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Hen uterine gene expression profiling during eggshell formation reveals putative proteins involved in the shell mineralization

Aurélien Brionne, Yves Nys, Christelle Hennequet-Antier, Joël Gautron

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INRA, « Function and regulation of egg proteins »

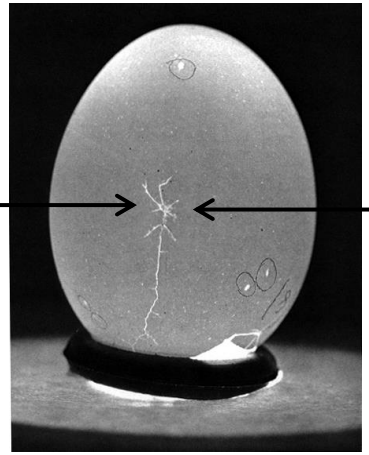
UR83 Recherches Avicoles, 37380 Nouzilly, France

The chicken eggshell

Natural envelope to ensure physical defence of egg

- *Protects the developing embryo*
- *Ensures that table eggs remains free of pathogens*

Cracks



Bacterial penetration

Eggshell mineralization in uterus

Microarrays in order to characterize gene products involved in shell mineralisation

Brionne *et al. BMC Genomics* 2014, **15**:220
http://www.biomedcentral.com/1471-2164/15/220

BMC Genomics

RESEARCH ARTICLE Open Access

Hen uterine gene expression profiling during eggshell formation reveals putative proteins involved in the supply of minerals or in the shell mineralization process

Aurélien Brionne, Yves Nys, Christelle Hennequet-Antier and Joël Gautron*

Supply of minerals for shell mineralization

Brionne et al., poster P235

95% of $CaCO_3$ (calcite)

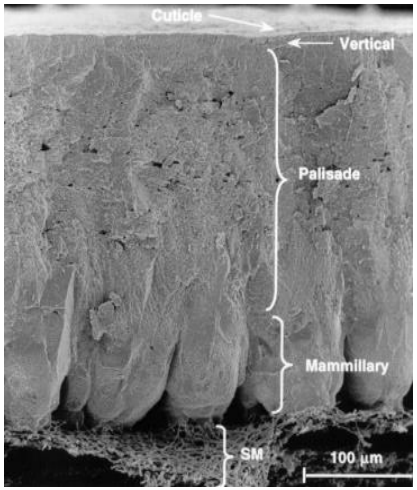
↑

Interaction

↓

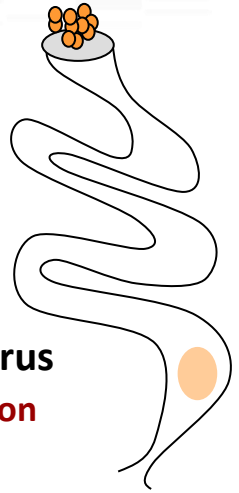
3.5% of organic matrix (proteins, proteoglycans)

Control of the calcification process



Eggshell mineralization in uterus

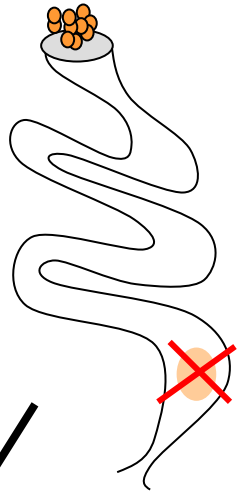
Microarrays in order to characterize gene products involved in shell mineralisation



Egg in uterus

Calcification

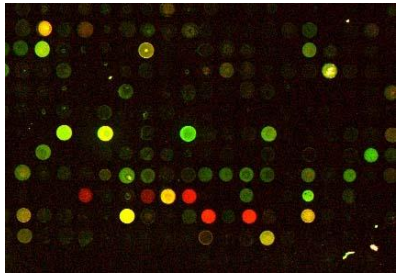
Stimulation of genes in presence or absence of egg



Expelled eggs

No calcification

Collection of uterine tissues
(16 hens)



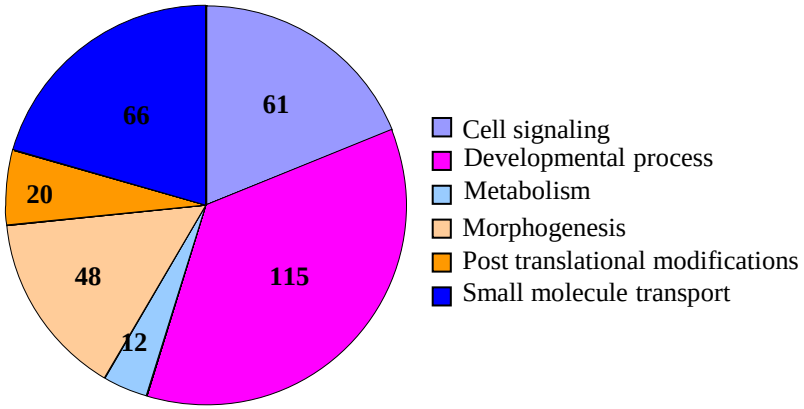
Eggshell mineralization in uterus

Microarrays in order to characterize gene products involved in shell mineralisation

302 Over-expressed genes during shell calcification

↓

Gene Ontology (GO) term enrichment analysis



The most over-represented proteins are related to reproductive functions
(163 Go parent terms, Morphogenesis, developmental process..)

→ Proteins potentially involved in shell mineralization ?

Proteins potentially involved in shell mineralization

Determination of secreted proteins

302 Proteins over-expressed by uterus during shell calcification

Intracellular
(Uterine metabolism)

Secreted to be functionally involved in eggshell deposition

Looking for Signal Peptide
(Short peptide sequence necessary to a protein to be secreted)

Non-classical way of secretion *(Yloc)*

Removal of transmembrane proteins

→ **Identification of 58 secreted proteins**

Potential roles in eggshell calcification? → Functional annotations

Functional Roles of secreted proteins

❑ Proteins associated to mineralization

✓Proteins involved in the **biomineralization** of bone tissue

- ***Osteopontin****, secreted phosphoprotein involved in bone mineralisation and chicken eggshell*
- ***Proenkephalin, Collagen, Decorin, SPARCL1,*** *Extracellular bone matrix proteins*

✓ **Calcium binding proteins (CaBPs)** interact with calcium

- ***SPARCL1, CD34, CRELD2, MCFD2, MATN2, SLIT2,*** *proteins with EF-hand and EGF-like calcium binding domains*

✓ **Proteoglycans** and proteoglycan binding proteins (negative charge to attract calcium)

- **HS6ST2** Heparan sulfate
- **EXT1, MG4T4B**, Glycosaminoglycans biosynthesis
- **TSKU,** Proteoglycan protein interaction
- **Glypican-1, ADAMTS1**

Functional Roles of secreted proteins

❑ Molecular chaperones

- ✓ Molecular chaperones are proteins that assist the non-covalent folding or unfolding and the assembly or disassembly of other macromolecular structures

- ✓ Proteins involved in the **proper folding of the eggshell matrix** to ensure template to the mineralized structure

- ✓ **Regulation of the activity of proteins** related to the shell deposit
- *Shell mineralisation in a non cellular milieu*
- *Molecular chaperone interact with proteins driving mineralisation*

6 Heat shock proteins
Clusterin, widely represented in secretory fluids
Ovocalyxin-21, eggshell specific protein

Functional Roles of secreted proteins

❑ Antimicrobial proteins

- ✓ The egg is formed in the lumen of oviduct.
- ✓ Many antimicrobial proteins are secreted in the lumen to keep the egg free of microbes

- ✓ 7 antimicrobial proteins identified in this study
  - 2 LBP/BPI plunc familly proteins (***OCX-36, BPIL3***)
    Binds to the lipolysaccharide (LPS) cell wall of the gram negative bacteria (death of bacteria)
  - **Pleiotrophin,** heparin binding protein
    Cluster (s) of exposed positives charges to interact with bacterial lipopolysaccharide petidoglycan
  - **Protein C,** Trypsin-like serin proteases
    Antimicrobial activities already demonstrated in other species
  - 3 molecules involved in immune response (**Lymphocyte antigen 86, Sema immunoglobulin domain, LOC422316)**

Conclusions-Perspectives

- Comparison of global gene expression in presence or absence of shell formation reveal 302 candidate proteins
  - Supply of minerals necessary to shel formation
  - Related to shell mineralization

- Determination of 58 proteins secreted in the uterine fluid and potentially involved in :
  - shell calcification
  - the regulation of activity of proteins driving mineralisation
  - The antimicrobial protection

Further physiological and genetics quantitative studies

↓

Identification of the prominent molecules controlling shell formation

Acknowledgments

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IMPACT (2013-2017)
(Identification of Matrix Proteins Affecting Calcite Texture in chicken and guinea fowls eggshells)

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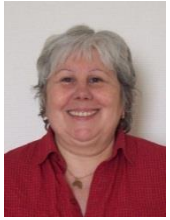
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SABRE
CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

SABRE
(2006-2010)