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Designing a Circuit to Tune Noise Tolerance in a Synthetic Device

Noise exists in all biological systems as fluctuations in gene expression due to stochasticity in transcription, translation, and molecular coupling processes. Molecular-level noise leads to phenotypically different cells in genotypically identical populations, an adaptive benefit allowing cells to adjust to variations in the external environment without altering the genetic makeup [1]. Furthermore, the cell's allowance of noise in certain pathways lowers the energetic cost of performing the process; while more stringent pathways require more time and energy. While this analog, or graded, response has clear benefits in biological systems, such variation in individual output is at odds with the digital, or binary "on/off", output desired in synthetic systems. Synthetic biologists attempt to minimize the noise level in synthetic devices, or more often, ignore the effects of noise altogether by averaging noisy output measurements. By addressing the noise in the synthetic design, one may be able to better control the output of the system, constructing a more finely tuned device. This proposed study aims to design a synthetic circuit that detects and responds to the noise level in a separate synthetic device in order to establish a noise tolerance threshold in the cell population.

The noise level in a pathway is calculated as the relative deviation from the average measured output. Factors affecting the noise level in a system or synthetic circuit pertain to the random formation and decay of single molecules and multi-component complexes. [1] Most factors displaying stochasticity are involved in the transcription and translation of genes. Because there is a limited amount of transcription factors, polymerases, and other gene expression machinery within the cellular space, variations in expression rate and protein abundance depend on the timing and kinetics of each protein. This manifestation of noise is known as the finite-number effect – with a smaller number of molecules, such as polymerases and plasmids, affecting protein abundance in a compartment, noise increases. Noise level is also affected by the degradation rate of proteins in the system. Longer cascades in synthetic systems have higher noise levels because there are more steps requiring molecular coupling. Negative feedback loops provide a noise-reduction mechanism in downstream processes. Negative feedback can also have a destabilizing effect that may increase noise level if it involves a transcriptional time delay. [1] Alternatively, positive feedback typically

amplifies fluctuations and population heterogeneity, increasing the system noise level. Amplification by positive feedback loops can even generate bimodal population distributions [2].

Positive feedback loops act as dynamic circuits in both biological and computational systems. Positive feedback, an autocatalytic circuit, underlies bistable or binary responses in both prokaryotes and eukaryotes (1,3]. In a bistable or binary system, reaching one of the two stable states depends on the system's input parameters. For example, Isaacs and colleagues [2] attempted to tune the fluorescence output of a synthetic circuit by varying the surrounding temperature, thereby destabilizing a repressor protein that controlled a positive feedback loop. They found, however, that noise plays a significant role in the tuning of the positive feedback loop. Stochasticity in the destabilization of the repressor either causes the cell to amplify the amount of GFP by positive feedback, or causes a minimal amount of GFP – "trademark bistability of the positive feedback architecture". [2] Here, the destabilization of the repressor element in the context of a positive feedback loop allows even minute differences in noise level to direct the creation of visually distinct bimodal populations. A similar system can be used to create bimodal populations in which one population within the desired noise threshold survives, and the other population outside of the noise threshold dies due to the rapid progression of a positive feedback loop.

The error catastrophe theory is a theory of aging that incorporates the idea of a feedback loop, that when triggered, leads to a relatively rapid accumulation of genetic errors causing the symptoms of aging, and eventually death [4]. Biomedicine has utilized the error catastrophe theory as a theoretical basis for treating viral infections, by creating drugs that increase the rate of DNA mutagenesis in the viral genome beyond a viable threshold [5]. In Orgel's model of error catastrophe, the error inducer must be a faulty protein involved in processing genetic information – like a mutated RNA polymerase –, that will lead to a reduced specificity and thus increased error frequency. In the proposed study, I would like to utilize the idea of positive feedback loops and error catastrophe to design an autocatalytic circuit triggered by noise level to induce a bimodal binary response. Cells within the desired noise level can persist, while the cell population outside of the noise threshold triggers the feedback loop to cause lethal error catastrophe. Thus, the noise level in the output of a synthetic device can be tuned to a desired threshold by killing all other cells.

The proposed noise tuner could utilize the TetA(C) gene to act as an alternative error catastrophe inducer. While low to moderate levels of TetA protein confer tetracycline resistance, over-expression of the gene is detrimental to cell growth and ultimately lethal [6]. Conceptually, I propose a circuit model in which noise levels above a threshold defined by the user of the synthetic

device cause either a destabilization event that directs, or the direct induction of a positive feedback loop causing the lethal over-expression of TetA. The Lux operon can be used to construct the positive feedback loop. The Lux system contains LuxR, a repressor that when activated by an autoinducer induces the pLux promoter. Induction causes expression of LuxI, the protein that produces the autoinducer to maintain the circuit [7]. The pLux promoter can coregulate LuxI and TetA in order to achieve lethal levels of tetracycline resistance. In order to design and construct this conceptual circuit, the following ideas must be further explored and addressed: **(1) What protein is needed to cause a destabilization that induces a positive feedback loop? (2) How can this destabilizing protein be tuned to tolerate different noise levels? (3) How can the noise level be detected so that this circuit can be applied to an existing synthetic device?**

The successful design and construction of a noise-tuning circuit could be applied to numerous existing and future synthetic devices. The benefit would be more predictable and controllable synthetic biology systems. Discovering how we can control the noise level in biological systems will provide a deeper understanding of noise, its compounding factors, and its effects.

References:

1. Kaern M, Elston TC, Blake WJ, Collins JJ. (2005). Stochasticity in Gene expression: from theories to phenotypes. Nature Rev Genet 6: 451–64.
2. Isaacs FJ, Hasty J, Cantor CR, Collins JJ. (2003). Prediction and measurement of an autoregulatory genetic module. PNAS 100: 7714 – 19.
3. Becskei A, Seraphin B, Serrano L. (2001). Positive feedback in eukaryotic gene networks: cell differentiation by graded to binary response conversion. EMBO 20: 2528 – 35.
4. Orgel LE. (1963). The maintenance of the accuracy of protein synthesis and its relevance to ageing. Biochem 49: 517 – 21.
5. Summers J, Litwin S. (2006). Examining the theory of error catastrophe. J Virol 80: 20 – 26.
6. Moyed HS, Nguyen TT, Bertrand KP. (1983). Multicopy Tn10 tet plasmid confer sensitivity to induction of tet gene expression. J Bacteriol 155: 549 – 56.
7. Wang J, Zhang J, Yuan Z, Zhou T. (2007). Noise-induced switches in network systems of the genetic toggle switch. BMC Sys Biol 1: 50.