Elucidating Signal Transduction Pathways in RNA Mediated Gene Regulation

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The rise of antibiotic resistance calls for immediate focus on identifying novel drug targets. RNAs are integral to cellular function, and a subset, called riboswitches, represent a distinct class of biomacromolecules that have already been validated as drug targets. Riboswitches take part in gene regulation in direct response to specific small-molecule effectors' levels. They usually reside in the 5´-leader sequence of bacterial mRNAs and exhibit a bipartite organisation. An aptamer domain senses the effector, while an expression platform regulates a gene or operon. Studies on riboswitches have mostly focused on their ligand binding abilities *in vitro*, outpacing our understanding of the underlying mechanisms that link effector-binding to gene-regulation.

To convincingly relate RNA structure to function, we analysed the $preQ_1$-II (class-2) riboswitch – a well-characterised HL_{out} pseudoknot that recognises the metabolite pre-queuosine$_1$ ($preQ_1$). A novel RNA-modifier called NAI was used to perform *in cell* (ic)SHAPE to compare flexibility changes of individual nucleotides in response to $preQ_1$. When mapped onto our crystal structure, our data showed excellent support of the gene-OFF conformational state. We developed a reporter assay in which GFPuv is controlled by a $preQ_1$-II riboswitch to study its gene regulatory function. Added effector showed a 10-fold repression of GFPuv expression (EC_{50} = 19.5±1.1 nM), consistent with binding studies done by Isothermal Titration Calorimetry (ITC) (K_D=17.9±0.6 nM). The functional relevance of *in vitro* observations thus established, we sought to identify molecular interactions that connect $preQ_1$ binding to gene-regulation. We used our reporter assay along with site-directed mutagenesis to study specific nucleobase interactions hypothesised to be on molecular signal-transduction pathways. Repression analyses were conducted on >10 mutants flanking the $preQ_1$-binding pocket and extending into the expression platform. Subsequently, we performed ITC on the mutants to compare affinity to GFPuv-repression.

Our findings indicate the need for strong base-pairing in the SDS-antiSDS region and maintenance of long-range base-triples for effective switching. The results also suggest that the A-minor bases flanking the binding pocket, and helix P4 of the pseudoknot, play an unexpected role in gene regulation. Finally, we have identified mutants showing 3 log-units of difference between K_D and EC_{50}, indicating decoupling of binding and gene expression.