Structure of a Small Riboswitch that Binds Two Metabolite Ligands in One Pocket

Riboswitches are non-coding (nc)RNA elements typically found in the 5′ leader sequences of bacterial messenger RNAs where they control the expression of a downstream gene in response to levels of a cognate cellular metabolite. Metabolite binding to riboswitch's aptamer domain triggers a conformational change that alters the accessibility of a gene-regulatory sequence known as the expression platform[1]. Riboswitches are believed to participate in biochemical feedback loops, often controlling genes essential for fitness and survival. One of the best-studied riboswitches is the class I prequeuosine$_1$ (preQ$_1$-I) riboswitch, which is broadly distributed in the biosphere. PreQ$_1$-I riboswitches are typically 30-35 nucleotides long[2] and represent a powerful platform to study RNA structure[3, 4], dynamics[5, 6] and small molecule-RNA interactions[7, 8]. This riboswitch regulates the biosynthesis of queuosine (Fig. 1a), the loss of which has been linked to reduced bacterial virulence[9], suggesting that these small riboswitches are viable antimicrobial targets.

Although over 55 riboswitch classes have been discovered, only a handful recognize more than one metabolite[10]. Recently, the Wedekind Lab at the University of Rochester School of Medicine and Dentistry structurally characterized a preQ$_1$-I riboswitch that senses two preQ$_1$ metabolites (Fig. 1b). Whereas all known multi-metabolite sensing riboswitches position their ligands in separate binding pockets, this small riboswitch recognizes its two ligands in a single binding pocket using novel interactions.

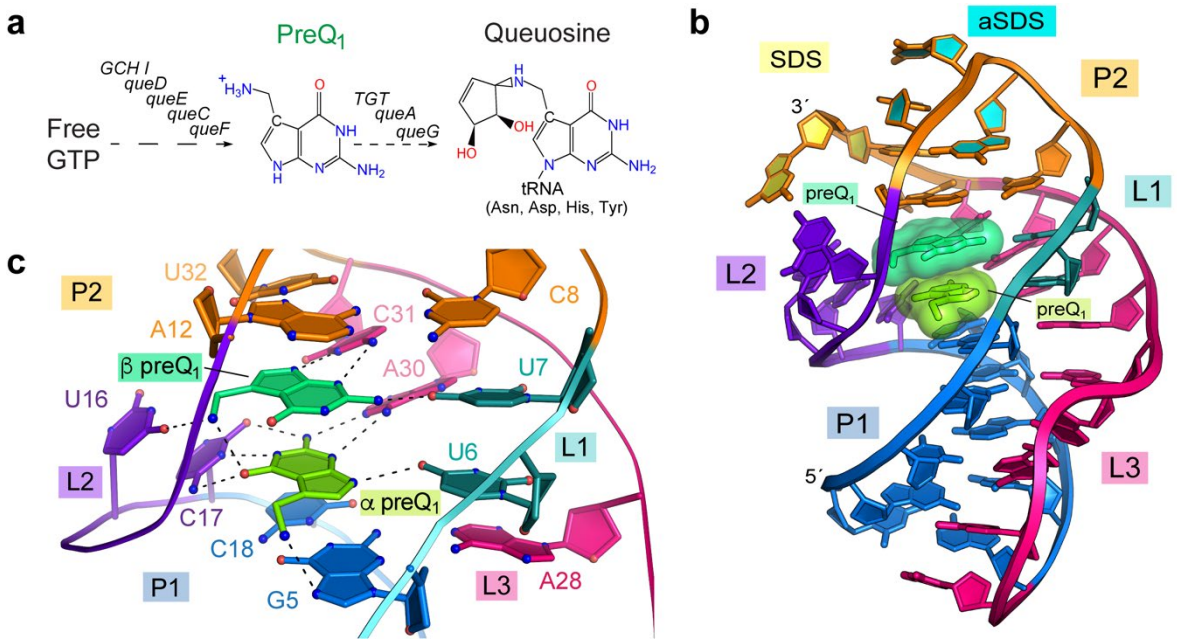

Figure 1: (**a**) The queuosine biosynthetic pathway proceeds through formation of the prequeuosine$_1$ (preQ$_1$) metabolite. (**b**) Ribbon diagram of the global, H-type pseudoknot fold of the preQ$_1$-I sensing riboswitch from *C. antarticus*. Bases in the gene regulatory Shine Dalgarno sequence (SDS) are highlighted yellow. Upon preQ$_1$ binding, these positions are hypothesized to pair with the anti(a)SDS, highlighted in cyan. (**c**) Overview of the fully occupied binding pocket featuring interacting preQ$_1$ metabolites, denoted alpha and beta. Dashed lines indicate hydrogen bonds.

Wedekind and co-workers discovered the 34-nucleotide $preQ_1$-I riboswitch in the genome of *Carnobacterium antarcticus* using an NCBI BLAST search. The riboswitch crystallized readily, and x-ray diffraction data were collected to 2.60 Å resolution on BL12-2, which revealed a pseudoknotted fold that engulfs two stacked metabolites. In addition to specific RNA interactions, the metabolites interact with each other via hydrogen bonding and aromatic stacking (Fig. 1c). The structure revealed key nucleotides that participate in $preQ_1$ recognition, which were validated experimentally by isothermal titration calorimetry and bacterial reporter assays in live cells. This work revealed that the two metabolites bind with positive cooperativity and that both $preQ_1$ molecules must bind for effective gene regulation.

The team also demonstrated that the mode of dual, stacked metabolite recognition is a hallmark of the most dominant $preQ_1$-sensing riboswitch class in terms of its presence in known bacterial DNA sequences. Moreover, existing riboswitches can provide insight into the capabilities of catalytic RNAs (ribozymes) that might have evolved in a prebiotic RNA world to carry out metabolism. The high conservation of this riboswitch across multiple bacterial phyla suggests that it has ancient origins. The team hypothesizes that this small aptamer could have arisen from a now-extinct ribozyme that held two metabolites close together for covalent bond formation or to exchange a chemical group. This finding changes our view of how RNA can interact with ligands and suggests a sophistication akin to single-domain proteins that bind multiple substrates in a one pocket.

Looking toward the future, $preQ_1$-I riboswitches are utilized by numerous bacteria to maintain homeostasis, including the antibiotic resistant pathogen *Nesseria. gonorrheae*[11]. The newly characterized, dual-metabolite binding pocket opens new opportunities to develop high-specificity small molecules that target this important gene-regulatory RNA, while averting cross-reactivity with the targets of natural metabolites.

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