Construct a push-on-push-off switch in *Escherichia coli*

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***Short Abstract* — We want to engineer the *E. coli* cell to behave as a push-on-push-off switch. The gene regulatory circuit was designed to sense Ultra-Violet (UV) irradiation as input signal. The circuit is composed of two associated parts: a NOR gate and a bistable switch. The NOR gate is controlled by both the input signal and the bistable switch. On the other hand, the bistable switch can be triggered by the output of the NOR gate and switch from one state to the other. Thus under intermittent UV irradiation, two reporter gene can be induced alternatively.**

***Keywords* — push-on-push-off switch, bistable switch, NOR gate, synthetic biology**

I. Introduction

SYNTHETIC biology is an emerging field that extends the methods of genetic engineering to integrate multiple gene regulation pathways to achieve certain complex functions [1]. A growing amount of modular genetic parts is being developed in bacteria, which can make up various cellular machines [2].

Our goal is to engineer the *E. coli* cell to behave as a push-on-push-off switch. We designed a gene regulatory circuit that senses UV irradiation as input signal. Under intermittent irradiation, the circuit gives alternative color as output. Five transcription regulatory genes are used: LexA, CI, CI434, CI(ind-) and LacI. LexA controls SOS response as a transcription repressor[3]. CI is an activator/repressor of lambda phage lysogeny-lysis transition[4]. CI434 is a repressor in 434 phage[5]. LexA, CI and CI434 can be degraded during SOS response. CI(ind-) is a mutant of CI which functions in a same way but is not degraded in SOS response. LacI repressor regulates lactose uptake[6].

II. Gene Circuit Design

The Bistable Switch is composed of CI and CI434 which represses each other in transcription. The NOR Gate is a promoter repressed by both LacI and LexA. These two parts are connected by LacI and CI(ind-). LexA is constitutively expressed in *E. coli*. LacI is in the same operon with CI. CI(ind-) is controlled by the NOR gate. GFP and mRFP are used as reporters of the two states respectively.

We substituted CRO with CI434 in wild type lambda switch, and modified PRM so that it can be repressed by CI434. We changed the SD signal of both repressors to make the switch bistable, and most cells go to state 2 automatically. We put the cassette in different copy-number plasmids to make sure the switch can be zeroized by SOS response.

We combine the SOS gene promoter with LacI binding site so that it can be repressed by both LacI and LexA. We tried binding sites in different position and with different affinity. As we got both parts function ideally, we will put them into one cell and adjust the system by changing the SD signal of LacI and CI(ind-).

III. Mathematical model

A set of ordinary differential equations (ODEs) are used to describe the dynamic behavior of this circuit after UV irradiation. Assuming initially the cell is expressing CI-GFP-LacI. CI434-mRFP is repressed by CI, and CI(ind-) is repressed by both LexA and LacI. We call this state as state 1. When the cell is bared to UV irradiation, LexA and CI are degraded. In the absence of CI, LacI transcription attenuates and the protein concentration decreases as cell growth. LexA, CI, and CI434 re-accumulate when SOS response shut off. During the SOS response and thereafter, CI(ind-) is always repressed, firstly by LacI and then by LexA. PR is stronger than PRM, thus CI434 accumulates faster and represses CI expression. The cell turns to state 2, in which CI434-mRFP is high and CI-GFP-LacI is low.

If the cell is bared to UV irradiation again, LexA is degraded. At this time, in the absence of both LexA and LacI, CI(ind-) is expressed. Before CI(ind-) is turned off by LacI and LexA, it helps CI to repress CI434, turning the cell to state 1.

IV. Conclusion

We have constructed and tested the bistable switch and NOR gate. The mathematical model can shed light on how to connect these two elements together to make the circuit work. With the push-on-push-off switch we may get better understand how cell differentiate on same input signal.

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