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CsrA enters Hfq's territory: Regulation of a base-pairing small RNA

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Abstract

Post-transcriptional regulatory networks in Gammaproteobacteria are to a large extent built around the two globally acting RNA-binding proteins (RBPs) CsrA and Hfq. Both RBPs interact with small regulatory RNAs (sRNAs), but the functional outcomes of these interactions are generally distinct. Whereas Hfq both stabilizes sRNAs and promotes their base-pairing to target mRNAs, the sRNAs bound by CsrA act as sequestering molecules that titrate the RBP away from its mRNA targets. In this issue of *Molecular Microbiology*, Lai et al. reveal that CsrA interacts with the Hfq-associated and base-pairing sRNA Spot 42. In this case, CsrA increases Spot 42 stability by masking a cleavage site for endoribonuclease RNase E, thereby promoting Spot 42-dependent regulation of *srlA* mRNA. Interestingly, the effect of CsrA on *srlA* expression is two-fold. In addition to affecting Spot 42-dependent regulation, CsrA directly inhibits translation of SrlM, an activator of *srlA* transcription. Together, this study reveals a new function for CsrA and indicates more intricate connections between the CsrA and Hfq networks than previously anticipated. Several recent studies have identified additional RBPs that interact with sRNAs. With new RBP identification methods at hand, it will be intriguing to see how many more sRNA-binding proteins will be uncovered.

KEYWORDS

CsrA, Hfq, post-transcriptional regulation, RBP, RNase E, spot 42

1 | INTRODUCTION

In their natural habitats, most bacteria are presented with an ever-changing environment where adaptation through rapid and precise gene expression changes is key to survival. In this light, it is not surprising that bacteria employ both transcriptional and post-transcriptional regulation to provide fast and accurate responses to extracellular signals. Two of the best studied post-transcriptional regulatory networks in the model bacterium *Escherichia coli* (*E. coli*) are centered around the carbon storage regulator (Csr) system and the many small regulatory RNAs (sRNA) that base-pair to mRNA targets and are assisted by the Hfq protein.

CsrA is a well-studied globally acting RNA-binding protein (RBP) that mediates post-transcriptional regulation by binding to a plethora of different mRNAs. Binding generally occurs at or near the ribosome binding site in an mRNA, which decreases ribosome accessibility, resulting in translation inhibition and, consequently,

destabilization of the mRNA (for a recent review see Romeo & Babitzke, 2018). CsrA is a small (14 kD) homo-dimeric RBP that preferably binds to GGA-containing motifs located in unstructured regions of target RNA molecules. Targets of CsrA include mRNAs encoding proteins involved in carbohydrate metabolism, biofilm formation, motility, and virulence (for reviews see Kusmierek & Dersch, 2018; Pourciau et al., 2020; Romeo & Babitzke, 2018). The regulatory activity of CsrA is controlled by sRNAs such as CsrB (Liu et al., 1997) and CsrC (Weilbacher et al., 2003), which carry multiple high affinity sites that titrate CsrA away from its lower affinity mRNA targets. CsrB/C levels are increased by various different nutritional stresses or metabolites, and decreased in the presence of preferred carbon sources such as glucose. Accordingly, CsrA is primarily active during exponential growth where it represses genes involved in stationary phase growth and stress responses.

The homohexameric Hfq protein accommodates at least four different RNA-binding surfaces. Although Hfq has been proposed to carry out a number of cellular functions, it is best known for facilitation of the imperfect base-pairing between sRNAs and their mRNA targets, which in turn leads to increased or decreased translation and/or stability (Hör et al., 2020). One of the first identified targets of Hfq was the 109 nt long sRNA Spot 42 (Figure 1a) (Møller et al., 2002). The expression of Spot 42 is activated by glucose and inhibited by the cAMP-CRP complex. Spot 42 appears to mainly target transcripts from genes encoding transporters and metabolic enzymes involved in utilization of non-glucose carbon sources (Beisel & Storz, 2011). This includes the *srlA* gene, encoding a component of a D-sorbitol-specific phosphotransferase system. Spot 42 base-pairs to, and negatively regulates translation of the *srlA* mRNA in an Hfq-dependent manner (Figure 1b).

2 | CONNECTIONS BETWEEN THE CsrA AND Hfq NETWORKS

Although both CsrA and Hfq are globally acting RBPs, they have been considered to belong to distinct regulatory systems, both regarding their RNA target repertoires and their function. Generally, the Csr system is believed to exert its function by direct binding of CsrA to mRNA targets, while Hfq is viewed as a matchmaking protein that guides the vast majority of known sRNAs to base-pair with their targets. Along these lines of thinking, previous reports of sRNAs that interact with CsrA suggested these interactions to function through regulation of CsrA activity, akin to CsrB/C (Jorgensen et al., 2013; Parker et al., 2017; Stenum et al., 2021). On a global level, CLIP-seq experiments have suggested that CsrA interacts with a handful of Hfq-associated sRNAs in both *Salmonella enterica* (*S. enterica*) and *E. coli* (Holmqvist et al., 2016; Potts et al., 2017), but it has remained unclear whether the interactions between CsrA and these additional sRNAs have any functional consequences.

In this issue of *Molecular Microbiology*, Lai et al. reveal that the sRNA Spot 42 interacts with both Hfq and CsrA in *E. coli*, and that both these interactions, independently of each other, affect expression of the Spot 42 target *srlA* (Lai et al., 2021). The Romeo and Babitzke labs had previously mapped a putative CsrA binding site in Spot 42 by CLIP-seq and verified the interaction in vitro by gel shift experiments, demonstrating that CsrA binds Spot 42 with high affinity (Potts et al., 2017). In the present study, this interaction is shown to be specific, as mutating the single GGA motif in Spot 42 substantially weakened CsrA binding. The authors then asked whether CsrA affects the cellular levels of Spot 42, and showed that CsrA promotes Spot 42 stability by protecting against RNase E-mediated decay; CsrA binding masks two RNase E cleavage sites which lie in direct vicinity of the GGA motif in Spot 42 (Figure 1b). The authors next asked how CsrA affects Spot 42-dependent regulation of *srlA* expression. In the absence of either CsrA or Spot 42, the *srlA* steady-state levels increased. This is expected, since Spot 42 is a direct negative regulator of *srlA* (Beisel & Storz, 2011), and CsrA is a positive regulator of Spot 42 (Lai et al., 2021). Interestingly, in the absence of both regulators, the *srlA* mRNA levels were elevated beyond those observed in the single deletion strains, indicating that CsrA also affects *srlA* expression through a mechanism independent of Spot 42. This effect disappeared when *srlA* was expressed from a heterologous promoter, suggesting that CsrA indirectly represses *srlA* transcription. A previous transcriptomic study from the same group had shown that *srlM* mRNA, encoding a transcriptional activator of *srlA*, was elevated in the absence of CsrA (Potts et al., 2017). Using a combination of in vitro and in vivo experiments, the authors now show that CsrA directly binds to a GGA motif in the translation initiation region of *srlM* mRNA, entailing inhibition of SrlM synthesis and decreased *srlM* mRNA levels (Figure 1c). Taken together, the present study reveals an intricate two-armed regulatory motif, one arm through which CsrA promotes Spot 42 stability, and one through which CsrA exerts translational repression at the *srlM* mRNA, but where both arms independently confer decreased expression of *srlA*.

3 | NEW FUNCTIONS OF CsrA

Previous studies investigating the function of interactions between CsrA and sRNAs have focused on how sRNAs can interfere with CsrA activity through sequestration, thereby counteracting regulation that CsrA would otherwise exert on mRNA targets. The classical CsrA antagonist RNAs CsrB and CsrC consist of multiple stem-loops each presenting a GGA motif for CsrA binding. That is, these sRNAs seem to have evolved to primarily, possibly exclusively, function as CsrA antagonists. In contrast, Spot 42 only contains one single GGA motif, suggesting it to be inefficient as a CsrA titrator. The additional sRNAs that have been identified by in vivo crosslinking follow the same pattern, each harboring between zero and four GGA sequences (Holmqvist et al., 2016; Potts et al., 2017), suggesting that binding of CsrA to these sRNAs also may serve other purposes than regulating CsrA activity.

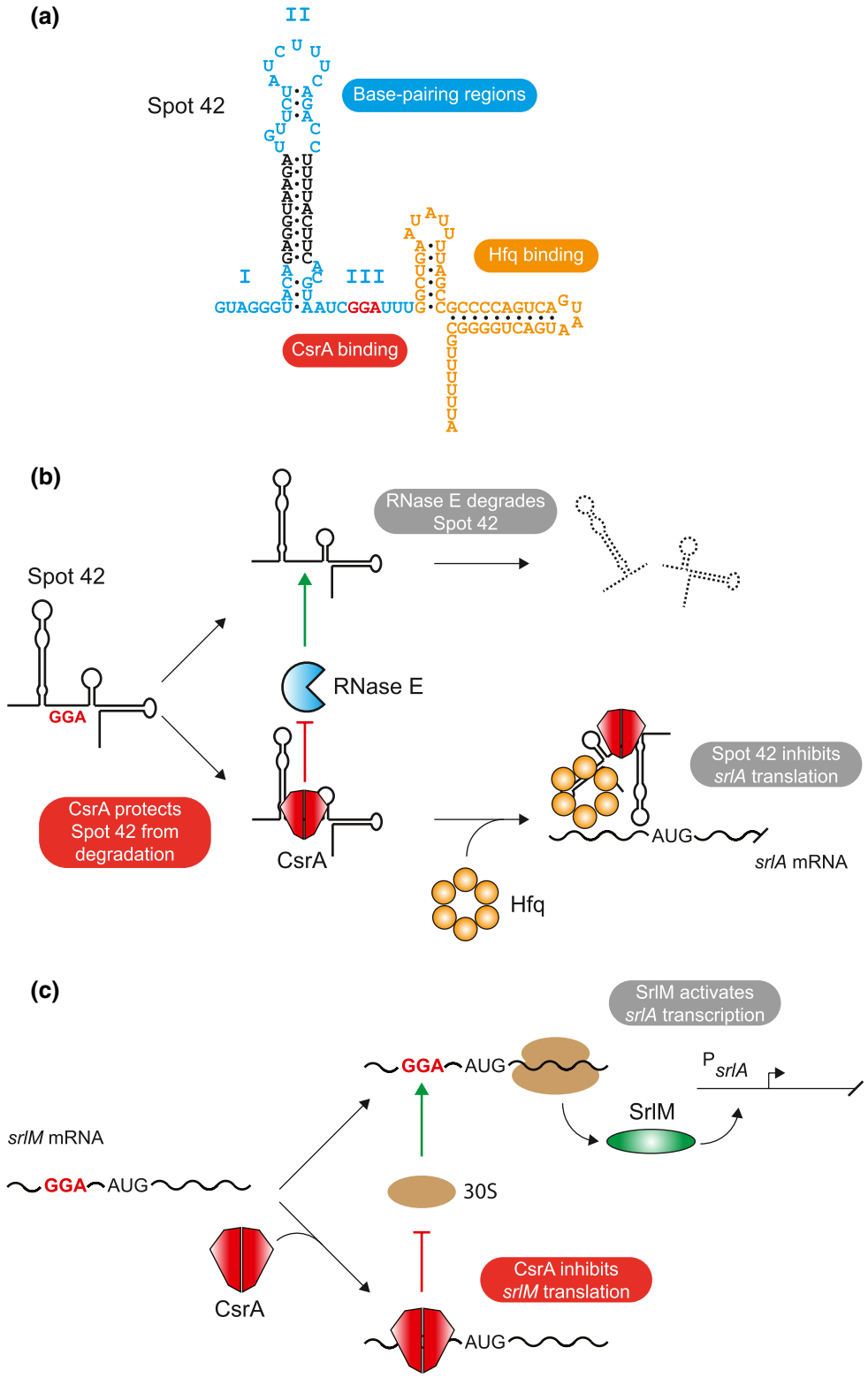

FIGURE 1 The small RNA Spot 42 interacts with both CsrA and Hfq. (a) Schematic representation of the secondary structure of Spot 42. Regions involved in base-pairing to mRNA targets are highlighted in blue and indicated with roman numerals. The GGA sequence required for CsrA binding is highlighted in red. The Hfq binding region is highlighted in orange. (b) Binding of CsrA to Spot 42 inhibits RNase E cleavage, leading to higher cellular stability of Spot 42 and increased repression of the Spot 42 target *srlA*. (c) CsrA binds to the *srlM* mRNA and inhibits translation of the SrlM protein, which activates transcription at the *srlA* promoter

The present study convincingly demonstrates that binding of CsrA protects Spot 42 from RNase E cleavage, but also indicates an additional possible functional outcome of this interaction. The Spot 42 RNA can be divided into two functional domains; the 5′

part encompasses three different single-stranded regions (regions I–III, Figure 1a) that participate in base-pairing to mRNA targets (Beisel & Storz, 2011), while the 3′ region contains an Hfq-binding module consisting of two stem-loop structures (Møller et al., 2002). Interestingly, the single GGA sequence in Spot 42, which is required for CsrA binding, overlaps with base-pairing region III. Although not a topic of the current study, this opens up interesting avenues for future studies. Possibly, CsrA may, in addition to acting as a stabilizing factor for Spot 42, mask region III to restrict the target repertoire of this sRNA. In this light it will be interesting to see whether uncharacterized interactions between CsrA and sRNAs involve protection from RNase activity (Lai et al., 2021), masking of base-pairing regions (suggested in Lai et al., 2021), or even CsrA-dependent involvement in sRNA-mRNA duplex formation, as recently shown in *Bacillus subtilis* (Müller et al., 2019).

4 | sRNA-BINDING PROTEINS BEYOND CsrA AND Hfq

The current study demonstrates that CsrA counteracts RNase E-dependent cleavage of Spot 42. This is in line with previous studies, in which CsrA was shown to protect both the *flhDC* mRNA, as well as its antagonist RNA CsrB, from RNase E cleavage (Vakulskas et al., 2016; Wei et al., 2001; Yakhnin et al., 2013). While it is too early to speculate about the extent to which CsrA exerts this role on other RNA targets, it is interesting to note that modulation of RNA stability seems to be a common function among bacterial sRNA-binding proteins.

A major example of this is the post-transcriptional regulator ProQ, a FinO-domain protein that acts as a global RBP in *S. enterica* and *E. coli* (Holmqvist et al., 2018; Melamed et al., 2020; Smirnov et al., 2016). As Hfq and CsrA, ProQ binds many mRNAs and sRNAs, and has been implicated in both RNA stabilization and sRNA-based regulation (Holmqvist et al., 2018; Melamed et al., 2020; Smirnov et al., 2017). However, for the majority of the ProQ targets, functional consequences are still largely unknown, and a full understanding of this protein and its effects on RNA remain to be determined. Additionally, several other FinO-domain proteins, including FopA from *Salmonella* (Gerovac et al., 2020) and RocC from *Legionella pneumophila* (Attaiech et al., 2016), have been identified and characterized as sRNA-binding proteins. Another recent example is the sRNA-binding protein Zea, which binds a variety of different RNAs in *Listeria monocytogenes* (Pagliuso et al., 2019). Zea is secreted into the cytosol of mammalian cells during infection, thereby affecting virulence traits. Other examples of sRNA-binding proteins include the cold shock proteins CspC/E from *Salmonella* that protect RNA targets from RNase E activity (Michaux et al., 2017), and RapZ from *E. coli*, which acts as an adaptor protein for sRNA degradation by RNase E (reviewed in Khan & Görke, 2020).

5 | DISCOVERY OF NEW RBP

Recent global studies in both *E. coli* and *S. enterica* have identified hundreds of proteins as potential RBPs, the majority of which were not previously known to bind RNA (Gerovac et al., 2020; Queiroz et al., 2019; Shchepachev et al., 2019; Urdaneta et al., 2019). This suggests that the investigated bacteria possess substantially more RBPs than anticipated. What are the functions of these new RBPs? In addition to many uncharacterized proteins, several of these putative RBPs have known functions unrelated to RNA-binding. For instance, metabolic enzymes participating in the TCA cycle and glycolysis have repeatedly been found to interact with RNA, consistent with RBP identification results in eukaryotic systems (for a recent review, see Curtis & Jeffery, 2021). One example is the eukaryotic glyceraldehyde-3-phosphate dehydrogenase (GAPDH) that "moonlights" as an RBP; binding of GAPDH to RNA increases during low glycolysis conditions and affects both translation and stability. These findings helped formulate the RNA-enzyme-metabolite (REM) hypothesis (Hentze & Preiss, 2010), which states that the RNA-binding activity of metabolic enzymes links the nutritional state of the cell to gene expression. One bacterial example congruent with the REM-hypothesis is the RNA-binding activity of the *E. coli* aconitases AcnA and AcnB, which catalyze the conversion of citrate to isocitrate in the TCA cycle. During low iron conditions their iron–sulfur clusters are disrupted, which enables them to bind and stabilize a number of transcripts (Tang & Guest, 1999). In this way the aconitases rapidly regulate gene expression as a result of changes in iron concentration.

Several additional hypotheses can be put forward to explain the remarkably high number of proteins with RNA-binding capacity: (a) The binding of RNA to protein could modulate the activity and/or stability of the protein, in a similar way that CsrB/C counteracts CsrA activity. (b) The RNA could drive localization of proteins or, vise-versa, a protein could drive localization of RNA. (c) Binding to RNA could provide a structural scaffold for proteins of related function and retain them in close proximity in the cell through liquid–liquid phase separation. This would be particularly beneficial for proteins working in cascades, for example where one enzyme is reliant on the product of another enzyme. It is possible that many of the newly found bacterial RBPs have fewer targets than the globally acting RBPs Hfq, CsrA, and ProQ, but it cannot be ruled out that more global post-transcriptional regulators still await discovery.

6 | CONCLUDING REMARKS

The seminal work on CsrA and Hfq during the last decades has been instrumental for our understanding of post-transcriptional networks built by RBPs and sRNAs as cornerstones of bacterial gene regulation. Decades of research on these networks notwithstanding, the present study shows that there is still much to learn,

and that regulation by CsrA and Hfq may be more connected than previously thought. Research on these two regulators has laid the ground for the discovery of additional RBPs, such as ProQ. It is now clear that many more proteins than previously assumed can function as RPBs, and that their functions may be more diverse than expected.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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REFERENCES

Attaiech, L., Boughammoura, A., Brochier-Armanet, C., Allatif, O., Peillard-Fiorente, F., Edwards, R.A. et al. (2016) Silencing of natural transformation by an RNA chaperone and a multitarget small RNA. *Proceedings of the National Academy of Sciences*, *113*(31), 8813–8818. Available from: https://doi.org/10.1073/pnas.1601626113

Beisel, C.L. & Storz, G. (2011) The base-pairing RNA spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in *Escherichia coli*. *Molecular Cell*, *41*, 286–297. Available from: https://doi.org/10.1016/j.molcel.2010.12.027

Curtis, N.J. & Jeffery, C.J. (2021) The expanding world of metabolic enzymes moonlighting as RNA binding proteins. *Biochemical Society Transactions*, *49*(3), 1099–1108. Available from: https://doi.org/10.1042/BST20200664

Gerovac, M., El Mouali, Y., Kuper, J., Kisker, C., Barquist, L. & Vogel, J. (2020) Global discovery of bacterial RNA-binding proteins by RNase-sensitive gradient profiles reports a new FinO domain protein. *RNA*, *26*, 1448–1463. Available from: https://doi.org/10.1261/rna.076992.120

Hentze, M.W. & Preiss, T. (2010) The REM phase of gene regulation. *Trends in Biochemical Sciences*, *35*, 423–426. Available from: https://doi.org/10.1016/j.tibs.2010.05.009

Holmqvist, E., Li, L., Bischler, T., Barquist, L. & Vogel, J. (2018) Global maps of ProQ binding *in vivo* reveal target recognition via RNA structure and stability control at mRNA 3′ ends. *Molecular Cell*, *70*, 971–982.e6. Available from: https://doi.org/10.1016/j.molcel.2018.04.017

Holmqvist, E., Wright, P.R., Li, L., Bischler, T., Barquist, L., Reinhardt, R. et al. (2016) Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking *in vivo*. *EMBO Journal*, *35*, 991–1011. Available from: https://doi.org/10.15252/embj.201593360

Hör, J., Matera, G., Vogel, J., Gottesman, S. & Storz, G. (2020) Trans-acting small RNAs and their effects on gene expression in *Escherichia coli* and *Salmonella enterica*. *EcoSal Plus*, *9*. Available from: https://doi.org/10.1128/ecosalplus.ESP-0030-2019

Jorgensen, M.G., Thomason, M.K., Havelund, J., Valentin-Hansen, P. & Storz, G. (2013) Dual function of the McaS small RNA in controlling biofilm formation. *Genes & Development*, *27*, 1132–1145. Available from: https://doi.org/10.1101/gad.214734.113

Khan, M.A. & Görke, B. (2020) A multifunctional small RNA binding protein for sensing and signaling cell envelope precursor availability in bacteria. *Microbial Cell*, *7*, 139–142. Available from: https://doi.org/10.15698/mic2020.05.717

Kusmierek, M. & Dersch, P. (2018) Regulation of host–pathogen interactions via the post-transcriptional Csr/Rsm system. *Current Opinion in Microbiology*, *41*, 58–67. Available from: https://doi.org/10.1016/j.mib.2017.11.022

Lai, Y., Yakhnin, H., Pannuri, A., Pourciau, C., Babitzke, P. & Romeo, T. (2022) CsrA regulation via binding to the base-pairing small RNA Spot 42. *Molecular Microbiology*, *117*, 32–53. Available from: https://doi.org/10.1111/mmi.14769

Liu, M.Y., Gui, G., Wei, B., Preston, J.F., Oakford, L., Yüksel, Ü. et al. (1997) The RNA molecule CsrB binds to the global regulatory protein CsrA and antagonizes its activity in *Escherichia coli*. *Journal of Biological Chemistry*, *272*, 17502–17510. Available from: https://doi.org/10.1074/jbc.272.28.17502

Melamed, S., Adams, P.P., Zhang, A., Zhang, H. & Storz, G. (2020) RNA-RNA interactomes of ProQ and Hfq reveal overlapping and competing roles. *Molecular Cell*, *77*, 411–425.e7. Available from: https://doi.org/10.1016/j.molcel.2019.10.022

Michaux, C., Holmqvist, E., Vasicek, E., Sharan, M., Barquist, L., Westermann, A.J. et al. (2017) RNA target profiles direct the discovery of virulence functions for the cold-shock proteins CspC and CspE. *Proceedings of the National Academy of Sciences*, 201620772. Available from: https://doi.org/10.1073/pnas.1620772114

Møller, T., Franch, T., Udesen, C., Gerdes, K. & Valentin-Hansen, P. (2002) Spot 42 RNA mediates discoordinate expression of the *E. coli* galactose operon. *Genes & Development*, *16*, 1696–1706. Available from: https://doi.org/10.1101/gad.231702

Müller, P., Gimpel, M., Wildenhain, T. & Brantl, S. (2019) A new role for CsrA: promotion of complex formation between an sRNA and its mRNA target in *Bacillus subtilis*. *RNA Biology*, *16*, 972–987. Available from: https://doi.org/10.1080/15476286.2019.1605811

Pagliuso, A., Tham, T.N., Allemand, E., Robertin, S., Dupuy, B., Bertrand, Q. et al. (2019) An RNA-binding protein secreted by a bacterial pathogen modulates RIG-I signaling. *Cell Host & Microbe*, *26*, 823–835.e11. Available from: https://doi.org/10.1016/j.chom.2019.10.004

Parker, A., Cureoglu, S., De Lay, N., Majdalani, N. & Gottesman, S. (2017) Alternative pathways for *Escherichia coli* biofilm formation revealed by sRNA overproduction. *Molecular Microbiology*, *105*, 309–325. Available from: https://doi.org/10.1111/mmi.13702

Potts, A.H., Vakulskas, C.A., Pannuri, A., Yakhnin, H., Babitzke, P. & Romeo, T. (2017) Global role of the bacterial post-transcriptional regulator CsrA revealed by integrated transcriptomics. *Nature Communications*, *8*, 1596. Available from: https://doi.org/10.1038/s41467-017-01613-1

Pourciau, C., Lai, Y.-J., Gorelik, M., Babitzke, P. & Romeo, T. (2020) Diverse mechanisms and circuitry for global regulation by the RNA-binding protein CsrA. *Frontiers in Microbiology*, *11*. Available from: https://doi.org/10.3389/fmicb.2020.601352

Queiroz, R.M.L., Smith, T., Villanueva, E., Marti-Solano, M., Monti, M., Pizzinga, M. et al. (2019) Comprehensive identification of RNA–protein interactions in any organism using orthogonal organic phase separation (OOPS). *Nature Biotechnology*, *37*, 169–178. Available from: https://doi.org/10.1038/s41587-018-0001-2

Romeo, T. & Babitzke, P. (2018) Global Regulation by CsrA and Its RNA Antagonists. In: *Regulating with RNA in Bacteria and Archaea*. ASM Press, pp. 339–354. Available from: https://doi.org/10.1128/9781683670247.ch19

Shchepachev, V., Bresson, S., Spanos, C., Petfalski, E., Fischer, L., Rappsilber, J. et al. (2019) Defining the RNA interactome by total RNA -associated protein purification. *Molecular Systems Biology*, *15*. Available from: https://doi.org/10.15252/msb.20188689

Smirnov, A., Förstner, K.U., Holmqvist, E., Otto, A., Günster, R., Becher, D. et al. (2016) Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. *Proceedings of the National Academy of*

Sciences, *113*, 11591–11596. Available from: https://doi.org/10.1073/pnas.1609981113

Smirnov, A., Wang, C., Drewry, L.L. & Vogel, J. (2017) Molecular mechanism of mRNA repression in trans by a ProQ-dependent small RNA. *EMBO Journal*, *36*, 1029–1045. Available from: https://doi.org/10.15252/embj.201696127

Stenum, T.S., Kongstad, M., Holmqvist, E., Kallipolitis, B., Svenningsen, S.L. & Sørensen, M.A. (2021) Three ribosomal operons of *Escherichia coli* contain genes encoding small RNAs that interact with Hfq and CsrA *in vitro*. *Frontiers in Microbiology*, *12*. Available from: https://doi.org/10.3389/fmicb.2021.625585

Tang, Y. & Guest, J.R. (1999) Direct evidence for mRNA binding and post-transcriptional regulation by *Escherichia coli* aconitases. *Microbiology*, *145*, 3069–3079. Available from: https://doi.org/10.1099/00221287-145-11-3069

Urdaneta, E.C., Vieira-Vieira, C.H., Hick, T., Wessels, H.-H., Figini, D., Moschall, R. et al. (2019) Purification of cross-linked RNA-protein complexes by phenol-toluol extraction. *Nature Communications*, *10*, 990. Available from: https://doi.org/10.1038/s41467-019-08942-3

Vakulskas, C.A., Leng, Y., Abe, H., Amaki, T., Okayama, A., Babitzke, P. et al. (2016) Antagonistic control of the turnover pathway for the global regulatory sRNA CsrB by the CsrA and CsrD proteins. *Nucleic Acids Research*, *44*, 7896–7910. Available from: https://doi.org/10.1093/nar/gkw484

Wei, B.L., Brun-Zinkernagel, A.-M., Simecka, J.W., Prüß, B.M., Babitzke, P. & Romeo, T. (2001) Positive regulation of motility and *flhDC* expression by the RNA-binding protein CsrA of *Escherichia coli*. *Molecular Microbiology*, *40*, 245–256. Available from: https://doi.org/10.1046/j.1365-2958.2001.02380.x

Weilbacher, T., Suzuki, K., Dubey, A.K., Wang, X., Gudapaty, S., Morozov, I. et al. (2003) A novel sRNA component of the carbon storage regulatory system of *Escherichia coli*. *Molecular Microbiology*, *48*, 657–670. Available from: https://doi.org/10.1046/j.1365-2958.2003.03459.x

Yakhnin, A.V., Baker, C.S., Vakulskas, C.A., Yakhnin, H., Berezin, I., Romeo, T. et al. (2013) CsrA activates *flhDC* expression by protecting *flhDC* mRNA from RNase E-mediated cleavage. *Molecular Microbiology*, *87*, 851–866. Available from: https://doi.org/10.1111/mmi.12136

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