Binding versus Triggering Riboswitches

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In this issue of *Chemistry & Biology*, Trausch and Batey report a discrepancy between ligand binding affinity and the effect of transcription termination in a THF riboswitch, raising some important questions about our current understanding of ligand-dependent RNA switches.

Riboswitches are gene-regulatory sequence motifs that are frequently found in 5′-UTRs of bacterial mRNAs (Breaker, 2012). Upon binding of the specific ligand to the aptamer domain of the riboswitch, gene expression is altered mainly by two common mechanisms: rearrangement of the expression platform that results in either transcriptional termination or control of translational initiation. In this issue of *Chemistry & Biology*, Trausch and Batey (2014) investigate tetrahydrofolate (THF) and a series of analogs with regard to riboswitch binding and transcription termination activities.

The THF riboswitch controls folate transport and synthesis in many Firmicutes (Ames et al., 2010). The THF riboswitch is special in several aspects. Although previously a matter of debate (Trausch et al., 2011; Huang et al., 2011), the present study demonstrates that typical THF aptamers possess two binding sites: one located adjacent to a three-way junction and the other located within a pseudoknot interaction. Importantly, although both sites show similar affinity for THF, population of the pseudoknot site seems to trigger termination of transcription. In addition, although the pterin moiety of THF is predominantly recognized by the RNA, the para-aminobenzoic acid residue is very important for regulation of transcription, but it does not contribute significantly to binding affinity. Along these lines, Trausch and Batey (2014) found adenine derivatives that bind with even higher affinities than THF but are unable to exert regulatory effects. The authors were able to discover such discrepancies, because they took into account not only structural data, affinity, and stoichiometry measurements, but they also correlated these with an in vitro activity assay that measures the effectiveness of the ligands to control transcription termination.

These findings have immediate consequences for strategies that aim to identify riboswitch ligand analogs as novel antibiotics. Targeting riboswitches with high-affinity derivatives of the native ligands has encountered some disappointing results with regard to their actual regulatory potential (Cressina et al., 2011). Hence, the present study highlights the need to involve functional assays when identifying novel antibiotic riboswitch effectors. In addition, the study is remarkable, because it reveals a hidden level of complexity that provokes further questions. As discussed by the authors, are the two binding sites of the THF riboswitch aptamer utilized for a more digital, positively cooperating response to the ligand as found in other riboswitch architectures (Breaker, 2012)? Or does the binding site near the three-way junction assist in riboswitch folding, a feature that is especially important in a scenario of kinetically controlled riboswitch action? Because many riboswitches are believed to be under kinetic control, knowledge of the ligand's affinity alone might often be insufficient to judge its regulatory potential (Haller et al., 2011). Moreover, is the identified purine binding to the pseudoknot site biologically significant in a manner that it is able to competitively counteract THF-mediated regulation?

The present study touches on some of the complicating aspects regarding the assignment of a biologically relevant riboswitch ligand; often, no appropriate in vivo assays for riboswitch control are available in the respective organisms. In addition, the exact levels of the diverse potential ligands are often unknown or hard to determine. Beyond knowing, it would be even more advantageous to be able to influence (genetically or chemically) the levels of potential ligands in vivo in order to identify relevant effectors of riboswitches. However, even if these criteria are met, riboswitch ligand assignment is error prone (Watson and Fedor, 2012; Nelson et al., 2013).

Riboswitches are very appealing because the underlying mechanisms appear to be easily comprehensible, making the redesign of RNA switches an ideal tool for synthetic biology purposes. They are often characterized as highly modular and conceptually simple designs of gene expression regulators. However, that this apparent simplicity is not always true is highlighted by some recent findings. For example, Schwalbe and coworkers described a riboswitch that operates a three-state instead of an anticipated two-state mechanism that senses the temperature in addition to adenine levels (Reining et al., 2013). It seems that RNA is well suited for implementing rather complex gene control devices, and it will be interesting to see to what extent nature makes use of such sophisticated complexities.

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