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Control of Protein Function by Reversible *N*^ε-lysine Acetylation in Bacteria

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Abstract

Recently published work indicates that reversible *N*_ε-lysine (*N*^ε-Lys) acetylation of proteins in bacteria may be as diverse, and as important for cellular function, as it has been reported in eukaryotes for the last five decades. In addition to biochemical and genetic approaches, proteomic studies have identified *N*^ε-Lys acetylation of proteins and enzymes involved in diverse cellular activities such as transcription, translation, stress response, detoxification, and especially carbohydrate and energy metabolism. These findings provide a platform for elucidating the molecular mechanisms behind modulation of enzyme activity by *N*^ε-Lys acetylation, as well as for understanding how the prokaryotic cell maintains homeostasis in a changing environment.

Introduction

Since the discovery of *N*^ε-lysine (*N*^ε-Lys) acetylation of the *S. enterica* Acs enzyme in 2002 [1], there have been several publications in recent years indicating a broader usage of *N*^ε-Lys acetylation by prokaryotes as a means of modulating enzyme activity, in particular, those enzymes involved in metabolism.

Until recently, the extent of *N*^ε-Lys acetylation in prokaryotes was unexplored. Unlike the acetylation of the α-amino group of lysine residues, *N*^ε-Lys acetylation is a reversible posttranslational modification, performed by acetyltransferases [2] and partner deacetylases [3], which has been extensively studied within the context of chromatin remodeling [4]. The probability that *N*^ε-Lys acetylation is an important regulatory mechanism of protein function in bacteria is high since known acetyl-Coenzyme A (Ac-CoA)-dependent acetyltransferases, Gcn5-like protein *N*-acetyltransferases (GNATs, COG1670) [5,6] and NAD⁺-dependent Sir2-like protein deacetylases (a.k.a. sirtuins, cd01412) [7], are conserved in all domains of life.

In the last two years, however, three independent studies utilizing a proteomics approach reported *N*^ε-Lys acetylation of diverse proteins in *E. coli* [8,9] and *S. enterica* [10]. These studies utilized immunoaffinity purification with anti-acetyllysine antibodies coupled with mass spectrometry analysis to identify the proteins affected by *N*^ε-Lys acetylation, and to reveal the number of acetylated residues per protein.

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The purpose of this review is to highlight recent reports that provide a basis for understanding the control of metabolic enzyme activity by Ac-CoA-dependent N^{ϵ} -Lys acetylation in prokaryotic physiology.

Acetyl-Coenzyme A dependent N^{ϵ} -Lys acetylation of metabolic enzymes by the protein acetyltransferase (Pat) enzyme in *S. enterica*

The *S. enterica* protein acetyltransferase (Pat; formerly YfiQ) enzyme is a GCN5-related N -acetyltransferase (GNAT) involved in the posttranslational regulation of central metabolic enzymes [10–12]. GNATs are a superfamily of histone acetyltransferases (HATs; now known as KATs for lysine acetyltransferases [13]) that perform acetyl transfers using Ac-CoA as donor. The kinetic and catalytic behavior of the GNAT family is predicted to be conserved, following a sequential mechanism where a ternary complex in which an active site glutamate deprotonates the substrate lysine, allowing it to directly attack the carbonyl carbon of Ac-CoA (Fig. 1) [2,14].

In *S. enterica*, acetyl-CoA synthetase (Acs; AMP-forming; EC 6.2.1.1; [15]) is inactivated upon Ac-CoA-dependent acetylation of a single active-site lysyl residue, Lys609, by Pat (Fig. 2) [11]. Acetylated Acs (Acs^{Ac}) then serves as substrate for the sirtuin-like deacetylase, CobB, which reactivates Acs^{Ac} by NAD^{+} -dependent deacetylation [1,16,17]. Acs is a ubiquitous enzyme important for the conversion of acetate to its high-energy intermediate Ac-CoA, a necessary step for its utilization for processes such as lipid synthesis and energy generation (Fig. 2) [18]. The activity of human homologues of Acs is also modulated by reversible N^{ϵ} -Lys acetylation [19,20].

Acs produces Ac-CoA via two half-reactions [21–23]. First, Acs converts acetate and ATP to acetyladenylate (Ac-AMP) and pyrophosphate (PP_i). In the second half-reaction, Acs transfers the acetyl group of Ac-AMP to CoASH to form Ac-CoA, releasing AMP. In both *S. enterica* and *E. coli*, Acs is considered an anabolic, high affinity pathway for acetate assimilation, serving mainly to scavenge for acetate when the concentration of acetate in the environment is low (<10 mM) [15,21,22]. In *S. enterica*, Pat inactivates Acs by acetylation of the active site residue Lys609, preventing the conversion of acetate into acetyl-AMP (Fig. 2) [11,22,23].

Because the lysyl residue modified in Acs is conserved in the AMP-forming family of acyl-CoA synthetases, it was suggested that the activity of these enzymes may be modulated by reversible N^{ϵ} -Lys acetylation [1]. In fact, both Pat and CobB were reported to modify propionyl-CoA synthetase (PrpE) by acylation, but in this case the activity of the PrpE enzyme was controlled by propionylation/depropionylation of a lysyl residue. The propionyltransferase activity of Pat and the depropionylase activity of the CobB sirtuin were studied *in vivo* [12,16] and *in vitro* [12]. The modified lysine in PrpE, Lys592, is located within the acetylation motif found in Acs, and like Acs, this residue is critical for its adenylation activity but not for thioester formation [23]. Interestingly, propionylated lysine residues have also been identified in histones [24].

More recently, Pat was reported to regulate the activities of three other metabolic enzymes by N^{ϵ} -Lys acetylation in *S. enterica*: glyceraldehyde phosphate dehydrogenase (GapA), isocitrate lyase (AceA), and isocitrate dehydrogenase kinase/phosphatase (AceK) [10]. These targets were identified by Wang *et al.* [10] using a proteomics approach via affinity immunoseparation with anti-acetyllysine antibodies, followed by mass spectrometry analysis. These authors reported that approximately 50% of the 191 acetylated proteins (235 acetylated peptides) identified by these approaches were involved in metabolism. Reversible N^{ϵ} -Lys acetylation of some enzymes (*i.e.*, GapA, AceA, AceK) was monitored *in vivo* and *in*

vitro. The authors concluded that Pat and CobB control GapA, AceA, and AceK activities in positive and negative ways. It is important to note that while Wang *et al.* identified Acs to be acetylated, the acetylated lysyl residue (Lys68) was far from the active site, and was not the known target of acetylation by Pat, *i.e.*, Lys609. This discrepancy may be a reflection of the method used to identify these sites, which is discussed in the next section.

***N*^ε-Lys acetylation of metabolic enzymes in *E. coli* – a proteomics approach**

Proteomics analyses of *N*^ε-Lys acetylation were also performed in *E. coli* [8,9]. Like the study in *S. enterica*, the majority of the *N*^ε-Lys acetylation modifications [~49% of 85 proteins (125 acetylation sites) [8] and ~53% of 91 proteins (138 acetylation sites)] [9] were identified in enzymes involved in metabolism, particularly those involved in the metabolism of energy, fatty acids, and nucleotides (Fig. 3A). This is significant because there was not much overall consensus on proteins modified by acetylation (Fig. 3B), and even among the same proteins identified, the site(s) assigned as acetylated sometimes did not match. This difference is likely due to the different strains (in the case of the *E. coli* studies, W3110 [8] and DH5 [9]) and growth conditions used, as well as the different anti-acetyllysine antibodies used, which likely have dissimilar binding affinities. Further, all three proteomics studies identified trypsin-generated peptides from experimentally generated tandem mass spectral data matched to the theoretical best match from a protein database. Although this is the typical approach, it has been reported to be inefficient, generating less positive hits, as well as generating false positives [25] even when score filtering criteria are used. Nonetheless, all three studies found the bulk of acetyl modifications in enzymes involved in metabolism, suggesting that the primary role of *N*^ε-Lys acetylation is to control the metabolic fates of carbon and energy sources.

***N*^ε-Lys acetylation of metabolic enzymes in other bacteria**

Metabolic enzymes whose activities are modulated by reversible *N*^ε-Lys acetylation were recently reported in the Gram-negative, purple non-sulfur α -proteobacterium, *Rhodospseudomonas palustris* [26]. In this bacterium, the *in vitro* activities of four acyl-CoA synthetases [*i.e.*, acetyl-CoA synthetase (Acs), benzoyl-CoA synthetase (BadA), 4-hydroxybenzoyl-CoA synthetase (HbaA) and cyclohexanecarboxyl-CoA (AliA)] were reported to be inactivated upon acetylation by the *R. palustris* Pat enzyme [26] at the conserved lysine located within the acetylation motif present in the AMP-forming family of acyl-CoA synthetases. Reactivation of BadA^{Ac}, HbaA^{Ac} and AliA^{Ac} was achieved upon deacetylation by LdaA (lysine deacetylase A), an acetate-forming protein deacetylase. In the same study, *S. enterica* CobB sirtuin was also reported to reactivate BadAAc. The activity of *R. palustris* Acs was not tested. Since *R. palustris* converts aromatic compounds into Ac-CoA for carbon and energy under anaerobic conditions, these findings suggest that reversible *N*^ε-Lys acetylation is important for the metabolism of these compounds. Further, one can extrapolate that in addition to Ac-CoA and propionyl-CoA serving as acyl-donors for the enzymes that modify Acs and PrpE, respectively, other acyl donors could behave in similar fashion [26].

In the Gram-positive, common soil bacterium *B. subtilis*, AcsA (acetyl-Coenzyme A synthetase) is also regulated by acetylation [27,28]. The acetyltransferase was identified as acetoin catabolism protein AcuA, a GNAT-like protein that appears to be just slightly larger than the predicted conserved GNAT catalytic fold [14]. However, the control of AcsA activity appears to be more complex than that reported in *S. enterica* since there were two deacetylases involved in the deacetylation of AcsA^{Ac}, SrtN, an NAD⁺-dependent protein deacetylase sirtuin, and AcuC, a deacetylase that does not require NAD⁺ as co-substrate

[29]. Like the other AMP-forming enzymes investigated, acetylation of the conserved active-site lysine inactivated the enzyme, while deacetylation restored its activity.

***N*^ε-Lys acetylation of *E. coli* CheY and CobB-mediated deacetylation of CheY^{Ac} reveals a link between chemotaxis and the carbon and energy state of the cell**

Chemotaxis is not a metabolic process, but it affects the overall cellular physiology and metabolism of the cell. Recently, the *E. coli* response regulator CheY was reported to be acetylated *in vivo* [30]. Other reports showed that acetylation of CheY had a negative effect on binding to its targets [31], and that CobB was able to regulate chemotaxis by deacetylation of CheY^{Ac} [32]. Thus, like the AMP-forming enzymes discussed here, CheY activity appears to be directly affected by sirtuin-dependent, reversible *N*^ε-Lys acetylation. The identity of the acetyltransferase that modifies CheY remains unknown.

Reversible *N*^ε-Lys acetylation of transcription factors in bacteria – a likely conserved mode of control

In response to a changing environment, the ability to regulate gene expression is just as crucial as that of metabolic control. In eukaryotes, the effect of *N*^ε-Lys acetylation on gene expression has been studied since the discovery of this posttranslational modification in histones in the 1960's [33,34], and has been expanded to include non-histone proteins such as transcriptional regulators and DNA-binding proteins (recently reviewed in [35]). Subsequently, this mode of regulation has been reported in archaea [36,37].

Until recently, the effect of *N*^ε-Lys acetylation on gene expression in bacteria was largely overlooked. However, two developments have suggested that *N*^ε-Lys acetylation may directly affect gene expression in bacteria. First, all three proteomics studies discussed above identified *N*^ε-Lys acetylation in proteins involved in transcriptional and translational regulation [8–10]. However, the effects of these modifications, both *in vitro* and *in vivo*, have yet to be reported. The second development was the report of the *in vitro* acetylation of *E. coli* RcsB (regulator capsule synthesis B), a bacterial response regulator involved in the activation of colanic acid capsule synthesis and cell division (reviewed in [38]), by both *E. coli* and *S. enterica* Pat [39]. Using an *E. coli* proteome microarray [40], these authors showed that RcsB and several other transcription factors were acetylated by *S. enterica* Pat. Further analysis showed that RcsB was acetylated at a single Lys residue, Lys180, located within the DNA-binding, helix-turn-helix motif of the protein [41]. *In vitro* and *in vivo* analyses showed that RcsB^{Ac} and variant RcsB proteins with substitutions at Lys180 were defective in their DNA-binding activity. However, *in vivo* acetylation of RcsB has yet to be reported. Nonetheless, acetylation was reversed by the *E. coli* and *S. enterica* CobB sirtuin, demonstrating the conserved, reversible nature of this modification.

Conclusions

The recent data reviewed here indicate that reversible *N*^ε-Lys acetylation of metabolic proteins is conserved in bacteria. Cells of all domains of life monitor their energy and carbon states to maintain a balance between energy generation and consumption. Two indicators of the energy and carbon states of the cell are the NAD⁺:NADH and Ac-CoA:CoA ratios, respectively. Hence it is not surprising that bacterial cells have evolved a posttranslational modification system that consists of enzymes responsive to fluctuating levels of NAD⁺ (sirtuins) and Ac-CoA (acetyltransferases). It is also not surprising that most bacterial proteins under *N*^ε-Lys acetylation control are enzymes involved in metabolic

processes. Although there have been recent advances suggesting widespread acetylation in bacteria, the identity of substrates of bacterial protein acetyltransferases and deacetylases is largely unknown, and our understanding of the roles of these enzymes in bacterial cell physiology is limited. Future work should address the broader impact of *N*^ε-Lys acetylation in prokaryotic cell physiology.

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Figure 1. Proposed mechanism of GNAT-catalyzed acetyl transfer

Biochemical and structural evidence suggest that the GNAT family of HATs use a sequential mechanism of acetyl transfer. In this mechanism, Ac-CoA binds first, followed by the other substrate to form a ternary complex. The target lysine is deprotonated by an active site residue acting as a base, triggering a nucleophilic attack on the carbonyl carbon of Ac-CoA. Figure is adapted from Berndsen and Denu [42]. The acetyl group is highlighted in blue; E = enzyme; S = substrate.

Figure 2. Posttranslational regulation of Acs activity by Ac-CoA-dependent Pat acetylation
 Acetate cannot be utilized by the cell until it is converted to the high-energy intermediate, Ac-CoA. Acs (AMP-forming; EC 6.2.1.1) converts acetate to Ac-CoA via two half-reactions. In the first half-reaction, Acs converts acetate and ATP to the enzyme-bound intermediate acetyladenylate (Ac-AMP). In the second half-reaction, Acs reacts Ac-AMP with CoASH to form Ac-CoA, releasing AMP. Pat inactivates Acs by acetylation of an active site lysine, Lys609, preventing catalysis of the first half-reaction.

Figure 3. Graphical representation of metabolic targets identified by two proteomic studies

A. The majority of the *N*^ε-Lys acetylation modifications [125 acetylation sites in 85 proteins (*left*), and 138 acetylation sites in 91 proteins (*right*)] were identified in enzymes involved in metabolism. **B.** Of the ~40 targets shared by both studies, only 6 targets shared consensus on the site(s) assigned as acetylated.