Translational Repression Contributes Greater Noise to Gene Expression than Transcriptional Repression

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ABSTRACT Stochastic effects in gene expression may result in different physiological states of individual cells, with consequences for pathogen survival and artificial gene network design. We studied the contributions of a regulatory factor to gene expression noise in four basic mechanisms of negative gene expression control: 1), transcriptional regulation by a protein repressor, 2), translational repression by a protein; 3), transcriptional repression by RNA; and 4), RNA interference with the translation. We investigated a general model of a two-gene network, using the chemical master equation and a moment generating function approach. We compared the expression noise of genes with the same effective transcription and translation initiation rates resulting from the action of different repressors, whereas previous studies compared the noise of genes with the same mean expression level but different initiation rates. Our results show that translational repression results in a higher noise than repression on the promoter level, and that this relationship does not depend on quantitative parameter values. We also show that regulation of protein degradation contributes more noise than regulated degradation of mRNA. These are unexpected results, because previous investigations suggested that translational regulation is more accurate. The relative magnitude of the noise introduced by protein and RNA repressors depends on the protein and mRNA degradation rates, and we derived expressions for the threshold below which the noise introduced by a protein repressor is higher than the noise introduced by an RNA repressor.

INTRODUCTION

It was first postulated by theory (1–3), and later confirmed by numerous experiments (4–10), that a low number of molecules taking part in the regulation of gene expression results in significant random fluctuations in this process, and that the magnitude of random fluctuations depends on the mechanism of gene regulation (11–14). It was also demonstrated that stochastic effects in gene expression may result in different physiological states of individual cells, leading to heterogeneous cellular populations, with important consequences for pathogen survival strategies (15,16) and the design of artificial gene networks applicable in biotechnology and gene therapy (17). These results prompted numerous studies about the origins of stochastic fluctuations in gene expression processes (10,14,18–21), and one of the most important advances was the development of experimental methods allowing a determination of extrinsic and intrinsic components of the total gene expression noise (9,19). Intrinsic noise is measured as the stochastic variability between two identical genes expressed in the same single cell. It represents sources of noise located within transcriptional and translational machineries of a single gene, such as DNA conformational changes and the movement of polymerases and ribosomes during elongation processes. The remaining component of total gene expression noise is referred to as the extrinsic noise, and represents fluctuations in a single gene expression attributable to random changes in concentrations, states, and locations of molecules required for the expression of two identical genes in the same single cell. Therefore, extrinsic noise is determined by random fluctuations in the quantity of active polymerases, ribosomes, and transcription and translation factors available for a single gene located in a single cell. Another important result, obtained first as a theoretical prediction and subsequently confirmed by experimental studies, is the relationship between gene expression noise and the frequency of transcription and translation initiation (5,11,12). If genes are expressed at the same low level, where stochastic fluctuations are significant, the gene expressed with a lower translation initiation frequency exhibits lower variance in the number of protein molecules. These results imply an evolutionary tradeoff between the accurate and the energetically efficient gene expression control. The gene can be expressed at a very low level without large stochastic fluctuations if it is downregulated at the translation level. This, however, means that a lot of mRNA molecules are synthesized but not translated, which effectively raises the number of ATP molecules consumed to produce a single protein molecule.

We studied the contributions of a regulatory factor produced by one gene to the variance in the number of protein molecules produced by another gene. Therefore, we studied the contributions of a particular regulatory factor to the extrinsic noise of gene expression. We focused on regulatory factors that decrease the activity of a regulated

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gene by interfering with its expression machinery, and studied the effects of the nature of a regulatory factor (protein or RNA), and whether this factor interferes with gene expression on the transcription or translation level. The problem of the contributions of noise in regulatory gene expression to the extrinsic noise of a regulated gene has not been systematically studied. To the best of our knowledge, previous studies (19–22) involved the modeling of protein repressor action in which the number of repressor molecules was modeled using a mean field approximation, without any consideration of the noise of regulatory gene expression. In an interesting frequency domain analysis of gene expression noise (23–25), extrinsic noise sources were included in the analysis, but the authors focused on single-gene and autoregulated single-gene systems, and different configurations of the two gene regulatory cascades were not separately analyzed. Moreover, our work provides a different analysis of transcriptional and translational regulation than did previous contributions to the field (11,12): Instead of comparing the noise of genes with the same expression level, but different transcription and translation initiation frequencies, we compared the noise of genes with the same effective initiation frequencies (and expression levels) established by different external regulatory factors. Therefore, we studied the dependence of gene expression noise on the mechanism by which the product of an external gene controls the transcription and translation initiation frequencies, rather than on the initiation rates themselves. The reference system in our studies is a single gene in which transcription and translation initiation rates result from the intrinsic properties of the promoter and ribosome binding site, i.e., the initiation reactions are modeled as pseudo-first-order processes. The noise from the expression of a reference gene is compared with the noise of the two-gene regulatory cascade in which the repressor produced by a regulatory gene determines transcription or translation initiation rates.

The repression of gene expression by the product of another gene is a frequently occurring interaction in gene regulatory networks. There are four general mechanisms by which the product of one gene can interfere with the expression of other genes: I), protein synthesized by a gene can act as a transcriptional repressor; II), protein synthesized by a gene can act as a translational repressor; III), RNA synthesized by a gene can act as a transcriptional repressor; and IV), RNA synthesized by a gene can act as a translational repressor. The repression of transcription by a protein repressor (mechanism I) is one of the most frequently encountered mechanisms of gene regulation, and molecular biology textbooks mention numerous classic examples, such as the lactose operon and phage-λ repressors. Examples of translational protein repressors (mechanism II) include the binding of iron-regulating proteins to the 5′ untranslated regions of eukaryotic transcripts (26). RNA interference (27) is the best-known example of a mechanism by which RNA regulatory factors act on the mRNA of a regulated gene (mechanism IV). This mechanism was used to develop gene-silencing protocols, with applications in both research and possibly therapeutic strategies. Another example of an RNA translational repressor is the product of the *OxyS* gene (28) in *Escherichia coli*. Finally, some RNA regulators interfere with the transcription of a regulated gene (mechanism III). For example, the 6S RNA (28) in *E. coli* interacts with a housekeeping form of an RNA polymerase and inhibits transcription, most probably by acting as a DNA mimic and competitive inhibitor. The expression of B2 RNA (28) in mice was induced during an environmental stress response, and this RNA molecule was shown to inhibit the action of RNA polymerase II by preventing the formation of active preinitiation complexes. The discovery of thousands of noncoding RNAs in eukaryotic transcriptomes further motivates the study of gene regulation by RNA molecules, because it suggests that RNA molecules acting as gene regulatory factors may be much more common in gene regulatory networks than was previously thought. It was postulated that the increased fraction of nonprotein-coding to protein-coding transcripts is a major feature that distinguishes the human genome from that of other organisms, and the network of RNA regulatory interactions may play a key role in brain development (29). Taking into account the effort dedicated to studies of stochastic effects in gene expression, it is surprising that these effects have so far been analyzed only in the first two of the four basic mechanisms of gene expression interference, with only one work dedicated to posttranscriptional events (30). Stochastic effects in gene-expression interference by regulatory RNA molecules have attracted little attention, despite their recognized importance in the regulation of cellular processes and the design of gene-silencing tools. To the best of our knowledge, studies have been limited to the comparison of a transcriptional protein repressor and translational RNA repressor with the same mean numbers of mRNA molecules, in a work that focused on the quantitative characterization of threshold-linear response in small RNA regulatory systems (31).

To draw general conclusions about the contributions of regulatory factors to the extrinsic noise in the four basic mechanisms of gene expression interference, we focused on a mathematical analysis of models involving only the basic steps of the gene expression process. We argue that noise characteristics of very strongly or very weakly repressed genes do not differ from those in well-studied single gene expression, and we introduce the notion of switching noise (SN), defined as the difference between the noise of a repressed gene and the noise of a reference, unregulated gene expressed with the same transcription and translation initiation frequencies. The switching noise, defined in this way, describes the variance in single gene expression emerging because of the action of a regulatory gene product, and represents a contribution of the regulatory factor to the extrinsic noise of a regulated gene. We derive

analytical formulas describing the dependence of SN on the mechanism of gene expression interference, and test their validity by computer simulations. We identify properties of switching noise that are determined by a mechanism of expression interference, and that are robust with respect to values of quantitative parameters. Our results show that translational repression results in a higher gene expression noise than repression at the promoter level, and that this relationship does not depend on quantitative parameter values. The magnitude of noise introduced by protein and RNA repressors depends on the protein and mRNA degradation rates, and we derive expressions for the threshold below which the noise introduced by a protein repressor is higher than the noise introduced by an RNA regulatory factor. We demonstrate the implications of our results in understanding the evolution of complex gene regulatory networks and a construction of artificial genetic circuits.

THEORY

Model of single gene expression

We use a very general model in which a single gene expression is modeled by four pseudo-first-order processes, lumping together elementary reactions corresponding to transcription, translation, and mRNA and protein degradation. Numerous studies demonstrated that equations governing the stochastic behavior of this model can be solved exactly, and used to derive analytical formulas describing the dependence of stochastic effects in gene expression on the fundamental parameters of this process (1,2,12,19–22,32–36). Analytical solutions of the general gene expression model were successfully used to generate predictions about stochastic effects in gene expression that were verified by experimental work (5,9,12,19). Following a well-established methodology (12,19), a state of the system is defined by the number of mRNA (r) and protein (p) molecules. The probability $f_t(r,p)$ of a system being in the state (r,p) at time t is investigated to study the stochastic dynamics of the system. The rate of change of $f_t(r,p)$ attributable to the production and degradation of protein and mRNA molecules is given by the following Master equation:

$$\begin{aligned}\frac{df_t}{dt}(r,p) &= k_r f_t(r-1,p) + \gamma_r(r+1)f_t(r+1,p)\\ &\quad + k_p r f_t(r,p-1) + \gamma_p(p+1)f_t(r,p+1)\\ &\quad - \big(k_r + \gamma_r r + k_p r + \gamma_p p\big)f_t(r,p), \qquad (1)\end{aligned}$$

where k_r and k_p denote the transcription and translation initiation frequencies, and γ_r and γ_p are the mRNA and protein degradation rates. Closed analytical formulas for means and variances of r and p can be obtained using the generating function approach. Here, noise strength is quantified as a Fano factor (the ratio of the variance to the mean). We used the Fano factor for the sake of analytic convenience, but qualitative conclusions are also valid if the magnitude of noise is quantified by a variation coefficient (the ratio of the standard deviation to the mean). The noise in a single gene expression, expressed as the steady-state Fano factor of the number of protein molecules, is given by the familiar equation (12) derived with the moment generating function approach:

$$\frac{var(p)}{\langle p\rangle} = 1 + \frac{k_p}{\gamma_r + \gamma_p}. \qquad (2)$$

Four mechanisms of gene expression interference

We investigated a two-gene regulatory network in which an RNA or a protein produced by a regulatory gene decreases the transcription or translation initiation rate of a regulated gene. The four possible regulatory schemes are shown in Fig. 1. One state of the system is given by (r_1, p_1, r_2, p_2), where r_1 and p_1 are the numbers of RNA and protein molecules produced by the regulatory gene, and r_2 and p_2 are the numbers of mRNA and protein molecules produced by the regulated gene. The $f_t(r_1, p_1, r_2, p_2)$ is the probability of the system being in the state (r_1, p_1, r_2, p_2) at time t. The model involves the following state transitions and their rates, common to all four regulatory mechanisms:

$$\begin{aligned}
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{r1}} f_t(r_1+1,p_1,r_2,p_2),\\
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{p1}r_1} f_t(r_1,p_1+1,r_2,p_2),\\
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{\gamma_r r_1} f_t(r_1-1,p_1,r_2,p_2),\\
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{\gamma_p p_1} f_t(r_1,p_1-1,r_2,p_2),\\
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{\gamma_r r_2} f_t(r_1,p_1,r_2-1,p_2),\\
f_t(r_1,p_1,r_2,p_2) &\xrightarrow{\gamma_p p_2} f_t(r_1,p_1,r_2,p_2-1), \qquad (3)
\end{aligned}$$

where k_{r1} and k_{p1} are transcription and translation initiation frequencies of the regulatory gene, and k_{r2} and k_{p2} are transcription and translation initiation frequencies of the regulated gene, respectively. Here, we study the case where RNA and protein degradation rates γ_r and γ_p are equal for both genes.

Interactions between the product of a regulatory gene and a promoter or an mRNA of the regulated gene are modeled by Hill functions, $F_r(x) = vk_{r2}/(1 + (x/H)^n)$ and $F_p(x) = vk_{p2}/(1 + (x/H)^n)$, where H is the dissociation constant of a regulatory factor and the DNA or RNA binding site, n is the number of regulatory factor molecules taking part in an active initiation complex, and $v > 1$ is an additional parameter used to compare the noise strength of different regulatory mechanisms that have the same effective transcription and translation initiation frequencies.

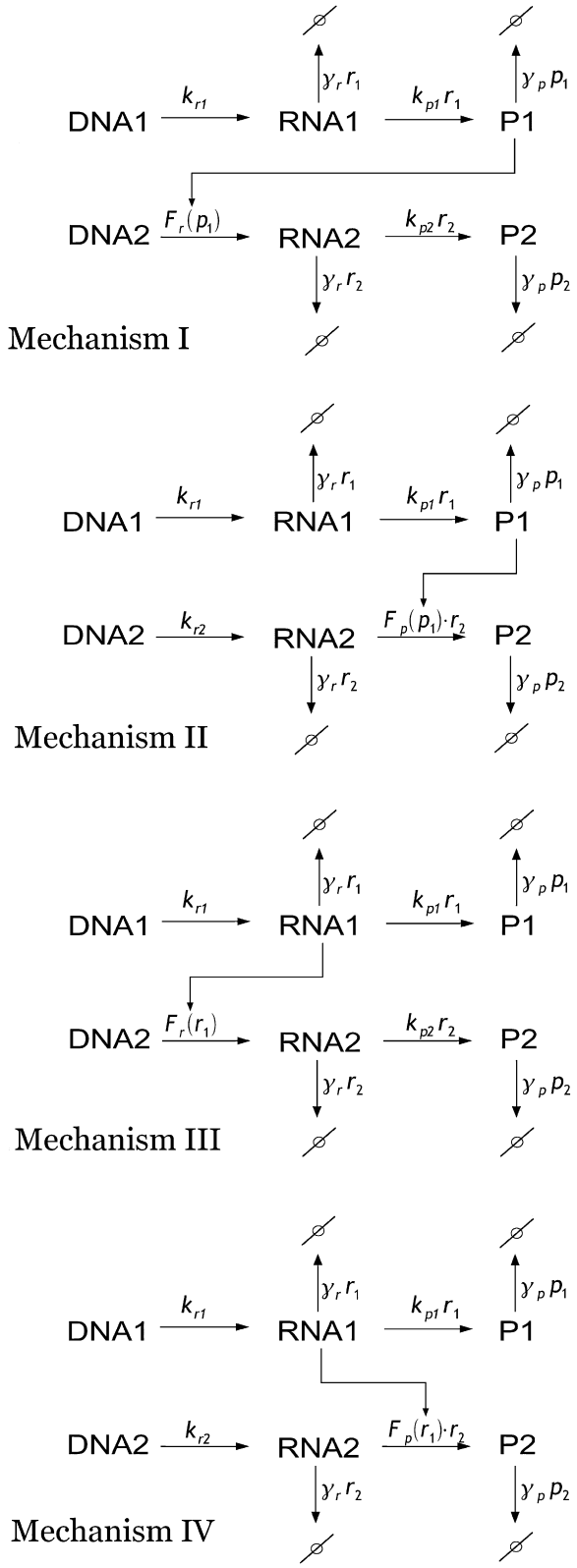

FIGURE 1 Four basic mechanisms of gene expression interference. Models involve the following substances: DNA1 and DNA2, the promoter regions of the regulatory and regulated gene; RNA1 and RNA2, transcripts of the regulatory and regulated gene; and P1 and P2, proteins produced by the regulatory and regulated gene. Parameters for describing interactions include: k_{r1} and k_{r2}, transcription initiation rates of the regulatory and regulated gene; k_{p1} and k_{p2}, translation initiation rates of the regulatory and regulated gene; γ_r and γ_p, the RNA and protein degradation rates; and $F_r(r_1)$, $F_r(p_1)$, $F_p(r_1)$, and $F_p(p_1)$, Hill equations describing effective rates of transcription or translation initiation as a function of the number of protein or RNA regulatory factor molecules.

Transcriptional regulation by a protein repressor (mechanism I) is modeled as the following state transitions and rates:

$$\begin{aligned} f_t(r_1,p_1,r_2,p_2) &\xrightarrow{F_r(p_1)} f_t(r_1,p_1,r_2+1,p_2),\\ f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{p2}r_2} f_t(r_1,p_1,r_2,p_2+1). \end{aligned} \tag{4}$$

In mechanism II, the protein acts as a translation factor, with the following transitions and rates:

$$\begin{aligned} f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{r2}} f_t(r_1,p_1,r_2+1,p_2),\\ f_t(r_1,p_1,r_2,p_2) &\xrightarrow{F_p(p_1)r_2} f_t(r_1,p_1,r_2,p_2+1). \end{aligned} \tag{5}$$

The action of RNA regulatory factors is modeled as the following transitions and rates. In mechanism III (the transcriptional regulation):

$$\begin{aligned} f_t(r_1,p_1,r_2,p_2) &\xrightarrow{F_r(r_1)} f_t(r_1,p_1,r_2+1,p_2),\\ f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{p2}r_2} f_t(r_1,p_1,r_2,p_2+1). \end{aligned} \tag{6}$$

In mechanism IV (the RNA interference):

$$\begin{aligned} f_t(r_1,p_1,r_2,p_2) &\xrightarrow{k_{r2}} f_t(r_1,p_1,r_2+1,p_2),\\ f_t(r_1,p_1,r_2,p_2) &\xrightarrow{F_p(r_1)r_2} f_t(r_1,p_1,r_2,p_2+1). \end{aligned} \tag{7}$$

Solution of Master equation for repressor-regulated gene networks

If a system contains reactions where rates depend on more than one molecular species, the closed solution of the Master equation cannot be obtained by the moment generating function approach. However, in a stationary state, distributions of the number of mRNA and protein molecules have a finite width, and sample only small regions of the domains of corresponding Hill functions. We may therefore approximate Hill functions by their linearizations about mean values of the numbers of either mRNA, $\langle r_1\rangle$, or protein molecules, $\langle p_1\rangle$, and obtain a closed solution of the Master equation using moment generating functions. Linearized forms of Hill functions, Master equations, and their solutions for mechanisms I–IV are presented in detail in the Supporting Material. Here we present only final solutions for the steady-state Fano factors of the reporter protein (var(p_2)/$\langle p_2\rangle$):

$$
\begin{aligned}
FF_I &= 1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)}\left(1 + \frac{1}{2}\frac{k_{p1}k_1^{I^2}k_{r1}\left((\gamma_r + k_{p1})(\gamma_r + \gamma_p)^2 + \gamma_r\gamma_p(\gamma_r + \gamma_p + k_{p1})\right)}{\gamma_p\gamma_r\left(k_{pr}\gamma_r\gamma_p + k_1^I k_{p1}k_{r1}\right)(\gamma_r + \gamma_p)^2}\right),\\
FF_{II} &= 1 + \frac{1}{2}\frac{k_{r1}k_1^{II^2}\left((\gamma_r + \gamma_p)(k_{p1^2}) + k_{p1^2}\left(\gamma_p k_{p1} + (\gamma_r + \gamma_p)^2\right)\right) + k_{r2}(\gamma_r + \gamma_p)2k_2^{II^2}\gamma_p^2}{\gamma_p(\gamma_r + \gamma_p)^2\left(\gamma_p k_2^{II}k_{r2}\right)},\\
FF_{III} &= 1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)}\left(1 + \frac{1}{2}\frac{k_1^{III^2}k_{r1}(2\gamma_r + \gamma_p)}{\gamma_r(\gamma_r + \gamma_p)\left(\gamma_r k_{rr} + k_1^{III}k_{r1}\right)}\right),\\
FF_{IV} &= 1 + \frac{k_{r1}k_1^{IV^2} + k_{r2}k_2^{IV^2}}{(\gamma_r + \gamma_p)k_2^{IV}k_{r2}},
\end{aligned}
\tag{8}
$$

where FF_{I}, FF_{II}, FF_{III}, and FF_{IV} are steady-state Fano factors for the number of reporter proteins in mechanisms I–IV, respectively, and k_i^{I}, k_i^{II}, k_i^{III}, and k_i^{IV} are linearization constants for Hill functions in these mechanisms (see the Supporting Material).

Switching noise

In this work we are interested in the contributions of a repressor to the noise of a regulated reporter gene. To establish the noise arising because of the action of a repressor, we need to compare the noise strength of two genes that differ exclusively in terms of being influenced by the repressor. To ensure that the only difference between two genes is the presence or absence of a repressor in their regulatory mechanism, we need to compare genes that not only have the same mean mRNA and protein levels, but also the same effective rates of transcription and translation initiation frequencies. Therefore, we are interested in the noise of a reporter gene, whose transcription or translation initiation frequency is determined by the action of the repressor, compared with the noise of the unregulated reporter gene expressed with the same effective transcription and translation initiation frequencies. Hence, we introduce the notion of switching noise (SN), defined as the difference between the Fano factor of a regulated gene and the Fano factor of an unregulated gene expressed with the same transcription and translation initiation frequencies. For the unregulated gene, the intrinsic contribution to total noise strength is given by Eq. 2. Thus, $\mathrm{SN_I}$, $\mathrm{SN_{II}}$, $\mathrm{SN_{III}}$, and $\mathrm{SN_{IV}}$, the switching noise expressions for mechanisms I–IV, are given as:

$$
\begin{aligned}
SN_I &= FF_I - \left(1 + \tfrac{k_{p2}}{\gamma_r + \gamma_p}\right),\\
SN_{II} &= FF_{II} - \left(1 + \tfrac{F_p(\langle p_1\rangle)}{\gamma_r + \gamma_p}\right),\\
SN_{III} &= FF_{III} - \left(1 + \tfrac{k_{p2}}{\gamma_r + \gamma_p}\right),\\
SN_{IV} &= FF_{IV} - \left(1 + \tfrac{F_p(\langle r_1\rangle)}{\gamma_r + \gamma_p}\right).
\end{aligned}
\tag{9}
$$

Validity of analytical linearized models

To validate our analytical models, we ran exact stochastic simulations, using the Gillespie algorithm (37), of all four regulatory mechanisms with nonlinear Hill functions, and compared the results with analytical solutions. The simulations, as run for a variety of different parameter sets, are presented in Fig. 2 and the Supporting Material (Fig. S3 and Fig. S4). Plots of noise strength as a function of the amount of a regulatory factor (Fig. 2) and mRNA of a regulated protein (Fig. S3) clearly show that analytical model is very accurate, even if the expression of regulatory gene is as low as ~20 protein molecules (see the Supporting Material for details). Therefore, the analytical model is valid for a very wide range of parameters. Only if the number of regulatory molecules is extremely low does the model slightly overestimate the noise, in which case, exact stochastic simulations should be used instead. This renders our conclusions valid for most gene regulatory interactions, because cases in which there are only a few molecules of regulatory factors are rare, especially in eukaryotic cells.

Here we use the Hill function to model interactions between a regulator and a nucleic-acid binding site. Therefore, we assume that a regulator-binding site complex is in quasisteady state, and do not explicitly model its reversible formation. To test to what extent this assumption influences our results, we ran Gillespie-algorithm simulations of mechanism I, in which the reversible formation of a protein-DNA complex was modeled by second-order association and first-order dissociation reactions. The model, quantitative parameters, and results are given in the Supporting Material. The qualitative shape of the Fano factor curves is the same for the simulation of the detailed model and analytical solution using a linearized Hill function. In particular, maximum Fano factor values correspond to very close mean gene expression values in both numerical simulations and analytical solutions. However, the analytical model underestimates the noise. The numerical result obtained for faster association/dissociation rates is closer to the analytical solution, which is expected as the Hill equation is derived at the limit of infinitely fast reversible complex formation. We conclude that our results are exact in terms of the limit of te infinitely fast association/dissociation of the regulator-binding site complex, and that the Hill equation approximation is accurate enough to justify the qualitative conclusions presented below.

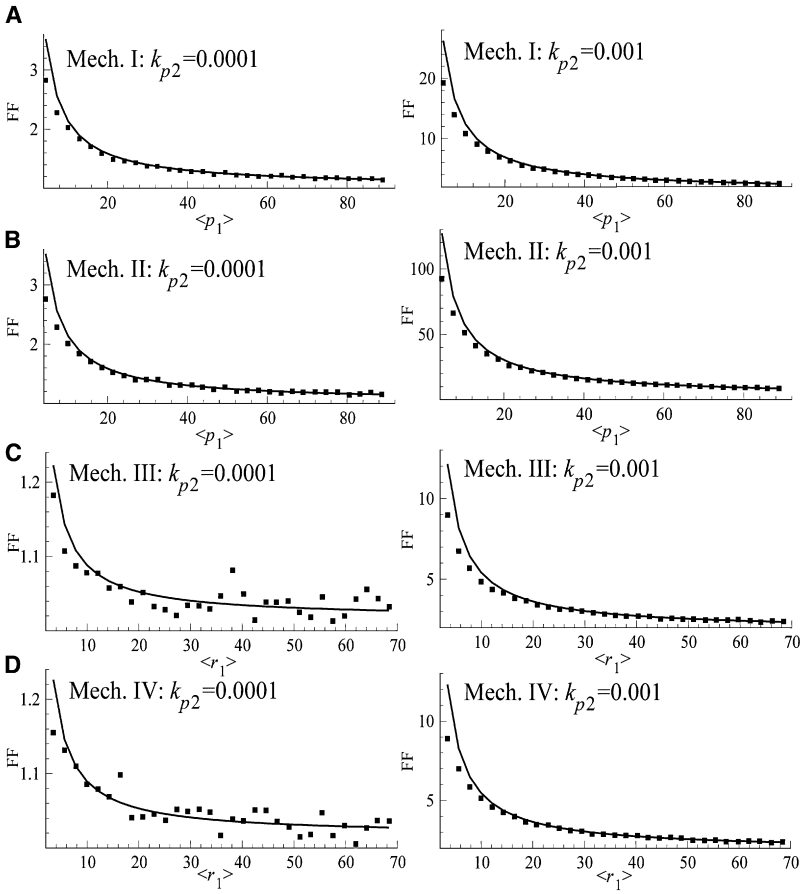

FIGURE 2 Comparison of linearized analytical model with exact stochastic simulations. Plot shows noise of a regulated gene as a function of mean number of regulatory factor molecules at steady state. Analytical solution is plotted as solid lines; squares represent results of Gillespie-algorithm simulation (see the Supporting Material). For each mechanism (*Mech.*), simulations were performed for two systems differing in basal expression level of the regulated gene ($k_{p2} = 10^{-4}$, *left*; $k_{p2} = 10^{-3}$, *right*). These parameters were used in all simulations: $n = 2$, $v = 2$, $\gamma_R = \log(2)/120$, and $\gamma_P = \log(2)/3600$. Transcription rate k_{r1} varies from 0.006–0.4. Analytical curves and numerical simulations overlap when the number of regulatory-factor molecules is larger than ~20.

RESULTS AND DISCUSSION

Properties of switching noise

To study the relationship between repression strength and reporter gene expression noise in the four basic mechanisms of gene expression interference, we used Eqs. 8 and 9 to compute the Fano factor and the switching noise for different values of the dissociation constant H. For each mechanism, the values of dissociation constant H were varied to sample the entire range of regulated gene expression levels, from the fully repressed gene to the gene unaffected by the repressor. For each value of H, Eq. 8 was used to calculate the Fano factor of repressed gene expression. Subsequently, Eq. 9 was used to calculate the SN, i.e., the difference between the Fano factor of the repressed gene and of the unregulated gene, with transcription and translation initiation rates equal to the effective, steady-state initiation rates of the gene controlled by the repressor acting with given value of H.

The plots of the Fano factor (Fig. 3) and the SN (Fig. 4) as functions of the mean number of reporter protein molecules show the following properties of SN: i), SN > 0; ii), for a very weak (very large H) and a very strong (very small H) repression, SN converges to 0; iii), SN has a maximum as the function of the mean number of the regulated gene protein molecules; and iv), SN curves for different mechanisms do not intersect. These properties can be also directly derived from Eq. 9 (see the Supporting Material). Properties i and ii show that in a certain region of the Hill function, the variance of a repressed reporter gene becomes significantly larger than the variance of an unregulated reporter gene expressed with the same transcription and translation initiation frequencies. Within the limits of very high and low H, the noise of repressed reporter gene expression converges to the noise of the reference, unregulated gene. There exists an intermediate value of the dissociation constant for which the difference between the noise of the repressed and the unregulated reporter gene is maximal.

The shape of the Fano factor curve as the function of repression strength was experimentally determined for mechanism I, and the results are presented in Fig. 4 of Blake et al. (13). Blake et al. (13) studied a genetic circuit where *yEGFP* expression in yeast is controlled by the Tet repressor, whose activity was modulated by the inducer. The effect of inducer on the Tet repressor corresponds to the change in the effective promoter-DNA dissociation constant, represented by H in our models. The experimentally determined FF curve shows a clear maximum for intermediate values of repression strength, in agreement with our theoretical predictions for mechanism I.

Property iv of the SN is essential for further analyses presented here. The fact that SN curves do not intersect means that for every mean value of the reporter protein, the SN values for different mechanisms are ordered in the same sequence. Therefore, we may choose an arbitrary point along the curves to characterize a qualitative relationship between SN values for different mechanisms. For the sake of convenience, we choose the dissociation constant H equal to the mean steady-state number of regulatory-factor molecules. Because this value is close to the maximum (Fig. 4), we refer to it as an approximated maximal switching noise (AMSN). The AMSNs for the four mechanisms of gene expression interference are given as:

$$\begin{aligned}
\mathrm{AMSN}_I &= \frac{k_{p2}}{(\gamma_r + \gamma_p)}\left(\frac{1}{8}n^2\frac{k_{r2}}{k_{r1}}\left(1 + \frac{1}{k_{p1}/\gamma_r} + \frac{\gamma_p}{(1 + \gamma_p/\gamma_r)k_{p1}} + \frac{\gamma_p/\gamma_r}{(1 + \gamma_p/\gamma_r)^2}\right)\right),\\
\mathrm{AMSN}_{II} &= \frac{k_{p2}}{(\gamma_r + \gamma_p)}\left(\frac{1}{8}n^2\frac{k_{r2}}{k_{r1}}\left(1 + \frac{\gamma_p/\gamma_r}{1 + \gamma_p/\gamma_r} + \frac{1 + \gamma_p/\gamma_r}{k_{p1}/\gamma_r}\right)\right),\\
\mathrm{AMSN}_{III} &= \frac{k_{p2}}{(\gamma_r + \gamma_p)}\left(\frac{1}{8}n^2\frac{k_{r2}}{k_{r1}}\left(1 + \frac{1}{1 + \gamma_p/\gamma_r}\right)\right),\\
\mathrm{AMSN}_{IV} &= \frac{k_{p2}}{(\gamma_r + \gamma_p)}\frac{1}{4}n^2\frac{k_{r2}}{k_{r1}}.
\end{aligned} \tag{10}$$

Translational repressor introduces more noise into reporter gene expression than the transcriptional repressor

It follows from Eq. 10 that for every parameter set, the following inequalities hold:

$$\begin{aligned}
\mathrm{AMSN_{II}} &\geq \mathrm{AMSN_{I}}\\
\mathrm{AMSN_{IV}} &\geq \mathrm{AMSN_{III}}.
\end{aligned} \tag{11}$$

Property iv of the SN and inequalities 11 imply that for every parameter set, translational repression results in a higher SN than the transcriptional repression, independent of whether the protein or RNA acts as the repressor. Therefore, if two genes are expressed with the same effective transcription and translation initiation rate, the gene regulated by a translational repressor has a higher variance in the number of protein molecules than the gene regulated by a transcriptional repressor. In other words, the translational repressor introduces a larger contribution to the extrinsic noise of gene expression than the transcriptional repressor. This seems to contradict the results of previous research demonstrating that gene-expression noise can be decreased by decreasing the translation initiation rate (5,11,12). However, those previous studies compared genes with the same mean expression level but different transcription and translation initiation rates, whereas this study compares genes with the same transcription and translation initiation rates set by different repression mechanisms. Previous studies demonstrated that if two genes produce a protein at the same low level, the gene with a lower translation initiation rate exhibits smaller variance. On the other hand, our results show that if two genes are expressed with the same effective rates of transcription and translation, the gene regulated by a translational repressor exhibits a larger variance than the gene regulated by a transcriptional repressor. This conclusion remains unchanged for the whole biologically relevant range of gene expression levels, because SN curves do not intersect.

Relationship between magnitudes of noise introduced by RNA and protein repressors

Equation 10 implies the following inequalities when comparing the noise introduced by RNA and protein repressors. In the case of both transcriptional and translational repression, the protein transcription factor introduces more noise than its RNA counterpart if a regulator's translational initiation frequency is smaller than the level that depends exclusively on protein and mRNA degradation rates. Because the SN curves in Fig. 4 do not intersect, inequalities 12 hold for the entire range of reporter gene expression levels:

$$\begin{aligned}
\mathrm{AMSN}_I \geq \mathrm{AMSN}_{III} &\Leftrightarrow k_{p1} \leq \frac{(\gamma_r(\gamma_r + \gamma_p) + \gamma_r\gamma_p)(\gamma_r + \gamma_p)}{\gamma_r^2},\\
\mathit{A}\mathrm{MSN}_{II} \geq \mathrm{AMSN}_{IV} &\Leftrightarrow k_{p1} \leq \frac{(\gamma_r + \gamma_p)^2}{\gamma_r}.
\end{aligned} \tag{12}$$

To understand inequalities 12, one should note that the translation initiation rate of the regulator k_{p1} influences the number of regulatory proteins, but does not influence the number of regulatory RNAs. A decrease of k_{p1} implies a decrease in the number of regulatory factor molecules in mechanisms I and III with respect to the number of regulatory factor molecules in mechanisms II and IV. The increase in the number of regulatory factor molecules makes the reporter gene noise smaller (Fig. 5). Expressions on the right-hand side of equalities 10 provide threshold k_{p1} values at which the relative amounts of protein and mRNA factors are such that relations between mechanisms I and III versus II and IV change. Therefore, the relationship between magnitudes of noise introduced by RNA and protein repressors depends on the relative amounts of these regulators in the gene expression cascades being compared.

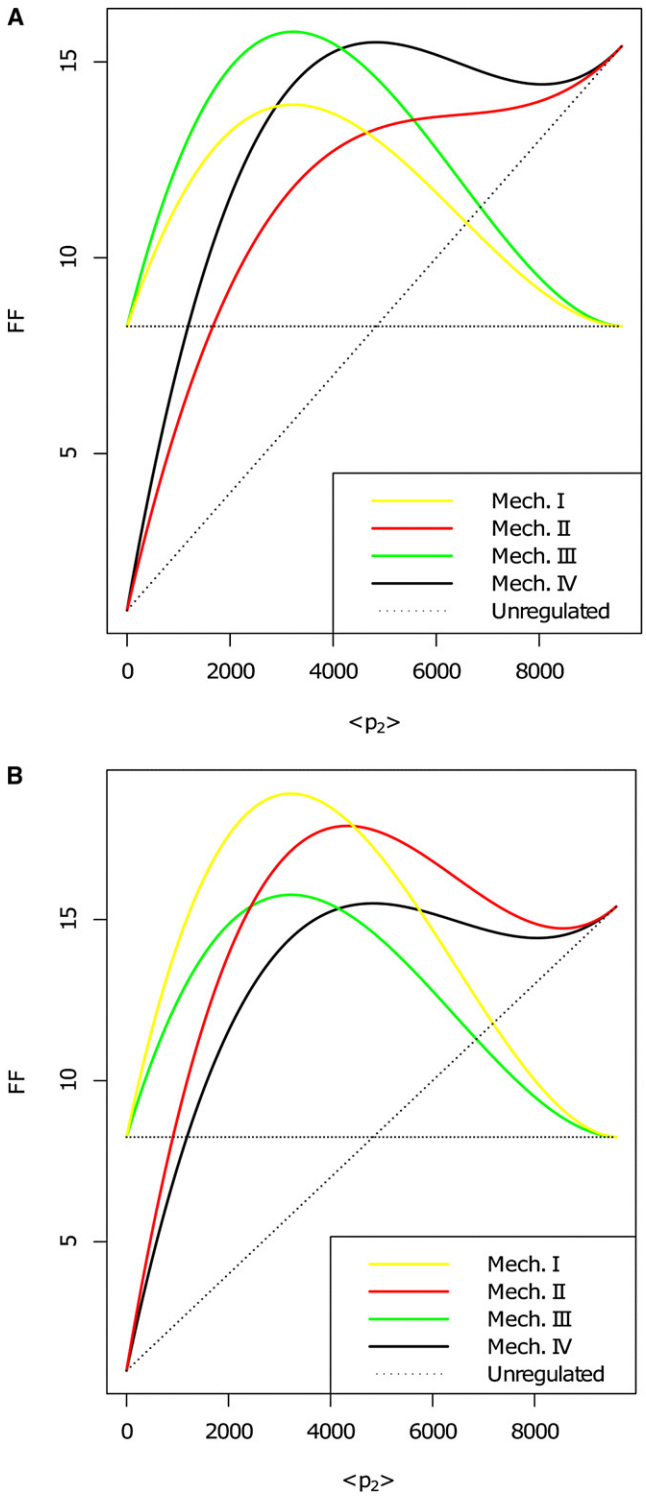

FIGURE 3 Noise strength in four gene-regulatory mechanisms (*Mech.*). The Fano factor (FF; ratio of variance to mean) is plotted as a function of the steady-state mean number of protein molecules produced by a regulated gene. Mechanisms I, II, III, and IV are plotted as yellow, red, green, and black solid lines, respectively. Plots *A* and *B* were calculated for different values of k_{P1} resulting in different mean levels of regulatory protein in mechanisms I and II. The mean protein level of the regulated gene is increased by changing the dissociation constant H in the Hill function, and decreasing the repression strength. The H was varied in a range of 3–2000 for mechanisms III and IV (*A*, *B*). For mechanisms I and II, H was varied from 9 to 58×10^3 (*A*) and 0.9 to 58×10^2 (*B*). The remaining parameters were set as: $n = 2$, $v = 2$,$\gamma_r = 0.0006$, $\gamma_p = 0.0002$, $k_{r1} = k_{r2} = 0.1$, $k_{p2} = 0.0058$, $k_{p1} = 0.0058$ (*A*), and $k_{p1} = 0.00058$ (*B*). For very highly and very weakly repressed genes, the FF of the regulated gene converges to the dotted black line representing an unregulated single gene. In agreement with previous results, the FF of a single gene regulated on the translation level (mechanisms II and IV) grows with the mean number of protein molecules, whereas the FF of the transcriptionally regulated single gene remains constant. The shape of FF curve obtained for mechanism I is in agreement with published experimental data (13), where *yEGFP* expression in yeast was controlled by a Tet repressor, whose activity was modulated by the inducer.

Regulation of protein and mRNA degradation

To explore the role of degradation rates further, we analyzed four additional regulatory cascades in which the RNA or protein product of the regulatory gene controls degradation of the mRNA or the protein produced by the regulated gene. The effective degradation rates were rendered dependent on the regulatory factor via the Michaelis-Menten equation, with the product of the regulatory gene acting as an enzyme (protease or RNAase). The models, derivations, and results are presented in the Supporting Material. The main conclusion of this analysis is that the regulation of protein degradation introduces more noise into reporter gene expression than degradation of the mRNA. Regardless of whether the synthesis or degradation steps of mRNA or protein synthesis are regulated, the regulation of gene expression at the protein (translation) level introduces more extrinsic noise than the regulation of mRNA (transcription). Henceforth we will consider only the four basic mechanisms shown in Fig. 1.

Implications for the synthetic biology

The design of artificial genetic circuits is an emerging field with potential applications in biotechnology and gene therapy (17). An understanding of gene expression noise is important in the design of gene regulatory cascades controlling transgene expression, especially in the context of gene therapy, where precise control may be essential. The results presented here are relevant to the situation where a gene is placed under a negative control of another gene, but its expression level does not change. In the context of artificial genetic circuit design, an engineer may want to maintain the native expression level of a gene, but may also want the ability to increase it by administering an inducer. For example, the Pip-KRAB (17) system is used to construct cells or animals in which an essential gene is placed under the control of a repressor protein, and may be overexpressed by the administration of an inducing chemical that decreases repressor activity. Our results show that under these circumstances, the choice of translational repressor does not offer any advantage in terms of expression accuracy. Moreover, if the mRNA of a regulatory gene is more stable than the

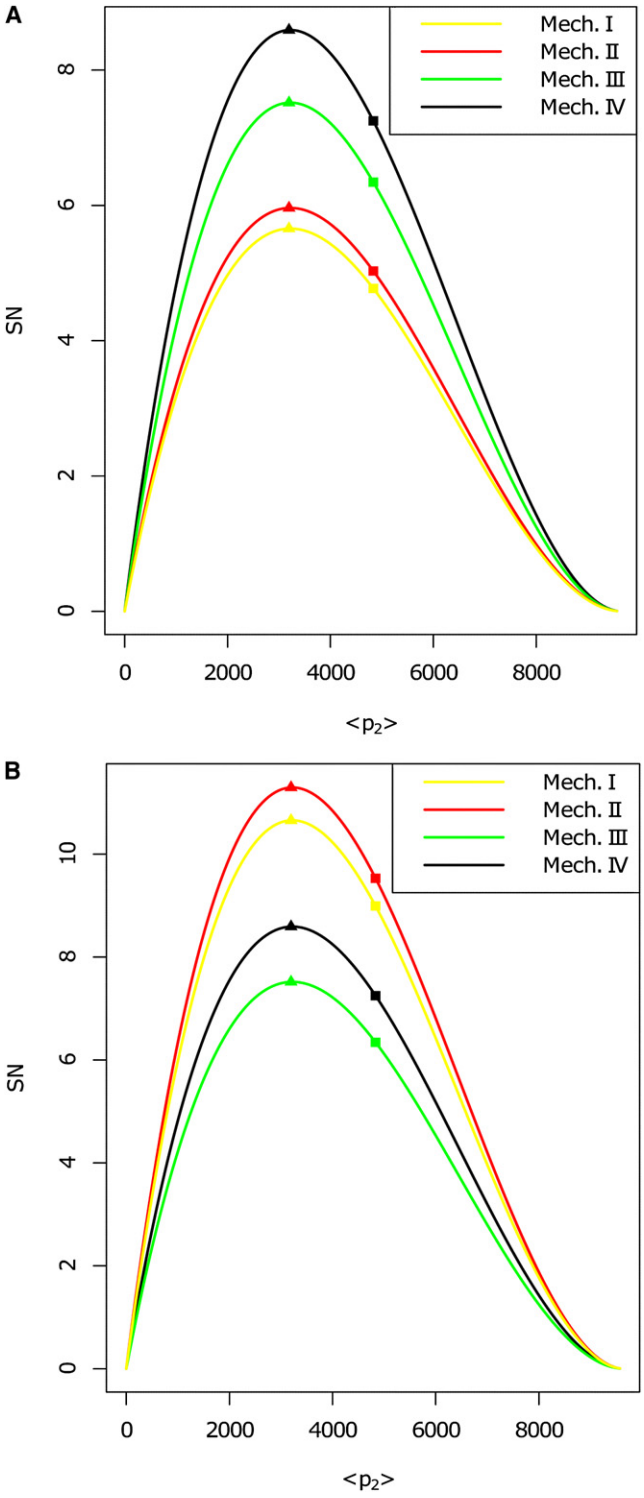

FIGURE 4 Switching noise in four gene-regulatory mechanisms (*Mech.*). Switching noise is plotted as a function of the steady-state mean number of protein molecules produced by a regulated gene. Each curve represents the difference between the FF of a repressed gene and an unregulated gene expressed with the same effective transcription and translation initiation rates. Mechanisms I, II, III, and IV are plotted as yellow, red, green, and black solid lines, respectively. Maximal and approximated maximal switching noise (AMSN) are marked as triangles and squares, respectively. The mean protein level of a regulated gene is increased by changing the dissociation constant H in the Hill function and decreasing the repression strength. Plots *A* and *B* were calculated for different values of k_{p1}, resulting in different mean levels of regulatory protein in mechanisms I and II. The ranges of dissociation constant H and other parameters were set at the same values as in Fig. 3. The SN curves converge to 0 for very highly and very weakly repressed genes. For intermediate values of H, their order remains the same for all values of $<p_2>$. For each regulatory factor, the curve corresponding to the translational regulation is always above the curve corresponding to the transcriptional regulation. The order of curves corresponding to protein and RNA regulatory factors depends on the expression level of a regulated gene, and differs between plots *A* and *B*.

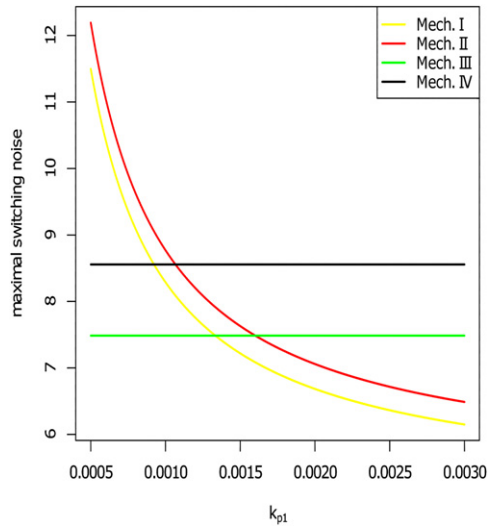

FIGURE 5 Maximal switching noise in four gene regulatory mechanisms (*Mech.*) as a function of k_{p1}. Maximal switching noise was calculated for all gene-regulatory mechanisms, using the same parameters as in Figs. 3 and 4. k_{p1} was changed in the range of 0.005–0.003. The maximal switching noise of mechanisms II (*green*) and IV (*black*), in which RNA is a regulator, does not depend on k_{p1}. If k_{p1} is low and the number of protein regulator molecules is low, mechanisms II (*red*) and III (*yellow*) result in a higher switching noise. Below a certain critical value of translational efficiency, the number of protein regulator molecules becomes sufficiently high to make the maximal switching noise of mechanisms II and III lower than the maximal switching noise of mechanisms I and II.

protein, a transcriptional repressor to maintain the transcription initiation frequency at a certain level will introduce less extrinsic noise than a translational repressor used to maintain the translation initiation frequency.

The relationship between the quantitative parameters of the model and the noise contribution of protein and RNA repressors is another finding of this work that is applicable in the context of artificial gene network design. Although accurate values for kinetic parameters are usually not available for the biological parts used in genetic circuit design, order-of-magnitude estimations can be usually undertaken. If the approximate values of translation initiation and degradation rates are known, an engineer can use inequalities 12 to decide whether a protein or RNA repressor will provide more accurate control in the genetic circuit to be constructed. Even in a case where no estimations of translation initiation and

degradation rates can be made, inequalities 12 still demonstrate that an increase in the regulatory gene translation initiation rate will increase the accuracy of a protein repressor with respect to the RNA repressor.

Finally, synthetic gene circuits are a major tool for studying stochastic effects in molecular interaction networks of a cell. In the circuits used to study the effect of translation initiation, the translational initiation rate was varied by changing the intrinsic properties of the ribosome binding site (5,13,38). Our study shows that interesting phenomena can be discovered by studying an alternative design in which the circuit contains a translational repressor whose expression is under experimental control. Such tunable, translational repressors have been used in genetic circuit design (17,31,39), but none of the systems has been observed at a single-cell level. Our work also prompts comparisons of designed gene regulatory systems in which the same initiation rates are established by the use of different extrinsic regulatory factors.

Implications for the natural selection of gene regulatory cascades

Analysis of the transcription regulatory networks reconstructed from full-genome sequences shows that transcription factors are less conserved than their target genes, which indicates an evolutionary plasticity of the gene regulatory networks (40). It is very likely that the gene regulatory networks evolved via tinkering with transcriptional interactions, and that many evolutionary events resulted in a gene to acquire another gene product as its regulator, thus gaining the ability to respond to a new set of environmental signals. The gene expression noise is one of the natural-selection factors determining the survival of new regulatory networks in the population.

In the evolutionary process, genes can become negatively regulated by product of the other gene in one of four ways depending on whether the protein or RNA acts as transcriptional or translational regulatory factor (Fig. 6). Our results show that if the acquisition of a new negative regulator strongly decreases the mean expression level of a regulated gene, the noise strength converges to the values calculated for single-gene expression (Fig. 3). Therefore, in accordance with previous reports, the noise strength of the transcriptional regulator will become constant, whereas the noise strength of a translational regulator will decrease linearly with the mean expression level. Our results further show that for strongly repressed genes, the noise strength depends exclusively on whether the regulator acts on transcription or translation, and does not depend on the protein/RNA nature of the repressor. The accuracy of gene expression is frequently measured in terms of the variation coefficient (VC, i.e., the ratio of the standard deviation and the mean). Contrary to the noise strength, expressed as a Fano factor, which is independent of the mean, the VC decreases with decreasing expression level. However, previous works demonstrated that if two genes in which basal expression

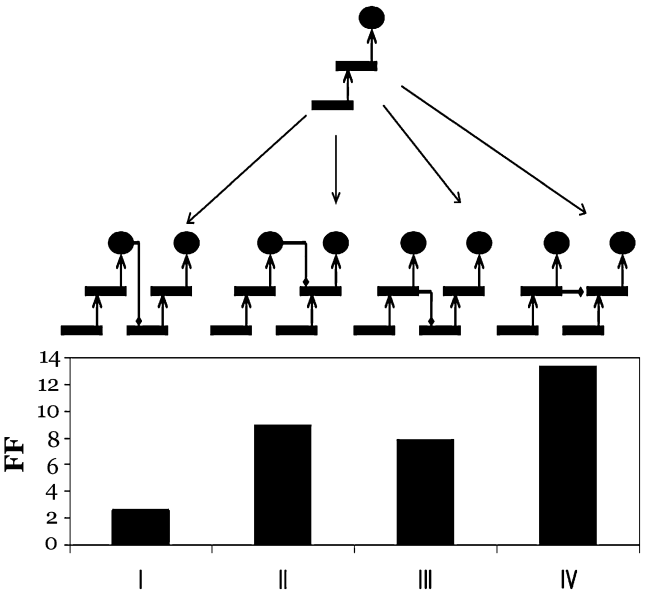

FIGURE 6 Example of scenario of alternative evolutionary events. The gene acquires a negative regulator in one of the four ways described here (mechanisms I–IV). The repressor does not change the effective transcription or translation initiation rate of the regulated gene under conditions most frequently encountered by the cell. The natural-selection advantage of the new regulatory link involves an ability to respond toa new environmental signal. The plot shows the Fano factor of the four alternative networks for the parameter set, with mRNA more stable than the protein, resulting in pronounced differences between transcriptional and translational repression. These rate constants were used: $\gamma_R = 0.0006$, $\gamma_P = 0.02$, $k_{r1} = 1$, $k_{r2} = 0.2$, $k_{p1} = 0.000384$, and $k_{p2} = 0.0232$. In mechanisms III and IV, k_{r1} was set to 0.02 to make the average, steady-state level of p_2 equal to 386.7 in all four regulatory networks.

levels are decreased to the same low value are compared, the one that is regulated at the translation level is expressed with a lower VC. Our results also confirm that the effect does not depend on the protein/RNA nature of the repressor (data not shown). The evolutionary scenario described above, in which the acquisition of a new repressor strongly decreases basal gene expression level, leads to the natural-selection tradeoff between the accuracy and efficiency of the gene expression process. The gene can be accurately expressed at a low level when a repressor acts on translation, with the result that a lot of mRNA molecules are unnecessarily synthesized and not translated. The tradeoff between gene expression accuracy and efficiency was described previously (5,11,12). The notion that translational repression is more accurate than transcriptional repression, although it is energetically wasteful, was further reinforced by the comparison of transcriptional and translational negative-feedback loops (30).

The evolutionary event in which a gene acquires a repressor that strongly decreases its basal expression level may bring about dramatic changes in the system as the activity of the gene product is suddenly removed from the network. Therefore, many regulatory links are likely to emerge, producing interactions that do not significantly

change the level of gene expression under normal environmental conditions, but that provide a selective advantage as the gene gains the ability to respond to new environmental signals. This scenario implies that a new regulatory link does not significantly change the effective transcription or translation initiation rate, but simply makes this rate dependent on an external factor. The unexpected finding of our study is that in this case, the acquisition of a translational repressor is no longer advantageous in terms of gene-expression accuracy. Our results show that if two genes are expressed with the same effective initiation rates, the gene in which the translation initiation step is controlled by an external repressor exhibits a higher noise strength than a gene in which the repressor controls promoter activity. The magnitude of this difference varies, depending on the rate constants, but the noise strength of translational repression is never smaller that the noise strength of transcriptional repression. More specifically, the ratio of the noise strength of the translational and transcriptional regulation involving the same kind of regulator (RNA or protein) is proportional to the quotient of degradation rates: $\gamma_R/(\gamma_P + \gamma_R)$. The difference between noise strengths of transcriptional and translational repression is most pronounced when the mRNA is more stable than a protein. Therefore, in prokaryotes, where most of the transcripts are short-lived, there is very little difference between the noise strength related to the acquisition of a translational or transcriptional repressor. However, even in this case, translational regulation does not confer any advantage in terms of expression accuracy, contrary to what was expected based on previous studies. The difference between the two evolutionary scenarios may be more pronounced in eukaryotes, where most of the transcripts are stable, and many proteins are quickly degraded. Fig. 6 gives an example of an evolutionary scenario for the parameter set resulting in pronounced differences of noise strength between translational and transcriptional repressors. The difference attributable to the RNA/protein nature of the regulator is described by inequalities 12, and Fig. 6 demonstrates a scenario where regulation by an RNA repressor contributes more extrinsic noise than regulation by a protein repressor.

Our findings have additional implications for understanding the evolvability of gene regulatory networks. We conclude that gene expression noise is not an important natural-selection factor discriminating between transcriptional and translational repressors if the protein is more stable than the mRNA. In addition, genes may acquire repressors without a substantial change in the effective basal initiation rate. Under these circumstances, the regulatory network is flexible in its choice of transcriptional or translational regulatory links, thus increasing its evolvability. Moreover, this finding further reinforces the hypothesis that acquisition of a repressor, without a change in effective transcription or translation initiation rates, is a frequent event during the evolution of gene regulatory networks.

To summarize, the dependence of gene-expression noise on basic gene regulatory network architectures is more complex than previously thought. Evolutionary scenarios must be compared very carefully, and conclusions will be different depending on whether the acquisition of new regulatory factors decreases the basal gene expression level or maintains this level under the most frequently occurring environmental conditions, while enabling control by a new set of environmental signals. The complexity of factors determining the natural selection of transcriptional or translational repressors under different circumstances may be responsible for the fact that neither of the two regulatory scenarios examined can be considered predominant. Most of the repressors described in detail so far are proteins interfering with the function of an RNA polymerase on the promoter level. However, in light of recent discoveries of the abundance of noncoding RNAs within genomes, one cannot exclude the possibility that RNA interference on the translation level is equally abundant.

CONCLUSIONS

We compared the contribution of regulatory factors to the extrinsic gene expression noise in four basic mechanisms of gene expression interference. We compared genes with the same effective transcription and translation initiation rates, but with different mechanisms by which these rates are established. Our reference system was an unregulated gene in which initiation frequencies were modeled by first-order reactions and were therefore treated as intrinsic properties of the gene expression machinery. We studied SN, defined as a Fano-factor difference between the reference gene and a gene in which one of the initiation reactions (transcription or translation) has been made dependent on an external repressor without changing its effective rate. Thus, SN represents the contribution of a gene encoding a single regulatory factor to the extrinsic noise of the expression of a regulated gene. Here the stochastic effects in RNA-based gene regulatory networks were analyzed for the first time, to the best of our knowledge. Our results show that translational repression introduces more noise into regulated gene expression than repression at the promoter level, independent of whether a protein or RNA acts as the repressor. Hence, we demonstrate that translational repression is leakier than transcriptional repression. Importantly, these conclusions do not depend on quantitative parameters of the system, although the difference between the expression noise introduced by protein and RNA repressors acting on the same regulation level (transcription or translation) does depend on rate constants. We derived a formula for the threshold value of the translational initiation rate of a regulatory gene below which the protein repressor introduces more noise than the RNA repressor. This threshold value of the regulatory gene translation rate depends only on the protein and mRNA degradation rates. The general inequalities (Eqs.

11 and 12) describing relationships between SNs and gene regulatory mechanisms are also true if a VC instead of a Fano factor is used. Therefore, our conclusions do not depend on a particular measurement of noise magnitude, and we use the Fano factor for an analytical convenience.

Our results may appear to contradict previous studies showing that a decrease in translational efficiency decreased the magnitude of stochastic fluctuations in gene expression (5,11,12). However, those previous studies considered the situation when the gene expression level is changed, and this change may be accomplished by changing either a transcription or a translation initiation frequency. In contrast, the results presented here are relevant to the situation where a gene is placed under the negative control of another genem, but its expression level does not change. The gene continues to play the same role in the system, but under specific conditions now regulated by the product of another gene. This scenario is relevant for the natural selection of genetic circuits that emerge when a gene acquires a new regulator, and for the design of artificial gene networks in which the native gene expression level is maintained by a repressor that allows controlled overexpression.

Here we present, to the best of our knowledge, the first comprehensive comparison of magnitudes of gene expression noise introduced by four basic mechanisms of repression. Our results thus contribute significantly to understanding the complex factors that determine the design principles of gene regulatory networks in the contexts of their function in living cells, natural selection, and synthetic biology.

SUPPORTING MATERIAL

Supplementary material is available at http://www.biophysj.org/biophysj/supplemental/S0006-3495(08)00069-6.

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