Conscious uncoupling of riboswitch functions

DOI 10.1074/jbc.H120.012787

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Riboswitches alter gene expression in response to ligand binding, coupling sensing and regulatory functions to help bacteria respond to their environment. The structural determinants of ligand binding in the prequeuosine (7-aminomethyl-7-deazaguanine, preQ1) bacterial riboswitches have been studied, but the functional consequences of structural perturbations are less known. A new article combining biophysical and cell-based readouts of 15 mutants of the preQ1-II riboswitch from Lactobacillus rhamnosus demonstrates that ligand binding does not ensure successful gene regulation, providing new insights into these shapeshifting sequences.

Riboswitches are segments within mRNA sequences that switch between two conformations in a ligand-dependent manner to regulate translation of the encoded gene (1). Maintaining sufficient pools of preQ1, then, is critical to bacterial homeostasis. Three types of riboswitches that bind preQ1 have been identified. The folding pathways, dynamics, and specific cation and ligand binding of preQ1 riboswitches have been explored through X-ray (5) and NMR (6) structures and other biochemical and biophysical studies (6). The new paper (4) concerns the preQ1-II riboswitch. All identified preQ1-II riboswitches contain Shine–Dalgarno sequences (SDSs) at their 3’-end, which-base pair with a highly conserved pyrimidine track to form an unusual H-type pseudoknot upon preQ1 binding (Fig. 1). Binding of preQ1 within this domain is stabilized by hydrogen bonding and stacking with highly conserved nucleotides in the riboswitch binding pocket, confirmed via mutational analysis of relevant bases. But how do these ligand-binding interactions translate to functional outcomes, and how can we use that knowledge?

The paper presented by Dutta and Wedekind (4) partially fills this gap of information. The authors prepared 15 mutants of the preQ1-II riboswitch containing changes within several critical structural elements of the pseudoknot forming the ligand-binding pocket, including the A-minor motif, the pseudoknot-insertion helix P4, U–A–U base triples, and canonical G–C pairs in the anti-SDS (aSDS) (Fig. 1). To determine how preQ1 is communicating through space with the distal region of riboswitch, the authors used a unique combined approach in which they paired isothermal titration calorimetry (ITC) to measure preQ1 binding with a cell-based analysis to monitor gene regulation. For the latter, they developed a cell-based system in which the riboswitch of interest controls a downstream GFPuv-reporter gene dependent on the addition of its ligand, preQ1.

With their experimental strategy prepared, the authors began investigating each of the mutations. First, the authors tested mutations of A70, which inserts into the minor groove to form an A-minor motif, and is proximal to preQ1 in the binding pocket. Each of the three riboswitch mutants tested fold similarly to WT, but the GFPuv-reporter gene expression was disrupted. The authors also tested mutations of A55, which stacks on A70 and interacts with the U31-A71-U40 triple in the binding pocket. The effects of the three A55 mutants were not as significant as mutations to A70; indeed, A55G and A55C mutants showed WT levels of gene repression. Dutta and Wedekind conclude that these bases are more important for gene regulation than preQ1 binding.

The second investigated region was the short P4 helix next to the preQ1 binding pocket. A mutant with the entire P4 hairpin deleted (ΔP4) showed gene-repression levels near WT; however, preQ1 binding was 25-fold weaker than WT. The authors

The authors declare that they have no conflicts of interest with the contents of this article.

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2. The abbreviations used are: preQ1, prequeuosine; Q, queuosine; SDS, Shine–Dalgarno sequence; aSDS, anti-SDS; ITC, isothermal titration calorimetry.
conclude that the short P4 hairpin length is sufficient for preQ₁ binding but leads to inefficient gene regulation.

The next region tested was the binding pocket itself, including the “floor” triple, U31·A71·U40, and the “sub-floor” triple, U32·A72·U39. The authors examined four models, including single and compensatory double mutants. The A71G mutant caused a strong change in both preQ₁ binding and gene regulation. The rescue mutant A71G/U31C was able to restore binding pocket structure, although gene regulation was still diminished. Looking at the sub-floor triple, the single mutation A72G did not inhibit preQ₁ binding or disrupt gene expression as much as A71G. The rescue mutant A72G/U32C improved preQ₁ binding compared with A71G, but gene regulation was weak. Together, these mutants suggest that interactions are needed at the Hoogsteen edges of A71 and A72 to support preQ₁ binding.

Finally, Dutta and Wedekind looked at G-C base pairing between the SDS and aSDS using single and double mutants. Experiments with the double mutants demonstrated a strong disruption of preQ₁-binding ability for C37G/C38G, but only a partial disruption for C37U/C38U. The single mutations C37U or C38U bound preQ₁ strongly, but their ability to regulate expression of the GFPuv-reporter gene was weak. These mutants suggest that the aSDS is important for regulatory switching and to promote a functional riboswitch fold.

Overall, the data presented by Dutta and Wedekind indicate that preQ₁-binding ability is not directly related with gene expression regulation, decoupling these actions into potentially separable steps. It would be interesting to identify a mutant for which ligand binding was altered but gene regulation was intact, to complement the insights gained here regarding specific interactions necessary for communication. The data also reinforce that hydrogen bonding and stacking interactions in the regions adjacent to the binding pocket influence binding of preQ₁, information that could be applied to the design of synthetic ligands (potentially therapeutic) that bind tightly to riboswitches or for the preparation of a preQ₁-II riboswitch with modified nucleotides to improve ligand binding and increase the efficiency of the riboswitch (7). Moreover, the data suggest that researchers seeking new riboswitch ligands should employ functional screens rather than ligand-binding assays, in case ligand binding does not properly activate the switch. One challenge for the field to enable these extensions will be improving computational analysis of RNA folds to include pseudoknots and ligand-bound structures. Finally, it will be interesting to see how general these findings are for other riboswitches, including the class I preQ₁ riboswitch that uses a much shorter binding domain, and how they might inform our understanding of other non-coding RNA structures. As with all scientific research, we’ll have to take it one step at a time.

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