

慶應 SFC 学会

(A) 研究成果発表 (学会発表)

第 28 回国際 RNA 学会 シンガポール 成果報告書

伊藤らら (慶應義塾大学 政策・メディア研究科 修士課程 1 年)

学会概要

研究タイトル: *In vitro* synthesis of (G4C2)_n RNA for the study of G-quadruplex gel formation in ALS

発表形式: ポスター

学会名: 第 28 回国際 RNA 学会 (The 28th Annual Meeting of the RNA Society)

開催場所: シンガポール・Suntec Convention Centre

参加期間: 2023 年 5 月 29 日~2023 年 6 月 4 日

開催形式: 現地対面

学会報告

この度 2023.05.29-06.04 の期間シンガポールで開催された第 28 回国際 RNA 学会 (The 28th Annual Meeting of the RNA Society) に研究の進捗をポスター形式にて発表するべく、学会に参加した。本学会は、年に 1 度世界中で RNA に関する研究を専門的に行う研究者が一同に集まり、研究の成果や進捗を発表する場である。昨年の開催まで新型コロナウイルス感染拡大の影響を受け、学会もオンライン開催をしていたが、第 5 類に下げられたことで今年の国際学会はオンサイトにて行う運びとなった。28 回目の開催であるため、学会として歴史は浅いが、第 28 回の学会では 1000 人以上がシンガポールへと足を運び、世界トップランクの大学の学生、研究者、PI と交流することができた。国際学会のため、言語は英語で自身の研究成果をポスターで発表した。この度の学会では私の研究テーマに類似した研究、グアニン四重鎖 (G-quadruplex) や筋萎縮性硬化症 (ALS) に関する研究成果を報告している方も多かったことから自身のポスターにも同様の研究を行う学生、PI が発表を聞きにきてくれた。特に台湾大学の研究室からは共同研究のオファーをいただいたり、John Hopkins 大学やスイス大学の PI とは研究手法に関するアドバイスもいただいたり、テーマの類似性から海外研究室インターンのオファーなど海外を拠点する研生活も視野に入れることができた。賞などは受賞できなかったものの、自身の研究と研生活の今後の可能性について考えさせられ、大きく進歩することができた有意義な時間だった。他にも若手研究者(ポスドクの学生まで)の集う会や海外での就職支援会、他国の PI と話しながら食事ができる会など学会では研究を聞く以外にも研究者と交流する機会や支援に関する催しものも多数用意しており、大変興味深く且つ日本国内以外の同年代くらいで世界で研究をする人々と知り合いになることができ、大変貴重な体験をすることができた。

In vitro synthesis of (G4C2)_n RNA for the study of G-quadruplex gel formation in ALS

○Lala Ito^{1,2}, Josephine Galipon^{1,3}

1) Systems Biology Program, Graduate School of Media and Governance, Keio University
 2) Institute for Advanced Biosciences, Keio University 3) Faculty of Engineering, Yamagata University
 contact ✉: lalaitoh@sfc.keio.ac.jp

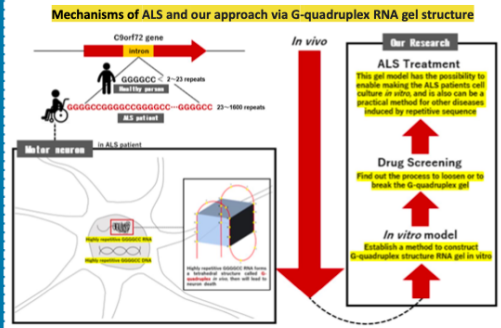


P1-156

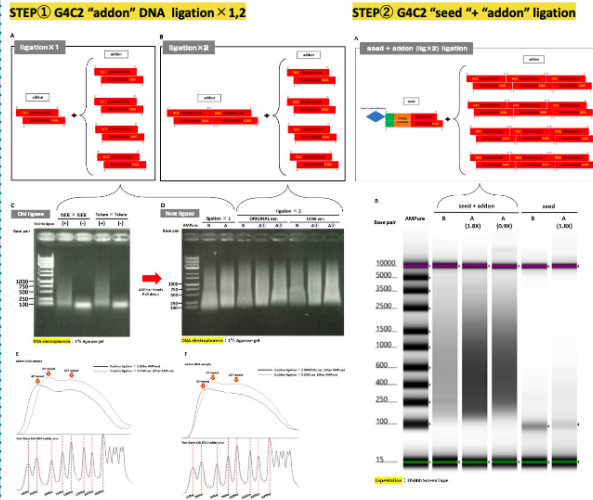
Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease induced by G4C2 hexanucleotide repeats in the non-coding region of the C9orf72 gene, numbering anywhere from 23 to 1600 repeats. The repeated DNA sequence is transcribed to RNA, which then folds into a tetrahedral structure called a G-quadruplex (RNA-G4). This structure aggregates with cytosolic proteins, leading to the formation of an intracellular gel which impairs motor neuron function, and gradual loss of muscle mass. However, the synthesis and cloning of repetitive GC-rich sequences, such as (G4C2)_n, ranging more than 100 repeats remains technically challenging due to its structural character and cellular toxicity. Indeed, the current record for cloning of uninterrupted (G4C2)_n is n = 71 repeats. Our research aims to establish a method for the in vitro synthesis of super-repeated (G4C2)_n RNA. Here, we present our original synthesis method that successfully generates uninterrupted DNA templates up to (G4C2)₃₁₁, based on the sequential ligation of sticky-end (G4C2)_n DNA. T7 transcription was successful on these longer templates, and we obtained (G4C2)₃₁₁ RNA for the first time, which is most likely the longest uninterrupted artificial (G4C2)_n RNA to date. These results are expected to contribute to research on diseases caused by repetitive sequences by providing an easy-to-use cell-free method for investigating the RNA-G4 formation by (G4C2)_n and its gelting mechanism, and RNA-G4-protein interactions, providing a platform for the future screening RNA-G4-binding drugs.

Overview



Result ① Able to synthesize DNA template (G4C2)₄₂₋₃₃₃



Workflow

Target of this research

DNA cloning and synthesis of a repetitive G4C2 sequence (ranging in the n > 1000 of repeats) remains extremely challenging, and the current state-of-art is 71 repeats. My research explores the possibilities of synthesizing n > 1000 repetitive G4C2, obtaining RNA from this sequence and to achieve a cell-free-system, with the suggested workflow below.

Protocol & Result of 2021

① Synthesis of blunt-end G4C2 DNA

● (G4C2)_n DNA
 17 nt (blunt-end) (GGGGCC)17
 17 nt (blunt-end) (CCCCGG)17

● (G4C2)_n DNA
 17 nt (blunt-end) (GGGGCC)8
 17 nt (blunt-end) (CCCCGG)8

● (G4C2)_n DNA
 17 nt (blunt-end) (GGGGCC)4
 17 nt (blunt-end) (CCCCGG)4

Result ① DNA (G4C2)_{17,8,4} synthesis

Agarose gel electrophoresis image showing the synthesis of DNA templates with 17, 8, and 4 repeats of G4C2. Molecular weight markers are indicated on the left (300, 200, 100 bp).

Result ② RNA transcription of (G4C2)_{17,8,4}

Agarose gel electrophoresis image showing the RNA transcription of DNA templates with 17, 8, and 4 repeats of G4C2. Molecular weight markers are indicated on the left (300, 200, 100 bp).

Conclusion

- We found that T7 RNA polymerase is able to transcribe up to 17 repeats of 100% GC highly structured RNA.
- This time (in 2022-2023) we want to make longer repeats (more than 17 repeats).

Protocol of 2022~2023

① Synthesis of sticky-end G4C2 DNA

To achieve a longer G4C2 sequence (like the ALS repeated G4C2), we want to produce a > 23 G4C2 DNA template. In this method, there are 2 types of DNA designed. One is called "seed", which includes biotin, a random sequence, T7 RNA promoter, and (G4C2)_n. The other type, called the "addon", only includes a (G4C2)_n. We added a biotin 5'-end modification, allowing us to pull down the DNA template connected with the seed only.

● Sticky-end G4C2 DNA (n > 23)

② Magnetic beads pull down

③ RNA transcription

④ G-quadruplex formation

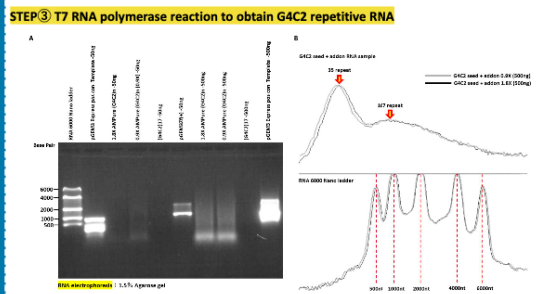
G4C2 RNA G-quadruplexes are stabilized by specific ions like potassium (K⁺), sodium (Na⁺), or magnesium (Mg²⁺) by adding these specific ions, and investigating the conditions, we were able to define the condition to loosen or break the gel, or to harden the gel. To evaluate the G-quadruplex formation, we are using the Circular Dichroism (CD).

⑤ RNA gel in vitro generation

We want to form a gel from RNA G4C2 G-quadruplex in vitro, to reproduce the condition of ALS patients cell environment.

(G4C2)_n RNA forming a structure to be a gel

Result ② Able to transcribe 100% (G4C2)₃₅₋₃₃₃ RNA template



Result ③ G4C2 RNA G-quadruplex structure checked in CD

