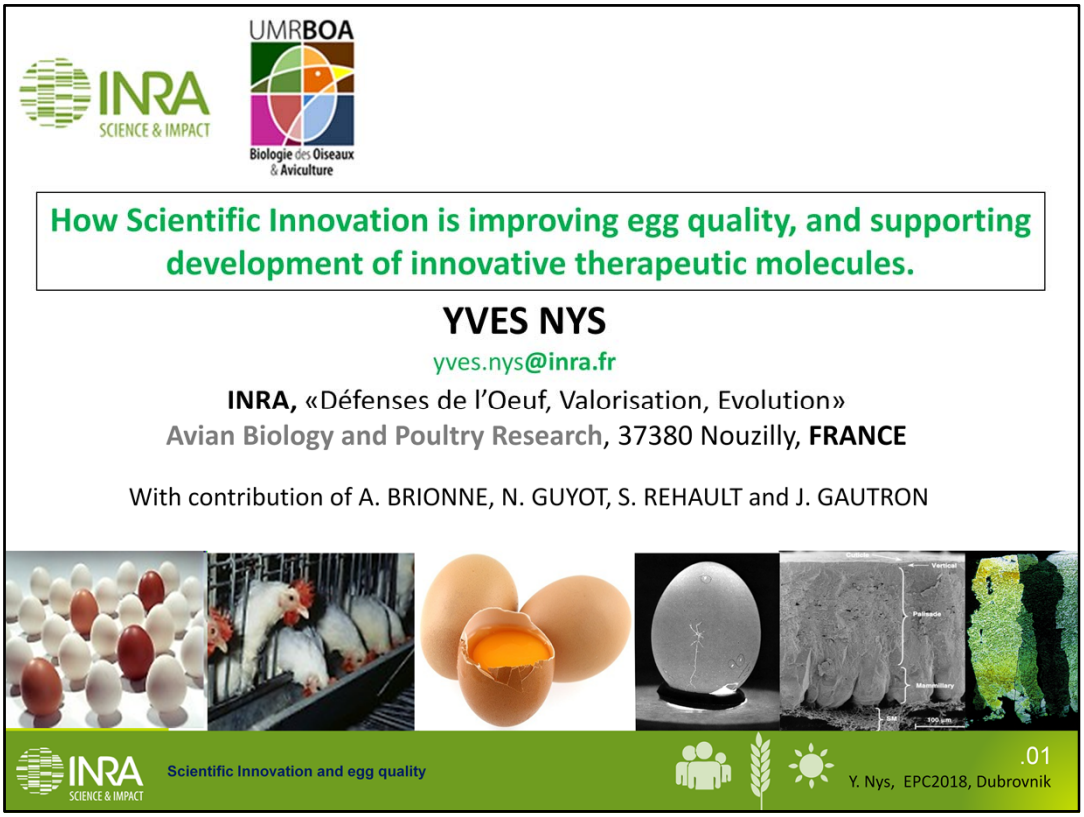
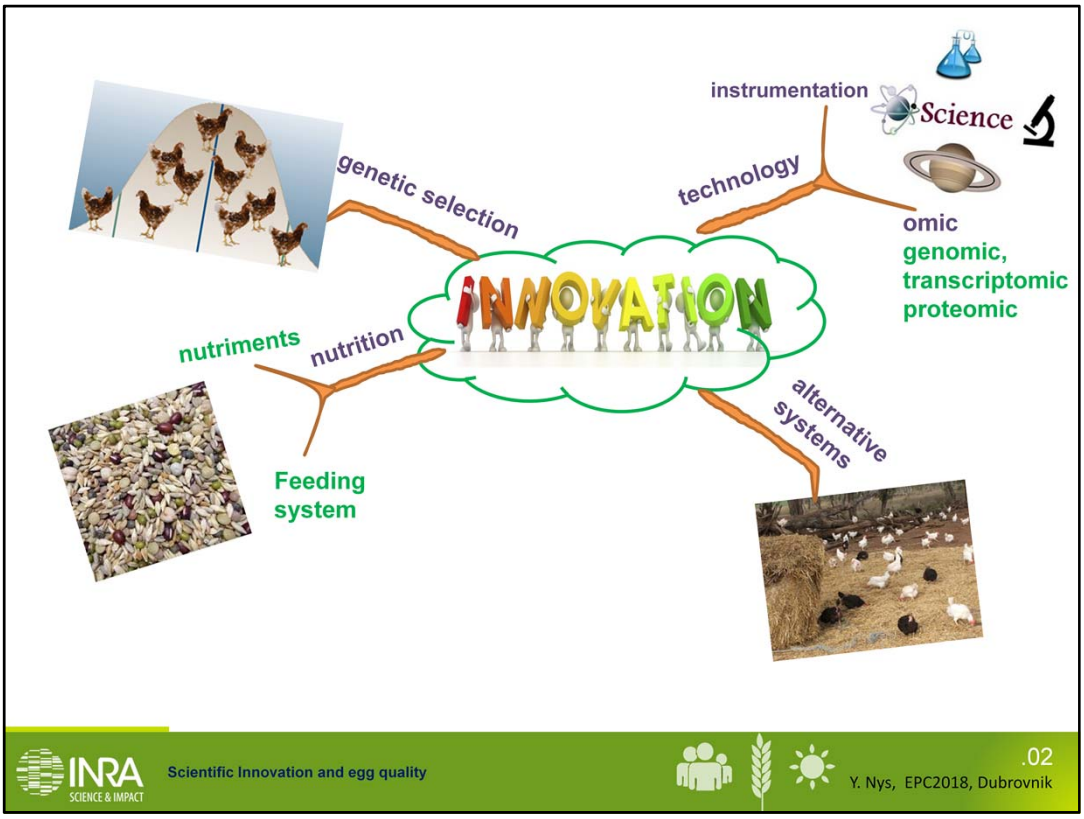
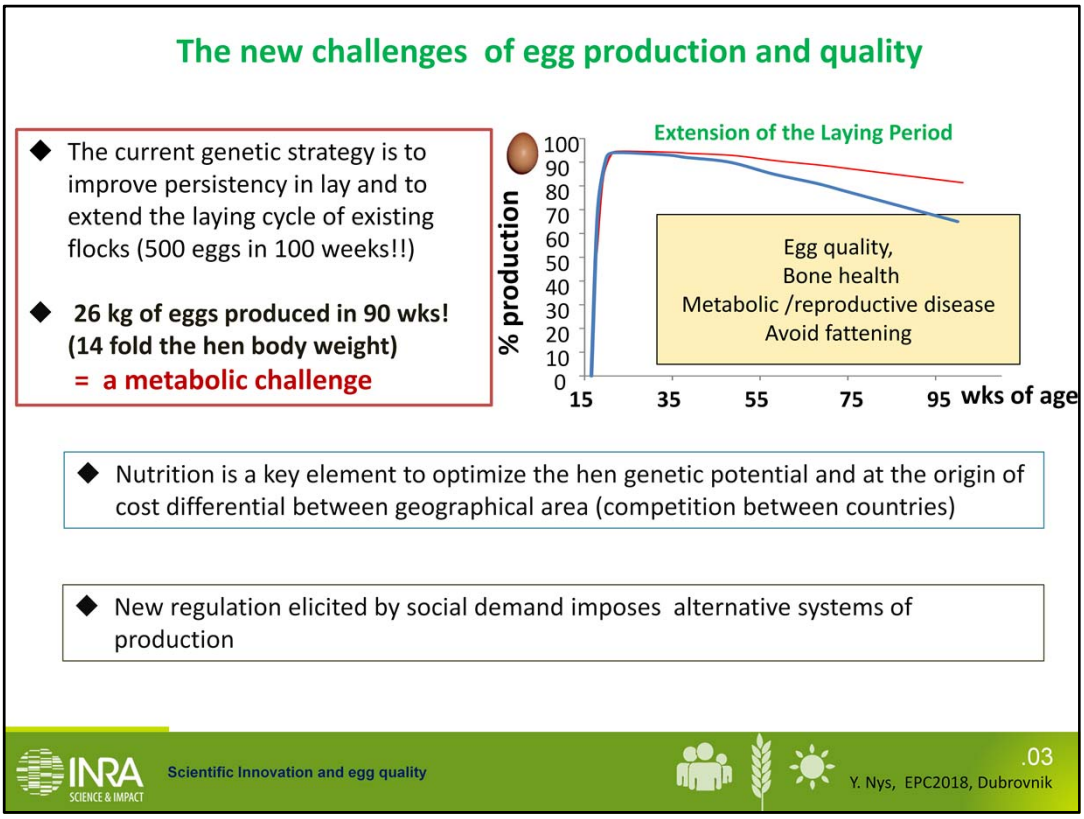


I would like to thank the organizing committee to give me the opportunity to present a review on egg. My title is probably ambitious because I cannot in 30 minutes present all innovations concerning egg production and quality. I will briefly introduce the major innovation concerning egg production and quality but will underline how hen genome sequencing and proteomic and genomic approaches have contributed to better understand mechanisms of egg formation and have revealed the numerous novel egg proteins with interesting biological activities.


instrumentation
Science
technology
genetic selection
omic
genomic,
transcriptomic
proteomic
INNOVATION
nutriments
nutrition
alternative
systems
Feeding
system
INRA
SCIENCE & IMPACT
Scientific Innovation and egg quality
.02
Y. Nys, EPC2018, Dubrovnik



The recent evolution of egg production is mainly due to the spectacular extension of the laying period due to the genetic strategy of selecting for persistency combined with selection for better egg quality in old hens. Hens should be able to produce more 500 eggs in 100 weeks. All improvement in egg quality in age hens is used to extend the period of egg production therefore all problems of egg quality in aged hens is just delayed. In addition hens are exporting 26 kg of eggs in 90 weeks, challenging hen metabolism with problems of bone quality, metabolic disease in the liver but also with welfare issues (feather picking).

Nutrition is a key element because it is challenge by genetic as nutrition should ensure the genetic potential and because it corresponded to too third of the cost and is at the origin of the difference in cost between the various geographical areas

The egg production is also challenge by new regulations concerning the system of production or ban of antibiotics rule which has imposed to innovate in production systems.

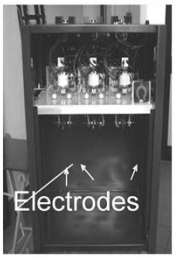
Nutrition has to be control at each step of pullet and egg production

- Need to better take into account pullet period and to optimize feeding techniques (empty feeder, particle size) and to continuously adapt feed composition (lipids) to hen dietary consumption to prevent metabolic disorders (liver, bone, feather) and optimize egg quality
- Innovation issued from black box approaches: optimize one dietary factor for higher performance but mechanisms?
- Some innovations mainly in feeding systems (sequential feeding) and in novel additive
- Control of feedstuff value facilitated by development of IR spectroscopy
- Modelling of nutritional requirements, nutrients digestibility or metabolism in hens is promising to understand complexity of interactions in hen feeding

NYS, Y. (2017a, b). Laying hen nutrition: optimizing energy intake, egg size and weight: Vol 2. Chap1:3-28; optimizing hen performance and health, bone and eggshell quality, Vol 2. Chap2: 29-56. In Roberts J eds Achieving sustainable production of eggs-. Burleigh Dodds Science publishing.
MOLNÁR A., HAMELIN C., DELEZIE E. and NYS Y., 2018. Sequential and choice feeding in laying hens. World's Poultry Science Journal, Vol. 74, June 2018

Nutrition is crucial for long production cycle to control egg quality and hen heath: we know how to feed hens but we have to control each step during the pullet and egg production period. The difficulty is to continuously optimize the feed supply to maintain egg production and quality egg. Feed composition and feeding techniques are crucial to properly feed all the birds in the flock and to prevent metabolic disorders or feather picking. Birds have to ingest enough protein and amino acid to maintain the high level of production even when there is flock heterogeneity at the end of the laying period.

Innovation in nutrition generally result from a black box approach: an additive is evaluated by analysing its efficiency on performance often without exploring in details the mechanisms.
There are still some innovation mainly in feeding system such as the sequential feeding and of course novel additives are frequently proposed.
One successful development is the use of IR spectroscopy to rapidly analyse feedstuff composition and therefore optimise the diet formulation.
Finally i would like to underline that modelling of nutrient and of their digestibility are promising tools to understand the complexity of interactions in hen feeding.
There are also questions concerning the potential of using big data: collection of flock parameters and hen performance in real time might be useful to reveal and correct any abnormalities and to analyse at large scale factors influencing egg performance and egg quality



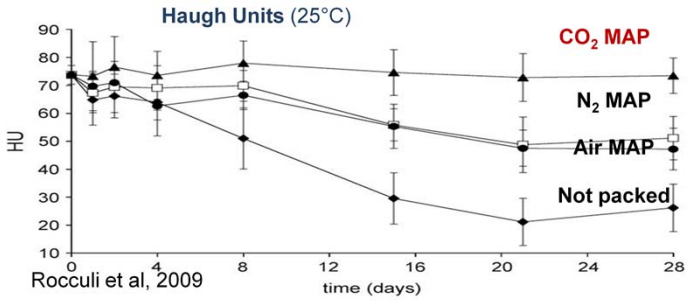
Innovative technology to control Egg quality

- **By improving initial hygienic quality**
  - Egg washing: improves initial hygienic status of eggs
  - Resistive barrier discharge (RBD) for generation of gas plasma (reactive oxygen or nitrogen), pulsed light, hot air and microwave pasteurization

By improving egg storage

Egg Modified-Atmosphere Packaging (MAP) to extend shelf life of eggs CO2, O2, N2 might limit chemical and microbial spoilage:

- Can improve some technological properties of egg white
- Compatible with perception of freshness??



Novel technologies techniques are available to improve hygienic quality of egg and to maintain its technological properties during egg storage

Egg washing is allowed in some countries to improve initial statute of eggs and new technologies such as resistance barrier discharge, pulsed light, hot atmosphere or microwave pasteurisation are explored to reduce microbial egg contamination.

Alternatively, modified atmosphere packaging with is used for human food might be of interest for egg storage. Such approach can improve some technological properties of egg white as demonstrated for CO2 or even might avoid some defect in internal egg quality or vitellin membrane but that remains to be explored. It is noteworthy that the producers are a bit reluctant toward this technology which might facilitate the international commercialisation of eggs and make more difficult the evaluation of egg freshness which remain one of the main criteria of the consumer demand.

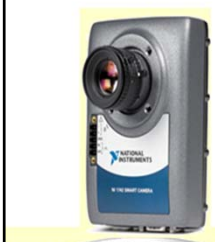
Finally, we know that whatever our effort, hen will always produced some down graded eggs so it is important to develop new technologies to sort the egg with lower quality.

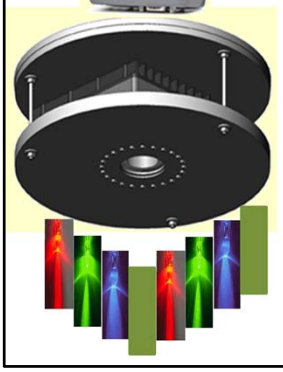
One interesting approach is the development of non invasive methods to sort all individual eggs at high speed and the acoustic approach listening to the sound of the eggshell crystal is a very elegant method developed by the university of Leuven and currently used at high scale in eggplant.

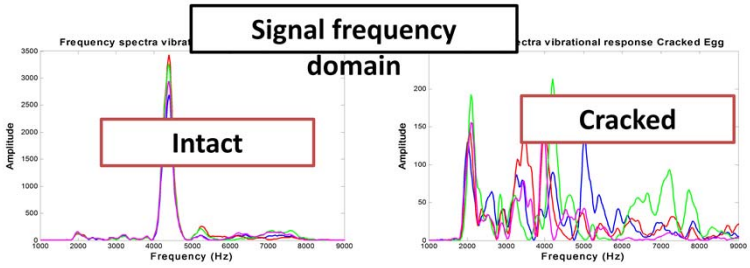
Transmitted light through the egg using NIR spectroscopy has been explored successfully for measuring shell colour or presence of blood spot. The results are promising for evaluating internal quality of egg white but the prediction remain too uncertain to be used at an industrial level.

So there is a large potential of innovation in these technologies concerning egg storage or egg grading but in my opinion, the main innovation in the last 15 year result from the hen genome sequencing and development of transcriptomic and proteomic technologies. These approaches contributed to better understand the process of egg formation and reveals numerous novel bioactive molecules in the egg as I will show you in the following part of my talk.

Innovative technology to control Egg quality

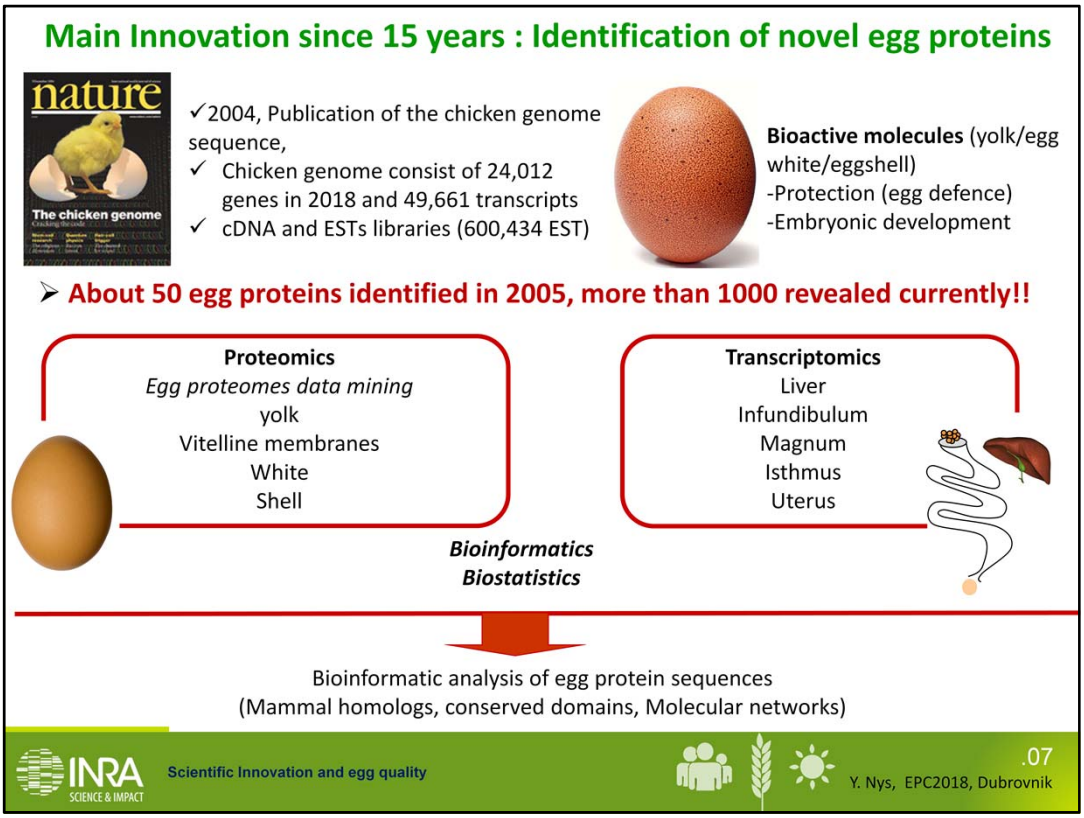




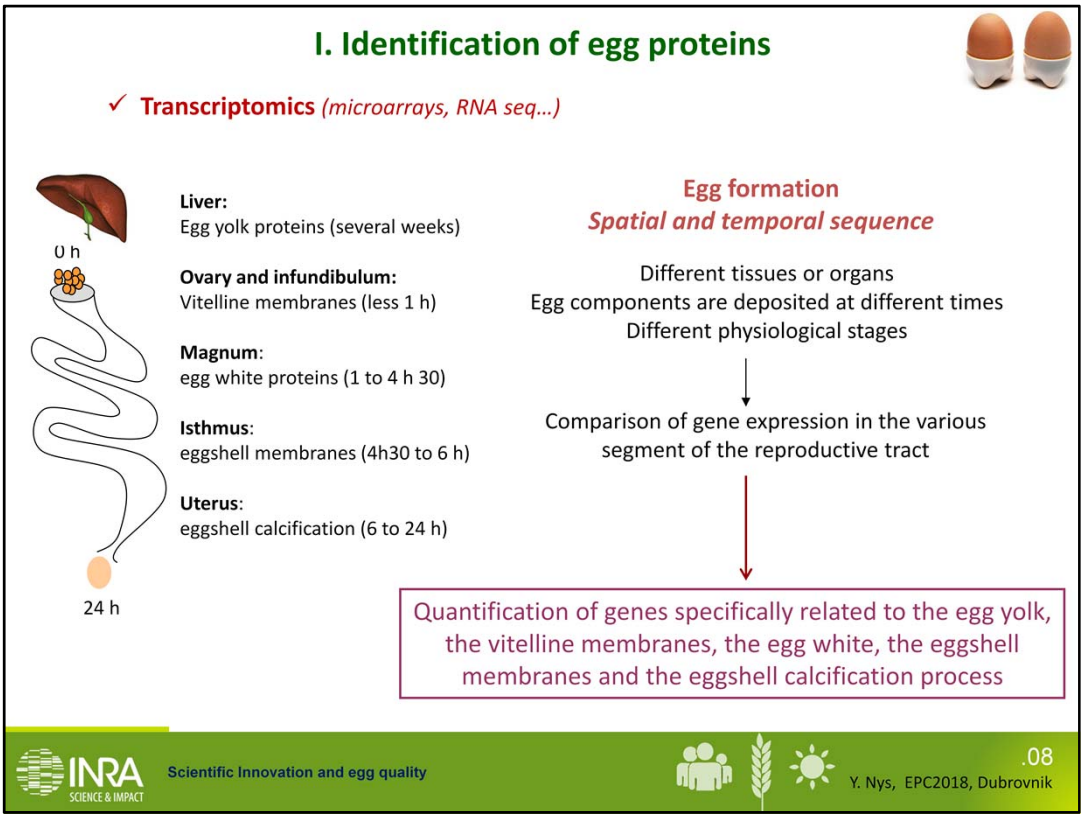


➢ **By Improving the technology for egg grading (University of Leuven)**

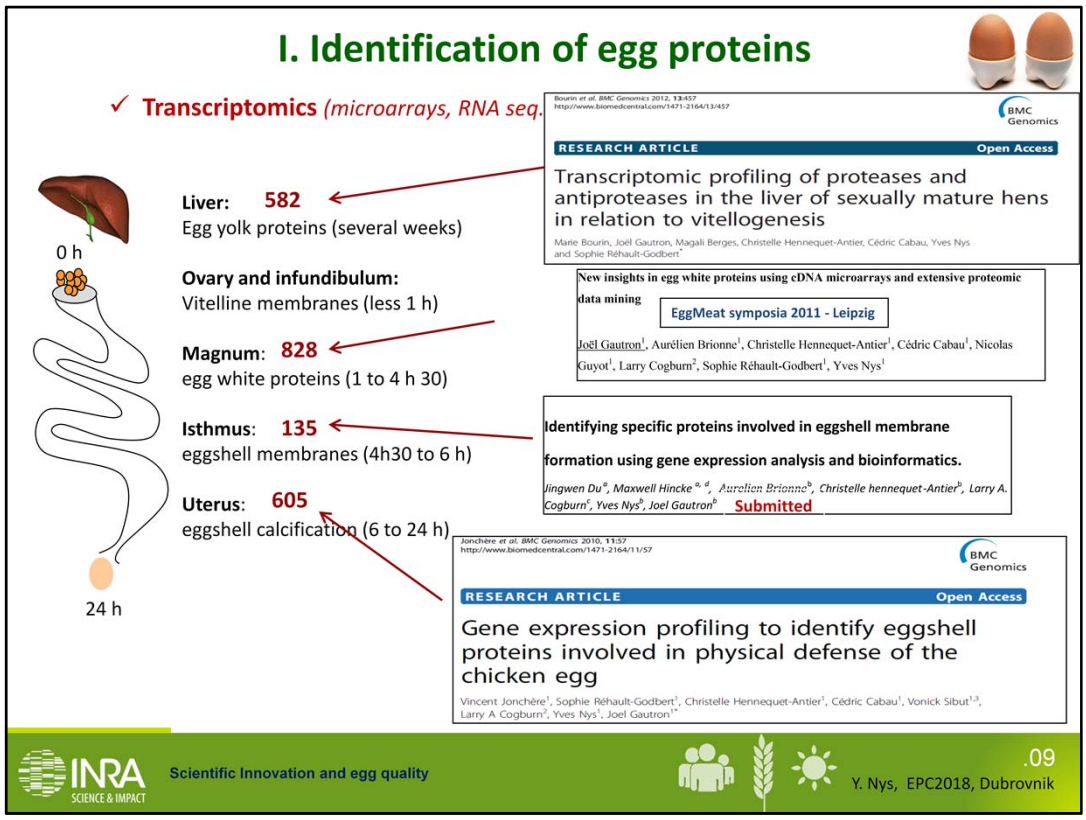
- **Development of non invasive methods for detection of shell defect (acoustic methods) currently used in eggplants**
- **Spectral measurements (NIR spectroscopy) for evaluation of shell color or internal quality (blood spot, pH, Haugh unit)**
- **Image analysis for detection of dirt on eggshells**



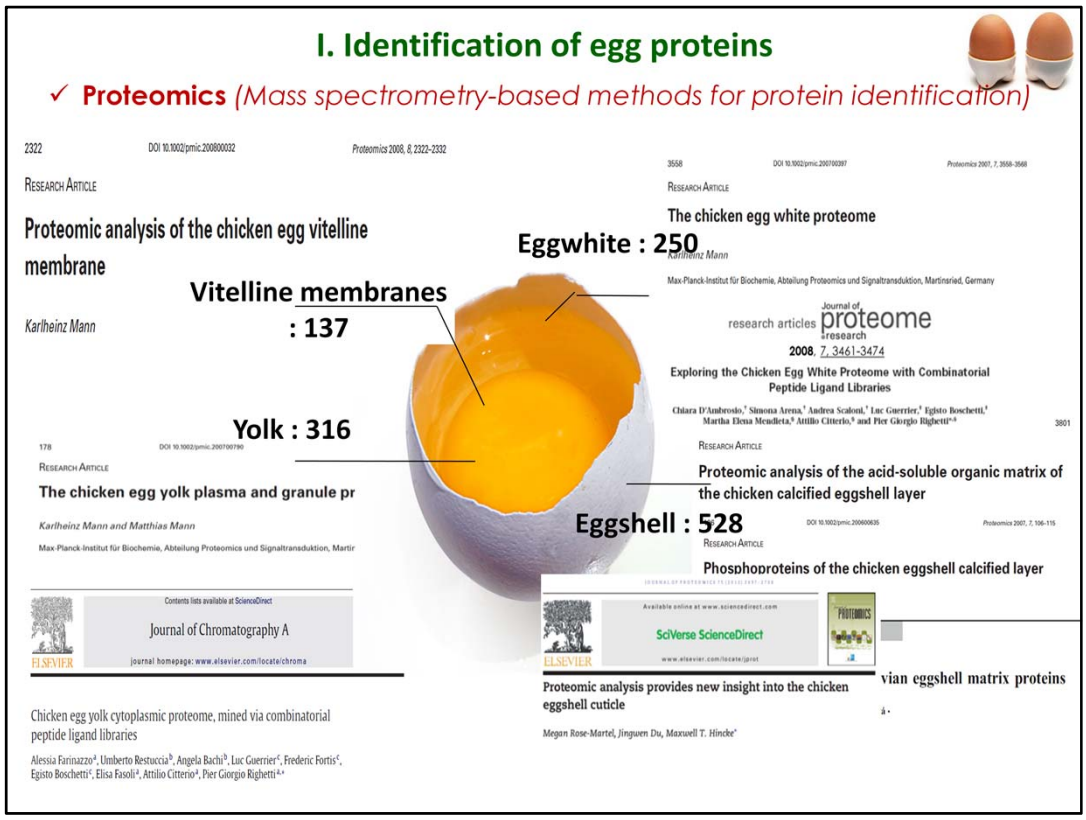
So numerous innovation in genetic selection, hen nutrition or technologies applied to egg storage and egg grading contribute to improve hen performance and egg quality but in my opinion, the main innovation in the last 15 years result from the hen genome sequencing and development of transcriptomic and proteomic technologies.
In 2005, about 50 proteins was identified in the egg using classical biochemistry, the publication of the chicken genome in 2004 allow to identify more than 1000 novel proteins , some showing some interesting biological activities which is not really surprising when considering that the hens should anticipate all protection for the embryo which has to grow in the egg in an external environment.
Proteomic has been used in all egg compartments and transcriptomic in all tissues implicated in synthesis and secretion of egg proteins.
Use of bioinformatic allows to analyse egg protein sequences and to predict by analogy with mammals putative function of proteins



Hen reproductive organs involved in egg formation are a wonderful model when using transcriptomic approaches because egg formation show a spatial and temporal sequence. The liver and different parts of the oviduct are successively synthesising and secreting the egg components so the comparison of gene expression in the various segments or at different physiological stages allow to identify the genes and proteins which are specific to an egg part . Numerous proteins specific to one step of egg formation have been therefore identified.



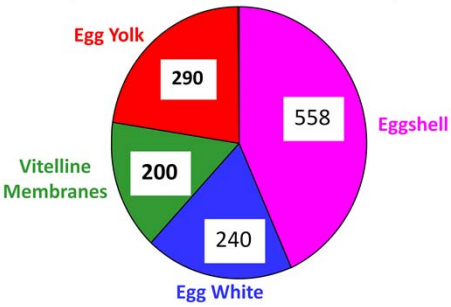
By this approach numerous genes are overexpressed in one segment of the oviduct and code for proteins involved in the synthesis of organic or mineral precursors of egg compartment. Therefore a large number of specific proteins have been revealed by this transcriptomic appraoches



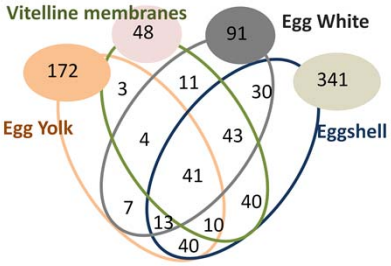
On the other hand, mass spectrometry analysis which was mainly carried out by Karheinz Mann in Germany revealed a large number of proteins present in yolk and vitellin membrane, in egg white and in eggshell. Some of the proteins can be found in different egg compartments but some are observed in only one part.
More than 1000 different proteins are present but you should keep in mind that mass spectrometry is mainly qualitative and is a very sensitive method allowing to detect trace of molecules.
Some proteins are at a very low level! In the eggwhite 12 proteins represents 95% of the eggwhite mass and 240 corresponded to 5 % of the white dry mater!
It is therefore important to identify amongst proteins those functionally important for egg formation, properties or interest for human health and that has been the main objectives of our egg group at INRA

I. Identification of egg proteins

Repartition in egg compartments



Common, shared and specific proteins



- More than 1000 eggs proteins identified but qualitative data
- Some proteins being at very low level (in egg white 12 proteins = 95 % of DM!, 240 = 5%!
- Need to identify amongst proteins those functionally important for egg formation, properties or interest for human health

- Our objectives : Characterize function of main novel egg proteins

II. Function: Identification of Transporter Proteins involved in the Uterine Supply of Minerals

Gene expression:

Microarrays
RNAsec
Real time PCR

Experimental models:

a. Uterus *vs* other parts of the oviduct
b. Uterus presence *vs* absence calcification

Data integration:

Bioinformatic analysis

a.

RESEARCH ARTICLE Open Access

Gene expression profiling to identify eggshell proteins involved in physical defense of the chicken egg

Vincent Jonchère[1], Sophie Réhault-Godbert[1], Christelle Hennequet-Antier[1], Cédric Cabau[1], Vonick Sibut[1,3], Larry A Cogburn[2], Yves Nys[1], Joel Gautron[1*]

a.
b.

RESEARCH ARTICLE Open Access

Identification of uterine ion transporters for mineralisation precursors of the avian eggshell

Vincent Jonchère, Aurélien Brionne, Joël Gautron and Yves Nys*

b.

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

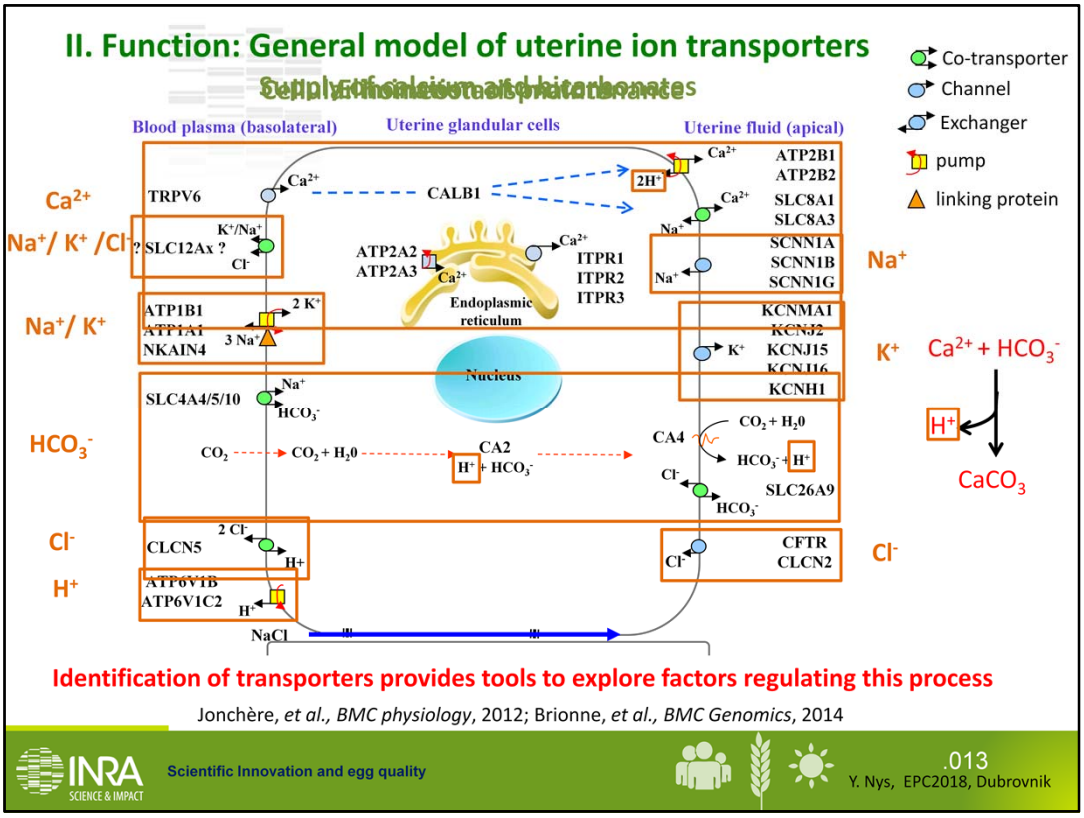
Aurélien Brionne, Yves Nys, Christelle Hennequet-Antier and Joël Gautron*

General model describing the uterine ion transporters during eggshell calcification

- Co-transporter
- Channel
- Exchanger
- pump
- linking protein

12

The first example I would like to present concern the Identification of transport proteins involved in the uterine supply of minerals.This process control the amount of eggshell which largely influences eggshell strength.
The comparison of gene expression in the uterus relative to other oviduct parts or during the period without or with eggshell formation, using microarrays, RNAseq or real time PCR and bioanalysis allowed us to identify a large number of ionic transporters and to define a model for uterine Ca secretion.



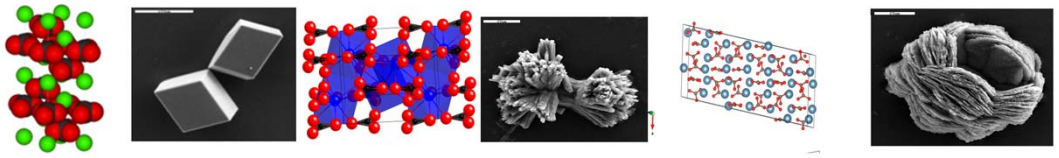
- 3 compartments are involved in the transfer of ions : the blood, the uterine glandular cell, and the uterine fluid where shell calcification takes place.

- For the calcium there is a passive input into the cell through a calcium channel, followed by calcium intracellular transport linked to a calcium binding protein (CALB1), and followed by an active secretion to the uterine fluid by calcium pumps or exchangers.
  - Bicarbonates mainly originate from the blood by diffusion of dissolved carbon dioxide across the plasma membranes, followed by the formation of bicarbonates catalyzed by the carbonic anhydrase 2 enzyme, and followed by an output of bicarbonates to the uterine fluid by anion exchangers.
  - Additional ionic fluxes are needed for the maintenance of cellular homeostasis (cellular volume, electro neutrality, and electrochemical gradients). it requires absorption or secretion of other ions such as chloride, potassium and sodium between the three compartments.
  - it is also necessary to remove the protons produced during the calcification by an active transport back to the blood involved the H+ ATPase

This description is indeed not exhaustive and we still identify additional ionic transporters. The identification of ionic transporters provide however tools for exploring factors regulating shell mineral precursors the amount of which controls shell strength

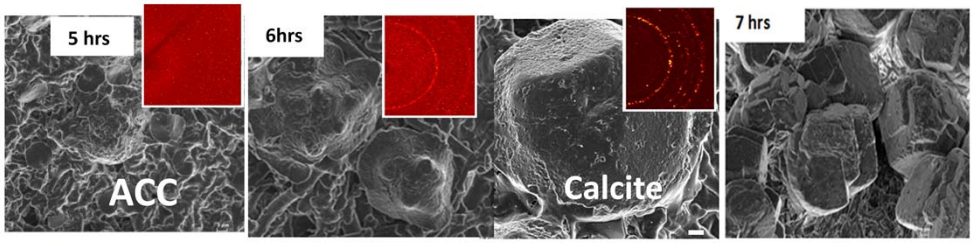
II. Function: Mechanisms of Eggshell Biomineralization

✓ The non crystallized amorphous calcium carbonate is transformed to 100 % calcite not to aragonite nor to vaterite in presence of shell matrix extract



Specific organic matrix components (proteins) control calcium carbonate polymorphism

• **early stages**: presence of amorphous calcium carbonate (transient phase) shown by SEM, FTIR, XRD



Eggshell mineralization occurs in the uterus on specific nucleation site present on the surface of the eggshell membrane. The shell formation is following a temporal sequence including the nucleation phase, the rapid crystal growth phase and the arrest of shell mineralization.

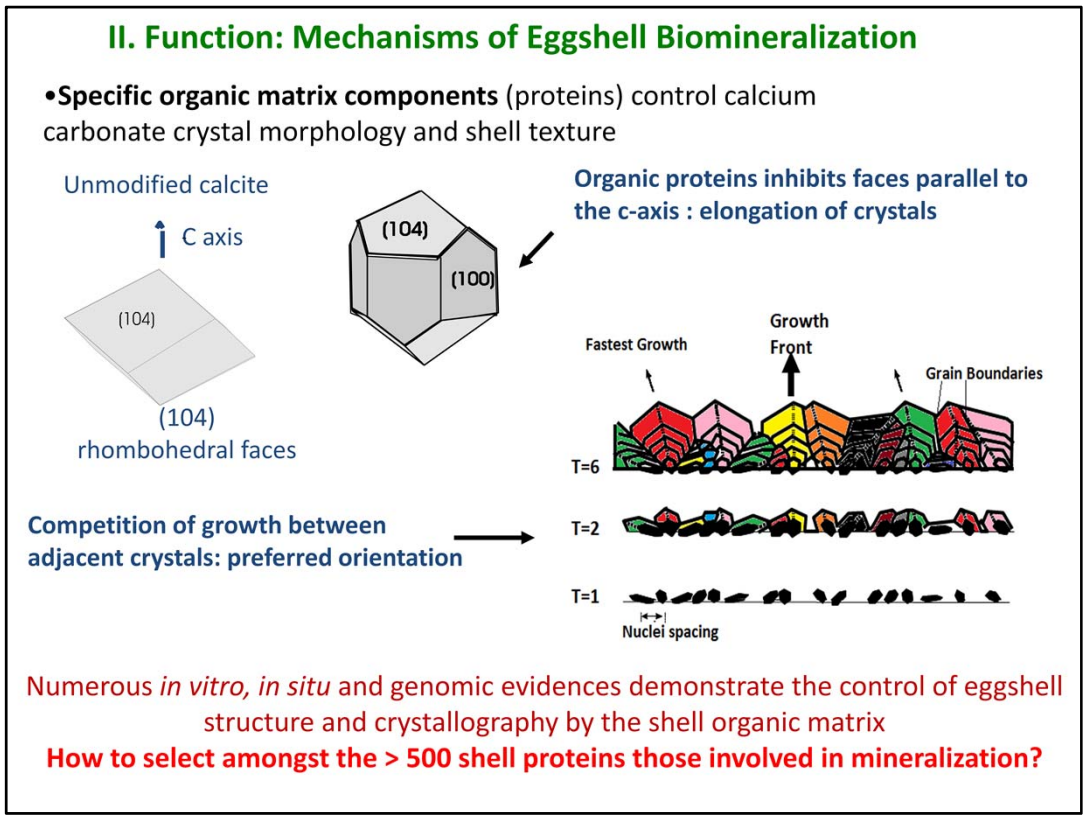
The Calcium carbonate is deposited only in the form of **calcite** from the **uterine fluid** which is hyper saturated relative to calcite for (Ca- HCO_3^-)). The polymorphism of the crystal is controlled by the shell organic matrix as demonstrated in vitro.

Recently the presence of Amorphous calcium carbonate as a preliminary phase of calcite formation has been demonstrated by scanning electronic microscopy, infra red spectroscopy and Xray analysis

Shell organic molecule interact directly with the face parallel to the C axis of the calcite and elicited an elongation of the crystal. This elongation of crystal explain the increase in size of crystal and appearance of preferred orientation because only crystal roughly parallel to the egg surface will growth due to competition of space between adjacent growing crystals

Numerous in vitro, in situ and genomic evidences demonstrate the control of eggshell structure and crystallography by the organic matrix

Our current priority is to select amongst the more than 500 proteins those involved in the process of mineralization of the shell



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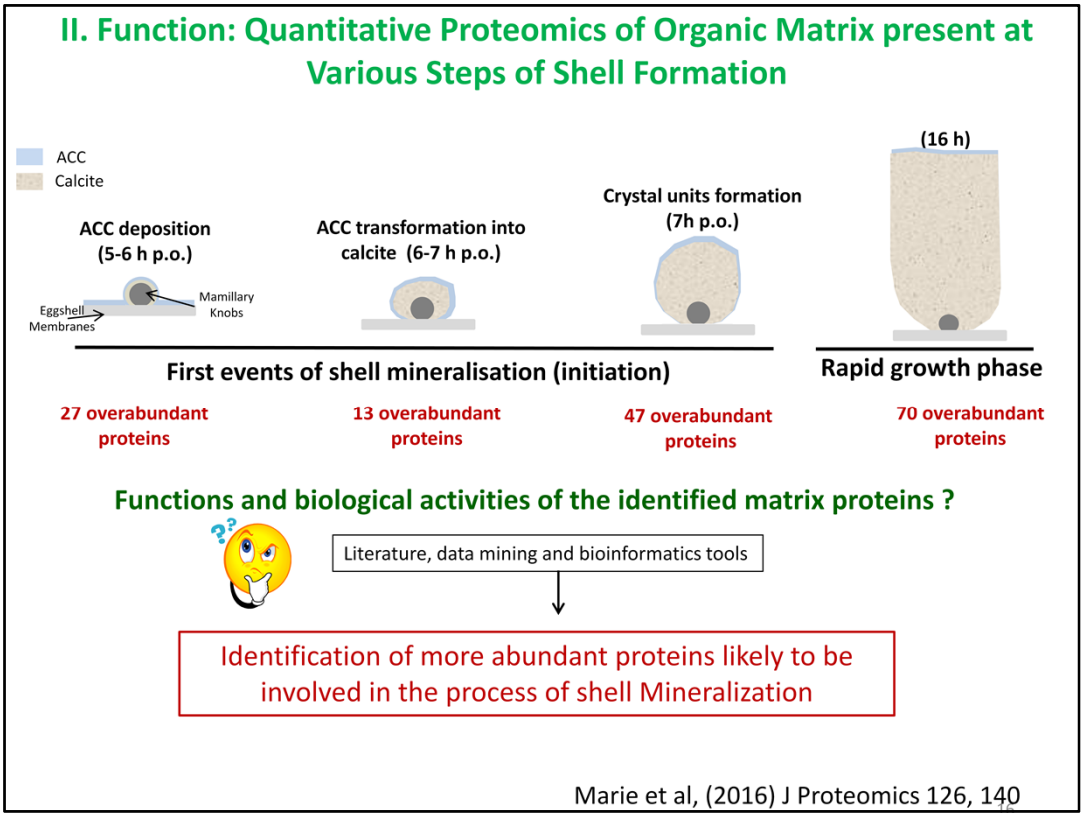
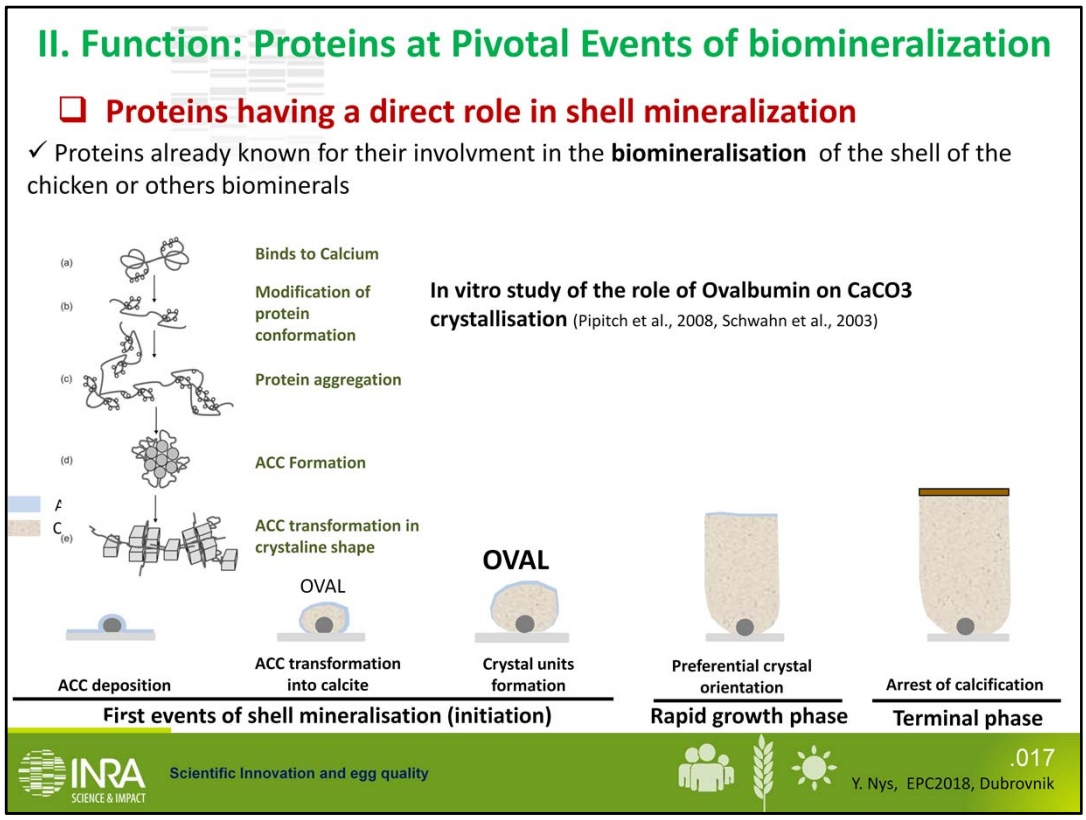


Recently, we used some semi quantitative proteomic approaches to identify the proteins involved at a particular stage of eggshell formation. We compared the abundance of 200 proteins at four stages of eggshell formation, the initial step of amorphous calcium carbonate deposition, its transformation to calcite, the formation of crystal units and the phase of rapid growth. We observed that some proteins were more abundant at a particular phase then analyzed their function and biological activities using bio informatics tools. This approach allow to identify the likely to be involved to the process of shell mineralization.



Amongst the Proteins having a direct involvement in shell mineralization, we observed the presence of ovalbumin at the earlier stage of shell formation. Recently In vitro studies of the role of Ovalbumin on CaCO3 crystallization suggest that this proteins bind to Ca which induce a change in its conformation and favor its aggregation in a shape favoring the formation of amorphous calcium carbonate and its transformation to calcite crystal.

II. Function: Proteins at Pivotal Events of biomineralization

❑ Proteins having a direct role in shell mineralization

✓ Proteins already known for their involvment in the **biomineralisation** of the shell of the chicken or others biominerals

Freeman et al, 2010

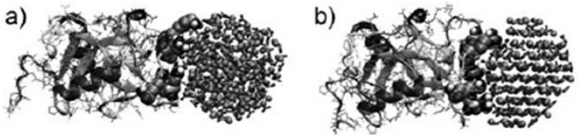
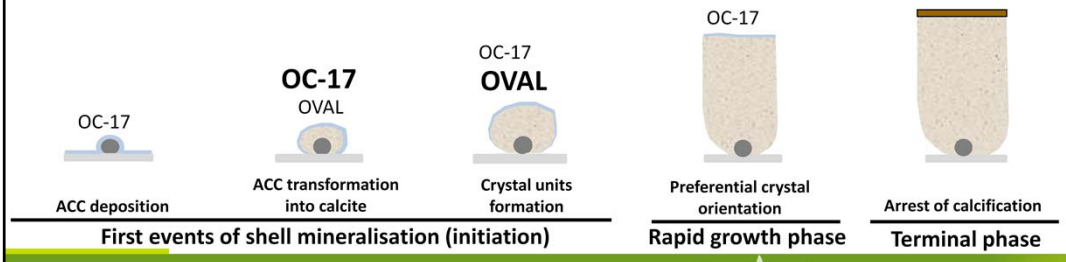
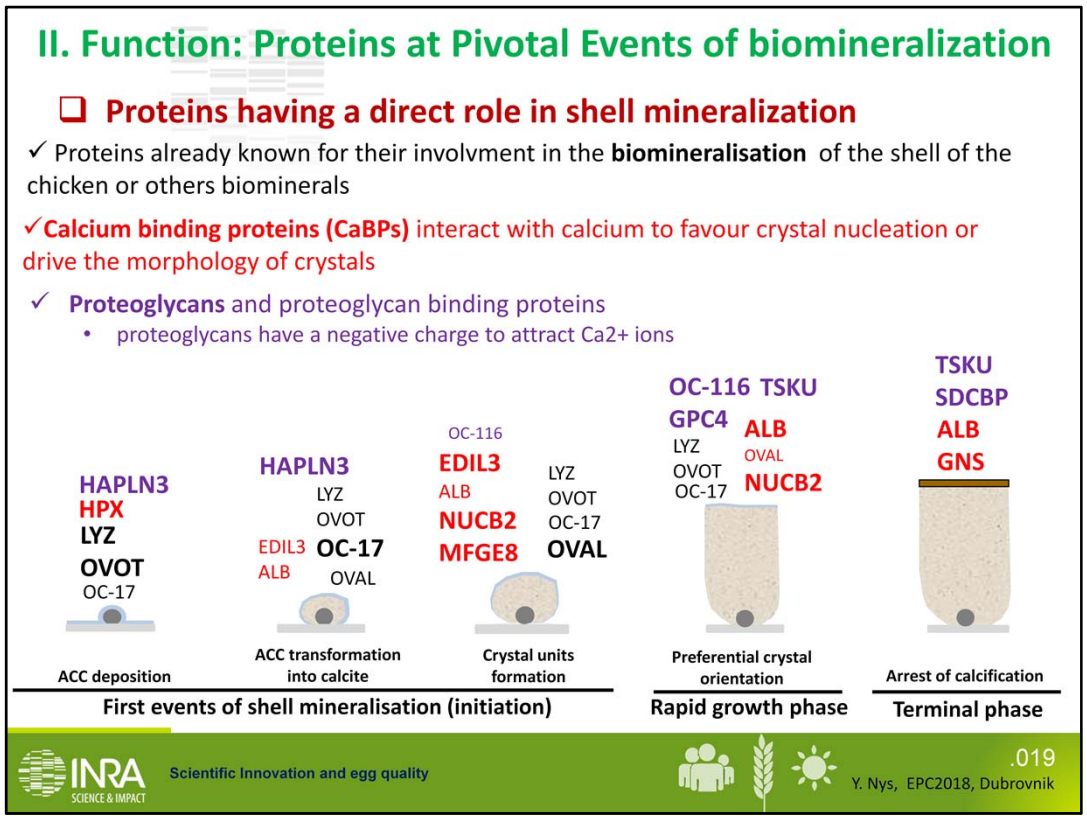


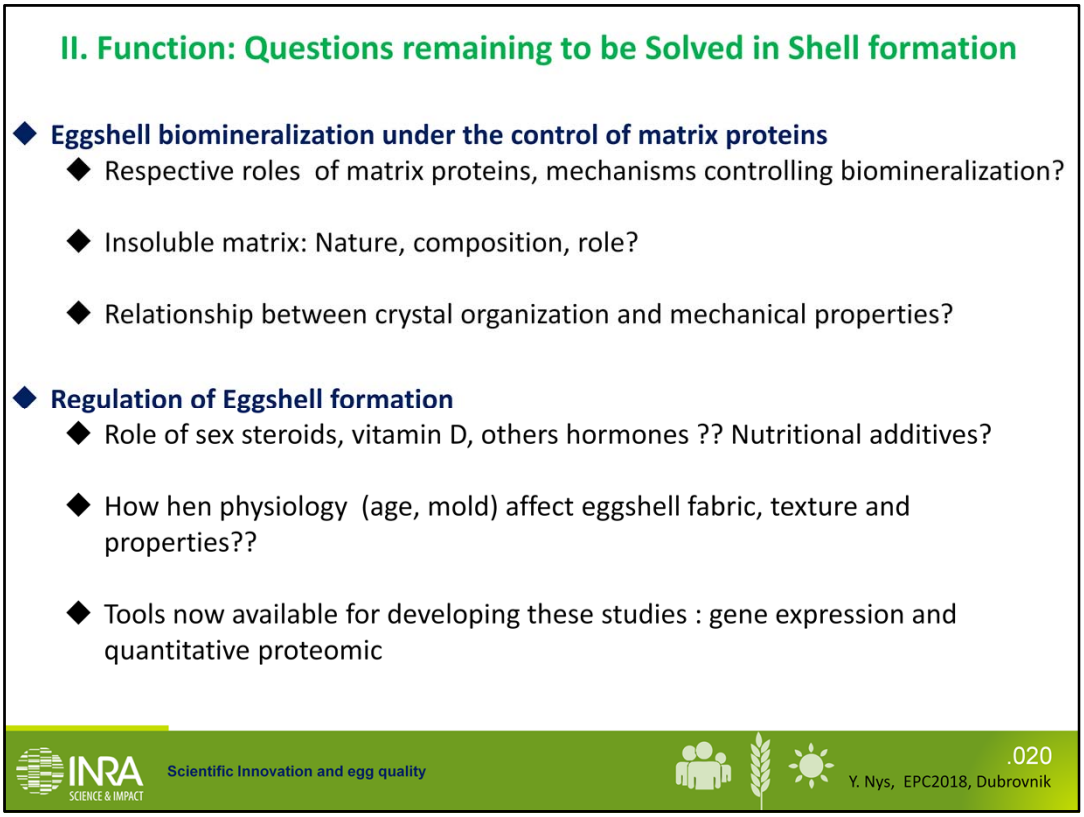
Figure 1. Ovocleidin-17 bound to an amorphous (a) and a crystallized (b) calcium carbonate nanoparticle containing 192 formula units. T'



Ovocleidin 17 is also present at higher concentration when ACC is transformed to calcite and might be involved in this process as suggested by Freeman using a modelling approach.

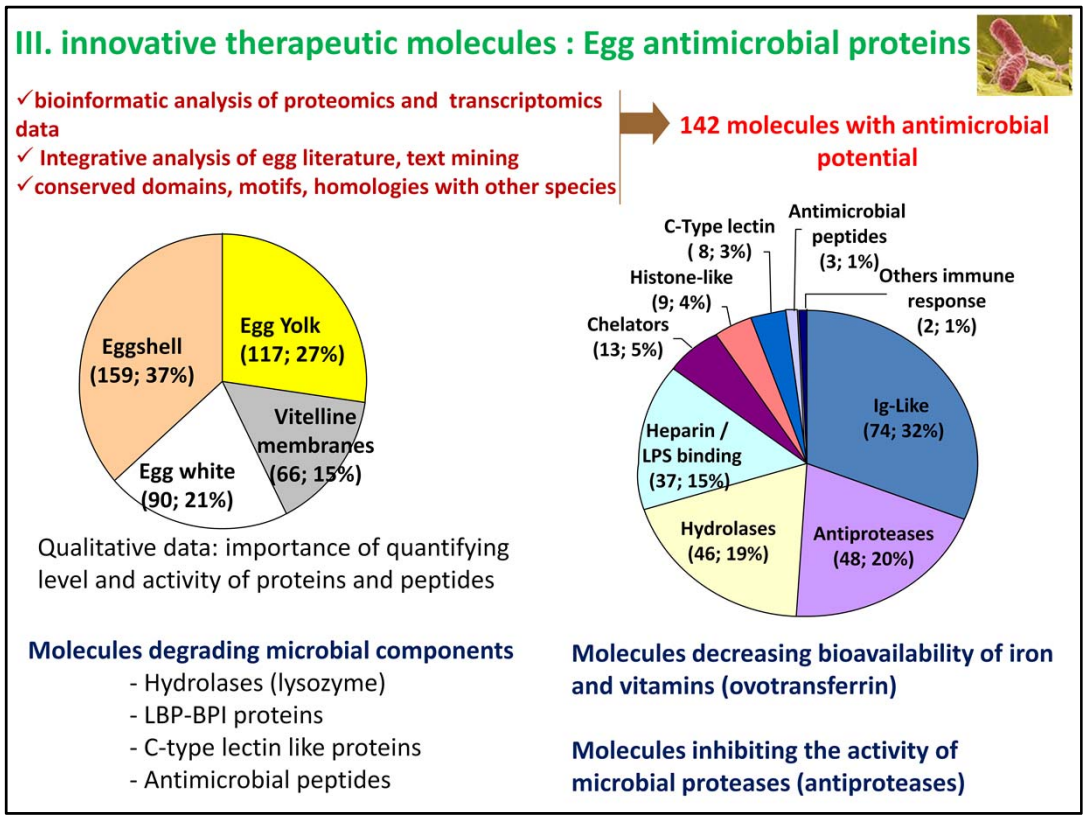


Some Calcium binding proteins are present at high level and are suspected to be involved in the process. Similarly Proteoglycans have the capacity to bind calcium, they are present at high level either at the initial stage or the rapid growth phase of shell mineralization and are suspected to play a key role as suggested initially by the group of Arias in Chili. Numerous of these proteins currently are under study to analyze their function and some evidence for secretion of amorphous calcium carbonate from uterine cells will be presented tomorrow early afternoon
in the session on egg safety and quality



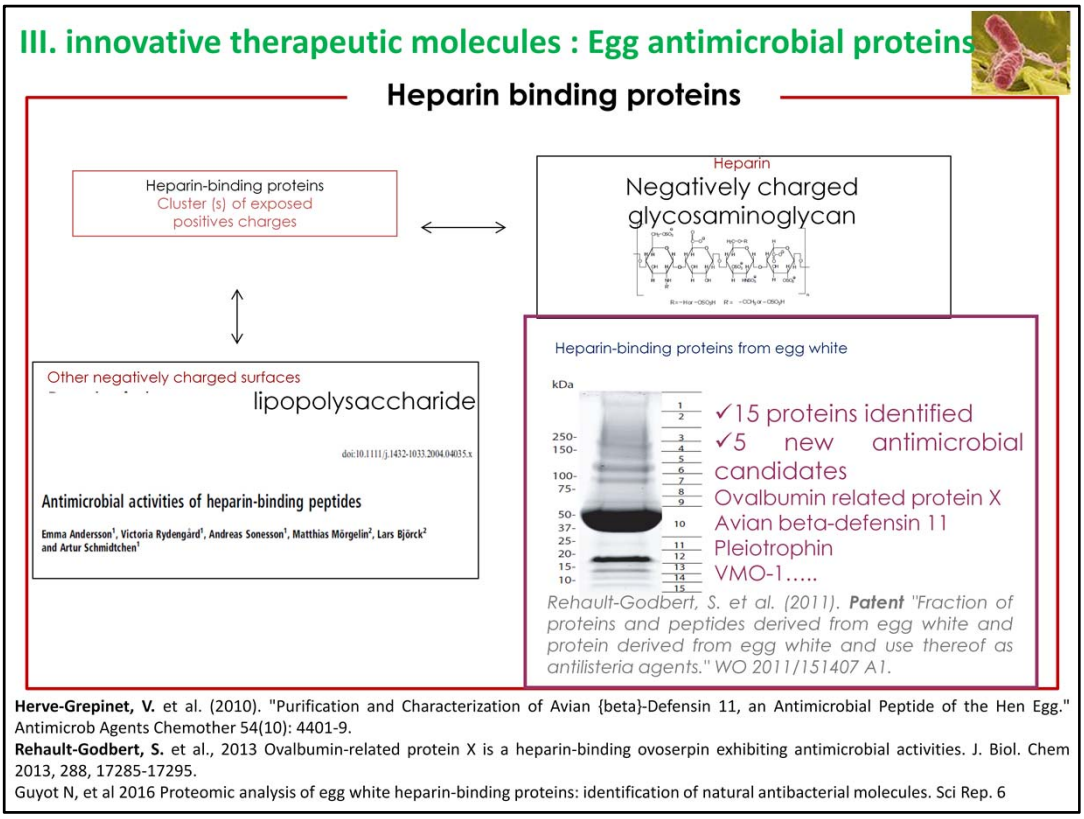
In conclusion, there is clearly a better understanding of the mechanism of eggshell formation and of the control of the shell ultrastructure and crystallography by organic matrix. It is well established that genetic, physiology and nutrition affect either the eggshell mass or fabric and the identification of the mechanisms of uterine ionic transporters and of eggshell mineralization should help to identify origin of shell defect and reduce their impact.

The role of matrix proteins is clearly established but many questions remained to be solved:
What are the respective roles of the numerous matrix proteins, by which mechanisms they controlled the biomineralization
Little information are available on the Insoluble matrix components
Are there post translational modification of active proteins?
What are the relationship between eggshell crystallography and mechanical properties.
Finally we have limited information on the regulation of the process of eggshell formation or on regulation of the proteins involved in uterine mineral secretion or eggshell mineralization :
What are the role of sex steroids , vitamins, nutritional factors?
How hen physiology influence the process of eggshell formation?



Proteomic and transcriptomic approaches revealed a large number of bioactive molecules in the egg. Bioinformatics using text mining, and search for active domains in other species allow to identify more than 140 antimicrobial proteins in the different compartments of the egg. The larger number was observed in the eggshell but you have to keep in mind that proteomic provides qualitative data. Indeed it is well establish when considering the concentration of protein that the egg white is the more active compartment against microbial contamination. In the eggshell the level of antimicrobial protein is very low but will be active only when protein are solubilized which is also an important prerequisite for a protein to be biologically active.

A large range of families are present amongst the antimicrobial proteins: we observed some hydrolase such as lysozyme, some Lipopolysaccharide Binding and Bactericidal Permeability Increasing proteins C type lectin like proteins and antimicrobial peptides, numerous of them being present in the fraction obtained by affinity chromatography using heparin. We revealed also some molecules decreasing bioavailability of nutrient such as ovotransferin of course but also novel one and numerous proteins inhibiting the activity of protease.



Herve-Grepinet, V. et al. (2010). "Purification and Characterization of Avian {beta}-Defensin 11, an Antimicrobial Peptide of the Hen Egg." Antimicrob Agents Chemother 54(10): 4401-9.
Rehault-Godbert, S. et al., 2013 Ovalbumin-related protein X is a heparin-binding ovoserpin exhibiting antimicrobial activities. J. Biol. Chem 2013, 288, 17285-17295.
Guyot N, et al 2016 Proteomic analysis of egg white heparin-binding proteins: identification of natural antibacterial molecules. Sci Rep. 6

One elegant methodology to concentrate antimicrobial proteins in an egg white fraction is the use of affinity chromatography using heparin. This glycoaminoglycan has a structure and negative charge mimicking the negatively charged molecules present at the surface of some bacteria and contributing to adhesion to the host cells such as bacterial peptidoglycan or lipopolysaccharides. this technic has allowed to concentrate 15 antimicrobial proteins present at low concentration in the egg white, including ovalbumin related protein X, avian beta defensins, pleiotrophin and VMO1.

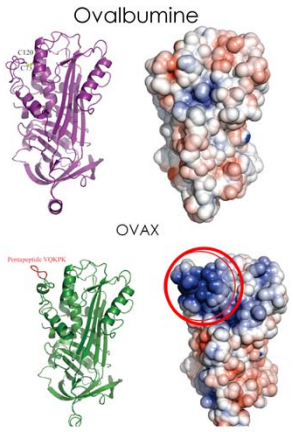
III. innovative therapeutic molecules : Egg antimicrobial proteins

ANTIMICROBIAL COMPONENTS

Ovalbumin-related protein X (OVAX)
Purified from egg white using various chromatographic steps
Present in all compartments but mainly in eggwhite
- 77% of protein sequence homology with ovalbumin

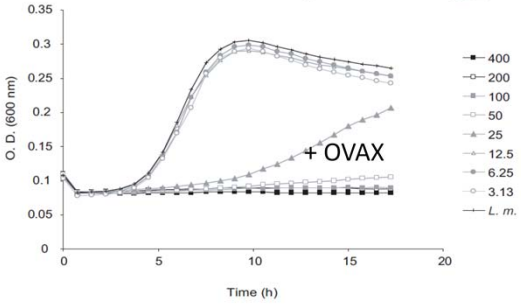
Ovalbumin 50 mg/mL
OVAX 0.1-0.3 mg/mL

- predicted sequence : belongs to the serine protease inhibitor family (serpin) but Ovalbumin and OVAX express no antiprotease activity



Antimicrobial activity of OVAX (in contrast to ovalbumin)
- *Salmonella enterica* Enteritidis
- *Listeria monocytogenes (as low as 25 µg/mL)*
- *activity blocked by heparin and reduced by an increased pH observed during egg storage)*

(Akasawa et al, 2018)



Cluster of positive charge only present on OVAX able to interact with LPS (O)

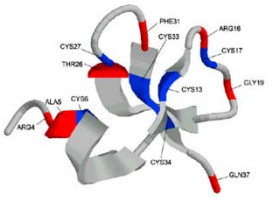
This approach revealed the ovalbumin-related protein X, a glycoprotein of about 45-50 kDa. This protein has 77% of protein sequence homology with ovalbumin and is at a concentration 100 fold lower than ovalbumin which is the major protein od the eggwhite (>50%).
It belongs to the serine protease inhibitor family but both ovalbumin and ovalbumin related protein X shows no anti-protease activity in vitro.

OVAX in contrast to ovalbumine show antimicrobial against Salmonella enteritidis and at a lower concentration against listeria monocytogen. The comparison of the spatial conformation of ovalbumin and ovalbumin related protein X revealed the presence only in OVAX of a cluster of positive charge able to interact with lipopolysaccharide of gram negative bacteria. This conformation change of the protein is at the origin of the antimicrobial activity as demonstrated by the bmockage of the antimicrobial activity of OVAX by heparin
This antimicrobial activity is blocked by heparin demonstrating the importance of the positive cluster to interact with bacteria wall.

III. innovative therapeutic molecules : Egg antimicrobial proteins

Avian beta defensins: host innate defence

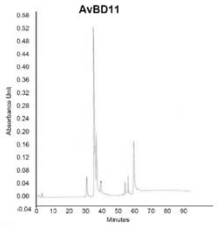
Cationic peptides (2-6 kDa)
6 cysteines involved in 3 disulfide bonds (very stable)
Broad spectrum of activity



Beta-defensins

Protein Name	Localization
AvBD-11	ES, EW,VM
AvBD-10	ES
Gallin	EW
AvBD-9	Ut

Purification (HPLC)



Gallin/OvoDA1 is active against pathogenic and non-pathogenic *E. coli* strains, but not against *Salmonella*
AvD11 long size beta-defensin (9.2 kDa) composed of two beta-defensin motifs.

Antimicrobial tests (Lehrer)

Bacterial group, species	MIC[a] (μM) (95% confidence interval)	
	MSI-94[b]	AvBD11
Gram positive		
S. aureus ATCC 29740	0.33 (0.19–0.48)	0.90 (0.27–1.7)
L. monocytogenes	0.28 (0.13–0.43)	0.18 (0.08–0.27)
Gram negative		
S. Enteritidis ATCC 13076	0.31 (0.25–0.35)	0.35 (0.27–0.46)
S. Enteritidis LA5	0.15 (0.10–0.21)	0.40 (0.29–0.49)
S. Typhimurium ATCC 14028	0.25 (0.11–0.40)	0.32 (0.31–0.32)
E. coli ATCC 25922	0.37 (0.23–0.52)	0.05 (0.04–0.05)

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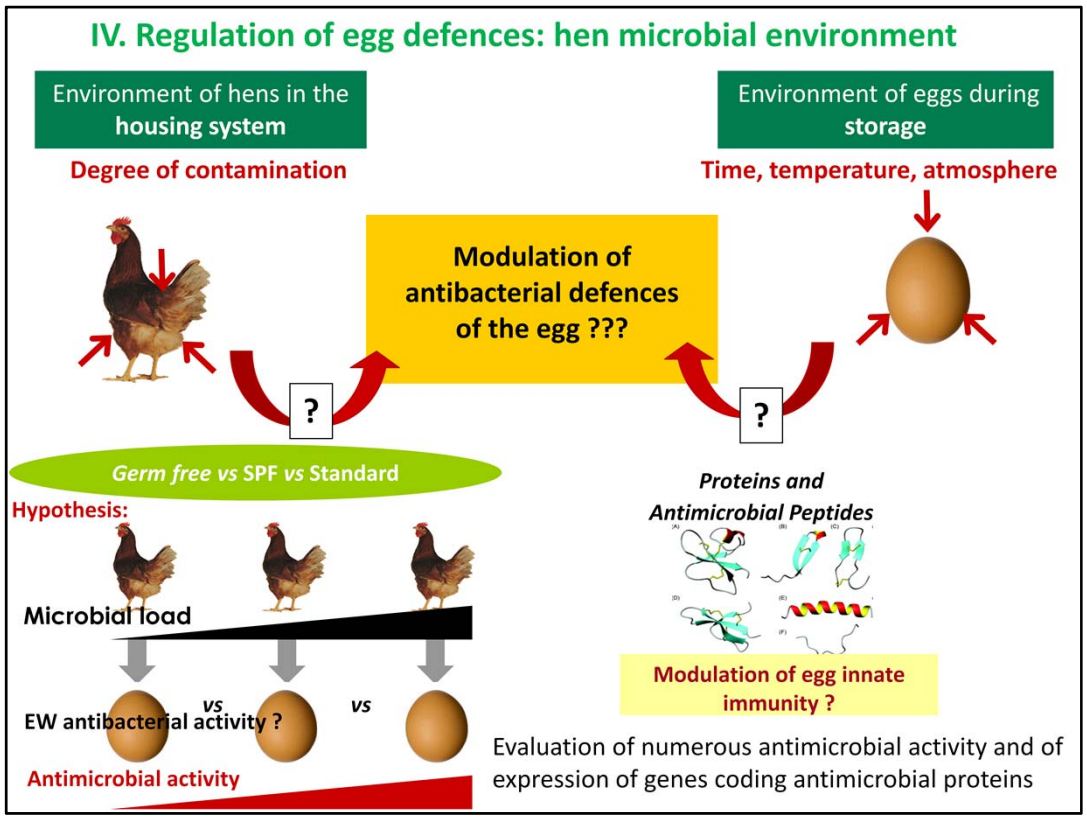
Defensins are cationic peptides of 2-6 kDa involved in the innate defence of organism found in many species, vertebrate, invertebrate and plants. This antimicrobial peptides contain 6 cysteins involved in 3 disulfide bond and are therefore very stable. The majority of these peptides show a broad spectrum of activity against gram positive and gram negative bacteria but also against fungi or viruses. They directly interact with the bacterial cell walls inducing disruption of membrane. In birds only beta defensins are present In the egg the main defensin are AvBD 11 present in three egg compartment , AvBD 10 and 9 in shell and gallin in Egg white.
Gallin or ovodefensin 1 is present in egg white of different bird species and also in vitelline membrane is active against E Coli but not against Salmonella Enteritidis. Its activity decrease during egg storage.
Of particular interest if AvBD 11 because it is a unique long size beta defensin with two active defensin motifs. It is active against a large range of gram positive and gram negative bacteria including Salmonalla Entiritidis and Typhimurium and against E Coli. Its activity is inhibited by heparin suggesting that this binding site interact with bacteria membrane.



The hens are anticipating the protection of the embryo in the egg by supplying in the egg white numerous antibacterial molecules. We therefore explored if the degree of contamination of the hen environment will stimulate the innate protection of the egg as it has been demonstrated for yolk antibodies. On the other hand, it is well established that egg storage modify the physicochemical properties of egg white (Ph and viscosity) and the question arise if these changes induced by duration and temperature of storage will modulate the antimicrobial potential of egg white.

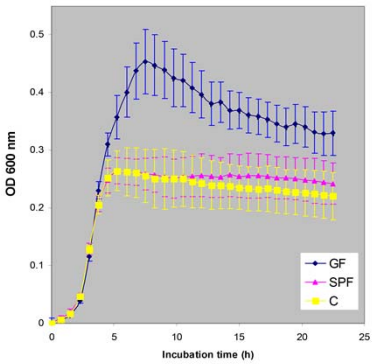
We use a very powerful model to study the effect on passive immunity of the egg of hen microbial environment by comparing three extreme breeding conditions, Germ-free (GF), specific pathogen free (SPF) and conventional (C) hens. We measured the egg antibacterial activity but also putative changes in the activity of numerous antibacterial molecules involved in sequestration of nutrient, in inactivation of exogenous protease or in hydrolysing bacteria wall.

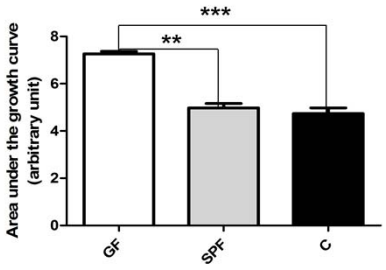
The difference in their immunological status was confirmed by the high stimulation of IL-1β, IL-8 and TLR4 genes in the intestine of C and SPF groups.

IV. Regulation of egg defences: hen microbial environment

Antibacterial activity : *Streptococcus uberis*

Growth of *Streptococcus uberis*





Germ free/ Specific Pathogen Free /conventional

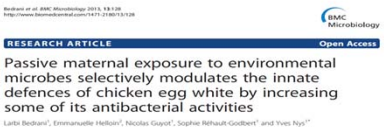
Growth inhibition of *Streptococcus uberis*

C> GF(35%) SPF> GF (26%) C= EOPS

Also observed for *Staphylococcus aureus but not for Salmonella* Enteritidis, *Salmonella* Gallinarum, *Escherichia coli* and *Listeria monocytogenes , no difference in antimicrobial activities of egg white*

★EW antibacterial activities can be enhanced by an immunostimulation of the hen (also observed in hen treated with LPS) but

- Moderate effect
- Dependent on the bacterial strain (Gram+ vs Gram-)
- Dependent on the nature of the immunostimulant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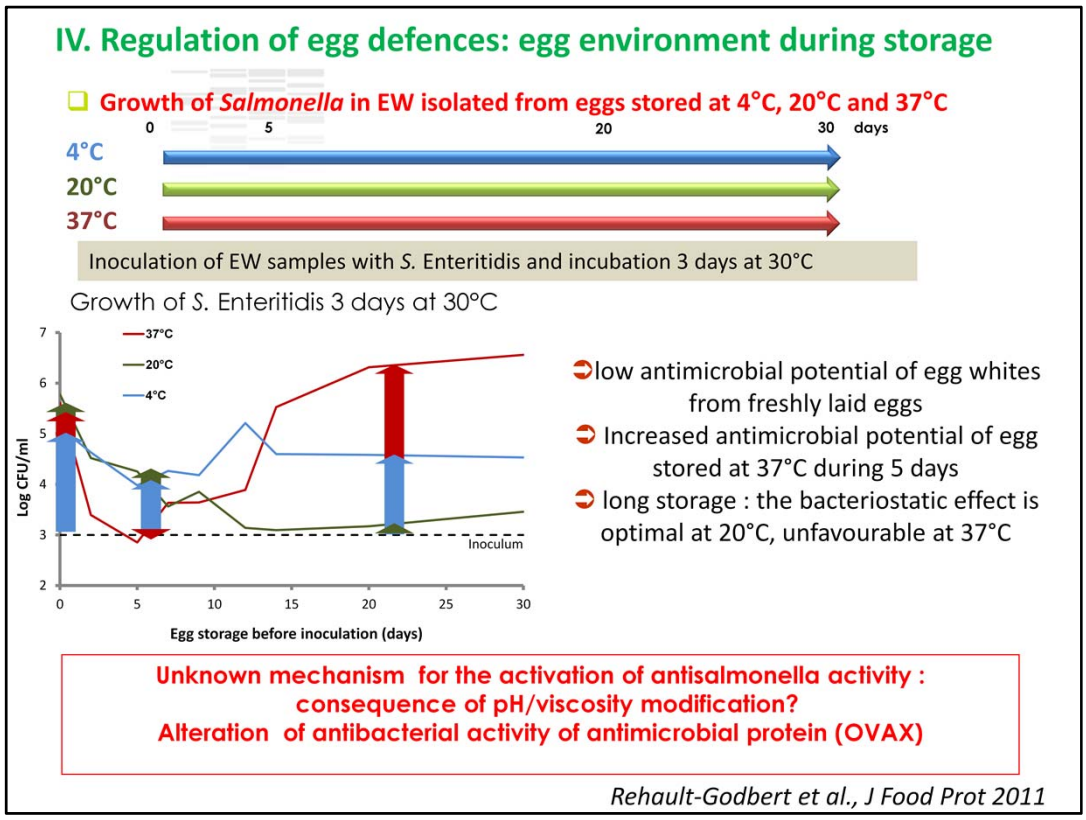
Passive maternal exposure to environmental microbes selectively modulates the innate defences of chicken egg white by increasing some of its antibacterial activities

EW from C and SPF groups demonstrated higher inhibitory effect against *Streptococcus uberis* (31 to 35%) and against *Staphylococcus aureus (*13 to 18%) as compared to GF but did not revealed any change between the three experimental groups (germ free and conventional or SPF hens) when comparing antimicrobial activities measured directly or by quantifying gene expression in the magnum. We observed similar activity against *Salmonella* Enteritidis, *Salmonella* Gallinarum, *Escherichia coli* and *Listeria monocytogenes between the three experimental groups.* Similarly when using a different experimental model, the injection of LPS we observed moderate effect on some antibacterial activity of eggwhite. In conclusion, the microbial environment of hens seems to have moderate influence on the egg innate immunity of eggs.

the degree of environmental microbial exposure of the hen moderately stimulated the egg innate defence, by reinforcing some specific antimicrobial activities to protect the embryo

Lysozyme activity, chymotrypsin-, trypsin- and papain-inhibiting potential of EW and the expression of numerous antimicrobial genes and IL-1β, IL-8 and TL4 were at similar levels in the EW or magnum tissue between the three experimental groups.



It is well established that temperature during egg storage influence the change in physicochemical properties of egg white therefore we explored if it also affected the antimicrobial potential of egg white against salmonella enteritidis. The graph represent the growth of Salmonella in egg white when incubated for three days at 30 °C. We observed that the growth of salmonella was important in freshly laid egg demonstrating a low antimicrobial potential of the egg white . Egg Storage at 37°C activated rapidly the antimicrobial activity but then inhibited this activity. At 20°C the activation was slower but then remain at high level. No activation was observed at low temperature.

Similar observation were observed for anti listeria and anti streptococcus egg white activity.

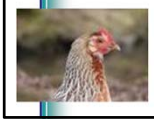
The mechanisms remain understudy , it can be explain by a direct effect of change in Ph and viscosity but it has also be observed that some antimicrobial protein such as OVAX is altered during egg white storage..

These data clearly demonstrated a regulation of antimicrobial potential of egg white by egg storage conditions.









Conclusions

- **The recent development of high-throughput technologies**
  - characterisation of hundreds of novel proteins involved in egg formation or with interesting biological properties
  - Need to quantify level and activity for evaluating their respective role
- **New tools for exploring the mechanisms and regulation of egg formation and of its biological properties**
  - Tools for exploring mechanisms affected by nutritional additives (nutrigenomic) or by hen physiology
  - Tools for analysing origin of gg defect and reinforce its biological and technological properties
  - Biological markers for genomic selection: phenotyping of the egg quality (eggshell breaking strength, internal quality, egg white antimicrobial activity) and localization of genes on genome and search for related SNPs.
- **New impulse in egg research and large opportunities to develop non-food use of egg for human/animal health:**
  - high diversity of functions to be further explored: antimicrobials, antiviral, antiparasitic but also antioxidant , anti-hypertensive, anticancer, tissue remodeling or diagnosis molecules
  - reluctance of pharmacological companies to use animal product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