Supporting Information

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SI Text

Mathematical Model of sRNA and mRNA Dynamics. The coupling between an sRNA (s) and its target mRNA (m) is described through the coupled equations (1-4):

$$\frac{ds}{dt} = \alpha_s - \frac{s}{\tau_s} - \delta \cdot s \cdot m\,, \qquad [1]$$

$$\frac{dm}{dt} = \alpha_s - \frac{m}{\tau_m} - \delta \cdot s \cdot m\,, \qquad [2]$$

where α_s and α_m set the production rates for the 2 RNAs, and τ_s and τ_m define the background degradation times. The reaction rate of the coupled degradation is given by the parameter δ. By measuring time in units of the background sRNA degradation time, $t \rightarrow t/\tau_s$ and measuring concentrations in units of $\alpha_m\tau_s$, we rescale $s \rightarrow s/(\alpha_m\tau_s)$ $m \rightarrow m/(\alpha_m\ \tau_s)$ and obtain Eqs. **3** and **4**:

$$\frac{ds}{dt} = \alpha - s - \gamma \cdot s \cdot m\,, \qquad [3]$$

$$\frac{dm}{dt} = 1 - \frac{m}{\tau} - \gamma \cdot s \cdot m\,, \qquad [4]$$

where $\alpha = \alpha_s/\alpha_m$, $\gamma = \delta\alpha_m\tau_s^2$, and $\tau = \tau_m/\tau_s$. The behavior of the system is thus entirely determined by the values of these 3 dimensionless parameters.

The regulatory input via small RNA is typically done through transcriptional regulation of α, as for example seen in the Fur–RyhB system in *E. coli*, or the CRP–Spot42 coupling in the galactose system in *E. coli*. For example in case of a single repressor R acting with Hill coefficient one, then $\alpha \propto 1/(1 + R)$, where the repressor concentration R is measured in units of its binding constant. To understand the importance of α, notice that if $\alpha < 1$, then it will be impossible to produce enough sRNA to degrade all targets. As a consequence, the target will not be repressed completely. In contrast to this, a value of $\alpha > 1$ allows efficient active degradation of the target, which can be nearly completely eliminated. In real systems, the dynamics of regulation will be associated with a change in α that will produce a response on a time scale that will also depend on the parameter γ.

In contrast to α, γ is not easily changed, as it is related to the irreversible association of sRNA to a given mRNA. This process is mediated by the abundant protein Hfq. Not much is understood about the details of this reaction, but it is likely that each individual sRNA–mRNA pair would have a specific value of γ. In general, one would expect that a higher degree of base-pair matching between the sRNA and its target would correlate with higher value of γ.

In the above equation there is a third parameter, τ, which sets the passive degradation time of the mRNA in the absence of sRNA. In most of the cases, sRNAs have a longer natural half-life than mRNAs, partly because Hfq often protects the sRNAs (5–7). Accordingly, we mostly expect τ to be < 1. The value of τ determines the steady-state level ($m = \tau$ in dimensionless units) of mRNAs in the absence of sRNA. In the following, we use $\tau = 0.2$ unless otherwise specified, as this value reflect the *sodB* target degradation in units of the relative stable RyhB (8).

Effect of Multiple Targets in Estimation of γ for RyhB–*sodB* System. In the estimation, we used the degradation data of *sodB* by induction of RyhB, but we do not have the degradation data for other target mRNAs. Therefore, we cannot estimate the differences in γ between targets. Our computation in Fig. 3 assumed that γ values for all target mRNAs are in a similar range. To see how much this assumption affect the result, we simulated degradation kinetics in the cases where γ for *sodB* is 400 but the other targets have 10-fold higher or lower γ; the difference in degradation time T for *sodB* in these simulations was within 1 min.

Measurement of Promoter Activities in the Gal Operon/Spot42 System. Promoter activities were measured by quantitation of RNA band intensities obtained by in vitro transcription assays. Transcription reactions were performed as described (9) using pSEM2008 and pSEM2008spf plasmid DNA templates. The pSEM2008 plasmid was obtained by inserting the DNA fragment containing the *rrnBT1T2* terminators (nucleotides 4141–4559) from pKK223–3 (Amersham Pharmacia; GenBank M77749) between the KpnI and EcoRI sites of pSA850 (10), upstream of the $P1_{galE}$ and $P2_{galE}$ promoters. Transcription of $P1_{galE}$ and $P2_{galE}$ is terminated by the *rpoC* terminator 125 nt downstream of the $P2_{galE}$ start site. The pSEM2008spf plasmid was created by the insertion of the −147 to + 33 region (using + 1 as a transcription start site in the numbering) of the *spf* gene between the EcoRI and PstI sites of pSEM2008. The in vitro transcription reaction mixture (50 μL) contained 20 mM Tris acetate (pH 7.8), 10 mM magnesium acetate, 200 mM potassium glutamate, and 2 nM supercoiled plasmid DNA template. RNA polymerase (20 nM) was added before incubating the reactions at 37 °C for 5 min. Transcription was started by the addition of 1.0 mM ATP, 0.1 mM GTP, 0.1 mM CTP, 0.01 mM UTP, and 5 mCi of [α-^{32}P]UTP (3,000 Ci/mmol). Reactions were terminated after 10 min by addition of an equal volume of transcription loading buffer (0.025% bromophenol blue, 0.025% xylene cyanol, 0.01 M EDTA and 90% deionized formamide). After heating at 90 °C for 3 min, the samples were loaded onto 7% polyacrylamide-urea DNA sequencing gels. RNA bands were quantified by using the ImageQuant PhosphorImager (Molecular Dynamics). Band intensities were normalized to the RNA1 transcript level and corrected with the background and the number of uracils incorporated into the different RNA species.

Measurement of Promoter Activities by β-Galactosidase in the RyhB System. Derivatives of *E. coli* MG1655 were used in all experiments. DH5α strain was used for routine cloning procedures. EM1055 (MG1655 Δ_{lac}) and EM1238 (EM1055 *ryhB*::*cat*) strains have been described (8). Transcriptional *lacZ* fusions were constructed by inserting a PCR product (chromosomal DNA as template) into pFRΔ as described (11). PCR products containing the promoter region of selected genes were generated by using oligos (see Table S1 for sequences) EM423 and EM424 (*sodB*), EM107 and EM117 (*sdh*), EM531 and EM551 (*acnB*), EM533 and EM534 (*fumA*), and EM487 and EM488 (*ryhB*). PCR products were digested by EcoRI and BamHI and ligated into *Eco*RI/*Bam*HI-digested pFRΔ to generate *sodB-lacZ*, *sdh-lacZ*, *acnB-lacZ*, *fumA-lacZ*, and *ryhB-lacZ*, respectively. Transcriptional *lacZ* fusions were delivered in single copy into the bacterial chromosome at the λ *att* site as described (12). Stable lysogens were screened for single insertion of recombinant λ by PCR (13).

Kinetic assays for β-galactosidase activity were performed as described (14) by using a SpectraMax 250 microtiter plate reader (Molecular Devices). Briefly, overnight bacterial cultures incu-

bated in LB media at 37 °C were diluted 1,000-fold into 50 mL of fresh LB media at 37 °C. Cultures were grown with agitation to an OD_{600} of 0.5 before inducing RyhB expression by adding 200 μM of 2,2′-dipyridyl (for strains EM1055 or EM1238). Specific β-galactosidase activities were calculated by using the formula V_{max}/OD_{600}. The results reported represent data typical of at least 3 experimental trials.

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Table S1. Sequence of oligonucleotides used for strain construction in the RyhB system

Oligo name	Sequence	Description
EM423	CAGTTGAATTCGGCGTGTATGTCGGCAACGG	*sodB* forward
EM424	GCTAGGGATCCTCGATGGTTTCCGCAGAAATG	*sodB* reverse
EM107	CCGATGAATTCTAACTGTCCCGAATGAATTGGTC	*sdh* forward
EM117	GCTAGGGATCCAGGACGATAGCGGTAGCG	*sdh* reverse
EM531	GCTAGGGATCCTAGACCATCCTTAACGATTCAG	*acnB* forward
EM551	GCTAGGGATCCATTTGGTTTGCATCCAGGGG	*acnB* reverse
EM533	CAGTTGAATTCCTATTAAAGCAAGAATCCTACGG	*fumA* forward
EM534	GCTAGGGATCCTCAGTATCATCTTTTTTGAGTG	*fumA* reverse
EM487	CAGTTGAATTCCCGTGTTTCTGCGTGGCG	*ryhB* forward
EM488	GCTAGGGATCCGCGAGACAATAATAATCATTC	*ryhB* reverse