

Noise in gene expression may be a choice of cellular system

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Gene expression and its regulation is a nonequilibrium stochastic process. Different molecules are involved in several biochemical steps in this process with low copies. It is observed that the stochasticity in biochemical processes is mainly due to the low copy number of the molecules present in the system. Several studies also show that the nonequilibrium biochemical processes require energy cost. But cellular system has developed itself through natural evolution by minimizing energy cost for optimum output. Here, we study the role of stochasticity qualitatively in a network of two genes using stochastic simulation method and approximately measure the energy consumption for the gene expression process. We find that the noise in gene expression process reduces the energy cost of protein synthesis. Therefore, we argued that the stochasticity in gene expression may be a choice of cellular system for protein synthesis with minimum energy cost.

Keywords: Gene expression and regulation; nonequilibrium biochemical processes; stochastic simulation.

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1. Introduction

Gene expression (GE) is a basic cellular process whereby proteins are synthesized according to the nucleotide sequences in the gene. Gene expression involved several biochemical reactions, the kinetics of which determine how the number of participating biomolecules changes as a function of time. There are two major steps in gene expression, transcription and translation. In the process of transcription mRNAs are synthesized. During the process of translation, the sequence of mRNA molecule is translated into the proteins.¹ Regulation is ubiquitous in complex living system. Gene expression is a regulatory process which can take place either at transcription, translation or degradation levels. Many regulatory molecules are involved in the gene expression process. There are two major types of regulatory molecules: activator and repressor.² Both types of regulatory molecules are also proteins and synthesized from some other genes. The regulatory molecules which regulate the transcription process

are called the transcription factors (TFs). They bind the specific binding site/sites on the gene and drive the gene into a state called the “ON” state or active state. The unbound state is called the “OFF” state or inactive state. Under the regulation by TFs, the gene can be either in the ON or OFF state depending on whether the TFs are bound to the gene or not.^{1–4} If the gene is in the ON (OFF) state then the transcription process can take place and mRNAs are synthesized with higher (low/basal) rate.⁴ From the newly born transcripts/mRNAs, proteins are synthesized and the process is called the translation. In the synthesis of proteins, ribosomes play the important roles. There are specific binding sites on the newly born mRNAs where ribosomes can bind and synthesize proteins. Newly born proteins have a specific degradation rate and the RNase molecules do that job. Each and every biomolecule does not exist forever to work rather they have a specific degradation rate. It is theoretically and experimentally well established that the biochemical events in gene expression are inherently stochastic in nature.^{5–19} The timing of the biochemical events cannot be predicted with certainty. The stochasticity in biochemical events lead to the fluctuation in mRNA and protein levels about a mean value. That fluctuation is called the noise in mRNA/protein level. Several studies show that the noise in gene expression appears due to low copy number of molecules (e.g. small number of regulatory molecules, very low gene copy number etc.) involved in the gene expression and regulation process.^{11–13,16–19} This stochastic nature of the biochemical reactions may be ignored in the limit of large number of biomolecules. The noise in gene expression appears from the random switching between the ON and OFF states, random production and degradation of mRNAs and proteins.^{5–9,20,23} It has also been shown that the stochastic effects due to random transitions between ON and OFF states of a gene are much stronger than the stochastic effects caused by random production and degradation of single mRNA and protein molecules.^{9,10,14,15} That happens because low copy number of regulatory molecules are involved to regulate one (haploid) or two (diploid) copies of a gene.

Several studies show that the stochasticity in gene expression produces two types of responses: graded and binary.^{24–30} In graded response, the mean protein level changes gradually and the distribution of protein level is shown to be unimodal. In binary response, gene expression occurs either at low level or at high level and the distribution of proteins will be bimodal. The bimodal distribution of protein level generally is observed with positive feedback network.^{31,32} But, stochasticity itself can produce bimodal distribution without any positive feedback loop. Random switching between the ON and OFF states of the gene play the important role in generation of bimodal distribution of protein level when there is no feedback loop.^{26–30}

The presence of noise or fluctuation in protein level gives rise to different phenomena in biological system.^{10,21,22} At the macroscopic level, we see that the biological systems are very much fine-tuned and deterministic. When a cell grows and divide from its embryonic stage, each and every event occurs at the right time with certainty. The event is controlled by some proteins since they are the functional molecules in the cells. Each and every cellular event is executed by some proteins.

They are required for structure, function, regulation of the body's tissues and organs. Many theoretical and experimental investigations show that the reduction of protein level may give rise to different diseases called haploinsufficiency.^{18,33–36} In diploid systems, proteins are produced from two copies of the same gene. If one of the two copies is mutated, the protein level gets reduced by 50%. That reduced amount of proteins are insufficient to carryout their specific job and give rise to several diseases called haploinsufficiency. This shows that for the proper functioning of the proteins, they have to stay above a critical level. But, there is a chance that the protein level may fall below the critical level because of the noise present in it.^{18,36–40} Thus, the noise in protein level has a detrimental role and therefore is undesirable.

The biological system or rather living system is basically a nonequilibrium system. To maintain the state of nonequilibrium in such system, they need energy from external sources. That is, for the formation of complex cellular structure and to maintain its activity, living cell has a cost.^{42–49} Again, the living system tries to optimize its function by minimizing the cellular cost through evolution and natural selection.^{45–47} The important cellular process like gene expression consists of several biochemical events e.g. gene activation–inactivation, transcription, translation, degradation, etc., which is not equilibrium rather nonequilibrium process.^{41,43,50–52} Huang *et al.*⁴³ studied the fundamental principle of energy consumption in gene expression. They showed that the speed of stochastic transitions between the ON and OFF states of the gene is at the cost of energy. That happens because many regulatory molecules need to accommodate at the promoter sites to get the ON state of the gene^{1–4} and that requires energy. The fluctuations in the number of regulatory molecules modulate the stochastic transitions between the ON and OFF states of a gene. Again, protein synthesis from the ON state of the gene also requires energy consumption. Study shows that a major part of the cellular energy is used for amino acid polymerization in protein synthesis process.⁵³ So, cell consumes energy to maintain a specific protein level and the consumption amount increases with the increase of mean protein level and random switching between ON-OFF states of gene. It is customary to think that there might be some mechanism of optimization of energy consumption in naturally evolving complex gene expression process.

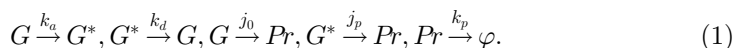
The cellular system achieved a complex structure by evolution and organized its contents according to its requirement. Now, one can raise the question: why cellular system has evolved with low copy number of regulatory molecules and noisy gene expression? Is it a choice or an accident? Does the undesirable noise have any role in the optimization of energy consumption in gene expression? In this work, we address such questions related to gene expression and show that introducing stochastic fluctuations in protein level cell can reduce the energy consumption efficiently. We show that the fluctuation in regulatory molecules has a crucial role behind it. We consider a stochastic model of simple gene regulatory network with two genes, a TF gene and a functional gene, and study the time evolution of protein's number from each gene with different noise level in TFs. We observe that the different amount of noise in TF level determines the noise and average protein level from the functional

gene. We also show from our stochastic simulation result that high noise in TF level reduces the cost of energy consumption to keep the protein level above some critical value from the functional gene rather than the less noisy TF level. Our general view is that the stochasticity or noise has a detrimental role in cellular functions as in the electronic system. But in this study, we find that the stochasticity can play the beneficial role in the cellular functions by saving energy, and therefore, may be a choice of cellular system.

2. Stochastic Model and Analysis

2.1. Two-state stochastic model of single gene expression

In two-state stochastic model of gene expression, a gene can be in two possible states: ON (active) and OFF (inactive). Genes make random transitions between the ON and OFF states with specific rate constants. The protein synthesis takes place in burst from both the states, active and inactive, of the gene with different rate constants. Let k_a and k_d be the activation and deactivation rate constants for the gene. In the active (inactive) state, protein production and degradation occurs with the rate constants j_p (j_0) and k_p , respectively. Here transcription and translation are lumped together into a single step.^{32,54,55} The biochemical steps of gene expression from a single gene is shown in Eq. (1).



Let $p_1(n, t)$ ($p_0(n, t)$) be the probability that at time t , gene is in the active (inactive) state G^* (G) with n number of protein molecules. The rate of change of probability with respect to the time is given by the Master equation

$$\begin{aligned} \frac{\partial p_0(n, t)}{\partial t} = & k_d p_1(n, t) - k_a p_0(n, t) + j_0 [p_0(n-1, t) - p_0(n, t)] \\ & + k_p [(n+1)p_0(n+1, t) - np_0(n, t)] \end{aligned} \quad (2)$$

$$\begin{aligned} \frac{\partial p_1(n, t)}{\partial t} = & k_a p_0(n, t) - k_d p_1(n, t) + j_p [p_1(n-1, t) - p_1(n, t)] \\ & + k_p [(n+1)p_1(n+1, t) - np_1(n, t)] \end{aligned} \quad (3)$$

For each rate constant, there is a gain term which adds to the probability and a loss term which subtracts from the probability. The Master Equation is a rate equation in which probability replaces concentration as the relevant variable.

We use the standard approach in the theory of stochastic processes to determine the steady state probability density function.⁵⁶ We define the generating functions

$$\begin{aligned} F_0(z, t) = \sum_n z^n p_0(n, t), \quad F_1(z, t) = \sum_n z^n p_1(n, t), \quad \text{and} \\ F(z, t) = \sum_n z^n p(n, t), \end{aligned} \quad (4)$$

where

$$\begin{aligned} F(z, t) &= F_0(z, t) + F_1(z, t), \\ p(n, t) &= p_0(n, t) + p_1(n, t), \end{aligned} \quad (5)$$

where $F(z, t)$ and $p(n, t)$ are the total generating function and total probability density function, respectively.

In terms of the generating functions given in Eq. (4), the Master equations (2) and (3) can be written as

$$\frac{\partial F_0(z, t)}{\partial t} = k_d F_1(z, t) - k_a F_0(z, t) + j_0(z-1)F_0(z, t) + k_p(1-z)\frac{\partial F_0(z, t)}{\partial z}, \quad (6)$$

$$\frac{\partial F_1(z, t)}{\partial t} = k_a F_0(z, t) - k_d F_1(z, t) + j_p(z-1)F_1(z, t) + k_p(1-z)\frac{\partial F_1(z, t)}{\partial z}. \quad (7)$$

In the steady state ($\frac{\partial F_0}{\partial t} = 0$ and $\frac{\partial F_1}{\partial t} = 0$), adding Eqs. (6) and (7), we get

$$j_p F_1(z, t) + j_0 F_0(z, t) = k_p \frac{\partial F(z, t)}{\partial z}. \quad (8)$$

Solving Eqs. (5) and (8), we get

$$F_1(z, t) = \frac{k_p}{J} \frac{\partial F(z, t)}{\partial z} - \frac{j_0 F(z, t)}{J}, \quad (9)$$

$$F_0(z, t) = \frac{j_p F(z, t)}{J} - \frac{k_p}{J} \frac{\partial F(z, t)}{\partial z}, \quad (10)$$

where $J = j_p - j_0$.

Now, in the steady state, using Eqs. (9) and (10), Eq. (6) can be written as

$$(a_2 z + b_2)F''(z) + (a_1 z + b_1)F'(z) + (a_0 z + b_0)F(z) = 0, \quad (11)$$

where $a_2 = 1$, $b_2 = -1$, $a = -(r_3 + r_4)$, $b_1 = (r_1 + r_2 + r_3 + r_4)$, $a_0 = r_3 r_4$, $b_0 = -(r_1 r_3 + r_2 r_4 + r_3 r_4)$, $r_1 = \frac{k_a}{k_p}$, $r_2 = \frac{k_d}{k_p}$, $r_3 = \frac{j_p}{k_p}$ and $r_4 = \frac{j_0}{k_p}$.

The exact solution of Eq. (11) is given by (using Mathematica)

$$F(z) = N e^{Kz} F_1\left(a_3; b_3; \left(\frac{z-\mu}{\lambda}\right)\right), \quad (12)$$

where $\mu = -\frac{b_2}{a_2}$, $K = \frac{\sqrt{D}-a_1}{2a_2}$, $D = a_1^2 - 4a_0 a_2$, $a_3 = \frac{b_2 K^2 + b_1 K + b_0}{2a_2 K + a_1}$, $b_3 = (a_2 b_1 - a_1 b_2) a_2^{-1}$ and $\lambda = -\frac{a_2}{2a_2 K + a_1}$. ${}_1F_1(a; b; z)$ is the confluent hypergeometric function and N is the normalization constant. N is determined from the condition $F(1) = 1$ and is given by $N = \{e^K F_1(a_3; b_3; (\frac{1-\mu}{\lambda}))\}^{-1}$.

Differentiating Eqs. (4) and (12) n times w.r.t. z at $z = 0$ and then comparing both sides we have the total probability density function

$$p(n) = N \sum_{m=0}^n \frac{K^{n-m} (1/\lambda)^m \Gamma(a_3 + m) \Gamma(b_3)}{(n-m)! m! \Gamma(a_3) \Gamma(b_3 + m)} {}_1F_1(a_3 + m; b_3 + m; -(1/\lambda)). \quad (13)$$

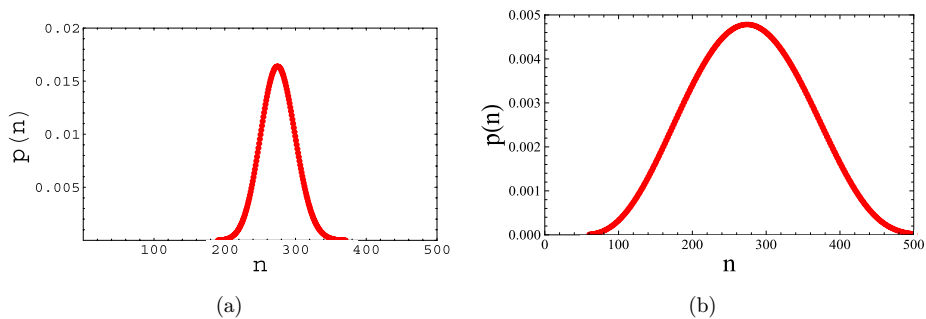


Fig. 1. (Color online) Unimodal responses. In figure (a) the rate constants are $k_a = 80.0$, $k_d = 80.0$, $J_p = 500.0$, $J_0 = 50.0$, $k_p = 1.0$. In figure (b) $k_a = 4.0$, $k_d = 4.0$ and remaining are the same. As the value of rate constants k_a and k_d decrease the width of the distribution increase. Equal values of k_a and k_d make the distribution symmetric about the mode.

Figure 1 shows the distribution of proteins ($p(n)$) vs number of proteins (n) plot for $r_3 = 500$, $r_4 = 50$ and for $r_1, r_2 > 1$. Figure 2 shows the same plot but with different values of r_1 and r_2 with $r_1, r_2 < 1$. It is seen that the distribution is bimodal (Fig. 2) for $r_1, r_2 < 1$ and unimodal (Fig. 1) for $r_1, r_2 > 1$. For $r_1, r_2 = 1$, the distribution becomes uniform. The unimodal responses can also be obtained either for $r_1 > 1$ or $r_2 > 1$, only with mode towards higher value or lower value, respectively. The rate constants r_3 and r_4 determine the positions of the upper and lower modes of the bimodal distribution, respectively.

Using Eq. (11) one can easily derive the expression for mean ($\langle n \rangle$) and variance (var) and are given by

$$\langle n \rangle = \frac{r_1}{r_1 + r_2} r_3 + \frac{r_2}{r_1 + r_2} r_4, \quad (14)$$

$$\text{var} = \langle n \rangle \left(1 + \frac{r_1 r_2 (r_3 - r_4)^2}{(r_1 + r_2)(r_1 + r_2 + 1)(r_1 r_3 + r_2 r_4)} \right), \quad (15)$$

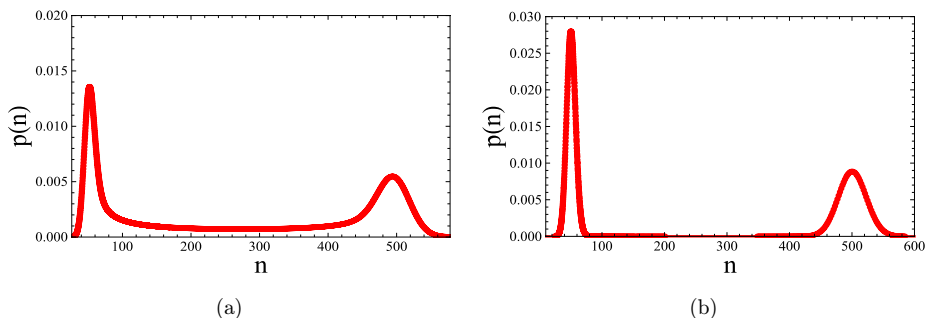


Fig. 2. (Color online) Bimodal responses. In figure (a) the rate constants are $k_a = 0.2$, $k_d = 0.2$, $J_p = 500.0$, $J_0 = 50.0$, $k_p = 1.0$. In figure (b) $k_a = 0.002$, $k_d = 0.002$ and remaining are the same. For the bimodal responses, the protein level fluctuates between low and high level among with the noise due to random birth and death of the proteins in the respective levels.

In Eq. (15), the first term appears due to the random birth and death of proteins and the second term appears due to the random transitions between the ON and OFF states of the gene. When r_1 and r_2 are very high i.e. the number of transitions between ON and OFF states of the gene is very large,⁵⁹ the noise in protein level about the mean is very low. Now, as r_1 and r_2 decreases, the number of transitions between ON and OFF states of the gene also decreases, the noise or fluctuation about mean level increases. The width of the distributions correctly reflects that in Figs. 1 (a) and 1(b). The graded responses of proteins are always less noisy compared to the binary responses for fixed average level of proteins. Sometimes, mean independent fluctuation or noise is measured by a quantity called Fano Factor (FF). The FF is defined by $\text{var}/\langle n \rangle$.^{5,7} Figure 3 shows the variation of FF with r_1 and r_2 for fixed value of r_3 and r_4 . FF increases as r_1 and r_2 are decrease.

The conditions of unimodal (either $r_1 > 1$ or $r_2 > 1$ or $r_1, r_2 > 1$) and bimodal ($r_1, r_2 < 1$) responses are actually given in Ref. 13 with approximate solution of probability density function for protein number. The bimodal distribution of protein level is also known as all-or-none phenomena in cellular system and can be observed without any feedback processes also.^{13,30} It can be shown that the gene expression response depends on the relative values of the parameters rather than the absolute values. If all the rate constants are multiplied by the same factor, the distribution will remain unchanged.

2.2. Stochastic model of two-gene network

In the two genes model, we consider a simple gene regulatory network consisting of two genes: transcription factor (TF) gene and functional gene (Fig. 4). The proteins from the TF gene activate the protein synthesis from the functional gene.⁴⁰ The proteins from the functional gene execute some important functions in the cell as *G6PC* gene in liver.¹² Each gene of the network follows the basic biochemical steps considered in Sec. 2.1. The steps are shown in Eqs. (16) and (17) along with the rate

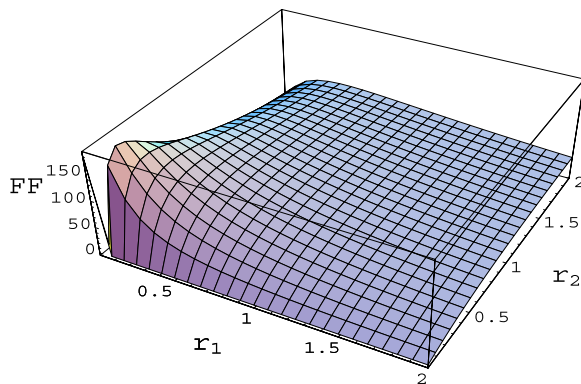


Fig. 3. (Color online) Variation of FF with r_1 and r_2 for $r_3 = 500$ and $r_4 = 50$.

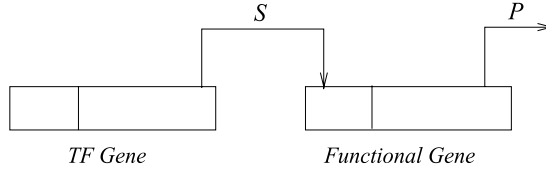
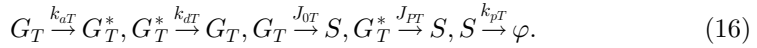


Fig. 4. Schematic diagram of gene network consisting of two genes: TF gene and Functional gene, Protein S (TF) from TF gene is regulating the synthesis of functional protein P from functional gene.

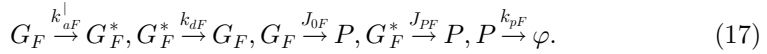
constant for the respective reaction. We also assume that the activation of functional gene requires n number of TFs. That n TF molecules bind the promoter sites of the functional gene through n steps to activate the functional gene. The n steps ($n = 1, 2, 3, 4$, etc.) activation process of functional gene by TFs can be, mapped by the Hill function and can be represented as single step process.^{35,36,40,57} This is shown in Eq. (17).

The biochemical reactions are considered as follows:

For TF gene



For Functional gene



The genes can be in two possible states: OFF (G_i) or ON (G_i^*) ($i = T$ or F). Protein synthesis takes place from the ON state of the gene with higher rate (J_{Pi}) than that from the OFF state (J_{0i}). Both the proteins have some degradation rate constant, k_{pi} . k_{aF}' (k_{aT}) and k_{dF} (k_{dT}) are the activation and deactivation rate constants, respectively for the functional gene (TF gene). The activation rate constant for the functional gene is given by $k_{aF}' = k_{aF}f$, where f is the Hill function and is given by $f = \frac{(S/K)^n}{1+(S/K)^n}$.^{40,52} S is the TF number and at $K = S$, the Hill function is $f = 0.5$, n is the Hill coefficient. The Hill function is a nonlinear sigmoidal shape for $n \geq 2$. A small fluctuation in TF numbers about $S = K$ gives rise to large fluctuations in k_{aF}' .³⁵ The expression for mean TF level ($\langle S \rangle$) and mean functional protein level ($\langle P \rangle$) is given by Eqs. (18) and (19), respectively. The mean functional protein level depends on the instantaneous value of TF number since k_{aF}' depends on S .

$$S_{\text{mean}} = \langle S \rangle = \left(\frac{k_{aT}}{k_{aT} + k_{dT}} \right) \frac{J_{pT}}{k_{pT}} + \left(\frac{k_{dT}}{k_{aT} + k_{dT}} \right) \frac{J_{0T}}{k_{pT}} \quad (18)$$

$$P_{\text{mean}} = \langle P \rangle = \left(\frac{k_{aF}'}{k_{aF}' + k_{dF}} \right) \frac{J_{pF}}{k_{pF}} + \left(\frac{k_{dF}}{k_{aF}' + k_{dF}} \right) \frac{J_{0F}}{k_{pF}} \quad (19)$$

2.3. Stochastic simulation, results and analysis

We simulate the biochemical processes of gene expression using Gillespie algorithm.⁵⁸ The rate constants of different biochemical steps in gene expression determine the dynamics of gene expression. We choose the protein synthesis and degradation rate constants for TF gene as $J_{pT} = 500.0$, $J_{0T} = 50.0$ and $k_{pT} = 1.0$. The choice of the value of rate constants J_{pT} and J_{0T} is arbitrary and any value can be chosen for the study. For chosen value and for $k_{aT} = k_{dT}$, the mean TF level is 275. We varied the noise in TFs level keeping S_{mean} fixed by varying k_{aT} and k_{dT} from a very high value (low noise) to a very low value (large noise) to observe the impact of noise of TF on the functional protein level. We divide wide region of parameters space for k_{aT} and k_{dT} into four different regions with different noise profiles of TF gene (Fig. 3) and responses from functional gene. We call them four different major Strategies. They are chosen as: Strategy I: Low noise in TF level ($\frac{k_{aT}}{k_{pT}}$ and $\frac{k_{dT}}{k_{pT}} \gg 10$), Strategy II: Moderate noise in TF level ($1 < \frac{k_{aT}}{k_{pT}}$ and $\frac{k_{dT}}{k_{pT}} < 10$), Strategy III: High noise in TF level ($0.1 < \frac{k_{aT}}{k_{pT}}$ and $\frac{k_{dT}}{k_{pT}} < 1$), Strategy IV: Very high noise in TF level ($\frac{k_{aT}}{k_{pT}}$ and $\frac{k_{dT}}{k_{pT}} \ll 0.1$). The functional proteins do some important job and therefore should not degrade too early after synthesis and the noise should be small in it. So, the rate constants are chosen as: $k'_{aF} = k_{aF}f$, $K = S_{\text{mean}}$, $k_{aF} = 8.0$, $k_{dF} = 4.0$, $J_{pF} = 5.0$, $J_{0F} = 0.5$, $k_{pF} = 0.005$. That gives noisy graded protein level for large TF numbers ($S > K$). For the assumption $k_{aF} = 2k_{dF}$, the fluctuating S with $K = S_{\text{mean}}$ gives $k'_{aF} = k_{dF}$.⁴⁰ The important point is that the protein synthesis and degradation dynamics for functional gene is assumed to be slower than the TF gene. The rate constants chosen here are almost similar to the study of Kaern *et al.*¹⁰ The above values of the rate constants give $P_{\text{mean}} = 550$ for $S = S_{\text{mean}} = 275$ and $K = S_{\text{mean}}$ and are kept fixed throughout the study. As already discussed, the 50% reduction in protein number for a gene can create problems (haploinsufficiency) in its functioning. This suggests that protein level must lie above a critical or threshold level for proper execution of its task. But there is no study showing the accurate value of the critical level. In our study, we choose 60% of the value of $P_{\text{mean}} = 550$ is the critical value i.e. $P_{\text{crit}} = 330$, for the functioning of the protein from functional gene. The estimation of energy consumption for the gene expression of two-gene network requires the exact amount of energy cost for each step in Eqs. (16) and (17). But, the exact value of energy cost per transition from OFF to ON state of a gene is not known. We can only say that to make transitions from OFF to ON state, energy consumption is essential for the assembling of different regulatory molecules at the promoter sites and it increases when number of transition increases.⁴³ That transition is determined by the activation and deactivation rate constants k_{aT} and k_{dT} (k'_{aF} , k_{dF}) for the TF gene (functional gene). Similarly, the exact value of energy cost for the synthesis of proteins per unit average value is not known. We can only say that as the mean protein level increases the energy consumption also increases.^{43,53} In our simulation study, we have noted the number of OFF to ON state transitions for TF (n_T) and functional

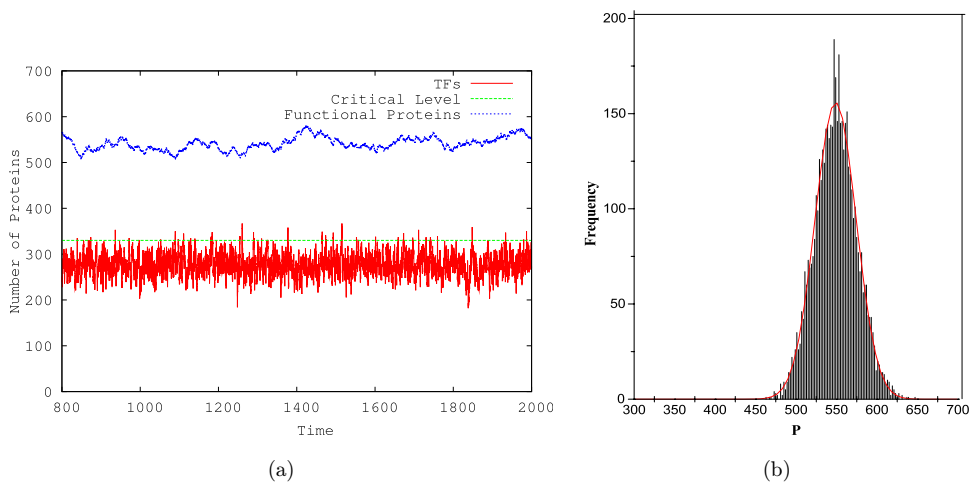


Fig. 5. (Color online) (a) Evolution of protein molecules corresponding to Strategy I (at the steady state). For the TFs (red solid line), the rate constants are $k_{aT} = 80.0$, $k_{dT} = 80.0$, $J_{pT} = 500.0$, $J_{0T} = 50.0$, $k_{pT} = 1.0$. For the functional proteins (blue dotted line), the rate constants are $k_{aF} = 8.0$, $k_{dF} = 4.0$, $J_{pF} = 5.0$, $J_{0F} = 0.5$, $k_{pF} = 0.005$, $n = 4$ and $K = S_{\text{mean}} = 275$. The functional protein level is well above the critical value (green dashed line). (b) Histogram for functional proteins at the steady state. The Gaussian fit gives $\langle P \rangle = 548$ and Standard Deviation = 25.44.

gene (n_F) and then presented an approximate calculation of energy cost for the network over a fixed time at the steady state. This helps us to compare the energy consumption of gene expression for different strategies. The simulation runs for $t = 2000$ units for the evolution of proteins and the steady state is considered at $t \geq 800$.

Strategy I: The random transitions between the active and inactive states of the TF gene is very fast with respect to TF degradation rate ($k_{aT} = 80.0$, $k_{dT} = 80.0$, $k_{pT} = 1.0$). The time evolution of TFs and functional proteins are shown in Fig. 5(a). Protein level from TF gene is now unimodal in nature with less noisy expression level with mean value at 275 (Fig. 1(a)). Because of the less noisy level of regulatory molecules, low fluctuating functional protein level (with the steady state average value $\langle p \rangle = 548$ and Standard Deviation (SD) = 25.44 (Fig. 5(b))) arises from the functional gene and that lies always above the critical value (330) shown by a dash-dot line in figure (Fig. 5(a)). The number of transitions between inactive to active state n_T is 47842 ($n_F = 2356$). Let us consider the energy cost for each transition from inactive to active state of both the genes is approximately H units. Let us also assume that the approximate average energy cost to produce per unit mean protein level from functional gene is K units. Therefore, the total energy cost in Strategy I is $E_1 = A + (n_T + n_F)H + \langle p \rangle K = A + 50198H + 548K$. Here, A is the average energy cost for all other processes in protein synthesis of the two-gene network.

Strategy II: Here, the random transitions between the active and inactive states of TF gene is moderate with respect to the degradation rate ($k_{aT} = 4.0$, $k_{dT} = 4.0$, $k_{pT} = 1.0$) of TF proteins. The time evolution of TFs and functional proteins are

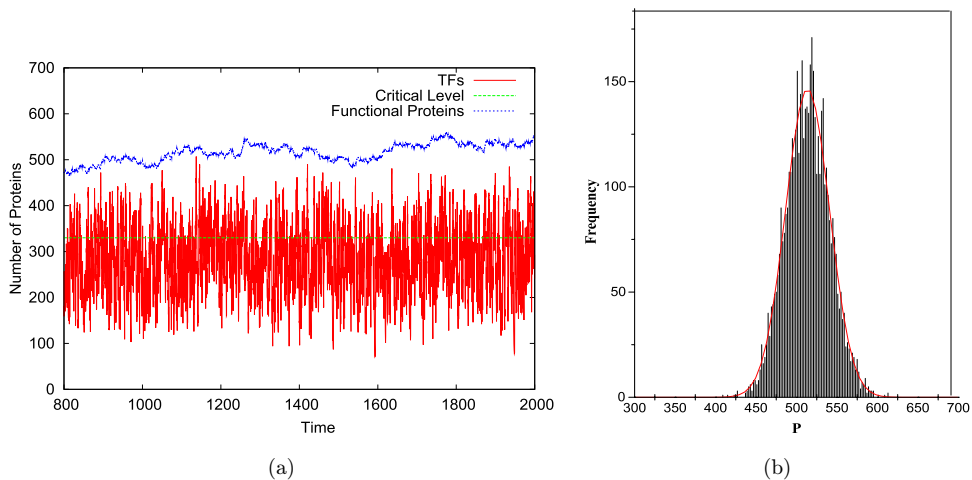


Fig. 6. (Color online) (a) Evolution of protein molecules corresponding to Strategy II (at the steady state). For the TFs (red solid line), the rate constants are $k_{aT} = 4.0$, $k_{dT} = 4.0$, $J_{pT} = 500.0$, $J_{0T} = 50.0$, $k_{pT} = 1.0$. For the functional proteins (blue dotted line), the rate constants are $k_{aF} = 8.0$, $k_{dF} = 4.0$, $J_{pF} = 5.0$, $J_{0F} = 0.5$, $k_{pF} = 0.005$, $n = 4$ and $K = S_{\text{mean}} = 275$. The functional protein level is well above the critical value (green dashed line). (b) Histogram for functional proteins at the steady state. The Gaussian fit gives $\langle P \rangle = 513$ and $(SD) = 27.25$.

shown in Fig. 6(a). The unimodal response of TFs is shown in Fig. 1(b). We got the value of $n_T = 2376$ ($n_F = 2206$) with low fluctuating functional protein level (with the steady state average value $\langle p \rangle = 513$ and $SD = 27.25$ (Fig. 6(b))). The total energy consumption is $E_2 = A + 4582H + 513K$. It is seen $E_2 < E_1$ because of the lower number of transitions to active states of both the genes and a bit low value of mean protein level from functional gene. In Strategy II (Fig. 6), the noise in TF protein level and also in functional protein level is greater than that in Strategy I (Fig. 5).

Strategy III: We consider here the slow transition rate constants between the active and inactive states of TF gene than the degradation rate constant ($k_{aT} = 0.2$, $k_{dT} = 0.2$, $k_{pT} = 1.0$). The time evolution of TFs and functional proteins are shown in Fig. 7(a). The protein level from TF gene is now bimodal in nature though the mean level remains same as before (Fig. 2(a)). The protein level from the functional gene is now more fluctuating about a mean value 420 with $SD = 40.05$ (Figs. 7(a) and 7(b)). The number of transitions between inactive to active state n_T is 130 ($n_F = 1612$). The average approximate energy cost of protein synthesis is $E_3 = A + 1742H + 420K$. E_3 is lower than the E_1 and E_2 . Here, the noise in TF and functional protein levels are more than that in Strategy I and Strategy II.

Strategy IV: Here, we consider the slower transition rate constants between the active and inactive states of TF gene compared to the protein's degradation rate constant ($k_{aT} = 0.002$, $k_{dT} = 0.002$, $k_{pT} = 1.0$). The time evolution of TFs and functional proteins are shown in Fig. 8(a). The time evolution shows that both genes

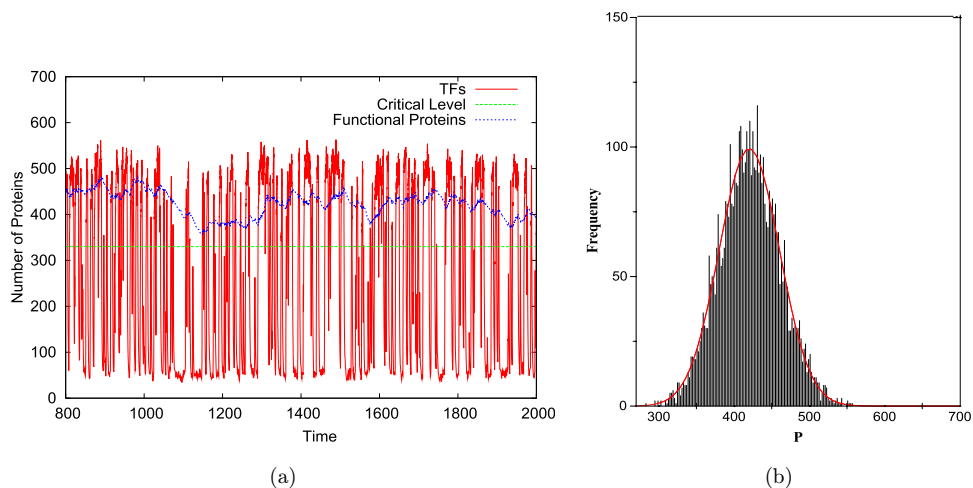


Fig. 7. (Color online) (a) Evolution of protein molecules corresponding to Strategy III (at the steady state). For the TFs (red solid line), the rate constants are $k_{aT} = 0.2$, $k_{dT} = 0.2$, $J_{pT} = 500.0$, $J_{0T} = 50.0$, $k_{pT} = 1.0$. For the functional proteins (blue dotted line), the rate constants are $k_{aF} = 8.0$, $k_{dF} = 4.0$, $J_{pF} = 5.0$, $J_{0F} = 0.5$, $k_{pF} = 0.005$, $n = 4$ and $K = S_{\text{mean}} = 275$. The functional protein level is well above the critical value (green dashed line). (b) Histogram for the functional proteins at the steady state. The Gaussian fit gives $\langle P \rangle = 420$ and $\text{SD} = 40.05$.

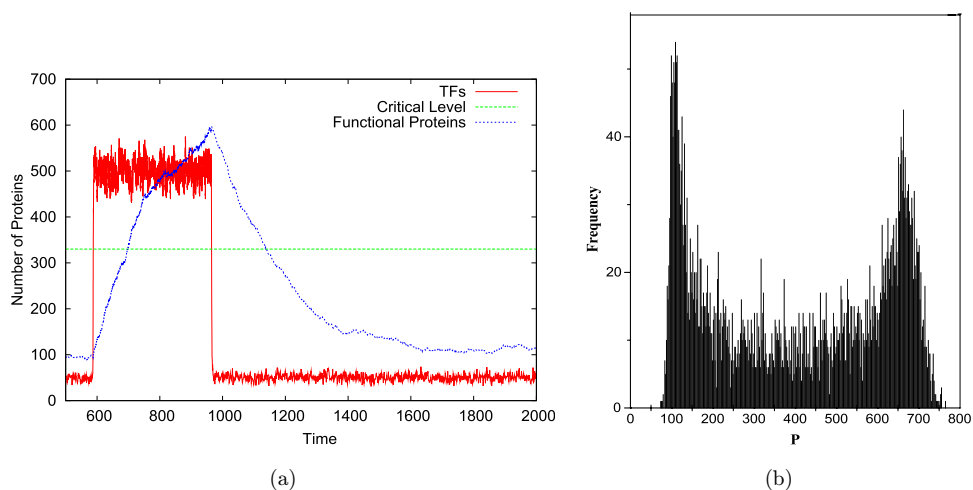


Fig. 8. (Color online) (a) Evolution of protein molecules corresponding to Strategy IV (at the steady state). For the TFs (red solid line), the rate constants are $k_{aT} = 0.002$, $k_{dT} = 0.002$, $J_{pT} = 500.0$, $J_{0T} = 50.0$, $k_{pT} = 1.0$. For the functional proteins (blue dotted line), the rate constants are $k_{aF} = 8.0$, $k_{dF} = 4.0$, $J_{pF} = 5.0$, $J_{0F} = 0.5$, $k_{pF} = 0.005$, $n = 4$, and $K = S_{\text{mean}} = 275$. The functional protein level falls below the critical level (green dashed line) (b) Histogram for functional proteins at the steady state. The Histogram is fitted with a bimodal distribution which gives $\langle P \rangle = 350$ and $\text{SD} = 187.2$.

remain silent for a long period with very small active period. The protein level from TF and functional genes are now bimodal in nature (Figs. 2(b) and 8(b)). At the steady state, the functional protein level stays for very short period above the critical level and a very long period below the critical level (Fig. 8). The number of transitions between the inactive and active states ($n_T = 0, n_F = 408$) of the genes give the amount of energy cost $E_4 = A + 408H + 350K$. E_4 is much lower than that in other strategies.

Numerical values of different quantities associated with the noise properties in four strategies are shown in Table 1. The average cost of energy is lowest in Strategy IV but the fluctuations in protein level from functional gene is too high. The protein level falls below the critical value and stays there for longer time. That kind of protein synthesis is not suitable in cellular processes as observed in the case of haploinsufficiency.^{34,38} The Strategy IV is energetically suitable but functionally unsuitable for cases when protein level has to stay above the critical level. But, Strategy IV may be helpful for cases when the functional proteins are not required for longer period of time. In the Strategy III, the energy consumption is little bit higher but protein level from functional gene always lies above the critical level. Therefore, the Strategy III is most suitable compared to others. We found that bimodal response of TFs with slow transition rates in Strategy III is suitable to keep the protein level from functional gene above a critical value with minimum consumption of energy. The dynamics of TFs modulate the dynamics of functional gene states. Because of the slower dynamics of transcription and degradation of functional proteins and moderate transitions between ON and OFF states, the functional proteins never come to very low level (basal level) rather always stay above the critical level. The assumption that the protein synthesis and degradation dynamics for functional gene is slower than the TF gene is crucial for our result. Many studies show that the synthesis and degradation dynamics of proteins are slower than the dynamics of gene states.^{8,10,11}

The four different strategies considered here basically represent four different probable situations of regulatory molecules or TFs in the cell. Strategy I (Strategy II) represent the situation such that the regulatory molecules are always present with large number with a little (large) noise about a steady value. Again, regulatory

Table 1. Numerical values in that table are obtained from our simulation using GA for both the genes for four different strategies. The Hill coefficient is set at $n = 4$. The counting is started from $t = 800$. n_T is the number of transitions from the inactive to active state for the TF gene. n_F is the number of transitions from inactive state to active states for the functional gene. The mean functional protein (FP) level for the different Strategy is gradually decreasing.

Strategy	k_{aT}	k_{dT}	TF response	TFs FF (SD)	n_T	n_F	Mean FP (SD)
I	80	80	Unimodal	2.14 (24.2)	47846	2343	548 (25.44)
II	4	4	Unimodal	21.4 (76.8)	2372	2206	513 (27.25)
III	0.2	0.2	Bimodal	132.5 (190.8)	130	1612	420 (40.05)
IV	0.002	0.002	Bimodal	184.3 (225.1)	0	583	350 (187.20)

molecules for a gene may not be present with large number continuously and throughout the time rather than they remain present for regulation for a short period followed by a short period of absent or low/basal value. That situation is represented by Strategy III (Fig. 7(a)). It may also happen that regulatory molecules remain absent for regulation for a longer period and become available only for a very short period. That situation is represented by Strategy IV. The results show that the short duration of availability and unavailability of regulatory molecules (i.e. large noise) for the regulation of functional gene is suitable to keep the protein level above the critical level with low energy consumption. The high and low levels of TFs considered here are arbitrary. The low level can be zero and high level can be a small number but greater than the Hill coefficient n . Small variation in the number of regulatory molecules gives rise to large fluctuations when the copy number of that molecule is low. Thus, cell can produce fluctuating protein level with minimum cost of energy by noisy low copy number of regulatory molecules.

In liver, *G6PC* gene plays an important role in glucose homeostasis. In the fasting condition, the blood glucose level becomes low. The proteins from *G6PC* gene then helps to convert the stored energy in the liver and release it into the bloodstream to raise the blood glucose level. Whereas in fed condition, the blood glucose level is high and then liver removes extra glucose from bloodstream to store it again in the liver. Halpern *et al.* found that in fed condition, the *G6PC* gene expression is very infrequent with very large OFF period and very small ON period of protein synthesis, whereas in the fasting condition, the random switching between ON and OFF period is higher than the fed condition.^{12,60} In the fed condition, the protein synthesis from the *G6PC* are no longer essential with higher level and cell shuts it down for longer period. In the fasting condition, bloodstream requires glucose and *G6PC* gene does that job by converting and transforming it from the liver. The behavior of *G6PC* gene in the experiment can be compared with the behavior of functional gene in our simulation study. In fed condition, the gene adopts the Strategy IV, whereas in fasting condition the cell may adopt any strategies between I and III depending on the situation. In the experiment, they also observed that the burst of mRNA synthesis from the *G6PC* gene increases and the degradation rate decreases to raise the accumulation of mRNA in the cell. This experimental observation clearly indicates the existence of critical or threshold level of protein for its proper functioning. In the fasting condition, the *G6PC* gene is not ON or active continuously, rather switching between ON and OFF states so that protein level can also fluctuate. Since, cell itself changes the production and degradation of mRNA synthesis to convert the glucose from liver to bloodstream, so it is desirable that mRNA level should not be too high rather be very close to the threshold value. So, in fasting situation, the *G6PC* gene may follow the Strategy III.

Acar *et al.*⁶¹ showed that, in a rapid fluctuating environment, cell population's growth rate is higher for fast promoter switching of the gene rather than the slow switching cells. In fast switching process, cells take more foods i.e. consume more energy and respond faster than the slow switching process in rapidly changing

environment. The signal from environment determines the TFs level in cell through series of biochemical events.^{13,30} Fluctuating environment also gives rise to fluctuation in TF numbers. The study of Acar *et al.* and Halpern *et al.* showed that cell can adopt any strategy depending on its situation and environmental conditions.

3. Conclusion

The cell has an energy cost for the synthesis of proteins from a gene.^{43–46} The cost has different values for the different steps depending on the complexity of the steps.¹⁰ It is shown by Huang *et al.*¹⁰ that the gene activation and deactivation is also costlier. The energy input is necessary and important to carryout each step of the GE process. It is also known that the cellular system has evolved itself in such a way so that it can minimize the energy cost for its activity and maximize the outcome.^{45–47} The strategy of optimization principle is followed in cellular processes to carryout its functional activity. The cellular system can choose or adjust the reaction rates to save energy consumption during protein synthesis and also works reliably.¹²

We studied a simple gene regulatory network with two genes qualitatively. The functional gene is regulated by the proteins from the TF gene. Different expression and noise level of proteins (different strategies) are possible depending on the rate constants of different steps of gene expression of the two genes. We observed the dependence of noisy expression level of functional proteins on the fluctuation of TF proteins. We consider four different strategies depending on low to high fluctuation in TF proteins. In Strategy I (Fig. 5), we see that TF molecules are always present with high level and with a little fluctuation about a steady level. As a result, the functional gene has more number of flips between active and inactive states and high mean protein level. This behavior is the utmost beneficial for the functioning of the network though that requires maximum energy cost. In Strategy II (Fig. 6), TF molecules are always present though with higher fluctuation about a steady level. That decreases the number of flips between the gene states and the mean functional protein level thereby lowering the energy cost of the cell. In Strategy III (Fig. 7), the TF proteins are not available always with high level to regulate the functional gene rather they are present in high level with very short period followed by low level (or absent) with very short period also. When TF molecules are at low level, the functional gene turns into OFF state and as a result, the accumulated proteins only degrade. Now, as the TF molecules move to high state, the functional gene also turns into ON state and synthesis starts. Thus, the flip number and mean protein level from functional gene is controlled by high randomness of the regulatory molecules. The energy cost for protein synthesis using this strategy is lowered than the Strategy I and Strategy II. Though the functional protein level is fluctuating but always lies well above the critical level. So, the TF molecules need not to be present always at the higher level to maintain the functional protein level above the threshold value. That became possible due to the assumption of very low degradation rate constant of the proteins from the functional gene. The protein level from functional gene does not

come down to very low level during the OFF period of TF gene and functional gene. The Strategy IV (Fig. 8) is not suitable to maintain the protein level from functional gene above some threshold value because it goes down below the threshold level and stays there for longer time due to long time inactivity of the TF gene. But, this is suitable for cases when long OFF periods are essential.¹² The scenario in Strategy III is similar like that of the modern day's refrigerator. The refrigerator automatically switches off its power supply when not required, thereby reducing the energy consumption. In gene expression and regulation processes, the transcription factors are shared by multiple genes for their regulation and that kind of sharing also creates noise in mRNA and protein level.⁶² The involvement or binding of some TFs for the regulation of one gene means their unavailability for the regulation of some others genes. This is also a kind of 'switch-off' condition of the regulatory molecules of the gene to whom that TFs are essential for its regulation. Thus, cells can efficiently maintain a required protein level with fewer number of regulatory molecules. Employing two different kinds of regulatory molecules of opposite nature (activator and repressor), the noise and mean level of functional molecules can also be controlled.^{24,25,29} Therefore, our important observation is that, cellular system produces fluctuating protein level to save energy consumption simply by employing low copy number of regulatory molecules. Thus, by creating low copy number of regulatory molecules in the cells the protein level and the noise in target gene expression can be controlled efficiently. The cell can adjust the protein level from functional gene above the critical level by adjusting the synthesis and degradation rate constants as well as the number or noise in regulatory molecules with minimum energy cost.

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