

Chapter 42

Gene Expression Patterns in the Mantle and Pearl Sac Tissues of the Pearl Oyster *Pinctada fucata*



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Abstract The shell of pearl oysters consists of two distinct layers, nacre and prismatic. Mantle is the tissue involved in the shell formation, and its ventral part (mantle edge) forms the prismatic layers, whereas the dorsal part (pallium) forms the nacre. In pearl culture, mantle grafts from the pallium of donor are transplanted into the recipient. Then pearl sac is formed by proliferation of epithelial cells from the grafted mantle to form pearls. It has been reported that gene expression patterns are different between mantle edge and pallium in accordance with their distinct functions in the shell formation. However, it is not well addressed whether gene expression is identical or not between two nacre-forming tissues, pallium and pearl sac. Here, we examined expression patterns of known genes related to nacre and prismatic layer formation in mantle edge, pallium, and pearl sac of *Pinctada fucata*. Although the pallium and pearl sac have the same function in terms of nacre formation, various genes were not expressed identically to the respective tissues, suggesting that shell matrix proteins differently function in the formation of shell nacre and pearls.

Keywords *Pinctada fucata* · Pearl · Shell nacre · Prismatic layer · Gene expression · RNA-seq

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42.1 Introduction

The shell of pearl oysters consists of two distinct layers: inner nacre and outer prismatic layers composed of aragonite and calcite crystals, respectively. The forming processes of these different shell layers are thought to be regulated by proteins secreted from epithelial cells in mantle tissues. The ventral part of the mantle (mantle edge) forms the prismatic layers, whereas the dorsal part (pallium) forms the nacreous layers. These two regions secrete different repertoire of shell matrix proteins. In pearl culture, mantle grafts from the pallium region of donor oyster are transplanted with spherical nuclei into the recipient oysters. Pearl sac is formed by proliferation of mantle epithelial cells originating from the mantle graft from which various proteins are secreted to form the nacre surrounding nuclei.

Pearl consists of nacre or “mother of pearl” and is formed inside the body of pearl oysters; thus the nature of shell nacre and pearls is considered to be identical. On the other hand, our previous study clearly showed differences in the expression levels of several shell matrix protein genes between pallium and pearl sac (Wang et al. 2009). Inoue et al. (2010) investigated the expression of six shell matrix protein genes and showed that some of them were not expressed identically in pearl sac and mantle center (nacre-forming region), although a significant correlation was observed in their expression patterns between the two tissues. To analyze molecular mechanisms underlying the shell and pearl formation, it is important to examine whether gene expression patterns are different or not between pearl sac and mantle. Here, we compared expression pattern of known genes related to nacre (nacreous genes) and prismatic layer formation (prismatic genes) in mantle edge, pallium, and pearl sac of pearl oyster *Pinctada fucata* by using our previous RNA-seq data (Kinoshita et al. 2011).

42.2 Materials and Methods

42.2.1 Sample Preparation

Mantle and pearl sac tissues were collected from four individuals of *P. fucata* maintained at the Mikimoto Pearl Research Laboratory, Mie, Japan. Mantle pieces were grafted to all individuals for pearling 5 months before sampling. The mantle edge and pallium regions were separated from the mantle. Pearl sacs were collected from gonad of pearl oysters, and contaminated recipient tissues were carefully trimmed. All tissues, pallium, mantle edge, and pearl sacs used in this study were collected at the same time.

42.2.2 RNA-Seq Analysis

mRNAs were purified from tissues. 3'-fragment sequencing was performed using the GS FLX 454 system (Kinoshita et al. 2011). After quality trimming of raw reads, a de novo assembly using MIRA assembler ver. 2.9.45x1 and the BLAST Clust program from NCBI was used to assemble the reads. Known nacreous and prismatic gene sequences were searched from assembled contig data set using the local blastn and tblastn algorithms. Expression levels of gene sequences were expressed by transcripts per million (TPM). Hierarchical cluster analysis was performed by CLUSTER3.0 using Euclidean distance.

42.3 Results and Discussion

42.3.1 Clustering of Expression Patterns in Three Shell-Formation Tissues for the Known Nacreous and Prismatic Genes

Although many studies have identified genes and proteins that are related to nacre and prismatic layer formation, the information on their functions has been limited. Among the genes previously reported, we selected 10 nacreous and 14 prismatic genes (Table 42.1). Based on the expression patterns of these nacreous and prismatic genes, hierarchical clustering analysis among three tissues, mantle edge, pallium, and pearl sac, was performed. As shown in Fig. 42.1, pallium and pearl sac were clustered in the same node and separated from the mantle edge, well in accordance with their functions in the nacre formation or prismatic layer formation.

42.3.2 Expression of Known Nacreous and Prismatic Genes

Most nacreous genes examined in this study were expressed predominantly in the pallium (Table 42.1). Among them, MSI60, MSI25, and Pif177 showed the highest expression in the pallium, suggesting their importance in the nacre formation. In contrast, two nacreous genes, *ACCBP* and *CaLP*, were expressed in the mantle edge more than in the pallium, suggesting their additional roles in the prismatic layer formation. On the other hand, N66, PFMG1, and N19 family members were detected only in pallium or pearl sac.

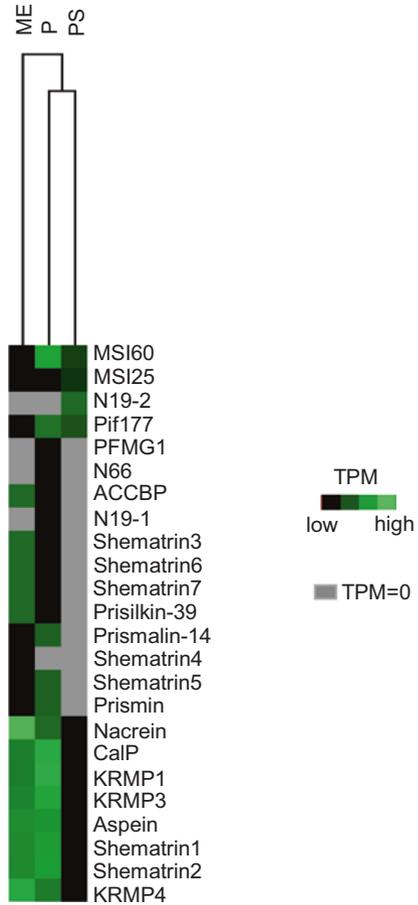
Table 42.1 The expression levels of known nacreous and prismatic genes

	TPM		
	ME	P	PS
Nacreous genes			
<i>MSI60</i>	131	2115	259
<i>MSI25</i>	29	406	120
<i>Pif177</i>	44	872	37
<i>Nacrein</i>	1019	2623	56
<i>N66</i>	0	36	0
<i>PFMG1</i>	0	36	0
<i>ACCBP</i>	146	96	0
<i>CalP</i>	553	466	56
<i>N19-1</i>	0	48	0
<i>N19-2</i>	0	0	28
Prismatic genes			
<i>Aspein</i>	4062	4146	28
<i>Prismalin-14</i>	1252	1577	0
<i>KRMP1</i>	3217	1972	13
<i>KRMP3</i>	3494	2844	65
<i>KRMP4</i>	1907	2629	194
<i>Prismin</i>	3669	3967	0
<i>Prisilkin-39</i>	641	442	0
<i>Shematin1</i>	1456	1386	93
<i>Shematin2</i>	4833	4672	1351
<i>Shematin3</i>	379	36	0
<i>Shematin4</i>	29	0	0
<i>Shematin5</i>	408	1517	0
<i>Shematin6</i>	4906	4756	0
<i>Shematin7</i>	903	370	0

TPM transcripts per million, ME mantle edge, P pallium, PS pearl sac

Meanwhile, the expression levels of most known prismatic genes did not differ between the pallium and mantle edge (Table 42.1). Shematin5 was expressed in the pallium much greater than in the mantle edge. These prismatic genes may also play roles in the nacreous layer formation. This is consistent with our previous report that knockdown of prismatic genes affects the shell nacre formation in *P. fucata* (Funabara et al. 2014). Jackson et al. (2010) also showed that prismatic genes such as shematrins and KRMPs are important in the formation of the shell nacre in *P. maxima*. These prismatic genes except for shematin2 were marginally detected in pearl sacs (Table 42.1).

Fig. 42.1 Hierarchical clustering of expression patterns in three shell-formation tissues for known nacreous and prismatic genes. *ME* mantle edge, *P* pallium, *PS* pearl sac. Explanation about color is needed



Although the pallium and pearl sac have the same function in terms of nacre formation, our data indicate that the expression patterns of various nacreous genes are not identical, though similar to each other, between the two tissues. In addition, most prismatic genes analyzed in this study showed high expression levels in both mantle edge and pallium but only marginally in pearl sac. One possibility is that contaminating tissues surrounding the pearl sac decreased the expression of shell formation-related genes in our pearl sac preparation. Nevertheless our data suggest different composition of shell matrix proteins between the shell nacre and pearls, and the importance of the gene expression analysis in the pearl sac to address molecular mechanisms underlying the pearl formation.

References

- Funabara D, Ohmori F, Kinoshita S, Koyama H, Mizutani S, Ota A, Osakabe Y, Nagai K, Maeyama K, Okamoto K, Kanoh S, Asakawa S, Watabe S (2014) Novel genes participating in the formation of prismatic and nacreous layers in the pearl oyster as revealed by their tissue distribution and RNA interference knockdown. *PLoS One* 9:e84706
- Inoue N, Ishibashi R, Ishikawa T, Atsumi T, Akoki H, Komaru A (2010) Gene expression patterns and pearl formation in the Japanese pearl oyster (*Pinctada fucata*): a comparison of gene expression patterns between the pearl sac and mantle tissue. *Aquaculture* 308:68–74
- Jackson DJ, McDougall C, Woodcroft B, Moase P, Rose RA, Kube M, Reinhardt R, Rokhsar DS, Montagnani C, Joubert C, Piquemal D, Degnan BM (2010) Parallel evolution of nacre building gene sites in molluscs. *Mol Biol Evol* 27:591–608
- Kinoshita S, Wang N, Inoue H, Maeyama K, Okamoto K, Nagai K, Kondo H, Hirono I, Asakawa S, Watabe S (2011) Deep sequencing of ESTs from nacreous and prismatic layer producing tissues and a screen for novel shell formation-related genes in the pearl oyster. *PLoS One* 6:e21238
- Wang N, Kinoshita S, Riho C, Maeyama K, Nagai K, Watabe S (2009) Quantitative expression analysis of nacreous shell matrix protein genes in the process of pearl biogenesis. *Comp Biochem Physiol B Biochem Mol Biol* 154:346–350

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