New insights on egg proteins and their biological activities

Joël Gautron, Aurélien Brionne, Sophie Réhault-Godbert, Yves Y. Nys

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INRA

« Function and regulation of egg proteins »

UR83 Recherches Avicoles

37380 Nouzilly

FRANCE

The Chicken Egg

Au niveau mondial en 2008

→ 60.7 millions de tonnes par an (1140 milliards d'œufs)
→ Accroissement du marché de 2.4 % (période 1998-2008)
→ Premier producteur est la Chine (22.7 MT – 37 % de la production mondiale)

Filière française en 2008

→ 14.3 milliards d'œufs (45 millions de poules pondeuses)
→ 1.11 milliards d'Euros
→ 4 % de la valeur des productions animales
→ 1.7 % de la valeur des productions agricoles

Consommation

→ 145 œufs par an et par habitant dans le monde
→ 248 œufs par an et par personne en France
→ 172 sous forme d'œufs en coquille (69 %)
→ 76 sous forme d'ovoproduits (31 %)

Source ITAVI

Microbiologie de l'œuf et des ovoproduits

→ Au moment de la ponte, le contenu des œufs est généralement stérile
→ Le pourcentage d'œufs frais contaminés reste souvent inférieur à 1 %
→ Contamination verticale est rare

→ La contamination horizontale est beaucoup plus fréquente
→ Se produit après la ponte par contact avec les microorganismes

* fientes
* environnement élevage
* centre de conditionnement
* circuit de commercialisation
* consommateur…

→ Les œufs et produits d'œufs sont impliqués dans 25% des salmonelloses

Le risque de contamination par les microorganismes et notamment *Salmonella* est donc une préoccupation pour la filière œufs et ovoproduits

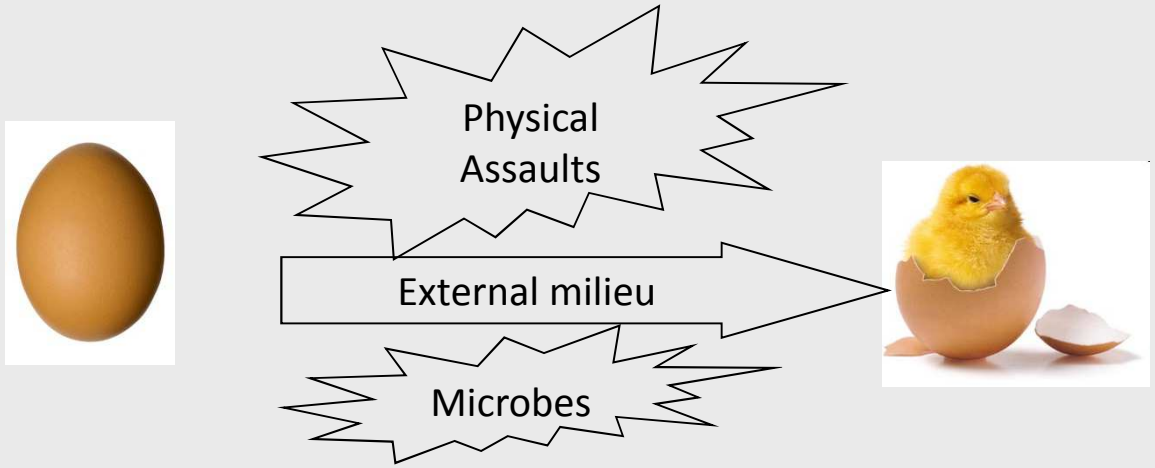
The Chicken Egg

Egg = Basic food for humans all around the world

- Almost perfect nutritional value
- Cheap and well balanced nutrients

Egg = Container for extra-uterine development of the embryo

- Major source of compounds with a broad range of biological activities



- Protective systems (Egg natural defenses)
  - Physical (eggshell as a barrier)
  - Chemical (molecular defense of the egg, components of innate immunity)

Biological activities in the egg ? → Pivotal role of egg proteins

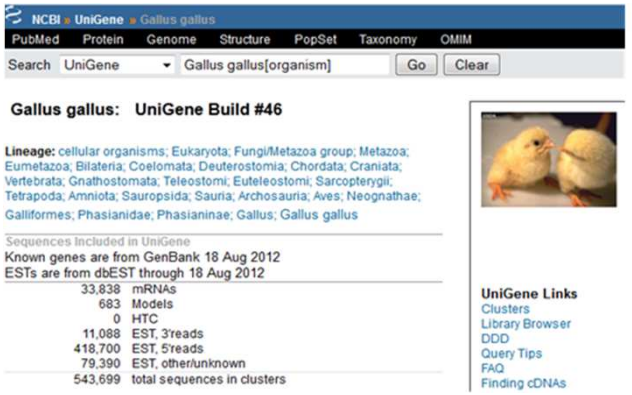
Characterization of egg proteins

- **About 50 egg proteins were identified in 2006**
- **Major advances came recently**

✓2004, Publication of the chicken genome sequence

Availability of « omics » high-throughput techniques and data mining tools to identify novel egg proteins

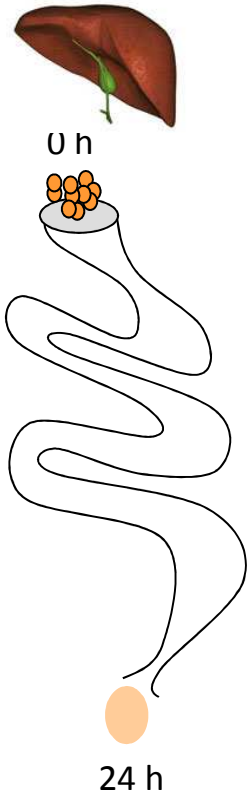
✓ Genome-wide non redundant catalog of 33 838 different genes



✓ cDNA and ESTs libraries
(Identification of 600 434 functional genes in chickens)

« omics » to identify novel egg proteins

✓ **Transcriptomics** *(microarrays, RNA seq…)*



Liver:
Egg yolk proteins (several weeks)

Ovary and infundibulum:
Vitelline membranes (less 1 h)

Magnum:
egg white proteins (1 to 4 h 30)

Isthmus:
eggshell membranes (4h30 to 6 h)

Uterus:
eggshell calcification (6 to 24 h)

Egg formation
Spatial and temporal regulation

Different tissues or organs
Egg components are deposited at different times
Different physiological stages

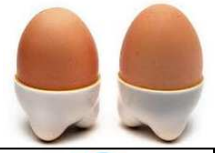
↓

Comparison of gene expression in the various segment of the reproductive tract

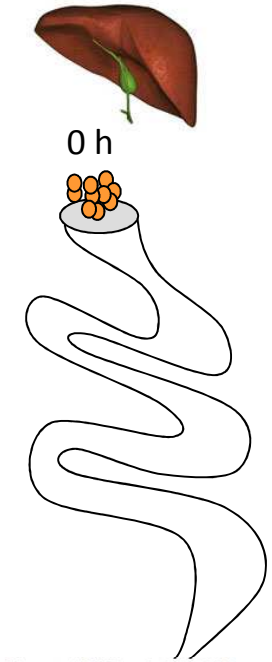
↓

Quantification of genes specifically related to the egg yolk, the vitelline membranes, the egg white, the eggshell membranes and the eggshell calcification process

« omics » to identify novel egg proteins



✓ **Transcriptomics** *(microarrays, RNA seq.*



Liver: **582**
Egg yolk proteins (several weeks)

Ovary and infundibulum:
Vitelline membranes (less 1 h)

Magnum: **828**
egg white proteins (1 to 4 h 30)

Isthmus: **135**
eggshell membranes (4h30 to 6 h)

Uterus: **605**
eggshell calcification (6 to 24 h)

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*

Bourin *et al. BMC Genomics* 2012, **13**:457
http://www.biomedcentral.com/1471-2164/13/457

BMC Genomics

RESEARCH ARTICLE Open Access

Transcriptomic profiling of proteases and antiproteases in the liver of sexually mature hens in relation to vitellogenesis

Marie Bourin, Joël Gautron, Magali Berges, Christelle Hennequet-Antier, Cédric Cabau, Yves Nys and Sophie Réhault-Godbert*

New insights in egg white proteins using cDNA microarrays and extensive proteomic data mining

EggMeat symposia 2011 - Leipzig

Joël Gautron[1], Aurélien Brionne[1], Christelle Hennequet-Antier[1], Cédric Cabau[1], Nicolas Guyot[1], Larry Cogburn[2], Sophie Réhault-Godbert[1], Yves Nys[1]

Identifying specific proteins involved in eggshell membrane formation using gene expression analysis and bioinformatics.

Jingwen Du[a], Maxwell Hincke[a, d], Aurelien Brionne[b], Christelle hennequet-Antier[b], Larry A. Cogburn[c], Yves Nys[b], Joel Gautron[b] **Submitted**

Jonchère *et al. BMC Genomics* 2010, **11**:57
http://www.biomedcentral.com/1471-2164/11/57

BMC Genomics

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*]

« omics » to identify novel egg proteins

✓ **Proteomics** *(Mass spectrometry-based methods for protein identification)*

178 DOI 10.1002/pmic.200700790 *Proteomics* 2008, *8*, 178–191

RESEARCH ARTICLE

The chicken egg yolk plasma and granule proteomes

Karlheinz Mann and Matthias Mann

Max-Planck-Institut für Biochemie, Abteilung Proteomics und Signaltransduktion, Martinsried, Germany

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

ELSEVIER

Chicken egg yolk cytoplasmic proteome, mined via combinatorial peptide ligand libraries

Alessia Farinazzo[a], Umberto Restuccia[b], Angela Bachi[b], Luc Guerrier[c], Frederic Fortis[c], Egisto Boschetti[c], Elisa Fasoli[a], Attilio Citterio[a], Pier Giorgio Righetti[a,*]

2322 DOI 10.1002/pmic.200800032 *Proteomics* 2008, *8*, 2322–2332

RESEARCH ARTICLE

Proteomic analysis of the chicken egg vitelline membrane

Karlheinz Mann

yolk

Vitelline membranes

egg white

shell

3558 DOI 10.1002/pmic.200700397 *Proteomics* 2007, *7*, 3558–3568

RESEARCH ARTICLE

The chicken egg white proteome

Karlheinz Mann

Max-Planck-Institut für Biochemie, Abteilung Proteomics und Signaltransduktion, Martinsried, Germany

research articles Journal of proteome research

2008, 7, 3461-3474

Exploring the Chicken Egg White Proteome with Combinatorial Peptide Ligand Libraries

Chiara D'Ambrosio,† Simona Arena,† Andrea Scaloni,† Luc Guerrier,‡ Egisto Boschetti,‡ Martha Elena Mendieta,§ Attilio Citterio,§ and Pier Giorgio Righetti*,§

Proteomics 2006, *6*, 3801–3810 DOI 10.1002/pmic.200600120 3801

RESEARCH ARTICLE

Proteomic analysis of the acid-soluble organic matrix of the chicken calcified eggshell layer

106 DOI 10.1002/pmic.200600635 *Proteomics* 2007, *7*, 106–115

RESEARCH ARTICLE

Phosphoproteins of the chicken eggshell calcified layer

JOURNAL OF PROTEOMICS 75 (2012) 2697–2706

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/jprot

ELSEVIER

PROTEOMICS

Proteomic analysis provides new insight into the chicken eggshell cuticle

*Megan Rose-Martel, Jingwen Du, Maxwell T. Hincke**

vian eggshell matrix proteins

« omics » to identify novel egg proteins

✓ **Proteomics** *(Mass spectrometry-based methods for protein identification)*

Thousands of protein identifiers in the different egg proteomes from 3 different databases
IPI (closed), GeneBank and UniProt

↓

Lot of redundancies
Majority of them were not annotated

↓

How many in each compartments ?
What is common, what is compartment specific ?
Functions in the egg ?

↓

Data mining and bioinformatics tools

↓

Loading of the sequences, multi alignment to eliminate redundancies

↙ ↘

Repartition in individual egg compartments

Update of functional annotations

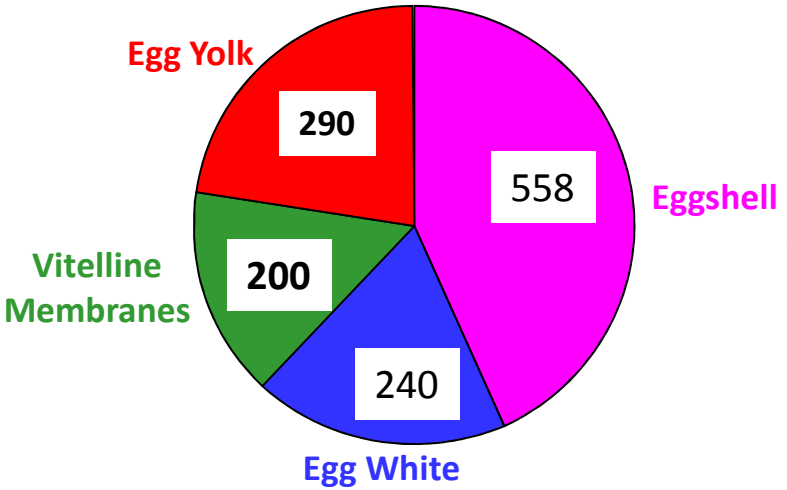
Novel egg proteins

Hundreds of genes encode egg proteins

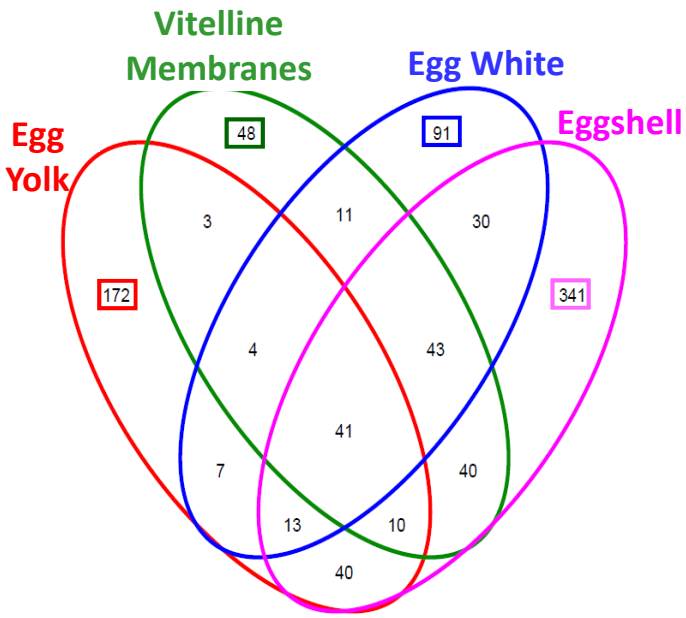
Protein variants
and isoforms

More than 1000 non redundant proteins in the egg

Repartition in egg compartments



Common, shared and specific proteins



Novel egg proteins

Functions and biological activities of the 1174 egg proteins ?

Protein families shared conservative domains and signatures which can be representative of their functions

Egg proteins grouped in 1062 different Interpro Ids

TOP 3 domains

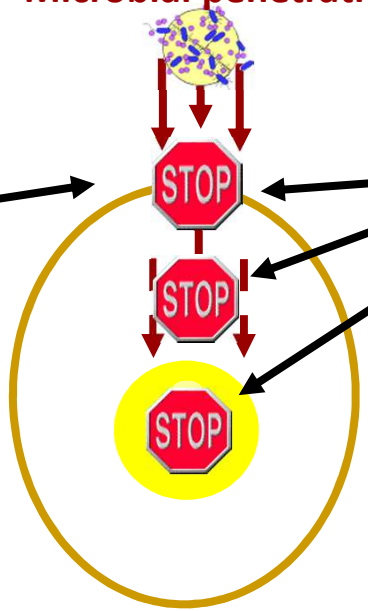
- Ig-like fold, the most important group (75 egg proteins, 2/3 in yolk and shell)
  - ✓ *Immunoglobulins*
  - ✓ *Protein folding ?*
  - ✓ *Antimicrobial defence ?*
- P-loop containing nucleotide triphosphate hydrolase (53 egg proteins)
- Calcium binding proteins (64 proteins, 38% in the shell)

Still a lot to do to have a global overview of the represented functions

Novel egg proteins and egg defences

Microbial penetration

Physical
(Eggshell as a barrier)



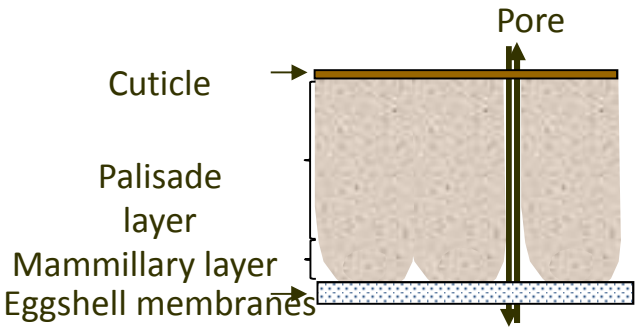
Chemical
Antimicrobial activities
(White, shell, yolk)

Microbial quality of eggs ?

Human foodborne disease

Eggshell proteins and physical defence

➢ **calcification process**



Calcium carbonate
95%

quantity

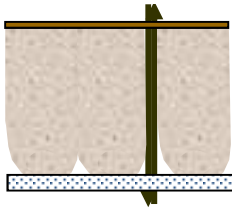
Interactions

Ultrastructure
Mechanical properties

control of biomineralisation process
(crystal polymorphism, morphology)

Organic matrix
(proteins and proteoglycans)
3.5 %

Eggshell proteins and physical defence



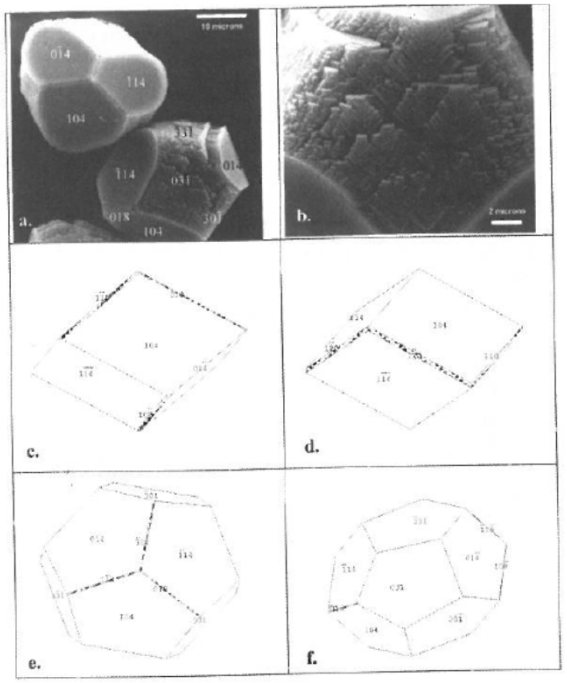
➢ calcification process

✓ Proteins involved in the **biomineralization** of shell

- *OC-17*
- *OC-116*
- *Ovotransferrin*
- *OCX-32*
- *Osteopontin*
- *Lysozyme*

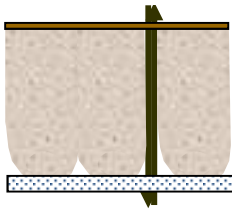
Ovotransferrin is a Matrix Protein of the Hen Eggshell Membranes and Basal Calcified Layer

J. GAUTRON[a], M.T. HINCKE[b], M. PANHELEUX[a], J.M. GARCIA-RUIZ[c], T. BOLDICKE[d] and Y. NYS[a,*]



Proteins interact with minerals to influence the morphology of crystals

Eggshell proteins and physical defence



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- *Ovotransferrin*
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- *Osteopontin*
- *Lysozyme*

Angewandte International Edition Chemie

Biomineralization

DOI: 10.1002/anie.201000679

Structural Control of Crystal Nuclei by an Eggshell Protein**

*Colin L. Freeman, John H. Harding, David Quigley, and P. Mark Rodger**

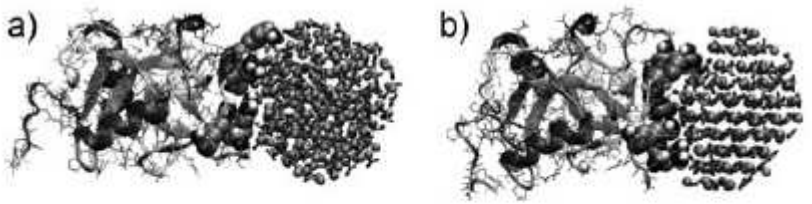
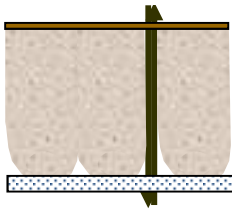


Figure 1. Ovocleidin-17 bound to an amorphous (a) and a crystallized (b) calcium carbonate nanoparticle containing 192 formula units. The

Eggshell proteins and physical defence



➢ **calcification process**

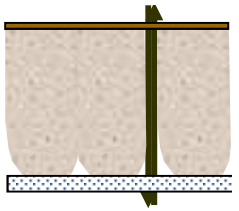
✓ Proteins involved in the **biomineralization** of shell

- *OC-17*
- *OC-116*
- *Ovotransferrin*
- *OCX-32*
- *Osteopontin*
- *Lysozyme*

✓**Calcium binding proteins (CaBPs)** to interact with calcium to favour crystal nucleation or drive the morphology of crystals

- Identification of numerous novel CaBPs
  - *A total of 23 proteins with EF-hand and EGF-like calcium binding domains are present in the shell*

Eggshell proteins and physical defence



➢ calcification process

➢ Proteins involved in the **proper folding of the eggshell matrix**

✓*An appropriate conformation of proteins is required to ensure calcium and matrix interactions and to ensure template to the mineralized structure*

- *Molecular chaperone*
- *Protein assisting folding*
- *Proteins with interactive properties related to proteoglycans*

➢ **Regulation of the activity of proteins** related to the shell deposit

✓*Shell mineralisation occurs in a non cellular milieu*

✓*In situ by direct action of proteins to inhibit or activate the molecular actors present in the milieu.*

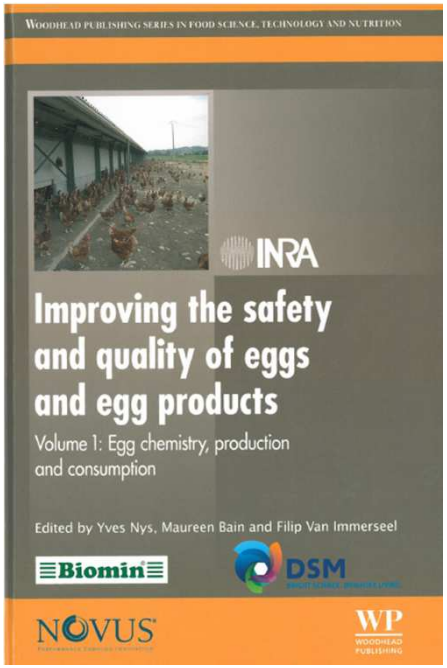
- *Molecular chaperone which can interact with proteins might be involved in this regulation process.*
- *Proteases and proteases inhibitors (specific and controlled role during calcification process, either by degrading proteins or regulating processing of proteins into their mature forms)*

Egg antimicrobial proteins (chemical defence)

2011

Molecules involved in chemical defence of the chicken egg

S. Réhault-Godbert, V. Hervé-Grépinet, J. Gautron, C. Cabau and Y. Nys, Institut National de la Recherche Agronomique, France and M. Hincke, University of Ottawa, Canada



➢ **Examination of egg protein sequences for specific domains related to molecular defence**

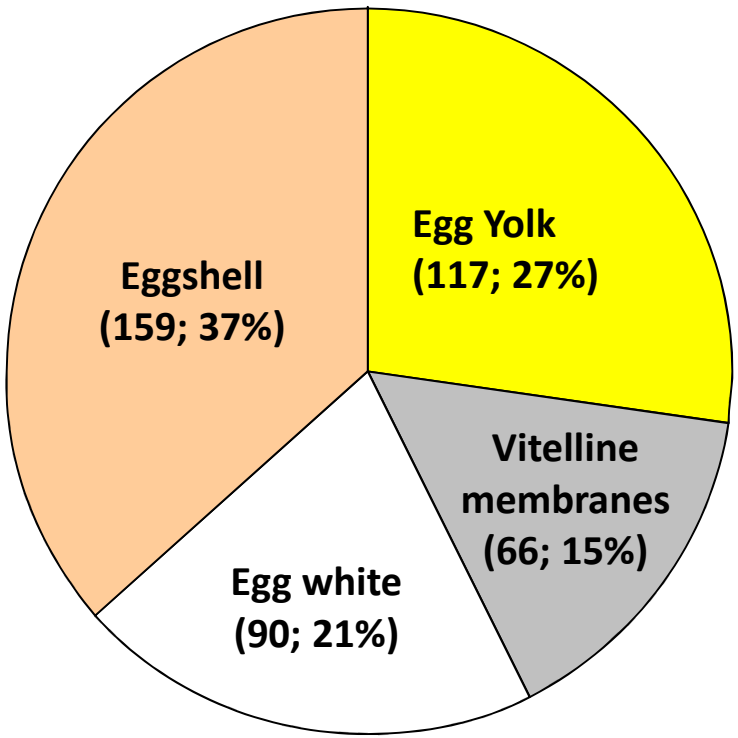
➢ **Identification of 142 molecules which could potentially degrade microbial components**

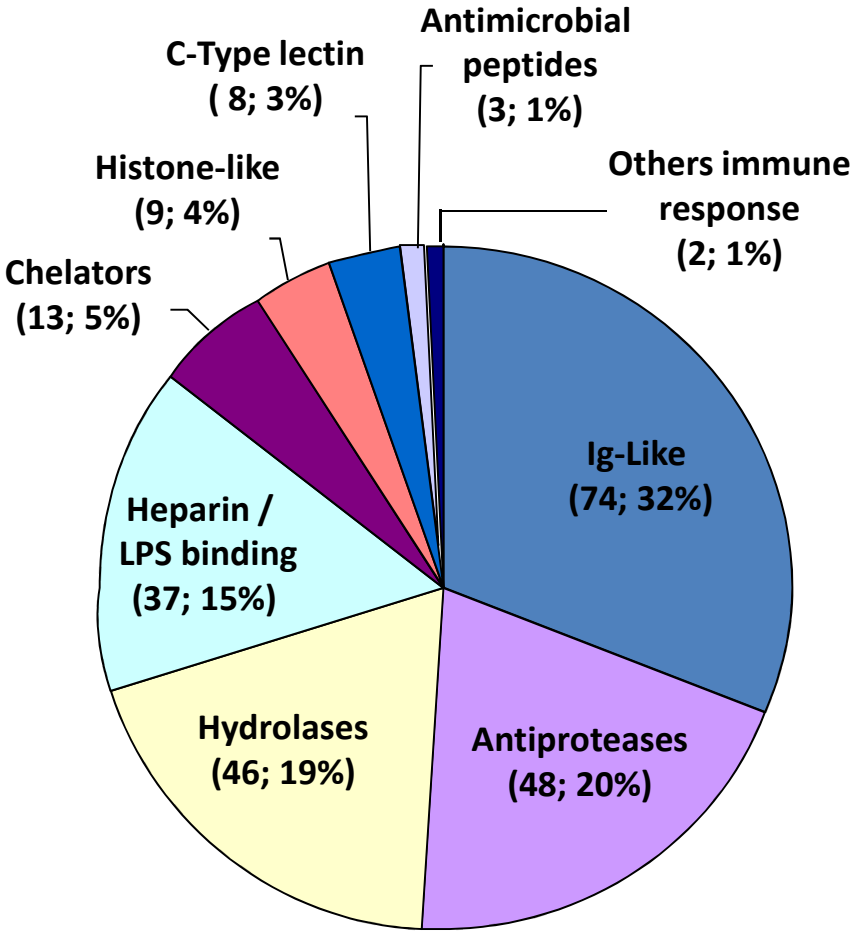
Use of the same InterPro domains and signature
→ 149 functional domains grouped in 9 families

Use of the most recent egg protein inventory
→ 1174 protein sequences

219 different proteins potentially involved in the chemical protection of the egg

Egg antimicrobial proteins (chemical defence)





Egg antimicrobial proteins (chemical defence)

Immunoglobulins superfamily (74 in egg)

Immunoglobulins

Yolk IgY most predominant in egg (>1mg/ml). Also IgM and IgA in yolk (20 µg/ml).
IgY, IgM and IgA in egg white (10 µg/ml). Evidences for IgD and IgE.

Proteins with Ig-like Domains

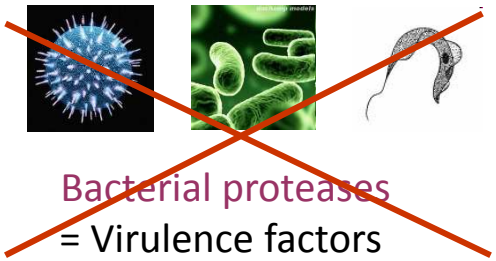
Widespread domain. Ig fold : antiparallel b-strands arranged into two sheets linked by a disulfide bond
Various tissue distribution.

Protein name	Localization
CEPU-Se alpha 2 isoform	EW, VM, ES
CEPU-1	EW, VM, ES
Protein CEPU-1	ES
Neogenin	ES
Basement membrane-specific heparan sulfate proteoglycan core protein	ES
Neuroplastin	Ut
Pro-neuregulin-1, membrane-bound isoform	EW
Semaphorin-3C	VM, ES
Muscle, skeletal receptor tyrosine protein kinase	EW
Butyrophilin subfamily 1 member A1	Ut
Basigin	ES
VH1 protein	ES
ICOS ligand	Ut
Beta-2-microglobulin (IR)	ES, Ut
T-cell surface glycoprotein CD1b4	EW

Egg antimicrobial proteins (chemical defence)

Protease inhibitors

•Highly represented in egg (48)



Bacterial proteases
= Virulence factors

Inactivation/hydrolysis of host proteins

Characterization of Ovoinhibitor

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY | J. Agric. Food Chem. 2011, 59, 12368–12374 ARTICLE pubs.acs.org/JAFC

Antimicrobial Potential of Egg Yolk Ovoinhibitor, a Multidomain Kazal-like Inhibitor of Chicken Egg

Marie Bourin,*,† Joël Gautron,† Magali Berges,† Sylvie Attucci,§ Gwenaelle Le Blay,# Valérie Labas,△ Yves Nys,† and Sophie Rehault-Godbert*,†

Antimicrobial activity

→ **Activity against *Bacillus thuringiensis***

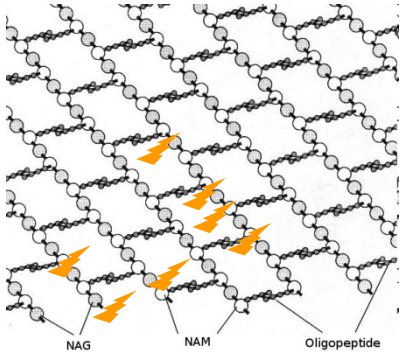
Egg antimicrobial proteins (chemical defence)

Hydrolases (46 egg proteins)

Lysozyme

Similar to acyloxylhydrolase (Egg white)

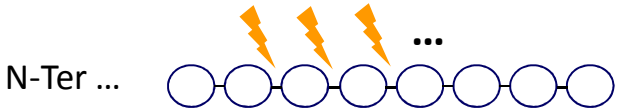
Hydrolyze bacterial peptidoglycan



Food preservative E1105 (Cheese, wine…)

Tablets for pain and inflammation of sore throat

Proteases



Several proteases in eggs

Antimicrobial activities already demonstrated in other species. No experimental evidences for chickens

Direct action (Degradation of antimicrobial proteins)

Indirect action
(Activation of antimicrobial precursors, production of antimicrobial peptides)

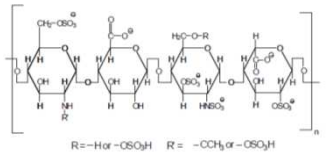
Egg antimicrobial proteins (chemical defence)

Heparin and Lipopolysaccharide binding proteins (37 proteins)

Heparin-binding proteins
Cluster (s) of exposed positives charges

Heparin

Negatively charged glycosaminoglycan



Other negatively charged surfaces

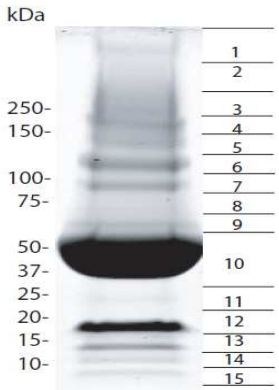
Bacterial lipopolysaccharide Peptidoglycan

Eur. J. Biochem. **271**, 1219–1226 (2004) © FEBS 2004 doi:10.1111/j.1432-1033.2004.04035.x

Antimicrobial activities of heparin-binding peptides

Emma Andersson[1], Victoria Rydengård[1], Andreas Sonesson[1], Matthias Mörgelin[2], Lars Björck[2] and Artur Schmidtchen[1]

Heparin-binding proteins from egg white



✓15 proteins identified
✓5 new antimicrobial candidates

Rehault-Godbert, S. et al. (2011). ***Patent*** *"Fraction of proteins and peptides derived from egg white and protein derived from egg white and use thereof as antilisteria agents." WO 2011/151407 A1.*

Egg antimicrobial proteins (chemical defence)

Heparin and Lipopolysaccharide binding proteins

Ovalbumin-related protein X *(Réhault-Godbert et al. 2013)*

Table 1 Minimal Active Concentration (MAC) of purified OVAX, Ovalbumin and Av-BD11 (positive control)

Bacterial group, strains	MAC (µM) ± std		
	AvBD11	Ovalbumin	OVAX
L. monocytogenes (Gram +)	0.58 ± 0.36	> 28	2.31 ± 0.00
S. Enteritidis ATCC 13076 (Gram -)	0.50 ± 0.20	> 51	10.04 ± 0.02
S. aureus ATTCC 29740 (Gram +)	7.73	> 37	> 37
E.Coli ATCC 25922 (Gram -)	1.6	> 37	> 37

MAC, Minimum Active Concentration (corresponding to a 0.5 mm-clear zone)

Egg antimicrobial proteins (chemical defence)

Heparin and Lipopolysaccharide binding proteins

Lipopolysaccharide Binding (LBP) and Bactericidal Permeability Increasing (BPI) proteins

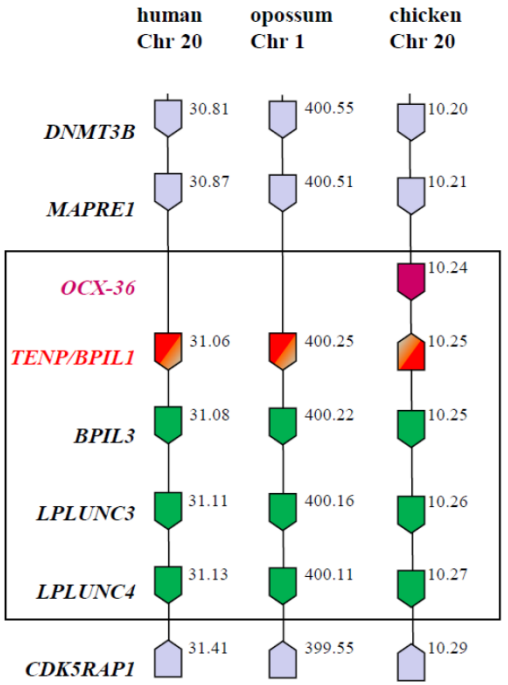
Key components of the innate immune system (permeabilization of LPS, initiation of inflammation upon infection etc.)

➢ Ovocalyxin-36 *(Gautron et al., 2007)* (eggshell and vitelline membranes)

- Identity and similarity with LBP/BPI and Plunc families proteins
- Binds to the lipolysaccharide (LPS) cell wall of the gram negative bacteria (death of bacteria)
- Early recognition of bacterial product
- OCX-36 binds to E. Coli LPS *(Correiro, Hincke et al., 2010)*
- Modestly inhibit the bacterial growth of Bacillus subtilis, Staphylococcus aureus, E. Coli, Pseudomonas aeruginosa

➢Other candidates

TENP (all compartments)
BPIL2 (Egg White, Vitelline membrane)
Similar to BPI (Egg white)



(Tian et al., 2010)

Egg antimicrobial proteins (chemical defence)

Chelators (13 proteins)

Molecules decreasing bioavailability of irons and vitamins

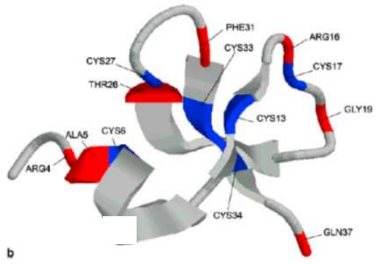
→Diminish bioavailability for microbes
→Affect growth and survival of bacteria

Name	Localization	Antimicrobial activity
Ovotransferrin	ES, EW, MV, EY	+
RBP	ES, EW, MV, EY	+
Avidin	ES, EW, MV	+
Similar to avidin	EY	?
Vitellogenin 1	EY	?
Vitellogenin 2	EY	?

Antimicrobial peptides

Avian beta-defensins

Cationic peptides
Broad spectrum of antimicrobial activity



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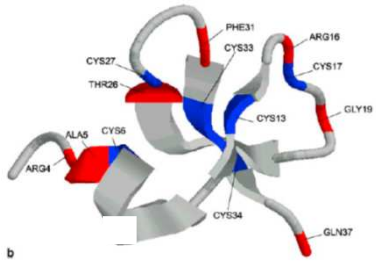


Histones

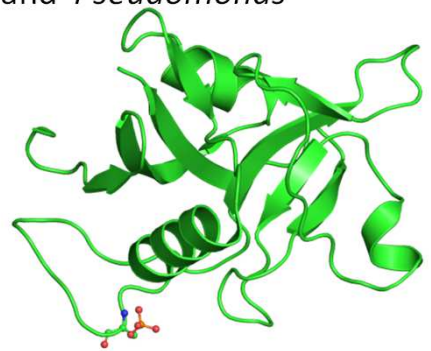
Basic proteins from nucleosomes
Agent of innate host defense (Gram + and Gram – strains)

Egg antimicrobial proteins (chemical defence)

C-type lectin-like proteins

Major components of the calcified eggshell of multiple avian species
C-type lectin domain : 110 to 130 residues including four cysteines involved in two disulfide bonds

→ Ovocleidin 17 *(Hincke et al., 1995)* (eggshell, eggwhite, vitelline membrane)
Bactericidal activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* *(Wellman-Labadie et al. 2008)*



→ Other candidates *(Jonchere et al., 2009, Bourin et al., 2012)*
Tetranectin (egg yolk)
Collagen XVIII (eggshell)
DEC-205 protein (eggshell)
Mannose binding protein (eggshell, uterus)

Conclusion

Global and exhaustive data mining of egg proteins

Physical defense of egg

Determinants of eggshell mechanical properties

Chemical defense of the egg

Proteases/antiproteases system

Identification of new antibacterial proteins

Other Biological activities
(antiviral, antioxidants, anti-hypertensive…)

New egg components with bioactive properties

- **Food use of egg**
- **Non food use of egg proteins**

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