Bacterial protein acetylation: mechanisms, functions, and methods for its study

Sergio Encarnación[1] and Jocelin Rizo[1]

[1]Universidad Nacional Autonoma de Mexico Centro de Ciencias Genomicas

August 4, 2023

Abstract

Lysine acetylation is an evolutionarily conserved protein modification that regulates different cellular pathways. The acetyl moiety can be transferred to the lysine side chain in two ways, enzymatically by lysine acetyltransferases and non-enzymatic. Usually, acetyl coenzyme A is the donor agent, although acetyl phosphate is the main regulator of acetylation in bacteria. The removal of the acetyl group occurs exclusively enzymatically. In prokaryotes, lysine acetyltransferases are grouped by a core structural domain architecture in the protein superfamily Gcn5-related N-acetyltransferase (GNAT). With different implications, these enzymes can acetylate the amino group of small molecules, metabolites, peptides, and proteins. As well as mitochondria, chemical acetylation has also been demonstrated as a global regulatory mechanism in bacteria. This review presents current knowledge of acetylation mechanisms and functional implications in bacteria metabolism. Additionally, the advances in mass spectrometry for studying this PTM, including relative quantification and stoichiometry quantification, and how these methods have allowed researchers to elucidate the biological significance in bacteria are reviewed.

Introduction

Unlike the human genome, whose total length is approximately 6.2 Gbp in which only 3% encode approximately 20,000 proteins, bacteria have smaller genome (<0.5 to 10 Mbp) encoding only 1,500-7,500 proteins (Fredens et al., 2019; Piovesan et al., 2019). Even with such a low number of proteins, microorganisms can carry out complex cellular functions by increasing the functional diversity of proteins through post-translational modifications (PTMs).

Post-translational modifications are covalent and generally enzymatic modifications of amino acid residues in a protein after the synthesis of the polypeptide chain. The modifications of the amino acid side chains range from small chemical groups (e.g., methylation, acetylation, and phosphorylation), to more complex modifications such as the addition of oligosaccharides or small peptides (e.g., glycosylation and ubiquitylation) (Heuts et al., 2009; Macek et al., 2019; Mann & Jensen, 2003). Through protein PTMs, cells regulate their functions and metabolic pathways and increase the variety and complexity of target proteins. The PTMs most widely distributed and frequently reported are phosphorylation, glycosylation, ubiquitination, methylation, and acetylation, among other types of alkylations. (Khoury et al., 2011).

A wide variety of amino acid residues are susceptible to post-translational modification. In particular, the epsilon amino group of the side chain of lysine residues is the target of many of these modifications, including acetylation (Gil et al., 2017). Based on the acetylation site in the protein, three different types have been described:

(1) The irreversible acetylation of the alpha-amino group in the N-terminal amino acid of proteins (Nα-acetylation), a common modification in eukaryotes (50-70% of yeast proteins and 80-90% of human proteins).

(2) The reversible acetylation of the hydroxyl side chain of serine or threonine (O-acetylation), is detected only in a few eukaryotic organisms.

(3) The reversible acetylation of the epsilon-amino group of the lysine residue (Ne-acetylation). The Nα- and Ne-acetylation may occur on different amino acids residues (Lys, Ala, Arg, among others) with different frequencies, being the acetylation of lysine residues the most reported (Diallo et al., 2019; Khoury et al., 2011; Ramazi & Javad Zahiri, 2021).

Ne-acetylation occurs through two distinct mechanisms: enzymatic by the lysine acetyltransferases (KATs) and non-enzymatic (by chemical acetylation). In both cases, the acetyl group from a donor molecule, typically in the form of acetyl coenzyme A (AcCoA) or acetyl phosphate (AcP), is transferred to a target lysine residue. Both types of acetylation are reversible by the action of deacetylase enzymes (Figure 1A) (Hentchel & Escalante-Semerena, 2015).

The acetylation of lysine residues was discovered more than 50 years ago in histones and was linked to the regulation of transcription (Allfrey et al., 1964). Extensive studies in eukaryotic cells have shown that acetylation is an essential protein modification that influences many cellular processes, including protein-protein interaction, protein stability, protein folding, cellular localization, and enzymatic activity. Also, this PTM regulated different biological pathways, such as cell cycle control, cell metabolism, DNA repair, DNA replication, ribosome biogenesis in the nucleus, nuclear transport, translation, and transcription, among others. (Berrabah et al., 2011; Gil et al., 2017; Oliveira & Sauer, 2012; Tarazona & Pourquié, 2020). In contrast, research on acetylation in prokaryotes is relatively new, primarily focused on describing global acetylation through a proteomic approach. From these studies, it has been found that in some bacteria, the acetylated proteins on lysine constitute more than 10% of the proteome. This PTM influences several fundamental cellular pathways, including cellular function, cellular differentiation, and bacterial metabolism.

1. Enzymatic acetylation

Ne-acetylation (K-acetylation) refers to an addition of an acetyl group from a donor molecule, like acetyl coenzyme A, to an epsilon amino group of lysine side chains, the reaction produces an increase in the size of the side chain and neutralizes the positive charge. K-acetylation is a reversible process where the acetate is enzymatically added and removed by lysine acetyltransferases and lysine deacetylases (KDACs), respectively (Alonso & Serra 2012; Christensen et al., 2019).

KATs are grouped into three major families based on amino acid sequence homology and biochemical characteristics of catalysis: (i) Gcn5- related N-acetyltransferases (GNATs); (ii) the p300/CBP family; and (iii) MYST family. The MYST and p300/CBP families are presented only in eukaryotic cells, while the Gcn5-related N-acetyltransferase (GNAT) family, contains orthologous proteins among bacteria, eukaryotes, and archaea (Favrot et al., 2016; Finkel et al., 2009; Gu & Roeder, 1997; Lee & Workman, 2007; Vetting et al., 2005).

KATs families differ in sequence similarity, domain organization, substrate specificity, and catalytic mechanism. The GNAT family uses a sequential mechanism where the acetyl-CoA and the protein substrate bind to form a ternary complex in which active site glutamate acts as a general base to deprotonate the amino group of the lysine, allowing it a nucleophilic attack on the carbonyl carbon of the acetyl moiety of CoA (Liu et al., 2008) (Figure 1B). Members of the MYST family can either use this ternary complex mechanism or a ping-pong mechanism, where the acetyl group is covalently attached to the enzyme to form an acyl-enzyme intermediate before being transferred to the substrate (Yan et al., 2002). In contrast, the p300/CBP family does not use a catalytic base to initiate the transfers of the acyl moiety, the mechanism for this family is categorized as "hit and run" (Theorell–Chance), a sequential mechanism where the ternary complex formed is kinetically irrelevant for the catalysis. In this catalytic mechanism, a tyrosine in the active site residue acts as a catalytic acid to increase the nucleophilicity of the lysine side chain (Liu et al., 2008). The protein substrate-AcCoA association binds transiently to the enzyme surface, allowing the lysine residue to receive the acetyl group, followed by rapid protein dissociation (Zhang et al., 2014).

1.2. GNAT family in prokaryotes

The first two reported members of what is now termed the Gcn5- related N-acetyltransferases (GNATs) were the aminoglycoside N-acetyltransferase from multidrug-resistant *Serratia marcescens* and the histone acetyltransferase (HAT1) from *Saccharomyces cerevisiae* (Dutnall et al., 1998; Wolf et al., 1998). So far, GNATs comprise one of the largest enzyme superfamilies identified with more than 870 000 members through all kingdoms and more than 200 three-dimensional structures, mainly of bacteria, deposited in RCSB Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do).

Bacteria and archaea present more annotated genes in the NCBI database than animals and plants, suggesting that the number of GNATs may be related to the environments inhabited by each organism and may reflect their metabolic complexity. For example, the genome of the nitrogen-fixing bacteria *Rhizobium leguminosarum* encodes 82 GNATs, the genome of the endophytic bacterium *Pantoea agglomerans*encodes 39 GNATs, the genome of the extremely halophilic archaea*Halapricum desulfuricans* and *Haloferax mediterranei*encoded 85 and 68 GNATs, respectively. These enzymes are involved in diverse cellular processes such as transcription control, antibiotic resistance, and stress regulation, among others (Salah et al., 2016; Xie et al., 2014). However, many GNATs still need to be characterized, so, their physiological role, substrate specificity, and structure of these enzymes are unknown.

GNAT members can acetylate the amino group of small molecules, metabolites, peptides, and proteins, with different implications (Table 1). The kinetically and structurally characterization of the aminoglycoside 6'-N-acetyltransferase from *Enterococcus faecium*and *Salmonella enterica* showed that the enzyme can acetylate several aminoglycosides in solution. The presence of these proteins could be related to the increase of antibiotic resistance in some pathogen bacteria (Hegde et al., 2002; Magnet et al., 2001; Wright & Ladak,1997). The acetylation of the spermidine prevents polyamine toxicity at low temperatures and may play a similar physiological role in response to other stressful conditions (Limsuwun & Jones, 2000).

Interestingly, some acetyltransferases can acetylate different substrates; for example, the Eis protein from *M. tuberculosis* was initially described as an aminoglycoside acetyltransferase (Chen et al., 2011; Houghton et al., 2013; Zaunbrecher et al., 2009), but it also acetylates proteins (Ghosh et al., 2016; Kim et al., 2012). Both types of activities have different implications for the bacterium.

1.2.1. Overall structure of GNATs

Although the homology in the primary sequence between GNATs is moderate (3%–23%), they are folded into a highly conserved three-dimensional structure. The core secondary elements of the GNAT proteins consist of six or seven β-strands and four α-helices, connected in order β0-β1-α1-α2-β2-β3-β4-α3-β5-α4-β6. Four conserved motifs, known as the N-acetyltransferase domain, are found in this core arranged in order C-D-A-B (Figure 1C) (Dyda et al., 2000; Hentchel & Escalante-Semerena, 2015; Tercero et al., 1992).

Motifs C and D play an essential role in protein stability, while motifs A and B contain the residues involved in acyl-CoA and acceptor substrate binding, respectively. (Dyda et al., 2000; Vetting et al., 2005). Motif A contains a six-residue segment (Q/R)-X-X-G-X-(G/A), known as P-loop (phosphate-binding loop), for substrate recognition and binding and glutamic or aspartic residue to deprotonate the target lysine (Liu et al., 2008; Lu et al., 2017; Vetting et al., 2005). Across the entire GNAT superfamily, there is structure divergence in the loop β1β2, the α-4 helix of motif B, and strand β6 at the C-terminal, which together form the binding site for the acceptor substrate. Specifically, the C-terminal region contains a loop of varying length and position, and the residues in the motif B are not well conserved. These structural variations allow the recognition of various substrates (Dyda et al., 2000; Salah et al., 2016). For example, in the mycothiol synthase (Rv0819) from *Mycobacterium tuberculosis,* the β-strand 1 in the N-terminal domain is missing, the position of helix 2, and a long loop inserted between α3' and β5' (Figure 1D), while aminoglycoside 6´-N-acetyltransferase from *Enterococcus faecium* has an additional α-helix between the strands β1 and β2 (Vetting et al., 2003; Wybenga-Groot et al., 1999).

The binding of the enzyme to the substrate is through interactions with the pantetheine and pyrophosphate

moieties of CoA (Clements et al., 1999; Dutnall et al., 1998; Hentchel & Escalante-Semerena, 2015; Lin et al., 1999; Rojas et al., 1999; Wang et al., 2008;). The pantetheine binding is based on hydrogen bonds with the main chain of β4 and β 5, and the pyrophosphate is coordinated mostly by the main chain nitrogen atoms of the conserved phosphate binding loop between β4 and α3 (Majorek et al., 2017).

1.2.2. GNAT classification

Different works have proposed a classification system for prokaryotic KATs of the GNAT family (Figure 1E) (Christensen et al., 2019; Hentchel & Escalante-Semerena, 2015; Lu et al., 2017; VanDrisse & Escalante-Semerena, 2019). This classification system recognized that the GNAT family exhibits different sequence lengths, domain architecture, and types. Recently, Christensen and collaborators proposed a new system of three main classes of KATs based on sequence length, several GNAT domains present, and five different types of KATs based on domain identities and arrangements. Class I consists of a large (¿80 KDa) multidomain enzyme, where only the GNAT catalytic domain is conserved. Class II encompasses most bacterial acetyltransferases, smaller enzymes with a single GNAT domain. Class III has a dual arrangement of GNAT domains. Depending on domain position, these classes are further categorized into five types: types I and II contain a domain homologous to nucleotide-diphosphate (NDP)-forming acyl-CoA ligase/synthetase (700-900 aa) but with a lack in activity and a GNAT domain (˜200 aa), at the N-terminal or C-terminal. Type III KATs have a smaller regulatory domain (˜300–400 aa) at the N-terminal binds to an effector (e.g., cAMP, NADP, or amino acids) and a C-terminal GNAT domain. Type IV and V do not contain any regulatory domain and consist only of one GNAT domain (400 aa) and multiple GNAT domains, respectively (Figure 1E). (Christensen et al., 2019; Hentchel & Escalante-Semerena, 2015; Lu et al., 2017; VanDrisse & Escalante-Semerena, 2019).

The diversity of domain architectures and organization of GNATs indicate that in bacteria, lysine acetylation is regulated by diverse metabolic signals in response to physiological conditions and environmental changes. However, more studies are needed to elucidate the substrates and structures of these enzymes and their role in these organisms.

2. Non-enzymatic acetylation

Acetyl-CoA is synthesized by different reactions: oxidative decarboxylation of pyruvate during glycolysis; catabolism of some amino acids (isoleucine, leucine, and threonine); and β-oxidation of fatty acids. Several lysine acetyltransferases use this molecule to acetylate proteins (previously mentioned) and can modify the amino group of lysine side chains non-enzymatically. The chemical acetylation was first mentioned in histone proteins by Phillips in 1963. A few years later, it was demonstrated that purified histones, albumin, and synthetic lysine homopolymer are acetylated *in vitro* at a pH ˜9 and in the presence of acetyl-CoA without additional enzymes (Paik et al., 1970). In eukaryotic cells, specifically in the mitochondria, the absence of acetyltransferases and the unique conditions, such as the high concentration of acetyl-CoA (1.5 mM steady-state) and alkaline pH (7.9–8.0), suggests that lysine acetylation occurs chemically. For example, many mitochondrial proteins are acetylated under conditions mimicking those of the mitochondria matrix in a mechanism that is generally pH- and acyl-CoA concentration-dependent (Wagner & Payne, 2013). The proposed mechanism for mitochondrial non-enzymatic acetylation appears to occur by a nucleophilic attack on the acetyl carbonyl by the side-chain amine of a lysine residue (Wagner & Payne, 2013). However, under the physiological characteristic of the mitochondrial matrix, the reaction would be slow, so it has been proposed that protein acetylation occurs via S-acetylation cysteine thiol with the subsequent transfer of the acetyl group to a nearby deprotonated lysine residue (Christensen et al., 2019; James et al., 2017).

Direct acetylation has also been observed in bacteria, where at elevated concentrations, acetyl phosphate (AcP) can non-enzymatically acetylate lysine residues on many proteins. AcP is a highly reactive molecule susceptible to hydrolyzing or acetylating without enzymes. It undergoes rapid hydrolysis under basic or acid conditions and in the presence of some ions (Mg_2^+ or Ca_2^+). However, in the presence of nucleophilic reagents such as thiols, hydroxyl groups, ε-amino or α-amino groups of lysine side chains or proteins, the chemical acetylation of these groups is favored (Kuhn et al., 2014).

3. Deacetylation

Enzymatic or non-enzymatic lysine acetylation can be reversed by the action of lysine deacetylase (KDAC), also known as histone deacetylases (HDAC), since histones were the first identified substrate of the enzymes (Inoue & Fujimoto, 1969). Based on pairwise sequence comparisons and cofactor requirement, HDACs are grouped into four classes: the metal-dependent class I, II, and IV HDACs that do not require cofactors to deacetylated; and class III HDACs, commonly referred to as sirtuins, that are evolutionarily and mechanistically distinct family of NAD-dependent enzymes (Gregorett et al., 2004; Hentchel & Escalante-Semerena, 2015; Lombardi et al., 2011).

3.1. Sirtuins

Sirtuins, also known as Sir2 proteins (silent information regulator 2), have a conserved catalytic core domain characterized by its requirement for nicotine adenine dinucleotide (NAD) to catalyze protein deacetylation by transferring the acetyl group from the lysine to NAD^+, resulting in deacetylated lysine, nicotinamide (NAM) and 2'-O-acetyl-ADP- ribose (Figure 2B) (Blander & Guarente, 2004). These proteins are evolutionarily conserved within the three domains of bacteria, archaea, and eukaryotes. The sirtuins have a conserved catalytic core domain, characterized by its requirement for nicotine adenine dinucleotide (NAD), to catalyze deacetylation by transferring the acetyl group from the lysine to NAD^+(Michan & Sinclair, 2007; Sauve et al., 2006).

Bacterial genomes and most archaea encode one or two sirtuins, except for *Escherichia coli* , which encodes three, whereas eukaryotes typically contain multiple sirtuins. Lammers (2021) suggests that the low number of sirtuins in bacteria allows them to control specific physiological processes where they can present a very narrow substrate range or may have a high degree of promiscuity in substrate recognition. An enzyme with high substrate promiscuity may also have specificity for certain substrates because they are processed more efficiently due to the region within the cell where the reaction occurs or by transcriptional regulation of their expression levels (Lammers, 2021).

3.1.1. Overall structure of sirtuin

The alignment of sirtuin´s primary sequence shows that they have a highly conserved catalytic core. At the same time, the regions corresponding to the N- and C-terminal are variable in length and sequence (Yuan & Marmorstein, 2012).

The catalytic core adopts an elongated shape containing a conserved large Rossmann-fold domain, and a smaller and more structurally diverse domain for acyl peptide and NAD+ binding, respectively, connected by a series of loops that contribute to the formation of a cleft between the large and small domains (North & Verdin, 2004; Sanders & Marmorstein, 2010; Zhao, et al., 2004).

The large domain comprises an inverted prototypical open α/β Rossmann fold structure, widely distributed in proteins that bind oxidized or reduced NAD or NADP. This domain comprises six parallel β strands forming a central β sheet packed between several α helices. The exact number of α helices depends on the protein. For example,*Escherichia coli* sirtuin CoB contains eight α helices. Also, a conserved Gly-X-Gly sequence important for phosphate binding, a pocket to accommodate an NAD+ molecule, and charged residues responsible for ribose group binding are found (Figure 2A) (Sanders & Marmorstein, 2010).

The structural Zn^{2+}-binding domain is composed of three antiparallel β-strands and a variable α helical region. A zinc ion is generally bound to four conserved lysine residues in the β-sheet module in a tetrahedral conformation, except for CobB, which is linked to two cysteine residues and contains three of the four expected zinc-coordinating cysteine residues according to sequence alignment (Sanders & Marmorstein, 2010; Yuan & Marmorstein, 2012). The zinc ion does not participate directly in the deacetylation, and it has been observed that it has an essential structural role in the integrity of the catalytic core domain (Figure 2A) (Blander & Guarente, 2004; Yuan & Marmorstein, 2012).

A binding site located between the sirtuin large and small domain, linked to each other by two flexible loops

(L1 and L2), forms a cleft that acts as the enzyme's active site. NAD+ and acetyl-lysine substrate bind at this cleft (Yuan & Marmorstein, 2012). The binding region is divided into three spatially distinct sites: site A, for an adenine-ribose moiety of NAD binding. Site B for the nicotinamide-ribose moiety binding. And site C site, located deep in the NAD-binding pocket for nicotinamide moiety binding (Figure 2A) (Sanders & Marmorstein, 2010).

3.2. Zinc-dependent deacetylases

The metal-dependent HDACs are hydrolases that cleave the acetyl group from the lysine to yield free lysine and acetate (Figure 2D). They belong to a large family of proteins, including acetylpolyamine amidohydrolases, acetoin utilization proteins, and histone deacetylases (Hernick & Fierke, 2006).

These enzymes are classified in eukaryotic organism based on their homology, domain organization, and cellular localization to yeast deacetylases. Class I HDACs are closely related to the transcriptional regulator RPD3 of the yeast *Saccharomyces cerevisiae* , with a length of 350–500 amino acids. They are principally localized in the nucleus and have a variable C-terminus with nuclear import and export signals. Class II HDACs are about 1,000 amino acids long, with a catalytic domain containing several conserved sequence motifs. These enzymes are primarily localized in the cytoplasm, have unique binding sites at their N-termini to control translocation of the protein in and out of the nucleus in response to specific cellular signals, and thus are at least in part cytoplasmic and, in some cases acting on non-histone protein substrates (Hildmann et al., 2006; Ruijter et al., 2003; Wagner et al., 2013). Class IV contains only a single enzyme which is localized to the nucleus.

In bacteria, some proteins have been identified as members of the family of zinc-dependent deacetylases. For example, the LpxC in gram-negative bacteria (*Escherichia coli, Aquifex aeolicus* , and*Pseudomonas aeruginosa*) (Hernick & Fierke, 2006; Jackman et al., 2000; Whittington et al., 2003;), the PA3774 of *Pseudomonas aeruginosa*(Majorek et al., 2013), the AcuC of *Bacillus subtilis* and*Aeromonas hydrophila* (Gardner et al., 2006; Jiang et al., 2017), the FB188 HDAH (histone deacetylase-like amidohydrolase) from*Bordetella /Alcaligenes* strain FB188 (Hildmann et al., 2004; Nielsen et al., 2005) and LdaA of *Rhodopseudomonas palustris* (Crosby et al., 2010).

3.2.1. Overall structure of zinc-dependent

The HDACs structure is characterized by having an α/β fold topology (Hentchel & Escalante-Semerena, 2015; Yang & Seto, 2008). In some family members, the crystallography analysis shows that one or two domains form the tertiary structure. For example, in the LpxC proteins, the two domains have an identical topology of secondary structural elements that includes a five-stranded parallel β-sheet and two principal α-helices connected by a 16-residue linker (Whittington et al., 2003). In contrast, the HDAC8 comprises a single domain consisting of an eight-stranded parallel β-sheet sandwiched between 13 α-helices (Figure 2C) (Somoza et al., 2004). Interestingly, the crystal structure of PA3774 from the human pathogen *P. aeruginosa* shares a high degree of homology with class IIb HDACs and consists of two dimers that are close to each other, forming a tetramer, which may be essential for substrate recognition and selectivity (Kramer et al., 2016). Despite these differences, the structural comparison shows that the structural difference is mainly restricted to the loop regions.

The catalytic center contains a zinc ion commonly pentacoordinate by two aspartic acids, a histidine, and a water molecule. In addition to the zinc ligands, two histidine, two aspartic acids, and one tyrosine are also present, which form hydrogen bonds with bound ligands. Single mutations of these residues abolish entirely the activity (Kramer et al., 2016). Furthermore, the surface of this site reveals the formation of a narrow pocket that probably serves to accommodate the acetylated lysine during the catalytic reaction (Vannini et al., 2004). The reaction requires that a conserved histidine residue act as a general base to activate a metal-bound water that attacks the carbonyl of the acetyl group (Figure 2C) (Finnin et al., 1999; Finnin, 2005; Hernick & Fierke, 2006).

4. Effects of acetylation on bacteria metabolism

To survive, and compete in their natural habitats, bacteria must be able to respond to environmental disturbances or nutrient availability fluctuations by adapting their metabolism. A quick response mechanism is through the regulation of the activity of metabolic enzymes, which can be controlled in three different aspects: the amount of enzyme, the catalytic activity, and the accessibility of substrates (Figure 3) (Xiong & Guan, 2012). Reversible post-translational modification, such as lysine acetylation, may be involved in at least one of these aspects.

Acetylation regulates the metabolic enzyme amount by promoting the assembly of functional multimeric structures that bind to the proteasome for the degradation of proteins or by targeting the substrate for ubiquitylation and proteasome-dependent degradation (Liu et al., 2021; Xiong & Guan, 2012). The enzymatic activity regulation is related to changes in the physical-chemical properties of the catalytic pocket. Several acetylated proteins have found a highly conserved lysine residue within the catalytic active site. The acetylation on this residue neutralizes its positive charge and increases its size, which causes a conformational change in the active site (Kim et al., 2006; Nakayasu et al., 2017). Also, acetylation can regulate substrate accessibility by hindering substrate entry into the active site (Figure 3).

4.1.1. Regulation of enzymatic activity

In prokaryotes, the role of N-lysine acetylation regulating enzymatic activity was first reported in the acetyl-CoA synthetase (Acs) of*S. enterica* (Starai et al., 2002). *In vivo* and *in vitro* assays showed that Pat interacts with leucine 641 of Acs, leading to acetylation of lysine-609, decreasing Acs's activity, the incubation of the acetylated enzyme with NAD^+-dependent deacetylase CobB results in its activation. A Leu-641 Acs mutant showed that position 641 has a structural contribution that allows the interaction of Acs with the protein acetyltransferase (Pat) (Starai & Escalante-Semerena, 2004; Starai et al., 2002; Starai et al., 2004). In other bacteria (*E. coli, B. subtilis, Saccharopolyspora erythraea, Rhodopseudomonas palustris,* and *M. smegmatis*) similar acetylation-dependent regulatory modes of Acs have been described (Castaño-Cerezo et al., 2015; Crosby et al., 2012; Gardner et al., 2006; Kim et al., 2013; Xu et al., 2011; You et al., 2014).

Interestingly, *Mycobacterium tuberculosis* employs acetylation instead of phosphorylation to regulate isocitrate dehydrogenase (ICDH). The enzyme is acetylated in two lysine residues by Rv2170, reducing the enzymatic activity to around 30% (Lee et al., 2017).

As demonstrated by Venkat et al. (2017), protein acetylation can also increase enzyme activity. The enzymatic activity of different acetylated variants of malate dehydrogenase (MDH) obtained by the expansion of the genetic code strategy showed that only the acetylation at positions K99 and K140 and the doubly acetylated MDH at both positions increased the enzyme activity. In other variants, no effect was observed. The authors also demonstrated that MDH acetylation can occur either enzymatically or non-enzymatically and that the level of MDH acetylation increases in a glucose-dependent manner (Venkat et a., 2017).

In other proteins, the effect on activity depends on the lysine residue that is acetylated. For example, the enzyme assay and kinetic analysis of different acetylated variants of *E. coli* citrate synthase (CS), showed that lysine acetylation could decrease the overall CS enzyme activity, mainly due to the acetylation of K295, which impaired the binding of acetyl-coenzyme A. However, acetylation at K283 increased the enzymatic activity since the binding of acetyl-coenzyme A is promoted (Venkat et al., 2019). The same result was observed in the isocitrate dehydrogenase (ICDH) (Venkat et al., 2018).

These studies prove that acetylation modulates the activity of central metabolic enzymes and eventually alters protein function for controlling competing pathways.

4.1.2. Carbon source-regulated protein acetylation

Comparative studies with different carbon sources have explored the effect of protein acetylation during growth under glycolytic and oxidative conditions. As Wang and collaborators demonstrated, *S. enterica* presents changes in cell growth and significant difference in the percentage of acetylated proteins in response to glucose or citrate. A total of 15 enzymes were identified with altered acetylation status in response to a carbon source, and all of them showed higher acetylation levels in cells grown in glucose (Wang et al., 2010).

The main targets of lysine acetylation were enzymes involved in central metabolism, and a correlation was observed in the cell growth and the acetylation levels on these proteins; in a medium containing glucose, cells grow faster, and an increase in the acetylation of central metabolic enzymes was observed, but when the acetylation decreases, opposite growth properties were observed (Wang et al., 2010). In *Bacillus subtilis* , different acetylation levels have also been reported in different carbon sources. in decreasing order, protein acetylation occurs in a medium containing only carbon source pyruvate, glucose, glycerol, or citrate. (Kosono et al., 2015). The main differences in the acyl modification patterns were observed in glucose and citrate. In fact, it was under these two conditions that changes in the acetylation sites were determined. Acetylation was upregulated at 13 sites in the glucose condition relative to the citrate condition, and as in *S. enterica* , acetylation positively modulated the growth of this bacterium in glucose-medium (Kosono et al., 2015). Western immunoblot analysis found that glucose and lactate produce global acetylation in *E. coli* K-12, which increased after the cells had entered the stationary phase. Induction of acetylation at this growth point requires continue exposure to carbon sources, specifically glucose (Schilling et al., 2015). The results suggest that the protein acetylation may have a physiological role in mediating adaptation to different carbon sources.

Another factor that could determine the lysine acetylation levels is the sugar amount. In *E. coli,* there is no difference in acetylation patterns during growth in glucose or xylose as the sole carbon source. However, an increase in the relative abundance of acetylation at high concentrations of these two carbohydrates has been observed, indicating that acetylation only sometimes has a regulatory role in obtaining specific carbon sources (Schilling et al., 2019). Also, acetylation increases in parallel with glucose consumption and acetate excretion (Schilling et al., 2015; Schilling et al., 2019). The results support the hypothesis that protein acetylation regulates metabolism in response to energy status, mainly when the carbon flux exceeds the capacity of the tricarboxylic acid (TCA) cycle, resulting in overflow metabolism (Schilling et al., 2015; Schilling et al., 2019).

4.1.2.1 Regulation of acetate metabolism by protein acetylation

Acetate overflow is a phenomenon that occurs when *E. coli* grows aerobically on glucose as the only carbon source. Under these conditions, there is an imbalance between glucose absorption and its conversion into biomass and products, causing the excretion of significant amounts of acetate (Bernal et al., 2016). The produced acetate can be used after depletion of the primary carbon source by the acetyl-CoA synthetase (Acs), which activates acetate to acetyl-coenzyme A (Ac-CoA), which is mainly metabolized by the glyoxylate shunt and the TCA cycle (Castaño-Cerezo et al., 2015). Acs is tightly controlled at the transcriptional level, and its activity, as mentioned above, is posttranslationally regulated by protein acetyltransferase.

The physiological role of Acs in acetate metabolism has been studied in*E. coli* strains with different genetic backgrounds. In knockout mutant [?]cobB and [?]acs, a similar phenotype was observed. Both strains produce acetate in glucose carbon-limited cultures, where yield is limited by its inability to scavenge overflown acetate (Castano-Cerezo et al., 2014). The [?]coB mutant shows a reduced growth rate dependent on acetate concentration, as well as a reduction in the enzymatic activity of Acs in acetate cultures (Castano-Cerezo et al., 2011; Castano-Cerezo et al., 2014; Peebo et al., 2014). The growth at high and low acetate concentrations is restored in the double cobB-yfiQ- mutant (Castano-Cerezo et al., 2011). The results demonstrate that protein acetylation plays an essential role in regulating overflow metabolism by modulating the activity of Acs. The inactivation of the enzyme limits acetyl-CoA synthesis, leading to acetate accumulation and growth inhibition.

Furthermore, acetylation of Acs may be involved in the control of the co-utilization of fermentable substrates. A random mutation in Leu-641 makes acetyl-CoA synthetase insensitive to acetylation even in high glucose concentrations (Starai & Escalante, 2005). The overexpression in *E. coli* W of this protein allows the efficient co-utilization of glucose and acetate. In a batch process containing glucose and high acetate concentrations, an increase of 2.7-fold in acetate up taken compared to a control strain was observed (Novak et al., 2018).

4.1.3. Regulation of metabolic flux

Most microorganisms have developed different strategies to co-metabolize a mixture of simple carbohydrates, favoring the utilization of glucose as a carbon and energy source to sustain a higher growth rate. The metabolic plasticity allows them to obtain efficiently specific carbon sources and survive in competitive environments (Vinuselvi et al., 2012). Most bacteria regulate their metabolism via carbon catabolite repression (CCR), which involves a complex interplay between metabolism, signaling by proteins and metabolites, and the regulation of gene expression (Kremling et al., 2015). Other mechanisms can be used to modulate carbon flux at critical metabolic nodes without this regulatory control.

Post-translational modifications affect flux distribution between important metabolic branches, such as glycolysis and gluconeogenesis, TCA cycle and glyoxylate shunt, and glycolysis and TCA cycle. Wang et al. (2010) demonstrated that carbon source-associated acetylation modulates metabolic flux profiles in *S. enterica* . In the presence of glucose, acetylation increases the glycolysis/gluconeogenesis flux ratio 2.07-fold, while acetylation reduces the glyoxylate bypass/TCA flux ratio under a citrate-based carbon source.

The isocitrate node is an important regulation point of carbon flux between the TCA cycle and the glyoxylate shunt. Isocitrate, the substrate of isocitrate dehydrogenase (ICDH) and isocitrate lyase (AceA), is converted to ∝-ketoglutarate by ICDH or is cleaved to succinate and glyoxylate by AceA, directing the carbon source flow to TCA cycle or to glyoxylate shunt, respectively. In *Mycobacterium tuberculosis,* this metabolic node is regulated by the acetylation of the ICDH. Acetylation suppresses enzyme activity in the presence of fatty acids reducing carbon flow into the TCA cycle (Lee et al., 2017). The activation of glyoxylate bypass allows the conversion of acetyl-CoA to the metabolic intermediate succinate to support the growth in the presence of non-carbohydrates substrates such as fatty acids or acetate (Cronan & Laporte 2005; Lee et al., 2017). For *S. enterica,* it has been reported that the node is controlled by modulating the activity of the bifunctional isocitrate dehydrogenase phosphatase/kinase (AceK) (Wang et al., 2010). However, in the analysis of *E. coli*proteome, acetylation of AceK was not detected, and not change in the metabolic fluxes was quantified (Castaño-Cerezo et al., 2014). Acetylation affects, the activity of isocitrate lyase (AceA) in this bacterium. Acetylation led to a decrease in Ace-A specific activity, and with the proteomic data, several acetylation sites were detected in the protein (Castaño-Cerezo et al., 2014).

Recently it was demonstrated that glyceraldehyde 3-phosphate dehydrogenase (GapA) and 2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (GpmA) were sensitive to non-enzymatic acetylation *in vitro* at physiological AcP concentrations. In both enzymes, acetylation reduced their activity, which could be reflected in reduced glycolytic/gluconeogenic flux in conditions with higher concentrations of AcP (Schastnaya et al., 2023).

The changing flux from glucose to glutamate is increased when the cell excretes glutamate. Factors like the depletion of biotin and the addition of detergents or antibiotics trigger glutamate overproduction and, therefore, a change in the flux of central carbon metabolism to favor glutamate production (Shirai et al., 2007). It has been proposed that in addition to the decrease in 2-oxoglutarate dehydrogenase complex (ODHC) activity, the regulation of phosphoenolpyruvate carboxylase (PEPC) activity by acetylation may be a mechanism involved in the change in metabolic flux during overproduction of glutamate. PEPC catalyzes the irreversible carboxylation of phosphoenolpyruvate to generate oxaloacetate, ensuring that the carbon flow is directed toward glutamate production via the Krebs cycle. Acetylation at the K653 site regulates enzyme activity and, therefore, the mechanism that maintains metabolic flux under glutamate-producing conditions (Mizuno et al., 2016; Nagano-Shoji et al., 2017).

Hence, acetylation may provide a new strategy for regulating protein activity and improving the utilization of different carbon sources.

5. ACP is the donor molecule in chemical acetylation

Many studies on protein acetylation in bacteria have focused on describing enzymatic acetylation, its implication in cellular physiology, pathogenesis, in bacterial response to environmental conditions, etc. However, evidence shows that non-enzymatic acetylation is also possible (Table 2).

The acetylation by AcP has been mainly described by proteomic studies, in which a comparative analysis of the acetylome of different strains allows us to determine if there is any difference in the protein acetylation levels and infer the acetylation mechanism. This has been achieved in *E. coli, N. gonorrhoeae* and *Borrelia burgdorferi* (Table 2) (Bontemps-Gallo et al., 2018; Kosono et al., 2015; Kuhn et al., 2014; Post et al., 2017; Reverdy et al., 2018; Schilling et al., 2015; Weinert et al., 2013). These studies have focused on comparing the proteomic data of the acetylated proteins of the wild-type strain with different isogenic mutant strains. The acetylome data using *Escherichia coli* as a model demonstrated that acetylation depends on acetyl-phosphate (AcP) formation and occurs at a low level in growth-arrested cells. Mutant cells unable to synthesize (*pta ackA* mutant) or metabolize (*ackA* mutant) AcP had the opposite behavior, while in the first one, significantly reduced acetylation levels were observed, and the accumulation of AcP significantly elevated acetylation levels. Also, the authors demonstrated that the AcP acetylate lysine residues *in vitro* at a concentration comparable to that found *in vivo* . These data establish AcP as a critical regulator of acetylation and suggest that AcP acts nonenzymatically to regulate acetylation levels in response to glucose (Table 2) (Kuhn et al., 2014; Schilling et al., 2015; Weinert et al., 2013).

Since AcCoA and AcP are derived from multiple metabolic pathways in other microorganisms, it is difficult to establish whether both or only one of these molecules is the acetyl group donor. The bacterium *B. burgdorferi* is characterized by producing AcCoA and AcP from a unique metabolic pathway, the acetate/mevalonate pathway. In this pathway, acetate is converted to Ac-P by acetate kinase (AckA), which is metabolized to acetyl-CoA by phosphotransacetylase (Pta) (Bontemps-Gallo et al., 2018; Richards et al., 2015). The acetylome analysis of different mutant strains and their respective complements showed that no acetylation is observed in the strain that does not synthesize either AcP nor AcCoA (ΔackA mutant). In the ackA complemented strain, an increase in acetylation was detected, and the Δpta complemented strain displayed similar levels of acetylation as the wild-type. Remarkably, hyper-lysine acetylation levels were detected in the Δpta due to the AcP accumulation. Together these results demonstrated that this molecule is the primary source of acetylation (Bontemps-Gallo et al., 2018) (Table 2).

To establish the metabolic processes that AcP acetylation regulates, the modified proteins can be analyzed with different software (PANTHER, DAVID, ERGO, and KEEG). From the proteomic data of *E. coli,* the functional analysis reveals that the elongation factors, most of the ribosomal subunits and aminoacyl-tRNA ligases, are acetylated in an AcP-dependent manner (Christensen et al., 2019; Kuhn et al., 2014; Post et al., 2017). In *Borrelia burgdorferi* , the acetylated proteins were involved in genetic information, metabolism and transport, protein folding and degradation, detoxification, motility, and chemotaxis (Bontemps-Gallo et al., 2018). Similarly, some metabolic pathways related to carbohydrate metabolisms (glycolysis/gluconeogenesis, pentose phosphate pathway, pyruvate metabolism, and the TCA cycle), fatty acid metabolism, and pantothenate metabolism are sensitive to AcP-dependent acetylation (Kuhn et al., 2014; Schilling et al., 2015).

5.1. Characteristics of lysine residues sensitive to chemical acetylation

As previously mentioned, in mitochondria, non-enzymatic acetylation is favored by its physicochemical conditions, where the pH value affects the protonation state of the lysine side chain; at basic pH, lysine is deprotonated, which increases its nucleophilicity (Figure 4). However, the cytoplasmatic pH is maintained at values close to neutrality in bacteria, e.g., in neutrophilic bacteria, the cytoplasmic pH is in a narrow range of ˜7.5–7.7, alkaliphilic bacteria maintain a constant internal basic pH value of 7.5–8.3, and the pH of acidophilic bacteria is close to 6.5 (Krulwich et al., 2011; Slonczewski et al., 2009). Under these conditions, lysine is not reactive since it has a high pKa, and the epsilon amino groups of most lysine side chains are protonated.

The analysis of different chemically acetylated proteins has shown that non-enzymatic acetylation in these organisms depends on the microenvironment of the protein that influences lysine reactivity toward AcCoA or AcP (Figure 4). The process preferentially occurs on lysine flanked by residues with positive charges (Lys or Arg), hydroxyls (Ser, Thr, or Tyr), or amides (Gln or Asn), which coordinate the AcP binding through ionic and hydrogen bonds (Hebert et al., 2013; Kuhn et al., 2014; Wagner and Payne, 2013). Also, a tendency of negatively charged glutamate (E) and/or aspartate (D) residues near the -1 or +1 position to an acetylated

lysine, which reduces the pKa of lysine and enhancement its reactivity, has been observed (Christensen et al., 2019; Kuhn et al., 2014; Post et al., 2017).

6. Methods for studying protein acetylation

To study the biological role of protein acetylation is necessary to apply appropriate methods to identify and quantify acetylated proteins and assign acetylation sites. For the same study, different techniques can be applied, depending on the aim of the investigation and sample complexity and type (Tables 3 and 4).

6.1. Western-blot analysis

As a first step in these analyses, a detection and semi-quantification of the targeted acetylated protein can be made by western blot analysis. For this reason, is necessary to extract the proteins from the cells or tissues using lysis buffers with chaotropic agents and detergents; in addition, to mechanical cell disruption methods such as heating, bead beating, ultrasonication, or mechanical agitation, to facilitate the protein extractions. Then, proteins can be purified and separated by gel electrophoresis for the subsequent transfer to nitrocellulose or polyvinylidene difluoride (PVDF) membranes to finally be detected using specific antibodies against acetylated proteins (Diallo et al., 2019; Yu et al., 2008; Hirano, 2012).

In western blot analysis, two types of antibodies can be used: (1) site-specific antibodies for recognition of a consensus amino acid sequences containing acetyl-lysine residue(s); (2) pan-specific antibodies for recognition of acetyl-lysine residues regardless of the surrounding amino acid sequence (Diallo et al., 2019; Komatsu et al., 2005).

The study of Schilling and coworkers on the effect of different carbon sources on protein acetylation in *E. coli* through western blot analysis showed that the global acetylation profile increased steadily independent of the carbon source used for their growth, with a higher number of acetylated proteins after the cells enter the stationary phase. Furthermore, immunoblot analysis revealed a higher number of acetylated proteins when the medium was supplemented with the carbon source after an incubation period. With these results, the authors suggest that carbon-induced protein acetylation in the stationary phase only occurs if cells are exposed to a supplemental carbon source in the stationary phase (Schilling et al., 2015). However, this approach usually presents a low sensitive and non-specific signal, the needs to introduce negative controls to ensure the anti-acetyl lysine antibody specificity, and it is not possible to know the number/assignment of specific acetylation sites and protein identification (Diallo et al., 2019)

6.2. Mass spectrometry analysis for the identification of acetylated lysins

Liquid chromatography coupled to mass spectrometry (LC-MS/MS) systems have become the method of choice for the sequencing and identification of thousands of proteins and also is essential, for the study PTMs, due to it is sensitivity, precision, and accuracy. Despite lysine acetylation emerging as a frequently occurring PTM, its low abundance compared to non-acetylated proteins requires enrichment steps before the detection in high-throughput proteomics. Protein affinity purification and prefractionation by immunoprecipitation are commonly used to reduce sample complexity and enrich acetylated proteins/peptides (Mischerikow & Heck, 2011). As demonstrated by Choudhary et al. (2009), the affinity enrichment of acetylated peptides allowed to increase in the number of acetylation sites identified since, in a separate experiment without affinity enrichment, the number of acetylation sites was 60-fold lower (Choudhary et al., 2009).

Most of the published studies on prokaryotic organisms use purification systems to the enrichment of acetylated peptides for the global characterization of protein acetylation (Table 3). For example, an integrated approach combining pan antibodies for protein enrichment with OrbitrapTM mass spectrometry (MS/MS) for comprehensive profiling of lysine acetylation in *E. coli* was developed by Zhang et al. (2013). This study identified 1070 acetylation sites on 349 proteins, along with the identification of peptide sequences and the assignment of acetylated sites. With this approach, not only previously reported acetylated proteins were identified, but also new target proteins were found. For example, the analysis identified isocitrate lyase as a novel acetylated protein. Additionally, many novel acetylation sites were discovered (Zhang et al., 2013). The acetylome study of *Vibrio parahemolyticus* , *S. eriocheiris,* and *Bacillusamyloliquefaciens* , showed

that the combination of immunoaffinity enrichment of lysine-acetylated peptides with LC-MS/MS (liquid chromatography–mass spectrometry), allows the identification of a greater number of acetylated proteins, which represent 13.6%, 44.69% and 32.9% of the total proteins in each bacterium (Lui et al., 2016; Meng et al., 2016; Pan et al., 2014).

As shown by Nakayasu and coworkers, protein enrichment is not an essential step in proteomics studies since the global proteome coverage of 48 organisms totaled 73,656 proteins (902,937 peptides) and 9,107 acetylated proteins identified, averaging ˜190 per organism (24,397 total acetylated peptides, ˜508 per organism). These numbers are substantially more extensive than several previous studies (Nakayasu et al., 2017).

Thus, mass spectrometry analysis is a powerful method to identify specific acetylated peptides and sites in a mixture of acetylated and non-acetylated peptides with a high confidence level. Also, it is possible to quantitatively characterize the acetylome and determine how the relative abundance of protein acetylation changes in response to different conditions (Diallo et al., 2019).

6.2.1. Mass spectrometry analysis for the relative quantification of acetylated lysins

Mass spectrometry is not inherently quantitative because proteolytic peptides have different physiochemical properties (size, charge, hydrophobicity, and more), which produce variations in the MS/MS spectra. Therefore, for accurate quantification, it is generally required to compare each peptide between experiments (Bantscheff et al., 2007). For this reason, different labeling techniques coupled to LC-MS have been developed: stable-isotope labeling with amino acids in cell culture (SILAC), isotope-coded affinity tags (ICAT), tandem mass tag (TMT), iTRAQ, multiplex isobaric tags, and heavy peptide AQUA are some examples (Gingras et al., 2007; Lindemann et al., 2017; Zhang & Elias, 2017). Isotope labels can be introduced into amino acids metabolically, chemically, or enzymatically. The labeled peptides are chemically identical to the corresponding native peptide, and therefore the difference in mass between the light and heavy peptides can be measured in the mass spectrometer. So, the quantification is achieved by comparing their respective signal intensities (Bantscheff et al., 2007; Zhang & Elias, 2017). However, these methods have some limitations as increased time and sample preparation complexity, high protein concentration is required, the reagents used are expensive, incomplete labeling, and the requirement for specific quantification software. So far, only TMT and iTRAQ allow the comparison of multiple samples simultaneously (Zhu et al., 2009).

An alternative strategy is the label-free quantification method for analyzing two or more experiments. The relative quantification can be made by comparing the direct mass spectrometric signal intensity for any given peptide or counting the number of peptide-to-spectrum matches (PSMs; spectral counting) obtained for each protein, as more abundant proteins are more likely to be observed in peptide spectra (Bantscheff et al., 2007; Lindemann et al., 2017; Zhu et al., 2009).

As shown in Table 4, using different labeling strategies, it has been possible to quantify acetylated sites and proteins robustly and precisely, which has allowed to elucidate the role of N-acetylation in processes such as biofilm formation and pathogenesis, determine how is the dynamics of acetylation during bacterial growth and if the carbon source influences the PTM rate. For example, the quantitative lysine acetylome analysis of the pathogen bacterium *Bacillus nematocida*B16 revealed that during pathogenesis proteins involved in the synthesis of nematode attractants and the secretion of the main virulence factors of B16 were acetylated and that the acetylation levels of different lysine sites were regulated significantly differently in the presence of nematodes. The results suggested that lysine acetylation may play a role in regulating B16-*C. elegans* interaction (Sun et al., 2018). For *E. coli* , this analysis showed that many acetylated lysine residues are regulated in an acetyl phosphate (acP)-dependent manner, demonstrating that chemical Nε-lysine acetylation is a viable mechanism (Kuhn et al., 2014). Gaviard et al. (2018) evidenced the importance of carrying out a quantitative study since, in the analysis of *P. aeruginosa*proteome, it was found that the number of acetylated peptides varies depending on the carbon source. However, the quantification of acetylated peptides did not show a significant abundance difference.

6.2.2. Stoichiometry of site-specific lysine acetylation

Although relative quantification is a powerful technique that allows to establish if there are variations in the acetylation levels in different conditions, it is limited by the fact that the changes are relative to the total protein abundance, which can vary from one condition to another, giving misinterpretations about the physiological significance of this PTM. Analysis of acetylation stoichiometry or occupancy can allow us to identify the critical acetylation sites whose changes in abundance are physiologically more important.

However, determining the acetylation stoichiometry is a complex task, mainly because the ionization efficiency of modified and unmodified peptides in a mass spectrometer is different. For this reason, various working groups have reported methods and workflows for the precise quantification of site-specific protein acetylation occupancy, which are based on the comparison of the proportion of endogenously acetylated lysine versus chemically labeled lysine that is not endogenously acetylated (Baeza et al., 2014; Gil et al., 2017; Miyagi, 2017; Weinert et al., 2014; Weinert et al., 2017; Wei et al., 2018).

The first protocol developed for directly quantifying stoichiometric of site-specific acetylation in bacteria was based on chemical acetylation of free lysine residues with isotopic acetic anhydride, followed by trypsin cleavage and MS analysis. The method was applied to analyze the entire proteome of *E. coli* , specifically to determine the role of deacetylases, CobB, on both site-specific and global acetylation (Baeza et al., 2014). In a similar study, Weiner et al. (2017) determined the absolute acetylation stoichiometry but used a serial dilution of SILAC-labeled peptides (SDSILAC). Although the methodologies differ in the way of labeling the peptides, with both approaches, it was shown that sirtuin deacetylase deficiency affects central metabolism and leads to both site-specific and global changes in protein acetylation stoichiometry (Baeza et al., 2014; Weiner et al., 2017).

Exploring the relationship of chemical acetylation and how it affects the enzymatic activity of glycolytic proteins, it was found that possibly a maximum of 10% of non-enzymatically acetylated proteins reach a stoichiometry that could inhibit their activity and that enzymes such as GapA and GpmA are acetylated at high stoichiometry (Schastnaya et al., 2023). The authors suggest that AcP-acetylation is specific and may exert control over metabolism.

Knowing the stoichiometry of acetylation can help to establish how it changes and whether it exerts a regulatory effect or only has a constitutive function necessary for protein folding or stable interaction.

Remarks

In recent years, the field of research on acetylation in bacteria has increased considerably, demonstrating its relevance in various cellular processes. Protein acetylation is a highly dynamic modification that occurs on short time scales (minutes to hours), where the acetylation levels are dictated by two closely related mechanisms, the addition and removal of the acyl group. (Baeza et al., 2020). This property allows the cell to respond quickly to different environmental changes by modifying the functionality of the protein to adjust metabolic processes.

The development of protein labeling and enrichment methods and technological advances in mass spectrometry for identifying and quantifying acetylated sites and proteins, have allowed the study of lysine acetylation in various organisms under different conditions. Thus, it has been shown that acetyl-lysines must result from one of the recognized acetylation mechanisms, whether enzymatic or non-enzymatic. Furthermore, it has been identified that the target proteins of acetylation participate in various cellular and metabolic processes, highlighting the acetylation of conserved lysines in central metabolism proteins. However, for many proteins, the biological relevance of this modification remains to be determined.

Another critical aspect that has yet to investigated deeply is the determination of the acetylation stoichiometry. Quantification of fold changes only sometimes has important biological significance. For example, if acetylation has functional consequences, such as loss of function, the modification should have a high stoichiometry. In comparison, a low percentage of acetylation may suggest a modification related to protein folding or the formation of a stable interaction (Carabetta & Cristea, 2016). So far, the techniques available for determining the stoichiometry have revealed that most acetylated proteins occur at low stoichiometry

(0-10%), and in approximately 4% of acetylated sites a >20% stoichiometry is observed (Baeza et al., 2014; Weinert et al., 2017). The aforementioned indicates that only a small fraction of the acetylome is of physiological importance. Also, essential to consider that proteomic analysis is based on experiments under a particular condition and in isolated organisms, so it would be convenient to consider performing a comparative analysis under different conditions (temperature, carbon sources, among others) and their behavior in co-culture or symbiosis for a correct interpretation of the data.

Several functional monitoring studies are still being carried out using*in vitro* assays, and although they allow us to know the effect of acetylation on the protein, only sometimes reflect the real consequence of this post-translational regulation process. Therefore, it is necessary to generate hybrid approaches that help us to identify and characterize not only the modified proteins but also in which metabolic pathways are involved and how this impacts the metabolism of the cell. Such approaches can integrate the analysis of proteomic, transcriptomic, and metabolomics data sets.

Incorporating synthetic biology can expand our understanding of how lysine acetylation regulates protein function. In this regard, genetic code expansion (GCE) has become essential for studing biological processes such as post-translational modifications. Rather than adding acetyl groups after protein translation, this approach relies on heterologous pairs of aminoacyl-tRNA-synthetases (aaRS) and its cognate tRNA from *Methanosarcinaceae* species that enable the co-translational incorporation of Nε-acetyllysine (AcK) in response to stop codon (Schmidt & Summerer, 2014). This approach has been used for some groups to evaluate the role of lysine acetylation using recombinant expressed proteins, finding that the enzymatic activity of several proteins is positively or negatively regulated. Once again, we believe this type of study should find an application for studies in bacteria*in vivo* .

Non-enzymatic acetylation is an inevitable consequence of the cell metabolic states. At high concentrations of AcP this mechanism is favored. However, it should be considered that this molecule can also be used as an acyl group donor for acetyltransferases catalyzing protein acetylation. Therefore, it cannot be ruled out that these mechanisms coexist. The roles of global acetylation and other acyl donors (succinyl-CoA, propionyl-CoA, and malonyl-CoA) for enzymatic or non-enzymatic protein acylation remain to be elucidated. Finally, it is essential to understand how lysine acetylation interferes with and cross-links with other post-translational modifications.

Funding

Part of this work was supported by the Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológica (PAPIIT-UNAM), grant IN-213522 to S. E-G.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

- Ali, I., Conrad, R. J., Verdin, E., & Ott, M. (2018). Lysine acetylation goes global: from epigenetics to metabolism and therapeutics. *Chemical reviews* , **118** (3), 1216-1252. doi: 10.1021/acs.chemrev.7b00181.
- Allfrey, V. G., Faulkner, R., & Mirsky, A. E. (1964). Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proceedings of the National Academy of Sciences* ,**51** (5), 786–794. doi: 10.1073/pnas.51.5.786.
- Alonso, V. L., & Serra, E.C. (2012). Lysine acetylation: elucidating the components of an emerging global signaling pathway in trypanosomes. *Journal of Biomedicine and Biotechnology* ,**12** . doi: 10.1155/2012/452934
- Baeza, J., Dowell, J. A., Smallegan, M. J., Fan, J., Amador-Noguez, D., Khan, Z., Denu, J. M. (2014). Stoichiometry of site-specific lysine acetylation in an entire proteome. *Journal of Biological Chemistry* , **289** (31), 21326-21338. doi: 10.1074/jbc.M114.581843.

- Baeza, J., Lawton, A. J., Fan, J., Smallegan, M. J., Lienert, I., Gandhi, T., ... Denu, J. M. (2020). Revealing dynamic protein acetylation across subcellular compartments. *Journal of proteome research* , **19** (6), 2404-2418. https://doi.org/10.1021/acs.jproteome.0c00088.
- Bai, L., Chang, M., Shan, J., Jiang, R., Zhang, Y., Zhang, R., & Li, Y. (2011). Identification and characterization of a novel spermidine/spermine acetyltransferase encoded by gene ste26 from*Streptomyces* sp. 139. *Biochimie* , **93** (9), 1401-1407. https://doi.org/10.1016/j.biochi.2011.04.014.
- Bantscheff, M., Schirle, M., Sweetman, G., Rick, J., & Kuster, B. (2007). Quantitative mass spectrometry in proteomics: a critical review. *Analytical and bioanalytical chemistry* , **389** (4), 1017-1031. doi: 10.1007/s00216-007-1486-6.
- Barrett, O. J., Pushechnikov, A., Wu, M., & Disney, M.D. (2008). Studying aminoglycoside modification by the acetyltransferase class of resistance-causing enzymes via microarray. *Carbohydrate research* , **343** (17), 2924-2931. doi: 10.1016/j.carres.2008.08.018.
- Bernal, V., Castaño-Cerezo, S., & Cánovas, M. (2016). Acetate metabolism regulation in Escherichia coli: carbon overflow, pathogenicity, and beyond. *Applied microbiology and biotechnology* , **100** , 8985-9001. doi: 10.1007/s00253-016-7832-x.
- Berrabah, W., Aumercier, P., Lefebvre, P., & Staels, B. (2011). Control of nuclear receptor activities in metabolism by post-translational modifications. *FEBS letters* ,**585** (11), 1640-1650. doi: 10.1016/j.febslet.2011.03.066.
- Blander, G., & Guarente, L. (2004). The Sir2 family of protein deacetylases. *Annual review of biochemistry* , **73** (1), 417-435. doi: 10.1146/annurev.biochem.73.011303.073651.
- Blasl, A. T., Schulze, S., Qin, C., Graf, L. G., Vogt, R., & Lammers, M. (2021). Post-translational lysine ac (et) ylation in health, ageing and disease. *Biological Chemistry* , 403 (2), 151-194. doi: 10.1515/hsz-2021-0139.
- Bontemps-Gallo, S., Gaviard, C., Richards, C. L., Kentache, T., Raffel, S. J., Lawrence, K. A., ... & Gherardini, F. C. (2018). Global profiling of lysine acetylation in *Borrelia burgdorferi*B31 reveals its role in central metabolism. *Frontiers in microbiology* , **9** , 2036. https://doi.org/10.3389/fmicb.2018.02036.
- Carabetta, V. J., Greco, T. M., Tanner, A. W., Cristea, I. M. & Dubnau, D. (2016). Temporal regulation of the *Bacillus subtilis*acetylome and evidence for a role of MreB acetylation in cell wall growth. *Msystems* , **1** (3), e00005-16. doi: 10.1128/mSystems.00005-16.
- Card, G. L., Peterson, N. A., Smith, C. A., Rupp, B., Schick, B. M., & Baker, E. N. (2005). The crystal structure of rv1347c, a putative antibiotic resistance protein from *Mycobacterium tuberculosis* , reveals a GCN5-related fold and suggests an alternative function in siderophore biosynthesis. *Journal of Biological Chemistry* , **280** (14), 13978-13986. DOI:https://doi.org/10.1074/jbc.M413904200.
- Castaño-Cerezo, S., Bernal, V., Blanco-Catala, J., Iborra, J. L., & Canovas, M. (2011). cAMP-CRP co-ordinates the expression of the protein acetylation pathway with central metabolism in Escherichia coli. *Molecular microbiology* , **82** (5), 1110-1128. https://doi.org/10.1111/j.1365-2958.2011.07873.x.
- Castano-Cerezo, S., Bernal, V., Post, H., Fuhrer, T., Cappadona, S., Sanchez-Diaz, N. C., & Canovas, M. (2014). Protein acetylation affects acetate metabolism, motility and acid stress response in*Escherichia coli* . *Molecular systems biology* , **10** (11), 762. doi: 10.15252/msb.20145227.
- Castano-Cerezo, S., Bernal, V., Rohrig, T., Termeer, S., & Canovas, M. (2015). Regulation of acetate metabolism in *Escherichia coli*BL21 by protein Nε-lysine acetylation. *Applied microbiology and biotechnology* , **99** (8), 3533-3545. doi: 10.1007/s00253-014-6280-8.
- Chen, W., Biswas, T., Porter, V. R., Tsodikov, O. V., & Garneau-Tsodikova, S. (2011). Unusual regioversatility of acetyltransferase Eis, a cause of drug resistance in XDR-TB.*Proceedings of the National Academy of Sciences* ,**108** (24), 9804 –9808. https://doi .org/10.1073/pnas.1105379108.
- Chen, Z., Luo, L., Chen, R., Hu, H., Pan, Y., Jiang, H., ... Gong, Y. (2018). Acetylome profiling reveals extensive lysine acetylation of the fatty acid metabolism pathway in the diatom *Phaeodactylum tricornutum* . *Molecular & Cellular Proteomics* , **17** (3), 399-412. DOI:https://doi.org/10.1074/mcp.RA117.000339.
- Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., ... Mann, M. (2009). Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*

, **325** (5942), 834-840. doi: 10.1126/science.1175371.

- Christensen, D. G., Baumgartner, J. T., Xie, X., Jew, K.M., Basisty, N., Schilling, B., ... Wolfe, A.J. (2019). Mechanisms, detection, and relevance of protein acetylation in prokaryotes. *MBio* , **10** (2), e02708-18. doi: 10.1128/mBio.02708-18.
- Christensen, D. G., Meyer, J. G., Baumgartner, J. T., D'Souza, A. K., Nelson, W. C., Payne, S. H., ... Wolfe, A. J. (2018). Identification of novel protein lysine acetyltransferases in *Escherichia coli* . *MBio* , **9** (5), e01905-18. doi: 10.1128/mBio.01905-18.
- Clements, A., Rojas, J. R., Trievel, R. C., Wang, L., Berger, S. L., & Marmorstein, R. (1999). Crystal structure of the histone acetyltransferase domain of the human PCAF transcriptional regulator bound to coenzyme A. *The EMBO journal* , **18** (13), 3521-3532. doi: 10.1093/emboj/18.13.3521.
- Cronan, Jr, J. E., & Laporte, D. (2005). Tricarboxylic acid cycle and glyoxylate bypass. *EcoSal Plus* , **1** (2). doi: 10.1128/ecosalplus.3.5.2.
- Crosby, H. A., Heiniger, E. K., Harwood, C. S., & Escalante-Semerena, J. C. (2010). Reversible Nε-lysine acetylation regulates the activity of acyl-CoA synthetases involved in anaerobic benzoate catabolism in*Rhodopseudomonas palustris* . *Molecular microbiology* , **76** (4), 874-888. doi: 10.1111/j.1365-2958.2010.07127.x.
- Crosby, H. A., Pelletier, D. A., Hurst, G. B., & Escalante-Semerena, J.C. (2012). System-wide studies of N-lysine acetylation in*Rhodopseudomonas palustris* reveal substrate specificity of protein acetyltransferases. *Journal of Biological Chemistry* ,**287** (19), 15590-15601. doi: 10.1074/jbc.M112.352104.
- DeLano, W. L. (2002). The PyMOL molecular graphics system. http://www. pymol. org/.
- Diallo, I., Seve, M., Cunin, V., Minassian, F., Poisson, J. F., Michelland, S., & Bourgoin-Voillard, S. (2019). Current trends in protein acetylation analysis. *Expert Review of Proteomics* , **16** (2), 139-159. doi: 10.1080/14789450.2019.1559061.
- Dutnall, R. N., Tafrov, S. T., Sternglanz, R., & Ramakrishnan, V. (1998). Structure of the histone acetyltransferase Hat1: a paradigm for the GCN5-related N-acetyltransferase superfamily. *Cell* ,**94** :427–438. 10.1016/S0092-8674(00)81584-6.
- Dyda, F., Klein, D. C., & Hickman, A. B. (2000). GCN5-related N-acetyltransferases: a structural overview. *Annual review of biophysics and biomolecular structure* , **29** (1), 81-103. doi: 10.1146/annurev.biophys.29.1.81.
- Favrot, L., Blanchard, J. S., & Vergnolle, O. (2016). Bacterial GCN5-related N-acetyltransferases: from resistance to regulation.*Biochemistry* , **55** ,989–1002. https://doi.org/10.1021/acs.biochem.5b01269. 59.
- Filippova, E. V., Kuhn, M. L., Osipiuk, J., Kiryukhina, O., Joachimiak, A., Ballicora, M. A., & Anderson, W. F. (2015). A novel polyamine allosteric site of SpeG from *Vibrio cholerae* is revealed by its dodecameric structure. *Journal of molecular biology* , **427** (6), 1316-1334. doi: 10.1016/j.jmb.2015.01.009.
- Finkel, T., Deng, C. X., & Mostoslavsky, R. (2009). Recent progress in the biology and physiology of sirtuins. *Nature* , 460(7255), 587-591. doi: 10.1038/nature08197.
- Finnin, C.A. (2005). Zinc hydrolases: the mechanisms of zinc-dependent deacetylases. *Archives of biochemistry and biophysics* , **433** (1), 71-84. doi: 10.1016/j.abb.2004.08.006.
- Finnin, M. S., Donigian, J. R., Cohen, A., Richon, V. M., Rifkind, R. A., Marks, P. A., ... Pavletich, N. P. (1999). Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* , **401** (6749), 188-193. doi: 10.1038/43710.
- Forouhar, F., Lee, I. S., Vujcic, J., Vujcic, S., Shen, J., Vorobiev, S. M., ... Tong, L. (2005). Structural and functional evidence for*Bacillus subtilis* PaiA as a novel N1 - spermidine/spermine acetyltransferase. *Journal of Biological Chemistry* ,**280** (48), 40328–40336. DOI:https://doi.org/10.1074/jbc.M505332200.
- Frankel, B. A. & Blanchard, J. S. (2008). Mechanistic analysis of*Mycobacterium tuberculosis* Rv1347c, a lysine Nε-acyltransferase involved in mycobactin biosynthesis. *Archives of biochemistry and biophysics* , **477** (2), 259-266. doi: 10.1016/j.abb.2008.05.013.
- Fredens, J., Wang, K., de la Torre, D., Funke, L. F. H., Robertson, W. E., Christova, Y., ... Chin, J. W. (2019). Total synthesis of*Escherichia coli* with a recoded genome. *Nature* ,**569** , 514–518. doi:

10.1038/s41586-019-1192-5.

- Fukuchi, J. I., Kashiwagi, K., Yamagishi, M., Ishihama, A., & Igarashi, K. (1995). Decrease in cell viability due to the accumulation of spermidine in spermidine acetyltransferase-deficient mutant of *Escherichia coli* . *Journal of Biological Chemistry* , **270** (32), 18831-18835. doi: 10.1074/jbc.270.32.18831.
- Gardner, J. G., Grundy, F. J., Henkin, T. M., & Escalante-Semerena, J.C. (2006). Control of acetyl-coenzyme a synthetase (AcsA) activity by acetylation/deacetylation without NAD+ involvement in*Bacillus subtilis* . *Journal of bacteriology* ,**188** (15), 5460–5468. https://doi.org/10.1128/JB.00215-06.
- Gaviard, C., Broutin, I., Cosette, P., Dé, E., Jouenne, T., & Hardouin, J. (2018). Lysine succinylation and acetylation in*Pseudomonas aeruginosa* . *Journal of proteome research* , **17** (7), 2449-2459. doi: 10.1021/acs.jproteome.8b00210.
- Ghosh, S., Padmanabhan, B., Anand, C., & Nagaraja V. (2016). Lysine acetylation of the *Mycobacterium tuberculosis* HU protein modulates its DNA binding and genome organization. *Molecular microbiology* , **100** (4):577–588. https:// doi.org/10.1111/mmi.13339.
- Gil, J., Ramírez-Torres, A., Chiappe, D., Luna-Peñaloza, J., Fernandez-Reyes, F. C., Arcos-Encarnación, B., ... Encarnación-Guevara, S. (2017). Lysine acetylation stoichiometry and proteomics analyses reveal pathways regulated by sirtuin 1 in human cells. *Journal of Biological Chemistry* , **292** (44), 18129-18144. DOI:https://doi.org/10.1074/jbc.M117.784546.
- Gingras, A. C., Gstaiger, M., Raught, B., & Aebersold, R. (2007). Analysis of protein complexes using mass spectrometry. *Nature reviews Molecular cell biology* , **8** (8), 645-654. doi:10.1038/nrm2208.
- Gregoretti, I., Lee, Y. M., & Goodson, H. V. (2004). Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *Journal of molecular biology* , **338** (1), 17-31. https://doi.org/10.1016/j.jmb.2004.02.006.
- Gu, W., & Roeder, R. G. (1997). Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* , **90** (4), 595-606. doi: 10.1016/s0092-8674(00)80521-8.
- Hebert, A. S., Dittenhafer-Reed, K. E., Yu, W., Bailey, D. J., Selen, E.S., Boersma, M. D., ... Coon, J. J. (2013). Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Molecular cell* , **49** (1), 186-199. doi: 10.1016/j.molcel.2012.10.024.
- Hegde, S. S., Dam, T. K., Brewer, C. F., & Blanchard, J. S. (2002). Thermodynamics of aminoglycoside and acyl-coenzyme A binding to the*Salmonella enterica* AAC(6‘)-Iy aminoglycoside N-acetyltransferase. *Biochemistry* , **41** (23), 7519-7527. doi: 10.1021/bi020190l.
- Hentchel, K. L., & Escalante-Semerena, J. C. (2015). Acylation of biomolecules in prokaryotes: a widespread strategy for the control of biological function and metabolic stress. *Microbiology and Molecular Biology Reviews* , **79** (3), 321-346. doi: 10.1128/MMBR.00020-15.
- Hernick, M., & Fierke, C. A. (2006). Catalytic mechanism and molecular recognition of *E. coli*UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase probed by mutagenesis. *Biochemistry* , **45** (51), 15240-15248. DOI: 10.1021/bi982339s.
- Heuts, D. P., Scrutton, N. S., McIntire, W. S., & Fraaije, M. W. (2009) What’s in a covalent bond? On the role and formation of covalently bound flavin cofactors. *The FEBS journal* , **276** (13), 3405-3427. doi: 10.1111/j.1742-4658.2009.07053.x.
- Hildmann, C., Ninkovic, M., Dietrich, R., Wegener, D., Riester, D., Zimmermann, T., ... Schwienhorst, A. (2004). A new amidohydrolase from*Bordetella* or *Alcaligenes* strain FB188 with similarities to histone deacetylases. *Journal of bacteriology* , **186** (8), 2328-2339. DOI: https://doi.org/10.1128/jb.186.8.2328-2339.2004.
- Hildmann, C., Wegener, D., Riester, D., Hempel, R., Schober, A., Merana, J., ... Schwienhorst, A. (2006). Substrate and inhibitor specificity of class 1 and class 2 histone deacetylases. *Journal of biotechnology* , **124** (1), 258-270. https://doi.org/10.1016/j.jbiotec.2006.01.030.
- Hirano, S. (2012). Western blot analysis. *Nanotoxicity: Methods and Protocols* , **926** ,87-97. DOI: 10.1007/978-1-62703-002-1_6.
- Houghton, J. L., Green, K. D., Pricer, R. E., Mayhoub, A. S., & Garneau-Tsodikova, S. (2013). Unexpected N-acetylation of capreomycin by *Mycobacterial* Eis enzymes. *Journal of Antimicrobial Chemo-*

therapy , **68** , 800–805. https://doi.org/10.1093/jac/dks497.

- Inoue, A., & Fujimoto, D. (1969). Enzymatic deacetylation of histone. *Biochemical and biophysical research communications* , **36** (1), 146-150. https://doi.org/10.1016/0006-291X(69)90661-5.
- Jackman, J. E., Fierke, C. A., Tumey, L. N., Pirrung, M., Uchiyama, T., Tahir, S. H., ... Raetz, C. R. (2000). Antibacterial agents that target lipid a biosynthesis in gram-negative bacteria: inhibition of diverse UDP-3-O-(r-3-hydroxymyristoyl)-N-acetylglucosamine deacetylases by substrate analogs containing zinc binding motifs. *Journal of Biological Chemistry* , **275** (15), 11002-11009. doi: 10.1074/jbc.275.15.11002.
- James, A. M., Hoogewijs, K., Logan, A., Hall, A. R., Ding, S., Fearnley, I. M., & Murphy, M.P. (2017). Non-enzymatic N-acetylation of lysine residues by acetylCoA often occurs via a proximal S-acetylated thiol intermediate sensitive to glyoxalase II. *Cell reports* , **18** (9), 2105-2112. doi: 10.1016/j.celrep.2017.02.018.
- Jiang, Q., Chen, W., Qin, Y., Huang, L., Xu, X., Zhao, L., & Yan, Q. (2017). AcuC, a histone deacetylase, contributes to the pathogenicity of *Aeromonas hydrophila* . *Microbiologyopen* , **6** (4), e00468. https://doi.org/10.1002/mbo3.468.
- Khoury, G., Baliban, R., & Floudas, C. (2011). Proteome-wide post-translational modification statistics: frequency analysis and curation of the swiss-prot database. *Scientific reports* ,**1** (1), 90. https://doi.org/10.1038/srep00090.
- Kim, D., Yu, B. J., Kim, J. A., Lee, Y. J., Choi, S. G., Kang, S., & Pan, J. G. (2013). The acetylproteome of Gram-positive model bacterium*Bacillus subtilis* . *Proteomics* , **13** (10-11), 1726-1736. doi: 10.1002/pmic.201200001.
- Kim, K. H., An, D. R., Song, J., Yoon, J. Y., Kim, H. S., Yoon, H. J., & Suh, S. W. (2012). *Mycobacterium tuberculosis* Eis protein initiates suppression of host immune responses by acetylation of DUSP16/MKP-7. *Proceedings of the National Academy of Sciences* , **109** (20), 7729-7734. doi: 10.1073/pnas.1120251109.
- Kim, S. C., Sprung, R., Chen, Y., Xu, Y., Ball, H., Pei, J., ... Zhao, Y. (2006). Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Molecular Cell* , **23** , 607–618. https://doi.org/10.1016/j.molcel.2006.06.026.
- Komatsu, Y., Iwabata, H., & Yoshida, M. Nova science publishers, inc (Eds.) (2005). Anti-acetyllysine antibody: a useful tool for listening to posttranslational language. Trends in monoclonal antibody research. 37–57 p.
- Kosono, S., Tamura, M., Suzuki, S., Kawamura, Y., Yoshida, A., Nishiyama, M., & Yoshida, M. (2015). Changes in the acetylome and succinylome of *Bacillus subtilis* in response to carbon source. *PloS one* , **10** (6), e0131169. https://doi.org/10.1371/journal.pone.0131169.
- Kramer, A., Wagner, T., Yildiz, O., & Meyer-Almes, F.J. (2016). Crystal structure of a histone deacetylase homologue from*Pseudomonas aeruginosa* . *Biochemistry* , **55** (49), 6858-6868. doi: 10.1021/acs.biochem.6b00613.
- Kremling, A., Geiselmann, J., Ropers, D., & de Jong, H. (2015). Understanding carbon catabolite repression in *Escherichia coli*using quantitative models. *Trends in microbiology,***23** (2), 99-109. doi: 10.1016/j.tim.2014.11.002.
- Krulwich, T. A., Sachs, G., & Padan, E. (2011). Molecular aspects of bacterial pH sensing and homeostasis. *Nature Reviews Microbiology* , **9** (5), 330-343. doi:10.1038/nrmicro2549.
- Kuhn, M. L., Zemaitaitis, B., Hu, L. I., Sahu, A., Sorensen, D., Minasov, G., ... Wolfe, A.J. (2014). Structural, kinetic and proteomic characterization of acetyl phosphate-dependent bacterial protein acetylation. *PloS one* , **9** (4), e94816. https://doi.org/10.1371/journal.pone.0094816.
- Lammers, M. (2021). Post-translational lysine ac(et)ylation in bacteria: a biochemical, structural, and synthetic biological perspective. *Frontiers in Microbiology* , 12 , 757179. doi: 10.3389/fmicb.2021.757179.
- Lee, K. K., & Workman, J. L. (2007). Histone acetyltransferase complexes: one size doesn't fit all. *Nature reviews Molecular cell biology* , **8** (4), 284-295. doi:10.1038/nrm2145.
- Lee, W., VanderVen, B. C., Walker, S., & Russell, D.G. (2017). Novel protein acetyltransferase,

Rv2170, modulates carbon and energy metabolism in *Mycobacterium tuberculosis* . *Scientific Reports* , **7** (1), 72. doi: 10.1038/s41598-017-00067-1.

- Lei, L., Zeng, J., Wang, L., Gong, T., Zheng, X., Qiu, W., ... Li, Y. (2021). Quantitative acetylome analysis reveals involvement of glucosyltransferase acetylation in *Streptococcus mutans* biofilm formation. *Environmental Microbiology Reports* , **13** (2), 86-97. doi: 10.1111/1758-2229.12907.
- Li, B., Maezato, Y., Kim, S. H., Kurihara, S., Liang, J., & Michael, A. J. (2019). Polyamine-independent growth and biofilm formation, and functional spermidine/spermine N-acetyltransferases in*Staphylococcus aureus* and *Enterococcus faecalis* . *Molecular Microbiology* , **111** (1), 159-175. doi: 10.1111/mmi.14145.
- Li, Y., Xue, H., Bian, D.R., Xu, G., & Piao, C. (2020). Acetylome analysis of lysine acetylation in the plant pathogenic bacterium*Brenneria nigrifluens* . *MicrobiologyOpen* , **9** (1), e00952. doi: 10.1002/mbo3.952.
- Liao, J. H., Tsai, C. H., Patel, S. G., Yang, J. T., Tu, I. F., Lo Cicero, M., ... Wu, S. H. (2017). Acetylome of *Acinetobacter baumannii* SK17 reveals a highly-conserved modification of histone-like protein HU. *Frontiers in molecular biosciences* , **4** , 77. https://doi.org/10.3389/fmolb.2017.00077.
- Limsuwun, K., & Jones, P.G. (2000). Spermidine acetyltransferase is required to prevent spermidine toxicity at low temperatures in*Escherichia coli* . *Journal of bacteriology* , **182** (19), 5373-5380. doi: 10.1128/jb.182.19.5373-5380.2000.
- Lin, Y., Fletcher, C. M., Zhou, J., Allis, C. D., & Wagner, G. (1999). Solution structure of the catalytic domain of GCN5 histone acetyltransferase bound to coenzyme A. *Nature* ,**400** :86–89. doi: 10.1038/21922.
- Lindemann, C., Thomanek, N., Hundt, F., Lerari, T., Meyer, H. E., Wolters, D., & Marcus, K. (2017). Strategies in relative and absolute quantitative mass spectrometry based proteomics. *Biological chemistry* , **398** (5-6), 687-699. doi: 10.1515/hsz-2017-0104.
- Liu, L., Wang, G., Song, L., Lv, B., & Liang, W. (2016). Acetylome analysis reveals the involvement of lysine acetylation in biosynthesis of antibiotics in *Bacillus amyloliquefaciens* . *Scientific reports* , **6** (1), 1-11. DOI:10.1038/srep20108.
- Liu, M., Guo, L., Fu, Y., Huo, M., Qi, Q., & Zhao, G. (2021). Bacterial protein acetylation and its role in cellular physiology and metabolic regulation. *Biotechnology Advances* , **53** , 107842. doi: 10.1016/j.biotechadv.2021.107842.
- Liu, X., Wang, L., Zhao, K., Thompson, P. R., Hwang, Y., Marmorstein, R., & Cole, P.A. (2008). The structural basis of protein acetylation by the p300/CBP transcriptional coactivator. *Nature,* **451** :846–850. doi: 10.1038/nature06546.
- Liu, Y.T., Pan, Y., Lai, F., Yin, X.F., Ge, R., He, Q. Y. & Sun, X. (2018). Comprehensive analysis of the lysine acetylome and its potential regulatory roles in the virulence of *Streptococcus pneumoniae* . *Journal of proteomics* , **176** , 46-55. https://doi.org/10.1016/j.jprot.2018.01.014.
- Lombardi, P. M., Cole, K. E., Dowling, D. P., & Christianson, D.W. (2011). Structure, mechanism, and inhibition of histone deacetylases and related metalloenzymes. *Current opinion in structural biology* , **21** (6), 735-743. doi: 10.1016/j.sbi.2011.08.004.
- Lu, Y. X., Liu, X. X., Liu, W. B., & Ye, B. C. (2017). Identification and characterization of two types of amino acid-regulated acetyltransferases in actinobacteria. *Bioscience reports* , **37** (4). doi: 10.1042/BSR20170157.
- Macek, B., Forchhammer, K., Hardouin, J., Weber-Ban, E., Grangeasse, C., & Mijakovic, I. (2019). Protein post-translational modifications in bacteria. *Nature Reviews Microbiology* , **17** (11), 651-664. https://doi.org/10.1038/s41579-019-0243-0.
- Magnet, S., Lambert, T., Courvalin, P., & Blanchard, J. S. (2001). Kinetic and mutagenic characterization of the chromosomally encoded*Salmonella enterica* AAC (6 ‘)-Iy aminoglycoside N-acetyltransferase. *Biochemistry* , **40** (12), 3700-3709. doi: 10.1021/bi002736e.
- Majorek, K. A., Kuhn, M. L., Chruszcz, M., Anderson, W. F., & Minor, W. (2013). Structural, functional, and inhibition studies of a Gcn5-related N-acetyltransferase (GNAT) superfamily protein PA4794: a new C-terminal lysine protein acetyltransferase from *Pseudomonas aeruginosa* . *Journal of*

Biological Chemistry , **288** (42), 30223-30235. https://doi.org/10.1074/jbc.M113.501353.

- Majorek, K. A., Osinski, T., Tran, D. T., Revilla, A., Anderson, W. F., Minor, W., & Kuhn, M. L. (2017). Insight into the 3D structure and substrate specificity of previously uncharacterized GNAT superfamily acetyltransferases from pathogenic bacteria. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* , **1865** (1), 55-64. https://doi.org/10.1016/j.bbapap.2016.10.011.
- Mann, M., & Jensen, O. N. (2003). Proteomic analysis of post-translational modifications. *Nature biotechnology* , **21** (3), 255-261. doi: 10.1038/nbt0303-255.
- Martini, C., Michaux, C., Bugli, F., Arcovito, A., Iavarone, F., Cacaci, M., ... Giard, J. C. (2015). The polyamine N-acetyltransferase-like enzyme PmvE plays a role in the virulence of*Enterococcus faecalis* . *Infection and Immunity* , **83** (1), 364-371. doi: 10.1128/IAI.02585-14.
- Meng, Q., Liu, P., Wang, J., Wang, Y., Hou, L., Gu, W., & Wang, W. (2016). Systematic analysis of the lysine acetylome of the pathogenic bacterium *Spiroplasma eriocheiris* reveals acetylated proteins related to metabolism and helical structure. *Journal of proteomics* , **148** , 159-169. doi: 10.1016/j.jprot.2016.08.001.
- Michan, S., & Sinclair, D. (2007). Sirtuins in mammals: insights into their biological function. *Biochemical Journal* , **404** (1), 1-13. doi: 10.1042/BJ20070140.
- Mischerikow, N., & Heck, A. J. (2011). Targeted large-scale analysis of protein acetylation. *Proteomics* , **11** (4), 571-589. doi: 10.1002/pmic.201000397.
- Miyagi, M. (2017). Site-specific quantification of lysine acetylation using isotopic labeling. In *Methods in Enzymology* ,**586** , 85-95. https://doi.org/10.1016/bs.mie.2016.09.029.
- Mizuno, Y., Nagano-Shoji, M., Kubo, S., Kawamura, Y., Yoshida, A., Kawasaki, H., ... Kosono, S. (2016). Altered acetylation and succinylation profiles in *Corynebacterium glutamicum* in response to conditions inducing glutamate overproduction. *Microbiologyopen* , **5** (1), 152-173. doi: 10.1002/mbo3.320.
- Mo, R., Yang, M., Chen, Z., Cheng, Z., Yi, X., Li, C., ... & Ge, F. (2015). Acetylome analysis reveals the involvement of lysine acetylation in photosynthesis and carbon metabolism in the model cyanobacterium *Synechocystis* sp. PCC 6803. *Journal of proteome research* , **14** (2), 1275-1286. doi: 10.1021/pr501275a.
- Mochalkin, I., Knafels, J. D., & Lightle, S. (2008). Crystal structure of LpxC from Pseudomonas aeruginosa complexed with the potent BB-78485 inhibitor. *Protein Science* , **17** (3), 450-457. https://doi.org/10.1110/ps.073324108.
- Nagano-Shoji, M., Hamamoto, Y., Mizuno, Y., Yamada, A., Kikuchi, M., Shirouzu, M., ... Kosono, S. (2017). Characterization of lysine acetylation of a phosphoenolpyruvate carboxylase involved in glutamate overproduction in *Corynebacterium glutamicum* . *Molecular Microbiology* , **104** (4), 677-689. oi.org/10.1111/mmi.13658.
- Nakayasu, E. S., Burnet, M. C., Walukiewicz, H. E., Wilkins, C. S., Shukla, A. K., Brooks, S., ... Payne, S. H. (2017). Ancient regulatory role of lysine acetylation in central metabolism. *MBio* , **8** (6), e01894-17. DOI: https://doi.org/10.1128/mbio.01894-17.
- Nielsen, T. K., Hildmann, C., Dickmanns, A., Schwienhorst, A., & Ficner, R. (2005). Crystal structure of a bacterial class 2 histone deacetylase homologue. *Journal of molecular biology* , **354** (1), 107-120. doi: 10.1016/j.jmb.2005.09.065.
- North, B. J., & Verdin, E. (2004). Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome biology* , **5** (5), 1-12. doi: 10.1186/gb-2004-5-5-224.
- Novak, K., Flöckner, L., Erian, A. M., Freitag, P., Herwig, C., & Pflügl, S. (2018). Characterizing the effect of expression of an acetyl-CoA synthetase insensitive to acetylation on co-utilization of glucose and acetate in batch and continuous cultures of *E. coli*W. *Microbial Cell Factories* , **17** (1), 1-15. DOI:10.1186/s12934-018-0955-2.
- Oliveira, A. P., & Sauer, U. (2012). The importance of post-translational modifications in regulating *Saccharomyces cerevisiae* metabolism. *FEMS yeast research* , **12** (2), 104-117. doi: 10.1111/j.1567-1364.2011.00765.x.
- Paik, W. K., Pearson, D., Lee, H. W., & Kim, S. (1970). Nonenzymatic acetylation of histones with

acetyl-CoA. *Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis* , **213** (2), 513-522. https://doi.org/10.1016/0005-2787(70)90058-4.

- Pan, J., Ye, Z., Cheng, Z., Peng, X., Wen, L., & Zhao, F. (2014). Systematic analysis of the lysine acetylome in *Vibrio parahemolyticus* . *Journal of proteome research* , **13** (7), 3294-3302. doi: 10.1021/pr500133t.
- Pang, H., Li, W., Zhang, W., Zhou, S., Hoare, R., Monaghan, S. J., ... Lin, X. (2020). Acetylome profiling of *Vibrio alginolyticus*reveals its role in bacterial virulence. *Journal of Proteomics* , **211** , 103543. doi: 10.1016/j.jprot.2019.103543.
- Peebo, K., Valgepea, K., Nahku, R., Riis, G., Oun, M., Adamberg, K., & Vilu, R. (2014). Coordinated activation of PTA-ACS and TCA cycles strongly reduces overflow metabolism of acetate in *Escherichia coli. Applied microbiology and biotechnology* , **98** , 5131-5143. doi: 10.1007/s00253-014-5613-y.
- Phillips, D. M. P. (1963). The presence of acetyl groups in histones. *Biochemical Journal* , **87** (2), 258. doi: 10.1042/bj0870258.
- Piovesan, A., Antonaros, F., Vitale, L., Strippoli, P., Pelleri, M.C. & Caracausi, M. (2019). Human protein-coding genes and gene feature statistics in 2019. *BMC research notes* , **12** :315. doi: 10.1186/s13104-019-4343-8.
- Post, D. M., Schilling, B., Reinders, L. M., D'Souza, A. K., Ketterer, M. R., Kiel, S. J., ... Gibson, B. W. (2017). Identification and characterization of AckA-dependent protein acetylation in*Neisseria gonorrhoeae* . *PLoS One* , **12** (6), e0179621. https://doi.org/10.1371/journal.pone.0179621.
- Ramazi, S., & Zahiri J. (2021). Post-translational modifications in proteins: resources, tools and prediction methods, *Database* , 2021, **2021** . https://doi.org/10.1093/database/baab012.
- Reverdy, A., Chen, Y., Hunter, E., Gozzi, K. & Chai, Y. (2018). Protein lysine acetylation plays a regulatory role in *Bacillus subtilis* multicellularity. *PLoS One* , **13** (9), e0204687. doi: 10.1371/journal.pone.0204687.
- Richards, C. L., Lawrence, K. A., Su, H., Yang, Y., Yang, X. F., Dulebohn, D. P., ... Gherardini, F. C. (2015). Acetyl-phosphate is not a global regulatory bridge between virulence and central metabolism in *Borrelia burgdorferi* . *PLoS One* 10:e0144472. doi: 10.1371/journal.pone.0144472.
- Rojas, J. R., Trievel, R. C., Zhou, J., Mo, Y., Li, X., Berger, S.L., ... Marmorstein, R. (1999). Structure of Tetrahymena GCN5 bound to coenzyme A and a histone H3 peptide. *Nature* ,**401** :93–98. https://doi.org/10.1038/43487.
- Ruijter, A. J. D., Gennip, A. H. V., Caron, H. N., Kemp, S., & Kuilenburg, A.B.V. (2003). Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochemical Journal* , **370** (3), 737-749. https://doi.org/10.1042/bj20021321.
- Salah Ud-Din, A. I. M., Tikhomirova, A., & Roujeinikova, A. (2016). Structure and functional diversity of GCN5-related N-acetyltransferases (GNAT). *International journal of molecular sciences* , **17** (7), 1018. ttps://doi.org/10.3390/ijms17071018.
- Sanders, B. D., Jackson, B., & Marmorstein, R. (2010). Structural basis for sirtuin function: what we know and what we don't. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* , **1804** (8), 1604-1616. doi: 10.1016/j.bbapap.2009.09.009.
- Sauve, A. A., Wolberger, C., Schramm, V. L., & Boeke, J. D. (2006). The biochemistry of sirtuins. *Annual Review of Biochemistry,***75** , 435-465. https://doi.org/10.1146/annurev.biochem.74.082803.133500.
- Schastnaya, E., Doubleday, P. F., Maurer, L., & Sauer, U. (2023). Non-enzymatic acetylation inhibits glycolytic enzymes in*Escherichia coli* . *Cell Reports* , **42** (1), 111950. doi: 10.1016/j.celrep.2022.111950.
- Schilling, B., Basisty, N., Christensen, D. G., Sorensen, D., Orr, J. S., Wolfe, A. J., & Rao, C.V. (2019). Global lysine acetylation in*Escherichia coli* results from growth conditions that favor acetate fermentation. *Journal of bacteriology* , **201** (9), e00768-18. DOI: https://doi.org/10.1128/jb.00768-18.
- Schilling, B., Christensen, D., Davis, R., Sahu, A. K., Hu, L. I., Walker-Peddakotla, A., ... Wolfe, A. J. (2015). Protein acetylation dynamics in response to carbon overflow in *Escherichia coli* . *Molecular microbiology* , **98** (5), 847-863. doi: 10.1111/mmi.13161.
- Schmidt, M. J., & Summerer, D. (2014). Genetic code expansion as a tool to study regulatory processes

of transcription. *Frontiers in chemistry* , **2** , 7. https://doi.org/10.3389/fchem.2014.00007.

- Shirai, T., Fujimura, K., Furusawa, C., Nagahisa, K., Shioya, S., & Shimizu, H. (2007). Study on roles of anaplerotic pathways in glutamate overproduction of *Corynebacterium glutamicum* by metabolic flux analysis. *Microbial cell factories* , **6** , 1-11. doi:10.1186/1475-2859-6-19.
- Slonczewski, J. L., Fujisawa, M., Dopson, M., & Krulwich, T. A. (2009). Cytoplasmic pH measurement and homeostasis in bacteria and archaea. *Advances in microbial physiology* , **55** , 1-317. doi: 10.1016/S0065-2911(09)05501-5.
- Somoza, J. R., Skene, R. J., Katz, B. A., Mol, C., Ho, J. D., Jennings, A. J., ... Tari, L. W. (2004). Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases.*Structure* , **12** (7), 1325-1334. doi: 10.1016/j.str.2004.04.012.
- Starai, V. J. & Escalante-Semerena, J. C. (2004). Identification of the protein acetyltransferase (Pat) enzyme that acetylates acetyl-CoA synthetase in *Salmonella enterica* . *Journal of molecular biology* , **340** (5), 1005-1012. https://doi.org/10.1016/j.jmb.2004.05.010.
- Starai, V. J., & Escalante-Semerena, J. C. (2004) Acetyl-coenzyme A synthetase (AMP forming). *Cellular and molecular life sciences CMLS* , **61** , 2020-2030. https://doi.org/10.1007/s00018-004-3448-x.
- Starai, V. J., Celic, I., Cole, R. N., Boeke, J. D., & Escalante-Semerena J. C. (2002). Sir2-dependent activation of acetyl-CoA synthetase by deacetylation of active lysine. *Science,* **298** (5602):2390–2392. doi: 10.1126/science.1077650.
- Starai, V. J., Gardner, J. G., & Escalante-Semerena, J. C. (2005). Residue Leu-641 of acetyl-CoA synthetase is critical for the acetylation of residue Lys-609 by the protein acetyltransferase enzyme of *Salmonella enterica* . *Journal of Biological Chemistry* , **280** (28), 26200-26205. DOI:https://doi.org/10.1074/jbc.M504863200.
- Sun, L., Yao, Z., Guo, Z., Zhang, L., Wang, Y., Mao, R., ... Lin, X. (2019). Comprehensive analysis of the lysine acetylome in*Aeromonas hydrophila* reveals cross-talk between lysine acetylation and succinylation in LuxS. *Emerging microbes & infections* , **8** (1), 1229-1239. doi: 10.1080/22221751.2019.1656549.
- Sun, X. L., Yang, Y. H., Zhu, L., Liu, F. Y., Xu, J. P., Huang, X. W., ... Zhang, K. Q. (2018). The lysine acetylome of the nematocidal bacterium *Bacillus nematocida* and impact of nematode on the acetylome. *Journal of proteomics* , **177** , 31-39. doi: 10.1016/j.jprot.2018.02.005.
- Tarazona, O. A., & Pourquie, O. (2020). Exploring the influence of cell metabolism on cell fate through protein post-translational modifications. *Developmental Cell* , **54** (2), 282-292. doi: 10.1016/j.devcel.2020.06.035.
- Tercero, J. C., Riles, L. E., & Wickner, R. B. (1992). Localized mutagenesis and evidence for post-transcriptional regulation of MAK3. A putative N-acetyltransferase required for double-stranded RNA virus propagation in *Saccharomyces cerevisiae* . *Journal of Biological Chemistry* , **267** (28), 20270-20276. https://doi.org/10.1016/S0021-9258(19)88696-9.
- Tsimbalyuk, S., Shornikov, A., Kuhn, M. L., & Forwood, J.,K. (2020). SpeG polyamine acetyltransferase enzyme from *Bacillus thuringiensis* forms a dodecameric structure and exhibits high catalytic efficiency. *Journal of structural biology* , **210** (3), 107506. doi: 10.1016/j.jsb.2020.107506.
- VanDrisse, C. M., & Escalante-Semerena, J. C. (2019). Protein acetylation in bacteria. *Annual review of microbiology* , **73** , 111-132. DOI: 10.1146/annurev-micro-020518-115526.
- Vannini, A., Volpari, C., Filocamo, G., Casavola, E. C., Brunetti, M., Renzoni, D., ... Di Marco, S. (2004). Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proceedings of the National Academy of Sciences* , **101** (42), 15064-15069. https://doi.org/10.1073/pnas.040460310.
- Venkat, S., Chen, H., McGuire, P., Stahman, A., Gan, Q. & Fan, C. (2019). Characterizing lysine acetylation of *Escherichia coli*type II citrate synthase. *The FEBS Journal* , **286** (14), 2799-2808. doi: 10.1111/febs.14845.
- Venkat, S., Chen, H., Stahman, A., Hudson, D., McGuire, P., Gan, Q., & Fan, C. (2018). Characterizing lysine acetylation of isocitrate dehydrogenase in *Escherichia coli* . *Journal of molecular biology*

, **430** (13), 1901-1911. https://doi.org/10.1016/j.jmb.2018.04.031.

- Venkat, S., Gregory, C., Sturges, J., Gan, Q., & Fan, C. (2017). Studying the lysine acetylation of malate dehydrogenase. *Journal of molecular biology* , **429** (9), 1396-1405. doi: 10.1016/j.jmb.2017.03.027.
- Vetting, M. W., de Carvalho, L. P. S., Yu, M., Hegde, S. S., Magnet, S., Roderick, S. L., & Blanchard, J. S. (2005). Structure and functions of the GNAT superfamily of acetyltransferases. *Archives of biochemistry and biophysics,* **433** , 212–226. 10.1016/j.abb.2004.09.003.
- Vetting, M. W., Roderick, S. L., Yu, M., & Blanchard, J. S. (2003). Crystal structure of mycothiol synthase (Rv0819) from*Mycobacterium tuberculosis* shows structural homology to the GNAT family of N-acetyltransferases. *Protein science* , **12** (9), 1954-1959. doi: 10.1110/ps.03153703.
- Vinuselvi, P., Kim, M. K., Lee, S. K., & Ghim, C. M. (2012). Rewiring carbon catabolite repression for microbial cell factory. *BMB Reports* , **45** , 59–70. doi: 10.5483/BMBRep.2012.45.2.59.
- Wagner, F. F., Weïwer, M., Lewis, M. C., & Holson, E. B. (2013). Small molecule inhibitors of zinc-dependent histone deacetylases. *Neurotherapeutics* , **10** (4), 589-604. doi: 10.1007/s13311-013-0226-1.
- Wagner, G. R., & Payne, R. M. (2013). Widespread and enzyme-independent n -acetylation and n -succinylation of proteins in the chemical conditions of the mitochondrial matrix*. *Journal of Biological Chemistry* , **288** (40), 29036-29045. https://doi.org/10.1074/jbc.M113.486753.
- Wang, L., Tang, Y., Cole, P.A., & Marmorstein, R. (2008). Structure and chemistry of the p300/CBP and Rtt109 histone acetyltransferases: implications for histone acetyltransferase evolution and function.*Current opinion in structural biology* , **18** :741–747. 10.1016/j.sbi.2008.09.004.
- Wang, Q., Zhang, Y., Yang, C., Xiong, H., Lin, Y., Yao, J., ... Zhao, G.P. (2010). Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux. *Science* ,**327** (5968), 1004-1007. doi: 10.1126/science.1179687.
- Wang, Y., Wang, F., Bao, X., & Fu, L. (2019). Systematic analysis of lysine acetylome reveals potential functions of lysine acetylation in*Shewanella baltica* , the specific spoilage organism of aquatic products. *Journal of proteomics* , **205** , 103419. doi: 10.1016/j.jprot.2019.103419.
- Wei, L., Meyer, J. G., & Schilling, B. (2018). Quantification of site-specific protein lysine acetylation and succinylation stoichiometry using data-independent acquisition mass spectrometry.*Journal of Visualized Experiments,* **4** (134),57209. doi: 10.3791/57209.
- Weinert, B. T., Iesmantavicius, V., Moustafa, T., Scholz, C., Wagner, S. A., Magnes, C., ... Choudhary, C. (2014) Acetylation dynamics and stoichiometry in *Saccharomyces cerevisiae* . *Molecular systems biology* , **10** (1), 716. doi: 10.1002/msb.134766.
- Weinert, B. T., Iesmantavicius, V., Wagner, S. A., Schölz, C., Gummesson, B., Beli, P., ... Choudhary, C. (2013). Acetyl-phosphate is a critical determinant of lysine acetylation in *E. coli* . *Molecular cell* , **51** (2), 265-272. 10.1016/j.molcel.2013.06.003.
- Weinert, B. T., Satpathy, S., Hansen, B. K., Lyon, D., Jensen, L. J., & Choudhary, C. (2017). Accurate quantification of site-specific acetylation stoichiometry reveals the impact of sirtuin deacetylase CobB on the *E. coli* acetylome. *Molecular & Cellular Proteomics* , **16** (5), 759-769. doi: 10.1074/mcp.M117.067587.
- Whittington, D. A., Rusche, K. M., Shin, H., Fierke, C. A., & Christianson, D. W. (2003). Crystal structure of LpxC, a zinc-dependent deacetylase essential for endotoxin biosynthesis. *Proceedings of the National Academy of Sciences* , **100** (14), 8146-8150. doi: 10.1073/pnas.
- Wolf, E., Vassilev, A., Makino, Y., Sali, A., Nakatani, Y., & Burley, S. K. (1998). Crystal structure of a GCN5-related *N* -acetyltransferase: *Serratia marcescens*aminoglycoside 3-N-acetyltransferase. Cell, **94** , 439–449. doi: 10.1016/s0092-8674(00)81585-8.
- Woolridge, D. P., Martinez, J. D., Stringer, D. E., & Gerner, E. W. (1999). Characterization of a novel spermidine/spermine acetyltransferase, BltD, from *Bacillus subtilis* . *Biochemical Journal* , **340** (3), 753-758.
- Wright, G. D., & Ladak, P. (1997). Overexpression and characterization of the chromosomal aminoglycoside 6'-N-acetyltransferase from *Enterococcus faecium* . *Antimicrobial agents and chemotherapy* , **41** (5), 956-960. doi: 10.1128/AAC.41.5.956.

- Wybenga-Groot, L. E., Draker, K., Wright, G. D., & Berghuis, A. M. (1999). Crystal structure of an aminoglycoside 6´-N-acetyltransferase: defining the GCN5-related N-acetyltransferase superfamily fold.*Structure* , **7** (5), 497–507. doi: 10.1016/s0969-2126(99)80066-5.
- Xie, L., Zeng, J., Luo, H., Pan, W., & Xie, J. (2014). The roles of bacterial GCN5-related N-acetyltransferases. *Critical Reviews in Eukaryotic Gene Expression* , **24** (1), 77-87. doi: 10.1615/critreveukaryotgeneexpr.2014007988.
- Xiong, Y., & Guan, K. L. (2012). Mechanistic insights into the regulation of metabolic enzymes by acetylation. *Journal of Cell Biology* , **198** (2), 155-164. doi: 10.1083/jcb.201202056.
- Xiong, Y., & Guan, K. L. (2012). Mechanistic insights into the regulation of metabolic enzymes by acetylation. *Journal of Cell Biology* , **198** (2), 155-164. doi: 10.1083/jcb.201202056.
- Xu, H., Hegde, S. S., & Blanchard, J. S. (2011). Reversible acetylation and inactivation of *Mycobacterium tuberculosis*acetyl-CoA synthetase is dependent on cAMP. *Biochemistry* , **50** (26), 5883-5892. doi: 10.1021/bi200156t.
- Xu, J. Y., Xu, Z., Liu, X., Tan, M., & Ye, B. C. (2018). Protein acetylation and butyrylation regulate the phenotype and metabolic shifts of the endospore-forming *Clostridium acetobutylicum* . *Molecular & Cellular Proteomics* , **17** (6), 1156-1169. doi: 10.1074/mcp.RA117.000372.
- Yan, Y., Harper, S., Speicher, D. W., & Marmorstein, R. (2002). The catalytic mechanism of the ESA1 histone acetyltransferase involves a self-acetylated intermediate. *Nature structural biology* , **9** (11), 862-869. doi: 10.1038/nsb849.
- Yang, X. J., & Seto, E. (2008). The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nature reviews Molecular cell biology* , **9** (3), 206-218. doi: 10.1038/nrm2346.
- You, D., Yao, L. L., Huang, D., Escalante-Semerena, J. C., & Ye, B. C. (2014). Acetyl coenzyme A synthetase is acetylated on multiple lysine residues by a protein acetyltransferase with a single Gcn5-type N-acetyltransferase (GNAT) domain in *Saccharopolyspora erythraea* . *Journal of bacteriology* , **196** (17), 3169-3178. doi: 10.1128/JB.01961-14.
- Yu, B. J., Kim, J., Moon, J. H., Ryu, S. E., & Pan, J. G. (2008). The diversity of lysine-acetylated proteins in *Escherichia coli* . *Journal of microbiology and biotechnology* , **18** (9), 1529-1536.
- Yuan, H., & Marmorstein, R. (2012). Structural basis for sirtuin activity and inhibition. *Journal of Biological Chemistry* , **287** (51), 42428-42435. doi: 10.1074/jbc.R112.372300.
- Zaunbrecher, M. A., Sikes, R. D., Metchock, B., Shinnick, T. M., & Posey, J. E. (2009). Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in*Mycobacterium tuberculosis* . *Proceedings of the National Academy of Sciences* , **106** (47), 20004-20009. https://doi.org/10 .1073/pnas.0907925106. 102.
- Zhang, K., Zheng, S., Yang, J. S., Chen, Y., & Cheng, Z. (2013). Comprehensive profiling of protein lysine acetylation in*Escherichia coli* . *Journal of proteome research* , **12** (2), 844-851. doi: 10.1021/pr300912q.
- Zhang, L., & Elias, J. E. (2017). Relative protein quantification using tandem mass tag mass spectrometry. In: Comai, L., Katz, J., Mallick, P. (eds) Proteomics. Methods in Molecular Biology.**1550** . Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-6747-6_14.
- Zhang, X., Ouyang, S., Kong, X., Liang, Z., Lu, J., Zhu, K., ... Luo, C. (2014). Catalytic mechanism of histone acetyltransferase p300: from the proton transfer to acetylation reaction. *The Journal of Physical Chemistry B* , **118** (8), 2009-2019. doi: 10.1021/jp409778e.
- Zhao, K., Chai, X., & Marmorstein, R. (2004). Structure and substrate binding properties of cobB, a Sir2 homolog protein deacetylase from*Escherichia coli* . *Journal of molecular biology* , **337** (3), 731-741. doi: 10.1016/j.jmb.2004.01.060.
- Zhu, W., Smith, J. W., & Huang, C. M. (2009). Mass spectrometry-based label-free quantitative proteomics. *Journal of Biomedicine and Biotechnology* , **2010** , 840518. doi: 10.1155/2010/840518.

Figure Legends.

Figure 1 . Types of lysine acetylation in prokaryotes. (A) Enzymatic and chemical acetylation and their respective acetyl donor molecule. In bacteria, enzymatic acetylation is catalyzed by Gcn5-related N-

acetyltransferase (GNAT). (B) The catalytic mechanism exerted by GNATs is a sequential mechanism where glutamate acts as a general base to deprotonate the amino group of the lysine, enabling nucleophilic attack of the Acetyl-CoA carbonyl, followed by the formation of a transient tetrahedral intermediate, that is resolved to yield the acetylated substrate amino group and coenzyme A. Overall structure of GNATs: (C) Cartoon representation of GNATS topology. The secondary structure elements are colored and represent the different motifs. Motifs C (β1–α1), D (β2–β3), A (β4–α3), and B (β5–α4) are colored orange, green, aquamarine, and blue, respectively. The least conserved secondary structure elements (strands β0 and β6 and helix α2) absent in some GNAT proteins are colored purple. (D) Crystal structure of the M. tuberculosis GNAT acetyltransferase Rv0819 in complex with Acetyl-CoA. The characteristic secondary structures of these enzymes are shown in different colors (PDB: 1OZP) (Vetting et al., 2003) [the figure was generated with PyMOL v.2.3.4 (DeLano, 2002). (E). Classification of GNATs in prokaryotes based on their domain organization and the arrangement of the GNAT domain [figure redrawn and modified from Blasl et al., 2021; Lammers, 2021].

Figure 2 . Lysine deacetylases (KDAC) can reverse enzymatic or non-enzymatic lysine acetylation. Sirtuins are NAD+-dependent lysine-deacetylases (A). Crystal structure of *E. coli* sirtuin deacetylase CobB in a complex with a lysine acetylated substrate (PDB: 1S5P). The characteristic secondary structures of these enzymes are shown in different colors (Zhao et al., 2004) [the figure was generated with PyMOL v.2.3.4 (DeLano, 2002)]. Catalytic mechanisms used by sirtuins: (B) Sirtuins are NAD^+-dependent lysine deacetylases as a co-substrate for catalysis. In the reaction, the carbonyl-oxygen of the acetyl group of lysine performs a nucleophilic attack on the electrophilic C-1' of the NAD^+ ribose, resulting in the fast release of nicotinamide and the formation of a C-1´-*O* -alkylamidate intermediate. The intermediate is hydrolyzed, forming the deacetylated lysine and 2´-*O* -acetyl-ADP-ribose, which is in a non-enzymatic equilibrium with 3´-*O* -acetyl-ADP ribose. Zinc-dependent classical deacetylase (HDAC/KDAC). are metalloenzymes. (C) Crystal structure of*Pseudomonas aeruginosa* zinc-dependent deacetylase LpxC in a complex with the potent BB-78485 inhibitor (PDB: 2ves). LpxC domain consists of two homologous domains, I (colored in magenta) and II (colored in purple) (Mochalkin et al., 2008) [the figure was generated with PyMOL v.2.3.4 (DeLano, 2002). Catalytic mechanisms used by classical deacetylases (D). HDACs use a catalytic water molecule that is coordinated and polarized by the catalytic Zn^{2+} ion. The ion, together with a histidine residue, interacts with the carbonyl oxygen of the acetyl group. The histidine is polarized and oriented by an aspartic residue (Asp). It acts as a general base to deprotonate the water molecule, thereby increasing the nucleophilicity for attacking the carbonyl carbon of the acetyl group. A second histidine, again polarized and oriented by another Asp, acts as electrostatic catalysis. A tetrahedral oxyanion intermediate is formed, which is stabilized by a histidine and the Zn^{2+} ion, to release acetate and the deacetylated lysine finally [figure redrawn and modified from the decomposition of the oxyanionic tetrahedral intermediate [figure redrawn and modified from Ali et al., 2018; Blasl et al., 2021; Lammers, 2021].

Figure 3 . Regulation of the metabolic enzyme activity. Acetylation regulates: 1) The number of metabolic enzymes by promoting their degradation through the ubiquitin–proteasomal system; 2) The catalytic activity through a) neutralizing the positive charge of lysine residues in the active site or b) causing allosteric changes and; 3) The substrate accessibility to metabolic enzymes by modifying the conserved lysine residues to hinder the entry of substrate (Xiong & Guan, 2012; Liu et al., 2021). Created with BioRender.com

Figure 4. Factors contributing to non-enzymatic acetylation. Acetyl-CoA and acetyl-phosphate are very reactive molecules that, when increasing intracellular concentrations, can non-enzymatically acetylate lysine residues on many proteins. The pH value also plays an essential role in chemical acetylation since, at basic pH, deprotonation of the lysine side chain is favored, increasing its nucleophilicity. In addition to these factors, the efficiency of chemical acetylation also depends on the microenvironment of the protein.

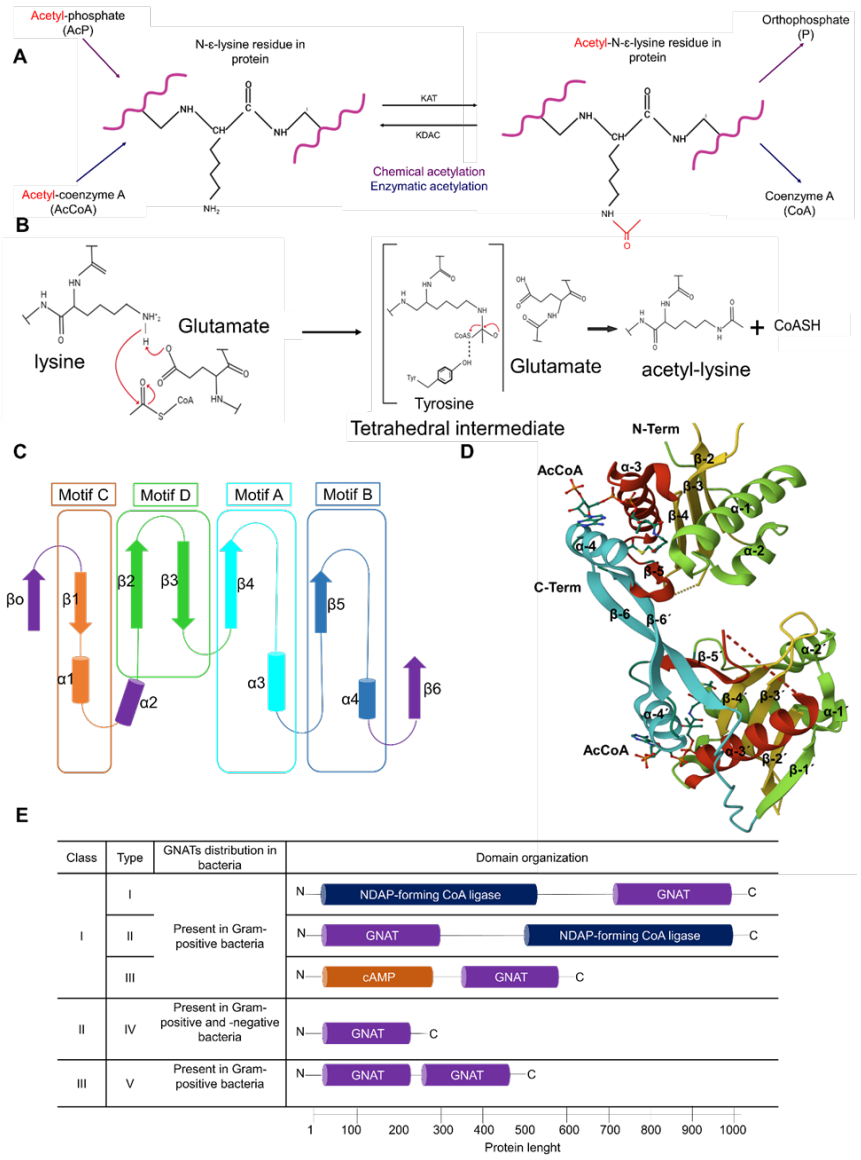

Figure 1.

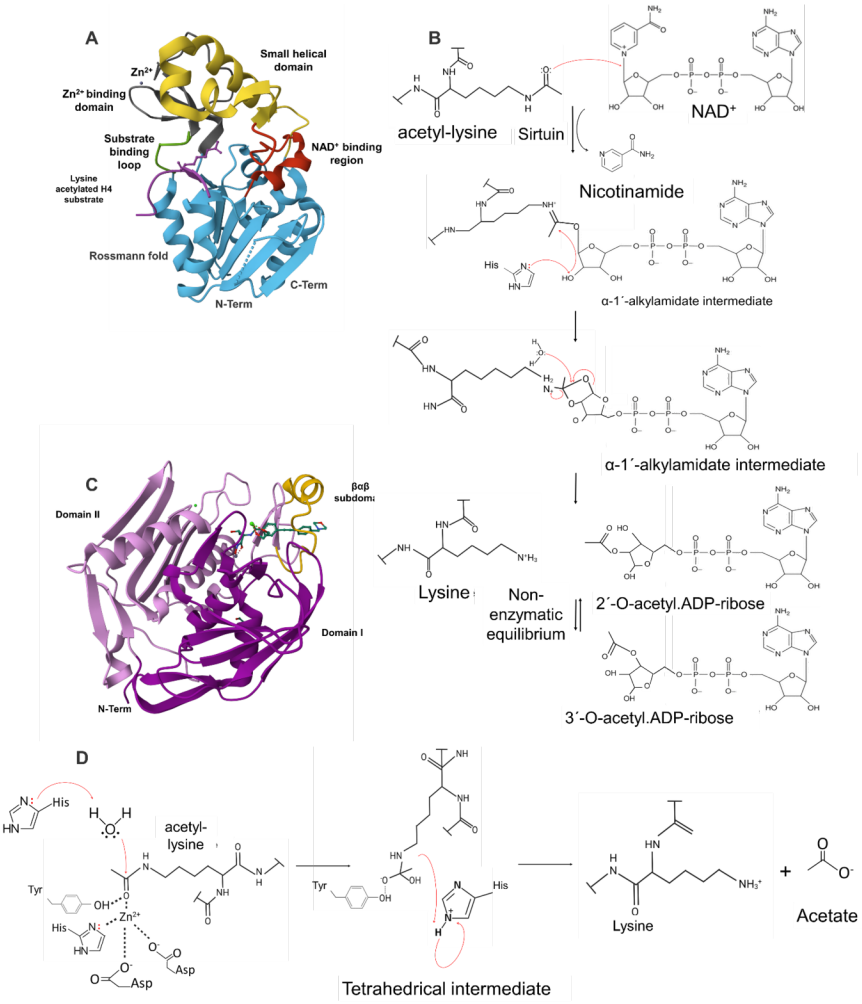

Figure 2.

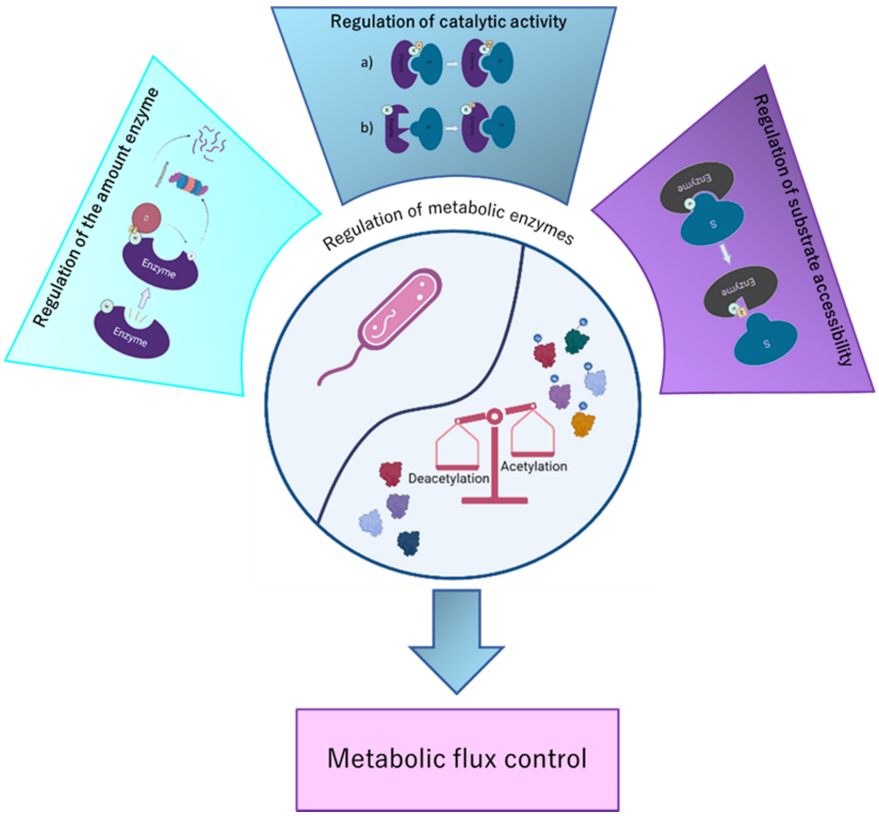

Figure 3.

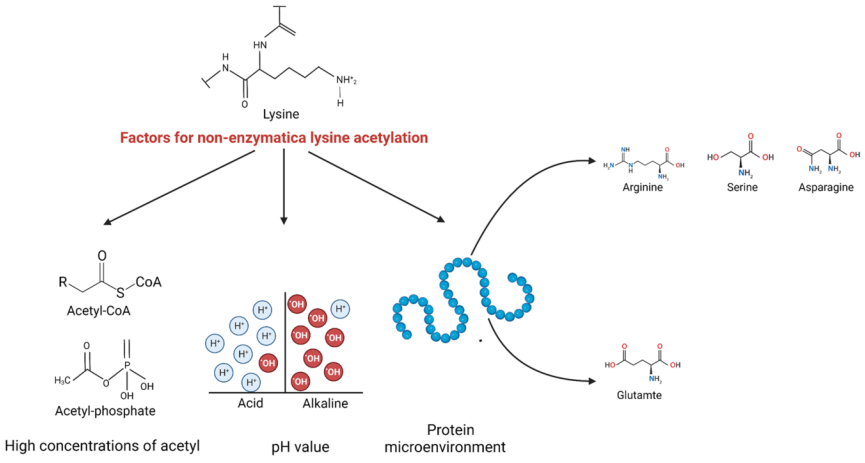

Figure 4.

Tables

Table 1. Some examples of GNATs enzymes, their targets and the effect produced by the acetylation are shown.

Family	Name	Bacteria	Substrate	Effect	Reference
Aminoglycoside N-acetyltransferase	MtAAC(2')-Ic	*M. tuberculosis*	Aminoglycosides	Antibiotic resistance	Barrett et al., 2008
	EfAAC(6')-Ii	*Enterococcus faecium*			Hegde et al., 2002; Magnet et al., 2001; Wright & Ladak,1997
	SeAAC(6')Ie	*Salmonella enterica*			
	MtbEis	*M. tuberculosis*	Aminoglycosides	Antibiotic resistance	Chen et al., 2011; Houghton et al., 2013; Zaunbrecher et al., 2009;
Spermidine/spermine N-acetyltransferase	BltD	*B. subtilis*	Polyamines (spermidine /spermine)	Unknown	Woolridge et al., 1999
	PaiA	*B. subtilis*			Forouhar et al., 2005
	SpeG	*Vibrio cholerae*		Possibly involved in biofilm formation	Filippova et al., 2015
		Staphylococcus aureus			Li et al., 2019
		E. coli		Inhibition of spermidine accumulation	Fukuchi et al., 1995; Limsuwun & Jones, 2000
		Bacillus thuringiensis		Unknown	Tsimbalyuk et al., 2020
	Ste26	*Streptomyces* sp.		Unknown	Bai et al., 2011
	PmvE	*Enterococcus faecalis*	Putrescine/spermine	Contributes to the virulence of the bacteria. Possibly involved in biofilm formation	Martini et al., 2015

Family	Name	Bacteria	Substrate	Effect	Reference
Lysine Nε-acyltransferase	MtRv1347c	*M. tuberculosis*	Fatty acyl-CoA with longer chain lengths	Possible role in siderophore biosynthesis (mycobactin T)	Card et al., 2005; Christensen et al., 2019; Frankel & Blanchard, 2008; Hentchel & Escalante-Semerena, 2015;
Non-histone protein N-acetyltransferases	YfiQ and its homologs	*E. coli, Vibrio cholerae, Salmonella entérica, Yersinia pestis, Rhodopseudomonas palustris, Streptomyces lividans,* and *Streptomyces griseus*	Acetyl-CoA synthetase	Loss of enzyme activity	Starai et al., 2004
	MSMEG_5458	*M. tuberculosis*			Xu, et al., 2011
	PA4794	*P. aeruginosa*	C-terminal lysine residue of a peptide	No reported	Majorek et al., 2013
	MtbEis	*M. tuberculosis*	Nucleoid-associated protein (MtHU)	May cause reduced interaction with DNA and altered DNA compaction ability of nucleoid-associated proteins	Ghosh et al., 2016
			Dual-specificity protein phosphatase 16/mitogen-activated protein kinase phosphatase-7 (DUSP16/MKP-7)	Inhibition of JNK-dependent autophagy, phagosome maturation, and ROS generation	Kim et al., 2012

Table 2. Acetylome studies show that AcP is the primary source of acetylation.

		Tested condition	Tested condition			
Bacteria	Mutant	Time/growth phase	Medium	Acetylation sites	Acetylated proteins	Reference
E. coli	Wild-type	EP	Not mentioned			Weinert et al., 2013
	ackA			Increase of 8-8-fold of global acetylation	Increase of 8-8-fold of global acetylation	
	pta			Reduce by about 40% of global acetylation	Reduce by about 40% of global acetylation	
	Wild-type	SP	TB7/ TB7+glucose	780/ 1204	355/ 446	Kuhn et al., 2014
	ackA			1149/ 2473	448/ 751	
	pta ackA			320/ —	166/ —	
	Wild-type	12 h	TB7/ TB7+glucose	451/ 2338	216/ 705	Schilling et al., 2015
Neisseria gonorrhoeae 1291	*Wild-type*	Overnight	IsoVitaleX-supplemented GC broth	1612	542	Post et al., 2017
	ackA			2401	604	
Borrelia burgdorferi B31	Wild-type	SP	BSK-II media	104	64	Bontemps-Gallo et al., 2018
	ΔackA			No acetylation	No acetylation	
	Δpta			242	164	
	ackA complemented strain			206	115	
	Δpta complemented strain			103	78	
Bacillus subtilis 3610	Wild-type	SP	LB with 1% glycerol (v/v) and 100 μM $MnSO_4$	No significant increase or decrease in the overall acetylation level was observed	No significant increase or decrease in the overall acetylation level was observed	Reverdy et al., 2018
	acuA					
	Pta					

acKa: AcP accumulation

pta: No conversion of acetyl-CoA into AcP

ΔackA (Ac-P$^-$, acetyl-CoA$^-$)

Δpta (Ac-P$^+$, acetyl-CoA$^-$)

acuA: mutant in lysine acetyltransferase

Exponential growth phase (EP) and stationary phase (SP).

Table 3. Some global acetylome studies in prokaryote organisms.

Bacteria	Method	No. of acetylation sites	No. of acetylation proteins	Reference
Escherichia coli	Immunoaffinity enrichment with nano-HPLC/mass spectrometric analysis	1070	349	Zhang et al., 2013
Streptococcus pneumoniae	Immunoaffinity enrichment with LC-MS/MS	653	392	Lui et al., 2018
Vibrio parahemolyticus		1413	656	Pan et al., 2014
Bacillus amyloliquefaciens		3268	1254	Lui et al., 2016
Spiroplasma eriocheiris		2567	555	Meng et al., 2016
Acinetobacter baumannii SK17-S and SK17-R		145	125	Liao et al., 2017
Phaeodactylum tricornutum		2324	1220	Chen et al., 2018
Bacillus nematocida		529	349	Sun et al., 2018
Vibrio alginolyticus		2883	1178	Pang et al., 2020
Aeromonas hydrophila		3189	1013	Sun et al, 2019
Brenneria nigrifluens		1866	737	Li et al., 2020
Shewanella baltica		2929	1103	Wang et al., 2019
Synechocystis	Peptide prefractionation, antibody enrichment, and LC-MS/MS	776	513	Mo et al., 2015

Bacteria	Method	No. of acetylation sites	No. of acetylation proteins	Reference
48 bacteria form six phyla: Proteobacteria, *Firmicutes, Bacteroidetes, Actinobacteria, Cyanobacteria*, and *Fibrobacteres*	None/LC-MS/MS	Not reported	9,107 acetylated proteins, averaging ˜190 per organism	Nakayasu et al., 2017

Table 4. Some quantitative acetylome studies in prokaryote organisms.

Bacteria	Quantification method	No. of acetylation sites	No. of acetylation proteins	Acetylome changes	Reference
Bacillus nematocida	TMT labeling/ Immunoaffinity enrichment with LC-MS/MS	129	349	During pathogenesis, the acetylation levels of 18 lysine sites were upregulated and 19 lysine sites in were downregulated	Sun et al., 2018
Streptococcus mutans (Bacterial biofilm growth (SMB) vs planktonic growth (SMP))		973	445	In SMB glucosyltransferase-SI, glucosyltransferase-I, and glucosyltransferase-S were downregulated	Lei et al., 2021
C. acetobutylicum	Stable isotope dimethyl labeling/ Immunoaffinity enrichment with nano-HPLC/mass spectrometric analysis	458	254	No differences were observed in the overall acetylation levels of whole cell lysates during the exponential and stationary phases. During the transitional phase, slight acetylation was observed	Xu et al., 2018

Bacteria	Quantification method	No. of acetylation sites	No. of acetylation proteins	Acetylome changes	Reference
Pseudomonas aeruginosa	Label-free/2D immunoaffinity approach coupled to nano HPLC/MS/MS	1102	522	The carbon source does not produce significant changes in the number of acetylated peptides.	Gaviard et al., 2018
Escherichia coli	Label-free/ Immunoaffinity enrichment with LC-MS/MS	2730	806	For cells grown in the presence of glucose the number of upregulated acetyl lysine sites is substantially larger in the ackA mutant than in the cobB mutant (>8-fold for acetyl sites, >5-fold for proteins) Acetyl lysine sites decreased slightly in the yfiQ mutant. Acetylation is both non-enzymatic and specific	Kun et al., 2014

Bacteria	Quantification method	No. of acetylation sites	No. of acetylation proteins	Acetylome changes	Reference
Bacillus subtilis		2372	841	In the stationary phase 271 acetylation sites were significantly downregulated and 92 were upregulated. In the transition from the log to the stat phase, DNA-mediated transformation proteins were upregulated, and proteins involved in translation were downregulated	Carabetta et al., 2016
Escherichia coli		818	434	KATs overexpression significantly increased the number of acetylated proteins and lysine residues.	Christensen et al., 2018