MS93.O05

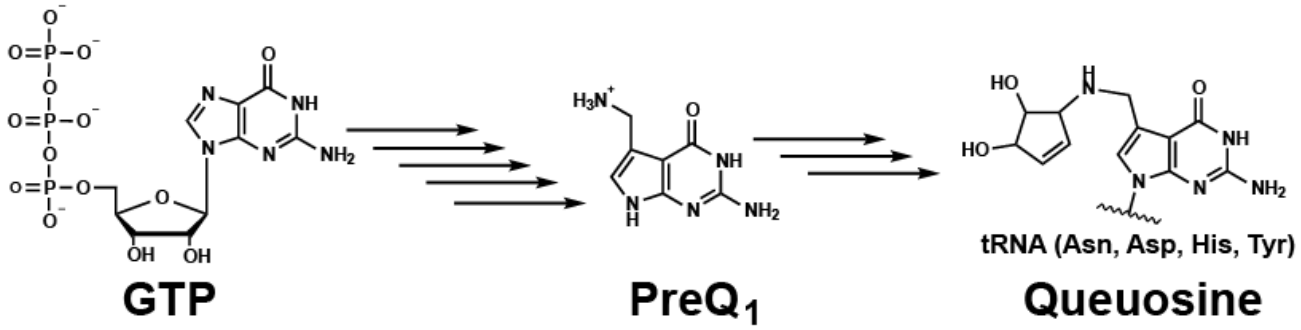
Translational control by diverse RNA folds that recognize the metabolite preQ$_1$

J. Liberman[1], M. Salim[1], I. Belashov[1], J. Wedekind[1]

[1]*University of Rochester, School of Medicine and Dentistry, Department of Biochemistry & Biophysics and Center for RNA Biology, Rochester, USA*

Typical paradigms of translational regulation utilize proteins, whereas riboswitches are economical, RNA-based elements that govern gene expression without protein partners. Present in all domains of life, riboswitches are commonly located in the 5´-untranslated regions of bacterial mRNAs where they establish feedback loops that respond to cellular levels of a specific small-molecule effector. Here we present the novel crystal structure of the recently discovered preQ$_1$-III (class 3) riboswitch at 2.85 Å resolution. The 101-nucleotide switch features an internal loop pseudoknot (iPK) that coaxially stacks upon flanking helices P2 and P3. PreQ$_1$ (7-aminomethyl-7-deazaguanine) − the last soluble intermediate in the biosynthesis of the hypermodified tRNA base queuosine (Scheme I) − binds to form a major groove base triple of the form U•preQ$_1$•C that utilizes highly conserved pyrimidine bases that help define this riboswitch class. This mode of effector binding involves non-canonical Watson-Crick pairing reminiscent of the phylogenetically unrelated preQ$_1$-II (class 2) riboswitch. Remarkably, both riboswitch classes utilize dual U•A-U base triples that stack against the ligand. The respective nine-nucleotide motifs and their preQ$_1$ ligands superimpose with an rmsd of 0.4 Å; the class 2 and 3 structures are otherwise unrelated. The class 3 preQ$_1$ binding pocket also includes contributions from junctions that connect the P2-iPK-P3 coaxial stack with perpendicularly positioned helix P4. Of special interest is the observation that P4 can form a hairpin-loop pseudoknot (hPK) with the ribosome binding site (RBS) of the mRNA, suggesting a mode of ligand-dependent RBS sequestration. Biochemical experiments describing the preQ$_1$ dependence of hPK formation will be presented, as well as a comparison to known, translational class 1 and 2 preQ$_1$ riboswitch structures [1-3]. Implications for bacterial translational control will be discussed based on this diverse riboswitch family.

[1] *Spitale R.C., Torelli A.T., Krucinska J., et al., (2009) The Structural Basis for Recognition of the PreQ$_0$ Metabolite by an Unusually Small Riboswitch Aptamer Domain. J. Biol. Chem. 284, 11012-6.,* ***[2]*** *Jenkins J.L., Krucinska J., McCarty R.M., et al., (2011) Comparison of a preQ$_1$ riboswitch aptamer in metabolite-bound and free states with implications for gene regulation. J. Biol. Chem. 286, 24626-37.,* ***[3]*** *Liberman J.A., Salim M., Krucinska J. et al. (2013) Structure of a class II preQ$_1$ riboswitch reveals ligand binding by a new fold. Nat. Chem. Biol. 9, 953-955.*



Scheme I: Bacterial biosynthesis of queosine at the tRNA wobble position

Keywords: riboswitch RNA structure, translation, ligand binding