氏名（本籍・生年月日）	Han Leng (韓 冷)（中華人民共和国・1982 年　1 月 28 日）
学位の種類	博士（　工学　）
学位記番号	乙　第　２２４号
学位授与の日付	平成２５年９月３０日
学位授与の要件	信州大学学位規程第５条第２項該当
学位論文題目	Study on spider silk genes and transgenic silkworm including the spider silk gene
論文審査委員	主査　教　授　中垣 雅雄 教　授　林田 信明 教　授　梶浦 善太 准教授　塩見 邦博 教　授　蜷木 理　（東京農工大学）

論　文　内　容　の　要　旨

Spider silk receives the most interest from scientists for its unique combination of high tensile strength and elasticity. Previously, we cloned and characterized several novel spider silk genes and observed their expression in *Bombyx mori* cell lines and larval bodies.

In this study, we focus on two research topics: one is that keeping on analysis those novel spider silk genes (Chapter I). The other is that analysis the transgenic silkworm and the silk from them (Chapter II).

Firstly, we have isolated a unique *MaSp* gene (Chapter 1 paragraph 1). We describe a new short type of dragline silk gene, *Cyrtophora moluccensis* MaSp1s. The full-length gene is only 1320 base pairs (bp), which encodes 439 amino acids that includes the intact non-repetitive N-terminal (149 residues), C-terminal (98 residues) and so-called repetitive regions (192 residues); the deduced molecular weight is approximately 40 kDa. It deviates from the large-size rule and is different from the previously reported MaSp1 (10 kb). The sequence analysis demonstrated that the two termini are highly homologous to the other characterized dragline silk genes but that the so-called repetitive region is different. The motif analysis suggested that MaSp1s included all of the MaSp1 characteristic motifs, such as GGX (X =A, Q, or Y), GX (X =Q, A, or R), and poly-A, Our results suggest that *MaSp1s* is a possible new characteristic dragline gene; the discovery of this gene should enhance our understanding of the Major Ampullate spider silk genes. The function of MaSp1s is presently unclear. But, the N and C termini of MaSp1s are highly homologous with those of other MaSp proteins. It is reported that the non-repetitive termini contribute to storage and assembly of spider silk proteins. This rationale supports the hypothesis that MaSp1s would be partially responsible for the storage and assembly of spider silk proteins, particularly with regard to the proteins assembling together with MaSp1 and MaSp2. Perhaps MaSp1s plays a role similar to p25 in the silkworm. Further research is needed to elucidate the function of MaSp1s.

Secondly, in the Chapter 1 paragraph 2, we reanalyzing the CySp2 gene of Wasp Spider *Argiope bruennich*i. The reason is that to gain further understanding of egg case silk proteins gene family, A. C. Zhao et al. (2006) isolated two full-length cDNAs for egg case silk proteins, cylindrical silk protein 1 (CySp1) and cylindrical silk protein 2 (CySp2), from the wasp spider, *Argiope bruennichi*. CySp2 was reported to contain no apparent signal peptide sequences , and the CySp1-CySp2 complex, which would possess a signal peptide, would be transported across the endoplasmic reticulum and secreted to the Golgi. According to a report by Hayashi, genomic DNA sequencing is one approach that can be successfully utilized to retrieve 5' ends of silk genes; using this method, we retrieved the 5' end of CySp2. We found that CySp2 contained a typical signal peptide similar to that found in CySp1; thus,

due to technical limitations, an artificial error had occurred in the CySp2 sequence reported by Zhao et al. (2006). And what' more, we found the mechanism, which lead to the error had happen. We hypothesize that the excision event occurred during cDNA library construction. When the double-strand DNA fragments produced by RT-PCR were linked into a plasmid and transformed into *E.coli*., an intermolecular homologous recombination occurred during the plasmid self-replicating process.

Chapter 2 was divided into three sections. In the first section (Chapter 2 paragraph 1), we further attempted the expression of a spider dragline gene: Cm.MaSp1s by germline transformation of the silkworm *B. mori*. using the *piggyBac* transposon-derived vector with the *B. mori* Fib H promoter. We chose to clone the repetitive unit of *MaSp1s*. And it was inserted into the *piggyBac* transposon-derived vector. In order to simplify the screening steps we chose the N4 white-eye strain. The G1 eggs were screened by detecting DsRed-fluorescence under the fluorescent stereomicroscope. And the other three stages (larvae, pupa and imago.) were also monitoring with DsRed fluorescence and visible light. G1 positive transgenic individuals were mated within the same family to generate the G2 descendents for the following analysis.

Although, many other researchers have done a lot work about the transgenic silkworm, there has been very limited information on the biological properties of silkworm carried the foreign silk protein. So in the second section (Chapter 2 paragraph 2), we analyzed the the relationship between the foreign gene and three major components of silk fibroin (FibH, FibL and p25), systematically. At the DNA level, the iPCR result showed that foreign gene MaSp1s inserted site located in scaffold868. At the RNA level, the Real-time PCR results showed that there was not significant difference between the wide type silkworm (WT) and transgenic silkworm (TG) on the transcriptional level of the three major genes (FibH, Fib-L and p25), particularly the FibH. It means that the expression of the foreign gene MaSp1s didn't affect three major genes expression at RNA level. At the protein level, the SDS-PAGE data showed that the abundance the foreign protein (about 2% among the total fibroin proteins) is much lower than that of FibH and Fib-L, but richer than p25. At last, we used the Co-IP (Co-immunoprecipitation) for detecting the interaction relationship between MaSp1s and FibL. The results showed that the MaSp1s and FibL do formed the protein complex as expected. And the fibroin complex also contains the FibH.

In the last section (Chapter 2 paragraph 3), we have analysis the physical properties and structure of silk from the transgenic silkworm. The reason is that, to data, there is little report about the physical properties of the transgenic silk including spider dragline silk protein. To study the properties and structure of the silkworm silk including the spider silk protein, the silk was characterized by Micro tensile testing, Fourier transform infrared spectroscopy (FT-IR), Wide angle X-ray diffraction (WAXD), and Differential scanning calorimetry (DSC). The tensile testing data showed that the silk's average tensile strength is 340 MPa and its strain is about 45%. FT-IR measurements suggested the existence of binary structure containing random coil conformation and β-sheet structure. DSC result indicated that the melting temperature of the silk is around 326℃. The molecular orientation calculating from WAXD pattern is 0.9403. We hope that the results obtained are promising as a basis for future industry applications of this kind of silk.

In conclusion, in this study, we focus on two research topics, one is spider silk genes, and the other is silkworm including spider silk gene. We hope that those results presented here would be a little helpful to this research field.