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BACTERIAL TRANSCRIPTION

Rho gets to grips with the riboswitch

Riboswitches have emerged as an important class of regulatory elements that control the fate of bacterial mRNAs. These RNA structures are located upstream of the coding region of many mRNAs and, in response to the binding of specific metabolites or ions, the mRNA structure is altered, typically blocking expression of the encoded protein. Two general mechanisms of riboswitch action have previously been described; the first relies on the formation of an intrinsic transcription terminator, and the second on sequestration of the ribosome-binding site or start codon. Hollands *et al*. now show that a third general mechanism exists in both *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Escherichia coli* that uses the RNA helicase, Rho, to attenuate transcription.

The *mgtA* mRNA of *S.* Typhimurium contains a riboswitch that responds to intracellular Mg^{2+}; at low concentrations the stem–loop formed in the leader region permits transcription of the downstream gene, but at high concentrations the mRNA leader adopts a conformation that impedes transcription elongation. However, because the leader lacks a consensus intrinsic terminator sequence, the authors postulated that Rho is required for silencing transcription. To investigate this idea, they constructed a plasmid containing a transcriptional fusion of the *mgtA* leader sequence and the *lacZ* gene, under the control of a Mg^{2+}-insensitive promoter. The activity of β-galactosidase was then monitored in wild-type cells grown under high Mg^{2+} concentrations in the presence and absence of the Rho-specific inhibitor bicyclomycin (BCM). BCM induced a tenfold increase in β-galactosidase activity, indicating that BCM counteracts the transcriptional inhibition that occurs when cytoplasmic Mg^{2+} levels are high. Further evidence of Rho-mediated transcriptional silencing was obtained by measuring transcription of the *mgtA* leader sequence *in vitro*; in the absence of Rho, RNA polymerase (RNAP) stalled but failed to terminate transcription, whereas the addition of Rho stimulated termination at the stall site, and this was enhanced by high Mg^{2+} levels.

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Rho interacts directly with its target mRNAs, usually at sites rich in cytosine residues. Binding to such sites stimulates the ATPase activity of Rho, which provides the energy required for Rho to translocate along the mRNA and induce dissociation of RNAP. Thus, the authors postulated that the structural conformation promoted by high Mg^{2+} levels facilitates Rho binding. Indeed, a mutation that locked the leader sequence in the high Mg^{2+} conformation stimulated the ATPase activity of Rho *in vitro*, and this effect was abolished when a stretch of cytosine residues flanking the stem–loop were mutated.

The authors also examined the *ribB* mRNA of *E. coli*, as it also lacks a canonical intrinsic terminator sequence but contains a flavin mononucleotide (FMN)-sensing riboswitch. Analogous to the results for the *mgtA* mRNA of *S.* Typhimurium, BCM suppressed transcriptional attenuation in the presence of the FMN precursor riboflavin, and FMN stimulated the ability of Rho to terminate transcription *in vitro*.

Both of these riboswitches repress transcription by shifting the structural conformation of the mRNA to induce Rho activity. Together, these data not only identify a previously unrecognized role of Rho but also support an entirely new general model of riboswitch action that could be common to many different bacterial species.

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ORIGINAL RESEARCH PAPER Hollands, K. Riboswitch control of Rho-dependent transcription termination. *Proc. Natl Acad. Sci. USA* 19 Mar 2012 (doi: 10.1073/pnas.1112211109)