DETERMINE PROTEOME OF THE OYSTER SHELL ON THE BASIS OF OYSTER GENOME SEQUENCE FOR SHELL FORMATION

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ABSTRACT

Because the majority of molluscan species (Conchifera) create shells that vary in size and form, their abundance demonstrates the variety of mineralized tissues. Mollusk shells are one of the most striking instances of matrix-mediated mineralisation occurring outside of living tissues. The calcifying matrix is a complex combination of proteins, glycoproteins, and polysaccharides that self-assemble and regulate the CaCO3 polymorph (calcite, aragonite), crystallite size and shape, and shell texture. The shell proteome data was combined with organ transcriptome data in this work, and we discovered that shell proteins may be generated by many organs, while the mantle remains the most significant organ for shell creation. We conducted a shell damage experiment and determined the shell-related gene set to discover the various transport pathways from different organs to the shell formation front to identify the transport pathways of these shell proteins not in the standard model of shell formation.

Keywords: Genome, Shell, Formation, Oyster, Protein.

I. INTRODUCTION

Molluscs have created exterior calcified structures to maintain their live tissues and defend themselves from predators since they are soft-bodied metazoans. These protective biominerals come in a variety of shapes and sizes. For the most basic 'worm-like' solenogaster and caudofoveate molluscs, they can be small spicules or scales, or calcareous plates in the case of polyplacophorans, such as the renowned living fossil chiton. In the majority of situations, however, they are actual hard protections, shells. Conchiferans, or shell-bearing mollusks, are the biggest category, since they contain the ancient monoplacophorans (Neopilina), bivalves (mussels, oysters, clams, scallops), scaphopods (tusk-shells), gastropods (snails), and cephalopods (snails) (Nautilus). In the marine, lacustrine, and terrestrial habitats, about 100 000 live mollusk species produce a shell. After coral mineralization, molluscan shell secretion is arguably one of the most prevalent and prolific biomineralization processes in the metazoan universe.

Molluscan shells have traditionally been utilised by humans for aesthetic purposes. Hundreds of punctured shells, initially stitched on their garments, were buried with the children of Grimaldi cave thirty thousand years ago. Several centuries ago, the Mayas implanted nacre pieces in jaws where teeth were missing. The nautilus shell was regarded a goldsmith's masterpiece in the 17th century when delicately set with gold and silver. Mother-of-pearl was commonly utilised for producing buttons and inlays in wooden furniture during the same time period till recently. D'Arcy Thompson, a notable naturalist, was inspired by the regular form of nautilus shells in the early twentieth century.

In general, the molluscan shell is composed of 95 percent calcium carbonate and 1–5% organic material. Under natural circumstances, CaCO3 occurs in several crystal polymorphs (e.g., calcite, aragonite, and vaterite) and is stacked in layers with a specific pattern to produce complex biomineral microstructures in molluscan shells. Scanning electron microscopy (SEM) investigations have shown more than 30 various biomineral microstructures of mollusk CaCO3, including nacre,

foliate, prismatic, cross lamellar, and homogeneous microstructure.

A typical molluscan shell (i.e., mussel, oyster, abalone, and nautilus shells) has a three-layered structure: the periostracum (a thin organic leathery layer) on the outside, and two calcified layers on the inside (the outer prismatic layer and the inner nacreous layer). Elongated calcitic crystals in the shape of prisms perpendicular to the periostracum make up the prismatic layer. The nacreous layer, also known as the inner glossy shell layer, is made up of aragonite crystals that are structured in a brick wall-like pattern. Nacre is one of the most intriguing mollusc shell microstructures because of its exceptionally high fracture resistance. Molluscan shell is made up of inorganic minerals (mostly CaCO3) and an organic matrix made up of proteins, peptides, lipids, and carbohydrates that is released by the mantle epithelium. Despite decades of research, the collaboration mechanism between these different components in generating a highly organised biomineralized shell remains unknown.

II. MATERIALS AND PROCEDURES

Animals

The genome of the Pacific oyster that was sequenced was created by four generations of brother–sister mating. Pro. Dennis Hedgecock of the University of Southern California provided her, and she was cultured in the tank at the Chinese Academy of Sciences' Institution of Oceanology (IOCAS). Two-year-old Pacific oysters with shell lengths of 9–12 cm was utilised to extract shell proteins, sequence the transcriptomes of seven organs, and conduct the shell damage experiment. They were bought from a farm in Weihai, China, and raised in the IOCAS aquarium.

Identification of shell protein genes

The oyster genome sequence provides a once-in-a-lifetime chance to research molluscan shell production. We extracted proteins from oyster shells, both soluble and insoluble, acquired peptidase sequences, and utilised them to discover 259 shell protein genes in the oyster genome in a prior work. The requirement for protein identification is that the unique peptides must be at least 6 amino acids long. Mascot software was used to search peptide sequences against the oyster protein collection. The protein is considered identified in the oyster shell only when the peptide matches a unique gene and the score is more than or equal to the mascot identity score.

The RNA-seq experiment procedure

Total RNA was extracted according to the manufacturer's instructions using the guanidinium thiocyanatephenol-chloroform extraction technique (Trizol, Invitrogen). Poly-A RNA was extracted from 20 mg total RNA of each sample using oligo-dT-coupled beads, then sheared, and the isolated RNA samples were utilised for first-strand cDNA synthesis using random hexamers and Superscript II reverse transcriptase (Invitrogen). E. coli DNA PolI was used to make the second strand (Invitrogen). Qiaquick PCR purification kit was used to purify double-stranded cDNA (Qiagen, Germantown, MD). The cDNA was ligated to Illumina paired-end adaptor oligo mix and size chosen to roughly 200 bp fragments by gel purification after end repair and inclusion of a 3' dA overhang. The libraries were sequenced using the Illumina sequencing technology and the paired-end sequencing module after 15 PCR cycles.

Shell protein genes are distributed throughout the body.

The transcriptomes of seven organs, including the mantle, digestive gland, gill, adductor muscle, hemolymph, labial palps, female and male gonad, were obtained using 90 bp paired-end RNA-seq from one person (excluding the male gonad, which was obtained from another individual). Based on their RPKM values, the gene expression levels of shell proteins were evaluated in several tissues (reads per kilobase of gene model per million mapped reads). In each organ, the number of expressed shell protein genes (at least RPKM. 5) was counted.

Shell matrix proteins GO enrichment analysis

We used EnrichPipeline to identify GO concepts that were abundant in shell proteins based on functional annotation using Gene Ontology (GO).

Treatment for shell damage

Six participants were assigned to each of the eight groups, which included one control group and seven treatment groups that suffered shell damage for 1, 3, 5, 9, 13, 17, and 21 days. To create shell damage, a section of the left side of the shell was carefully removed without injuring the mantle using an electric drill with a flat sanding head.

Left and right mantle gene expression profiles

The left and right mantles of each oyster were harvested on each sampling day, and their total RNAs were

extracted separately. The total RNAs from the left mantles of six people in the same group were mixed evenly to make a sample for 49 bp single-end RNA-seq analysis, and the right mantles were treated the same way. In samples obtained on each of the seven sampling days, the number of upregulated and downregulated genes in the right and left mantles relative to the control were tallied.

Construction of a novel shell formation model

Based on a combination of the organ distribution of shell proteins, the functional features of shell proteins, the pathways likely involved in shell creation, and some published studies, a new model for shell formation was presented.

III. DISCUSSION AND FINDINGS

Shell protein genes are found in several organs.

According to the conventional paradigm of shell formation, the mantle was the primary organ engaged in shell creation (Fig. 1). However, majority of the discovered shell protein genes were expressed extensively across all organs (Fig. 2), suggesting that most shell proteins had a multi-organ origin. The 27 genes that were not expressed in the mantle (RPKM, 5 was classified as unexpressed) had at least twofold expression in other organs (RPKM. 5), suggesting that certain shell proteins are generated by organs other than the mantle. Furthermore, SMP genes not expressed in the mantle or hemolymph exhibited at least twofold expression in other organs (RPKM. 5), suggesting that shell proteins might be generated by organs other than the mantle and hemolymph. More crucially, the finding ruled out the possibility of hemocytes being dispersed in other organs, resulting in shell protein dispersion throughout numerous organs.

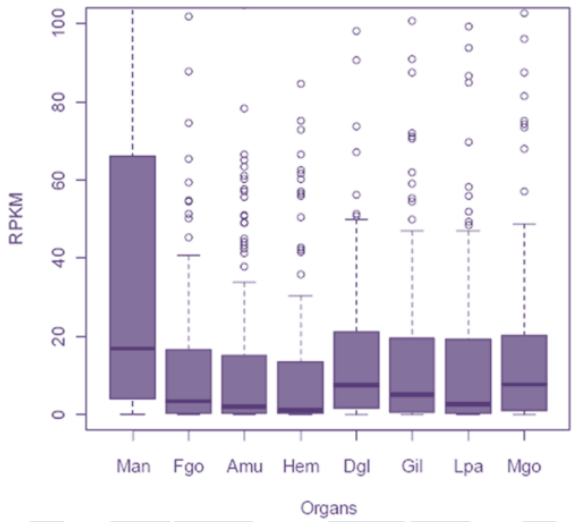

Figure 1. The gene expression level of shell proteins in the mantle was the highest among all organs.

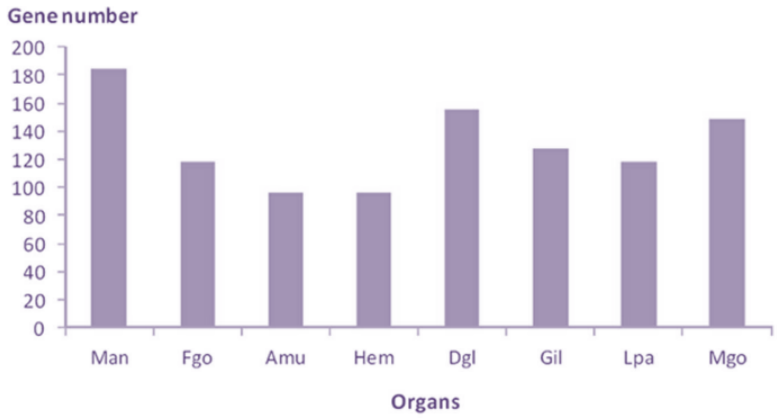

Figure 2. The number of expressed shell protein genes in the eight organs

Osteopontin, a fundamental component of the mineralized extracellular matrix of bones and teeth, is produced by a variety of tissue types. Many tissues have also been reported to have a 48 kDa shell matrix protein. Our findings further reveal that in species with mineralized tissues, biomineralization proteins may be

found in several organs. Shell protein genes discovered in other organs but not the mantle or hemolymph demonstrated that these shell proteins are transported to the shell development front via distinct pathways.

The GO enrichment of shell proteins suggests shell remodelling.

Because chitin is one of the shell organic framework's major components, several of the GO enrichment results for oyster shell proteins are considered to be connected to the deconstruction and creation of the shell organic framework, such as chitin binding and chitin metabolic process. As a result, a rebuilding process comparable to bone remodelling may exist during shell formation. During this process, some components of the old shell are catabolized and rebuilt in order to connect with the new shell as it forms. The Eastern oyster shell has been observed remodelling its inorganic component, calcite crystals, and shell proteins are assumed to be responsible for this. As a consequence, the prevalence of GO keywords related to chitin binding and metabolism in shell proteins indicated that the organic structure of the shell could be regenerated during shell formation, guaranteeing a close linkage between the old and new shell. The occurrence of shell disintegration in mollusks showed the presence of shell remodelling. The GO enrichment data might give molecular evidence for the shell disintegration event.

Table 1. GO enrichment results for oyster shell proteins

GO_ID	GO_Term	GO_Class	P-value
GO:0009308	amine metabolic process	BP	0.000149
GO:0006022	aminoglycan metabolic process	BP	7.26E-07
GO:0030246	carbohydrate binding	MF	3.77E-05
GO:0005975	carbohydrate metabolic process	BP	5.98E-06
GO:0008061	chitin binding	MF	8.90E-06
GO:0006030	chitin metabolic process	BP	1.98E-06
GO:0004866	endopeptidase inhibitor activity	MF	6.69E-07
GO:0030414	peptidase inhibitor activity	MF	5.83E-10
GO:0004867	serine-type endopeptidase inhibitor activity	MF	1.83E-05
GO:0030234	enzyme regulator activity	MF	5.22E-06
GO:0005576	extracellular region	CC	6.02E-08

The discovery of genes involved in shell formation

In samples obtained on each of the seven sampling days, the number of upregulated and downregulated genes in the right and left mantles relative to the control right and left mantles were tallied (P,1023). We chose genes associated with shell damage that were elevated in the left mantle on at least six of the seven sample days. This strategy was based on the assumption that the number of upregulated genes on one sampling date should be the highest, while those on all seven sampling dates should be the lowest, and that if the expression of genes was not affected by shell damage, the decrease should follow a gradual random distribution. The numbers of genes in the right mantle were distributed randomly, as predicted, and the distribution of downregulated genes in the left mantle was equally random (Fig. 3). The very high number of upregulated genes on the six sample dates was most likely due to shell damage, indicating that the findings were reliable. As a result, the genes associated to shell formation that were elevated

on 6/7 sampling dates in the left mantle were chosen.

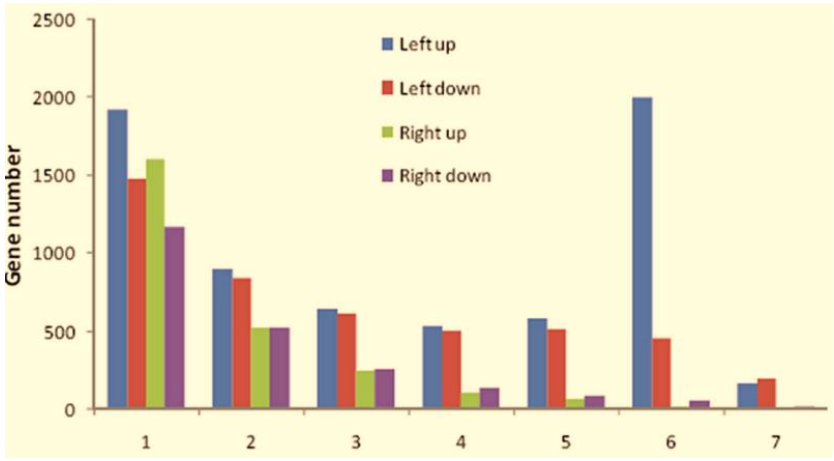

Figure 3. On each of the seven sample days, the number of upregulated and downregulated genes in the right and left mantles were compared to the control right and left mantles.

Furthermore, only the left mantle responded to injury to the left shell, suggesting that the left and right mantles are functionally independent, at least in terms of their biomineralization function, while being connected as a whole.

IV. CONCLUSION

The presence of complex life processes during shell formation is suggested by our novel findings, which include the multiple-organ origin of shell proteins, the probable proteins related to shell remodelling, the diverse functions of genes related to shell formation, and the possible pathways for shell protein transport. Many physiological experiments need be conducted in the future to prove the immature model given in this work.

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