Engineering a 1:2 Bio-multiplexer for controlled stem cell differentiation

Sairam Subramanian
Dept. of Electrical Engineering, Princeton University
sairams@princeton.edu

Prof. Ihor Lemischka
Dept. of Molecular Biology, Princeton University
ilemischka@molbio.princeton.edu

Prof. Ron Weiss
Dept. of Electrical Engineering and Dept. of Molecular Biology, Princeton University
rweiss@princeton.edu

Abstract

Precise control of stem cell differentiation offers tremendous potential for tissue engineering. Synthetic gene networks provide a framework for understanding and engineering life. We propose to use synthetic gene networks to engineer circuits that dictate the cell fate of embryonic stem (ES) cells by controlling gene expression. These networks will be capable of turning on cell fate regulator genes in stem cells at precise times and under well-controlled and well-defined conditions based on external stimuli and the internal state of the cell. The over-expression of these cell fate regulator genes is sufficient to trigger particular differentiation pathways in ES cells. We have implemented a lentivirus delivered 1:2 multiplexer circuit for programmed differentiation. This network uses a transactivator, a repressor and one small molecule input Doxycycline (Dox). Dox selects to activate one of two cell fate regulator gene outputs, thereby pushing the ES cell along one of two differentiation pathways. Preliminary results demonstrate the ability to switch between the expression of two fluorescent proteins – EGFP and DsRed-Express based on the external input Dox. Upon integration of the cell fate regulators MyoD and Nanog into this circuit, ES cells will either differentiate into muscle or maintain their undifferentiated state. Characterization of this simple network in mammalian cells is an important first step as this circuit will serve as a basis for building more complex networks that can select between many outputs using only a few inputs to form structures that resemble complex tissues like the spinal cord.