Integration of Bacterial Small RNAs in Regulatory Networks

Mor Nitzan,[1,2,*] Rotem Rehani,[1] and Hanah Margalit[1]

[1]Department of Microbiology and Molecular Genetics, Institute for Medical Research Israel-Canada, Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 9112102, Israel; email: hanahm@ekmd.huji.ac.il

[2]Racah Institute of Physics, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel

Annu. Rev. Biophys. 2017. 46:131–48

The *Annual Review of Biophysics* is online at biophys.annualreviews.org

https://doi.org/10.1146/annurev-biophys-070816-034058

*Current address: School of Computer Science, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel

Keywords

small RNA, regulatory network, feed-forward loop, competing RNAs

Abstract

Small RNAs (sRNAs) are central regulators of gene expression in bacteria, controlling target genes posttranscriptionally by base pairing with their mRNAs. sRNAs are involved in many cellular processes and have unique regulatory characteristics. In this review, we discuss the properties of regulation by sRNAs and how it differs from and combines with transcriptional regulation. We describe the global characteristics of the sRNA–target networks in bacteria using graph-theoretic approaches and review the local integration of sRNAs in mixed regulatory circuits, including feed-forward loops and their combinations, feedback loops, and circuits made of an sRNA and another regulator, both derived from the same transcript. Finally, we discuss the competition effects in posttranscriptional regulatory networks that may arise over shared targets, shared regulators, and shared resources and how they may lead to signal propagation across the network.

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INTRODUCTION

Small RNAs (sRNAs) are major posttranscriptional regulators of gene expression in bacteria (86, 95). These 50- to 400-nucleotide-long RNA molecules are encoded in the genomes of many bacteria and play central roles in various cellular processes, especially under specific growth and stress conditions (62, 97). Most sRNAs exert their regulatory function by base pairing with the mRNA of their target genes in *trans* (86, 95, 97). Base pairing with the mRNA of the *trans* targets is often incomplete and affects the transcript stability and/or the translation rate (**Figure 1**) (95). It is often mediated by the RNA chaperone protein Hfq, which stabilizes the sRNA and facilitates its interaction with the target RNA (reviewed in 93). *Trans*-base-pairing sRNAs act mostly as negative regulators of their targets but can also function as positive regulators. In fact, an sRNA can act as both a positive and a negative regulator, downregulating some targets and upregulating others (e.g., DsrA, RprA, and RyhB in *Escherichia coli*). As negative regulators, sRNAs can destabilize the target mRNA by inducing RNA cleavage and degradation by an RNase (e.g., 57, 81), and/or inhibit translation by blocking the ribosome binding site (e.g., 5, 81). As positive regulators, they can either stabilize the mRNA by interfering with cleavage by an RNase or enhance translation by preventing intramolecular base pairing that otherwise blocks the ribosome binding site (79). In case of negative regulation, it was suggested that there are various possible fates for the sRNA and target mRNA in the complex: (*a*) they are both degraded (27, 58), (*b*) only one of them is degraded (27, 29), or (*c*) none of them are specifically targeted for degradation and the regulatory effect is manifested at the protein level (27). An sRNA can regulate different targets by different mechanisms (13, 27) and even the same target by more than one mechanism (81). Recently, it was shown that *trans*-base-pairing sRNAs can also affect the expression level of some targets by interfering with premature Rho-dependent transcription termination (83).

In recent years, the important roles of sRNAs in the cellular networks and their integration with transcriptional regulation in regulatory circuits have been increasingly recognized (10). In this review, we focus on *trans*-base-pairing sRNAs and their implications in the cellular circuitry. Several aspects of the involvement of sRNAs in regulatory networks were discussed in previous excellent reviews (10, 55, 95). Here we attempt to provide a comprehensive conceptual view on the principles of sRNA-mediated regulation and the special contribution of sRNAs to the local and global regulatory circuitry of the cell. We first summarize the current understanding of the kinetic properties of this type of regulation and how it differs from transcriptional regulation. Second,

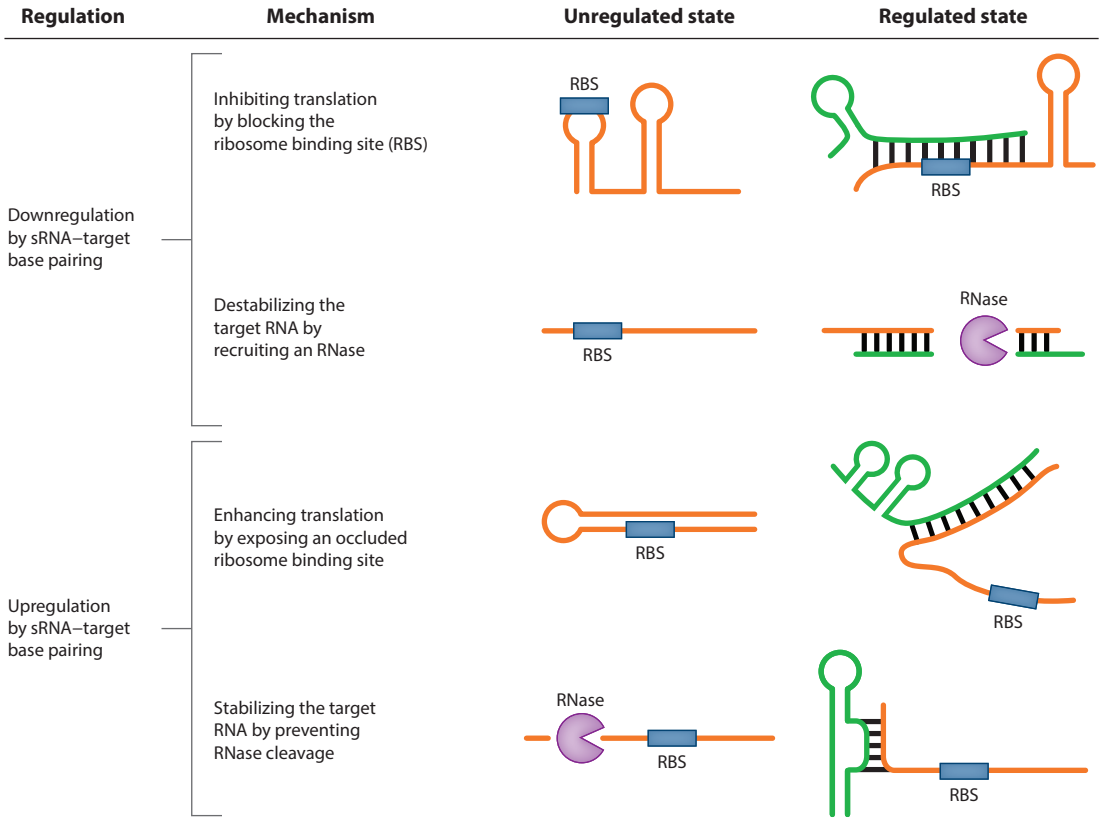

Figure 1

Mechanisms of regulation by *trans*-base-pairing sRNAs. (*Top row*) sRNA base pairing with the target mRNA in the vicinity of the ribosome binding site (RBS) inhibits translation by preventing ribosome binding [e.g., the base pairing between the sRNA ChiX and the mRNA of *chiP* in *Salmonella* (29) and *Escherichia coli* (77)]. (*Second row*) sRNA base pairing with the target mRNA establishes a double-strand substrate for RNase III [e.g., the base pairing between RNA III and the mRNA of its target, *spa*, in *Staphylococcus aureus* (39)]. (*Third row*) sRNA exposes an occluded ribosome binding site by base pairing with a region in the mRNA that otherwise would have base paired with the RBS [e.g., the base pairing between RNA III and the mRNA of its target, *hla*, in *S. aureus* (71)]. (*Bottom row*) sRNA stabilizes the mRNA of the target by preventing single-strand cleavage by an RNase [e.g., the base pairing between FasX and the mRNA of its target, *ska*, in group A *Streptococcus* (82)].

we characterize the network of sRNA–target interactions and how it rewires across internal and external conditions. Third, we describe how sRNAs are integrated in specific regulatory circuits along with transcriptional regulators. This is exemplified by identified circuits in various bacteria, mostly *E. coli* and *Salmonella enterica* serovar Typhimurium (hereinafter, *Salmonella*). Finally, we discuss the effects of competition between sRNAs and between targets in posttranscriptional regulatory networks and their global effects on network dynamics.

PROPERTIES OF REGULATION BY sRNAs

To better understand the kinetic properties of posttranscriptional regulation of gene expression by sRNAs, it is useful to compare it to the widely studied and well-established transcription regulation by transcription factors (TFs) (41, 59, 85, 86). Generally, TFs, which regulate transcription initiation at the promoter of the target gene, cause a greater effect on the target gene expression

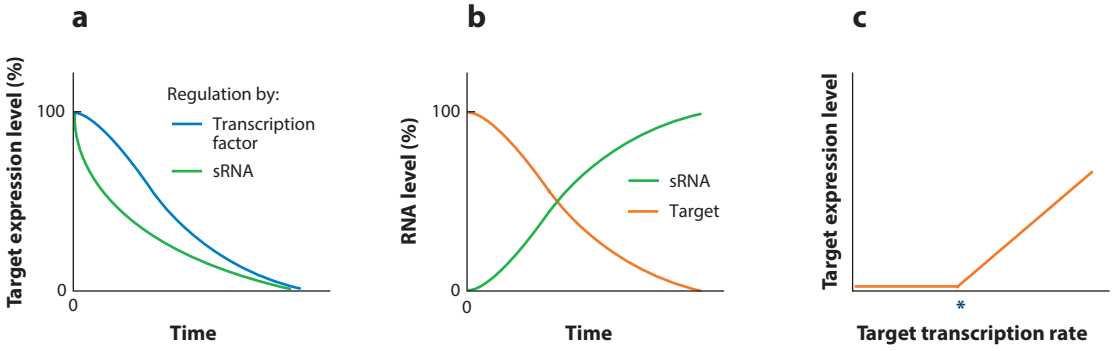

Figure 2

Kinetic properties of regulation by sRNA. (*a*) Regulation by an sRNA leads to a faster decrease in the level of the target's protein product than regulation by a transcription factor (TF) (schematic representation adapted from Reference 85). (*b*) Inverse relations between the levels of free sRNA and free target mRNA (schematic representation based on results reported in References 8 and 11). (*c*) sRNAs establish a threshold-linear response. As long as the transcription rate of the target gene is lower than the transcription rate of the sRNA, the target is not expressed. Once the target's transcription rate exceeds that of the sRNA (marked by *), the target is expressed and its expression level is linearly proportional to the difference between the two transcription rates (schematic representation adapted from Reference 50).

than sRNAs do (41). In addition, the dynamics of sRNA and TF regulation differ. Computational analysis by Shimoni et al. (85) showed that the change in the level of the target's protein product following the activation of a negative regulator is faster for regulation by sRNAs than by TFs (**Figure 2*a***). They suggested that this may stem from two possible reasons: (*a*) sRNA expression involves only the transcription process, as opposed to transcription and translation for TF expression, and (*b*) sRNAs act posttranscriptionally directly on the mRNA level. In the case of negative regulation, for example, a TF represses the transcription of the target, yet available mRNAs keep being translated until their pool is depleted (depending on their half-life), whereas an sRNA leads to a faster depletion of the mRNA pool available for translation because it directly destabilizes the mRNA or blocks translation. However, in a paper by Hussein and Lim (41), it was claimed that these two intuitive mechanistic details of regulation by sRNAs have relatively little impact on the target's dynamics, and instead, it is primarily determined by clearance rates, steady-state levels and response curves of the regulators. Both studies agreed that target gene expression changes with a minimal delay when the transcription of the sRNA is turned on or off. Hence, sRNAs often play important roles in biological processes that require quick regulatory responses, such as stress response (32, 95). The drawback is that under sRNA repression, the synthesis of new mRNAs persists, implying that for sustained downregulation, continuous production of sRNAs is required (assuming the sRNA is degraded or sequestered).

TFs and sRNAs exhibit distinct types of interactions with targets. In TF–target interaction, one molecule of the TF (or a few, in the case of a multimer) binds the DNA, usually upstream of the transcription start site of the target gene. In contrast, sRNA–target interaction may involve many pairs formed between RNA molecules of the regulator and the target, and the levels of free molecules of the two types are inversely related (**Figure 2*b***). This property of regulation by sRNAs has a couple of implications:

1. An increase in the level of free sRNA leads to more sRNA–target mRNA complexes and to a decrease in the level of free target mRNA. Likewise, a change in the free–target-mRNA level has an inverse effect on the level of free sRNA. Thus, there are bidirectional effects between the sRNA and target RNA, which might have local and even global effects on other interactions in

the regulatory network (75). Consequently, it was suggested that expression noise (both internal and external) of a few principal targets of sRNAs can be buffered through the regulation of many other weak targets (44). It is of note that in case the RNAs in the complex are degraded as a result of the interaction, there are inverse effects between the sRNA and target not only on the levels of free molecules but on their total levels.

2. sRNAs establish a threshold-linear response for their targets' expression with ultrasensitivity near the threshold (41, 51, 59, 66; for review, see 50). If the target's transcription rate is lower than that of the sRNA, then all target RNAs are sequestered by the sRNA. If the transcription rate of the sRNA is lower than that of the target, the target's effective transcription rate is linearly proportional to the difference between its own and the sRNA transcription rate (**Figure 2*c***). This mechanism can aid in avoiding target expression following weak or transient internal or external transcription signals, can lead to faster target response following changes in conditions that require derepression of the target, and promotes homogenous target kinetics by, for example, filtering transcriptional noise (6).

POSTTRANSCRIPTIONAL REGULATORY NETWORKS INVOLVING sRNAs

It is illuminating to describe the sRNA–target interactions in the cell as a network, where nodes represent either sRNAs or their targets and edges represent regulatory interactions between them. This representation allows the characterization of both structural and dynamical properties of the regulatory system, as discussed below. Regulatory networks are usually represented as directed networks, with edges pointing from the regulator node to the target node. However, the bidirectional effects between the sRNA and target (**Figure 2*b***) suggest that the sRNA–target networks can be better represented as undirected (or bi-directed) networks (15, 28, 75). In the discussion below, we treat and analyze the sRNA–target networks as undirected. Because the number of known sRNA–sRNA interactions is very small (60, 67), these networks have been considered essentially bipartite, with interconnections mainly between the two node groups of sRNAs and targets.

Experimentally determined sRNA–target interactions have been gradually accumulating (see table S3 in Reference 60). Recently, a few studies described experimental approaches to globally capture sRNA–target interactions in bacteria (36, 49, 60, 98), enabling the description of sRNA–target networks at a transcriptome-wide scale. Here, we characterize the properties and dynamics of sRNA–target networks using the networks deciphered in *E. coli* by Melamed et al. (60) under three different conditions (log phase, stationary phase, and iron limitation), because they are the most extensive networks deciphered and allow comparison between different conditions. Pairs of sRNA–target included in these networks were determined using a new experimental-computational methodology, RIL-seq (RNA interaction by ligation and sequencing), by which Hfq-mediated sRNA–target interactions are identified. RIL-seq involves co-immunoprecipitation of Hfq and bound RNAs, ligation of RNAs, sequencing, and identification of chimeric fragments, which represent RNA pairs interacting while bound to Hfq (60).

In **Figure 3** we present the unified network involving the interactions in all three conditions (~2,800 interactions), and in the discussion below, we refer to the three networks in the three conditions. Approximately two-thirds of the interactions in the three networks involve well-established Hfq-bound sRNAs, whereas the additional interactions involve newly identified Hfq-bound sRNAs. For simplicity, we analyze the subnetworks involving the well-established sRNAs and their target mRNAs (analysis of the full networks yielded very similar characteristics). We first analyze the degree distribution of the nodes, where a node's degree refers to the number of edges

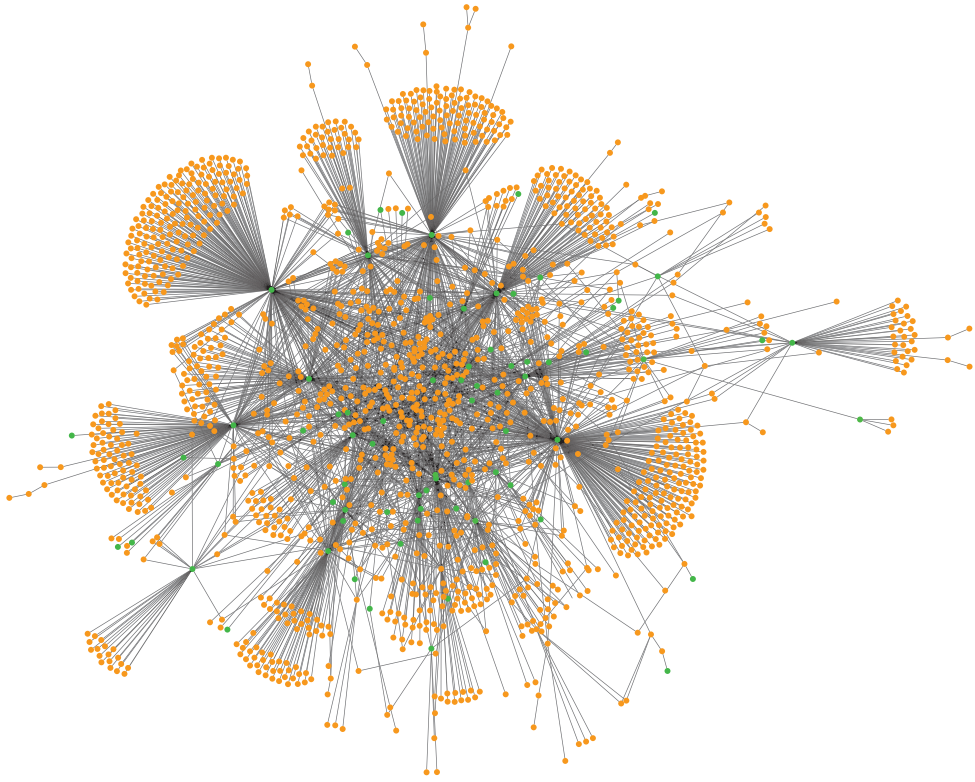

Figure 3

Network of sRNA–target interactions in *Escherichia coli* (based on data from Reference 60). sRNA nodes are shown in green and targets in orange. Edges connect between sRNAs and their targets. The network comprises ~2,800 interactions in total, unifying the interactions in the three studied growth conditions.

connecting it to other nodes. Analysis of the degree distributions of the three networks revealed that they are scale-free, meaning that they exhibit a power-law degree distribution, characterized by $P(k) \sim k^{-\alpha}$, where $P(k)$ is the probability of a node to have degree k and α is 2.32, 2.08, and 2.04 for log phase, stationary phase, and iron limitation, respectively. These results are in accord with many other real-world networks, specifically biological networks, in which α is usually in the range $2 < \alpha < 3$ (2, 3; for review, see 9). This means that most of the RNAs in the networks are sparsely connected, yet there is a small fraction of hub nodes that are connected to many other RNAs. These hubs refer mostly to sRNAs that have many targets but also to several targets that are regulated by multiple sRNAs [such as *flhD* (23)]. Whereas the sRNA–target network rewires substantially between conditions, 34% of hub nodes retained their high connectivity across conditions.

Biological networks are exposed to various perturbations. These include external perturbations (e.g., drugs or chemicals) and internal perturbations (e.g., mutations), which may affect the nodes and/or edges. Our computational analysis of the sRNA–target networks under all three conditions revealed their robustness to mutations or perturbations applied to random nodes. However, they were found to be highly vulnerable to targeted hub perturbations, highlighting the important role that hub nodes have in maintaining the structural integrity of the networks. These results are consistent with findings for other scale-free networks (17, 35, 43).

Another network measure of interest is the shortest path length between two nodes. When computing the average shortest path length over all node pairs in a network, we find that it is 3.89, 3.34, and 3.63 for log phase, stationary phase, and iron limitation, respectively. Consistent with findings for the miRNA–target network (75, based on data from 37), the distributions of shortest path lengths are sharply peaked around these averages, suggesting the potential for cross-talk between most nodes in these networks (75). This implies that perturbations of a node may propagate in the network and influence distant nodes, as discussed in the final part of this review.

REGULATORY CIRCUITS INVOLVING sRNAs

The availability of large-scale data enumerating regulatory interactions has paved the way to the identification of regulatory circuits—distinct connected patterns of regulators and targets in the network. Often, a regulatory pattern is recurring in different parts of the network and in different contexts, suggesting the wide functional use of those circuit structures. Such patterns in the sRNA–target network include, for example, the single input module, where an sRNA regulates several targets, and the dense overlapping regulon, where multiple sRNAs coregulate several targets. These regulatory patterns were comprehensively reviewed in Reference 10. Here we focus on distinct structures of mixed regulatory circuits that involve transcription regulation by a TF and posttranscriptional regulation by an sRNA. With the accumulation of data of regulatory interactions, various configurations of such mixed circuits were deciphered, and it would be illuminating to exemplify them and discuss their properties and functionality.

Mixed Feed-Forward Loops

A feed-forward loop (FFL) is a three-node circuit in which a regulator regulates the expression of a target gene both directly and indirectly via another regulator (**Figure 4*a***, *top*). FFLs can exhibit different logic gates for their target's dual regulation; two of these are the AND logic gate, where both regulators are required to exert the regulatory function on the target, and the OR logic gate, where one regulator suffices. FFLs can also be characterized as coherent or incoherent. In coherent FFLs, the two regulation paths lead to the same overall effect on the target (activation or repression), whereas in incoherent FFLs, the two paths lead to different results, one to activation and the other to repression. Because each of the three edges in the FFL can represent either positive or negative regulation, there are eight (2^3) possible configurations of FFLs, four coherent and four incoherent. Mangan & Alon (56) classified them into types (coherent types 1–4 and incoherent types 1–4), and we follow this classification in our discussion (**Figure 4*a***, *middle*).

Systematic analyses of the transcriptional regulatory networks of *E. coli* and *Saccharomyces cerevisiae* identified the FFL as a main building block of those networks (65, 84; for review, see 4). Both the coherent and the incoherent transcriptional FFLs were shown to have unique properties. Coherent FFLs were shown to function as sign-sensitive delay elements, where the FFL structure leads to a delay in the response of the target following a stimulus to the top regulator in only one direction but not the other (e.g., OFF to ON step but not ON to OFF step), a property that can protect against fluctuations or noise by avoiding a response to transient inputs. Incoherent FFLs, on the other hand, were shown to function under suitable conditions as pulse generators and as sign-sensitive accelerators (56 and reviewed in 4).

Given the characteristics of regulation by sRNAs, inclusion of sRNAs in FFLs has several benefits. It allows an enhanced response and provides a tighter, double-layered regulation. A double-layered repression of a target prevents possible transcriptional leakage at the posttranscriptional level, whereas a double-layered upregulation enhances simultaneously the target's transcription and translation rates (either directly or as a result of the increase in the target

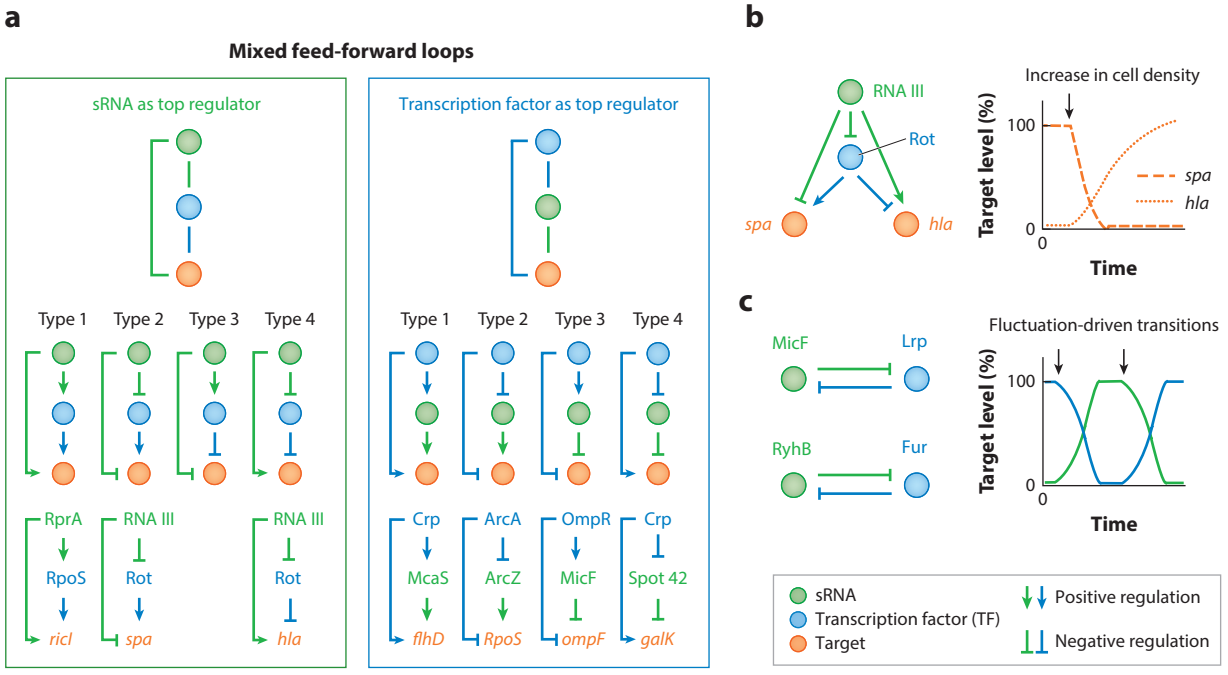

Figure 4

Mixed regulatory circuits involving an sRNA and a transcription factor. (*a*) Mixed feed-forward loops (FFLs) can involve either an sRNA or a transcription factor (TF) as top regulator. Shown are all possible configurations of coherent mixed FFLs along with biological examples, when available. The examples are from various bacteria: *Salmonella*, type 1 with top sRNA; *Staphylococcus aureus*, types 2 and 4 with top sRNA; *Escherichia coli*, types 1–4 with top TF. The numbering of FFL types follows Reference 56. Arrows indicate positive regulation, and truncated arrows indicate negative regulation. (*b*) Double selector switch in *S. aureus* composed of two combined mixed FFLs, controlling the inverse expression of *spa* and *hla* (*left*). Upon a quorum-sensing signal, *spa* is quickly downregulated, whereas the upregulation of *hla* is delayed (*right*) (73). (*c*) Mixed negative–negative feedback loops in *E. coli* (*left*), showing bistable dynamics, exhibiting two stable states with fluctuation-driven transitions between them (*right*) (74).

mRNA's stability). Because the TF and sRNA regulate the target at two different layers, the logic gate controlling the multiple inputs to the target can be considered (for an ideal leakage-free system) as an AND gate if both regulation paths lead to activation of the target and as an OR gate if both regulation paths lead to repression of the target. However, in more realistic scenarios, combination of regulation by TFs and sRNAs can result in more elaborate logic gates (96).

It was shown that the transcription of quite a few sRNAs is regulated by TFs and/or various σ factors [e.g., regulation of *ryhB* by Fur (58), *micF* by OmpR (22), and *ryhB* by σ^E (31); reviewed in 55], and many sRNAs regulate TF-encoding genes or σ factor–encoding genes [e.g., regulation of *fhlA* by OxyS (5), *csgD* by RprA (63), and *rpoS* by RprA (53); reviewed in 55]. This implies there are two possible node architectures of mixed FFLs, depending on the locations of the TF and sRNA—either an sRNA occupies the top layer, regulating the target both directly and indirectly through a TF, or a TF is at the top layer, regulating the target both directly and indirectly through an sRNA (**Figure 4*a***). Data from studies focusing on various specific sRNAs led to identification of different configurations of mixed FFLs arising in different contexts. Interestingly, in several identified mixed FFLs, the same two regulators are included and regulate many different targets (such circuits were termed multi-output FFLs) (11, 47, 84). Examples of mixed FFLs are illustrated in **Figure 4** and discussed below.

The dynamics of mixed FFLs can be studied both experimentally and computationally. Experimentally, one of the interactions in the FFL is artificially abolished and the change in expression of the target is compared to the change governed by the wild-type circuit upon change in conditions (11). Computationally, the dynamics are usually investigated by mathematical modeling and simulations, achieved by describing the change in target protein level over time by rate equations, taking into account the rates of generation and degradation of the regulators and target and the relevant binding and dissociation constants (25, 59, 73, 85). The dynamics obtained in the simulations depend to a large extent on the parameter values used, but for widely acceptable biological ranges of those parameters, general conclusions can be drawn for the various configurations. However, comprehensive comparisons of circuit dynamics between mixed FFLs of identical architectures that differ in the location of the sRNA and TF over a wide range of parameters are not yet available. Here, for the sake of space, we focus on mixed coherent FFL configurations (**Figure 4*a***). We describe the qualitative dynamic properties of several identified coherent mixed FFLs in bacteria, referring also to experimental measurements when available.

Feed-forward loop type 1. FFL type 1 filters transient signals by delaying the response of the target. With either sRNA or TF at the top, expression of the target is enabled when its transcription is activated by the TF and its translation is enhanced by the sRNA, whereas the expression of the bottom regulator depends on its activation/enhancement by the top regulator. The FFL comprising RprA–RpoS–*ricI*, recently discovered in *Salmonella*, provides an example for type 1 FFL with sRNA at the top (78). In this example, membrane stress and Rca phosphorelay activate RprA, which enables the translation of *rpoS* posttranscriptionally. RpoS transcribes *ricI*, and RprA enables its translation. The end result is prevention of plasmid conjugation by RicI. RicI protein is expressed only when both RprA and RpoS activate it. Thus, the circuit ensures that upon persistent membrane stress, plasmid conjugation is prevented. When the signal is not present (OFF state), the activity of *ricI* is quickly diminished because RprA is inactive. The circuit involving Crp–McaS–*flhD*, identified in *E. coli*, represents a type 1 FFL with TF at the top, but its physiological role is not yet clear (88).

Feed-forward loop type 2. In FFL type 2, the bottom regulator activates the target, and upon external signal activation, the top regulator suppresses both the bottom regulator and the target, enhancing the downregulation of the target. When the external signal to the top regulator is turned off, the target activation is expected to be delayed, because the pool of active RNA transcripts of the bottom regulator needs to be refilled. An example for this FFL with sRNA as a top regulator is the circuit RNA III–Rot–*spa* in *Staphylococcus aureus* (14). Rot is a TF that activates *spa* when local cell density is low. Upon increase in cell density, a quorum-sensing signal leads to activation of the regulatory RNA, RNA III, which suppresses posttranscriptionally both *rot* and *spa*. Abolishing the activation of the transcription of *spa* by Rot and repressing it posttranscriptionally by RNA III, downregulates *spa* and prevents transcription leakage (73). Upon decrease in cell density (OFF signal), there is a delay in the upregulation of *spa* because its expression requires Rot's protein pool to refill. The circuit with TF at the top is exemplified by ArcA–ArcZ–*rpoS* (54, 64), where, under anaerobic conditions, phosphorylated ArcA represses the transcription of both *rpoS* and the gene encoding the sRNA ArcZ, which activates *rpoS* posttranscriptionally (54, 83). Under aerobic conditions (OFF), ArcA does not act as a repressor and *rpoS* is expressed.

Feed-forward loop type 3. In FFL type 3, the top regulator represses the target and activates the bottom regulator that also represses the target. Several such circuits with TF as the top regulator were identified in *E. coli*. Although no such circuit with an sRNA as a top regulator has yet been

identified, it will conceivably be deciphered when more regulatory interactions are revealed along with their regulation sign (positive or negative). An example of a circuit with a top TF is provided by OmpR–MicF–*ompF* in *E. coli* (22, 34, 69, 85). Upon high osmotic stress, phosphorylated OmpR represses the transcription of *ompF* and activates the sRNA *micF*, which represses ompF posttranscriptionally. This double-layered regulation enhances the downregulation of *ompF* and prevents its transcriptional leakage. In this FFL, the transition to OFF state is delayed and depends on the half-life of MicF.

Feed-forward loop type 4. In FFL type 4, the bottom regulator represses the target and the top regulator represses the bottom regulator and activates the target, leading to an increase in the target level. An example of such an FFL with a top TF is provided by the CRP–Spot 42–*galK* circuit (11). Other genes encoding metabolic enzymes are also targets in this multi-output FFL, controlled by CRP–Spot 42 (11). In the presence of glucose, enzymes involved in metabolism of other sugars are not activated and any leaked transcripts are repressed by Spot 42 (OFF state). In the absence of glucose (ON state), metabolism of other sugars, such as galactose, takes place. When Crp is active (ON state), it directly enables the transcription of *galK* and indirectly enables its translation by the downregulation of Spot 42. Consistent with computational predictions for transcriptional coherent FFL type 4, it was demonstrated experimentally that this circuit provides a delayed increase in the target protein level when Crp is activated (ON) (11). In addition, experiments also revealed an accelerated decrease in target protein level when Crp was deactivated, which was attributed to the posttranscriptional downregulation performed by Spot 42 (11). An example of a coherent type 4 FFL with an sRNA on top is provided by the RNA III–Rot–*hla* circuit in *S. aureus*. Upon increase in cell density and activation of RNA III, the expression of *hla* is enabled, as RNA III represses posttranscriptionally the gene encoding its transcriptional repressor Rot and enhances its translation by releasing its otherwise-blocked ribosome binding site (71). Therefore, the repression of *hla* is enforced at two regulation layers, and its expression upon ON step is delayed because it requires the depletion of Rot's protein pool (73).

Elaborate Circuits Made by Combinations of Feed-Forward Loops

As an FFL structure has been considered to be one of the building blocks of the transcriptional regulatory network (65), a mixed FFL can be considered as one of the building blocks of the integrated regulatory network of transcriptional and posttranscriptional regulatory interactions. Two such FFLs can combine in the network to establish more elaborate regulatory circuits, such as sophisticated switches. One example for such a switch is between *ompF* and *ompC*, encoding two porins that are required under different osmotic stresses. This switch is formed by combining a type-3 FFL with a type-4 FFL, where a common regulator, the TF OmpR, is at the top of the two FFLs (21, 22, 34, 69, 85). Under high osmotic stress, the FFL OmpR–MicF–*ompF* (**Figure 4*a***, *bottom*) leads to repression of *ompF*, whereas the second FFL, OmpR–MicC–*ompC*, leads to delayed accumulation of OmpC. Another intriguing double-layered regulatory switch was identified in *S. aureus* and termed the double selector switch (DSS) (73) (**Figure 4*b***). The DSS controls the ability of *S. aureus* to switch between a defensive and an offensive state according to a quorum-sensing signal. It coherently controls two targets, *spa*, encoding protein A (which supports the ability of the bacterium to defend itself against phagocytosis), and *hla*, encoding the toxin Hla (which creates pores and tunnels in targeted cells) (76). The DSS is formed by combining a type-2 FFL and a type-4 FFL, sharing a top sRNA regulator, RNA III, and a bottom TF regulator, Rot. Compared to the simplest switch composed of a single regulator, either a TF or an sRNA, the DSS was shown both computationally and experimentally to induce asymmetric response of its

targets (a delay in the induction of *hla* upon ON step and a delay in the induction of *spa* upon OFF step; see **Figure 4*b***, *right*, for the ON step), guarantee tight regulation, and filter transient external signals (73).

Mixed Feedback Loops

Another circuit involving both transcriptional and posttranscriptional regulations is the mixed feedback loop, where a TF regulates an sRNA, which in turn regulates the TF. Two prominent configurations of this circuit are discussed below:

1. Positive–negative, where one regulation is positive and the other is negative (e.g., the TF–sRNA circuit LuxO-(Qrr1-5) in *Vibrio harveyi* and *V. cholerae*; reviewed in 10, 72). In a positive–negative mixed feedback loop, oscillatory dynamics may arise (48, 52).
2. Negative–negative, where a TF and an sRNA repress each other [e.g., the circuit Lrp–MicF (38), or Fur–RyhB, where the binding of RyhB is to a transcriptionally coupled upstream gene of *fur* (92); see **Figure 4*c***, *left*]. Dynamically, a negative–negative mixed feedback loop was shown to exhibit bistability under suitable parameter values, where two stable states can coexist, one dominated by the sRNA and the other by the TF (74). Because of stochasticity, transitions between the two stable states can occur (**Figure 4*c***, *right*). The frequency of such transitions and the regulators' levels characterizing the two stable states are functions of the parameters of the synthesis and interactions of the two regulators. Thus, suitable adjustment of external conditions may lead to dynamical states that best fit the current biological condition (7, 74).

Mixed Circuits of Two Regulators Derived from the Same Transcript

Circuits involving two regulators derived from the same transcript can be established in at least two ways: (*a*) when the sRNA includes an open reading frame that is translated into a protein product (91) and (*b*) when an mRNA of a protein-encoding gene is processed to produce an sRNA [frequently, from its 3′ untranslated region (UTR)] (19, 60; reviewed in 68). When the two products derived from the same transcript function in the same or complementary cellular pathways, they make up intriguing regulatory circuits, where the two regulators are inherently coordinated (**Figure 5**). The first scenario is exemplified by SgrS in *E. coli* and *Salmonella* (94) and RNA III in *S. aureus* (42). SgrS regulates genes that play a role in the response to glucose–phosphate stress by base pairing with their mRNAs through its 3′ region. SgrS also encodes in its 5′ region a small protein, SgrT, which is translated and participates in the response to the same stress. RNA III in *S. aureus* plays a role as a regulatory RNA in the virulence pathways of the bacterium, as described above, and also encodes a small protein, Hld, which is a toxin that plays a role in the virulence of the bacterium (42). In both examples, it seems that the two manifestations of the sRNA (as a protein-encoding gene and as a base-pairing regulatory RNA) are mutually exclusive (12, 94). The second scenario is exemplified by the CpxP–CpxQ circuit in *Salmonella* and *E. coli* (20, 33) and the GadE–GadF circuit in *E. coli* (60). CpxQ sRNA is generated from the 3′ UTR of *cpxP* (a gene encoding a chaperone that plays a role in the response to inner-membrane stress) and was shown to repress many inner-membrane proteins by base pairing to their mRNAs. GadF sRNA is generated from the 3′ UTR of *gadE*, which encodes a transcriptional activator that regulates many genes involved in the response to acidic stress (60). Because GadF was found to base pair with mRNAs of genes in the same pathway (and among them with three targets of GadE), the GadE–GadF circuit provides an example of a regulatory circuit comprising a transcriptional and a posttranscriptional regulator derived from the same transcript. As additional sRNAs were

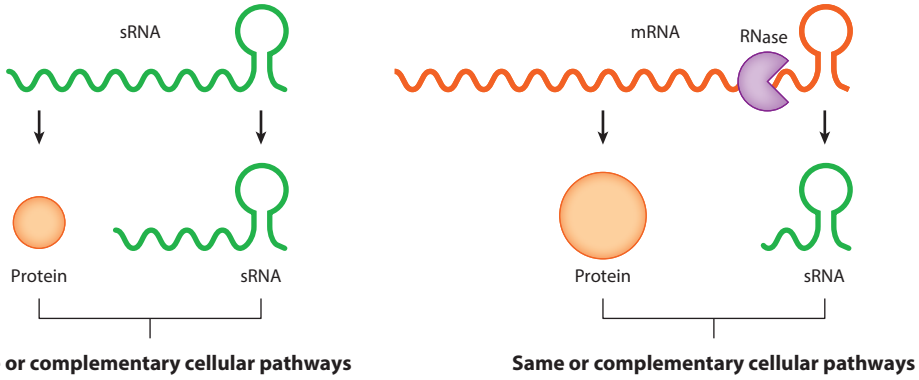

Figure 5

Mixed circuits involving two regulators derived from the same transcript. (*Left*) An sRNA that includes an open reading frame and is translated into a protein. (*Right*) An mRNA that is processed by an RNase to produce an sRNA. In both cases, the resulting sRNA and protein function in the same or complementary cellular pathways. A curly green line represents an sRNA, and a curly orange line represents an mRNA. An orange circle represents a protein.

recently discovered in the 3′ UTR of mRNAs (e.g., 19, 60), other such circuits are expected to be revealed.

COMPETITION EFFECTS IN sRNA–TARGET NETWORKS

The inverse relation between the levels of free sRNA molecules and free target RNA molecules for a given sRNA–target interaction (**Figure 2*b***) implies that targets that are coregulated by the same sRNA compete over its binding. Hence, common targets of an sRNA affect each other's binding to the sRNA and, consequently, their level of free molecules. If two targets, A and B, bind an sRNA regulator, an increase in the expression level of free A will lead to more complexes of A with the sRNA, lowering the number of complexes with B and leading to an increase in the level of free B. In such a scenario, target A is often regarded as sponging the sRNA and relieving its regulatory effect from other targets. Examples of this phenomenon were demonstrated for miRNAs in eukaryotes and sRNAs in prokaryotes, where the sponges span a wide range of RNA molecules [e.g., circular RNA (61); long noncoding RNA (18); and pseudogene transcripts (80) in eukaryotes, and mRNA (29, 77); sRNA (60, 67); and tRNA (49) in prokaryotes; for review see 16, 26, 79]. In eukaryotes, there has been a debate about the physiological implications of such miRNA sponging (89). Although sponging was shown to play a role in the physiological response to cellular stress in plants (30) and was shown to play a role in cancer in humans [e.g., (45, 46, 80, 87)], the general physiological relevance of miRNA sponges in eukaryotes was challenged by demonstrating that high nonphysiological concentrations of an mRNA acting as a sponge are needed to observe an effect on the expression level of other targets of the miRNA (24).

In bacteria, a few sponging interactions were shown to play a role in cellular physiology. The first example is of ChiX sRNA, which conventionally represses the expression of the chitoporin ChiP. In the presence of chitosugars, ChiX is sponged by an intergenic region of the chbBCARFG transcript, encoding genes involved in the same pathway. The *chbB*–ChiX interaction leads to ChiX degradation and relieves the repression of *chiP* (29, 77). It is interesting that conventionally, *chbB* transcription is repressed and ChiX prevents its possible leaky transcription. It is only in the

presence of chitosugars that the level of *chbB* transcript increases and enables it to function as a sponge of ChiX. This exemplifies that the sponging capability depends on the relative levels of the sRNA and its RNA interactor. Another example of sponging interaction is provided by SroC–GcvB. SroC is an sRNA generated from the 3′ UTR of one of the targets of GcvB, *gltI*. The interaction SroC–GcvB leads to degradation of GcvB and to relief of its targets' repression (mostly involved in amino acid metabolism and transport), including *gltI* (67). SroC is processed from *gltI* transcript and acts as an Hfq-bound sRNA to establish sRNA–sRNA interaction with GcvB. Interestingly, in enterohemorrhagic *E. coli*, it was found that a bacteriophage-encoded sRNA antagonizes GcvB in a similar manner, granting the bacteria a growth advantage (90). Recently, another sRNA, PspH (derived from the 3′ UTR of *pspG*), was shown to sponge Spot 42 and upregulate its targets, but the physiological context of this sponging is not yet clear (60). Finally, the 3′ end of a tRNA was shown to sponge two sRNAs in *E. coli*—RyhB and RybB. This region is cleaved from the pre-tRNA and base pairs with the two sRNAs under conditions in which they are not needed, buffering any unnecessary transcriptional noise (49).

The inverse relationship between the levels of free sRNA and target suggests there may also be competition between sRNAs over shared targets and, hence, mutual effects between sRNAs through their shared targets (15, 28, 75). Combination of the two competition types—of sRNAs over shared targets and of target RNAs over common regulatory RNAs—suggests that perturbations can gradually propagate in the network through such posttranscriptional regulatory paths, as demonstrated in **Figure 6** (1, 15, 28, 75). It was found that the extent of such propagation is a function of the network topology as well as the parameters of synthesis, degradation, and interactions of the sRNAs and targets composing the chains along which information can flow (75). This suggests a potential for distant effects between genes in the posttranscriptional regulatory network, with possible implications for indirect effects of mutations and off-target effects of drugs. The physiological implications of this type of information flow in cellular networks are yet to be discovered. sRNAs do not compete only over shared targets but also over shared resources, most prominently over binding to Hfq (40, 70). Hence, a change in the expression of a certain

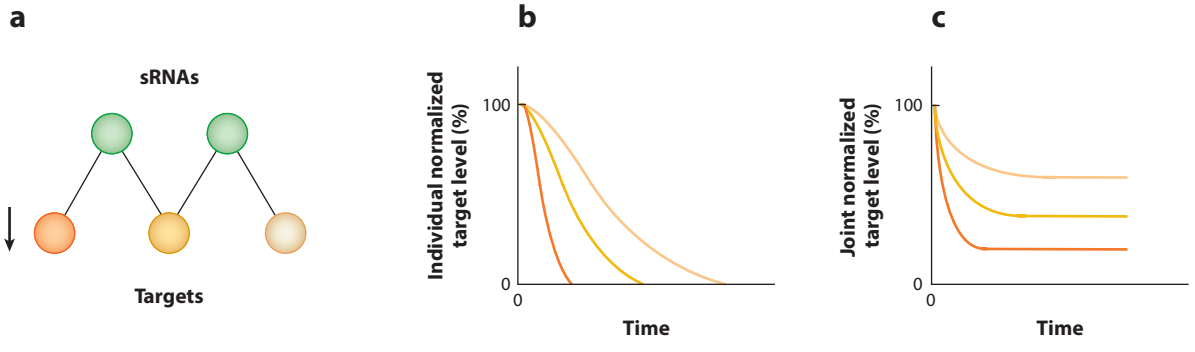

Figure 6

Propagation of a perturbation effect along a chain of sRNA–target interactions. (*a*) Schematic representation of a chain of interactions comprising recurrent interacting sRNAs (*green circles*) and target RNAs (*orange circles*). The network in panel *a* is perturbed out of steady state by decreasing the level of the leftmost target ("source"), marked by a downward black arrow. The colors of targets get lighter with increasing distance from the source. Shown are the changes in target levels over time, normalized (*b*) by the maximal and minimal levels within each individual RNA component and (*c*) by the maximal level of the source. The perturbation effect is delayed (*b*), and its amplitude decreases (*c*) with the increase in distance from the perturbed source (schematic representation adapted from Reference 75). Colors of the lines in panels *b* and *c* correspond to the colors of circles in panel *a*.

sRNA could affect the ability of other sRNAs to bind to Hfq and hence affect the regulation of genes that are not direct targets of the perturbed sRNA. It is the combination of competition over binding to Hfq and binding to shared targets that determines the relative amounts of Hfq-bound sRNA molecules available for interaction with target RNAs. A major challenge is to quantify the effects of these two types of competitions and to take into account the mutual effects of RNAs in the network, which would enhance our understanding of the functionality of the sRNA–target network and its dynamic response to perturbations.

CONCLUDING REMARKS

sRNAs play important roles in the control of gene expression in central cellular pathways in bacteria. They are integrated in regulatory circuits that participate in the adaptation of bacteria to new environmental conditions and in the response to various stresses. The special properties of regulation by sRNAs and by TFs and their combination in regulatory circuits award each circuit configuration with distinct dynamic properties. As more sRNAs and sRNA–target interactions become available, the analysis and implications on the posttranscriptional regulatory network are expected to broaden, both at the global scale (affecting the statistical and dynamical properties of the network as a whole) and the local scale (revealing additional regulatory circuits and their biological function, dynamics, and regulatory context). Identifying the interactions by themselves is still insufficient for revealing the functionality of regulatory circuits, as knowledge about the properties of regulation (positive or negative) is required for this. There is a challenge therefore to develop efficient methods not only for interaction determination but also for determining whether the sRNA acts as a repressor or activator. It is also intriguing to understand how the sRNAs are integrated with additional regulatory interactions, such as protein–RNA interactions. Taking a more global view, extension of the network analyses to additional posttranscriptional networks (including other regulatory mechanisms in addition to sRNA–target interactions, networks deciphered under additional conditions, additional high-resolution dynamic phases, and other organisms) could enable extended comparative analysis and highlight the consistencies, inconsistencies, and their significance across the different networks.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We apologize to the many researchers whose work we could not cite due to space limitation. We thank the members of our laboratory for their good suggestions and comments. Work from our lab described in this review was supported by the European Research Council Advanced Grant #322920, I-CORE Programs of the Planning and Budgeting Committee and The Israel Science Foundation (grants 1796/12 and 41/1), the Israel Science Foundation administered by the Israeli Academy for Sciences and Humanities (grant no. 1411/13), and the High Council for Scientific and Technological cooperation between France and Israel. M.N. is grateful to the Azrieli Foundation for the award of an Azrieli Fellowship. We thank Maayan Harel for the drawings in **Figure 1** and Raya Faigenbaum-Romm for the network view in **Figure 3**.

LITERATURE CITED

1. Ala U, Karreth FA, Bosia C, Pagnani A, Taulli R, et al. 2013. Integrated transcriptional and competitive endogenous RNA networks are cross-regulated in permissive molecular environments. *PNAS* 110:7154–59
2. Albert R. 2005. Scale-free networks in cell biology. *J. Cell Sci.* 118:4947–57
3. Albert R, Barabasi A-L. 2002. Statistical mechanics of complex networks. *Rev. Mod. Phys.* 74:47
4. Alon U. 2007. Network motifs: theory and experimental approaches. *Nat. Rev. Genet.* 8:450–61
5. Altuvia S, Zhang A, Argaman L, Tiwari A, Storz G. 1998. The *Escherichia coli* OxyS regulatory RNA represses *fhlA* translation by blocking ribosome binding. *EMBO J.* 17:6069–75
6. Arbel-Goren R, Tal A, Friedlander T, Meshner S, Costantino N, et al. 2013. Effects of post-transcriptional regulation on phenotypic noise in *Escherichia coli*. *Nucleic Acids Res.* 41:4825–34
7. Arbel-Goren R, Tal A, Parasar B, Dym A, Costantino N, et al. 2016. Transcript degradation and noise of small RNA-controlled genes in a switch activated network in *Escherichia coli*. *Nucleic Acids Res.* 44:6707–20
8. Argaman L, Altuvia S. 2000. *fhlA* repression by OxyS RNA: kissing complex formation at two sites results in a stable antisense-target RNA complex. *J. Mol. Biol.* 300:1101–12
9. Barabasi A-L, Oltvai ZN. 2004. Network biology: understanding the cell's functional organization. *Nat. Rev. Genet.* 5:101–13
10. Beisel CL, Storz G. 2010. Base pairing small RNAs and their roles in global regulatory networks. *FEMS Microbiol. Rev.* 34:866–82
11. Beisel CL, Storz G. 2011. The base-pairing RNA spot 42 participates in a multioutput feedforward loop to help enact catabolite repression in *Escherichia coli*. *Mol. Cell* 41:286–97
12. Benito Y, Kolb FA, Romby P, Lina G, Etienne J, Vandenesch F. 2000. Probing the structure of RNAIII, the *Staphylococcus aureus agr* regulatory RNA, and identification of the RNA domain involved in repression of protein A expression. *RNA* 6:668–79
13. Bobrovskyy M, Vanderpool CK. 2016. Diverse mechanisms of post-transcriptional repression by the small RNA regulator of glucose-phosphate stress. *Mol. Microbiol.* 99:254–73
14. Boisset S, Geissmann T, Huntzinger E, Fechter P, Bendridi N, et al. 2007. *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev.* 21:1353–66
15. Bosia C, Pagnani A, Zecchina R. 2013. Modelling competing endogenous RNA networks. *PLOS ONE* 8:e66609
16. Bossi L, Figueroa-Bossi N. 2016. Competing endogenous RNAs: a target-centric view of small RNA regulation in bacteria. *Nature Rev. Microbiol.* 14:775–84
17. Callaway DS, Newman ME, Strogatz SH, Watts DJ. 2000. Network robustness and fragility: percolation on random graphs. *Phys. Rev. Lett.* 85:5468–71
18. Cesana M, Cacchiarelli D, Legnini I, Santini T, Sthandier O, et al. 2011. A long noncoding RNA controls muscle differentiation by functioning as a competing endogenous RNA. *Cell* 147:358–69
19. Chao Y, Papenfort K, Reinhardt R, Sharma CM, Vogel J. 2012. An atlas of Hfq-bound transcripts reveals 3′ UTRs as a genomic reservoir of regulatory small RNAs. *EMBO J.* 31:4005–19
20. Chao Y, Vogel J. 2016. A 3′ UTR-derived small RNA provides the regulatory noncoding arm of the inner membrane stress response. *Mol. Cell* 61:352–63
21. Chen S, Zhang A, Blyn LB, Storz G. 2004. MicC, a second small-RNA regulator of Omp protein expression in *Escherichia coli*. *J. Bacteriol.* 186:6689–97
22. Coyer J, Andersen J, Forst SA, Inouye M, Delihas N. 1990. *micF* RNA in *ompB* mutants of *Escherichia coli*: different pathways regulate *micF* RNA levels in response to osmolarity and temperature change. *J. Bacteriol.* 172:4143–50
23. De Lay N, Gottesman S. 2012. A complex network of small non-coding RNAs regulate motility in *Escherichia coli*. *Mol. Microbiol.* 86:524–38
24. Denzler R, Agarwal V, Stefano J, Bartel DP, Stoffel M. 2014. Assessing the ceRNA hypothesis with quantitative measurements of miRNA and target abundance. *Mol. Cell* 54:766–76
25. Duk MA, Samsonova MG, Samsonov AM. 2014. Dynamics of miRNA driven feed-forward loop depends upon miRNA action mechanisms. *BMC Genom.* 15(Suppl. 12):S9

26. Ebert MS, Sharp PA. 2010. Emerging roles for natural microRNA sponges. *Curr. Biol.* 20:R858–61
27. Feng L, Rutherford ST, Papenfort K, Bagert JD, van Kessel JC, et al. 2015. A Qrr noncoding RNA deploys four different regulatory mechanisms to optimize quorum-sensing dynamics. *Cell* 160:228–40
28. Figliuzzi M, Marinari E, De Martino A. 2013. MicroRNAs as a selective channel of communication between competing RNAs: a steady-state theory. *Biophys. J.* 104:1203–13
29. Figueroa-Bossi N, Valentini M, Malleret L, Fiorini F, Bossi L. 2009. Caught at its own game: regulatory small RNA inactivated by an inducible transcript mimicking its target. *Genes Dev.* 23:2004–15
30. Franco-Zorrilla JM, Valli A, Todesco M, Mateos I, Puga MI, et al. 2007. Target mimicry provides a new mechanism for regulation of microRNA activity. *Nat. Genet.* 39:1033–37
31. Gogol EB, Rhodius VA, Papenfort K, Vogel J, Gross CA. 2011. Small RNAs endow a transcriptional activator with essential repressor functions for single-tier control of a global stress regulon. *PNAS* 108:12875–80
32. Gottesman S, McCullen CA, Guillier M, Vanderpool CK, Majdalani N, et al. 2006. Small RNA regulators and the bacterial response to stress. *Cold Spring Harb. Symp. Quant. Biol.* 71:1–11
33. Grabowicz M, Koren D, Silhavy TJ. 2016. The CpxQ sRNA negatively regulates Skp to prevent mistargeting of beta-barrel outer membrane proteins into the cytoplasmic membrane. *mBio* 7:e00312-16
34. Hall MN, Silhavy TJ. 1981. The *ompB* locus and the regulation of the major outer membrane porin proteins of *Escherichia coli* K12. *J. Mol. Biol.* 146:23–43
35. Han J-D, Bertin N, Hao T, Goldberg DS, Berriz GF, et al. 2004. Evidence for dynamically organized modularity in the yeast protein–protein interaction network. *Nature* 430:88–93
36. Han K, Tjaden B, Lory S. 2016. GRIL-seq provides a method for identifying direct targets of bacterial small regulatory RNA by in vivo proximity ligation. *Nature Microbiol.* 2:16239
37. Helwak A, Kudla G, Dudnakova T, Tollervey D. 2013. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. *Cell* 153:654–65
38. Holmqvist E, Unoson C, Reimegard J, Wagner EG. 2012. A mixed double negative feedback loop between the sRNA MicF and the global regulator Lrp. *Mol. Microbiol.* 84:414–27
39. Huntzinger E, Boisset S, Saveanu C, Benito Y, Geissmann T, et al. 2005. *Staphylococcus aureus* RNAIII and the endoribonuclease III coordinately regulate *spa* gene expression. *EMBO J.* 24:824–35
40. Hussein R, Lim HN. 2011. Disruption of small RNA signaling caused by competition for Hfq. *PNAS* 108:1110–15
41. Hussein R, Lim HN. 2012. Direct comparison of small RNA and transcription factor signaling. *Nucleic Acids Res.* 40:7269–79
42. Janzon L, Arvidson S. 1990. The role of the delta-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. *EMBO J.* 9:1391–99
43. Jeong H, Mason SP, Barabasi A-L, Oltvai ZN. 2001. Lethality and centrality in protein networks. *Nature* 411:41–42
44. Jost D, Nowojewski A, Levine E. 2013. Regulating the many to benefit the few: role of weak small RNA targets. *Biophys J.* 104:1773–82
45. Kalyana-Sundaram S, Kumar-Sinha C, Shankar S, Robinson DR, Wu Y-M, et al. 2012. Expressed pseudogenes in the transcriptional landscape of human cancers. *Cell* 149:1622–34
46. Karreth FA, Reschke M, Ruocco A, Ng C, Chapuy B, et al. 2015. The BRAF pseudogene functions as a competitive endogenous RNA and induces lymphoma in vivo. *Cell* 161:319–32
47. Kashtan N, Itzkovitz S, Milo R, Alon U. 2004. Topological generalizations of network motifs. *Phys. Rev. E* 70:031909
48. Lahav G. 2008. Oscillations by the p53-Mdm2 feedback loop. *Adv. Exp. Med. Biol.* 641:28–38
49. Lalaouna D, Carrier M-C, Semsey S, Brouard J-S, Wang J, et al. 2015. A 3′ external transcribed spacer in a tRNA transcript acts as a sponge for small RNAs to prevent transcriptional noise. *Mol. Cell* 58:393–405
50. Levine E, Hwa T. 2008. Small RNAs establish gene expression thresholds. *Curr. Opin. Microbiol.* 11:574–79
51. Levine E, Zhang Z, Kuhlman T, Hwa T. 2007. Quantitative characteristics of gene regulation by small RNA. *PLOS Biol.* 5:e229
52. Liu D, Chang X, Liu Z, Chen L, Wang R. 2011. Bistability and oscillations in gene regulation mediated by small noncoding RNAs. *PLOS ONE* 6:e17029

53. Majdalani N, Chen S, Murrow J, St John K, Gottesman S. 2001. Regulation of RpoS by a novel small RNA: the characterization of RprA. *Mol. Microbiol.* 39:1382–94
54. Mandin P, Gottesman S. 2010. Integrating anaerobic/aerobic sensing and the general stress response through the ArcZ small RNA. *EMBO J.* 29:3094–107
55. Mandin P, Guillier M. 2013. Expanding control in bacteria: interplay between small RNAs and transcriptional regulators to control gene expression. *Curr. Opin. Microbiol.* 16:125–32
56. Mangan S, Alon U. 2003. Structure and function of the feed-forward loop network motif. *PNAS* 100:11980–85
57. Massé E, Escorcia FE, Gottesman S. 2003. Coupled degradation of a small regulatory RNA and its mRNA targets in *Escherichia coli*. *Genes Dev.* 17:2374–83
58. Massé E, Gottesman S. 2002. A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *PNAS* 99:4620–25
59. Mehta P, Goyal S, Wingreen NS. 2008. A quantitative comparison of sRNA-based and protein-based gene regulation. *Mol. Syst. Biol.* 4:221
60. Melamed S, Peer A, Faigenbaum-Romm R, Gatt YE, Reiss N, et al. 2016. Global mapping of small RNA-target interactions in bacteria. *Mol. Cell* 63:884–97
61. Memczak S, Jens M, Elefsinioti A, Torti F, Krueger J, et al. 2013. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495:333–38
62. Michaux C, Verneuil N, Hartke A, Giard J-C. 2014. Physiological roles of small RNA molecules. *Microbiology* 160:1007–19
63. Mika F, Busse S, Possling A, Berkholz J, Tschowri N, et al. 2012. Targeting of *csgD* by the small regulatory RNA RprA links stationary phase, biofilm formation and cell envelope stress in *Escherichia coli*. *Mol. Microbiol.* 84:51–65
64. Mika F, Hengge R. 2005. A two-component phosphotransfer network involving ArcB, ArcA, and RssB coordinates synthesis and proteolysis of sigma^S (RpoS) in *E. coli*. *Genes Dev.* 19:2770–81
65. Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U. 2002. Network motifs: simple building blocks of complex networks. *Science* 298:824–27
66. Mitarai N, Benjamin J-A, Krishna S, Semsey S, Csiszovszki Z, et al. 2009. Dynamic features of gene expression control by small regulatory RNAs. *PNAS* 106:10655–59
67. Miyakoshi M, Chao Y, Vogel J. 2015. Cross talk between ABC transporter mRNAs via a target mRNA-derived sponge of the GcvB small RNA. *EMBO J.* 34:1478–92
68. Miyakoshi M, Chao Y, Vogel J. 2015. Regulatory small RNAs from the 3′ regions of bacterial mRNAs. *Curr. Opin. Microbiol.* 24:132–39
69. Mizuno T, Chou MY, Inouye M. 1984. A unique mechanism regulating gene expression: Translational inhibition by a complementary RNA transcript (micRNA). *PNAS* 81:1966–70
70. Moon K, Gottesman S. 2011. Competition among Hfq-binding small RNAs in *Escherichia coli*. *Mol. Microbiol.* 82:1545–62
71. Morfeldt E, Taylor D, von Gabain A, Arvidson S. 1995. Activation of alpha-toxin translation in *Staphylococcus aureus* by the *trans*-encoded antisense RNA, RNAIII. *EMBO J.* 14:4569–77
72. Ng W-L, Bassler BL. 2009. Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* 43:197–222
73. Nitzan M, Fechter P, Peer A, Altuvia Y, Bronesky D, et al. 2015. A defense-offense multi-layered regulatory switch in a pathogenic bacterium. *Nucleic Acids Res.* 43:1357–69
74. Nitzan M, Shimoni Y, Rosolio O, Margalit H, Biham O. 2015. Stochastic analysis of bistability in coherent mixed feedback loops combining transcriptional and posttranscriptional regulations. *Phys. Rev. E* 91:052706
75. Nitzan M, Steiman-Shimony A, Altuvia Y, Biham O, Margalit H. 2014. Interactions between distant ceRNAs in regulatory networks. *Biophys. J.* 106:2254–66
76. Novick R. 2000. Pathogenicity factors and their regulation. In *Gram-Positive Pathogens*, ed. VA Fischetti, RP Novick, JJ Ferretti, DA Portnov, JJ Rood, pp. 392–407. Washington, DC: ASM Press
77. Overgaard M, Johansen J, Møller-Jensen J, Valentin-Hansen P. 2009. Switching off small RNA regulation with trap-mRNA. *Mol. Microbiol.* 73:790–800

78. Papenfort K, Espinosa E, Casadesus J, Vogel J. 2015. Small RNA-based feedforward loop with AND-gate logic regulates extrachromosomal DNA transfer in *Salmonella*. *PNAS* 112:E4772–81
79. Papenfort K, Vanderpool CK. 2015. Target activation by regulatory RNAs in bacteria. *FEMS Microbiol. Rev.* 39:362–78
80. Poliseno L, Salmena L, Zhang J, Carver B, Haveman WJ, Pandolfi PP. 2010. A coding-independent function of gene and pseudogene mRNAs regulates tumour biology. *Nature* 465:1033–38
81. Prévost K, Desnoyers G, Jacques J-F, Lavoie F, Massé E. 2011. Small RNA-induced mRNA degradation achieved through both translation block and activated cleavage. *Genes Dev*. 25:385–96
82. Ramirez-Peña E, Treviño J, Liu Z, Perez N, Sumby P. 2010. The group A *Streptococcus* small regulatory RNA FasX enhances streptokinase activity by increasing the stability of the *ska* mRNA transcript. *Mol. Microbiol*. 78:1332–47
83. Sedlyarova N, Shamovsky I, Bharati BK, Epshtein V, Chen J, et al. 2016. sRNA-mediated control of transcription termination in *E. coli*. *Cell* 167:111–21
84. Shen-Orr SS, Milo R, Mangan S, Alon U. 2002. Network motifs in the transcriptional regulation network of *Escherichia coli*. *Nat. Genet.* 31:64–68
85. Shimoni Y, Friedlander G, Hetzroni G, Niv G, Altuvia S, et al. 2007. Regulation of gene expression by small non-coding RNAs: a quantitative view. *Mol. Syst. Biol.* 3:138
86. Storz G, Vogel J, Wassarman KM. 2011. Regulation by small RNAs in bacteria: expanding frontiers. *Mol. Cell* 43:880–91
87. Sumazin P, Yang X, Chiu H-S, Chung W-J, Iyer A, et al. 2011. An extensive microRNA-mediated network of RNA-RNA interactions regulates established oncogenic pathways in glioblastoma. *Cell* 147:370–81
88. Thomason MK, Fontaine F, De Lay N, Storz G. 2012. A small RNA that regulates motility and biofilm formation in response to changes in nutrient availability in *Escherichia coli*. *Mol. Microbiol.* 84:17–35
89. Thomson DW, Dinger ME. 2016. Endogenous microRNA sponges: evidence and controversy. *Nat. Rev. Genet.* 17:272–83
90. Tree JJ, Granneman S, McAteer SP, Tollervey D, Gally DL. 2014. Identification of bacteriophage-encoded anti-sRNAs in pathogenic *Escherichia coli*. *Mol. Cell* 55:199–213
91. Vanderpool CK, Balasubramanian D, Lloyd CR. 2011. Dual-function RNA regulators in bacteria. *Biochimie* 93:1943–49
92. Večerek B, Moll I, Bläsi U. 2007. Control of Fur synthesis by the non-coding RNA RyhB and iron-responsive decoding. *EMBO J.* 26:965–75
93. Vogel J, Luisi BF. 2011. Hfq and its constellation of RNA. *Nat. Rev. Microbiol.* 9:578–89
94. Wadler CS, Vanderpool CK. 2007. A dual function for a bacterial small RNA: SgrS performs base pairing-dependent regulation and encodes a functional polypeptide. *PNAS* 104:20454–59
95. Wagner EG, Romby P. 2015. Small RNAs in bacteria and archaea: who they are, what they do, and how they do it. *Adv. Genet.* 90:133–208
96. Wang D, Yan K-K, Sisu C, Cheng C, Rozowsky J, et al. 2015. Loregic: a method to characterize the cooperative logic of regulatory factors. *PLOS Comput. Biol.* 11:e1004132
97. Waters LS, Storz G. 2009. Regulatory RNAs in bacteria. *Cell* 136:615–28
98. Waters SA, McAteer SP, Kudla G, Pang I, Deshpande NP, et al. 2017. Small RNA interactome of pathogenic *E. coli* revealed through crosslinking of RNase E. *EMBO J.* 36:374–87