# Societal impact of biomineralization in chicken eggshells

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science for people, life & earth

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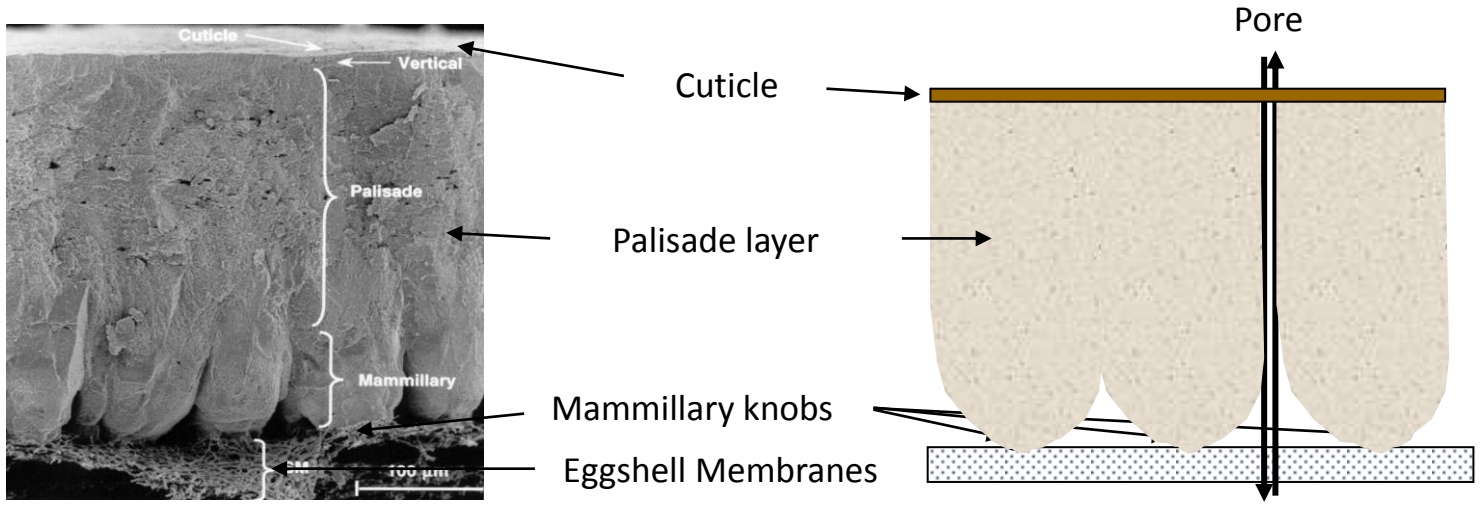
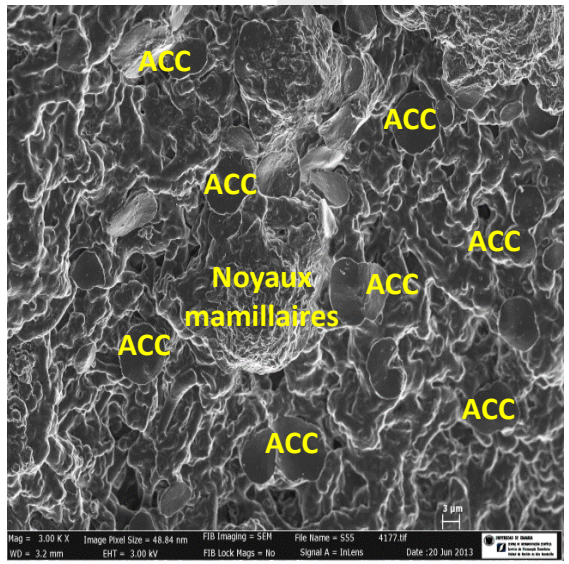
# The chicken eggshell formation

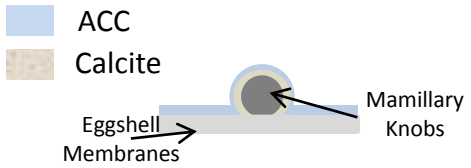
- ✓ Eggshell biomineralization in uterus (fast process)
- ✓ 5-6 g of mineral (calcium carbonate) are deposited within a 20 h period



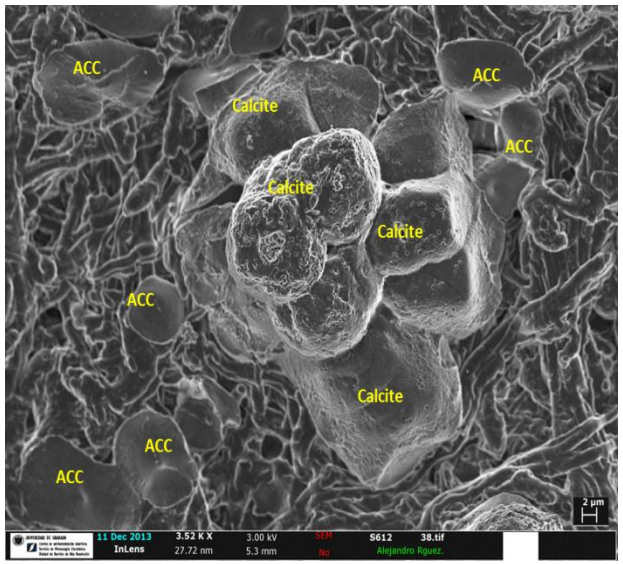
# Eggshell biomineralization



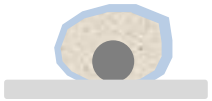

**First events of nucléation**



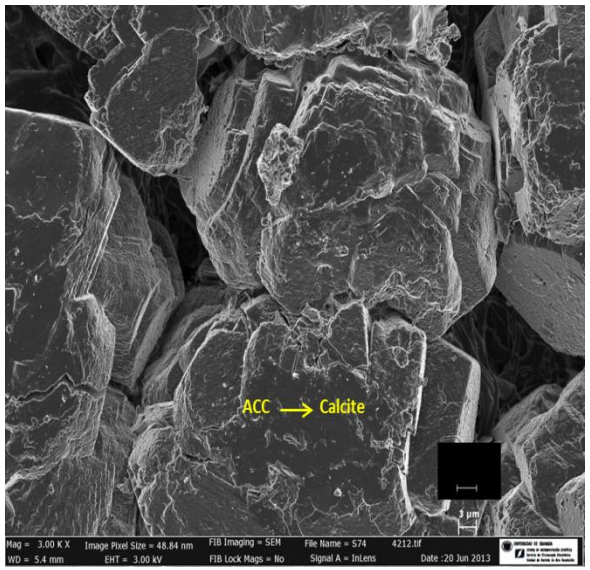

**Time 1 (5-6 h Post ovulation)**:
ACC particles nucleate on the whole eggshell membranes.
Form massive deposits



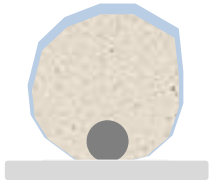

**Calcite formation**



**Time 2 (6-7 h post ovulation)**:
Interface-coupled dissolution precipation process
Direct transformation of ACC into calcite aggregates on mammillary knobs



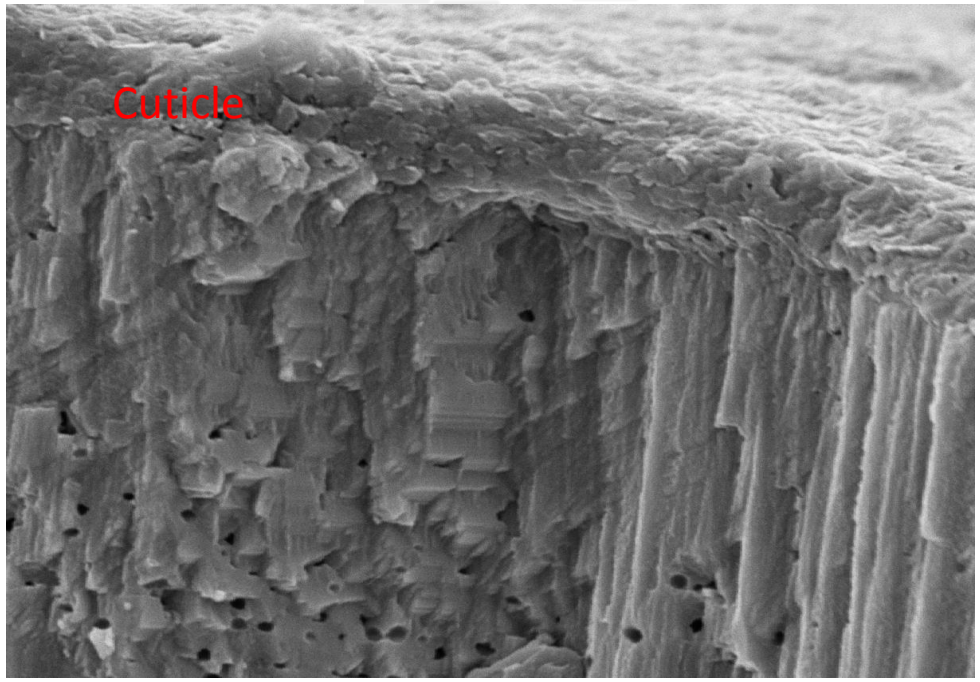

**Larger calcite crystal units deposition**

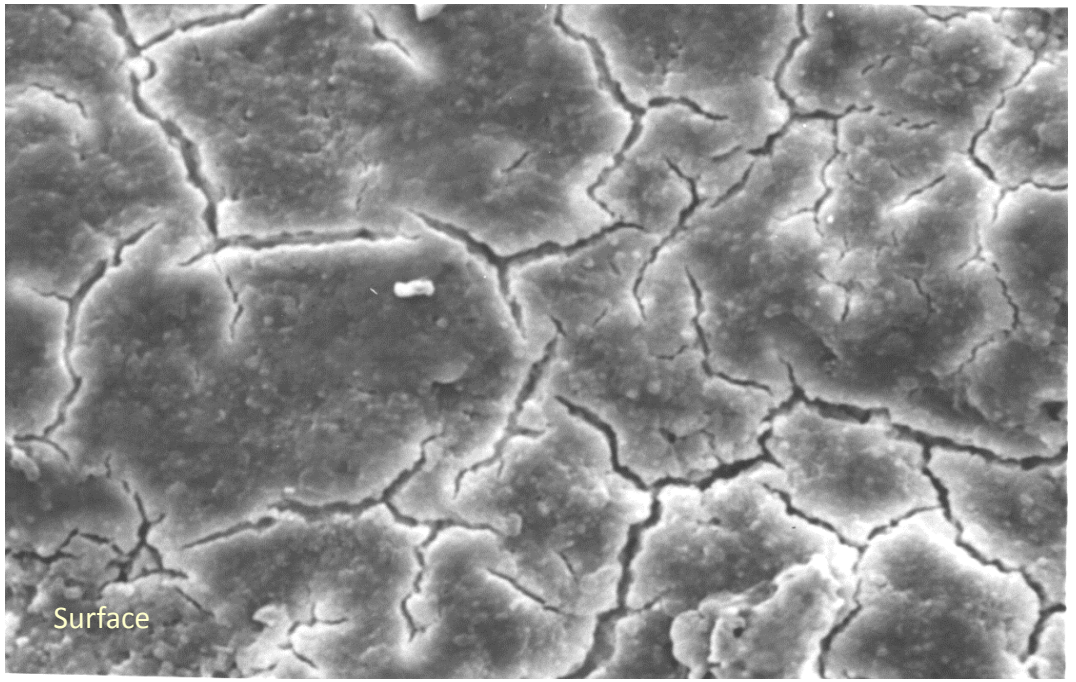


**Time 3 (>7h post ovulation):**
Additional cristallisation events on calcite template

Rodriguez-Navarro et al., Journal of structural Biology, 2015

# Avian eggshell biomineralization




**Time 5 (11 to Oviposition):**

- Formation of palisade layer. Generation of a compact layer with crystals all oriented perpendicular to the surface
- Deposition of a thin layer of vertical structure
- Cuticle deposition
- Oviposition, drying and cracking of cuticle

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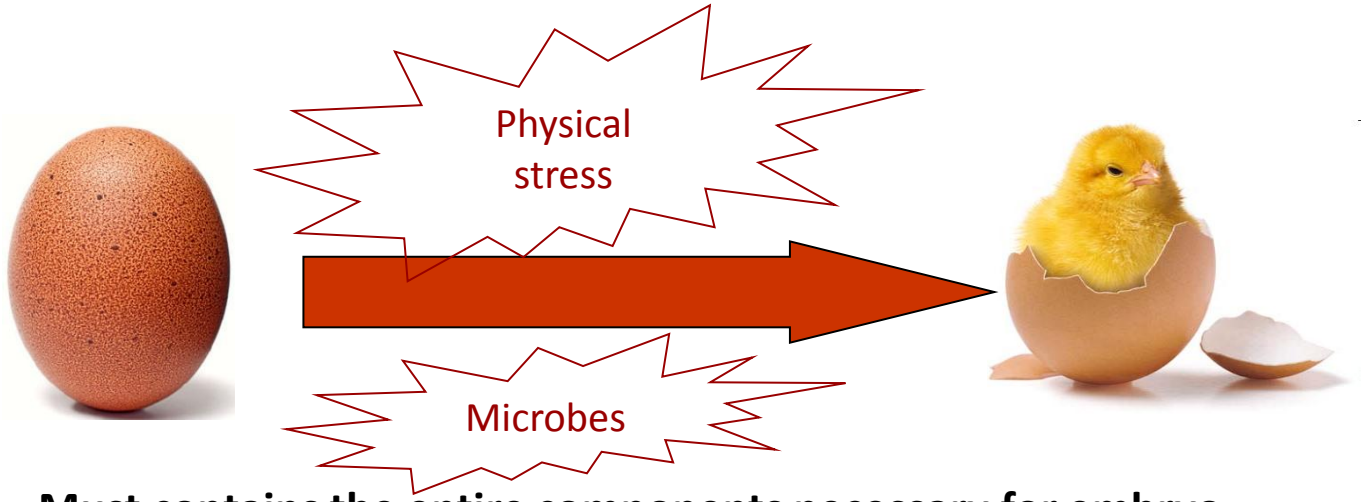
# Socio-economic context

## Eggs

**An autonomous close chamber to allow the embryo development**




**Must contains the entire components necessary for embryo**

- Well-balanced nutritious ingredients
- Lot of compound (> 1000) with a broad range of biological activities
- Protective systems (natural defenses)

Physical defense (Mainly shell)
Chemical defense (Proteins with antimicrobial activities)

## Table eggs

**A basic ingredient for human food**

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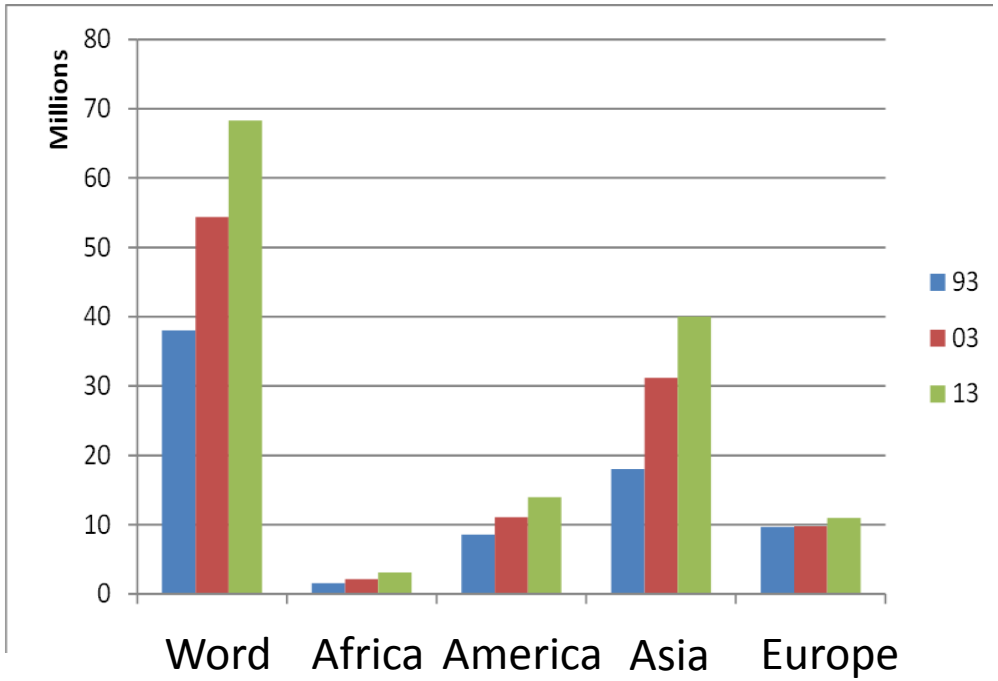
# Socio-economic context

## Table eggs

### A basic ingredient for human food

- 68.2 MT of egg produced each year in the word > 1200 billions eggs each year
- 14,7 billions eggs in France each year
- The cheapest animal food of high nutritional quality
- No religious prohibition

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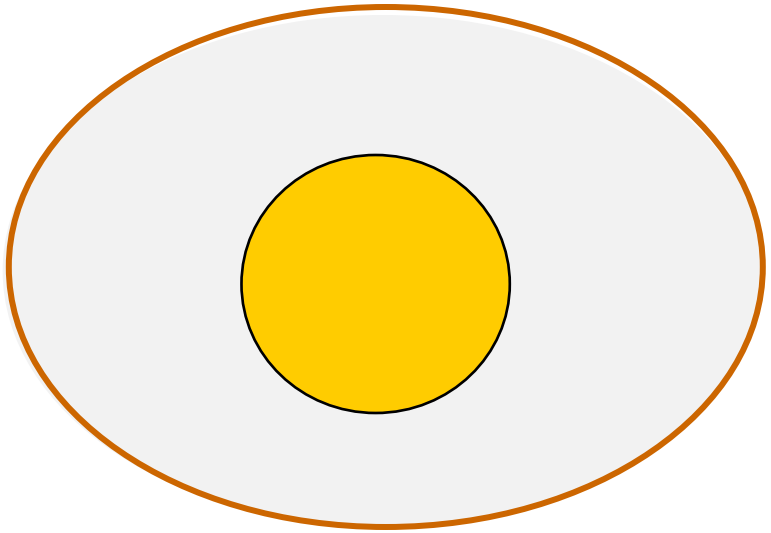
# Eggshell in the socio-economic context of the egg

**Eggshell is the only non-consumable part of an egg…..**

<u>Mineral eggshell</u>

- *Ensure the physical protection*
- *Avoid bacterial penetration*
- *Ensure a thermic protection*
- *Allow gaz exchanges*
- *Calcium source for embryo*



**… but its quality is crucial for the marketing of the egg**

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# Socio-economic context

## Economic issues

**Downgraded eggs due to deteriorated egg quality**

poor internal qualities leading to white/yellow separation problems

Dirty, cracked or broken shells

**Hatchability of the chick**

Shell allows gas exchanges during embryo development

## Health issues

**Risks of toxi-infections for the consumer (Salmonellosis)**

Eggshell as a physical barrier

**SHELL QUALITY**

**Shell mechanical properties**

## Ethical issues

**Housing systems and societal demand**

Consumer demand for non cage and free-range systems → Lower shell quality

Long life cycle (shell quality decreases with age of birds)

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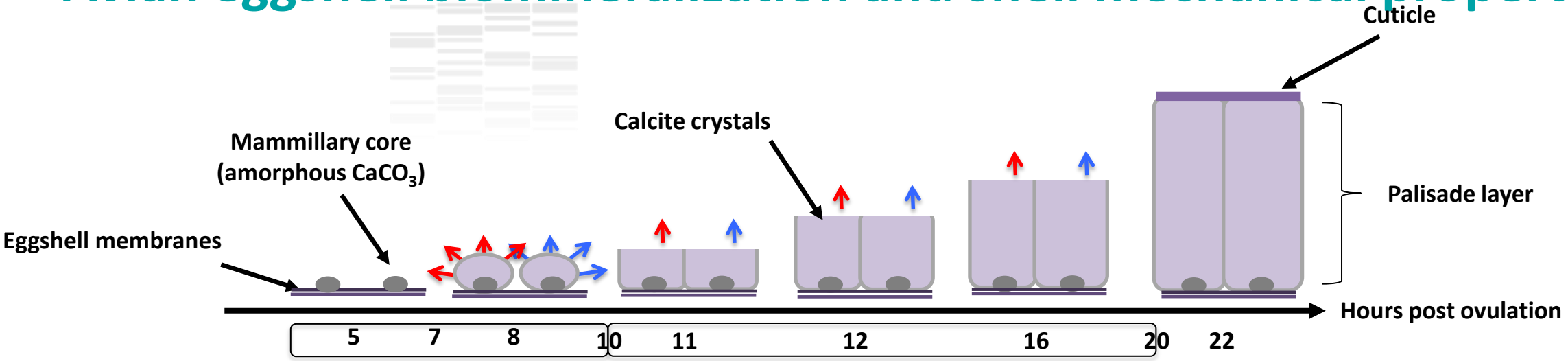
# Avian eggshell biomineralization and shell mechanical properties




**95 % of calcium carbonate (calcite) ⟵ Interaction ⟶ 3.5 % organic matrix (proteins, proteoglycans)**

**Role of organic matrix proteins at pivotal events**

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

**Ultrastructure, Mechanical properties**

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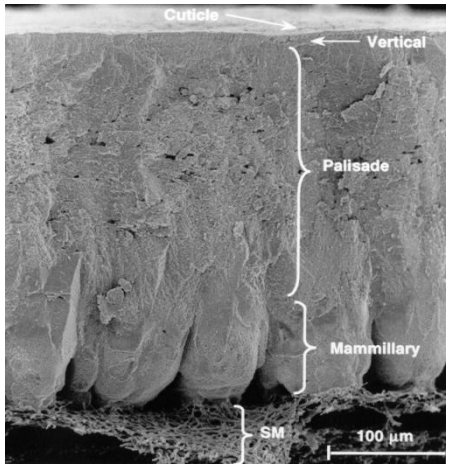
# Societal impact of avian eggshell biomineralization

## Maintain and improve shell quality

Calcium Carbonate (calcite) 95 %

Organic matrix 3.5 %




Quantity

Control of the shell calcification process

Mechanical properties

**Mass or fabric ?**

☞ Mass: nutrition, génetic, environemment, lighting programs

☞ fabric : Regulation of shell matrix proteins and genetic selection

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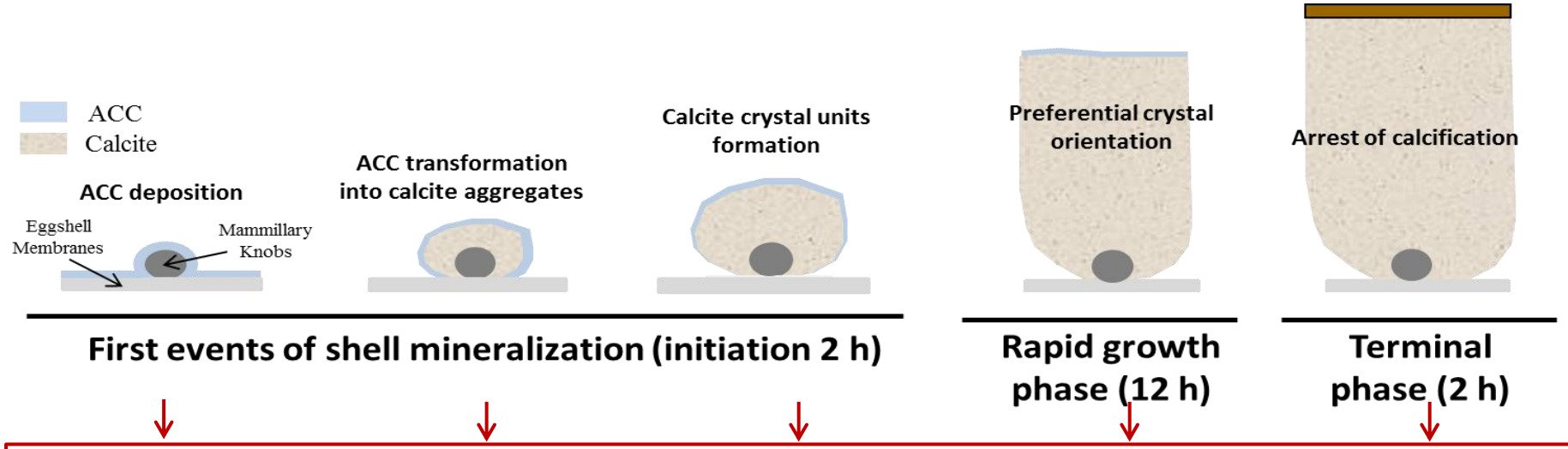
# Eggshell biomineralization




**444 Main candidate proteins to be involved in the shell biomineralization process**

## Predicted functional activities of the identified matrix proteins ?

(Marie et al., 2014, 2015a,b)

**Classification in 3 different groups according to their potential functions**

- **Associated to mineralization process**
- **Involved in the regulation of activity of proteins**
- Antimicrobial and other proteins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# And now ? How to improve shell quality ?

**Physiology**

**Understand the mechanisms of shell manufacturing and determine the origin of its weaknesses**

**Genetics**

**Classical and genomic selection**

**Recent Developments and Future Prospects :**

- ✓ Genomic selection to taking into account scientific advances in the knowledge of mechanisms
  - Candidate gene approach


Candidate Genes of eggshell calicification in laying hens (CACAO)

Défenses de l'œuf DOVE Valorisation Evolution


Eggshell Calcification Polymorphism Candidates (POLCACAO)

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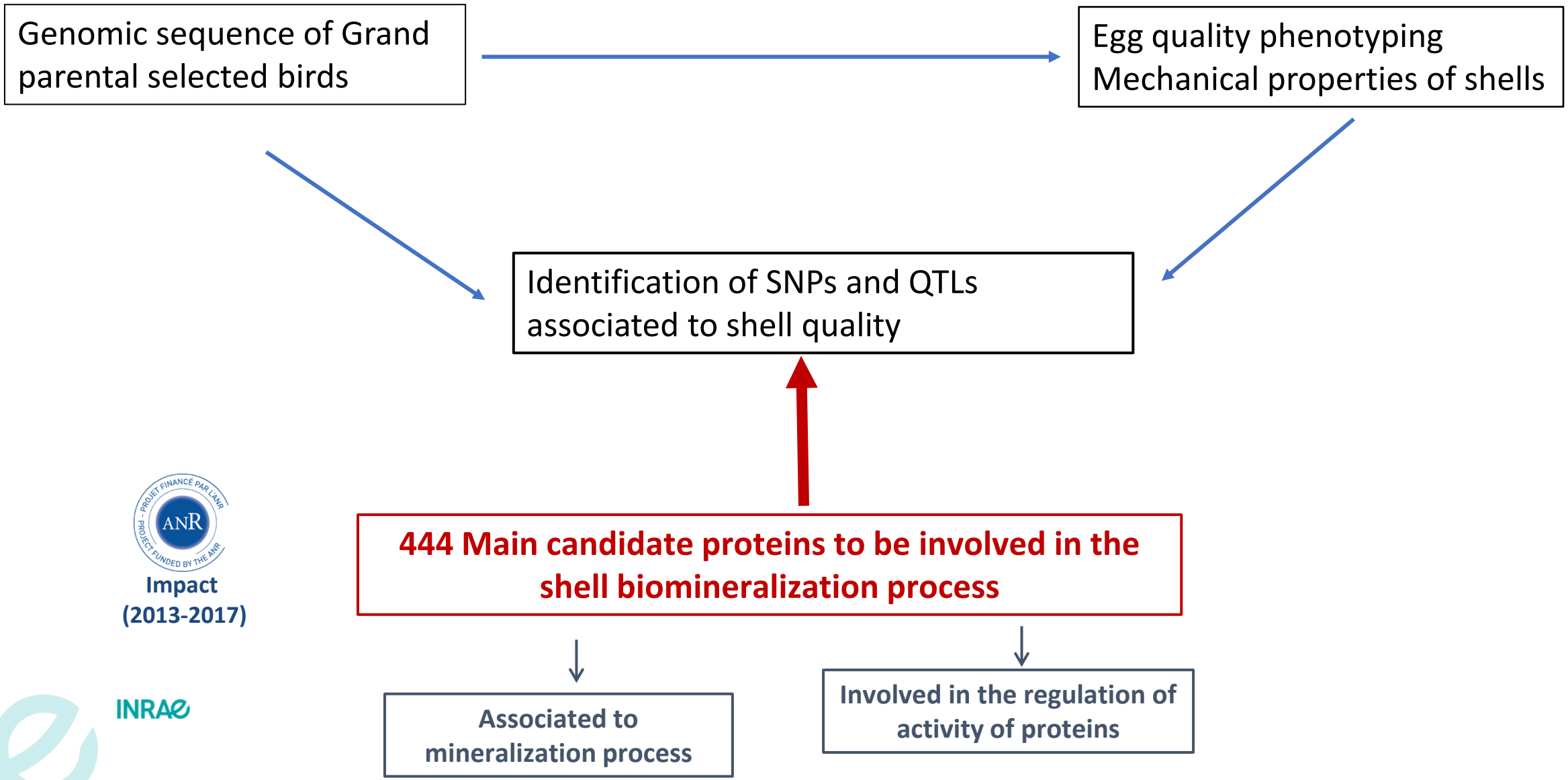
# Using recent advances in genetics and genomics to improve eggshell strength

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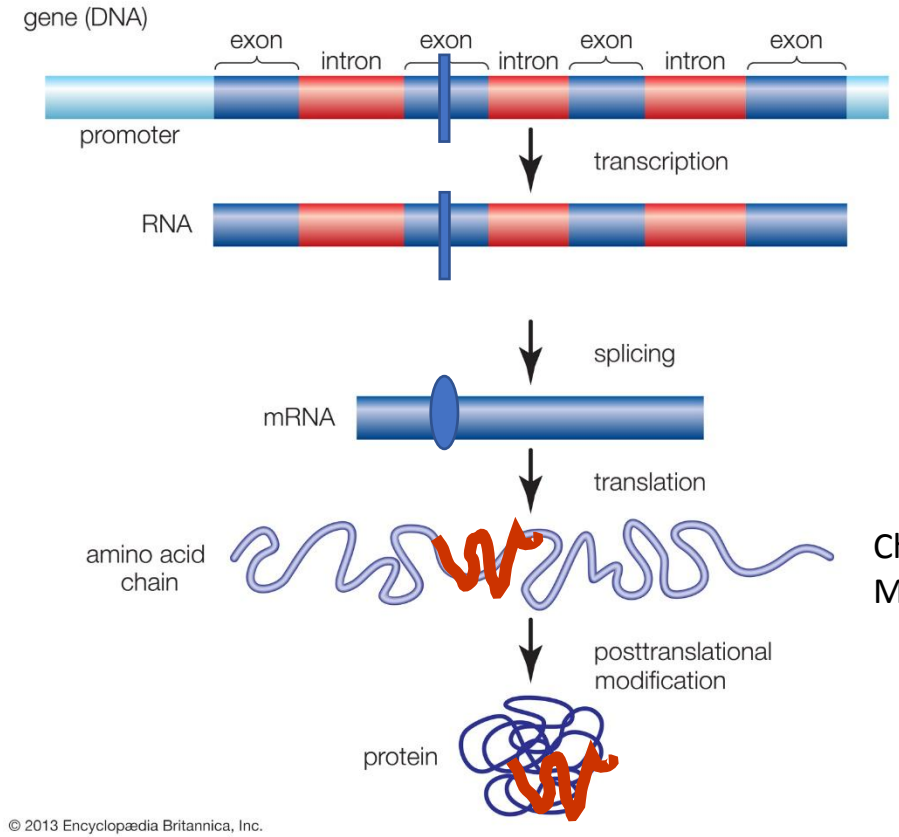
# Using recent advances in genetics and genomics to improve eggshell strength

## Marker-assisted genomic selection




Single nucleotide polymorphisms (SNP) (ex: G/C)

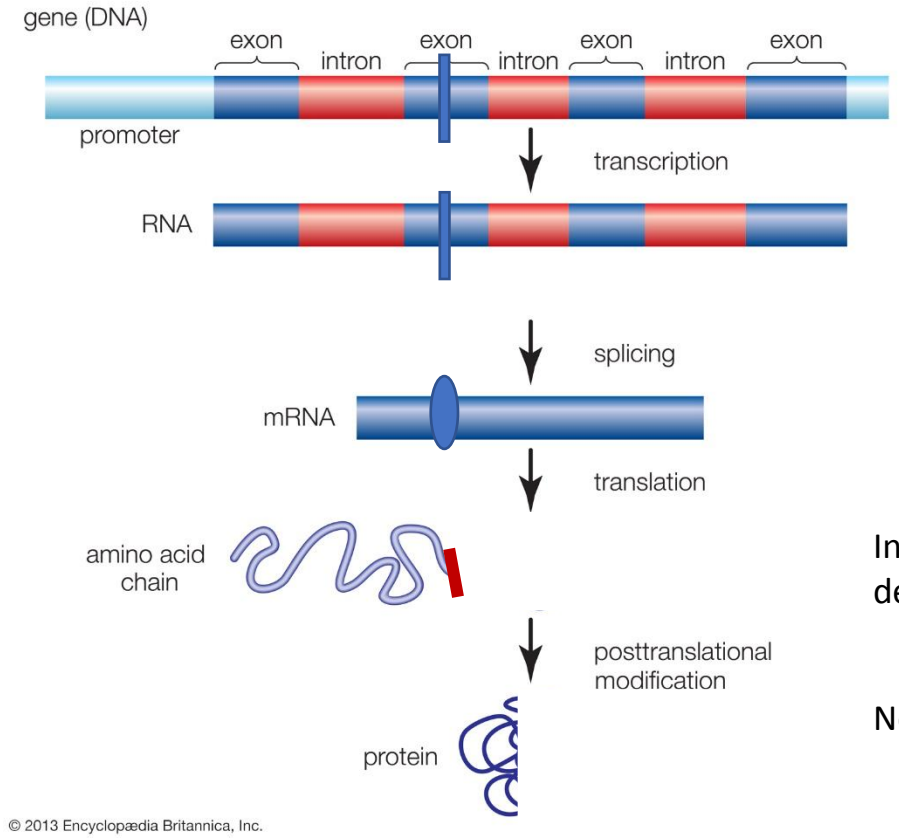
Single nucleotide polymorphisms (SNP) (ex: G/C)

Changes in amino acid sequence
Modification of the function of protein

**Polymorphism usable in genetic selection**

# Using recent advances in genetics and genomics to improve eggshell strength

## Marker-assisted genomic selection




Single nucleotide polymorphisms (SNP) (ex: G/C)

Single nucleotide polymorphisms (SNP) (ex: G/C)

Insertion of stop codon
deleterious mutation

New protein

**Polymorphism usable in genetic selection**

# Using recent advances in genetics and genomics to improve eggshell strength

## Marker-assisted genomic selection

### GENOTYPING

Blood collection from laying hens

Analysis of SNPs (Genotyping chips)

### PHENOTYPING

Collection of eggs laid by layers

Measurement of biomechanical properties

- Number of broken eggs
- Eggshell breaking strength
- Eggshell thickness
- Eggshell elastic modulus
- Eggshell toughness

**STATISTICS**

**Association test**

**Statistical test to identify the presence of SNPSs related to greater shell mechanical properties**

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# Conclusions- Take home messages

- Biomineralization mechanisms in birds' eggs determine the mechanical properties of the shell
- The societal and socio-economic challenges of this mineralization process are important
  - Economic, health and ethical issues

- Using the information on eggshell biomineralisation, genetic improvement can be achieved

- This component involves many actors in the egg sector:
  - ✓ Egg producers
  - ✓ Genetic selection companies
  - ✓ Nutritionists and formulators
  - ✓ Decision-makers (political, economic, food industry, supermarket sales)

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