Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Multiple conformational states of riboswitches fine-tune gene regulation

Boris Fürtig, Senada Nozinovic, Anke Reining and Harald Schwalbe

Riboswitches are structured regions of mRNAs that modulate gene expression in response to specific binding of low molecular-weight ligands. They function by induced transitions between different functional conformations. The standard model assumed that the two functional states, the ligand-bound and ligand-free state, populated only two stable conformations. Recent discoveries of multiple conformations for the apo-state and holo-state of riboswitches challenge this model. Moreover, it becomes evident that detected conformational heterogeneity — mostly in the apo-state — provides sensitivity to multiple environmental inputs for riboswitch-based gene-regulation.

Address
Institute of Organic Chemistry and Chemical Biology, BMRZ, Johann Wolfgang Goethe-Universität, Max von Laue-Straße 7, 60438 Frankfurt am Main, Germany

Corresponding author: Schwalbe, Harald (schwalbe@nmr.uni-frankfurt.de)

Current Opinion in Structural Biology 2015, **30**:112–124

This review comes from a themed issue on **Nucleic acids and their protein complexes**

Edited by **Hashim al Hashimi** and **Fred Allain**

http://dx.doi.org/10.1016/j.sbi.2015.02.007

0959-440X/© 2015 Elsevier Ltd. All rights reserved.

Introduction

Riboswitches are RNA structural elements that sense the change in concentration of intracellular metabolites. Through direct binding of these metabolites to the RNA, riboswitches regulate gene expression [1]. They are found in non-coding regions of mRNAs, primarily in the 5′-untranslated region (5′-UTR) [2]. Riboswitches can regulate gene expression at different levels: at translation initiation [1], premature transcription termination [3], auto-catalytic cleavage [4], splicing [5] and control of rho-dependent transcription termination [6]. Low molecular weight ligands typically bind to riboswitches with high affinity and specificity and they span a large variety of chemical space. Riboswitches for purine nucleo-bases, nucleotides and nucleosides [7], vitamins [1], second messengers [8], amino acids [9], magnesium cations [10] and even fluoride anions [11] have been reported. Riboswitch-mediated regulation can either enhance or suppress gene expression. The two functions are referred to as ON-switch and OFF-switch, respectively.

Although control mechanisms and ligands are diverse, the modular assembly is maintained as common architectural principle for all riboswitches. This modular assembly of riboswitches comprises an aptamer domain and an effector platform. Ligand binding induces a conformational transition in the aptamer that is transmitted to the effector platform where it induces a structural rearrangement of functional sequence elements [12]. Commonly, this signal transduction involves mutually exclusive base pairing interactions within the part of the mRNA that connects the aptamer domain and the expression platform. With respect to the sequential arrangement of both functional units, we wish to introduce the following concept: in coupled riboswitches, aptamer and expression platform overlap, whereas in decoupled riboswitches both domains are separated by a number of nucleotides that are neither part of the aptamer nor the expression platform.

The simplest biophysical model to account for the function of a riboswitch under thermodynamic control assumes that the RNA can adopt two mutually exclusive conformations, representing the apo-state and holo-state of the molecule. For an ON-switch, the ligand-free apo-state represented by a single unbound RNA conformation is the inactive state, gene expression is repressed. For such an ON-switch, ligand binding leads to the formation of the metabolite-bound riboswitch (the holo-state) and gene expression is activated through formation of a single stable RNA–ligand complex conformation. Ligand binding to the aptamer domain induces an allosteric conformational transition and triggers a downstream functional effect. The apo-conformation and holo-conformation are at chemical equilibrium. The position of this equilibrium depends on the affinity of the interaction of metabolite and the riboswitch RNA and their concentrations. For an ON-switch the potential intrinsic equilibrium, in which the riboswitch adopts a functional ON-state even in the absence of ligand, is (exclusively) shifted towards the inactive conformation. The description of such a two-state model can be easily delineated from the law of mass action. For this simple model and under the assumption that binding has reached equilibrium, the parameter characterizing the system is the dissociation constant

K_D, describing at which ligand concentration half saturation of the riboswitch RNA is reached. In order to progress from a biophysically controlled system to the cellular situation, it is essential to know the intracellular concentrations of mRNA, of Mg^{2+} and the difference in ligand concentrations inducing the change from gene repression to gene activation.

It is noteworthy to realize that translation-regulating riboswitches, for example, the adenine-dependent switch found in the 5′-UTR of the *add* gene in *Vibrio vulnificus*, are under thermodynamic control. After initial coupled transcription and translation, in which the newly synthesized mRNA chain is directly bound by the ribosome and translated, the produced message subsequently binds ribosomes multiple times, and equilibrium between states can be achieved given the average distance between translating ribosomes of 100 nucleotides [13] and the typical sizes for riboswitches. Under these conditions, the dissociation constant equals the concentration of ligand at which half maximal regulatory response is achieved, $EC_{50} \approx K_D$. By contrast, transcription-regulating riboswitches, for example, the adenine-dependent riboswitch found in the 5′-UTR of the *pbuE* gene in *Bacillus subtilis*, employ a fundamentally different mechanism. Those switches operate at least partially under kinetic control [14–16], as the adaptation of a functional state has to occur in a time window defined by the velocity of transcription, and therefore under these conditions it is often found that $EC_{50} \gg K_D$.

Nevertheless, in both cases the dissociation constant can sufficiently describe the relation of the two conformational states for a simple two-state mechanism. However, under translational control, K_D is the important parameter defining regulation efficiency. For transcriptional riboswitches operating under kinetic control, we wish to stress that two kinetic processes determine the efficiency of the switch: k_{on}.[Metabolite] determines the rate of binding to the aptamer domain. Furthermore, regulation further depends also on the subsequent refolding rate k_{refold} describing the structural rearrangement of the RNA [17,18].

The deceptive beauty of aptamer holo-state conformations

Numerous X-ray structures of ligand-bound aptamer domains of riboswitches have fueled the concept that the apo-state and holo-state can best be described by a single RNA conformation. Immediately after the first reports describing riboswitches as novel RNA regulation elements, numerous structural studies on holo-state aptamer domains have been published [10,19–26]. These studies revealed how low-molecular weight ligands could be recognized by natural riboswitch aptamers. While previously targeted ligands designed to bind RNAs with high affinity in the context of drug discovery often had only limited success, the discovered natural riboswitches showed that specific binding of metabolites is in fact possible, and the underlying principles of molecular recognition were unexpected and remain astounding. Besides this success in explaining the specificity and the source of affinity, however, these initial high-resolution structural studies on holo-state aptamer domains lacked — firstly, the description of the apo-state and secondly, the description of full-length riboswitches including both aptamer and effector domain. Chemical probing experiments on riboswitches revealed that the accessibility and hence the secondary and tertiary structure is modulated in a ligand-dependent manner. The extent of variations in the in-line probing pattern between ligand-free and ligand-bound forms differs from riboswitch to riboswitch, for example, up to nine different sites of structural modulation are found in the coenzyme B12dependent riboswitch from *btuB* mRNA in *Escherichia coli* [27] whereas only nucleotides adjacent to the central five-way junction in the lysine-dependent riboswitch in the *lysC* mRNA of *B. subtilis* exhibit different accessibilities [9]. From the differences in the in-line probing pattern one expects that the conformations representing the apo-state of riboswitches show different three-dimensional structure and tertiary interactions that rely on a modulation of distinct secondary structures, at least in part.

Meanwhile, the apo-state crystal structures for at least four different riboswitch aptamer domains are available including the preQ1-dependent riboswitch regulating transcription found in the organism *Thermatoga tengcongensis*; the SAM-I dependent riboswitch from *T. tencongensis*; the lysine-dependent riboswitch from *Thermatoga maritima* and the glycine-dependent riboswitch from *V. cholerae* [28,29,30•,31•]. It is striking to realize that the differences between the apo-structure and holo-structure are small and the aptamer domains share the same secondary structure in both states. Overall, the structures align with relatively minute differences, for the riboswitches the RMSD lies between 0.3 Å and 0.5 Å. Most prominent changes are restricted to the ligand-binding pocket. In the crystallized apo-state it is often occupied by internal residues, a behavior that is also reported for mutants of the purine riboswitches, where an remote nucleotide is able to mimic the purine ligand in the ligand-free state [32].

The very small differences between these structures raises the question whether they truly represent the ligand-dependent difference between the functional conformations of ON-state and OFF-state and how an allosteric conformational switch can be induced in the effector domain by these small amplitude changes within the aptamer domain. Crystal formation could favor compact structures. For SAM-I, however, the identity of secondary structure in absence and presence of ligand

Download English Version:

https://daneshyari.com/en/article/8320021

Download Persian Version:

https://daneshyari.com/article/8320021

Daneshyari.com