NON-CODING RNA

Sequestration by riboswitches

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Riboswitches are non-coding regulatory mRNA elements found in the 5′ untranslated regions of bacterial transcripts. In response to metabolic ligands, riboswitches typically control RNA expression by inducing conformational changes in downstream regions. Two new studies show that riboswitches can also mediate gene expression by controlling the transcription of non-coding RNAs (ncRNAs) and by regulating the sequestration of specific proteins. This regulation contains an additional level of control, as it requires the presence of specific cofactors.

Both studies investigated the ethanolamine utilization (eut) pathway, which is widely conserved across various pathogens. The upregulation of this pathway is frequently associated with pathogenesis.

Mellin *et al.* focused on a vitamin B12-sensitive riboswitch in *Listeria monocytogenes* upstream of the *eut* locus. Using RNA sequencing and quantitative real time PCR, they showed that *eut* gene expression requires both the cofactor vitamin B12 and ethanolamine, which suggests that this vitamin B12-sensitive riboswitch regulates *eut* gene expression. To investigate this possibility, the team examined the transcription of the riboswitch locus using Northern blotting with a probe complementary to the riboswitch. In the absence of vitamin B12 and ethanolamine, a 450-nucleotide ncRNA transcript (*Rli55*) was produced, whereas a shorter 200-nucleotide transcript was generated in the presence of these compounds. This finding suggests that the vitamin B12-sensitive riboswitch mediates transcriptional termination of *Rli55*. Furthermore, they showed that *eut* gene expression levels are negatively correlated with *Rli55* expression. In particular, mutant strains containing deletions of the *Rli55* locus showed high levels of *eut* gene expression, whereas mutant strains that had a mutation in the riboswitch (and thus constitutively produced *Rli55*) had low *eut* gene expression levels irrespective of the presence or absence of vitamin B12 and ethanolamine.

How does *Rli55* regulate *eut* gene expression? The researchers identified ANTAR (AmiR and NasR transcriptional antiterminator regulator) elements within *Rli55*, and showed that these elements are necessary and sufficient to regulate the *eut* genes. Specifically, ANTAR elements are required for *Rli55* to act as a 'sponge', binding to and sequestering the EutV protein, which is required for *eut* gene expression. Interestingly, using several *L. monocytogenes* mutants in a mouse infection model, Mellin and co-workers showed that defects in ethanolamine utilization attenuate virulence of this pathogen.

In parallel to the findings in *L. monocytogenes,* DebRoy *et al.* discovered a small regulatory RNA, *EutX*, that mediates *eut* expression by sequestering EutV proteins in *Enterococcus faecalis*. This small RNA contains an adenosyl cobalamine (AdoCbl, the active form of vitamin B12)-sensitive riboswitch. Binding of the cofactor AdoCbl to the riboswitch in the presence of ethanolamine prevents formation of hairpin structures in *EutX*, which are required for EutV binding. Thus, in the presence of AdoCbl, *EutX* cannot bind to and sequester EutV, leaving it free to activate expression of the *eut* genes. Conversely, in the absence of AdoCbl, *EutX* is able to form these hairpin structures, and can therefore bind to and sequester EutV, leading to downregulation of *eut* gene expression.

Together, these studies extend the role of riboswitches as regulators of both ncRNA and protein availability, and provide further insights into RNA regulation in bacteria, as well as new links between metabolite sensing, RNA structure and protein sequestration.

Isabel Lokody

ORIGINAL RESEARCH PAPERS Mellin, J. R. *et al.* Sequestration of a two-component response regulator by a riboswitch-regulated noncoding RNA. *Science* **345**, 940–943 (2014) | DebRoy, S. *et al.* A riboswitch-containing sRNA controls gene expression by sequestration of a response regulator. *Science* **345**, 937–940 (2014)