Riboswitches: Fold and Function

Riboswitches in the 5′-untranslated regions of mRNAs cotranscriptionally couple ligand binding and gene regulation. In this issue of *Chemistry & Biology*, Lemay et al. [1] describe folding of a key tertiary interaction in the adenine riboswitch and its mechanistic consequences.

Folding from a nascent chain into a biologically functional entity is an essential stage in the life of every macromolecule. For most proteins and RNAs, a crucial feature of folding is that all pathways lead to a single productive conformation, limiting misfolded or inactive species. Only after acquiring native structure do these molecules begin to respond to their cellular environment, making “decisions” such as whether a repressor will bind to a DNA operator or a kinase will phosphorylate its target protein. Riboswitches, a recently discovered type of genetic regulatory element found in the 5′-untranslated regions (5′-UTR) of a number of bacterial mRNAs (reviewed in [2]), differ from this theme in that the folding process leads to one of two distinct biological outcomes: gene expression or repression. These regulatory sequences function in the absence of associated proteins and in response to cellular levels of particular small-molecule metabolites. Complicating this process is the fact that coupled folding and “decision-making” occurs cotranscriptionally such that the final outcome is set before the polymerase escapes the 5′-UTR, a critically important feature for riboswitches that regulate at the transcriptional level. In the current issue of *Chemistry & Biology*, Lafontaine and colleagues provide new insights into the first stage of this process through their investigation of the coupling between folding of the adenine riboswitch ligand recognition domain and its ability to productively bind adenine [1].

Riboswitches are composed of two overlapping domains: a 5′ aptamer domain responsible for binding a specific metabolite and a downstream expression platform that controls gene expression. Switching between expression and repression is accomplished by the expression platform, which adopts one of two mutually exclusive secondary structures (in the case of transcriptional regulation, either an antiterminator or a terminator element), depending upon whether the aptamer domain has bound the appropriate ligand. The aptamer domain of the adenine riboswitch, as well as the structurally related guanine riboswitch, comprises a compact three-way junction structure ([Figure 1A](#)) [3–6]. Loops capping the P2 and P3 helices, which contain a number of highly phylogenetically conserved nucleotides, interact through two base quadruples and a noncanonical base pair ([Figure 1A](#), cyan). The ligand binding pocket is formed by a three-way junction in which the purine nucleobase is the keystone for another set of tertiary interactions between the joining regions ([Figure 1A](#), J1/2, J2/3, and J3/1). Purine specificity is dictated by a single pyrimidine residue (denoted by an asterisk in Figure 1) that interacts with the ligand through Watson-Crick base pairing to yield a ~20,000-fold discrimination between guanine and adenine [3, 4].

A key feature of the adenine riboswitch is a tertiary interaction between the terminal loops (L2 and L3) whose proper formation is essential for ligand binding [3]. As a first step toward understanding how this element of architecture serves to establish the global fold of the aptamer domain, Lafontaine and coworkers [1] extensively mutagenized residues in the loops to determine their relative importance for riboswitch function. Despite the universal conservation of many of these nucleotides, most can be altered with only a small impact upon ligand binding. The structurally important core of this interaction appears to be two G-C Watson-Crick pairs (G37-C61 and G38-C60 in Figure 1A) that form part of each quartet. Even more surprising from a structural perspective is that the sequence of the two loops can be swapped without loss of affinity for adenine. Together, these results indicate that the loop-loop interaction is plastic and able to compensate for many mutations, despite the fact that over half these nucleotides are phylogenetically invariant.

One of the primary questions about the folding of the adenine riboswitch is how does the acquisition of tertiary architecture facilitate ligand binding? Single-molecule FRET studies provide a unique perspective into this process. Lemay et al. report results obtained by labeling the loops with a FRET donor-acceptor pair (stars in Figure 1) [1]. Under low magnesium ion conditions (<50 μM) the RNA experiences three folding states, described by the authors of the current study as “U,” “I,” and “F” [1]. The unfolded state (U) represents the RNA with all of its secondary structural elements formed (P1, P2, and P3), but with no tertiary structure. In this state, the three helices are likely to be spayed apart, similar to what is observed in other three-way junctions (Figure 1B) [7–9]. The folded state (F), conversely, represents a globally organized state in which the loop-loop interaction brings P2 and P3 together, priming the RNA for ligand recognition. However, in-line probing [4], NMR [6], and mechanistic studies [10] have indicated that the three-way junction remains locally disordered to allow for ligand binding.

Surprisingly, there is an intermediate folding state (“I”) that is observed. While the authors do not speculate as to the nature of this conformation, when considered in the light of other studies, a reasonable model for this process can be proposed. In the absence of ligand, the three-way junction is not completely disordered; rather, one strand of the three-way junction, J3/1, is pre-organized to present a pyrimidine residue that forms the Watson-Crick pair with exogenous adenine or guanine [10]. Only upon productive pairing between the ligand and this pyrimidine (U65 of the riboswitch studied by the Lafontaine group) do J1/2 and J2/3 close around the ligand, forming the extensive set of interactions...
that make the nucleobase almost completely solvent inaccessible [3, 6]. One possibility is that the "I" state represents the coaxial stacking of the P1 and P3 helices along with the bases in J3/1 in the absence of the L2-L3 interaction (Figure 1B). In this state, L2 and L3 would be brought closer together, leading to the detected intermediate FRET signal. Formation of the loop-loop interaction would stabilize the P1-J3/1-P3 stacking, thereby promoting ligand binding.

During transcription, the aptamer domain is first synthesized by RNA polymerase and folds independently of the expression platform. A stretch of uridines following the aptamer domain causes the polymerase to stall temporarily, enabling temporal separation of the two folding events [11, 12]. Transcriptional pausing is important for the riboswitch's ability to bind metabolite; an RNA that does not experience this temporal decoupling of the two folding events binds adenine very

Figure 1. Structure of the Adenine Riboswitch and a Proposed Mechanism for Folding and Ligand Binding

(A) Secondary (left) and three-dimensional (right) structures of the add adenine riboswitch from *V. vulnificus* (PDB ID 1Y26). The cyan region represents the two loops (L2 and L3) that were studied in the work of Lemay et al. [1]. The three-way junction (green) contains the adenine (magenta) binding pocket. The red and yellow stars denote the two uridines that were labeled with FRET probes.

(B) A proposed mechanism by which folding and ligand binding are coupled in the adenine riboswitch. The U, I, and F/F* states were observed directly in single-molecule FRET studies. The elements of structure highlighted by orange circles are those that are organized in each step of the folding/binding mechanism. If the aptamer domain does not bind ligand, the RNA takes a second pathway leading to the F' state containing an antiterminator stem loop (AT).
poorly, if at all [3, 6]. In other cases where ligand binding is kinetically slow compared to the rate of transcription (e.g., for the FMN [12] and purine [11] riboswitches), pausing allows the aptamer domain sufficient time to bind ligand if the metabolite’s intracellular concentration is sufficiently high. This binding interaction allows the RNA to proceed from the F to F* state (Figure 1B). For the pbuE adenine riboswitch [5], acquisition of the F* state determines that transcription will be aborted and thus turns off gene expression.

In the absence of ligand, the 3′ side of the P1 helix dissociates from the aptamer domain and is used to form a stem-loop structure in the expression platform that dictates the second genetic outcome, upregulation of transcription (Figure 1B). A second pause site, usually found in the middle of the expression platform, enables riboswitches to perform the intrinsically slow secondary structural rearrangement (F → F* in Figure 1B) that leads to active transcription of the downstream coding sequence [12]. Previous studies have noted the necessity for the loop-loop interaction in ligand recognition, but they were unable to provide a plausible connecting mechanism. This coupling is an important issue, as three-way junctions are a ubiquitous structural motif for higher-order organization of RNA, involved in forming protein binding and catalytic active sites [13]. Characterization of the natural ("fast") hammerhead ribozyme revealed that a loop-loop interaction facilitates organization of the catalytic site located in a three-way junction, allowing it to cleave at greatly accelerated rates in physiologically relevant magnesium concentrations [14, 15]. Strikingly, this RNA has strong architectural similarities to the adenine riboswitch, illustrating that organization of functional RNA junctions is often facilitated by other elements of tertiary structure [16]. These recent studies underscore that we are still just beginning to understand how the ability of RNA to fold into intricate three-dimensional structures allows them to execute their diverse cellular functions.

Sunny D. Gilbert1 and Robert T. Batey1
1Department of Chemistry and Biochemistry
University of Colorado, Boulder
Campus Box 215
Boulder, Colorado 80309

Discovering New MAP Kinase Inhibitors

The current study by Kim et al. [1] (in this issue of Chemistry & Biology) uses a genetic approach with the yeast Schizosaccharomyces pombe to identify a highly specific inhibitor of Spc1 MAP kinase that competes with protein substrates for Spc1 interactions, but not with ATP binding.

Mitogen activated protein (MAP) kinases are key signaling proteins that mediate cellular responses to extracellular signals. Overactivation of the MAP kinase signaling pathways, primarily through genetic mutations of upstream regulatory proteins, is thought to play a prominent role in inflammatory diseases and tumor cell proliferation [2]. Thus, there is wide interest in the development of specific and effective MAP kinase inhibitors. In this issue of Chemistry & Biology [1], Kim et al. describe the identification of a highly specific, small molecular weight isoquinolinium compound that appears to inhibit only a specific MAP kinase isoform found in the yeast Schizosaccharomyces pombe (S. pombe) but has little effect on the activity of other yeast MAP kinases, MAP kinase activators, or homologous MAP kinases from mammalian species. These findings may help set the stage for the development of highly specific and hopefully more clinically effective MAP kinase inhibitors.