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Riboswitches exert genetic control through metabolite-induced conformational change

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Conserved RNA structures have traditionally been thought of as potential binding sites for protein factors and consequently are regarded as fulfilling relatively passive albeit important roles in cellular processes. With the discovery of riboswitches, RNA no longer takes a backseat to protein when it comes to affecting gene expression. Riboswitches bind directly to cellular metabolites with exceptional specificity and affinity, and exert control over gene expression through ligand-induced conformational changes in RNA structure. Riboswitches now represent a widespread mechanism by which cells monitor their metabolic state and facilely alter gene expression in response to changing conditions.

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Abbreviations

FMN	flavin mononucleotide
GlcN6P	glucosamine-6-phosphate
SAM	*S*-adenosylmethionine
TPP	thiamine pyrophosphate
UTR	untranslated region

Introduction

Regardless which kingdom of life one examines, the notion that RNA is only a target and never an instigator of post-transcriptional genetic regulatory mechanisms represents a bygone era. For example, it has become increasingly evident that both prokaryotic and eukaryotic organisms possess a multitude of endogenous small RNA transcripts that function as modulators of gene expression through antisense-mediated mRNA recognition [1,2]. Although such *trans*-acting 'riboregulators' illustrate RNA's remarkably widespread role in establishing genetic regulatory circuits that are vital to cellular function, their effects are largely exerted through processes or pathways with requisite protein activities.

Within the past two years, a novel mode of RNA-mediated genetic regulation has been discovered that requires no auxiliary protein factor. Here, RNA serves as the sole molecular switch that modulates transcription, translation or RNA processing through conformational changes prompted by direct interaction with a specific cellular metabolite [3,4]. A stunning array of such 'riboswitches' have been characterized that respond to coenzyme B_{12} [5••,6–8], flavin mononucleotide (FMN) [9,10,11••], thiamine pyrophosphate (TPP) [11••,12•,13,14••,15], *S*-adenosylmethionine (SAM) [16•,17,18], lysine [19–23], guanine [24•], adenine [24•,25•] or glucosamine-6-phosphate (GlcN6P) [26••] (Table 1). Each of these *cis*-acting regulatory elements is largely found in the mRNAs of genes that comprise the biosynthetic pathway responsible for producing the cognate metabolite. Therefore, riboswitches afford an elegant mechanism for feedback regulation of biosynthetic pathways.

The biological importance of riboswitches is made evident by the fact that they are widely distributed and highly conserved among prokaryotes and, in the case of the TPP riboswitch, among certain eukaryotes [14••]. Furthermore, certain riboswitch classes are represented numerous times throughout a single organism's genome. For example, 69 genes in *Bacillus subtilis* appear to be under the control of any one of the eight known riboswitch elements [24•,26••], which corresponds to approximately 2% of the organism's genome. Consequently, riboswitches represent a frequently utilized form of genetic control that allows organisms to respond to the metabolic state of the cell. The purpose of this review is to examine the structural characteristics of each known riboswitch with regard to consensus sequence, molecular recognition, structural modulation and resulting effects on gene expression.

The architecture of riboswitches

To exert control over gene expression, riboswitches must couple the task of ligand recognition with that of modulating a requisite aspect of gene expression. Consequently, riboswitches are generally composed of two interdependent but otherwise distinguishable domains: a natural ligand-binding or aptamer domain [27] and an 'expression platform' [4] whose precise conformation impacts gene expression. Like most artificial aptamers, natural aptamers exhibit a property termed 'adaptive binding' [28], a type of induced fit whereby conformational change is concomitant with ligand interaction. Metabolite binding to the aptamer domain therefore provides the impetus for altering the conformation of the expression platform and gene expression.

Table 1

Properties of known riboswitches.

Riboswitch/metabolite	Apparent K_D	Representation	Proposed mechanism(s)	References
Adenosylcobalamin (coenzyme B_{12})	300 nM	Prokaryotes	Transcription termination Translation initiation	[5••,6–8]
TPP	100 nM	Prokaryotes Eukaryotes	Transcription termination Translation initiation RNA processing	[11••,12•,13,14••,15]
FMN	5 nM	Prokaryotes	Transcription termination Translation initiation	[9,10,11••]
SAM	4 nM	Prokaryotes	Transcription termination	[16•,17,18]
Guanine	5 nM	Prokaryotes	Transcription termination	[24•]
Adenine	300 nM	Prokaryotes	Transcription termination	[24•,25•]
L-lysine	1 μM	Prokaryotes	Transcription termination	[19–23]
GlcN6P	200 μM	Prokaryotes	RNA cleavage	[26••]

Natural aptamers, which range in length from 70 to 170 nucleotides, appear to be truly modular structures that retain the ability to bind ligand, and exhibit conformational changes in secondary and tertiary structure in the absence of an expression platform. Consequently, it is the aptamer domain sequence that is highly conserved among evolutionarily divergent organisms (Figure 1). By contrast, expression platforms vary in sequence, structure and mechanism, and usually affect prokaryotic gene expression through the formation of Rho-independent transcriptional terminators or through sequestration of the Shine–Dalgarno sequences required for translation initiation. In fact, expression platforms can vary among members of the same riboswitch class, as observed for TPP riboswitches [11••,12•,14••], underscoring the modularity and versatility of riboswitch aptamer domains.

Evidence of riboswitch function

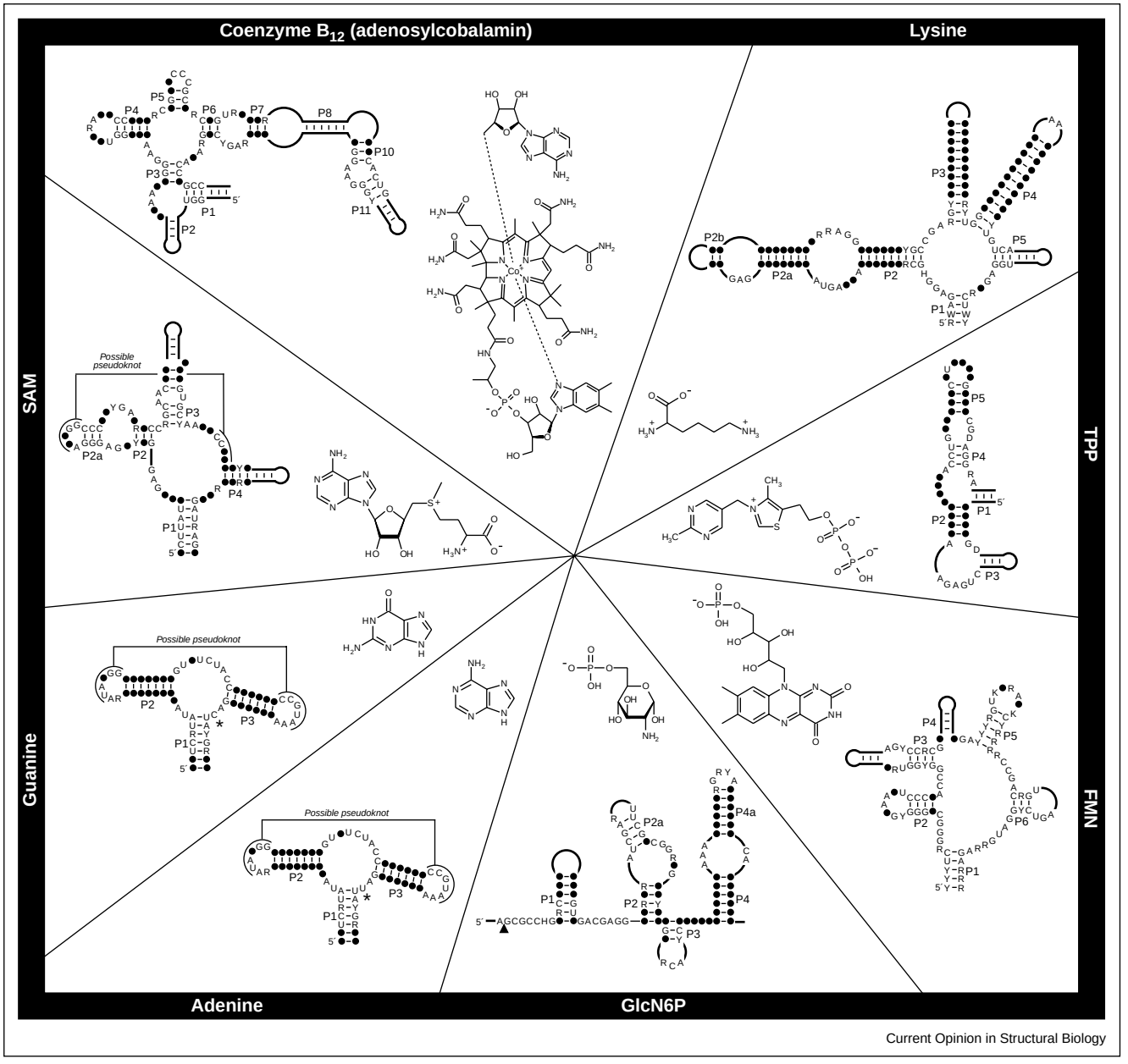
Each riboswitch aptamer class, excluding that which binds GlcN6P, had previously been recognized as a conserved RNA element required for proper metabolic regulation of associated genes [3]. Although extensive genetic and biochemical analyses of such RNA elements led to suggestions that metabolite might interact directly with RNA [13,29,30], evidence of direct binding has only been recently provided. Methodology for demonstrating metabolite interaction with riboswitch RNA has included the use of RNase H probing [11••,16•,17,21], 'in-line' probing [5••,8,9,12•,14••,18,22,24•,25•,26••] and equilibrium dialysis [5••,12•,18,22,24•,25•]; the latter two techniques have been most widely applied. In-line probing monitors the spontaneous cleavage of each phosphodiester bond in a structured RNA, whereby the rate of cleavage is dependent upon conformation and flexibility [31]. For example, internucleotide linkages in base-paired regions are structurally constrained, so as to preclude the in-line conformation required to achieve cleavage via transesterification, and therefore exhibit relatively low rates of strand scission. In-line probing of riboswitch RNA in both the absence and presence of metabolite reveals conformational changes that ensue from ligand interaction as changes in the observed cleavage pattern. Thus, in-line probing provides evidence of both metabolite binding and conformational changes that might affect gene expression. Direct binding of metabolites to their cognate riboswitch aptamers has been further corroborated through equilibrium dialysis experiments. Such studies have revealed the exquisite specificity and affinity of each riboswitch aptamer for its cognate metabolite.

Molecular recognition by riboswitch aptamers

Most riboswitch aptamers possess an affinity and specificity for their ligands that rival the precision of small molecule–protein interactions. With regard to affinity, most riboswitch aptamers exhibit apparent dissociation constants (K_D) for their ligands in the range from low nanomolar to low micromolar (Table 1). One exception is the GlcN6P riboswitch, which exhibits an apparent K_D of ~200 μM, but begins to respond to GlcN6P at concentrations as low as 200 nM [26••]. With regard to specificity, the use of metabolite analogs has revealed the exquisite sensitivity of riboswitch aptamers in molecular recognition.

For example, the B_{12} aptamer discriminates against analogs that lack the 5′-deoxy-5′-adenosyl moiety, modify the N1, N3 and N6 of the adenosyl moiety, or alter the stereochemistry of the corrin ring [5••]. The TPP aptamer favors binding of TPP 1000-fold over binding to thiamine phosphate or thiamine, and analogs of thiamine further interfere with molecular recognition [12•]. The FMN aptamer exhibits 1000-fold discrimination against riboflavin, which lacks a phosphate group [9]. The SAM aptamer discriminates against analogs that modify the methionine or 5′-deoxy-5′-adenosyl moieties, as demonstrated by reduced binding of *S*-adenosylhomocysteine, which lacks a single methyl group and a positive charge [18]. The lysine aptamer binds stereospecifically to L-lysine, whereby contacts are made to each amino and carboxy group [22]. Furthermore, the aptamer forms a highly discriminating binding pocket that can sense the length and character of the alkyl sidechain.

Figure 1



The known riboswitch aptamers. The consensus sequence and secondary structure of each aptamer are depicted with its corresponding metabolite. Nucleotide identities that are more than 80–90% conserved among representative sequences are shown, where D designates A, G or U; H designates A, C or U; K designates G or U; R designates A or G; W designates A or U; and Y designates C or U. Filled circles represent positions of variable nucleotide sequence and dashes indicate base pairing. Thick lines denote regions of variable nucleotide sequence and length. For the guanine and adenine aptamers, the nucleotide affecting metabolite specificity is denoted with an asterisk.

The guanine and adenine aptamers are identical except for a single nucleotide difference to which they owe their ligand specificity (Figure 1) [24•,25•]. A cytidine in the guanine aptamer versus a uridine in the adenine aptamer consensus sequence suggests that each aptamer recognizes its ligand in part by forming a Watson–Crick base pair. However, alteration of almost any functional group on the purine heterocycle causes a substantial loss of binding affinity, indicating that the entire ligand interfaces with the RNA.

The GlcN6P riboswitch breaks the mold of typical riboswitches in that it is also a ribozyme [26••]. The RNA performs self-cleavage, exhibiting a 1000-fold rate enhancement in the presence of GlcN6P. The riboswitch also exhibits excellent specificity, as analogs including

glucose-6-phosphate and glucosamine do not stimulate accelerated cleavage. Presently, it is unclear what precise role GlcN6P plays in catalysis. However, it is likely that the GlcN6P-dependent ribozyme represents a natural form of previously engineered allosteric ribozymes [32].

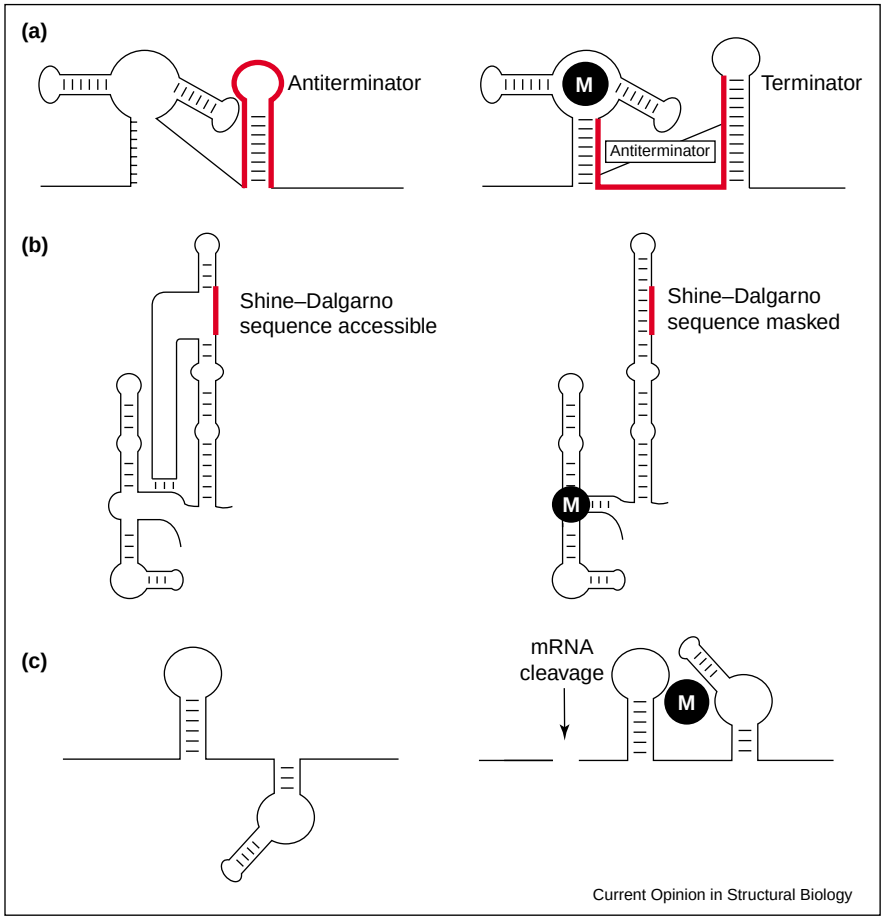
An interesting commonality among certain riboswitches is their recognition of metabolite phosphate groups. The TPP, FMN and GlcN6P riboswitches create productive binding interactions with negatively charged phosphate moieties despite the fact that RNA itself is a polyanion. Whether RNA might accomplish this task through a common motif is one of many details that further structural investigations will reveal.

Conformational change drives riboswitch function

The riboswitch aptamer must communicate with the expression platform to affect gene expression and it is the conformational change that ensues from ligand interaction that thus drives riboswitch function. Often times, probing reveals the more precise mechanisms of gene regulation. For example, RNase H probing of the FMN riboswitch demonstrated that metabolite binding modulates the formation of an antiterminator stem [11••] and in-line probing of the TPP riboswitch shows that metabolite binding reduces the conformational flexibility of the Shine–Dalgarno sequence [12•]. Such studies illustrate the major expression platforms available to riboswitches: regulation of transcription termination or translation initiation.

Regulation of transcription termination is utilized by nearly every riboswitch class (Table 1) and typically involves metabolite-dependent formation of a terminator stem, which prevents transcription elongation and inhibits gene expression (Figure 2a). One exception is the adenine riboswitch, wherein metabolite binding prevents terminator stem formation and activates gene expression. Regulation of translation initiation is less widely utilized (Table 1) and involves altering the accessibility of the Shine–Dalgarno sequence (Figure 2b). In this case,

Figure 2



Mechanisms of riboswitch function. **(a)** Transcription termination induced by metabolite (M) binding to nascent RNA, as observed for a guanine riboswitch. **(b)** Translation initiation modulated by metabolite-dependent sequestration of a Shine–Dalgarno sequence, as observed for a TPP riboswitch. **(c)** RNA processing regulated by metabolite-dependent self-cleavage, as observed for a GlcN6P riboswitch.

metabolite binding masks the Shine–Dalgarno sequence within secondary structure to prevent ribosome binding and inhibit gene expression. Interestingly, riboswitches in Gram-negative bacteria seemingly prefer regulation of translation initiation, whereas Gram-positive bacteria favor transcription termination, a correlation that probably reflects the higher frequency of polycistronic genes in Gram-positive bacteria [3,4].

A third expression platform that can be utilized by riboswitches to affect gene expression is regulation of RNA processing events. A conceptually simplistic manifestation of this expression platform is represented by the GlcN6P riboswitch, for which ligand binding induces catalytic self-cleavage of the mRNA and inhibition of gene expression (Figure 2c) [26••]. However, it seems unlikely that the aptamer and expression platform (ribozyme) are separable functionalities, as they are for other riboswitches. Interestingly, the discovery of TPP riboswitches in eukaryotic genes has unveiled other possibilities for riboswitch control of RNA processing [14••,15]. For example, the presence of TPP aptamers within introns or 3′ untranslated regions (UTRs) suggests that riboswitches might regulate splicing or 3′ end formation, respectively.

Conclusions

Riboswitches represent a facile means for cells to monitor their metabolic state and alter gene expression in response to changing conditions. Riboswitches exhibit exceptional specificity and affinity in molecular recognition, and remarkable versatility with regard to how conformational changes are utilized to modulate gene expression. The conservation and distribution of riboswitch aptamers among evolutionarily divergent organisms suggest that the origin of at least one motif (the TPP aptamer) predates the evolutionary split of prokaryotic and eukaryotic organisms some 1.5 billion years ago. Regardless of whether riboswitches actually represent held-over components from a hypothesized RNA world [4,14••], it is certainly clear that contemporary biology has made good use of this paradigm in genetic control. Possibly, riboswitches continue to afford modern organisms some measure of genetic streamlining by precluding the evolutionary and metabolic expense of manufacturing other regulatory mechanisms that include protein. The advantage of such streamlining to microorganisms that must often flourish with meager resources might explain the prevalence of riboswitches among prokaryotes. Furthermore, it is interesting to note that a diversity of stimuli can affect RNA-mediated genetic control, as tRNA-responsive [33] and thermoregulated [34] RNA switches that modulate gene expression have been characterized. Consequently, roles for RNA receptors as genetic regulatory elements might be more widespread than the present collection of riboswitches suggests.

As the identification of riboswitches seems to have exhausted the known set of metabolically related RNA elements, bioinformatics approaches will undoubtedly aid the identification of novel riboswitch motifs. This has already been the case in the identification of the GlcN6P riboswitch, which was identified as a conserved sequence element within intergenic regions preceding a gene involved in GlcN6P synthesis [26••]. However, a considerable challenge for future riboswitch candidates might be the identification of ligands that are not intuitively obvious.

Although the foundation for riboswitch function via conformation change has been firmly laid, each riboswitch motif uniquely solves a molecular recognition problem. Thus, in riboswitches resides a wealth of information regarding the intricacies of RNA structure and ligand interaction waiting to be explored through further biochemical and biophysical analyses.

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