Synthetic 'switches': a new way to tackle complex diseases and biotechnological innovation

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Motivation

This project is aimed at engineering a synthetic network for in vivo delivery of mRNA/protein. The final intent will be to create a stable synthetic oscillatory network in a mammalian system, which is able to express mRNA/protein levels with a pre-determined frequency and amplitude. The rhythm of delivery should be corresponding to the normal nutrient uptake.

Methods

There are many problems to overcome when trying to build artificial networks in living cells; inefficient inducibility, instability, stochastic effects and background activity (leakiness) have been highlighted by scientists as the most important ones. Previous studies indicate as design guidelines the use of strong promoters and efficient ribosome binding sites, as well as making sure there are tight transcriptional repression and a comparative protein and m-RNA decay. To take into account all the possible variables it is necessary to perform a systematic examination of the effects of parameter variation with quantitative modeling and analysis to evaluate ranges of parameters for the experiments and to predict possible out-comes. In this way several synthetic networks constructed by rearranging regulatory components in a cell have been characterized. In this project we will follow the experimental method described by Gardener et al. [1] and Kramer et al.

[2]. These two groups used first a mathematical model to design a toggle to be implemented respectively in E. Coli and Chinese hamster ovary cells and then built the synthetic toggle in vivo. We are planning to build a switch based on the toggle designed by Collins and his group, in which each promoter is inhibited by the repressor that is transcribed by the opposing promoter; in the absence of inducers, two stable states are possible:

A. promoter 1 transcribes repressor 2 B. promoter 2 transcribes repressor 1

The switch is obtained by introduction of an external inducer of the currently active repressor (1 or 2). The inducer permits the opposing repressor to be maximally transcribed until it stably represses the original active promoter. The novelty is that we will use shRNA to express either a siRNA to silence transcription by increasing degradation level of mRNA [3], or to express a microRNA to block translation of the protein [3]. In both cases, the shRNA will act as a repressor so that, having a control at the level of transcription/translation should avoid problems with leakiness of the promoters, as the proteins produced would be silenced. The use of lentiviral vectors throughout the study will allow testing the circuit on primary cell and in animal models. Specifically, once built, the toggle will be used to produce a therapeutic protein in animal model of genetic diseases to test the possibility of inducing the protein in vivo only when needed. In this latter system, individual cellular oscillators will be synchronized to fulfill the macroscopic function of a protein delivery device.

Results

The final goal of the project is to use synthetic inducible switches for therapeutic application.

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