Science and Technology Group
Annual Report FY2016

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1 Introduction

I engaged in two independent research projects during FY2016, namely, engineering of catalytic RNAs (ribozymes) and investigations on natural fibers.

1) Ribozyme Engineering: Several new screening methods to discover ribozymes and RNA aptamers were investigated. A new method to screen active self-cleaving ribozymes directly in mammalian cells was developed.

2) Natural Fibers: I was awarded a Kakenhi Kiban C grant to discover peptide sequences that can bind to silk fibers and confer antibiotic resistance. I also started a new project to study the traditional Okinawan textile *Basho-fu* from materials perspective.

2 Activities and Findings

1) Ribozyme Engineering

<u>High-throughput assay of RNA ligase mutants</u>

We recently developed a method that allows high-throughput assay of self-cleaving ribozymes using deep sequencing (1). I adapted this method to study another class of ribozyme with ligase activity for the first time. I selected a small ligase ribozyme discovered by the Joyce group (2) and assayed activities of ~2000 mutants simultaneously. The sequence-activity relationship provided a high-resolution map of the critical and non-critical nucleotides and base-base interactions.

<u>Direct screening of self-cleaving ribozyme activity in mammalian cells</u>

Ribozymes can be used to control gene expression in mammalian cells, but their intracellular activity is difficult to predict. I developed a new method to screen thousands of natural and synthetic ribozymes for their activity directly in mammalian cells. The screening method is outlined in Figure 1. A library of dsDNA encoding ~3000 ribozymes were transfected into HEK 293 cells. Uncleaved (UC) and cleaved (C) RNAs were isolated from the total RNA extract of the transfected cells. Amplified cDNA from the isolated RNAs (UC, C) were analyzed by deep sequencing. Few of the sequences shown to be active by deep sequencing were individually cloned in the 3'UTR of an mRNA encoding EGFP to confirm their activity. Additional sequences will be tested in the coming year to validate this methodology.

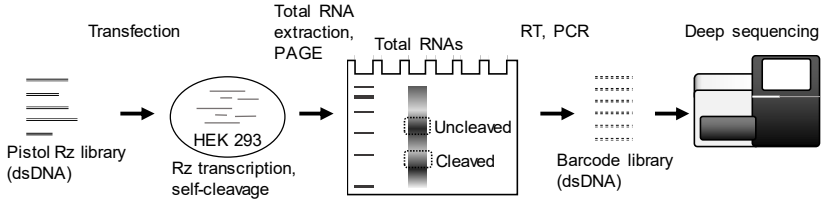

Figure 1. Outline of the ribozyme screening strategy in mammalian cells

In addition to the projects described above, I have explored new methods to identify small molecule aptamers using natural aptamer scaffolds in vitro and in E. coli. However, no promising results were obtained during the fiscal year.

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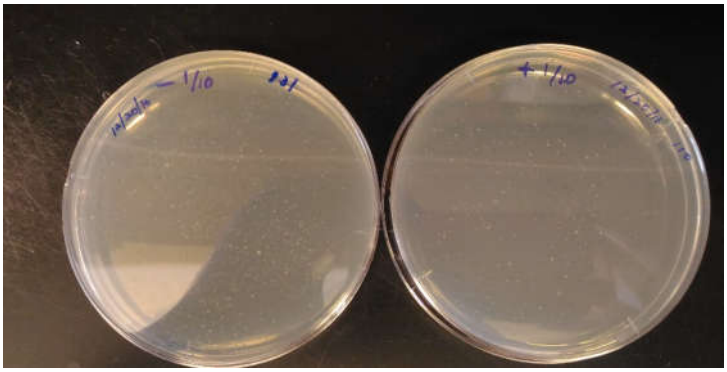
2) Natural Fibers

Antibiotic silk fiber using peptides (Kakenhi project)

I was awarded a Kakenhi Kiban C grant for this project (see 4. Publication and other output). As the first experiment, I fused fibroin binding peptide having QSWS motif (YN42 and YN43 in ref 3) to several short antibacterial peptides. Ten peptides were designed and tested against *E.coli* Top10. The best combination was YN42 fused to RRRWWW-NH2. Then, the linker between YN42 and RRRWWW-NH3 was adjusted. Fiber test using YN42 G3SG3 RRRWWW-NH2 showed the difference between with (110 cfu) and without (831cfu) this peptide (photo). Also, binding of this peptide to silk fibroin fiber was confirmed by ELISA. Furthermore, to obtain even stronger silk-binding peptides for practical applications, I performed M13 phage selection using a modified protocol. However, no promising sequences were obtained. On the other hand, YN42 fused repeated QSWS motif showed stronger binding to fibroin fiber than YN42 only. To obtain new silk fibroin binding peptides, I will use a more effective selection method and using deep sequencing next year.



Basho-fu project

I started a new natural fiber project. *Basho-fu* is a typical traditional textile made from *Itobasho* (banana plant) stem fiber. Scientific analysis of *Basho-fu* has not seen much progress since the 1980's. Especially, the traditional production process has not been studied scientifically despite decline of *Basho-fu* production recently. OIST Business Development Section and I set up a collaboration system with other organizations for *Basho-fu* research project including *Itobasho* cultivation. I started analyzing *Basho-fu* materials provided from Kijoka Basho-fu Association in Ogimi village.

References

1. M. P. Robertson, G. F. Joyce, *Chem. Biol.,* 21, 238-245, (2014).
2. S. Kobori, Y. Nomura, A. Miu, Y. Yokobayashi, *Nucleic Acids Res.*, 43, e85 (2015).
3. Y. Nomura, V. Sharma, A. Yamamura, Y. Yokobayashi, *Biotechnol. Lett.*, 33, 1069-1073, (2011).

3 Collaborations

Yokobayashi Unit
Basho-fu project collaborator: Kijoka Basho-fu association, University of the Ryukyus

4 Publications and other output

Kakenhi Kiban C, FY 2016-2018 (3 years)
Antibiotic silk fibroin using peptides
Direct expenses 3,300, 000 yen and overhead expenses 990, 000 yen
In total 4,290,000 yen