Cloning and characterization of the shell matrix protein Shematrin in scallop

Ya Lin, Ganchu Jia, Guangrui Xu, Jingtian Su, Liping Xie, Xiaoli Hu, and Rongqing Zhang

Institute of Marine Biotechnology, School of Life Sciences, Tsinghua University, Beijing 100084, China
Protein Science Laboratory of the Ministry of Education, Tsinghua University, Beijing 100084, China
Key Laboratory of Marine Genetics and Breeding (MGB), Ministry of Education, College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China
*Correspondence address. Tel/Fax: +86-10-62772899; E-mail: lpxie@mail.tsinghua.edu.cn

The Shematrin family is unique to the organic matrices of pearl oyster shells, containing repetitive, low-complexity domains designated as XGnX (where X is a hydrophobic amino acid). Current studies suggested that Shematrins are framework proteins in the prismatic layer of Pinctada fucata; however, the exact function of Shematrin during shell formation is unclear. In this study, we cloned and characterized Shematrin, a protein highly homologous to Shematrin-2, from the mantle tissue of scallop (Chlamys farreri). Semi-quantitative reverse transcript polymerase chain reaction analysis showed that Shematrin is exclusively expressed in the mantle. Knocking down the expression of Shematrin in adult scallops via double-stranded RNA injection led to an abnormal folia surface. After the shell was notched, the expression level of Shematrin remarkably increased and then gradually decreased, suggesting that Shematrin is critically involved in the shell repair process. Injection of Shematrin double-stranded RNA reduced the speed of shell regeneration and caused abnormal surface morphology of the regenerated shell. The RNA interference and shell notching experiments indicated that Shematrin plays a key role in the folia formation of C. farreri. Structure prediction showed that Shematrin may be an intrinsically disordered protein, with high flexibility and elasticity of the molecular conformation, which facilitate binding multiple protein partners. Based on the structure features, we hypothesized that Shematrin may participate in framework organization via binding with several specific acidic proteins, functioning as a molecular hub in the protein interaction networks.

Keywords: Shematrin; shell; framework; Chlamys farreri; folia

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Matrix protein Shematrin in scallop Chlamys farreri

Materials and Methods

Materials

The scallops (C. farreri) were collected and transported from Taiping Corner (Yellow Sea, Qingdao, China). The animals were cultured in aerated artificial seawater (Sude instant sea salt, 3%) at a temperature of 20°C. The experiments started after the animals had acclimatized to our laboratory conditions for a week or more.

Total RNA extraction

Total RNA was extracted from the mantle tissue of C. farreri following the instructions of the TRIzol reagent (Invitrogen, Carlsbad, USA).

Rapid amplification of cDNA ends

RACE was performed using a SMARTer™ RACE cDNA amplification kit (Clontech, Mountain View, USA). The gene-specific primers for 5'-RACE (gene-specific primer race-5' and the nested primer race-5'N, Table 1) and 3'-RACE (gene-specific primer race-3' and the nested primer race-3'N, Table 1) were designed based on the nucleotide sequences of the two fragments identified from the tBLASTn search between the Shematrin family and C. farreri mantle transcriptome datasets. Both the 5'-RACE and 3'-RACE products were cloned into pEASY-T3 vector and sequenced. The full-length sequence was confirmed using the primers Full-1 and Full-2 (Table 1) and high fidelity Probest™ DNA Polymerase (Takara, Dalian, China).

Bioinformatics analysis

The entire nucleotide sequence was analyzed using the BLAST program at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). Primer Premier 5.0 (PRIMER Biosoft International, Palo Alto, USA) was used to identify the encoded protein. SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP-3.0/) predicted the signal peptide. Phyre² (http://www.sbg.bio.ic.ac.uk/phyre2/) predicted the secondary structure and the tertiary structure.

Semi-quantitative RT-PCR

Using the methods described above, total RNA was extracted from the mantle, foot, gonad, viscus, gill, and adductor muscles of adult C. farreri. Equal quantities (2 μg) of total RNA from different tissues were reverse-transcribed into first strand cDNA (as the template) using Quant Reverse Transcriptase (Tiangen, Beijing, China), following the manufacturer’s instructions. RT-PCR was conducted using the primer pairs RT-1/RT-2 (Table 1) and β-actin-1/β-actin-2 (Table 1) to amplify Shematrin and β-actin (as a positive control for DNA template) gene fragments, respectively. To
avoid false-positive results and cross-contamination of different samples, a negative control was conducted in the absence of cDNA template. All PCR products were subcloned and confirmed by sequencing.

### RNAi experiments and real-time quantitative PCR

The RNAi assay was performed as described in Suzuki et al. [20], with some modifications. Primer pairs RNAi-1 and RNAi-2 (Table 1) were used to amplify a specific fragment from the cDNA template. The vector pEGFP-C1 (Clontech) was used as the template to amplify a green fluorescent protein (GFP) coding sequence to construct a control double-stranded RNA (dsRNA). A Wizard PCR PrepDNA purification system (Promega, Beijing, China) was used to purify the PCR products, which were then used as the template to synthesize dsRNA following instructions of the RiboMAXTM large scale RNA production system (T7) kit (Promega). RNase-free DNase I (Takara) was used to digest any residual DNA in the dsRNA samples. A UV-spectrophotometer (UV-9000) was used to detect the purity and OD260/OD280 value of 1.8–2.0 were reserved and then diluted to 40 μg/100 μl or 80 μg/100 μl using phosphate buffered saline. The dsRNAs were injected into the adductor muscle of adult individuals with a shell length of 45–55 mm. Five individuals were used for each treatment. Total RNA from the mantle tissue of each scallop was extracted 6 days after injection and then reverse-transcribed into cDNA, as described in ‘semi-quantitative RT-PCR’.

Real-time quantitative PCR was used to quantify the expression level of Shematrin, where β-actin was used as the internal reference. The quantitative PCR was performed using the Mx3000PTM (Stratagene, Palo Alto, USA) with an SYBR Premix Ex Taq™ II kit (Takara), according to the manufacturer’s instructions (for primer details see Table 1). The cycling parameters were 95°C for 30 s (1 cycle), followed by 95°C for 5 s, 55°C for 30 s, and 72°C for 45 s (40 cycles). Dissociation curves were analyzed to determine the purity of the product and the specificity of amplification. The relative expression levels were calculated using the 2^{-ΔΔCt} method [21] and the expression level of the phosphate buffered saline group was normalized to a relative value of 1.0 as the control. The experiment was repeated three times, with the data presented as mean ± standard deviation. Significant differences between different groups were analyzed by Paired-samples T test (SPSS 19.0).

### Scanning electron microscopy observation and Fourier transform infrared spectroscopy detection of the shell

The microstructure of normal shells and the shells from the dsRNA-injected group were studied using a scanning electron microscopy (SEM). First, the internal soft tissues of the shell were cleared away, and the shells were allowed to dry naturally after washing with distilled water thoroughly. The shell was cut into pieces of <3 mm by mechanical means, and then coated with gold-palladium by using the ion sputtering system. SEM (FEI Quanta 200) was used to observe the microstructures of the samples. The SEM observation showed that the microstructure of the inner surface corresponding to the adductor differs from the other parts; therefore, powdered samples were scraped from the inner surface corresponding to the adductor and the other parts of the shell, respectively. The samples were milled into powders with a particle size of ~150 μm. Fourier transform infrared (FT-IR) spectroscopy (Nicolet 6700FTIR) was used to identify the phase composition of the samples.

### Shell notching experiments

The shell notching experiments were performed according to the method described in Mount et al. [22], with some

### Table 1. Sequences of designed primers

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>race-5'</td>
<td>5'‐CCGCCACCTAAACACCCACCTCCCAA‐3'</td>
</tr>
<tr>
<td>race-5'N</td>
<td>5'‐GCGAGTCCATAACCGACATTCCG‐3'</td>
</tr>
<tr>
<td>race-3'</td>
<td>5'‐CCCATACCTTACGGGACGACCTGC‐3'</td>
</tr>
<tr>
<td>race-3'N</td>
<td>5'‐GGGGACGTGCTGTTGTCAGTGC‐3'</td>
</tr>
<tr>
<td>Full-1</td>
<td>5'‐TAAAGAAGTACCCATCCACA‐3'</td>
</tr>
<tr>
<td>Full-2</td>
<td>5'‐GGCAAATCCCATAACATCAA‐3'</td>
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<tr>
<td>RT-1</td>
<td>5'‐GGAGGACTTATCTCGTTGGA‐3'</td>
</tr>
<tr>
<td>RT-2</td>
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</tr>
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</tr>
<tr>
<td>RNAi-2</td>
<td>5'‐GCCTATACGACTCATATAGGGAGAACCATAACCTCCTCCCAA‐3'</td>
</tr>
</tbody>
</table>
modifications. A flat notch was cut on the shell margin of adult individuals with a shell length of 45–55 mm (Fig. 1A), and then the shell-injured scallops were randomly divided into ten groups, each group containing three individuals. The eight groups were returned to seawater tanks in an aquarium at 20°C for 0, 6, 12, 18, 24, 36, 48, 72, 96, and 120 h, respectively, and were then sacrificed. An \( \approx 3 \)-cm\(^2\) area of the mantle tissue around the cut was separated. Total RNA from the separated mantle tissue of each individual was extracted and reverse transcribed into cDNA. Real-time quantitative PCR was performed to quantify the expression level of Shematrin at different time points after shell injury, with \( \beta \)-actin as the internal reference. The average expression level of the 0 h group individuals was normalized to a relative value of 1.0 as the control. The RNA extraction and real-time quantitative PCR experiments were conducted as described in ‘real-time quantitative PCR’.

About 20 days after shell notching, newly formed very thin shell could be observed with the naked eyes (Fig. 1B). The shell regeneration speeds in scallops subject to various times of Shematrin dsRNA treatment were measured. Fifty adult scallops were subjected to shell notching and then randomly separated into five groups, each group containing 10 individuals: group 0 was subjected to no treatment for normal regeneration speed determination; group 1 was subjected to 40 \( \mu \)g dsRNA injection on the first day after shell notching; group 2 was subjected to 40 \( \mu \)g dsRNA injection on the first and seventh day after shell notching; group 3 was subjected to 40 \( \mu \)g dsRNA injection on the 1st, 7th, and 13th day after shell notching; group 4 was subjected to 40 \( \mu \)g dsRNA injection on the 1st, 7th, 13th, and 19th day after shell notching. Thirty days after shell notching, the length of the regenerated shell lamella was measured at the longest point using a vernier caliper (Fig. 1B). Some of their shells were collected for SEM observation.

Results

cDNA cloning and sequence analysis

After RACE cloning based on the DNA fragments identified by tBLASTn searching between \( P. \ fucata \) Shematrin family and \( C. \ farreri \) mantle transcriptome datasets, we obtained a 1372-bp transcript including a 5'-untranslated region of 57 bp, an open reading frame of 1182 bp (encoding a deduced 393 amino acid protein) and a 3'-untranslated region of 133 bp (GenBank\(^{\text{TM}}\) accession number KF419188) (Fig. 2). The deduced mature protein had a calculated molecular mass of 35.3 kDa, a pI of 9.51, a signal peptide and was referred to as Shematrin. A BLASTx search against the GenBank\(^{\text{TM}}\) database indicated that the amino sequence of Shematrin was 100% identical to that of \( P. \ fucata \) Shematrin-2 (GenBank\(^{\text{TM}}\) accession number AB244420), suggesting that Shematrin-2 may be a highly conserved protein among different species.

Gene expression analysis

To preliminarily explore the function of Shematrin in \( C. \ farreri \), the mRNA expression pattern of Shematrin was examined via semi-quantitative RT-PCR. The RT-PCR result showed that the Shematrin was exclusively expressed in the mantle (Fig. 3). Combined with the result of signal peptide prediction, it suggested that Shematrin was synthesized in the mantle and secreted into the shell to exert its function.

Microstructural changes in shell after in vivo silencing of Shematrin

An RNAi assay was conducted to further explore the role of Shematrin in shell formation. Compared with the control group, the Shematrin expression levels decreased to \( \approx 45\%\)
in the 40 μg injected group and to ~30% in the 80 μg injected group 6 days after treatment. In contrast, the expression level of Shematrin was not affected by injection of the GFP dsRNA (Fig. 4).

To detect the effect of Shematrin on shell formation, we compared the microstructures of the shells between the GFP-injected group and Shematrin dsRNA-injected group. SEM observation showed that the normal shell of *C. farreri* comprises two kinds of microstructures: the inner surface corresponding to the adductor presents the prismatic structure, while the other part of the shell presents the foliated structure (Supplementary Fig. S1). FT-IR spectroscopy showed that the foliated layer comprises calcite crystals while the prismatic layer comprises aragonite crystals (Supplementary Fig. S2). The shell microstructure and phase composition was very similar to that of scallop *Patinopecten yessoensis* [23].

Compared with the normal shell, obvious morphological changes on the inner surface of the foliated layer were observed in the Shematrin dsRNA-injected group. Figure 5A,B shows the inner surface of a normal shell: blade-like elongated parallel laths are arranged side by side, forming overlapping sheets, rather like tiles on a roof. The terminal end of the lath has a

Figure 2. cDNA sequence and deduced amino acid sequence of Shematrin from the scallop *Chlamys farreri* (GenBank™ accession number KF419188) The start codon and stop codon are boxed; an asterisk at the end of the amino acid sequence also indicates the stop codon. The nucleotide sequence contains one complete open reading frame encoding a protein of 393 amino acids. Shading marks the putative site of the signal peptide.

![Matrix protein Shematrin in scallop Chlamys farreri](https://academic.oup.com/abbs/article-abstract/46/8/709/1712)
Matrix protein Shematrin in scallop Chlamys farreri

Shematrin expression after shell notching
To detect the response of Shematrin during the shell regeneration process, the expression level of Shematrin was assayed at each time point during shell regeneration after shell notching. As shown in Fig. 6, the expression level remarkably increased after the shell was notched and reached its highest point (about 13 times of the normal level) 12 h after shell injury. The expression level then gradually decreased to a stable value, ~1.6 times of the normal level. Thus, the expression level of Shematrin increased rapidly at the initial stage of shell regeneration and retained at a relatively high level during the regeneration process. This changing trend of expression level indicated that Shematrin is essential for shell regeneration.

Inhibitory effect of Shematrin during shell regeneration process
Figure 7 shows the lengths of the new formed shell lamella from five groups with different times of Shematrin dsRNA-injection, 30 days after shell notching, thus the average speed of shell regeneration could be calculated respectively. The normal regeneration speed of the shell was ~0.13 mm/day, while the regeneration speed reduced to ~0.11 mm/day in group 1, which was treated once with Shematrin dsRNA-injection. The regeneration speed reduced respectively to 0.09, 0.07, and 0.06 mm/day in groups 2, 3, and 4 respectively, which corresponded to two, three, and four Shematrin dsRNA injections every 6 days. This result showed that the more times Shematrin was silenced in vivo, the more severe the inhibition of the regeneration process, indicating that Shematrin plays a significant role in shell regeneration. To detect the effect of Shematrin on shell regeneration, we compared the microstructure of the inner surface between the normally regenerated shell and the regenerated shell from scallops injected with Shematrin dsRNA. The SEM images of the normally regenerated shell from group 0 were shown in Fig. 8A,B. All the SEM images from the four groups treated with Shematrin dsRNA-injection showed varying degrees of abnormal microstructures, among which the degree of abnormality of group 4 was the most significant. The normally regenerated shell presented the typical foliated structure growth pattern with blade-like elongated parallel crystals arranged side by side, forming overlapping sheets (Fig. 8A), and a clear multiple-layer structure was observed from the side edge (Fig. 8B). For group 4 individuals, the terminal free edges of the laths were strongly deformed, similar to those observed in the inner surface of normal shells treated with Shematrin dsRNA injection (Figs. 5C-F and 8C). In addition, the side edge of the regenerated shells from group 4 failed to form the multiple-layer structure, and instead, different layers seemed to stick together and fused to a whole piece (Fig. 8D).

Secondary and tertiary structure prediction
The RNAi and shell notching experiments indicated that Shematrin is critically involved in the folia formation in C. farreri. To further discover Shematrin’s function, we performed secondary and tertiary structure predictions.

Figure 3. Tissue-specific gene expression of Shematrin by RT-PCR analysis Total RNA extracted from mantle (Man), gill (Gill), foot (Foot), Visceral mass (Vis), adductor (Add), and gonad (Gon) was used for RT-PCR. RT-PCR without a template was used as a negative control (Control). The housekeeping gene β-actin was used as a positive control.

Figure 4. RNAi decreased the expression levels of Shematrin For the control, the expression levels of the phosphate buffered saline (PBS)-injected group were set at a relative value of 1.0. The star represents a significant difference compared with the PBS-injected group (P < 0.001).

Figure 5A-E. Inhibitory effect of Shematrin during shell regeneration process (A) Normal shell with normally regenerated shell from group 0, (B) Shell notching (C) Normal shell showing the lengths of the new formed shell lamella, (D) Shell notching and treated with PBS injection, (E) Shell notching and treated with Shematrin dsRNA injection. The regeneration speed could be calculated respectively from five groups with different times of Shematrin dsRNA injection, 30 days after shell notching, thus the average speed of shell regeneration could be calculated respectively. The normal regeneration speed of the shell was ~0.13 mm/day, while the regeneration speed reduced to ~0.11 mm/day in group 1, which was treated once with Shematrin dsRNA-injection. The regeneration speed reduced respectively to 0.09, 0.07, and 0.06 mm/day in groups 2, 3, and 4, respectively, which corresponded to two, three, and four Shematrin dsRNA injections every 6 days. This result showed that the more times Shematrin was silenced in vivo, the more severe the inhibition of the regeneration process, indicating that Shematrin plays a significant role in shell regeneration. To detect the effect of Shematrin on shell regeneration, we compared the microstructure of the inner surface between the normally regenerated shell and the regenerated shell from scallops injected with Shematrin dsRNA.

Figure 6. Inhibitory effect of Shematrin during shell regeneration process. The normal regeneration speed of the shell was ~0.13 mm/day, while the regeneration speed reduced to ~0.11 mm/day in group 1, which was treated once with Shematrin dsRNA-injection. The regeneration speed reduced respectively to 0.09, 0.07, and 0.06 mm/day in groups 2, 3, and 4, respectively, which corresponded to two, three, and four Shematrin dsRNA injections every 6 days. This result showed that the more times Shematrin was silenced in vivo, the more severe the inhibition of the regeneration process, indicating that Shematrin plays a significant role in shell regeneration. To detect the effect of Shematrin on shell regeneration, we compared the microstructure of the inner surface between the normally regenerated shell and the regenerated shell from scallops injected with Shematrin dsRNA. The SEM images of the normally regenerated shell from group 0 were shown in Fig. 8A,B. All the SEM images from the four groups treated with Shematrin dsRNA-injection showed varying degrees of abnormal microstructures, among which the degree of abnormality of group 4 was the most significant. The normally regenerated shell presented the typical foliated structure growth pattern with blade-like elongated parallel crystals arranged side by side, forming overlapping sheets (Fig. 8A), and a clear multiple-layer structure was observed from the side edge (Fig. 8B). For group 4 individuals, the terminal free edges of the laths were strongly deformed, similar to those observed in the inner surface of normal shells treated with Shematrin dsRNA injection (Figs. 5C-F and 8C). In addition, the side edge of the regenerated shells from group 4 failed to form the multiple-layer structure, and instead, different layers seemed to stick together and fused to a whole piece (Fig. 8D).

Secondary and tertiary structure prediction
The RNAi and shell notching experiments indicated that Shematrin is critically involved in the folia formation in C. farreri. To further discover Shematrin’s function, we performed secondary and tertiary structure predictions.
The secondary structure prediction showed that Shematrin has an exceptionally low proportion of α-helixes and β-sheets, but a very high proportion of disordered loops (Fig. 9). The abundant glycine repeats in Shematrin were assumed to form multiple ‘glycine loops’, as suggested in keratins, loricrins, and lustrin A [24,25]. The tertiary structure prediction showed that Shematrin mainly consists of disordered loops and exhibits no stable three-dimensional structure (Fig. 10). The tertiary structure prediction corresponding to the secondary structure prediction collectively indicated that Shematrin may be an intrinsically disordered protein (IDP) with no stable spatial structure. Many IDPs are promiscuous binders that interact with multiple partners and frequently function as hubs in protein interaction networks [26]. We hypothesized that Shematrin may function as a molecular hub during construction of the organic framework, thus playing a key role in shell formation.
In this study, RACE based on two previously identified Shematrin-2-like sequences produced a full-length cDNA of Shematrin from the mantle issue of *C. farreri*. The deduced amino sequence of Shematrin gene was 100% identical to that of Shematrin-2 gene from *P. fucata*. Considering that the matrix proteins reported previously are usually diverse in different species, this uncommonly high similarity of these two molecules was unexpected. The full-length cDNA sequence of *C. farreri* Shematrin has been confirmed using cDNA templates from different scallop individuals. According to previous studies and our own work, we presume that Shematrin-2 (Shematrin) may be a highly conserved protein with particularly important functions. Although as many as seven molecular species of Shematrin were identified in *P. fucata*, Shematrin-2 was detected to be particularly abundant in the mantle by northern blotting, and only Shematrin-1 and Shematrin-2 were detected in the prismatic layer of *P. fucata* by MALDI-TOF/TOF MS analysis. Among the seven molecular species, Shematrin-1 and Shematrin-2 share significant homology over their entire length and are much more weakly homologous with the other members of the Shematrin family [11]. In *C. farreri*, only two DNA fragments homologous to Shematrin-2 were identified when using all the molecular species of Shematrin as queries to interrogate the mantle transcriptome datasets via tBLASTn [19]. In our study, we designed several primer pairs (these designed primers were able to amplify the other molecular species of Shematrin from *P. fucata*) based on the conserved sequences of the Shematrin family to identify further molecular species from the mantle tissue of *C. farreri*. However, we failed to amplify any, perhaps because of their low abundances in the cDNA template. We cannot rule out the existence of other molecular species of Shematrin in *C. farreri*; however, we are quite sure that Shematrin-2 is particularly highly expressed in the mantle, indicating its significant role in shell formation.

Unlike Shematrin-2, which was detected to be expressed slightly in the adductor muscle and highly in the mantle tissue [11], Shematrin was exclusively expressed in the mantle of *C. farreri*. This slight difference in the gene expression pattern between the two molluscan classes implied a difference in protein function or may reflect a difference of experiment accuracy, like the degree of RNA degradation in the total RNA extraction step. Combined with the result of signal peptide prediction, it seemed certain that Shematrin was synthesized in the mantle and secreted into the shell to exert its function.

The RNAi experiments were designed to detect the *in vivo* effect of Shematrin during shell formation. RNAi is a powerful reverse genetic tool that has been employed widely to silence gene expression in mammalian and human cells [27]. In biomiminar research, it is also an effective way to detect the *in vivo* function of matrix proteins, such as PfP, PfN23, and PfN44 [20,28,29]. To avoid the potential down-regulation of other Shematrin molecules by RNAi, the dsRNA fragment chosen was highly specific. In our study, the expression of Shematrin could be effectively knocked down in a dose-dependent way (Fig. 4) and the knock-down of Shematrin expression resulted in an abnormal morphology of the foliated structure (Fig. 5). The morphology of
the aragonite prismatic layer showed no changes, indicating that Shematrin functions only in the foliated layer in C. farreri.

Shell notching experiments were carried out to detect the \textit{in vivo} effect of Shematrin during shell repair process. The results showed that high levels of Shematrin were induced soon after the shell injury and the expression level of Shematrin then decreased gradually to a relatively stable value 50%–80% higher than normal level (Fig. 6). Similar to the normal shell formation, the procedure of shell repair starts with the deposition of a chitin matrix framework [30,31]. KRMP, another framework protein isolated in the prismatic layer in P. fucata, was also induced rapidly after shell injury [29]. The similar changes in expression levels of Shematrin and KRMP may provide evidence in support of their similarity in function during shell regeneration: accumulation in the shell to facilitate framework formation. The expression level of Shematrin decreased gradually after the rapid increase up to 12 h, implying negative feedback control of the expression of Shematrin in C. farreri. RNAi silencing of Shematrin slowed the speed of shell regeneration and the degree of decrease corresponded to the number of Shematrin dsRNA injections. In addition, these regenerated shells showed abnormal morphology under SEM observation (Fig. 8C,D). This abnormal morphology looked very similar to the inner surface of the normal shells treated with Shematrin dsRNA injection (Fig. 5C–F), as we expected.

The Shematrins were demonstrated to be shell framework proteins, containing high proportions of glycine repeats [11]. Previous studies showed that the structure framework was composed of a thin layer of β-chitin, sandwiched by layers of proteins adopting the antiparallel β-sheet conformation. Many acidic proteins, together with various polysaccharides, bind on the surface of the framework [32]. Several framework proteins identified from mollusks were predicted to possess a chitin-binding motif in a β-strand conformation, such as Prismalin-14 [8] and Prisilkin-39 [9]. However, in this study, the result of the secondary structure prediction implied that Shematrin might have no typical chitin-binding motif with a β-strand structure (Fig. 9). Sequence analysis revealed the existence of many glycine residues in Shematrin: glycine is notorious for its role in disrupting stable secondary structures, such as α-helices and β-sheets [24,33]. The abundant glycine loops may contribute to the elasticity and flexibility of the molecular conformation, thus facilitating

Figure 8. SEM images of the inner surface of the regenerated shells (A, B) SEM images of the inner surface of the regenerated shell from the individuals of group 0; (C, D) SEM images of the inner surface of the regenerated shell from the individuals of group 4.
protein binding [24,35]. The tertiary structure prediction corresponded with the secondary structure prediction collectively indicated that Shematrin may be an IDP with no stable spatial structure. It has been proved that the IDPs usually contain a one-to-many binding ability via disorder-to-order transition of molecule conformation. Backbone and side chain torsion angle rotations may be used to bring about the conformational changes needed to enable close fits between a single molecular and distinct partners [25,35]. In addition, Shematrin is a basic protein, with a pI value of 9.51. This basic nature, which is different from many other macromolecules isolated and characterized from various biominerals, combined its disordered structure, suggested that Shematrin may interact with several specific acidic macromolecules in the framework and function as a molecular hub in the protein interaction networks, thereby playing a key role in the framework construction process. If Shematrin functions as a molecular hub in protein interaction networks, the in vivo silencing of Shematrin may disrupt the normal organization of the framework, which is responsible for supporting and limiting the size, shape, and spatial orientation of crystal growth, resulting in abnormal morphology of the foliated structure, as observed in both RNAi and shell notchting experiments.

In conclusion, it is the first time that the shell framework protein Shematrin has been cloned from the mantle tissue of the scallop Chlamys farreri. Shematrin was proved to be significantly involved in the folia formation and shell regeneration process in C. farreri. On the basis of the structural characteristics of Shematrin, we hypothesized that Shematrin may participate in the organic framework construction by interacting with several specific acidic macromolecules, functioning as a molecular hub in the protein interaction networks.

Figure 9. Secondary structure prediction of Shematrin  Based on the protein sequence of Shematrin, the secondary structure prediction was performed. The amino acids are colored based on the physiochemical properties of the side chains. The regions adopting putative α-helix and β-sheet conformations are represented as green spiral and blue arrow, respectively. The degrees of confidence 0–9 are also indicated by a rainbow color gradient.

Figure 10. Three-dimensional structure prediction of Shematrin  The tertiary structure prediction of Shematrin was performed by Phyre2. The structure consists of a substantial portion of disordered loop regions and a small fractions ordered secondary structures of α-helix and β-sheet conformation. The left and right panels are related by a 90° rotation around a horizontal axis. The figure is prepared with PyMol [34].
Obviously, more studies at the protein level will help to further understand the function of Shematrin during shell formation.

**Supplementary Data**

Supplementary data is available at *ABBS* online.

**Funding**

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