論文の内容の要旨

Comparisons of larval and adult shell proteomes in molluscs
(軟体動物の成体殻と幼生殻における貝殻プロテオームの比較)

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Marine molluscs produce larval shells before settling down on the substrate to start precipitating adult shells. Although the functional importance of shell matrix proteins (SMPs) in shell formation, and their importance in understanding shell evolution have been generally recognized, proteomes of molluscan larval shells have been poorly known.

First, we report for the first time comparisons of adult and larval shell matrix proteins between different molluscan species. In this study, by proteomic analysis combined with genomic and transcriptomic analyses, we identified 111 and 31 larval SMPs from the two bivalve species, *Crassostrea gigas*, and *Pinctada fucata*, respectively, and they were compared with their adult counterparts. Comparisons between larval and adult SMPs in those species revealed that the larval SMPs are surprisingly different from the adult SMPs with only four common SMPs shared between larval and adult SMPs in each species. Expression patterns of the genes

encoding the SMPs containing the same domains, such as Nacrein and Pif, clearly showed that, within those gene families, some members are highly expressed at early developmental stages while others are adult shell specific, despite the fact that they contain the same domains. Pfam domain searches showed that von Willebrand factor type A (VWA), carbonic anhydrase (CA), the carbohydrate-binding module CBM_14 and EF-hand domains exist in both larval and adult SMPs for both species, indicating their indispensable roles in shell formation. The differences of the components between larval and adult SMPs suggest that the larval and adult shells originated and evolved independently.

Second, phylogenetic analyses performed on the SMPs containing the domain share by larval and adult shells indicated a surprising fact that the recruitment of CAs, one of the most important SMPs for both larval and adult shell formation, occurred to molluscan shells rather late than expected. Also, lineage-specific evolutions of SMPs independently occurred to the larval and adult shells were suggested.

Last, in order to establish a transgenic molluscan lineage for functional analyses of SMPs, the method for introducing *green fluorescent protein* (*GPF*) expression plasmids were tested on *Pinctada. fucata*. The delivery of foreign DNA by electroporation and chemicals were confirmed by PCR amplification, yet fluorescent embryos cannot be detected under microscope, which indicated possible failures caused by promoters or the GFP-coding sequence. Also, RNA constructs of CRISPR/Cas-9 towards the SPARC gene of the limpet, *Nipponacmea fuscoviridus* were generated and introduced into the fertilized egg for a test. By far, no conclusive results of a knockout effect has been obtained.