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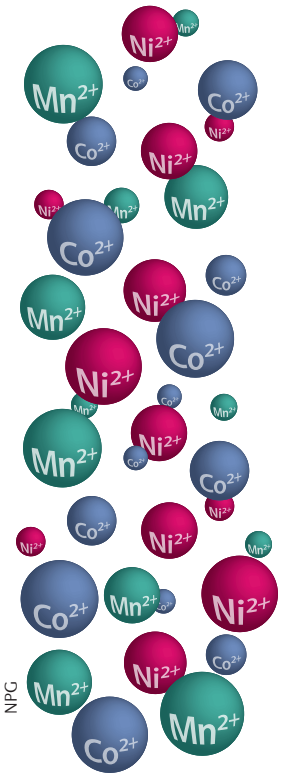
BACTERIAL GENETICS

Metalloregulatory riboswitches



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Transition metal ions are essential cofactors and structural components of proteins and nucleic acids; however, at high concentrations, they pose a threat to bacterial cells owing to their toxicity. Therefore, metal ion homeostasis is tightly controlled by metal-sensing proteins that regulate the expression of the corresponding transporters. Three new studies now identify a new mode of regulation of transition metal ion homeostasis, which involves riboswitches that selectively bind to transition metal ions to regulate the expression of metal ion transporters.

Riboswitches are regulatory RNA structures located upstream of the coding region of mRNAs; binding of a specific ligand to the riboswitch alters the mRNA structure, generally controlling either transcription or translation. The *yybP-ykoY* riboswitch is widely distributed in bacterial genomes, and poorly characterized downstream genes are predicted to have a role in cation transport; however, the identity of its ligand has been unclear for the past decade. In *Escherichia coli*, the *yybP-ykoY* riboswitch is located in the 5′ UTR of the *mntP* gene, which was recently shown to encode a manganese (Mn^{2+}) exporter. Dambach *et al.* showed that MntP is induced by Mn^{2+} at both the transcriptional and translational levels. *mntP* transcription is activated because the transcription factors MntR and Fur antagonize the repressive effects of the nucleoid-binding protein H-NS, which binds to the *mntP* promoter. MntP translation is regulated by the *yybP-ykoY* riboswitch, which directly binds to Mn^{2+}; this induces structural changes in the riboswitch that prevent the formation of a stem–loop structure that sequesters the ribosome-binding site of *mntP* and thus enables translation initiation. Other *E. coli* and *Bacillus subtilis* genes that have the *yybP-ykoY* motif are also specifically induced by Mn^{2+}, suggesting that the riboswitch functions as an Mn^{2+}-sensing 'on' switch of downstream genes that have roles in metal homeostasis.

In agreement with this, Price *et al.* showed that the *Lactococcus lactis yybP-ykoY* riboswitch, which is found upstream of the ATPase-encoding *yoaB* gene, binds Mn^{2+} with high affinity. In the absence of Mn^{2+}, RNA polymerase produces terminated transcripts, whereas high concentrations of Mn^{2+} lead to transcriptional readthrough. The authors determined the crystal structure of the Mn^{2+}-bound *L. lactis yybP-ykoY* riboswitch aptamer domain (which is the ligand-sensing domain) at 2.85 Å resolution and report that two sets of coaxially stacked helices form a hairpin structure and are held in place by two sets of contacts: a four-way junction and a distal hand-shaking interaction between two conserved loops, termed L1 and L3. The interface between these two loops accommodates two metal-binding sites: one site has low metal-binding specificity and the second site is highly selective for Mn^{2+}. Additional structures revealed that removal of Mn^{2+} weakens the L1–L3 interface owing to conformational changes. Based on these observations, the authors propose that the *L. lactis yybP-ykoY* riboswitch regulates gene expression at the transcriptional level: a low Mn^{2+} concentration leads to the formation of a premature terminator structure in the riboswitch, whereas a high Mn^{2+} concentration stabilizes the L1–L3 interface, resulting in disruption of the terminator structure and transcriptional readthrough. Finally, growth inhibition of a Mn^{2+}-sensitive *B. subtilis* strain was rescued when *L. lactis yoaB* was integrated into the genome, suggesting that its product (a riboswitch-controlled protein) functions as an Mn^{2+} exporter.

In a third study, Furukawa *et al.* identified a novel structured RNA class in *Clostridiales* that selectively binds and responds to the low-abundance metal ions cobalt (Co^{2+}) and nickel (Ni^{2+}). These NiCo riboswitches are located upstream of *czcD* genes, which encode proteins involved in cobalt, zinc and cadmium efflux. X-ray crystallography studies of these riboswitches revealed two sets of coaxially stacked helices with interhelical nucleotides forming clustered Co^{2+}- or Ni^{2+}-binding sites. The authors propose that this clustering facilitates cooperative binding of several Co^{2+} or Ni^{2+} cations by the riboswitch aptamer domain. Finally, the authors found that a NiCo riboswitch-containing gene encoding a putative cation exporter was induced in the presence of Ni^{2+}, whereas Mn^{2+} or Zn^{2+} had no effect, supporting the notion that NiCo riboswitches selectively regulate transition metal homeostasis.

In summary, these data suggest that bacteria use selective transition metal-sensing riboswitches to balance the intracellular levels of potentially toxic metal ions.

Andrea Du Toit

> "selective transition metal-sensing riboswitches … balance the intracellular levels of potentially toxic metal ions"

ORIGINAL RESEARCH PAPERS
Dambach, M. *et al.* The ubiquitous *yybP-ykoY* riboswitch is a manganese-responsive regulatory element. *Mol. Cell* **57**, 1099–1109 (2015) | Price, I. R. *et al.* Mn^{2+}-sensing mechanisms of *yybP-ykoY* orphan riboswitches. *Mol. Cell* **57**, 1110–1123 (2015) | Furukawa, K. *et al.* Bacterial riboswitches cooperatively bind Ni^{2+} or Co^{2+} ions and control expression of heavy metal transporters. *Mol. Cell* **57**, 1088–1098 (2015)