Riboswitches: Discovery of Drugs That Target Bacterial Gene-Regulatory RNAs

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CONSPECTUS

Riboswitches are messenger RNA (mRNA) domains that regulate gene function in response to the intracellular concentration of a variety of metabolites and second messengers. They control essential genes in many pathogenic bacteria, thus representing an inviting new class of biomolecular target for the development of antibiotics and chemical—biological tools. In this Account, we briefly review the discovery of riboswitches in the first years of the 21st century and their ensuing characterization over the past decade. We then discuss the progress achieved so far in using riboswitches as a focus for drug discovery, considering both the value of past serendipity and the particular challenges that confront current researchers.

Five mechanisms of gene regulation have been determined for riboswitches. Most bacterial riboswitches modulate either transcription termination or translation initiation in response to ligand binding. All known examples of eukaryotic riboswitches, and some bacterial riboswitches, control gene expression by alternative splicing. The *glmS* riboswitch, which is widespread in Gram-positive bacteria, is a catalytic RNA activated by ligand binding: its self-cleavage destabilizes the mRNA of which it is part. Finally, one example of a trans-acting riboswitch is known.

Three-dimensional structures have been determined for representatives of 13 structurally distinct riboswitch classes, providing atomic-level insight into their mechanisms of ligand recognition. While cellular and viral RNAs have attracted widespread interest as potential drug targets, riboswitches show special promise due to the diversity of small-molecule recognition strategies that are on display in their ligand-binding pockets. Moreover, riboswitches have evolved to recognize small-molecule ligands, which is unique among known structured RNA domains.

Structural and biochemical advances in the study of riboswitches provide an impetus for the development of methods for the discovery of novel riboswitch activators and inhibitors. Recent rational drug design efforts focused on select riboswitch classes have yielded a small number of candidate antibiotic compounds, including one active in a mouse model of *Staphylococcus aureus* infection. The development of high-throughput methods suitable for riboswitch-specific drug discovery is ongoing. A fragment-based screening approach employing equilibrium dialysis that may be generically useful has demonstrated early success. Riboswitch-mediated gene regulation is widely employed by bacteria; however, only the thiamine pyrophosphate-responsive riboswitch has thus far been found in eukaryotes. Thus, riboswitches are particularly attractive as targets for antibacterials. Indeed, antimicrobials with previously unknown mechanisms have been found to function by binding riboswitches and causing aberrant gene expression.
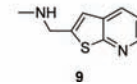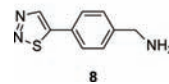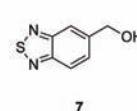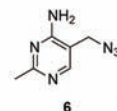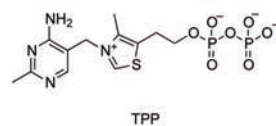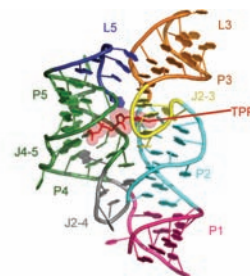
1. RNA as a Drug Target

Although RNA might appear to lack chemical diversity and thus not be a promising drug target, closer inspection reveals that it has many attractive characteristics. Like proteins, RNA can fold into intricate 3D structures with pockets and cavities that have the potential to bind ligands specifically.[1] Many RNAs undergo extensive structural rearrangement upon associating with small molecules, RNAs,

TABLE 1. Riboswitches with Available Structural Information

class	ligands	bound structures[a] [X,N,S]	free structures [X,N,S][a]	fold[b]	expression platform structural info [X,N,S][a]	refs
cyclic diguanylate (c-di-GMP)	c-di-GMP	X,S	S	3HJ		64, 65
flavin mononucleotide (FMN)	FMN	X,S	S	6HJ		3, 32
glmS	glucosamine-6-phosphate (GlcN6P)	X	X	3Ψ, 3HJ		66−69
glycine	glycine	X,S	X,S	3HJ		70, 71
lysine	lysine	X,S	X,S	5HJ		3, 50, 72
m-box (magnesium)	Mg^{2+}	X, S	S	$2 \times$ 3HJ		73
preQ$_1$	7-aminomethyl-7-deazaguanine (preQ$_1$), 7-cyano-7-deazaguanine (preQ$_0$)	X, N	N	Ψ	N	74−77
purine	adenine, guanine, 2′-deoxyguanosine (dG)[c], hypoxanthine	X, N	N	3HJ		51, 52, 78, 79
SAH	*S*-adenosylhomocysteine	X		Ψ		80
SAM-I	*S*-adenosylmethionine (SAM)	X, S	S	4HJ, Ψ		3, 22, 81
SAM-II	SAM	X		Ψ	X	23
SAM-III (S$_{MK}$ box)	SAM	X,N	N,S	3HJ	N,S	24, 82
thi-box (TPP)	thiamine pyrophosphate (TPP)	X, N, S	N, S	3HJ		3, 38, 83−86

[a]X, X-ray crystallography; N, NMR; S, SAXS (small-angle X-ray scattering). [b]HJ, helix-junction; Ψ, pseudoknot. [c]dG recognition analyzed with an engineered guanine riboswitch with reduced affinity.

or proteins (e.g., refs 2−5), and therefore exist in multiple distinct conformations that can be targeted. In addition, many cellular RNAs are subject to extensive post-transcriptional modification, further increasing their chemical versatility (reviewed in ref 6).

The majority of antibiotics in clinical use target the ribosome, and structures of ribosome−antibiotic complexes reveal that nearly all bind the rRNAs, rather than ribosomal proteins,[7] thereby validating RNA as a drug target. The aminoglycoside neomycin has been shown to bind several RNAs in addition to rRNA. It prevents charging of tRNA[Phe] by its cognate aminoacyl-tRNA synthetase at concentrations well below those of other aminoglycoside inhibitors.[8,9] It also binds the trans-activating response (TAR) and Rev response element (RRE) RNAs of HIV-1. Neomycin inhibits the interaction between TAR and the protein Tat that enhances transcription of full-length HIV-1 RNA[10] and binds to RRE with $K_D \approx 100$ nM to disrupt the Rev−RRE interaction that facilitates nuclear−cytoplasmic export of the viral RNA.[11] Neomycin has also been shown to inhibit catalytic RNAs, such as group I introns, RNase P, and the hammerhead and hepatitis δ virus ribozymes,[12−15] by displacing specific Mg^{2+} ions.

2. Discovery and Characterization of Riboswitches

Isolation of aptamers preceded discovery of riboswitches. Aptamers are nucleic acids selected *in vitro* to bind specific ligands (small molecules, nucleic acids, proteins) by subjecting pools of as many as 10^{15} different RNAs or DNAs to iterative rounds of selection and amplification. A variety of aptamers and catalytic nucleic acids have been discovered thus. The ligand selectivity of aptamers has led to their application as biosensors and therapeutics (reviewed in ref 16). In addition, several aptamers have been shown to function as small-molecule-responsive *in vivo* genetic switches, that is, as artificial riboswitches.[17,18]

To date, natural riboswitches responsive to 17 different small-molecule metabolites, one divalent cation, and one second messenger have been described (Table 1, reviewed in ref 19). Biochemical and phylogenetic analyses show that many of these have a substructure that is necessary and sufficient to bind specifically to their cognate ligand and is also typically the element with the highest sequence conservation. This is often called the "aptamer domain" of the riboswitch.

In order to regulate gene expression, binding of ligand to the aptamer domain must be communicated to the machinery of gene expression. The majority of known bacterial riboswitches function by transcriptional or translational attenuation, in which the aptamer domain and either a terminator stem−loop or a Shine−Dalgarno element compete for folding and accessibility. Mechanisms unique to specific riboswitches have also been described. Eukaryotic and some bacterial riboswitches can modulate alternative splicing through ligand binding. A single bacterial class I SAM riboswitch is trans-acting.[20] Finally, the *glmS* riboswitch undergoes ligand binding-induced self-cleavage that destabilizes the mRNA in which it resides, leading to mRNA degradation by RNase J1 (reviewed in refs 19 and 21). In

most cases, transduction of the ligand binding signal to a change in gene expression relies on an RNA segment that has been termed the "expression platform". Depending on the riboswitch, the expression platform can be an RNA segment downstream of the aptamer domain or can overlap partially or wholly with it.

Three-dimensional structural information is available for 13 different riboswitch classes (Table 1). The majority of studies have elucidated the structure of ligand-bound aptamer domains, although some have characterized ligand-free states or the conformation of expression platforms. Overall, the structures available at present do not suggest phylogenetic relationships between riboswitch classes, except in the instances where structurally near-identical riboswitches bind closely related ligands (e.g., the purine riboswitches, Table 1). From a purely structural standpoint, riboswitch aptamer domains have been classified based on whether they fold around a multihelical junction, a pseudoknot, or combinations of these. Consistent with the diversity of RNA folds employed by different riboswitch classes, the molecular details of their ligand recognition also vary between classes. Even riboswitches of different classes that bind the same ligand (e.g., the SAM-binding riboswitches, Table 1) recognize their ligand differently. For instance, the SAM-I riboswitch flanks SAM with two helical stacks, the SAM-II riboswitch encapsulates SAM inside an RNA triplex, and the SAM-III riboswitch intercalates SAM in a three-way junction.[22−24] The riboswitches differ in their recognition of the methionine moiety of SAM: SAM-I forms extensive contacts with it, SAM-II forms fewer, and SAM-III makes no contacts.

3. Riboswitches as Drug Targets

Riboswitches are attractive targets for new drugs for three principal reasons. First, riboswitches evolved to recognize small molecules. Although numerous RNA domains have been experimentally targeted for drug discovery, small molecule binding by these RNAs is often fortuitous, not their physiologic function.[25] Ligands targeting such RNAs can exhibit poor selectivity.[26] Second, with the exception of the TPP riboswitch, known riboswitches occur predominantly in bacteria, not eukaryotes. If eukaryotes employ riboswitches, it is likely that these will be distinct from those of bacteria, minimizing cross-reactivity of bacterial riboswitch-targeted ligands. Third, known riboswitches respond to ubiquitous and essential metabolites and second messengers and are often associated with mRNAs encoding proteins essential for survival or pathogenesis. Often, a riboswitch and its association with specific genes are highly
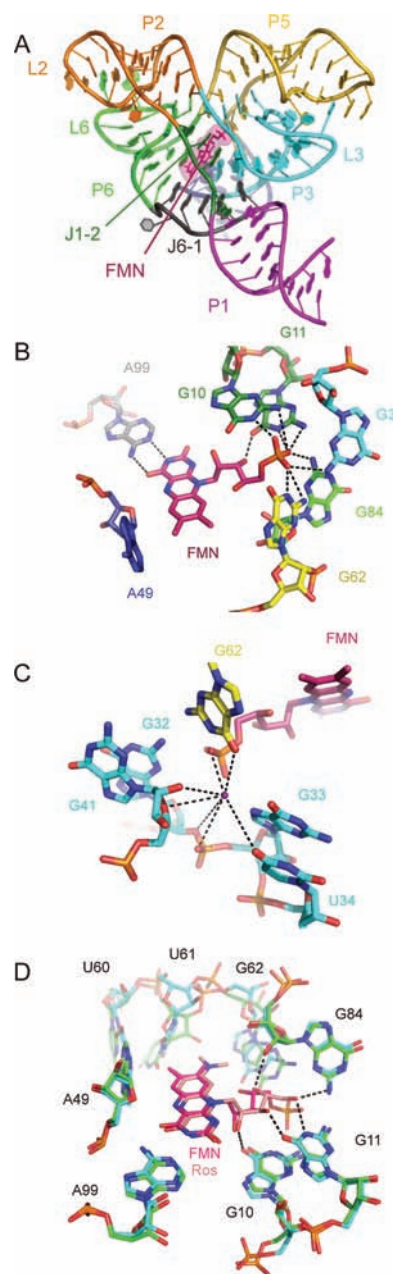
FIGURE 1. (A) The *Fusobacterium nucleatum* FMN riboswitch[32] (PDB 3F4E) and (B) its FMN binding site. (C) Mg^{2+} (purple sphere) mediates FMN−RNA interactions. (D) Superposition of FMN (RNA, green; FMN, magenta) and roseoflavin (RNA, cyan; roseoflavin, pink, PDB 3F4H) bound to the riboswitch.

conserved across phylogeny[27] underscoring their physiologic importance.

4. Serendipitously Discovered Riboswitch-Targeting Compounds

Recent studies on four antibacterials and antifungals for which the mechanism of action was unknown have revealed that they target riboswitches.[25] These serendipitously

discovered riboswitch-targeting compounds are roseoflavin, pyrithiamine, L-aminoethylcysteine and DL-4-oxalysine.

Roseoflavin, an analog of riboflavin and FMN, is a pigment from *Streptomyces davawensis* with antimicrobial activity.[28] Roseoflavin inhibits the growth of several Gram-positive bacteria, and roseoflavin-resistant mutants overproduce riboflavin.[29] In Gram-positive bacteria, all genes involved in riboflavin synthesis are under the control of a single FMN riboswitch.[30,31] Roseoflavin has been shown directly to bind the aptamer domain of the FMN riboswitch[32] (Figure 1) and to reduce the expression of a reporter gene placed under control of an FMN riboswitch in *Bacillus subtilis*.[33,34] Mutations within the aptamer domain of the FMN riboswitch are present in roseoflavin-resistant bacteria, disrupt roseoflavin binding, and result in an impaired regulatory response in the presence of either riboflavin or roseoflavin, reducing bacterial survival.[33]

Pyrithiamine (PT) is an analog of thiamine toxic to fungi and bacteria in which the thiazole ring is replaced by a pyridinium ring.[35,36] Like thiamine, PT is pyrophosphorylated *in vivo* to pyrithiamine pyrophosphate (PTPP).[37] PTPP binds the TPP riboswitch (Figure 2) with an affinity similar to that of TPP. *In vivo*, PT (presumably acting as PTPP) inhibits the expression of reporter genes placed under the control of a TPP riboswitch.[35] PT-resistant bacteria have mutations within the TPP riboswitch that disrupt ligand binding to the aptamer domain and result in the aberrant regulation of genes involved in TPP metabolism.[35] Crystallographic studies show that while PT binds the TPP riboswitch, it stabilizes the bound conformation of the aptamer domain poorly.[38] In contrast, the PTPP-bound aptamer domain is highly stabilized.[39] Interestingly, PTPP binding leads to a local rearrangement in the ligand-binding pocket of the TPP riboswitch. In the TPP complex, the riboswitch does not recognize the central thiazole ring of TPP. In contrast, in the PTPP complex, the RNA rearranges to make specific contacts with the central pyridinium ring of PTPP. Thus, PTPP binding drives the RNA into a conformation that it does not adopt in the cognate complex, reminiscent of the recognition of a unique inactive conformation of the Abl kinase by the anticancer drug Gleevec.[40] This demonstrates that drug specificity may arise from RNA conformations absent in the cognate complex of a riboswitch.

L-Aminoethylcysteine (AEC) and DL-4-oxalysine are lysine analogs that inhibit the growth of some Gram-positive bacteria. They bind to the *lysC* riboswitch of *B. subtilis* (Figure 3) and repress expression of a lysine riboswitch-regulated reporter gene in *B. subtilis*.[41] Mutations within
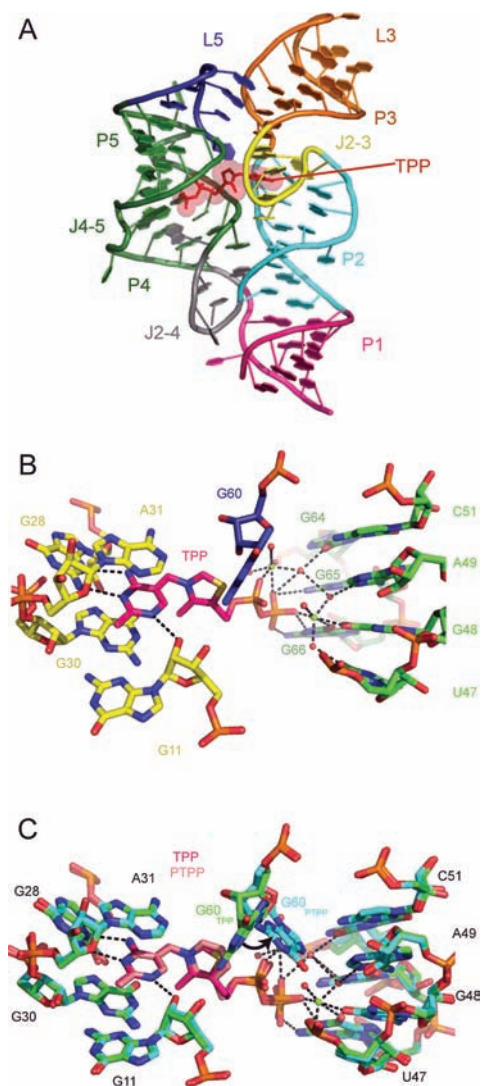
FIGURE 2. (A) The *Arabidopsis thaliana* TPP riboswitch[39] with TPP (red) bound (PDB 3D2G). (B) Mg^{2+} and waters (green and red spheres, respectively) in the ligand binding site. (C) Superposition of TPP (RNA, green; TPP, magenta) and PTPP (RNA, cyan; PTPP, pink, PDB 3D2V) bound to the RNA. Black arrow denotes movement of G60 between the two structures.

the *lysC* riboswitch are present in AEC-resistant mutants of *B. subtilis*[42] and *Escherichia coli*.[43] While these studies suggest that AEC and DL-4-oxalysine have an antibacterial effect by targeting the lysine riboswitch,[25] it is possible that the primary antimicrobial action of these analogs is a riboswitch-independent mechanism in which the lysyl-tRNA synthetase (LysRS) is inhibited by AEC, inhibiting protein synthesis. In this scenario, AEC resistance conferred by mutations to the riboswitch leads to more effective competition of lysine with AEC for binding to LysRS, reflecting an indirect effect of the lysine riboswitch-mediated increase in aspartokinase levels and cellular concentration of lysine.[44]
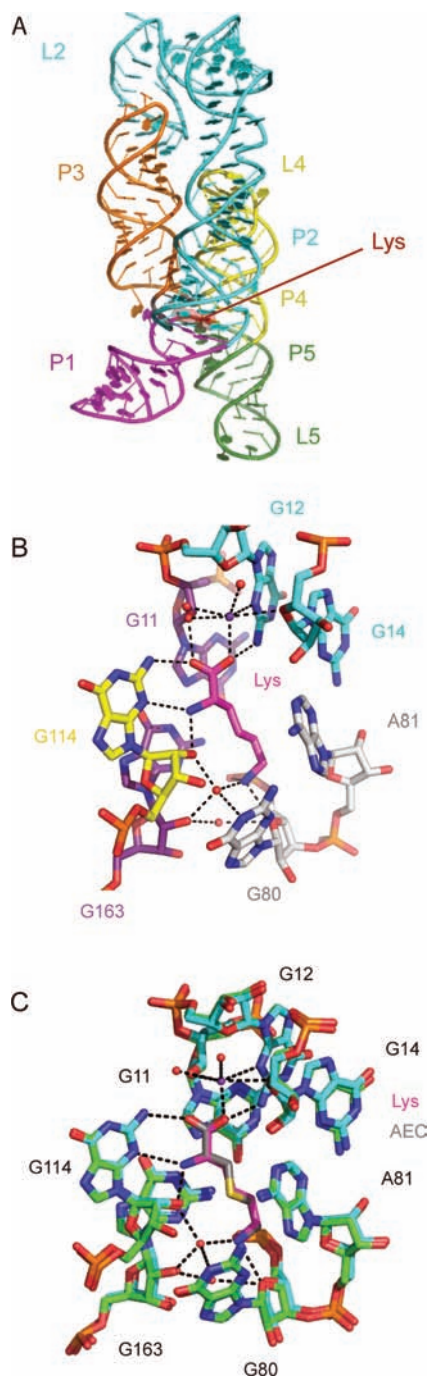
FIGURE 3. (A) The *Thermotoga maritima* lysine riboswitch[50] (PDB 3DIL) bound to lysine (red). (B) K$^+$ and waters (purple and red spheres, respectively) mediate lysine−RNA interactions. (C) Superposition of the lysine (RNA, green; lysine, magenta) and AEC (RNA, cyan; AEC, gray, PDB 3DIG) bound riboswitch structures.

5. Challenges for Riboswitch-Targeted Drug Discovery

A challenge in anti-riboswitch drug discovery is to establish whether compounds that bind riboswitches *in vitro* are deleterious to pathogens *in vivo* and, if so, that they exert their effect through riboswitch binding. This is nontrivial because riboswitches respond to essential, often abundant, metabolites and second messengers. Thus, the importance of riboswitches (and the effect of their inhibitors) cannot be evaluated by depleting cells of their cognate ligands. For instance, the *glmS* ribozyme-riboswitch responds to intracellular glucosamine-6-phosphate (GlcN6P) levels. Since GlcN6P is essential for bacterial cell wall synthesis, depleting bacterial cell strains incapable of its biosynthesis of exogenous GlcN6P causes death, without providing evidence for the effect of *glmS* riboswitch inactivation. Conversely, replacing the single genomic copy of the riboswitch with a version that is incapable of GlcN6P-induced activation impairs sporulation in *B. subtilis*. While important, this experiment does not establish whether *glmS* riboswitch hyperactivation (and consequent down-regulation of the mRNA whose stability it controls) results in a decrease in phenotypic fitness for the bacterium (reviewed in ref 21). Other riboswitches are present in multiple genomic loci in a given organism or occur in tandem, making experimental verification of their physiologic importance and effectiveness as drug targets even more difficult. Orthogonal riboswitch mutants that are not activated by natural compounds but respond specifically to a synthetic ligand[45] represent a potential means to tackle this challenge.

The ubiquity of the cognate ligands for riboswitches raises the issues of cross-reactivity and toxicity for potential antiriboswitch drugs. Compounds that target riboswitches have been shown to interact with mammalian proteins. The TPP riboswitch ligand PT inhibits rat thiamine pyrophosphokinase.[46] The lysine riboswitch ligand AEC is incorporated into proteins following transport into mammalian cells.[47] Two features of known riboswitches mitigate toxicity concerns. First, riboswitches employ recognition mechanisms different from those of proteins that recognize the same small molecules. Second, while there are eukaryotic gene regulatory RNAs that switch conformation in a ligand-dependent manner (e.g., ref 48), none of the known riboswitches are present in human, lowering the likelihood of cross-reactivity of new riboswitch-targeted compounds.

The emergence of resistance is a concern with any antibiotic. Bacteria express proteins that act on metabolites recognized by riboswitches. Mutations to these proteins or their regulators can confer resistance to anti-riboswitch compounds that resemble the natural ligand.[25] For instance, *B. subtilis* can evolve resistance to PT by overexpressing a thiaminase.[49] Resistance to compounds known to interact with riboswitches has been witnessed to evolve through

mutations to the riboswitch.[35] However, this resistance mechanism may be less prevalent than for protein targets. A point mutation would only confer resistance in cases in which a riboswitch of one class does not regulate multiple genes or operons essential for survival.[25] In addition, if the drug candidates were chemical analogs of the natural ligand, such mutations would likely disrupt binding of the natural ligand. This would impact riboswitch function, potentially decreasing bacterial fitness.

A consideration in targeting of riboswitches is that many of them appear to be under kinetic, rather than thermodynamic control. Many assays employed for drug discovery, especially those that are high-throughput, screen for candidates with high equilibrium association constants. Optimal assays for the discovery of novel riboswitch ligands may have to monitor directly the effect of candidates on the expression of genes under riboswitch control, rather than measuring the free energy of the small molecule−RNA interaction (reviewed in ref 19).

6. Progress in Riboswitch-Targeted Drug Discovery

Structure-guided rational design, high-throughput screening methods, and riboswitch-specific assays have been applied to the discovery of novel riboswitch-targeted drugs. These efforts have produced compounds with antibacterial activity *in vivo* that appear to function by targeting riboswitches.

Blount and colleagues identified L-lysine analogs that bind to the lysine riboswitch and inhibit *B. subtilis* growth.[41] They evaluated the affinity of 12 L-lysine analogs to the riboswitch and found that the RNA is intolerant to changes in most of the amino acid, except at the Cγ position. In addition, the Nε could also be modified, as long as the amine still carried a positive charge and could function as a hydrogen bond donor. Five of the 12 analogs tested bound to the *lysC* riboswitch from *B. subtilis*. Of these five, three (L-4-oxalysine and compounds **1** and **2** in Figure 4) inhibited *B. subtilis* growth in minimal medium, one (compound **3**) only inhibited a lysine auxotroph strain, and one did not inhibit growth. Two additional analogs did not bind the riboswitch and inhibited bacterial growth through riboswitch-independent mechanisms. The three compounds that bound the riboswitch and inhibited *B. subtilis* growth were determined to function at least in part by riboswitch-mediated repression of the *B. subtilis* enzyme aspartokinase II.[41] Structure determination of AEC, L-4-oxalysine, and L-homoarginine (compound **3**) bound to the riboswitch shows how the environment of Cγ and Nε can accommodate substituents
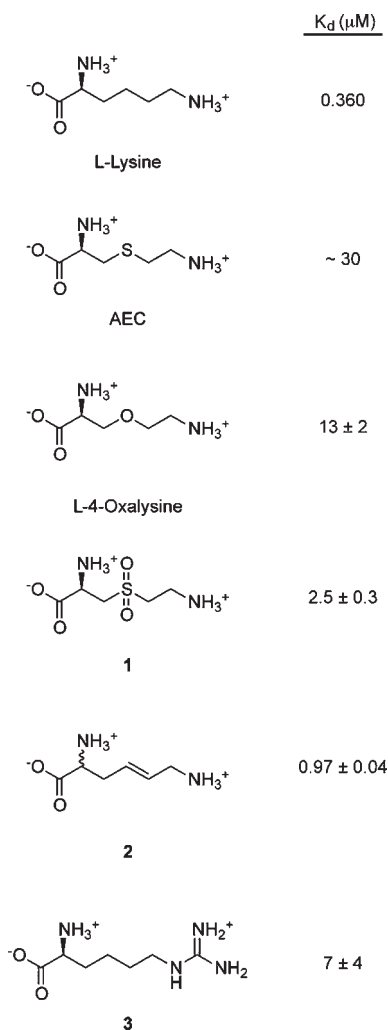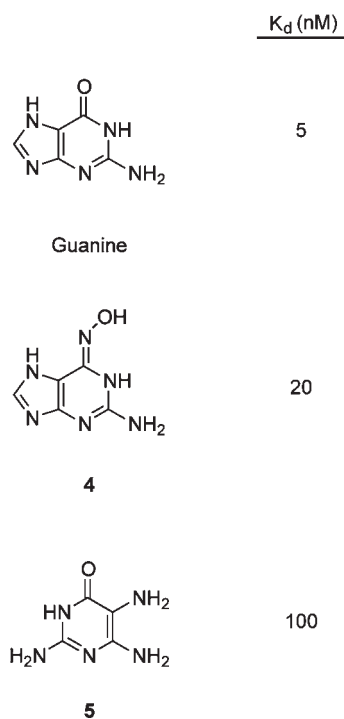

FIGURE 4. L-Lysine analogs that bind the lysine riboswitch: L-aminoethylcysteine (AEC), L-4-oxalysine, L-3-[(2-aminoethyl)-sulfonyl]-alanine (**1**), DL-*trans*-2,6-diamino-4-hexenoic acid (**2**), and L-homoarginine (**3**). K_D values from refs 41 and 63.

(Figure 3). In the case of the latter, the substituents displace water molecules and induce subtle rearrangements of the binding pocket.[50]

Structures of the ligand-bound guanine riboswitch[51,52] suggested that substitutions at guanine positions C2 and C6 would be tolerated[53] (Figure 5). Sixteen analogs with modifications at these positions were tested for binding, inhibition of bacterial growth, and repression of a reporter gene. Assessment of affinity yielded K_D values between 500 pM and 3.3 μM; one analog was found to have a K_D lower than that of guanine. These results confirm that the guanine C2 and C6 positions are available for modification. Five analogs were found to reduce bacterial growth by greater than 50%, and three were found to completely inhibit growth. Only one of these (compound **4**, Figure 5), however, was found to repress expression of a guanine riboswitch-regulated

K_d (nM)

5

Guanine

20

4

100

5

FIGURE 5. Guanine analogs that bind the guanine riboswitch: (*Z*)-2-amino-1*H*-purin-6(9*H*)-one oxime (**4**) and 2,5,6-triaminopyrimidin-4-one (**5**). K_D values from refs 53 and 54.

reporter gene. The poor correlation between *in vitro* binding and bacterial growth inhibition may reflect bioavailability and compound stability.[53]

A second study targeting the guanine riboswitch indicates that this RNA is a viable target for new antibiotics. Mulhbacher et al.[54] devised two pyrimidine analogs that would be predicted, on the basis of the crystal structures, to bind to the RNA. *In vitro* binding was demonstrated; both pyrimidines were found to repress *B. subtilis* growth in minimal media and to modulate reporter gene expression. However, only one of these pyrimidines (compound **5**, Figure 5) inhibited growth of *Staphylococcus aureus*, a bacterium that possesses a four-ORF operon under the control of the guanine riboswitch. When tested against a broad range of Gram-positive bacteria, compound **5** was found to inhibit growth of organisms that have the gene *guaA* (encoding GMP synthetase) under riboswitch control. Specificity was supported by lack of growth inhibition of an *E. coli* strain lacking guanine riboswitches. Encouragingly, it was found that multidrug-resistant bacterial strains were sensitive to the compound, suggesting that its antimicrobial action employs a mechanism not shared with previously known antibiotics. Finally, the compound was tested *in vivo* using a mouse model with *S. aureus*-infected mammary glands, where it was found to inhibit bacterial growth.[54]

The T-box discriminates between charged and uncharged tRNAs and modulates transcriptional antitermination (reviewed in ref 55). Oxazolidinones have been known to bind to rRNA, and Means et al. screened a small library of derivatives for their ability to bind and modulate the conformation of an RNA that mimics the antiterminator stem−loop of a T-box.[56] In a subsequent study, they optimized some candidates to find compounds with increased solubility.[57] *In vitro* transcription antitermination assays identified one compound that led to reduced tRNA-dependent antitermination and another that led to increased tRNA-independent antitermination. An interesting feature of these compounds is that they are polar but not ionic, suggesting that specificity does not arise from interactions with the RNA backbone.

Two assays suitable for high-throughput discovery of activators of the *glmS* ribozyme-riboswitch have been described. One monitors *glmS* ribozyme cleavage through fluorescence polarization. Fluorescein is attached covalently 5′ to the self-cleavage site of a *cis*-acting *glmS* riboswitch. Ligand-induced self-cleavage results in a short fluorescent oligonucleotide. The smaller size of the latter results in increased decorrelation of polarization.[58] The second utilizes FRET (fluorescence resonance energy transfer). Ligand-induced ribozyme cleavage of an oligonucleotide containing FRET donor and acceptor groups on either end releases the FRET acceptor at the 5′ end of the oligonucleotide, while the FRET donor-containing fragment remains bound to the ribozyme. This method was employed to screen ∼1000 compounds. However, only glucosamine, an analog of the natural ligand GlcN6P, was found to induce efficient cleavage.[59]

Development of a generic, high-throughput method for screening compounds for identification of small-molecule riboswitch ligands is highly desirable. A promising approach is fragment-based screening using equilibrium dialysis.[60,61] Screening with chemically and structurally diverse fragments instead of larger molecules allows for chemical space to be explored more efficiently using relatively small libraries (on the order of 100−1000 fragments) and increases the likelihood of finding a hit.[62] When employed against the *E. coli thiM* TPP riboswitch, this method identified 20 hits out of a screen of ∼1300 fragments, all of which were confirmed with water−LOGSY NMR and ITC (select fragment hits, compounds **6**−**9** in Figure 6). However, in an *in vitro* translation screen, these fragments failed to affect gene expression significantly. The authors propose that this may be due to the small size of the fragments, which likely only occupy a

K$_d$ (µM)

TPP   0.050

PTPP   0.160

6   49 ± 4

7   56 ± 6

8   103 ± 6

9   60 ± 5
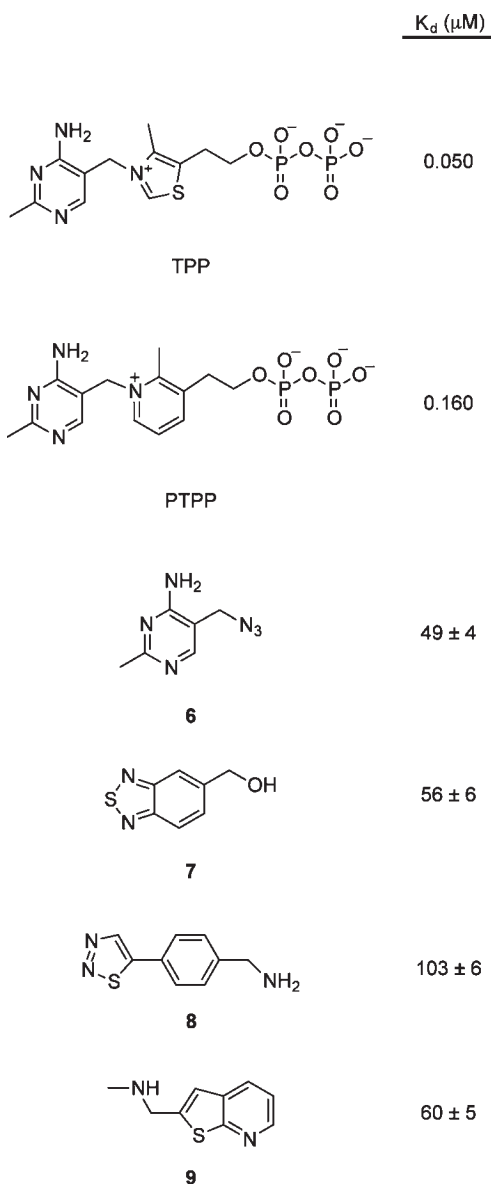
FIGURE 6. The TPP analog PTPP and select fragments that bind the TPP riboswitch: 5-(azidomethyl)-2-methylpyrimidin-4-amine (**6**), benzo[c]-[1,2,5]thiadiazol-5-ylmethanol (**7**), (4-(1,2,3-thiadiazol-5-yl)phenyl)-methanamine (**8**), and *N*-methyl-1-(thieno[2,3-*b*]pyridin-2-yl)-methanamine (**9**). K_D values from refs 35 and 61.

portion of the ligand binding site and are insufficient to induce the switching necessary to activate the riboswitch, or decreased affinity of ligands to riboswitches in their mRNA context. Further development of the fragments into larger and more potent molecules may lead to viable new ligands for the TPP riboswitch, making fragment-based screening a generic method for discovering new riboswitch-targeting ligands.

RNA has been considered a drug target since the discovery that aminoglycosides target rRNA. Riboswitches are exciting targets for novel antibiotics and chemical tools due to their structural sophistication, specificity, and function as genetic regulators of essential bacterial genes. Previously reported antibiotics and newly identified compounds that function through riboswitches emphasize progress made in the field and provide a foundation for future discovery of new riboswitch-targeting compounds.

BIOGRAPHICAL INFORMATION

Katherine Deigan received her B.A at the University of North Carolina—Chapel Hill under the direction of Kevin Weeks and her M.Phil. at the University of Cambridge in the lab of Ian Brierley as a Churchill Scholar. She is currently pursuing a Ph.D. at the University of Cambridge and the National Institutes of Health as an NIH-Oxford-Cambridge Scholar under the supervision of Adrian Ferré-D'Amaré and Chris Abell.

Adrian Ferré-D'Amaré received his Ph.D. from The Rockefeller University. After a postdoctoral stay at Yale University, he joined the faculty of the Fred Hutchinson Cancer Research Center (FHCRC) in 1999. In 2008, he was appointed Investigator of the Howard Hughes Medical Institute (HHMI). He resigned from the FHCRC and HHMI in 2011 to pursue opportunities in molecular biophysics and riboregulation at the National Heart, Lung and Blood Institute.

FOOTNOTES

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