Variability in cellular gene expression profiles and homeostatic regulation

Author: Yuriy Mishchenko

Affiliation: Toros University

Address: Toros University, 45 Evler Campus, Mersin 33140 Turkey

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Corresponding Author:

Dr. Yuriy Mishchenko

45 Evler Campus Toros University Mersin 33140 Turkey

Email: yuriy.mishchenko@gmail.com

Phone:+90 (530) 244 62 40

Abstract

One of surprising recent discoveries in biology is that the gene and protein expression profiles in cells with identical genetic and environmental makeup can exhibit large variability. The nature and the significance of this variability had been posed as one of the current fundamental questions in biology. In this letter, we argue that the observed variability in cellular gene and protein expression can be understood as an outcome of homeostatic regulation mechanisms controlling the gene and protein expression profiles.

INTRODUCTION

One of recent findings in biology is that the gene and protein expression profiles in cellular systems with identical genetic and environmental makeup can often exhibit a large stochastic variability (*1–16*). The nature and the significance of this variability had been stated as one of the fundamental problems in biology. In this letter, we argue that the observed gene and protein expression variability emerges naturally if one considers the regulation of the gene and protein expression in biological cells in the context of homeostatic regulation mechanisms.

One of the main challenges faced by biological systems is maintaining a stable physiological state in the face of the fluctuations in the parameters of internal and external environments. One of the best mechanisms available to biological systems for countering such disruptive influences is homeostatic regulation. Homeostasis is the property of biological systems to maintain a stable physiological state by means of various feedbacks mechanisms sensitive to the changes in that state (*17*). Homeostatic regulation responds directly to the changes in the controlled physiological parameter and by doing so can be very effecting in maintaining the necessary value of that parameter. In this letter, we show that this property of the homeostatic regulation to be sensitive directly to a system's physiological state also leads with necessity to large variability in the observed internal configurations of that system. This conclusion is general and is based broadly on two properties of biological systems – the possibility of implementing the same physiological state via different internal configurations and the reliance of homeostatic regulation on physiological state for feedback. We present a general argument towards this point and demonstrate the emergence of this phenomenon using a simulation.

MATERIALS AND METHODS

We consider a simple model to inspect the impact of homeostatic regulation on the internal configuration states of populations of biological cells. More specifically, we consider the model of the control of the expression levels of a single protein in cell, whereas the protein production is regulated via multiple pathways. The time-evolution of the protein concentration in a cell is given by the following relationship,

$$\frac{dX_k}{dt} = -aX_k + ru_k + e\varepsilon_k, \quad (1)$$

where X_k is the concentration in the cell of the protein produced via the pathway *k*, aX_k is the rate of natural degradation of the protein, ru_k is the rate of the protein production in pathway *k*, and ε_k is the standard normal noise variable with zero mean and unit variance. a, r, e are constants, and *k* is the index talking on the values from 1 to N and enumerating protein concentration regulation pathways, where N is the total number of such pathways. u_k is understood as the variable used to control the concentration of the

protein in the cell and affecting specifically the pathway *k*. The key value of interest is the total protein concentration in the cell, given by $X = \sum_{k=1}^{k=N} X_k$.

When solving the model (1), it is necessary to define how the control variables u_k depend on the internal state of the cell, $\{X_k, k = 1 \dots N\}$. We inspect three possibilities: (i) feed-forward regulation, (ii) individual regulation, and (iii) homeostatic regulation. In feed-forward regulation, the values of u_k are fixed at a constant value $u_k = u = a\bar{X}/Nr$, implying the rate of protein production fixed in feed-forward manner, where $\bar{X}$ is the target protein concentration. The passive equilibrium is then achieved when $aX_k = ru_k$ or $X_k = \bar{X}/N$ and, thus, $X = \bar{X}$. In the individual regulation, the pathways are regulated by the cell using individual feedback modeled as $u_k = \bar{X} - NX_k$. In this case, each pathway is driven by the cell towards a fixed point $X_k = \bar{X}/N$, also resulting in $X = \bar{X}$. Finally, in homeostatic regulation, the pathways are regulated via a homeostatic feedback sensitive to the final protein concentration X, $u_k = u = \bar{X} - X$. In this model, the regulating pathways are driven to a suitable configuration point by relying directly on the realized final protein concentrations.

RESULTS

We argue that the large variability in the gene and protein expression levels observed in otherwise identical biological cells can be a direct consequence of homeostatic regulation mechanisms affecting the gene and protein networks in such cells. One of the main challenges faced by biological systems in maintaining their physiological state are the disruptive fluctuations in their internal and external environment conditions. One of the best mechanisms available to biological systems for countering such changes is the homeostatic regulation. Homeostasis is the property of biological systems to maintain stable internal physiological condition by means of feedbacks sensitive to the changes directly in the systems' physiological state parameters (*17*). By responding to the changes in the physiological state, such mechanisms are highly effective in maintaining the state necessary for a biological system to perform its functions. When compared to other regulation strategies, homeostatic regulation both allows achieving a significant reduction in the variability of the resulting physiological state in the face of noise as well as offers the possibility of recovery from critical component failures, Figure 1.

The capacity of homeostatic regulation to respond directly to the changes in a controlled physiological parameter, which confers to it these important properties, however also makes it insensitive to the perturbations in the system's parameters that do not enact a significant change in the physiological state, such as a random up-regulation of one and a simultaneous down-regulation of another pathway regulating a physiological parameter. Whenever such a perturbation results in no net change in the physiological state, homeostatic feedbacks likewise will not trigger and will enact no corrective actions.

To consider this peculiar point further, we investigate a model of cellular regulation of the expression level of one internal protein X, which can be controlled via two complementary production pathways X_1 and X_2, so that $X = X_1 + X_2$. If the production of such protein is affected by a substantial amount of noise, such as due to random protein degradation or molecular noise in the protein transcription processes, there can be multiple approaches that the cell can pursue to ensure the required concentration of the protein in its cytoplasm. One of the most basic such approaches is to fix the rate of the protein production in each channel, so that a given final concentration $\bar{X}$ is achieved in the equilibrium. Of course such a simple open-loop approach can be heavily disrupted by noise. Alternative approach includes the closed-loop feedback regulation affecting production pathways individually, ensuring that the cell remains at a specific internal state $(\bar{X}_1, \bar{X}_2)$, which guarantees the required final protein concentration $\bar{X}$. Yet another alternative approach is to regulate the protein production using feedbacks relying directly on the realized final protein concentration, measured in some way. The latter, of course, is the homeostatic regulation. Of the three approaches, the homeostatic regulation offers the highest degree of robustness for the cell.

It is clear that, should we choose the homeostatic regulation to control the protein production, such regulation will be insensitive to the changes in the expression profiles of individual pathways X_1 and X_2 that do not affect the protein's final concentration $X = X_1 + X_2$. This corresponds to up-regulation of one pathway and simultaneous down-regulation of the other, leaving the sum $X_1 + X_2$ intact. This insensitivity has a dramatic consequence for the system's internal configurations that can be realized. In Figure 2, we show the distribution of the internal configurations (X_1, X_2) in such a model over different times. One can clearly see the spread of the internal states along the direction $X_1 + X_2 = const$ where homeostatic regulation provides no feedback. This spread is caused by random "neutral" perturbations, that is, such that produce no net change in the protein concentration X, accumulating over time without causing any action from homeostatic feedback and resulting in a large variability in the realized internal states under otherwise identical external conditions.

The above phenomenon can have dramatic effects when the number of contributing regulating pathways is large. In Figure 3, we show the example of the same model with $N = 10$ protein concentration regulating pathways. The individual pathways' expression levels X_k in this case are spread widely, filling an entire region of space. The variation in the expression levels of individual pathways under otherwise identical conditions is up to 10 times greater than that realized in the model using non-homeostatic regulation mechanisms.

Thus, we arrive at the following conclusion. The reliance of homeostatic feedbacks on the systems' physiological state can make such feedbacks insensitive to the perturbations in the internal state that do not result in a net change of the physiological

parameters. This makes it possible for such “neutral” perturbations to accumulate over time, resulting in a large spread of the internal configurations of such systems without any significant differences in their phenotype, genotype, or external environment.

DISCUSSION AND CONCLUSIONS

We argue that the puzzling stochastic variability observed in the gene and protein expression of otherwise identical biological cells can be understood as a result of the homeostatic feedbacks in such cells’ internal regulation mechanisms. Homeostatic regulation is ubiquitous in biology. Compared with the other regulation mechanisms, homeostatic regulation offers superior capacity for reducing stochastic fluctuations in the physiological state parameters as well as allowing recovery from failures. The key property of the homeostatic regulation to respond to the changes in the controlled physiological parameter also makes them insensitive to biological systems’ perturbations that do not result in a change of such parameter, for example, such as a random up-regulation of one and a simultaneous down-regulation of another regulating pathways. Accumulation of such neutral perturbation over time can lead to large variations in a biological system’s internal states without any accompanying genetic, phenotypic or environmental changes. The wide spread of homeostatic regulation in biology implies that this phenomenon is likely to emerge as a generic property of biological systems rather than be limited to gene and protein expression in cellular populations. Indeed, we note that possibly other examples of this phenomenon had been already observed in neuronal axonal and dendritic ion-channel compositions and the neuronal circuits such as the central pattern generator circuits in lobster somatogastric ganglion (*17–22*). One can expect more examples of this phenomenon to be observed in the future.

If homeostatic regulation is the primary cause of the stochastic variability observed in cellular gene and protein expression, then the discussion above allows making several experimentally testable predictions. For example, we should expect that higher amounts of stochastic variability will be recorded in the systems with a larger number of internal degrees of freedom, that is, where more complementary pathways contribute to the regulation of their physiological state. Moreover, such stochastic variability should be decoupled from the variability in the phenotype, that is, large variations in gene or protein expression will not correlate with the variations in the phenotype. Quite contrary, the variations in the individual expression levels will not be independent and will exhibit a substantial degree of correlation among them. Most interestingly, however, is that we predict that the gene and protein expression profiles in cells change over time, that is, the stochastic variability can be observed even in a single cell measured over different moments of time. Finally, we expect the examples of stochastic variability to be found in many different biological systems ranging from single cell to multicellular organisms as well as complex biological systems such as neuronal circuits.

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FIGURE 1

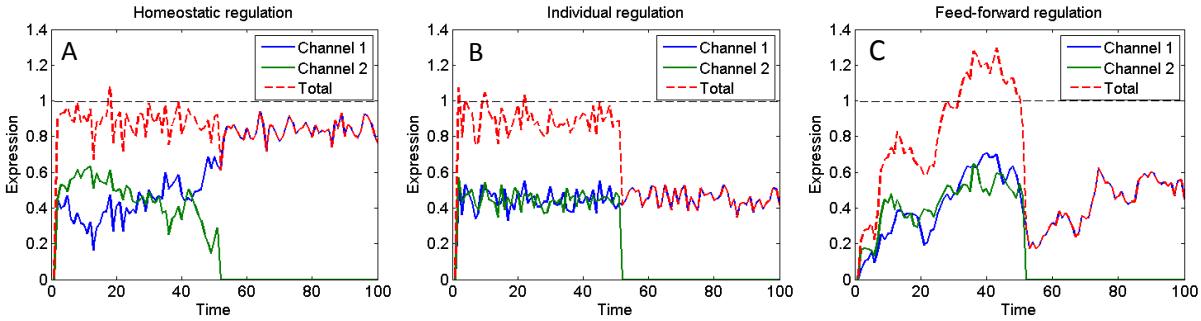

Figure 1: Time evolution of the total protein concentration in a cell, controlled via complementary regulation pathways, as discussed in the paper. A) Homeostatic regulation offers a high level of noise suppression in the final protein concentrations as well as allows recovery from a failure in one of the pathways (blockage introduced at time 50 units in channel 2). B) Individual control of the protein production pathways, driving them towards a given fixed operating point, also offers a high level of noise suppression in the final protein concentrations, but fails to recover from the blockage in one of the pathways. C) Feed-forward control both is highly vulnerable to noise and fails to recover from the failure in one of the regulation pathways. Simulation parameters $e = 0.05, a = 0.1, r = 0.5$. The target concentration $\bar{X} = 1$ is indicated with dashed line.

FIGURE 2

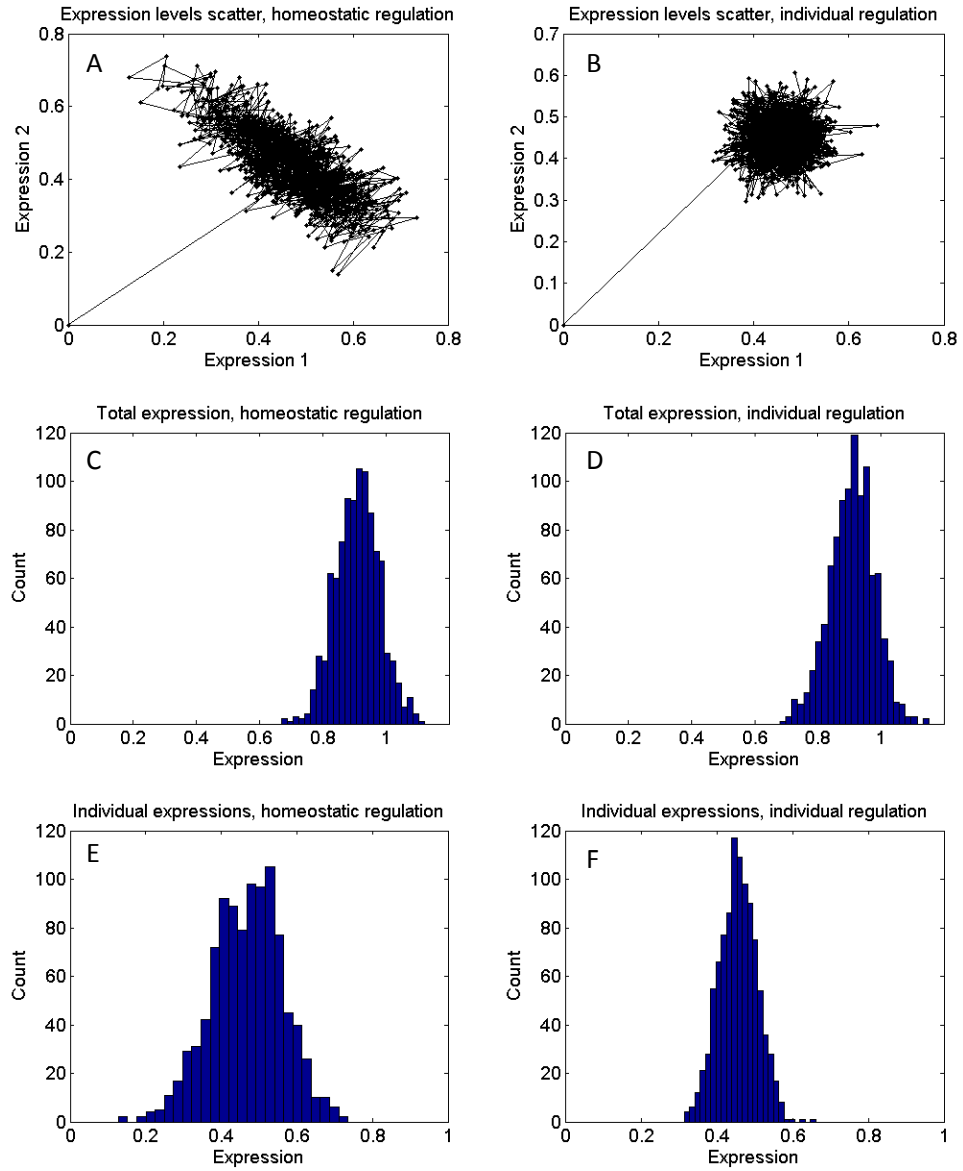

Figure 2: The insensitivity of homeostatic regulation to the perturbations of the biological system leaving its final physiological state unaffected results in a large spread of the system's internal configurations, due to the unobstructed accumulation of such "neutral" perturbations. A) Realized internal configuration states (X_1,X_2) in the model (1) for homeostatic regulation. The spread of the internal states along the direction of constant X=X_1+X_2 is clearly visible. B) Realized internal configuration states for the model (1) with individually controlled pathways X_1 and X_2. C-D) In both examples, the variation of the final protein concentration in homeostatic regulation (C) and individual regulation (D) is the same. E-F) In homeostatic regulation the spread in the individual pathways' expression levels, X_1 and X_2, (E) is at least two times greater than that in individual regulation (F).

FIGURE 3

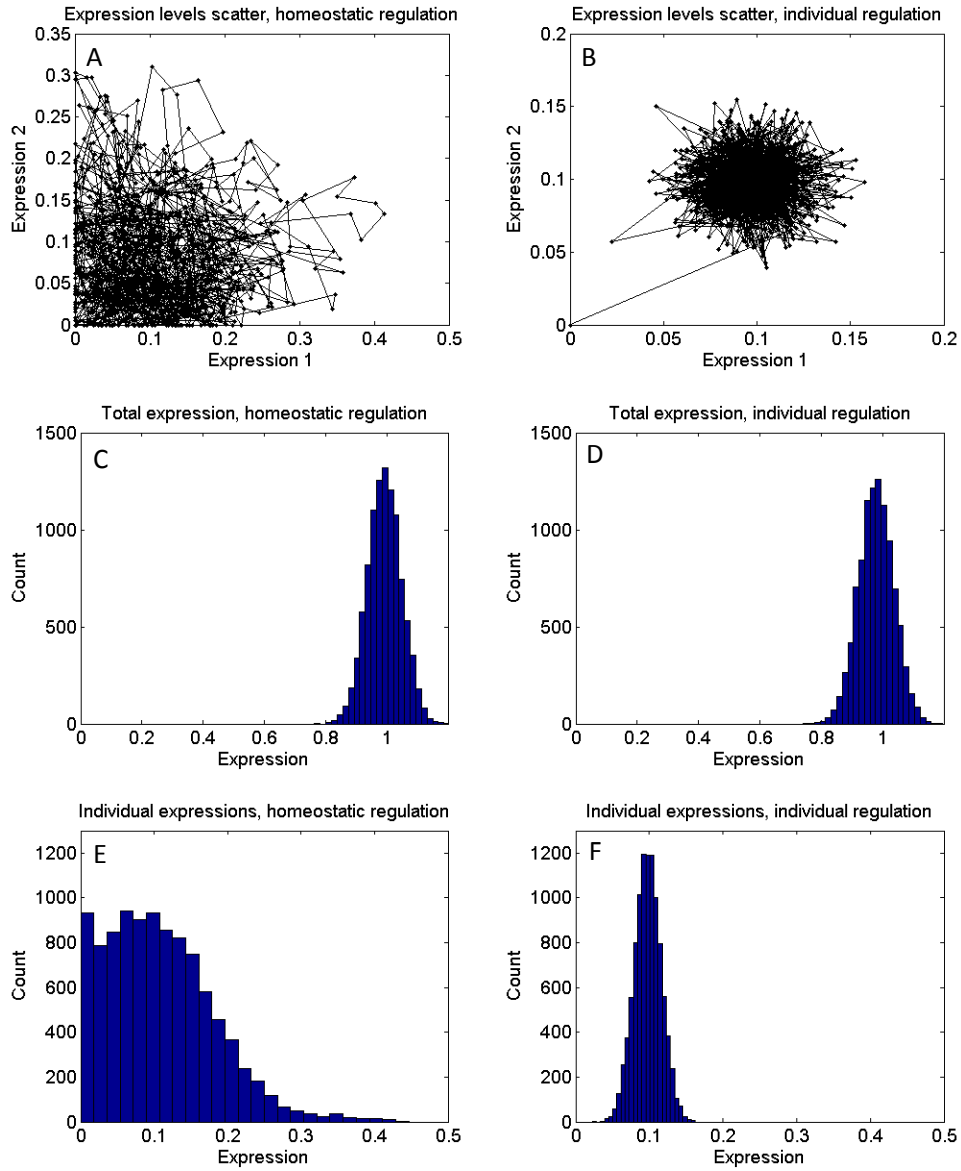

Figure 3: The stochastic variation in a biological system's internal configurations may reach dramatic levels when the number of internal degrees of freedom, that is, the number of pathways regulating a physiological parameter, is large. A) Realized internal states (X_1,X_2) of the model system (1) with homeostatic regulation and *N*=10 regulation pathways. B) Realized internal states for the model system (1) with *N*=10 and individually controlled pathways. C-D) The variation of the final total protein concentration is the same in homeostatic (C) and individual regulation (D). E-F) The spread in the individual pathways' expression levels X_k in the homeostatic regulation (E) is dramatically greater than that in the individual regulation (F).