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Small RNAs establish gene expression thresholds

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Abstract

The central role of small RNAs in regulating bacterial gene expression has been elucidated in the last 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]. In other cases, the RNA complex may be highly stable, trapping both RNAs in an inert state [22,23]. Thus,

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unlike regulatory proteins, which exert their effect on gene expression in a catalytic fashion, these sRNAs are *consumed* as they exert their regulatory effects.

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 [25–30] and experimentally [25–27]. 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 transcription of the sRNA (α_s) is more frequent than that of the mRNA ($\alpha_m < \alpha_s$), then most of the targets are expected to pair rapidly with the sRNAs, and the target will not be expressed. Conversely, if $\alpha_m > \alpha_s$ 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, i.e., be linearly proportional to $\alpha_m - \alpha_s$. This qualitative expectation is summarized by the schematic response shown in Figure 1, where the target transcription rate α_m is taken to be continuously tunable at a given sRNA transcription rate α_s . This behavior is referred to as a *threshold-linear* response [25]. A key feature of this form is that different values of α_s shift the position of the threshold, without changing the slope of the linear rise beyond it. Associated with the threshold at $\alpha_m \approx \alpha_s$ is the ultrasensitivity of the response, namely an abrupt switch-on of target expression via a small change in target transcription [16,24]. This type of behavior has been observed experimentally in *E. coli* using a synthetic reporter target [25].

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, and thus reflects the level of tolerance to superoxidative stress [32]. Indeed, RyhB sets a threshold for the expression of the superoxide dismutase SodB [25], which responds to that 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 – immediately 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 [25,27]. Together, these effects can keep the expression of a gene repressed by a small RNA at a *steady* low level; compare figures 2a and 2b. Small RNAs may also repress protein bursts that stem from possible bursts of transcriptional initiation events [30,46].

This difference in the noise properties may be very important in genetic circuits where a large burst of proteins will switch a cell from one stable state to another; see Box 2 for an illustrative example. In cases such as stress responses where unintentional entry into the alternative state may be harmful and spontaneous switching is to be avoided, sRNA-mediated regulation might possess a distinct advantage.

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 (so that $\alpha_s > \alpha_m$) to one where its expression is no longer required ($\alpha_s = 0$). The response in target gene expression will occur via two steps [26]. First, the sRNA pool present in the cell at the time of the transition is depleted. Only then can target mRNA and proteins can accumulate. Under the assumptions mentioned above, the transition time (t_x) between these two steps can be shown to be approximately by $t_x = \beta_s^{-1} \log(\alpha_s/\alpha_m)$. 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, which make the de-repression kinetics in the two cases very different. First the response time t_x 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 α_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 [47]. 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 [28].

The other feature is that changes in the target transcription level do not propagate to changes in protein levels at times shorter than t_x . Transient changes in the signals that control α_m that persist for shorter periods are filtered by the small RNA regulator (figure 3b) [26]. 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 [48].

Coordinated response of sRNA targets

Many of the known small RNAs have more than one mRNA target [49–51]. 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 consequence 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 α_s , the steady state may be such that either both targets are expressed (low α_s), only target 2 is expressed (intermediate α_s), 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 (α_1 and α_2) but smaller than their sum ($\alpha_1 + \alpha_2$). Due 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.

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 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 which only accounts for the free small RNA and mRNA molecules. This model takes the form [16,25,31]

$$\begin{aligned}\frac{d}{dt}s &= \alpha_s - \beta_s - pkm_s \\ \frac{d}{dt}m &= \alpha_m - \beta_m - kms\end{aligned}\tag{1}$$

where α_s , α_m are the transcription rates of the sRNA, mRNA, β_s , β_m are the turnover rates for the RNA species alone, and k is the rate of coupled degradation of sRNA with its target. The parameter p 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 β_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 k can depend both on sequence complementarity and secondary structure of the RNA molecules. The value of k has been estimated for several sRNA/target pairs to be around $1/50 \text{ (nM min)}^{-1}$ [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 can be much higher *in vivo*. In any case, the results presented here remain valid as long as $k \gg \beta_m \beta_s / \alpha_{\text{threshold}} \sim 1/500 \text{ (nM/min)}^{-1}$.

Box 2: small RNAs stabilize multi-stable genetic systems

We consider as an example an auto-activated gene (*R*), that may be (slightly) repressed by a small RNA (*s*), as depicted on the left. In some cases, this system may sustain two stable states, of high or low expression. Leakiness in the expression of the target is the main reason why a low-expression state is typically unstable. **(a)** A bifurcation diagram for this system, with (red) and without (blue) the small RNA. Solid and dashed lines represent protein concentrations that are stable and unstable, resp. Note that in the presence of the small RNA, bi-stability is achieved over a wider range of leakiness. Conversely, the presence of the small RNA has almost no effect on the high state. **(b)** Even when the system is bi-stable, random bursts of protein may take the system from the low-expression state to the high-expression one. Thus, the probability to remain in the low state decreases with time. However, since small RNA serve to suppress these bursts, it decreases much slower in the presence of sRNA (red) than in its absence (blue).

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Figure 1. Threshold linear response

The expression of a target gene is silenced as long as its transcription rate, α_m , is smaller than that of the small RNA, α_s . Below this dynamically defined threshold, the small RNAs buffer translational noise and transient changes in environmental conditions. Above the threshold, the effect of the small RNA become negligible.

Figure 2. 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 based on 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.

Figure 3. the kinetic of de-repression

(a) Consider a case where at $t=0$ the cells are transferred into an environment which suppressed expression of the repressor, either a protein transcription factor (blue) or a sRNA (red). Dynamics in the sRNA case is predicted to be more abrupt, with a delay period t_X that can be tuned by α_s . (b) Transient signals that suppress sRNA expression are not expected to have significant effects, if their duration is smaller than t_X (≈ 30 min in this case).