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Molecular Recognition and Function of Riboswitches

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Abstract

Regulatory mRNAs elements termed riboswitches respond to elevated concentrations of cellular metabolites by modulating expression of associated genes. Riboswitches attain their high metabolite selectivity by capitalizing on the intrinsic tertiary structures of their sensor domains. Over the years, riboswitch structure and folding have been amongst the most researched topics in the RNA field. Most recently, novel structures of single- and cooperative double-ligand sensors have broadened our knowledge of architectural and molecular recognition principles exploited by riboswitches. The structural information has been complemented by extensive folding studies, which have provided several important clues on the formation of ligand-competent conformations and mechanisms of ligand discrimination. These studies have greatly improved our understanding of molecular events in riboswitch-mediated gene expression control and provided the molecular basis for intervention into riboswitch-controlled genetic circuits.

Keywords

X-ray crystallography; Ligand-binding pockets; NMR; RNA structure and folding

Introduction

Regulatory mRNA elements termed riboswitches have evolved for specific sensing of cellular metabolites and modulation of gene expression in species from all three kingdoms of life. Following their landmark discovery in 2002 as missing links in the feedback control of vitamin-related genes [1,2], riboswitches in the subsequent decade have materialized into one of the most important mechanisms of gene expression control in bacteria. Most bacterial riboswitches are located within the 5′-untranslated regions of genes associated with metabolism and transport of their cognate metabolites. Typically, riboswitches are composed of two domains, an evolutionary conserved metabolite-sensing module and a variable expression platform that contains RNA elements required for gene repression or activation. Depending on metabolite availability, riboswitches can adopt mutually exclusive metabolite-bound and metabolite-free conformations that oppositely affect gene expression (Figure 1a,b). This conformational interplay is often based on the ability of a segment that

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forms the closing helix P1 of the sensing domain to alternately pair with an expression platform region.

Riboswitches respond to a variety of metabolites ranging from large coenzymes to small cations and anions [3,4]. Given the low complexity of RNA molecules, the intriguingly high selectivity of riboswitches has been the subject of intensive structural research [5]. This review focuses on the three-dimensional structures and mode of action of seven additional riboswitch classes investigated recently. Aside from the direct structural approach, several laboratories have pursued biochemical and biophysical studies, also discussed in this review, which have uncovered folding trajectories of several small and medium sized riboswitches, thereby demonstrating the variety of approaches implemented by RNA for adopting binding-competent conformations. These recent structural and structure-guided studies have provided a more comprehensive overview of factors underlying the molecular mechanisms of riboswitches action, thereby establishing a solid foundation for the development of metabolite-like antibacterials and re-engineered RNA-based molecular sensors.

Variety of purine riboswitch folds

Purine and related riboswitches constitute a diverse group of RNA elements responding to purine bases and their derivatives. The first reported structures of riboswitches, adenine and guanine sensors [6,7], have been recently supplemented with structures of natural [8] and hybrid [9] riboswitches specific to the nucleoside 2'-deoxyguanosine (dG), structures [10–13] of the class I riboswitch responding to the modified purine base pre-queuosine-1 (preQ₁-I), and structures of two classes of sensors selective for the cyclic purine dinucleotide c-di-GMP [14–16]. The above riboswitches adopt either three-way junctional or pseudoknot folds reinforced by long-distance tertiary interactions and, in addition, stabilized by ligand binding.

Despite shortened hairpins P2 and P3 and numerous nucleotide changes [17], the dG riboswitch adopts a three-way junctional fold reminiscent of the tuning fork-like conformation of the canonical guanine riboswitch (Figure 1c, left). Not surprisingly, several mutations in the conserved guanine riboswitch core can switch the guanine selectivity to a modest specificity for dG [9] by providing room for the deoxyribose moiety of the ligand. The most critical difference between guanine and dG riboswitches is the U58C substitution, which causes sliding of the pyrimidine along the ligand and alternative base pairing with dG (Figure 1c, bottom) [8,9]. Additional dissimilarities, that likely account for the specificity and affinity of the dG riboswitch, have been observed in regions outside of the ligand-binding pocket. They include (i) involvement of a purine (G33) in formation of the (G34•U52)•G33 triple within stem P2, thereby replacing an unpaired stacked purine (A24) within stem P3 of the guanine riboswitch [8,18] (Figure 1c, middle) and (ii) novel 'key-and-lock' inter-loop interactions formed by adenine A71 insertion into the L3 loop [8], thereby replacing base quartets observed in the guanine riboswitch (Figure 1c, top).

The smallest riboswitch to date, which binds to preQ₁, forms a tight H-type pseudoknot conformation (Figure 1a) where loops L1 and L3 are positioned in the major and minor grooves of co-axially stacked stems S2 and S1, respectively (Figure 1d, top) [10–13]. PreQ₁ is intercalated into the interhelical interface and maintains continuous stacking of the two stems. Similar to purine riboswitches [6,7], preQ₁ is almost completely encapsulated in the ligand-binding pocket. As in guanine [6,7] and dG [8] riboswitches, preQ₁ forms a Watson-Crick pair with a cytosine (C17), whereas other edges are specifically recognized by base and backbone residues of the riboswitch (Figure 1d, bottom), facilitated by bound Ca²⁺ cations [12,19].

Like *S*-adenosyl-(L)-methionine (SAM) riboswitches [5], the two riboswitch classes responding to the secondary messenger c-di-GMP exemplify alternative structural solutions for recognition of the same ligand. The c-di-GMP-I riboswitch adopts a Y-shaped three-way junctional structure, with helices P2 and P3 joined together by tertiary tetraloop-tetraloop receptor interactions (Figure 1e, top) [14,15]. In the c-di-GMP-II riboswitch [16], the P2–P3 stem is bent towards J2/4 where it forms tertiary pseudoknot base pairing (P4), which could be considered as a third helix of the pseudo three-way junction (Figure 1f, top). In both riboswitches, ligands bind the core regions through intensive stacking interactions that involve invariant adenines A47 and A70 sandwiched between ligand bases. Nevertheless, both ligand bases and the phosphodiester backbone provide more extensive hydrogen bonding to the RNA in the c-di-GMP-I riboswitch, thereby contributing to the higher affinity of this class for the ligand [20,21] (Figures 1e,f, bottom).

S-adenosylhomocysteine riboswitch: infrequent pseudoknot fold

The *S*-adenosyl-(L)-homocysteine (SAH)-specific riboswitch appears to exclusively up-regulate expression of genes involved in utilization of the by-product of SAM-dependent chemical reactions. The SAH riboswitch adopts an infrequent “LL-type” pseudoknot fold where the P1 helix lies perpendicular to coaxially stacked P2, P2b and P4 helices (Figure 1g, top) [22]. The fold is stabilized by docking J1/4 into the minor groove of P1 and by ligand binding in the cleft created by the minor grooves of P2b and P1 (Figure 1g, bottom). The adenine ring participates in stacking and specific base pairing with guanine G15, whereas the methionine moiety interacts primarily with the RNA backbone.

Structural solutions for cooperative metabolite binding by RNA

RNA molecules usually employ cooperativity for folding/unfolding and binding of proteins or Mg^{2+} cations. Surprisingly, glycine riboswitches that are composed of two adjacent glycine-sensing domains showed cooperative binding of two glycine molecules [23] (Figure 1b). The structure of the *Vibrio cholerae* sensing domain II [24] and the moderate resolution structure of the *Fusobacterium nucleatum* tandem domain glycine riboswitch [25] shed light on the cooperative nature of glycine recognition.

The glycine riboswitch features a three-way junctional architecture with glycine positioned above the junction in the widened Mg^{2+} -stabilized helical region of P3 (Figure 2a, top). Glycine is specifically recognized by conserved U69 and purine bases with the help of a Mg^{2+} cation, which similarly to other riboswitches [5], neutralizes the negative charge of the ligand (Figure 2a, bottom). The junction and regulatory helix P1 are stabilized by tertiary intercalation of an adenine (A33) extruded from the glycine-binding pocket.

Intermolecular interactions in the asymmetric unit of the domain II structure [24] and the interdomain interactions in the tandem riboswitch structure [25] (Figure 2b, top and middle), coupled with biochemical data [26,27], strongly suggest the formation of three interdomain contact regions (quaternary interactions) likely involved in the cooperative response. Two pseudo-symmetrical contacts, designated α – α' and β – β' , are formed by the insertion of non-paired adenine-rich segments into the minor groove of helices P1 (Figure 2b, top and bottom). Additional interactions, designated γ – γ' , involve a non-canonical U•A base pair. Since the contact regions are located in proximity to the glycine binding pockets, it is conceivable that glycine binding to one domain could induce structural rearrangements that facilitate ligand binding to the second domain.

The structure of the *Sterptococcus mutants* tetrahydrofolate (THF) riboswitch uncovered another cooperative system, which, in contrast to the tandem glycine riboswitch, employs binding of two ligands to one RNA sensor [28]. The THF riboswitch adopts an ‘inverted’

junctional architecture where the three-way junction and long-distance tertiary contacts have switched places [29] (Figure 2c, top). One of the THF ligands stabilizes the three-way junction by intercalating into the adjacent widened helix, whereas the other ligand is sandwiched between two helical stems in the vicinity of tertiary base pairing interactions, stabilizing the pseudoknot and helix P1. Ligand binding is similar in both sites and, in contrast to many other riboswitches, mostly involves the recognition of a small part of the ligand moiety by conserved pyrimidines (Figure 2c, middle and bottom), whereas the glutamate moiety appears not to interact with the RNA. Cooperative ligand binding was observed at physiological Mg^{2+} concentration of 0.5 mM, whereas higher concentrations (6 mM) prompted independent binding [28].

The *Eubacterium siraeum* THF riboswitch structure has captured a conformation with a disrupted helix P1, which could be considered an intermediate state of riboswitch folding [29]. This structure lacks the ligand positioned adjacent to the pseudoknot, and additional studies are required to clarify whether this riboswitch binds only one ligand or alternately whether the second binding site is disrupted by crystal packing interactions.

Ligand discrimination: rules and exceptions

Riboswitches demonstrate high selectivity towards their cognate ligands mostly through shape complementarity and specific interactions. Recent riboswitch structures have revealed several variations on these themes. The dG riboswitch discriminates against guanosine because its ribose adopts the favorable C3'-*endo* conformation instead of the C2'-*endo* conformation, eliminating a hydrogen bond with RNA (Figure 3a) [8]. The potential for strong interactions between the dG riboswitch and dGTP is possibly prevented by the flexibility of the dGTP sugar-phosphate moiety and the lack of a dedicated binding pocket on the riboswitch [8]. The SAH riboswitch forms a tight pocket so that SAM, which has an additional methyl group, would clash with the RNA (Figure 1g, bottom) [28]. In the preQ₁ riboswitch, the preQ₁ ligand forms hydrogen bonds that would be eliminated when its methylamine moiety is replaced by the cyano moiety of the related preQ₀ compound [11–13].

Contrary to the conventional view that a riboswitch responds to just a single cognate metabolite, the *glmS* riboswitch can respond to an array of chemical compounds related to glucosamine N6-phosphate (GlcN6P), thereby assessing information of the overall metabolic state of the cell [30]. Additionally, more than one effector could possibly participate in preQ₁ and THF riboswitch control [11,28,29].

Along with these observations, several riboswitches were reported to interact with metabolite-like compounds including antibiotics. Efforts have been undertaken for the structure-guided design of novel riboswitch ligands [31,32], which may exert antimicrobial properties through gene expression suppression. Such studies have been successful in the case of the guanine riboswitch, where artificial compounds were found to inhibit growth of pathogenic bacteria in rich media [33,34] and in a mammalian model of staphylococcal infection [34].

An additional advance was contributed by Dixon et al., who re-engineered an adenine riboswitch for binding to the non-natural compounds ammeline and azacytosine, using a limited number of core mutations [35] (Figure 3b). The resulting riboswitches discriminated against purines and responded to synthetic ligands *in vivo*. Structural studies of the novel riboswitch-ligand pair demonstrated small adjustments within the ligand-binding pocket, including a lateral movement of C51, reminiscent of the dG riboswitch.

Riboswitch dynamics and metabolite sensing

Since many ligands are encapsulated in the ‘closed’ binding pockets, riboswitches should adopt a ligand-competent ‘open’ conformation prior to metabolite binding. The surprising resemblance of ligand-free and ligand-bound states described for lysine and *glmS* riboswitches [36–38] was also observed in the novel structures of THF [29], glycine [24] and FMN [39] riboswitches. In the SAM-I [40] (Figure 3c), preQ₁ [11] and mutant adenine [41,42] riboswitches, internal residues occupy sites of bound ligands in the free state. Nevertheless, ‘closed’ and ‘blocked’ conformations can open up for metabolites soaked into the unliganded riboswitch crystals [40] and, therefore, are in equilibrium with open riboswitch states. However, local conformational dynamics of RNA cannot adequately explain small-angle X-ray scattering (SAXS) [40], fluorescent [43] and other data, which suggest that the apo-form of riboswitches may adopt an ensemble of substantially dissimilar interconverting states in the absence of ligands [40]. These results imply that riboswitches likely employ ‘conformational selection’ to recognize and bind their cognate ligands. The conformational selection concept postulates that the ligand selects ligand-binding competent conformation(s) from the dynamic ensemble of multiple pre-existing conformations and shifts the equilibrium towards the bound state via favorable binding energetics. The closing of the ligand-binding pockets also suggests post-binding conformational adjustments, consistent with the ‘induced fit model’.

Some riboswitches, for example TPP [44] and c-di-GMP-I [14] riboswitches, appear to populate near ligand-bound conformations less frequently than other riboswitch classes, therefore their free and bound states are considerably distinct. The free states also differ amongst representatives of the same riboswitch class [11,40,45,46] and it would be interesting to understand whether the similarity of the free and bound states correlate better with environmental factors (for instance, growth temperature) or mechanisms of gene expression control (transcription or translation).

In solution, ligand-bound riboswitches are not frozen in a single conformation and demonstrate motions on different time scales [19]. RNA dynamics is not restricted to flexible loop regions and can be observed within the ligand-binding pocket. According to time-resolved fluorescence spectroscopy of purine-sensing riboswitches [47], crystal structures represent the global minimum that accounts for only 50–60% of the population, thereby suggesting the elastic nature of the ligand recognition mode that provides high affinity and specificity for the sensor.

Riboswitch gymnastics

Most riboswitches have to commit to the metabolite-bound or metabolite-free folding pathway in a short temporal window of riboswitch transcription. Therefore, much attention has been devoted to understanding the global folding of metabolite-sensing domains and entire riboswitches. Recent studies performed on purine [43,48–50], preQ₁ [46,51], SAM-I [40,45], SAM-II [52], and SAM-III [53] riboswitches have revealed various folding trajectories for different riboswitch classes, as well as species within the same class. The vast majority of these studies emphasize the importance of Mg²⁺ cations for the pre-formation of ligand-binding competent conformations, by for instance, aligning helical segments and promoting base pairing, as in the SAM-I riboswitch (Figure 4) [40,45]. Ligand binding further contributes to the stabilization of the bound riboswitch fold through either local adjustments or global changes, for instance, stabilization of stacking and tertiary pairing contacts [40,45,54,55]. A full understanding of the mechanics of riboswitch folding will require studies performed under cotranscriptional conditions.

Conclusions

Many critical questions in the riboswitch field, such as how riboswitches sense cognate ligands and how they choose folding pathways, have been recently addressed by numerous studies on many riboswitch systems. These studies have provided the structural basis for cooperative ligand binding by glycine and THF riboswitches, discrimination of closely related compounds by dG, preQ₁ and SAH riboswitches, and differential ligand binding by c-di-GMP-I and c-di-GMP-II riboswitches. Despite various strategies adopted by riboswitches for binding of cognate ligands, many experimental results converge on the ‘conformational selection’ principle of metabolite recognition, possibly with some impact from ‘induced fit’ events after initial sensing. Future studies should explore and address the sensing and folding processes in a cotranscriptional context.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Mironov AS, Gusarov I, Rafikov R, Lopez LE, Shatalin K, Kreneva RA, Perumov DA, Nudler E. Sensing small molecules by nascent RNA: a mechanism to control transcription in bacteria. *Cell*. 2002; 111:747–756. [PubMed: 12464185]
 2. Winkler W, Nahvi A, Breaker RR. Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression. *Nature*. 2002; 419:952–956. [PubMed: 12410317]
 3. Baker JL, Sudarsan N, Weinberg Z, Roth A, Stockbridge RB, Breaker RR. Widespread genetic switches and toxicity resistance proteins for fluoride. *Science*. 2012; 335:233–235. [PubMed: 22194412]
 4. Breaker RR. Prospects for riboswitch discovery and analysis. *Molecular cell*. 2011; 43:867–879. [PubMed: 21925376]
 5. Serganov A. The long and the short of riboswitches. *Curr Opin Struct Biol*. 2009; 19:251–259. [PubMed: 19303767]
 6. Batey RT, Gilbert SD, Montange RK. Structure of a natural guanine-responsive riboswitch complexed with the metabolite hypoxanthine. *Nature*. 2004; 432:411–415. [PubMed: 15549109]
 7. Serganov A, Yuan YR, Pikovskaya O, Polonskaia A, Malinina L, Phan AT, Hobartner C, Micura R, Breaker RR, Patel DJ. Structural basis for discriminative regulation of gene expression by adenine- and guanine-sensing mRNAs. *Chem Biol*. 2004; 11:1729–1741. [PubMed: 15610857]
 - 8. Pikovskaya O, Polonskaia A, Patel DJ, Serganov A. Structural principles of nucleoside selectivity in a 2′-deoxyguanosine riboswitch. *Nat Chem Biol*. 2011; 7:748–755. The molecular basis of the dG riboswitch selectivity and involvement of the regions outside of the binding pocket into riboswitch specificity and affinity. [PubMed: 21841796]
 9. Edwards AL, Batey RT. A structural basis for the recognition of 2′-deoxyguanosine by the purine riboswitch. *J Mol Biol*. 2009; 385:938–948. [PubMed: 19007790]
 - 10. Kang M, Peterson R, Feigon J. Structural insights into riboswitch control of the biosynthesis of queuosine, a modified nucleotide found in the anticodon of tRNA. *Mol Cell*. 2009; 33:784–790. NMR structure of the preQ₁ riboswitch. [PubMed: 19285444]

11. Jenkins JL, Krucinska J, McCarty RM, Bandarian V, Wedekind JE. Comparison of a preQ₁ riboswitch aptamer in metabolite-bound and free states with implications for gene regulation. *J Biol Chem*. 2011; 286:24626–24637. [PubMed: 21592962]
- 12. Klein DJ, Edwards TE, Ferre-D'Amare AR. Cocystal structure of a class I preQ₁ riboswitch reveals a pseudoknot recognizing an essential hypermodified nucleobase. *Nat Struct Mol Biol*. 2009; 16:343–344. X-ray structure of the preQ₁ riboswitch. [PubMed: 19234468]
- 13. Spitale RC, Torelli AT, Krucinska J, Bandarian V, Wedekind JE. The structural basis for recognition of the preQ₀ metabolite by an unusually small riboswitch aptamer domain. *J Biol Chem*. 2009; 284:11012–11016. X-ray structure of the preQ₁ riboswitch bound to preQ₀. [PubMed: 19261617]
- 14. Kulshina N, Baird NJ, Ferre-D'Amare AR. Recognition of the bacterial second messenger cyclic diguanylate by its cognate riboswitch. *Nat Struct Mol Biol*. 2009; 16:1212–1217. X-ray structure of the preQ₁ riboswitch. [PubMed: 19898478]
- 15. Smith KD, Lipchock SV, Ames TD, Wang J, Breaker RR, Strobel SA. Structural basis of ligand binding by a c-di-GMP riboswitch. *Nat Struct Mol Biol*. 2009; 16:1218–1223. X-ray structure of the c-di-GMP-I riboswitch. [PubMed: 19898477]
- 16. Smith KD, Shanahan CA, Moore EL, Simon AC, Strobel SA. Structural basis of differential ligand recognition by two classes of bis-(3′–5′)-cyclic dimeric guanosine monophosphate-binding riboswitches. *Proc Natl Acad Sci U S A*. 2011; 108:7757–7762. X-ray structure of the c-di-GMP-II riboswitch. [PubMed: 21518891]
17. Kim JN, Roth A, Breaker RR. Guanine riboswitch variants from *Mesoplasma florum* selectively recognize 2′-deoxyguanosine. *Proc Natl Acad Sci U S A*. 2007; 104:16092–16097. [PubMed: 17911257]
18. Wacker A, Buck J, Mathieu D, Richter C, Wohnert J, Schwalbe H. Structure and dynamics of the deoxyguanosine-sensing riboswitch studied by NMR-spectroscopy. *Nucleic acids research*. 2011; 39:6802–6812. [PubMed: 21576236]
19. Zhang Q, Kang M, Peterson RD, Feigon J. Comparison of solution and crystal structures of preQ₁ riboswitch reveals calcium-induced changes in conformation and dynamics. *Journal of the American Chemical Society*. 2011; 133:5190–5193. [PubMed: 21410253]
20. Shanahan CA, Gaffney BL, Jones RA, Strobel SA. Differential analog binding by two classes of c-di-GMP riboswitches. *J Am Chem Soc*. 2011; 133:15578–15592. [PubMed: 21838307]
21. Smith KD, Lipchock SV, Livingston AL, Shanahan CA, Strobel SA. Structural and biochemical determinants of ligand binding by the c-di-GMP riboswitch. *Biochemistry*. 2010; 49:7351–7359. [PubMed: 20690679]
- 22. Edwards AL, Reyes FE, Heroux A, Batey RT. Structural basis for recognition of *S*-adenosylhomocysteine by riboswitches. *RNA*. 2010; 16:2144–2155. X-ray structure and folding of the SAH riboswitch. [PubMed: 20864509]
23. Mandal M, Lee M, Barrick JE, Weinberg Z, Emilsson GM, Ruzzo WL, Breaker RR. A glycine-dependent riboswitch that uses cooperative binding to control gene expression. *Science*. 2004; 306:275–279. [PubMed: 15472076]
- 24. Huang L, Serganov A, Patel DJ. Structural insights into ligand recognition by a sensing domain of the cooperative glycine riboswitch. *Mol Cell*. 2010; 40:774–786. X-ray structure and biochemical data that suggested quarternary interactions involved in cooperative response of the glycine riboswitch. [PubMed: 21145485]
- 25. Butler EB, Xiong Y, Wang J, Strobel SA. Structural basis of cooperative ligand binding by the glycine riboswitch. *Chem Biol*. 2011; 18:293–298. X-ray structure of the tandem sensing domain glycine riboswitch that showed interdomain interactions involved in the cooperative response. [PubMed: 21439473]
26. Erion TV, Strobel SA. Identification of a tertiary interaction important for cooperative ligand binding by the glycine riboswitch. *RNA*. 2011; 17:74–84. [PubMed: 21098652]
27. Kwon M, Strobel SA. Chemical basis of glycine riboswitch cooperativity. *RNA*. 2008; 14:25–34. [PubMed: 18042658]
- 28. Trausch JJ, Ceres P, Reyes FE, Batey RT. The structure of a tetrahydrofolate-Sensing riboswitch reveals two ligand binding sites in a single aptamer. *Structure*. 2011; 19:1413–1423. The THF

riboswitch structure and biochemical data that revealed cooperative binding of two ligands to a single sensing domain. [PubMed: 21906956]

- 29. Huang L, Ishibe-Murakami S, Patel DJ, Serganov A. Long-range pseudoknot interactions dictate the regulatory response in the tetrahydrofolate riboswitch. *Proc Natl Acad Sci U S A*. 2011; 108:14801–14806. The THF riboswitch structure in partially unfolded conformation. [PubMed: 21873197]
- 30. Watson PY, Fedor MJ. The *glmS* riboswitch integrates signals from activating and inhibitory metabolites *in vivo*. *Nat Struct Mol Biol*. 2011; 18:359–363. Genetic response to different ligands by the *glmS* riboswitch *in vivo*. [PubMed: 21317896]
- 31. Daldrop P, Reyes FE, Robinson DA, Hammond CM, Lilley DM, Batey RT, Brenk R. Novel ligands for a purine riboswitch discovered by RNA-ligand docking. *Chemistry & biology*. 2011; 18:324–335. [PubMed: 21439477]
- 32. Chen L, Cressina E, Leeper FJ, Smith AG, Abell C. A fragment-based approach to identifying ligands for riboswitches. *ACS chemical biology*. 2010; 5:355–358. [PubMed: 20158266]
- 33. Kim JN, Blount KF, Puskarz I, Lim J, Link KH, Breaker RR. Design and antimicrobial action of purine analogues that bind guanine riboswitches. *ACS Chem Biol*. 2009; 4:915–927. [PubMed: 19739679]
- 34. Mulhbach J, Brouillette E, Allard M, Fortier LC, Malouin F, Lafontaine DA. Novel riboswitch ligand analogs as selective inhibitors of guanine-related metabolic pathways. *PLoS Pathog*. 2010; 6:e1000865. Guanine analogs inhibit staphylococcal infection in a mammalian model through the riboswitch mechanism. [PubMed: 20421948]
- 35. Dixon N, Duncan JN, Geerlings T, Dunstan MS, McCarthy JE, Leys D, Micklefield J. Reengineering orthogonally selective riboswitches. *Proc Natl Acad Sci U S A*. 2010; 107:2830–2835. [PubMed: 20133756]
- 36. Garst AD, Heroux A, Rambo RP, Batey RT. Crystal structure of the lysine riboswitch regulatory mRNA element. *J Biol Chem*. 2008; 283:22347–22351. [PubMed: 18593706]
- 37. Serganov A, Huang L, Patel DJ. Structural insights into amino acid binding and gene control by a lysine riboswitch. *Nature*. 2008; 455:1263–1267. [PubMed: 18784651]
- 38. Klein DJ, Ferre-D'Amare AR. Structural basis of *glmS* ribozyme activation by glucosamine-6-phosphate. *Science*. 2006; 313:1752–1756. [PubMed: 16990543]
- 39. Vicens Q, Mondragon E, Batey RT. Molecular sensing by the aptamer domain of the FMN riboswitch: a general model for ligand binding by conformational selection. *Nucleic Acids Res*. 2011; 39:8586–8598. [PubMed: 21745821]
- 40. Stoddard CD, Montange RK, Hennelly SP, Rambo RP, Sanbonmatsu KY, Batey RT. Free state conformational sampling of the SAM-I riboswitch aptamer domain. *Structure*. 2010; 18:787–797. Elegant demonstration of the SAM-I riboswitch folding and characterization of the ligand-unbound state. [PubMed: 20637415]
- 41. Delfosse V, Bouchard P, Bonneau E, Dagenais P, Lemay JF, Lafontaine DA, Legault P. Riboswitch structure: an internal residue mimicking the purine ligand. *Nucleic Acids Res*. 2010; 38:2057–2068. [PubMed: 20022916]
- 42. Tremblay R, Lemay JF, Blouin S, Mulhbach J, Bonneau E, Legault P, Dupont P, Penedo JC, Lafontaine DA. Constitutive regulatory activity of an evolutionarily excluded riboswitch variant. *J Biol Chem*. 2011; 286:27406–27415. [PubMed: 21676871]
- 43. Brenner MD, Scanlan MS, Nahas MK, Ha T, Silverman SK. Multivector fluorescence analysis of the *xpt* guanine riboswitch aptamer domain and the conformational role of guanine. *Biochemistry*. 2010; 49:1596–1605. [PubMed: 20108980]
- 44. Ali M, Lipfert J, Seifert S, Herschlag D, Doniach S. The ligand-free state of the TPP riboswitch: a partially folded RNA structure. *J Mol Biol*. 2010; 396:153–165. [PubMed: 19925806]
- 45. Heppell B, Blouin S, Dussault AM, Mulhbach J, Ennifar E, Penedo JC, Lafontaine DA. Molecular insights into the ligand-controlled organization of the SAM-I riboswitch. *Nat Chem Biol*. 2011; 7:384–392. Insights into the folding trajectory of the SAM-I riboswitch. [PubMed: 21532599]

- 46. Rieder U, Kreutz C, Micura R. Folding of a transcriptionally acting preQ₁ riboswitch. *Proc Natl Acad Sci U S A*. 2010; 107:10804–10809. Insights into the folding trajectory of the preQ₁ riboswitch. [PubMed: 20534493]
47. Jain N, Zhao L, Liu JD, Xia T. Heterogeneity and dynamics of the ligand recognition mode in purine-sensing riboswitches. *Biochemistry*. 2010; 49:3703–3714. [PubMed: 20345178]
48. Lee MK, Gal M, Frydman L, Varani G. Real-time multidimensional NMR follows RNA folding with second resolution. *Proc Natl Acad Sci U S A*. 2010; 107:9192–9197. [PubMed: 20439766]
49. Leipply D, Draper DE. Effects of Mg²⁺ on the free energy landscape for folding a purine riboswitch RNA. *Biochemistry*. 2011; 50:2790–2799. [PubMed: 21361309]
50. Lemay JF, Desnoyers G, Blouin S, Heppell B, Bastet L, St-Pierre P, Masse E, Lafontaine DA. Comparative study between transcriptionally- and translationally-acting adenine riboswitches reveals key differences in riboswitch regulatory mechanisms. *PLoS Genet*. 2011; 7:e1001278. [PubMed: 21283784]
51. Feng J, Walter NG, Brooks CL 3rd. Cooperative and directional folding of the preQ₁ riboswitch aptamer domain. *Journal of the American Chemical Society*. 2011; 133:4196–4199. [PubMed: 21375305]
- 52. Haller A, Rieder U, Aigner M, Blanchard SC, Micura R. Conformational capture of the SAM-II riboswitch. *Nat Chem Biol*. 2011; 7:393–400. Insights into the folding trajectory of the SAM-II riboswitch. [PubMed: 21532598]
53. Wilson RC, Smith AM, Fuchs RT, Kleckner IR, Henkin TM, Foster MP. Tuning riboswitch regulation through conformational selection. *J Mol Biol*. 2011; 405:926–938. [PubMed: 21075119]
54. Hennelly SP, Sanbonmatsu KY. Tertiary contacts control switching of the SAM-I riboswitch. *Nucleic acids research*. 2011; 39:2416–2431. [PubMed: 21097777]
55. Lu C, Smith AM, Ding F, Chowdhury A, Henkin TM, Ke A. Variable sequences outside the SAM-binding core critically influence the conformational dynamics of the SAM-III/SMK box riboswitch. *Journal of molecular biology*. 2011; 409:786–799. [PubMed: 21549712]
56. Heppell B, Lafontaine DA. Folding of the SAM aptamer is determined by the formation of a K-turn-dependent pseudoknot. *Biochemistry*. 2008; 47:1490–1499. [PubMed: 18205390]

Highlights

Riboswitches utilize different conformations and pockets for ligand binding

Riboswitch structures provide molecular basis underlying the cooperative response

Ligand selectivity is achieved by shape complementarity and specific interactions

Riboswitches employ 'conformational selection' to bind their cognate ligands

Different riboswitch classes follow distinct folding trajectories

Figure 1.

Gene expression control by riboswitches and three-dimensional structures of metabolite-sensing domains (top panels) and their metabolite-binding pockets (bottom panels). The RNA backbone is in a ribbon or stick representation. Ligands are in red stick representation. Green and blue nucleotides use their bases and backbones, respectively, for hydrogen bonding (dashed lines) with ligands. (a) Repression of transcription by the preQ₁ riboswitch. In the absence of ligand, mRNA forms an antiterminator hairpin that allows transcription to proceed. In the presence of preQ₁ the metabolite sensor adopts a stable metabolite-bound conformation that triggers formation of transcription terminator and premature termination of transcription. Key complementary sequences are colored. (b) Activation of transcription

by the *gcvT*-type glycine riboswitch. Two sensing domains of the riboswitch are arranged in tandem and are followed by a single expression platform. **(c)** dG riboswitch (PDB ID: 3SKI). Peripheral nucleotides important for dG recognition are in violet. Top, middle and bottom panels in the middle column show involvement of A71 in the loop-loop interactions, (G•U)•G triple formation and dG binding pocket, respectively, from the dG riboswitch, whereas the right column presents corresponding regions from the guanine riboswitch. **(d)** preQ₁-I riboswitch (PDB ID: 2KFC and 3FU2). **(e)** c-di-GMP-I riboswitch (PDB ID: 3IRW). A water molecule is shown as a red sphere. **(f)** c-di-GMP-II riboswitch (PDB ID: 3Q3Z). PK stands for pseudoknot. **(g)** SAH riboswitch (PDB ID: 3NPQ). Double-headed arrow shows a short distance between the ligand sulfur atom and RNA.

Figure 2. Three-dimensional structures of riboswitches that exhibit cooperativity. RNA representation and color codes in panels (a) and (c) are as in Figure 1. **(a)** Glycine-sensing domain II of the *Vibrio cholerae* riboswitch (PDB ID: 3OWI). Mg^{2+} coordination bonds are depicted in green sticks. **(b)** Secondary structure schematic (top) and structure (middle) of the tandem *Fusobacterium nucleatum* glycine riboswitch (PDB ID: 3P49). Sensing domains I and II are in light green and orange, respectively, with quaternary interdomain interactions shaded, indicated with double arrows, and highlighted in brighter colors. Bottom: contacts between P1 helix and J3a/3b region. **(c)** Structure of the THF riboswitch bound to two molecules of folinic acid (FA_I and FA_{II}) (PDB ID: 3SUH).

Figure 3.

Ligand-sensing and discrimination by riboswitches. Arrows indicate conformational differences between structures. **(a)** Discrimination of dG and guanosine (top panels) by the dG riboswitch revealed by superposition of the dG-bound (green and blue) and guanosine-bound (orange) binding pockets (PDB IDs: 3SKI and 3SKZ) [8]. **(b)** Re-engineering of the adenine riboswitch to discriminate against adenine and interact with ammeline and azacytosine (top panels). Conformational and bonding differences are shown on the adenine-bound (green and blue, PDB ID: 1Y26) and azacytosine-bound (orange, PDB ID: 3LA5) pockets superimposed on the discriminatory pyrimidine 74 [35]. **(c)** Blocking of the SAM-binding site by A46 (highlighted in bright colors) in the free structure shown by superposition of the SAM-bound (orange, PDB ID: 3IQP) and free (green, PDB ID: 3IQR) pockets [40].

Figure 4.

A model of the SAM-I riboswitch folding [56]. In the unfolded state, the riboswitch adopts a Y-shaped conformation. Addition of Mg^{2+} facilitates P2-P3 stacking, the pseudoknot interactions (PK), and the close juxtaposition of P1 and P3. SAM binding to this preorganized structure stabilizes P1-P4 stacking and rotates P1 along its axis.