Positive regulatory dynamics by a small noncoding RNA: speeding up responses under temperature stress†

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Received 21st November 2011, Accepted 9th March 2012
DOI: 10.1039/c2mb05479e

Recent discoveries of noncoding regulatory RNAs have led to further understanding of the elements controlling genetic expression. In E. coli, most of those ncRNAs for which functional knowledge is available were shown to be dependent on the Hfq RNA chaperone and to act as inhibitors of translation by base pairing with their mRNA target. Nevertheless, there are also some examples where the sRNA plays a role of a translational activator, structurally enhancing ribosome binding to mRNA. In this work, we seek to understand the dynamics of DsrA-based positive regulation of rpoS mRNA, encoding the σS RNA polymerase subunit, and to understand how it helps to mitigate environmental stress in bacteria. Our analysis is based on the first absolute quantification of the copy number of both the sRNA and of its corresponding mRNA in combination with mathematical models for post-transcriptional regulation. We show that on average, DsrA is present at a ratio of 3 to 24 copies per cell, while an rpoS transcript is present at a level of 1 to 4 copies per cell, both levels increasing when temperature is decreased. Our analysis supports the idea that temperature dependency of DsrA degradation is not a crucial condition for the attainment of observed DsrA steady levels, but highlights when temperature is decreased. Our analysis also reveals how reversibility of RNA complex formation and σS-regulated degradation act to reduce intrinsic noise in σS induction. Taking into account the importance of this master regulator, which allows E. coli as well as other important pathogens to survive their environment, the present work contributes to complete the panel of multiple signals used to regulate bacterial transcription.

Introduction

First considered as intermediates of gene expression, RNAs are now recognized as key players in various cellular processes. For instance, RNA–RNA interactions play important roles in both prokaryotic and eukaryotic cells, where noncoding RNAs are used for post-transcriptional regulation. One advantage of using an RNA for genetic regulation comes from its faster synthesis and decay compared to that of a protein, thus allowing a more rapid and transient response in order to adapt to changes in physiological conditions. In E. coli, there are around 150 known noncoding RNAs, which are evolutionary conserved and 50–300 nucleotides long (hence the term sRNA for small RNA2–4). Bacterial sRNAs often act by base pairing with an mRNA to regulate diverse pathways in response to environmental stresses or virulence. The transcription of the sRNA is itself regulated under physiological conditions; for instance, SgrS sRNA is induced upon phosphosugar stress,5 whereas DsrA is expressed when temperature is reduced6 and MicA is produced after a membrane stress.7 Nevertheless, in contrast to classical antisense RNA, stress-related bacterial sRNAs usually form imperfect duplexes with their targets such that base pairing needs to be stimulated by the RNA chaperone Hfq.8,9

Most sRNAs for which knowledge is available were shown to bind to their target mRNAs to inhibit translation and, as a consequence, to down-regulate the level of the encoded protein. There are, however, also some examples where the sRNA plays a role as a translational activator, structurally enhancing ribosome binding of the mRNA target.10,11 In the present study, we have used the model of DsrA sRNA to address major questions about how positive regulation by Hfq-dependent sRNA occurs in vivo. DsrA is an 87 nucleotide sRNA. Two of its targets are important transcriptional regulators: the σS RNA polymerase subunit, a stress response...
master regulator encoded by rpoS and H-NS, a nucleoid protein and transcriptional repressor. The action of DsrA stems from RNA–RNA interactions as it base pairs with hns and rpoS mRNAs. Even if the mechanism used for hns regulation is still unclear, DsrA binds the hns mRNA in order to inhibit its translation. This regulation is still unclear, DsrA binds the hns mRNA in order to inhibit its translation.15 This self-assembly could result in structures that inhibit reverse transcription and/or amplification of a part of the RNA. Therefore, we first confirmed that the polymeric form of DsrA (sliced-out from a gel) could be reverse-transcribed and amplified (not shown). Note that taking into account the constraints for reverse transcription and amplification by quantitative-PCR of the small sRNA (87 nt), we consider that DsrA quantification measures copies of full length DsrA (including polymers) but not processed DsrA fragments.

The validity of our absolute quantification was established by adding a known amount of the DsrA transcript (3 × 10^7 copies) to 2 μg of total RNA extracted from a dsrA-deletion mutant strain. Under these conditions, our quantification method allowed the detection of the total amount of exogenous sRNA added in each sample (with 15% standard deviation), which validates our assay.

In all, as shown in Fig. 1, our results indicate that DsrA was present at a ratio of 3 to 24 copies per cell on average, and that its levels increased when the temperature decreased (37 to 16 °C). Our results are thus fully in agreement with those reported previously6,22,25 but provide the first absolute quantification of this noncoding RNA in the cell.

Analogous results were obtained for an rpoS transcript, indicating that the mRNA is present at levels of 1 to 4 copies per cell, a level which increases as temperature decreases. In agreement with previous reports, we also observe that chromosomally-expressed DsrA is unstable, and rpoS mRNA less abundant, in the absence of Hfq26,27 further, rpoS mRNA is nearly absent in the cell in the absence of DsrA (not shown).28 Our quantification clearly indicates that the increase of DsrA sRNA level during stationary phase is limited to ~30%.

Results and discussion

Absolute quantification of DsrA sRNA

Absolute quantification of DsrA sRNA was performed by using reverse transcription quantitative-PCR (RT-qPCR). Taking into account the fact that DsrA can fold into different structures (Fig. S1, ESI†), oligonucleotides used for reverse transcription and amplification were optimized to allow the detection regardless of these structures. Indeed, it was shown previously that DsrA is able to self-assemble21,24 and this self-assembly could result in structures that inhibit reverse transcription and/or amplification of a part of the RNA. Therefore, we first confirmed that the polymeric form of DsrA (sliced-out from a gel) could be reverse-transcribed and amplified (not shown). Note that taking into account the constraints for reverse transcription and amplification by quantitative-PCR of the small sRNA (87 nt), we consider that DsrA quantification measures copies of full length DsrA (including polymers) but not processed DsrA fragments.

In this study, we investigate the dynamics of rpoS positive regulation by DsrA noncoding RNA. Our analysis is based on the first absolute quantification of the bacterial sRNA and of its mRNA target for various temperatures and growth conditions, in combination with mathematical models of post-transcriptional regulation. Measuring simultaneously sRNA and mRNA levels allows a clear estimation of unknown kinetic parameters and a validation of previous experimental results. Quantitative modeling reveals some unique features of this system. In contrast to other well characterized sRNA regulatory systems acting as repressors, a response to a cold shock is induced by base pairing to activate target mRNA, but also by stabilization of the active complex. DsrA amounts seem to be under a tight temperature control both at the level of production and degradation.22 While temperature control of degradation is not necessary to adjust DsrA steady-state levels, we show by modeling that this control has a marked influence on dynamics, specifically speeding up recovery times to normal levels after removal of stress, thus allowing external stimuli to actively control transcriptional and translational efficiency. In contrast, intrinsic noise in σ^5 induction (variability in protein levels due to the low copy numbers of the RNAs involved) is barely affected by temperature control mechanisms, since sRNA and mRNA fluctuations are uncorrelated and noise in protein production depends mainly on sRNA:mRNA complex and protein half-lives. We discuss how reversibility of complex formation and σ^5-regulated degradation act to reduce intrinsic noise. Finally, we used the quantification of DsrA in the cell and extended models to draw conclusions about the relevance of DsrA polymers in vivo.21

![Fig. 1 RT-qPCR quantification of DsrA and rpoS RNAs under various growth conditions. Error bars indicate the standard error of the measurement.](image-url)
Modeling of DsrA-based rpoS regulation

Several features of the mechanism by which DsrA regulates the response to a cold temperature stress have been investigated before (recently reviewed by Battesti et al.\textsuperscript{12}). In particular, it is known that transcription of DsrA is enhanced as temperature decreases, and this temperature control seems to be dependent only on promoter structure, without the need for additional trans-acting factors for temperature regulation.\textsuperscript{25} Moreover, degradation of DsrA seems to be also under temperature control with stabilization of DsrA at lower temperature.\textsuperscript{22} Recently, McCullen et al.\textsuperscript{28} have shown that DsrA plays a dual role in the positive regulation of the $\sigma^5$ factor: on the one hand, DsrA stimulates translation by annealing to the inactive mRNA. On the other hand, DsrA–rpoS pairing has also a stabilizing effect on rpoS mRNA, which is otherwise very quickly degraded.\textsuperscript{28} This is in contrast to what happens in other sRNA systems that repress translation, for example iron metabolism control by RyhB, in which pairing with the target mRNA favors its rapid degradation.\textsuperscript{29,30} These facts hint at a tight temperature tuning in which pairing with the target mRNA favors its rapid degradation.

Are there other mechanisms controlling DsrA amounts? One possibility is self-assembly of DsrA to form polymers. DsrA multimerization has been observed and was established that degradation of polymers is around five times faster than DsrA monomers in vitro.\textsuperscript{21} Since both activation and degradation of rpoS are coupled to DsrA, simultaneous quantification of both DsrA and its target mRNA levels provides a unique means to thoroughly characterize this system in vivo, to validate some of the conclusions reached previously and to address other major questions, such as the existence of additional control mechanisms for DsrA (e.g., polymerization) and the effect of RNA turnover control on both DsrA and its mRNA target.

To gain further insight into these questions, we set up a mathematical model based on mass action kinetics for the positive regulatory dynamics of rpoS by DsrA, taking into account the condensed regulatory interactions between sRNA, target mRNA and protein (Fig. 2). This model is similar to previous existing models of negative regulation by bacterial sRNAs\textsuperscript{31–33} and microRNAs in mammalian cells.\textsuperscript{34} In a first step we assume that self-assembly of DsrA is negligible in vivo, so that the model will consist of four ordinary differential equations describing the dynamics of DsrA (D), its target rpoS mRNA (r), the DsrA–rpoS complex (c) and the rpoS-encoded $\sigma^5$ (RpoS) factor (R):

$$\frac{dD}{dt} = a_D(T) + k_{\cdot c} - k_+ Dr - \delta_D D$$  \hspace{1cm} (1)

$$\frac{dr}{dt} = a_r + k_{\cdot c} - k_+ Dr - \delta_r r$$  \hspace{1cm} (2)

$$\frac{dc}{dt} = k_+ Dr - k_{\cdot c} - \delta_c c$$  \hspace{1cm} (3)

$$\frac{dR}{dt} = \beta_R c - \delta_R R$$  \hspace{1cm} (4)

Fig. 2 Schematic representation of the basic regulatory reactions in the DsrA–rpoS system used for modeling: DsrA and rpoS are independently transcribed from two genes (with DsrA transcription rate $a_D(T)$ under temperature control) and can base pair to form a complex which activates translation at a rate $\beta_R$. We take into account degradation of all the species and, possibly, self-assembly of DsrA at a rate $k_o$.

We included transcription of DsrA (through the temperature dependent rate $a_D(T)$ and rpoS (with rate $a_r$), degradation reactions for DsrA, rpoS, DsrA–rpoS complex and $\sigma^5$ (with rates $\delta_D$, $\delta_r$, $\delta_c$ and $\delta_R$ respectively), association and dissociation reactions for the DsrA–rpoS complex ($k_+$ and $k_-$) and rpoS translation from the active complex (with rate $\beta_R$).

As in other models of post-transcriptional regulation, we don’t explicitly take into account intermediate steps of complex association mediated by Hfq for which we assume approximately constant concentration in the cell (which seems appropriate taking into account the very low copy numbers of DsrA and rpoS and the abundance of Hfq hexamers in the cell) and describe the duplex association through a single second-order kinetic constant $k_+$ lumping Hfq-dependent reactions. Note that in contrast to previous work where quasi-equilibrium or irreversibility of the sRNA–mRNA complex is assumed,\textsuperscript{30–32} we take into account the full dynamics of the system, eqn (1–4).

From the model we can extract relevant quantitative information in two ways. On the one hand, we can study equilibrium properties by setting time derivatives equal to zero and solving the algebraic equations to get steady state values of D, r, c and R. Using the absolute quantification data of total DsrA and rpoS levels, and known kinetic parameters, we estimated unknown kinetic constants and validated previous experimental observations. On the other hand, eqn (1)–(4) can be numerically integrated to investigate several dynamic features of the $\sigma^5$ temperature induction system, such as activation and recovery times, or noise levels.

Equilibrium properties of positive rpoS regulation by DsrA

RT-qPCR is able to detect DsrA and rpoS mRNA both in their monomeric (free) and in their heteroduplex (DsrA–rpoS) forms; thus experimental abundances for each species are given.
by the sum of monomer and heteroduplex amounts. By solving eqn (1–4) under equilibrium conditions, these can be related to the different kinetic parameters and fitted simultaneously (using multiobjective optimization routines, Model fitting) as a function of temperature (Fig. 3). To avoid parameter dependencies, we used known values of degradation rates and fitted only transcription rates and an effective complex association constant 
\( K = \delta_{\text{h}} k_w (k_b + \delta_b) \) (ESI†). We checked consistency and estimated fitting errors by varying initial conditions and ranges for optimization of the fitted parameters (Model fitting). Moreover, by allowing \( \delta_i \) and \( \delta_b \) to vary in a wide range in the optimization procedure, we confirmed that the observed experimental values for free \( rpoS \) and DsrA–\( rpoS \) complex degradation rates are fully consistent with our absolute quantification data. We found that accurate fitting to the \( rpoS \) experimental data was only possible using \( rpoS \) half-lives of less than 1 min while simultaneously increasing the DsrA–\( rpoS \) complex half-life at least 4–5 fold. Thus, our analysis supports the conclusion that stabilization of \( rpoS \) by pairing with DsrA, in addition to translational activation, is required \textit{in vivo} to induce \( \sigma^8 \) response.

Further, we checked whether DsrA polymerization was possible \textit{in vivo} given our quantification data. The low levels of DsrA found \textit{in vivo}, even at low temperatures, suggest that polymers should not be present and that only limited oligomers can form. To verify this, we extended the model in eqn (1–4) to take into account DsrA multimerization (ESI†). We then assumed that total DsrA concentration measured in experiments came from monomers, heteroduplexes with \( rpoS \), and polymers. We found that only very short polymers (dimers–tetramers at most) are compatible with the experimental data, assuming that polymers are degraded much faster than monomers (as it has been observed \textit{in vitro}21). Such short length oligomers have been observed \textit{in vitro}, but only for concentrations above 1 \( \mu \text{M} \).21 Taking into account DsrA physiological concentration we therefore conclude that self-assembly cannot play a major function for DsrA-based regulation. Indeed, our analysis rather supports the possibility that misfolded RNA formed during transcription is at the origin of the self-assembly previously observed, rather than the hypothesis that an excess of sRNA allows this self-assembly. RNA is known to have a strong propensity to adopt misfolded conformations and non-functional misshaped sRNA could be harmful for the cell; molecular self-assembly could thus appear as a potential quality control of sRNA structure where misfolded RNA can self-assemble and be quickly degraded. Supporting this hypothesis, we observed that the use of a synthetic 87 nt-DsrA RNA (produced under denaturing conditions) does not form polymers \textit{in vitro} (not shown).

Finally, we note that our quantification data were compatible with both the stable DsrA half-life and temperature-dependent DsrA degradation (dashed lines in Fig. 3). Why, then, is a temperature control of the sRNA at the degradation level even necessary? In the next section we show that the dynamics of \( rpoS \) induction may provide an explanation for this.

**Dynamics of temperature response**

The advantage of sRNA-mediated regulation comes from the faster induction–repression of the regulated genes (most of them involved in response to physiological stress) compared to transcriptional regulation. Additionally, fast recovery mechanisms could be beneficial for cells, under certain conditions where stress duration is short, as it might be the case in a temperature shock. We thus used our models and kinetic rates obtained from the experimental data and curve fittings to study the dynamics of \( rpoS \) induction and recovery. As a measure of the response speed, we used the time needed to reach 50% of the final steady state value in \( \sigma^8 \) after a sudden decrease/increase in temperature (activation/deactivation times). We numerically calculated the activation–deactivation times as a function of the temperature decrease/increase (Fig. 4). For the regulatory model with stable DsrA (solid lines), activation times are relatively fast (~16 min) and almost independent of the temperature drop-off (solid line, panel A). Recovery times, however, are slowed down for large temperature decreases, and can be on the order of one cell cycle (~22 min, solid line in panel B). When DsrA is under temperature control (dashed lines) we see that \( \sigma^8 \) induction becomes moderately faster (activation time at large temperature reduction ~14 min, dashed line in panel A) but, importantly, deactivation times decrease more than two-fold (~10 min, dashed line in panel B). This is due to the fact that, under optimal growth conditions \((T > 35 ^\circ C)\), DsrA half-life is shorter (around 4 min) and this makes the dynamics of the response under perturbations faster. In Fig. 4, we used values of complex association–dissociation constants giving nearly irreversible duplex formation (Fig. S3A, ESI†). The conclusions reached here do not change in the regime where the heteroduplex is irreversible, although it can be seen that reversibility of complex formation further reduced response times (ESI† and Fig. S3B and C).
Noise in protein induction

Another intriguing feature of the *rpoS*-DsrA regulation is the low number of active transcripts involved (around 1–2 copies of DsrA-*rpoS* complex per cell). Kinetic rate equations, such as eqn (1–4), are only a bulk or average description of the cellular dynamics, which is subject to random fluctuations of intrinsic and extrinsic origin. In particular, large intrinsic noise can be expected when molecular species are at low copy number, due to the probabilistic nature of biochemical reactions. A recent genome-wide study of protein and mRNA noise with single molecule sensitivity in *E. coli* has shown that for low copy numbers (<10), most of the protein variability has an intrinsic origin, and is proportional to the inverse of the mean copy number of protein.

We thus calculated the intrinsic noise in *σ^S* induction associated with the regulatory reactions by DsrA, using both numerical simulations with a Monte Carlo method (Gillespie algorithm) and a theory based on linear noise approximations (ESI†). Two quantities are usually employed to characterize both intracellular and cell-to-cell variability of molecular levels in a clonal population: the coefficient of variation (CV) or standard deviation over mean (SD/μ), giving the relative variability in molecular abundance, and the noise strength or Fano factor (variance over mean, SD^2/μ). The Fano factor is important to discriminate different mechanisms contributing to intrinsic noise in protein abundance and also to reveal correlations among different molecular species and deviations from the Poisson behavior.

In Fig. 5A, we plot the relative variability in *σ^S* levels (CV) after temperature induction, comparing the temperature dependent and independent control mechanisms of DsrA degradation (dashed and solid lines, respectively, in Fig. 5A). A faster decay rate of DsrA (at higher temperatures) increases noise. However, this increase in noise occurs at normal growth temperature, where *σ^S* abundance is low and has no functional consequences. Therefore, a temperature control of DsrA half-life has the benefit of accelerating response dynamics without substantially increasing noise in the relevant response regime.

When we consider the Fano factor, we see that for the active DsrA-*rpoS* complex SD^2/μ = 1 (red squares in Fig. 5B) and is independent of temperature (the same behavior is obtained when DsrA degradation depends on temperature; data not shown). This indicates that fluctuations in DsrA and *rpoS* are uncorrelated, and noise in the active complex is Poissonian (the variance equals the mean). Then fluctuations in the active complex can be supposed to be transmitted independently of the other species to the protein *S*. Under this assumption, the noise strength or Fano factor in *σ^S* can be shown to take the form (see ESI†):

$$\frac{SD^2}{\mu} = 1 + \frac{\beta_R}{\delta_R + \delta_S}$$  

(5)

where β_R and δ_R are the translation and degradation rates of *σ^S* protein, respectively, and δ_eff = δ_c + k is the effective turnover rate constant for an active DsrA-*rpoS* complex, including active degradation (δ_c) by RNAses and the complex dissociation constant k (see eqn (1–3)†). In Fig. 5B we plot the Fano factor for protein numerically obtained from Monte Carlo simulations (black circles) and the prediction given by eqn (5) (blue line). Although approximate, eqn (5) shows that, for a fixed value of the effective complex association constant (eqn (8) below), determining steady state levels of the molecular species, the larger the dissociation constant k, the smaller the noise in protein. We show this behavior in Fig. S4 (ESI†), calculating noise as a function of temperature and k using the linear noise approximation. Thus, reversibility of the DsrA-*rpoS* complex acts to reduce the noise in *σ^S* protein expression, although it is not possible to estimate the value of the dissociation constant from current experiments. Accordingly, *in vitro* experiments hint to the possibility that complex formation mediated by Hfq may be reversible at the level of DsrA-*rpoS* annealing before they are released from Hfq.

We finally also note that for stable proteins, where the mRNA degradation rate is much larger than that of protein, the Fano factor takes the usual form SD^2/μ = 1 + b, where b = β_R/δ_c is the translational efficiency or translational ‘burst'
(average number of proteins synthesized per mRNA), which is \( \sim 15 \) in our case. \( \sigma^S \) production, however, is regulated by proteolysis,\(^{12}\) with rapid degradation during exponential phase, thus effectively reducing the noise due to translational bursting (see eqn (5) and Fig. 5B).

**Stochastic response times**

Due to the low copy numbers of the molecular species involved, protein levels may exhibit large fluctuations and thus response times under a sudden temperature change can be highly variable. A typical stochastic trajectory for \( \sigma^S \) abundance after a cold shock and recovery is shown in Fig. 6A, together with the deterministic approximation (grey line). Protein is produced in large translational bursts following pairing of DsrA–rpoS. It is thus pertinent to ask whether the speed up of response time discussed above with the deterministic model (Fig. 4) is also observed when the stochastic dynamics is taken into account. For random variables, response times are better defined as mean first passage times\(^{45}\) (in our case, average times for \( \sigma^S \) protein to reach a specified level after a temperature shock). Since protein expression takes place in ‘bursts’ Fig. 6A), we adopted a more functional definition of response times: activation times are calculated as the mean time needed to produce the first burst of protein above initial average levels after a temperature shock (blue circle in Fig. 6A). Similarly, we defined recovery times as average times to completely shut off protein expression after temperature returns to normal values (red square in Fig. 6A). Average times for activation (blue lines) and recovery (red lines) calculated from ensembles of 10 000 stochastic trajectories are shown in Fig. 6B. Solid lines correspond to temperature-independent DsrA degradation, while dashed lines are calculated with temperature control of DsrA degradation. Upon comparing with Fig. 4, we see that qualitatively the same behavior is observed: activation times are slightly lower with temperature-dependent DsrA degradation, and recovery times, slower for stable DsrA, are almost halved with temperature-dependent DsrA decay.

**Conclusion**

In this paper, we have used the simultaneous quantification of DsrA and rpoS RNA levels in vivo, together with the analysis of a mathematical model to increase our understanding of the mechanisms leading to induction of \( \sigma^S \) under cold shock stress. Our quantification of rpoS mRNA agrees with previous reports showing that change in rpoS transcription is quite limited (2–3 fold) and that the main \( \sigma^S \) regulation occurs at the translational and proteolysis levels.\(^{12}\)

Furthermore, the particularly low levels of rpoS measured, even at low temperature, imply that mRNA stabilization, and not only the activation by DsrA binding, is necessary to induce a sufficient response to express \( \sigma^S \). This response needs to be fast, which is achieved by translational control by an sRNA rather than by transcription, but also moderate, avoiding excessive \( \sigma^S \) levels (achieved also by rpoS turnover control). Several studies suggest that accumulation of \( \sigma^S \) might not be beneficial for the cell. For instance, King et al.\(^{46}\) found that \( \sigma^S \) controlled metabolic capabilities of the cell, and strains with high \( \sigma^S \) levels were more resistant to stress, but had poor nutritional capacity. This suggests that a fast recovery mechanism once environmental conditions have returned to normal values is important for cell viability in the longer term. We have shown here that the temperature control of DsrA degradation (shorter half-life at higher temperatures) favors this fast recovery. We note that degradation control is not necessary to keep the proper DsrA steady-state levels at different temperatures, since this is achieved transcriptionally by the DsrA promoter, but that it has a pronounced effect on the dynamics of rpoS deactivation. Temperature degradation control, however, barely affects variability (intrinsic noise amplitude) in \( \sigma^S \) induction during exponential phase, since this variability mainly depends on active complex and protein turnover, and on duplex reversibility. Note that, despite that our analysis focuses on temperature regulation specifically, other sRNA systems with stress control of degradation can also have noticeable outcomes on rpoS deactivation.\(^{47}\)

Finally, we hypothesized that autoassembly of DsrA observed in vitro to form polymers that can be quickly degraded may constitute a quality control mechanism against misfolded RNA, since experimental data and modeling rule out the possibility of appreciable amounts of polymers in vivo.

Similar quantification of different sRNAs involved in \( \sigma^S \) regulation and theoretical modeling can help to illuminate other posttranscriptional mechanisms in bacteria still under debate. For instance, in the transition from cold-shock to oxidative stress, OxyS sRNA is known to switch off rpoS translation in an Hfq-dependent manner when oxidative stress occurs, but a direct annealing between the RNAs is not yet clear.\(^{29}\) Our quantification is thus in favour of a model where a competition between DsrA sRNA (24 copies) and OxyS (4500 molecules per cell during oxidative stress\(^{48}\)) occurs,

![Fig. 6](image-url)
thus resulting in sequestration of Hfq. The regulatory mechanisms that are predicted herein may be recurrent in regulatory networks of noncoding RNAs which remain to be tested.

**Methods**

**Experimental**

Cell cultures, RNA extraction and reverse transcription. *E. coli* MC4100 WT and MC4100 Δhfiq::cm<sup>99</sup> were grown in Luria–Bertani (LB) broth at different temperatures. Total RNA preparation was performed as described previously.<sup>21</sup>

Briefly, 20 mL of culture were transferred into a pre-chilled microcentrifuge tube and centrifuged at 12 000 g for 1 min at 4 °C. After centrifugation, the recovered cells were resuspended in 3 mL of chilled 10 mM Tris–HCl pH 7.5 buffer containing 10 mM KCl and 5 mM MgCl<sub>2</sub>. 3 mL of prewarmed 20 mM Tris–HCl pH 8 containing 200 mM NaCl, 40 mM, EDTA and 1% SDS was added. After a vortex step, the mixture was incubated at 100 °C for 2 min and then immediately chilled in ice. Prior to RNA extraction, 3 pmol of a fragment of kan<sup>R</sup> transcript (105 nt, T7 *in vitro* transcription) was added to the bacterial lysate for normalization. RNAs were then extracted by hot-phenol and ethanol precipitated. Finally, samples were subjected to a DNase treatment (RNase-free, Fermentas) for 1 hour at 37 °C followed by phenol–chloroform extraction.

The cDNA synthesis was performed at 37 °C for 60 min followed by a step at 85 °C for 5 min (for reverse-transcriptase inactivation) by using the M-MuLV RT (Fermentas, 20U/μL) and following the manufacturer’s instructions. Primers used were the forward primers indicated in Table 1 for the corresponding RNAs. Negative controls include omission of reverse transcriptase.

**RT-qPCR analysis.** We used a SYBR Green based RT-qPCR method to determine the average copy number of sRNAs in bacterial cells. Four sequences were amplified: dsrA, rpoS, kan<sup>R</sup> and rrsB. Δhfiq strain (NM 317, -10 to stem-loop 3 deletion) was kindly provided by N. Majdalani (NCI/NIH, Bethesda, MD, USA), *rrsB* housekeeping gene was used to normalize RNA quantity in each reaction and *kan<sup>R</sup>* was used to calculate the efficiency of RNA extraction. The primer 3 program (http://frodo.wi.mit.edu/primer3/) was used to design the primers. The primers (ordered from Eurogentec) are shown in Table 1. Other functional forms for the temperature-dependent DsrA transcription rate can be empirically well reproduced using a sigmoidal function (Fig. 3A). We assumed for simplicity a Hill-like temperature dependence for *D*<sub>T</sub> and *r*<sub>T</sub> is a Hill-like coefficient giving the steepness of the activation curve. We fitted these three parameters to estimate the transcription rate *z*<sub>D</sub>(*T*), *z*<sub>r</sub>, and the effective association constant *K*. Moreover, the temperature-dependence of *z*<sub>D</sub> is not known, but we noticed that the experimental temperature dependence for *D*<sub>T</sub> can be empirically well reproduced using a sigmoidal function (Fig. 3A). We assumed for simplicity a Hill-like temperature dependence for *z*<sub>D</sub> of the form:

\[
\frac{z_0}{1 + \left( \frac{T}{T_\text{th}} \right)^\theta_T}
\]

where *z*<sub>0</sub> is the maximum transcription rate for DsrA (at low temperatures), *θ*<sub>T</sub> is the threshold for temperature activation, and *n*<sub>T</sub> is a Hill-like coefficient giving the steepness of the activation curve. We fitted these 3 parameters for the temperature-dependent DsrA transcription rate can be considered. For instance, we also used a hyperbolic tangent function (see eqn (12) below) widely employed to describe sigmoidal dependencies. The temperature threshold and steepness of the activation curve changed very little, as well as the rest of the optimized parameters, with fitted curves similar to

Amplification was followed by analysis on denaturing PAGE and melting curve analysis using the default program of a ROCHE Light Cycler 480 qPCR.

**Model fitting**

The experimentally accessible variables measured by RT-qPCR are the total DsrA and *rpoS* amounts, *D*<sub>T</sub> and *r*<sub>T</sub>, given by:

\[
D_T = D^* + c^* + e^* (6)
\]

\[
r_T = r^* + c^* (7)
\]

where asterisks denote concentrations at equilibrium. Eqn (1–4) are set equal to zero and analytically solved to get the free DsrA, *D*<sup>+</sup>, *rpoS*, *r*<sup>+</sup> complex, *c* concentrations in terms of six kinetic constants: transcription rates of *D* and *r*, degradation rates of *D*, *r* and *c*, and the effective complex association constant *K* (see ESI†):

\[
K = \frac{\delta_+ k_+}{k_- + \delta_c} (8)
\]

*D*<sub>T</sub> and *r*<sub>T</sub> are simultaneously fitted to the experimental points by minimizing the root mean square error (rmse) as a function of temperature, using a multiobjective optimization method (Goal Attainment method in MATLAB, Mathworks) with nonlinear constraints, to restrict the parameter ranges to physiologically relevant values.

We note that for steady state conditions there are parameter dependencies, for instance between transcription and degradation rates, and fitted values are not unique if we let all kinetic parameters float. We thus used known values for the degradation rates from previous experiments (Table 2), and fitted only the transcription rates *z*<sub>D</sub>(*T*), *z*<sub>r</sub>, and the effective association constant *K*. Moreover, the temperature-dependence of *z*<sub>D</sub> is not known, but we noticed that the experimental temperature dependence for *D*<sub>T</sub> can be empirically well reproduced using a sigmoidal function (Fig. 3A). We assumed for simplicity a Hill-like temperature dependence for *z*<sub>D</sub> of the form:

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\]

\[
r_T = r^* + c^* (7)
\]

where asterisks denote concentrations at equilibrium. Eqn (1–4) are set equal to zero and analytically solved to get the free DsrA, *D*<sup>+</sup>, rpoS, *r*<sup>+</sup> and complex, *c* concentrations in terms of six kinetic constants: transcription rates of *D* and *r*, degradation rates of *D*, *r* and *c*, and the effective complex association constant *K* (see ESI†):

\[
K = \frac{\delta_+ k_+}{k_- + \delta_c} (8)
\]

*D*<sub>T</sub> and *r*<sub>T</sub> are simultaneously fitted to the experimental points by minimizing the root mean square error (rmse) as a function of temperature, using a multiobjective optimization method (Goal Attainment method in MATLAB, Mathworks) with nonlinear constraints, to restrict the parameter ranges to physiologically relevant values.

We note that for steady state conditions there are parameter dependencies, for instance between transcription and degradation rates, and fitted values are not unique if we let all kinetic parameters float. We thus used known values for the degradation rates from previous experiments (Table 2), and fitted only the transcription rates *z*<sub>D</sub>(*T*), *z*<sub>r</sub>, and the effective association constant *K*. Moreover, the temperature-dependence of *z*<sub>D</sub> is not known, but we noticed that the experimental temperature dependence for *D*<sub>T</sub> can be empirically well reproduced using a sigmoidal function (Fig. 3A). We assumed for simplicity a Hill-like temperature dependence for *z*<sub>D</sub> of the form:

\[
\frac{z_0}{1 + \left( \frac{T}{T_\text{th}} \right)^\theta_T}
\]

where *z*<sub>0</sub> is the maximum transcription rate for DsrA (at low temperatures), *θ*<sub>T</sub> is the threshold for temperature activation, and *n*<sub>T</sub> is a Hill-like coefficient giving the steepness of the activation curve. We fitted these three parameters to estimate the transcription rate *z*<sub>D</sub>(*T*) (Table 2). Other functional forms for the temperature-dependent DsrA transcription rate can be considered. For instance, we also used a hyperbolic tangent function (see eqn (12) below) widely employed to describe sigmoidal dependencies. The temperature threshold and steepness of the activation curve changed very little, as well as the rest of the optimized parameters, with fitted curves similar to

**Table 1** Primers used for qRT-PCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Product size(bp)</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>dsrA</td>
<td>69</td>
<td>CACATCAGATTTCCCTGGTGTAACGC</td>
<td>GGGGTCGGGATGAAACTTGC</td>
</tr>
<tr>
<td>rpoS</td>
<td>75</td>
<td>CATCCTGGCCGGATGAAAAA</td>
<td>TTGACGATGCTCTGCTTCATA TC</td>
</tr>
<tr>
<td>kan&lt;sup&gt;R&lt;/sup&gt;</td>
<td>103</td>
<td>GGGAGTGATTTTGTGAGCCA</td>
<td>CATGAGTGACCTGAATCC</td>
</tr>
<tr>
<td>rrsB</td>
<td>130</td>
<td>TGTCCGTCAGCTGCTGTGTTGTG</td>
<td>ATCCCCACCTTCCTCCAGTT</td>
</tr>
</tbody>
</table>
the solid black lines shown in Fig. 2. We checked also that response times were unaltered.

We estimated fitting errors by optimizing unknown parameters starting from different initial conditions, and also considered the experimental uncertainty in rpos degradation (which is too fast to be measured, and thus we sampled in the range 60–300 h⁻¹ to calculate errors, Table 2). For temperature-independent DsrA degradation, δD was also allowed to vary in the range 1–3 h⁻¹ due to uncertainty in cell division times, see below. We selected 500 optimization runs with high accuracy (rmse < 0.1) and calculated standard deviations for each of the fitted parameters, shown in Table 2. We note that although the rpos transcription rate, x₀ has a large uncertainty due to the wide range of possible δa values, the rest of the parameters have narrow bounds indicating consistency of the optimization procedure.

**Estimation of model parameters from experimental data**

Several values of the model parameters can be estimated from previous experiments. In particular, we have access to half-lives of all the species involved. As our quantification measurements are in vivo, degradation rates have two contributions: active degradation (by RNases or proteases), and degradation due to growth and dilution:

\[
\delta_i = \frac{\ln 2}{\tau_i} + \frac{\ln 2}{\tau_{cc}}
\]

where \(\tau_i\) is the half-life of the \(i^{th}\) species due to active degradation and \(\tau_{cc}\) is the cell cycle doubling time, which we take as ~30 min for *E. coli* in exponential phase. For stable species, \(\delta_i \approx \ln 2/\tau_{cc}\), while for rapid active degradation \(\delta_i \approx \ln 2/\tau_i\). The half-life of DsrA has been measured by stopping transcription with the antibiotic rifampin under different experimental conditions: in the study of Majdalani *et al.*, DsrA was found to be stable at \(T = 32\) °C, thus we take \(\delta_i \approx \ln 2/30\) min⁻¹ in the set of numerical experiments assuming a temperature independent degradation rate for DsrA. In a later experiment, Repoila and Gottesman measured the half-life of DsrA at different temperatures, and found that it varied in the range 4–23 min from \(T = 42°\) C to \(T = 25°\) C. For the temperature dependent fitting of parameters, we used \(\delta_D\) values interpolated from these half-lives using a sigmoidal temperature dependence with a sharp threshold at ~35 °C. Specifically, we used two different functional forms to interpolate from the experimental data of ref. 22, giving slightly different temperature dependence for DsrA degradation:

\[
\delta_D(T) = a + b\frac{T^b}{K_T^b + T^b}
\]

(11)

\[
\delta_D(T) = a + b\tanh\left(\frac{T^b}{K_T}\right)
\]

(12)

with \(a = 2\) h⁻¹, \(b = 10\) h⁻¹, \(K_T = 35°\) C, \(h = 21\) for eqn (11) and \(a = 2\) h⁻¹, \(b = 10\) h⁻¹, \(K_T = 36.7°\) C, \(h = 13\) for eqn (12). Both functional forms changed very little the rest of the parameters fitted to experimental data, much less than expected variations due to the experimental uncertainty of estimated degradations or optimization errors (Table 2).

The half-lives of rpos mRNA and DsrA–rpos complex have been recently measured by McCullen *et al.* again after blocking transcription. In strains where DsrA–rpos pairing was disrupted, rpos degradation was extremely fast and became undetectable immediately after addition of rifampin, suggesting that the rpos half-life is very short, <1 min. In the fittings shown in Fig. 3 we used \(\tau_i = 0.5\) min. Pairing of rpos and DsrA stabilized the mRNA, increasing rpos half-life to about 3 min.

Translation and degradation rates for \(\sigma^S\) can be estimated from experiments of quantification of protein abundance giving between 170–200 copies of protein in stationary phase and around 15–20 copies of protein in exponential phase at 30 °C. From our quantification data (Fig. 1) we deduce that there should be 2–3 copies of the active DsrA–rpos complex in stationary phase, and 1–2 copies of complex in exponential phase. On the other hand, \(\sigma^S\) was found to be stable in

**Table 2 Model parameters: definitions and estimated values**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Meaning</th>
<th>Estimated value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a^0)</td>
<td>Maximum transcription rate of DsrA</td>
<td>(74 \pm 17) nM h⁻¹</td>
<td>&quot;</td>
</tr>
<tr>
<td>(\theta_T)</td>
<td>Threshold for temperature activation of DsrA</td>
<td>(100 \pm 7) nM h⁻¹</td>
<td>&quot;</td>
</tr>
<tr>
<td>(n_T)</td>
<td>Steepness of DsrA temperature activation</td>
<td>(26.3 \pm 0.4) °C⁻¹</td>
<td>&quot;</td>
</tr>
<tr>
<td>(\sigma_r)</td>
<td>ropos transcription rate</td>
<td>(98 \pm 48) nM h⁻¹</td>
<td>&quot;</td>
</tr>
<tr>
<td>(\beta_R)</td>
<td>(\sigma^S) translation rate</td>
<td>(220) h⁻¹</td>
<td>From Jishage and Ishihama</td>
</tr>
<tr>
<td>(\delta_D)</td>
<td>DsrA degradation rate</td>
<td>(1.4) h⁻¹</td>
<td>From Majdalani <em>et al.</em></td>
</tr>
<tr>
<td>(\delta_c)</td>
<td>ropos degradation rate</td>
<td>(120) h⁻¹</td>
<td>From McCullen <em>et al.</em></td>
</tr>
<tr>
<td>(\delta_R)</td>
<td>(\sigma^S) degradation rate</td>
<td>(15) h⁻¹</td>
<td>From McCullen <em>et al.</em></td>
</tr>
<tr>
<td>(K)</td>
<td>Effective association rate of a DsrA–ropos complex</td>
<td>(4.6 \pm 0.5) nM⁻¹ h⁻¹</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

" Estimated from the fitting of the model to the quantification data (Fig. 3). Errors are standard deviations taking into account experimental uncertainties in DsrA and ropos degradations (see Model fitting in the Methods section). b Parameters for the DsrA–rpos system taking into account temperature control of DsrA degradation. Numbers in brackets are the expected physiological ranges used to estimate errors in the fitted parameters (Model fitting).
stationary phase\textsuperscript{31,32} but had a half-life of 2–6 min in exponential phase\textsuperscript{31,32} suggesting that σ\textsuperscript{53} induction under stress conditions is both under translational and degradation control. Using a half-life of 60 min and 2 min for σ\textsuperscript{53} in stationary and exponential phases, respectively, using eqn (4) we estimate translation rates β\textsubscript{p} of 1–1.5 min\textsuperscript{−1} (stationary phase) and 3–7 min\textsuperscript{−1} (exponential phase). Taking into account the rpoS length, this corresponds to rates of ∼5–8 aa s\textsuperscript{−1} in stationary phase and ∼15–35 aa s\textsuperscript{−1} in exp. phase, which is in agreement with general estimates of translational efficiency in E. coli\textsuperscript{33,54}.

The rest of the parameters were not directly available from experiments and were estimated from the simultaneous fitting of DsrA and rpoS quantification using the procedure specified in Model fitting.

Note that in order to convert to the proper units in the macroscopic kinetic rates dependent on concentrations, such as in the transcription rates and complex association constant, using quantification data in molecule copy numbers, we assumed that in E. coli 1 molecule per cell ∼1 nM.

Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>rbs</td>
<td>ribosome binding site</td>
</tr>
<tr>
<td>sRNA</td>
<td>small noncoding RNA</td>
</tr>
<tr>
<td>RT-qPCR</td>
<td>reverse transcription quantitative-PCR</td>
</tr>
</tbody>
</table>

Acknowledgements

This work was supported by University Paris Diderot, CEA, CNRS, and EC FP7 DIVINOCCELL Health-F3-2009-223431 (VA) and MEC (Spain) under project BFU2008-03632/BMC (RG). We are very grateful to R. A. Lease (OSU, Columbus, OH, USA) for critical reading of the manuscript.

References