379c Ultrasensitivity in Genetic Networks Is a Key Requirement for Noise Mitigation

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Introduction

The objective of all designs — whether evolved or engineering– is robust performance in uncertain environments. The inherent robustness of different biological systems has been attributed to the myriad configurations of feedback loops (Freeman, 2000). Among these, we consider the multiple feedback paradigm for regulation of serially arranged sub-systems commonly found in biological systems including genetic networks (Venkatesh et al., 2004), signaling pathways (Angeli et al., 2004) and metabolic regulation (Hohmann, 2002). The multiple feedback loops arise from the distribution of the output from the downstream sub-system to regulate the upstream sub-systems. The tryptophan system of *Escherichia coli* can also be conceptualized as 3 sub-systems in series namely, transcription, translation, and tryptophan synthesis. Regulation of tryptophan concentration is achieved by three distinct negative feedback loops namely, genetic regulation, mRNA attenuation, and enzyme inhibition (Yanofsky, 1984). The multiple feedback structure results due to feedback of tryptophan to all three regulators (Venkatesh et al. 2004). The regulators have been modeled using the nonlinear Hill equations (Bhartiya et al, 2003), which take the form,

$$u = \frac{K_i^{h_H}}{K_i^{h_H} + y^{h_H}}$$

Here, K_i represents the half-saturation constant and h_H is the Hill coefficient. Variables y and u represent the input and output to the regulator, respectively. A value of h_H in excess of unity implies ultrasensitive regulation, while less than unity leads to a sub-sensitive regulator. In this work, we focus our attention on the noise mitigation properties of such networks using the tryptophan system of *Escherichia coli* as a test bed. Noise is inherent in regulatory networks due to a) the very low copies of regulatory molecules – anywhere between a few tens to a few hundred molecules per cell (Elowitz et al., 2002; McAdams and Arkin, 1999); and b) a noisy environment. Thus, it is of interest to identify the design components that endow the system with noise mitigation properties.

Venkatesh et al. (2004) demonstrated that the multiple feedback loop design is necessary for rapid synthesis of tryptophan from a tryptophan-starved state to its in vivo level in about 5 minutes. Delays in meeting the regulatory system targets often translate to starvation or toxicity, which may result in cell death. Moreover, this rapid synthesis feature is maintained despite large variations in process (rate constant, specific growth rate) or regulator parameters (half-saturation constant, Hill coefficient), implying robust performance. Multiple feedback loops results in phase lead due to addition of system zeros relative to a single feedback system. This feature is evident by comparing the frequency response of a *linearized* single feedback and *linearized* multiple feedback designs. The higher bandwidth of multiple feedback designs is responsible for the smaller rise time as the system becomes sensitive to inputs at larger frequencies (Venkatesh et al., 2004). However, as the roll-off rates of the single and multiple feedback systems are similar in magnitude, the multiple feedback system with linear regulators is more susceptible to noise relative to a single feedback loop design. Thus, the multiple feedback loop architecture essential for rapid synthesis appears to have a detrimental effect on the ability of the system to cope with noise. However, simulation studies with the tryptophan model show that the tryptophan system using ultrasensitive genetic regulation performs superior noise rejection than its linearized version. How does the nonlinear tryptophan system, therefore, simultaneously accomplish noise mitigation while retaining the beneficial dynamic attributes seen in the tryptophan system?

Methods

To address the above question, we use the describing function method to examine the role of nonlinearity of the regulators in noise mitigation. In the describing function method, the nonlinear Hill equations in the tryptophan system are replaced by their quasi-linear equivalents (Gelb and Vander Velde, 1968). The fundamental harmonic of the system response to a sinusoidal input by the nonlinearity and its quasi-linear equivalent are identical. We investigated the performance of the nonlinear system for two scenarios: a) comparing the in vivo multiple feedback loop design with a mutated single feedback loop design wherein the regulators representing attenuation and inhibition are absent, and b) identifying the role of ultrasensitivity in the multiple feedback loop design on the overall ability of the system to reject noise by varying the Hill coefficient of the regulators. Conclusions on the ability to reject noise for the above two cases were based on comparison of peak resonance, bandwidth, and roll-off rates. We also describe a non-trivial extension of the describing function method necessary for representing the asymmetrically nonlinear Hill equation.

Results

a) Comparison of multiple and single feedback loop designs

The analysis using the describing function method indicates that noise (high frequency inputs) impact the tryptophan system differently depending on the status of the system. For a tryptophan rich system, the presence of multiple feedback loops results in a larger bandwidth, while the ultrasensitive genetic regulation is responsible for a larger roll-off rate. Thus, the combination of multiple feedback loops and the ultrasensitive regulation results in a faster rise time as well as superior noise mitigation. For a tryptophan lean system, the multiple feedback loop design shows marginal degradation relative to the tryptophan rich system characterized by a slightly enhanced peak resonance. On the other hand, the large peak resonance of the single feedback loop design compared to the multiple feedback design indicates poor dynamic response. Also the lower roll-off rate shows that the single feedback design is more susceptible to noise.

b) Impact of ultransensitivity on roll-off rate

The Hill coefficient of the genetic regulator may be varied to result in sub-sensitive regulation ($h_H<1$), to a Michelis-Mentin response ($h_H=1$) and an ultrasensitive response ($h_H>1$). Our results indicate that with sub-sensitive genetic regulation, a multiple feedback loop design is more sensitive to noise than a single feedback loop design as indicated by a small roll-off rate. On the other hand, an ultrasensitive genetic regulator shows an enhanced roll-off rate with the multiple feedback architecture than the single feedback design.

The results therefore demonstrate that the ultrasensitivity observed in the genetic regulation of the tryptophan system as well as reported in various other biological systems is a design feature that endows the system with superior noise mitigation properties, while the multiple feedback loop architecture is responsible for the rapid synthesis of tryptophan.

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