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Bacterial riboswitches and RNA thermometers: Nature and contributions to pathogenesis

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ABSTRACT

Bacterial pathogens are always challenged by fluctuations of chemical and physical parameters that pose serious threats to cellular integrity and metabolic status. Sudden deprivation of nutrients or key metabolites, changes in surrounding pH, and temperature shifts are the most important examples of such parameters. To elicit a proper response to such fluctuations, bacterial cells coordinate the expression of parameter-relevant genes. Although protein-mediated control of gene expression is well appreciated since many decades, RNA-based regulation has been discovered in early 2000s as a parallel level of regulation. Small regulatory RNAs have emerged as one of the most widespread and important gene regulatory systems in bacteria with rare representatives found in Archaea and Eukarya. Riboswitches and thermosensors are *cis*-encoded RNA regulatory elements that employ different mechanisms to regulate the expression of related genes controlling key metabolic pathways and genes of temperature relevant proteins including virulence factors. The extent of RNA contributions to gene regulation is not completely known even in well-studied models such *E. coli* and *B. subtilis*. In depth understanding of riboswitches is promising for opportunity to discover a narrow spectrum antibacterial drugs that target riboswitches of essential metabolic pathways.

1. Introduction

Shifting of pathogenic bacterial species from an environmental niche to a living host or vice versa is a challenging condition to acclimate to. Changes in nutrients availability, pH, and temperature are the most important parameters to be monitored constantly. Dramatic changes of these parameters may lead to deleterious effects on cellular physiology or resources wasting in synthesis of already available metabolites. To prevent such problems, bacteria have established a panel of signaling networks to coordinate gene expression programs to respond to its surroundings. Proteins play the major role in signals sensing and mounting the regulatory responses by acting as signal receivers at membrane sensors and cytoplasmic effector molecules activate or repress transcription of relevant genes. It is well-appreciated that proteins coordinate gene expression at various phases, at transcription, translation, or post-translational levels. For two decades, bacterial RNA-based regulatory strategies are being discovered in accelerating fashion (reviewed elsewhere [1–3]). A plethora of RNA elements were found not to encode for proteins or proteins synthesizing machinery, but to execute regulatory functions in controlling gene expression. Such non-coding RNA elements reside in the intergenic regions of open reading frames (ORFs).

Regulatory RNAs are classified into two major categories; *cis*-encoded elements which are located mostly upstream genes they regulate and *trans*-encoded elements that are transcribed from other locations in the genome. Pathogenic lifestyle of some bacterial species prioritized the sensing and responding to changes of physical and chemical signals especially key metabolites and temperature to avoid synthesizing available metabolites or proteins in the absence of their substrates. Riboswitches and RNA thermometers (RNATs) are regulatory elements contained within the 5′-untranslated region (5′-UTRs) of bacterial mRNA transcripts for genes they regulate at the transcriptional and translational levels. The tertiary structure of such leader sequences are formed or

Abbreviations: FMN, Flavin mononucleotide; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; SD, Shine-Dalgarno; AdoCbl, adenosylcobalamine; RNAP, RNA polymerase; RNAT, RNA thermometer; TPP, Thiamine pyrophosphate; RBS, Ribosomal Binding Site; ORFs, open reading frames; 5′-UTRs, 5′-untranslated region; CSPs, Cold Shock Proteins.

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disrupted in response to chemical or physical signals which lead to activation or inhibition of downstream genes [4]. Such RNA regulators exert their regulatory effects without obligate involvement of other factors. It is hard to accept as true that the number of riboswitches in *Bacillus subtilis* outpaces the number of validated metabolite-binding proteins coordinating gene expression [5].

Riboswitches participate in regulation of diverse cellular physiologies and their ligands range from diverse metabolites of different molecular weights to uncharged tRNAs and ions. Such regulatory RNA elements can sense a plethora of cellular metabolites such as amino acids and their derivatives [lysine, glycine, S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH)], carbohydrates [glucosamine6-phosphate (Glcn6P)], coenzymes [flavin mononucleotide (FMN), thiamin pyrophosphate (TPP), coenzyme B12], nucleobases and their derivatives (adenine, guanine, cyclic di-GMP, cyclic di- AMP) [6,7], metal ions (Magnesium, Nickel, and Cobalt) [8,9], uncharged tRNA [10] and pH [11]. Temperature is another physical parameter that also found to be monitored by RNA elements called RNA thermometers (RNATs), which are considered by many authors as riboswitches [12,13].

The diversity of ligands and sensing RNA sequences has been exploited as criteria to classify riboswitches into currently ~40, different classes [14]. Indeed, not only the exact number of riboswitches classes is unknown, but also rough estimation is difficult to draw even in completely sequenced bacterial genomes [5]. Each class of riboswitches has a high degree of conserved nucleotides comprising the sensory domains in different bacterial species or, in some instances, among riboswitch variants of the same class in the same species. Bioinformatic studies and high throughput sequencing approaches accompanied by biochemical and genetic characterization continued to reveal the complexity and diversity of RNA-based gene regulation in various bacterial genomes.

The aim of this review is to discuss the nature and characteristics of riboswitches and RNATs reported in bacterial pathogens (overt or opportunistic) and their regulatory contributions to pathogenesis to appreciate the roles and importance behind such elements to bacterial cell physiology. Roles of other ncRNA elements in virulence and pathogenicity are excellently reviewed elsewhere [15–18].

2. Riboswitches

2.1. Structure and secondary foldings

Bacterial riboswitches reside mostly at the 5′ untranslated regions (UTRs) of metabolic and transport genes which they regulate in *cis*-fashion after direct binding of a specific metabolite ligand [19–23]. Typical riboswitch sequence contains two functional domains, the aptamer and the expression platform with a region of overlap called switching sequence between the two domains [24]. Folding of the aptamer into distinctive secondary and tertiary structures, result in scaffolding of the ligand docking site. The expression platform responds to ligand-induced folding at the aptamer region by adopting the functioning structure which interfaces with the transcription or translation processes of downstream sequences to elicit a regulatory response.

After synthesis of aptamer, it undergoes a folding pathway in order to achieve its effective configuration. Sequences and structural studies of many bacterial riboswitches have deciphered the molecular architecture of aptamers at atomic levels. The folding events of riboswitch follow the common principles governing other RNA molecules [25]. Various RNA structural configurations have been reported in riboswitches including helices, loops, and bulges. The configuration of these loops and turns is dictated by the sequence motifs of nucleotides. Such motifs can interact with each other to form higher level of packing. These structural motifs include, but not limited to, GA3 tetraloop, kink-turns (K-turns), kissing-loop (KL), sarcin-ricin loops, T-loops, and pseudoknots which facilitate the global folding of RNA molecules (reviewed in Ref. [25]). Disrupting the sequences of these structural themes renders or markedly hinders riboswitch function [26–32]. Real-time folding of an aptamer is determined by sophisticated techniques other than crystallography, which shows the final configuration of the interrogated molecule. Local folding of the purine riboswitch, *xpt-pbuX* of *B. subtilis* was tracked over time by a chemical footprinting technique called Selective 2′-Hydroxyl Acylation analyzed by Primer Extension (SHAPE) that exploits the attacking reactivity of N-methylisatoic anhydride (NMIA) against the 2′-hydroxyl groups of aptamer nucleotides [31]. This technique revealed conformational changes of nucleotides at the binding pocket in response to ligand binding in time window. Folding dynamics of the TPP riboswitch, *thimM*, of *E. coli* in the presence and absence of the ligand (thiamine pyrophosphate) and Mg^{2+} ions have also been studied at the molecular basis by another imaging technique called single-molecule Fluorescence Resonance Energy Transfer (smFRET) [33]. In smFRET technique, the targeted parts of the aptamer are labeled differently and folding transitions are correlated with changes in the detected FRET value. The same study shows high degree of plasticity and dynamics of riboswitch parts configurations as a result of ligand docking.

Having its binding site organized, the aptamer domain can specifically bind the proper metabolite with a great discrimination power against closely related compounds. For instance, adenine riboswitch achieves ~10.000-fold level of discrimination between adenine and guanine [34,35], however, lysine riboswitch has at least 5000-fold level of discrimination between lysine and ornithine; amino acids that differ in their R group by a single methylene group [24]. The virtue of high specificity is attributed to the fact that all functional groups and polar parts of the ligand are engaged in interactions with the nucleobases of the binding pocket, in some instances, mediated by positive ions. The experimental proofs of high selectivity came initially from synthetic aptamers designed to sense different ligand metabolites with affinity and specificity [36]. Strikingly, these synthetic elements failed to exhibit the discrimination power of naturally occurring counterparts. This is not surprising because natural aptamers have been and continue to be sharpened by persistent and stringent evolutionary constraints for billions of years.

2.2. Mechanism of genetic regulation

The conformational structures of bacterial riboswitches are triggered by folding in response to ligand biding which directly modulate gene transcription either to seize or to proceed through formation of terminator or antiterminator structures respectively [24]. Interestingly, riboswitches in Gram-positive bacteria exert their action most commonly via transcriptional inhibition, while translation inhibition is the frequent mechanism in Gram-negative due to Shine-Dalgarno (SD) sequence sequesteration. The preference of transcriptional arrest mechanism in Gram-positive may be linked to the fact that their genomes are embedded with large biosynthetic operons where more resources would be wasted if a full-length mRNA is synthesized.

Despite the fact that premature transcription termination is the most common mechanism employed by riboswitches [6], transcription activation, translation initiation inhibition, and ribozyme-like cleavage mechanisms have also been documented in certain riboswitch classes [37]. Such diversity in mechanisms, alongside with protein-mediated mechanisms, enables bacterial cells to finely tune its metabolic status and pathogenic lifestyle by

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