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REVIEW

Small-Molecule Acetylation by GCN5-Related *N*-Acetyltransferases in Bacteria

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SUMMARY Acetylation is a conserved modification used to regulate a variety of cellular pathways, such as gene expression, protein synthesis, detoxification, and virulence. Acetyltransferase enzymes transfer an acetyl moiety, usually from acetyl coenzyme A (AcCoA), onto a target substrate, thereby modulating activity or stability. Members of the GCN5-*N*-acetyltransferase (GNAT) protein superfamily are found in all domains of life and are characterized by a core structural domain architecture.

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These enzymes can modify primary amines of small molecules or of lysyl residues of proteins. From the initial discovery of antibiotic acetylation, GNATs have been shown to modify a myriad of small-molecule substrates, including tRNAs, polyamines, cell wall components, and other toxins. This review focuses on the literature on small-molecule substrates of GNATs in bacteria, including structural examples, to understand ligand binding and catalysis. Understanding the plethora and versatility of substrates helps frame the role of acetylation within the larger context of bacterial cellular physiology.

KEYWORDS GCN5-related acetyltransferases, acetyltransferases, acylation, antibiotic resistance, chemical modification, histone acetylation, metabolic control, toxin-antitoxin

INTRODUCTION

Overview of Acetylation

Acetylation as a regulatory mechanism was first reported over 50 years ago, when the acetylation of histone tails was shown to regulate gene expression in eukaryotes (1). Only later did the importance of acetylation in bacteria become clear. Small-molecule acetylation by bacteria was first described in 1965 within the context of antibiotic resistance (2). Since then, our knowledge of the acetylation capabilities of bacteria has expanded to include proteins, polyamines, toxins, tRNAs, and cell wall components (3–7).

Acetylation is a ubiquitous modification, and acetyltransferase enzymes are found in all domains of life (3). The three main classes of acetyltransferases include (i) the GCN5-*N*-acetyltransferase (GNAT) family (Pfam 00583), (ii) the MYST family (Pfam 01853), and (iii) the p300/CBP family (Pfam 06466). Only the GNAT family of acetyltransferases is found in both eukaryotes and prokaryotes, while the MYST and p300/CBP families are found exclusively in eukaryotes (3). This review focuses on the acetylation of small molecules by bacterial GNATs. Recent reviews on bacterial protein acetylation and other posttranslational modifications can be found in references 8 to 10.

Bacterial GNATs

The GNAT superfamily is one of the largest families of proteins, containing more than 300,000 proteins (11). GNATs share little sequence homology and are instead characterized based on the core GNAT domain. As mentioned above, GNATs can acetylate a wide variety of small molecules. The largest group is the antibiotic-modifying GNATs, especially GNATs modifying aminoglycoside antibiotics. Bacterial GNATs also have roles in detoxification, synthesis, and transcription. In this review, we address each of these topics and describe the GNATs involved. An overview of the GNATs and their targets is provided in Table 1.

General topology of GNATs. The GNAT domain consists of six or seven β-strands and four α-helices (12). A cartoon representation of the GNAT topology is shown in Fig. 1A. GNATs contain four conserved motifs, with motif A being the most conserved (13). Another conserved aspect of GNATs is the binding of acetyl coenzyme A (AcCoA) via the pyrophosphate binding loop, referred to as the "P loop," between β-4 and α-3. This conserved sequence [(R/Q)-X-X-G-X-(A/G)] allows for multiple sites of hydrogen bonding to the pyrophosphate of AcCoA to the amine backbone (14). The AcCoA molecule sits in the β-bulge, a V-shaped splaying of β-4 and β-5, where the pantothenate moiety of AcCoA interacts with the C-terminal end of β-4 (15). Neither the acetyl group nor the adenine ring of AcCoA makes many interactions with the GNAT; thus, GNATs may bind other acyl-CoAs as well as coenzyme A (CoA) nonspecifically (14). Structurally, GNATs have a conserved site for CoA binding and catalysis but display structural variations that accommodate other acyl-CoA substrates (e.g., propionyl-CoA [PrpCoA], succinly-CoA [SucCoA]) (16).

Catalytic mechanism of GNATs. GNATs usually act as dimers and use a sequential mechanism to catalyze the transfer of an acetyl moiety from AcCoA to the substrate

TABLE 1 GNATs and their targets

GNAT function and GNAT	Major acetylated small-molecule substrate(s)	Reference(s)
GNATs modifying antibiotics		
AAC(1)	Apramycin, butirosin, lividomycin, and paromomycin	30, 31
AAC(3)	Gentamicin, tobramycin, and sisomicin	27, 36, 37
AAC(2′)	Broad range of aminoglycosides with 2′-amino groups (e.g., tobramycin, gentamicin)	48, 49, 55
AAC(6′)	Broad range of 4,6-disubstituted aminoglycosides (e.g., kanamycin, amikacin) and 4,5-disubstituted aminoglycosides (e.g., neomycin)	4
Hpa2	Kanamycin and streptomycin	70
Eis	Kanamycin A, amikacin, and capreomycin	76, 77
PA3994	Polymyxin B and polymyxin E (colistin)	82, 83
SatA	Streptothricin	87, 90–93, 95–97
MccE	Microcin C7	103
RimL	Microcin C7	104
YhhY	Leucine sulfamoyl adenylates	104
GNATs modifying polyamines		
SSAT, SpeG	Spermidine and spermine	107
SpeG	Spermidine and spermine	114, 119
PmvE	Putrescine and spermine	116
PaiA	Spermidine and spermine	106
BltD	Spermidine and spermine	121
SnaB	Spermidine, spermine, cadaverine, and putrescine	122
GNATs modifying glutamate-mimicking substrates		
PitA (PA4866), AcePitA (ACIAD1637), and MddA (STM1590)	Methionine sulfoximine and methionine sulfone	134–136
Bar, Pat	Phosphinothricin	123, 127, 128
PhoN1	Phosphinothricin	139
PhoN2	Methionine sulfoximine	139
Ttr	Tabtoxinine-β-lactam	143–146
Antitoxin-GNAT toxin systems		
AtaT	Initiator methionine-tRNA^{fMet}	153, 154
ItaT	Isoleucine-tRNA^{Ile}	155
TacT, TacT2, TacT3	Amino acids of charged tRNAs, with a preference for glycine and isoleucine/leucine	156, 157
KacT	Unknown, possibly tRNAs	161
GmvT	Unknown, possibly tRNAs	162
GNATs involved in anabolism		
TmcA	Wobble base (position 34) of tRNA^{Met}	6
MshD	1-D-*myo*-Inosityl-2-L-cysteinyl-amido-2-deoxy-α-D-glucopyranoside	175
CBG	Unknown, predicted involvement in clavulanic acid synthesis	182, 183
PseH	UDP-4-amino-4,6-dideoxy-β-L-AltNAc	185
WecD	dTDP-4-amino-4,6-dideoxy-α-D-galactose	192, 193
Aminoacyl GNATs		
MurM	Transfers the first alanine or serine to the cross-link in *S. pneumoniae*	205, 209, 210
MurN	Transfers the second alanine to the cross-link in *S. pneumoniae*	206, 209
BppA1	Transfers the first alanine of the cross-link in *E. faecalis*	211
BppA2	Transfers the second alanine of the cross-link in *E. faecalis*	211
FemX (also called FmhB)	Transfers the first glycine of the cross-link in *S. aureus* or *Weissella viridescens*	203, 204
FemA	Transfers the second and third glycine residues of the cross-link in *S. aureus*	213, 215
FemB	Transfers the fourth and fifth glycine residues of the cross-link in *S. aureus*	213, 216
MprF, LysX, L-PGS	Transfers a lysine residue onto phosphatidylglycerol	229–231, 233
A-PGS	Transfers an alanine residue onto phosphatidylglycerol	233
LFT	Transfers a leucine or phenylalanine residue to the N-terminal arginyl or lysyl residue of a protein	235
VlmA	Transfers serine in the biosynthesis of valanimycin	237
PacB	Transfers alanine in the biosynthesis of pacidamycin	238

(Continued on next page)

TABLE 1 (Continued)

GNAT function and GNAT	Major acetylated small-molecule substrate(s)	Reference(s)
DhpH	Transfers leucine in the biosynthesis of dehydrophos	240
DhpK	Transfers glycine in the biosynthesis of dehydrophos	240
FmzI	Transfers valine in the biosynthesis of fosfazinomycin	241
Orf11	Transfers glycine in the biosynthesis of BD-12	242
GNATs involved in small-molecule-dependent transcription		
BadL	Aminobenzoates	247
OatA	*O*-Acetylserine	248
CD1211	*O*-Acetylserine, L-serine, L-threonine, and L-methionine	249

(Fig. 2) (15). GNATs use a general acid/base mechanism, where a catalytic glutamate or aspartate acts as a general base to abstract a proton from the amine. The nucleophilic amine attacks the thioester carbonyl carbon, resulting in a direct transfer of the acetyl group. A general acid (usually a tyrosine or a serine) reprotonates the thiolate of coenzyme A (9). A conserved tyrosine that acts as a general acid is found in 62% of characterized GNATs, while 36% have a conserved glutamate as a general base (17). In lieu of a residue acting as a general base, a water molecule(s) deprotonates before the nucleophilic attack occurs (11). GNATs may follow a random kinetic mechanism, where the binding order does not matter, or an ordered kinetic mechanism, where the binding order of the substrate and AcCoA is dependent on the other (18, 19).

GNAT-DEPENDENT ANTIBIOTIC INACTIVATION

Aminoglycoside-Modifying GNATs

Aminoglycosides are hydrophilic sugars with several amino and hydroxyl moieties that vary by number and location based on the specific antibiotic (20) (Fig. 3A). These antibiotics are produced mainly by actinomycetes and inhibit protein synthesis by interacting with the 16S rRNA of the bacterial ribosome and block the A site (4, 21–24). A detailed review on the mechanism of aminoglycosides can be found in references 25 and 26. The most common means of aminoglycoside resistance arises from aminoglycoside-modifying enzymes that can phosphorylate, adenylate, or acetylate the antibiotic (20).

Aminoglycoside acetyltransferases, called AACs, belong to the GNAT superfamily (4). They are classified based on the site of acetylation (Fig. 3A) and further subclassified based on the antibiotics that they modify (20, 27). AAC(1) and AAC(3) modify the position 1 and 3 amino groups, respectively, of the 2-deoxystreptamine ring, and AAC(2′) and AAC(6′) modify the 2′ and 6′ amino positions, respectively, of the 2,6-dideoxy-2,6-diaminoglucose ring (26, 28). An example of AAC(6′) acetylation is shown in Fig. 3B. Acetylation of the aminoglycoside impairs its ability to bind the rRNA because of unfavorable steric or electrostatic interactions, thereby reducing the cytotoxicity of the antibiotic (20, 29).

The first AAC was described by Okamoto and Suzuki in 1965, marking the first time that this family of antibiotics was reported to be inactivated in such a manner (2). Since then, more than 70 AACs have been reported (26). A brief summary of each class is discussed below.

AAC(1) family. The AAC(1) family is comprised of only two GNATs. AAC(1)-I was originally found in *Escherichia coli* to monoacetylate apramycin, butirosin, lividomycin, and paromomycin and to diacetylate ribostamycin and neomycin (30). Later, AAC(1)-I was discovered in *Campylobacter* to be the first reported example of an AAC(1) enzyme found in a clinical isolate (4). In contrast, AAC(1)-II was identified in an *Actinomycetes* isolate and shown to have a broader substrate range than AAC(1)-I (31). However, these studies showed that AAC(1) modifications did not significantly reduce the antibacterial effect of the aminoglycoside.

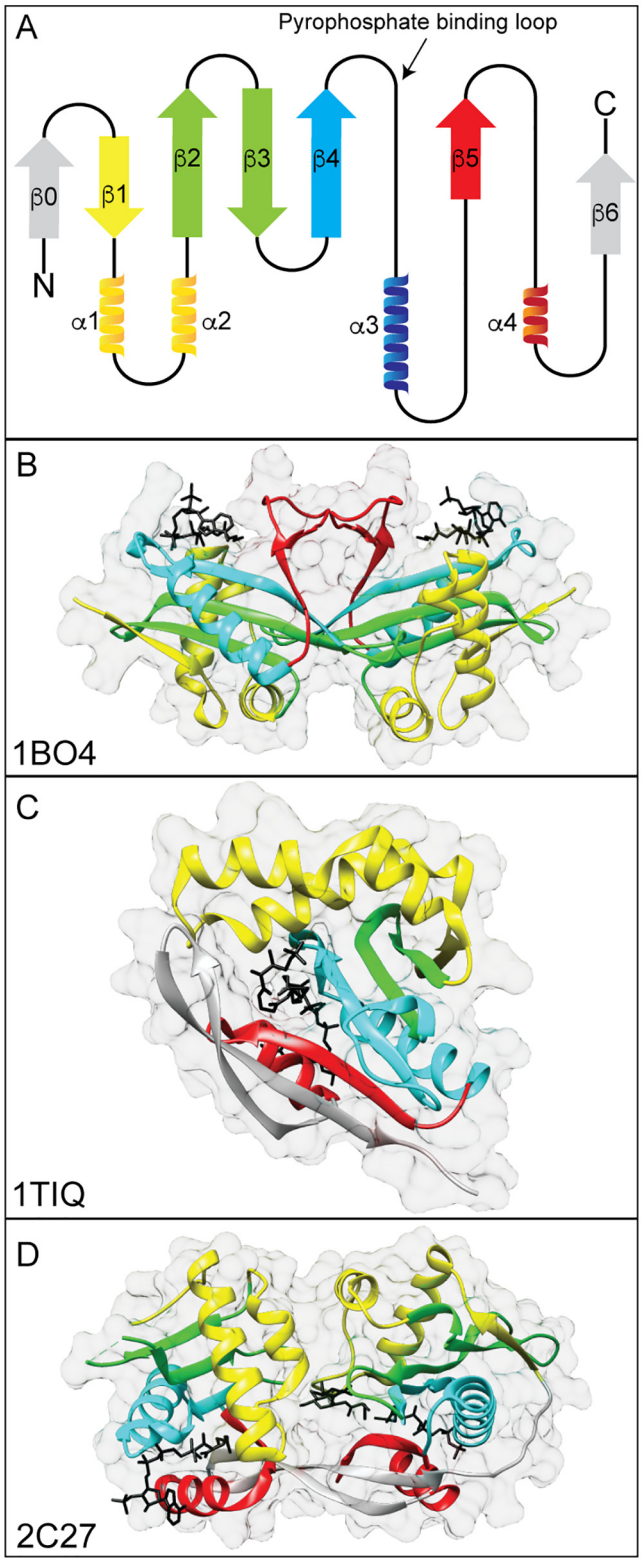

FIG 1 General structure of GNATs. (A) Cartoon representation of GNAT topology. GNATs contain six or seven β-sheets and four α-helices. The β-sheets are shown as arrows, and the α-helices are shown as ribbons. The four motifs are colored (yellow, motif C; green, motif D; blue, motif A; red, motif B), and this coloring applies to panels B to D. The least-conserved elements are colored gray. Examples of GNAT structures are shown with substrates in black. (B) AAC(3)-Ia dimer from *Serratia marcescens* in complex with AcCoA (PDB accession number 1BO4). (C) PaiA from *Bacillus subtilis* in complex with an oxidized CoA dimer (PDB accession number 1TIQ). (D) MshD from *Mycobacterium tuberculosis* in complex with AcCoA, CoA, and desacetylmycothiol (PDB accession number 2C27).

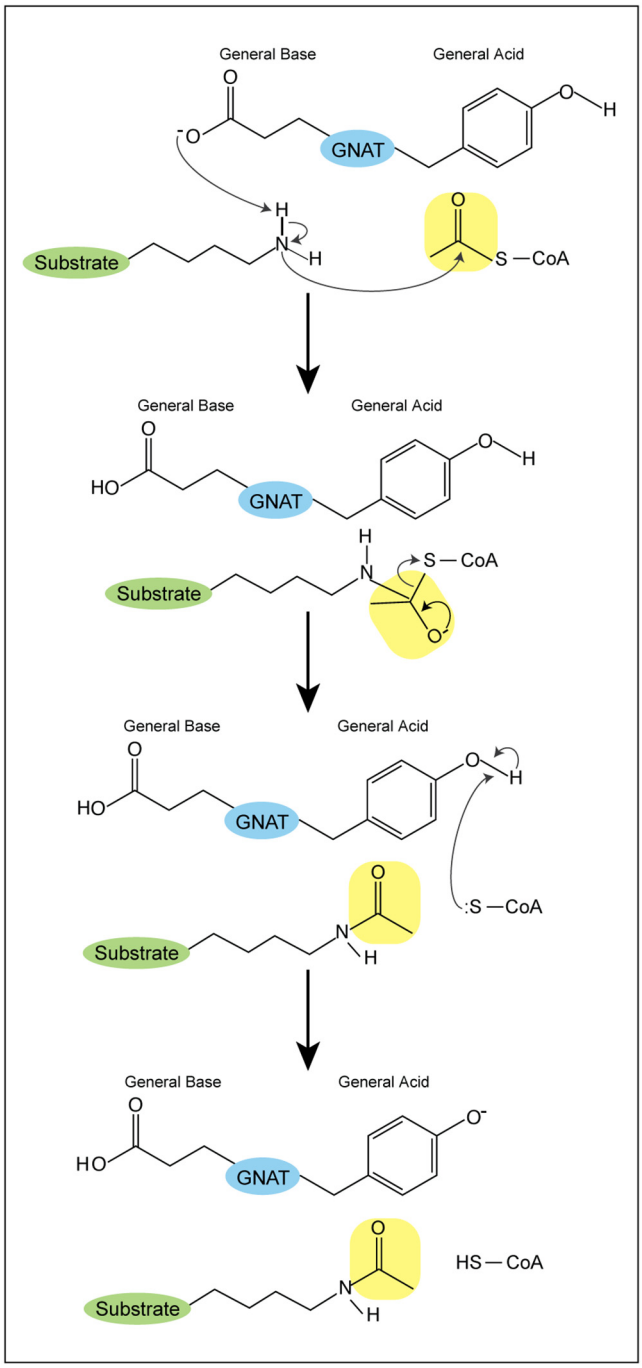

FIG 2 GNAT acetylation mechanism. A catalytic glutamate or aspartate acts as a general base to abstract a proton from an amine. The nucleophilic amine attacks the carbonyl carbon of the acetyl moiety, allowing for a direct transfer of the acetyl group. A general acid (usually a tyrosine) reprotonates the thiolate of CoA.

AAC(3) family. The AAC(3) family includes nine subclasses of GNATs (4) and includes the first aminoglycoside-modifying enzyme to be purified (gentamicin acetyltransferase) (32). Kinetic studies with AAC(3) enzymes showed that these enzymes use a sequential kinetic mechanism and prefer AcCoA as the acyl donor, helping to establish the mechanism used by most GNATs in general (33–35).

(i) Members of the AAC(3)-I subfamily confer resistance to gentamicin, sisomicin, and astromicin and are found in the integrons of Gram-negative bacteria (4, 36, 37). The first

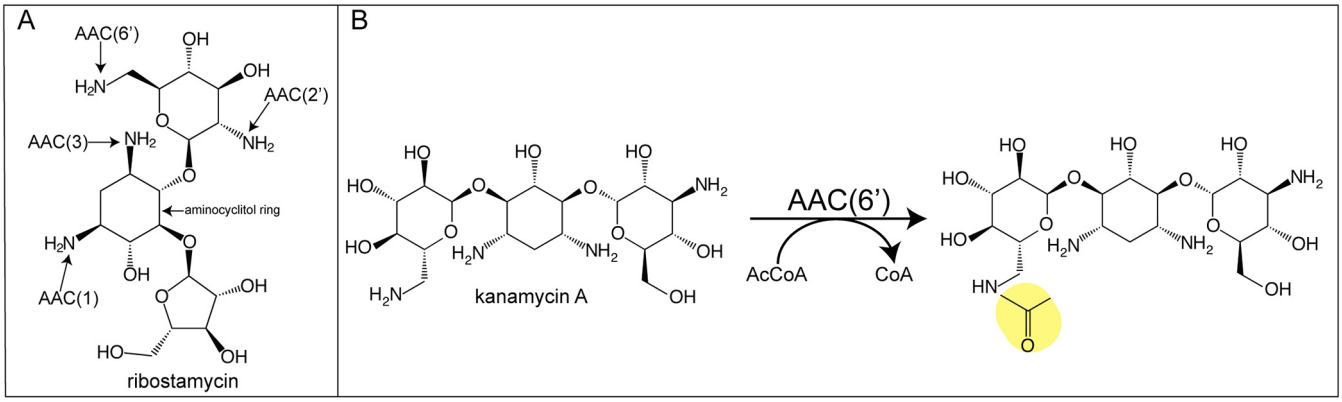

FIG 3 Acetylation of aminoglycoside antibiotics. (A) Chemical structure of the aminoglycoside ribostamycin showing the sites of acetylation (1, 3, 2′, 6′). (B) Example of acetylation of kanamycin A by AAC(6′). The transferred acetyl moiety is highlighted in yellow.

AAC-type enzyme to be crystalized, AAC(3)-Ia from *Serratia marcescens* (Fig. 1B), was also from this family of GNATs (38). The crystal structure of AAC(3)-Ia in complex with CoA displays the typical GNAT architecture with a V-shaped splay between β-4 and β-5 for CoA binding. Acidic residues found in motif B are important for binding the positively charged antibiotic (38). This first AAC crystal structure allowed for structure-based sequence comparisons for other GNATs as well as a means to define structurally the four GNAT motifs.

(ii) The AAC(3)-II subfamily includes three GNATs [AAC(3)-IIa to -IIc] that confer resistance to gentamicin, netilmicin, tobramycin, sisomicin, 2′-*N*-ethylnetilmicin, 6′-*N*-ethylnetilmicin, and dibekacin (4, 27). The AAC(3)-III and AAC(3)-IV GNAT subfamilies have a broader substrate range than the AAC(3)-I and AAC(3)-II enzymes (35). The AAC(3)-III subfamily includes three enzymes [AAC(3)-IIIa to -IIIc], all of which were isolated from *Pseudomonas aeruginosa* isolates (4, 39). There is only one example of a AAC(3)-IV enzyme, which is found in *E. coli*, *Campylobacter jejuni*, and *Pseudomonas stutzeri* strains, and only one example of a AAC(3)-VI enzyme, which is found in enteric bacteria (40–43).

(iii) Members of the AAC(3)-VII, AAC(3)-VIII, AAC(3)-IX, and AAC(3)-X subclasses are all chromosomally encoded in Gram-positive actinomycetes (4, 44–46). Surprisingly, the AAC(3)-X enzyme also displays AAC(3″) activity, since it can acetylate the 3″-amino group of arbekacin and amikacin, though only 3″-*N*-acetylamikacin lost antimicrobial activity when acetylated (47).

AAC(2′) family. All the aminoglycoside acetyltransferases in the AAC(2′) family are chromosomally encoded (28). AAC(2′)-Ia from *Providencia stuartii* was the first of this family to be described (48). This GNAT shows broad specificity for aminoglycosides with 2′-amino groups (49) and can acetylate the aminoglycoside tobramycin using an acetyl group from AcCoA, soluble fragments of peptidoglycan, or *N*-acetylglucosamine (50). However, kinetic and phenotypic analyses suggest that the physiological role of AAC(2′)-Ia appears to be in cell wall metabolism, such as *O*-acetylation of peptidoglycan (50–52).

The four other GNATs belonging to this family [AAC(2′)-Ib to -Ie] are found in the *Mycobacterium* species, though recently, an AAC(2′)-Ib enzyme was detected in *Acinetobacter baumannii* (53, 54). AAC(2′)-Ib from *Mycobacterium fortuitum* FC1K showed homology to AAC(2′)-Ia (38.5% identity), and expression of the *aac(2′)-Ib* gene in *M. smegmatis* provided increased resistance to gentamicin, tobramycin, dibekacin, netilmicin, and 6-*N*-ethylnetilmicin compared to that for the vector-only control (55). Use of the *aac(2′)-Ib* gene as a probe in DNA hybridization experiments indicated the universal distribution of aminoglycoside 2′-*N*-acetyltransferase genes throughout the *Mycobacterium* genus and facilitated the identification of the *aac(2′)-Ic*, *aac(2′)-Id*, and *aac(2′)-Ie* genes from *M. tuberculosis* H37Rv, *M. smegmatis*

mc²155, and *M. leprae*, respectively (56). All the encoded *Mycobacterium* proteins share ~60% to 80% identity, while they are only ~30% to 40% identical to AAC(2′)-Ia from *P. stuartii* (56).

A deletion of the *aac(2′)-Id* gene in *M. smegmatis* results in decreased MIC values for aminoglycosides and a lack of aminoglycoside acetyltransferase activity in crude extracts, indicating a role for AAC(2′)-Id in aminoglycoside inactivation (56). Besides a sensitivity to aminoglycosides, the *aac(2′)-Id*-deficient strain also displayed increased sensitivity to lysozyme (56). This suggests that another physiological role of AAC(2′)-Id is involvement in the synthesis of the cell wall, similar to that of AAC(2′)-Ia. Also similar to AAC(2′)-Ia, AAC(2′)-Ic has been shown to *O*-acetylate kanamycin A and amikacin, suggesting that some AAC(2′) enzymes are capable of *N*- and *O*-acetylation (57, 58) and may play a role in cell wall synthesis (59).

The AAC(2′)-Ic protein has been crystalized as a dimer, displaying the typical GNAT fold with an atypical P-loop sequence (60). The C-terminal aminoglycoside-binding site is lined with acidic residues (D35, D40, E82, D152, and D179) and the carboxyl terminus of W181, all of which are conserved among AAC(2′) enzymes (60). These residues interact with the hydroxyl and amino substituents of the aminoglycoside either directly or through water molecules. The hydroxyl of Y126 is 3.6 Å away from the sulfur moiety of CoA and may act as a general acid, while residue E82 or W181 may act as a remote general base (60).

AAC(6′) family. The AAC(6′) family is the largest family and is found in Gram-positive and Gram-negative bacteria alike (4). An example of the reaction catalyzed by this family is shown in Fig. 3B, which shows how the enzyme acetylates kanamycin A. There are two subclasses, based on the resistance profiles for amikacin and gentamicin C1. AAC(6′)-I enzymes have low levels of activity against gentamicin C1 and high levels of activity against amikacin and gentamicin C1a and C2, while AAC(6′)-II enzymes have high levels of activity against all forms of gentamicin but low levels of activity against amikacin (4). Though the AAC(6′)-Ib-cr enzyme can acetylate fluoroquinolones, it is not considered a third subclass because only two amino acid substitutions (W102R and D179Y) differentiate it from AAC(6′)-Ib (61).

The AAC(6′)-I subfamily is the largest, with over 50 unique enzymes organized into three distinct subclasses characterized by structure and the catalytic efficiencies as a function of their aminoglycoside substrates (4, 62). Subclass A contains AAC(6′)-Ig, -Ih, and -Iy as well as *Acinetobacter*-specific AAC(6′)-I enzymes. These proteins act as dimers and are the least catalytically efficient (62). Subclass B contains AAC(6′)-Ii, is characterized by dimerization and intermediate catalytic efficiency, and exhibits subunit cooperativity (62, 63). The final subclass, subclass C, contains AAC(6′)-Ib and -Ie, which are monomers and which have the highest known catalytic efficiency toward aminoglycosides (62).

AAC(6′)-Ib is the most clinically relevant AAC(6′) enzyme and is found in over 70% of AAC(6′)-I-positive Gram-negative bacterial clinical isolates (4, 64). Because of their medical importance, many AAC(6′)-Ib enzymes have been crystalized to identify active-site residues and understand molecular mechanisms. The crystal structure of AAC(6′)-Ib from *E. coli* shows that D115 acts as a general base and that Y164 may be a general acid (65). Structures also exist for a close homologue, AAC(6′)-Ib$_{11}$, which has a broader range of antibiotic substrates than AAC(6′)-Ib from *E. coli* (66). Both structures display the conserved pyrophosphate binding loop as well as the V shape to accommodate AcCoA binding (65, 67). Unique to AAC(6′)-Ib enzymes is an extended α-helical flap that forms a lid over the antibiotic-binding pocket and that may account for the ordered kinetic mechanism where AcCoA binds first (19, 65, 67). Surprisingly, AAC(6′)-Ib acts as a monomer, though *Salmonella enterica* AAC(6′)-Ib$_{11}$ acts as a mixture of monomers and dimers (65, 67). Comparing the structures of AAC(6′)-Ib and AAC(6′)-Ib$_{11}$ reveals that the active-site residues of AAC(6′)-Ib (Q106 and L107) are replaced by leucine and serine, respectively, in AAC(6′)-Ib$_{11}$. These substituted residues induce a structural change that creates a gaping of the active site to allow larger aminoglycosides to bind and explains the broader substrate range of AAC(6′)-Ib$_{11}$ (66, 67). Many

other AAC(6′)-Ib variants with changes in the N terminus have been described, indicating flexibility in this portion of the protein, since these variants still confer resistance (4).

In addition to the subfamilies, there is a variety of fusion proteins with the AAC(6′) portion at the N or C terminus of the protein (68). These fusion proteins can have aminoglycoside adenylylase (ANT) or phosphotransferase (APH) activity (4), highlighting how bacteria can become resistant to multiple antibiotics. A single tyrosine substitution (D179Y) of AAC(6′)-Ib-cr appears to allow for π-stacking interactions with the quinolone ring, enabling fluoroquinolone binding and acetylation (65). Some fusion proteins are a fusion of two AAC proteins, such as AAC(3)-Ib/AAC(6′)-Ib′ of *P. aeruginosa* (69). This enzyme has two GNAT domains, both of which are active and use an ordered sequential kinetic mechanism where AcCoA binds first (19).

Recently, the histone acetyltransferase Hpa2 of *A. baumannii* was reported to acetylate and inactivate kanamycin and streptomycin (70). *A. baumannii* Hpa2 (*Ab*Hpa2) shows a topology similar to the crystal structure of *Salmonella enterica* AAC(6′)-Iy, which has been shown to acetylate both histone peptides and aminoglycosides (71). A virtual substrate screen also indicated that aminoglycosides may be a possible substrate of *Ab*Hpa2 (72). Similar to AAC(6′)-Iy, apo-*Ab*Hpa2 acts as a dimer in solution (73); however, the addition of AcCoA converts the Hpa2-AcCoA complex into monomers (70). The binding of kanamycin induces structural changes in *Ab*Hpa2, and isothermal titration calorimetry experiments demonstrate that *Ab*Hpa2 binds aminoglycosides kanamycin and streptomycin well (K_d [dissociation constant], 8 μM and 79 μM, respectively) (70). Importantly, noncanonical AAC GNATs are emerging as reservoirs of aminoglycoside resistance.

The Eis GNAT. The enhanced intracellular survival (Eis) protein is a unique aminoglycoside-acetylating enzyme that is divergent from other AACs but that is still capable of acetylating multiple amino groups (74). Eis was originally described in *M. tuberculosis* as a secreted protein that enhances survival in macrophages (75), and Eis homologues are found in other mycobacteria and several nonmycobacterial prokaryotes (76). Eis has been reported to acetylate many second-line antituberculosis drugs, such as kanamycin A, amikacin, and capreomycin, with this being reported as the first instance of inactivation of capreomycin via acetylation (76, 77). *M. tuberculosis* Eis (*Mt*Eis) can acetylate the 3-amino and 6′-amino groups of kanamycin, the 3-amino- and 4-amino-2-hydroxybutyryl groups of amikacin, and the ε-amino group of the β-lysine side chain of capreomycin (76, 77). The physiological role of Eis aminoglycoside acetylation is unclear since the K_m is high for both substrates (154 μM for kanamycin and 112 μM for amikacin), and the authors of these studies suggested that Eis may acetylate host factors during *M. tuberculosis* infection (77). Recently, it was shown that Eis from *M. tuberculosis*, but not that from *M. smegmatis*, acetylates K55 of dual-protein phosphatase 16/mitogen-activated protein kinase phosphatase 7 (DUSP16/MKP-7), thereby suppressing cytokine production in macrophages (78). Inspecting the crystal structure of the two Eis homologues, *Mt*Eis contains a narrow channel in the active site that is absent in *M. smegmatis* Eis (*Ms*Eis), and this channel may act as a binding site for DUSP16/MKP-7 (78).

Eis monomers contain three structural domains and form hexamers in solution (74, 78). Surprisingly, both domain 1 (residues 1 to 150) and domain 2 (residues 151 to 291) adopt a GNAT fold, even though domain 2 shows little sequence similarity to other GNATs (78). Domain 3 (residues 292 to 402) shares homology to sterol carrier protein 2 (78). Domain 1 can bind AcCoA or CoA, but domain 2 cannot, as it lacks the R/Q residue needed in the P loop to bind the pyrophosphate of CoA, indicating that domain 2 is an inactive GNAT (74, 78). The active site is formed between the N-terminal domain 1 and central domain 2 GNAT domains, creating a negatively charged pocket for aminoglycosides of different sizes to bind (74). The substrate range and multiacetylation patterns of *Mt*Eis and *Ms*Eis differ, which is proposed to be the result of substitutions to several nonconserved residues lining the aminoglycoside-binding pocket (79). Residue H119 positions the aminoglycoside amino group for direct attack, while F402

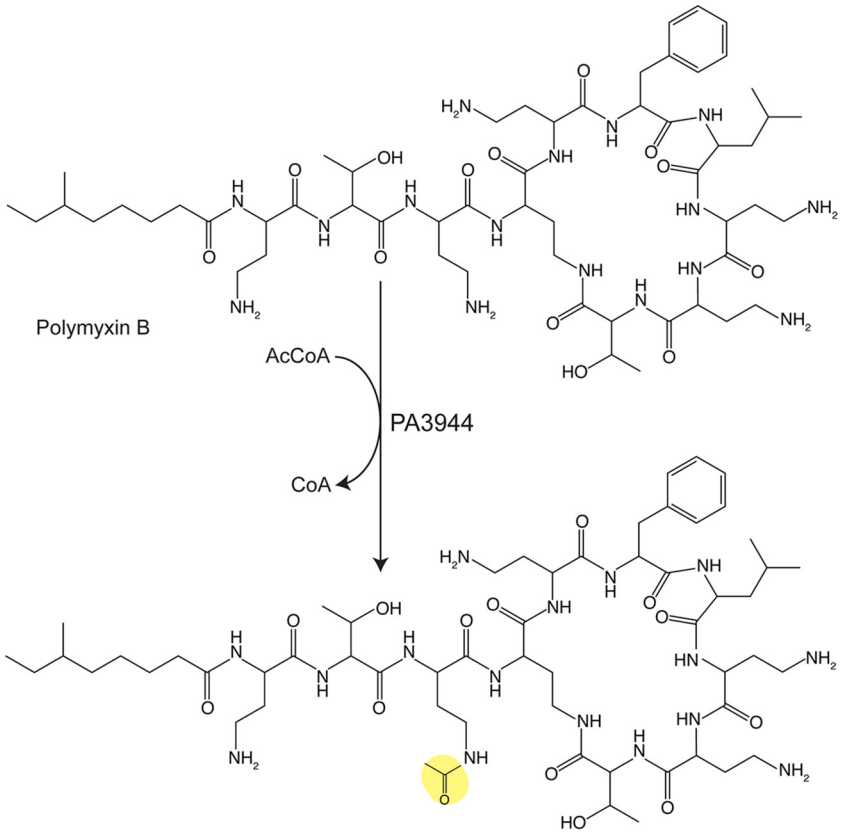

FIG 4 Acetylation of the antibiotic polymyxin B. PA3994 acetylates the γ-amino group of α,γ-diaminobutryic acid proximal to the cyclic peptide. The transferred acetyl moiety is highlighted in yellow.

is proposed to be a remote base via a water molecule. Mutational analysis confirmed the role of residue Y126 as a general acid, which is 3.4 Å away from the thiolate of CoA (74). A series of substituted 1,2,4- triazino-[5,6*b*]indole-3-thioether molecules was found to inhibit Eis acetylation (80). Structural data indicate that these inhibitor compounds bind in the kanamycin binding pocket and are competitive inhibitors that may have clinical use in the future (80).

Polymyxin-Modifying GNATs

Polymyxin antibiotics are cyclic lipodecapeptide antimicrobial molecules composed of a cyclic heptapeptide, a linear tripeptide, and a fatty acyl tail (Fig. 4). Polymyxins are basic because they contain five free amino groups, thereby facilitating interaction with the anionic lipopolysaccharide (LPS) of Gram-negative bacteria. These interactions lead to cracks in the outer membrane and the leakage of cytoplasmic contents (81). Currently, polymyxins are clinically used as a last-resort drug in multidrug-resistant cases (81), and the sources of resistance are of particular medical interest.

Pseudomonas aeruginosa PAO1 encodes a GNAT (PA3994) that acetylates polymyxin B and polymyxin E (colistin) (82). Acetylation by PA3994 occurs on a single diaminobutyric acid (residue 3) closest to the cyclic peptide, with PA3994 being reported to be the first instance of a polymyxin-modifying enzyme (Fig. 4) (83). The crystal structure displays the classic GNAT domain, which acts as a monomer in solution and in the crystal (83). The authors note that polymyxin acetylation is probably not the physiological role of PA3994, since the K_m for the antibiotics are in the millimolar range, but that this function can be exploited for biotechnology applications to modify selectively structurally similar antibiotics (83). PA3994 was also found to acetylate aspartame (83), though more work must be done to understand the physiological function of that modification by PA3994.

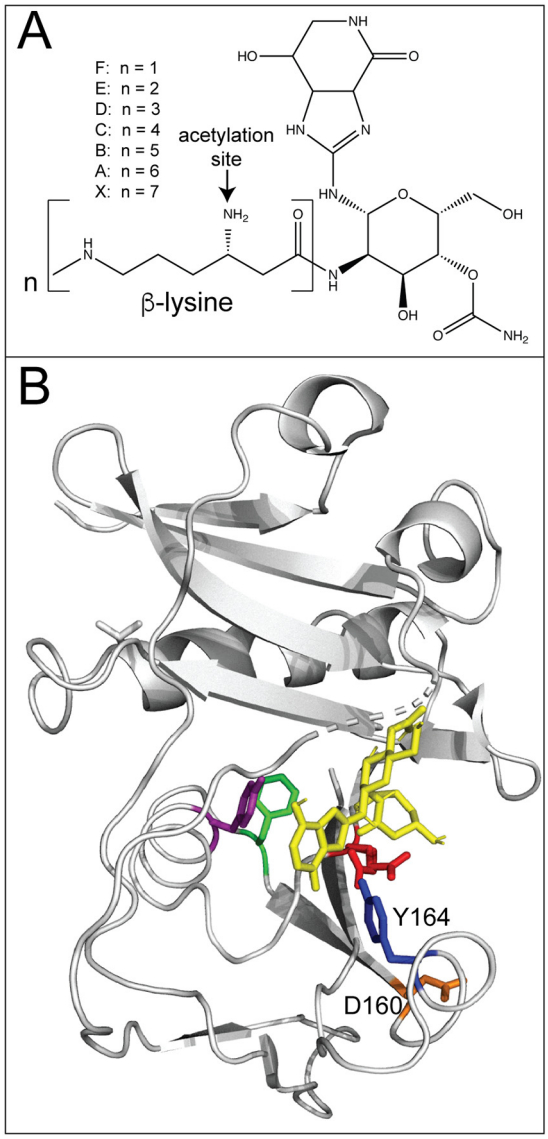

FIG 5 Acetylation of streptothricin. (A) Chemical structure of streptothricin showing the site of acetylation on the β-lysine. (B) Model of SatA (PDB accession number 3PP9) in complex with streptothricin (yellow). A conserved tyrosine (Y164, blue) appears to be in close proximity to a hydrogen bond with streptothricin. A conserved aspartate (D160, orange) is important for dimerization. (Adapted from reference 98.)

Streptothricin-Modifying GNATs

Besides aminoglycoside antibiotics, bacteria have evolved ways to acylate and inactivate other antimicrobial agents. Streptothricin is one of the most commonly found antibiotics in the soil and is composed of a streptolidine, a gulosamine sugar, and a β-lysine that varies from 1 to 7 repeating units (Fig. 5A) (84, 85). It is produced by *Streptomyces* species and acts as a broad-spectrum antimicrobial that inhibits protein synthesis by interacting with the bacterial ribosome (86–89). To protect themselves, many streptothricin-producing species encode a streptothricin acetyltransferase, as shown in *Streptomyces lavendulae* (87, 90), *Streptomyces noursei* (91, 92), and *Streptomyces rochei* (93). These GNATs monoacetylate the β-lysine, inhibiting the cytotoxicity of the antibiotic (94). Since the initial discovery of streptothricin acetyltransferases in *Streptomyces* species, other Sat (streptothricin acetyltransferase) enzymes have been characterized in *Campylobacter coli* (95), *E. coli* (96), and *Bacillus subtilis* and *Bacillus*

anthracis (97), suggesting a selective pressure on soil-dwelling bacteria for the acquisition of streptothricin resistance.

To gain insights into streptothricin binding to SatA, a detailed study of the *B. anthracis* SatA (*Ba*SatA) enzyme was performed (98). The crystal structure of *Ba*SatA displays a typical GNAT architecture with AcCoA situated in a V-shaped cleft. Examination of the structure indicated a putative C-terminal streptothricin-binding pocket. Through the use of a combination of site-directed mutagenesis, isothermal titration calorimetry (ITC), and docking software, the authors concluded that a conserved tyrosine (Y164) may interact with the streptothricin ligand (Fig. 5B). In addition, a conserved aspartate (D160) was found to be necessary for dimerization (98). Together, these data help shape our understanding of how, in general, GNATs recognize and bind their ligand.

McC-Modifying GNATs

Microcin C7 (McC) is a small-molecule antimicrobial produced by the *Enterobacteriaceae* (99). Microcin is the product of the 21-bp *mccA* gene (100). It is produced from a ribosomally synthesized heptapeptide with a C-terminal aspartate linked to AMP and a formylated N-terminal methionine (101). Inside sensitive cells, the nonhydrolyzable aspartyl adenylate analogue inhibits aspartyl-tRNA synthetase, thereby inhibiting translation (102). As a protection mechanism, the genomes of McC producers encode the *mccE* gene, which encodes a two-domain protein with an N-terminal domain homologous to decarboxylases and a C-terminal domain with homology to GNATs (103, 104). Overexpression of the C-terminal GNAT domain, but not the N-terminal domain, leads to cells with resistance to McC, albomycin, as well as other nonhydrolyzable aminoacyl adenylates with primary amines (103). Mass spectrometry analysis indicated the McC-dependent acetylation of the primary amino group of the aminoacyl moiety, rendering McC nontoxic (103).

E. coli MccE (*Ec*MccE) was crystalized with CoA or acetyl-CoA and a variety of substrates to understand its broad substrate specificity (105). *Ec*MccE displays typical GNAT domain folds, consisting of seven β-sheets and four α-helices, and binds the pyrophosphate and pantetheine of CoA with hydrogen bonds similar to those of other known GNATs (105). *Ec*MccE crystalized as a monomer, which is consistent with size exclusion chromatography data. Residues S553 and E572 are implicated to serve as the general acid/base for catalysis, respectively, since replacement of both of these residues by alanine results in a protein with an $\sim$25-fold lower acetyltransferase activity (105). The adenine ring of the substrate predominantly determines specificity. The substrate binding pocket contains several hydrophobic residues that participate in van der Waals interactions with the adenine ring, with Try453 and Phe466 allowing for strong π-stacking interactions with the aromatic adenine ring (105).

Interestingly, RimL of *E. coli* was found to provide basal resistance to McC through acetylation, similar to MccE (104). Overexpression of RimL leads to McC resistance, as seen with MccE overexpression (104, 106). RimL can also acetylate other aminoacyl adenylates, like MccE (104). Another *E. coli* GNAT, YhhY, was shown to acetylate and detoxify other nonhydrolyzable aminoacyl adenylates, though it did not process McC (104). YhhY conferred full resistance to leucine sulfamoyl adenylates (LSA) and partial resistance to alanine sulfamoyl adenylates (ASA) and phenylalanine sulfamoyl adenylate (FSA) (104). More work is needed to understand the physiological role of the acetylation of aminoacyl adenylates by these other GNATs.

GNAT-DEPENDENT DETOXIFICATION

As in the case of antibiotics, acetylation of toxins is a common mechanism for detoxification. GNATs have evolved to recognize and inactivate a series of toxic substrates.

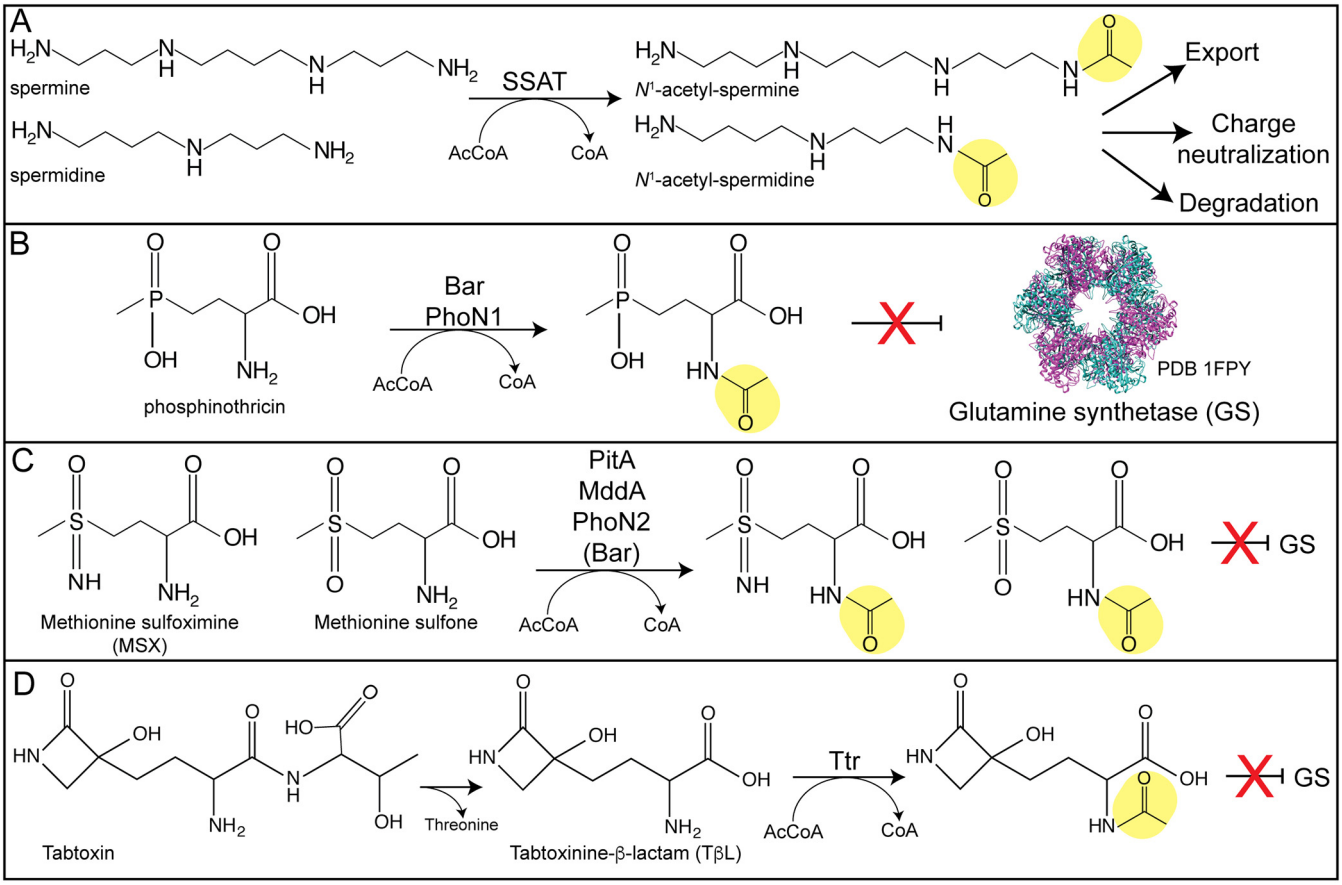

FIG 6 Acetylation of toxic compounds. (A) Acetylation of spermine or spermidine by a spermidine/spermine acetyltransferase (SSAT) signals for the N^1-acetylated product's export or degradation. (B to D) Acetylation of glutamine synthetase (GS) inhibitors blocks the toxic effect of phosphinothricin (B), methionine sulfoximine or methionine sulfone (C), or tabtoxinine-β-lactam (D).

Spermidine/Spermine-Acetylating GNATs

Polyamines (e.g., spermine, spermidine) are low-molecular-weight molecules with more than two amino groups (Fig. 6A), making them good targets for acetylation. Spermidine and spermine are found in cells of all domains of life and play a variety of roles, including the regulation of transcription, ion channels, and enzymatic activity (107, 108). Thus, the regulation of polyamine levels is strictly regulated in cells.

One means of targeting polyamines for degradation is acetylation by a spermidine/spermine acetyltransferase (SSAT). These enzymes acetylate spermidine or spermine under stress conditions to promote degradation via the polyamine oxidase, thereby preventing polyamine toxicity (109–112). Acetylation also neutralizes the positive charge of polyamines to inhibit their interaction with other macromolecules (5). SSAT enzymes are induced by polyamines and help maintain proper cellular polyamine concentrations (107).

The involvement of SSATs in virulence has been documented (113). *Staphylococcus aureus* and *Enterococcus faecalis* do not synthesize polyamines and are hypersensitive to them. The virulent *S. aureus* USA300 strain has acquired a SSAT (*speG*) on a mobile element, which the authors suggest makes the strains resistant to high polyamine levels inside a human host and thus may aid in overall virulence (114). High polyamine levels can inhibit biofilm formation, but *S. aureus* and *E. faecalis* use their respective SSATs to acetylate and neutralize host polyamines, allowing for effective biofilm formation and infection (115). PmvE (polyamine metabolism and virulence of *Enterococcus faecalis*) acetylates putrescine and spermine, and overexpression of *pmvE* leads to higher virulence (116). The authors suggest that this PmvE-dependent viru-

lence phenotype may be in part linked to a better ability to survive conditions in the macrophage, especially since *pmvE* appears to be essential in *E. faecalis* (116). In contrast, inactivation of *speE* in *Shigella* increases intracellular spermidine levels and enhances survival under oxidative stress in the macrophage (117). Besides the role of SSATs in virulence, polyamine inhibition is exploited in the development of chemotherapeutics. Since the activities of some polyamine analogues mimic SSAT activity, this leads to a decrease in polyamine levels and, thus, a decrease in cell growth of cancerous tissue (107).

Because of their role in virulence and cell viability, several SSAT enzymes have been crystalized. PaiA from *Bacillus subtilis* (*Bs*PaiA) crystalized as a dimer, though it acts as a monomer in solution (106). Surprisingly, an oxidized CoA dimer was crystalized in the active site, suggesting that a long, linear molecule—such as a polyamine—would be the physiological substrate (Fig. 1C) (106). Residue Y142 was determined to act as a general acid, and a water molecule was determined to act as a general base (106). PaiA (locus tag, Ta0374) from *Thermoplasma acidophilum* (*Ta*PaiA) also displays the GNAT architecture, though the loops between β-4/β-5 and β-6/β-7 are structurally different from the *Bs*PaiA structure (118). Like *Bs*PaiA, *Ta*PaiA acts as a monomer with a putative polyamine-binding cleft made up of residues highly conserved in PaiA enzymes (106, 118). Cocrystallization of *Ta*PaiA and CoA shows that the conserved Y138 is located close to the sulfur atom of CoA and may act as a general acid (118). In contrast to PaiA proteins, the spermidine *N*-acetyltransferase SpeG from *Vibrio cholerae* (*Vc*SpeG) acts as a dodecamer, formed by a dimer of hexamers that sit on top of each other (119). A monomer from one hexamer interacts with a corresponding monomer from the other hexamer to form the classic GNAT dimer (119). This oligomerization allows for an allosteric site that binds spermidine/spermine and that results in a conformational change that allows for AcCoA and polyamine binding (120). The *Vc*SpeG crystal shows that residue Y134 is located in close proximity to the sulfur atom of AcCoA and may act as a possible general acid (119).

Other SSATs include BltD, a SSAT that helps export polyamines via the multidrug Blt transporter (121). *Bacillus subtilis* BltD acetylates spermine and spermidine with a high affinity (K_m, $<$67 μM and 200 μM, respectively). BltD can also diacetylate spermine, using N^1-acetylspermine as a substrate (5). The spermidine *N*-acetyltransferase A (SnaA) from *Corynebacterium glutamicum* modifies a variety of polyamines, including spermidine, spermine, cadaverine, and putrescine (122). SnaA can use AcCoA, PrpCoA, and SucCoA, with a preference for AcCoA (122). Surprisingly, a *snaA* deletion did not cause a growth defect in *C. glutamicum* under the conditions tested, and the physiological role of polyamines in this organism is not well understood (122).

Phosphinothricin and Methionine Derivatives Inactivating GNATs

Streptomyces species produce the herbicide bialaphos, which is a tripeptide of two L-alanine residues and a glutamate mimic called phosphinothricin (PPT) (Fig. 6B) (123, 124). Once inside cells, the two alanine residues are cleaved by peptidases and release free PPT. As an analogue of glutamate, PPT inhibits glutamine synthetase and leads to ammonium toxicity in plants (125, 126). To protect themselves, *Streptomyces* species encode a PPT-modifying GNAT to acetylate and inactivate PPT (Fig. 6B) (123, 127, 128).

The first reported characterization of a PPT-modifying GNAT is the Bar (for bialaphos resistance) protein from *Streptomyces hygroscopicus* (123). Thompson et al. demonstrated that strains overexpressing the *bar* gene had 15-fold more acetylated PPT or demethyl-PPT than the vector control (123). The Bar protein of *Streptomyces viridochromogenes*, which is 73% identical to *S. hygroscopicus* Bar, also acetylates and detoxifies PPT (129).

The use of phosphinothricin acetyltransferases to make crops resistant to bialaphos, thereby allowing the antibiotic to be used selectively in the field, has received much attention in agriculture (128, 130). Phosphinothricin resistance was first introduced into plants (e.g., canola) in 1995, and since then, its use has been expanded to other crops (131). Recently, it was discovered that Bar can acetylate aminoadipate and tryptophan,

leading to *N*-acetyl-aminoadipate and *N*-acetyl-tryptophan accumulation in plants (132). To understand its mechanism, Bar with CoA and PPT was crystalized. The crystal structure shows that E88 and Y107 act as a general base and an acid, respectively (132). The structure also highlights residues that interact with PTT, including F36, G127, and V161, which stabilize the methylphosphoryl group, as well as K78, R80, and Y83, which hydrogen bond with the two phosphoryl oxygen molecules (132). Based on structural data, Christ et al. engineered Bar variants that target only PPT, highlighting the importance of enzymological studies, needed to improve biotechnologies (132).

Besides PPT, another potent inhibitor of glutamine synthetase is methionine sulfoximine (MSX) (125, 133). As with PPT, acetylation of the amine of MXS blocks its inhibitory effects (Fig. 6C). Many GNATs annotated as PPT-modifying GNATs have been shown to acetylate MSX or the structurally related compound methionine sulfone. The Bar protein of *S. hygroscopicus* can also acetylate MSX, although it does so with a 3-order-of-magnitude decreased efficiency relative to that of PPT (123). The PitA protein (encoded by PA4866) of *Pseudomonas aeruginosa* (*Pa*PitA), the PitA protein of *Acinetobacter baylyi* (*Ace*PitA; encoded by ACIAD1637), and MddA (methionine derivative detoxifier A, encoded by the gene formerly known as *yncA*) of *Salmonella enterica* have all been shown to acetylate MSX and methionine sulfone but not PPT (134–136). The structures of *Pa*PitA and *Ace*PitA both showed the GNAT α/β fold of β-sheets and α-helices and a V shape for AcCoA binding (135, 137). *Pa*PitA was cocrystalized with MSX in the active site, and R75 appears to hydrogen bond with the oxygen, while residues I30 and F77 help stabilize the methyl portion of MSX (134). In the *S. hygroscopicus* Pat and *S. viridochromogenes* Pat enzymes, I30 is replaced by an asparagine, which may be a reason for the different substrate specificity. In comparison to the apoenzyme, it appears that *Pa*PitA undergoes a conformational change upon binding the substrate (134). All MSX- and methionine sulfone-modifying GNATs act as dimers (134–136). In an effort to predict the substrate specificity of annotated PPT-modifying GNATs, one study used a combination of *in vivo*, *in vitro*, and bioinformatics approaches to determine that phylogeny is a key criterion for substrate specificity (PPT, MSX, or methionine sulfone) of Bar homologues (138). Some organisms contain two separate GNATs, with one for PPT and one for MSX. This is the case in *Pseudomonas putida*, where PhoN1 is a PPT-modifying GNAT and PhoN2 is an MSX-modifying GNAT (139). The physiological reason why some organisms have different numbers of PPT-like modifying enzymes is unclear.

Tabtoxin-Inactivating GNATs

As mentioned above, inhibition of glutamine synthetase leads to cell death via ammonium toxicity. Another inhibitor of glutamine synthetase is tabtoxin (140). This toxin is a dipeptide made up of a threonine linked by a peptide bond to a monocyclic β-lactam (tabtoxinine-β-lactam [TβL]) (141) (Fig. 6D). This toxin is produced by *Pseudomonas syringae*, and once inside cells, the cleavage of threonine releases the active TβL moiety, which inhibits glutamine synthetase and causes wildfire disease of tobacco plants (140, 142). To protect themselves, *P. syringae* carries a tabtoxin resistance (*ttr*) gene that encodes a GNAT to monoacetylate the α-amino group, which inactivates TβL (143–146). Tobacco plants expressing *ttr* were resistant to TβL and infection by *P. syringae* pv. *tabaci* (143). Ttr acetylates TβL but not the dipeptide tabtoxin (146).

The crystal structure of Ttr in complex with AcCoA shows typical GNAT folding with a V shape, facilitating AcCoA binding via 10 direct hydrogen bonds and 3 water-mediated hydrogen bonds (17). Ttr crystalized as a dimer, consistent with other GNATs (17). Residue Y141 is highly conserved among other crystalized GNATs, and the crystal structure shows a hydrogen bond between Y141 and the carbonyl of AcCoA (17). TβL interacts with a water molecule stabilized by residues E92 and D130, prompting the authors to suggest that these residues may act together to promote a water-mediated general base, while Y141 may be a general acid (17).

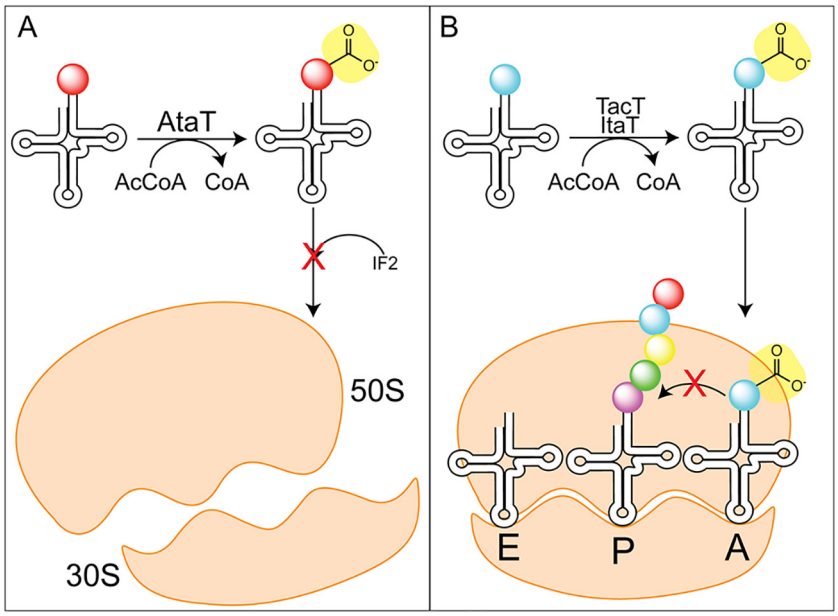

FIG 7 GNAT toxins acetylate tRNAs to halt translation. (A) AtaT acetylates the initiator tRNA, preventing binding to the initiation factor 2 (IF2), thus impairing initiation of translation. (B) TacT and ItaT acetylate charged tRNAs to prevent elongation.

GNATs AS TOXINS IN TA SYSTEMS

Overview of TA Systems

Toxin-antitoxin (TA) systems are a common mechanism for cells to cope with stress. TA systems are usually encoded by a two-gene operon; one gene encodes a toxin protein (~100 amino acids), and the other one encodes the antitoxin, which is a small protein (~80 amino acids) or regulatory antisense RNA that neutralizes the activity of the toxin, hence the name antitoxin (147). Usually the antitoxin is more labile than the toxin and degraded by specific proteases or RNases (147, 148). Thus, under stressful conditions, the antitoxin is degraded so as to let the toxin inhibit growth until conditions are more favorable. The role of TA systems in establishing a persister state has been extensively studied (148).

There are currently six types of TA systems, classified based on how the antitoxin neutralizes the toxin (148, 149). Below we discuss only type II systems because some of the described toxins contain a GNAT domain. Type II systems are comprised of a two-domain protein antitoxin in which one domain binds the cognate toxin protein, resulting in the inhibition of toxin activity, and the second domain binds DNA (148). Usually, the antitoxin acts as a repressor for the TA operon, with the toxin acting as a corepressor (147, 148). Recently, a TA system with a toxin with a GNAT fold was reported (150, 151). Since then, more GNAT toxins with a ribbon-helix-helix fold antitoxin have been reported, establishing a new subfamily of type II TA systems (149, 152).

Toxin GNATs Inhibit Translation

Toxins with a GNAT domain acetylate charged tRNAs and halt translation (152). AtaT (aminoacyl tRNA acetyltransferase; locus tag, Z4832) of *E. coli* O157:H7 specifically acetylates the initiator methionine-tRNA^{fMet}, blocking the interaction with initiation factor II and thus halting translation initiation (Fig. 7A) (153, 154). ItaT (locus tag, EcHs_A0501) from *E. coli* acetylates isoleucine-tRNA^{Ile} specifically, blocking elongation (155). In contrast to the highly specific AtaT and ItaT, TacT (tRNA-acetylating toxin; locus tag, STM3651) from *Salmonella enterica* acetylates amino acids on charged tRNAs, thereby inhibiting elongation (Fig. 7B) (156). Two other GNAT toxins were found in *S. enterica* and were named TacT2 and TacT3 (157). All TacT-like toxins acetylate a variety

of charged tRNAs with a preference for glycine and isoleucine/leucine (157). Another group reported that TacT acetylates its cognate antitoxin (TacA) as well, thereby increasing the activity of TacT and leading to decreased protein synthesis (158). The authors report that this is the first instance of a GNAT acetylating both a protein and a small molecule (158), and more work is needed to understand how the acetylation of both small-molecule and protein targets affects persistence.

The TacT2 proteins from two *Salmonella enterica* serovars, *Salmonella enterica* serovars Typhimurium and Enteritidis, show differences in toxicity and persister state formation, as well as the profile of acetylated charged tRNAs (157). Comparing the sequences, there is a single substitution (E29K) that reverses the charge of the residue at this position (157). The lysyl residue is near the active site and appears to hydrogen bond with neighboring residues F17 and Y15, helping make the active site more accessible to the substrate and thus helping make the protein more potent as a toxin (157). The TacT structure and mutational analysis demonstrate that the active site is a positively charged patch at the dimer interface, and the above-mentioned stabilization bonds are present in the TacT structure (156); this groove is necessary for binding a tRNA molecule (156). The structure of TacT3 and the modeled structure of TacT2 also reveal a groove of positively charged residues that, when substituted, resulted in a lack of toxicity (157). However, the orientation of AcCoA is different between the TacT3 structure and the TacT structure (156, 157). In TacT3, the bulky W142 prevents full engagement with the adenine base in the binding pocket, while residue G145 outside the binding pocket hydrogen bonds with the base for stabilization (157).

The TacT structure shows a conserved tyrosine, residue Y140, coordinating an oxygen molecule of AcCoA to allow for correct positioning. The Y140F variant is inactive (156). When the corresponding residue of AtaT (Y144) was replaced by a phenylalanine, this variant was also inactive (159). As with TacT-type toxins, AtaT was shown to have a positive patch formed by the dimers that interacts with methionine-tRNA^{fMet} (160). The antitoxin AtaR binds AtaT in a heterohexameric complex (AtaT-AtaT$_2$-AtaR$_2$-AtaT), trapping it from its toxic form as a dimer (160). The GNAT domain-containing toxin KacT (locus tag, KPHS_05890) of *Klebsiella pneumoniae* is also neutralized by forming a heterohexamer with its cognate ribbon-helix-helix antitoxin, KacA (161). The transcription of *kacT* increased significantly when *K. pneumoniae* bacteria were challenged with the antibiotic meropenem (10-fold increase) or tigecycline (40-fold increase), suggesting that this TA system is used for entering into a persister state during antibiotic stress (161). The crystal structure of KacT complexed with AcCoA displays typical GNAT folding and has a basic region like TacT does, suggesting that it may bind tRNAs (161). The sulfur of CoA is within hydrogen bonding distance of residue Y145 and is hypothesized by the authors to act as a general acid (161). Though having low sequence homology (~20% to 30%), these similarities in structure and activity suggest a common mechanism for GNATs in tRNA acetylation and TA systems in general.

The TA systems that involve a GNAT may have additional physiological roles other than triggering the persistent state. In *Shigella flexneri*, GmvT (GNAT maintenance of virulence toxin) halts protein synthesis in an AcCoA-dependent manner, possibly via tRNA acetylation (162). The activity of GmvT is sufficient for virulence plasmid stability at environmental temperatures (162). Bioinformatics studies have annotated other GNAT-dependent TA systems with unknown roles (163–166). In *Acinetobacter baumannii*, a TA system is comprised of a helix-turn-helix toxin protein and a GNAT antitoxin, thus named CheTA (switched-element toxin-antitoxin system) because it is the opposite of other TA systems with a GNAT toxin (167). Thus, validation will be needed to determine the common mechanisms and structures of these GNAT-dependent TA systems.

GNATs INVOLVED IN ANABOLISM

Besides inactivating toxic compounds, GNATs have roles in the synthesis of a variety of compounds. Acetylation of biosynthetic intermediates results in molecules central to

many necessary cellular processes, including protein synthesis, antigen production, flagellar modifications, and cell wall synthesis.

TmcA-Dependent Acetylation for tRNA Synthesis

GNATs found in toxin-antitoxin (TA) systems have been shown to acetylate charged tRNAs in order to block translation. In contrast, TmcA (tRNA^{Met} cytidine acetyltransferase) acetylates the wobble base of tRNA^{Met} (6). First identified in 1972 by Oashi et al., N^4-acetylcytidine (ac^{4}C) is a modified nucleoside found in a variety of tRNAs and rRNAs (168). This modification of position 34 of tRNA^{Met} has been shown to prevent misreading of the isoleucine AUA codon in favor of the correct methionine AUG codon (169).

E. coli strains lacking *tmcA* did not produce ac^{4}C, though no phenotype was observed for this strain, while in *Saccharomyces cerevisiae* yeast, the *tmcA* homologue is essential (6, 170). *In vitro* assays show that ac^{4}C formation is dependent on TmcA, ATP/GTP, and AcCoA (6). These data show that ATP/GTP hydrolysis is important for acetylation and that AcCoA binding stimulates hydrolysis and tRNA binding (6, 171). The structure of the *E. coli* TmcA shows an N-terminal ATP-binding RNA helicase-like domain and a C-terminal GNAT domain. The C terminus is composed of helices that form a positively charged groove that may facilitate tRNA binding (171). Mutational analysis demonstrates that the anticodon stem of tRNA^{Met} is important for TmcA recognition (6). The crystal was solved to be complexed with AcCoA and ADP, with AcCoA binding occurring in the traditional V-shaped β-bulge in the middle of the structure (171). The two domains interact, though surprisingly, the two active sites are ~30 Å apart (171). The authors suggest that the RNA helicase activity is needed to remodel the tRNA molecule to allow the C-34 amino group to make a direct nucleophilic attack since no residues corresponding to a general acid or base were observed (171). These data highlight how, in different contexts, GNATs can positively or negatively regulate translation.

MshD-Dependent Acetylation for MSH Synthesis

Mycothiol (MSH) is a redox-active molecule that is found only in actinomycetes (172). MSH appears to act analogously to glutathione; that is, MSH is needed to maintain the redox balance of the cell (173); actinomycetes do not synthesize glutathione. Mycobacteria produce high levels of MSH, and *M. smegmatis* strains lacking MSH become more sensitive to alkylating agents, oxidizing agents, and antibiotics (172, 174). The final step in MSH biosynthesis involves the acetylation of the amino group of cysteine present in 1-D-*myo*-inosityl-2-L-cysteinyl-amido-2-deoxy-α-D-glucopyranoside by the GNAT mycothiol synthase MshD (Fig. 8A) (175). An *M. tuberculosis mshD* strain displays decreased survival in the macrophage and higher sensitivity than the wild type to hydrogen peroxide (176, 177). These data highlight the importance of MSH for actinomycetes, potentially making MSH biosynthesis a good drug target.

To understand the mechanism of MshD, the protein from *M. tuberculosis* was crystalized both in complex with AcCoA and in complex with CoA and desacetylmycothiol (Fig. 1D) (178, 179). Both structures showed that MshD acts as a monomer that contains two GNAT domains linked by a random coil (178, 179). This double GNAT domain is similar to that of *Staphylococcus aureus* FemA and possibly resulted from gene duplication (175, 180). Each GNAT domain can bind AcCoA via the pyrophosphate binding loop between β-4 and β-5, though the authors propose that the C-terminal domain is active, while the N-terminal domain is a structural element because AcCoA is not positioned correctly to donate an acetyl group (178). Desacetylmycothiol binds in the central region between the GNAT domains and interacts with both domains, while only the C-terminal GNAT domain transfers an acetyl moiety (179). The N-terminal GNAT domain retains an AcCoA molecule, confirming its lack of catalytic ability (179). The authors suggest that residues E234 and Y294 may act as a general base and acid, respectively (179). Comparison of the binary and ternary complex shows a substantial movement of the N-terminal domain toward the C-terminal domain upon binding of the substrate (179).

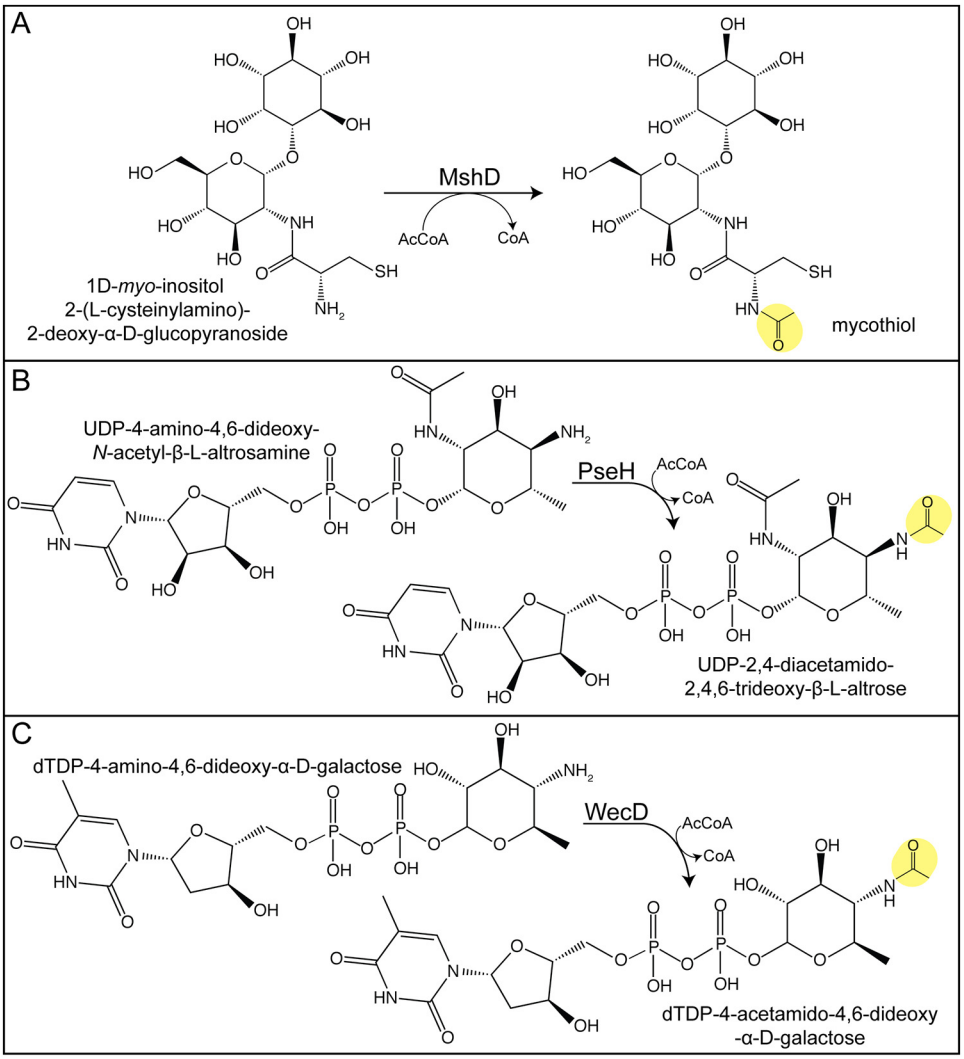

FIG 8 Acetylation involved in anabolism. (A) MshD acetylates the final step in mycothiol synthesis. (B) PseH-dependent acetylation is involved in pseudaminic acid production. (C) WecD acetylates intermediates in the enterobacterial common antigen (EAC) biosynthetic pathway. Acetyl moieties are highlighted in yellow.

CBG-Dependent Acetylation for CA Synthesis

Clavulanic acid (CA) is a secondary metabolite produced by *Streptomyces clavuligerus* that inhibits β-lactamases by binding irreversibly to an active-site serine (181). Because of the unique chemistry required to produce CA and its acylated versions, understanding the enzymes involved in its biosynthesis is of interest. The CA biosynthesis GNAT (CBG) is predicted to be involved in the synthesis of CA, since *orf14* of *S. clavuligerus* produced notably less CA, though the specific role of CBG in CA synthesis is still unknown (182, 183).

The structure and gel filtration of CBG show that it is mainly a monomer containing two tandem GNAT domains linked by a β-strand, similar to the structure of the MshD and Fem proteins (178, 180, 184). While AcCoA was bound to the N-terminal GNAT domain in the crystal, the authors suggest that this may be a structural domain since the AcCoA is buried and not accessible for acetyl transfer (182). The C-terminal GNAT domain is most likely responsible for acyl transfer, containing a hydrophobic pocket that may accommodate other CoA analogs (182). Other acyl-CoAs (palmitoyl-CoA, lauroyl-CoA, myristoyl-CoA, succinyl-CoA) bind CBG, indicating that this enzyme may use other acyl-CoAs as substrates.

PseH-Dependent Acetylation for Pseudaminic Acid Synthesis

Pseudaminic acid (5,7-diacetamido-3,5,7,9-tetradeoxy-L-*glycero*-α-L-*manno*-nonulosonic acid) is used by some bacteria to modify their flagella (185). In the biosynthetic pathway, PseH acetylates UDP-4-amino-4,6-dideoxy-β-L-AltNAc to produce UDP-2,4-diacetamido-2,4,6-trideoxy-β-L-altropyranose using AcCoA as the acetyl donor (Fig. 8B) (185). Glycosylation is essential for flagellum synthesis and motility in *Helicobacter pylori* and *Campylobacter jejuni*, and *pseH* mutants are nonmotile (186, 187). Because pseudaminic acid production is unique to bacteria and acts as a flagellum decoration in pathogenic bacteria, this pathway is a good target for antimicrobial agents (185). The importance of glycosylation for virulence has been shown, thus providing an understanding of the mechanism of PseH that may make it a good therapeutic target (187, 188).

H. pylori PseH (*Hp*PseH) in complex with AcCoA shows the typical GNAT domain with AcCoA binding between the splayed β-4 and β-5 sheets, though *Hp*PseH has an additional C-terminal helix (α-5) in comparison to the structures of other GNATs (189, 190). The proximity of AcCoA to a conserved tyrosine, Y138, suggests that this residue may be a general acid, though no obvious residues appear to act as a general base (190). The acetyl group is located at the bottom of an active-site pocket made up of polar and aromatic residues (190). Modeling of UDP-4-amino-4,6-dideoxy-β-L-AltNAc in the pocket shows extensive interactions with the substrate and places the uracil ring between R30 and F52, allowing for π-stacking interactions (190). The authors suggest that a water molecule hydrogen bonded to S78 and T80 acts as a general base to deprotonate the 4-amino group for the nucleophilic attack (190). The dimer interface is composed of a β-sheet from each monomer to form a continuous β-sheet (190). In contrast, PseH from *C. jejuni* (*Cj*PseH) acts as a monomer, though it also shows typical GNAT folding with a splayed AcCoA binding pocket (191). In *Cj*PseH, residue Y128 is proposed to be the general acid because it is close to the sulfur atom of AcCoA (~4.4 Å) and D70 may be a remote base in a mechanism similar to that described for *Hp*PseH (191).

WecD-Dependent Acetylation for Antigen Synthesis

The outer membrane glycolipid enterobacterial common antigen (ECA) is found in many *Enterobacteriaceae*. The final step of synthesis involves acetylation of the dTDP-4-amino-4,6-dideoxy-α-D-galactose to TDP-4-acetamido-4,6-dideoxy-D-galactose by the TDP-fucosamine acetyltransferase WecD (Fig. 8C) (192, 193). ECA contributes to maintaining resistance to bile salts and thus plays an important role in virulence (194). *Salmonella enterica wecD* mutants have decreased virulence in a mouse model, highlighting the importance of the acetylation of this glycolipid (194). ECA may also be involved in biofilm formation, since an ECA-deficient strain of nontypeable *Haemophilus influenzae* produced smaller biofilms (195), and transcriptomics analysis of *Actinobacillus pleuropneumoniae* shows that *wecD* expression is upregulated in a biofilm (196). The production of ECA is also necessary for flagellar biosynthesis and motility in *Serratia marcescens* (197).

The crystal structure of *E. coli* WecD (*Ec*WecD) with AcCoA has been solved (193). *Ec*WecD acts as a dimer both in gel filtration chromatography experiments and in the crystal (193). *Ec*WecD lacks 43 N-terminal residues compared to the sequences of other GNATs, thus lacking two α-helices and two β-strands (193). Like PseH, WecD acetylates the 4-amino group of a nucleotide-linked sugar, and the two crystals are similar, though *Ec*WecD has an extra 70 amino acids at the N terminus compared to the sequence of *Hp*PseH (190, 193). Residue Y208 is positioned near (~3.0 Å) the sulfur atom of AcCoA and may act as a general acid, while residue E68 may be a remote base via a water-mediated deprotonation (193).

Aminoacyl-transferases Use Aminoacyl-tRNA as an Acyl Donor

Some GNATs can use an aminoacyl-tRNA as a donor instead of AcCoA. In these cases, the GNAT transfers the aminoacyl group to the substrate molecule. Aminoacyl-

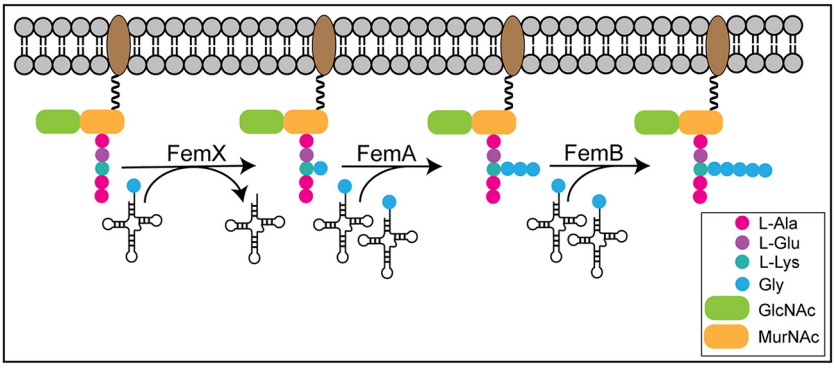

FIG 9 Fem protein aminoacyl peptide cross-bridges in cell wall synthesis. Fem aminoacyl-transferase enzymes transfer an aminoacyl group from aminoacyl-tRNA to create a peptide cross-link. In *Staphylococcus aureus*, FemX transfers the first glycine residue, FemA transfers the second and third glycine residues, and FemB transfers the fourth and fifth glycine residues.

transferases are involved in the synthesis of various bacterial structures, including the cell wall, cell membranes, and secondary metabolites.

Fem aminoacyl-transferases are involved in cell wall synthesis. Peptidoglycan is made up of repeating units of *N*-acetylglucosamine (GlcNAc) and *N*-acetyl muramic acid (MurNAc) glycans with pentapeptide stems cross-linked by peptide bridges (198–200). In some bacteria, the FemABX aminoacyl-transferase enzymes are responsible for synthesis of the peptide cross-bridges, transferring an aminoacyl group from aminoacyl-tRNA to the growing peptide cross-link (Fig. 9) (7). In bacteria with Fem proteins, the pentapeptide stem (L-Ala)-(D-Glu)-X-(D-Ala)-(D-Ala) is modified on the X residue, which can be lysine, ornithine, or *meso*-diaminopimelic acid (DAP) (7). These cross-links are important for cell structure and antibiotic resistance, with Fem proteins first being recognized for their importance for methicillin resistance, hence the name factors essential for the expression of methicillin resistance (200, 201). Fem proteins from different species form different peptide cross-links (7, 202). Fem proteins have been reported in a variety of organisms, including *Staphylococcus*, *Streptococcus*, and *Enterococcus* species (203–207). Bioinformatics studies indicate the presence of Fem proteins in diverse bacteria, including *Borrelia burgdorferi*, *Streptomyces coelicolor*, *Streptomyces toyocaensis*, and *Clostridium perfringens*, though experimental validation is needed (202, 208). Thus, the ability to aminoacylate the peptide cross-link is a conserved feature important for overall cell viability.

Streptococcus pneumoniae produces a dipeptide cross-bridge of L-Ser–L-Ala or L-Ala–L-Ala (202). MurM in *S. pneumoniae* transfers an alanine or a serine to the lysine of the peptide stem (205, 209, 210). MurN then adds the second residue (alanine), showing a greater than 10-fold higher catalytic efficiency for the L-Ala cross-link than for the L-Ser cross-link (206, 209). In *Enterococcus faecalis*, BppA1 adds the first alanine and BppA2 transfers the second alanine to form the dipeptide cross-link of L-Ala–L-Ala (207, 211).

Staphylococcus aureus produces pentaglycine cross-links that connect L-Lys (position 3) to a neighboring D-Ala (position 4) and that have been shown to be vital for cell integrity (212). FemX (also called FmhB) is essential and adds the first glycine residue of the cross-link (203). FemAB were found to add the next set of residues, with FemA adding the second and third glycine residues and FemB adding the last two (213–216). Though the essentiality of *femAB* in *S. aureus* is in question (212), *femAB* mutants were more susceptible to antibiotics than the wild type (214, 215, 217). The crystal structure of FemA from *S. aureus* is a monomer that adopts a globular fold made up of two GNAT domains with an L-shaped channel in the second GNAT domain, similar to the structure of CBG and MshD (180). The authors suggest that this channel is the binding location of the disaccharide hexapeptide substrate. Additionally, two antiparallel helices extend out, creating a coiled-coil domain similar to the coiled-coil domains found in seryl-tRNA synthetases, suggesting that this domain binds tRNA (180, 218).

Instead of the pentaglycine cross-link used by *S. aureus*, *Weissella viridescens* (also known as *Lactobacillus viridescens*) uses an L-Ala–L-Ser cross-link (202). *W. viridescens* FemX (*Wv*FemX) was shown to transfer an amino acid (alanine, serine, or glycine) to a lysine residue of the UDP-MurNAc pentapeptide precursor (204). Kinetic analysis shows that *Wv*FemX uses an ordered sequential binding mechanism (18). Mutational analysis suggests that D109 acts as a general base, while E320 is important for overall catalysis (18). The structure shows that *Wv*FemX contains two GNAT domains separated by a deep cleft (~15 Å deep and ~20 Å wide) (184). Unlike *S. aureus* FemA (*Sa*FemA), *Wv*FemX does not contain a coil-coil region (180, 184). The UDP-MurNAc pentapeptide binds in the cleft, making contact with domain 1 mostly, with residues Lys36, Arg211, Tyr215, and Tyr256 being shown to be important for substrate binding (184, 219). The structure of *Wv*FemX in complex with a peptidoglycan analogue and an RNA molecule mimicking Ala-tRNA^{Ala} shows that peptidyl-tRNA binding is also found in this cleft (220). The tRNA makes contact with a long channel spanning domain 2, interacting with Ile208 and Leu301 (220). Mutational analysis indicates that Lys305 is important for stabilizing the negative charge of L-Ala after the nucleophilic attack of Ala-tRNA^{Ala}, while Phe304 is important for π-stacking with the substrate (220).

Because peptide cross-links are important for cell viability and antibiotic resistance in a variety of bacteria, Fem proteins are a good target for antimicrobial agents. Studies showing the specificity of Fem proteins for their tRNA and lipid substrates have been reported (210, 221). Villet et al. demonstrate that posttranscriptional modifications and recognition of the acceptor stem of the tRNA are important criteria for the recognition by Fem proteins (222). Mutational analysis of *S. pneumoniae* MurM chimeras with substitutions in a 30-amino-acid region in the coil-coil domain demonstrates that this region determines residue specificity (Ala versus Ser) (223). Analogues of aminoacyl-tRNA substrates that inhibit Fem proteins have been reported (224–226). More work is needed to understand the mechanisms and specificity of Fem proteins in order for these proteins to be a possible future drug target.

Aminoacyl-phosphatidylglycerol synthases are involved in cell membrane synthesis. Besides peptide stems, phospholipids can be aminoacylated. The addition of an amino acid reduces the overall negative charge of the bacterial membrane to help cells cope with cationic antimicrobial agents released by the immune system (227). A recent review of lipid aminoacylation can be found in reference 228. MprF (multiple-peptide resistance factor) and its homologue, LysX, transfer a lysyl residue from Lys-tRNA^{Lys} onto phosphatidylglycerol (229–231). *Staphylococcus aureus*, *Listeria monocytogenes*, and *Mycobacterium tuberculosis* cells lacking *mprF* or *lysX* were more sensitive to antimicrobial agents than the wild type and had attenuated virulence in mouse models (230–232). These aminoacyl-phosphatidylglycerol synthases are bifunctional enzymes composed of a GNAT cytosolic C-terminal domain (to modify the lipid) and an integral membrane N-terminal domain (to flip the lipids into the periplasm) (228).

The structures of the GNAT domains of *Pseudomonas aeruginosa* Ala-tRNA^{Ala}-dependent alanyl-phosphatidylglycerol synthase (*Pa*A-PGS; PA0920) and *Bacillus licheniformis* Lys-tRNA^{Lys}-dependent lysyl-phosphatidylglycerol synthase (L-PGS; *yfix*) have been solved (233). The structures show that the C-terminal domain is made up of a tandem GNAT fold, similar to that of *S. aureus* FemA (*Sa*FemA) and *Weissella viridescens* FemX (180, 184, 233). Like *Wv*FemX, the crystal structure and mutational analysis of *Pa*A-PGS demonstrate that residues Lys840 and Phe839 (corresponding to Lys305 and Phe304 of *Wv*FemX) are important for binding tRNA (184, 233). The crystal structure of *Pa*A-PGS shows that only the aminoacyl moiety is in direct contact with the main-chain atoms of the enzyme, consistent with work with MprF paralogs from *Clostridium perfringens* that indicates that the aminoacyl moiety is the main determinant for aminoacyl-tRNA specificity (233, 234). Docking and site-directed mutagenesis experiments show that a tunnel connected to but opposite the aminoacyl-tRNA binding pocket may act as a binding site for the lipid substrate in the membrane, with residues D765 and R768 being important for catalysis (233).

LFTs tag proteins for degradation. The leucyl/phenylalanyl-tRNA protein transferase (LFT) also shows high homology to Fem proteins, with both enzymes transferring a residue from their cognate aminoacyl-tRNA. These proteins transfer a leucine or a phenylalanine residue to the N-terminal arginyl or lysyl residue of a protein (235). This N-end rule modification marks the protein for degradation (236). The structure of *E. coli* LFT shows that it contains a C-terminal GNAT domain, similar to that of *Staphylococcus aureus* FemA and *Weissella viridescens* FemX, while the N-terminal domain is not similar to a GNAT domain (180, 184, 235). The N-terminal domain appears to be important for stabilizing the aminoacyl-tRNA substrate via π-stacking with residue W49 (235). Site-directed mutagenesis demonstrates that the C-terminal GNAT domain is important for recognizing the correct aminoacyl-tRNA via a hydrophobic pocket that recognizes the aminoacyl moiety (235). The authors suggest that residues in the GNAT domain (E156 and Q188) are also important for orienting the substrate protein in close proximity of the aminoacyl-tRNA, promoting aminoacyl transfer (235).

GNAT aminoacyl-transferases are involved in antibiotic synthesis in *Streptomyces*. Many *Streptomyces* species produce a variety of antibiotics. The synthesis of some of these antibiotics involves a tRNA-dependent GNAT aminoacyl-transferase (228). VlmA from *Streptomyces viridifaciens* transfers serine from Ser-tRNA^{Ser} in the biosynthesis of valanimycin (237). In *Streptomyces coeruleorubidus*, PacB transfers alanine from Ala-tRNA^{Ala} in the production of pacidamycin, which inhibits cell wall synthesis (238). The antibiotic dehydrophos is produced by *Streptomyces luridus* and inhibits pyruvate dehydrogenase (239). DhpH and DhpK use Leu-tRNA^{Leu} and Gly-tRNA^{Gly}, respectively, to synthesize the tripeptide dehydrophos (240). FzmI uses Val-tRNA^{Val} to synthesize fosfazinomycin (241). In *Streptomyces luteocolor*, the protein encoded by *orf11* transfers glycine from Gly-tRNA^{Gly} to produce the streptothricin-like antibiotic BD-12, which has a glycine side chain instead of a β-lysine (Fig. 5A). This is the first reported instance of a tRNA-dependent peptide bond-forming enzyme capable of using an amino sugar as a substrate (242).

SMALL-MOLECULE ACETYLATION-DEPENDENT TRANSCRIPTION

Modulation of transcription is another way in which acetylation regulates bacterial cellular processes. Acetylation was first discovered on eukaryotic histones as a means to modulate transcriptionally active areas of the genome. In bacteria, direct acetylation of lysine residues of transcription factors has been shown to interfere with DNA binding. In *E. coli*, the GNAT Pat acetylates RcsB on residue K180 in the DNA-binding helix-turn-helix motif (243). Acetylation inhibits RcsB binding to the *flhDC* promoter, thereby modulating RcsB-dependent gene expression (243). Since this initial report, other acetylated bacterial transcription factors have been reported, such as RutR, RpsD, McbR, HilD, GntR, and RprY (243–246). Besides direct acetylation of transcription factors, acetylation of small molecules has been shown to modulate transcription.

BadL Acetylation of Aminobenzoates Regulates the Benzoate Degradation Operon via BadM

In *Rhodopseudomonas palustris*, the GNAT BadL acetylates the amino group of aminobenzoates, which bind to the repressor BadM (247). This BadM/acetylated aminobenzoate complex can no longer repress transcription of the benzoate degradation operon *badDEFGAB*, allowing the bacterium to catabolize benzoate (247). Acetylated aminobenzoates were also shown to be involved in the transcriptional control of light-harvesting complexes I and II in this purple nonsulfur photosynthetic bacterium (247). BadL is suggested to sense aminobenzoates in the environment to connect carbon metabolism and proton motor force generation via photosynthesis (247).

OatA Acetylation of *O*-Acetylserine Regulates Cysteine Biosynthesis Genes

In *S. enterica*, the GNAT OatA (OAS acetyltransferase A; formerly YjgM) acetylates the N$^{\alpha}$-amino group of *O*-acetylserine (OAS) to produce *N*,*O*-diacetylserine (DAS) (248). OatA is a monomer that acetylates OAS and not *N*-acetylserine, indicating that it is an

N-acetyltransferase (248). Overexpression of *oatA* resulted in increased cysteine biosynthesis gene expression, leading to shorter lag times in sulfate-limiting medium (248). Critically, the authors suggested that DAS, and not OAS, is the physiological signal that alters cysteine regulator CysB DNA binding to its regulon, though the addition of DAS does not alter binding *in vitro* (248). Possibly under physiological conditions, DAS may bind to CysB to activate the cysteine regulon, or the positive effect of DAS on the *cys* regulon is indirect (248). A GNAT from *Clostridium difficile* 630 (CD1211) has also been shown to acetylate OAS (82). Kinetic characterization of the CD1211 enzyme showed that the enzyme acetylated OAS, L-Ser, L-Thr, and L-Met with similar catalytic efficiencies and displayed positive cooperativity with AcCoA in the presence of each of the four substrates (249). The physiological role of this GNAT, possibly in cysteine biosynthesis, remains to be answered.

CONCLUSIONS

As schematized in Fig. 10, GNATs affect many vital cellular processes, ranging from detoxification and degradation to the synthesis of proteins or cell wall components. The plethora of substrates modified by the same GNAT fold displays the high versatility of this protein family.

Characterization and Validation of Physiologically Relevant Substrates of GNATs

While the physiological role of many GNATs has been identified, many predicted GNATs have yet to be studied or validated to be bona fide GNATs. Currently, bioinformatic annotation is the main mechanism for the identification and classification of GNATs. There are more than 300,000 proteins that belong to this family (11), suggesting that there are many novel GNATs that are waiting to be discovered. This review focused mainly on GNATs that catalyze the transfer of acetyl moieties onto small molecules, but the transfer of other acyl moieties from acyl-CoA molecules should be explored in the future. In *S. aureus*, the GNAT AcuA (*Sa*AcuA) is the first reported case of a bacterial GNAT that can succinylate a protein target (250). *Sa*AcuA can also acetylate or propionylate, using acetyl-CoA or propionyl-CoA, respectively, suggesting that the aforementioned GNATs in this review may be capable of using other acyl donors. There is a great need for more *in vitro* and *in vivo* studies with predicted GNATs to validate their role as acetyl/acyltransferases.

With the great number of predicted GNATs, more work is also needed to elucidate the substrates of various GNATs. High-throughput studies have found possible substrates (82), but validation of each enzyme for those substrates is needed, as is a wider variety of substrates to be tested. Crystallization studies of annotated GNATs have given more structural information (249, 251), but the physiological role of these proteins must be explored *in vivo*.

Interestingly, many amino acids have been reported to be acetylated (82). *Staphylococcus aureus* SACOL1063 acetylates L-threonine and L-tryptophan, though it acetylates L-Trp with an order-of-magnitude lower catalytic efficiency than it does L-Thr (249). The Bar GNATs have been reported to acetylate tryptophan, in addition to phosphinothricin (132). However, the physiological role of amino acid acetylation by these proteins and others has not yet been determined and should be a focus of future study.

GNATs as Antimicrobial Targets

Acetylation of antibiotics is a major source of antibiotic resistance in bacteria. With the worldwide increase in the incidence of antibiotic-resistant bacteria, elucidating the molecular mechanisms of antibiotic-acetylating proteins can aid the development of new drugs and therapies. The crystal structures of medically relevant GNATs that have modified or expanded substrate specificity can help elucidate the structural reasons for the change in substrate range. The crystal structure of AAC(6′)-Ib$_{11}$ highlighted how a few residue substitutions induced structural changes that led to a larger active site for larger aminoglycosides to bind (66, 67). The crystallization of other AAC(6′)-Ib

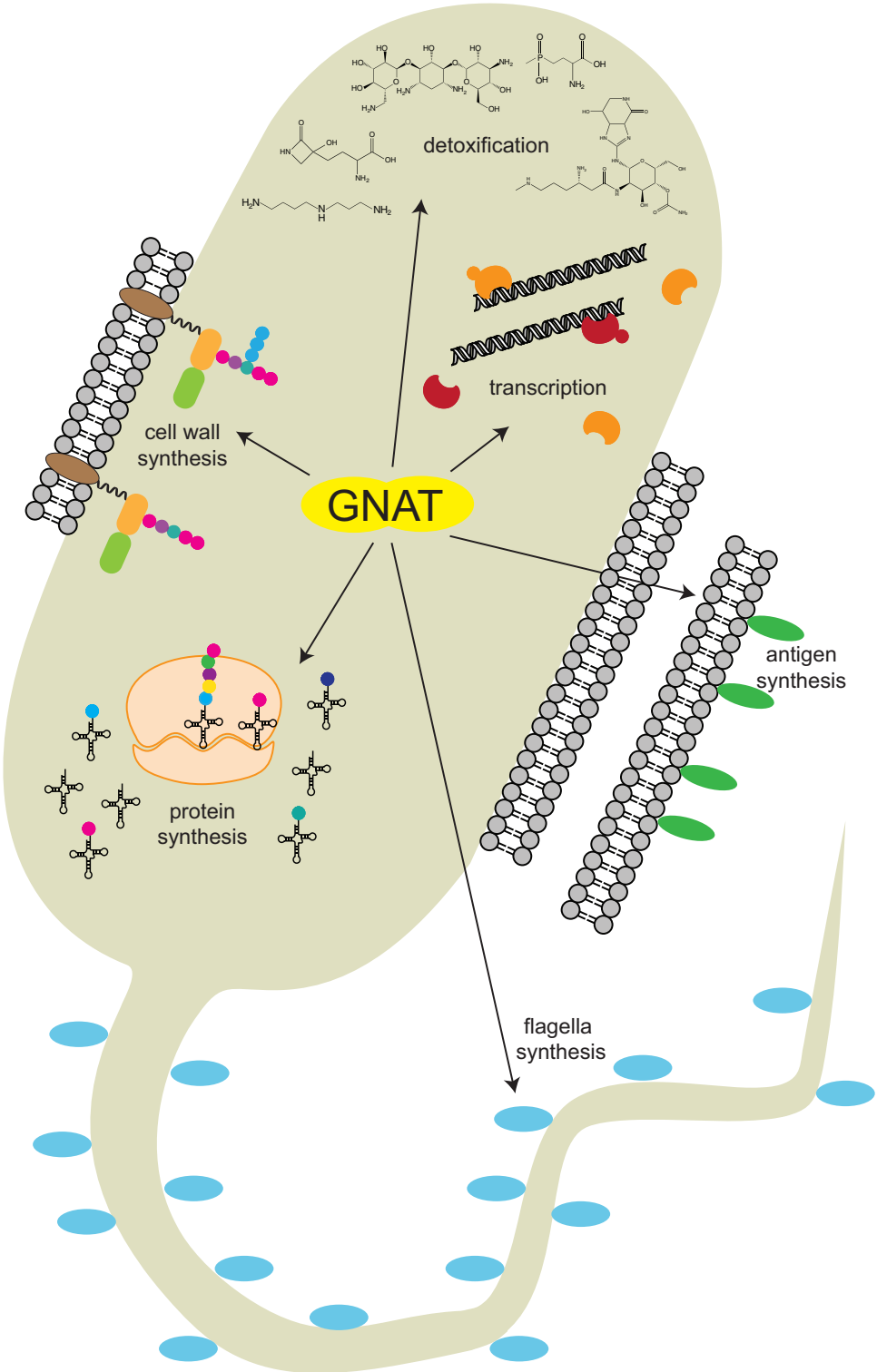

FIG 10 GNAT-dependent acylation modulates diverse cellular processes.

N-terminus variants may lead to a better understanding of how bacterial GNATs adapt to the human use of antibiotics. These structural data may also aid in the development of GNAT inhibitors that can bind and block the active site. It is hoped that these inhibitors can be used in combination with aminoglycoside antibiotics clinically in the future (80).

Since GNATs are conserved in all domains of life, understanding the wide variety of acylated substrates can foster the identification of targets in various other organisms. Acetylation of numerous small molecules modulates many cellular processes specific to bacteria. Thus, GNATs may serve as good therapeutic targets, and recent work has focused on inhibitors of GNATs (80, 226). For example, mycobacteria exclusively use mycothiol as a redox-balancing molecule, and mycothiol synthesis is dependent on the GNAT MshD (175). An *M. tuberculosis mshD* strain displays decreased survival in the macrophage and higher sensitivity to hydrogen peroxide, suggesting that future work should explore exploiting MshD as a drug target (176, 177). Research understanding the wide variety of acylated substrates through genetic, biochemical, and structural means will facilitate a better comprehension and appreciation of the physiological impact of acetylation in all domains of life.

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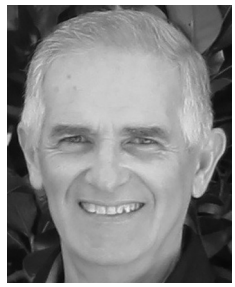

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