Report on MERIT Long-term Overseas Dispatch

Takuma Sueoka
2nd year PhD student, Okamoto Group
Department of Chemistry and Biotechnology

Overview

I stayed at Prof. Hanbin Mao's Group in Kent State University (Ohio, USA) from April 27 to July 6 2016, supported by the MERIT long-term overseas dispatch program. Prof. Mao and I met and discussed several issues at an academic meeting last year, and he got interested in my research area in nucleosome. He kindly accepted my visit to his laboratory and we could start a collaborative research project.

Background and research

Prof. Mao's group performs biophysical research to analyze dynamics of biomolecules through optical laser tweezers. Recently he has published a number of impressive papers about G-quadruplex in DNA or RNA. G-quadruplex is a quartet-strand structure formed in nucleic acids whose sequences are rich in guanine. It is mainly located in telomere region, which is the end of chromosome. Due to the fact that this unique structure is related with diverse functions such as protection of genes or repression of aging, many researchers have been trying to investigate it by using biochemical and physical way. In order to conduct analysis of higher-order structure in nucleic acids, laser tweezers are one of the powerful methods. Tweezers equipment can trap micrometer-scale beads by laser light and manipulate them with pN order force. Therefore, attachment of both ends of a target sample to two different beads enables us to track slight changes of folding or unfolding of the target by just pulling the beads. In addition to that, magnet can produce "torsion" in samples by rotating beads if we use a magnetic bead as a reagent.
My research specialty is chemical synthesis of proteins and investigation of nucleosome structure. In eukaryotic cells DNA and histone proteins form higher-order structure called nucleosome, whose structural changes regulate gene expression. It is commonly accepted that histone posttranslational modifications affect electrostatic interaction and steric hindrance between DNA and histones, and these changes result in changes of nucleosome structure. I planned this collaborative project in which we can estimate the stability of DNA-histone interaction by applying physical force with laser tweezers in Prof. Mao's group. In proceeding this project, histone octamer (complex of eight histone proteins) was prepared in Okamoto laboratory and mailed to Prof. Mao's laboratory. After starting this dispatch, I tried to reconstitute nucleosome *in vitro* by using the octamer and DNA prepared in Prof. Mao's laboratory.

During the stay at Prof. Mao's group I performed mainly three types of experiments: learning how to use tweezers equipment, analysis of DNA in replication process, and reconstitution of nucleosome. At first I learned a method to prepare a tiny flow path, which is needed to supply beads and reagents. Although it is just composed of glass plate, Parafilm M®, and glass capillary, you need much experience and skill to make good coverslips. It is because slight difference of the width of the flow path or tiny dusts cause failure

of trapping beads. Also I learned how to deal with tweezers equipment composed of laser microscopy and analysis machines.

After understanding the basic procedure for tweezers experiment, I assisted the other member's project by acquiring the data and analyzing it. In this time, I used a construct which imitates DNA replication.

Third, I did nucleosome reconstitution experiments. A high affinity sequence to histone octamer called 601DNA is generally utilized for nucleosome reconstitution. We prepared an approximately 5000 bp construct containing biotin and digoxigenin at the both end of the construct respectively and containing 601 sequence at the center of the construct. Such kind of several thousand sequence is required in order to set tweezers equipment. On the other hand, I optimized the reconstitution condition of nucleosome by using short DNA construct. Unfortunately, it took long time to make the 5000 bp DNA and we could not conduct enough analysis of nucleosome. Therefore, we decided to continue this collaborative project after the dispatch.

Daily life in USA

Kent State University is located in Kent city, one-hour drive from Cleveland. Cleveland is a main city in Ohio and is prosperous at the south shore of Lake Erie (one of the Great Lake). In Kent city, it is balmy and comfortable weather from spring to summer, and then in winter it is severe cold. I stayed there during spring and early summer, so it was very nice place to live in. Moreover, it is quiet and beautiful city: the lawn in every houses is cared carefully and there are many forests around the city. In contrast, there is little public transportation. There are also not many grocery stores or restaurants, so the students in the group is joking about Kent city "This is rural, there is nothing but the university". I keenly realized that I needed automobile to live in USA.

All of the members are working analytical research with laser tweezers. They engage in researches of the same category, while the members in our group have individual research topic of their own. I felt this difference of the style quite interesting. Their style enables them to share detailed knowledge about experiments, and that would be efficient way to produce results in short period.

Few Japanese students are studying in Kent State. In addition to that, the most of them are undergraduate, so I had never seen Japanese people during the summer holiday season. There are especially many Chinese students, and students from other Asian countries such as India or South Korea are studying here. Prof. Mao's group also mainly consists of Asian students. As they attach special importance to their lifestyle, they adjust their research schedule without strict rules in the group. I realized they have different values from those of Japanese students. I was impressed that an academic field in USA is broad-minded for widely accepting foreign people to do research.

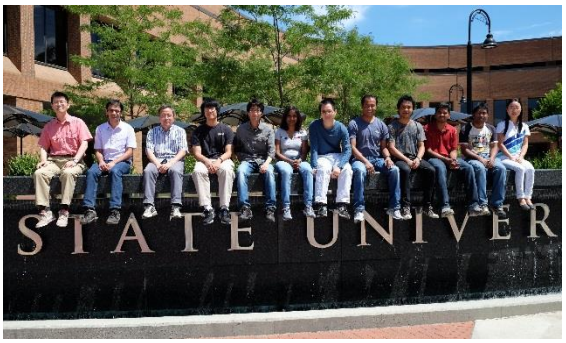

Fig. Group photo

Acknowledgements

I would like to express my sincere gratitude to Prof. Mao for kindly accepting my visit to his group. I also would like to thank Prof. Mao and the members in the group for supporting my experiments and daily life. I appreciate Prof. A. Okamoto and Prof. M. Fujita for letting me study in Kent State. Finally, I am grateful to MERIT program for giving me a great opportunity to conduct this collaborative research.