



HAL
open science

What makes a small RNA work?

Saleh Alquethamy, David Lalaouna, Jai J Tree

► **To cite this version:**

Saleh Alquethamy, David Lalaouna, Jai J Tree. What makes a small RNA work?. Nucleic Acids Research, 2025, 53 (12), <10.1093/nar/gkaf563>. <hal-05190376>

HAL Id: hal-05190376

<https://hal.science/hal-05190376v1>

Submitted on 28 Jul 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY-NC 4.0 - Attribution - Non-commercial use - International License

What makes a small RNA work?

Saleh Alquethamy¹, David Lalaouna², Jai J. Tree^{1,*}

¹School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney 2052, NSW, Australia

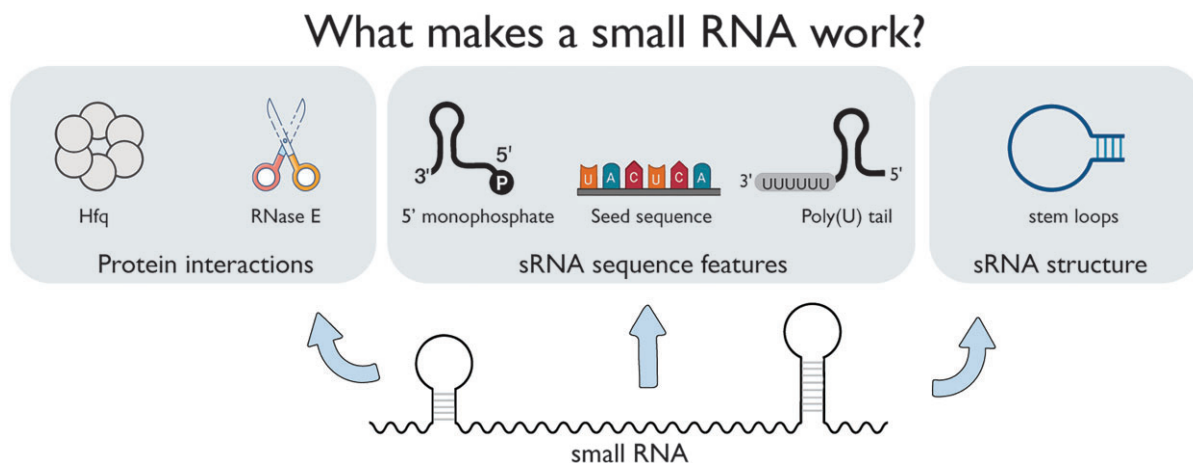
²Université de Strasbourg, CNRS, Architecture et Réactivité de l'ARN, UPR9002, Strasbourg 67000, France

*To whom correspondence should be addressed. Email: j.tree@unsw.edu.au

Abstract

Bacterial small RNAs (sRNAs) are key regulators of gene expression, interacting with target messenger RNAs (mRNAs) through imperfect base pairing. Unlike other non-coding RNAs such as microRNAs and PIWI-interacting RNAs, bacterial sRNAs exhibit significant sequence and structural diversity, complicating functional predictions. Recent high-throughput profiling of the sRNA interactome has accentuated this problem by revealing a highly complex network of sRNA interactions. It is clear that there is an incredible diversity of sRNA interactions with different RNA classes *in vivo*, including different interaction modes with mRNAs. In this review, we attempt to summarize the known sequence and structural features that contribute to sRNA function in bacteria. As many of these features drive recruitment of protein partners, we necessarily focus on interactions with chaperones and ribonucleases, the best studied being Hfq and RNase E. Where possible, we have included examples outside this well-studied system as diversity and rule breaking appear to be central themes of sRNA biology. Understanding the sequences and structures that drive sRNA function will enhance our ability to predict regulatory outcomes, and this may inform the development of effective RNA therapeutics that are inspired by bacterial sRNA mechanisms.

Graphical abstract



Introduction

A diversity of regulatory RNA classes in a wide phylogeny have been described that control RNA through sequence-specific base pairing with target RNAs. Several classes of regulatory RNA, including eukaryotic microRNA (miRNA) and PIWI-interacting RNA, as well as the single guide RNA central to the CRISPR–Cas9 system, have very well-defined length, structure, and sequence requirements that promote interactions with protein partners and present specific nucleotides for target pairing [1, 2]. Often, the target spectrum and function can be predicted from the sequence with relative confidence. Conversely, bacterial regulatory small RNAs (sRNAs) exhibit remarkable sequence and structural diversity, compli-

cating the prediction of their function in comparison to other classes of RNA [3, 4]. For this class of RNA, sequence and structural diversity is *de rigueur*, and functional predictions routinely produce false positives.

Recent high-throughput profiling of the sRNA interactome has accentuated the problem. It is clear that there is an incredible diversity of sRNA interactions with different RNA classes *in vivo*, including different interaction modes with messenger RNAs (mRNAs). The lack of restrictions placed on sRNAs by their interaction partners, coupled with a lack of clearly defined functional categories, contributes significantly to their sequence and structural diversity. In this review, we aim to summarize our current understanding of the sequence and

Received: February 27, 2025. Revised: May 8, 2025. Editorial Decision: May 9, 2025. Accepted: June 10, 2025

© The Author(s) 2025. Published by Oxford University Press on behalf of Nucleic Acids Research.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License

(https://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.

structural features of sRNAs that contribute to interactions with binding partners and functional outcomes.

In recent years, a number of new mechanisms of sRNA action have been identified, many acting on processes beyond translation initiation and ribonuclease recruitment [5–9]. Similarly, it is now appreciated that regulatory sRNA can be generated through processing of longer transcripts or transcription of sub-regions of genes [10]. In the interest of space, this review does not cover the multitude of sRNA mechanisms or diversity of new locations that sRNAs can be found within the transcriptome.

As more sophisticated algorithms become available to interrogate ever-increasing amounts of high-throughput data, our hope is to provide a summary of sRNA features that contribute to function and might be incorporated into predictive models, or for the design of synthetic gene regulators.

Sequences and structures that functionalize sRNAs

Several algorithms have been developed to predict sRNA interactions by constraining predictions based on base-pairing patterns between the seed region of the sRNA and the target site of the mRNA. These predictions commonly use free energy (ΔG) as a proxy for interaction strength and functional relevance. Some algorithms also restrict predictions based on a minimum number of consecutive base pairs, RNA accessibility, restricting seeds to Watson–Crick base pairs, or evolutionary conservation [11–15]. All of these variables have been suggested from case-by-case analysis of functional sRNA–mRNA pairs or extrapolated from the more extensively studied miRNA systems, where the functional determinants of miRNA seeds have been more exhaustively explored and are better understood. Recently, machine learning has been applied to sRNA prediction that uses the features above and local sequence features like k -mer frequency [15, 16].

The strength of base pairing between sRNA and mRNAs is often used as a proxy for predicting the functionality of sRNA–mRNA interactions, with several studies demonstrating a correlation between ΔG of these pairs and their function. While this serves as a useful indicator, it is clear that many strong interactions (low ΔG) are not functional, while weaker interactions (higher ΔG) have clear effects on gene expression. Faigenbaum-Romm *et al.* recently used RNA-seq and RNA interaction by ligation and sequencing (RIL-seq) to identify parameters that more accurately differentiate functional sRNA–mRNA interactions from non-functional ones [17]. The authors found that while ΔG is not a reliable proxy for function, hybrid read count (the frequency of interactions detected by RIL-seq), and Hfq binding were able to segregate functional and non-functional interactions reasonably well. These findings indicate that local Hfq binding is an important driver of sRNA function but several other factors, including local sequence motifs, RNA structure, base-pairing patterns, and protein interactions, are likely important and are explored below.

sRNA binding to the RNA chaperone, Hfq

The sRNA chaperone, Hfq, plays a critical role in facilitating the activity of many sRNAs in Gram-negative bacteria by increasing the kinetics of sRNA–mRNA annealing to biologically useful rates [18]. Hfq binding is also important to stabilize many sRNAs. Canonically, Hfq recruits mRNAs to its

distal face, sRNAs to the proximal face, and facilitates an interaction between the RNAs at the rim (or lateral) sites [19].

The distal face of Hfq in *Escherichia coli* accommodates a repeated ARN motif, where A represents adenine, R any purine (adenine or guanine), and N any nucleotide, typically with one repeat per Hfq monomer and three or more repeats overall [20]. Maximal binding is achieved with a repeated AAN_{*n*} motif [21]. The Shine–Dalgarno (SD) sequence is neatly accommodated within this motif, which is consistent with it being a major target for sRNA repression. *In vitro*, the A-rich distal site motif can facilitate sRNA pairing when positioned both 5' and 3' of the seed region, but is slightly more efficient when positioned 3' of the seed [22]. In contrast, using cross-linking and immunoprecipitation sequencing (CLIP-seq), Hfq was found to preferentially bind 5' of mRNA target site *in vivo* [23]. sRNAs were found to have preferential binding 3' of the target site, a result that was also reported in pathogenic *E. coli* [6] and predicts that Hfq is ‘sandwiched’ between the mRNA (bound 5' of the target site) and sRNA (bound 3' of the seed), positioning the complementary sequence at the rim for annealing (Fig. 1, left). This *in vivo* preference is also seen in Hfq RIL-seq data where the mRNA is more frequently first (5') in the chimeric read and sRNA second (3') [24]. The latter authors rationalize that the sRNA 3' OH end is obstructed by proximal face binding (see below) and not available for ligation, so it may bias the chimeric reads towards this order.

The Hfq distal binding site is often closely associated with the mRNA target site [6, 25] and restricting predictions of sRNA complementarity to the Hfq footprint has been one approach to filter for functional sRNA–mRNA interactions *in silico* [26]. In many cases, the distal ARN_{*n*} motif overlaps with the mRNA target region, suggesting a mechanism whereby pairing of the sRNA would envelop the ARN_{*n*} binding motif in a duplex and promote dissociation of the sRNA–mRNA duplex from Hfq [6].

The proximal face of Hfq binds to poly-uridine tracts and can accommodate an RNA 3' OH end at the central ‘pore’ [27], making intrinsic terminators the major binding targets for the proximal face. Given the relative ubiquity of intrinsic terminators on bacterial transcripts, how Hfq specifically recognizes subsets of sRNA terminators has been an open question. Using CLIP-seq to compare Hfq binding sites with the sRNA chaperone ProQ (discussed below), Holmqvist *et al.* demonstrated that the poly(U) tract of Hfq-bound sRNAs was longer than ProQ targets, suggesting that the length of the poly(U) may influence proximal face binding [28]. This study mirrors results demonstrating that the length of the 3' poly(U) tract influences Hfq and ProQ binding to sRNAs and mRNAs such as SgrS, RyhB, *malM* 3'-UTR, and *cspE* [29–31].

Hfq also encodes a disordered C-terminal tail that varies in length depending on the species. The disordered tail does not contain an RNA-binding motif, but its acidic tip is thought to mimic nucleic acid interactions with the arginine-rich rim, thereby providing competition and increasing specificity [32–34]. By providing competitive interactions with the rim, the C-terminal is thought to promote dissociation of sRNA–mRNA duplexes from Hfq. Interestingly, deletion of the C-terminal tail significantly reduces RydC binding to Hfq, suggesting that the flexible C-terminal may also help ‘fish’ for substrates by increasing the molecular encounter radius [35]. Alternative roles for the C-terminal domain in promoting G-quadruplex formation [36] and membrane insertion [37, 38] have also been proposed.

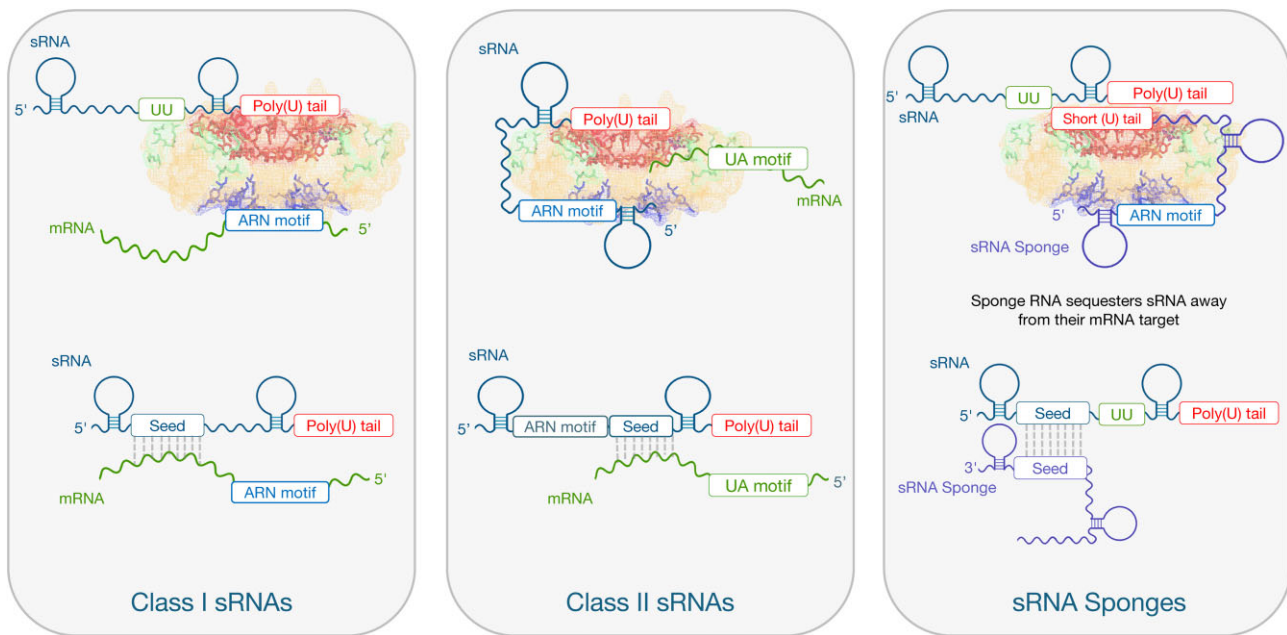


Figure 1. Schematic representation of how sRNA can bind Hfq. (Left panel) Class I sRNA encodes for a poly(U) tail that binds to the Hfq proximal face (red sticks) and UA-rich motif that binds to the lateral rim (green sticks). (Central panel) Class II sRNAs also contain a poly(U) tail but instead of a UA-rich motif they contain an ARN motif that binds to Hfq distal face (blue sticks). (Right panel) RNA sponges act similarly to Class II sRNAs but regulate other sRNAs rather than mRNA targets. The Hfq structure (PDB: 1HK9) [128] is represented in a yellow mesh format.

sRNA binding to Hfq rim site positions the sRNA seed for mRNA interactions

Many sRNAs also have internal uridines that are thought to contact the arginine-rich rim binding site of Hfq [39], potentially positioning the sRNA seed for base pairing with mRNA targets bound to the distal face (Fig. 1, left). Structural analysis of the RydC–Hfq complex indicates that an internal U-rich motif 3' of the sRNA seed interacts with the positively charged rim of Hfq to present the last four nucleotides of the seed in an extended conformation [35]. The nucleotides extending from the rim of Hfq are positioned to initiate base pairing with a target mRNA presented on the distal side of Hfq. Mutation of these nucleotides is likely to have a disproportionately large effect on sRNA–mRNA duplex disruption and likely explains the importance of the seed core (discussed in the next section).

The important role of an internal U/A-rich region in sRNA interaction with the Hfq rim site has been demonstrated for a number of sRNAs, including Spot42 [40], MicA [41], SgrS [29], OxyS [42], and DsrA [43]. A UU dinucleotide motif located immediately upstream (5') of a structured stem region was also identified at Hfq cross-linking sites within sRNAs by *in vivo* UV cross-linking and sequencing (CRAC) in pathogenic *E. coli* [6], further supporting its important role in Hfq binding *in vivo*.

For many sRNAs, it is likely that a U-rich motif adjacent (3') to the sRNA seed is important for Hfq rim binding and presentation of the seed to mRNAs on the distal face. In the case of SgrS, a U-rich motif positioned between an internal stem-loop and the 3' end of the seed regions was found to enhance stability and target repression [29]. Similarly, systematic mutation and Sort-seq analysis found that U182 downstream of the SgrS seed region is required for function [44].

Additional evidence for the importance of internal uridines in sRNA function has also been found in *Vibrio harveyi* [45]. In the sRNA Qrr4, U43 and U44 are positioned adjacent to the

sRNA seed and are required for function, even though they are not part of the sRNA seed sequence. These internal uridines are also likely to interact with the rim motif of Hfq to position the sRNA seed for mRNA interactions.

While most mRNA target sites are closely associated with distal face binding motifs, a significant number of target sites are positioned far downstream of the Hfq binding sites, which would seem to be problematic for target site presentation at the rim [25]. For these distant sites, it has been proposed that the positively charged rim allows scanning of downstream U-rich sites that induce compaction of the mRNA and draw distant sites into proximity of the sRNA seed [25].

Alternative Hfq interaction modes have also been described. Those sRNAs that bind the proximal and rim sites are termed Class I sRNAs, but exceptions exist that have been classified as Class II sRNAs (Fig. 1, central panel). Using a series of point mutations in the distal, proximal, or rim binding sites, the Storz and Gottesman labs demonstrated that ChiX, CyaR, MgrR, and McaS bound both the proximal and distal surfaces of Hfq [46]. These sRNAs contain both poly(U) tails that occupy the proximal face and ARN_n motifs that bind the distal face. Because Class II sRNAs occupy the distal face, their target mRNAs bind the rim of Hfq and carry U-rich sequences upstream of the mRNA target site [46, 47]. Class II sRNAs are generally more stable and are not degraded with their mRNA targets [48]. Their stability and ability to interact with both faces of Hfq allow Class II sRNAs to exert pleiotropic effects on the sRNA network by sequestering Hfq [48].

sRNA sponges represent another class of sRNA that deviate from the Class I binding pattern involving proximal/rim binding (Fig. 1, right). Sponges target other sRNAs rather than mRNAs. While many sponges have now been described, relatively little has been documented on their interactions with Hfq. Malecka *et al.* demonstrated that the sRNA sponges AgvB and 3'ETS^{leuZ} associate with both the proximal and dis-

tal faces, similar to Class II sRNAs [49, 50]. The cryo-electron tomography (cryo-EM) structure of 3'ETS^{leuZ} in complex with Hfq and PNPase also indicates that the sponge makes Class II contacts with Hfq [50]. Both AgvB and 3'ETS^{leuZ} have short poly(U) tails. Extension of the U-rich 3' end of AgvB enhanced proximal face binding and target sRNA annealing, suggesting that proximal face binding is a mechanism to tune the strength of target RNA silencing [49]. Like Class II sRNAs, AgvB interacts with an sRNA that is predominately associated with the rim of Hfq, in this case GcvB [49].

This raises the interesting question of how Class II sRNAs and sponges—that do not bind the rim—present the seed for base pairing. For sRNA sponges, distal face interactions have been proposed to anchor the sRNA 5' end and position the seed region for base pairing with target RNAs at the rim [49]. A similar role for distal face binding might also be expected for Class II sRNAs that do not utilize the rim for seed presentation.

sRNA–mRNA base pairing initiates at a seed core

Several studies have demonstrated that not all base pairs in the seed duplex contribute equally to sRNA function. High-density libraries of single nucleotide mutations have been generated for the sRNAs Qrr4, SgrS, DsrA, and RyhB using a Sort-seq approach to map the nucleotides that contribute to target repression [44, 45, 51]. In the sRNA Qrr4 and SgrS, a 'core' region of ~5 nt has been identified within the seed region where single nucleotide changes dramatically reduce sRNA function. This core region is likely presented in an extended conformation at the rim of Hfq (discussed earlier) to nucleate the interaction with an mRNA target site. For example, in *V. harveyi*, the sRNA Qrr4 was shown to control quorum sensing and regulates multiple mRNA targets through an 18-nt seed region. Only five individual nucleotides within the seed region appear to be essential for regulation of the mRNAs *luxR* or *luxO* (i.e. single point mutations disrupt sRNA function) [45].

A similar result was found for the sRNA SgrS in *E. coli*, where only three nucleotides within the 3' end (G176–G178) of the predicted 20-nt seed region [52] are individually required for SgrS repression of *ptsG* mRNA [44]. Using single molecule fluorescence *in situ* hybridization (sm-FISH), SgrS kinetics for the seed core mutants were calculated and revealed a 24%–31% reduction in association rate (k_{on}) between the sRNA and mRNA compared to wild type and a 14%–25% increase in the dissociation rate (k_{off}) [44]. These results indicate that the seed core plays an important role in the sRNAs' ability to find and bind target mRNAs. In *Pseudomonas aeruginosa*, the sRNA PhrS was disrupted by introducing tiled dinucleotide mutations across part of the seed region [53]. Similar to Qrr4 and SgrS, these authors found that three nucleotides (positions C174–C176) and four nucleotides (positions A178–C181) within the seed region are disproportionately required for repression of *hmgA* and *antR* mRNAs, respectively.

Base pairing outside of the seed core remains important for sRNA function (although individual nucleotides make smaller contributions) and is suggested to play a supporting role in stabilizing the interaction [45]. This is conceptually analogous to supplementary or compensatory pairing that can stabilize imperfect miRNA–mRNA interactions [54] and an sRNA

seed interaction of 12 nt has been suggested to provide optimal repression [55].

A more extensive seed core appears to be present in the sRNAs RyhB and DsrA when assayed using qSort-seq [51]. Single base changes throughout the 9–23-nt seed regions disrupt sRNA function, although the authors note that mutation of some bases in the seed region has only a minimal effect on sRNA function. Within the seed region of RyhB, the identity of specific base pairs (AU or GC) influences sRNA function and appears to tune repression of the mRNA target. Using a series of complementary mutations in RyhB and *sodB*, Hao *et al.* demonstrated that the identity of the base-paired nucleotides within the seed region tunes repression strength [56]. These changes are qualitatively correlated with changes in interaction strength predicted by the change in free energy (ΔG).

The picture that has emerged from both high-throughput mutagenesis and systematic introduction of point mutations within the seed region is that a short seed core of 3–5 nt may be required to nucleate the sRNA–mRNA interactions. Duplex formation at the seed core may be stabilized by more extensive supplementary base pairing that is less sensitive to single base changes.

Base-pairing patterns matter

Perhaps more surprising is that the base-pairing pattern can affect the function of the sRNA–mRNA interaction. A limited number of studies have explored how the base-paired nucleotides drive function and found that seed identity and base-pairing patterns can affect sRNA and mRNA stability. In elegant work from the Bassler lab, Feng *et al.* showed that the sRNA Qrr3 can regulate mRNA targets through catalytic degradation of the target mRNA, coupled degradation, sequestration, and activation [57]. The different mechanisms of repression appear to be driven by both the location of interactions within the sRNA and mRNA and the extent of base pairing with the mRNA. Coupled degradation appears to occur when mRNA base pairing invades a stabilizing 5' stem-loop (SL1) in Qrr3. Catalytic degradation is mediated by mRNA base pairing that preserves the protective 5' stem-loop of Qrr3. Sequestration (where both sRNA and mRNA are stable) is promoted by extensive base pairing between the sRNA and mRNA, although the mechanism is not clear.

The functional importance of the base-pairing pattern and position is also highlighted by work from the Massé lab. Using the mRNA *hdeD*, they studied interactions with CyaR and RprA sRNAs [58]. While both sRNAs bind to the 5'UTR near the SD of *hdeD* and interfere with the recruitment of the 30S ribosomal subunit, the fate of the transcript is different. RprA inhibits translation initiation by occluding initiating ribosomes, whereas CyaR also actively recruits RNase E to *hdeD* for degradation. Data suggest that most of the information necessary to recruit RNase E is stored in the seed sequence, as swapping the seed region of the CyaR and RprA sRNAs allows the degradation mechanisms to be interchanged. Interestingly, RprA uses a slightly longer and imperfect seed base pairing compared to CyaR [58].

In Gram-positive bacteria, many sRNAs use a C-rich seed sequence positioned within an unstructured region (e.g. within a loop or between two stem-loops). This was first observed in *Staphylococcus aureus* [59] and then extended to many other

Gram-positive bacteria [60]. This leads to a preferential targeting of G-rich SD sequences, resulting in inhibition of translation initiation. However, the SD is not the only G-rich target as exemplified by the sRNA FasX, which uses a C-rich seed sequence to bind to the 5' end of *skd* mRNA to protect it from degradation [61]. Interestingly, CU-rich seeds are also found in some Gram-negatives. For example, Cjnc190 sRNA uses a CU-rich loop region to target the SD of *ptmG* mRNA in *Campylobacter jejuni* [62], as do CiaRH-dependent sRNAs (csRNAs) in *Streptococcus pneumoniae* [63].

It is easy to imagine that the length of the binding site or the GC% should be higher in Gram-positive bacteria to compensate for the absence or depreciation of an RNA chaperone protein such as Hfq. Indeed, many long interactions have been predicted for sRNA–mRNA duplexes in Gram-positive bacteria. However, few of them have been validated experimentally. While an ≈ 60 -nt-long pairing site between RoxS sRNA and *ppnKB* mRNA has been predicted in *Bacillus subtilis* [64], the mutation of only 4 nt abolished the RoxS-dependent repression of *ppnKB* translation. In addition, these long pairing sites are far from representative of the diversity found in Gram-positive bacteria. Similarly, the GC% varies widely from one seed sequence to another. For example, the staphylococcal sRNA RsaC binds to *sodA* mRNA and inhibits its translation using an ≈ 16 -nt-long seed sequence with 25% GC content [65]. Conversely, in *Streptomyces coelicolor* the sRNA Scr5239 interacts with *dagA* mRNA using an ≈ 20 -nt-long single-stranded region with 75% GC content [66].

Several sRNAs also use multiple pairing sites to control their targets. In *Staphylococcus aureus*, RsaA uses two seed regions, both of which are essential to control the translation of MgrA [67]. A short C-rich motif is used to target the SD of *mgrA*, while a second seed sequence forms a loop–loop interaction with the coding sequence of *mgrA*, which stabilizes the RsaA–*mgrA* interaction. This is not an isolated case, as the staphylococcal sRNA RNAIII uses multiple loop–loop interactions to regulate its mRNA targets [68–70], and a second seed site contributes to repression for the Gram-negative sRNAs RprA and StxS [58, 71].

Internal stems contribute to sRNA function, seed presentation, and stability

Many sRNAs encode internal stem-loops (in addition to the 3' terminator loop) that play important roles in stability, seed presentation, or interactions with the degradosome. High-density libraries of single nucleotide mutations have revealed the importance of internal stems for sRNA function in both RyhB [51] and SgrS [44].

The sRNA RyhB has three stem-loops (SL1, SL2, and SL3) with the seed region positioned within the loop of SL2. Mutations in SL1 have varying effects on function, including complete inactivation [51]. For many of the point mutants, stable folding of the sRNA (ΔG) is correlated with sRNA efficiency, suggesting that sRNA structure—even beyond the terminator and Hfq binding motifs—is important for function.

Similarly, the sRNA SgrS encodes a short 4-nt stem-loop adjacent to the terminator that is required for function [44]. Both the terminator and short stem-loop are required for Hfq binding and a point mutation in the 4-nt stem-loop destabilizes SgrS consistent with reduced Hfq binding. Interestingly, the 4-nt stem is preceded by a U-rich region that is also involved in Hfq binding and likely interacts with the rim (dis-

cussed earlier) [29]. The stem may play a role in positioning the U-rich motif (and sRNA seed) at the correct distance and orientation in three-dimensional space from the poly(U) tail (that is bound to the proximal face) so that the seed is presented at the rim [35].

Internal stems also play an important role in binding and presentation of the sRNA–mRNA duplex to the catalytic site of RNase E. The N-terminal domain of RNase E contains an internal entry site that binds structured RNA [72]. Internal stem-loops within the sRNA MicC and/or the cognate target *ompD* mRNA have been proposed to bind the internal entry site to present the mRNA for cleavage [72, 73]. This site may also bind the sRNA–mRNA duplex directly.

Internal stems can also guide RNase E cleavage of the sRNA. The sRNA MicL is transcribed from the 3'UTR of *cutC* mRNA [74]. MicL is processed by RNase E into an 80-nt sRNA and cleavage is directed by a stem-loop in MicL that binds the internal entry site of RNase E to direct processing [74].

Collectively, internal stems position the sRNA seed in three-dimensional space, contribute to sRNA stability, and direct processing of both sRNA and mRNA by recruiting the RNA degradosome. Some of the features that promote sRNA function are illustrated in Fig. 2.

sRNA delivery of mRNA to the RNA degradosome

sRNA pairing with an mRNA target can have several functional outcomes, including recruitment of the sRNA–mRNA pair to the RNA degradosome. In *E. coli*, the degradosome is scaffolded by the endoribonuclease, RNase E. Five RNA binding sites have been identified on RNase E and likely allow a diverse range of RNA substrates to bind the complex (Fig. 3A and B). The catalytic site recognizes a single-stranded RN \downarrow WUU motif (A/G, A/U/C/G, A/U, U, and U) [75]. Catalysis can be enhanced by binding of a free 5' monophosphate to the 'sensor pocket' [76, 77]. The free 5' monophosphate can be provided by the mRNA or a duplexed sRNA and induces a conformational change in RNase E that closes the complex and orients the RNA for cleavage, improving binding and cleavage [75–77]. Many sRNAs are processed from longer transcripts and carry a 5' monophosphate end, which likely plays a key role in stimulating RNase E activity on a single-stranded region of the duplexed mRNA [76]. Interestingly, for those sRNAs 'activated' by RNase E cleavage, they appear to be cleaved, dissociate, and re-associate again when base-paired with a target mRNA, indicating that different functional outcomes are achieved by presenting the sRNA to RNase E in different configurations [78].

RNase E can also be recruited to sites within RNA targets (distant from the 5' monophosphate end) using the direct entry site (also termed internal entry site) at the inter-protomer surface within the N-terminal domain [72]. The direct entry site binds the phosphate backbone of RNA duplexes (or stems) to position RNAs for cleavage. The lack of sequence specificity at this site is suggested to allow broad substrate recognition. The sRNA RprA is processed by RNase E and contains three stem-loops. Binding of the first stem-loop to the internal entry site positions RprA for cleavage and activation [72]. A similar situation has been reported for the 3' UTR sRNA, MicL (discussed earlier) [74]. The direct entry site provides a route for recognition of RNA without a 5' monophosphate end, but these pathways are not mutually ex-

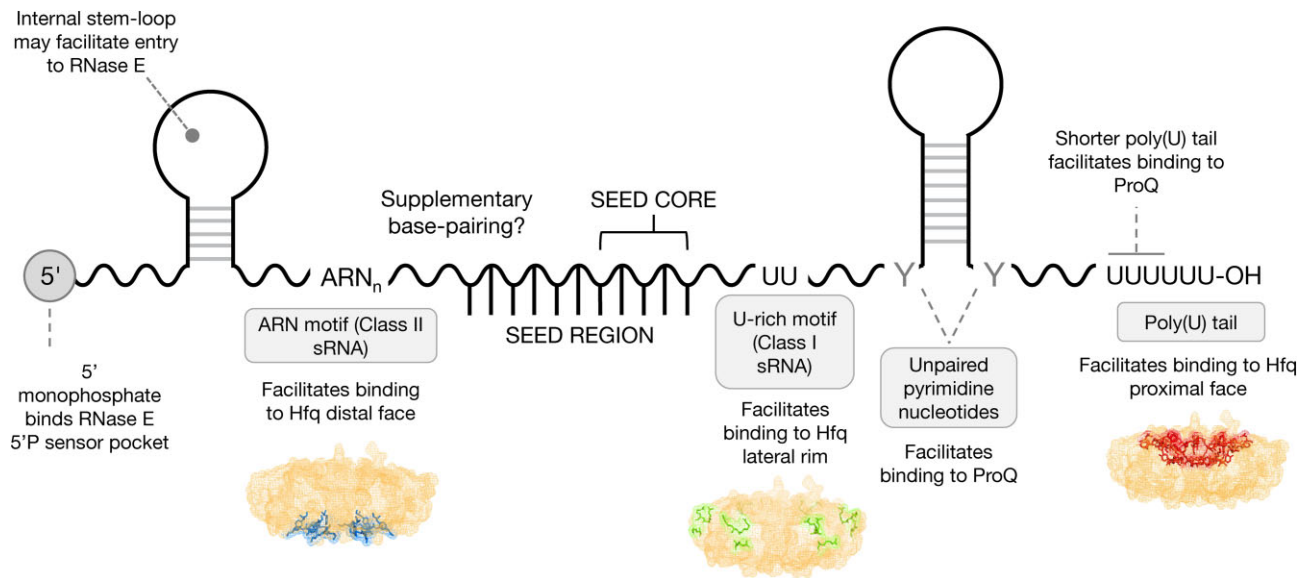


Figure 2 Sequences and structural elements that drive sRNA function. sRNAs encode for specific sequences and structural motifs that enable their interaction with RNA-binding proteins (e.g. Hfq and ProQ) and ribonucleases (e.g. RNase E). The Hfq structure (PDB: 1HK9) [128] is represented in a yellow mesh format.

clusive and for the sRNA–mRNA pair *MicC–ompD* it seems that the sRNA provides a 5' monophosphate while an adjacent stem in *ompD* binds the internal entry site to enhance RNase E binding [72].

In *E. coli*, the C-terminal domain of RNase E is unstructured and provides a scaffold for components of the RNA degradosome, including the core components PNPase, enolase, and RhlB [79] (Fig. 3B). Two RNA-binding sites have been identified within the C-terminal domain termed RBD (also known as ARRB, 604–688 residues in *E. coli*) and AR2 (796–819 residues) [79–83]. The ternary Hfq–sRNA–mRNA complex interacts with the C-terminal domain of RNase E through bridging interactions with the RNAs rather than protein–protein interactions between Hfq and RNase E [80, 84]. Removing the C-terminal domain reduces sRNA–mRNA processing indicating that it is required for duplex recruitment to the degradosome [85–88]. Bruce *et al.* have demonstrated that the sRNA *MicC* can mediate an interaction between Hfq and the C-terminal domain of RNase E *in vitro* and have proposed a model where sRNAs recruit target mRNAs to the N-terminal catalytic core [80]. Indeed, cryo-EM of the degradosome complex indicates that RNA binding is accompanied by compaction of the C-terminus towards the catalytic N-terminal domain [89].

In contrast, Sinha *et al.* have analysed the contributions of the RBD and AR2 domains to recruitment of CyaR and RyhB sRNA–mRNA duplexes to RNase E *in vivo*. They propose that the AR2 domain interacts with the mRNA target rather than the sRNA [90]. Deletion of the AR2 domain or mutation of the distal (mRNA binding) face of Hfq decreases interactions with RNase E, indicating that the mRNA is responsible for RNase E recruitment to the Hfq–sRNA–mRNA complex *in vivo* [90].

Recent work has found that the C-terminal unstructured domain of RNase E allows sRNA-directed cleavage of mRNAs engaged with the 30S translation initiation complex [73] and an early expressosome complex consisting of paused RNA polymerase linked to 30S by NusG [91]. This co-

translational silencing requires a portion of the RNase E C-terminal domain that contains AR2 [73]. The sequences or structures within the mRNA target that recruit the AR2 domain of RNase E are still unknown, but likely play an important role in determining the functional outcome of the sRNA–mRNA interaction (Fig. 3C).

The seed pairing sequence or position also seems to contribute to RNase E recruitment. As mentioned earlier, the sRNAs RprA and CyaR both repress the mRNA *bdeD*, although only the latter recruits RNase E to promote degradation [58]. Switching the seed regions of RprA appears to affect RNase E recruitment to the sRNA–mRNA duplex but is dependent on an adjacent AU-rich region that is bound by Hfq.

In most Gram-positive bacteria, RNase E is replaced by RNase Y [92]. RNase E is only present in some Bacillota (formerly Firmicutes) (e.g. *Listeria* and *Clostridia*) and Actinomycetota (formerly Actinobacteria) (e.g. *Mycobacteria*). Both enzymes have similar cleavage specificities, but RNase Y does not appear to play as dominant a role as RNase E in sRNA-dependent regulation. The recruitment of a ribonuclease to actively degrade the targeted mRNA seems less prevalent than in Gram-negative bacteria, with regulation generally taking place at the level of translation initiation.

RNase Y is the main endonuclease for mRNA decay in Gram-positive bacteria. The deletion of *rny* gene affects from 13% to 23% of transcripts in *B. subtilis* and *Clostridium perfringens* [93]. While not as prevalent as Gram-negatives, RNase Y is involved in sRNA-mediated cleavage of some mRNAs (e.g. VR-RNA-*colA* in *C. perfringens* [94] and RoxS-*ppnKB* in *B. subtilis* [64]).

RNase III is not part of the Gram-positive RNA degradosome but appears to play a prominent role in sRNA–mRNA regulation and degradation. RNase III recognizes double-stranded substrates in a highly specific manner and key RNA structural elements (helix length, helix-budge motifs) and sequence elements have been identified in *E. coli* [95, 96]. When stacked coaxially, intermolecular interactions can also generate a structure that is recognized by RNase III [67, 97]. Be-

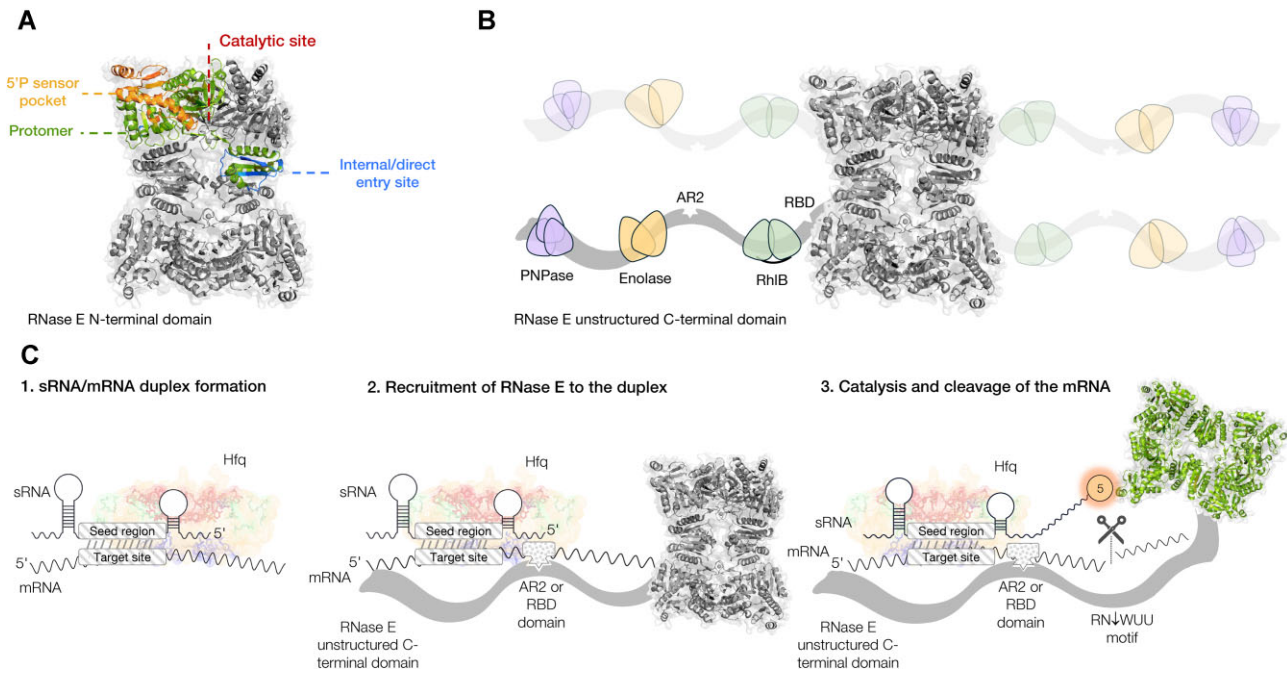


Figure 3 RNase E architecture and interaction with sRNAs. **(A)** The structural features that allow RNase E (PDB: 6G63) to bind sRNA. RNase E forms a tetramer that can bind sRNAs via a 5' phosphate sensor pocket (orange) and an internal/direct entry site (blue). **(B)** The unstructured C-terminal domain of RNase E contains multiple sites that allow sRNA binding, i.e. AR2 and RBD domains. **(C)** Illustration of how sRNA can guide RNase E to process mRNA targets in *E. coli*.

sides its major role in the control of type I toxin–antitoxin system and pervasive transcription, the double-strand-specific endoribonuclease RNase III is involved in *trans*-acting sRNA-dependent regulation [64, 67, 98]. A greater involvement in sRNA-dependent regulation is suggested by different copurification approaches in *S. aureus* [99–101].

RNase Y [64, 102], RNase III [62, 65], and RNase J [103] also play important roles in maturation of many sRNAs in Gram-positives. Similar to RNase E-dependent activation of sRNAs, this suggests that pre-sRNAs are recruited to the Gram-positive degradation machinery and then released to bind target mRNAs, before becoming RNase substrates once again.

Collectively, sequences and structures within both the sRNA and mRNA promote processing of the sRNA–mRNA duplex. The duplexed sRNA can provide a 5' monophosphate *in trans* to promote catalysis of the mRNA and either sRNA or mRNA might provide adjacent stems to bind the internal entry site of RNase E. The mRNA appears to play an active role in initial recruitment of RNase E to the ternary Hfq–sRNA–mRNA complex through interactions with the AR2 domain, and ultimately presents a suitable cleavage site, suggesting that the sRNA augments degradation signals already present within the mRNA.

Functional interactions with sRNA-binding proteins

Several alternative sRNA-binding proteins have now been identified largely through high-throughput analysis of the RNA-binding proteome and these appear to modulate sRNA functions by stabilizing or destabilizing the sRNA or sRNA–mRNA duplex.

Using Grad-seq, Smirnov *et al.* demonstrated that ProQ was a global sRNA-binding protein [104]. ProQ has a FinO RNA binding domain and results from CLIP-seq, structural

modelling, and genetic screening suggest that it binds the 3' end of the terminator stem on a subset of sRNAs [28, 105]. ProQ appears to bind structured RNA and targets that tend to have shorter poly(U) tails than Hfq ligands. This may allow ProQ interactions with the terminator stem while bound at the 3' [28, 105]. Two further FinO domain proteins have been described that bind sRNAs, the archetype FinO that controls the copy number of F plasmid through FinP sRNA binding [106] and RocC that controls competence in *Legionella* through RocR sRNA binding [107]. Both appear to bind sRNAs through intrinsic terminators with short polyuridine tails and related structures may bind different length tails [107]. ProQ and FinO achieve further specificity by recognizing the unpaired nucleotides flanking the terminator stem [108]. For a few sRNA–mRNA pairs, ProQ seems to promote annealing, but for many targets ProQ appears to associate and stabilize the duplex after annealing, or stabilize individual sRNAs by blocking RNase access. ProQ has been shown to inhibit RNase II and RNase III degradation of sRNAs and sRNA–mRNA duplexes, and PAPI-dependent polyadenylation of an mRNA 3'UTR [28, 109, 110].

Extending Grad-seq to a range of bacteria has identified KhpA and KhpB as additional sRNA-binding proteins that modulate sRNA stability in a wide range of bacteria [111–114]. The sequences or structures that drive KhpA/B recruitment are still not clear but binding may play a major role in sRNA function, particularly in species where Hfq or ProQ does not play a major role in sRNA regulation. A similar function is suggested for YhgF, an sRNA-binding protein identified using an adapted RNA interactome (RIC) approach, and it appears to stabilize RNA targets through an unknown mechanism [115].

Recently, a global sRNA-binding protein, RbpB, was identified in *Bacteroides thetaiotaomicron* through CLIP-seq analyses [116, 117]. This protein binds to numerous cellular RNAs,

including a family of sRNAs termed FopS [116, 117]. These FopS sRNAs repress the translation of SucC glycan transporters. In contrast to other RNA-binding proteins that facilitate sRNA repression of mRNA targets, RbpB binds the FopS sRNA and prevents their silencing activity (functionally analogous to an sRNA sponge), thereby promoting glycan transporter expression and enabling transcriptional adaptation to nutrient availability [116, 117].

While ProQ, KhpAB, and YhgF typically stabilize sRNAs, recent studies have identified several ‘adapter’ proteins that promote sRNA degradation by chaperoning them to ribonucleases [9, 118]. The adapter RapZ binds the sRNA GlmZ and chaperones it to RNase E for degradation [9, 118]. In contrast to other global sRNA chaperones, RapZ seems to have a narrow target spectrum. In a recent high-throughput screen for proteins that modulate the activity of RyhB, Chen *et al.* identified YicC that enhances PNPase degradation of RyhB [119]. In *S. pneumoniae*, this idea is taken a step further where the 3'→5' exoribonuclease Cbf1 was found to trim the 3' of sRNAs and remain stably associated with the trimmed 3' end to protect from further degradation [120]. These studies hint at an abundance of protein interactions that both stabilize and destabilize sRNAs to modulate function.

Several proteins have been shown to moonlight, i.e. perform accessory roles, as sRNA chaperones. For example, the carbon storage regulator CsrA was thought to predominately act independently of sRNAs, but appears to promote duplex formation for select sRNA–mRNA pairs [121, 122]. Similarly, the DNA-binding transcription factors, SpoVG and CcpA, appear to have accessory functions in sRNA binding in Gram-positive bacteria [123, 124]. Modulation of sRNA stability or interactions may be a relatively accessible pathway for altering gene expression. With the recent development of generative AI software like AlphaFold 3 [125] and RosettaFoldNA [126] that can predict protein–RNA interactions, the sRNA-binding proteome is likely to expand further in the near future.

Functional motifs within sRNA interactome data

Several techniques are now available to profile sRNA–mRNA interactions *in vivo* and a wealth of data is accumulating on the sRNA interactome [127]. Within these datasets, it is clear there are many interactions that fall outside of our current understanding of sRNA function and a proportion may be non-functional or provide non-specific buffering interactions to the network. While we can now see the complexity of the sRNA interactome, predicting the strength and functions of individual interactions remains difficult and often requires case-by-case investigation. This is in part due to the diversity of sRNA sequences, structures, protein interactions, and functions compared to other non-coding RNA classes. As we map the architecture of the transcriptome, RNA–RNA interactions, and protein–RNA interactions, the individual context for each sRNA–RNA interaction is coming into focus and provides clues to the function of each interaction. Machine learning approaches seem well suited to the task of categorizing sRNA–mRNA interactions and identifying features that predict known and new function.

The sRNA regulatory network is at least as large as the transcriptional network in *E. coli* and may be much larger, particularly in those bacteria with reduced genomes and fewer transcription factors. A predictive framework for sRNA function will allow integration with transcriptional networks and

a more complete understanding of how bacteria control gene expression.

Acknowledgements

The work of the Interdisciplinary Thematic Institute IMCBio, as part of the ITI 2021–2028 program of the University of Strasbourg, CNRS, and INSERM, was supported by IdEx Unistra (ANR-10-IDEX-0002), by SFRI-STRAT'US project (ANR 20-SFRI-0012), and by EUR IMCBio (IMCBio ANR-17-EURE-0023) under the framework of the French Investments for the Future Program.

Author contributions: Saleh Alquethamy (Writing—original draft [supporting], Writing—review & editing [equal]), David Lalaouna (Writing—original draft [supporting], Writing—review & editing [equal]), and Jai J. Tree (Writing—original draft [lead], Writing—review & editing [equal]).

Conflict of interest

None declared.

Funding

J.J.T. was supported by grants from the National Health and Medical Research Council (GNT2028572) and Australian Research Council (DP220101938). D.L. was supported by the Agence Nationale de la Recherche (ANR-20-CE12-0021). Funding to pay the Open Access publication charges for this article was provided by National Health and Medical Research Council.

Data availability

There are no new data in this review article.

References

- Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116:281–97. [https://doi.org/10.1016/S0092-8674\(04\)00045-5](https://doi.org/10.1016/S0092-8674(04)00045-5)
- Siomi MC, Sato K, Pezic D *et al.* PIWI-interacting small RNAs: the vanguard of genome defence. *Nat Rev Mol Cell Biol* 2011;12:246–58. <https://doi.org/10.1038/nrm3089>
- Storz G. Unexpected richness of the bacterial small RNA world. *J Mol Biol* 2025;437:169045. <https://doi.org/10.1016/j.jmb.2025.169045>
- Gottesman S. Bacterial regulatory circuits are linked and extended by small RNAs. *J Mol Biol* 2025;437:169059. <https://doi.org/10.1016/j.jmb.2025.169059>
- Sedlyarova N, Shamovsky I, Bharati BK *et al.* sRNA-mediated control of transcription termination in *E. coli*. *Cell* 2016;167:111–21. <https://doi.org/10.1016/j.cell.2016.09.004>
- Tree JJ, Granneman S, McAteer SP *et al.* Identification of bacteriophage-encoded anti-sRNAs in pathogenic *Escherichia coli*. *Mol Cell* 2014;55:199–213. <https://doi.org/10.1016/j.molcel.2014.05.006>
- Lalaouna D, Carrier MC, Semsey S *et al.* A 3' external transcribed spacer in a tRNA transcript acts as a sponge for small RNAs to prevent transcriptional noise. *Mol Cell* 2015;58:393–405. <https://doi.org/10.1016/j.molcel.2015.03.013>
- Jagodnik J, Chiaruttini C, Guillier M. Stem-loop structures within mRNA coding sequences activate translation initiation

- and mediate control by small regulatory RNAs. *Mol Cell* 2017;68:158–70. <https://doi.org/10.1016/j.molcel.2017.08.015>
9. Khan MA, Durica-Mitic S, Göpel Y *et al.* Small RNA-binding protein RapZ mediates cell envelope precursor sensing and signaling in *Escherichia coli*. *EMBO J* 2020;39:e103848. <https://doi.org/10.15252/embj.2019103848>
 10. Adams PP, Storz G. Prevalence of small base-pairing RNAs derived from diverse genomic loci. *Biochim Biophys Acta Gene Regul Mech* 2020;1863:194524. <https://doi.org/10.1016/j.bbagr.2020.194524>
 11. Wright PR, Richter AS, Papenfort K *et al.* Comparative genomics boosts target prediction for bacterial small RNAs. *Proc Natl Acad Sci USA* 2013;110:E3487–96.
 12. Mückstein U, Tafer H, Hacker Müller J *et al.* Thermodynamics of RNA–RNA binding. *Bioinformatics* 2006;22:1177–82. <https://doi.org/10.1093/bioinformatics/btl024>
 13. Busch A, Richter AS, Backofen R. IntaRNA: efficient prediction of bacterial sRNA targets incorporating target site accessibility and seed regions. *Bioinformatics* 2008;24:2849–56. <https://doi.org/10.1093/bioinformatics/btn544>
 14. Tafer H, Hofacker IL. RNAplex: a fast tool for RNA–RNA interaction search. *Bioinformatics* 2008;24:2657–63. <https://doi.org/10.1093/bioinformatics/btn193>
 15. Naskulwar K, Peña-Castillo L. sRNARFTarget: a fast machine-learning-based approach for transcriptome-wide sRNA target prediction. *RNA Biol* 2022;19:44–54. <https://doi.org/10.1080/15476286.2021.2012058>
 16. Tjaden B. TargetRNA3: predicting prokaryotic RNA regulatory targets with machine learning. *Genome Biol* 2023;24:276. <https://doi.org/10.1186/s13059-023-03117-2>
 17. Faigenbaum-Romm R, Reich A, Gatt YE *et al.* Hierarchy in Hfq chaperon occupancy of small RNA targets plays a major role in their regulation. *Cell Rep* 2020;30:3127–38. <https://doi.org/10.1016/j.celrep.2020.02.016>
 18. Hopkins JF, Panja S, Woodson SA. Rapid binding and release of Hfq from ternary complexes during RNA annealing. *Nucleic Acids Res* 2011;39:5193–202. <https://doi.org/10.1093/nar/gkr062>
 19. Santiago-Frangos A, Woodson SA. Hfq chaperone brings speed dating to bacterial sRNA. *Wiley Interdiscip Rev RNA* 2018;9:e1475. <https://doi.org/10.1002/wrna.1475>
 20. Link TM, Valentin-Hansen P, Brennan RG. Structure of *Escherichia coli* Hfq bound to polyribadenylate RNA. *Proc Natl Acad Sci USA* 2009;106:19292–7. <https://doi.org/10.1073/pnas.0908744106>
 21. Robinson KE, Orans J, Kovach AR *et al.* Mapping Hfq–RNA interaction surfaces using tryptophan fluorescence quenching. *Nucleic Acids Res* 2014;42:2736–49. <https://doi.org/10.1093/nar/gkt1171>
 22. Panja S, Woodson SA. Hfq proximity and orientation controls RNA annealing. *Nucleic Acids Res* 2012;40:8690–7. <https://doi.org/10.1093/nar/gks618>
 23. Holmqvist E, Wright PR, Li L *et al.* Global RNA recognition patterns of post-transcriptional regulators Hfq and CsrA revealed by UV crosslinking *in vivo*. *EMBO J* 2016;35:991–1011. <https://doi.org/10.15252/embj.201593360>
 24. Melamed S, Peer A, Faigenbaum-Romm R *et al.* Global mapping of small RNA–target interactions in bacteria. *Mol Cell* 2016;63:884–97. <https://doi.org/10.1016/j.molcel.2016.07.026>
 25. Malecka EM, Woodson SA. RNA compaction and iterative scanning for small RNA targets by the Hfq chaperone. *Nat Commun* 2024;15:2069. <https://doi.org/10.1038/s41467-024-46316-6>
 26. Sy BM, Tree JJ. Small RNA regulation of virulence in pathogenic *Escherichia coli*. *Front Cell Infect Microbiol* 2020;10:622202. <https://doi.org/10.3389/fcimb.2020.622202>
 27. Sauer E, Schmidt S, Weichenrieder O. Small RNA binding to the lateral surface of Hfq hexamers and structural rearrangements upon mRNA target recognition. *Proc Natl Acad Sci USA* 2012;109:9396–401. <https://doi.org/10.1073/pnas.1202521109>
 28. Holmqvist E, Li L, Bischler T *et al.* Global maps of ProQ binding *in vivo* reveal target recognition via RNA structure and stability control at mRNA 3' ends. *Mol Cell* 2018;70:971–82. <https://doi.org/10.1016/j.molcel.2018.04.017>
 29. Ishikawa H, Otaka H, Maki K *et al.* The functional Hfq-binding module of bacterial sRNAs consists of a double or single hairpin preceded by a U-rich sequence and followed by a 3' poly(U) tail. *RNA* 2012;18:1062–74. <https://doi.org/10.1261/rna.031575.111>
 30. Otaka H, Ishikawa H, Morita T *et al.* PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq action. *Proc Natl Acad Sci USA* 2011;108:13059–64. <https://doi.org/10.1073/pnas.1107050108>
 31. Stein EM, Kwiatkowska J, Basczok MM *et al.* Determinants of RNA recognition by the FinO domain of the *Escherichia coli* ProQ protein. *Nucleic Acids Res* 2020;48:7502–19.
 32. Panja S, Santiago-Frangos A, Schu DJ *et al.* Acidic residues in the Hfq chaperone increase the selectivity of sRNA binding and annealing. *J Mol Biol* 2015;427:3491–500. <https://doi.org/10.1016/j.jmb.2015.07.010>
 33. Santiago-Frangos A, Jeliakov JR, Gray JJ *et al.* Acidic C-terminal domains autoregulate the RNA chaperone Hfq. *eLife* 2017;6:e27049.
 34. Santiago-Frangos A, Kavita K, Schu DJ *et al.* C-terminal domain of the RNA chaperone Hfq drives sRNA competition and release of target RNA. *Proc Natl Acad Sci USA* 2016;113:E6089–96. <https://doi.org/10.1073/pnas.1613053113>
 35. Dimastrogiovanni D, Fröhlich KS, Bandyra KJ *et al.* Recognition of the small regulatory RNA RydC by the bacterial Hfq protein. *eLife* 2014;3:e05375.
 36. Parekh VJ, Wien F, Grange W *et al.* Crucial role of the C-terminal domain of Hfq protein in genomic instability. *Microorganisms* 2020;8:1598.
 37. Malabirade A, Morgado-Brajones J, Trépout S *et al.* Membrane association of the bacterial riboregulator Hfq and functional perspectives. *Sci Rep* 2017;7:10724. <https://doi.org/10.1038/s41598-017-11157-5>
 38. Turbant F, Waeytens J, Campidelli C *et al.* Unraveling membrane perturbations caused by the bacterial riboregulator Hfq. *Int J Mol Sci* 2022;23:8739.
 39. Panja S, Schu DJ, Woodson SA. Conserved arginines on the rim of Hfq catalyze base pair formation and exchange. *Nucleic Acids Res* 2013;41:7536–46. <https://doi.org/10.1093/nar/gkt521>
 40. Møller T, Franch T, Højrup P *et al.* Hfq: a bacterial Sm-like protein that mediates RNA–RNA interaction. *Mol Cell* 2002;9:23–30. [https://doi.org/10.1016/S1097-2765\(01\)00436-1](https://doi.org/10.1016/S1097-2765(01)00436-1)
 41. Andrade JM, Pobre V, Arraiano CM. Small RNA modules confer different stabilities and interact differently with multiple targets. *PLoS One* 2013;8:e52866. <https://doi.org/10.1371/journal.pone.0052866>
 42. Zhang A, Wassarman KM, Ortega J *et al.* The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. *Mol Cell* 2002;9:11–22. [https://doi.org/10.1016/S1097-2765\(01\)00437-3](https://doi.org/10.1016/S1097-2765(01)00437-3)
 43. Brescia CC, Mikulecky PJ, Feig AL *et al.* Identification of the Hfq-binding site on DsrA RNA: Hfq binds without altering DsrA secondary structure. *RNA* 2003;9:33–43. <https://doi.org/10.1261/rna.2570803>
 44. Poddar A, Azam MS, Kayikcioglu T *et al.* Effects of individual base-pairs on *in vivo* target search and destruction kinetics of bacterial small RNA. *Nat Commun* 2021;12:874. <https://doi.org/10.1038/s41467-021-21144-0>
 45. Rutherford ST, Valastyan JS, Taillefumier T *et al.* Comprehensive analysis reveals how single nucleotides contribute to noncoding RNA function in bacterial quorum sensing. *Proc Natl Acad Sci USA* 2015;112:E6038–47. <https://doi.org/10.1073/pnas.1518958112>

46. Schu DJ, Zhang A, Gottesman S *et al.* Alternative Hfq-sRNA interaction modes dictate alternative mRNA recognition. *EMBO J* 2015;34:2557–73. <https://doi.org/10.15252/embj.201591569>
47. Kwiatkowska J, Wroblewska Z, Johnson KA *et al.* The binding of class II sRNA MgrR to two different sites on matchmaker protein Hfq enables efficient competition for Hfq and annealing to regulated mRNAs. *RNA* 2018;24:1761–84. <https://doi.org/10.1261/rna.067777.118>
48. Roca J, Santiago-Frangos A, Woodson SA. Diversity of bacterial small RNAs drives competitive strategies for a mutual chaperone. *Nat Commun* 2022;13:2449. <https://doi.org/10.1038/s41467-022-30211-z>
49. Malecka EM, Sobanska D, Olejniczak M. Bacterial chaperone protein Hfq facilitates the annealing of sponge RNAs to small regulatory RNAs. *J Mol Biol* 2021;433:167291. <https://doi.org/10.1016/j.jmb.2021.167291>
50. Dendooven T, Sinha D, Roeselová A *et al.* A cooperative PNPase-Hfq-RNA carrier complex facilitates bacterial riboregulation. *Mol Cell* 2021;81:2901–13. <https://doi.org/10.1016/j.molcel.2021.05.032>
51. Peterman N, Lavi-Itzkovitz A, Levine E. Large-scale mapping of sequence–function relations in small regulatory RNAs reveals plasticity and modularity. *Nucleic Acids Res* 2014;42:12177–88. <https://doi.org/10.1093/nar/gku863>
52. Bobrovskyy M, Vanderpool CK. The small RNA SgrS: roles in metabolism and pathogenesis of enteric bacteria. *Front Cell Infect Microbiol* 2014;4:61. <https://doi.org/10.3389/fcimb.2014.00061>
53. Gebhardt MJ, Farland EA, Basu P *et al.* Hfq-licensed RNA–RNA interactome in *Pseudomonas aeruginosa* reveals a keystone sRNA. *Proc Natl Acad Sci USA* 2023;120:e2218407120. <https://doi.org/10.1073/pnas.2218407120>
54. Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215–33. <https://doi.org/10.1016/j.cell.2009.01.002>
55. Brück M, Köbel TS, Dittmar S *et al.* A library-based approach allows systematic and rapid evaluation of seed region length and reveals design rules for synthetic bacterial small RNAs. *iScience* 2024;27:110774. <https://doi.org/10.1016/j.isci.2024.110774>
56. Hao Y, Zhang ZJ, Erickson DW *et al.* Quantifying the sequence–function relation in gene silencing by bacterial small RNAs. *Proc Natl Acad Sci USA* 2011;108:12473–8. <https://doi.org/10.1073/pnas.1100432108>
57. Feng L, Rutherford ST, Papenfort K *et al.* A Qrr noncoding RNA deploys four different regulatory mechanisms to optimize quorum-sensing dynamics. *Cell* 2015;160:228–40. <https://doi.org/10.1016/j.cell.2014.11.051>
58. Lalaouna D, Prévost K, Laliberté G *et al.* Contrasting silencing mechanisms of the same target mRNA by two regulatory RNAs in *Escherichia coli*. *Nucleic Acids Res* 2018;46:2600–12. <https://doi.org/10.1093/nar/gkx1287>
59. Geissmann T, Chevalier C, Cros MJ *et al.* A search for small noncoding RNAs in *Staphylococcus aureus* reveals a conserved sequence motif for regulation. *Nucleic Acids Res* 2009;37:7239–57. <https://doi.org/10.1093/nar/gkp668>
60. Pitman S, Cho KH. The mechanisms of virulence regulation by small noncoding RNAs in low GC Gram-positive pathogens. *Int J Mol Sci* 2015;16:29797–814. <https://doi.org/10.3390/ijms161226194>
61. Ramirez-Peña E, Treviño J, Liu Z *et al.* The group A *Streptococcus* small regulatory RNA FasX enhances streptokinase activity by increasing the stability of the *ska* mRNA transcript. *Mol Microbiol* 2010;78:1332–47. <https://doi.org/10.1111/j.1365-2958.2010.07427.x>
62. Svensson SL, Sharma CM. RNase III-mediated processing of a *trans*-acting bacterial sRNA and its *cis*-encoded antagonist. *eLife* 2021;10:e69064. <https://doi.org/10.7554/eLife.69064>
63. Schnorpfeil A, Kranz M, Kovács M *et al.* Target evaluation of the non-coding csRNAs reveals a link of the two-component regulatory system CiaRH to competence control in *Streptococcus pneumoniae* R6. *Mol Microbiol* 2013;89:334–49. <https://doi.org/10.1111/mmi.12277>
64. Durand S, Braun F, Lioliou E *et al.* A nitric oxide regulated small RNA controls expression of genes involved in redox homeostasis in *Bacillus subtilis*. *PLoS Genet* 2015;11:e1004957. <https://doi.org/10.1371/journal.pgen.1004957>
65. Lalaouna D, Baude J, Wu Z *et al.* RsaC sRNA modulates the oxidative stress response of *Staphylococcus aureus* during manganese starvation. *Nucleic Acids Res* 2019;47:9871–87. <https://doi.org/10.1093/nar/gkz728>
66. Vockenhuber MP, Suess B. *Streptomyces coelicolor* sRNA scr5239 inhibits agarase expression by direct base pairing to the *dagA* coding region. *Microbiology (Reading)* 2012;158:424–35. <https://doi.org/10.1099/mic.0.054205-0>
67. Romilly C, Lays C, Tomasini A *et al.* A non-coding RNA promotes bacterial persistence and decreases virulence by regulating a regulator in *Staphylococcus aureus*. *PLoS Pathog* 2014;10:e1003979. <https://doi.org/10.1371/journal.ppat.1003979>
68. Boisset S, Geissmann T, Huntzinger E *et al.* *Staphylococcus aureus* RNAPIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev* 2007;21:1353–66. <https://doi.org/10.1101/gad.423507>
69. Chabelskaya S, Bordeau V, Felden B. Dual RNA regulatory control of a *Staphylococcus aureus* virulence factor. *Nucleic Acids Res* 2014;42:4847–58. <https://doi.org/10.1093/nar/gku119>
70. Chevalier C, Boisset S, Romilly C *et al.* *Staphylococcus aureus* RNAPIII binds to two distant regions of *coa* mRNA to arrest translation and promote mRNA degradation. *PLoS Pathog* 2010;6:e1000809. <https://doi.org/10.1371/journal.ppat.1000809>
71. Sy BM, Lan R, Tree JJ. Early termination of the Shiga toxin transcript generates a regulatory small RNA. *Proc Natl Acad Sci USA* 2020;117:25055–65. <https://doi.org/10.1073/pnas.2006730117>
72. Bandrya KJ, Wandzik JM, Luisi BF. Substrate recognition and autoinhibition in the central ribonuclease RNase E. *Mol Cell* 2018;72:275–85. <https://doi.org/10.1016/j.molcel.2018.08.039>
73. Bandrya KJ, Fröhlich KS, Vogel J *et al.* Cooperation of regulatory RNA and the RNA degradosome in transcript surveillance. *Nucleic Acids Res* 2024;52:9161–73. <https://doi.org/10.1093/nar/gkae455>
74. Updegrove TB, Kouse AB, Bandrya KJ *et al.* Stem-loops direct precise processing of 3' UTR-derived small RNA MicL. *Nucleic Acids Res* 2019;47:1482–92. <https://doi.org/10.1093/nar/gky1175>
75. Chao Y, Li L, Girodat D *et al.* *In vivo* cleavage map illuminates the central role of RNase E in coding and non-coding RNA pathways. *Mol Cell* 2017;65:39–51. <https://doi.org/10.1016/j.molcel.2016.11.002>
76. Bandrya KJ, Said N, Pfeiffer V *et al.* The seed region of a small RNA drives the controlled destruction of the target mRNA by the endoribonuclease RNase E. *Mol Cell* 2012;47:943–53. <https://doi.org/10.1016/j.molcel.2012.07.015>
77. Callaghan AJ, Marcaida MJ, Stead JA *et al.* Structure of *Escherichia coli* RNase E catalytic domain and implications for RNA turnover. *Nature* 2005;437:1187–91. <https://doi.org/10.1038/nature04084>
78. Chao Y, Papenfort K, Reinhardt R *et al.* An atlas of Hfq-bound transcripts reveals 3' UTRs as a genomic reservoir of regulatory small RNAs. *EMBO J* 2012;31:4005–19. <https://doi.org/10.1038/emboj.2012.229>
79. Callaghan AJ, Aurikko JP, Ilag LL *et al.* Studies of the RNA degradosome-organizing domain of the *Escherichia coli* ribonuclease RNase E. *J Mol Biol* 2004;340:965–79. <https://doi.org/10.1016/j.jmb.2004.05.046>
80. Bruce HA, Du D, Matak-Vinkovic D *et al.* Analysis of the natively unstructured RNA/protein-recognition core in the *Escherichia coli* RNA degradosome and its interactions with

- regulatory RNA/Hfq complexes. *Nucleic Acids Res* 2018;46:387–402. <https://doi.org/10.1093/nar/gkx1083>
81. Chandran V, Poljak L, Vanzo NF *et al.* Recognition and cooperation between the ATP-dependent RNA helicase RhlB and ribonuclease RNase E. *J Mol Biol* 2007;367:113–32. <https://doi.org/10.1016/j.jmb.2006.12.014>
 82. Leroy A, Vanzo NF, Sousa S *et al.* Function in *Escherichia coli* of the non-catalytic part of RNase E: role in the degradation of ribosome-free mRNA. *Mol Microbiol* 2002;45:1231–43. <https://doi.org/10.1046/j.1365-2958.2002.03104.x>
 83. Vanzo NF, Li YS, Py B *et al.* Ribonuclease E organizes the protein interactions in the *Escherichia coli* RNA degradosome. *Genes Dev* 1998;12:2770–81. <https://doi.org/10.1101/gad.12.17.2770>
 84. Worrall JA, Gorna M, Crump NT *et al.* Reconstitution and analysis of the multienzyme *Escherichia coli* RNA degradosome. *J Mol Biol* 2008;382:870–83. <https://doi.org/10.1016/j.jmb.2008.07.059>
 85. Fei J, Singh D, Zhang Q *et al.* RNA biochemistry. Determination of *in vivo* target search kinetics of regulatory noncoding RNA. *Science* 2015;347:1371–4. <https://doi.org/10.1126/science.1258849>
 86. Massé E, Escorcia FE, Gottesman S. Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli*. *Genes Dev* 2003;17:2374–83. <https://doi.org/10.1101/gad.1127103>
 87. Massé E, Gottesman S. A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proc Natl Acad Sci USA* 2002;99:4620–5. <https://doi.org/10.1073/pnas.032066599>
 88. Morita T, Maki K, Aiba H. RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. *Genes Dev* 2005;19:2176–86. <https://doi.org/10.1101/gad.1330405>
 89. Dendooven T, Paris G, Shkumatov AV *et al.* Multi-scale ensemble properties of the *Escherichia coli* RNA degradosome. *Mol Microbiol* 2022;117:102–20. <https://doi.org/10.1111/mmi.14800>
 90. Sinha D, De Lay NR. Target recognition by RNase E RNA-binding domain AR2 drives sRNA decay in the absence of PNPase. *Proc Natl Acad Sci USA* 2022;119:e2208022119. <https://doi.org/10.1073/pnas.2208022119>
 91. Luisi B, Roske J, Paris G *et al.* Structure of the 30S translation initiation complex coupled to paused RNA polymerase and its potential for riboregulation. *Research Square*, 2025.
 92. Condon C, Pellegrini O, Gilet L *et al.* Walking from *E. coli* to *B. subtilis*, one ribonuclease at a time. *C R Biol* 2021;344:357–71. <https://doi.org/10.5802/crbior.70>
 93. Durand S, Condon C. RNases and helicases in Gram-positive bacteria. *Microbiol Spectr* 2018;6:RWR-0003-2017. <https://doi.org/10.1128/microbiolspec.RWR-0003-2017>
 94. Obana N, Nakamura K, Nomura N. Role of RNase Y in *Clostridium perfringens* mRNA decay and processing. *J Bacteriol* 2017;199:e00703-16. <https://doi.org/10.1128/JB.00703-16>
 95. Altuvia Y, Bar A, Reiss N *et al.* *In vivo* cleavage rules and target repertoire of RNase III in *Escherichia coli*. *Nucleic Acids Res* 2018;46:10530–1. <https://doi.org/10.1093/nar/gky816>
 96. Pertzev AV, Nicholson AW. Characterization of RNA sequence determinants and antideterminants of processing reactivity for a minimal substrate of *Escherichia coli* ribonuclease III. *Nucleic Acids Res* 2006;34:3708–21. <https://doi.org/10.1093/nar/gkl459>
 97. Franch T, Thisted T, Gerdes K. Ribonuclease III processing of coaxially stacked RNA helices. *J Biol Chem* 1999;274:26572–8. <https://doi.org/10.1074/jbc.274.37.26572>
 98. Huntzinger E, Boisset S, Saveanu C *et al.* *Staphylococcus aureus* RNAlII and the endoribonuclease III coordinately regulate *spa* gene expression. *EMBO J* 2005;24:824–35. <https://doi.org/10.1038/sj.emboj.7600572>
 99. Lioliou E, Sharma CM, Caldelari I *et al.* Global regulatory functions of the *Staphylococcus aureus* endoribonuclease III in gene expression. *PLoS Genet* 2012;8:e1002782. <https://doi.org/10.1371/journal.pgen.1002782>
 100. McKellar SW, Ivanova I, Arede P *et al.* RNase III CLASH in MRSA uncovers sRNA regulatory networks coupling metabolism to toxin expression. *Nat Commun* 2022;13:3560. <https://doi.org/10.1038/s41467-022-31173-y>
 101. Mediati DG, Wong JL, Gao W *et al.* RNase III-CLASH of multi-drug resistant *Staphylococcus aureus* reveals a regulatory mRNA 3'UTR required for intermediate vancomycin resistance. *Nat Commun* 2022;13:3558. <https://doi.org/10.1038/s41467-022-31177-8>
 102. Marincola G, Schäfer T, Behler J *et al.* RNase Y of *Staphylococcus aureus* and its role in the activation of virulence genes. *Mol Microbiol* 2012;85:817–32. <https://doi.org/10.1111/j.1365-2958.2012.08144.x>
 103. Desgranges E, Barrientos L, Herrgott L *et al.* The 3'UTR-derived sRNA RsaG coordinates redox homeostasis and metabolism adaptation in response to glucose-6-phosphate uptake in *Staphylococcus aureus*. *Mol Microbiol* 2022;117:193–214. <https://doi.org/10.1111/mmi.14845>
 104. Smirnov A, Förstner KU, Holmqvist E *et al.* Grad-seq guides the discovery of ProQ as a major small RNA-binding protein. *Proc Natl Acad Sci USA* 2016;113:11591–6. <https://doi.org/10.1073/pnas.1609981113>
 105. Stein EM, Wang S, Dailey KG *et al.* Biochemical and genetic dissection of the RNA-binding surface of the FinO domain of *Escherichia coli* ProQ. *RNA* 2023;29:1772–91. <https://doi.org/10.1261/rna.079697.123>
 106. Olejniczak M, Storz G. ProQ/FinO-domain proteins: another ubiquitous family of RNA matchmakers? *Mol Microbiol* 2017;104:905–15. <https://doi.org/10.1111/mmi.13679>
 107. Kim HJ, Black M, Edwards RA *et al.* Structural basis for recognition of transcriptional terminator structures by ProQ/FinO domain RNA chaperones. *Nat Commun* 2022;13:7076. <https://doi.org/10.1038/s41467-022-34875-5>
 108. Mamońska MD, Basczok MM, Stein EM *et al.* Different RNA recognition by ProQ and FinO depends on the sequence surrounding intrinsic terminator hairpins. *RNA* 2025;31:692–708.
 109. Melamed S, Adams PP, Zhang A *et al.* RNA–RNA interactomes of ProQ and hfq reveal overlapping and competing roles. *Mol Cell* 2020;77:411–25. <https://doi.org/10.1016/j.molcel.2019.10.022>
 110. Bergman S, Birk C, Holmqvist E. ProQ prevents mRNA degradation through inhibition of poly(A) polymerase. *Nucleic Acids Res* 2025;53:gkaf103. <https://doi.org/10.1093/nar/gkaf103>
 111. Lamm-Schmidt V, Fuchs M, Sulzer J *et al.* Grad-seq identifies KhpB as a global RNA-binding protein in *Clostridioides difficile* that regulates toxin production. *Microlife* 2021;2:uqab004. <https://doi.org/10.1093/femsml/uqab004>
 112. Michaux C, Gerovac M, Hansen EE *et al.* Grad-seq analysis of *Enterococcus faecalis* and *Enterococcus faecium* provides a global view of RNA and protein complexes in these two opportunistic pathogens. *Microlife* 2023;4:uqac027. <https://doi.org/10.1093/femsml/uqac027>
 113. Riediger M, Spät P, Bilger R *et al.* Analysis of a photosynthetic cyanobacterium rich in internal membrane systems via gradient profiling by sequencing (Grad-seq). *Plant Cell* 2021;33:248–69. <https://doi.org/10.1093/plcell/koaa017>
 114. Zhu Y, Ponath F, Cosi V *et al.* A global survey of small RNA interactors identifies KhpA and KhpB as major RNA-binding proteins in *Fusobacterium nucleatum*. *Nucleic Acids Res* 2024;52:3950–70. <https://doi.org/10.1093/nar/gkac010>
 115. Stenum TS, Kumar AD, Sandbaumhüter FA *et al.* RNA interactome capture in *Escherichia coli* globally identifies RNA-binding proteins. *Nucleic Acids Res* 2023;51:4572–87. <https://doi.org/10.1093/nar/gkad216>

116. Rüttiger A-S, Ryan D, Spiga L *et al.* The global RNA-binding protein RbpB is a regulator of polysaccharide utilization in *Bacteroides thetaiotaomicron*. *Nat Commun* 2025;16:208. <https://doi.org/10.1038/s41467-024-55383-8>
117. Adams AND, Azam MS, Costliow ZA *et al.* A novel family of RNA-binding proteins regulate polysaccharide metabolism in *Bacteroides thetaiotaomicron*. *J Bacteriol* 2021;203:e0021721. <https://doi.org/10.1128/jb.00217-00221>
118. Durica-Mitic S, Göpel Y, Amman F *et al.* Adaptor protein RapZ activates endoribonuclease RNase E by protein–protein interaction to cleave a small regulatory RNA. *RNA* 2020;26:1198–215. <https://doi.org/10.1261/rna.074047.119>
119. Chen J, To L, de Mets F *et al.* A fluorescence-based genetic screen reveals diverse mechanisms silencing small RNA signaling in *E. coli*. *Proc Natl Acad Sci USA* 2021;118:e2106964118.
120. Hör J, Garriss G, Di Giorgio S *et al.* Grad-seq in a gram-positive bacterium reveals exonucleolytic sRNA activation in competence control. *EMBO J* 2020;39:e103852. <https://doi.org/10.15252/embj.2019103852>
121. Müller P, Gimpel M, Wildenhain T *et al.* A new role for CsrA: promotion of complex formation between an sRNA and its mRNA target in *Bacillus subtilis*. *RNA Biology* 2019;16:972–87. <https://doi.org/10.1080/15476286.2019.1605811>
122. Rojano-Nisimura AM, Simmons TR, Leistra AN *et al.* CsrA selectively modulates sRNA–mRNA regulator outcomes. *Front Mol Biosci* 2023;10:1249528. <https://doi.org/10.3389/fmolb.2023.1249528>
123. Burke TP, Portnoy DA. SpoVG is a conserved RNA-binding protein that regulates *Listeria monocytogenes* lysozyme resistance, virulence, and swarming motility. *mBio* 2016;7:e00240. <https://doi.org/10.1128/mBio.00240-16>
124. Chu LC, Arede P, Li W *et al.* The RNA-bound proteome of MRSA reveals post-transcriptional roles for helix-turn-helix DNA-binding and Rossmann-fold proteins. *Nat Commun* 2022;13:2883. <https://doi.org/10.1038/s41467-022-30553-8>
125. Abramson J, Adler J, Dunger J *et al.* Accurate structure prediction of biomolecular interactions with AlphaFold 3. *Nature* 2024;630:493–500. <https://doi.org/10.1038/s41586-024-07487-w>
126. Baek M, McHugh R, Anishchenko I *et al.* Accurate prediction of protein–nucleic acid complexes using RoseTTAFoldNA. *Nat Methods* 2024;21:117–21. <https://doi.org/10.1038/s41592-023-02086-5>
127. Cohen S, Maximof E, Rokach S *et al.* sInterBase: a comprehensive database of *Escherichia coli* sRNA–mRNA interactions. *Bioinformatics* 2023;39:btad172. <https://doi.org/10.1093/bioinformatics/btad172>
128. Sauter C, Basquin Jrm, Suck D. Sm-like proteins in Eubacteria: the crystal structure of the hfq protein from *Escherichia coli*. *Nucleic Acids Res* 2003;31:4091–8. <https://doi.org/10.1093/nar/gkg480>