Published in final edited form as:

Microbiol Spectr. 2018 July ; 6(4): . doi:10.1128/microbiolspec.RWR-0026-2018.

Proteins that chaperone RNA regulation

Sarah A. Woodson^{1,*}, Subrata Panja¹, and Andrew Santiago-Frangos²

¹T. C. Jenkins Department of Biophysics, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218 USA

²Program in Cell, Molecular and Developmental Biology and Biophysics, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218 USA

Abstract

RNA-binding proteins chaperone the biological functions of non-coding RNA by reducing RNA misfolding, improving matchmaking between regulatory RNA and targets, and exerting quality control over RNP biogenesis. Recent studies of *E. coli* CspA, HIV NCp and *E. coli* Hfq, are beginning to show how RNA-binding proteins remodel RNA structures. These different protein families use common strategies for disrupting or annealing RNA double helices, which can be used to understand the mechanisms by which proteins chaperone RNA-dependent regulation in bacteria.

INTRODUCTION

Non-coding RNA sequences fold into useful structures that regulate gene expression as ribozymes, metabolite binding sensors, or antisense RNAs (1–5). These regulatory RNAs are chaperoned by diverse families of RNA binding proteins, and the loss of RNA chaperone proteins can lead to impaired growth, reduced tolerance to stress, and reduced virulence (6–11). RNA chaperones also facilitate conformational rearrangements during ribosome biogenesis (12) and eukaryotic pre-mRNA splicing (13).

These housekeeping and regulatory functions of RNA chaperones are particularly important at cold temperatures that hyperstabilize RNA structures. For example, the up-regulation of cold shock domain RNA binding proteins during low temperature growth destabilizes RNA structures that would otherwise impair transcription elongation and translation initiation at low temperatures (14). Moreover, over-expression of cold shock proteins and other RNA chaperones buffered deleterious mutations in *E. coli* (15), suggesting that such proteins broadly mitigate the effects of RNA misfolding.

RNA chaperones act by transiently binding and releasing RNA substrates, disrupting the RNA secondary and tertiary structure (unwinding and unfolding) or accelerating base pairing with a second RNA strand (annealing) (16, 17). RNA helices can be actively unwound by DEAD-box proteins, which couple unfolding of the RNA structure to ATP hydrolysis (18, 19). Bacteria typically encode a handful (0–12) of DEAD-box proteins that

*Correspondence to swoodson@jhu.edu; tel. 410-516-2015; FAX 410-516-7348.

mainly act in ribosome biogenesis and RNA turnover (12, 20). This chapter, however, will focus on RNA binding proteins that “passively” remodel RNA structures without hydrolyzing ATP. In bacteria, this type of passive RNA chaperone includes cold shock proteins (CSPs) (14), the Sm family protein Hfq (21), the FinO/ProQ family of RNA binding proteins (22), and ribosomal proteins S1 (23–25) and S12 (26). H-NS and StpA, which interact with the bacterial nucleoid, also possess RNA chaperone activity (17). Eukaryotic proteins with ATP-independent RNA chaperone properties include RNA recognition motif (RRM) proteins such as hnRNP A1 (27), viral proteins such as the well-studied retroviral nucleocapsid protein (NCp7) (28, 29), and La and Ro proteins (30, 31).

The diverse biological roles of the above examples highlight the broad importance of proteins that escort, facilitate or accelerate structural changes in non-coding RNA. This chapter will discuss how well-studied examples, such as *E. coli* CspA, HIV NCp7, ribosomal protein S1, and *E. coli* Hfq, are beginning to provide a physical picture of how proteins remodel RNA structures during RNA regulation.

RNA folding and the need for RNA chaperones

RNA double helices are stable and long-lived, yet able to interchange through the sequential migration of base pairs during strand transfer or branch migration reactions. These features of stability and interchangeability ideally suit RNA for creating metastable structures that can switch gene expression on or off. Owing to the stability of the RNA double helix, RNA regulatory elements may be as small as a single stem-loop or anti-sense helix, or involve more elaborate tertiary structures (32–34). The potential simplicity of RNA-based regulation offers microbes an expedient means of evolving new regulatory circuits (35). (See also chapter by R. Raghavan). It also presents synthetic biologists with an attractive platform for genetic engineering (36, 37). (Also see chapter by M. Hammond).

Although stable RNA base pairs create good switches, the small number of natural nucleobases limits the specificity of RNA regulatory interactions and increases the chance of RNA misfolding (16). For example, the free energy of forming a 10 bp stem-loop typically ranges from -10 to -20 kcal/mol at 37 C, yet a mismatched helix of the same length may be only 1–2 kcal/mol less stable than the fully matched helix. As an RNA grows longer, the odds that it can form more than one stable secondary structure increases substantially. Tertiary interactions between double helices make folding more specific by favoring folding intermediates in which the double helices are correctly aligned (38, 39). Nevertheless, interactions between nucleotides far apart in the RNA sequence are entropically less favorable than those between nearby nucleotides. As a result, stable local interactions outcompete long-range interactions, frustrating the search for the native conformation and allowing incorrect base pairing patterns to persist (40, 41).

The potential for misfolding in RNA is exacerbated by the varied time scales for forming secondary and tertiary interactions. In a typical folding pathway (Figure 1), local stem-loops form in ~10 μ s while individual domains of tertiary structure or intermolecular interactions typically form in 1–100 ms, depending on the folding conditions (41–46). This is far less time than the 0.5 to 4 s needed for a bacterial RNA polymerase to synthesize a 50–100 nt RNA domain, allowing the 5' end of a long RNA to form intermediate structures before the

3' end has been transcribed (47). Because non-native structures must partially unfold to reach the native structure, refolding can take 1–100 s or longer (41). Thus, RNA chaperones are needed to disrupt incorrect secondary or tertiary structure and increase the likelihood that the RNA will achieve its native conformation (16, 48).

Similar challenges complicate the formation of anti-sense interactions between transacting regulatory RNAs and their targets. During the association of unstructured oligonucleotides, slow nucleation of the double helix ($\sim 10^5\text{--}10^6\text{ M}^{-1}\text{s}^{-1}$) is followed by more rapid zippering ($\sim 1\text{ }\mu\text{s/bp}$) of the remaining base pairs (49, 50). The secondary structure of natural anti-sense RNAs alters their association kinetics, in some cases necessitating the assistance of a chaperone protein (51). For example, anti-sense RNAs from colE1 and R1 plasmids initially base pair at exposed hairpin loops, forming unstable “kissing complexes” (52, 53) (Figure 2, middle). Stable anti-sense binding depends on the rearrangement of adjacent nucleotides to extend the intermolecular base pairing (52, 54, 55), which for colE1 is facilitated by the Rop/Rom protein (56). In an analogous fashion, trans-acting small RNAs depend on chaperone proteins like Hfq to initiate base pairing, facilitate strand exchange, and destabilize self-structure that can mask complementary regions in the sRNA and the target RNA (9, 21).

Thermodynamic limits to passive unfolding of RNA

To unfold RNA, chaperones must bind single-stranded RNA more strongly than they bind double-stranded RNA (16). This concept was supported by early experiments on hnRNP A1 (57) and StpA (58, 59), which preferentially interact with RNAs that have some unstructured regions. Simple thermodynamic arguments suggest that the more strongly a chaperone binds the unfolded RNA, the more potent its unfolding activity. If binding is too strong, however, the RNA will not be released, limiting the range of structures on which the chaperone can act (60). In practice, RNA chaperones unfold both native and misfolded substrates, sometimes with little discrimination (61). This suggests that thermodynamic models are inadequate to describe chaperone mechanisms, and that most chaperones take advantage of differences in the dynamics of folded and unfolded RNA to increase the flux through RNA folding pathways over the short term.

Transient interactions drive iterative chaperone cycles

To facilitate the formation of regulatory RNA structures, molecular chaperones must interact transiently with their substrates, releasing the folded RNA to perform its normal function in the cell. In the classic iterative annealing model for protein or RNA chaperones (62, 63), the chaperone binds and partially unfolds the substrate before releasing it to fold again (Figure 1). Repeated rounds of chaperone-induced unfolding and refolding provide the substrate many chances to fold correctly, ultimately resulting in a higher yield of native protein or RNA. On each round of folding, the RNA randomly partitions between folding pathways that lead to the native structure or non-native structures (41). If the pool of native RNA is depleted through a subsequent biochemical reaction or the addition of other proteins, this cycling will increase the throughput of RNA folding and assembly, even if the chaperone does not discriminate between native and non-native structures.

The iterative annealing mechanism was validated by experiments on GroEL protein chaperone (62) and on the CYT-19 DEAD-box RNA chaperone (19), which both use ATP hydrolysis to carry out multiple cycles of substrate unwinding. A recent theoretical analysis of the reaction kinetics of GroEL and CYT-19 ATPases led to the proposal that active protein and RNA chaperones operate far from equilibrium, increasing the amount of native substrate in the short term even if some native protein or RNA is also unfolded by the chaperone (64). In this framework, repeated rounds of unfolding accelerate the rate at which substrates reach the native structure, although the efficiency of the chaperone depends on its ability to discriminate between native and misfolded protein or RNA.

Passive chaperones also cycle on and off their substrates to transiently unfold (and refold) the RNA (60). Analogous cycles of binding and release facilitate annealing between trans-acting regulatory RNAs and their targets. The main difference is that the chaperone must simultaneously bind the regulatory or guide RNA and the target RNA, bringing them together in a ternary complex that allows the two RNAs to base pair (65). After the RNAs base pair, the chaperone releases the RNA duplex or is recycled when the RNA complex is turned over (66).

Whether the RNA folds upon itself or with another RNA, rapid binding and release of the chaperone is essential for allowing the RNA to search out its most stable structure (17). This has been borne out by experiments on variants of HIV NCp7 (67) and on *E. coli* StpA (59, 68). Similarly, Hfq protein that promotes annealing between sRNAs and their mRNA targets in vitro (59, 69–71), cycles off the sRNA-mRNA duplex (70, 72). Efficient matchmaking requires that the sRNA Hfq complex can rapidly search among candidate targets until a complementary site is found (73).

RNA unfolding by capturing single-strands

Recent biophysical studies illuminate how passive RNA chaperones such as CspA and HIV NCp7 weaken RNA secondary structures. CspA is the major cold shock protein in *E. coli*, and is strongly induced upon cold shock at 10 C (74). Among other functions, CspA and its relative CspE are proposed to destabilize mRNA secondary structures that may terminate transcription or interfere with mRNA translation at low temperature (75–77). The cold shock domains of CspA and CspE are related to eukaryotic Y-box proteins, and forms a beta barrel structure that binds RNA along one surface (78, 79). Three aromatic side chains protruding from the surface of the cold shock domain were found to be crucial for RNA chaperone activity (80, 81).

Rennella et al used time-resolved NMR spectroscopy to observe how multiple CspA proteins facilitate the dimerization of two complementary RNA hairpins (82). The slowest unfolding rate equaled the rate of RNA dimerization, suggesting that each hairpin must unzip before the two strands can anneal. CspA increased the hairpin unfolding rates, surprisingly by destabilizing base pairs closest to the loop or at a helix junction. As in previous studies, exposed aromatic residues contributed to destabilization of the RNA. The authors concluded that base pairs in the RNA hairpins are progressively disrupted by stacking interactions with the aromatic side chains, accompanied by hydrogen bonding with surrounding basic residues. This combination of aromatic and basic side chains, found in

other RNA binding proteins (83), seems to offer a flexible yet energetically favorable interaction surface for single-stranded RNA. Importantly, side chains on the exposed surface of CspA are dynamic (84), but are stabilized upon binding to the RNA hairpin (82). Thus, increased motion in the RNA base pairs is accompanied by reduced motion in the protein. As discussed below, many chaperones contain disordered regions, leading to the idea that entropy is transferred from the chaperone to the substrate (85).

The RNA chaperone activities of nucleocapsid proteins are mainly understood from extensive studies of HIV nucleocapsid (NCp7), which can both disrupt RNA structures and promote interactions between RNA strands (29). These chaperone properties contribute to many stages of the retroviral life cycle, including dimerization and packaging of the genomic RNA, tRNA priming, and trans activation (86, 87). Processed from the longer gag polyprotein, NCp7 contains two small Zn finger domains that bind and destabilize RNA structures (88–91). Each Zn finger contains a hydrophobic pocket capable of binding an unpaired guanosine nucleobase. A disordered, positively charged N-terminal domain is associated with NCp7's aggregating properties (92).

Like CspA, many copies of NCp7 bind a single RNA, destabilizing its structure (93, 94). Recent force-stretching experiments provided additional insight into how HIV NCp7 destabilizes the trans activation TAR RNA hairpin (95). During reverse transcription of the HIV genome, the TAR RNA hairpin must be destabilized and annealed to a cDNA hairpin (96). In the force-stretching experiments, NCp7 increased the probability of TAR unfolding about 10,000 times. This acceleration was accomplished by moving the position of the transition state for unfolding, so that fewer base pairs must open at one time before the entire TAR hairpin unzips (Figure 2, top). In agreement with earlier studies (97, 98), the authors concluded that TAR mainly acts by preferentially binding guanosines near local defects in the RNA such as G U wobble pairs, bulges, or loops, disrupting their base pairing interactions. This explains how NCp7 progressively destabilizes large RNA structures at the moderate loading ratios of 1 protein per 7–15 nt, at which its chaperone activity is most prominent (99). Moreover, NCp7 interacts weakly with most of its binding sites at moderate protein:RNA ratios (67). This ensures that NCp7 dissociates frequently enough that the RNA has a chance to form more stable interactions.

Multi-domain interactions between chaperones and RNA

Many RNA chaperones form multiple weak interactions with the RNA that are individually exchangeable, yet collectively have a large effect on the RNA's structure. This distributive binding strategy offers several advantages: First, contacts between the chaperone and the RNA can rearrange without the chaperone losing its grip on the RNA substrate. Second, multiple RNA binding surfaces can be used to flexibly recognize different RNA structures or to select a particular class of substrates. Third, chaperones with annealing activity require multiple surfaces to bring together two RNA strands. Many RNA-binding proteins use multiple domains or multiple copies of the same protein to chaperone RNA refolding or improve substrate selection, although the three-dimensional structure of each protein family is different.

Small chaperone proteins, such as CspA, NCp7, and StpA, restructure the RNA by having many copies of the protein bind at the same time, explaining why these proteins are most effective at moderately high protein:RNA ratios (59, 75, 91). Each polypeptide also possesses multiple RNA binding domains that must work together to unwind or anneal the RNA (68). For example, the chaperone activity of HIV NCp7 requires the combined action of each Zn finger and the basic N-terminal region (88–90, 97, 100). Ribosomal protein S1 also has a well-characterized propensity to unwind RNA helices (23–25). Of the six Oligonucleotide Binding (OB) domains in *E. coli* protein S1, at least three are needed for the 30S ribosome to accommodate structured mRNAs (101), suggesting that multiple OB domains must work together to unfold the mRNA. Consistent with this idea, optical tweezer experiments showed that each protein S1 unwinds 10 base pairs in a multi-step fashion that requires less energy than would be needed to unfold the entire helix at once (102).

Eukaryotic proteins with chaperone activity also typically contain multiple RNA binding domains joined by flexible linkers. This “pearl necklace” organization enables more specific recognition of RNA sequence motifs (103) and accommodates conformational changes in the RNA. For example, yeast Prp24 facilitates annealing between U6 and U4 snRNA, an essential step for recycling spliceosomal complexes (104). The four RNA recognition motif (RRM) domains of Prp24 wrap around spliceosomal U6 snRNA, making extensive contacts with double and single-stranded regions of the RNA (105). Reorientation of these domains is presumably needed to allow remodeling of U6 base pairs (106). Yeast La protein binds the 3′ ends of pol III transcripts (107, 108) and chaperones misfolded pre-tRNAs (109) and other small nuclear RNAs (110). When La binds the RNA 3′ end, a flexible linker between the La motif and an RRM domain becomes ordered, orienting the two domains and greatly increasing the fidelity of substrate selection (111).

Ring-shaped chaperones for small RNAs

Ring-shaped proteins that bind RNA offer a different solution for the requirement that RNA chaperones contact more than one RNA segment at a time (112). Unlike multi-domain RNA binding proteins, ring-shaped proteins form rigid three-dimensional structures that bind RNA segments on different surfaces. As a result, the RNA is wrapped around or even through the ring, distorting the RNA’s structure or aligning two different strands of RNA. The *Bacillus subtilis* *trp* RNA-binding attenuator protein (TRAP) is a particularly dramatic example, wrapping up to 33 nt of RNA around a large 11 subunit wheel (113). A smaller example is the Ro antigen, which uses a ring of HEAT repeats to bind and chaperone eukaryotic Y box small RNAs (31). (See also chapter by S. Wolin).

Hfq (Host Factor phage Qbeta) is the best characterized chaperone for small RNAs (sRNA) in bacteria (114). A member of the Sm/Lsm protein family (115), Hfq assembles into a six-subunit ring that binds sRNAs, protects them from turnover, and aids their annealing with target mRNAs (21) (Figure 2, bottom). In addition to supporting post-transcriptional regulation by sRNAs, Hfq directly regulates the translation of its own and other mRNAs (116–120) and among other regulatory roles (121).

Hfq uses a mixture of RNA binding surfaces and disordered domains to recognize a broad family of RNA substrates (122), while ignoring other RNA in the cell. The “proximal” face

of Hfq is conserved among Sm/Lsm proteins and interacts with single-stranded U's at the 3' ends of sRNAs (123–126). These interactions were shown to be important for sRNA stability in the cell (127–130). The opposite “distal” face of *E. coli* Hfq binds a single-stranded AAN triplet motif present in mRNA targets of Hfq and sRNA regulation (131–133), as well as a minor class of sRNA (130). AAN recognition was found to be important for sRNA up-regulation of *rpoS* translation in *E. coli* (132, 134, 135), and down-regulation of many targets by Spot 42 (136). Thus, one Hfq ring can bring together an sRNA and mRNA, promoting their association (69, 115, 131, 137). In addition to these sequence-specific binding surfaces, the lateral edge or rim of the Hfq hexamer contains patches of basic residues (usually arginines) that interact with complementary regions of each RNA (138, 139). The importance of these multi-lateral interactions for Hfq-dependent sRNA regulation have been supported by additional mutational analyses and in-cell crosslinking and co-immunoprecipitation (*e.g.*, (118, 131, 140)(130, 141–143)).

A variety of biophysical experiments showed that Hfq accelerates base pairing between complementary RNA strands (70, 71, 144, 145), and this annealing activity requires the basic patch on the rim of the hexamer (139, 146). Like other RNA chaperone proteins, Hfq binds substrate RNA within a few seconds, but then cycles off the sRNA-mRNA duplex once base pairing is complete (70, 72). Single-molecule FRET studies using short RNA substrates showed that stable annealing is preceded by transient RNA binding to Hfq, and that Hfq dissociates from the RNA duplex soon after base pairing occurs (147). Although Hfq is reported to bind ATP (148, 149), Hfq lacks a RecA-like domain, and ATP hydrolysis is not needed for its annealing activity (70, 144, 145).

It is not known precisely how the arginine side chains on the rim of the Hfq hexamer facilitate RNA base pairing, but several features of the annealing reaction have been established. First, interactions between the rim and UA motifs in the body of the sRNA (128) predispose the seed region for base pairing with a complementary strand (138), perhaps by increasing the flexibility of the bound sRNA (150). Second, the arginines directly stabilize a helix initiation complex, either by overcoming electrostatic repulsion of the two RNA strands or by hydrogen bonding (151). Third, by simultaneously interacting with the RNA via its proximal, distal and lateral rim surfaces, Hfq can refold the RNA into a structure that is more amenable to pairing with its complement. For example, the distal face of Hfq is recruited to an (AAN)₄ motif in an upstream domain of the *rpoS* mRNA 5' UTR (132, 152), while the rim interacts with a U-rich loop downstream of the sRNA target site (142). SHAPE modification and SAXS experiments showed that this multi-site recognition folds the *rpoS* 5' UTR into distorted structure that is poised to base pair with sRNAs that regulate *rpoS* expression (153). Other mRNA targets of sRNA and Hfq regulation similarly contain multiple Hfq recognition sites (118, 154–156), suggesting that distortion of the mRNA conformation is a common strategy for enhancing the efficiency of sRNA regulation.

RNA chaperones drive sRNA competition and target selection

Small non-coding RNAs must locate their proper targets among a myriad of spurious candidates in the cell. The target search kinetics depends on co-localization, a search among potential targets, and accurate recognition of the cognate target sequence. CRISPR guide

annealing) while recycling Hfq to bind another RNA. Second, it creates a hierarchy of sRNA regulation because the CTDs do not displace AAN RNA from the distal face of Hfq, enabling RNAs containing AAN motifs to outcompete other sRNAs for access to Hfq in the cell (177). Because the length of the CTD and number of acidic residues vary among bacterial species, these features may be used to fine-tune the permissiveness and turnover rate of Hfq in different organisms (181).

CONCLUSION

Proteins that bind and chaperone non-coding RNA sequences are ubiquitous in biology and are necessary for many forms of gene control, stress response, viral replication, and normal homeostasis of RNA metabolism. This chapter has aimed to outline some of the common physical mechanisms by which such proteins help refold regulatory RNA structures or facilitate anti-sense interactions between regulatory RNAs and their targets. Further understanding of RNA chaperone mechanisms will require not only additional high resolution structures, but advanced physical methods for tracking the dynamical motions of the RNA-protein complexes in real time.

Acknowledgments

The authors acknowledge the support of their research from the National Institutes of Health (R01GM120425) and thank G. Storz and S. Gottesman for helpful discussion and comments on the manuscript.

References

1. Serganov A, Patel DJ. Ribozymes, riboswitches and beyond: regulation of gene expression without proteins. *Nat Rev Genet.* 2007; 8:776–790. [PubMed: 17846637]
2. Grundy FJ, Henkin TM. From ribosome to riboswitch: control of gene expression in bacteria by RNA structural rearrangements. *Crit Rev Biochem Mol Biol.* 2006; 41:329–338. [PubMed: 17092822]
3. Winkler WC, Breaker RR. Regulation of bacterial gene expression by riboswitches. *Annu Rev Microbiol.* 2005; 59:487–517. [PubMed: 16153177]
4. Beisel CL, Storz G. Base pairing small RNAs and their roles in global regulatory networks. *FEMS Microbiol Rev.* 2010; 34:866–882. [PubMed: 20662934]
5. Wagner EG, Romby P. Small RNAs in bacteria and archaea: who they are, what they do, and how they do it. *Adv Genet.* 2015; 90:133–208. [PubMed: 26296935]
6. Chao Y, Vogel J. The role of Hfq in bacterial pathogens. *Curr Opin Microbiol.* 2010; 13:24–33. [PubMed: 20080057]
7. Gottesman S, McCullen CA, Guillier M, Vanderpool CK, Majdalani N, Benhammou J, Thompson KM, FitzGerald PC, Sowa NA, FitzGerald DJ. Small RNA regulators and the bacterial response to stress. *Cold Spring Harb Symp Quant Biol.* 2006; 71:1–11. [PubMed: 17381274]
8. Romeo T, Vakulskas CA, Babitzke P. Post-transcriptional regulation on a global scale: form and function of Csr/Rsm systems. *Environ Microbiol.* 2013; 15:313–324. [PubMed: 22672726]
9. Sobrero P, Valverde C. The bacterial protein Hfq: much more than a mere RNA-binding factor. *Crit Rev Microbiol.* 2012; 38:276–299. [PubMed: 22435753]
10. Lucchetti-Miganeh C, Burrowes E, Baysse C, Ermel G. The post-transcriptional regulator CsrA plays a central role in the adaptation of bacterial pathogens to different stages of infection in animal hosts. *Microbiology.* 2008; 154:16–29. [PubMed: 18174122]
11. Romby P, Vandenesh F, Wagner EG. The role of RNAs in the regulation of virulence-gene expression. *Curr Opin Microbiol.* 2006; 9:229–236. [PubMed: 16529986]

12. Redder P, Hausmann S, Khemici V, Yasrebi H, Linder P. Bacterial versatility requires DEAD-box RNA helicases. *FEMS Microbiol Rev.* 2015; 39:392–412. [PubMed: 25907111]
13. Staley JP, Woolford JL Jr. Assembly of ribosomes and spliceosomes: complex ribonucleoprotein machines. *Curr Opin Cell Biol.* 2009; 21:109–118. [PubMed: 19167202]
14. Phadtare S, Severinov K. RNA remodeling and gene regulation by cold shock proteins. *RNA Biol.* 2010; 7:788–795. [PubMed: 21045540]
15. Rudan M, Schneider D, Warnecke T, Krisko A. RNA chaperones buffer deleterious mutations in *E. coli*. *Elife.* 2015; 4
16. Herschlag D. RNA chaperones and the RNA folding problem. *J Biol Chem.* 1995; 270:20871–20874. [PubMed: 7545662]
17. Doetsch M, Schroeder R, Furtig B. Transient RNA-protein interactions in RNA folding. *Febs J.* 2011; 278:1634–1642. [PubMed: 21410645]
18. Jankowsky E, Gross CH, Shuman S, Pyle AM. Active disruption of an RNA-protein interaction by a DEXH/D RNA helicase. *Science.* 2001; 291:121–125. [PubMed: 11141562]
19. Bhaskaran H, Russell R. Kinetic redistribution of native and misfolded RNAs by a DEAD-box chaperone. *Nature.* 2007; 449:1014–1018. [PubMed: 17960235]
20. Iost I, Bizebard T, Dreyfus M. Functions of DEAD-box proteins in bacteria: current knowledge and pending questions. *Biochim Biophys Acta.* 2013; 1829:866–877. [PubMed: 23415794]
21. Updegrove TB, Zhang A, Storz G. Hfq: the flexible RNA matchmaker. *Curr Opin Microbiol.* 2016; 30:133–138. [PubMed: 26907610]
22. Olejniczak M, Storz G. ProQ/FinO-domain proteins: another ubiquitous family of RNA matchmakers? *Mol Microbiol.* 2017; 104:905–915. [PubMed: 28370625]
23. Bear DG, Ng R, Van Derveer D, Johnson NP, Thomas G, Schleich T, Noller HF. Alteration of polynucleotide secondary structure by ribosomal protein S1. *Proc Natl Acad Sci U S A.* 1976; 73:1824–1828. [PubMed: 778845]
24. Hajnsdorf E, Boni IV. Multiple activities of RNA-binding proteins S1 and Hfq. *Biochimie.* 2012; 94:1544–1553. [PubMed: 22370051]
25. Kolb A, Hermoso JM, Thomas JO, Szer W. Nucleic acid helix-unwinding properties of ribosomal protein S1 and the role of S1 in mRNA binding to ribosomes. *Proc Natl Acad Sci U S A.* 1977; 74:2379–2383. [PubMed: 329281]
26. Coetzee T, Herschlag D, Belfort M. *Escherichia coli* proteins, including ribosomal protein S12, facilitate in vitro splicing of phage T4 introns by acting as RNA chaperones. *Genes Dev.* 1994; 8:1575–1588. [PubMed: 7958841]
27. Herschlag D, Khosla M, Tsuchihashi Z, Karpel RL. An RNA chaperone activity of non-specific RNA binding proteins in hammerhead ribozyme catalysis. *Embo J.* 1994; 13:2913–2924. [PubMed: 8026476]
28. Levin JG, Guo J, Rouzina I, Musier-Forsyth K. Nucleic acid chaperone activity of HIV-1 nucleocapsid protein: critical role in reverse transcription and molecular mechanism. *Prog Nucleic Acid Res Mol Biol.* 2005; 80:217–286. [PubMed: 16164976]
29. Rein A, Henderson LE, Levin JG. Nucleic-acid-chaperone activity of retroviral nucleocapsid proteins: significance for viral replication. *Trends Biochem Sci.* 1998; 23:297–301. [PubMed: 9757830]
30. Bayfield MA, Yang R, Maraia RJ. Conserved and divergent features of the structure and function of La and La-related proteins (LARPs). *Biochim Biophys Acta.* 2010; 1799:365–378. [PubMed: 20138158]
31. Sim S, Wolin SL. Emerging roles for the Ro 60-kDa autoantigen in noncoding RNA metabolism. *Wiley Interdiscip Rev RNA.* 2011; 2:686–699. [PubMed: 21823229]
32. Furtig B, Nozinovic S, Reining A, Schwalbe H. Multiple conformational states of riboswitches fine-tune gene regulation. *Curr Opin Struct Biol.* 2015; 30:112–124. [PubMed: 25727496]
33. Lau MW, Ferre-D'Amare AR. Many Activities, One Structure: Functional Plasticity of Ribozyme Folds. *Molecules.* 2016; 21
34. Krajewski SS, Narberhaus F. Temperature-driven differential gene expression by RNA thermosensors. *Biochim Biophys Acta.* 2014; 1839:978–988. [PubMed: 24657524]

35. Peer A, Margalit H. Evolutionary patterns of Escherichia coli small RNAs and their regulatory interactions. *Rna*. 2014; doi: 10.1261/rna.043133.113
36. Kang Z, Zhang C, Zhang J, Jin P, Zhang J, Du G, Chen J. Small RNA regulators in bacteria: powerful tools for metabolic engineering and synthetic biology. *Appl Microbiol Biotechnol*. 2014; 98:3413–3424. [PubMed: 24519458]
37. Trausch JJ, Batey RT. Design of modular "plug-and-play" expression platforms derived from natural riboswitches for engineering novel genetically encodable RNA regulatory devices. *Methods Enzymol*. 2015; 550:41–71. [PubMed: 25605380]
38. Behrouzi R, Roh JH, Kilburn D, Briber RM, Woodson SA. Cooperative tertiary interaction network guides RNA folding. *Cell*. 2012; 149:348–357. [PubMed: 22500801]
39. Chauhan S, Woodson SA. Tertiary interactions determine the accuracy of RNA folding. *J Am Chem Soc*. 2008; 130:1296–1303. [PubMed: 18179212]
40. Thirumalai D, Hyeon C. RNA and protein folding: common themes and variations. *Biochemistry*. 2005; 44:4957–4970. [PubMed: 15794634]
41. Thirumalai D, Woodson SA. Kinetics of folding of protein and RNA. *Accounts of Chemical Research*. 1996; 29:433–439.
42. Crothers DM. RNA conformational dynamics. In: Söll D, Nishimura S, Moore P, editors *RNA*. Elsevier; Oxford, UK: 2001. 61–70.
43. Draper DE. Strategies for RNA folding. *Trends Biochem Sci*. 1996; 21:145–149. [PubMed: 8701472]
44. Zarrinkar PP, Williamson JR. Kinetic intermediates in RNA folding. *Science*. 1994; 265:918–924. [PubMed: 8052848]
45. Pan T, Sosnick TR. Intermediates and kinetic traps in the folding of a large ribozyme revealed by circular dichroism and UV absorbance spectroscopies and catalytic activity. *Nat Struct Biol*. 1997; 4:931–938. [PubMed: 9360610]
46. Sclavi B, Sullivan M, Chance MR, Brenowitz M, Woodson SA. RNA folding at millisecond intervals by synchrotron hydroxyl radical footprinting. *Science*. 1998; 279:1940–1943. [PubMed: 9506944]
47. Lai D, Proctor JR, Meyer IM. On the importance of cotranscriptional RNA structure formation. *RNA*. 2013; 19:1461–1473. [PubMed: 24131802]
48. Schroeder R, Barta A, Semrad K. Strategies for RNA folding and assembly. *Nat Rev Mol Cell Biol*. 2004; 5:908–919. [PubMed: 15520810]
49. Craig ME, Crothers DM, Doty P. Relaxation kinetics of dimer formation by self complementary oligonucleotides. *Journal of Molecular Biology*. 1971; 62:383–401. [PubMed: 5138338]
50. Porschke D. A direct measurement of the unzipping rate of a nucleic acid double helix. *Biophys Chem*. 1974; 2:97–101. [PubMed: 4433688]
51. Nordstrom K, Wagner EG. Kinetic aspects of control of plasmid replication by antisense RNA. *Trends Biochem Sci*. 1994; 19:294–300. [PubMed: 8048170]
52. Tomizawa J. Control of ColE1 plasmid replication: the process of binding of RNA I to the primer transcript. *Cell*. 1984; 38:861–870. [PubMed: 6207934]
53. Persson C, Wagner EG, Nordstrom K. Control of replication of plasmid R1: formation of an initial transient complex is rate-limiting for antisense RNA--target RNA pairing. *EMBO J*. 1990; 9:3777–3785. [PubMed: 1698622]
54. Tamm J, Polisky B. Characterization of the ColE1 primer-RNA1 complex: analysis of a domain of ColE1 RNA1 necessary for its interaction with primer RNA. *Proc Natl Acad Sci U S A*. 1985; 82:2257–2261. [PubMed: 2581244]
55. Kolb FA, Engdahl HM, Slagter-Jager JG, Ehresmann B, Ehresmann C, Westhof E, Wagner EG, Romby P. Progression of a loop-loop complex to a four-way junction is crucial for the activity of a regulatory antisense RNA. *EMBO J*. 2000; 19:5905–5915. [PubMed: 11060041]
56. Tomizawa J. Control of ColE1 plasmid replication. Interaction of Rom protein with an unstable complex formed by RNA I and RNA II. *J Mol Biol*. 1990; 212:695–708. [PubMed: 1691791]

57. Herschlag D, Khosla M, Tsuchihashi Z, Karpel RL. An RNA chaperone activity of nonspecific RNA-binding proteins in hammerhead ribozyme catalysis. *EMBO J.* 1994; 13:2913–2924. [PubMed: 8026476]
58. Grossberger R, Mayer O, Waldsich C, Semrad K, Urschitz S, Schroeder R. Influence of RNA structural stability on the RNA chaperone activity of the *Escherichia coli* protein StpA. *Nucl Acids Res.* 2005; 33:2280–2289. [PubMed: 15849314]
59. Mayer O, Rajkowitsch L, Lorenz C, Konrat R, Schroeder R. RNA chaperone activity and RNA-binding properties of the *E. coli* protein StpA. *Nucl Acids Res.* 2007; 35:1257–1269. [PubMed: 17267410]
60. Woodson SA. Taming free energy landscapes with RNA chaperones. *RNA Biol.* 2010; 7:1–10.
61. Tijerina P, Bhaskaran H, Russell R. Nonspecific binding to structured RNA and preferential unwinding of an exposed helix by the CYT-19 protein, a DEAD-box RNA chaperone. *Proc Natl Acad Sci U S A.* 2006; 103:16698–16703. [PubMed: 17075070]
62. Todd MJ, Lorimer GH, Thirumalai D. Chaperonin-facilitated protein folding: optimization of rate and yield by an iterative annealing mechanism. *Proc Natl Acad Sci U S A.* 1996; 93:4030–4035. [PubMed: 8633011]
63. Hyeon C, Thirumalai D. Generalized iterative annealing model for the action of RNA chaperones. *J Chem Phys.* 2013; 139:121924. [PubMed: 24089736]
64. Chakrabarti S, Hyeon C, Ye X, Lorimer GH, Thirumalai D. Molecular chaperones maximize the native state yield on biological times by driving substrates out of equilibrium. *Proc Natl Acad Sci U S A.* 2017; 114:E10919–E10927. [PubMed: 29217641]
65. Storz G, Opdyke JA, Zhang A. Controlling mRNA stability and translation with small, noncoding RNAs. *Curr Opin Microbiol.* 2004; 7:140–144. [PubMed: 15063850]
66. Wagner EG. Cycling of RNAs on Hfq. *RNA Biol.* 2013; 10:619–626. [PubMed: 23466677]
67. Cruceanu M, Gorelick RJ, Musier-Forsyth K, Rouzina I, Williams MC. Rapid kinetics of protein-nucleic acid interaction is a major component of HIV-1 nucleocapsid protein's nucleic acid chaperone function. *J Mol Biol.* 2006; 363:867–877. [PubMed: 16997322]
68. Cusick ME, Belfort M. Domain structure and RNA annealing activity of the *Escherichia coli* regulatory protein StpA. *Mol Microbiol.* 1998; 28:847–857. [PubMed: 9643551]
69. Moll I, Leitsch D, Steinhäuser T, Blasi U. RNA chaperone activity of the Sm-like Hfq protein. *EMBO Rep.* 2003; 4:284–289. [PubMed: 12634847]
70. Lease RA, Woodson SA. Cycling of the Sm-like protein Hfq on the DsrA small regulatory RNA. *J Mol Biol.* 2004; 344:1211–1223. [PubMed: 15561140]
71. Rajkowitsch L, Schroeder R. Dissecting RNA chaperone activity. *RNA.* 2007; 13:2053–2060. [PubMed: 17901153]
72. Hopkins JF, Panja S, Woodson SA. Rapid binding and release of Hfq from ternary complexes during RNA annealing. *Nucleic Acids Res.* 2011; 39:5193–5202. [PubMed: 21378124]
73. Adamson DN, Lim HN. Essential requirements for robust signaling in Hfq dependent small RNA networks. *PLoS Comput Biol.* 2011; 7:e1002138. [PubMed: 21876666]
74. Goldstein J, Pollitt NS, Inouye M. Major cold shock protein of *Escherichia coli*. *Proc Natl Acad Sci U S A.* 1990; 87:283–287. [PubMed: 2404279]
75. Jiang W, Hou Y, Inouye M. CspA, the major cold-shock protein of *Escherichia coli*, is an RNA chaperone. *J Biol Chem.* 1997; 272:196–202. [PubMed: 8995247]
76. Phadtare S, Inouye M, Severinov K. The nucleic acid melting activity of *Escherichia coli* CspE is critical for transcription antitermination and cold acclimation of cells. *J Biol Chem.* 2002; 277:7239–7245. [PubMed: 11756430]
77. Phadtare S, Tadigotla V, Shin WH, Sengupta A, Severinov K. Analysis of *Escherichia coli* global gene expression profiles in response to overexpression and deletion of CspC and CspE. *J Bacteriol.* 2006; 188:2521–2527. [PubMed: 16547039]
78. Newkirk K, Feng W, Jiang W, Tejero R, Emerson SD, Inouye M, Montelione GT. Solution NMR structure of the major cold shock protein (CspA) from *Escherichia coli*: identification of a binding epitope for DNA. *Proc Natl Acad Sci U S A.* 1994; 91:5114–5118. [PubMed: 7515185]

79. Schindelin H, Jiang W, Inouye M, Heinemann U. Crystal structure of CspA, the major cold shock protein of *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1994; 91:5119–5123. [PubMed: 8197194]
80. Phadtare S, Tyagi S, Inouye M, Severinov K. Three amino acids in *Escherichia coli* CspE surface-exposed aromatic patch are critical for nucleic acid melting activity leading to transcription antitermination and cold acclimation of cells. *J Biol Chem*. 2002; 277:46706–46711. [PubMed: 12324471]
81. Phadtare S, Inouye M, Severinov K. The mechanism of nucleic acid melting by a CspA family protein. *J Mol Biol*. 2004; 337:147–155. [PubMed: 15001358]
82. Rennella E, Sara T, Juen M, Wunderlich C, Imbert L, Solyom Z, Favier A, Ayala I, Weinhaupl K, Schanda P, Konrat R, Kreutz C, Brutscher B. RNA binding and chaperone activity of the *E. coli* cold-shock protein CspA. *Nucl Acids Res*. 2017; 45:4255–4268. [PubMed: 28126922]
83. Hall KB. RNA and Proteins: Mutual Respect. *F1000Res*. 2017; 6:345. [PubMed: 28408981]
84. Feng W, Tejero R, Zimmerman DE, Inouye M, Montelione GT. Solution NMR structure and backbone dynamics of the major cold-shock protein (CspA) from *Escherichia coli*: evidence for conformational dynamics in the single-stranded RNA-binding site. *Biochemistry*. 1998; 37:10881–10896. [PubMed: 9692981]
85. Tompa P, Kovacs D. Intrinsically disordered chaperones in plants and animals. *Biochem Cell Biol*. 2010; 88:167–174. [PubMed: 20453919]
86. Darlix JL, de Rocquigny H, Mely Y. The multiple roles of the nucleocapsid in retroviral RNA conversion into proviral DNA by reverse transcriptase. *Biochem Soc Trans*. 2016; 44:1427–1440. [PubMed: 27911725]
87. Rein A, Datta SA, Jones CP, Musier-Forsyth K. Diverse interactions of retroviral Gag proteins with RNAs. *Trends Biochem Sci*. 2011; 36:373–380. [PubMed: 21550256]
88. Guo J, Wu T, Kane BF, Johnson DG, Henderson LE, Gorelick RJ, Levin JG. Subtle alterations of the native zinc finger structures have dramatic effects on the nucleic acid chaperone activity of human immunodeficiency virus type 1 nucleocapsid protein. *J Virol*. 2002; 76:4370–4378. [PubMed: 11932404]
89. Heath MJ, Derebail SS, Gorelick RJ, DeStefano JJ. Differing roles of the N- and C-terminal zinc fingers in human immunodeficiency virus nucleocapsid protein-enhanced nucleic acid annealing. *J Biol Chem*. 2003; 278:30755–30763. [PubMed: 12783894]
90. Williams MC, Gorelick RJ, Musier-Forsyth K. Specific zinc-finger architecture required for HIV-1 nucleocapsid protein's nucleic acid chaperone function. *Proc Natl Acad Sci U S A*. 2002; 99:8614–8619. [PubMed: 12084921]
91. Williams MC, Rouzina I, Wenner JR, Gorelick RJ, Musier-Forsyth K, Bloomfield VA. Mechanism for nucleic acid chaperone activity of HIV-1 nucleocapsid protein revealed by single molecule stretching. *Proc Natl Acad Sci U S A*. 2001; 98:6121–6126. [PubMed: 11344257]
92. Le Cam E, Coulaud D, Delain E, Petitjean P, Roques BP, Gerard D, Stoylova E, Vuilleumier C, Stoylov SP, Mely Y. Properties and growth mechanism of the ordered aggregation of a model RNA by the HIV-1 nucleocapsid protein: an electron microscopy investigation. *Biopolymers*. 1998; 45:217–229. [PubMed: 9465785]
93. Azoulay J, Clamme JP, Darlix JL, Roques BP, Mely Y. Destabilization of the HIV-1 complementary sequence of TAR by the nucleocapsid protein through activation of conformational fluctuations. *J Mol Biol*. 2003; 326:691–700. [PubMed: 12581633]
94. Heilman-Miller SL, Wu T, Levin JG. Alteration of nucleic acid structure and stability modulates the efficiency of minus-strand transfer mediated by the HIV-1 nucleocapsid protein. *J Biol Chem*. 2004; 279:44154–44165. [PubMed: 15271979]
95. McCauley MJ, Rouzina I, Manthei KA, Gorelick RJ, Musier-Forsyth K, Williams MC. Targeted binding of nucleocapsid protein transforms the folding landscape of HIV-1 TAR RNA. *Proc Natl Acad Sci U S A*. 2015; 112:13555–13560. [PubMed: 26483503]
96. You JC, McHenry CS. Human immunodeficiency virus nucleocapsid protein accelerates strand transfer of the terminally redundant sequences involved in reverse transcription. *J Biol Chem*. 1994; 269:31491–31495. [PubMed: 7989315]
97. Godet J, Ramalanjaona N, Sharma KK, Richert L, de Rocquigny H, Darlix JL, Duportail G, Mely Y. Specific implications of the HIV-1 nucleocapsid zinc fingers in the annealing of the primer

- binding site complementary sequences during the obligatory plus strand transfer. *Nucl Acids Res.* 2011; 39:6633–6645. [PubMed: 21543454]
98. Grohman JK, Gorelick RJ, Lickwar CR, Lieb JD, Bower BD, Znosko BM, Weeks KM. A guanosine-centric mechanism for RNA chaperone function. *Science.* 2013; 340:190–195. [PubMed: 23470731]
99. Darlix JL, Godet J, Ivanyi-Nagy R, Fosse P, Mauffret O, Mely Y. Flexible nature and specific functions of the HIV-1 nucleocapsid protein. *J Mol Biol.* 2011; 410:565–581. [PubMed: 21762801]
100. Wu H, Mitra M, Naufer MN, McCauley MJ, Gorelick RJ, Rouzina I, Musier-Forsyth K, Williams MC. Differential contribution of basic residues to HIV-1 nucleocapsid protein's nucleic acid chaperone function and retroviral replication. *Nucl Acids Res.* 2014; 42:2525–2537. [PubMed: 24293648]
101. Duval M, Korepanov A, Fuchsbaauer O, Fechter P, Haller A, Fabbretti A, Choulier L, Micura R, Klaholz BP, Romby P, Springer M, Marzi S. Escherichia coli ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation. *PLoS Biol.* 2013; 11:e1001731. [PubMed: 24339747]
102. Qu X, Lancaster L, Noller HF, Bustamante C, Tinoco I Jr. Ribosomal protein S1 unwinds double-stranded RNA in multiple steps. *Proc Natl Acad Sci U S A.* 2012; 109:14458–14463. [PubMed: 22908248]
103. Clery A, Blatter M, Allain FH. RNA recognition motifs: boring? Not quite. *Curr Opin Struct Biol.* 2008; 18:290–298. [PubMed: 18515081]
104. Raghunathan PL, Guthrie C. A spliceosomal recycling factor that reanneals U4 and U6 small nuclear ribonucleoprotein particles. *Science.* 1998; 279:857–860. [PubMed: 9452384]
105. Montemayor EJ, Curran EC, Liao HH, Andrews KL, Treba CN, Butcher SE, Brow DA. Core structure of the U6 small nuclear ribonucleoprotein at 1.7-Å resolution. *Nat Struct Mol Biol.* 2014; 21:544–551. [PubMed: 24837192]
106. Didychuk AL, Montemayor EJ, Brow DA, Butcher SE. Structural requirements for protein-catalyzed annealing of U4 and U6 RNAs during di-snRNP assembly. *Nucl Acids Res.* 2016; 44:1398–1410. [PubMed: 26673715]
107. Belair C, Sim S, Wolin SL. Noncoding RNA Surveillance: The Ends Justify the Means. *Chem Rev.* 2017; doi: 10.1021/acs.chemrev.7b00462
108. Maraia RJ, Lamichhane TN. 3' processing of eukaryotic precursor tRNAs. *Wiley Interdiscip Rev RNA.* 2011; 2:362–375. [PubMed: 21572561]
109. Pannone BK, Xue D, Wolin SL. A role for the yeast La protein in U6 snRNP assembly: evidence that the La protein is a molecular chaperone for RNA polymerase III transcripts. *Embo J.* 1998; 17:7442–7453. [PubMed: 9857199]
110. Blewett NH, Maraia RJ. La involvement in tRNA and other RNA processing events including differences among yeast and other eukaryotes. *Biochim Biophys Acta.* 2018; doi: 10.1016/j.bbagr.2018.01.013
111. Kotik-Kogan O, Valentine ER, Sanfelice D, Conte MR, Curry S. Structural analysis reveals conformational plasticity in the recognition of RNA 3' ends by the human La protein. *Structure.* 2008; 16:852–862. [PubMed: 18547518]
112. Wilusz CJ, Wilusz J. Eukaryotic Lsm proteins: lessons from bacteria. *Nat Struct Mol Biol.* 2005; 12:1031–1036. [PubMed: 16327775]
113. Babitzke P. Regulation of transcription attenuation and translation initiation by allosteric control of an RNA-binding protein: the *Bacillus subtilis* TRAP protein. *Curr Opin Microbiol.* 2004; 7:132–139. [PubMed: 15063849]
114. Waters LS, Storz G. Regulatory RNAs in bacteria. *Cell.* 2009; 136:615–628. [PubMed: 19239884]
115. Zhang A, Wassarman KM, Ortega J, Steven AC, Storz G. The Sm-like Hfq protein increases OxyS RNA interaction with target mRNAs. *Mol Cell.* 2002; 9:11–22. [PubMed: 11804582]
116. Vecerek B, Moll I, Afonyushkin T, Kaberdin V, Blasi U. Interaction of the RNA chaperone Hfq with mRNAs: direct and indirect roles of Hfq in iron metabolism of *Escherichia coli*. *Mol Microbiol.* 2003; 50:897–909. [PubMed: 14617150]

117. Vecerek B, Moll I, Blasi U. Translational autocontrol of the *Escherichia coli* hfq RNA chaperone gene. *RNA*. 2005; 11:976–984. [PubMed: 15872186]
118. Desnoyers G, Masse E. Noncanonical repression of translation initiation through small RNA recruitment of the RNA chaperone Hfq. *Genes Dev*. 2012; 26:726–739. [PubMed: 22474262]
119. Chen J, Gottesman S. Hfq links translation repression to stress-induced mutagenesis in *E. coli*. *Genes Dev*. 2017; doi: 10.1101/gad.302547.117
120. Ellis MJ, Trussler RS, Haniford DB. Hfq binds directly to the ribosome-binding site of IS10 transposase mRNA to inhibit translation. *Mol Microbiol*. 2015; 96:633–650. [PubMed: 25649688]
121. Kavita K, de Mets F, Gottesman S. New aspects of RNA-based regulation by Hfq and its partner sRNAs. *Curr Opin Microbiol*. 2017; 42:53–61. [PubMed: 29125938]
122. Weichenrieder O. RNA binding by Hfq and ring-forming (L)Sm proteins: A trade-off between optimal sequence readout and RNA backbone conformation. *RNA Biol*. 2014; 11:537–549. [PubMed: 24828406]
123. Schumacher MA, Pearson RF, Moller T, Valentin-Hansen P, Brennan RG. Structures of the pleiotropic translational regulator Hfq and an Hfq-RNA complex: a bacterial Sm-like protein. *Embo J*. 2002; 21:3546–3556. [PubMed: 12093755]
124. Brescia CC, Mikulecky PJ, Feig AL, Sledjeski DD. Identification of the Hfq-binding site on DsrA RNA: Hfq binds without altering DsrA secondary structure. *Rna*. 2003; 9:33–43. [PubMed: 12554874]
125. Otaka H, Ishikawa H, Morita T, Aiba H. PolyU tail of rho-independent terminator of bacterial small RNAs is essential for Hfq action. *Proc Natl Acad Sci U S A*. 2011; 108:13059–13064. [PubMed: 21788484]
126. Sauer E, Weichenrieder O. Structural basis for RNA 3'-end recognition by Hfq. *Proc Natl Acad Sci U S A*. 2011; 108:13065–13070. [PubMed: 21737752]
127. Sledjeski DD, Whitman C, Zhang A. Hfq is necessary for regulation by the untranslated RNA DsrA. *J Bacteriol*. 2001; 183:1997–2005. [PubMed: 11222598]
128. Ishikawa H, Otaka H, Maki K, Morita T, Aiba H. 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; 18:1062–1074. [PubMed: 22454537]
129. Fei J, Singh D, Zhang Q, Park S, Balasubramanian D, Golding I, Vanderpool CK, Ha T. RNA biochemistry. Determination of in vivo target search kinetics of regulatory noncoding RNA. *Science*. 2015; 347:1371–1374. [PubMed: 25792329]
130. Zhang A, Schu DJ, Tjaden BC, Storz G, Gottesman S. Mutations in Interaction Surfaces Differentially Impact *E. coli* Hfq Association with Small RNAs and Their mRNA Targets. *J Mol Biol*. 2013; 425:3678–3697. [PubMed: 23318956]
131. Mikulecky PJ, Kaw MK, Brescia CC, Takach JC, Sledjeski DD, Feig AL. *Escherichia coli* Hfq has distinct interaction surfaces for DsrA, rpoS and poly(A) RNAs. *Nat Struct Mol Biol*. 2004; 11:1206–1214. [PubMed: 15531892]
132. Soper TJ, Woodson SA. The rpoS mRNA leader recruits Hfq to facilitate annealing with DsrA sRNA. *RNA*. 2008; 14:1907–1917. [PubMed: 18658123]
133. Link TM, Valentin-Hansen P, Brennan RG. Structure of *Escherichia coli* Hfq bound to polyribadenylate RNA. *Proc Natl Acad Sci U S A*. 2009; 106:19292–19297. [PubMed: 19889981]
134. Soper T, Mandin P, Majdalani N, Gottesman S, Woodson SA. Positive regulation by small RNAs and the role of Hfq. *Proc Natl Acad Sci U S A*. 2010; 107:9602–9607. [PubMed: 20457943]
135. Updegrove T, Wilf N, Sun X, Wartell RM. Effect of Hfq on RprA-rpoS mRNA pairing: Hfq-RNA binding and the influence of the 5' rpoS mRNA leader region. *Biochemistry*. 2008; 47:11184–11195. [PubMed: 18826256]
136. Beisel CL, Updegrove TB, Janson BJ, Storz G. Multiple factors dictate target selection by Hfq-binding small RNAs. *EMBO J*. 2012; doi: 10.1038/emboj.2012.52
137. Moller T, Franch T, Hojrup P, Keene DR, Bachinger HP, Brennan RG, Valentin-Hansen P. Hfq: a bacterial Sm-like protein that mediates RNA-RNA interaction. *Mol Cell*. 2002; 9:23–30. [PubMed: 11804583]

138. 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 U S A*. 2012; 109:9396–9401. [PubMed: 22645344]
139. Panja S, Schu DJ, Woodson SA. Conserved arginines on the rim of Hfq catalyze base pair formation and exchange. *Nucleic Acids Res*. 2013; 41:7536–7546. [PubMed: 23771143]
140. Papenfort K, Said N, Welsink T, Lucchini S, Hinton JC, Vogel J. Specific and pleiotropic patterns of mRNA regulation by ArcZ, a conserved, Hfq-dependent small RNA. *Mol Microbiol*. 2009; 74:139–158. [PubMed: 19732340]
141. Tree JJ, Granneman S, McAteer SP, Tollervey D, Gally DL. Identification of bacteriophage-encoded anti-sRNAs in pathogenic *Escherichia coli*. *Mol Cell*. 2014; 55:199–213. [PubMed: 24910100]
142. Peng Y, Soper TJ, Woodson SA. Positional effects of AAN motifs in rpoS regulation by sRNAs and Hfq. *J Mol Biol*. 2014; 426:275–285. [PubMed: 24051417]
143. Schu DJ, Zhang A, Gottesman S, Storz G. Alternative Hfq-sRNA interaction modes dictate alternative mRNA recognition. *Embo j*. 2015; doi: 10.15252/embj.201591569
144. Hopkins JF, Panja S, McNeil SA, Woodson SA. Effect of salt and RNA structure on annealing and strand displacement by Hfq. *Nucleic Acids Res*. 2009; 37:6205–6213. [PubMed: 19671524]
145. Arluison V, Hohng S, Roy R, Pellegrini O, Regnier P, Ha T. Spectroscopic observation of RNA chaperone activities of Hfq in post-transcriptional regulation by a small non-coding RNA. *Nucl Acids Res*. 2007; 35:999–1006. [PubMed: 17259214]
146. Zheng A, Panja S, Woodson SA. Arginine Patch Predicts the RNA Annealing Activity of Hfq from Gram-Negative and Gram-Positive Bacteria. *J Mol Biol*. 2016; 428:2259–2264. [PubMed: 27049793]
147. Hwang W, Arluison V, Hohng S. Dynamic competition of DsrA and rpoS fragments for the proximal binding site of Hfq as a means for efficient annealing. *Nucleic Acids Res*. 2011; 39:5131–5139. [PubMed: 21357187]
148. Sukhodolets MV, Garges S. Interaction of *Escherichia coli* RNA polymerase with the ribosomal protein S1 and the Sm-like ATPase Hfq. *Biochemistry*. 2003; 42:8022–8034. [PubMed: 12834354]
149. Wang W, Wang L, Zou Y, Zhang J, Gong Q, Wu J, Shi Y. Cooperation of *Escherichia coli* Hfq hexamers in DsrA binding. *Genes Dev*. 2011; 25:2106–2117. [PubMed: 21979921]
150. de Ribeiro EA Jr, Beich-Frandsen M, Konarev PV, Shang W, Vecerek B, Kontaxis G, Hammerle H, Peterlik H, Svergun DI, Blasi U, Djinovic-Carugo K. Structural flexibility of RNA as molecular basis for Hfq chaperone function. *Nucleic Acids Res*. 2012; 40:8072–8084. [PubMed: 22718981]
151. Panja S, Paul R, Greenberg MM, Woodson SA. Light-Triggered RNA Annealing by an RNA Chaperone. *Angew Chem Int Ed Engl*. 2015; 54:7281–7284. [PubMed: 25959666]
152. Updegrove TB, Wartell RM. The influence of *Escherichia coli* Hfq mutations on RNA binding and sRNA*mRNA duplex formation in rpoS riboregulation. *Biochim Biophys Acta*. 2011; 1809:532–540. [PubMed: 21889623]
153. Peng Y, Curtis JE, Fang X, Woodson SA. Structural model of an mRNA in complex with the bacterial chaperone Hfq. *Proc Natl Acad Sci U S A*. 2014; 111:17134–17139. [PubMed: 25404287]
154. Lease RA, Belfort M. Riboregulation by DsrA RNA: trans-actions for global economy. *Mol Microbiol*. 2000; 38:667–672. [PubMed: 11115103]
155. Bordeau V, Felden B. Curli synthesis and biofilm formation in enteric bacteria are controlled by a dynamic small RNA module made up of a pseudoknot assisted by an RNA chaperone. *Nucleic Acids Res*. 2014; 42:4682–4696. [PubMed: 24489123]
156. Salim NN, Feig AL. An upstream Hfq binding site in the fhlA mRNA leader region facilitates the OxyS-fhlA interaction. *PLoS One*. 2010; 5:e13028. [PubMed: 20927406]
157. Gorski SA, Vogel J, Doudna JA. RNA-based recognition and targeting: sowing the seeds of specificity. *Nat Rev Mol Cell Biol*. 2017; 18:215–228. [PubMed: 28196981]
158. Hussein R, Lim HN. Disruption of small RNA signaling caused by competition for Hfq. *Proc Natl Acad Sci U S A*. 2011; 108:1110–1115. [PubMed: 21189298]

159. Moon K, Gottesman S. Competition among Hfq-binding small RNAs in *Escherichia coli*. *Mol Microbiol.* 2011; 82:1545–1562. [PubMed: 22040174]
160. Olejniczak M. Despite similar binding to the Hfq protein regulatory RNAs widely differ in their competition performance. *Biochemistry.* 2011; 50:4427–4440. [PubMed: 21510661]
161. Fender A, Elf J, Hampel K, Zimmermann B, Wagner EG. RNAs actively cycle on the Sm-like protein Hfq. *Genes Dev.* 2010; 24:2621–2626. [PubMed: 21123649]
162. Boehr DD, Nussinov R, Wright PE. The role of dynamic conformational ensembles in biomolecular recognition. *Nat Chem Biol.* 2009; 5:789–796. [PubMed: 19841628]
163. Uversky VN. The multifaceted roles of intrinsic disorder in protein complexes. *FEBS Lett.* 2015; 589:2498–2506. [PubMed: 26073257]
164. Vo MN, Barany G, Rouzina I, Musier-Forsyth K. HIV-1 nucleocapsid protein switches the pathway of transactivation response element RNA/DNA annealing from loop-loop "kissing" to "zipper". *J Mol Biol.* 2009; 386:789–801. [PubMed: 19154737]
165. Grohman JK, Del Campo M, Bhaskaran H, Tijerina P, Lambowitz AM, Russell R. Probing the mechanisms of DEAD-box proteins as general RNA chaperones: the C-terminal domain of CYT-19 mediates general recognition of RNA. *Biochemistry.* 2007; 46:3013–3022. [PubMed: 17311413]
166. Mohr G, Del Campo M, Mohr S, Yang Q, Jia H, Jankowsky E, Lambowitz AM. Function of the C-terminal domain of the DEAD-box protein Mss116p analyzed in vivo and in vitro. *J Mol Biol.* 2008; 375:1344–1364. [PubMed: 18096186]
167. Busa VF, Rector MJ, Russell R. The DEAD-Box Protein CYT-19 Uses Arginine Residues in Its C-Tail To Tether RNA Substrates. *Biochemistry.* 2017; 56:3571–3578. [PubMed: 28650145]
168. Russell R, Jarmoskaite I, Lambowitz AM. Toward a molecular understanding of RNA remodeling by DEAD-box proteins. *RNA Biol.* 2013; 10:44–55. [PubMed: 22995827]
169. Kucera NJ, Hodsdon ME, Wolin SL. An intrinsically disordered C terminus allows the La protein to assist the biogenesis of diverse noncoding RNA precursors. *Proc Natl Acad Sci U S A.* 2011; 108:1308–1313. [PubMed: 21212361]
170. Koculi E, Lee NK, Thirumalai D, Woodson SA. Folding of the Tetrahymena ribozyme by polyamines: importance of counterion valence and size. *J Mol Biol.* 2004; 341:27–36. [PubMed: 15312760]
171. Koculi E, Thirumalai D, Woodson SA. Counterion charge density determines the position and plasticity of RNA folding transition states. *J Mol Biol.* 2006; 359:446–454. [PubMed: 16626736]
172. Hauk G, McKnight JN, Nodelman IM, Bowman GD. The chromodomains of the Chd1 chromatin remodeler regulate DNA access to the ATPase motor. *Mol Cell.* 2010; 39:711–723. [PubMed: 20832723]
173. Kozlov AG, Cox MM, Lohman TM. Regulation of single-stranded DNA binding by the C termini of *Escherichia coli* single-stranded DNA-binding (SSB) protein. *J Biol Chem.* 2010; 285:17246–17252. [PubMed: 20360609]
174. Tretter EM, Berger JM. Mechanisms for defining supercoiling set point of DNA gyrase orthologs: I. A nonconserved acidic C-terminal tail modulates *Escherichia coli* gyrase activity. *J Biol Chem.* 2012; 287:18636–18644. [PubMed: 22457353]
175. Wang C, Uversky VN, Kurgan L. Disordered nucleome: Abundance of intrinsic disorder in the DNA- and RNA-binding proteins in 1121 species from Eukaryota, Bacteria and Archaea. *Proteomics.* 2016; 16:1486–1498. [PubMed: 27037624]
176. Santiago-Frangos A, Jeliaskov JR, Gray JJ, Woodson SA. Acidic C-terminal domains autoregulate the RNA chaperone Hfq. *Elife.* 2017; 6 pii 27049.
177. Santiago-Frangos A, Kavita K, Schu DJ, Gottesman S, Woodson SA. C-terminal domain of the RNA chaperone Hfq drives sRNA competition and release of target RNA. *Proc Natl Acad Sci U S A.* 2016; 113:E6089–e6096. [PubMed: 27681631]
178. Beich-Frandsen M, Vecerek B, Konarev PV, Sjoblom B, Kloiber K, Hammerle H, Rajkowitsch L, Miles AJ, Kontaxis G, Wallace BA, Svergun DI, Konrat R, Blasi U, Djinovic-Carugo K. Structural insights into the dynamics and function of the C-terminus of the *E. coli* RNA chaperone Hfq. *Nucleic Acids Res.* 2011; 39:4900–4915. [PubMed: 21330354]

179. Vincent HA, Henderson CA, Stone CM, Cary PD, Gowers DM, Sobott F, Taylor JE, Callaghan AJ. The low-resolution solution structure of *Vibrio cholerae* Hfq in complex with Qrr1 sRNA. *Nucleic Acids Res.* 2012; 40:8698–8710. [PubMed: 22730296]
180. Qualley DF, Stewart-Maynard KM, Wang F, Mitra M, Gorelick RJ, Rouzina I, Williams MC, Musier-Forsyth K. C-terminal domain modulates the nucleic acid chaperone activity of human T-cell leukemia virus type 1 nucleocapsid protein via an electrostatic mechanism. *J Biol Chem.* 2010; 285:295–307. [PubMed: 19887455]
181. Santiago-Frangos A, Woodson SA. Hfq chaperone brings speed dating to bacterial sRNA. *Wiley Interdiscip Rev RNA.* 2018; doi: 10.1002/wrna.1475:e1475

Figure 1. Iterative annealing of RNA by chaperones

Typical kinetic mechanism for forming RNA secondary structure (left) and tertiary structure (right). Assembly of the double helices (cylinders) into compact intermediates is followed by further reorganization of tertiary interactions to produce the native RNA. Because the RNA may adopt many secondary structures, some molecules fold directly to the native structure (top path) while others become trapped in non-native structures. In the classic iterative annealing model, chaperones (gold, bottom) bind and partially unfold misfolded intermediates, then release the unfolded RNA to fold again. Adapted from (60) with permission.

Figure 2. Chaperone-assisted annealing of anti-sense RNA

Annealing of anti-sense or trans acting sRNAs with a complementary RNA target typically begins with base pairing between two hairpin loops (kissing complex) or a loop and a single strand (middle path). This is followed by extension of base pairing, which often requires refolding of adjacent sequences. HIV nucleocapsid (NCp7) and Rom/Rop promote annealing by disrupting secondary structure in each RNA, lowering the energetic barriers for extending the anti-sense interactions (top path). NCp7 can also aggregate RNA strands to speed up initiation of base pairing. Hfq facilitates sRNA-mRNA base pairing by forming a ternary complex with both RNAs that increases the rate of helix nucleation (bottom path). Hfq can also favor anti-sense base pairing by restructuring one or both RNAs.