Bacterial riboswitches and RNA thermometers: Nature and contributions to pathogenesis

Jameel M. Abduljalil

Microbiology Section, Department of Biological Sciences, Faculty of Applied Sciences, Thamar University, PO Box: 87246, Dhamar, Yemen

ARTICLE INFO

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

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

modulating relevant genes and their products. Heterogeneity of genetic control mechanisms is attributed to different ligand-induced structural reorganization of the aptamer region which consequently determines the folding pathway of the downstream expression platform region (Fig. 1). Such mutually exclusive secondary and tertiary structures are harnessed to control the expression of downstream coding sequence(s). A strong intrinsic terminator stem has two features; high G-C content followed by a run of U residues that cause RNA polymerase (RNAP) to stall transcription and dissociate from the DNA and nascent RNA stretch [38]. In ligand-free state of riboswitch working by transcription termination, aptamer nucleobases are engaged in certain base pairing that masks key bases necessary to construct the terminator stem (Fig. 1). In other words, some key bases of the terminator stem are incorporated in certain structural motifs, but ligand docking establishes new interactions between binding site' nucleobases and downstream bases. These new interactions favor terminator stem formation. Similarly, in translation inhibiting mechanism, ligand binding causes the Shine-Dalgarno (SD) sequence or translation initiation site to be masked in stem formation.

For a typical riboswitch to achieve optimally its regulatory goal,

it should bind the correct ligand, adopt the final and stable conformation of ligand-bound status, and arrest the transcriptional machinery before the RNAP passes the 3' boundary of the riboswitch. Global folding in timescale of adenine riboswitches, *pbuE* of *B. subtilis*, and *add* of *Vibrio vulnificus*, has been determined to be around 1 s using smFRET and force spectroscopy [29,40]. Interestingly, both techniques demonstrated that tertiary structure play a key role in pre-organizing the binding pocket before ligand binding.

Considering that the elongation rate of bacterial RNAP is around 45 nucleotides per second at 25 °C, transcription of such aptamers would require around 1.5 s, thereby transcription and folding should occur within the same time as expected for a cotranscriptional process. Experimental studies on purine and FMN riboswitches suggest that at 1 μM intracellular concentration of ligand, 10 s at least would be required for an aptamer to bind the ligand correctly [41,42]. If transcription machinery progresses at constant rate for this duration, a segment of nearly 500 nucleotides would be produced downstream the riboswitch terminus. How cells resolve this problem? One of the solutions is the programmed pause sites located within the expression platform domain that effectively decreasing transcription rate allowing time for the aptamer to fold
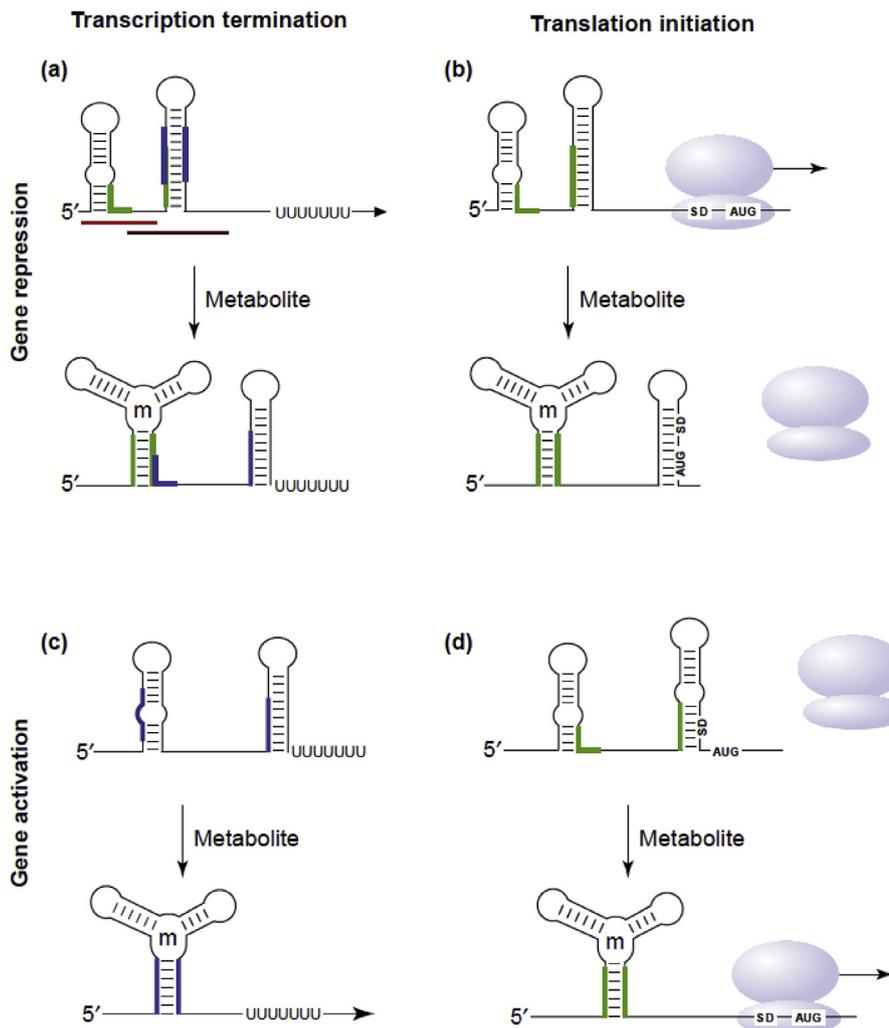
Fig. 1. Diverse regulatory strategies of riboswitch elements [39]. Structurally, a riboswitch element precedes a coding sequence and comprises of two domains; a sensor part called an aptamer (corresponding to red bar) and an output domain called an expression platform (brown bar). In a transcription repressor riboswitches (a), binding of ligand induces conformational changes to trap the antiterminator sequence thus forming a terminator loop. However, translation inhibiting riboswitches sequester the SD sequence and/or start codon AUG in response to ligand binding (b). In transcription activation, ligand binding prevents the formation of terminator loop (c), while translation permissive riboswitches free SD sequence and/or start codon AUG for ribosomal units loading (d). Complementary sequences are color coded.

properly [43]. It is also worth pointing out that different riboswitch sequences will require different amounts of time to fold properly before binding the target ligand. Of note, other factors also play important roles in this regards include; speed of folding, RNA structural stability, and ligand concentration inside bacterial cell (R.R. Breaker, personal communication).

Transcription termination, via intrinsic structures, and translation inhibition are the two previously known mechanisms of riboswitches regulation. However, recently, a new general mechanism was demonstrated in Mg^{2+}-sensing (*mgtA*) and FMN-sensing riboswitches in *Salmonella* Typhimurium and *E. coli* respectively [44]. Interestingly, *mgtA* and *ribB* riboswitches lack typical intrinsic transcription terminator sequence [45–47] and shuts off the transcription process by Rho-dependent mechanism [44]. Rho factor is a homohexameric RNA helicase that binds to nascent RNA and translocates along the strand to reach the RNAP and cause dissociation of the newly synthesized mRNA from elongation complex at specific cytosine-rich sequence [48]. Surprisingly, few riboswitches show additional novel mechanism of gene regulation by acting in *trans*. In principle, premature termination of transcription produces short segments of RNA corresponding to the riboswitches sequence. Additionally, aptamer segments also produce by RNase cleavage of transcriptionally generated riboswitches full segments [9,49]. An intriguing question is; what is the fate(s) of these RNA segments? Degradation and recycling of building blocks is one of these fates. However, it was found that high aptamer concentration effectively maintain homeostasis by titration of ligand metabolites, in other words, by decreasing its cellular free concentrations [50]. A subsequent study detected an interesting finding that, in certain cases, these segments act in *trans* similarly to small regulatory RNAs [51]. This study showed, for the first time, that two S-adenosylmethionine (SAM) riboswitches (*SreA* and *SreB*) in *Listeria monocytogenes* target several mRNA transcripts of virulence genes through base pairing leading to translation inhibition and mRNA degradation (see contribution to pathogenicity).
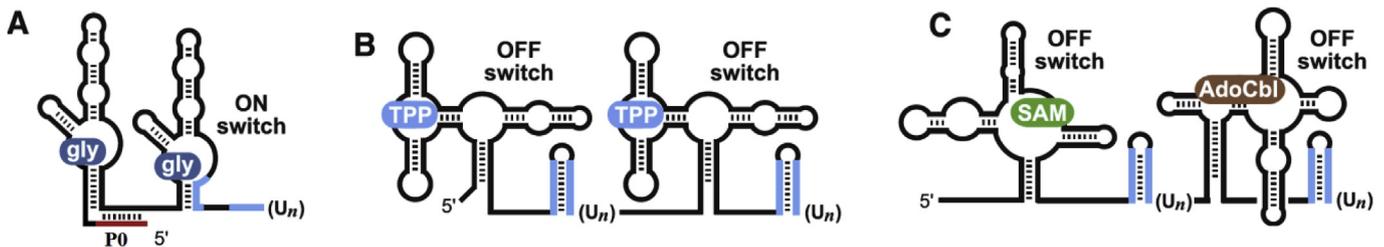
2.3. Tandem architecture

Composite riboswitches come in two versions (Fig. 2), two-aptamer-one expression platform riboswitches (e.g. Glycine riboswitches in *Vibrio cholerae* and *Bacillus anthracis*) and two complete riboswitches reside adjacent to each other within the same mRNA transcript (TPP riboswitches in *Bacillus anthracis*). Tandem arrangement of binding domains produces more complex characteristics of interacting with ligand metabolites and gene expression control. The two-aptamer version either possesses the same specificity for a particular ligand or the two aptamers differ markedly in their target metabolites. The latter case was found in *metE* mRNA in *Bacillus clausii* tandem riboswitches sensing SAM and Adenosylcobalamin (AdoCbl or coenzyme B_{12}) [52]. The double

riboswitches version was also characterized biochemically and genetically in TPP riboswitches located in the 5′-UTR of *tenA* operon in *Bacillus anthracis* encoding for thiaminase II involved in the regeneration of the thiamine [53].

Similarly to all riboswitches, interactions of binding domains with cognate ligands rely on many chemical strategies including hydrogen bonding, van der Waals, electrostatic, and stacking interactions [54]. Intradomain interactions have been proposed in glycine riboswitches, *gcvT*, of *V. cholerae* [55] and the saprophytic species *B. subtilis* [54]. Having two-aptamer one expression platform architecture, these riboswitches are neither independent nor cooperative however. The benefit of such architecture was a topic of debate until recently. Indeed, studies of ligand binding kinetics revealed a sigmoidal curve which was interpreted as indications of cooperative nature between the aptamers [55]. Nucleotide Analog Interference Mapping (NAIM) approaches and mutagenesis studies suggest cooperative nature inferred by specific interaction between specific regions of both aptamers [55]. In fact, these studies have employed and characterized glycine riboswitches lacking P0 double helix linker at the 5′end of the riboswitch (Fig. 2a). Hence, the earlier observed cooperativity was an artifact of the truncated constructs used for biochemical characterization. The discovery of this k-turn motif precludes the proposed cooperativity between aptamers in glycine riboswitches from *V. cholerae* [56]. This small segment base pairs with a small stretch of ribonucleotides connecting the two aptamers (Fig. 2a). A revised model for ligand binding in such riboswitches was proposed explaining that aptamers dimerization, rather than double ligand-site occupancy, is crucial for high ligand binding affinity. In other words, dimerization and stability of the second aptamer results from ligand binding in the first aptamer [56]. These in vitro analyses have been supported very recently by evidences from *in vivo* interrogation of Glycine riboswitch folding behavior [57].

Tandem T-box riboswitches (regulating amino acid metabolic genes) are known in several pathogenic species; *B. anthracis*, *C. perfringens*, and *M. pneumoniae* [58]. In order to trigger the expression of amino acids metabolic operons, high levels of uncharged t-RNAs are required to saturate all copies of T-boxes upstream the operon. What advantage has been gained from harboring identical successive riboswitches? This, presumably, would ensure nearly complete repression of the subsequent operon through the combined action of both riboswitches with a nearly half-ligand concentration needed for single riboswitch to reach the same outcome (transcription termination). TPP Riboswitches were found in tandem as in *tenA* RNAs encoding thiaminase (EC. 3.5.99.2) in *Bacillus anthracis* [53]. TPP riboswitches in *tenA* are composed of two complete versions of riboswitches linked by a stretch of repetitive U residues. These tandem riboswitches control gene transcription termination independently as a natural Boolean NOR logic gate, where ligand docking to either aptamer yields an output of

Fig. 2. Tandem architecture of riboswitches. (A) Glycine (Gly) riboswitch from *Bacillus* with a double-aptamer and single expression platform [52]. The newly characterized kink-turn (P0) is dipected in red. This configuration is found in many glycine riboswitches in Gram-positive speciesas well as in *V. cholerae*. (B) Two similar complete TPP riboswitches from *B. anthracis* and (C) two different complete riboswitches; SAM and Adenosylcobalamine (AdoCbl) precede methionine metabolic gene in *Bacillus* [52]. Expression platforms sequences are colored in blue.

gene regulation. NOR logic function does not require that the two riboswitches influence each other [52]. Such conclusion was drawn from genetic experiments in which mutated and wild-type tandem TPP riboswitch was fused with beta-galactosidase reporter gene in various combinations and tested at different concentrations of thiamine, the precursor of TPP. Furthermore, the absence of the second riboswitch was found negligible and one riboswitch was sufficient to impart transcription termination of the reporter gene construct in transformed *B. subtilis* [53].

3. RNA thermometers

The activities of enzymes and many biological molecules are affected by changes in ambient temperature. Drastic fluctuations of temperature pose a serious threat to cell physiology, therefore, bacterial cell has to monitor and respond efficiently and rapidly to such heat perturbations. Elevated temperature causes protein denaturation, weakening structural integrity of DNA and RNA, and increase fluidity of plasma membrane to an extent that disrupts its selective permeability. In contrast, decrease in temperature leads to dramatic decrease of enzymes activities, membranes rigidification, and formation of stable RNA structures that obscure or interfere with transcription and translation leading eventually to growth arrest. Different physiological responses are mounted to face such physiological deleterious alterations among of which is an RNA-based immediate response. Accumulation of denatured proteins serves as a signal that trigger the expression of hundreds genes employed to respond to and limit the heat-triggered damages to maintain normal metabolism. Three categories of genes are subjected to thermoregulation; heat shock genes, cold shock genes, and virulence genes.

Prompt change in temperature is most efficiently sensed and transduced by RNA structures, called RNA Thermometers (RNATs) because other signal transduction systems, protein- or DNA-mediated, require much time to come into effect. The virtue of RNATs immediate response is attributed to their mode of regulation in which translation initiation of existing mRNA is controlled in temperature-dependent manner. It has been reported that as minor as 1 °C variation of temperature is detected by such RNA-based system [59]. However, the mechanism employed by RNATs to detect such subtle variations of temperature is still largely unknown. The exploitation of mismatch pairing in the effector hairpins of RNATs seems to be the major strategy. Pathogenic bacterial species of environmental origin seem to depend on RNATs to confirm that a living host has been reached in order to initiate virulence factors production.

These complex RNA structures are mostly found in the 5′-UTR of temperature-responsive genes, and alter their conformation between two distinct structures in response to temperature [59]. In low temperature, the "closed" configuration is adopted, in which the SD sequence and/or AUG codon are occluded due to base pairing with complementary nucleotides in the same element. Melting of these secondary structures results in the alternative "open" conformation exposing SD sequence and/or AUG to the ribosome [60]. Although the first RNAT element was found in plague agent, *Yersinia pestis*, in 1993 [61], the best understood RNAT is the thermometer of prfA transcript in *Listeria monocytogenes* controlling, with other activators, the synthesis of PrfA protein, a transcriptional global activator of virulence genes [62]. This thermosensor (127 nt.) folds into hairpin structure at low temperature to inhibit translation initiation, but the hairpin stem is melted upon increase of temperature to 37 °C [63]. In general, these riboregulators are highly dynamic motifs in a narrow temperature ranges to modulate heat and cold shock response proteins [64,65]. To clarify the fundamentals of RNAT-based regulation, two classes of these thermosensors are discussed herein.

3.1. High temperature RNA thermosensors

3.1.1. ROSE elements

*R*epression *O*f hear *S*hock gene *E*xpression (ROSE) is the most common class of RNATs encountered in rhizobia, alphaproteobacteria, and gammaproteobacteria [66—68]. ROSE elements range from 60 to >100 nucleotides (nt.) upstream the coding sequences of mRNA coding for heat shock chaperone proteins. Structurally, ROSE elements are usually composed of 2—4 hairpins (Fig. 3a). The hairpin of the 5′-end is structurally stable irrespectively of the temperature [69], whereas the 3′-end hairpin looses its tertiary and secondary structures gradually as temperature increase, much like a zipper. The SD sequence is trapped by base pairing with conserved U(U/C)GCU motif in the 3′-end hairpin which is the functional part of the RNAT. The other hairpins, however, are thought to confer scaffolding purposes and ensure proper folding of the ROSE element [70,71]. *In vivo* analysis of ROSE elements, it was proved that at 30 °C, the ribosomal binding site (RBS) at the 3′-end hairpin is masked but partial liberation of SD sequence occurs at 37 °C, whereas an increase of temperature to around 42 results in full liberation of RBS leading to complete translation of downstream gene in a zipper like fashion [70].

Expression of heat shock genes occurs when sigma factor 32 (σ^{32}) assists RNAP to identify their promoters. In turn, genes of σ^{32}, *rpoH*, in *E. coli*, are controlled by four different promoters which respond to different environmental signals [72] alongside with ROSE-like RNATs at the level of transcription and translation respectively. Unlike other RNATs, the RBS of *rpoH* is not completely occluded, but partially exposed [12,13]. Nevertheless, its translation is prevented at non-stress temperature by inhibitory structure created by folding of two distinct segments in the coding sequence that obscure ribosome units loading. At high temperature the inhibitory structure is weakened and ribosome entry becomes feasible [12,72]. Two of sigma 32- activated genes, *IbpA* and *IbpB* encode for heat shock proteins participating in multichaperone network that stabilizes heat-denatured proteins have been found to possess a 96-nt ROSE-like RNATs at their 5′-UTRs [73—75].

3.1.2. Four uredine thermometers

A stretch of four uredines (a.k.a. fourU) was firstly found at the 5′-UTR of *agsA* gene in *Salmonella enterica* serovar Typhimurium. *AgsA* gene encodes for Aggregation Suppression A (AgsA) protein [76]. Several heat shock and virulence genes were also found to harbor this sequence which exploits the strategy of mismatch base pairing that would be disrupted easily in response to temperature increase. Such sequence sequesters the SD sequence and control translation initiation of mRNA at low temperatures. The 5′-UTR containing the fourU element folds into two stem-loops; the 5′-hairpin is heat stable, as in ROSE elements (Fig. 3b), while the second hairpin containing the fourU is temperature-sensitive [76]. At high temperatures (40sº C), the mismatch pair A29. G52 is easily disrupted due to their weak bonding and the melting of the fourU hairpin ensues. However, the entire melting of the stem-loop is prevented by the stable base pair G34. C46 at the top of the stem. Substitution of A29. G52 with a typical C-G base pair results in complete repression of the gene *in vivo* due to difficulty in opening the stem [76]. On the other hand, substitution of G34. C46 by A-U pair decreases the melting point by 5 °C and expressed the downstream gene at non-stress conditions. As in riboswitches, Mg^{2+} ions participate in tertiary structure stabilization of the 3′-hairpin [77]. Additionally, translation of *htrA* gene in *Salmonella* Typhyimurium, encoding for periplasmic serine protease (a.k.a. Do or DegP protein), is controlled by a fourU-type RNAT composed of only one
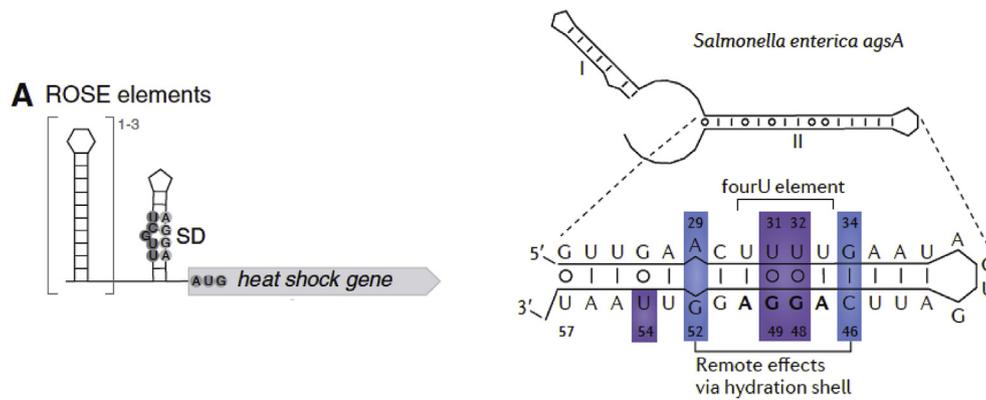
Fig. 3. Structural features of two RNA thermometers (RNATs). (A) ROSE elements are composed of 2–4 hairpin structures, which sequester the SD sequence and in some cases the AUG start codon in their 3′ proximal hairpins. The SD sequence is predicted to be paired by the characteristic U(U/C)GCU motif (marked in dark gray). The actual structure, however, can be different than the ROSE represented herein. All known ROSE elements are found in the 5′-UTR of bacterial small heat shock genes [79]. (B) FourU element upstream aggregation suppressing A (agsA) gene in Salmonella enterica. It is made of two hairpins (I and II), with the functional fourU thermosensor placed in the 3′-most stem–loop structure. Experimentally validated base pairs involved in hydration shell-based intramolecular communication are indicated. Hydration shell is a layer of water molecules that is formed around intracellular macromolecules (for example, DNA, RNA and proteins), with a possible effect on their functionality [59]. Purple shaded bases are Mg^{2+} binding sites.

hairpin [78].

3.2. Cold RNA thermosensors

Cold Shock Proteins (CSPs) are protective proteins produced under cold conditions to mitigate the damage or harmful effects at membranes and undesirable RNA structures that impede translation initiation and elongation [80]. In *L. monocytogenes*, three genes encoding CSPs are preceded by in-built relatively long (198–363 nt.) sequences predicted to have hairpin structures [81]. This pathogen endures refrigerator temperature which reflects its physiological ability to acclimate to such low temperature. In 4 °C incubation, the expression levels of these genes elevate by 4–27-fold compare to incubation at 37 °C [81]. The best characterized representative of cold RNATs is the 160 nucleotides 5′-UTR of *cspA* in *E coli*. Similar to riboswitch, the arrangement of this thermosensor results from mutually-exclusive structures. At 10 °C, the RNAT is stable and adopted a secondary structure with exposed SD sequence. Whereas at 37 °C, stem-loop formation traps the ribosome binding site which limits translation process. Interestingly, the functional sequence of *cspA* RNAT in *E coli* extended into ~60 nucleotides of the downstream coding sequence [65].

4. Contributions of riboswitches and RNATs to pathogenicity

Though the aforementioned examples of RNATs reside in the 5′-UTRs of either monocistronic or polycistronic mRNAs, new levels of RNAT-based sophisticated loci and regulation were documented in human pathogens yersiniae and *Neisseria meningitidis* [82,83]. In *Yersinia pestis*, an RNAT at the 5′-UTR of *lcrF* gene was identified in the intergenic region of two-gene operon, *yscW-lcrF* operon [82]. LcrF is a global transcriptional activator of many virulence genes including outer membrane proteins (Yops) employed for evasion of antibacterial immune response [84] and type III secretion system [85–87]. Furthermore, transcription of the whole operon is also regulated by thermosensitive histone-like protein that only when melted at 37 °C allows RNAP to read-through the operon [82,88]. *LcrF* RNAT of human pathogenic yersiniae is composed of two conserved hairpins that sequester SD sequence in its 3′ end loop by a typical fourU motif. The partially occluded start AUG codon in secondary structure is liberated at 37 °C when G-C mismatches of SD/fourU are disrupted [82].

In *Bartonella hanselae*, the causative agent of Cat-scratch disease, and also in other species of *Bartonella*, the coding sequences of numerous transcriptional regulators, trp family, are preceded by putative riboswitches named *Brt1-9*, with unidentified ligands [89]. From biochemical experiments, it is likely that in the absence of its ligand, *Brt1* terminates the transcription of the ensuing *trp 1* sequence. Trp 1 is a helix-turn-helix DNA binding protein that positively regulates *badA* gene transcription [89]. BadA is a major virulence surface protein involved in autoaggregation, biofilm formation, and phagocytosis inhibition [90,91]. Interestingly, besides being a putative riboswitch, experimental evidence inferred a possible *trans*-acting mechanism for *Brt1* to downregulate badA by base paring with *badA* mRNA transcript [89].

Ecologically, *Listeria monocytogenes* is a widely distributed species that survives primarily as a saprophytic species in soil [92]. The ability of *L. monocytogenes* to switch to pathogenic lifestyle upon ingestion by a susceptible human host is mediated by the activation of number of genes collectively known as virulence genes. Products of virulence genes are implicated in host cell invasion, intracellular growth, and spread to neighboring cells [93]. The majority of such virulence factors, thus far, are known to be regulated by PrfA protein, a transcriptional activator, which harbors a thermometer regulatory sequence [92]. After successful access to human host, increase of temperature serves as a confirmation signal to the listerial cell to switch to parasitism. Intriguingly, PrfA biosynthesis is subjected to regulation by a thermometer located in the 5′-UTR. At 30 °C, this regulatory region folds into hairpin secondary structure masking the SD sequence which only freed from the hairpin if temperature elevated to 37 °C when RNA secondary structures denatured [51]. At 37 °C, the virulence factors modulate the pathogenicity after the thermometer permits the translation of PrfA mRNA [92]. In presence of SAM, *SreA* riboswitch responds by transcription termination of methionine metabolic genes. The produced stretch of RNA corresponding to the 5′-UTR of *SreA* engages in base pairing with the sequence upstream the SD sequence of *prfA* mRNA transcript at 37 °C. This *trans*-acting regulatory effect of *SreA* does not take place at 30 °C, because the SD sequence is incorporated in a thermometer stem [51].

Other examples in which a riboswitch plays a role in regulating virulence factors are reported in *Enterococcus faecalis* [94] and also in *Listeria monocytogenes* [95]. In *L. monocytogenes,* the *eut* operon encoding for ethanolamine utilization is regulated by two-

component regulatory system (EutVW). In this protein system, EutW is the membrane sensor kinase and EutV is its associate DNA-binding response regulator. EutV functions as antiterminator via disrupting intrinsic terminator hairpins produced in the Rli55 segment during 5′-end transcription of the operon [96–98]. The enzymes of ethanolamine utilization pathway require vitamin B$_{12}$ as a coenzyme. If ethanolamine is available (as in the intestines during infection), EutW signals the EutV to aid transcription of the operon. However, if B$_{12}$ is absent, Rli55 is fully transcribed with its terminator loops leading to EutV sequestration and failure to express *eut* genes. Yet binding of B$_{12}$ to its riboswitch at the 5′-UTR of Rli55 masks the sequences that sequester EutV allowing for *eut* operon to be expressed [95]. Such integrative multifaceted (protein and RNA) regulation emphasizes the idea that pathogenic species have engineered efficient systems to express pathogenicity-related genes only under correct optimal conditions. Ethanolamine is abundant molecule in the intestines of vertebrates and its utilization was found to be part of listerial pathogenesis in mice model [99].

Shigella dysenteriae has an outer membrane heme receptor, ShuA, important for acquisition of iron from complex molecules in human body. Nutrient iron is required for pathogenicity initiation and progression [100,101]. *ShuA* transcription is regulated by an iron-dependent transcriptional repressor, Fur [102,103] while its translation is subjected to fourU RNAT located in its 300 nucleotides 5′-UTR [104]. In iron-poor condition, ShuA is synthesized only at 37 °C when translation of *ShuA* mRNA is permitted, however, at low temperature; fourU RNAT occludes the SD sequence regardless of iron concentration.

In *V. cholerae*, the causative agent of life-threatening diarrheal cholera disease, the production of cholera toxin (CT) and toxin-coregulated pili (TCP) occurs when the bacterium reaches human intestines [105]. The successful arrival to host is confirmed by rise in temperature from less than 20 °C of the contaminated water to the 37 °C of the body. The mentioned toxin and pili are the important major virulence factors for pathogenicity (reviewed in Ref. [106]). The expression of these factors is subjected to transcriptional activator ToxT. The ToxT genes harbor a fourU RNAT element that masks SD sequence under low temperature conditions [107].

5. Evolution of riboswitches and RNATs

Before evolutionary emergence of DNA as genetic material, life thought to have relayed on RNA for the mission. Furthermore, life of the RNA world might have depended on RNA for catalysis of chemical reactions and for modulation of biological processes, at least the essential ones. RNA viruses are considered as evidences to support the hypothesis of RNA being the only medium of genetic material [108–110]. On the other hand, ribozymes are thought to have been the biocatalysts in the RNA life, much like modern enzymes before protein existence. This is supported by the discovery of a carbohydrate-sensing riboswitch element that employe self-cleavage mechanism. The key metabolites thought to have been existed are amino acids and nucleobases, from which modern co-enzyme may have evolved. Despite the fact that primordial RNA world hypothesis is controversial, at least currently, successive discoveries in the arena of RNA continue to provide testimonials one after another. Riboswitches are robust evidences that RNA has the ability to selectively bind specific molecules under low concentration with great discrimination power, much like contemporary metabolite-binding proteins. If riboswitches are real fossils from ancient RNA life, it is remarkable that they have retained conserved sequences during billions of years of evolutionary pressure to serve in modern organisms. Indeed, TPP riboswitches

have been found in the three domains of life and representatives of different ribozymes are distributed in the three domains of life and viruses.

On the other hand, an intriguing question is that; if modern RNATs are remnants of the primordial RNA world, then what were they employed for in the absence of DNA or protein molecules? If their existence in RNA world is proven, then their contributions were likely to regulate the expression and protection of genetic material (RNA) resembling the modern roles in the modern DNA-protein world. The catalysis capabilities of RNA molecules (such as ribozymes) are affected by temperature raise which necessitated the existence of a protective/regulatory aid mostly fulfilled by RNATs.

Bioinformatic analyses continue to identify not only new classes but also variants of already characterized classes [111–114]. A new computational study employing multiple-sequence alignments, atomic-resolution structural information, and riboswitch gene association have identified many variants that changed their ligand specificities much like protein enzymes [114]. Some variants completely lost their ability to bind the primary ligand and the ligands of such variants are still unknown. Such changes are direct results of mutations in key nucleotides at the binding pockets reflecting continuous genome evolution. From gene regulation standpoint, the associated genes with such variants are most probably relying on other more effective regulatory mechanisms beside the original riboswitch versions.

6. Conclusions and perspectives

Riboswitches and RNATs are important RNA-based regulators that respond to key metabolites and temperature, respectively, via different mechanisms and to different extent. Some riboswitches precede a single gene while others control a polycistronic transcript encoding more than one gene. Unlike other noncoding RNA elements that are devoted to interact with other RNA transcripts via restricted typical base pairing, riboswitches and RNAT are more developed to compete with proteins to bind chemical compounds and sense temperature shifts, respectively, to coordinate gene expression and cope up with pathogenic lifestyle. The distribution and levels of complexity reflect the paramount role of RNA domains to participate in maintenance of cell physiology and homeostasis. The extent of RNA-based regulatory networks is far from being fully appreciated. In occasions, some riboswitches have been found to interact with other riboregulators that control different physiological process. Cells relay on riboswitches and RNA thermosensors to avoid synthesizing unnecessary proteins or to induce the immediate production of others in a much rapid response than protein-mediated one.

Levels of RNA-based regulation of cell physiology are getting expanded and sophisticated. Understanding of simple prokaryotic model systems will provide the knowledge and principles to explore higher eukaryotic RNA regulatory networks. Additionally, discovery of selective drugs that compete with ligand to bind riboswitch is a promising approach to fight pathogenic species and lowering the burden of antibiotics resistant infections. The continuous sequencing and annotation of bacterial genomes will aid the research to delve deeply into mechanistic, biological function, and evolutionary questions regarding riboswitches and RNA thermosensors. Additionally, characterization of a riboswitch upstream the coding sequence of a hypothetical gene helps in deciphering the identity and function of that gene product.

Conflicts of interest

The author declares the absence of any actual or potential

conflict of interest.

References

[1] K. Papenfort, J. Vogel, Regulatory RNA in bacterial pathogens, Cell Host Microbe 8 (2010) 116–127. https://doi.org/10.1016/j.chom.2010.06.008.

[2] G. Storz, J. Vogel, K.M. Wassarman, Regulation by small RNAs in bacteria: expanding frontiers, Mol. Cell. 43 (2011) 880–891. https://doi.org/10.1016/j.molcel.2011.08.022.

[3] L.S. Waters, G. Storz, Regulatory RNAs in bacteria, Cell 136 (2009) 615–628. https://doi.org/10.1016/j.cell.2009.01.043.

[4] A. Serganov, E. Nudler, A decade of riboswitches, Cell 152 (2013) 17–24. https://doi.org/10.1016/j.cell.2012.12.024.

[5] R.R. Breaker, Prospects for riboswitch discovery and analysis, Mol. Cell 43 (2011) 867–879. https://doi.org/10.1016/j.molcel.2011.08.024.

[6] J.E. Barrick, R.R Breaker, The distributions, mechanisms, and structures of metabolite-binding riboswitches, Genome Biol. 8 (2007) R239. https://doi.org/10.1186/gb-2007-8-11-r239.

[7] N. Sudarsan, E.R. Lee, Z. Weinberg, R.H. Moy, J.N. Kim, K.H. Link, Riboswitches in eubacteria sense the second messenger cyclic di-GMP, Science 321 (2008) 411–413. https://doi.org/10.1126/science.1159519.

[8] E.E. Regulski, R.H. Moy, Z. Weinberg, J.E. Barrick, Z. Yao, W.L. Ruzzo, R.R. Breaker, A widespread riboswitch candidate that controls bacterial genes involved in molybdenum cofactor and tungsten cofactor metabolism, Mol. Microbiol. 68 (2008) 918–932. https://doi.org/10.1111/j.1365-2958.2008.06208.x.

[9] S.V. Spinelli, L.B. Pontel, E. GarciaVescovi, F.C. Soncini, Regulation of magnesium homeostasis in Salmonella: Mg(2+) targets the mgtA transcript for degradation by RNaseE, FEMS Microbiol. Lett. 280 (2008) 226–234. https://doi.org/10.1111/j.1574-6968.2008.01065.x.

[10] T.M. Henkin, F.J. Grundy, Sensing metabolic signals with nascent RNA transcripts: the T box and S box riboswitches as paradigms, Cold Spring Harbor Symp. Quant. Biol. 71 (2006) 231–237. https://doi.org/10.1101/sqb.2006.71.020.

[11] G. Nechooshtan, M. Elgrably-Weiss, A. Sheaffer, E. Westhof, S. Altuvia, A pH-responsive riboregulator, Genes Dev. 23 (2009) 2650–2662. https://doi.org/10.1101/gad.552209.

[12] M. Morita, M. Kanemori, H. Yanagi, T. Yura, Heat-induced synthesis of σ32 in Escherichia coli: structural and functional dissection of rpoH mRNA secondary structure, J. Bacteriol. 181 (1999) 401–410. PMC93392.

[13] M.T. Morita, Y. Tanaka, T.S. Kodama, Y. Kyogoku, H. Yanagi, T. Yura, Translational induction of heat shock transcription factor σ32: evidence for a built-in RNA thermosensor, Genes Dev. 13 (1999) 655–665. PMC316556.

[14] P.J. McCown, K.A. Corbino, S. Stav, M.E. Sherlock, R.R. Breaker, Riboswitch diversity and distribution, RNA 23 (2017) 995–1011, https://doi.org/10.1261/rna.061234.117.

[15] J. Gripenland, S. Netterling, E. Loh, T. Tiensuu, A. Toledo Arana, J. Johansson, RNAs: regulators of bacterial virulence, Nat. Rev. Microbiol. 8 (2010) 857–866, https://doi.org/10.1038/nrmicro2457.

[16] A.K. Heroven, A.M. Nuss, P. Dersch, RNAbased mechanisms of virulence control in Enterobacteriaceae, RNA Biol. 14 (5) (2016) 471–487. https://doi.org/10.1080/15476286.2016.1201617.

[17] Y. Caldelari, P. Chao, J. Romby, Vogel, RNA-mediated regulation in pathogenic bacteria, Cold Spring Harb Perspect Med (2013), https://doi.org/10.1101/cshperspect.a010298.

[18] J.J. Quereda, P. Cossart, Regulating bacterial virulence with RNA, Annu. Rev. Microbiol. 71 (2017) 263–280. https://doi.org/10.1146/annurev-micro-030117-020335.

[19] B.A. McDaniel, F.J. Grundy, I. Artsimovitch, T.M. Henkin, Transcription termination control of the S box system: direct measurement of S-adenosylmethionine by the leader RNA, Proc. Natl. Acad. Sci. 100 (2003) 3083–3088. https://doi.org/10.1073/pnas.0630422100.

[20] A.S. Mironov, I. Gusarov, R. Rafikov, L.E. Lopez, K. Shatalin, R.A. Kreneva, D.A. Perumov, E. Nudler, Sensing small molecules by nascent RNA: a mechanism to control transcription in bacteria, Cell 111 (2002) 747–756. https://doi.org/10.1016/S0092-8674(02)01134-0.

[21] A. Nahvi, N. Sudarsan, M.S. Ebert, X. Zou, K.L. Brown, R.R. Breaker, Genetic control by a metabolite binding mRNA, Chem. Biol. 9 (1043) (2002) 952–956. https://doi.org/10.1016/S1074-5521(02)00224-7.

[22] W. Winkler, A. Nahvi, R.R. Breaker, Thiamine derivatives bind messenger RNAs directly to regulate bacterial gene expression, Nature 419 (2002) 952–956. https://doi.org/10.1038/nature01145.

[23] W.C. Winkler, S. Cohen-Chalamish, R.R. Breaker, An mRNA structure that controls gene expression by binding FMN, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 15908–15913. https://doi.org/10.1073/pnas.212628899.

[24] A.D. Garst, A.L. Edwards, R.T. Batey, Riboswitches: structures and mechanisms, Cold Spring Harb Perspect Biol 3 (2011) a003533. https://doi.org/10.1101/cshperspect.a003533.

[25] D.K. Hendrix, S.E. Brenner, S.R. Holbrook, RNA structural motifs: building blocks of a modular biomolecule, Q. Rev. Biophys. 38 (2005) 221–243. https://doi.org/10.1017/S0033583506004215.

[26] S. Blouin, D.A. Lafontaine, A loop loop interaction and a K-turn motif located in the lysine aptamer domain are important for the riboswitch gene regulation control, RNA 13 (2007) 1256–1267. https://doi.org/10.1261/rna.

[27] S.D. Gilbert, C.E. Love, A.L. Edwards, R.T. Batey, Mutational analysis of the purine riboswitch aptamer domain, Biochemistry 46 (2007) 13297–13309. https://doi.org/10.1021/bi700410g.

[28] B. Heppell, D.A. Lafontaine, Folding of the SAM aptamer is determined by the formation of a K-turn-dependent pseudoknot, Biochemistry 47 (2008) 1490–1499. https://doi.org/10.1021/bi701164y.

[29] J.F. Lemay, J.C. Penedo, R. Tremblay, D.M. Lilley, D.A. Lappntaine, Folding of the adenine riboswitch, Chem. Biol. 13 (2006) 857–868. https://doi.org/10.1016/j.chembiol.2006.06.010.

[30] B.A. McDaniel, F.J. Grundy, T.M. Henkin, A tertiary structural element in S box leader RNAs is required for S-adenosylmethionine directed transcription termination, Mol. Microbiol. 57 (2005) 1008–1021. https://doi.org/10.1111/j.1365-2958.2005.04740.x.

[31] C.D. Stoddard, S.D. Gilbert, R.T. Batey, Ligand-dependent folding of the three-way junction in the purine riboswitch, RNA 14 (2008) 675–684. https://doi.org/10.1261/rna.736908.

[32] W.C. Winkler, A. Nahvi, N. Sudarsan, J.E. Barrick, R.R. Breaker, An mRNA structure that controls gene expression by binding S-adenosylmethionine, Nat. Struct. Biol. 10 (2003) 701–707. https://doi.org/10.1038/nsb967.

[33] A. Haller, R.B. Altman, M.F. Soulière, S.C. Blanchard, R. Micura, Folding and ligand recognition of the TPP riboswitch aptamer at single-molecule resolution, Proc. Natl. Acad. Sci. U.S.A. 110 (2013) 4188–4193. www.pnas.org/cgi/doi/10.1073/pnas.1218062110.

[34] M. Mandal, B. Boese, J.E. Barrick, W.C. Winkler, R.R. Breaker, Riboswitches control fundamental biochemical pathways in Bacillus subtilis and other bacteria, Cell 113 (2003) 577–586. https://doi.org/10.1016/S0092-8674(03)00391-X.

[35] M. Mandal, R.R. Breaker, Adenine riboswitches and gene activation by disruption of a transcription terminator, Nat. Struct. Mol. Biol. 11 (2004) 29–35. https://dx.doi.org/10.1038/nsmb710.

[36] R.R. Breaker, Complex riboswitches, Science 319 (2008) 1795–1797. https://doi.org/10.1126/science.1152621.

[37] A. Serganov, D.J. Patal, Molecular recognition and function of riboswitches, Curr. Opin. Struct. Biol. 22 (2012) 279–286. https://doi.org/10.1016/j.sbi.2012.04.005.

[38] R.R. Breaker, Riboswitches and the RNA world, Cold Spring Harb Perspect Biol 4 (2012) a003566. https://doi.org/10.1101/cshperspect.a003566.

[39] E. Nudler, A.S. Mironov, The riboswitch control of bacterial metabolism, Trends Biochem. Sci. 29 (2004) 11–17. https://doi.org/10.1016/j.tibs.2003.11.004.

[40] W.J. Greenleaf, K.L. Frieda, D.A. Foster, M.T. Woodside, S.M. Block, Direct observation of hierarchical folding in single riboswitch aptamers, Science 319 (2008) 630–633. https://doi.org/10.1126/science.1151298.

[41] S.D. Gilbert, C.D. Stoddard, S.J. Wise, R.T. Batey, Thermodynamic and kinetic characterization of ligand binding to the purine riboswitch aptamer domain, J. Mol. Biol. 359 (2006) 754–768. https://doi.org/10.1016/j.jmb.2006.04.003.

[42] J.K. Wickiser, M.T. Cheah, R.R. Breaker, D.M. Crothers, The kinetics of ligand binding by an adenine-sensing riboswitch, Biochemistry 44 (2005) 13404–13414. https://doi.org/10.1021/bi051008u.

[43] J.K. Wickiser, W.C. Winkler, R.R. Breaker, D.M. Crothers, The speed of RNA transcription and metabolite binding kinetics operate an FMN riboswitch, Mol. Cell. 18 (2005) 49–60. https://doi.org/10.1016/j.molcel.2005.02.032.

[44] K. Hollands, S. Proshkind, S. Sklyarovad, V. Epshteine, A. Mironovd, E. Nudlere, E.A. Groisman, Riboswitch control of Rho-dependent transcription termination, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 5376–5381. https://doi.org/10.1073/pnas.1112211109.

[45] A.G. Vitreschak, D.A. Rodionov, A.A. Mironov, M.S. Gelfand, Regulation of riboflavin biosynthesis and transport genes in bacteria by transcriptional and translational attenuation, Nucleic Acids Res. 30 (2002) 3141–3151. https://doi.org/10.1093/nar/gkf433.

[46] J. Vogel, V. Bartels, T.H. Tang, G. Churakov, J.G. Slagter-Jäger, A. Hüttenhofer, E.G.H. Wagner, RNomics in Escherichia coli detects new sRNA species and indicates parallel transcriptional output in bacteria, Nucleic Acids Res. 31 (2003) 6435–6443. http://doi.org/10.1093/nar/gkg867.

[47] M.J. Cromie, Y. Shi, T. Latifi, E.A. Groisman, An RNA sensor for intracellular Mg(2+), Cell 125 (2006) 7184. https://doi.org/10.1016/j.cell.2006.01.043.

[48] J.M. Berg, J.L. Tymoczko, G.J. Gatto Jr., L. Stryer, Biochemistry Eighth Ed, W.H-Freeman, New York, 2015. ISBN: 10: 1–4641-2610-0.

[49] K. Shahbabian, A. Jamalli, L. Zig, H. Putzer, RnaseY, a novel endoribonuclease, initiates riboswitch turnover in Bacillus subtilis, EMBOJ 28 (2009) 3523–3533. https://doi.org/10.1038/emboj.2009.283.

[50] M. Kawano, A.A. Reynolds, J. Miranda-Rios, G. Storz, Detection of 5′-and 3′-UTR-derived small RNAs and cis-encoded antisense RNAs in Escherichia coli, Nucleic Acids Res. 33 (2005) 1040–1050. https://doi.org/10.1093/nar/gki256.

[51] E. Loh, O. Dussurget, J. Gripenland, K. Vaitkevicius, T. Tiensuu, P. Mandin, F. Repoila, C. Buchrieser, P. Cossart, J. Johansson, A trans-acting riboswitch controls expression of the virulence regulator PrfA in Listeria monocytogenes, Cell 139 (2009) 770–779. https://doi.org/10.1016/j.cell.2009.08.046.

[52] N. Sudarsan, M.C. Hammond, K.F. Block, R. Welz, J.E. Barrick, A. Roth, R.R. Breaker, Tandem riboswitch architectures exhibit complex gene control functions, Science 314 (2006) 300–304. https://doi.org/10.1126/science.1130716.

[53] R. Welz, R.R. Breaker, Ligand binding and gene control characteristics of tandem riboswitches in Bacillus anthracis, RNA 13 (2007) 573–582. https://

560307.

doi.org/10.1261/rna.407707.

[54] M. Mandal, M. Lee, J.E. Barrick, Z. Weinberg, G.M. Emilsson, W.L. Ruzzo, R.R. Breaker, A glycine-dependent riboswitch that uses cooperative binding to control gene expression, Science 306 (2004) 275–279. https://dx.doi.org/10.1126/science.1100829.

[55] L. Huang, A. Serganov, D. Patel, Structural insights in to ligand recognition by a sensing domain of the cooperative Glycine riboswitch, Mol. Cell 40 (2010) 774–786. https://doi.org/10.1016/j.molcel.2010.11.026.

[56] K.M. Ruff, S.A. Strobel, Ligand binding by the tandem glycine riboswitch depends on aptamer dimerization but not double ligand occupancy, RNA 20 (2014) 1775–1788. https://doi.org/10.1261/rna.047266.114.

[57] A.M. Babina, N.E. Lea, Meyer, in vivo behavior of the tandem glycine riboswitch in Bacillus subtilis, mBio 8 (2017) e01602–e01617. https://doi.org/10.1128/mBio.01602-17.

[58] A. Gutiérrez-Preciado, T.M. Henkin, F.J. Grundy, C. Yanofsky, E. Merino, Biochemical Features, Functional, Implications of the RNA-based t-box regulatory mechanism, Microbiol. Mol. Biol. Rev. 73 (2009) 36–61. https://doi.org/10.1128/MMBR.00026-08.

[59] J. Kortmann, F. Narberhaus, Bacterial RNA thermometers: molecular zippers and switches, Nat. Rev. Microbiol. 10 (2012) 255–265, https://doi.org/10.1038/nrmicro2730.

[60] F. Narberhaus, T. Waldminghous, S. Chowdhury, RNA thermometers, FEMS Microbiol. Rev. 30 (2006) 3–16. https://doi.org/10.1111/j.1574-6976.2005.004.x.

[61] N.P. Hoe, J.D. Goguen, Temperature sensing in Yersinia pestis: translation of the LcrF activator protein is thermally regulated, J. Bacteriol. 175 (1993) 7901–7909. PMID: 7504666 PMCID: PMC206968.

[62] M. Scortti, H.J. Monzó, L. Lacharme-Lora, D.A. Lewis, J.A. Vázquez-Boland, The PrfA virulence regulon, Microb. Infect. 10 (2007) 1196–1207. http://doi.org/10.1016/j.micinf.2007.05.007.

[63] J. Johansson, P. Mandin, A. Renzoni, C. Chiaruttini, M. Springer, P. Cossart, An RNA thermosensor controls expression of virulence genes in Listeria monocytogenes, Cell 110 (2002) 551–561. https://doi.org/10.1016/S0092-8674(02)00905-4.

[64] J. Rinnenthal, B. Klinkert, F. Narberhaus, H. Schwalbe, Direct observation of the temperature-induced melting process of the Salmonella fourU RNA thermometer at base-pair resolution, Nucleic Acids Res. 38 (2010) 3834–3847. https://doi.org/10.1093/nar/gkq124.

[65] A.M. Giuliodori, F. Di Pietro, S. Marzi, B. Masquida, R. Wagner, P. Romby, C.O. Gualerzi, C.L. Pon, The cspA mRNA is a thermosensor that modulates translation of the cold shock protein CspA, Mol. Cell 37 (2010) 21–33. https://doi.org/10.1016/j.molcel.2009.11.033.

[66] A. Nocker, T. Hausherr, S. Balsiger, N.P. Krstulovic, H. Hennecke, F. Narberhaus, A mRNA-based thermosensor controls expression of rhizobial heat shock genes, Nucleic Acids Res. 29 (2001) 4800–4807. PMCID: PMC96696.

[67] A. Nocker, N.P. Krstulovic, X. Perret, F. Narberhaus, ROSE elements occur in disparate rhizobia and are functionally interchangeable between species, Arch. Microbiol. 176 (2001) 44–51. PMID: 11479702.

[68] T. Waldminghaus, A. Fippinger, J. Alfsmann, F. Narberhaus, RNA thermometers are common in α- and γ-proteobacteria, Biol. Chem. 386 (2005) 1279–1286. https://doi.org/10.1515/BC.2005.145.

[69] T. Waldminghaus, L.C. Gaubig, B. Klinkert, F. Narberhaus, The Escherichia coli ibpA thermometer is comprised of stable and unstable structural elements, RNA Biol. 6 (2009) 455–463. https://doi.org/10.4161/rna.6.4.9014.

[70] S. Chowdhury, C. Ragaz, E. Kreuger, F. Narberhaus, Temperature-controlled structural alterations of an RNA thermometer, J. Biol. Chem. 278 (2003) 47915–47921. https://doi.org/10.1074/jbc.M306874200.

[71] D. Lai, J.R. Proctor, I.M. Meyer, On the importance of cotranscriptional RNA structure formation, RNA 19 (2013) 1461–1473. http://doi.org/10.1261/rna.037390.112.

[72] T. Yura, K. Kanemori, M.T. Morita, The heat shock response: regulation and function, in: G. Storz, R. Hengge-Aronis (Eds.), Bacterial Stress Responses, ASM Press, Washington, 2000, pp. 3–18.

[73] L.C. Gaubig, T. Waldminghaus, F. Narberhaus, Multiple layers of control govern expression of the Escherichia coli ibpAB heat-shock operon, Microbiology 157 (2011) 66–76, https://doi.org/10.1099/mic.0.043802-0.

[74] M. Matuszewska, D. Kuczynska-Wisnik, E. Laskowska, K. Liberek, The small heat shock protein IbpA of Escherichia coli cooperates with IbpB in stabilization of thermally aggregated proteins in a disaggregation competent state, J. Biol. Chem. 280 (2005) 12292–12298. https://doi.org/10.1074/jbc.M412706200.

[75] L. Veinger, S. Diamant, J. Buchner, P. Goloubinoff, The small heat-shock protein IbpB from Escherichia coli stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network, J. Biol. Chem. 273 (1998) 11032–11037. https://doi.org/10.1074/jbc.273.18.11032.

[76] T. Waldminghaus, N. Heidrich, S. Brantl, F. Narberhaus, FourU: a novel type of RNA thermometer in Salmonella, Mol. Microbiol. 65 (2007) 413–424. https://doi.org/10.1111/j.1365-2958.2007.05794.x.

[77] J. Rinnenthal, B. Klinkert, F. Narberhaus, H. Schwalbe, Modulation of the stability of the Salmonella fourU-type RNA thermometer, Nucleic Acids Res. 39 (2011) 8258–8270. https://doi.org/10.1093/nar/gkr314.

[78] B. Klinkert, A. Cimdins, L.C. Gaubig, J. Rossmanith, U. Aschke-Sonnenborn, F. Narberhaus, Thermogenetic tools to monitor temperature-dependent gene expression in bacteria, J. Biotechnol. 160 (2012) 55–63. https://doi.org/10.1016/j.jbiotec.2012.01.007.

[79] S.S. Krajewski, F. Narberhaus, Temperature-driven differential gene expression by RNA thermosensors, Biochim. Biophys. Acta 1839 (2014) 978–988, https://doi.org/10.1016/j.bbagrm.2014.03.006.

[80] B. Lim, C.A. Gross, Cellular response to hear shock and cold shock, in: G. Storz, R. Hengge (Eds.), Bacterial Stress Responses, second ed., ASM press, Washington, 2011, pp. 93–114.

[81] B. Schmid, J. Klumpp, E. Raimann, M.J. Loessner, R. Stephan, T. Tasara, Role of cold shock proteins in growth of Listeria monocytogenes under cold and osmotic stress conditions, Appl. Environ. Microbiol. 75 (2009) 1621–1627. https://doi.org/10.1128/AEM.02154-08.

[82] K. Böhme, R. Steinmann, J. Kortmann, S. Seekircher, A.K. Heroven, E. Berger, F. Pisano, T. Thiermann, H. Wolf-Watz, F. Narberhaus, P. Dersch, Concerted actions of a thermo-labile regulator and a unique intergenic RNA thermo-sensor control Yersinia virulence, PLoS Pathog. 8 (2012) e1002518.

[83] E. Loh, E. Kugelberg, A. Tracy, Q. Zhang, B. Gollan, H. Ewles, R. Chalmers, V. Pelicic, C.M. Tang, Temperature triggers immune evasion by Neisseria meningitidis, Nature 502 (2013) 237–240. https://doi.org/10.1038/nature12616.

[84] B. Grabowski, M.A. Schmidt, C. Rüter, Immunomodulatory Yersinia outer proteins (Yops)–useful tools for bacteria and humans alike, Virulence 8 (7) (2017) 1124–1147. https://doi.org/10.1080/21505594.2017.1303588.

[85] G.R. Cornelis, A. Boland, A.P. Boyd, C. Geuijen, M. Iriarte, C. Neyt, M.P. Sory, I. Stainier, The virulence plasmid of Yersinia, an antihost genome, Microbiol. Mol. Biol. Rev. 62 (1998) 1315–1352.

[86] N.P. Hoe, F.C. Minion, J.D. Goguen, Temperature sensing in Yersinia pestis: regulation of yopE transcription by lcrF, J. Bacteriol. 174 (1992) 4275–4286. PMID: 1624422 PMCID: PMC206210.

[87] L. de Rouvroit, C. Sluiters, G.R. Cornelis, Role of the transcriptional activator, VirF, and temperature in the expression of the pYV plasmid genes of Yersinia enterocolitica, Mol. Microbiol. 6 (1992) 395–409. https://doi.org/10.1111/j.1365-2958.1992.tb01483.x.

[88] M.W. Jackson, E. Silva-Herzog, G.V. Plano, The ATP-dependent ClpXP and Lon proteases regulate expression of the Yersinia pestis type III secretion system via regulated proteolysis of YmoA, a small histone-like protein, Mol. Microbiol. 54 (2004) 1364–1378.

[89] N. Tu, R.K. Carroll, A. Weiss, L.N. Shaw, G. Nicolas, S. Thomas, A. Lima, U. Okaro, B. Anderson, A family of genus- specific RNAs in tandem with DNA-binding proteins control expression of the badA major virulence factor gene in Bartonella henselae, Microbiol. Open 00 (2016) 1–14. https://doi.org/10.1002/mbo3.420.

[90] N.F. Müller, P.O. Kaiser, D. Linke, H. Schwarz, T. Riess, S. Schäfer, J.A. Eble, V.A.J. Kempf, Trimeric autotransporter adhesin- dependent adherence of Bartonella henselae, Bartonella quintana, and Yersinia enterocolitica to matrix components and endothelial cells under static and dynamic flow conditions, Infect. Immun. 79 (2011) 2544–2553. https://dx.doi.org/10.1128/IAI.01309-10.

[91] T. Riess, S.G. Andersson, A. Lupas, M. Schaller, A. Schafer, P. Kyme, J. Martin, J. Wälzlein, U. Ehehalt, H. Lindroos, M. Schirle, A. Nordheim, I.B. Autenrieth, V.A. Kempf, Bartonella adhesin mediates a proangiogenic host cell response, J. Exp. Med. 200 (2004) 1267–1278. https://doi.org/10.1084/jem.20040500.

[92] N.E. Freitag, G.C. Port, M.D. Miner, Listeria monocytogenes — from saprophyte to intracellular pathogen, Nat. Rev. Microbiol. 7 (2009) 623–628. https://doi.org/10.1038/nrmicro2171.

[93] M. Hamon, H. Bierne, P. Cossart, Listeria monocytogenes: a multifaceted model, Nat. Rev. Microbiol. 4 (2006) 423–434. https://doi.org/10.1038/nrmicro1413.

[94] S. DebRoy, M. Gebbie, A. Ramesh, J.R. Goodson, M.R. Cruz, A. vanHoof, W.C. Winkler, D.A. Garsin, Riboswitches. A riboswitch-containing sRNA controls gene expression by sequestration of a response regulator, Science 345 (2014) 937–940. https://doi.org/10.1126/science.1255091.

[95] J.R. Mellin, M. Koutero, D. Dar, M.A. Nahori, R. Sorek, P. Cossart, Sequestration of a two-component response regulator by a riboswitch-regulated non-coding RNA, Science 345 (2014) 940–943. https://doi.org/10.1126/science.1255083.

[96] Y. Bertin, J.P. Girardeau, F. Chaucheyras-Durand, B. Lyan, E. Pujos-Guillot, J. Harel, C. Martin, Enterohaemorrhagic Escherichia coli gains a competitive advantage by using ethanolamine as a nitrogen source in the bovine intestinal content, Environ. Microbiol. 13 (2011) 365–377. https://doi.org/10.1111/j.1462-2920.2010.02334.x.

[97] P. Thiennimitr, S.E. Winter, M.G. Winter, M.N. Xavier, V. Tolstikov, D.L. Huseby, T. Sterzenbach, R.M. Tsolis, J.R. Roth, A.J. Bäumler, Intestinal inflammation allows Salmonella to use ethanolamine to compete with the microbiota, Proc. Natl. Acad. Sci. U.S.A. 108 (2011) 17480–17485. https://doi.org/10.1073/pnas.1107857108.

[98] O. Tsoy, D. Ravcheev, A. Mushegian, Comparative genomics of ethanolamine utilization, J. Bacteriol. 191 (2009) 7157–7164. https://doi.org/10.1128/JB.00838-09.

[99] C. Archambaud, M. Nahori, G. Soubigou, C. Bécavin, L. Laval, P. Lechat, T. Smokvina, P. Langella, M. Lecuit, P. Cossart, Impact of lactobacilli on orally acquired listeriosis, Proc. Natl. Acad. Sci. U.S.A. 109 (2012) 16684–16689. https://doi.org/10.1073/pnas.1212809109.

[100] S.M. Payne, E.E. Wyckoff, E.R. Murphy, A.G. Oglesby, M.L. Boulette, N.M. Davies, Iron and pathogenesis of Shigella: iron acquisition in the intracellular environment, Biometals 2 (2006) 173–180. https://doi.org/10.

1007/s10534-005-4577-x.

[101] Y. Wei, E.R. Murphy, *Shigella* iron acquisition systems and their regulation, Front. Cell. Infect. Microbiol. 6 (2016) 18. http://doi.org/10.3389/fcimb.2016.00018.

[102] M. Mills, S.M. Payne, Genetics and regulation of heme iron transport in *Shigella dysenteriae* and detection of an analogous system in *Escherichia coli* O157:H7, J. Bacteriol. 177 (1995) 3004–3009. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC176986/.

[103] E.E. Wyckoff, D. Duncan, A.G. Torres, M. Mills, K. Maase, S.M. Payne, Structure of the *Shigella dysenteriae* haem transport locus and its phylogenetic distribution in enteric bacteria, Mol. Microbiol. 28 (1998) 1139–1152. https://doi.org/10.1046/j.1365-2958.1998.00873.x.

[104] A.B. Kouse, F. Righetti, J. Kortmann, F. Narberhaus, E.R. Murphy, RNA-mediated thermoregulation of iron-acquisition genes in *Shigella dysenteriae* and pathogenic *Escherichia coli*, PLoS One 8 (2013) e63781, https://doi.org/10.1371/journal.pone.0063781.

[105] D.J. DiRita, C. Parsot, G. Jander, J.J. Mekalanos, Regulatory cascade controls virulence in Vibrio cholerae, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 5403–5407.

[106] A.J. Silva, J.A. Benitez, *Vibrio cholerae* biofilms and cholera pathogenesis, PLoS Neglected Trop. Dis. 10 (2016), https://doi.org/10.1371/journal.pntd.0004330 e0004330.

[107] G.G. Weber, J. Kortmann, F. Narberhaus, K.E. Klose, RNA thermometer controls temperature-dependent virulence factor expression in *Vibrio cholerae*, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 14241–14246. www.pnas.org/cgi/doi/10.1073/pnas.1411570111.

[108] P. Forterre, The two ages of the RNA world, and the transition to the DNA world: a story of viruses and cells, Biochimie 87 (2005) 793–803. https://doi.org/10.1016/j.biochi.2005.03.015.

[109] P. Forterre, D. Prangishvili, The origin of viruses, Res. Microbiol. 160 (2009) 466–472. https://doi.org/10.1016/j.resmic.2009.07.008.

[110] E.C. Holmes, The Evolution and Emergence of RNA Viruses, Oxford University Press, Oxford, 2009. ISBN-10: 0199211132.

[111] Z. Weinberg, J.X. Wang, J. Bogue, J. Yang, K. Corbino, R.H. Moy, R.R. Breaker, Comparative genomics reveals 104 candidate structured RNAs from bacteria, archaea, and their metagenomes, Genome Biol. 11 (2010) R31, https://doi.org/10.1186/gb-2010-11-3-r31.

[112] C.A. Kellenberger, S.C. Wilson, S.F. Hickey, T.L. Gonzalez, Y. Su, Z.F. Hallberg, T.F. Brewer, A.T. Iavarone, H.K. Carlson, Y.F. Hsieh, M.C. Hammond, GEMM-I riboswitches from Geobacter sense the bacterial second messenger cyclic AMP-GMP, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 5383–5388, https://doi.org/10.1073/pnas.1419328112.

[113] J.W. Nelson, N. Sudarsan, G.E. Phillips, S. Stav, C.E. Lünse, P.J. McCown, R.R. Breaker, Control of bacterial exoelectrogenesis by c-AMP-GMP, Proc. Natl. Acad. Sci. U.S.A. 112 (2015) 5389–5394, https://doi.org/10.1073/pnas.1419264112.

[114] Z. Weinberg, J.W. Nelson, C.E. Lünse, M.E. Sherlock, R.R. Breaker, Bioinformatic analysis of riboswitch structures uncovers variant classes with altered ligand specificity, Proc. Natl. Acad. Sci. U.S.A. 114 (2017) E2077–E2085, https://doi.org/10.1073/pnas.1619581114.