PROSPECTS
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Prokaryotic Nε-Lysine Acetylomes and Implications for New Antibiotics

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ABSTRACT

Protein Nε-acetylation, a post-translational modification widespread in eukaryotes and prokaryotes, has been intensively explored due to its crucial roles in multitudinous physiologic events including transcriptional regulation, metabolic regulation, etc. The particular hotspot is the relationship between acetylation and metabolic regulation. Protein acetylation major types and functions thereof, prokaryotic acetyltransferase, deacetylases, and acetylation sites of enzymes related to glycometabolism, together with the cross-talk between acetylation and other modification, such as the phosphorylation were summarized, with emphases on those from Mycobacteria. J. Cell. Biochem. 113: 3601–3609, 2012.

KEY WORDS: Nε-ACETYLATION; LYSINE ACETYLTRANSFERASE; LYSINE DEACETYLASE

Post-translational protein acetylation was firstly reported by Phillips [1963], and focused primarily on eukaryotic histones and some transcription-associated proteins. This was expanded to archaea and bacterial proteins subsequently, such as other DNA-dependent nuclear proteins and proteins involved in many other complex biological processes. Two archaea proteins [Hase et al., 1978, 1980; Marsh et al., 2005], namely Alba (*Sulfolobus solfataricus*) and a 2Fe–2S ferredoxin (*Halobacterium halobium* and *Halobacterium marismortui*), can be Nε-acetylation targets. Acetylated proteins found in mitochondrion [Zhao et al., 2010], a close relative of α-proteobacteria [Gray et al., 1999], suggest that extensive protein acetylation might exist in bacteria. Two bacterial proteins [Starai and Escalante-Semerena, 2004; Gardner et al., 2006; Yan et al., 2008], acetyl-CoA synthetase (AcSA; *Escherichia coli* K-12 and *Bacillus subtilis*) and CheY (*E. coli* K-12), were found to be acetylated at lysine residue. Proteins involved in diverse cellular processes, including protein synthesis, detoxication responses, and metabolism have been found to be acetylated in three independent proteomic studies in *E. coli* [Yu et al., 2008; Zhang et al., 2009] and *Salmonella enterica* [Wang et al., 2010] (Fig. 1). Nε-lysine acetylation might play much broader critical physiological roles.

THE DEFINITION AND TYPES OF ACETYLATION

Acetylation might exert regulatory role commensurate to phosphorylation in response to both intracellular and extracellular signals fluctuation [Kouzarides, 2000; Soppa, 2010]. Protein acetylation, catalyzed by diverse acetyltransferases, is the transfer of acetyl moiety from an acetyl donor molecule, for example, acetyl-coenzyme A (acetyl-CoA) to either the α-amino groups of amino terminus or to the ε-amino groups of lysine residues in proteins [Polevoda and Sherman, 2002]. Protein acetylation has two major types: Nα- and Nε-lysine acetylation. Nα-acetylation is the irreversible modification of the N-terminal amino acid (methionine or the penultimate amino acid after methionine removal) [Arnesen, 2011]. In eukaryotes, Nα-acetylation is considered to be an exceedingly common protein modification, occurring co-translationally on approximately 85% of eukaryotic proteins, whereas very rare in prokaryotes with post-translationally modification on ribosomal proteins [Polevoda and Sherman, 2002]. For example, *E. coli* ribosomal proteins S5, S18, and L12 and mycobacterial ribosomal protein L12 are all amino-terminal acetylated proteins [Yoshikawa et al., 1987; Tanaka et al., 1989]. Unlike Nα-acetylation,

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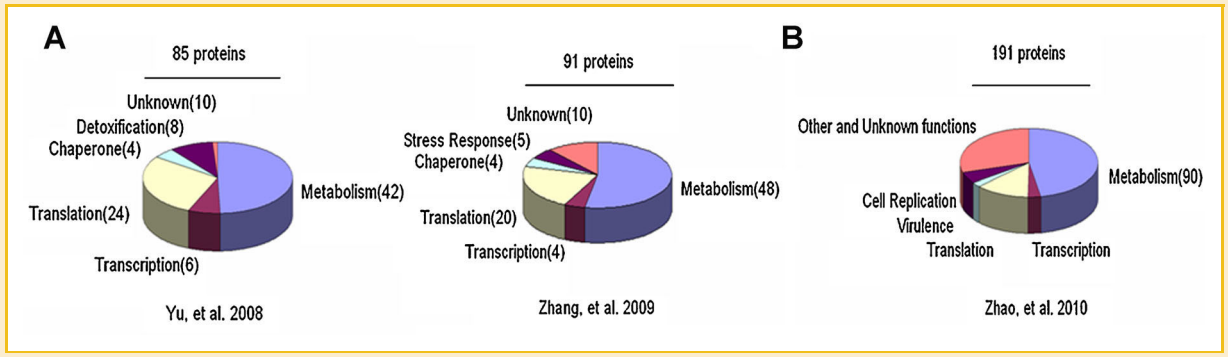

Fig. 1. The functionally annotated lysine-acetylated proteins from three proteomic studies. Functional classification of lysine-acetylated proteins in *E. coli* (A) and *S. enterica* (B). [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

post-translational Nε-acetylation is highly reversible and dynamic [Glozak et al., 2005], which can change the size, charge, and/or conformation of the protein to alter DNA-binding affinity, protein stability, protein–protein interaction, protein localization, and protein function [Kouzarides, 2000; Choudhary et al., 2009]. In addition, this modification can cross-talk with phosphorylation, methylation, ubiquitination, or other post-translational modifications under different circumstances [Yang and Seto, 2008]. Huge bulk of proteins in *E. coli* K-12 and *S. enterica* are reported to bear this modification. Gcn5-like protein *N*-acetyltransferases (GNATs) and Sir2-like protein deacetylase (sirtuins) are conserved in all three kingdoms. Therefore, Nε-lysine acetylation might be a vital regulatory mechanism in prokaryotes. The following sections will focus on prokaryotic Nε-lysine acetylation.

LYSINE ACETYLTRANSFERASE AND LYSINE DEACETYLASE

Nε-acetylation is catalyzed by lysine acetyltransferases (KATs or LATs) and reversed by "partner" lysine deacetylases (KDACs or HDACs) [Lee and Workman, 2007]. Proteins with KAT activity can be categorized into five major families: (i) the MYST family, which contains MOZ, Ybf2/Sas3, Sas2, Tip60; (ii) Gcn5-like protein GNAT family; (iii) the CBP/p300 co-activators; (iv) the SRC family of co-activators; (v) the TAFII family of transcriptional activators [Lee and Workman, 2007; Berndsen and Denu, 2008]. GNATs using Ac-CoA as donor for acetyl transfer are the largest enzyme superfamily, with more than 10,000 members across all kingdoms of life [Vetting et al., 2005]. Compared to the numerous eukaryotic GNATs, only one protein acetyltransferase (Pat) similar to the GNAT enzymes was found in *S. enterica* [Starai and Escalante-Semerena, 2004], and one GNAT family acetyltransferease AcuA in *B. subtilis* [Gardner et al., 2006] demonstrating Nε-acetylation. Moreover, two mycobacterial GNATs (MSMEG_5458 in *Mycobacterium smegmatis* and Rv0998 in *Mycobacterium tuberculosis*) also undergo lysine acetylation [Nambi et al., 2010]. But some GNATs, for example, RimI, RimL, and RimJ of *E. coli*, undergo Nα-acetylation [Yoshikawa et al., 1987; Tanaka et al., 1989].

Proteins with KDACs activity can be broadly divided into two families: simple hydrolases, which removes the acetyl group from acetyl-lysine and releases acetate, and sirtuins (silent information regulator 2) utilizing NAD^+ as a coenzyme to generate *O*-acetyl-ADP-ribose and nicotinamide [Shahbazian and Grunstein, 2007; Hu et al., 2010]. Sirtuins are very conserved between the eukaryotes and prokaryotes [Zhao et al., 2004]. In eukaryotes, the sirtuins play important roles in DNA repair, the determination of cell fate, metabolic regulation, and aging [Sauve et al., 2006; Finkel et al., 2009]. Putative prokaryotic homologs for each KDAC class have been predicted, only a few proteins have been shown to serve as deacetylases and very few protein substrates have been identified [Hildmann et al., 2007]. CobB, a Sir2 homolog protein deacetylase, exists in *S. enterica* and *E. coli* [Starai et al., 2002; Zhao et al., 2004]. Both in vivo and in vitro data have established the presence of a *B. subtilis* NAD^+-dependent protein deacetylase (encoded by the *yhdZ* gene) homologous to the yeast Sir2 protein [Gardner and Escalante-Semerena, 2009].

THE FUNCTION OF PROKARYOTIC ACETYLATION

The diverse cellular function demonstrated by proteins with acetylated lysines, including transcription, chemotaxis, stress responses, central metabolism, suggests that widespread prokaryotic Nε-acetylation might play fundamental roles. Some are enumerated as following.

REGULATING TRANSCRIPTION

Prokaryote can control gene expression by modulating the acetylation state of DNA-binding protein, transcription factor (TFs), or other proteins associated with transcription. *Sulfolobus solfataricus* P2 Alba, the major archaeal chromatin protein, can be acetylated on lys16 by a homolog of Pat, characterized in *Salmonella*, resulting in significant reduced binding affinity for DNA. ssSir2, an archaeal Sir2 homolog, can deacetylate Alba and depress transcription in a reconstituted in vitro transcription system [Bell et al., 2002]. Bacterial Gcn5-like protein acetyltransferase (YfiQ/Pat) can acetylate on the K180 of RcsB, a global regulatory protein involved in cell division, and capsule and flagellum biosynthesis. K180 located within the DNA binding, helix-turn-helix motif of RcsB. In vitro and in vivo analyses showed that the

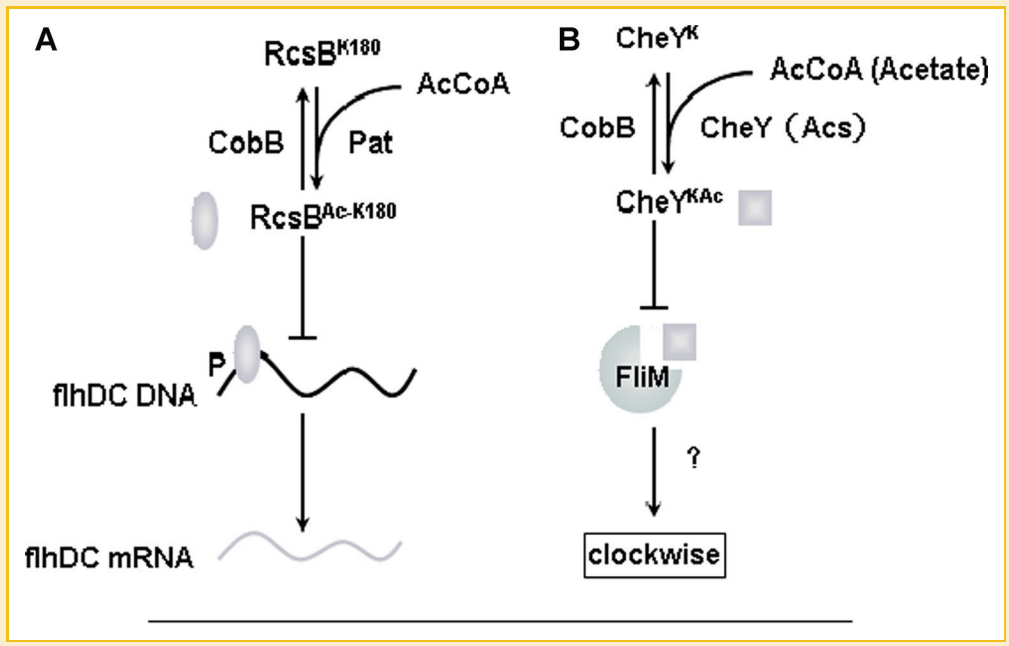

Fig. 2. Acetylation regulatory modes. A: The ellipse represents RcsBAc-K180. B: The square represents CheY in acetylated phase.

acetylation of RcsB caused defective binding of RcsB to the flhDC promoter and upregulated flhDC gene expression. Meanwhile, the NAD^+-dependent Sir2 (sirtuin)-like protein deacetylase (CobB) can deacetylate acetylated RcsB ($RcsB^{Ac}$) [Thao et al., 2010; Fig. 2]. Lysine acetylation sites present in the transcription-related enzymes, such as RNA polymerase, ssDNA-binding protein, CRP transcriptional dual regulator, and Rho protein [Yu et al., 2008].

MODIFYING STRESS RESPONSES

Nε-acetylation enables *E. coli* to withstand environmental stress. Upregulation of acetyltransferase YfiQ led to higher cell densities and more heat and oxidative stress resistant. Reduced acetylation via overexpression of deacetylase CobB resulted in totally different scenario. Both transcriptome and quantitative, reverse-transcription polymerase chain reaction (qRT-PCR) showed that CobB can repress the expression of *katG* under oxidative stress. Moreover, the two-component regulator proteins, such as CpxA, UvrY, PhoP, and BasR involved in the catalase activity might be the targets of acetylation, and implicated in the stress reaction partly due to acetylation [Ma and Wood, 2011].

CONTROLLING CHEMOTAXIS

CheY, the response regulator of bacterial chemotaxis, can transduce sensory signals from receptors to the flagellar motor and binds to a switch protein FliM, altering the rotation of the flagellum from counterclockwise to clockwise [Jones and O'Connor, 2011]. Both phosphorylation/dephosphorylation and acetylation/deacetylation can modulate *E. coli* CheY [Barak et al., 1992]. Two mechanisms underlie CheY acetylation: autoacetylation using acetyl-CoA as the acetyl donor and ACS-catalyzed acetylation utilizing acetate as the acetyl donor [Barak et al., 2006]. In vivo, the acetylation of CheY resulted largely from CheY autoacetylation [Yan et al., 2008]. Acetylation can repress the binding of CheY to FliM target protein, and activates CheY to generate clockwise rotation [Liarzi et al., 2010]. CobB might mediate *E. coli* chemotaxis by deacetylating CheY in vivo, since CheY acetylation is elevated in cobB deleted mutants [Li et al., 2010; Fig. 2].

REGULATING INTERMEDIARY METABOLISM

Approximately 50% of the 191 *S. enterica* acetylated proteins (totally 235 acetylated sites) involved in metabolism [Wang et al., 2010]. Likewise, the majority of Nε-acetylation [49% of 85 acetylation substrates (125 acetylation sites; [Yu et al., 2008]) and 53% of 91 acetylation proteins (138 acetylation sites; [Zhang et al., 2009])] from *E. coli* involved in metabolism, such as TCA cycle, glycolysis, glyoxalate bypass, gluconeogenesis, the metabolism of fatty acid, nucleotides, and amino acids (Fig. 3). These data implicate that the primary role of Nε-acetylation might be regulating the metabolic fates of carbon and energy sources [Thao and Escalante-Semerena, 2011]. Several prominent examples are listed.

ACETYL-CoA SYNTHETASE (ACS)

ACS is a ubiquitous enzyme important for the conversion of acetate to its high-energy intermediate Ac-CoA for processes, such as lipid synthesis and energy generation. In *S. enterica*, Pat inactivates Acs upon Ac-CoA-dependent acetylation of the highly conserved lysine (K609) [Starai and Escalante-Semerena, 2004]. Then acetylated ACS (ACS^{Ac}), serving as substrate for the sirtuin-like deacetylase (CobB), is reactivated by NAD^+-dependent deacetylation, producing niacinamide and 2′(3′)-*O*-acetyl-ADP-ribose [Starai et al., 2002; Fig. 4]. This mode of regulation may be a general phenomenon in

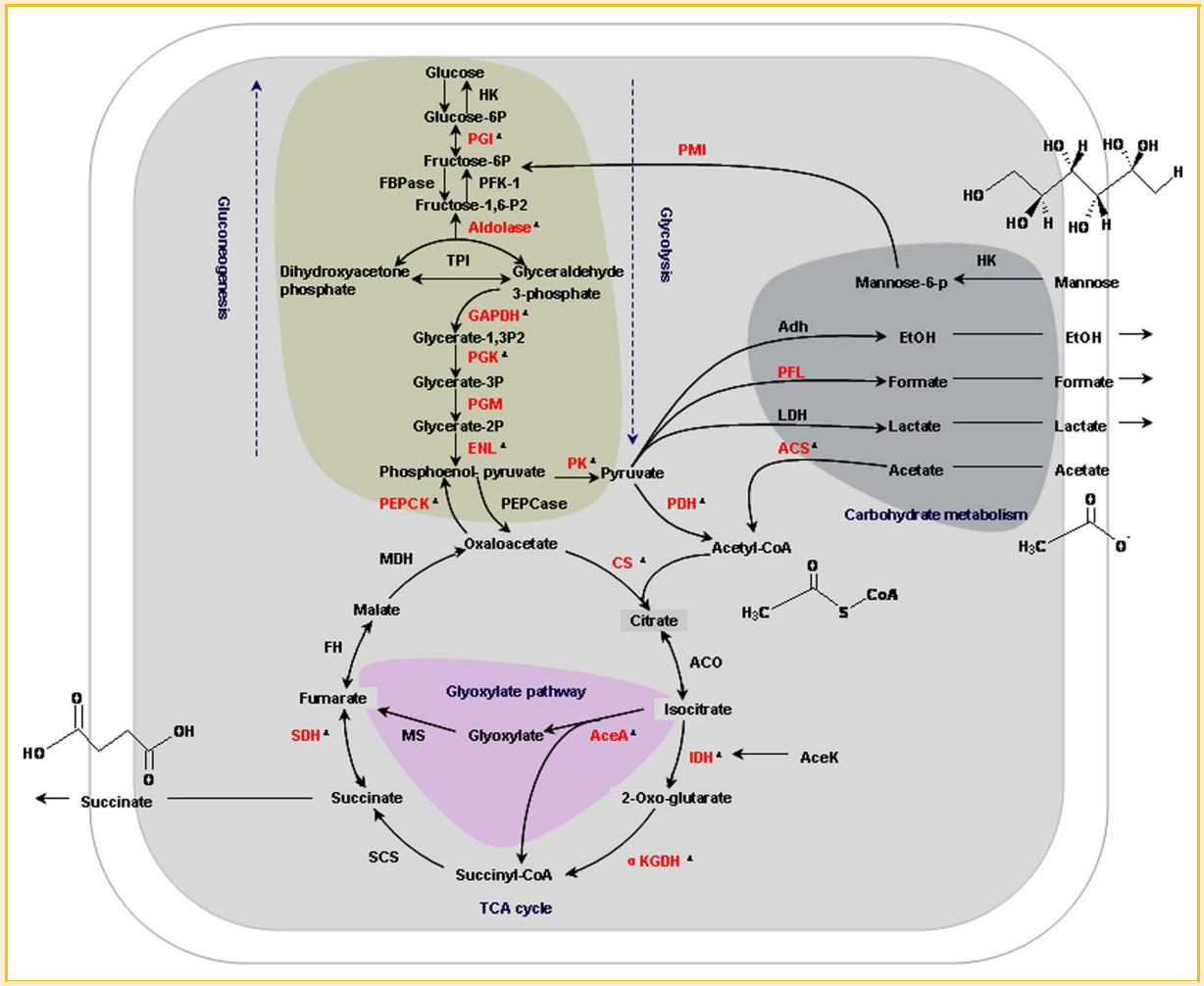

Fig. 3. Lysine acetylated enzymes involved in glycolysis, gluconeogenesis, glyoxylate pathway, TCA cycle, and carbohydrate metabolism in *E. coli*. Enzymes identified as lysine-acetylated in the *E. coli* are colored in red. Those identified in a screen of *S. enterica* cells are marked with an asterisk. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

bacteria. For example, in *E. coli* K-12, YfiQ, and NpdA are orthologs of Pat and CobB, the corresponding ACS also carries K609. In *B. subtilis*, AcsA is also regulated by acetylation/deacetylation, but this process might be much complex due to the two lysine deacetylases (SrtN, an NAD^+-dependent deacetylase sirtuin, and AcuC, a deacetylase not requiring NAD^+ as co-substrate) involved in the deactylation of $AcsA^{Ac}$ [Gardner and Escalante-Semerena, 2009]. Furthermore, the activity of *Rhodopseudomonas palustris* ACS can be inactivated upon acetylation by its Pat enzyme and be reactivated upon deacetylation by lysine deacetylase A (LdaA) [Crosby et al., 2010].

GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GAPDH)

GAPDH, encoded by *GapA*, can catalyze the reversible conversion between glyceraldehyde 3-phosphate and 1,3-bisphosphoglycerate in both glycolysis process and gluconeogenesis pathway [Guan and Xiong, 2011]. In *S. enterica*, Pat and CobB are the major enzymes responsible for the reversible acetylation of GAPDH [Wang et al., 2010]. In a *cobB* null mutant (Δ*cobB*), GAPDH is acetylated and its acetylation is significantly increased, whereas acetylation of this enzyme is reduced in a *pat* null mutant (△*pat*) compared with that of the wild-type strain. Furthermore, GAPDH acetylation may mediate the adaptation to different carbon sources. Under glucose-rich conditions, the increased GAPDH acetylation can promote glycolysis but inhibit gluconeogenesis. In contrast, under acetate-rich conditions, the decreased GAPDH acetylation favors the gluconeogenesis but inhibits glycolysis (Fig. 4). This shows that acetylation might play a key role in regulating the directivity of carbon flux in response to nutrient availability, but the underlying precise mechanism remains obscure. One possibility might be the transcriptional regulation of *pat* and *cobB* gene expression in response to different carbon sources [Wang et al., 2010].

ISOCITRATE LYASE (AceA) AND ISOCITRATE DEHYDROGENASE (IDH) KINASE/PHOSPHATASE (AceK)

Besides ACS, GAPDH, *S. enterica* AceA and AceK are regulated by acetylation too. Anti-acetylysine immunoblot showed that acetyla-

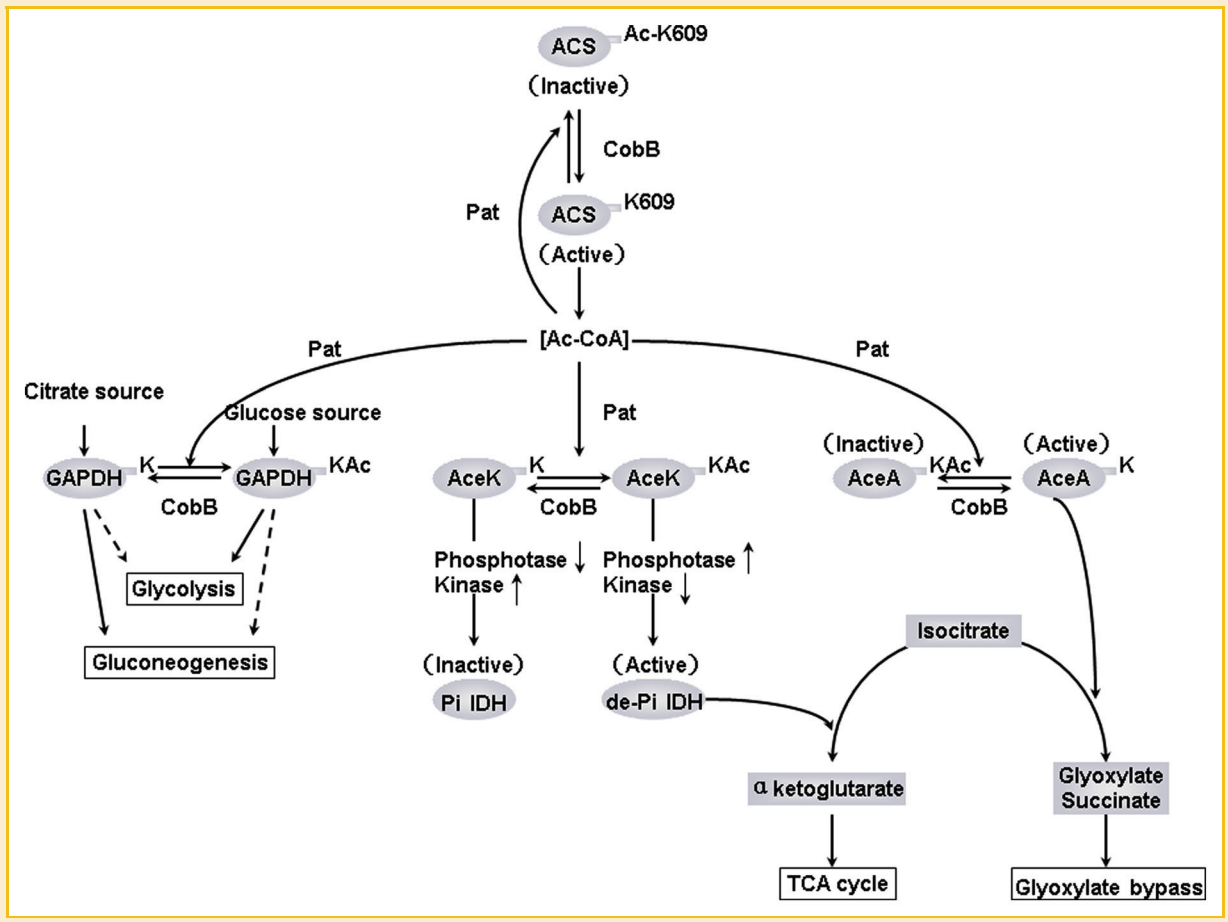

Fig. 4. Acetylation regulates the activity of ACS, GAPDH, AceA, and AceK, and channels the flux direction of glycolysis versus gluconeogenesis or the distribution of isocitrate between citrate cycle and glyoxylate bypass in *S. enterica*: the solid line represents positive regulation, the dash line represents negative regulation.

tion of the two enzymes decreased in the △*pat* strain, but increased in the △*cobB* strain in the presence of oxidative carbon source—citrate [Wang et al., 2010]. In vitro, acetylation of AceA by treatment with Pat increased AceA-specific activity, on the other hand, deacetylation of AceA by CobB decreased its enzymatic activity; acetylation of AceK by Pat caused the reduction of its ability to inactivate ICDH, namely, phosphatase activity increased, kinase activity decreased, whereas deacetylation of AceK by CobB led to the converse result [Wang et al., 2010; Fig. 4]. These results demonstrate that acetylation can control activities of AceA, and AceK enzymes which in turn may direct the distribution of isocitrate between the TCA cycle and glyoxylate bypass.

PHOSPHOENOLPYRUVATE CARBOXYKINASE (Pck1p)

Saccharomyces cerevisiae Pck1p involved in gluconeogenesis can be acetylated by nucleosome acetyltransferase of H4 (NuA4) complex at Lys514. Pck1p acetylation increases enzymatic activity and promotes the ability of yeast cells to grow on nonfermentable carbon sources. Furthermore, Pck1p is deacetylated by Sir2p both in vitro and in vivo, which can depress Pck1p activity and block the extension of yeast chronological life span caused by water starvation [Lin et al., 2009]. Two Lys acetylation sites of Pck1p have been identified in *E. coli* [Zhang et al., 2009]. One acetylation site can be found in the Pck1p of *S. enterica* [Wang et al., 2010]. However, the relationship between prokaryotic Pck1p acetylation and gluconeogenesis remains to be determined.

OTHER METABOLIC ENZYMES

Apart from isocitrate dehydrogenase (IDH), three enzymes involved in TCA cycle are also lysine-acetylated, such as citrate synthase, succinic dehydrogenase, and 2-ketoglutarate dehydrogenase [Zhang et al., 2009]. In addition, enzymes involved in amino acid and nucleotide metabolism are also lysine-acetylated. These proteins include seven proteins involvement in these two metabolisms, respectively. Deoxyribosephosphate aldolase catalyzing the reversible reaction between glyceraldehyde-3-phosphate and acetaldehyde to form 2-deoxyribose-5-phosphate in nucleotide metabolism is acetylated at Lys167 which is catalytic residue involved in Schiff base formation. It is possible that acetylation of this active site lysine could inhibit the activity of enzyme [Zhang et al., 2009].

CROSS INFLUENCE BETWEEN Lys ACETYLATION AND OTHER POST-TRANSLATIONAL MODIFICATIONS (PTMs)

Lys acetylation can involve in complicated cross-talk and communication with other PTMs to maintain fine regulation within the cell. By mutating lysine to glutamine or leucine and predicting the impact on neighboring phosphorylation sites, Lu et al. demonstrated that most lysine acetylation has the potential to influence the surrounding protein phosphorylation, ubiquitination, and methylation status (over 4,000 phosphorylation sites, more than 600 ubiquitination sites and less than 80 methylation sites are potentially be influenced), leading the change on gene expression, cellular degradation pathways [Lu et al., 2011]. In bacterium *Mycoplasma pneumoniae* with small known genome, deleting two putative GNAT would affect protein phosphorylation [van Noort et al., 2012]. Reciprocally, deletion of its only one phosphatase (PrpC|Mpn247) and two protein kinases (HprK|Mpn223 and PknB|PrkC|Mpn248) not only has an effect on the phosphorylation network but also controls the abundance of proteome and the patterns of lysine acetylation.

M. TUBERCULOSIS Nε-ACETYLATION

M. tuberculosis, the causative agent of tuberculosis (TB), is a major threat to public health worldwide. HIV-associated TB and multi-drug-resistant TB (MDR-TB) further exacerbate the situation. Insight into the metabolism regulation system of the pathogen could offer new clues for treating TB. Protein lysine acetylation exists in Mycobacteria [Nambi et al., 2010]. At least one Mycobacteria acetyltransferase is cAMP-dependent and targeted by Nε-acetylation [Xu et al., 2011]. An overview of the *M. tuberculosis* Nε-acetylation was proposed.

M. TUBERCULOSIS ACETYLTRANSFERASE AND DEACETYLASE

There are 20 predicted GNAT's in *M. tuberculosis*, but only five GNAT (Rv0819, Rv2747, AAC(2′)-Ic, Rv1347c, and Rv0998) have been characterized (Table I). Rv0998, whose structural feature is different from other acetyltransferase, comprises a C-terminal GNAT domain that exhibits highest sequence identity to Pat *S. enterica* and an N-terminal cyclic nucleotide (cNMP)-binding domain that is most similar to the cNMP domain of eukaryotic protein kinases. Rv0998 and MSMEG_5458 (the homologous protein of Rv0998 in *M. smegmatis*) acetylate the universal stress protein (USP) at K107 dependent on cAMP, an important second messenger essential for pathogenesis in *M. tuberculosis*, and the increased amount of cAMP enhances the acetylation of USP by MSMEG_5458 [Nambi et al., 2010]. Moreover, MSMEG_5458 has been considered a new downstream effector of the intracellular cAMP signaling pathway. When the level of cAMP is elevated, the activity of acetyltransferase is upregulated, catalyzing the acetylation of a highly conserved lysine residue in ACS [Xu et al., 2011]. These provide evidences for the possible key role of Rv0998 or MSMEG_5458 in an entirely new signal pathway mediated by cAMP exclusive to Mycobacteria so far.

Meanwhile, Rv1151c, a sirtuin-like deacetylase in *M. tuberculosis*, SUP>-dependent deacetyla/SUP>-dependent deacetylation [Xu et al., 2011]. Therefore, mycobacterial Pat and the sirtuin-like deacetylase constitute a reversible acetylation system that regulates the activity of ACS. BLAST searches indicate that the homologous proteins of MSMEG_5458 and Rv1151c are broadly distributed in other Mycobacterium. Furthermore, in *S. enterica*, Pat and CobB control the activities of three extra central metabolic enzymes (GAPDH, AceA, and IDH kinase/phosphatase) through lysine acetylation, thus coordinating carbon source utilization and metabolic flux [Zhang et al., 2009]. It is possible that mycobacterial Pat and the sirtuin-like deacetylase constituting a reversible acetylation system also regulate the orthologs of the three enzymes or other enzymes involved in metabolism in Mycobacteria and play new roles in regulating the central carbon metabolic processes. If this hypothesis is reasonable, it will be extremely significant to identify the glycometabolic enzymes that might be modified by this acetylation system and the putative lysine acetylated sites.

THE PREDICTED LYSINE ACETYLATED SITES OF GLYCOMETABOLIC ENZYMES IN MYCOBACTERIA (*M. SMEGMATIS*, *M. BOVIS* BCG, *M. TUBERCULOSIS*, AND *M. LEPRAE*)

By tryptic digestion for immunoprecipitation with an anti-acetyl lysine antibody and subsequent nano-HPLC/MS/MS, 125 lysine

TABLE I. The Similarities and Differences Among Five Characterized GNAT in *M. tuberculosis*

Name	Structural feature	Acetylation site	Acyl donor	Acyl accepter	Physiological functions	Refs.
Rv0998	GNAT domain, cAMP-binding domain	Lys	Ac-CoA	USP, ACS	A new cAMP signaling pathway	Nambi et al. [2010], Xu et al. [2011]
Rv0819	2 Repeated GNAT domain	Cysteinyl amine	Ac-CoA, CoA	Cysteine–glucosamine–inositol (Cys–GlcN–Ins)	Mycothiol biosynthesis process that maintains cellular redox state and detoxifies powerful electrophiles	Vetting et al. [2003]
Rv2747	GNAT domain	Glutamate	Ac-CoA	L-Glutamate	Arginine synthesis pathway	Errey and Blanchard [2005]
AAC(2′)-Ic	GNAT domain	2′-OH, 2′-NH_2	Ac-CoA	Aminoglycosides and therapeutically antibiotics, such as kanamycin A and amikacin	Unidentified	Hegde et al. [2001]
Rv1347c	GNAT domain	Lys	ACP, acyl-CoA	Mycobactin siderophores	Mycobactin biosynthetic pathway directly related to the virulence	Frankel and Blanchard [2008]

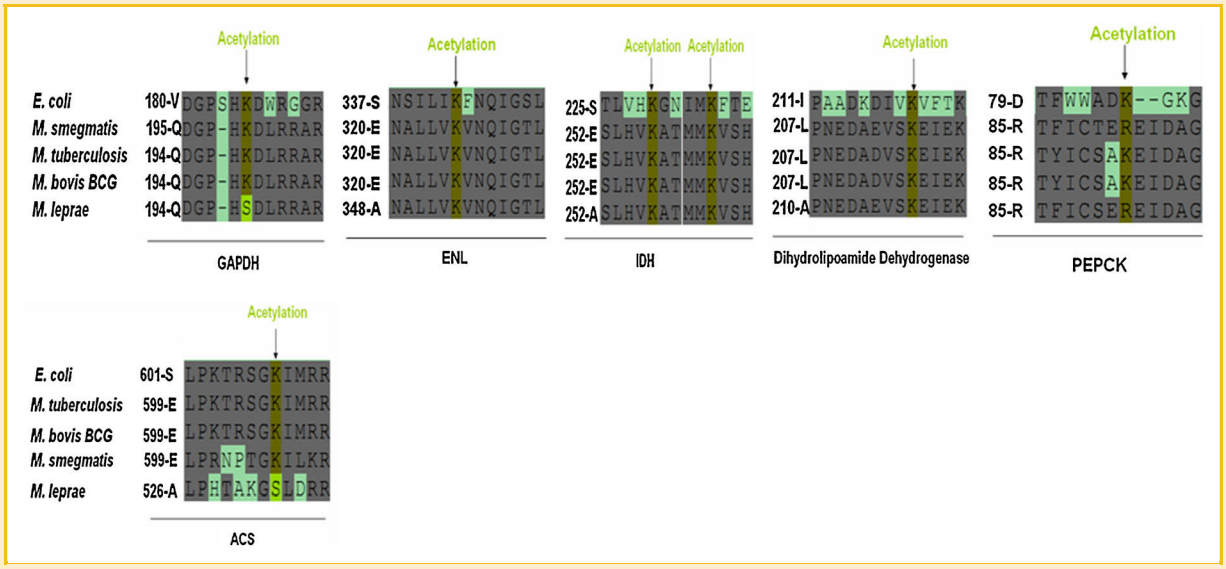

Fig. 5. The sequence alignment of glycometabolic enzymes in Mycobacteria. The possible conserved Lys acetylation site in Mycobacteria is boxed in green and marked by an arrow. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/jcb]

acetylated sites in 85 proteins among proteins derived from *E. coli* were identified [Yu et al., 2008], many lysine-acetylated proteins identified were involved in glycometabolism, thus, lysine acetylation might modify or regulate the activities of these proteins. For example, when 1 of 6 acetylated lysine residues in IDH, K230, was substituted by methionine, the overall conformation of this enzyme is not largely influenced, but its k_{cat} markedly decreases to 1.1% of wide-type activity [Lee et al., 1995]. We align amino acid sequences of diverse glycometabolic enzymes whose lysine acetylation sites have been identified in *E. coli* with the sequences of the related proteins in Mycobacteria (*M. smegmatis*, *M. bovis*, *M. tuberculosis*, and *M. leprae*), thus finding that only five enzymes, namely, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), IDH, enolase (ENL), dihydrolipoamide dehydrogenase, phosphoenolpyruvate carboxykinase (PEPCK) comprise conserved lysine acetylated sites. It is possible that acetylated sites of the five enzymes from *M. tuberculosis* are K199, K325, K257, or/and K260, K216, K87, respectively (Fig. 5). Moreover, as it has been reported that acetylation of a key K residue at the active center inactivates ACS enzymes from bacteria to humans, we align the amino acid sequences of ACS in *M. tuberculosis* with that in *E. coli*. The result of sequence alignment is that ACS of *M. tuberculosis* contains a highly conservative Lys-acetylation site-K617, which just agrees with the fact that K617 is acetylated by Mspat and deacetylated by Rv1151c. Therefore, it is very likely that GAPDH, IDH, ENL, dihydrolipoamide dehydrogenase, and PEPCK are modified by Nε-acetylation in Mycobacteria. Observation suggests that GAPDH involved in glycolytic steps is apparently dispensable for growth of *M. tuberculosis* in mice, whereas the level of PEPCK is upregulated in the lungs of infected mice, inducing strong cell-mediated immune responses [Sassetti and Rubin, 2003; Liu et al., 2006]. Meanwhile, PEPCK is essential for virulence of *M. bovis*, when deleting PEPCK-encoding gene *pckA*, the capacity of the bacteria to infect and survive in macrophages is reduced [Liu et al., 2003]. Fuethermore, dihydrolipoamide dehydrogenase, a member of pyruvate dehydrogenase complex, can control virulence in *M. tuberculosis*, inducing dendritic cell maturation and Th1 polarization [Heo et al., 2011; Venugopal et al., 2011]. This implies that acetylation might play a role in the pathogenesis of these *M. tuberculosis* key regulators.

PROSPECT

Nε-acetylation represents a largely untapped treasure trove for both fundamental biology and new antibiotics. For example, *Candida albicans* cells in the absence of fungal-specific histone acetyltransferase Rtt109, whose pathogenicity is significantly attenuated in mice are more susceptible to DNA-damaging reactive oxygen species (ROS)-mediated killing by macrophages than are wild-type cells in vitro, indicating that Rtt109 might be a potential target for therapeutic antifungal agent [Lopes da Rosa et al., 2010]. Bacterial KATs such as Pat could also be targets for novel antibiotics. However, the extent of Nε-acetylation in the prokaryotes, the roles of acetylation in pathogens and the reasons udderlying the multiple acetylation sites of some proteins remain to be addressed. The clarification of these scientific questions will be conducive to both basic biology and clinical application.

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