Using NF-κB modules and DNA elements to engineer combinatorial and dynamic gene regulation

Qiuyue Wang[1,3], Jun Jiang[2], Huaiqiu Zhu[1,3], Ping Wei[1,2,4]*

In response to large variety of stimuli, mammalian NF-κB transcriptional factors (TFs) promote diversified series of genes expression. The unique DNA-binding properties of NF-κB dimers is a key signal integration step. To investigate these properties, we build a library of NF-κB binding DNA elements (κB sites) in a synthetic promoter and use flow cytometer to evaluate the transcriptional activity in yeast. We further characterize these dimer-specific promoters and then rebuild genetic circuits, such as logic gates etc. These study provides us new insight of how NF-κB dimerization act as regulatory function and become a powerful toolbox for synthetic biology practice.

I. Introduction

LIVING cells regulate gene expression by well-designed gene circuits. After the process of upstream signal transduction pathway, the delicate interaction between DNA and TFs play an important role[1].

Several modular tools, such as zinc finger [2], TALE (transcription activator-like effector) [3], and CRISPR [4] system have been used to regulate gene expression in synthetic biology. Their excellent orthogonality and few off-target rate make them a broad application prospect.

In this paper, we use NF-κB natural and refabricated protein to control programed promoters. First, our TFs derived from nature, which will be true reflection of real gene regulation mechanism. Second, NF-κB family proteins can form homo- and hetero- dimers, which exhibit specific DNA binding properties. Third, we can program extended logics into transcriptional regulation through controlling NF-κBs' dimerization process.

II. Results

A. Rapid Identification of NF-kB dimer specific gene activation DNA elements in yeast

Based on database [5] and results from high throughput technology [6], we build a library of 260 different NF-κB binding sites and insert them in front of minimal CYC1 promoter. Then we transform all the plasmids into 11 kinds of yeast with different combination of NF-κB proteins.

The sequence specificity of NF-κB dimers is obvious, which helps us to rediscover the specificity of NF-κB dimers. We also have got powerful toolbox and orthogonal pairs for synthetic genetic circuits. Well-conserved sequence motifs are shown for those dimers.

B. Programmable gene regulation with rational designed synthetic promoters and refabricated NF-kB proteins.

Combining NF-κB proteins with 18 other co-factors, such as med6 and hda3, the properties of natural NF-κB proteins are widened. A quicker repressor is born, which will dynamically change the regulation of downstream genes.

Systematically engineering NF-κB binding position makes promoters, such as ADH1 and CYC1, have different basal expression and fold of activation and repression, which provide us an abundant source of material.

C. Synthetic devises and logic gates coded by NF-kB protein dimerization

Through changing different kinetic κB sites, we build different self-activation and inhibition curves, which will fit well with computation models. Taking use of the dimerization process of NF-κB protein, we have rebuilt almost all the logic gates with a single promoter, which is simpler and more modularized.

III. Conclusion

The ability to manipulate gene regulation is the most fundamental business in genetic engineering. Here, we rigorously measure the specificity of eukaryotic TF, NF-κB. We find that properties of TFs and promoters can be fine-tuned. Beyond these, with the dimerization process of NF-κB proteins, we simply rebuild useful synthetic devises.

[1]Center for Quantitative Biology, and [2]Peking-Tsinghua Center for Life Sciences, Academy for Advanced Interdisciplinary Studies, [3]College of Engineering, [4]School of Life Sciences, Peking University, Beijing 10086, China.

*Corresponding to: pwei@pku.edu.cn

References

[1] Purvis JE, & Lahav G. (2013). Encoding and decoding cellular information through signaling dynamics. *Cell*, **152(5)**, 945–56

[2] Khalil AS, etal. (2012). A Synthetic Biology Framework for Programming Eukaryotic Transcription Functions. *Cell*, **150(3)**, 647–658.

[3] Maeder ML, et al. (2013). Targeted DNA demethylation and activation of endogenous genes using programmable TALE-TET1 fusion proteins. *Nature Biotechnology*, **31(12)**, 1137–42.

[4] Gilbert LA, et al. (2013). CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. *Cell*, **154(2)**, 442–51

[5] Gosselin K, Touzet H, Abbadie C (2004) Institut de Biologie de Lille et LIFL,

[6] Siggers T, et al. (2011). Principles of dimer-specific gene regulation revealed by a comprehensive characterization of NF-kB family DNA binding. *Nature Immunology*, **13(1)**, 95–102.