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

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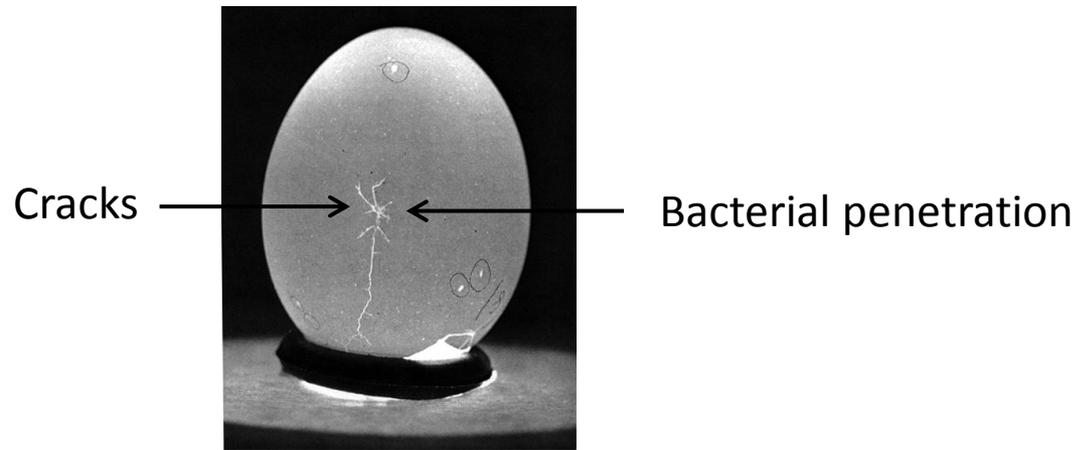
joel.gautron@tours.inra.fr

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*



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>



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

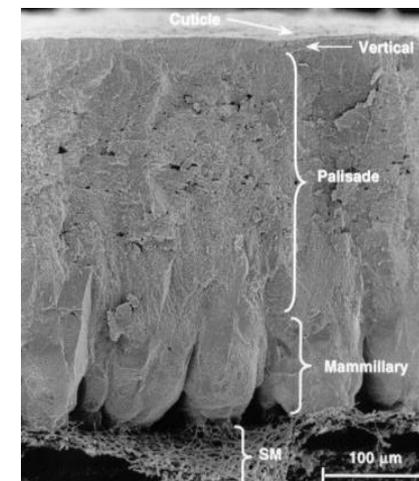
**Brionne et al.,
poster P235**

95% of CaCO_3 (calcite)

↑
Interaction

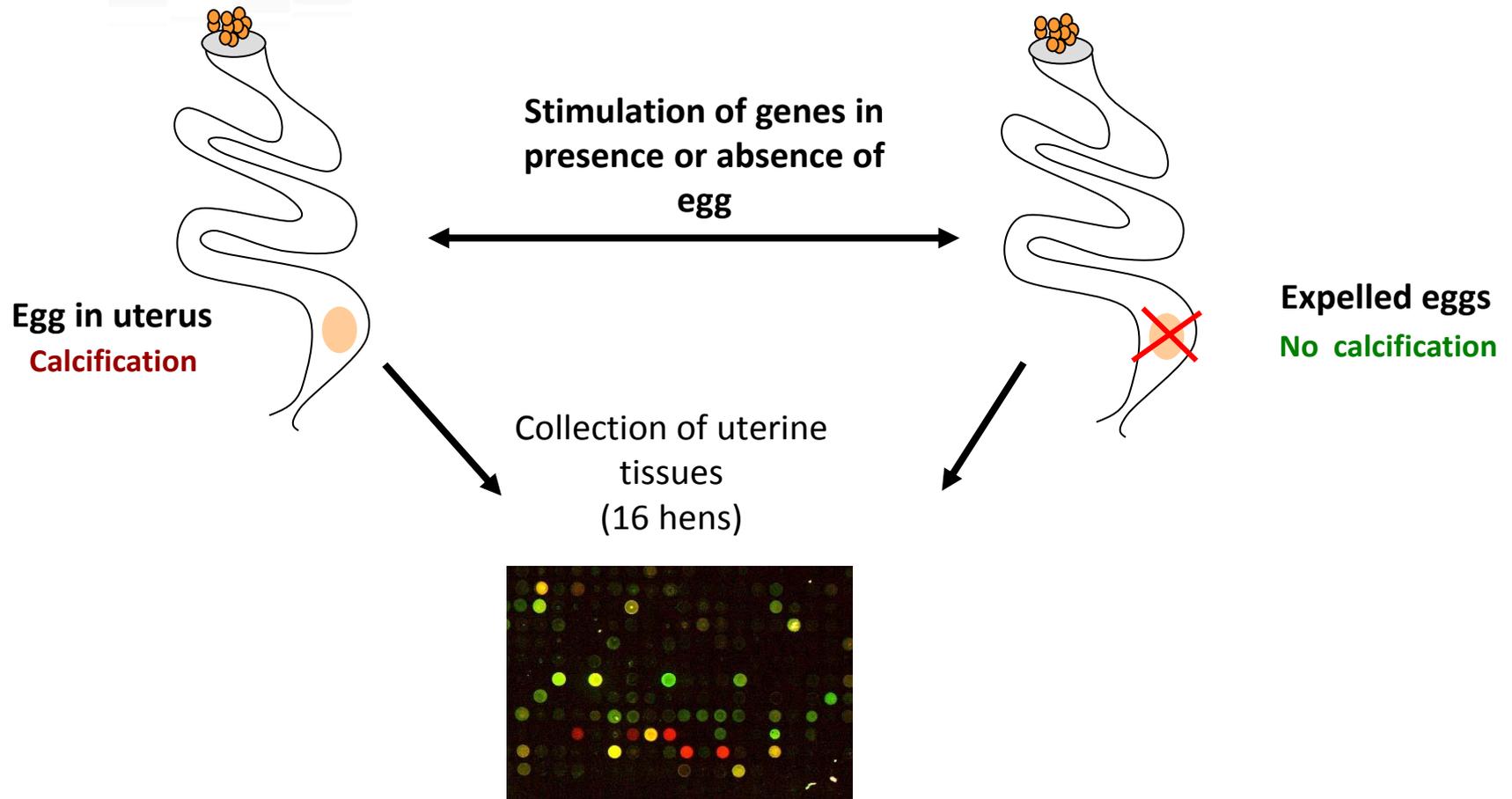
Control of
the
calcification
process

↓
3.5% of organic matrix (proteins,
proteoglycans)



Eggshell mineralization in uterus

Microarrays in order to characterize gene products involved in shell mineralisation



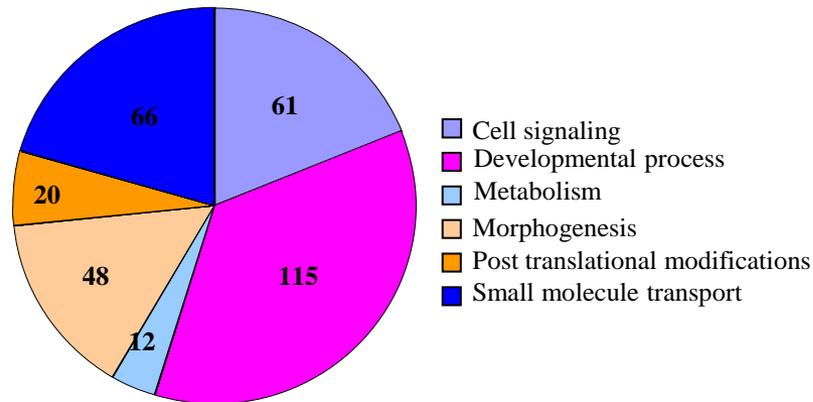
Eggshell mineralization in uterus

Microarrays in order to characterize gene products involved in shell mineralisation

302 Over-expressed genes during shell calcification



Gene Ontology (GO) term enrichment analysis

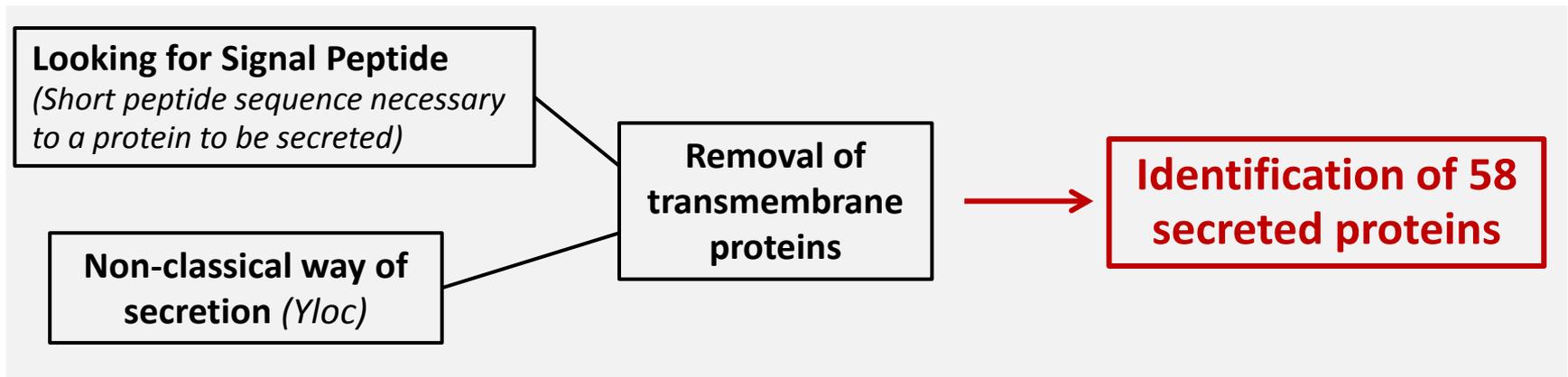
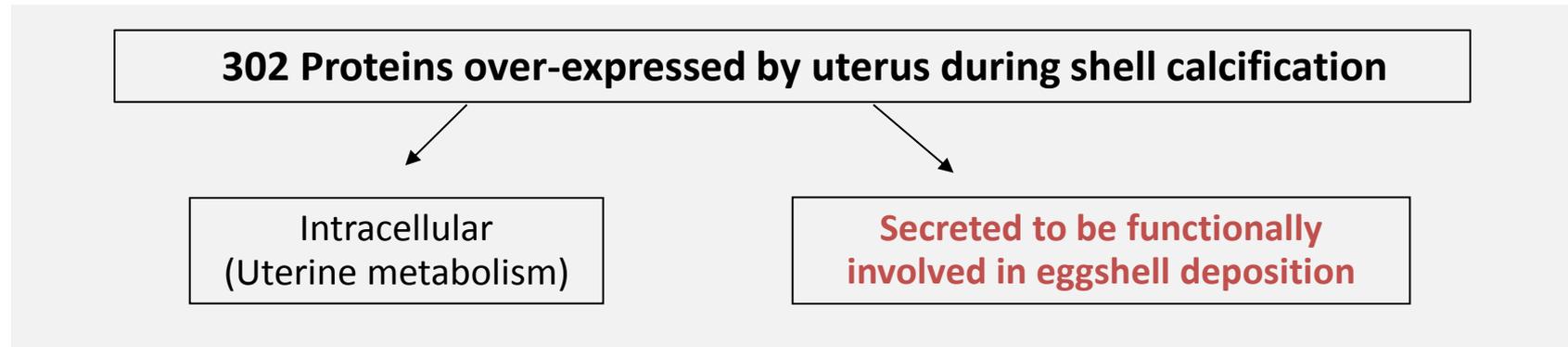


The most over-represented proteins are related to reproductive functions (163 Go parent terms, Morphogenesis, developmental process..)

→ Proteins potentially involved in shell mineralization ?

Proteins potentially involved in shell mineralization

Determination of secreted proteins



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 family proteins (**OCX-36, BPIL3**)
Binds to the lipopolysaccharide (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 shell 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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