Molecular cloning and functional characterization of *Hc-fibrillin* in shell biomineralisation of the triangle sail mussel *Hyriopsis cumingii*

Yongbin Yuan [a,c,1], Honghui Hu [a,c,1], Jinyan Zhong [a,c] , Ling Yan [a,c], Zhiyi Bai [a,b,c,*], Jiale Li [a,b,c,*]

[a] Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture and Rural Affairs, Shanghai Ocean University, Shanghai 201306, China

[b] Shanghai Engineering Research Center of Aquaculture, Shanghai Ocean University, Shanghai 201306, China

[c] Shanghai Collaborative Innovation Center for Cultivating Elite Breeds and Green-culture of Aquaculture animals, Shanghai Ocean University, Shanghai 201306, China

* Corresponding author at: Key Laboratory of Freshwater Aquatic Genetic Resources, Ministry of Agriculture and Rural Affairs, Shanghai Ocean University, Shanghai 201306, China.

Corresponding author. E-mail addresses: zybai@shou.edu.cn (Z. Bai), jlli2009@126.com (J. Li).

Abstract

Fibrillin(FBNs) are key relay molecules that form the skeleton of microfibrils, which integrate a biological network of structural and guidance information, play an important role in a variety of physiological processes, including osteoblast differentiation in higher animals. However, its biomineral function in invertebrates remains poorly understanding. In this study, a full-length cDNA of *Hc-fibrillin* from the triangle sail mussel *Hyriopsis cumingii* was obtained, and its function in shell formation was investigated. The full-length cDNA of *Hc-fibrillin* was 1952 bp with an open reading frame (ORF) of 1389 bp encoding apolypeptide of 462 amino acids, including eight EGF-CA domains. We also found high homology of *Hc-fibrillin* with *M.coruscus*, accounting for 44.32%, consistent to the most closed phylogeny with *M. coruscus*. The tissue expression pattern showed that *Hc-fibrillin* was expressed in all tested tissues, highly in the mantle and gill, suggesting its role in absorption and transport of calcium ion. In the shell repaired assay, there was a significantly higher expression of *Hc-fibrillin* in shell repair group from day 16 to day 25. After *Hc-fibrillin* was silenced, the shape of aragonite flakes in the pearl layer gradually changed to round, while the surface of the calcium carbonate crystals in the prismatic layer got rougher with great size differencr, and the organic matrix between the crystals appeared skeletonized, indicating the important function in biomineralization. In vitro calcium carbonate crystallization assay showed that in spite of calcite, fibrillin peptide can also induce calcium carbonate crystals to vaterite, probably suggesting the reason of low-luster pearl produced by freshwater mussels. The results provide new insights about the biomineralization in freshwater molluscs and lay a theoretical basic for pearl quality improvement.

Keywords: *Hyriopsis cumingii*, Biomineralization, Matrix protein, *Hc-fibrillin*

Materials and methods

1. The total RNA was extracted using Trizol method referred to the instruction of RNAiso Plus* (TaKaRa, Dalian, China).
2. *Hc-fibrillin* cloning and sequence analysis. Bioinformatics analysis for *Hc-fibrillin* was carried out by Bai's method(Bai, et al., 2017)
3. The *Hc-fibrillin* RNA silencing assay. The dsRNA was diluted to 60 μg/100 μL using RNase-free water, and 100 μL solutions were injected into the adductor of *H. cumingii*. and qRT-PCR were performed to analyze the silencing effect. Then the shell was washed and dried, with the nacre and prismatic layer of the shell were observed by scanning electron microscopy (SEM).

Results

1. Cloning and sequence analysis of Hc-fibrillin. The full-length of *Hc-fibrillin* was 1952 bp, with a ORF of 1389 bp, encoding 462 amino acids.
2. Tissue distribution of *Hc-fibrillin* and in situ hybridization

(Figure1)

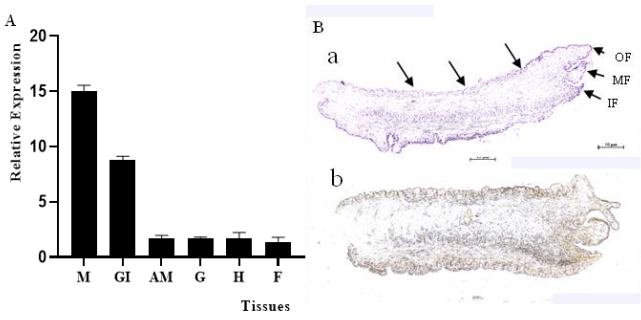

Figure 1 Tissue-specific expression of *Hc-fibrillin*. A: Real-time PCR; B: In situ hybridization anaylsis of *Hc-fibrillin*.

3. The expression analysis following silencing of *Hc-fibrillin* (Figure2). SEM results showed that the normal growth of nacre, while the disturbed aragonite flakes were irregular in shape, no longer hexagonal in shape, and gradually round. the normal prism layer of the calcium carbonate crystal after interference is rougher, with great size differences, and the organic matrix between the crystals appears hollow.

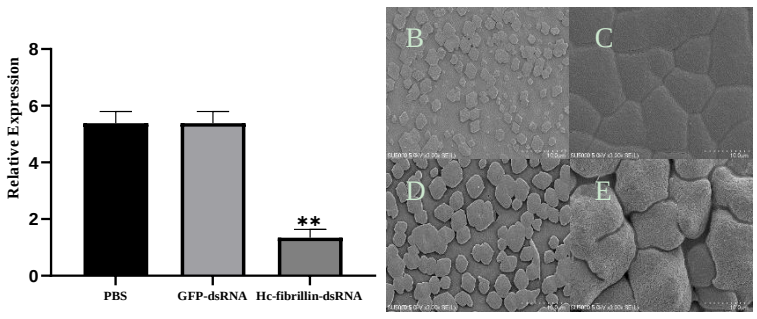

Figure 2 Effects of Hc-fibrillin silence by RNAi. B, C, D, and E show the shells at the box magnified 3000 times.

4. Calcium carbonate crystallization assay(Figure3). The spicules and spikes group showing typical shape of vaterite, it presented a radial needling shape.

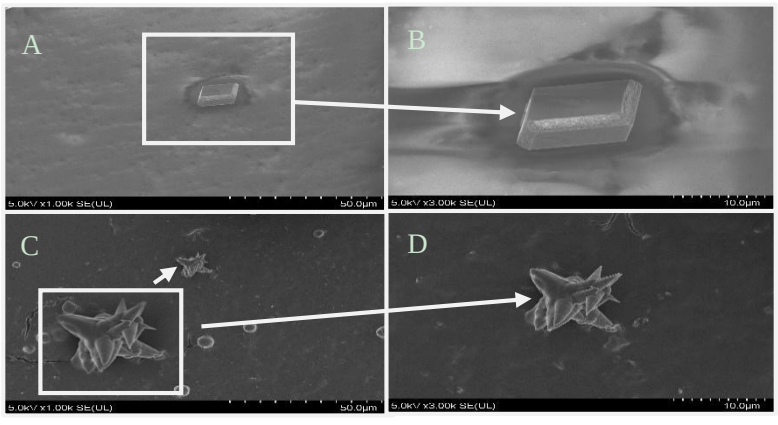

Figure 3 Result of in vitro crystallization experiments in the presence of Hc-fibrillin. A-D, SEM images of in vitro crystallization experiments.

Conclusion

In conclusion, *Hc-fibrillin* has been cloned, and the high expression of *Hc-fibrillin* in the mantle and gills suggests a role in mineralization. Both shell repair experiment and RNAi experiments have confirmed the involvement in shell biomineralization. And In vitro calcium carbonate crystallization assay, *Hc-fibrillin* polypeptide could induce the conversion of calcium carbonate crystals to calcite and vaterite, probably suggesting the reason of low-luster pearl produced by freshwater mussels.

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