Impact
(2013-2017)

Quantitative proteomics provides new insight into chicken eggshell matrix protein functions during pivotal stages of shell mineralization

Joël Gautron, P. Marie (phD)

A. **Brionne, C. Hennequet-Antier, Y. Nys**

INRA, « Function and regulation of egg proteins »

UR83 Recherches Avicoles, 37380 Nouzilly, France

B. **Rodriguez-Navarro**

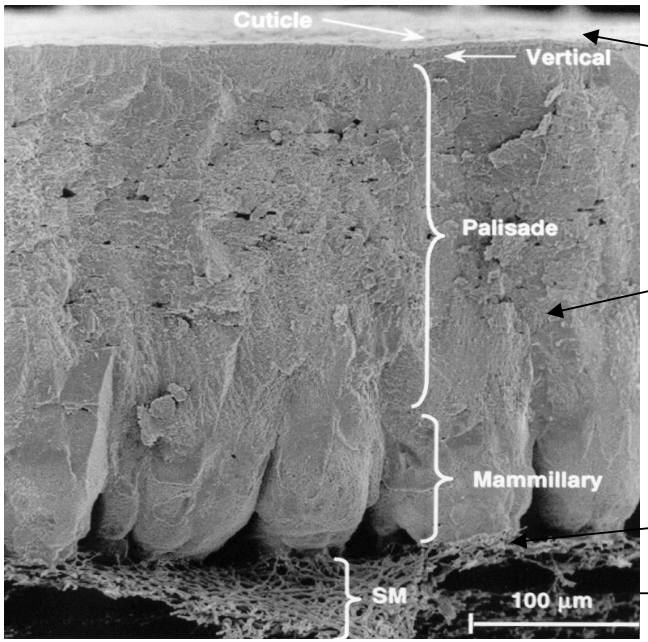
University of Granada, **CSIC**, Spain

V. Labas, G. Harichaux

UMR INRA 85, Proteomics facilities, 37380 Nouzilly France

The chicken eggshell

- Eggshell biomineralization in uterus (fast process)
- 5-6 g of mineral are deposited within a 20 h period



SEM

Cuticle

Palissadic layer

Mammilary knobs

Eggshell membranes

Cross-polarized light

Images: J.M. Garcia-Ruiz, Granada

95 % calcium carbonate (calcite polymorph)
3.5 % proteins and proteoglycans (organic matrix)

Interaction

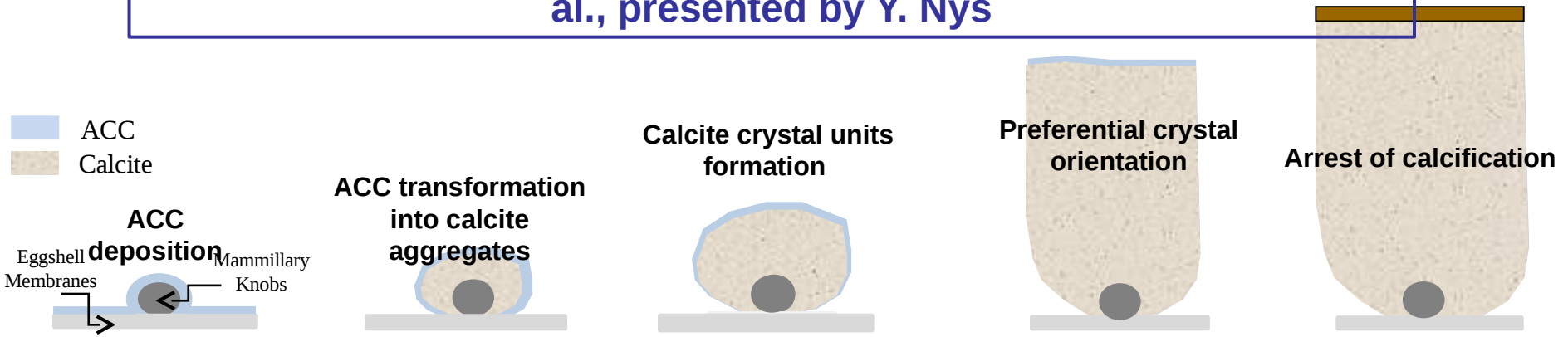
Ultrastructure
Mechanical properties

The chicken eggshell formation

Role of amorphous calcium carbonate (ACC) at pivotal stages of shell formation

Rodriguez-Navarro et al., Journal of Structural Biology, 2015, 190: 291-303

Friday 18: « 654. Evolution of calcium carbonate mineralogy during rapid avian eggshell calcification » Rodriguez-Navarro et al., presented by Y. Nys



First events of shell mineralization (initiation 2 h) | **Rapid growth phase (12 h)** | **Terminal phase (2 h)**

Role of organic matrix proteins at pivotal events

- ✓ *Stabilization of amorphous calcium carbonate (ACC)*
- ✓ *Polymorphs, morphology and size of crystals*

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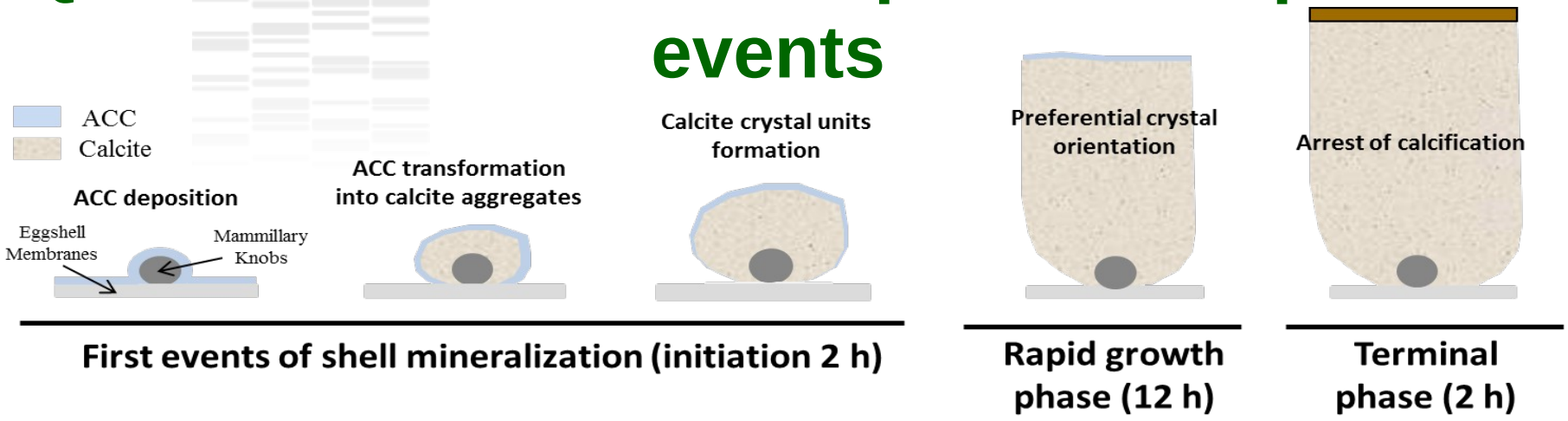
Quantification of matrix proteins at pivotal events



High-throughput quantitative proteomics, statistical and bioinformatic functional analyses of matrix proteins

Marie et al., Journal of Proteomics, 2015, 113: 178-193
Marie et al., Journal of Proteomics, 2015, 126: 140-154

To sort major protein candidates involved in particular key points of the eggshell mineralization

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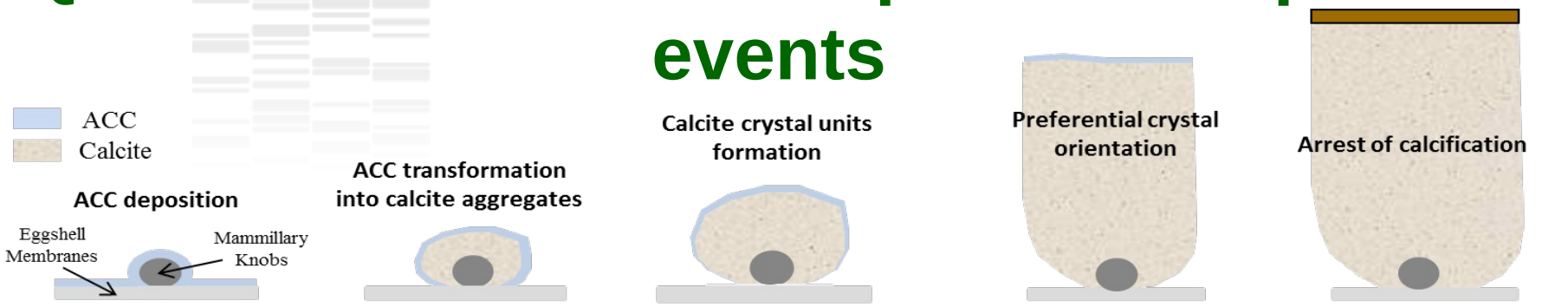
Quantification of matrix proteins at pivotal events



Distribution and variation of abundance of about 300 matrix proteins

Predicted functional activities of the identified matrix proteins ?

Classification in 3 different groups according to their potential functions

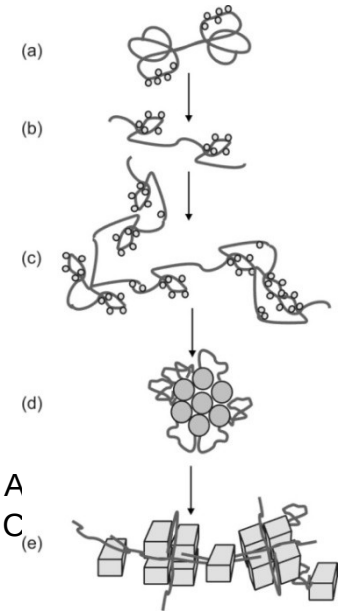
- **Associated to mineralization process**
- **Involved in the regulation of activity of proteins**
- Antimicrobial proteins

Highlight on 22 matrix proteins suspected to have predominant roles in the control of the different stages of shell calcification

Proteins at pivotal events

Proteins having a direct involvement in eggshell mineralizatio

- Proteins with established role in the **biomineralisation**



Binds to Calcium

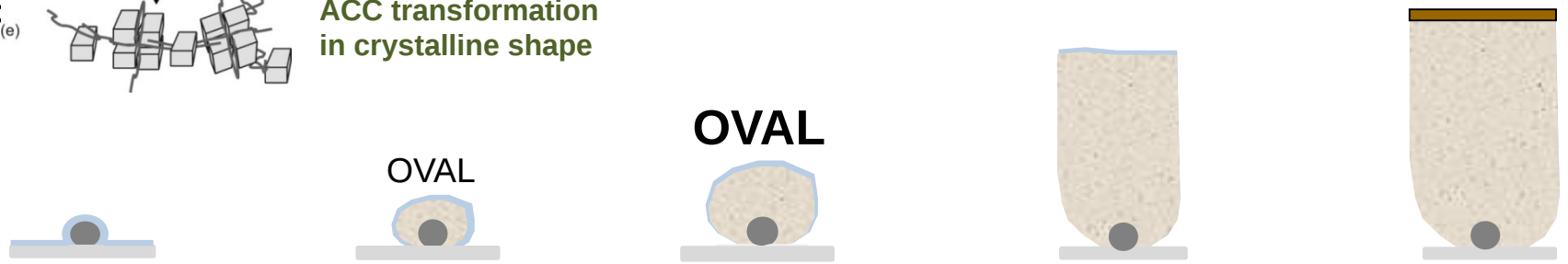
Modification of protein conformation

Protein aggregation

ACC Formation

ACC transformation in crystalline shape

In vitro study of the role of Ovalbumin on CaCO3 crystallization (Pipitch et al., 2008, Schwahn et al., 2003)



ACC deposition

ACC transformation into calcite

Crystal units formation

Preferential crystal orientation

Arrest of calcification

First events of shell mineralization (initiation)

Rapid growth phase

Terminal phase

Proteins at pivotal events

Proteins having a direct involvement in eggshell mineralizatio

- Proteins with established role in the **biomineralisation**

Freeman et al, 2010

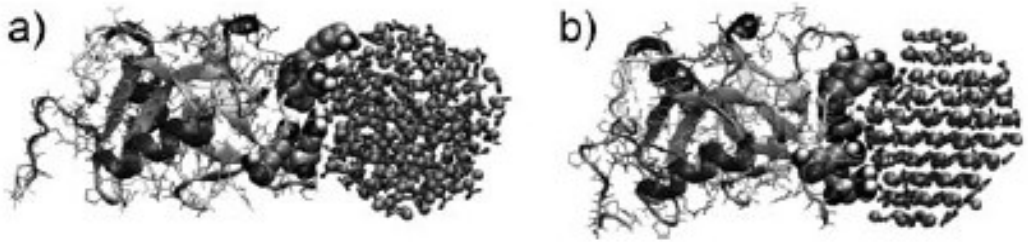
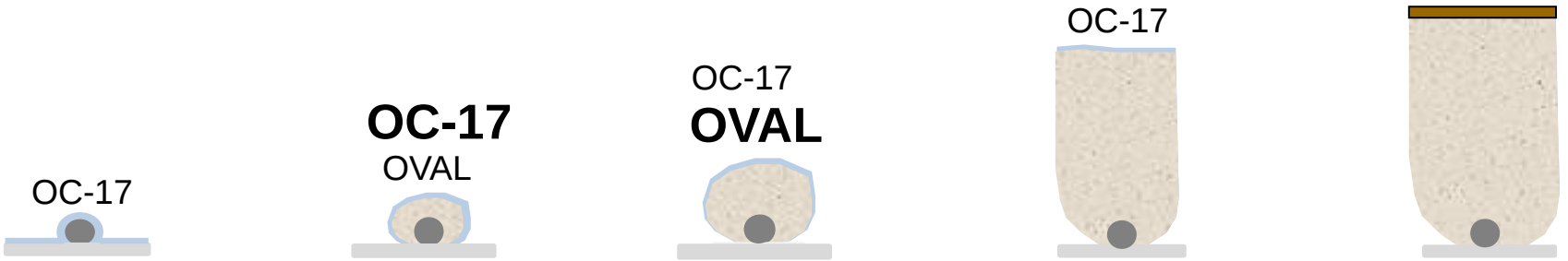


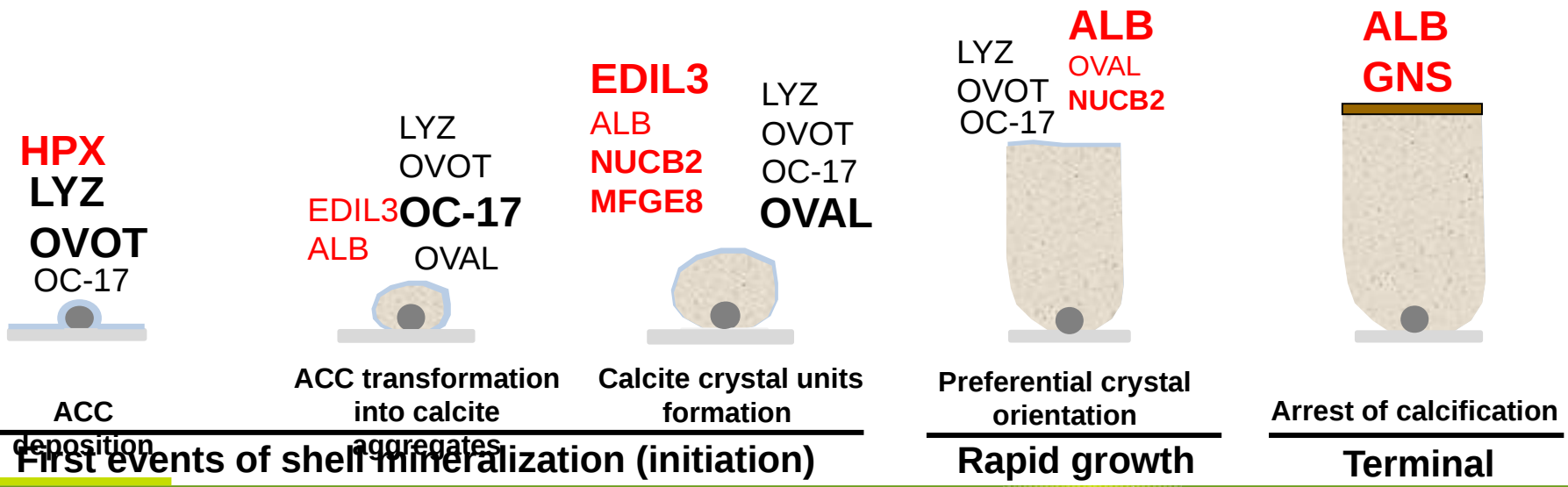
Figure 1. Ovocleidin-17 bound to an amorphous (a) and a crystallized (b) calcium carbonate nanoparticle containing 192 formula units.



Proteins at pivotal events

Proteins having a direct involvement in eggshell mineralizatio

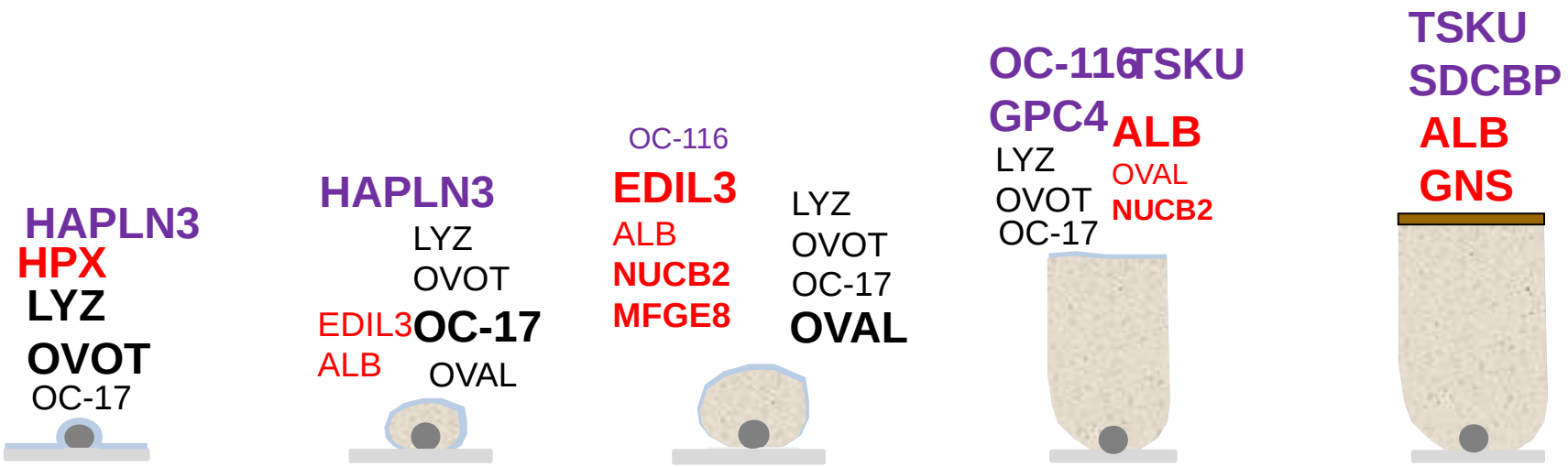
- Proteins with established role in the **biomineralisation**
- **Calcium binding proteins (CaBPs)** interacting with calcium, favoring crystal nucleation and driving the morphology of crystals
  - *Proteins with EF-hand and EGF-like calcium binding domains*



Proteins at pivotal events

Proteins having a direct involvement in eggshell mineralizatio

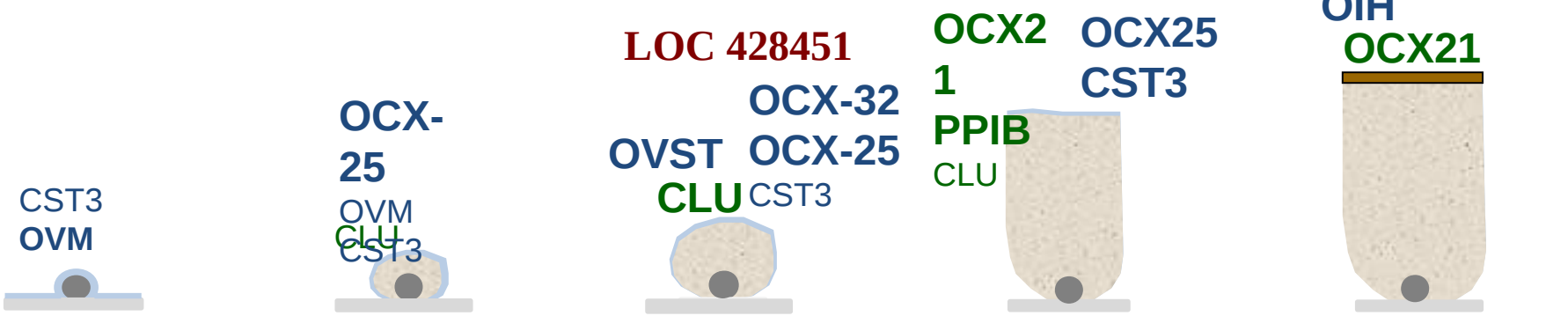
- ✓ Proteins with established role in the **biomineralisation**
- ✓ **Calcium binding proteins (CaBPs)** interacting with calcium, favoring crystal nucleation and driving the morphology of crystals
  - *Proteins with EF-hand and EGF-like calcium binding domains*
- ✓ **Proteoglycans** and proteoglycan binding proteins
  - proteoglycans have a negative charge to attract Ca2+ ions



Proteins at pivotal events

Proteins involved in the regulation of proteins driving mineralization

- Proteins involved in the **proper folding of the eggshell matrix** to ensure calcium and mineral interactions and to ensure template to the mineralized structure
- Proteins **inhibiting or activating proteins present in the mineralization milieu (non cellular).**
  - *Molecular chaperone interacting with proteins driving mineralization*
  - *Proteases and protease inhibitors (specific and controlled role during calcification process, either by degrading proteins or regulating processing of proteins into their mature forms)*
- Mineralization depends of the **degree of protein phosphorylation**
  - ***Kinases and Phosphatases***



ACC deposition | ACC transformation into calcite aggregates | Calcite crystal units formation | Preferential crystal orientation | Arrest of calcification

First events of shell mineralization (initiation) | Rapid growth phase | Terminal phase

Chicken eggshell calcification

- **Quantitative proteomics to classify and determine a hierarchy of proteins driving the mineralization Restricted list of 22 pivotal candidate proteins**

- **Direct role in shell mineralization**
  - ***Ovocleidin-17*** **ACC transformation into calcite**
  - ***Nucleobindin, MFGE8 and EDIL3*** **calcite crystal units formation and generation of a preferential crystal orientation**

- **Regulation of activity of proteins driving the mineralization**
  - ***Glypican-4, TSKU Ovocalyxin-21*** **Regulation of activity of proteins during the active growth and terminal phases of shell calcification**
  - ***Ovocalyxin-25*** **Initiation and rapid growth phases of eggshell calcification**

Experimental Validation

(Purification, interaction with mineral, Regulation according to physiological stages, polymorphisms related to eggshell quality, Biological markers ….)

THANK YOU FOR YOUR ATTENTION