Form 1-5-2

Japan-U.S. Brain Research Cooperation Program
Researchers Dispatched to the U.S. Program FY2019: Report

Field:__①Cell and molecule__

1. Researcher

Name Yusuke Komi
Title Researcher
Affiliation: RIKEN Center for Brain Science, Laboratory for Protein Conformation Disease

2. Research Title:

Investigation of co-translational folding of protein relevant to neurodegenerative diseases using optical tweezers

3. U.S. Joint Researchers/Institutes
Please give the name, title and affiliation.

Bustamante Carlos, Professor, University of California Berkeley Department of Mol and Cell Biol

4. Research Period, from/to (mm/dd/yyyy):

06/13/2019 to 01/14/2020

5. Abstract, Results, and Research Significance (300 Words):

Neurodegenerative diseases often arise from polypeptide misfolding and aggregation in the brain. However, comparatively much less attention has been directed to characterizing the sequential folding steps of proteins as they are being synthesized in the ribosome. The recent studies suggest that nascent chains adopt folding intermediates whose stability and lifetime depend on interactions of the polypeptide with the ribosome, making them particularly vulnerable to misfolding and aggregation.

Dr. Bustamante's laboratory has established and demonstrated measurement of translation in real-time using the prokaryotic translation system. During this period, I tried to establish a technique to examine the structure of a nascent chain using the human translation system. First, I confirmed whether we can use the human translation system for optical tweezers experiment. I succeeded to measure translation in real-time using optical tweezers with the human translation system. However, this experiment cannot be used for measurement of co-translational protein folding, therefore I made a new mRNA construct to directly pull on the nascent chain for the measurement of co-translational folding. The double hairpin structure of the mRNA construct containing a valine codon used in a translation system without valine allows for visual confirmation of the stalled translation complex. If the translation stops in the proper position, one hairpin structure is disrupted by ribosome-mediated destabilization. I succeeded to make the double hairpin mRNA construct and when I partially translated this mRNA using the human translation system without valine I succeeded in grabbing the nascent chain and mRNA to form the tethers. However, I was unable to find an individual rip in the mRNA structure to confirm the properly stalled complex.

My project aimed to measure the protein folding and mis-folding on the human ribosome which would useful for revealing structural alterations potentially associated with neurodegenerative diseases.

6. Other (Research concerns, particular points of note):

*Please attach any reference materials as necessary.