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Regulation of bacterial physiology by lysine acetylation of proteins

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Post-translational modification of proteins is a reversible mechanism of cellular adaptation to changing environmental conditions. In eukaryotes, the physiological relevance of N-ε-lysine protein acetylation is well demonstrated. In recent times, important roles in the regulation of metabolic processes in bacteria are being uncovered, adding complexity to cellular regulatory networks.

The aim of this mini-review is to sum up the current state-of-the-art in the regulation of bacterial physiology by protein acetylation. Current knowledge on the molecular biology aspects of known bacterial protein acetyltransferases and deacetylases will be summarized. Protein acetylation in *Escherichia coli*, *Salmonella enterica*, *Bacillus subtilis*, *Rhodopseudomonas palustris* and *Mycobacterium tuberculosis*, will be explained in the light of their physiological relevance. Progress in the elucidation of bacterial acetylomes and the emerging understanding of chemical acylation mechanisms will be discussed together with their regulatory and evolutionary implications. Fundamental molecular studies detailing this recently discovered regulatory mechanism pave the way for their prospective application for the construction of synthetic regulation networks.

Introduction – concept and historical overview

Post-translational modification (PTM) of proteins is an evolutionarily conserved strategy used by organisms for the efficient control of their biological activities, allowing them to exert rapid adaptive responses to environmental changes. Several types of PTMs exist in Nature. Among the more prominent are serine, threonine, and tyrosine phosphorylation; lysine ubiquitination, sumoylation, and neddylation; lysine acylation; lysine and arginine methylation; proline isomerization; and N- and O-glycosylation, which occurs on several residues, but mainly on serine, threonine and asparagine [1].

In the past years, protein acylation is receiving increasing attention because its involvement in several mitochondrial, nuclear and cytosolic processes [2–5]. Protein acylation can be defined as the transfer of an acyl group from a convenient biochemical donor molecule to an amino group on a protein. Many proteins can be acylated by activated acyl groups such as acyl-CoAs and acyl-phosphates. Thus, proteins can be acetylated, propionylated, butyrylated or succinylated [6,7]. Although some of these processes are catalyzed by specific transferases, they can also occur non-enzymatically. It is not clear yet whether these events are spontaneous or auto-catalytical. Among all these acylation modifications, protein acetylation is by far the best known.

Acetylation of lysine residues of proteins was first identified in histones more than 40 years ago. Recent reports have shown that a high number of metabolic enzymes are acetylated, which has led to suggesting an evolutionarily conserved mean of regulation of intermediary metabolism [8,9]. Protein acetylation and deacetylation is catalyzed by protein acetyltransferases and deacetylases, respectively, of which several families exist. In addition, several proteins acetylate in the presence of reactive

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acetyl derivatives, such as acetyl-phosphate, acetyl-CoA or acetyl-AMP [10–12].

Based on the chemical nature of the acetylated amino group, two types of protein acetylation can be considered, each one exhibiting specific characteristic features. The acetylation of the α-amino group of the N-terminal amino acid of proteins is very rare in bacteria [13] but frequent in eukaryotes (30–80% of proteins) and archaea (14–29% of proteins) [14]. On the other hand, the acetylation of proteins at the ε-amino group of internal lysine residues is a widely distributed PTM, frequent in all domains of life. This is a reversible post-translational process which has now been found on over 1500 eukaryotic proteins with diverse functions and locations [4,15,16]. Lysine acetylation exerts various effects on many of their critical functional properties, including interference with catalytic mechanisms, interaction with other proteins and DNA, stability, and sub-cellular distribution [2,4].

Until very recently, few bacterial proteins were known to be acetylated. However, the increasing power of high throughput proteomic techniques is starting to change this view [4,5]. Acetylation of microbial proteins has been excellently reviewed [14–16]. The aim of this mini-review is to sum up the current state of the art in the roles of protein acetylation in bacteria, with a special focus on the implications of chemical and enzymatic acetylation of proteins on cellular physiology.

Diversity of bacterial protein acetyltransferases and deacetylases

Protein acetyltransferases

N-acetyltransferases catalyze the transfer of an acetyl group from acetyl-CoA to a primary amine on a substrate. The acetylation of the ε-amino group of lysine residues of proteins is catalyzed by lysine acetyltransferases (often abbreviated to KATs or LATs), formerly known as histone acetyltransferases (HATs). Several KATs are known in eukaryotes, which are structurally diverse. All families contain a homologous acetyl-CoA binding core segment, which raises the possibility that they have evolved from a common ancestral protein [17].

Enzymes belonging to the Gcn5-related N-acetyltransferase (GNAT) superfamily (pfam00583) are conserved in all domains of life and are best described as enzymes that utilize acyl coenzyme A (CoA) as a donor for the acylation of the ε-amino group of lysine residues of proteins and small molecules. Many of the bacterial KATs described belong to this group. This is the case of *Salmonella enterica*: in 2004, the gene encoding the KAT *Se*Pat was identified in this organism. This protein is responsible for the acylation of acetyl-CoA and propionyl-CoA synthetases [18,19]. There is controversy on the acetylation of other metabolic proteins by *Se*Pat in *S. enterica*. Wang and col. described that the metabolic enzymes GapA, AceA and AceK are acetylated by *Se*Pat [9], but it has been claimed that these results cannot be reproduced [20].

*Se*Pat possesses two domains: a C-terminal domain homologous to GNAT acetyltransferases and a N-terminal domain with high homology to NDP-forming acetyl-CoA synthetase. However, it lacks acetyl-CoA forming activity because the replacement of a histidine residue which is critical for catalysis [19,21]. *Se*Pat shows sigmoidal kinetics and positive cooperativity for acetyl-CoA and a biphasic interaction with acetyl-CoA, which are unusual in GNATs. The structural basis for this behavior is the tetramerization of the protein in the presence of acetyl-CoA [21]. This biphasic acetyl-CoA binding relies on the acetyl-CoA synthetase-like domain, which allows it to respond to small variations in the acetyl-CoA concentration. This characteristic contributes to maintaining acetyl-CoA homeostasis during acetate uptake [21,22].

Other microorganisms contain KATs from the GNAT superfamily that lack the putative acetyl-CoA synthetase domain found in *Se*Pat (such as AcuA from *B. subtilis*) or have a regulatory domain absent in the *Se*Pat (such as *Mt*Pat from *Mycobacterium tuberculosis* which is responsive to cyclic nucleotides). In addition, many organisms that contain homologs to protein lysine deacetylases lack Pat homologs, an observation that suggests the existence of functional orthologs with distinct evolutionary lineage [19].

Protein deacetylases

Lysine deacetylases (KDACs) catalyze the cleavage of N-acetyl amide moieties. Eukaryotic KDACs are subdivided into four classes on the basis of phylogenetic criteria [23]. According to their reaction mechanism, two types of deacetylases can be discerned: simple hydrolases, which release the acetyl group as free acetate, and sirtuins, which are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases. Sirtuins use NAD^+ as a co-substrate, which is cleaved in the catalytic cycle, generating nicotinamide and 2′-O-acetyl-ADP-ribose (OAADPr) as co-products. KDACs are conserved proteins with an ancient origin, and they are expected to participate in basic processes well conserved across organisms [23].

Sirtuins typically consist of a conserved catalytic core domain (~250 aa) and two optional and highly variable N- and C-terminal domain (50–300 aa) [23,24]. It is now evident that sirtuin protein sequences are broadly conserved within the three domains of bacteria, archaea and eukaryotes. Of special relevance is the involvement of sirtuins in the response to nutrients availability (calorie restriction response) and, therefore, in metabolic regulation [2].

Both types of KDACs are found in bacteria. In bacteria, the CobB protein in *S. enterica* is the best characterized sirtuin [25], although they have also been found in *E. coli* [26], *B. subtilis* [27], *Rhodopseudomonas palustris* [28], *Streptomyces coelicolor* [29] and other genera. Phylogenetic analysis reveals that bacterial sirtuins are related to the mitochondrial Sirt4 and Sirt5 from humans, while archaeal sirtuins are closer to eukaryotic Sirt6 and Sirt7. Eukaryotic Sirt1, Sirt2 and Sirt3 cluster separately, together with yeast sirtuins [24]. No recognizable hydrolytic (non-sirtuin) deacetylases have been found in many individual eubacterial species (e.g. *E. coli*) [23], although they have been described in *B. subtilis* [30] and *R. palustris* [28]. *M. smegmatis* possesses a predicted non-sirtuin deacetylase although its role has not been experimentally demonstrated [31].

Protein acetylation in bacteria: targeted pathways

Modulation of the activity of bacterial proteins by site-specific acetylation has long been known. One classical example is CheY, which regulates chemotaxis through the acetylation of two specific lysine residues which impairs binding to components of the flagellar motor switch complex and chemotaxis machinery [32,33]. Nevertheless, it was not until 2002, that the regulation of a bacterial enzyme by reversible acetylation/deacetylation was described for the first time [25]. Major advances in the identification of acetylated proteins have been done in *E. coli*, *S. enterica*, *B.*

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