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PIN-AP: an epigenetic synthetic "toggle switch"

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Date and Time: Monday, 13 October 2008, 2:30pm – 3:30pm
Venue: Room **B6605**, City University of Hong Kong
Reception starts at 2:15pm
(Language: **English**)

Abstract

This project is aimed at building a synthetic circuit in mammalian cells in the form of a "toggle switch" for in vivo delivery of mRNA/protein. This synthetic network enables to "switch on" and "off" expression of a protein at will, without the need of external inducer molecules, but transiently.

The network has already been built, it comprises of a transcription factor (TF) that inhibits transcription of shRNA silencing the TF itself. This reciprocal inhibition enables the toggle to be in one of two possible states, high TF and low shRNA, or vice-versa. The switch between the two possible states is obtained by the transient introduction of an external inducer of the currently active repressor. Once the network has switched, the new epigenetic state is maintained indefinitely or until the application of the other inducer.

We are using a lentiviral backbone, pLVUTH, containing a Tet-repressor-KRAB based TET-ON system under the control of the Ubiquitin promoter, and a TRE-H1 promoter expressing an aptamer-fused shRNA (silencing TTR) in a TTR responsive manner. The switch transition is provoked by theophylline that inhibits the aptamer-fused shRNAs or doxycycline that inhibits the TTR protein. The use of an aptamer fused shRNA responsive to theophyllline to silence tran-

scription is a major novelty which also allows for the whole circuit to be contained in a single lentiviral vector. The same vector is utilized throughout the study and it will permit testing the circuit on stably integrated primary cell and later animal models. So far both the inducible systems have been tested with positive results and the virus expressing the circuit has been produced in Hek293T cells.

In order to investigate design constraints and parameters, we derived a semi-quantitative model based on ordinary differential equations. The mathematical variables of this model are the concentrations of the protein tTR/KRAB, the mRNA tTR and the shRNA tTR. Transcription was assumed to follow Hill kinetics and translation was modeled as a first order process. The shRNA-mRNA interaction was modeled based on mass-conservation principles and kinetic rate laws. The model predictions were in good agreement with the experiments.

The presented toggle is envisioned not only as a device with applications for gene therapy, but as a double inducible system which could be of use in perturbations experiments for functional genomics and systems biology.

About the Speaker

December 2006 - present: Post-doctoral appointment looking at unraveling genetic networks with the use of synthetic biology. (Telethon Institute of Genetics and Medicine, Via P. Castellino 111, 80100, Naples, Italy, website: www.tigem.it)

June 2006 - November 2006: Research assistant position looking at confirming the predictions of a time-course based algorithm for p63 gene-network using chromatin IP and microarrays. (Telethon Institute of Genetics and Medicine, Via P. Castellino 111, 80100, Naples, Italy, website: www.tigem.it)

November 2004 - August 2005: Post-doctoral appointment as responsible for molecular markers development and transfer technology on a project for the construction of a linkage map for oil palm (*Elaeis guineensis* Jacq.). (School of Biological Sciences, Lyle Building, The University of Reading, Whiteknights, Reading RG6 6AS, UK)

January 2005, The University of Reading – Department of Biological Sciences Ph.D in Molecular Biology – Title: "Investigating self-incompatibility in Brassica rapa at molecular level and its population genetics impact"

October 1999 BSc Biology and Genetics Class: 1st (105/110) University of Padua, Italy

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