos (*32*), which were summarized in a recent review paper (*27*). Here we will focus on an overview of the recent advances in the field of bacterial phosphoproteome, highlighting recent methods in phosphoproteomics, connectivity of the phosphorylation networks, as well as the correlation between pathological potentials and the known bacterial phosphoproteomes.

Table 1 The bacterial phosphoproteomes identified so far

Bacterium*	No. of phosphopeptides	No. of phospho sites	% of serine	% of threonine	% of tyrosine
Escherichia coli (*17*)	105	81	67.9	23.5	8.6
Bacillus subtilis (*16*)	103	78	69.2	20.5	10.3
Latococcus lactis (*18*)	102	79	46.5	50.6	2.7
Pseudomonas putida (*22*)	56	53	52.8	39.6	7.5
Pseudomonas aeruginosa (*22*)	57	55	52.7	32.7	14.5
Campylobacter jejuni (*13*)	58	35	30.3	72.7	9.1
Streptococcus pneumoniae (*19*)	102	163	47.2	43.8	9.0
Streptomyces coelicolor (*24*)	44	44	34.1	52.3	13.6
Klebsiella pneumoniae (*20*)	117	93	31.2	15.1	25.8
Mycoplasma pneumoniae (*21*)	15	15	53.3	46.7	0
Helicobacter pylori (*25*)	80	124	42.8	38.7	18.5

*Bacterial name followed by the reference in brackets.