BACTERIAL PHYSIOLOGY

Magic mechanisms

New research in *Cell* has identified a role for specific base-pairing between magic spot — the nucleotide guanosine tetraphosphate (ppGpp) — and cytosines in the mechanism of ppGpp transcription regulation.

ppGpp controls bacterial stringent control — the adaptive response of bacteria to amino-acid starvation. On binding to RNA polymerase, ppGpp inhibits the transcription of one set of genes (those that encode components of the translation machinery) and promotes the transcription of another (those that encode components of amino-acid biosynthetic pathways). Although a GC-rich 'discriminator' sequence has been identified in the promoters of negatively regulated genes, the mechanism of ppGpp transcription regulation is unknown.

Artsimovitch *et al.* obtained high-resolution X-ray crystal structures of ppGpp bound to RNA polymerase in the vicinity of the active site and found that ppGpp binds to the two independent RNA polymerase molecules in alternative orientations — designated as 5′ or 3′ according to the proximity of the 5′ and 3′ ppGpp diphosphates to the active site of RNA polymerase. This observation suggests that different modes of ppGpp binding are related to its activity. But how might the alternative orientations of ppGpp contribute to transcription regulation?

Two catalytic Mg^{2+} ions have been identified as necessary for the catalytic activity of RNA polymerase. Interestingly, the structures revealed two catalytic Mg^{2+} ions in the active site of RNA polymerase when ppGpp was bound in the 5′ orientation, but only one when ppGpp was bound in the 3′ orientation, indicating that transcription might be inhibited by this conformation.

Moreover, the authors hypothesized that substrate-specific contacts between ppGpp and the non-template DNA strand could be important, and used the crystal structures to model the RNA polymerase open complex. They found that base-specific contacts are likely between the cytosines of the non-template DNA strand and ppGpp in the 3′, but not the 5′, orientation. To determine whether this predicted base-specific interaction contributes to the effect of ppGpp, they mutated a promoter sequence to show that mutating the cytosine at positions –1 or –2 to thymine or guanine abolished the effect of ppGpp on the open complex stability, further highlighting the importance of cytosine at negatively regulated promoters.

Taken together, this work provides details of the mechanism of ppGpp and reveals the 'magic' behind the action of magic spot — knowledge that could lead to the design of new or improved antimicrobials.

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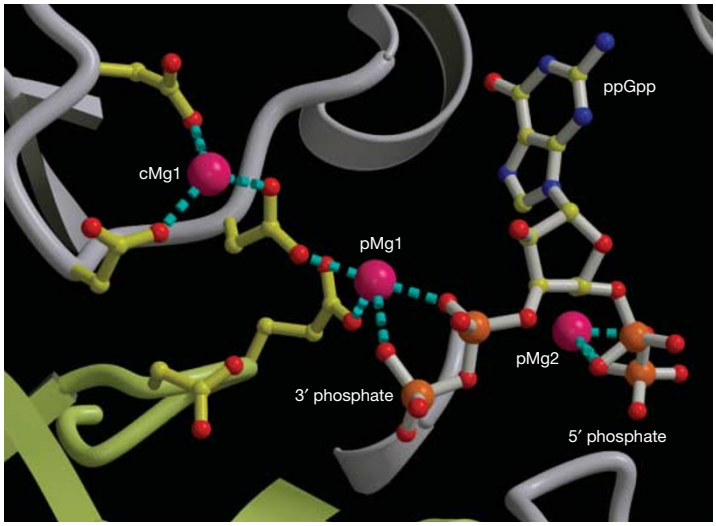
References and links

ORIGINAL RESEARCH PAPER Artsimovitch, I. *et al*. Structural basis for transcription regulation by alarmone ppGpp. *Cell* **117**, 299–310 (2004)

WEB SITES

Irina Artsimovitch's laboratory:
http://www.osumicrobiology.org/faculty/iartsimovitch.htm

RIKEN Harima Institute:
http://www.riken.jp/engn/r-world/research/lab/harima/signaling/index.html



ppGpp bound to RNA polymerase in the 3′ orientation. cMg, catalytic Mg^{2+}; pMg, ppGpp-bound Mg^{2+}. Image courtesy of Dmitry Vassylyev, RIKEN Institute, Japan.