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BACTERIAL TRANSCRIPTION

Finding the magic spot on RNAP

> ppGpp locks RNAP in either the closed or the open conformation

Guanosine tetraphosphate (ppGpp) and its precursor, guanosine pentaphosphate (pppGpp), known collectively as magic spot, are crucial for reprogramming bacterial transcription in response to nutritional stress. Three studies now reveal that these nucleotides bind at the interface between the ω-subunit and the β′-subunit of RNA polymerase (RNAP), providing the first direct evidence for their role as allosteric regulators of transcription.

The accumulation of ppGpp and pppGpp during starvation results in extensive alterations in gene expression owing to the direct interaction of these nucleotides with RNAP. These interactions are thought to destabilize open complexes formed between RNAP and the DNA, thereby inhibiting transcription. However, the exact binding site of these small molecules on RNAP has remained elusive, which has hindered an understanding of their mechanism of action. Furthermore, although it is generally assumed that ppGpp and pppGpp have an equal regulatory potential, few studies have assessed this.

Mechold *et al.* measured the potential differential regulatory effects of ppGpp and pppGpp in *Escherichia coli* using genetic manipulation to direct preferential accumulation of one nucleotide over the other. They found that ppGpp is more potent than pppGpp at regulating five nutritional stress responses, including growth rate and inhibition of rRNA transcription. Furthermore, crystal structures showed that both nucleotides bind to a site ~30 Å from the catalytic pocket of RNAP, at the interface between the ω-subunit and the β′-subunit, which form part of the shelf and core of RNAP, respectively.

Using crosslinking and protease mapping, Ross *et al.* also found that ppGpp spans the interface of these two *E. coli* RNAP subunits. By introducing several amino acid substitutions in this region and measuring the sensitivity of the mutant RNAPs to ppGpp, residues involved in binding were identified and localized to between 30 Å and 35 Å from the active site. To determine the effect of an altered ppGpp-binding site on ppGpp function *in vivo,* two key binding residues in the β′-subunit were mutated. Although the mutant strain displayed a similar growth phenotype to the wild-type strain in both rich and minimal media, it was delayed in the restoration of maximal growth rate after a nutritional downshift. This is consistent with previous observations that ppGpp is required for rapid adaptation to nutritional transitions and provides further evidence that the identified residues are crucial for ppGpp binding to RNAP.

Zuo *et al.* generated a 4.5 Å crystal structure of the *E. coli* RNAP in complex with ppGpp, which also showed that this nucleotide binds the interface between the ω-subunit and the β′-subunit. Moreover, the structure revealed that both pyrophosphates of ppGpp interact mainly with the ω-subunit, whereas the G base interacts with the β′-subunit. Many of the binding residues identified in the crystal structure overlapped with those found by Ross *et al.* to be crucial for ppGpp crosslinking.

These three studies solve the mystery of the ppGpp-binding site on RNAP and suggest an allosteric mechanism for ppGpp modulation of RNAP activity. The shelf and core domains of RNAP form a clamp around the DNA, and the junction of these two domains forms a cleft, within which the catalytic site is deeply buried. Both the shelf and core are mobile modules that undergo a ratcheting motion to facilitate opening and closing of the clamp during transcription. Zuo *et al.* propose that by binding at the shelf and core interface, far from the catalytic site, ppGpp locks RNAP in either the closed or the open conformation and thereby inhibits ratcheting and RNA synthesis. Ross *et al.* also suggest that restricted movement of the clamp weakens the network of contacts between RNAP and DNA to inhibit transcription. Owing to the modular nature of RNAP, this type of allosteric regulation might represent a more general mechanism of transcriptional control.

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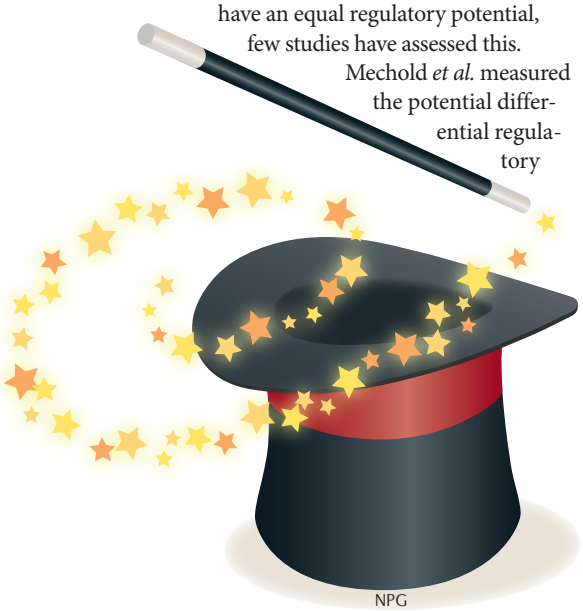

ORIGINAL RESEARCH PAPERS Mechold, U. *et al.* Differential regulation by ppGpp versus pppGpp in *Escherichia coli*. *Nucleic Acids Res.* 25 Apr 2013 (doi:10.1093/nar/gkt302) | Ross, W. *et al.* The magic spot: a ppGpp binding site on *E. coli* RNA polymerase responsible for regulation of transcription initiation. *Mol. Cell* **50,** 420–429 (2013) | Zuo, Y. *et al.* The mechanism of *E. coli* RNA polymerase regulation by ppGpp is suggested by the structure of their complex. *Mol. Cell* **50,** 430–436 (2013)