Supporting Material

Translational repression contributes greater noise to gene expression than transcriptional repression

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1. Derivation of analytical results, transcriptional and translational repression

We analyze here four basic models of regulatory networks of gene expression: a transcriptional or a translational repression either by mRNA or protein regulatory molecules. Our regulatory networks are schematically presented in Fig.1. We model the repression by taking transcription or translation initiation rates to be decreasing Hill functions of the number mRNA or protein regulatory molecules. The strength of the repression depends on the fluctuating number of regulatory molecules and this is a source of an additional
variance of the number of molecules of the regulated protein. Let us present formally our models.

The state of our regulatory network is specified at each time \( t \) by a vector of integers \((r_1, p_1, r_2, p_2)\), where \( r_1 \) and \( r_2 \) denote numbers of mRNA molecules related to a regulatory and a regulated gene respectively, similarly \( p_1 \) and \( p_2 \) are numbers of protein molecules coded by the first and the second gene. We assume that our biochemical processes are birth and death processes. It means that the probability of a production or a degradation of one mRNA or one protein molecule in a small time interval is proportional the length of that interval and coefficients of proportionality are corresponding reaction rates: \( k_{r1}, k_{r2}, k_{p1}, k_{p2}, \gamma_{r1}, \gamma_{r2}, \gamma_{p1}, \gamma_{p2} \). Probability that more than one event will take place in a small time interval is of the higher order with respect to the length of the interval. Finally, we assume that events taking place in disjoint time intervals are independent. Let \( f_t(r_1, p_1, r_2, p_2) \) be the probability that the system is in the state \((r_1, p_1, r_2, p_2)\) at the time \( t \).

We discuss four possible regulatory schemes which are shown in Fig.1. Our model involves the following state transitions and their rates common
to all four regulatory mechanisms:

\[
\begin{align*}
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{k_{r_1}} \text{ft}(r_1 + 1, p_1, r_2, p_2), \\
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{k_{p_1}r_1} \text{ft}(r_1, p_1 + 1, r_2, p_2), \\
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{\gamma_r r_1} \text{ft}(r_1 - 1, p_1, r_2, p_2), \\
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{\gamma_p p_1} \text{ft}(r_1, p_1 - 1, r_2, p_2), \\
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{\gamma_p p_2} \text{ft}(r_1, p_1, r_2 - 1, p_2), \\
\text{ft}(r_1, p_1, r_2, p_2) & \xrightarrow{\gamma_p p_2} \text{ft}(r_1, p_1, r_2, p_2 - 1),
\end{align*}
\] (1)

where \(k_{r_1}\) and \(k_{p_1}\) are transcription and translation initiation frequencies of the regulatory gene, \(k_{r_2}\) and \(k_{p_2}\) are transcription and translation initiation frequencies of the regulated gene. In this work we study the case where RNA and protein degradation rates, \(\gamma_r\) and \(\gamma_p\), are equal for both genes.
Figure 1: Schematic and matrix representations of four basic mechanisms of the gene expression interference studied in this work. Entries of the matrices contain parameters of the linearized model.
Interactions between products of the regulatory gene and a promoter or mRNA molecules of the regulated gene are modelled 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 nucleic acid or an RNA and \( n \) is the number of regulatory factor molecules taking part in an active initiation complex. An additional parameter \( v > 1 \) in the Hill function is used to compare noisiness of different regulatory mechanisms that have the same effective transcription and translation initiation frequencies.

Transcriptional regulation of the regulated gene by the protein repressor in **Mechanism I** is modelled by the following state transitions and their rates:

\[
\begin{align*}
  f_t(r_1, p_1, r_2, p_2) & \xrightarrow{F_r(p_1)} f_t(r_1, p_1, r_2 + 1, p_2), \\
  f_t(r_1, p_1, r_2, p_2) & \xrightarrow{k_{p2}r_2} f_t(r_1, p_1, r_2, p_2 + 1).
\end{align*}
\]  

(2)

In **Mechanism II**, the protein acts as a translation factor:

\[
\begin{align*}
  f_t(r_1, p_1, r_2, p_2) & \xrightarrow{k_{r2}} f_t(r_1, p_1, r_2 + 1, p_2), \\
  f_t(r_1, p_1, r_2, p_2) & \xrightarrow{F_p(p_1)r_2} f_t(r_1, p_1, r_2, p_2 + 1).
\end{align*}
\]  

(3)

The action of RNA regulatory factors is modelled by the following transitions and rates.
In Mechanism III (transcriptional regulation):

\[
\begin{align*}
    f_t(r_1, p_1, r_2, p_2) & \xrightarrow{F_r(r_1)} f_t(r_1, p_1, r_2 + 1, p_2), \\
    f_t(r_1, p_1, r_2, p_2) & \xrightarrow{k_{p2}r_2} f_t(r_1, p_1, r_2, p_2 + 1).
\end{align*}
\]  

(4)

In Mechanism IV (RNA interference):

\[
\begin{align*}
    f_t(r_1, p_1, r_2, p_2) & \xrightarrow{k_{r2}} f_t(r_1, p_1, r_2 + 1, p_2), \\
    f_t(r_1, p_1, r_2, p_2) & \xrightarrow{F_p(r_1)r_2} f_t(r_1, p_1, r_2, p_2 + 1).
\end{align*}
\]  

(5)

In a stationary state, distributions of the number of mRNA and protein molecules have finite widths and sample only small regions of the domain of the Hill functions. We may therefore approximate Hill functions by their linearizations around mean values of the numbers of either mRNA, \( \langle r_1 \rangle \), or protein molecules, \( \langle p_1 \rangle \). Hence the corresponding repressed reaction rates in (2-5) are replaced respectively by

\[
\begin{align*}
    k_{pr} & + k_1^I p_1, \\
    k_{pp} & + k_1^{II} p_1 + k_2^{II} r_2, \\
    k_{rr} & + k_1^{III} r_1, \\
    k_{rp} & + k_1^{IV} r_1 + k_2^{IV} r_2.
\end{align*}
\]  

(6)

where linearization constants for corresponding regulatory mechanisms are
Mechanism I:

\[ k_1^I = \frac{\partial F_r}{\partial p_1}( < p_1 >_I), \quad k_{pr} = F_r(< p_1 >_I) - k_1^I < p_1 >_I . \]

Mechanism II:

\[ k_1^{II} = \frac{\partial F_p}{\partial p_1}( < p_1 >_{II} < r_2 >_2), \quad k_2^{II} = F_p(< p_1 >_{II}), \quad k_{pp} = -k_1^{II} < p_1 >_{II} . \]

Mechanism III:

\[ k_1^{III} = \frac{\partial F_r}{\partial r_1}( < r_1 >_{III}), \quad k_{rr} = F_r(< r_1 >_{III}) - k_1^{III} < r_1 >_{III} . \]

Mechanism IV:

\[ k_1^{IV} = \frac{\partial F_p}{\partial r_1}( < r_1 >_{IV} < r_2 >_{IV}), \quad k_2^{IV} = F_p(< r_1 >_{IV}), \quad k_{rp} = -k_1 < r_1 >_{IV} . \]

In all mechanisms, \(< r_1 >_I, < r_1 >_{II}, < r_1 >_{III}, < r_1 >_{IV} \) and \(< p_1 >_I, < p_1 >_{II}, < p_1 >_{III}, < p_1 >_{IV} \) denote stationary means of \( r_1 \) and \( p_1 \). Thus, we can represent our models in the matrix form proposed in [1], see Fig.1.

We may now write Master equations (one for each regulatory scheme) for
the probability that the system will be in the state \((r_1, p_1, r_2, p_2)\) at the each time \(t\) [2, 1]:

\[
\frac{\partial f_t(r_1, p_1, r_2, p_2)}{\partial t} = \sum_{i,j=0}^{4} (E_{q_i}^{-1} - 1) A_{ij} q_j f_t(q_1, q_2, q_3, q_4) \\
+ \sum_{i,j=0}^{4} (E_{q_i}^{+1} - 1) \Gamma_{ij} q_j f_t(q_1, q_2, q_3, q_4),
\]

where \(q_0 = 1, q_1 = r_1, q_2 = p_1, q_3 = r_2, q_4 = p_2\),

\(E_{q_i}^{\pm 1}\) is a step operator [2] defined as

\[
E_{q_i}^{\pm 1} f_t(..., q_i, ...) = f_t(..., q_i \pm 1, ...),
\]

matrices \(A\) are given in Fig.1, and \(\Gamma\) is a matrix of degradation rates given below,

\[
\Gamma = \begin{bmatrix}
0 & 0 & 0 & 0 & 0 \\
0 & \gamma_r & 0 & 0 & 0 \\
0 & 0 & \gamma_p & 0 & 0 \\
0 & 0 & 0 & \gamma_r & 0 \\
0 & 0 & 0 & 0 & \gamma_p
\end{bmatrix}
\tag{7}
\]

We define now a moment generating function for each regulatory scheme,

\[
F(z_1, z_2, z_3, z_4) = \sum_{r_1, r_2, p_1, p_2 = 0}^{\infty} z_1^{r_1} z_2^{r_2} z_3^{p_1} z_4^{p_2} f_t(r_1, p_1, r_2, p_2).
\tag{8}
\]
We differentiate (8) with respect to time, use (7) and obtain a partial differential equation,

$$\frac{\partial F}{\partial t} = \sum_{i=0}^{4} (1 - z_i)(\gamma_{ii} \frac{\partial F}{\partial z_i} - \sum_{j=0}^{4} a_{ij} z_j \frac{\partial F}{\partial z_j}).$$

(9)

We differentiate (9) twice with respect to $z_i$, then set $z_1 = z_2 = z_3 = z_4 = 1$ and get ordinary differential equations for the first and second moments of $f_t(r_1, p_1, r_2, p_2)$. In a stationary state, the time derivative in the left-hand side of (9) is zero and after a tedious algebra we obtain stationary mean values of the number of molecules of the second protein, $<p_2>_i, i = I, II, III, IV$ for each regulation mechanism:

$$<p_2>_I = \frac{k_{p2} (k_{pr} \gamma_r \gamma_p + k_{1}^{p} k_{p1} k_{r})}{\gamma_r \gamma_p^2} = \frac{F_r(<p_1>_I) k_{p2}}{\gamma_r \gamma_p^2},$$

$$<p_2>_II = \frac{\gamma_r \gamma_p k_{pp}^2 + \gamma_p k_{r2} k_{p2} + k_{1}^{II} k_{p} k_{r}}{\gamma_r \gamma_p^2} = \frac{F_p(<p_1>_II) <r_2>_II}{\gamma_p},$$

$$<p_2>_III = \frac{k_{p2} (k_{rr} \gamma_r + k_{1}^{III} k_{r1})}{\gamma_r \gamma_p^2} = \frac{F_r(<r_1>_III) k_{p2}}{\gamma_r \gamma_p^2},$$

$$<p_2>_IV = \frac{\gamma_r k_{rp} + k_{1}^{IV} k_{r1} + k_{2}^{IV} k_{r2}}{\gamma_r \gamma_p} = \frac{F_p(<r_1>_IV) <r_2>_IV}{\gamma_p},$$

(10)
and Fano factors, $FF_i$ (ratios of stationary variances to stationary means):

\[
FF_I = 1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{2} \frac{k_{p1}k_{r1}^{I^2}k_{r1}}{\gamma_p \gamma_r (k_{p1}k_{r1} + k_{p1}k_{r1}^2) (\gamma_r + \gamma_p)^2} \right),
\]

\[
FF_{II} = 1 + \frac{1}{2} \frac{k_{r1}k_{I^2}^{II}((\gamma_r + \gamma_p)(\gamma_r + \gamma_p) + k_{r1}(\gamma_p k_{r1} + (\gamma_r + \gamma_p)^2)) + k_{r2}(\gamma_r + \gamma_p)2k_{II}^{II} \gamma_p^2}{\gamma_p (\gamma_r + \gamma_p)^2 (\gamma_p k_{II}^{II}k_{I^2})},
\]

\[
FF_{III} = 1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{2} \frac{k_{r1}^{II}k_{r1}(2\gamma_r + \gamma_p)}{\gamma_r (\gamma_r + \gamma_p)(\gamma_r k_{rr} + k_{II}^{III}k_{r1})} \right),
\]

\[
FF_{IV} = 1 + \frac{k_{r1}k_{I^2}^{IV} + k_{r2}k_{I^2}^{IV}}{(\gamma_r + \gamma_p)(k_{II}^{IV}k_{r2})}.
\]

The dependence of the Fano factor on the mean number of regulated protein molecules in four regulatory mechanisms is shown in Fig.3 in the main text.

To measure a contribution of the regulator fluctuations into the total noise of the number of molecules of the regulated protein, we introduce a switching noise, $SN$. It is defined as the difference between the noise of a repressed gene measured by the Fano factor and the noise of the same unregulated gene expressed with the same effective transcription and translation initiation frequencies. We know that for an unregulated gene, an intrinsic contribution to the total noise strength is $1 + \frac{k_{p}}{\gamma_r + \gamma_p} [1]$. Thus for our four regulatory types
we have:

\[
SN_{I} = FF_{I} - (1 + \frac{k_{p2}}{\gamma_{r} + \gamma_{p}}),
\]

\[
SN_{II} = FF_{II} - (1 + \frac{F_{p}(< p_{1} >)}{\gamma_{r} + \gamma_{p}}),
\]

\[
SN_{III} = FF_{III} - (1 + \frac{k_{p2}}{\gamma_{r} + \gamma_{p}}),
\]

\[
SN_{IV} = FF_{IV} - (1 + \frac{F_{p}(< r_{1} >)}{\gamma_{r} + \gamma_{p}}).
\]

(12)

Combining (11) and (12) we get

\[
SN_{I} = \frac{k_{p2}}{\gamma_{r} + \gamma_{p}} \left( \frac{1}{2} \frac{k_{p1}k_{1}^{I2}k_{r1}}{\gamma_{p}} \left( \gamma_{r} + k_{p1} \gamma_{r} \gamma_{p} + k_{1}^{I2}k_{r1} \right) \right),
\]

\[
SN_{II} = \frac{1}{2} \frac{k_{r1}k_{1}^{II2}}{\gamma_{p}^{2} \left( \gamma_{r} + \gamma_{p} \right)^{2} \left( \gamma_{r} + \gamma_{p} \right) \left( k_{r1}^{II}k_{r2} \right)^{2}},
\]

\[
SN_{III} = \frac{k_{p2}}{\gamma_{r} + \gamma_{p}} \left( \frac{1}{2} \frac{k_{r1}^{III2}}{\gamma_{r} \left( \gamma_{r} + \gamma_{p} \right) \left( \gamma_{r} + \gamma_{p} + k_{r1}^{III}k_{r1} \right)} \right),
\]

\[
SN_{IV} = \frac{k_{r1}k_{1}^{IV2}}{\gamma_{r} + \gamma_{p} \left( k_{2}^{IV}k_{r2} \right)}.
\]

(13)

The dependence of the SN on the mean value of the number of protein molecules of the regulated gene, \(< p_{2} >\), in four regulatory mechanisms, is shown in Fig.4 in the main text.

To compare noisiness of different regulatory mechanisms it would be desired to determine maximum values of the switching noise with respect to \(< p_{2} >\). Unfortunately, expressions for the maximum switching noise are very complicated so their analysis would hardly bring any useful insights.
Instead we propose to evaluate the switching noise for dissociation constants equal to the mean number of regulatory molecules. A switching noise evaluated for such values of $H$ will be called an approximately maximal switching noise ($AMSN$). When we put $H$ equal to the mean number of regulatory molecules in (12), we obtain the following expressions for $AMSN$:

\[
AMSN_{I} = \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( \frac{1}{16} n^2 \frac{v k_r 2}{k_r 1} \left( 1 + \frac{1}{k_{p1}/\gamma_r} + \frac{\gamma_p}{(1 + \gamma_p/\gamma_r) k_{p1}} + \frac{\gamma_p/\gamma_r}{(1 + \gamma_p/\gamma_r)^2} \right) \right),
\]

\[
AMSN_{II} = \frac{v k_{p2}}{(\gamma_r + \gamma_p)} \left( \frac{1}{16} n^2 \frac{k_r 2}{k_r 1} \left( 1 + \frac{\gamma_p/\gamma_r}{1 + \gamma_p/\gamma_r} + \frac{1 + \gamma_p/\gamma_r}{k_{p1}/\gamma_r} \right) \right),
\]

\[
AMSN_{III} = \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( \frac{1}{16} n^2 \frac{v k_r 2}{k_r 1} \left( 1 + \frac{1}{1 + \gamma_p/\gamma_r} \right) \right),
\]

\[
AMSN_{IV} = \frac{v k_{p2}}{(\gamma_r + \gamma_p)} \frac{1}{8} n^2 \frac{k_r 2}{k_r 1}.
\]

(14)

**Theorem:**

The sequence of $SN_{i}$ curves is determined by the sequence of $AMSN_{i}$ curves, i.e., if for a certain parameter set and a permutation $\pi$, $AMSN_{\pi 1} \geq AMSN_{\pi 2} \geq AMSN_{\pi 3} \geq AMSN_{\pi 4}$, and if dissociation constants $\{H_i\}_{i=I,II,III,IV}$ are taken such that $<p_2 >_I = <p_2 >_{II} = <p_2 >_{III} = <p_2 >_{IV}$, then $SN_{\pi 1} \geq SN_{\pi 2} \geq SN_{\pi 3} \geq SN_{\pi 4}$. 
Proof:

Equation (13) can be written as:

\[
SN_I = \frac{(k_I^I)^2}{F_p(< p_I >_I)} A_I, \\
SN_{II} = \frac{(k_{II}^I)^2}{F_p(< p_{II} >_{II})} A_{II}, \\
SN_{III} = \frac{(k_{III}^I)^2}{F_r(< r_{III} >_{III})} A_{III}, \\
SN_{IV} = \frac{(k_{IV}^I)^2}{F_r(< r_{IV} >_{IV})} A_{IV},
\]

(15)

and also in the following form:

\[
SN_I = \frac{< p_I >_{I}^{2(n-1)}}{H^3_I} \left(1 + \frac{< p_I >_{I}^{2(n-1)}}{H^4_{II}}\right)^3 B_I, \\
SN_{II} = \frac{< p_{II} >_{II}^{2(n-1)}}{H^3_{II}} \left(1 + \frac{< p_{II} >_{II}^{2(n-1)}}{H^4_{III}}\right)^3 B_{II}, \\
SN_{III} = \frac{< r_{III} >_{III}^{2(n-1)}}{H^3_{III}} \left(1 + \frac{< r_{III} >_{III}^{2(n-1)}}{H^4_{IV}}\right)^3 B_{III}, \\
SN_{IV} = \frac{< r_{IV} >_{IV}^{2(n-1)}}{H^3_{IV}} \left(1 + \frac{< r_{IV} >_{IV}^{2(n-1)}}{H^4_{IV}}\right)^3 B_{IV},
\]

(16)

where constants \(A_i\) and \(B_i\) are \(H_i\) independent.

To insure that \(< p_2 >_{I} = < p_2 >_{II} = < p_2 >_{III} = < p_2 >_{IV}\), following
conditions must be satisfied:

\[
\frac{<p_1>_I}{H_I} = \frac{<p_1>_II}{H_{II}} = \frac{<r_1>_III}{H_{III}} = \frac{<r_1>_IV}{H_{IV}}
\]

and this finishes the proof.

In the main text we have shown that \(SN_I \leq SN_{II}\) and \(SN_{III} \leq SN_{IV}\) for \(H\) equal to the average number of regulatory molecules in each mechanism and therefore it follows from the Theorem that the above inequalities hold for all \(<p_2>\).

2. Regulation of degradation

Here we discuss the situation in which the first gene encodes a protein that influences the degradation rate of the mRNA of the second gene (Mechanism V) or the second protein (Mechanism VI), see Fig.2. We can use formulae (13) to calculate the switching noise in these cases.

**Mechanism V:** protein \((p_1)\) regulates the degradation rate of mRNA \((r_2)\).

Transcription rates, \(k_{r1}, k_{r2}\), and translation rates, \(k_{p1}, k_{p2}\), are constant. We assume that the degradation rate of the second gene’s mRNA, \(F_{\gamma_r}(p_1)\), depends on the amount of the first protein, \(p_1\), through the Michaelis-Menten function,

\[
F_{\gamma_r}(p_1) = \frac{\gamma_{r2}^m (\frac{p_1}{H})^n}{1 + (\frac{p_1}{H})^n},
\]

where \(\gamma_{r2}^m\) is a new parameter describing the maximum intensity of degrada-
Figure 2: Mechanisms of degradation regulation.
tion. To compare the switching noise in Mechanism V (SN$_V$) with that in Mechanism I (SN$_I$), we ensure that all effective rates in both mechanisms are equal,

$$F_{\gamma r}(<p_1>) = \gamma r,$$

$$\frac{v k_{r2}}{1 + (<\frac{p_1}{H}>)^n} = k_{r2}. \quad (17)$$

This implies that $<p_2>_<p_2>_<p_2> <p_2> <r_2> + k_{r2}$.

To calculate the switching noise $SN_V$, we linearize $r_2 F_{\gamma r}$ around $<p_1>_<r_2>$ and $<r_2>$ and we use the expression for $SN_I$. Linearization constants are denoted by $k^V_{pr}, k^V_1$ and have the following form:

$$k^V_{pr} = \frac{\partial}{\partial p_1} F_{\gamma r}(<p_1>) <p_1> <r_2> + k_{r2},$$

$$k^V_1 = -\frac{\partial}{\partial p_1} F_{\gamma r}(<p_1>) <r_2>. $$

It follows from (17) that

$$k^V_1 = \frac{\gamma^m r}{v \gamma r} k^I_1. \quad (18)$$

Now we use (13) to get

$$SN_V = \left(\frac{\gamma^m r}{v \gamma r}\right)^2 SN_I = \left(\frac{H}{<p_1>}\right)^2 SN_I, \quad (19)$$

where the first equality follows from the first equality in (17). Hence we obtain the following
Proposition 1:

\[ SN_V \leq SN_I \iff < p_1 > \geq H. \]

The other mechanisms are treated in an analogous way.

**Mechanism VI**: protein \((p_1)\) regulates the degradation rate of protein \((p_2)\).

We now assume that the degradation rate of the second gene’s protein, \(F_{\gamma_p}(p_1)\), depends on the amount of the first protein, \(p_1\), through the Michaelis-Menten function,

\[ F_{\gamma_p}(p_1) = \frac{\gamma_p^{m_n} \left(\frac{p_1}{H}\right)^n}{1 + \left(\frac{p_1}{H}\right)^n}. \]

To compare the switching noise in Mechanism VI with that in Mechanism II, we set

\[ F_{\gamma_p}(< p_1 >) = \gamma_p, \quad (20) \]

\[ \frac{\nu k_{\gamma p} p_2}{1 + (\frac{< p_1 >}{H})^n} = k_{\gamma p}. \]

This implies that \(< p_2 >_{II} = < p_2 >_{VI}\). To get the switching noise \(SN_{VI}\), we linearize \(p_2 F_{\gamma_p}\) around \(< p_1 >_{VI}\) and \(< p_2 >_{VI}\). The appropriate linearization
constants are denoted by $k_{pp}^{VI}$, $k_1^{VI}$, and $k_2^{VI}$ and have the following form:

$$
\begin{align*}
  k_{pp}^{VI} &= \frac{\partial}{\partial p_1} F_{\gamma_p}(< p_1 >) < p_2 >_{VI} < p_1 >, \\
  k_1^{VI} &= -\frac{\partial}{\partial p_1} F_{\gamma_p}(< p_1 >) < p_2 >_{VI}, \\
  k_2^{VI} &= k_{p2}.
\end{align*}
$$

It follows from (20) that

$$
\begin{align*}
  k_2^{VI} &= k_2^{II} = k_{p2}, \\
  k_1^{VI} &= \frac{\gamma^{m}_{p2} k_1^{II}}{v_{\gamma_p}}.
\end{align*}
$$

From (13) we get

$$SN_{VI} = (\frac{\gamma^{m}_{p2}}{v_{\gamma_p}})^2 SN_{II} = (\frac{H}{< p_1 >})^2 SN_{II}.$$  \hspace{1cm} (21)

**Proposition 2:**

$SN_{VI} \leq SN_{II}$ iff $< p_1 > \geq H$.

**Mechanism VII:** RNA $(r_1)$ regulates the degradation rate of RNA $(r_2)$.

Transcription rates, $k_{r1}, k_{r2}$, and translation rates, $k_{p1}, k_{p2}$, are constant. We assume that the degradation rate of the second gene’s RNA, $F_{\gamma_r}(r_1)$, depends
on the amount of the first RNA, \( r_1 \), through the Michaelis-Menten function,

\[
F_{\gamma_r}(r_1) = \frac{\gamma_{r2}^m (\frac{r_1}{H})^n}{1 + (\frac{r_1}{H})^n},
\]

where \( \gamma_{r2}^m \) is a new parameter describing the maximum intensity of the degradation. To compare the switching noise in Mechanism VII (\( SN_{VII} \)) with that in Mechanism III (\( SN_{III} \)), we ensure that all effective rates in both mechanisms are equal,

\[
F_{\gamma_r}(<r_1>) = \gamma_r,
\]

\[
\frac{v k_{r2}}{1 + (\frac{<r_1>}{H})^n} = k_{r2}.
\]

This implies that \( <p_2>_{III} = <p_2>_{VII} \). To calculate the switching noise \( SN_{VII} \), we linearize \( r_2 F_{\gamma_r} \) around \( <r_1>_{VII} \) and \( <r_2>_{VII} \) and we use the expression for \( SN_{III} \). Linearization constants are denoted by \( k_{rr}^{VII}, k_1^{VII} \) and have the form:

\[
k_{rr}^{VII} = \frac{\partial}{\partial r_1} F_{\gamma_r}(<r_1>) <r_1> <r_2> + k_{r2},
\]

\[
k_1^{VII} = -\frac{\partial}{\partial r_1} F_{\gamma_r}(<r_1>) <r_2>.
\]

It follows from (22) that

\[
k_1^{VII} = \frac{\gamma_{r2}^m}{v \gamma_r} k_1^{III} \] (23)
and from (13) that

\[ SN_{VII} = \left( \frac{\gamma_{m}^{2}}{v\gamma_{r}} \right)^{2} SN_{III} = \left( \frac{H}{<r_{1}>} \right)^{2} SN_{III}. \]  \hspace{1cm} (24)

**Proposition 3:**

\[ SN_{VII} \leq SN_{III} \text{ iff } <r_{1}> \geq H. \]

**Mechanism VIII:** RNA \((r_{1})\) regulates the degradation rate of protein \((p_{2})\).

We now assume that the degradation rate of the second gene’s protein, \(F_{\gamma p}(r_{1})\), depends on the amount of the first RNA, \(r_{1}\), through the Michaelis-Menten function,

\[ F_{\gamma p}(r_{1}) = \frac{\gamma_{p_{2}}(r_{1})^{n}}{1 + (\frac{r_{1}}{H})^{n}}. \]

To compare the switching noise in Mechanism VIII \((SN_{VIII})\) with that in Mechanism IV \((SN_{IV})\), we set,

\[ F_{\gamma p}(<r_{1}>) = \gamma_{p}, \]
\[ \frac{vk_{p_{2}}}{1 + (\frac{<r_{1}>}{H})^{n}} = k_{p_{2}}. \]  \hspace{1cm} (25)

This implies that\( <p_{2}>_{IV} = <p_{2}>_{VIII} \). To get the switching noise \(SN_{VIII}\), we linearize \(F_{\gamma p}\) around \(<r_{1}>_{VII}\). The appropriate linearization constants
are denoted by $k_{pp}^{VIII}, k_1^{VIII},$ and $k_2^{VIII}$ and have the form:

\[ k_{pp}^{VIII} = \frac{\partial}{\partial r_1} F_{\gamma_p}(< r_1 >) < p_2 >_{VIII} < r_1 >, \]
\[ k_1^{VIII} = -\frac{\partial}{\partial r_1} F_{\gamma_p}(< r_1 >) < p_2 >_{VIII}, \]
\[ k_2^{VIII} = k_{p2}. \]

It follows from (25) that

\[ k_2^{VIII} = k_2^{IV} = k_{p2}, \]
\[ k_1^{VIII} = \frac{\gamma_{p2}^{m}}{v\gamma_p} k_1^{IV} \]

and from (13) we have

\[ SN_{VIII} = (\frac{\gamma_{p2}^{m}}{v\gamma_p})^2 SN_{IV} = (\frac{H}{< r_1 >})^2 SN_{IV}. \]  \hspace{1cm} (26)

**Proposition 4:**

$SN_{VIII} \leq SN_{IV}$ iff $< r_1 > \geq H.$

**Discussion**

We shown that mechanisms of degradation regulation are more noisy than their corresponding transcriptional and translational regulatory mechanism:

$SN_V > SN_I, SN_{VI} > SN_{II}, SN_{VII} > SN_{III}, SN_{VIII} > SN_{IV}$
for \( < p_1 >> H \) or \( < r_1 >> H \) (depending on the regulatory mechanism).

Now for a reasonable noise comparison we assume that

\[
\frac{\gamma_{p2}^m}{\gamma_p} = \frac{\gamma_{r2}^m}{\gamma_r}.
\]

It follows from first equalities in (19), (21), (24), and (26) and inequalities

\[
SN_{II} \geq SN_I, SN_{IV} \geq SN_{III}
\]

that

\[
SN_{VII} \geq SN_{V},
\]

\[
SN_{VIII} \geq SN_{VII}.
\]

**Proposition 5:**

*Protein degradation regulation introduces more noise into reporter gene expression than RNA degradation regulation.*

The above Proposition is analogous to the main result of our paper which says that the translational repressor introduces more noise into the reporter gene expression than the transcriptional one.

### 3. Validity of linearized models

**3.1 Non-linear Hill function**

To validate our analytical models, we run exact stochastic simulations using the Gillespie algorithm [3] of all four regulatory mechanisms with non-linear
Hill functions. Fano factors and mean values obtained by simulations are compared with those resulting from analytical derivations in Figs. 3-4 and in Fig. 2 in the main text. Each data point on the plots is a result of $10^4$ runs, steady state has been assumed to have been reached at 10 times protein half-life time. Since linearizations have been applied to both Hill functions $F_r(\cdot)$ and products $F_p(\cdot)r_2$, we checked if the accuracy of the analytical model is not affected by the low number of regulatory molecules and the large number of regulated gene’s RNA molecules. The plot of the Fano factor as a function of the amount of a regulatory factor (Fig. 2 in the main text) clearly shows that the analytical model is very accurate even if the expression of the regulatory factor is as low as 20 regulatory molecules. Nevertheless, in two mechanisms of translational regulation, this bound is only valid if the number of regulated gene’s RNA is smaller than few hundreds (see Fig. 3). If $< r_2 >$ is very large, more regulatory molecules are needed for the precise prediction. This is presented in Fig. 3, where the Fano factor is plotted as a function of $< r_2 >$ for Mechanism IV. Translational regulation by protein (Mechanism II) is not affected by the above condition since the number of protein regulators is usually very large. Above considerations demonstrate that our analytical model is sensible for a very wide range of parameters and makes our conclusions valid for most of gene regulatory interactions as the cases where there are only few molecules of regulatory factors are rare, especially in eukaryotic cells.
3.2 Genetic switch model

We discuss only Mechanism I here. We take into account stochasticity of the regulation process itself. A molecule of the first protein can bind to a gene regulatory region and stop the transcription. It means that the gene regulatory region can be in two states, the active state which we denote by 1 and the switched-off state denoted by 0. Let $f_t(0, r_1, p_1, r_2, p_2)$ be the probability that the system is in the state $(0, r_1, p_1, r_2, p_2)$ at the time $t$ and $f_t(1, r_1, p_1, r_2, p_2)$ be the probability that the system is in the state $(1, r_1, p_1, r_2, p_2)$. We have to include two additional processes in our stochastic model: forming a protein complex and its dissociation,

\[ f_t(0, r_1, p_1, r_2, p_2) \xrightarrow{\beta} f_t(1, r_1, p_1, r_2, p_2), \]
\[ f_t(1, r_1, p_1, r_2, p_2) \xrightarrow{\alpha p_1} f_t(0, r_1, p_1, r_2, p_2). \]

We run the Gillespie algorithm for Mechanism I with all 10 elementary processes and compare the results with ones obtained in the linearized model in Fig.5.

4. The noise strength ratio of translational and transcriptional regulation

To find out whether noise contribution related to the type of regulation is relevant factor influencing way how new regulatory connections are created we compare Fano factor ratios of transcriptional and transnational regula-
tion \( \frac{FF_{II}}{FF_{I}}, \frac{FF_{IV}}{FF_{III}} \) for parameters that ensure that effective transcription and translation rates are equal for all four mechanisms. If we set \( v = 2 \) and put \( H = < p_1 >, < p_1 >, < r_1 >, < r_1 > \) respectively, we satisfy above requirement and can easily use equations (9) and (10) from the main text. We obtain Fano factor ratios:

\[
\frac{FF_{II}}{FF_{I}} = \frac{1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{8} n^2 \frac{k_{r2}}{k_{r1}} \left( 1 + \frac{\gamma_r}{k_{p1}} + \frac{\gamma_p}{k_{p1}} + \frac{\gamma_r}{\gamma_r + \gamma_p} \right) \right)}{1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{8} n^2 \frac{k_{r2}}{k_{r1}} \left( 1 + \frac{\gamma_r}{k_{p1}} + \frac{\gamma_p}{k_{p1}} + \frac{\gamma_r}{\gamma_r + \gamma_p} \right) \right)}
\]

\[
\frac{FF_{IV}}{FF_{III}} = \frac{1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{8} n^2 \frac{k_{r2}}{k_{r1}} \left( 1 + \frac{\gamma_r}{\gamma_r + \gamma_p} \right) \right)}{1 + \frac{k_{p2}}{(\gamma_r + \gamma_p)} \left( 1 + \frac{1}{8} n^2 \frac{k_{r2}}{k_{r1}} \left( 1 + \frac{\gamma_r}{\gamma_r + \gamma_p} \right) \right)}
\]

It is reasonable to assume that a regulatory factor is significant if the corresponding ratio is big. To identify factors responsible for the magnitudes of these ratios, we can write them in the following form:

\[
\frac{FF_{II}}{FF_{I}} = \frac{1 + c_1 + c_1 (c_2 + c_3)}{1 + c_1 + c_1 (c_2 + c_3 U)}
\]

\[
\frac{FF_{IV}}{FF_{III}} = \frac{2 + 1/d}{(1 + U) + 1/d}
\]

where \( U = \frac{\gamma_r}{\gamma_r + \gamma_p} \) and constants, \( c_1, c_2, c_3, d \) correspond to coefficients present in the previous equations. We get that

- \( U \in (0, 1) \) is the crucial factor that determines the magnitude of considered ratios
- \( \frac{FF_{IV}}{FF_{III}} < 2 \)
\[ \frac{FF_{II}}{FF_{I}} \] is not bounded from the above.

In typical situations, \( \gamma_r \gg \gamma_p \), and therefore \( u \) is close to 1 and noises in transcriptional and translational regulatory schemes are similar. Since the upper bound for the ratio \( \frac{FF_{II}}{FF_{I}} \) does not exist, differences may be more significant in mechanisms with proteins as regulatory factors.

References


Figure 3: Comparison of the analytical linearized model with exact stochastic simulations for Mechanism IV for three different numbers of regulatory RNA molecules: \( < r_1 > = 17.3, k_{r1} = 0.1 \), a dotted line and triangles; \( < r_1 > = 34.6, k_{r1} = 0.2 \), a lashed line and diamonds; \( < r_1 > = 51.9, k_{r1} = 0.3 \), a solid line and boxes. If the number of regulatory RNA molecules is small (the dotted line), a discrepancy occurs if the number of regulated gene’s RNA molecules exceeds 200. The level for which this discrepancy occurs increases as the number of regulatory molecules increases (the lashed line). The inconsistency disappears for a large number of regulatory RNA molecules (the solid line and boxes). The following parameters has been used in simulations: \( n = 2, v = 2, \gamma_R = log(2)/120, \gamma_P = log(2)/3600, k_{p1} = 0.001, H = < r_1 > k_{r2} \in [0.02, 9.82] \).
Figure 4: Comparison of the analytical linearized model with exact stochastic simulations. Plots of analytical results presented in Fig. 3 in the main text together with results of computer simulations.
Figure 5: Comparison of the analytical solution of the linearized model and computer simulations for Mechanism I with the genetic switch. The computer simulations (solid lines) explicitly accounted for the protein/DNA complex formation. Two parameter sets have been simulated: diamonds $\alpha = 0.2, \beta = 0.1, 1, 10, 96, 500, 1000$; triangles - $\alpha = 0.05, \beta \in \{0.1, 1, 10, 24, 100, 1000\}$. In the linearized model, Fano factors were calculated using the Hill function to represent protein/DNA interaction. Two parameters set are shown: crosses $H \in \{0.5, 5, 50, 480, 2500, 5000\}$; squares $H \in \{2, 20, 200, 480, 2000, 20000\}$. In all simulations, other parameters of the models were set as follows: $k_{r1} = 0.1, k_{p1} = 0.00058, k_{p2} = 0.0058, \gamma_{r} = 0.006, \gamma_{p} = 0.0002$. 

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