Study of the processes of biomineralisation by global transcriptomic approaches and improvement of the pearl quality in the pearl oyster Pinctada margaritifera

Kevin MAGRÉ

7th Rendez-vous de Concarneau
October 16th 2015
1- Ifremer in French Polynesia

2- Ifremer Research contribution to pearl oyster farming
- Presentation of pearl oyster farming
- Transcriptomic analysis of mineralization mechanisms with the aim to increase superior pearl quality production
Joint Research Unit «Oceanian Island Ecosystem»
Analysis of the interactions between human and its environment
65 scientists / 4 research plateforms in Tahiti (French Polynesia)

42 PEOPLE
INCLUDING
6 RESEARCHER,
4 PHD STUDENTS
4 MASTERS STUDENTS
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LABORATORY OF EXCELLENCE «CORAL REEFS FACING GLOBAL CHANGE”

SOUTH PACIFIC INTEGRATED OBSERVATORY FOR ENVIRONMENT AND TERRESTRIAL AND MARINE BIODIVERSITY
Since 40 years, Ifremer research projects in French Polynesia answer to concerns for local development.

Emerging projects:
- Environment
- Marine renewable energy
- Biodiversity

Main partners:
- Fishery and pearl oyster offices
- University UPF, ILM, IRD, CNRS-EPHE

Diapositive M. Taquet
Pearl industry in French Polynesia

Pearl culture is a fundamental part of the Polynesian economy:
- largest export industry (60% ; ~ 70 million euros in 2010)
- employs 4000 people
- 400 pearl producers
- contributes to land management and reversal of migration from the atolls to Tahiti
- 80% of small farms (1-15 ha)

Major crises since 2001:
- uncontrolled development
- decline in pearl quality
- more and more competitive market
- disorganized commercialization
Overview of pearl oyster farming

The black lipped pearl oyster
Pinctada margaritifera
var. cumingii
First step: larvae collecting

Collectors are suspended at 6-8 meters under the sea to capture larvae and produced juveniles.
Pearl oysters growing

long line culture system of pearl oysters
Grafting

donor oyster

piece of mantle

graft

nucleus

pearl pouch

recipient oyster (3 years old)

Schéma : C. Montagnani
Pearl formation

Pearl sac epithelium development → ¾ days → Nacre deposition on nucleus → 45 days → pearl harvest → 18 months

Minimum nacre thickness 0,8mm
Ifremer’ researches in French Polynesia are organized in 3 axes

• (1) breeding families of graft donor oysters selected for their capacity to produce pearls of quality and/or particular colours and/or rapid growth

• (2) studying the grafting process and the mineralization mechanisms with the aim to increase superior pearl quality production

• (3) understanding bivalve larvae dispersal with the aim to optimize the spat collection strategy
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Impact of external factors on pearl quality


10 research laboratories with different expertise working together to study factors influencing pearl formation and quality
Impact of external factors on pearl quality

“Improve quality of pearl from French Polynesia”

10 research laboratories with different expertise working together to study factors influencing pearl formation and quality
Molecular tools to study pearl quality

Development of transcriptomic approach to study molecular mechanisms involved in biomineralization during pearl formation

mineralizing tissue

mineralizing cells
DNA genes and their regulation

graft (mantle)

pearl sac

Understand pearl formation, defects apparition and identify pearl quality and growth biomarkers
Transcriptomic program on mantle tissue

Pyrosequencing: 276,735 EST

12 pearl oysters

mantle

76,790 unique sequences
Mantle EST database

Homologies
- Annotation BlastX (nrdb NCBI)
  - 38% annotated sequences (29,479)
  - 44% unknown protein (13,064)

Function
- Annotation BlastX (GO)
  - 13% annotated sequences (10,004)
  - Cellular components (57%)
  - Biologic process (59%)
  - Molecular functions (68%)
    - Bindings (76%)
    - Catalytic activity (54%)

biomineralizing tissue profile
Research of genes encoding biomineralizing proteins in *P. margaritifera*

- 140 bivalve sequences
- 103 gasteropod sequences

BlastX

- 82 genes sequences encoding proteins potentially implicated in biomineralization in *P. margaritifera*

- 140 bivalve sequences
- 56 gasteropod sequences
Transcriptome and proteome analysis of *Pinctada margaritifera* calcifying mantle and shell: focus on biomineralization

Caroline Joubert, David Piquemal, Benjamin Marie, Laurent Manchon, Fabien Pierrat, Isabelle Zanella-Cléon, Nathalie Cochenneuc-Laureau, Yannick Gueguen, Caroline Montagnani

Abstract

**Background**: The shell of the pearl-producing bivalve *Pinctada margaritifera* is composed of an organic cell-free matrix that plays a key role in the dynamic process of biologically-controlled biomineralization. In order to increase genomic resources and identify shell matrix proteins implicated in biomineralization in *P. margaritifera*, high throughput Expressed Sequence Tag (EST) pyrosequencing was undertaken on the calcifying mantle, combined with a proteomic analysis of the shell.

**Results**: We report the functional analysis of 276 738 sequences, leading to the constitution of an unprecedented catalog of 82 *P. margaritifera* biomineralization-related mantle protein sequences. Components of the current "chitin-silk fibrin gel-acidic macromolecule" model of biomineralization processes were found, in particular a homolog of a biomineralization protein (Pifi-177) recently discovered in *P. fucata*. Among these sequences, we could show the localization of two other biomineralization protein transcripts, *pmarg-aspein* and *pmarg-pealin*, in two distinct areas of the outer mantle epithelium, suggesting their implication in calcite and aragonite formation. Finally, by combining the EST approach with a proteomic mass spectrometry analysis of proteins isolated from the *P. margaritifera* shell organic matrix, we demonstrated the presence of 30 sequences containing almost all of the shell proteins that have been previously described from shell matrix protein analyses of the *Pinctada* genus. The integration of these two methods allowed the global composition of biomineralizing tissue and calcified structures to be examined in tandem for the first time.

**Conclusions**: This EST study made on the calcifying tissue of *P. margaritifera* is the first description of pyrosequencing on a pearl-producing bivalve species. Our results provide direct evidence that our EST data set covers most of the diversity of the matrix protein of *P. margaritifera* shell, but also that the mantle transcripts encode proteins present in *P. margaritifera* shell, hence demonstrating their implication in shell formation. Combining transcriptomic and proteomic approaches is therefore a powerful way to identify proteins involved in biomineralization. Data generated in this study supply the most comprehensive list of biomineralization-related sequences presently available among protostomian species, and represent a major breakthrough in the field of molluskan biomineralization.
Proteomic approach: in parallel with transcriptomic approach

1. Proteins from nacre or prisms
   - Enzymatic digestion + LC-MS/MS analysis
   - Observed MS/MS spectra

2. Mantle ESTs
   - 76,790 sequences (putative proteins)
     - In silico enzymatic digestion + in silico MS/MS analysis
     - Theoretical MS/MS spectra

3. Comparison using MASCOT: MS/MS (observed vs theoretical)

4. Identification of mRNA encoding protein specific of nacre, prims and first deposit around nucleus
Proteomic approach enable us to identify matrix protein associated with mineralized shell and pearl.

- Shell prisms
- Shell nacre
- Pearl nacre
- First deposit on the nucleus

Shematrin
MNP88 protein
Prismalin-14
KRMP-7 et KRMP-11
Tyrosinase-like protein 1
Proteomic approach: identification of matrix protein associated with mineralized shell and pearl

Shell prisms
- Shematrin
- MNP88 protein
- Prismalin-14
- KRMP-7 et KRMP-11
- Tyrosinase-like protein 1

Shell nacre

Pearl nacre
- N66
- Pmarg-Pearlin
- MSI60, MRNP-34
- Linkine

First deposit on the nucleus
Proteomic approach: identification of matrix protein associated with mineralized shell and pearl

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- Linkine

First deposit on the nucleus
- N19
- Mantle protein 12

✓ Majoritary proteins of organic matrix are specific of each structure
Proteomic approach: identification of matrix protein associated with mineralized shell and pearl

- **Shell prisms**: Shematrin, MNP88 protein, Prisinalin-14, KRMP-7 et KRMP-11, Tyrosinase-like protein 1, Mantle protein 10
- **Shell nacre**: N66, $Pmarg$-Pearlin, MSI60, MRNP-34, Linkine, PIF 177
- **Pearl nacre**: N19, Mantle protein 12
- **First deposit on the nucleus**: Shem 8

- **Majority proteins of organic matrix are specific of each structure**
- **Some organic matrix proteins are common between structure**
Proteomic approach: identification of matrix protein associated with mineralized shell and pearl

- **Shell prisms**
  - Shematrin
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  - Mantle protein 10

- **Shell nacre**

- **Pearl nacre**
  - N66
  - Pmarg-Pearlin
  - MSI60, MRNP-34
  - Linkine
  - PIF 177

- **First deposit on the nucleus**
  - N19 Mantle protein 12
  - Shem 8

- **Majority proteins of organic matrix are specific of each structure**
- **Some organic matrix proteins are common between structure**
- **Shell and pearl nacre matrix proteins are common**
Different secretory repertoires control the biomineralization processes of prism and nacre deposition of the pearl oyster shell

Benjamin Marie\textsuperscript{a,b,1,2}, Caroline Joubert\textsuperscript{a}, Alexandre Tayalé\textsuperscript{a}, Isabelle Zanella-Cléon\textsuperscript{c}, Corinne Belliard\textsuperscript{a}, David Piquemal\textsuperscript{d}, Nathalie Cochennec-Laureau\textsuperscript{a,b}, Frédéric Marin\textsuperscript{b}, Yannick Guéguen\textsuperscript{a}, and Caroline Montagnani\textsuperscript{a,b,2}

\textsuperscript{a}Ifremer, Centre de Recherche du Pacifique, Unité Mixte de Recherche 241 Écosystèmes Insulaires Océaniens, Papeete, French Polynesia; \textsuperscript{b}Centre National de la Recherche Scientifique, Biogéosciences, Université de Bourgogne, 21000 Dijon, France; \textsuperscript{c}École Normale Supérieure, 34184 Montpellier, France; \textsuperscript{d}IFREMER, Station de la Trinité sur Mer, 56470 La Trinité-sur-Mer, France; \textsuperscript{1}Ifremer, Unité Mixte de Recherche 5119 Écologie des Systèmes Marin Côtiers, Université Montpellier II, 34095 Montpellier, France

Edited\textsuperscript{a} by Andrew H. Knoll, Harvard University, Cambridge, MA, and approved October 31, 2012 (received for review July 9, 2012)

Mollusca evolutionary success can be attributed partly to their efficiency to sustain and protect their soft body with an external biomineralized structure, the shell. Current knowledge of the protein set responsible for the formation of the shell microstructural polymorphism and unique properties remains largely patchy. In 	extit{Pinctada margaritifera} and 	extit{Pinctada maxima}, we identified 80 shell matrix proteins, among which 66 are entirely unique. This is the only description of the whole “biomineralization toolkit” of the matrices that, at least in part, is thought to regulate the formation of the prismatic and nacreous shell layers in the pearl oysters. We unambiguously demonstrate that prisms and nacre are assembled from very different protein repertoires. This suggests that these layers do not derive from each other.

The pearl oyster is characterized by an extremely high fracture resistance, accompanied by a higher ductility. Hence, the external layer rather constitutes a primary barrier, whereas nacre dissipates energy and stops cracks (11–13). Complex environmental selection pressures (biotic, abiotic) may have favored the appearance and maintenance of such structures (14, 15). However, the origin of both prisms and nacre remains enigmatic (16, 17). Even more elusive are the molecular processes involved in prisms and nacre deposition and the identification of the “molecular toolkit” required for the emergence of these microstructures from liquid/colloidal precursors.

To identify the proteinaceous “actors” that contribute to generate prisms and nacre, we performed a high-throughput comparison of the occluded shell protein repertoire—at transcript and protein levels—expressed during the deposition of these two calcified layers in the Polynesian pearl oyster 	extit{Pinctada margaritifera}. Our data provide strong evidence that the proteinaceous matrices associated with prism and nacre are extremely different. This observation supports the hypothesis that biomineralization is driven by protein-mediated processes.
Understanding the process of biomineralization by a global transcriptomic approach

Develop biomarker for «pearl growth and quality»
Selection of graft donor pearl oyster

The color, the size and the quality are determined by the donor oyster

Each set of pearls is coming from the same DONOR pearl oyster
Selection of pearl growth and quality candidate biomarkers

Global transcriptomic approaches
mineralizing tissues

- graft
- pearl pouch
- mantle

Digital Gene Expression

Identify genes differentially expressed in terms of functional areas associated with the quality

Candidate biomarkers

92

EST Databank

Identify genes encoding proteins involved in the biomineralization process

63

Annotation

proteomics approach
mineralized structures

- shell
- pearl

MS/MS databank

Identify proteins composing shell and pearl

113

Annotation

268 candidate biomarkers of pearl growth and quality were selected
Biomarkers identification and validation

Donor oyster

(1) Transcriptomic approaches provide \( \sim 110000 \) « genes from mineralizing tissue to analyze »

(2) 188 of the 268 candidates biomarkers were selected

(3) Experimental graft to validate biomarkers
Correlate biomarker expression ......
To pearl quality and/or growth

Experimental grafts

graft 
2000 grafting 
Graft sampling before the graft

100 samples/conditions tested on about 200 candidate biomarker

High scale expression analyses using Fluidigm (=20,000 analysis at the individual level)
Identify groups of donors pearl oysters among a population with high potential mineralizing with the set of 42 biomarkers.

42 BIOMARKERS ABLE TO PREDICT AFTER PEARL QUALITY 18 MONTHS LATER

To obtain high quality pearls 18 months later.
PATENT: SIGNATURE PREDICTIVE OF THE BIOMINERALIZATION CAPACITY OF A GRAFT-DONOR PEARL OYSTER

Title:
(EN) SIGNATURE PREDICTIVE OF THE BIOMINERALIZATION CAPACITY OF A GRAFT-DONOR PEARL OYSTER
(FR) SIGNATURE PREDICTIVE DE LA CAPACITÉ DE BIOMINÉRALISATION D'UNE HUITRE PERLIÈRE DONNEUSE DE GREFFONS

Abstract:
The present invention relates to a signature predictive of the biominalization capacity of a graft-donor pearl oyster, said signature comprising the expression profile of at least one biomarker comprising or consisting of a nucleotide sequence selected from SEQ ID NO 1 to SEQ ID NO 42, variants thereof and fragments thereof. Another subject of the invention is a graft-donor pearl oyster with a high biominalization capacity, characterized in that it has a predictive signature according to the invention. The invention also relates to a biomarker predictive of the biominalization capacity of a graft-donor pearl oyster, said biomarker being selected from the list consisting of SEQ ID NO 1 to SEQ ID NO 42.

Pub. No.: WO2014096688
International Application No.: PCT/FR2013/053144
Publication Date: 26.06.2014
International Filing Date: 17.12.2013
IPC: C12Q 1/08 (2006.01)
Inventors: JOUBERT, Caroline (CH), TAYALE, Alexandre (CH), MONTAGNANI, Caroleine (FR), SAULNIER, Denise (FR), GUEGUEN, Yannick (FR), PIQUEMAL, David (FR)
Agent: ICOSA, 83 avenue Dufert-Rochereau F-75014 Paris (FR)
Priority Data: 1262140 17.12.2012 FR

Increase knowledge on the molecular mechanisms involved in biomineralization during pearl formation in *P. margaritifera*.

Gene and proteins of mineralization

Identification of biomarker predictive of the biomineralization capacity of a graft donor pearl.
Thank you for your attention!
Merci de votre attention!
Mauruuru roa i te farereira’a!