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Bacterial GCN5-Related *N*-Acetyltransferases: From Resistance to Regulation

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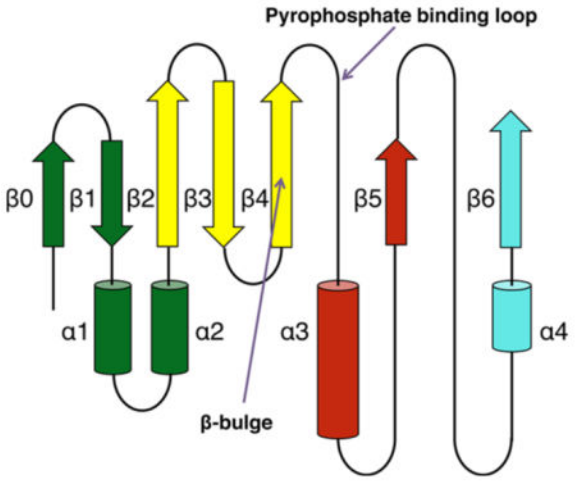
Abstract

The GCN5-related *N*-acetyltransferases family (GNAT) is an important family of proteins that includes more than 100000 members among eukaryotes and prokaryotes. Acetylation appears as a major regulatory post-translational modification and is as widespread as phosphorylation. *N*-Acetyltransferases transfer an acetyl group from acetyl-CoA to a large array of substrates, from small molecules such as aminoglycoside antibiotics to macromolecules. Acetylation of proteins can occur at two different positions, either at the amino-terminal end (αN-acetylation) or at the ε-amino group (εN-acetylation) of an internal lysine residue. GNAT members have been classified into different groups on the basis of their substrate specificity, and in spite of a very low primary sequence identity, GNAT proteins display a common and conserved fold. This Current Topic reviews the different classes of bacterial GNAT proteins, their functions, their structural characteristics, and their mechanism of action.

Graphical abstract



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Histone acetylation and aminoglycoside resistance caused by acetylation were both discovered 50 years ago.[1–3] Even though both discoveries were reported within a year of each other, most early work focused on histone acetylation and its consequences with respect to gene regulation.[4,5] With the development of high-resolution mass spectrometry and genome sequencing, it has become clear that acetylation of proteins has emerged as a major modiffer of their activities in eukaryotes and prokaryotes.[6] Acetylation can, however, occur in both non-enzymatic and enzyme-catalyzed processes. This is due to the extremely exothermic nature of the acetylation reaction. Acetyl-CoA (AcCoA) has an acetyl transfer potential higher than the phosphoryl transfer potential of ATP, and the acetylated amide product is extremely stable and considerably more so than the phosphomonoester product of the kinases.[7] Thus, the high intracellular concentration of acyl-CoA's, including AcCoA, will nonenzymatically react with any protein lysine residue with a reduced p*K* value. It is thus important in defining the "acetylome" to ensure that the acetylated protein be generated enzymatically by the action of an acetyltransferase and deacetylated by an appropriate deacetylase and that there be a catalytic or structural phenotype associated with the modification. Without this, the likelihood is that the protein simply contains a lysine residue that is accessible and is in an environment that reduces its p*K* value.

The use of the term GNAT to describe *N*-acetyltransferases was first derived from the yeast GCN5, which was shown to be a histone acetyltransferase.[4,8] However, the first GNAT member was described by Okamoto in 1965 as a bacterial aminoglycoside acetyltransferase conferring kanamycin and gentamicin antibiotic resistance.[3] Many aminoglycoside acetyltransferases have since been characterized and are clinically one of the most common reasons for aminoglycoside resistance.[9] The GNAT superfamily currently contains more than 100000 members found in eukaryotes, prokaryotes, and archea.[10]

Despite the fact that all the members of the GNAT superfamily possess a very low primary sequence identity, their structures display a particularly conserved fold.[11,12] The core secondary elements of all GNAT structures consist of six or seven β-strands and four α-helices (β0–β1–α1–α2–β2–β3–β4–α3–-β5–α4–β6) (Figure 1). The loop connecting β4 to α3, the pyrophosphate binding loop, appears as a characteristic structural feature of the GNAT superfamily, and the sequence is highly conserved between members (R/Q-X-X-G-X-A/G).[13] The pyrophosphate binding loop plays a central role in AcCoA binding because it forms several hydrogen bonds between a number of amide backbone nitrogens and the pyrophosphate group of AcCoA.[11,12] Another distinctive feature found in most structures of the GNAT members is a "β-bulge" located on β4, breaking apart parallel β-strands β4 and β5, resulting in a V-like shape that serves as a binding site for AcCoA. The pantothenate "arm" of AcCoA interacts with the C-terminal end of β4, while there are few interactions observed between the adenine ring and the active site. Typically, GNAT proteins function as dimers in solution, although we will discuss examples in which a monomeric unit is composed of two fused GNAT domains.

All members of the GNAT superfamily catalyze bireactant reactions, and the acetyltransferase reaction can proceed via either a ping-pong or sequential mechanism.[12] There is a single example of a reaction occurring via a ping-pong kinetic mechanism. The yeast ESA1 is a member of the MYST subfamily of histone acetyltransferases, and there are

compelling structural and mutagenesis data that support the kinetic necessity of an acetylated enzyme intermediate (C304 in the case of ESA1).[14] For all other GNAT superfamily members, sequential kinetic mechanisms have been observed. Once both substrates are bound, a residue located in the active site, typically an aspartate or glutamate, acts as a general base and deprotonates the amine prior to nucleophilic attack on the thioester carbonyl. Residues acting as a general base have been identified by site-directed mutagenesis studies, and many ternary complex structures have been determined in recent years.[11,12] In some cases, the deprotonation step involves an intervening water molecule connecting the side chain of a general base to the amine via hydrogen bonding.[15,16] Thus, with the exception of the MYST subfamily of histone acetyltransferases or those GNATs with an appropriately positioned active site cysteine, the sequential mechanism involving the formation of a ternary complex is observed in a majority of the cases.[11,12]

GNAT members can acetylate the amino group of a large range of substrates and so have been classified into three groups: (1) small molecule acetyltransferases, such as aminoglycosides and mycothiol; (2) peptide acetyltransferases such as the peptidoglycan that is part of the bacterial cell wall; and (3) protein acetyltransferases, for instance, the histone family. In this Current Topic, we will focus on the classes of GNAT commonly found in bacteria, because the histone acetyltransferases have been reviewed in detail.[17–19]

1. SMALL MOLECULES

1.1. Aminoglycosides *N*-Acetyltransferases

Amino-glycosides are broad-spectrum antibacterial compounds containing typically one aminocyclitol ring (the most common being 2-deoxystreptamine) linked by a glycosidic bond to one or more amino sugars (Figure 2). The first aminoglycoside to be discovered was streptomycin; it was isolated from *Streptomyces griseus* and originally used for the treatment of tuberculosis.[20] Typically, aminoglycosides work synergistically with β-lactams or other antibiotics that enhance their uptake.[21] More recently, aminoglycosides have also been investigated for the treatment of certain genetic disorders, for instance, cystic fibrosis[22,23] or Duchenne muscular dystrophy,[24,25] as well as HIV therapies.[26–28] Aminoglycosides inhibit protein translation in bacteria by binding to the 16S rRNA of the 30S ribosome.[29,30] The site of binding and mechanism of inhibition has been significantly clarified with the elucidation of the structures of 16S rRNA fragments and the 30S ribosome subunit with various bound AGs.[31–37]

Drug resistance to aminoglycosides emerged many decades ago. Several mechanisms have been identified, including mutation or methylation of certain 16S rRNA nucleotides involved in aminoglycoside binding, decreased aminoglycoside uptake, and chemical modification of aminoglycosides by aminoglycoside-modifying enzymes.[38,39] Enzymatic modification is the most common and clinically relevant, although a combination of different mechanisms is sometimes observed.[38–40] Three different classes of enzymes modify amino-glycosides: ATP-dependent phosphotransferases (O-phosphorylation, APH), ATP-dependent nucleotidyltransferases (O-nucleotidylation, ANT), and acetyl-CoA-dependent *N*-acetyl-transferases (N-acetylation, AAC), which are members of the GNAT family.

Aminoglycoside *N*-acetyltransferases were the first members of the GNAT family to be identified.[3] Shaw et al. have established a nomenclature for describing the different aminoglycoside *N*-acetyltransferases.[41] The enzymes are abbreviated by a three-letter code, AAC, followed by a number in parentheses that indicates the position of the acetylated amino group (Figure 2). A Roman numeral distinguishes between the different resistance profiles, and a lowercase letter specifically identifies the associated gene. Four types of AACs have been identified to date depending on the site of modification: AAC(1) when the 1-NH_2 is acetylated, AAC(3), AAC(2′), and AAC(6′). Examples in each category of AAC will be provided in this Current Topic.

AAC(1) was first identified in *Escherichia coli* (*E. coli*) and *Actinomycete* strains.[42,43] On the basis of high-performance liquid chromatography analysis, Lovering et al. demonstrated that *E. coli* AAC(1) could catalyze the transfer of one acetyl group from AcCoA to the N1 position of apramycin, lividomycin, paromomycin, and butirosin.[42] Additionally, the enzyme could diacetylate neomycin and ribostamycin. However, the enzyme does not appear to be clinically relevant. Later, Sunada et al. identified another AAC(1) from an actinomycetes strain that predominantly acetylates paromomycin at the 1-NH_2 position; however, the activity of the antibiotic was not significantly affected by this modification.[43]

The AAC(3) family includes nine subclasses of enzymes (I–X), but subclass V was later excluded after DNA analysis revealed that the genes encoding AAC(3)-II and -V were identical and conferred resistance to the same antibiotics.[41] The subclass I group can be subdivided into five groups (a–e) exhibiting resistance to gentamicin, sisomicin, and fortimicin.[41] Gentamicin acetyltransferase from *E. coli* catalyzing the acetylation of gentamicin at the 3-NH_2 position was the first purified and kinetically characterized aminoglycoside-modifying enzyme.[44–46] Kinetic analysis using a spectrophotometric assay revealed that the enzyme utilizes a random bi-bi mechanism. *Serratia marcescens* (*S. marcescens*) AAC(3)-Ia was the first aminoglycoside acetyltransferase GNAT structure to be determined, simultaneously with the first structure of the histone acetyltransferase, Hat1.[47,48] The enzyme harboring a C-terminal truncation was cocrystallized with CoA, and the structure was determined to 2.3 Å by multiwavelength anomalous dispersion.[47] The structure exhibits the typical GNAT fold that includes four α-helices and six β-strands (Figure 3A). The pyrophosphate group of CoA was found to form hydrogen bonds with the conserved motif R/Q-X-X-G-X-A/G, while the pantothenate group interacts with the end of strand β4. Although a dimer was observed in the asymmetric unit, the existence of a dimer in solution was not conclusively determined. AAC(3)-III enzymes confer resistance to gentamicin, kanamycin, neomycin, paromomycin, and tobramycin.[41] Recently, one enzyme of this subclass, AAC(3)-IIIb from *Pseudomonas aeruginosa*, has been extensively characterized thermodynamically and kinetically to provide insights into the binding mode of CoA and aminoglycosides.[49–51]

The AAC(2′) family includes only one subclass. The enzymes generally promote the acetylation of dibekacin, gentamicin, kanamycin, netilmicin, and tobramycin.[41] Initially, AAC(2′)-Ia was identified in *Providencia stuartii* in which overexpression of the acetyltransferase is observed in the presence of aminoglycosides.[52,53] The other AAC(2′) enzymes are found in only mycobacteria, including AAC(2′)-Ib in *Mycobacterium*

fortuitum,[54] AAC(2′)-Ic in *Mycobacterium tuberculosis* (*M. tuberculosis*), AAC(2′)-Id in *Mycobacterium smegmatis* (*M. smegmatis*), and AAC(2′)-Ie in *Mycobacterium leprae*.[55] AAC(2′)-Ic from *M. tuberculosis* has been very well characterized, both enzymatically and structurally.[56,57] Kinetic analysis reveals that the enzyme can acetylate the amino group at position 2′ of a broad range of AGs.[56] An interesting feature of AAC(2′)-Ic specificity is the demonstration that the enzyme can also perform O-acetylation and acetylate AGs such as kanamycin A or amikicin, each of which contains a 2′-hydroxyl group. Dead-end inhibition studies indicate that the acetylation reaction, like other AACs, follows a sequential kinetic mechanism in which AcCoA binds first, promoting the binding of the AG. The crystal structure of AAC(2′)-Ic was determined in an apo form and in complex with CoA and various aminoglycosides (kanamycin A, ribostamycin, and tobramycin) (Figure 3B).[57] The overall fold of the enzyme, in addition to the presence of the characteristic "*β*-bulge" (G83) and pyrophosphate binding loop, confirms that it belongs to the GNAT family. As observed for other GNAT proteins, AAC(2′)-Ic forms a dimer. The binding mode of CoA is similar to that observed in GNAT structures. However, this was the first reported structure of an aminoglycoside *N*-acetyltransferase in a ternary complex with CoA and aminoglycoside bound. The different aminoglycosides bind within a proximal site to the CoA binding pocket, in a region containing acidic residues and a tryptophan residue (W181) that are conserved among the AAC(2′) enzymes. Most of the interactions occur with the amino and hydroxyl groups from the 2-deoxystreptamine ring, which explains the specificity of the AAC(2′) enzymes for aminoglycosides containing this ring. The 2′-substituent, either an amino group or a hydroxyl group, is located 3.6–4.1 Å from the thiol group of CoA, supporting the direct nucleophilic attack mechanism.

AAC(6′) is the most common class of aminoglycoside *N*-acetyltransferases because the 6′-substituent is important in the binding of aminoglycosides to the 30S ribosome subunit.[31,33,36] Two different classes of enzymes have been determined on the basis of their ability to confer resistance, and both can acetylate netilmicin, tobramycin, and 2′-*N*-ethylnetilmicin. However, type I enzymes confer resistance to amikacin and gentamicin C1a and C2 but not to gentamicin C1, while type II enzymes exhibit activity with all types of gentamicin, but not amikacin.[58] AAC(6′)-I is the most common aminoglycoside *N*-acetyltransferase,[38] and an extensive list of enzymes belonging to the AAC(6′) family has recently been reviewed by Ramirez et al.[39] AAC(6′)-Ii from *Enterococcus faecium*[59–62] and AAC(6′)-Iy from *Salmonella enterica* (*S. enterica*)[63–65] were the first AAC(6′) enzymes to be extensively characterized, both kinetically and structurally. Interesting examples among the members of the AAC(6′) family are AAC(6′)-Ib and its two variants AAC(6′)-Ib-cr, a bifunctional enzyme that confers resistance to both aminoglycosides and fluoroquinolones,[66] and AAC(6′)-Ib_{11}, an enzyme that displays activity with both amikacin and gentamicin.[67] This enzyme is the most widespread AG-modifying enzyme and is found in more than 70% of Gram-negative clinical isolates that produce AAC(6′)-I enzymes.[68] Kinetic analysis and dead-end inhibition studies demonstrate that both AAC(6′)-Ib and AAC(6′)-Ib-cr use an ordered sequential kinetic mechanism in which AcCoA binds first, followed by the addition of either aminoglycoside or fluoroquinolone.[69] Both enzymes exhibit a bell-shaped pH–activity profile, suggesting that they use a catalytic base and a catalytic acid in the reaction. In the case of AAC(6′)-Ib-cr, the pH optima are different depending on the substrate used,

either aminoglycoside or fluoroquinolone. Both AAC(6′)-Ib and AAC(6′)-Ib$_{11}$ structures have been determined.[69,70] Both structures exhibit a characteristic GNAT fold. Surprisingly, AAC(6′)-Ib and AAC(6′)-Ib-cr are monomeric in solution, while AAC(6′)-Ib$_{11}$ is in a monomer/dimer equilibrium.[70] Variant AAC(6′)-Ib$_{11}$ possesses two mutations compared to wild-type AAC(6′)-Ib (Q106L and L107S). These two mutations are located in the vicinity of the binding pocket of AcCoA, more specifically where the pantothenate arm is positioned, and lead to a severe structural change compared to the AAC(6′)-Ib structure (Figure 3C).[70] The central β-strand is disrupted, and the α-helical flap, which forms a lid above the aminoglycoside binding site in AAC(6′)-Ib, is more flexible. This structural difference between the wild-type enzyme and its variant could explain the substrate specificity of both enzymes. Minor differences in the binding interactions were observed depending on the AG bound, either kanamycin or 4,5-disubstituted aminoglycosides (ribostamycin and paromomycin).[69] On the basis of the positions of the substrates, the structures of AAC(6′)-Ib ternary complexes suggest a direct nucleophilic attack with D115 and Y164 acting as the proposed catalytic base and acid.

It should also be noted that AAC enzymes can be fused into bifunctional enzymes and can be associated with either another AAC enzyme [e.g., AAC(3)-Ib/AAC(6′)-Ib′][71] or another AG-modifying enzyme [e.g., AAC(6′)/APH(2″)].[72,73]

1.2. Mycothiol Transferase

Mycothiol (MSH), whose function is analogous to that of glutathione in eukaryotes and eubacteria, is found only in actinomycetes. MSH is the major low-molecular weight thiol and serves as a cellular antioxidant to protect the bacteria against oxidative damage and electrophilic toxins.[74,75] High levels of MSH are found in mycobacteria, especially in *M. tuberculosis*.[76] Four unique enzymes conduct the biosynthesis of MSH in five steps (Figure 4A).[77] First, *N*-acetylglucosamine is transferred from UDP-*N*-acetylglucosamine to 1-L-*myo*-inositol-1-phosphate, leading to the formation of 3-phospho-1-D-*myo*-inositol-2-acetamido-2-deoxy-α-D-glucopyranoside (GlcNAc-Ins-P). This step is catalyzed by the glycosyltransferase MshA.[78] The intermediate is then dephosphorylated by a phosphatase, MshA2, yet to be identified.[79] The zinc-dependent metalloprotein MshB subsequently deacetylates the intermediate to generate 1-D-*myo*-inositol-2-amino-2-deoxy-α-D-glucopyranoside (GlcN-Ins).[80] MshC catalyzes the ATP-dependent ligation between L-cysteine and the 2-amino group from GlcN-Ins, yielding 1-D-*myo*-inosityl-2-L-cysteinylamino-2-deoxy-α-D-glucopyranoside (Cys-GlcN-Ins).[81] Finally, Cys-GlcN-Ins is acetylated by MshD at the cysteinyl amine, resulting in the formation of MSH.[82]

The enzyme MshD that catalyzes the final acetylation step in MSH biosynthesis is a GNAT protein. This enzyme was first identified in *M. smegmatis* and *M. tuberculosis*.[82] A comparison of the MshD sequence with other acetyltransferase proteins revealed that MshD possesses twice the number of amino acids compared to other GNAT proteins and appeared to include two GNAT motifs in its sequence. Because the C-terminal region displays the highest degree of similarity to acetyltransferases, Koledin and co-workers hypothesized that MshD is the result of gene duplication and the C-terminal region is involved in the acetylation reaction while the N-terminal region is inactive.[82]

M. tuberculosis MshD (Rv0819) was crystallized in the presence of both AcCoA and CoA.[83] The structure of a ternary complex of MshD cocrystallized with CoA and desacetylmycothiol (DAM) was also determined (Figure 4B).[16] The structure confirms the presence of two GNAT motifs with the N-terminal domain (residues 1–140) and the C-terminal domain (residues 151–315) linked by a random coil. While most members of the GNAT family form dimers in solution, dynamic light scattering experiments and gel filtration revealed that MshD is a monomer in solution.[83] Typically, the terminal β-strands (β6) of each subunit of the dimer exchange across the dimer interface to establish a continuous β-sheet. The same pattern is observed in the MshD structure. The two domains exhibit very similar structures as highlighted by a low root-mean-square (rms) displacement value for the Cα atom positions (1.7 Å) when superimposing one domain onto the other. However, the two domains appear to utilize different binding modes for AcCoA. In fact, the acetyl moiety of AcCoA is found buried in a hydrophobic pocket and is not correctly positioned to donate its acetyl group to Cys-GlcN-Ins in the N-terminal domain.[83] Additionally, the βbulge is lacking in the latter domain. These two structural observations support the hypothesis that only the C-terminal domain is catalytically active. The structure of the ternary complex confirmed this hypothesis.[16] In the binary complexes, a large cavity was observed between the two domains, and it was considered that upon binding of CoA and the acetyl acceptor, a conformational change between the two motifs might occur.[83] In the ternary complex structure, the N-terminal domain is rotated toward the C-terminal domain, resulting in a narrower central cavity where DAM is bound.[16] The amine moiety from DAM is oriented toward CoA bound in the C-terminal domain. Residues from both N-terminal and C-terminal domains participate in specific interactions with DAM. Interestingly, although only CoA and DAM were added to MshD prior to crystallization, an AcCoA molecule was found in the N-terminal domain, suggesting that AcCoA remained bound to the C-terminal domain during purification. The pH dependence of the maximal velocity suggested that MshD uses both a catalytic base and a catalytic acid. On the basis of their positions in the ternary complex structure, E234 (deprotonates a water molecule, which in turn accepts the proton from the amino group of DAM) and Y294 are believed to play those roles.[16]

1.3. Other Small Molecule Acetyltransferases

In addition to the aminoglycoside *N*-acetyltransferases and the mycothiol transferase that have been extensively studied, many other types of small molecule acetyltransferases have been partially or fully characterized. To give the reader a taste of acetyltransferase substrate diversity, a short list of diverse bacterial acetyltransferases is compiled in Table 1. This table highlights acetyltransferases involved in peptidoglycan recycling,[84] detoxification pathways,[85–88] production of virulence factor,[89,90] iron acquisition,[91,92] and redox balance.[93]

2. PEPTIDES

2.1. Fem Family

Members of the family of Fem amino-acyltransferases catalyze the addition of amino acids to the nascent peptidoglycan using aminoacylated tRNA as a substrate to form an elongated,

branched cell wall cross-link.[94] Fem family members are found primarily in Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Clostridium*, and *Streptomyces* and in few Gram-negative pathogenic spirochetes, including *Borrelia burgdorferi* and *Treponema pallidum*. The peptidoglycan, a critical component of the cell wall, is composed of alternating *N*-acetylglucosamine and *N*-acetylmuramic acid, on which the pentapeptide is linked via a lactyl group (Figure 5).[95] In organisms containing the Fem enzymes, the pentapep-ide (L-Ala)-(D-Glu)-X-(D-Ala)-(D-Ala) is further modified by the addition of up to five amino acids at position *X*, where *X* is either lysine, ornithine, or the D,L-diamino acid, meso-diaminopimelic acid (Dap) (Table 2). The enzymes that synthesize the interchain peptide were first discovered in a methicillin-resistant *Staphylococcus aureus* (*S. aureus*) strain (pentaglycine extension at position *X*) and were initially believed to be factors essential for methicillin resistance, from which the name Fem is derived.[96] In the 1990s, three *fem* genes were identified by insertional mutagenesis in *S. aureus: femX* (also known as *fmhB*), *femA*, and *femB. femX* was shown to be an essential gene in *S. aureus* that catalyzed the addition of the first glycine substituent onto the peptidoglycan precursor associated with the membrane (lipid II).[97] On the other hand, *femA* and *femB* insertional mutants were not detrimental and lead to the formation of a one-glycine extended branched peptide and a three-glycine extended branched peptide, respectively.[98] These results suggested that FemX adds the first glycine, FemA the second and third, and FemB the last two glycines. In the early 2000s, the first Fem was successfully expressed and assayed.[99] FemX from *Lactobacillus viridescens* [also known as *Weissella viridescens* (*W. viridescens*)] was shown to catalyze the amino acyl transfer of glycine from charged $tRNA^{Gly}$ to a soluble UDP-*N*-acetylmuramyl pentapeptide (Figure 5).[100] In the literature, several Fem homologues have been reported. In *Streptococcus pneumonia*, MurM adds L-alanine or L-serine to ε-L-lysine, and subsequently, MurN adds a second L-alanine residue.[101,102] In *Enterococcus faecalis*, BppA1 and BppA2 transfer L-alanine from Ala-tRNA to the first and second positions of the interchain peptide, respectively.[103] All Fem enzymes and homologues use an amino-acylated tRNA as an acyl donor. *S. aureus* encodes three $tRNA^{Gly}$ isoacceptors that bind poorly to elongation factor EF-Tu, thus preventing their use in ribosomal protein synthesis, and two "adequate" $tRNA^{Gly}$ molecules suitable for protein synthesis.[104] Specific nucleotide pairing changes or additional base pairing in the isoacceptor tRNA conserved loops led to the distortion of the anticodon loop and the inability to bind to the translational machinery.[105] Because FemX is essential for bacterial survival, the enzyme constitutes an ideal drug target. To date, analogues of nonribosomal tRNA containing an oxadiazole group mimicking the 3′-amino acyl ester of the tRNA and peptidyl-RNA bisubstrate analogues have been developed and shown to be effective FemX inhibitors *in vitro*.[106,107] The three-dimensional structures of two Fem enzymes have been determined, FemA from *S. aureus* and FemX from *W. viridescens* (Figure 6).[108,109] The FemA structure revealed a globular domain composed of two GNAT subdomains and a pair of antiparallel α-helix domains located between $\beta 2'$ and $\beta 3'$ of the second GNAT subdomain. The α-helical coiled coil arm was suggested to play a role in the binding of Gly-$tRNA^{Gly}$ during glycine transfer based on similar structural features found for seryl-tRNA synthetases.[110] The main difference between the FemX structure complexed with UDP-MurNac-pentapeptide and the FemA structure is the absence of the coiled coil domain, which is replaced by a tight five-residue loop. FemX is also composed of two GNAT

domains, separated by a cleft where the UDP-MurNac-pentapeptide is interacting primarily with the second GNAT domain.[109] A newer FemX structure in complex with a bisubstrate analogue revealed crucial mechanistic information.[111] The bisubstrate analogue is composed of a peptidoglycan precursor analogue linked by a 1,4-triazole ring to a RNA molecule mimicking the acceptor arm of $tRNA^{Ala}$. Superimposition of the FemX-UDP-MurNac-pentapeptide and FemX-peptidyl-RNA structures gave a rms displacement value of 0.4 Å for the Cα atom positions, indicating almost no change in the protein conformation. The only exception is the rearrangement of the loop between strands β5 and β6 (residues 136–140) toward the catalytic cavity, which is critical for maintaining the position of the pentapeptide lysine, which is ε-aminoacylated. The triazole ring of the pentapeptide is in the proximity of K305 and was proposed to be the residue participating in the chemical step. K305 and F304 are highly conserved in Fem enzymes, and mutation of those residues to alanine decreased the rate of turnover of FemX by 1.3×10^4- and 16-fold, respectively. On the basis of those results, a structure-based mechanism for substrate-assisted catalysis was proposed.

3. PROTEINS

GNAT enzymes also acetylate proteins, either at the N-terminus (αN-acetylation) or on the side chain of internal lysine residues (εN-acetylation). The largest family of protein *N*-acetyltransferases is the histone acetyltransferase family found in eukaryotes, which acetylate histones predominantly at the N-terminus.[11] Because this Current Topic is focused on GNAT proteins in bacteria, the histone acetyltransferase family will not be discussed here.

3.1. Rim Proteins

αN-Acetylation of proteins is a common post-translational modification in eukaryotes, but there are relatively few examples in prokaryotes.[112] Three ribosomal proteins, S5, S18, and L12, are known to be αN-acetylated in bacteria.[113] Using nitrosoguanine mutagenesis followed by two-dimensional gel electrophoresis, Isono and co-workers identified the three proteins responsible for the αN-acetylation of these ribosomal proteins: RimI, RimJ, and RimL acetylate S18, S5, and L12, respectively.[113–116] The function of αN-acetylation of these proteins is not fully understood.

The acetylation of L12 leads to the formation of L7, an acetylated version of L12. L12 and L7 are dimers that interact with the ribosome through an adaptor ribosomal protein, L10.[117] Contrary to other ribosomal proteins, multiple copies of L12/L7 are present in the ribosome.[118] More recently, Gordiyenko et al. demonstrated that the αN-acetylation of L12 allows for tighter binding between L12/L7 and L10, stabilizing the ribosomal stalk complex.[119] Three RimL structures from different organisms have been determined to date: RimL from *Salmonella typhimurium* LT2 (*S. typhimurium*),[120] RimL from *Thermus thermophilus* (*T. thermophilus*),[121] and YdaF (a homologue of RimL) from *Bacillus subtilis* (*B. subtilis*).[122] Although the YdaF structure exhibits a typical GNAT fold, its acetyltransferase activity has not been confirmed. Experiments including gel filtration and dynamic light scattering suggest that *S. typhimurium* RimL forms a dimer in solution.[120] In the RimL–CoA complex, CoA is bound between the splayed β4 and β5 strands, and few

specific interactions are observed between the adenine/ribose moieties and the protein (Figure 7A). Additionally, backbone amides from the pyrophosphate binding loop, as well as a conserved water molecule, interact with the pyrophosphate moiety through hydrogen bonds while the pantothenate "arm" interacts with residues located at the C-terminal end of strand β4. A conformational change involving the loop linking α1 to α2 is observed upon the binding of CoA. One characteristic structural feature of the GNAT family is the presence of a "β-bulge" on β4, responsible for the splaying of strands β4 and β5, but this structural feature is not observed in the *S. typhimurium* RimL structure. A cysteine (C134) proximal to the active site is involved in a disulfide bond with CoA in one of the *S. typhimurium* RimL–CoA structures, suggesting that the reaction catalyzed by RimL could exploit a ping-pong mechanism. However, this residue does not appear to be conserved among the other RimL enzymes. The mutant enzyme (C134A) displays kinetic properties similar to those of the wild-type enzyme, indicating that the enzyme utilizes a direct nucleophilic attack. S141 and E160 are hypothesized as the general acid and base, respectively. Finally, the cleft formed at the dimer interface could be the binding site for L12 as it is also observed in the *T. thermophilus* RimL and *B. subtilis* YdaF structures.[121,122]

The *S. typhimurium* RimI that acetylates S18 has been extensively characterized, both kinetically and structurally.[123,124] RimI utilizes an ordered kinetic mechanism involving the binding of AcCoA first, followed by the binding of S18.[123] Because full-length S18 from *S. typhimurium* was not successfully expressed, a peptide representing the first six amino acids of S18 was used for the kinetic and inhibition studies (abbreviated as $S18_{1-6}$). *S. typhimurium* RimI was crystallized in the presence of AcCoA, CoA, and a bisubstrate inhibitor.[124] The structures exhibit all the characteristic features of the GNAT fold. However, the enzyme appears to be a monomer in solution. The cocrystal structure with a bisubstrate inhibitor (CoA-S-acetyl-$S18_{1-6}$) reveals how the inhibitor binds to the active site (Figure 7B). The peptide from the bisubstrate inhibitor binds perpendicularly to the pantothenate group of CoA. Several residues of the active site are specifically interacting with the peptide portion through hydrogen bonds, as well as van der Waals interactions. Several conformational changes are observed upon the binding of the peptide compared to the structure containing CoA, the most significant involving the orientation of the loop connecting β6 and β7.

The structure of a putative RimJ protein from *Vibrio fischeri* has been determined (PDB entry 3IGR) and exhibits a structural fold similar to that of the RimI and RimL structures. In addition to acetylating S5, RimJ has been proposed to be involved in ribosome assembly[125] and to acetylate thymosin α1, an immunomodulating peptide.[126]

3.2. Protein Acetyltransferase, Pat

Acetylation at the ε-amino group of a lysine residue is a major post-translational protein regulation mechanism found in all kingdoms of life. Since the discovery of ε*N*-lysine-acetylated AcCoA synthase (ACS) in *S. enterica*[127] and acetylome studies in *E. coli*[128,129] and *S. enterica*,[130] ε*N*-lysine acetylation appears to be widespread in prokaryotes. The first ε*N*-lysine acetyltransferase was identified in *S. enterica* as *Se*Pat (formerly yhiQ) and is responsible for ACS acetylation leading to enzyme inhibition.[131] The *Se*Pat enzyme is

composed of two domains: a GNAT acetyltransferase domain at the C-terminus and a NDP-forming AcCoA synthetase domain at the N-terminus, which is 96% identical to *E. coli* PatZ.[132] However, the NDP-forming AcCoA domain is unable to produce AcCoA from acetate, ATP, and CoA because the catalytic histidine, present in PatZ, is replaced with an asparagine (N114) in *Se*Pat. *Se*Pat has been reported to acetylate several metabolic enzymes, including ACS,[131] glyceraldehyde-3-phosphate dehydrogenase (GapA), isocitrate lyase (AceA), and isocitrate dehydrogenase kinase (aceK),[130] and to propionylate propionyl-CoA synthetase (PprE).[133] It has been proposed that Pat acetylates AcCoA synthase at high intracellular concentrations of AcCoA to prevent further increases in its concentration, maintain the acetate pool, and prevent unnecessary ATP hydrolysis.[134]

Enzyme acetylation is reversed by deacetylase enzymes, including the NAD^+-dependent sirtuin-like deacetylases, which allows for the rapid response and adaptation to new metabolic needs or physiological changes. In *B. subtilis*, ACS activity is also post-translationally modified by AcuA, a GNAT protein acetyltransferase.[135] The ACS gene and AcuABC operon are adjacent to each other, with AcuA functioning as the acetylase and AcuC as an NAD^+-independent deacetylase. A second NAD^+-dependent deacetylase, CobB, is also able to deacetylate ACS, suggesting that depending on growth conditions, two independent acetylases could be differentially used.[135] Reversible ACS acetylation by Pat enzymes has been reported in many organisms, including *Rhodopseudomonas palutris*,[136] *Streptomyces coelicolor*,[137] *Streptomyces lividans* (*S. lividans*),[138] *M. smegmatis*,[139] and *M. tuberculosis*.[140] The recently determined structure of an active catalytic complex between the *S. lividans* Pat GNAT domain and *S. enterica* ACS revealed key determinants for protein substrate recognition and subsequent acetylation.[141] In addition to the conserved $PX_4G\mathbf{K}$ motif on the C-terminus of the ACS protein substrate, a trio of arginines located after the $PX_4G\mathbf{K}$ motif also conserved in ACS homologues was shown to interact with a negative patch on Pat. Those complementary ionic interactions contribute to Pat substrate specificity. A unique feature of *M. tuberculosis* Pat and *M. smegmatis* Pat acetyltransferase structures is the fusion of an N-terminal cyclic nucleotide binding domain to the GNAT domain.[142,143] This cyclic nucleotide binding domain specifically recognizes cAMP, allowing allosteric activation of Pat. Following macrophage phagocytosis of *M. tuberculosis*, a dramatic increase (50-fold) in cAMP concentration was observed in the macrophage cytoplasm as well as within the bacteria.[144] cAMP is a key regulatory molecule in mycobacterial physiological adaptation during infection and influences the host response. The *M. tuberculosis* Pat crystal structure reveals that after cAMP binds to the N-terminal domain, a conformational change causes *M. tuberculosis* Pat to switch from an autoinhibited state to a cAMP-activated state (Figure 8). Activated *M. tuberculosis* Pat acetylates and inhibits the activity of multiple downstream enzymes such as ACS, fatty acyl-CoA ligase FadD13, and fatty acyl-AMP ligase FadD33, which regulate fatty acid metabolism and siderophore biosynthesis, respectively.[140,145,146]

4. CONCLUSION

It has been 50 years since the discovery of the AcCoA-dependent *N*-acetyltransferases, and our understanding of their roles in antibiotic resistance, metabolism, and biosynthesis and most recently control and regulation of central carbon metabolism has improved

dramatically. Their kinetic and chemical mechanisms have been dissected, and numerous structures of the different "classes" of GNATs have been reported. Although originally thought to use exclusively AcCoA, there are examples shown in this review in which the acyl transfer substrate can be an aminoacylated tRNA molecule and other examples in which the nature of the fatty acyl substituent is proprionyl, and longer acyl chains (e.g., yeast myristoyltransferase,[147] also a monomeric enzyme composed of two GNAT domains). The size and nature of the eubacterial "acetylome" are largely unknown and, in some reported cases, unlikely to be correct due to nonenzymatic acetylation reactions. Today, a majority of the identifiable bacterial GNATs are of unknown biochemical function, and further efforts are necessary to unravel the full picture of a promising field of research.

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ABBREVIATIONS

AAC	aminoglycoside *N*-acetyltransferase
AcCoA	acetyl-coenzyme A
ANT	aminoglycoside nucleotidyltransferase
APH	aminoglycoside phosphotransferases
CoA	coenzyme A
Cys-GlcN-Ins	1-D-*myo*-inosityl-2-L-cysteinylamino-2-deoxy-αD-glucopyranoside
DAM	desacetylmycothiol
Dap	diaminopimelic acid
GlcNAc-Ins-P	3-phospho-1-D-*myo*-inositol-2-acet-amido-2-deoxy-α-D-glucopyranoside
GlcN-Ins	1-D-*myo*-inositol-2-amino-2-deoxy-α-D-glucopyranoside
GNAT	GCN5-related *N*-acetyltransferase
MSH	mycothiol
PDB	Protein Data Bank
rms	root-mean-square

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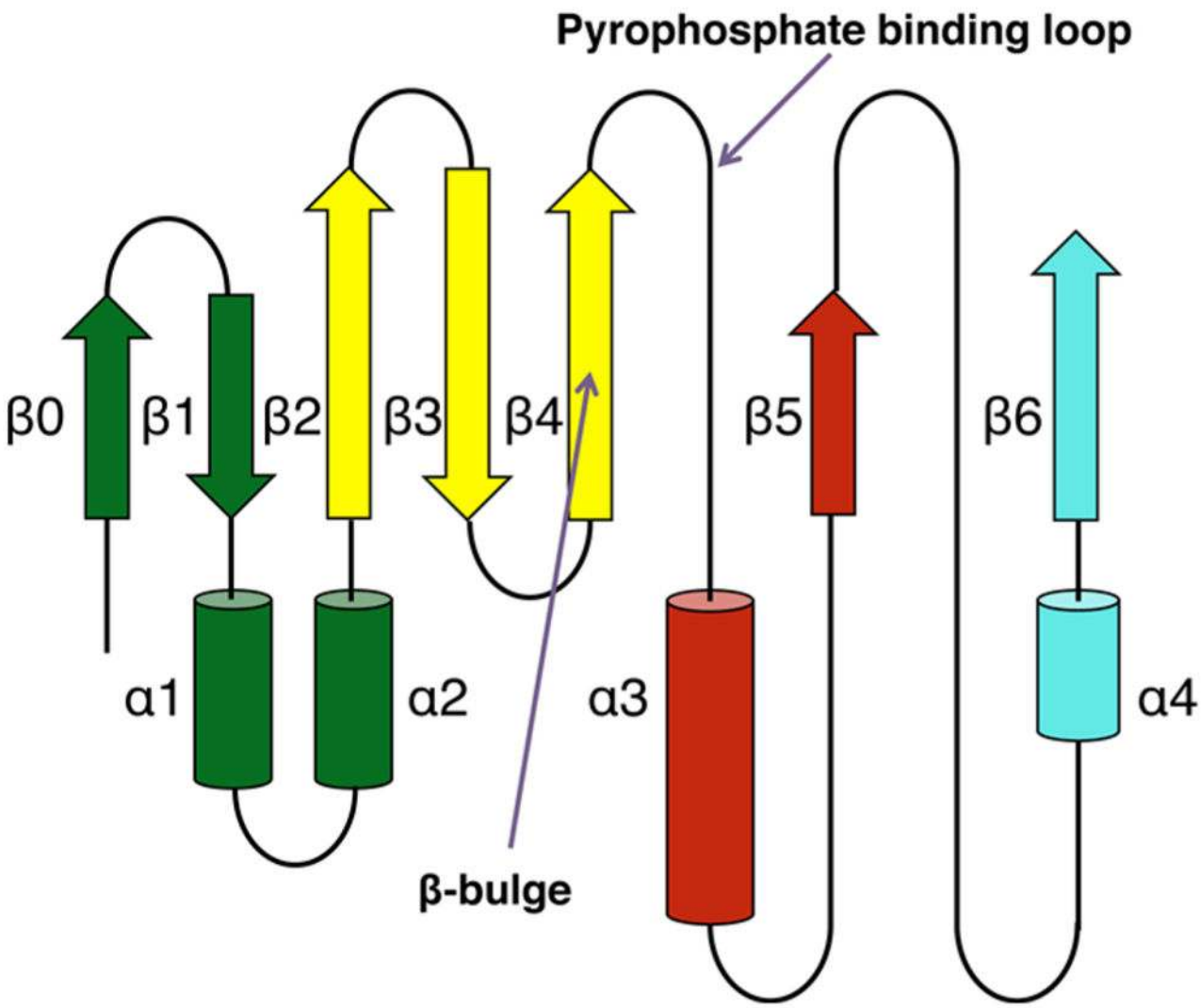

Figure 1.

Topology scheme of the GNAT proteins. Starting at the N-terminal end, the secondary structure elements are colored dark green (*β*0, *β*1, *α*1, and *α*2), yellow (*β*2–*β*4), red (*α*3 and *β*5), and cyan (*α*4 and *β*6).

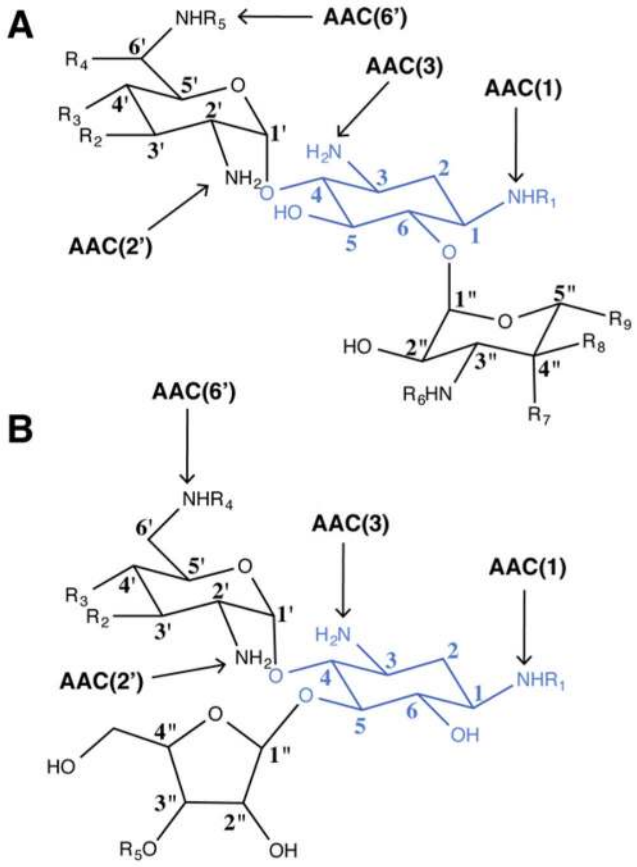

Figure 2.

General structures of various aminoglycosides. The amino-cyclitol (2-deoxystreptamine) is colored blue. Arrows indicate the positions that are commonly modified by AACs. (A) 4,6-Disubstituted deoxystreptamine aminoglycoside. Examples cited in this review are butirosin, paromomycin, and neomycin. (B) 4,5-Disubstituted deoxystreptamine aminoglycoside. Position 2′ can be either an amino group (kanamycin B, gentamicin C1a and C1, etc.) or a hydroxyl group (kanamycin A, amikacin, etc.).

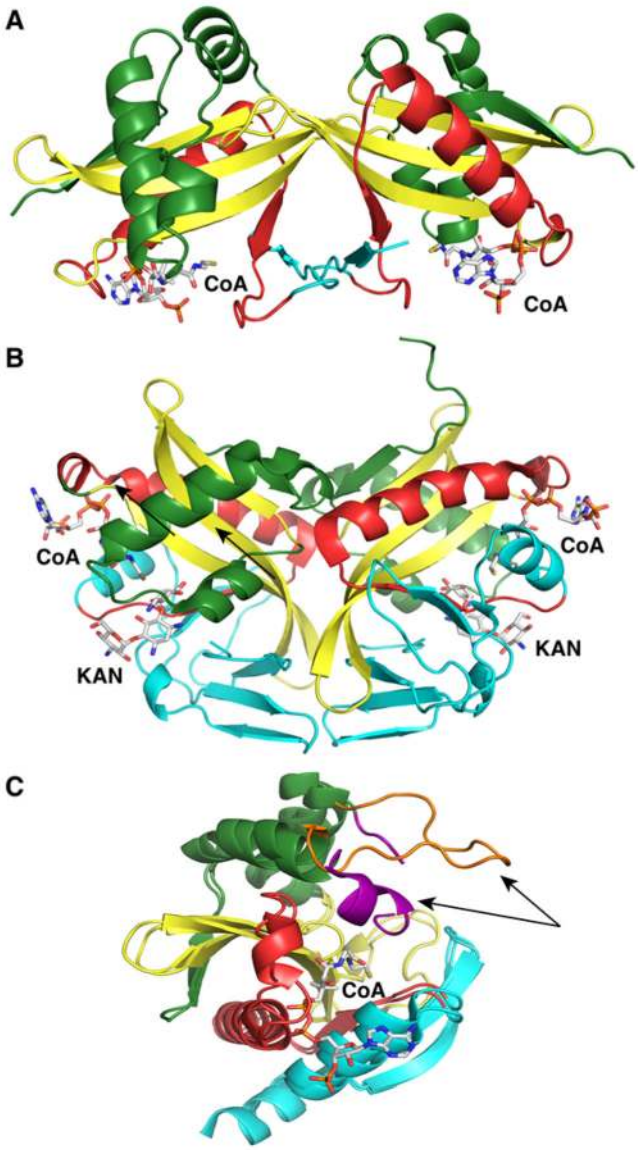

Figure 3.

Structures of aminoglycoside *N*-acetyltransferases. The secondary structure elements are colored like the topology scheme presented in Figure 1. The substrates or products bound are rendered as sticks with white carbons. (A) Structure of *S. marcescens* AAC(3)-Ia (PDB entry 1BO4). (B) Structure of *M. tuberculosis* AAC(2′)-Ic in complex with CoA and kanamycin A (KAN) (PDB entry 1M4I). (C) Superimposition of the *S. enterica* AAC(6′)-Ib structure in complex with CoA and its variant AAC(6′)-Ib_{11} (PDB entries 2PRB and 2PR8, respectively). The α-helical flap (highlighted with an arrow in each structure) located above the aminoglycoside binding site is colored purple in the AAC(6′)-Ib structure and orange in the AAC(6′)-Ib_{11} structure.

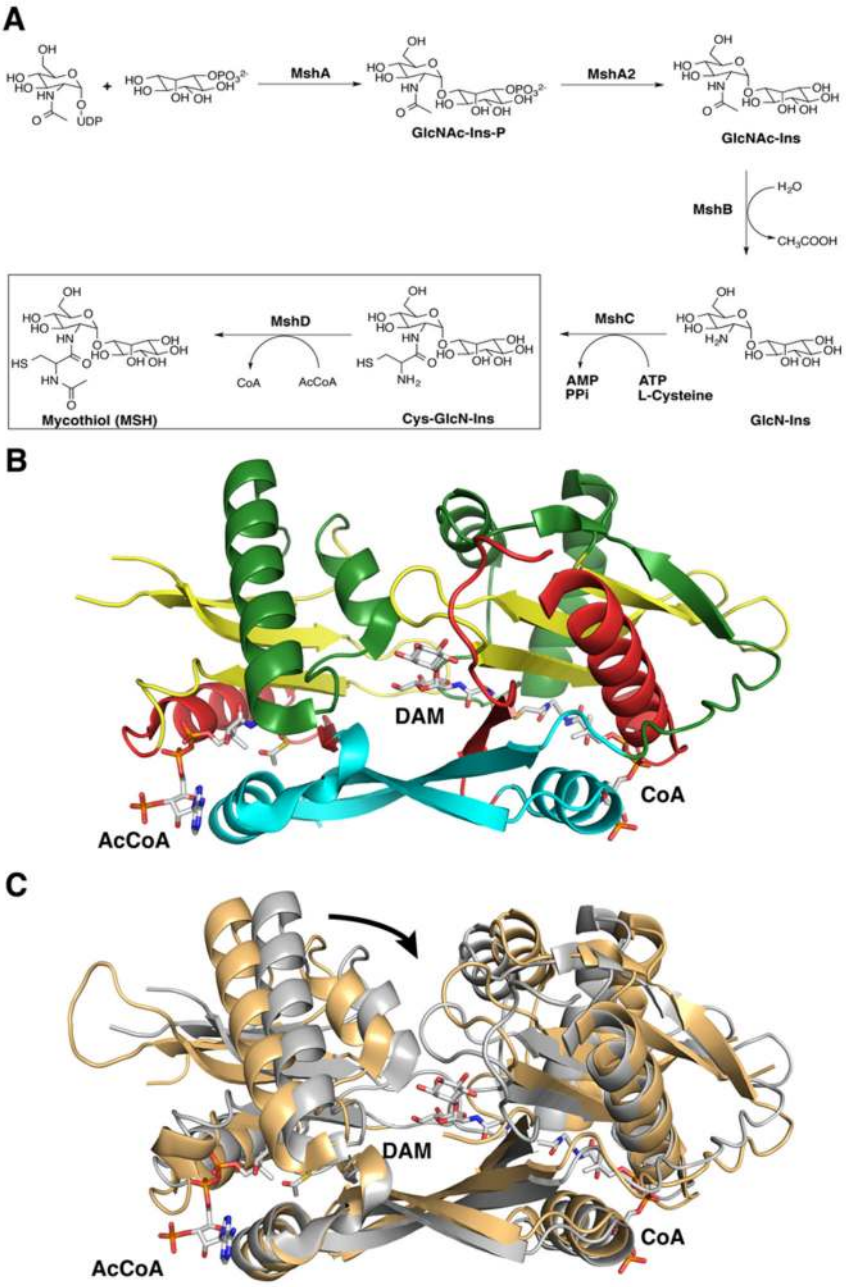

Figure 4.

Role and structure of MshD in mycothiol biosynthesis. (A) Biosynthetic pathway of mycothiol. The box highlights the reaction catalyzed by MshD. (B) Structure of *M. tuberculosis* MshD in complex with CoA and DAM (PDB entry 2C27). The secondary structure elements are colored like the topology scheme presented in Figure 1. The substrates or products bound are rendered as sticks with white carbons. (C) Superimposition of MshD in complex with CoA and DAM (PDB entry 2C27, gray) and MshD in complex with AcCoA (PDB entry 1OZP, beige). Upon substrate binding, the N-terminal domain rotates closer to the C-terminal domain to allow a narrower binding cavity for DAM as highlighted by the arrow.

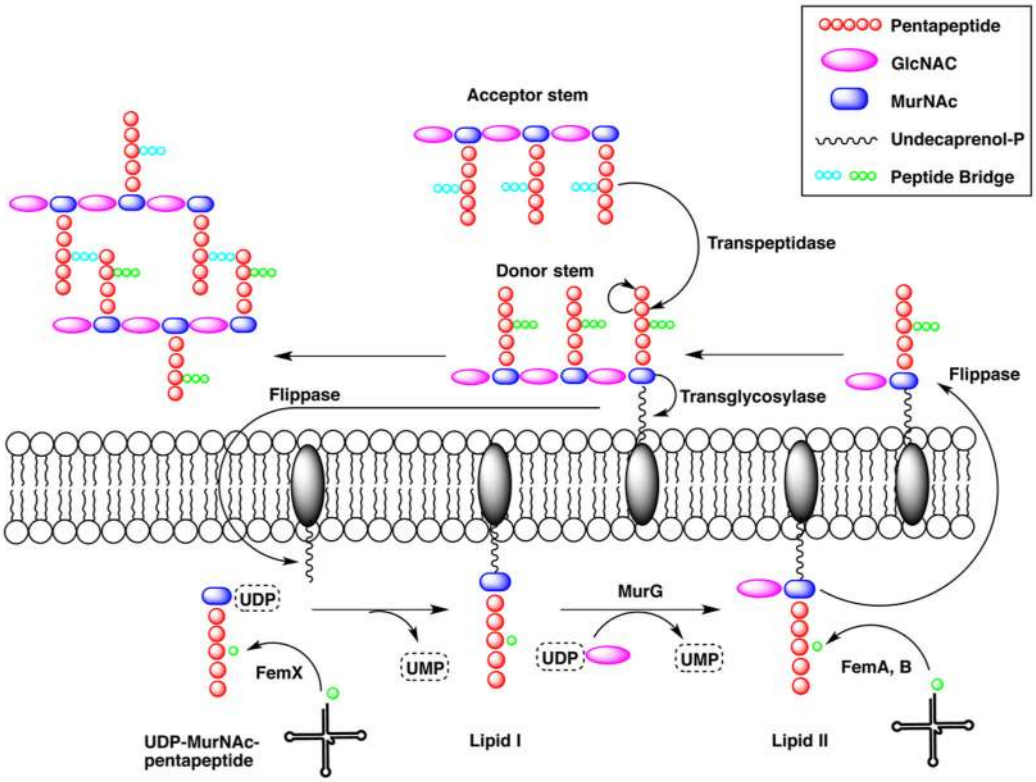

Figure 5.

Scheme of peptidoglycan layer formation. Fem enzymes act at different stages of the peptide bridge formation depending on the bacterial species. In *W. viridescens*, FemX adds only the first amino acid. In *S. aureus*, FemX adds the first glycine residue whereas FemA adds the second and third glycine residues and FemB the last two glycine residues. For the sake of simplicity, only three of five amino acids in the peptide bridge are represented in the figure. After peptide bridge formation, the terminal Gly_5 α-NH_2 group of the peptide bridge is cross-linked to the penultimate amino acid of the neighboring pentapeptide stem by transpeptidation leading to the release of the terminal amino acid from the stem peptide.

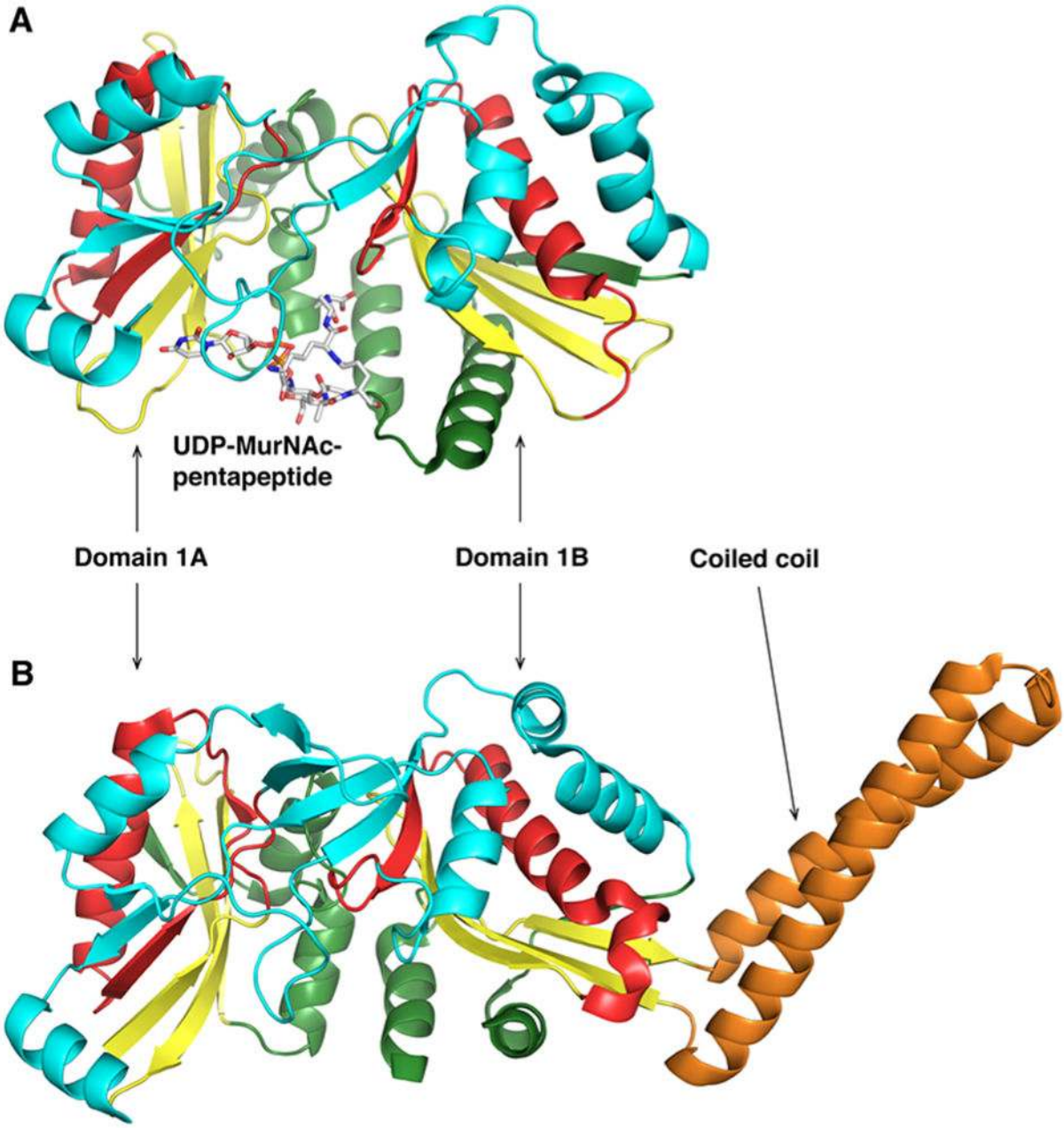

Figure 6.

Structural comparison of *W. viridescens* FemX and *S. aureus* FemA. The GNAT secondary structure elements are colored like the topology scheme presented in Figure 1. The bisubstrate is rendered as sticks with white carbons. (A) The *W. viridescens* FemX consists of two similar GNAT domains, 1A and 1B (PDB entry 1P4N). (B) The *S. aureus* FemX structure also displays domains 1A and 1B and contains an additional coiled coil segment that is colored orange (PDB entry 1LRZ).

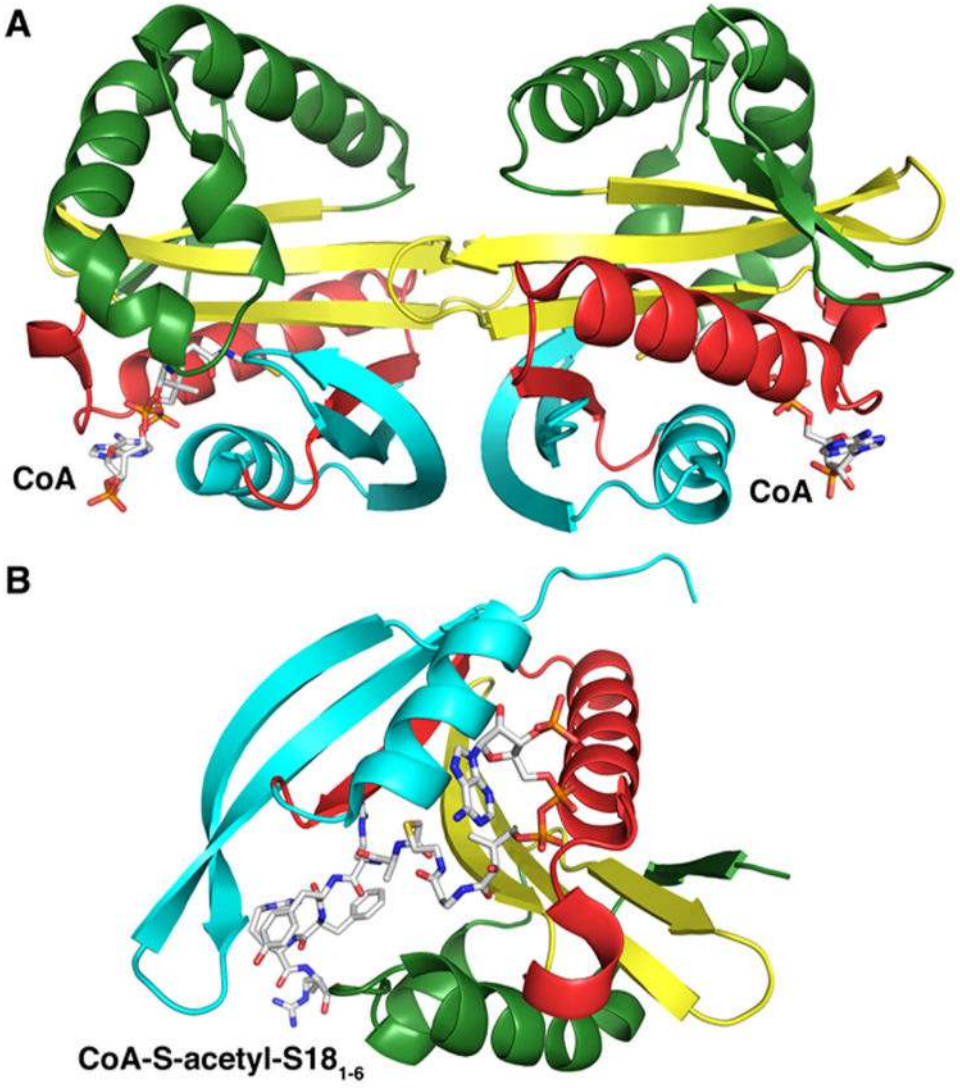

Figure 7.

Structures of Rim proteins. The secondary structure elements are colored like the topology scheme presented in Figure 1. CoA and the bisubstrate are rendered as sticks with white carbons. (A) Structure of *S. tphimurium* LT2 RimL in complex with CoA (PDB entry 1S7N). (B) Structure of *S. typhimurium* LT2 RimI in complex with a bisubstrate CoA-S-acetyl-S18$_{1–6}$ (PDB entry 2CNM).

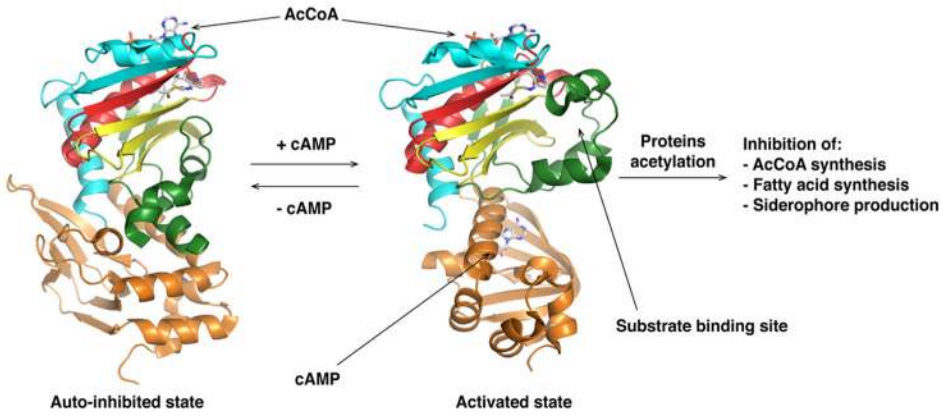

Figure 8.

Activation of *M. tuberculosis* Pat via cAMP binding (left structure, PDB entry 4AVA; right structure, PDB entry 4AVC). The N-terminal regulatory domain (orange) is linked to C-terminal catalytic GNAT domain. Upon cAMP binding, the Pat protein undergoes a conformational rearrangement allowing protein–substrate binding in the catalytic domain active site. The GNAT secondary structure elements are colored like the topology scheme presented in Figure 1. The substrates and activators bound are rendered as sticks with white carbons.

Table 1

Examples of GNAT Enzymes and Their Targets

names	targets	notes
PA4794[148]	potential protein	acetyltransferase
ApxC[89]	prototoxin HlyA	acyltransferase
GAT, SaGNAT[149,150]	unknown small molecule	acetyltransferase, herbicide
CBG[151]	clavaminic acid	acetyltransferase, tandem GNAT
MccE[90]	antibiotic microcin C7	acetyltransferase, bidomain
PseH[84]	UDP-4-amino-4,6-dideoxy-β-L-AltNAc	acetyltransferase
SpeG/PaiA[85,86]	spermidine	acetyltransferase, dodecamer GNAT
WecD[152]	4-amido-4,6-dideoxy-D-galactose	acetyltransferase
MddA/ADP1[87,88]	methionine sulfoxide, methionine sulfone	acetyltransferase
MbtK[91,92]	mycobactin	acyltransferase
PhnO[153]	aminoalkylphosphonic acid	acetyltransferase
Rv0802c[154]	unknown	succinyl transferase
GlmA[93]	glucosamine	acetyltransferase

Table 2

Interpeptide Bridge Composition of Various Bacterial Species

species	interpeptide bridge composition	position *X* of the pentapeptide
Lactobacillus coprophilus	$(L\text{-}Ala)_2$	ε-lysine[155]
S. aureus	$(Gly)_5$	ε-lysine[156]
Staphylococcus epidermidis	Gly-L-Ala	ε-lysine[157]
W. viridescens	L-Ala-L-Ser	ε-lysine[100]
Streptococcus pneumoniae	L-Ser-$(L\text{-}Ala)_2$	ε-lysine[158]
Borrelia burgdorferi	Gly	δ-L-ornithine[159]
Treponema palladium	Gly	δ-L-ornithine[159]
Clostridium perfringens	Gly	ω-L,L-diaminopimelic acid[160]
M. tuberculosis	Gly	ω-L,L-diaminopimelic acid[161]
B. subtilis	none	ω-L,L-diaminopimelic acid[162]
Streptomyces coelicolor	Gly	ω-L,L-diaminopimelic acid[163]