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The intricate world of riboswitches

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Abstract

Riboswitches are segments of the 5'-untranslated region of certain bacterial mRNAs that upon recognition of specific ligands modify the expression of a protein(s) encoded in the message. These proteins are responsible for the biosynthesis or transport of ligands, which are typically organic molecules but could also be metal ions. Riboswitch-mediated control of gene expression might be thermodynamic or kinetic, depending on the rate of transcription elongation by RNA polymerase and the structures adopted by the riboswitch RNA. Certain 5'-untranslated regions harbor two riboswitches in tandem that bind to different ligands. Thus, RNA sensors can respond to metabolic changes by modifying gene expression in ways previously thought to be exclusive of proteins.

Introduction

Most organisms have the ability to change their gene expression patterns in response to internal and external signals. This requires the presence of sensors whose function is to detect the levels of particular metabolites, and to alter the levels of proteins responsible for maintaining physiological levels of those metabolites. Whereas the sensing and regulation have been traditionally ascribed to proteins, it is becoming increasingly clear that these activities can be carried out by RNAs.

These RNA sensors, designated 'riboswitches', are part of the 5'-untranslated region (UTR) of selective mRNAs and have been identified in all three life domains [1,2]. Riboswitches function as sensors by binding to particular ligands and modifying the expression of biosynthetic and transport proteins for those ligands. Among the ligands that bind to specific riboswitches are amino acids (e.g. lysine), nuclear bases (e.g. guanine and adenine), and sugars (e.g. glucosamine-6-phosphate). Ligand-binding results in structural changes in the riboswitch that affect the ability of RNA polymerase to continue the process of transcription elongation through the formation of intrinsic transcription terminator structures (or in one, case cleavage of the mRNA), or the ability to translate an mRNA by sequestration of the ribosome binding site. This can result in turning a gene on or off.

To date, the best-characterized riboswitches consist of two domains: an aptamer region that is involved in ligand binding, and an expression platform that is responsible for bringing about the changes in gene expression (Figure 1). Studies of the crystal structure of the aptamer region are providing the first glimpses of both the changes resulting from ligand binding and the specificity with which aptamers can discriminate among chemically related ligands such as guanine and adenine [3*,4,5*,6,7*,8,9]. Moreover, it has enabled the construction of artificial riboswitches exhibiting predictable behaviors and having potential biotechnological

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applications [10]. However, recent data indicate that certain riboswitches lack a modular structure with discrete aptamer and expression domains. By contrast, these two domains seem to have merged to mediate ligand binding (Figure 1c) [11^{**}], indicative that evolution has produced different solutions to the problem of coupling ligand recognition by RNA to changes in gene expression.

In this review, articles published since January 2005 are discussed, including the identification and characterization of novel riboswitches with a particular emphasis on Mg^{2+} as primary ligand and its function in riboswitches that bind organic molecules, and the role that transcription elongation rate plays in determining the thermodynamic versus kinetic control of riboswitch-regulated transcripts. Readers are referred to several recent reviews for other aspects of riboswitches [1,2,12–14].

New riboswitch architectures

The vast majority of riboswitches sense single metabolites to modulate gene expression. However, Breaker's laboratory [15^{**}] has now provided the first examples of mRNAs harboring two riboswitches in tandem in their 5'-UTR regions, each responding to a different metabolite (Figure 1). They demonstrated that the *metE* mRNA from *Bacillus clausii* has, in its 5'-region, two distinct riboswitches: one responding to S-adenosylmethionine and another responding to coenzyme B₁₂ [15^{**}]. Because these two ligands can independently repress *metE* expression, the tandem riboswitch arrangement is proposed to function as a Boolean NOR gate that inhibits the production of the MetE protein whenever S-adenosylmethionine or coenzyme B₁₂ are present [15^{**}]. The ability to control *metE* expression in response to these two metabolites is conserved in *E. coli*, but through the 'traditional' protein binding to S-adenosylmethionine and coenzyme B₁₂. In addition, the Breaker laboratory reports the results of a genomic analysis predicting the existence of other mRNAs with tandem riboswitches, some of which are suggested to respond to different (undefined) ligands. These results indicate that extant bacterial species often rely on sophisticated RNA-based sensors to integrate multiple signals into the decision to express particular gene products, which had been the province of protein molecules.

Role of Mg^{2+} in riboswitch function and as a primary riboswitch ligand

The divalent cation Mg^{2+} plays a critical role in the structure and function of many RNAs [16–18]. Indeed, Mg^{2+} has been shown to stabilize RNA tertiary structures under conditions that only weakly affect the stability of the RNA secondary structure [19]. This begs the question: what is the role that Mg^{2+} plays in riboswitch function?

Gilbert *et al.* [20^{*}] investigated the effect that Mg^{2+} has on hypoxanthine binding to the guanine riboswitch and determined that increased Mg^{2+} levels enabled tighter metabolite binding. Taken together with structural data, this led to the proposal that the RNA aptamer for the guanine riboswitch must undergo a degree of organization before the metabolite can bind, and that this would be followed by a substantial conformational change that completely encloses the metabolite as is observed in the crystal structure. Gilbert *et al.* [20^{*}] went on to develop a two-state model for metabolite binding where only a fraction of the ensemble of riboswitch conformations is competent for metabolite binding. Metabolite binding then leads to a reorganization of the binding pocket in the guanosine riboswitch [20^{*}]. Similarly, through single-molecule fluorescence resonance energy transfer (FRET), Lemay *et al.* [21] observed discrete folding intermediates for the adenine riboswitch, supporting a model in which the aptamer region adopts multiple conformations in its pathway to become competent for ligand binding. Additionally, Yamauchi *et al.* [22] observed that increased levels of Mg^{2+} contributed to stronger metabolite binding by the thiamine pyrophosphate (TPP) riboswitch.

The *glmS* riboswitch present in certain Gram-positive bacteria is unique in several ways. First, it is the only riboswitch described to date in which binding to its specific ligand (glucosamine 6-phosphate) stimulates a self-cleaving ribozyme activity targeting its own mRNA [23]. Second, glucosamine 6-phosphate appears to bind to a pre-folded active site pocket in the RNA, suggesting that it acts as a coenzyme in the self-cleavage reaction [7*,24], which is in contrast to other riboswitches that act by inducing structural changes in the RNA. And third, Mg^{2+} only plays a structural role in the *glmS* ribozyme function and is not required for catalytic activity [25].

Mg^{2+} has been shown to be the primary ligand of a riboswitch located in the 5'-UTR of the mRNA for the MgtA Mg^{2+} uptake protein of the Gram-negative bacterium *Salmonella enterica* (Figure 1) [26**]. The DNA corresponding to the 264 nucleotide *mgtA* riboswitch confers Mg^{2+} regulation when cloned in front of a reporter gene and behind a derivative of the *lac* promoter. The *mgtA* riboswitch has the ability to adopt two alternative stem-loop structures: one that is favored in low Mg^{2+} concentrations and promotes transcription elongation into the *mgtA* coding region, and another that is favored in high Mg^{2+} concentrations and blocks elongation into the *mgtA* coding region [26**]. Unlike other riboswitches that affect transcription termination, an obvious Rho-independent transcription terminator was not identified in the *mgtA* riboswitch. This raises the question as to the mechanism preventing elongation into the coding region given the fact that the Rho protein was not necessary for Mg^{2+} -regulated transcription termination *in vitro*.

A distinctive aspect of the *mgtA* riboswitch is that its role as a sensor for cytoplasmic Mg^{2+} levels is superimposed onto the regulation of *mgtA* transcription initiation, which is controlled by a Mg^{2+} -responding protein-based regulatory system that senses the extracytoplasmic levels of Mg^{2+} [27]. This provides a singular example where the same ligand acts in two different cellular compartments to regulate different steps in gene transcription. That a truncated *mgtA* transcript is produced at intermediate Mg^{2+} concentrations raises the intriguing possibility of the truncated transcript functioning *in trans* by modifying the expression of genes other than *mgtA*.

Riboswitch mechanisms: kinetic versus thermodynamic control

The name 'riboswitch' carries with it the implication of a binary ON/OFF switch, and this concept has been applied to describe the effect of metabolite binding on riboswitch structure. Simply put, binding of the metabolite induces a conformational change of the mRNA, switching it from a conformation that favors expression of the encoded proteins to one that does not. In the case of riboswitches that operate by mechanisms in which binding of a metabolite alter the potential for base pairing of an anti-Shine–Dalgarno region with the Shine–Dalgarno region (i.e. the ribosome binding site), translation of the mRNA might dynamically respond to fluctuating levels of the metabolite by adopting one or another structure. However, the situation might be quite different for those riboswitches that function by affecting the ability of RNA polymerase to either proceed with transcription elongation or to terminate transcription. This is because an elongating mRNA that includes a riboswitch is capable of adopting many conformations as it becomes free of the RNA polymerase machinery. Of these conformations, only a fraction is competent to bind a metabolite and thus to become subjected to its regulation (Figure 2).

Metabolite binding can be controlled kinetically or thermodynamically. In essence, kinetic control occurs when the rate of metabolite binding to the RNA is rapid relative to the rate of riboswitch folding. By contrast, thermodynamic control occurs when the riboswitch structure formed upon metabolite binding is stabilized relative to other possible mRNA conformations. Another way to think of these two alternative mechanisms is in terms of competition:

kinetically, the rate of riboswitch folding competes with the time required for the aptamer region and its ligand to reach thermodynamic equilibrium. To drive formation of the metabolite-bound form of the riboswitch, the concentration of metabolite must exceed its dissociation constant. Therefore, the kinetics of RNA-folding compete (temporally) with ligand binding kinetics, whereas the thermodynamics of RNA folding compete (energetically) with the thermodynamics of ligand binding.

In addition to the role played by RNA folding, kinetic and thermodynamic control of riboswitches depends on the rate of transcription elongation by RNA polymerase. The speed at which RNA polymerase synthesizes an mRNA is dependent on several factors, including the concentrations of nucleotide triphosphates, Mg^{2+} ions, and the relevant transcription factor (s). Moreover, the sequence of the DNA template can also contribute to the speed of transcription because pause sites (i.e. certain DNA sequences and RNA secondary structural features) can cause RNA polymerase to briefly halt the transcription process [28]. These pauses have been shown to allow extra time for riboswitches to bind their specific metabolites and, thus, effectively control gene expression [29**]. Depending on the amount of time 'gained' at pause sites, the speed with which RNA polymerase elongates the mRNA can determine whether a riboswitch is kinetically or thermodynamically controlled.

The distinction between kinetic and thermodynamic control of riboswitches was initially made by Wickiser *et al.* [29**] who observed that the dissociation constant for metabolite binding to the flavin mononucleotide (FMN) aptamer region was inconsistent with the metabolite concentration required to exert control *in vivo*. When compared to the speed of transcription elongation alone, FMN binding to the riboswitch was slower than the time needed by RNA polymerase to reach its decision point for read-through/termination. However, RNA polymerase pausing provided the extra time required for FMN-binding to take place. Because the dissociation rate for the metabolite-RNA complex is slow, FMN-binding essentially commits the mRNA to transcription termination. Examination of the adenine riboswitch has revealed that the adenine association rate constant is slower than that of FMN, but that the dissociation rate constant is more rapid [30**]. The lifetime of the metabolite-RNA complex was estimated to reach equilibrium after 15 s, which is on the same time scale as that required for RNA polymerase to traverse the distance from the aptamer region to the expression platform where a functional decision must be made. Therefore, the adenine riboswitch is a candidate for either kinetic or thermodynamic control, depending on cellular factors contributing to the speed of transcription.

Pausing provides a window for RNA folding as well as metabolite binding, and the two could be coupled if binding stabilizes one riboswitch conformation over others. As discussed above for the adenine riboswitch, pause sites could slow transcription enough to facilitate thermodynamic control of the transcription termination decision. In the case of the Mg^{2+} -dependent *mgtA* riboswitch [26**], pausing could facilitate interconversion between alternative structures if the rates of the conformational transitions are of the same order as the pause. Gilbert *et al.* [20*] proposed that in the population of actively transcribed mRNAs, only a fraction is pre-organized in a conformation that is competent to bind its ligand. Therefore, a process that enables conformational rearrangements to occur, especially in the presence of Mg^{2+} ions that can influence the tertiary structure of an mRNA, could lead to more efficient ligand binding and hence, heightened gene expression control by the riboswitch.

Conclusions

The spectrum of metabolites that can be recognized by riboswitches has been expanded to include the metal ion Mg^{2+} , which governs the expression of a Mg^{2+} transporter. It is likely that, as suggested by computational predictions and genetic experiments, additional cation-

responding riboswitches will be uncovered. Moreover, the discovery of RNA sensors consisting of two riboswitches in tandem illustrates the complex type of input that can be provided by RNA molecules. Finally, the effective riboswitch control of gene expression will probably be determined by a delicate balance among the rates with which RNA folds, those with which a metabolite binds to the riboswitch, and the speed with which RNA polymerase transcribes its template.

Update

It is generally agreed that Mg^{2+} ions contribute to the co-transcriptional folding of riboswitches. However, the extent of that contribution appears to vary with each riboswitch. As described in the text, the purine and TPP riboswitches seem to require Mg^{2+} for metabolite binding. Indeed, Lipfert *et al.* [31**] published a three-state thermodynamic model for the glycine riboswitch, where millimolar concentrations of Mg^{2+} lead to compactation and partial folding, allowing the riboswitch to bind glycine cooperatively [31**]. Yet, NMR experiments carried out by Noeske *et al.* demonstrated that Mg^{2+} ions are not essential for metabolite binding, and only appear to further stabilize a pre-formed structure at higher temperatures [32*].

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Figure 1. Schematic structure of traditional riboswitches **(a)**, which bind single metabolites, and of new riboswitches **(b)** that can harbor tandem aptamers recognizing different metabolites, **(c)** where there are no distinct aptamer and expression platform domains, **(d)** and where Mg^{2+} is the physiological ligand. Aptamer regions are in red and expression platforms in blue.

Figure 2.

Flow chart depicting the fate of an elongating mRNA that harbors a riboswitch with a transcription termination mechanism. The aptamer region, synthesized first, is depicted as a blue square. The open shape inside the square represents the metabolite binding pocket. The different shapes reflect various folded conformations of the aptamer, only one of which is competent for binding its ligand. The genetic outcome is determined at two key intersections (highlighted boxes). In order for riboswitch to exert control, both of these events must take place before the polymerase reaches the coding region of the mRNA. Metabolite binding via kinetic or thermodynamic control leads to formation of an intrinsic terminator. Absence of binding prevents formation of the terminator and allows for read-through and expression.