学位論文　（要約）

Shell matrix proteins of brachiopods: Implications for the mechanisms of shell formation
（腕足動物の殻体タンパク質: 殻形成メカニズムの解明に向けて）

平成 27 年 12 月　博士（理学）申請

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Abstract

Brachiopods are sessile marine invertebrates with calcitic or phosphatic shells. The brachiopod shells contain proteins that play pivotal roles in shell formation and are important in understanding the evolution of biomineralization. In this study, I performed a large-scale exploration of shell matrix proteins in the rhynchonelliform brachiopod *Laqueus rubellus* using proteomic analysis and transcriptome analysis. A transcriptome analysis in mantle tissue of the closely related species, *Coptothyris grayi* was also performed. Then, comparative analysis of shell matrix proteins in brachiopods was performed using my results with proteome data of shell matrix proteins in the rhynchonelliform brachiopod *Magellania venosa* and linguliform brachiopod *Lingula anatina* published recently (Jackson et al., 2015; Luo et al., 2015). These results provided suggestions for possible molecular mechanisms and evolution of brachiopod shell.

In chapter 1, I reviewed previous studies related to this work. Firstly, I summarized the anatomical structure of brachiopod soft body and shells as well as phylogenetic position of Brachiopoda and phylogenetic relationship of the three brachiopod subphyla. Then some previous studies of shell matrix proteins in molluscs and brachiopods were shown to emphasize the importance of sequencing shell matrix proteins to understand the mechanisms and evolution of biomineralization. Finally, I discussed significance of this study to understanding of the evolution of biominerals and to paleontological applications.

In chapter 2, I performed proteome analysis in the shell matrix proteins in *Laqueus rubellus*. As a result, I identified a total of 77 shell matrix proteins. Many shell matrix proteins identified in this study have signal peptide and transmembrane region. I identified ICP-1, which is a chromoprotein detected in brachiopod shells in previous studies. ICP-1 has been sequenced completely for the first time, and showed the highest abundance in the shell. MSP130, which is a skeletal protein identified from sea urchins and oysters, was also identified. In addition, many extracellular proteins, digestive enzyme, and trypsin inhibitor were identified. Among these proteins, possible ion transporter domains, EGF-CA and EF-hand were also identified. On the other hand, 48 out of the 77 had no homologues in public databases. Among these unknown proteins, one shell matrix protein was identified with a domain architecture that includes a NAD(P) binding domain, a transmembrane region, and an aspartic acid rich region. The repertoire of brachiopod shell matrix proteins also contains a basic amino acid-rich

protein and proteins. These pieces of sequence information of shell matrix proteins in *Laqueus rubellus* suggest that some unique mechanisms exist in brachiopod biomineralization.

In chapter 3, I performed comparative analyses of shell matrix proteins in brachiopods. The comparison of the shell matrix proteins in rhynchonelliform brachiopods showed that about half of the shell matrix proteins in *Laqueus rubellus* and *Magellania venosa* share a high sequence similarity with each other. This conserved proteins contained some extracellular, trypsin and trypsin inhibitor as well as many rhynchonelliform brachiopod specific shell matrix proteins. These shared proteins are thought to have important functions in the calcitic brachiopod shell formation. I also carried out comparative analyses of shell matrix proteins between rhynchonelliform and linguliform brachiopods. The shell matrix proteins in the linguliform brachiopod *Lingula anatina* share almost all detected domains conserved between *Laqueus rubellus* and *Magellania venosa*, while the rhynchonelliform brachiopod specific shell matrix proteins were not identified in the shell matrix proteins in *Lingula anatina.* In addition, the comparative analysis between molluscs and brachiopods showed that Peroxidasin, P-selectin and MSP130 are shared.

Finally, in chapter 4, I discussed the possible molecular mechanisms of brachiopod shell formation, and the evolution of the shell in the ‘Cambrian explosion’ as well as future perspectives based on the results of this study. This study suggests unique mechanisms in brachiopod shell formation and provides a hypothesis of molecular evolution of the shell matrix proteins involved in the independent origin of the brachiopod shells among animals.

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Chapter 3
Comparative analysis of shell matrix proteins in brachiopods

本章については、5 年以内に雑誌等で刊行予定のため、非公開。

Chapter 4
General discussion

本章については、5 年以内に雑誌等で刊行予定のため、非公開。

Acknowledgements

I wish to express the deepest appreciation to Prof. Kazuyoshi Endo (The University of Tokyo) for his advice as my supervisor. I could perform my research thanks to his tremendous support. I also thank a lot to Dr. Isao Sarashina (The University of Tokyo) for his advice on many experiments. I am grateful to Dr. Keiji Kito (Meiji University) for the proteome analysis and reading the manuscript. I would like to thank Drs. Kenshiro Oshima, Elika Iioka, Misa Kiuchi and Prof. Masahira Hattori (The University of Tokyo) for the transcriptome analysis using Roche 454 GS junior. I also thank to Drs. Takeshi Kawashima (University of Tsukuba), Manabu Fujie and Prof. Nori Satoh (Okinawa Institute of Science and Technology) for the transcriptome analysis using Illumina HiSeq 2500.Thanks are due to Dr. Takanobu Tsuihiji and Dr. Takenori Sasaki (The University of Tokyo) for reading the manuscript and valuable comments in the palaeobiology seminar. I also thank Dr. Toshihiro Kogure and Dr. Yohey Suzuki (The University of Tokyo) for reading the manuscript. I wish to thank Drs. Beatrice Demarchi, Matthew Von Tersch, Keri Rowsell, and Prof. Matthew Collins (University of York) for the pilot study using fossil samples and their kind hospitality. I am grateful to Akito Ishikawa (The University of Tokyo) for the gene expression analysis. I thank the staff of the Misaki Marine Biological Station (The University of Tokyo) for collecting *Laqueus rubellus* samples. I also thank Satoru Yamamoto (Tsuruga fishermen's cooperative) for collecting *Coptothyris grayi* samples.

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Appendix 1 Accession numbers of the shell matrix proteins identified using Roche 454 GS Junior

Isotig no.	Accession no.	Homologous contigs generated by Illumina HiSeq 2500
isotig00046	FX982984	c67563_g1_i3_fr+3
isotig00149a	FX982985	-
isotig00213	FX982986	c74363_g1_i1_fr+1
isotig00227	FX982987	c71949_g1_i2_fr-3
isotig00281	FX982988	c63018_g1_i1_fr-3
isotig00337	FX982989	c63765_g1_i2_fr-1
isotig00341	FX982990	c66077_g1_i1_fr+3
isotig00435	FX982991	-
isotig00515	FX982992	c72664_g1_i1_fr-2
isotig00543	FX982993	c54324_g1_i1_fr-2
isotig00601	FX982994	-
isotig00776	FX982995	c65341_g1_i1_fr-3
isotig00914	FX982996	-
isotig00916	FX982997	c50794_g1_i1_fr+2
isotig00949	FX982998	c39088_g1_i1_fr-1
isotig00959	FX982999	c70658_g1_i1_fr-3
isotig00996	FX983000	-
isotig01016	FX983001	c60717_g1_i1_fr+2
isotig01095	FX983002	c54667_g1_i1_fr+3
isotig01124	FX983003	-
isotig01158	FX983004	c55851_g1_i1_fr-2
isotig01176	FX983005	c59536_g1_i1_fr-1
isotig01202	FX983006	c22988_g1_i1_fr-3
isotig01252	FX983007	c43779_g1_i1_fr+1
isotig01312	FX983008	-
isotig01382	FX983009	c58173_g1_i3_fr+1
isotig01414	FX983010	-
isotig01423	FX983011	c60034_g1_i1_fr-2
isotig01521	FX983012	-
isotig01556	FX983013	c58437_g2_i1_fr+3
isotig01587	FX983014	-

Appendix 1 Continued

Isotig no.	Accession no.	Homologous contigs generated by Illumina HiSeq 2500
isotig01670	FX983015	c66941_g1_i2_fr-3
isotig01886	FX983016	c58437_g3_i1_fr+2
isotig01967	FX983017	-
isotig02158	FX983018	-
isotig02447	FX983019	-
isotig02555	FX983020	c63594_g1_i3_fr+2
isotig02613	FX983021	-
isotig02671	FX983022	-
isotig00149b	FX983023	c71594_g1_i1_fr-1