

Science and Technology Group Annual Report FY2021

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1 Introduction

I continued to engage in two independent research projects during FY2021, namely, engineering of catalytic RNAs (ribozymes) and investigations on natural fibers (e.g., Bashofu).

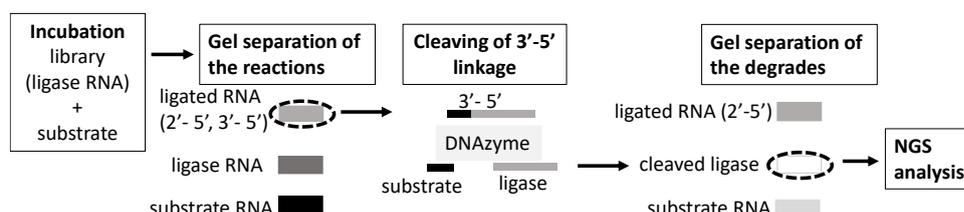
I continued to explore RNA ligase having a small catalytic core. Another project on catalytic RNA research I had partially been engaged in was published in a scientific journal (Section 4, #1).

For the natural fiber projects, I had to revise the original plans due to the COVID-19 pandemic and I eventually decided to extend the project period of one of the Kakenhi projects. Under this new plan, we will successfully extract fibers by a facile method from neglected materials in the premium Bashofu making. Some of the results were published in a professional journal (Section 4, #2). Furthermore, we started to explore another extraction method using biotechnological skills, and we applied this method to *Kariyushi wear project* funded by the Bank of Okinawa.

2 Activities and Findings

A. Engineering catalytic RNA projects with Yokobayashi unit

I continued to study RNA ligases with a small catalytic core that can self-ligate 3'-5' RNA sequences by the same manner as the present enzymatic ligation. We tried



Selection method for 3'-5' linkage RNA ligase

selecting 3'-5' RNA ligases from the libraries of short RNAs (7, 8, 9, 14, 16 nt) than the 18 nt sequence we reported ⁽¹⁾. However, we could not isolate RNA ligases that catalyze the formation of 3'-5' linkage. To select ligated RNA having 3'-5' linkage we added an extra step of cleaving 3'-5' linkage by a deoxyribozyme (DNAzyme) to our method (above figure). However, we were still unable to isolate ligases that catalyze 3'-5' linkage using this method. Therefore, we plan to characterize the ligase ribozymes that catalyze the formation of 2'-5' linkages with small catalytic cores.

About another project, we synthesized a new pistol ribozyme scaffold by circularly permutation, and we selected variants working as riboswitches with aptamers in mammalian cells. We obtained an excellent riboswitch with a guanine aptamer, resulting in ON/OFF ratios as high as 8.6. This work was published (Section 4, #1).

(1) Y. Nomura and Y. Yokobayashi, Systematic minimization of RNA ligase ribozyme through large-scale design-synthesis-sequence cycles, *Nucleic Acids Research*, 2019, 47, 8950-8960.

B. Natural fiber projects

The goal of the research of Kakenhi KibanC 19K02308 is to improve Bashofu production by using scientific investigations on the site of the Bashofu studio in Ogimi village. However, due to the COVID-19 pandemic, we could barely visit the studio because the artisans are over 70 year-old. Instead, we came up with a plan to use materials that are deemed worthless for the premium

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Bashofu making in the studio. Because the materials (*Itobasho* plants) are strictly selected for premium Bashofu making, most of *Itobasho* plants cannot be used for the production.

I performed a facile extraction of the fibers from *Itobasho* plants that are too young or too old for premium Bashofu making. Then our extracted fibers were compared to those of the sophisticated fibers made by the artisans. The thickness of the fibers was larger than those made by the artisans, so some skills will be needed to use the fibers for clothing textiles. The output from the collaboration research with Teijin Frontier on natural fibers could be applied to our Bashofu fibers obtained through the facile extraction method in the future.

Furthermore, we investigated applicable biotechnology skills to *Itobasho* fiber extraction for the *Kariyushi wear project*. Local Okinawan weaver Urasoe-ori Association wove the first textile made from our fibers (right photo).



**A test weaving at local studio
Urasoe-Ori Association.**

warp: silk yarn, weft: our *Itobasho* yarns,
A4 size (approx. 30 cm x approx. 20 cm),
weft 58 yarns/inch.

3 Collaborations

Scientific Imaging Section Dr. K. Koizumi,
Kijoka Bashofu Association, Urasoe-ori Association,
Japan Women's University, University of the Ryukyus,
Teijin Frontier Co. Ltd., Kato-Tech Co. Ltd., Lequio LTD.

4 Publications and other output

Peer reviewed publications:

1. K. Mustafina, Y. Nomura, R. Rotrattanadumrong, Y. Yokobayashi, Circularly-Permuted Pistol Ribozyme, A Synthetic Ribozyme Scaffold for Mammalian Riboswitches, ACS Synth. Biol. 2021, 10(8), 2040-2048.
2. F. Kakihara, K. Koizumi, T. Sasaki, Y. Nomura, Facile Extraction of Bashofu Fibers, J. of Home Economics of Japan, 2021, 72(12), 818-828. Corresponding author Y.N.

Books:

3. Y. Nomura and Y. Yokobayashi, Aptazyme-Based Riboswitches and Logic Gates in Mammalian Cells, Method Mol Biol, 2021, 2323, RNA Scaffolds, 213-220.

Peer reviewed presentation:

4. F. Kakihara, K. Koizumi, T. Sasaki, Y. Maehara, Y. Nomura, Microscopic Observations of Bashofu Yarn Fibers, 2021 May 30th. The Japan Society of Home Economics, annual meeting.

Invited presentation & author:

5. Y. Nomura, Scientific Analysis for Bashofu (科学者からみた芭蕉布－芭蕉布の新しい価値の発見－), The 111th Presentation at Okinawa Textile Meeting (第111回 沖縄染織研究会), 2021 May 21st.
6. Y. Nomura, Scientific Analysis for Bashofu, Okinawa Textile Meeting News (沖縄染織研究会通信). 2021, 105, 1-14.

Fundings:

- Kakenhi KibanC 19K02308 (PI, 4,420,000 yen, over 4 years, FY 2019-2022,)
Kakenhi KibanC 20K02354 (co-PI, 2,860,000 yen, over 3 years, FY 2020-2022)
Okigin Furusato Promotion Fund (*Kariyushi wear project*, co-PI, 1,000,000 yen for 1 year, 2021 July-2022 July)
Teijin Frontier Co. (PI, 1,820,000 yen for FY 2021)