

Discovery of a bacterial riboswitch class that binds metabolites in a stacked configuration for cooperative gene regulation

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Riboswitches are structured non-coding RNAs often located upstream of essential genes in bacterial messenger RNAs. Such RNAs regulate expression of downstream genes by recognizing a specific cellular metabolite. Although 55 riboswitch classes are known, only a handful recognize multiple effectors. Here, we describe the 2.60-Å resolution co-crystal structure of a class I type I preQ1-sensing riboswitch that reveals two metabolite effectors stacked atop one another in a single binding pocket (Figure 1). This observation is unprecedented in RNA biology and has gone undetected since the discovery of preQ1 riboswitches fifteen years ago. Using biochemical approaches, we determined that preQ1 metabolites bind the riboswitch with positive cooperativity (i.e., cooperativity constant, γ , of 7.7) and that recognition of both metabolites is necessary for efficient gene regulation in bacterial cells. The observed mode of stacked effector recognition appears to be a hallmark of the largest subgroup of preQ1 riboswitches (i.e., type I), which includes those from human pathogens such as *Neisseria gonorrhoeae* and *Haemophilus influenzae*. We postulate that recognition of stacked effectors arose in an ancient prebiotic RNA World to facilitate close positioning of substrates for RNA-mediated catalysis. These findings expand known effector recognition capabilities of RNAs and have implications for antimicrobial development.

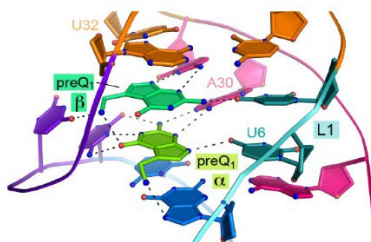


Figure 1