

July 3, 2019

Institute for Advanced Biosciences, Keio University

Secrets of “Spider Silk” Strength Revealed —Expectations in the Development of New Materials through the Discovery of New Genes—

Project Assistant Professor Nobuaki Kono and Associate Professor Kazuharu Arakawa’s group at the Institute for Advanced Biosciences, Keio University (Tsuruoka City, Yamagata Prefecture; Director of the Institute: Masaru Tomita) collaborated with a team from Spiber Inc. (Tsuruoka City, Yamagata Prefecture), including Hiroyuki Nakamura, and determined the genome of the orb-weaving spider *Araneus ventricosus*, shed light on the full picture of the various spider silk proteins and their related genes for the first time in the world, and succeeded in the phylogenetic analysis of silk genes. In this research, new spider silk genes and the existence of low molecular proteins that influence silk strength were also discovered, and it is thought that these findings will significantly contribute to the development of artificial spider silk in the future. The outcomes of this research were published in the international scientific journal “Scientific Reports” on June 10, 2019.

1. Background of Research

The recent trend of using artificial biomaterials derived from organisms as new materials has revealed clues for a solution to energy problems and is said to be a kind of industrial revolution. In particular, the artificial synthesis of spider silk has attracted attention, but to reproduce its natural properties, there were a lot of obstacles that had to be overcome. Spiders separately use about 7 different types of silks depending on the circumstances, all of which are made from different proteins. As a result, in order to use artificially synthesized spider silk in industrial fields, it becomes necessary to use different proteins and synthesize silks that correspond to the application purposes. However, the gene sequences encoding the silk proteins have very complex structures which were extremely difficult to determine through conventional methods. For this reason, a silk gene catalog, which is necessary for the artificial synthesis of silks, had not been developed to any extent. Therefore, while there was a great fascination with the strengths of the various types of silks used by spiders, it had not been possible to artificially produce and take advantage of the diverse potential of the material.

2. Results and Significance of Research

In this study, new genome sequencing and gene search techniques for the orb-weaving spider *Araneus ventricosus* were developed, and a search for the complex silk genes from the massive quantity of prevailing genomic information was attempted. The *Araneus ventricosus* is a species of a family of spiders that weave circular webs. They are large spiders that are found all over Japan (figure 1). Like other spiders, the *Araneus ventricosus* uses several types of silks through their behavior and lifestyle, from reproduction to feeding. For example, the silk they produce to suspend themselves, move, create egg sacs, and capture prey all have different characteristic properties (strength and extensibility). The genome size of spiders is known to be larger than that of humans, and the *Araneus ventricosus* is no exception. Traditionally, a genome was determined by a short DNA fragment sequencing and assembly, but when dealing with something so

substantial, mathematical and computational issues stand in the way, meaning useful genomic information cannot be attained practically from conventional methods alone. Therefore, the problem becomes how to sequence this large genomic DNA as it is. Kono and the other members combined a nanopore sequencer (Oxford Nanopore Technologies, UK)—a single molecule DNA sequencer—and Illumina sequencer (Illumina, USA) with the Chromium System (10x Genomics, USA), which enables long-range DNA analysis using emulsion technology. Through this, they were successful in obtaining a large amount of highly accurate long-range DNA sequence information, which in the end led to the successful determination of 3.66 Gb of draft genome (N50 scaffold size: 59,619 bp). This was the first time a spider genome was determined using these technologies, and a genome that can sufficiently satisfy the search for spider silk genes was also established. However, in order to reveal everything about the silk genes, it will be necessary to solve the problems related to the sequence structures of the silk genes.

The sequences of the silk genes have a highly repetitive structure (a structure in which the same sequence appears many times). With such repeated structures, it becomes very difficult to accurately determine the order and number of the sequences mathematically, and therefore, most research groups around the world had given up on getting the full picture of silk genes. In addition to the abovementioned genomic information, Kono and the other team members combined direct-RNA sequencing (Oxford Nanopore Technologies, UK), which directly sequences the expressed genes, with an independently developed analysis algorithm to successfully create a catalog of the 11 silk genes that are used to make all 7 types of spider silks (figure 2). This was the first time in the world that a complete catalog was created. Furthermore, the development of this catalog led to the discovery of new genes and helped in the understanding of the constituents that make up spider silk.

Dragline silk, the strongest silk which spiders use to move about, had been thought to be made up of two proteins, MaSp1 and MaSp2. However, from the detailed analysis of this study, it was revealed that in addition to these two, a new protein, MaSp3, is also used by *Araneus ventricosus*, which produces especially strong silk. The partial analyses which had been carried out up to now did not lead to the discovery of MaSp3 as its sequence is very similar to those of MaSp1 and MaSp2. Furthermore, from the constituent analysis, etc., of the silk, it became clear that the presence of a low molecular protein called SpiCE is also necessary in *Araneus ventricosus* silk. This means that in order to create really strong artificial spider silk, it is not enough to just use the silk proteins that had been industrially worked on in the past, and that the combination of new constituents, such as MaSp3 and SpiCE, is essential. For the first time in the world, this research successfully discovered ways to improve the physical properties of artificial spider silk.

At the time of publication, Project Assistant Professor Kono said that he was able to determine genomes at the human level and discover new spider silk genes, as well as factors related to silk strength thanks to the achievements of the Institute for Advanced Biosciences, Keio University, which up to now had acquired the best technologies available. He also mentioned that the outcomes of this research are likely to rapidly progress future research in new material development.

Original paper: Nobuaki Kono, Hiroyuki Nakamura, Rintaro Ohtoshi, Daniel A. Pedrazzoli Moran, Asaka Shinohara, Yuki Yoshida, Masayuki Fujiwara, Masaru Mori, Masaru Tomita & Kazuharu Arakawa, “Orb-weaving spider *Araneus ventricosus* genome elucidates the spidroin gene catalogue,” *Scientific Reports*, (2019) 9:8380.
<https://www.nature.com/articles/s41598-019-44775-2>
DOI: 10.1038/s41598-019-44775-2



Figure 1: *Araneus ventricosus* (Photo taken by Hitoshi Kawakami, 2nd-year student at the Keio University Faculty of Environment and Information Studies)

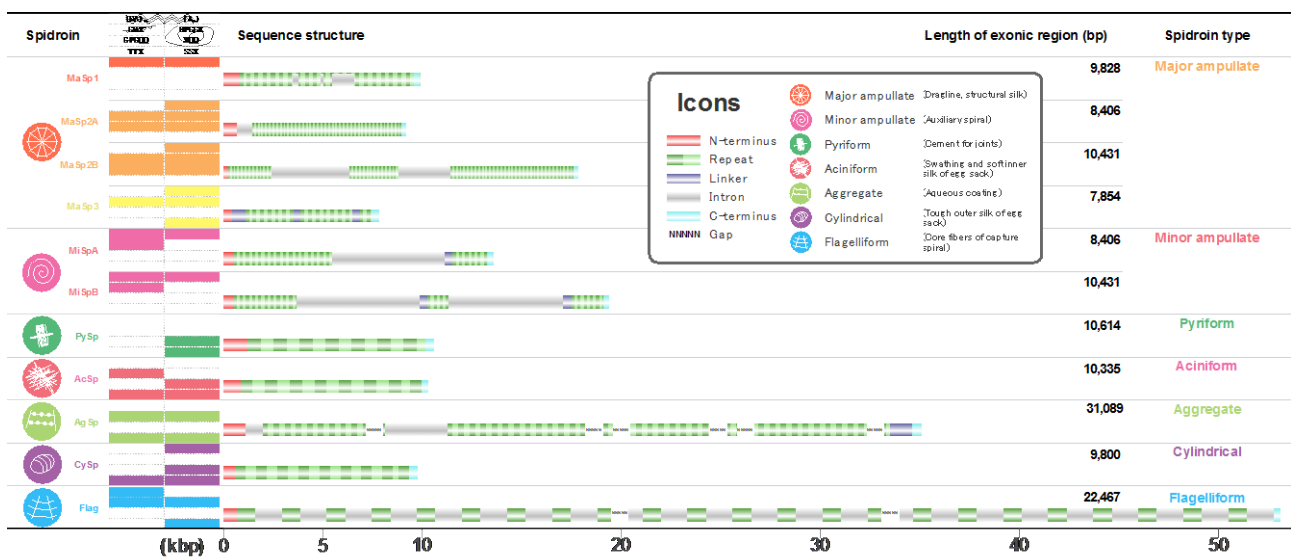


Figure 2: Complete gene catalog of *Araneus ventricosus*. The round icons on the left represent the 7 types of silks and their application. From the top, they are the web frame (orange), hub silk (pink), adhesive silk for joints (green), silk to capture prey (red), viscous silk (yellow-green), egg sac silk (purple), and crosswise silk (light blue). Each silk is made up of 1 to 4 silk genes and the lengths of the genes vary from 10 to 50 kbp. The newly discovered gene in this study is the MaSp3 in the orange area. Furthermore, the sequence structures of the silk genes are very similar, and the green stripes between the red and blue boxes at the ends indicate repeat sequences. It is clear that a large number of repeat sequences are present. (Taken from Figure 1 in Kono, *et al.*, 2019).

*Please direct any requests or inquiries to the contact information provided below in advance.

【Inquiries about research】

Institute for Advanced Biosciences, Keio University, Public Relations Office

Tel: +81-0235-29-0802 Fax: +81-0235-29-0809

E-mail: office@ttck.keio.ac.jp <http://www.iab.keio.ac.jp/>

【Inquiries about press release】

Keio University Office of Communications and Public Relations

Tel: +81-03-5427-1541 Fax: +81-03-5441-7640

E-mail: m-pr@adst.keio.ac.jp <https://www.keio.ac.jp/>