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Experimental measurements and mathematical modeling of biological noise arising from transcriptional and translational regulation of basic synthetic gene circuits

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HIGHLIGHTS

- Protein variability is lower under post-transcriptional control of gene expression.
- Noise reduction is due to control mechanisms acting on the efficiency of translation.
- Similar strategies are proposed to control noise in synthetic biology applications.

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ABSTRACT

The small number of molecules, unevenly distributed within an isogenic cell population, makes gene expression a noisy process, and strategies have evolved to deal with this variability in protein concentration and to limit its impact on cellular behaviors. As translational efficiency has a major impact on biological noise, a possible strategy to control noise is to regulate gene expression processes at the post-transcriptional level. In this study, fluctuations in the concentration of a green fluorescent protein were compared, at the single cell level, upon transformation of an isogenic bacterial cell population with synthetic gene circuits implementing either a transcriptional or a post-transcriptional control of gene expression. Experimental measurements showed that protein variability is lower under post-transcriptional control, when the same average protein concentrations are compared. This effect is well reproduced by stochastic simulations, supporting the hypothesis that noise reduction is due to the control mechanism acting on the efficiency of translation. Similar strategies are likely to play a role in noise reduction in natural systems and to be useful for controlling noise in synthetic biology applications.

1. Introduction

Phenotypic noise is defined as the cell-to-cell variability in gene expression within an isogenic population (Sanchez et al., 2013; Munsky et al., 2012; Li and Xie, 2011; Raj and van Oudenaarden, 2008; Maheshri and O'Shea, 2007). Noise affecting gene expression originates from two conceptually different processes, and may therefore be classified into two separate terms: (i) intrinsic and (ii) extrinsic noise. The former arises from the stochasticity of biochemical events, while extrinsic noise is caused by differences

Abbreviations: AU, arbitrary units; GFP, green fluorescent protein; IPTG, Isopropyl β-D-1-thiogalactopyranoside; LB, Luria Bertani broth; OD, optical density; RBS, ribosome binding site; CV, coefficient of variation; F, Fano factor

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in status among individual cells, e.g. concentration of RNA-polymerases, ribosomes and metabolites or local environmental conditions. Phenotypic variability might have positive effects on the overall survival rate of a cell population, as heterogeneous populations are better able to react to environmental changes (Wolf et al., 2005; Thattai and van Oudenaarden, 2004; Spudich and Koshland Jr., 1976). On the other hand, the variability in protein expression might be deleterious for cellular events that require a fine control of protein concentrations (Arias and Hayward, 2006). Therefore, in order to survive in a noisy environment, cells evolved strategies to deal with both intrinsic and extrinsic noise. The control of noise is also critical for the design of gene circuits with well-defined functionalities, which is the purpose of synthetic biology (Becskei and Serrano, 2000; Hasty et al., 2000). Thus, in order to better understand biological processes and to design more robust synthetic circuits, it is important to understand how phenotypic noise is affected by the different regulatory mechanisms that control gene expression. In this study, the noise on protein concentration was compared between two synthetic gene circuits, implementing a transcriptional and a post-transcriptional control of gene expression, respectively.

Transcriptional and translational processes induce different effects on protein variability. In gene circuits where gene expression is controlled by a transcriptional mechanism, the efficiency of transcription has only a marginal effect on noise, while translational efficiency is the major determinant of protein variability, with genes translated more efficiently usually affected by higher variability in expression levels. The main cause of this behavior is the higher molecular stability of proteins than mRNAs. A consequence of this different stability is that translational processes amplify the oscillations in the concentration of mRNAs, and, moreover, the resulting changes in protein concentrations persist long after the degradation of the coding mRNA molecules. This noise contribution is classified as an intrinsic one, being the result of stochastic events such as transcription, translation and degradation. Ozbudak et al. (2002) experimentally confirmed the different effect of transcriptional and translational processes on protein level variability, using a genetically modified bacterium. The rate of transcription was modified by an inducible promoter or by mutating the promoter sequence, while translational regulation was achieved by inserting point mutations in the ribosome binding site (RBS) and in the initiation codon of a fluorescent reporter. Mutations that decreased translational efficiency reduced the variability in protein concentration, while changes in transcription efficiency had little effect on protein level variability. This mechanism might explain why certain critical genes (e.g. *cya*, *malT*, and *nagC*) are translated from low-efficient RBS sequences (Chapon, 1982). The same strategy might be adopted to reduce noise in the design of synthetic gene circuits.

The control of noise by mutations in the RBS sequence is an optimal strategy when noise level is a static characteristic of the circuit, i.e. gene expression stochasticity does not need to be adapted to fluctuating environmental conditions. A classic example of this situation is the control in the expression levels of genes whose protein products need to be present in precise concentration in order to guarantee cell survival. However, an external control on noise strength might be useful to speed up cell dynamics, as a cell population with more dispersed distribution of expression levels is more prone to react to changes in environmental conditions (Chapon, 1982). Stochastic resonance is an example of how a noise level sensitive to an external signal might be exploited to implement complex cellular behaviors. In systems exhibiting stochastic resonance, oscillations might arise in response to a weak periodic input signal, if it is associated with a specific level of noise. If noise is abolished, the outcome of the system ceases to be periodic. Thus, contrary to common sense, the signal-to-noise ratio increases for increasing noise levels (Wiesenfeld and Jaramillo, 1998). Stochastic resonance has been proposed as a possible mechanism for explaining circadian rhythms in biological systems (Guantes and Poyatos, 2006; Goldbeter, 2002), which are indeed caused by a weak periodic signal (the day–night cycle) in a noisy environment. A mechanism similar to stochastic resonance takes part in stochastic focusing, where noise is used to improve the sensitivity of a detector (Paulsson et al., 2000). If the noise strength can be controlled by an external signal, circuits based on stochastic resonance (or stochastic focusing) might be turned on/off in response to changes in the environmental conditions. This possibility has two positive consequences. On one hand, tunable noise could be used to implement complex functionalities in synthetic gene circuits. At the same time, thanks to an external control on noise strength, it would be possible to directly test the role of mechanisms such as stochastic resonance or stochastic focusing, or more generally of noise itself, on cellular processes.

A possible strategy to modulate the strength of noise by an external signal is to act on the efficiency of translation that, as previously discussed, is a major determinant of protein variability. As a consequence, a post-transcriptional control of gene expression, interfering with protein translation, might modify the amplitude of noise. Post-transcriptional mechanisms are known to control gene expression both in eukaryotes and in prokaryotes. In bacteria, such as *Escherichia coli*, post-transcriptional control by small RNA (sRNA) molecules seems predominant in stress response pathways and in virulence genes regulation (Beisel and Storz, 2010). Theoretical analyses have revealed that a gene downregulated by a trans-acting sRNA might exhibit three regimes of expression (Levine et al., 2007). When sRNA molecules outnumber coding mRNA copies, expression is silenced. At the other side of the spectrum, i.e. in presence of a surplus of coding mRNA, the concentration of protein increases linearly with the rate of transcription of the target gene. In between these extreme conditions, there is a crossover regime, where variance on protein concentration is maximized (Mehta et al., 2008). Stochastic simulations have shown that, in the silenced regime, the post-transcriptional control mechanism has minimal noise, and that this noise level is lower than the one exhibited when the same average concentration of protein is synthesized through a transcriptional control mechanism (Mehta et al., 2008; Jost et al., 2011). The low level of noise in the silenced regime might be an explanation for the post-transcriptional control of critical genes, like e.g. the ones responsible for the response to oxidative stress (Zhang et al., 2002). However, experimental analyses on the iron homeostasis network of *E. coli* have not revealed any reduction in noise levels related to post-transcriptional control (Arbel-Goren et al., 2013). In this case, transcriptional control turned out to be less noisy, even at low protein concentrations (Lavi-Itzkovitz et al., 2014). This lack of experimental evidence for a decrease in noise due to post-transcriptional control might be explained by two factors. First, extrinsic noise might be predominant, thus masking any effect of the post-transcriptional control mechanism on intrinsic noise. Second, it is plausible that the post-transcriptional control mechanism of the iron homeostasis network cannot reach the silenced regime, as a consequence of toxic effects related to iron deprivation.

In this study, we compared transcriptional and post-transcriptional regulations in two synthetic gene circuits. Experimental measurements showed lower noise levels in presence of a post-transcriptional regulatory mechanism with respect to a transcriptional control, over the tested range of protein concentrations. Stochastic simulations were consistent with experimental results once cell division events were included.

2. Results and discussion

The noise that affects protein expression at steady state was studied in two synthetic gene circuits (Fig. 1): (i) one in which protein synthesis is controlled by a transcriptional mechanism (TC); and (ii) one implementing a post-transcriptional control of gene expression