トリプレットリピート病の原因となる RNA タンパク質凝集体形成機構の解明

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Mechanism of RNA Protein Aggregate Formation as a Cause of Triplet Repeat Disease (Institute of Advanced Energy, Kyoto University[1], Integrated Research Center for Carbon Negative Science, Institute of Advanced Energy, Kyoto University[2]) CHUAYCHOB, Surachada[1,2]; HOU, Wanqing[1]; SHIMIZU, Musashi[1]; NAKANO, Shun[1]; RAJENDRAN, Arivazhagan[1]; NAKATA, Eiji[1]; MORII, Takashi[1]

Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy caused by expansions of CTG repeats[1]. These repeats interfere with normal mRNAs by titrating the Muscleblind-like RNA binding protein 1 (MBNL1), leading to the formation of RNA-protein (RNP) aggregates[2]. However, the chemical nature and mechanism behind their aggregation are still unclear. To address this, we designed an in vitro system using DNA origami to create RNP aggregates from RNA molecules with over 1000 CUG repeats and MBNL1 proteins. High-speed AFM measurement was used to analyze the aggregates and provide mechanistic insights into their formation.

Herein, we transcribed CUG repeat RNA with different repeat numbers (n) of 10, 20, and 28 in vitro and investigated their purity and length using PAGE. The hairpin-like structure of RNA was successfully synthesized and confirmed by fluorescence enhancement of CUG repeat RNA-binding molecules such as thioflavin T (ThT)[3] upon binding to the RNA repeat unit. We then assembled $(CUG)_n$ RNAs on the rectangle DNA origami and examined their assembly using AGE. MBNL1 and MBNL1**Δ**105, a mutant of MBNL1 with the C-terminal 111 amino acid residues deleted, were cloned from pGEX plasmids, expressed in E. coli, and purified using His-tag and GST-tag chromatography. The purified proteins were examined using SDS-PAGE before being utilized for the aggregation formation study. The RNP aggregates were visualized using high-speed AFM measurement, and the decrease in height of RNP implied their successful binding on the DNA nanostructure. This preliminary study of CUG repeat RNA-MBNL1 protein aggregates shows the possibility of verifying their mechanism. Specifically, we seek to clarify what kind of RNP aggregates are formed depending on the number of CUG repeats.

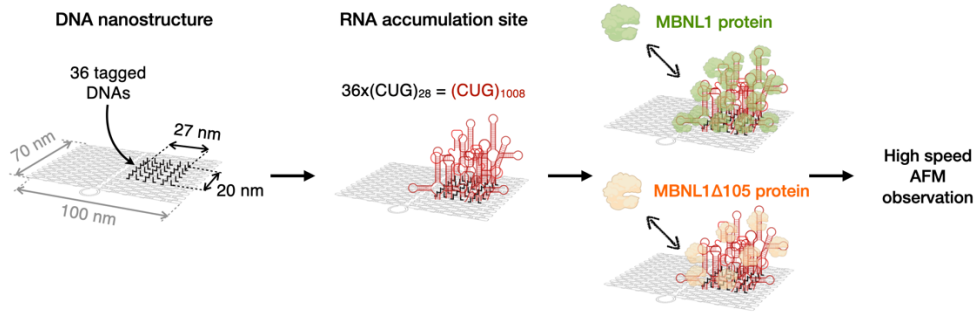

Fig. 1. Recapitulating CUG repeat RNA-MBNL1 protein aggregates using DNA nanostructure.

1) G. Ho, *World J Clin Pediatr.* **2015,** *4, 66.* 2) O. Pettersson, *Nucleic Acids Res.* **2015,** *43, 2433.* 3) S. Sugimoto, *Nucleic Acids Res.* **2015**, *43*, 14.