Two riboswitches that share a common ligand-binding fold show dramatic differences in the ability to accommodate mutations

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Riboswitches are naturally occurring, structured RNAs that directly "sense" the levels of cellular metabolites to regulate downstream genes. PreQ1-III (class III) riboswitches sense the metabolite preQ1 (7-deaza-7-aminomethyl-guanine) to control translation of a downstream transporter required for preQ1 salvaging. Most preQ1-III riboswitches belong to the Ruminococaceae family of commensal gut bacteria, which produces the hypermodified nucleobase queuosine that is necessary for genetic decoding by tRNAs with GUN anticodons. We showed previously that despite a distinctive overall HLout pseudoknot fold, the binding pocket of the preQ1-III riboswitch from Faecalibacterium prausnitzii (Fpr) shares ten identical nucleotides with the phylogenetically distinct preQ1-II (class II) riboswitches.1,2 Notably, preQ1-II riboswitches are present in several human pathogens, such as Streptococcus pneumoniae (Spn) - a leading cause of bacterial meningitis in adults and children. Given the goal of targeting RNA motifs with therapeutics, we asked whether commensal and pathogenic riboswitches respond similarly to mutations in or around their binding pockets that could cause loss of gene regulation or antibacterial resistance. Specifically, we prepared A52G, A84G, Δ84, U8C/A8G mutations in the Fpr class III binding pocket based on homologous mutations in the preQ1-II riboswitch that proved detrimental to preQ1-binding and gene regulation.3 X-ray crystallographic analysis of each class III mutant revealed compensatory chemical networks in the binding pocket, including large conformational changes, that maintain ligand binding. Indeed, preQ1 binding analysis revealed that each mutation is tolerated better by preQ1-III riboswitches than preQ1-II riboswitches. Chemical probing of riboswitch flexibility in solution suggests base-pairing of mutants is consistent with the crystal structures, although flexibility is altered compared to the wildtype sequence. A take home message is that the context of the global RNA fold impacts mutation tolerance. Accordingly, larger class III riboswitches from commensal bacteria appear more resilient to mutations compared to their smaller class II counterparts found in human pathogens.

References

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