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The magnitude and colour of noise in genetic negative feedback systems

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ABSTRACT

The comparative ability of transcriptional and small RNA-mediated negative feedback to control fluctuations or 'noise' in gene expression remains unexplored. Both autoregulatory mechanisms usually suppress the average (mean) of the protein level and its variability across cells. The variance of the number of proteins per molecule of mean expression is also typically reduced compared with the unregulated system, but is almost never below the value of one. This relative variance often substantially exceeds a recently obtained, theoretical lower limit for biochemical feedback systems. Adding the transcriptional or small RNA-mediated control has different effects. Transcriptional autorepression robustly reduces both the relative variance and persistence (lifetime) of fluctuations. Both benefits combine to reduce noise in downstream gene expression. Autorepression via small RNA can achieve more extreme noise reduction and typically has less effect on the mean expression level. However, it is often more costly to implement and is more sensitive to rate parameters. Theoretical lower limits on the relative variance are known to decrease slowly as a measure of the cost per molecule of mean expression increases. However, the proportional increase in cost to achieve substantial noise suppression can be different away from the optimal frontier—for transcriptional autorepression, it is frequently negligible.

INTRODUCTION

In order to understand life at the level of individual cells we must understand how cells control and exploit the stochasticity inherent in biochemical mechanisms (1). Feedback control is often proposed as an important means of suppressing biochemical fluctuations (2,3), although a stochastic negative feedback system can in theory suppress or amplify fluctuations. Recent work (4) has derived limits on the extent to which biochemical feedback control mechanisms could suppress fluctuations by characterizing their magnitude when the control is mathematically optimal. However, very little is known about how close biochemical systems come in practice to achieving such lower bounds.

Negative autoregulation of gene expression is widespread in both prokaryotes and eukaryotes (5). Such regulation occurs both transcriptionally at the level of mRNA synthesis and post-transcriptionally due to the action of small non-coding RNAs (termed sRNAs in bacteria and microRNAs in eukaryotes) (6–9). Approximately 40% of known transcription factors in *Escherichia coli* are subject to negative transcriptional autoregulation (NTAR) (10,11). Several functions have been proposed for the widespread NTAR motif, including speeding up the response time of transcription networks to reach steady-state (11), linearizing the dose–response relationship of a downstream gene (12), and the control of noise (13,14). The noise properties of regulation by small RNAs are still poorly understood (15), especially so in the case of negative autoregulation (termed NSAR here). Incoherent, microRNA-mediated feedforward loops can couple finetuning of protein means and noise control (16).

Previous theoretical work has reported that NTAR can suppress intrinsic noise (17,18). As we will see, relying exclusively upon one of the two commonly encountered summary noise measures would result in finding either noise 'suppression' or 'amplification' due to both NTAR and NSAR, depending on the choice of measure. The reason is that the autorepression typically reduces both the variance and the average (or mean) of protein levels, making it important to consider both effects individually. We find that the variance usually decreases strongly enough compared with the simultaneous decrease in the mean to decrease the relative variance of the number of protein molecules (RV) but not the coefficient of variation (CV). Experiments measuring the CV for expression levels from plasmid-borne genes observed U-shaped dependence of the CV on the strength of repressor binding (19,20). This was not explained by intrinsic noise alone but by

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the presence of extrinsic processes and, in particular, the variability of plasmid segregation at cell division.

What then do we expect to happen to gene expression noise when an autoregulatory negative feedback loop is added to an unregulated gene expression system? For example, the promoter of the gene may acquire the property of autorepression by the protein or the promoter for a complementary sRNA may acquire the property of activation by the protein (Figure 1). Such changes arise during evolution, during the lifetime of a cell due to a modification of the protein such as phosphorylation, or as the result of deliberate engineering of the gene circuit in synthetic biology and in experiments studying noise. To investigate the question, we must study the noise properties of negative autoregulation compared with the noise properties of the system that is identical except for the absence of the negative feedback loop—the system with all other rate parameters unchanged. Results holding the mean protein expression level constant are also given to facilitate comparison with some previous work. We provide results that are valid when all parameters are allowed to vary within broad, biologically plausible ranges and that are exact (up to Monte Carlo sampling error). By contrast, the accuracy of analytical approximations deteriorates for low numbers of molecules when reaction kinetics are non-linear due to the presence of bimolecular reactions such as promoter binding and complex formation by sRNA with mRNA.

We find that NTAR and translational, small RNA-mediated autoregulation (NSAR) affect noise properties very differently. Transcriptional autorepression robustly reduces both the relative variance and persistence (or lifetime) of fluctuations. We explain how both benefits combine to reduce noise in downstream gene expression. Autorepression via small RNA can achieve more extreme noise reduction and typically has less effect on the mean expression level. However, it is often more costly to implement and is more sensitive to rate parameters. Theoretical lower limits on the relative variance are known to decrease slowly as a measure of the cost per molecule of mean expression increases (4). However, it should not be concluded that biochemical noise suppression is always a costly business. The proportional increase in cost to achieve substantial noise suppression can be different away from the optimal frontier. Such settings are relevant because both naturally occurring and synthetic networks may be far from currently achieving the fundamental mathematical limits. Substantial noise control could then be implemented although incurring a negligible increase (or even a decrease) in the average cost per molecule of mean expression. We report that this is frequently the case for transcriptional autorepression.

METHODS

We study the stationary NTAR, NSAR and unregulated systems using standard Monte Carlo methods (21), as described in the Supplementary Data. The NTAR and NSAR reaction mechanisms we consider are depicted in Figure 1 A and B and detailed in Supplementary Table S1.

Definition of summary noise measures

We consider two summary measures of noise magnitude: the coefficient of variation (CV) of the protein level X and the relative variance (RV) or 'Fano factor' of X. They are related as follows:

$$CV_x^2 = \frac{\mathrm{Var}(X)}{E(X)^2} = RV_x \cdot E(X)^{-1}, \quad (1)$$

which indicates that the CV can increase when the mean decreases if the RV does not decrease sufficiently strongly. We recommend that when the RV is used to compare two systems, the dimensionless ratio of the two RVs is reported.

We define the autocorrelation time of the protein level X as:

$$\tau_x = \int_0^\infty \frac{\mathrm{Cov}(X_t, X_{t+u})}{\mathrm{Var}(X_t)}\,du = \int_0^\infty \mathrm{ACF}_x(u)du. \quad (2)$$

In practice, the upper limit of the integral was chosen to be $\Delta = 10^5$s. This value is almost one order of magnitude larger than any timescale we considered in our simulations. We expect the contributions to the integral beyond this time to be small. Futhermore, Δ is small enough compared with the total simulation time that accurate estimation of the autocorrelation function (ACF) is possible from the simulated data.

Lower bound on coefficient of variation and relative variance

We can apply the results of Lestas *et al*. [see (4), Box 1] to obtain the following lower bound on the coefficient of variation (squared) for any NTAR or NSAR system:

$$CV_x^2 \geq \left\{E(X) \cdot (1 + N\log\frac{N_{\max}}{N})\right\}^{-1}, \quad (3)$$

where N is the expectation of the number of 'signalling' molecules synthesized during a time interval equal to the inverse of the protein degradation rate, d_x. It should be noted that, while the squared CV is dimensionless, this lower bound has the same units as the reciprocal of protein levels. The bound is only valid when these units are numbers of molecules. Fluctuations in the signalling molecule convey information about the level of protein: this molecular species is the mRNA for the NTAR system and the sRNA for the NSAR system. $N_{\max}$ is the product of the inverse of the protein degradation rate and an upper bound on the intensity of the counting process for the birth of the signalling molecule. $N_{\max}$ is therefore given by k_m/d_x for NTAR and k_s/d_x for NSAR, where k_m and k_s are the rates of transcription from the *active* promoters for mRNA and sRNA, respectively.

Other bounds provided in (4) do not apply to our reaction networks because, rather than implicitly making assumptions about relative timescales, we include the reactions for the binding and unbinding of the signalling molecule to the promoter. Each of the promoters (for mRNA or sRNA) switches between two states corresponding to whether or not the signalling molecule is

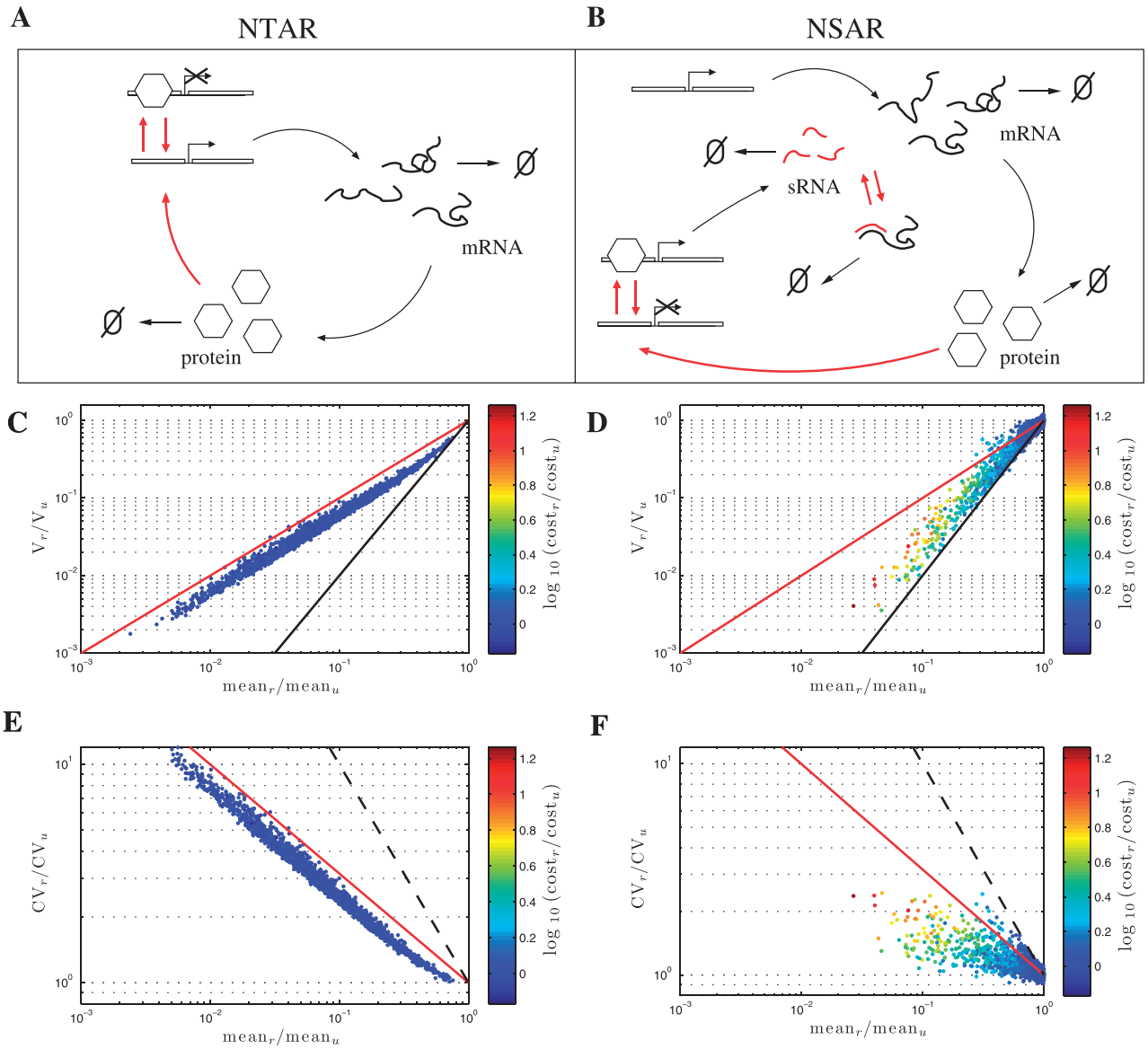

Figure 1. Comparing suppression of the mean and fluctuations of protein under transcriptional and sRNA-mediated negative autoregulation. Results for 2000 parametrizations of each feedback control system, selected uniformly from the biologically plausible rate parameter space (Supplementary Data). (**A**) NTAR with the reactions added to the unregulated system to obtain the corresponding NTAR system shown in red. (**B**) NSAR, with the reactions added again shown in red. Complete descriptions of both reaction networks are in Supplementary Table S1. (**C**) For NTAR, the ratio of the variances of the number of protein molecules in the regulated (r) and unregulated (u) systems, as a function of the ratio of the corresponding means (using logarithmic axes). Each point corresponds to a pair of rate parameter vectors (one each for r and u): rate parameters present in both systems are held constant. The colour bar shows the logarithm of the ratio of average cost per molecule of mean expression (per unit time) for the two systems (Equation 5). The red and black lines through the origin have gradients of 1 and 2 respectively. (**D**) As in C, but for NSAR. (**E**) For NTAR, as in C, but with the ratio of the CV of protein on the *y*-axis. Points below (above) the red line have a lower (higher) relative variance (or Fano factor) in the regulated case. Points below (above) the dashed line have a lower (higher) variance in the regulated case. (**F**) As in E, but for NSAR. Adding transcriptional or sRNA-mediated autorepression usually increases the protein noise measured by the CV.

bound. We avoid modelling transcriptional regulation using Hill functions, preferring to specify the reaction networks in terms of the underlying reactions.

Notice that Equation 3 implies (using Equation 1) a lower bound for the relative variance of X that does not depend on $E(X)$:

$$RV_x \geq \left\{1 + N\log\frac{N_{\max}}{N}\right\}^{-1}. \quad (4)$$

Average cost measure

In order to take into account the cost of mRNA, sRNA and protein synthesis, we define the average cost per molecule of mean expression (per unit time) for each genetic network as follows:

$$\text{cost} = \frac{1}{E(X)}[E(S_{\text{mRNA}})C_{\text{mRNA}} + E(S_X)C_X + E(S_{\text{sRNA}})C_{\text{sRNA}}], \quad (5)$$

where X is the number of protein molecules, E denotes a mean or expectation, S indicates the number of molecules synthesized per unit time and C is the energetic cost per molecule. For the NTAR network, which of course lacks sRNA, we set $E(S_{\mathrm{sRNA}}) = 0$. We assume an effective cost per monomer for each molecular species so that

$$C_{\mathrm{mRNA}} = L_{\mathrm{mRNA}} c_{nu},\ C_X = L_X c_{aa},\ C_{\mathrm{sRNA}} = L_{\mathrm{sRNA}} c_{nu}, \quad (6)$$

where L_{mRNA}, $L_X = L_{\mathrm{mRNA}}/3$ and L_{sRNA} are the lengths of the molecular species in terms of their constituent monomers, and c_{nu}, c_{aa} are the effective costs per nucleotide and per amino acid, respectively. For our calculations we used $L_{\mathrm{sRNA}}/L_{\mathrm{mRNA}} = 10^{-2}$, $c_{nu} = 49.3$ [corresponding to the median cost of precursor synthesis per nucleotide in yeast measured in numbers of high-energy phosphate bonds (22)], and $c_{aa} = 30.3$ [corresponding to the combined biosynthesis and polymerization cost per amino acid measured in numbers of high-energy phosphate bonds (22)].

Relative variance of downstream gene expression

The relative variance of a downstream gene Y whose transcription rate is proportional to the level of a protein, X, is given (Supplementary Data) by:

$$DG = 1 + \frac{v_y}{d_y + d_{m_y}} + RV_x \frac{k v_y}{d_y + d_{m_y}} \frac{(\tau_x^{-1} + d_y + d_{m_y})}{(\tau_x^{-1} + d_y)(\tau_x^{-1} + d_{m_y})}, \quad (7)$$

where the transcription rate of Y is equal to kX, τ_x is the autocorrelation time of the upstream protein, and (d_y, d_{m_y}) are the per molecule degradation rates of the protein Y and of its mRNA. Our Equation 7 is exact when the persistence (lifetime) of the fluctuations in X away from the mean is governed by an exponential decay over time and hence the ACF of X is exponential (Supplementary Data).

RESULTS

Comparing suppression of the mean and variance of protein levels under transcriptional and sRNA-mediated negative autoregulation

What happens to fluctuations in protein levels when either the transcriptional or sRNA-mediated negative feedback loop is added to a constitutive gene expression system to obtain the corresponding NTAR or NSAR system? Previous work has tended to characterize protein fluctuations using a single summary measure of noise—the ratio of either the standard deviation or variance of protein to its mean (17,23). These are called the CV, and the Fano Factor or RV, respectively ('Methods' section, Equation 1). However, undue reliance on any single, summary measure of noise can deliver misleading conclusions. Since introducing feedback control typically alters both the mean and variance of fluctuations, it is preferable to consider both effects individually.

Figure 1 shows the effect of adding the negative feedback loop on both the variance and the mean, relative to those of the unregulated system. We present results for 2000 different rate parameter vectors for each feedback mechanism, sampling the biophysically plausible parameter space randomly and uniformly (Supplementary Data). For each data point shown, reactions common to both the regulated (r) and unregulated (u) systems have identical rate parameters. The NTAR and NSAR systems we consider (Figure 1A and B) explicitly include the reversible, bimolecular reactions in which the protein binds to the relevant promoter. There is one binding site per promoter for the protein and hence no cooperativity in promoter binding.

For both mechanisms NTAR and NSAR (detailed in Supplementary Table S1), the mean of the protein level is always reduced on the addition of negative feedback compared to the unregulated mean, typically more so for NTAR than for NSAR. The strength of variance suppression by the two feedback mechanisms can be assessed by comparing the loci of points in panels C and D of Figure 1. For a given reduction in (the logarithm of) the mean, a greater reduction in (the logarithm of) the variance indicates stronger suppression. These reductions do not depend on the units in which protein levels are measured. Broadly speaking, NSAR suppresses the variance more strongly than NTAR. However, suppression by NSAR is more sensitive to rate parameters than NTAR for smaller reductions in the mean, where increases in the variance itself are sometimes observed. This larger sensitivity for NSAR is evident in the larger spread of points (for the smaller values of mean reduction) in panel D compared to panel C of Figure 1.

Interestingly, the reduction in the logarithm of the variance is approximately proportional to the reduction in the logarithm of the mean for both mechanisms. Linear regression lines through the origin have slopes of 1.16 and 1.58, and R^2 equal to 0.989 and 0.964, for NTAR and NSAR, respectively. The R^2 gives, in each case, the squared correlation between the fitted values from the regression and the actual data, with values closer to 1 therefore indicating a better fit for the linear regression. The larger slope for NSAR reflects the generally stronger variance suppression by NSAR. For an exactly proportional relationship, a slope >1 is equivalent to the RV decreasing when feedback is added and a slope >2 is equivalent to the CV decreasing when feedback is added. Finding increases or decreases in these summary noise measures thus depends on the rate at which the variance decreases as the mean decreases. We predict from Figure 1C and D, for both NTAR and NSAR that typically the RV will decrease but the CV will increase on adding the feedback (comparing to the red and black lines, respectively). These predictions are correct and discussed further below.

Figure 1 also shows the effect of adding the feedback on the average cost per molecule of mean expression (per unit time, see 'Methods' section, Equation 5). The effect is small and rather uniform for NTAR, but more variable for NSAR. Stronger variance suppression by NSAR than NTAR is associated with greater average cost (see the

points closer to the black line in panel D than panel C). We consider the costliness of the feedback controls in detail later on.

We also compared the NTAR and NSAR systems to the unregulated system, holding the mean protein level constant across the systems (Supplementary Figure S4). A difficulty with such comparisons is that there are many different parametrizations of both the regulated and unregulated system that result in (approximately) the same mean. Considering a wide range of means and without biasing the choice of parametrizations (e.g., by tuning only some subset of the parameters), we find that the variance is decreased in 42 and 52% of the cases for NTAR and NSAR, respectively. The results are clearly sensitive to the specific choice of parameter vectors for the regulated and unregulated systems being compared.

Adding negative autoregulation rarely suppresses fluctuations enough to reduce the coefficient of variation

Although some previous theoretical studies report that (in the absence of extrinsic fluctuations) transcriptional autorepression reduces noise (17,18), others report an increase in noise with increasing feedback strength (24,25). Comparisons are made by holding different features of the system constant, often including the mean protein level. The summary noise measure used also differs between studies.

The present context highlights particularly clearly the danger of undue reliance on any single summary noise measure such as the CV (Figure 1E and F). For our NTAR mechanism, which has one binding site for the protein in the promoter, the CV of the protein is never reduced and can increase as much as 10-fold. For NSAR, the increase in the CV is typically less pronounced (26) and lies between 0 and 10% in 50% of the cases sampled. We observed a decrease in the CV for NSAR of up to 10% in a minority of the cases sampled (18%).

Should we then conclude that neither negative feedback mechanism robustly 'suppresses noise'? Both mechanisms effectively reduce protein variance and standard deviation (Figure 1C and D). They usually reduce these strongly enough compared with the reduction in the mean to reduce the relative variance or Fano factor (compare to the red line in panels C and D) but not enough to reduce the CV (compare to the black line). This quantification of the strength of fluctuation suppression usefully goes beyond a statement about the sign of the change in the CV. (For the relationship between the two noise measures, see 'Methods' section Eqution 1). There is no reason to suppose that, when the mean is decreased, only reductions in the variance of the number of protein molecules that are sufficient to reduce the CV can be beneficial to the cell. Such a conclusion does not follow, for example, from the scale invariance of the CV. The reduction in the mean may be of primary phenotypic importance, and the associated reduction in the variance of additional benefit.

The non-decrease in the CV compared with the unregulated case for our NTAR system is consistent with the theoretical prediction of Singh *et al.* (27) for the limiting regime where protein degradation is much slower than mRNA degradation. They describe transcriptional repression using a Hill function and find that, in the absence of extrinsic noise, the minimum CV on varying the feedback strength while holding other kinetic parameters fixed is given by the unregulated system. The approach is an approximate analytical one, relying on a linearization of the Hill function around the stationary protein mean and a stochastic hybrid system description with continuously valued protein levels. They find that when a Hill coefficient >1 is used (to model cooperativity), the CV can decrease compared to the unregulated case.

The relative variance for NTAR and NSAR is rarely below one and often substantially exceeds a theoretical lower limit for feedback control

We find for both negative feedback mechanisms NTAR and NSAR that the protein variance is rarely reduced below the level of the mean—in other words, the RV of the number of protein molecules is always (approximately) ≥ 1 (colour bars Figure 2A and B). Of course, the (greatest) lower bound is equal to one for the RV of the 'unregulated', 2-stage gene expression system. Analysis of NTAR using a Hill function approximation and linearization around the steady-state indicates that a RV <1 is possible for NTAR (17); see also (28). We find when explicitly including promoter binding and unbinding reactions that such 'sub-Poissonian' noise is very rare. Furthermore, a recent system-wide study of protein levels in *Escherichia coli* cells provides some experimental evidence that regulatory mechanisms do not decrease the RV substantially below a value of one (29).

Motivated by the poor characterization of many biochemical systems, Lestas *et al.* (4) recently gave an elegant derivation of lower limits on the extent to which biochemical feedback control mechanisms could suppress fluctuations by characterizing the performance of mathematically optimal control. However, very little is yet known about how close biochemical systems come in practice to achieving such lower bounds. This question is clearly of interest in its own right, both for naturally occurring and synthetically designed systems. We therefore quantify the performance of the NTAR and NSAR systems relative to the performance derived in (4) for a hypothetical, optimal controller. We computed the lower bound on the coefficient of variation provided by (4) for our reaction networks ('Methods' section, Equation 3), and found that the actual CV is on average 8.0 times the magnitude of the lower bound for NTAR and 4.3 times the lower bound for NSAR (Figure 2). An actual CV exceeding the lower limit by a factor of ≥ 10 is not uncommon for NTAR (26% of cases sampled). These numbers are unchanged when we consider the lower bound for the square root of the relative variance of protein.

It should be noted that the lower bound for the CV depends not only on the expected rate of synthesis of the number of 'signalling' molecules, but also on a ratio of rate constants ($N_{\max}$) that varies across different parametrizations of the system. The larger this ratio, the less the optimal controller is constrained by the maximal rate of transcription from the promoter of the signalling molecule

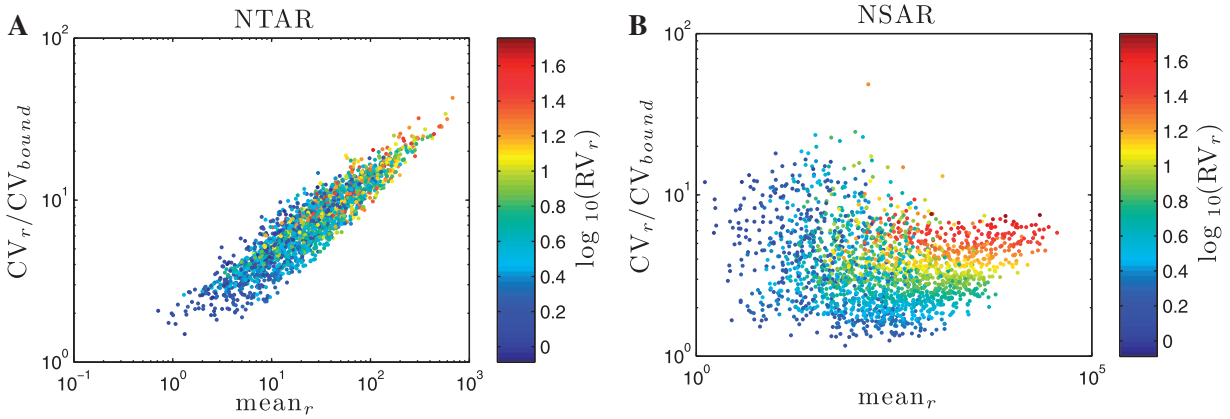

Figure 2. The magnitude of the discrepancy between the theoretical lower limits based on optimal control and the CV of protein for NTAR and NSAR. Comparison of the CV of the regulated system (r) with the lower bound of Lestas *et al.* (4) given by Equation 3, for 2000 parametrizations of each feedback control system (selected uniformly from the biologically plausible space). (**A**) For NTAR, the ratio of the CV to the lower bound equals that of the $RV^{\frac{1}{2}}$ to its bound and is plotted against the mean of protein under regulation (using logarithmic axes). The colour bar shows the logarithm of the magnitude of the relative variance of the number of protein molecules for the NTAR system. RV_r <0.95 for 0.4% of cases. (**B**) As in A, but for NSAR. RV_r <0.95 for 0.0% of cases.

(mRNA for NTAR and sRNA for NSAR). The lower bound for the squared CV is proportional to the reciprocal of the mean. It therefore provides a lower bound for the relative variance that does not depend on the mean ('Methods' section, Equation 4). This lower bound tends to estimate the actual RV better when the latter is closer to one, although we find that even then the bound can perform poorly as an approximation of the RV (Figure 2). While it is clear that the NTAR and NSAR systems must perform worse in terms of CV and RV than the lower limits based on optimal control, the magnitude of the discrepancy has not previously been quantified. The lower bounds in (4) are limits on the *level* of the noise for a given system (measured either by the CV or RV) expressed in terms of the costliness of control. They allow a relative comparison of the noise level of two systems in terms of the relative cost of control when both systems achieve (or get very close to) the optimal noise level for their respective costs. When this is not the case, the relationship between reduction of noise and increase in the cost of control may be very different from that implied by the form of the lower limits. We return to this point in a subsequent section.

Adding transcriptional autorepression whitens noise and reduces the relative variance of protein numbers

Adding transcriptional autorepression reduces the variance of protein measured relative to its mean (RV) in the overwhelming majority of cases sampled (99.95%). Figure 3 is analogous to Figure 1 but reports three additional summary statistics, again comparing them for NTAR and NSAR. We report the ratio of the RVs for the regulated and unregulated systems, which does not depend on the units in which protein levels are measured (although numbers of molecules is a natural choice in our context). The proportional reduction in the RV is quite stable across a wide range of resultant reductions in the mean (Figure 3A) and therefore a useful summarizing measure here, with 98% of cases reducing the RV to between 0.5 and 0.8 of its level in the unregulated system. We consider NSAR in the subsequent section. Two previous theoretical studies (17,18) used approximate methods to predict a decrease in the RV under NTAR, when comparing the regulated and unregulated system as we do.

NTAR also reliably whitens the noise in protein expression. The variance is the time-average of the squared deviation of the protein level from its mean over a long time (for an ergodic system). Biologically relevant timescales over which some form of time-averaging by downstream modules takes place may be considerably shorter, in which case the persistence over time (lifetime) of excursions away from the mean becomes important. Such persistence is measured by the autocorrelation function (ACF) for the protein level and its autocorrelation time (defined here as the time-integral of that function, see 'Methods' section Equation 2). For NTAR, the autocorrelation time is always reduced and is between 0.5 and 0.7 of that for the unregulated system in 69% of the cases sampled (Figure 3C).

Such a 'whitening' of the noise by transcriptional autoregulation was predicted by Simpson *et al.* (18) (NSAR is not considered) using a frequency domain analysis with Langevin and shot noise approximations. These approximations may however perform poorly for low numbers of molecules. We have demonstrated without approximation (beyond that due to Monte Carlo sampling error), and using a single 'chromosomal' copy of the gene, that NTAR always reduces the autocorrelation time for parameters in the biophysically plausible parameter space. A shift of the distribution of ACF 'half-lives' towards shorter half-lives due to NTAR has been observed experimentally for a gene circuit on high copy number plasmids (14). The distribution is across lineages of dividing cells.

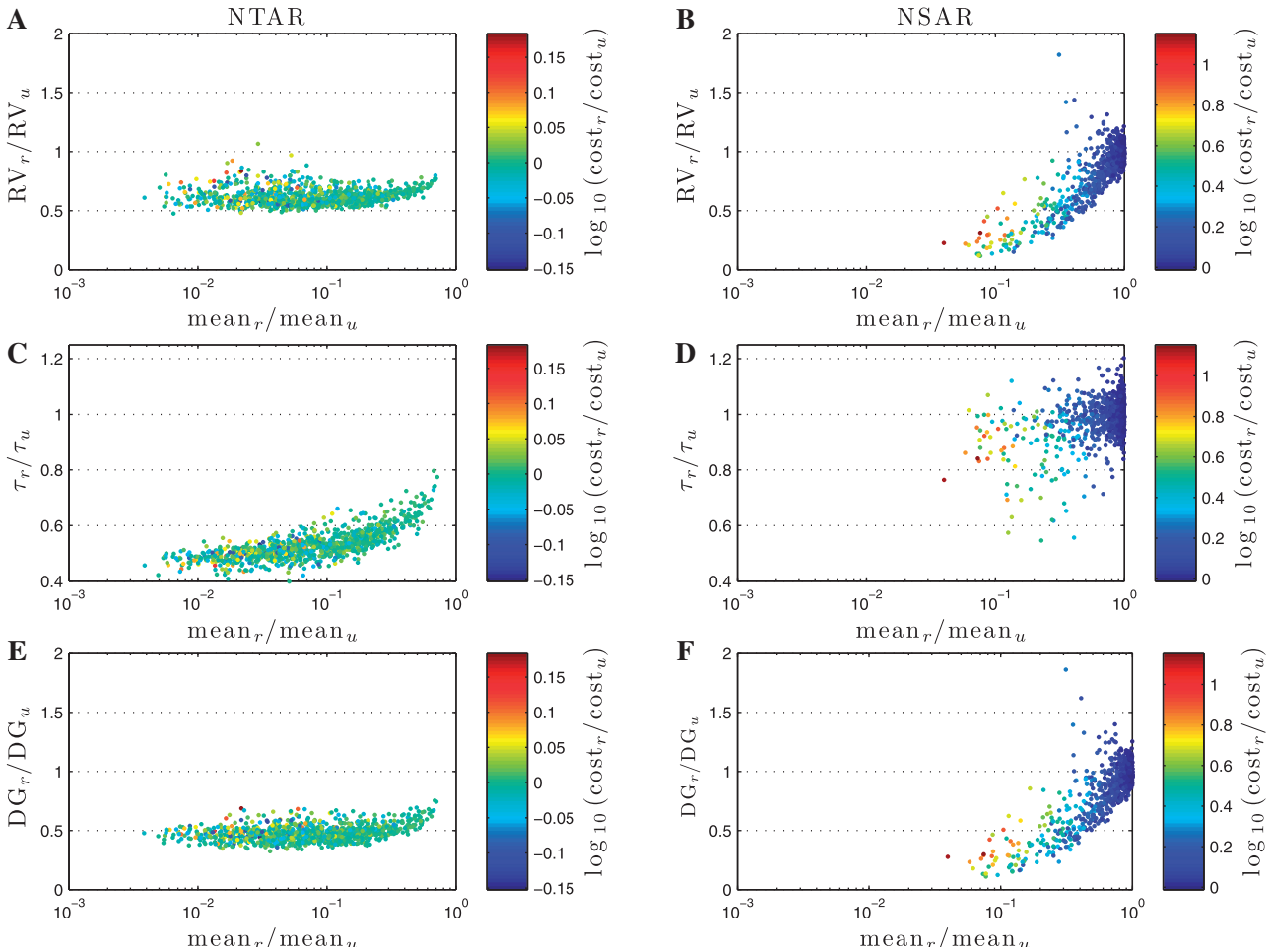

Figure 3. Comparing noise control by transcriptional and sRNA-mediated negative feedback. Adding transcriptional autorepression both whitens noise and reduces relative variance (RV), with both effects reducing variability in expression of an activated downstream gene. Autoregulation by sRNA typically suppresses the mean less. It is otherwise more sensitive to rate parameters, often more costly, and usually performs less noise whitening. First column is for NTAR, second column for NSAR. (**A**) and (**B**) The ratio of the RV in the regulated (r) and unregulated (u) systems, as a function of the ratio of the corresponding means (using logarithmic axes). As in Figure 1, each point corresponds to a pair of rate parameter vectors (one for the regulated and one for the unregulated system): rate parameters present in both systems are held constant. Colour bars for all panels show the ratio of the average cost per molecule of mean expression (per unit time) in the two systems (Equation 5). (**C**) and (**D**) As in (A) and (B), but for the ratio of the protein autocorrelation time (Equation 2). (**E**) and (**F**) As in (A) and (B), but for the ratio of the relative variance of a downstream gene (DG) transcriptionally activated by the autoregulated protein. Exact results (up to Monte Carlo sampling error) for 1000 parametrizations of each system, selected uniformly from the biologically plausible space.

We assess below the combined effect of the observed reductions in both the relative variance and the autocorrelation time by considering noise in expression of a downstream gene. We are able to explain analytically how the reductions in both noise measures combine to control the downstream noise, for the case of proportional transcriptional activation.

The noise properties of sRNA-mediated negative feedback are sensitive to rate parameters

Negative feedback mediated by sRNA sometimes increases and sometimes decreases the relative variance and the autocorrelation time (Figure 3B and D). Both noise measures are reduced relative to the unregulated system in 47% of cases sampled, compared to 100% for NTAR. NSAR has relative autocorrelation time between 0.8 and 1 in 56% of cases, and between 1 and 1.2 in 41% of cases. NSAR thus typically whitens protein fluctuations less than NTAR. We find that NSAR can be much more or much less effective than NTAR in reducing the RV, depending on the choice of rate parameters. Comparison of panels A and C (NTAR) with panels B and D (NSAR) in Figure 3 provides a bird's eye view of the differences between the effects of the transcriptional and translational control mechanisms.

Noise reduction in a downstream gene is the result of both noise whitening and variance reduction

In order to assess the combined effect of regulating the variance and the autocorrelation time, we consider the noise in expression of a downstream gene that is transcriptionally activated by the protein (X) that is under negative autoregulation. The transcription rate of the downstream gene is then itself stochastic. Figure 3E and F shows the

ratio of the relative variance of the downstream gene (DG) when X is regulated (by NTAR and NSAR, respectively) to its relative variance when X is unregulated. NTAR robustly reduces the RV of the downstream gene—the RV is on average 0.48 that for the unregulated case. (Again, the proportional reduction in the RV is quite stable and a useful summary measure here.) For NSAR, the ratio of the relative variances of the downstream gene is again more sensitive to rate parameters and is similar to the same ratio for the autoregulated protein itself (Figure 3B). In this setting, the addition of sRNA-mediated autoregulation is usually less effective in suppressing downstream noise, with only 10% of cases below the average ratio of 0.48 for NTAR.

When the rate of transcription of the downstream gene is proportional to the level of X, the relative variance of the downstream gene is given (see 'Methods' section) by

$$DG = k_0 + k_1 W(\tau_x) RV_x, \quad (8)$$

where the constants k_0 and k_1 depend only on the constant rate parameters governing the gene expression reactions for the downstream gene. The function W *decreases* as the autocorrelation time of X decreases ('Methods' section, Equation 7). Equation 8 thus quantifies how changes in the relative variance (RV_x) and autocorrelation time of the autoregulated protein combine to determine the relative variance of downstream gene expression. Decreases in both are beneficial, and the relative variance of the downstream gene therefore always decreases for NTAR (Figure 3E). The analytical expression makes precise the intuition that whiter noise is more readily filtered out by downstream gene circuits (18). It is exact when the persistence (lifetime) of the fluctuations in X away from the mean is governed by an exponential decay over time and hence the ACF of X is exponential (Supplementary Data). Comparison of plots obtained using Equation 8 to evaluate DG with the results for simulation of downstream expression shown in Figure 3E and F reveals that Equation 8 is in close agreement with the Monte Carlo estimates (see supplementary Figures S2 and S3). Suppose that the upstream protein X had the same relative variance under regulation as for NTAR but that the autocorrelation time was unchanged compared to the unregulated case. Then, using Equation 8, the average ratio of the RVs for the downstream gene would be 0.65 instead of 0.48, reflecting the importance of the noise whitening by NTAR.

The cost of feedback control

Motivated by recent interest in the costliness of noise suppression by feedback control (4), we quantified the cost of implementing the two types of negative autoregulation. In order to take into account mRNA, sRNA and protein synthesis, we defined the average cost per molecule of mean expression (per unit time) for each genetic network as follows (see 'Methods' section):

$$\text{cost} = \frac{1}{E(X)}[E(S_{\text{mRNA}})C_{\text{mRNA}} + E(S_X)C_X + E(S_{\text{sRNA}})C_{\text{sRNA}}], \quad (9)$$

where S indicates the number of molecules synthesized per unit time and C is the energetic cost per molecule. We have divided by the mean protein level in this definition in order to make comparisons across systems with widely differing means.

Lestas *et al.* (4) emphasize the importance of the expected number of births of the signalling molecule—in their framework, mRNA for the NTAR system and sRNA for the NSAR system—in assessing the costliness of noise suppression. In the case of the NSAR system, for example, we also include the effect of different values of the rate parameters on mRNA and protein births, as well as allowing for the difference in cost per molecule of mRNA and of the much shorter sRNA. Lestas *et al.* (4) consider the expected number of births of the signalling molecule (per molecule of mean expression) during the protein 'lifetime', that is the period of time equal to the reciprocal of the protein degradation rate.

We compared the average cost per molecule of mean expression for the system with the negative feedback loop added and for the unregulated system, computing the ratio of the cost in the former to the cost in the latter (colour bars in Figures 1 and 3). For NTAR, the average cost is *lower* for the regulated system than the unregulated in roughly half of the cases. The cost ratio varies between 0.68 and 1.53. There is no obvious relationship between any of the three measures of noise control in Figure 3 and the cost effect of adding the NTAR negative feedback. Figure 4 shows histograms for NTAR of the proportional increase in average cost associated with the average observed levels of reduction in the relative variance for the autoregulated protein and downstream gene expression (using the data from Figure 3). It is clear from Figures 3 and 4 that for NTAR, substantial reductions in the relative variance of both the regulated protein and downstream expression are very frequently observed with little increase in average cost—the cost ratio remains close to 1. As we discuss below, this runs counter to some recent interpretations (30) of the slow decline of the lower limits on the relative variance in (4) as the measure of the cost of control increases.

For NSAR, the cost ratio is more variable, with a minimum and maximum of 0.97 and 18.4, respectively (and a lower average cost under regulation in only 0.5% of cases). Adding the sRNA-mediated control tends to be more costly than adding transcriptional control, with a cost ratio for sRNA greater than the maximum for NTAR observed in 18% of cases. Broadly speaking, cost ratios much larger than one for NSAR tend to be associated with a greater decrease in the relative variance and in the relative variance of the downstream gene.

Praxis

Our results have practical implications on a number of fronts: for experimentalists comparing noise in gene expression across different systems, for biologists seeking to understand the role of negative feedback in controlling cellular variation, and for the construction of synthetic gene circuits. First, our results caution against comparing

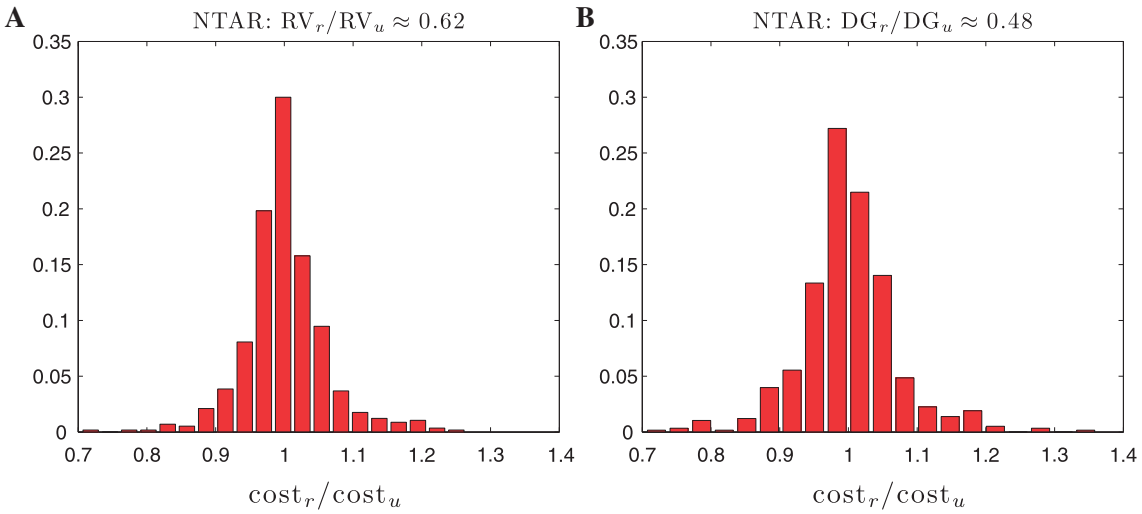

Figure 4. The increase in average cost per molecule of mean expression to substantially suppress noise is frequently negligible for transcriptional autorepression. (**A**) The histogram of the proportional increase (or decrease) in average cost (Equation 5) incurred in order to decrease the relative variance by 38 ± 5% compared with the unregulated system (38% is the average reduction observed for NTAR). Data from Figure 3A. (**B**) Histogram of proportional increase in average cost as in (**A**) but for a resultant decrease in the relative variance of the activated downstream gene (DG) of 52 ± 5% compared with the unregulated system (52% is the average reduction observed for NTAR). Data from Figure 3E.

systems using any single, summary measure of biochemical noise. Negative feedback mechanisms often have a substantial effect on the mean, the variance and the autocorrelation of the fluctuations. All of these effects should be considered. For each mechanism, plotting the logarithm of the variance against the logarithm of the mean (or the deviation of these variables from suitable reference values) for different parametrizations or experimental configurations, as we did, provides informative comparisons and better characterizes the strength of noise suppression. Reporting the dimensionless ratio of the relative variance for two systems or parametrizations, in addition to that of the CVs, is recommended.

Second, biologists concerned with understanding the role of negative feedback should likewise consider its effect on both the mean and the variance. As we have explained, it would be misleading to conclude that, since the CV increases for NTAR on adding negative feedback, transcriptional autorepression has no biological importance in controlling cellular variation due to intrinsic processes. Indeed, we found that fluctuations were almost always suppressed strongly enough by NTAR to reduce the relative variance. Separate consideration of effects on the mean and the variance is also important when the genetic system is subject to regulation by incoming signals. Negative autoregulation could impair information transfer (31) despite reducing the (conditional) variances as a result of also reducing the variability of the mean responses to the different signal values (the 'dynamic range'). Our finding that NSAR typically has less effect on the mean response than NTAR suggests that sRNA-mediated autoregulation could potentially perform better as a signal transduction mechanism. This deserves further investigation.

Third, our results suggest that NSAR may have more limited value than transcriptional autoregulation for noise reduction in some synthetic biology applications. The performance of NSAR relative to the unregulated system is considerably more sensitive to rate parameters, which may themselves be difficult to control in synthetic circuits. Furthermore, when NSAR suppresses noise more effectively than NTAR, we found that the metabolic cost imposed on the cell tends to be considerably higher. More generally, comparing the noise performance of a proposed synthetic gene circuit, for example *in silico*, with the bounds of Lestas *et al.* (4) in the way we did can guide a design process. We found that the NTAR and NSAR gene circuits rarely get close to the optimal performance.

DISCUSSION

Adding negative autoregulation typically suppresses both the mean and variance of protein levels compared to the unregulated gene expression system with the same rate parameters. We have demonstrated the importance of quantifying individually and relating both these effects when characterizing the impact of feedback on biochemical fluctuations. For the broad ranges of biologically plausible rate parameters we consider, the two effects usually combine to increase the coefficient of variation when transcriptional or small RNA-mediated autorepression is added to the system. However, the absolute magnitude of fluctuations is typically suppressed for both feedback mechanisms, often strongly enough to decrease the variance of the number of protein molecules measured relative to the mean. Such reductions in variance can still benefit the cell, discouraging sole reliance on the coefficient of variation (CV) as a summary noise measure. For example, the reduction in the mean may be of primary phenotypic importance, and the associated reduction in the variance of additional

benefit. By their construction both the CV and relative variance conflate effects on the mean and variance, effects that are best also considered individually.

Dynamic properties of protein fluctuations beyond their variance should be considered, in particular their autocorrelation properties (14). The variance is the time-average of the (squared) deviation of the protein level from the mean over a long time period (for an ergodic system). Biologically relevant timescales over which some form of time-averaging by downstream modules takes place may be considerably shorter, in which case the lifetime of excursions away from the mean becomes important. This lifetime can be measured by the autocorrelation function for the protein level and its autocorrelation time. We have quantified and explained analytically how beneficial reductions in both the autocorrelation time ('noise whitening') and in the relative variance of a transcription factor combine to control the noise in downstream gene expression, for the case of proportional transcriptional activation. We find that transcriptional autorepression whitens noise more substantially and reliably, compared to small RNA-mediated negative autoregulation.

We have provided an extensive bird's eye view and comparison of the properties of these two types of negative autoregulation, rather than relying on approximate analytical solutions or presenting simulation results for a limited number of choices of rate parameters. Transcriptional negative feedback mediated via an intermediate protein species is studied in the Supplementary Data (Supplementary Figure S1), and is found to offer no particular benefits compared to the unmediated case. Our results are exact except for the carefully controlled variability introduced by Monte Carlo sampling.

It has been previously suggested that translational autoregulation is a more effective means of attenuating noise in gene expression than transcriptional autoregulation (32) (for parametrizations of the systems having the same mean protein level and direct binding of the protein to its own mRNA). The noise properties of negative, small RNA-mediated translational control have, however, received little previous attention, despite the widespread occurrence we find of the feedback motif from bacteria to humans (Table 1). We report that adding transcriptional feedback to the existing system is a more robust and usually less costly means of controlling noise than adding translational feedback using small RNA—it is considerably less sensitive to rate parameters. Transcriptional autorepression both reduces relative variance and whitens the noise by decreasing its autocorrelation time. Autorepression by small RNA usually reduces the mean to a lesser extent. Both more extreme noise suppression than transcriptional control, and a simultaneous increase in the relative variance and autocorrelation time are possible.

It would be interesting to perform similarly detailed comparisons in the presence of extrinsic noise, although exactly how to specify the extrinsic processes is challenging. Previous work considering transcriptional autorepression and extrinsic noise points to the existence of an optimal feedback strength (20,27,33). Experiments using chromosomal rather than plasmid-borne genes should be performed to compare the effect of small RNA and transcriptional autorepression in living cells—as discussed, we predict a very different noise signature for small RNA-mediated negative feedback. Our theoretical analysis could be extended to include the effects of protein dimerization, multiple binding sites in promoters, delays from intracellular transport, noise attenuation due to macromolecular crowding inside the cell (34), and more detailed specification of the reactions involved in the processes of transcription and translation.

We have shown for both types of negative autoregulation considered that the relative variance of the number of protein molecules (RV) is rarely reduced below the Poissonian value of one, in contrast to predictions based on Hill function inhibition of transcription. The RV also often substantially exceeds a recently obtained theoretical lower limit (4) for general feedback control systems. To the best of our knowledge, we report the first quantitative comparison of such limits with realistic biochemical feedback mechanisms. A relative variance equal to one is a natural point of comparison because it is the (greatest) lower bound for the RV of unregulated, two-stage gene expression and is also the RV of a stationary birth–death process describing the synthesis and degradation of a molecular species.

The theoretical lower limits (lower bounds) on the RV discussed by Lestas *et al*. (4) decrease slowly as one moves along the 'frontier' of optimal control by increasing the cost of that control. Cost is measured by the expected number of births of signalling molecules (per molecule of mean expression) during the protein 'lifetime', and the lower bound on the relative variance is found to decrease with the square root of births (4). However, it should not be concluded that biochemical noise suppression is always a costly business. We find that for transcriptional autorepression, substantial reductions in the relative variance of both the autoregulated protein and of a downstream gene are very frequently observed with little increase in the average cost per molecule of mean expression—the average cost remains close to that of the more noisy, unregulated system (Figures 3 and 4). The reductions we report in relative variance are proportional ones, independent of the units in which protein levels are measured. The relationship between relative variance and the costliness of control is expected to be different away from the frontier of optimal control. Such settings are relevant because both naturally occurring and synthetic networks may be far from currently achieving the fundamental mathematical limits. Substantial noise control can then be implemented by the cell while incurring a negligible increase (or even a decrease) in the average cost per molecule of mean expression, as we have illustrated using a biochemically realistic feedback mechanism.

Our results challenge preconceptions concerning the strength and costliness of noise suppression by autoregulation in genetic networks, and call for experiments comparing transcriptional and small RNA-mediated control of chromosomal gene expression in living cells. The disparate signatures on protein noise

Table 1. Negative feedback loops between pairs of protein transcription factors and small non-coding RNAs in various organisms

Species	Pairs	Examples	Notes
Human	61	let-7a/TRIM32, miR-20a/E2F1, miR-195/E2F3, miR-19b/MYCN, miR-9/NFKB1, miR-25/PTEN, miR-27a/RUNX1, miR-21/STAT3, miR-410/CDKN2A, miR-302a/SOX2.	Predicted by us (see Supplementary Data) using bioinformatics databases (53 pairs identified using TransmiR (35) and microRNA.org databases) or found in the literature (36,37) (8 pairs).
Caenorhabditis elegans	23	miR-43/Lin-26, miR-48/DAF-3, miR-236/Lin-26, miR-80/ODR-7.	Predicted from bioinformatics data by (38). Negative and positive feedback loops not distinguished.
Escherichia coli	4	OmpA/OmpR, OmrB/OmpR, GadY/GadX, RybB/RpoE.	Identified by us using the RegulonDB dataset of regulatory interactions (39) (3 pairs) or in the experimental literature (40) (1 pair).

A full list of the pairs we identified is given in Supplementary Data (Supplementary Table S2).

properties, together with the high cost and sensitivity to rate parameters of the most effective noise control by small RNA suggest different functional roles for the two feedback architectures.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online: Supplementary Tables 1–2, Supplementary Figures 1–4, Supplementary information and Supplementary References [21,40–50].

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