Science and Technology Group
Annual Report FY2017

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1 Introduction

I continued to engage in two independent research projects during FY2017, namely, engineering of catalytic RNAs (ribozymes) and investigations on natural fibers (silk and *Basho-fu*). In particular, I made significant progress in the *Basho-fu* (traditional Okinawan textile) project this year.

1) Engineering of Catalytic RNAs: A new method to screen active self-cleaving ribozymes directly in mammalian cells was published. Furthermore, we tried to apply this method to RNA aptazyme screening. We also discovered minimized RNA ligases using high-throughput sequencing assay.

2) Natural Fibers: We characterized materials from key steps in the traditional production process of *Basho-fu* by SEM and chemical analysis. The findings were published in a fiber science journal. In the silk project, I tried to discover more effective antibiotic peptides that can bind to silk fibroin.

2 Activities and Findings

1) Engineering of Catalytic RNAs

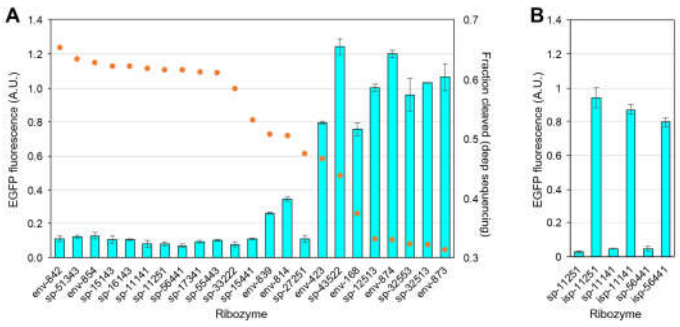
<u>High-throughput assay of RNA ligase mutants</u>

Last year, I adapted our high-throughput assay of self-cleaving ribozymes using deep sequencing (1) to another class of ribozyme with RNA ligase activity. Ribozyme-catalyzed RNA ligation is a fundamental reaction that could have played an important role in the origin of life. I chose a small ligase ribozyme discovered by the Joyce group (2) and assayed activities of ~2000 mutants simultaneously. This year, we designed new libraries consisting of shorter sequences based on the sequence-activity relationship that we revealed last year. We determined the sequences of minimized RNA ligases by our high-throughput assay. As a next step, we plan to use this highly active minimized RNA ligase to construct a self-replication system in vitro.

(1) S. Kobori, Y. Nomura, A. Miu, Y. Yokobayashi, *Nucleic Acids Res.*, 43, e85 (2015).
(2) M. P. Robertson, G. F. Joyce, *Chem. Biol.,* 21, 238-245, (2014).

<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 (c.f. figure in FY2016 annual report). To confirm the intracellular activities of the identified ribozymes, we cloned the ribozyme sequences in the 3'UTR of an mRNA encoding EGFP. Intracellular activities of the 23 ribozymes tested (right figure) confirmed that our method can identify active ribozymes. The findings were reported in an international symposium and published in *Chemical Communications.* (4. A1, A2).



(A) Ribozyme activity based on EGFP assay in HEK293 cells (light blue bars) and fraction cleaved (FC) values derived from deep sequencing (orange circles). EGFP fluorescence of HEK293 cells transfected with an EGFP-ribozyme plasmid was normalized to the fluorescence of the cells transfected with an empty (no ribozyme) plasmid. Top 10, worst 5, and 8 intermediate variants based on the deep sequencing were tested. (B) Inactivation of the ribozyme by two-base mutation restores EGFP expression in 3 selected ribozymes.

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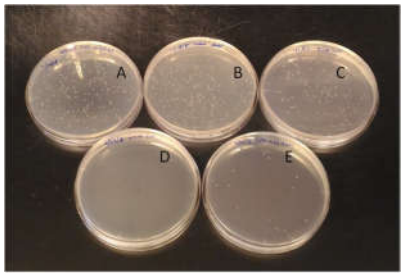
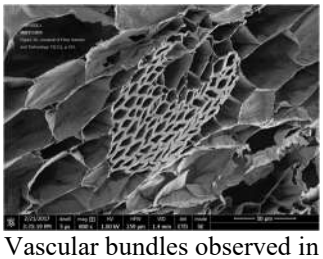
2) Natural Fibers

Antibiotic silk fiber using peptides (Kakenhi project, 4. B1)

I investigated antibiotic peptides fused to the fibroin binding peptide YN42 which I previously reported having a single QSWS motif. Because repeated QSWS motif derived from YN42 showed stronger binding to fibroin fiber compared to YN42 only, I measured the antibiotic activity of these peptides. However, antibiotic activity of the peptide was reduced when the repeated QSWS motif was used. As a next strategy, I performed deep sequencing analysis to obtain new fibroin binding peptides. I fused the newly selected peptides to a short antibiotic peptide RRRWWW-NH_2. I found that few of the peptides could strongly bind to fibroin fiber, and that the strongest binding peptide WVWanti kills both Gram negative and Gram positive bacteria (right photo D). I concluded that this WVWanti was the best candidate and I will optimize the binding conditions of WVWanti to fibroin fiber for practical applications next year.



WVWanti sequences showed strong antibacterial activity. Plates: *E.coli* colonies from silk fibroin fibers treated with antibacterial peptide.
A, DMSO (no-peptide) 302 CFU,
B, VHWanti 287 CFU,
C, TSWanti 172 CFU,
D, WVWanti 0 CFU,
E, YN42G3Santi 17 CFU.



Vascular bundles observed in the materials after the traditional degumming step.

Basho-fu 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. In collaboration with OIST Research Support Sections, I characterized materials from before and after traditional degumming process, a key step in *Basho-fu* production. The degumming step was mild, and small vascular bundles (left photo) of the thread's main component were well conserved in the materials after this procedure. The findings were published in a fiber science journal (4. B2a)

Next year, we will analyze the mechanical properties of threads and fibers from the later steps of *Basho-fu* production process. Because of the local cultural relevance of this project for Okinawa, OIST CPR and I prepared a press release to the local media (4. B2b). The reaserch was covered by the two major local newspapers as well as in other media. We are also planning the first scientific *Basho-fu* exhibition with a symposium related to this research open to local public next year.

3 Collaborations

Prof. Yokobayashi (Nucleic Acid Chemistry and Engineering Unit)
Basho-fu project collaborator: Kijoka Basho-fu Association, University of the Ryukyus,
OIST Imaging section (Mr. Toshio Sasaki & Dr. Koji Koizumi), OIST Mechanical Engineering and Microfabrication Support Section (Dr. Hyung-Been Kan)

4 Publications and other output

A1, Y. Nomura et al, International Symposium on Nucleic Acids Chemistry (Poster session, Tokyo, 14/Nov/2017-16/Nov/2017).
A2, Y. Nomura et al, *Chemical Communications,* 53, pp12540-12543, 2017.
B1, Kakenhi Kiban C, FY 2016-2018 (4,290,000 yen in 3 years) Antibiotic silk fibroin using peptides.
B2a, Y. Nomura* et al, *Fiber Science and Technology,* 71, pp317-326, 2017. * Corresponding author.
B2b, The press release talk about *Basho-fu* project (at Okinawa Prefectural Government, 22/Dec/2017).