Small RNAs establish gene expression thresholds
Erel Levine and Terence Hwa

The central role of small RNAs in regulating bacterial gene expression has been elucidated in the past years. Typically, small RNAs act via specific basepairing with target mRNAs, leading to modulation of translation initiation and mRNA stability. Quantitative studies suggest that small RNA regulation is characterized by unique features, which allow it to complement regulation at the transcriptional level. In particular, small RNAs are shown to establish a threshold for the expression of their target, providing safety mechanism against random fluctuations and transient signals. The threshold level is set by the transcription rate of the small RNA and can thus be modulated dynamically to reflect changing environmental conditions.

**Introduction**

In recent years, it has been noticed that small non-coding RNAs (referred to here as sRNAs) can exert significant regulatory effect on gene expression in bacteria [1,2]. Early on, sRNAs were mainly recognized for their specialized roles in controlling the transposition of insertion elements [3], in regulating plasmid copy number [4,5], and in mediating plasmid maintenance through the toxin–antidote systems [6]. Only a few cases involving chromosomally encoded sRNA were known [7,8]. The advent of genomic and bioinformatic methods has led to the identification and the subsequent verification of numerous chromosomal sRNAs in a variety of bacterial species during this decade [9–11]. These sRNAs are involved predominantly in stress response pathways [12] and pathogenesis [13], but they are also found in the regulation of metabolism [7,14], transport [15], quorum sensing [16], and more.

Small RNAs bind specifically to the mRNA molecules of their target, in a manner that relies both on sequence complementarity and on secondary structure. Like regulatory proteins, many of these sRNAs act in trans, regulate the expression of multiple target genes, and are themselves regulated by one or more factors. Small RNAs – like proteins – can act as activators or repressors of gene expression by modulating translational efficiency and mRNA stability.

A great deal of progress has been made in the past few years in elucidating the mechanisms of sRNA-mediated regulation of gene expression, especially for the major class of post-transcriptional repressors [2,17–20]. Typically, translational repression results from binding of the sRNA to the translational initiation region of the target mRNA. Importantly, this binding may lead to the rapid degradation of both the sRNA and the target mRNA [21]. Thus, unlike regulatory proteins, which exert their effect on gene expression in a catalytic fashion, these sRNAs are consumed as they exert their regulatory effects. (In some cases, the RNA complex may be highly stable, trapping both RNAs in an inert state [21,22]. From functional point of view, they are no different from rapid co-degradation and we will not distinguish the two in the discussion below.)

At the level of ‘Boolean logic’, namely when gene expression is only considered to be either ‘on’ or ‘off’, transcriptional and post-transcriptional regulators may seem redundant. Recent studies suggest that sRNA repressors are in fact distinct from transcriptional repressors at a quantitative level and that its unique characteristics may be closely related to the physiological roles of sRNA regulators. To establish these results, a simple mathematical model (Box 1) has been formulated [16,24,25], and different aspects of it have been studied theoretically [25,26] and experimentally [25,26]. Here we review and expand upon the current state of knowledge on the quantitative aspects of sRNA regulation.

**Small RNAs establish threshold for gene expression**

Consider the case of an sRNA with a single target at steady state. If the rate of sRNA transcription (αs) is larger than that of the mRNA (αm < αs), then most of the targets are expected to pair rapidly with the sRNAs, and the target will not be expressed. Conversely, if αs > αm then most of the sRNAs pair with the target while the unpaired mRNAs are free to direct protein translation. In the latter case, the expressed protein level would reflect the difference between the two transcription rates, that is, be linearly proportional to αs − αm. This qualitative expectation is summarized by the schematic response shown in

---

[Box 1: Mathematical Model for Small RNA Regulation]
Box 1 Quantitative model for gene regulation by small RNA.

A quantitative study of gene regulation by small RNA starts with a simple mathematical model. In constructing such a model, the most prominent feature is the possibility that the small RNA itself is consumed via the interaction between sRNAs and their mRNA targets. Two additional assumptions, namely that the association and dissociation rates occur rapidly and that the RNA complex cannot be translated, lead to a minimal model that focuses on the free small RNA and mRNA molecules. This model takes the form

\[
\frac{d}{dt} \frac{a}{C_1} = \alpha_s - \beta_s - \rho \kappa m
\]

\[
\frac{d}{dt} \frac{m}{C_1} = \alpha_m - \beta_m - \kappa m
\]

where \(\alpha_s, \alpha_m\) are the transcription rates of the sRNA, mRNA; \(\beta_s, \beta_m\) are the turnover rates for the RNA species alone, and \(\kappa\) is the rate of coupled degradation of sRNA with its target. The parameter \(\rho\) accounts for the possibility that the degradation of the small RNA molecule due to binding may not be the same as that of the mRNA; it is set to 1 throughout this review as it does not make a qualitative difference to the results [25**].

The transcription rates of the two RNAs can readily be changed dynamically by regulating the activity at their promoters. More hard-wired are the other parameters, namely the turnover rates of the two RNAs and their coupled degradation. Small RNAs that are involved in gene regulation are likely to be stable in the absence of their targets, as demonstrated for both cis and trans acting sRNAs [32–34]. Thus, the value of \(\beta_s\) is dictated by the growth rate of the cell. Messenger RNAs in E. coli are typically unstable, with degradation rate of a few minutes. It is possible that mRNAs that are targeted by sRNAs are on the more stable side [23,32–35], say \(\beta_m \leq (10 \text{ min})^{-1}\). The binding rate \(\kappa\) can depend both on sequence complementarity and secondary structure of the RNA molecules. The value of \(\kappa\) has been estimated for several sRNA/target pairs to be around 1/50 (nM min)\(^{-1}\) [21,25**,35,36]. Some authors have estimated this value to be larger; but since this value is already close to the diffusion-limited association rate for typical small proteins [37,38], we do not expect it to be much higher \(\text{in vivo}\). In any case, the results presented here remain valid as long as \(\kappa > \beta_m \beta_s \alpha_m \alpha_s / \text{threshold} \sim 1/500 (\text{nM/min})^{-1}\).

Many bacterial small regulatory RNAs are involved in regulating stress response pathways. It is often the case in stress responses that an external signal triggers the execution of a cellular response that may be expensive (in terms of energy and nutrients) or even toxic [39,40]. In such cases, small RNAs may define a threshold for activation of the response. This threshold may reflect the level of tolerance of the cell to that particular stress. For example, the transcription rate of the small RNA RyhB reflects the concentration of free Fe\(^{2+}\) ions in the cell, which potentially imposes a oxidative stress through the Fenton Reaction [32]. Indeed, RyhB sets a threshold for the expression of the superoxide dismutase FeSOD [25**], which ameliorates the stress.

Small RNAs suppress fluctuations

Gene expression is a stochastic process, involving synthesis of individual molecules through a series of orchestrated yet random events [41,42]. Repression of gene expression may result in a small number of proteins per cell, raising the possibility of significant temporal and cell-to-cell fluctuations.

Consider first a gene whose transcription is tightly repressed by a transcription factor. In this case, transcription events are rather rare (can occur for example only a few times every hour [43,44]). Every transcription event is then amplified by recurring translation events, resulting in a burst of proteins, see Figure 2a. The size of this burst is controlled by the rate of translation of this particular mRNA, multiplied by the lifetime of the RNA molecule. Thus, expression of a gene that is repressed transcriptionally is ‘bursty’, with long periods of silence punctuated with (sometimes large) bursts of proteins [45].

Suppressing the expression of a gene by a small RNA – rather than at the level of transcription – relieves the genetic system from the effect of rare transcription events. The continuous transcription of both the target and the sRNA makes the kinetics much more homogeneous. The two effects of a small RNA – to reduce the rate of translation and to decrease the lifetime of the mRNA molecules – serve to decrease the protein burst size [25**]. Together, these effects can keep the expression of a gene repressed by a small RNA at a steady low...
Small RNAs suppress fluctuations in gene expression. Computer simulations were performed for a gene that is repressed by (a) a transcription factor or (b) a small RNA. Upper panels show the cellular protein levels as a function of time, and lower panels show the individual transcription events. In the case of transcriptional repression (a), rare transcription events are accompanied by large protein bursts. In the case of post-transcriptional repression (b), transcription events are frequent, but the small RNA pool reduces the bursts. In these simulations, parameters for the first gene were chosen on the basis of the known parameters of the lac operon in *E. coli*. Required parameters for the second gene were used to ensure that the mean protein numbers are equal in both cases.

Small RNAs can provide rapid response while filtering transient signals

The threshold effect described above has a distinct temporal signature as well. Suppose that a cell is changed from an environment where the small RNA highly suppresses the target (i.e., with $\alpha_1 > \alpha_m$) to one where its expression is no longer required ($\alpha_1 = 0$). The response in target gene expression will occur via two steps. First, the small RNA pool present originally in the cell is depleted. Only after then can target mRNA and proteins accumulate. Under the assumptions mentioned above, the transition time ($t_\alpha$) between these two steps is approximately $t_\alpha = \beta_1^{-1}\log(\alpha_1/\alpha_m)$. This scenario is depicted in Figure 3a (red), where, for comparison, the de-repressing kinetics for an analogous protein-regulated gene is also shown (blue).

Let us focus on two salient features of sRNA dynamics, which make the de-repression kinetics very different from that of protein repressors. First the response time $t_\alpha$ can be reduced across the physiologically important range of time scales, from over an hour down to less than 10 min, simply by increasing the target expression $\alpha_m$. This is very different from the case of protein regulators, for which the waiting time is determined primarily by the protein turnover rate, which enforces a ‘speed limit’ for protein-mediated response. Small RNAs do not submit to this limit, since their targets can actively degrade them. Thus, sRNA-mediated regulation may be one of the viable regulatory strategies in situations where a rapid temporal response is physiologically important for the cell.

The other feature is that changes in the target transcription level do not propagate to changes in protein levels at times shorter than $t_\alpha$. Transient changes in the signals that control $\alpha_m$ that persist for shorter periods are filtered by the small RNA regulator (Figure 3b). This, for example, may be an important feature in SOS response, where the stress is transient in nature (persisting between the occurrence of DNA damage and its repair), and of regulation of iron homeostasis that must overcome iron pulses used by some hosts to fight pathogens.

Coordinated response of sRNA targets

Many of the known small RNAs have more than one mRNA target. These targets are expected to have different affinities for association with the small RNA, resulting from different lengths of the base-pairing regions and different secondary structure. Different interaction strengths between sRNA and its targets should...
have little effect on the threshold when the target is expressed alone; however, when expressed simultaneously, the different mRNA species will compete for association with the same pool of sRNA molecules. The outcome of this competition necessarily depends on both the affinities and the abundances of the different mRNA species involved.

Generalizing the model (Box 1) to the case of multiple targets is straightforward [25,29]. One predicted con-
sequence of this hierarchy is an ordering (or prioritization) of the targets. For example, consider a case of two targets, with $k_1 \gg k_2$. In this case, depending on the value of $\alpha$, the steady state may be such that either both targets are expressed (low $\alpha$), only target 2 is expressed (intermediate $\alpha$), or none. This prioritization is similar to the ordered transcriptional response of different genes controlled by a common transcription factor, where prioritization is governed by the equilibrium binding constants to various operator sites [52].

However, targets of a small RNA are not only affected by their regulator, they also deplete it. Thus, an increased expression of one target enhances the expression of another target, by reducing the level of its inhibitor [25**]. Consider for example the case where the transcription rate of a sRNA regulator is larger than that of either targets ($\alpha_1$ and $\alpha_2$) but smaller than their sum ($\alpha_1 + \alpha_2$). Owing to the threshold-linear nature of the response (Figure 1), either target is expected to be silenced when transcribed alone, but can be expressed if both are transcribed together.

A more quantitative analysis of this case reveals a hierarchy between the expression of different targets. Expression of a strongly bound target is predicted to have a strong effect on the expression of weakly bound targets, while the expression of a weakly bound target is predicted to have a much weaker effect on the former. This behavior has been observed for different targets of the small RNA RyhB in E. coli [25**]. The existence of this mechanism offers a possibility for sRNA regulatory systems to set up a complex hierarchy of responses based on the expression of the targets as well as the regulator.

Conclusions
Quantitative studies of gene regulation by small RNA in bacteria suggest that small RNAs establish a threshold for gene expression. This threshold can be dynamically tuned as a response to changes in environmental conditions. Consequently, fluctuations that stem from the stochastic nature of gene expression, as well as transient changes in environmental conditions, can be strongly suppressed.

Although a number of the novel features involving sRNA regulation have been demonstrated for specific systems, it remains to be shown how such features may be utilized in physiological responses. Regardless, these features and possibilities should be taken into account when analyzing the responses of systems involving sRNA regulation.

Acknowledgements
We thank Mathew Scott, Ned Wingreen, and Johan Paulsson for discussions, and the experimental members of the Hwa lab, in particular Zhongge Zhang and Tom Kuhlman, for initiating the experimental studies. Our sRNA work is supported by NIH (Grant RO1GM77296) and by the NSF (Grant PHY-0822283) through the PFC-sponsored Center for Theoretical Biological Physics.

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:
• of special interest
• of outstanding interest


A recent review of bacterial small RNA regulation, covering sRNAs encoded on plasmids, transposons, and the chromosome.


This is a comprehensive quantitative study of the role played by cis-acting small RNAs in controlling plasmid copy number. While the mechanism— and hence the details of the model—are somewhat different from those discussed here, small RNA also provide threshold behavior and noise suppression in this system.


In this review, Aiba presents a detailed picture of the mechanism by which the best studied class of small RNAs suppress the expression of their targets.


Small RNAs establish gene expression thresholds Levine and Hwa 579


This detailed quantitative study of small RNA regulation in E. coli demonstrates how interplay between theoretical modeling and quantitative experiments may provide a unique perspective on the features of small RNAs.


Legewie et al. use a simple model to interpret their previous experiments on the cyanobacterial sRNA isrR and suggest that sRNAs may filter transient signals.


