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BACTERIAL PHYSIOLOGY

A new chaperone for regulatory sRNAs

> the role of ProQ may be to chaperone a distinct class of RNAs that are characterized by a high degree of structure

In bacteria, two RNA-binding proteins (RBPs) — Hfq and CsrA — are known to be global post-transcriptional regulators that chaperone small RNAs (sRNAs). However, numerous sRNAs do not associate with either of these chaperones, which raises the possibility that these sRNAs bind to RBPs that have as-yet-undiscovered global regulatory roles. Now, Smirnov *et al.* identify ProQ as a third sRNA chaperone that functions as a global post-transcriptional regulator in the bacterial pathogen *Salmonella enterica*.

To separate classes of RNA according to RBP binding partner, a high-throughput profiling method ('Grad-seq') was developed that combines biochemical separation by sedimentation in a glycerol gradient with high-throughput sequencing of complementary DNA (cDNA). The sequencing data showed that RNAs that are associated with shared RBP complexes (such as the ribosome, RNA polymerase holoenzyme or chaperones) tended to coalesce in the same group of gradient fractions, owing to similar sedimentation coefficients. For example, most well-characterized Hfq-associated sRNAs coalesced in this way, as did CsrA-associated sRNAs, regardless of differences in length, sequence or specific regulatory role.

Interestingly, one large group of sRNAs that coalesced in the Grad-seq experiment was not associated with either Hfq or CsrA. Using 12 aptamer-tagged sRNAs as baits in pull-down experiments, together with confirmation by western blotting, the authors found that the sRNAs were associated with the RNA chaperone ProQ, which has previously been described as an osmoregulator that optimizes the expression of the proline channel ProP. However, the prevalence of sRNAs that were associated with ProQ in the Grad-seq dataset led the authors to propose that ProQ is a global post-transcriptional regulator, alongside Hfq and CsrA, rather than a specific regulator of ProP. Indeed, RNA immunoprecipitation experiments identified 98 sRNAs as associated with ProQ, which included type I antitoxins, attenuators of transcription, sponges that sequester other sRNAs, and *trans*-acting regulatory sRNAs that base-pair with target RNAs. These diverse regulatory roles further support a global regulatory function for ProQ. Finally, kinetic experiments demonstrated that the binding affinities of ProQ for its most enriched sRNAs were similar to those of Hfq and CsrA, and genetic experiments confirmed that ProQ stabilizes its associated sRNAs and thus acts as a chaperone. Together, these data suggest that ProQ and its associated RNAs form a novel class of bacterial RNA–chaperone complex.

The question of why the cell should require ProQ in addition to Hfq and CsrA may be answered by the finding that ProQ-associated sRNAs tend to be highly structured, whereas Hfq and CsrA bind to single-stranded regions of RNAs that also tend to have less overall structure than ProQ-associated RNAs. Consequently, only two of the 98 ProQ-associated RNAs are also known to associate with Hfq. Therefore, the role of ProQ may be to chaperone a distinct class of RNAs that are characterized by a high degree of structure. That this seemingly globally acting regulator had not previously been reported might seem remarkable, but the authors note that previous methods had limitations that impeded the global exploration of RNA–RBP interactions. As Grad-seq can be applied to any organism, the method represents an advance that may be applied in future studies to further illuminate the bacterial RBPome. The identification of ProQ as a global regulator in *S. enterica* is also expected to stimulate the investigation of ProQ homologues, which are widely distributed in proteobacterial chromosomes, plasmids and phages.

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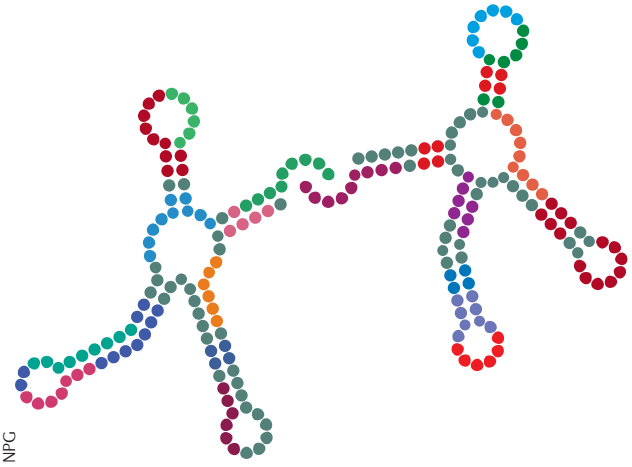

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ORIGINAL ARTICLE Smirnov, A. *et al.* Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. *Proc. Natl Acad. Sci. USA* http://dx.doi.org/10.1073/pnas.1609981113 (2016)