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The long and the short of riboswitches

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Regulatory mRNA elements or riboswitches specifically control the expression of a large number of genes in response to various cellular metabolites. The basis for selectivity of regulation is programmed in the evolutionarily conserved metabolite-sensing regions of riboswitches, which display a plethora of sequence and structural variants. Recent X-ray structures of two distinct SAM riboswitches and the sensing domains of the Mg^{2+}, lysine, and FMN riboswitches have uncovered novel recognition principles and provided molecular details underlying the exquisite specificity of metabolite binding by RNA. These and earlier structures constitute the majority of widespread riboswitch classes and, together with riboswitch folding studies, improve our understanding of the mechanistic principles involved in riboswitch-mediated gene expression control.

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Introduction

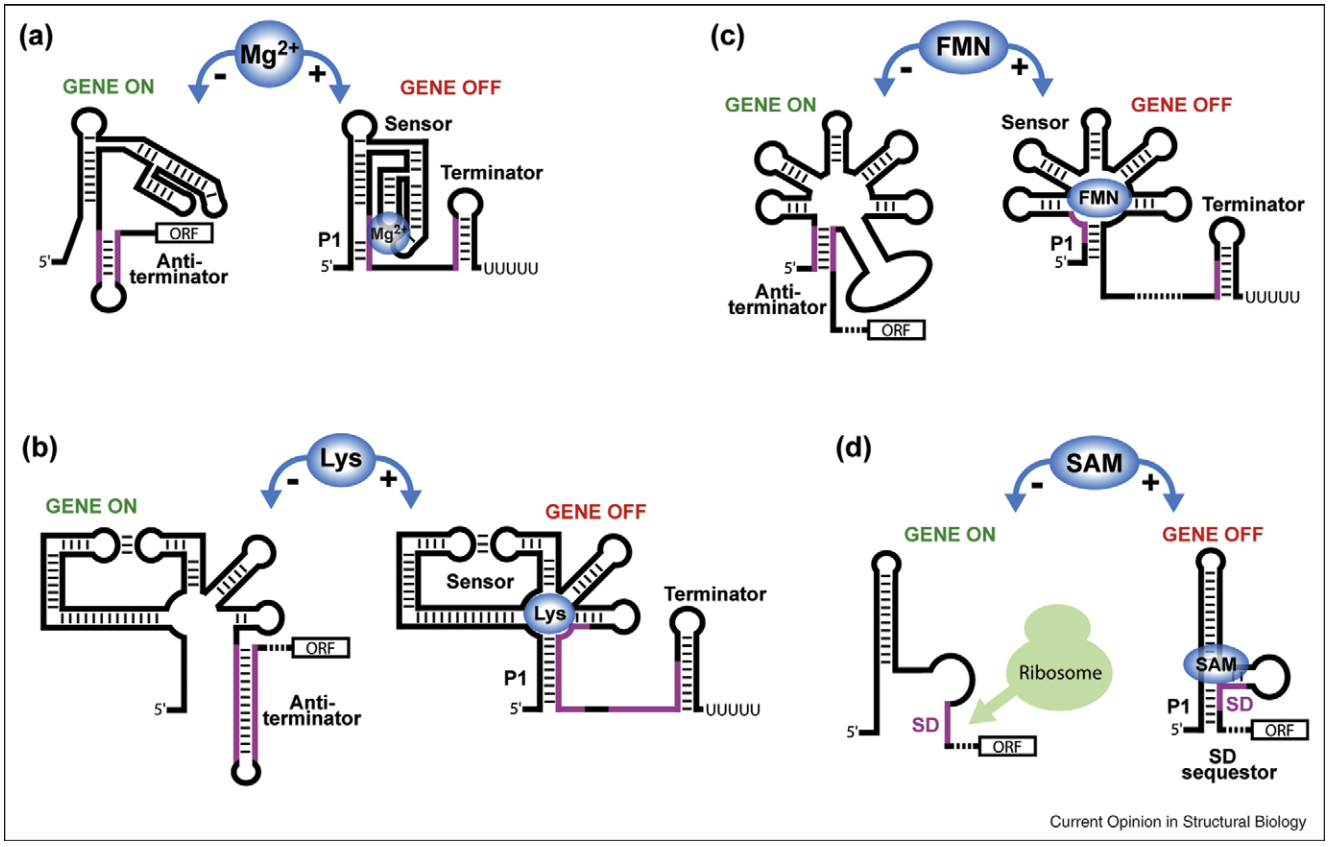
Since the discovery of riboswitches and related regulatory mRNA elements, it has become evident that the sensing and gene controlling functions traditionally attributed to proteins can be performed by RNAs [1,2]. Recent intensive biochemical and biophysical research has increased the appreciation of riboswitches as universal genetic factors that adjust gene expression in response to various chemical cues in evolutionarily diverse species [3,4]. The adaptation of protein biosynthesis to environmental conditions is achieved by riboswitches through the direct sensing of small-molecule metabolites present in cells at elevated concentrations. Most known riboswitches are located in the 5′-untranslated regions of bacterial genes associated with cognate metabolites and are involved in regulating gene expression at the levels of transcription attenuation and translation initiation. The regulation is typically based on the interplay between alternative conformations within an evolutionarily conserved metabolite-sensing domain and a variable expression platform bearing gene control elements (Figure 1). In some bacteria, the riboswitch-based feedback regulatory circuits exert control over a significant portion of the genome, competing in number with metabolite-sensing regulatory proteins [3]. Several thiamine pyrophosphate (TPP) sensing riboswitches have been also found in fungal and plant genes, where they modulate mRNA splicing and stability [5].

To date, riboswitches encompass more than a dozen classes specific to various types of metabolites. Not surprisingly, the metabolite-sensing domains of riboswitches demonstrate high diversity in composition, size, and complexity of their secondary and tertiary structures [6]. Principles underlying the exquisite selectivity of metabolite recognition became better understood after determination of the three-dimensional structures of guanine-, adenine-, TPP-, and *S*-adenosylmethionine (SAM)-sensing domains [7–11] and of the *glmS* riboswitch/ribozyme [12,13]. During the past two years, this list has been extended by five new riboswitches whose X-ray structures range from simple conformations, such as a small pseudoknot, to large multi-helical folds. These recent structural advances along with studies of molecular mechanisms of riboswitch function will be primarily discussed herein, thus complementing earlier reviews on mRNA recognition by metabolites and proteins [14,15].

The expanding world of riboswitches

After 2006, several new classes have been added to the existing collection of riboswitches responsive to adenine, guanine, TPP, SAM, flavin mononucleotide (FMN), adenosylcobalamin, lysine, glycine, and glucosamine-6-phosphate (GlcN6P) [3]. The most important discovery was the identification of the riboswitch specific for the second messenger cyclic di-guanosine monophosphate (di-GMP) in bacteria and bacteriophages [16••]. Cyclic di-GMP-sensing allows for the RNA-based control of genes that are responsible for wide-ranging physiological transformations within bacterial cells and are not directly involved in the metabolism of the compound. Furthermore, the riboswitch family receptive to purines and purine derivatives was expanded by two variants of the queuosine precursor $preQ_1$ sensor [17,18•], the shortest known riboswitch that controls biosynthesis of the hypermodified nucleoside present in certain tRNAs, and by the 2′-deoxyguanosine sensor [19] that deviates from the classical adenine/guanine riboswitch sequences. In addition, SAM metabolism, dependent on five riboswitch variants in different bacteria [20], has been found to rely

Figure 1



Gene expression control by various riboswitches. Transcriptional attenuation mechanism in the M-box Mg^{2+} sensor **(a)**, lysine riboswitch **(b)**, and flavin mononucleotide (FMN) riboswitch **(c)**. In the absence of ligand, mRNA forms an anti-terminator helix and transcription can proceed through the open reading frame (ORF). In the presence of ligand, the sensing domain binds the cognate metabolite, thereby stabilizing the P1 helix, triggering formation of a transcription terminator in the expression platform, and turning off expression of the downstream gene. Complementary anti-terminator sequences are shown in magenta. **(d)** Regulation of translation initiation by the type III *S*-adenosylmethionine (SAM) riboswitch. Without ligand, Shine–Dalgarno (SD) sequence is accessible for ribosome entry and translation initiation. In the presence of SAM, SD sequence is paired, thereby preventing ribosome binding.

on yet another type of mRNA segment targeted by *S*-adenosylhomocysteine (SAH) [21]. Lastly, the first metallosensor in *Salmonella enterica mgtA* mRNA [22] has been complemented by the findings of a different magnesium sensor [23••] and RNA motifs triggered by molybdenum and related tungsten cofactors Moco and Tuco [24].

New riboswitch architectures

Despite the continuous success in identification of new riboswitch classes, the determination of riboswitch structures remains a tedious and time-consuming process. However, recent structural studies have succeeded in the determination of challenging riboswitch structures, varying in size and complexity.

Intriguingly, one of the large ribosensors, called M-box, is specific for Mg^{2+} cations, one of the smallest and most abundant cellular components [23••]. The sensor controls Mg^{2+} homeostasis in *Bacillus subtilis* by attenuating the transcription of the Mg^{2+} transporter gene *mgtE* (Figure 1a). The crystal structure (Figure 2a) revealed that the M-box architecture comprises a short stem-loop P6 and two parallel composite stems P1–P2 and P3–P4 connected by a three-way junction. The P3–P4 helix, from which stem P5 branches off, forms a closely packed three-helical bundle P4/P5/P1–P2 stapled together by extensive tertiary contacts involving J1/2, L4, and L5 regions. Unlike in other riboswitches, Mg^{2+} cations play a central role in folding the bridging region and mediating long-distance interactions that stabilize base-pairing in the J1/2 region and in the adjacent P1 helix, thereby inducing the formation of the transcription terminator.

Since the structures of the M-box and other riboswitches are primarily built around three-way or four-way helical junctions, they are difficult to use for characterization of more complex riboswitches, such as lysine and FMN riboswitches (Figure 1b,c), thereby demanding their