BACTERIAL GENETICS

A hidden GEMM

The second messenger cyclic dimeric guanosine monophosphate (c-di-GMP) induces wide-ranging physiological changes in diverse bacterial species, but how it controls the transcription and translation of so many genes has been unclear. Now, reporting in *Science*, Sudarsan, Breaker and colleagues describe how, in many bacterial species, c-di-GMP is sensed by riboswitches.

The number of known protein interactions that involve c-di-GMP cannot explain its global cellular

effects, but it has been proposed that these effects could be explained by the existence of c-di-GMP riboswitches. Riboswitches are complex, structured RNA domains that function as receptors for specific metabolites. They are found in the non-coding regions of various mRNAs and control gene expression in response to metabolite binding.

Sudarsan *et al.* discovered that the upstream regions of several genes that are controlled by c-di-GMP contain a highly conserved RNA domain called GEMM (genes for the environment, for membranes and for motility). They subjected two GEMM motifs from *Vibrio cholerae* (Vc1 and Vc2) to an RNA-structure probing method in the absence and presence of c-di-GMP. This method identified nucleotides in these GEMM motifs that are important for c-di-GMP binding and showed that they bind c-di-GMP tightly and specifically.

Next, the authors investigated whether GEMM motifs from *V. cholerae* (Vc2), *Bacillus cereus* (Bc1 and Bc2) and *Clostridium difficile* (Cd1) could function as riboswitches in cells. They transformed cells using a construct that was composed of the 5'-untranslated region in which each GEMM motif was fused to a reporter gene. The construct that carried Vc2 showed high levels of reporter-gene

expression, and mutations that disrupted the c-di-GMP-binding properties of Vc2 reduced these levels. This indicates that Vc2 has an 'on'-switch function, which was also the case for Bc1, whereas an 'off'-switch function was identified for Bc2 and Cd1.

Modulation of gene expression by a c-di-GMP riboswitch was established by further analysing the off-switch function of Cd1. Sudarsan *et al.* used a c-di-GMP-degrading enzyme to manipulate the cellular concentration of c-di-GMP and showed that, in the presence of this enzyme, reporter-gene expression that was under the control of Cd1 was switched on.

Sudarsan *et al.* have therefore shown that c-di-GMP is sensed by riboswitches in eubacteria, which explains how it controls the expression of genes that are involved in numerous fundamental cellular processes from bacterial virulence to flagellum biosynthesis.

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ORIGINAL RESEARCH PAPER Sudarsan, N. *et al.* Riboswitches in eubacteria sense the second messenger cyclic di-GMP. *Science* **321**, 411–413 (2008)

FURTHER READING Winkler, W. C. & Breaker, R. R. Regulation of bacterial gene expression by riboswitches. *Ann. Rev. Microbiol.* **59**, 487–517 (2005)