Ovocalyxin-36 and other LBP/BPI/Plunc- like proteins as molecular actors of the mechanism of the avian egg natural defences

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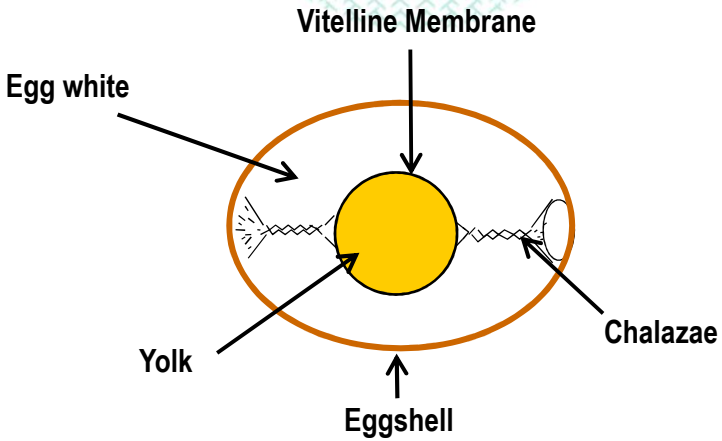
G. Pascal, INRA, UMR85, physiologie de la reproduction et des comportements, 37380 Nouzilly, France

M.T. Hincke, Department of cellular and molecular medecine, University of Ottawa, K1H8M5, Canada

The chicken egg



Container for extra-uterine development of the embryo

Must contains the entire components essential for the embryo development

Almost perfect nutritional value

Basic food for humans all around the world

World egg market (2008)

→ 60.7 millions tons per year (1140 billion eggs)
→ China is first producer (22.7 MT – 37 % of world production)
→ 145 eggs per year and habitant in the world
→ 248 eggs per year and habitant in France (31% as ovoproducts)

Protective systems (Egg natural defences)

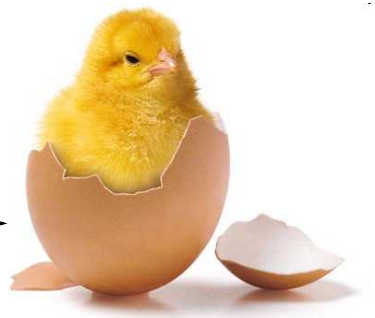
The egg's natural defences

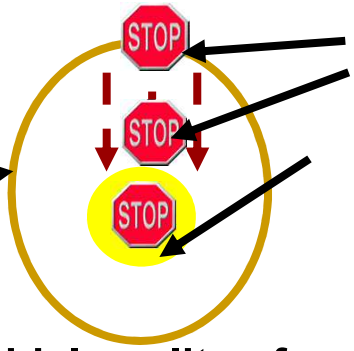
Container for extra-uterine development of the embryo

Physical damages

External milieu

Microbes





Physical
(Eggshell as a barrier)

Chemical
Antimicrobial activities
(White, shell, yolk)

Microbial quality of eggs ?

Humans toxi-infections

The biological activities of egg proteins

Container for extra-uterine development of the embryo

Wide spectrum of biological activities in the yolk, the vitelline membrane, the white and the shell

→ Transport lipids, ions ….

→ Inflammatory and antioxidant processes

→ Embryonic development

→ Natural egg defences

- ✓ Eggshell biomineralisation (physical defence)
- ✓ Antimicrobial molecules (chemical defence)

High potential for pharmaceutical, cosmetic, food industries and for human and animal health

→ Food and non food uses of eggs

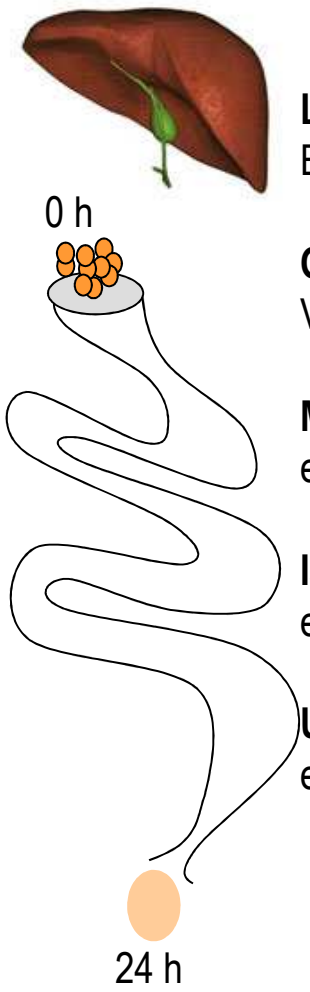
Understand and improve the egg's natural defences

↓

Identify and characterize egg proteins

Egg formation

Spatial and temporal regulation



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)

Different tissues or organs

↓

Egg components are deposited at different times

↓

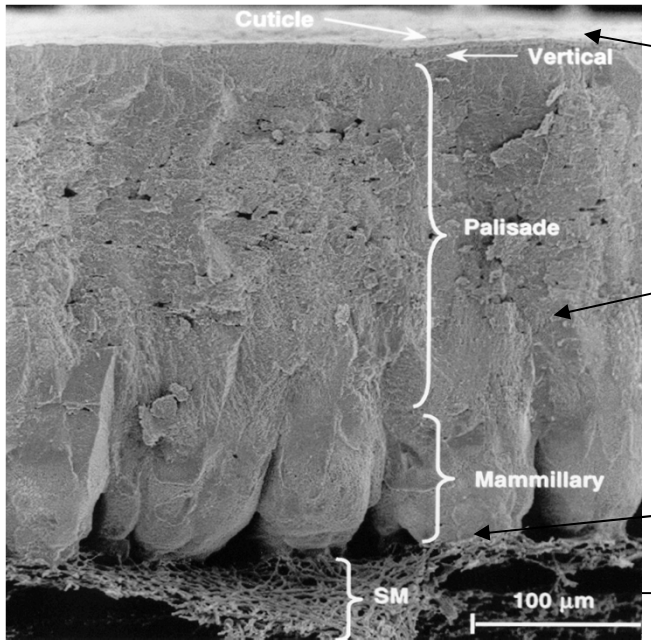
Different physiological stages

The eggshell (physical defence)

→ **Eggshell biomineralisation in uterus (one of the fastest on earth)**

→ **Into the uterine fluid with the appropriate physico-chemical conditions**

Hypersaturation of calcium and bicarbonates

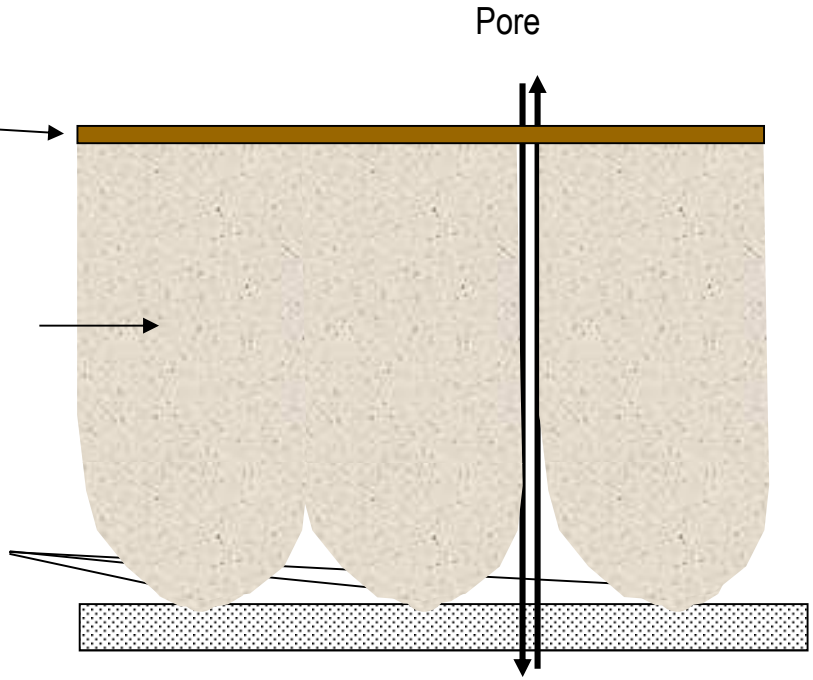


Cuticle

Palissadic layer

Mammilary knobs

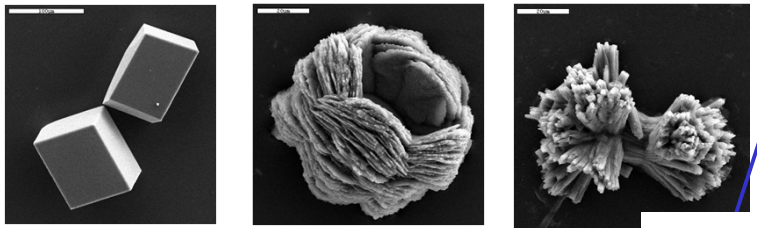
Eggshell membranes



The eggshell (physical defence)

→ **95% of calcium carbonate on calcitic polymorph**

Calcite Aragonite Vaterite



Quantity

Interaction

Mechanical properties
- about 300 µm
- eggshell breaking strength (about4 kg)

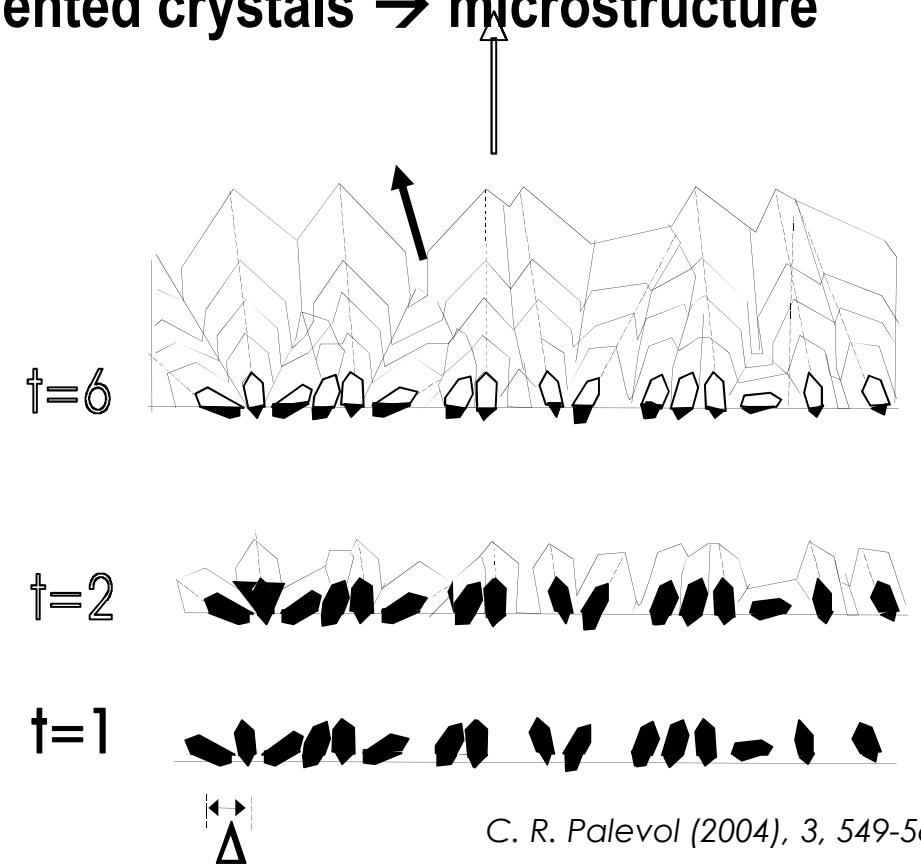
Control of the calcification process

→ **3,5% of organic matter (organic matrix)**
Proteins and proteoglycans

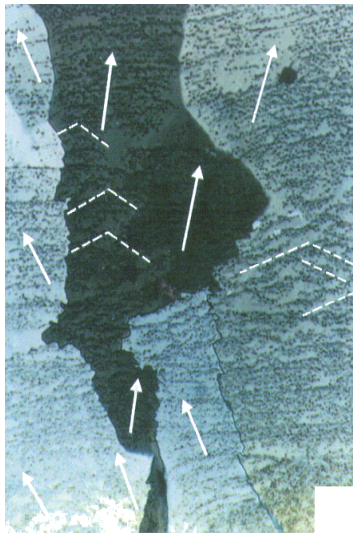
The eggshell (physical defence)

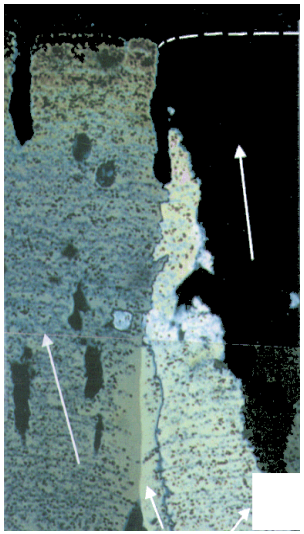
Temporal sequence of the nucleation, the growth

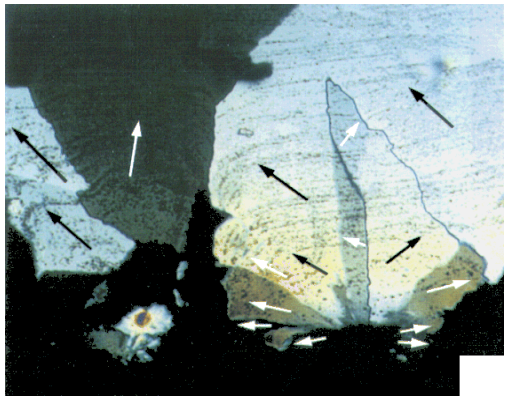
Oriented crystals → microstructure



C. R. Palevol (2004), 3, 549-562







→ Identification and characterization of organic matrix proteins

Ovocalyxin-36

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 8, pp. 5273–5286, February 23, 2007

Cloning of Ovocalyxin-36, a Novel Chicken Eggshell Protein Related to Lipopolysaccharide-binding Proteins, Bactericidal Permeability-increasing Proteins, and Plunc Family Proteins*

Received for publication, November 3, 2006, and in revised form, December 14, 2006 Published, JBC Papers in Press, December 19, 2006, DOI 10.1074/jbc.M610294200

Joël Gautron[‡1], Emi Murayama[§], Alain Vignal[¶], Mireille Morisson[¶], Marc D. McKee[‖], Sophie Réhault[‡], Valérie Labas[**], Maya Belghazi[**], Mary-Laure Vidal[‡], Yves Nys[‡], and Maxwell T. Hincke[‡‡]

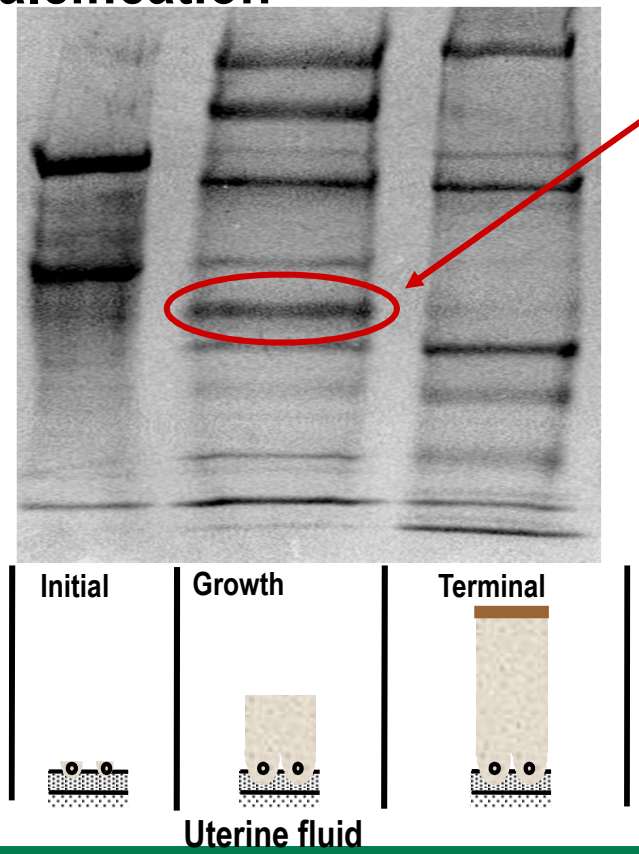
→ OCX-36, is a new eggshell matrix protein

→ OCX-36 is potentially involved in the antibacterial defense of the egg

Ovocalyxin-36

→ Variation of the organic composition of the uterine fluid during the process of shell calcification

→ Adaptation of the organic matrix at the different phases of shell calcification



N-terminal Amino acid sequencing :
VLGSGLSCAISPRAMQQVLSDAIIQTGGL

↓

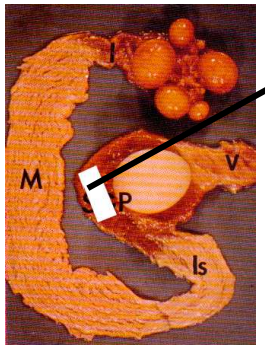
No correspondences in databases

↓

Production of specific antibodies

Ovocalyxin-36

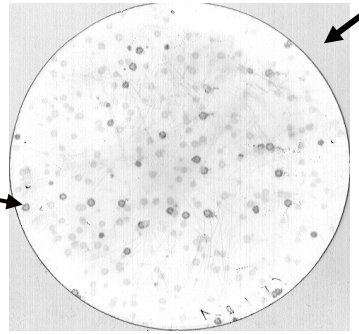
→ Expression screening



RNA prepared from uterus harvested during the shell calcification

Preparation of a bacteriophage cDNA expression library

OCX-36 specific antibodies



Expression screening of the cDNA

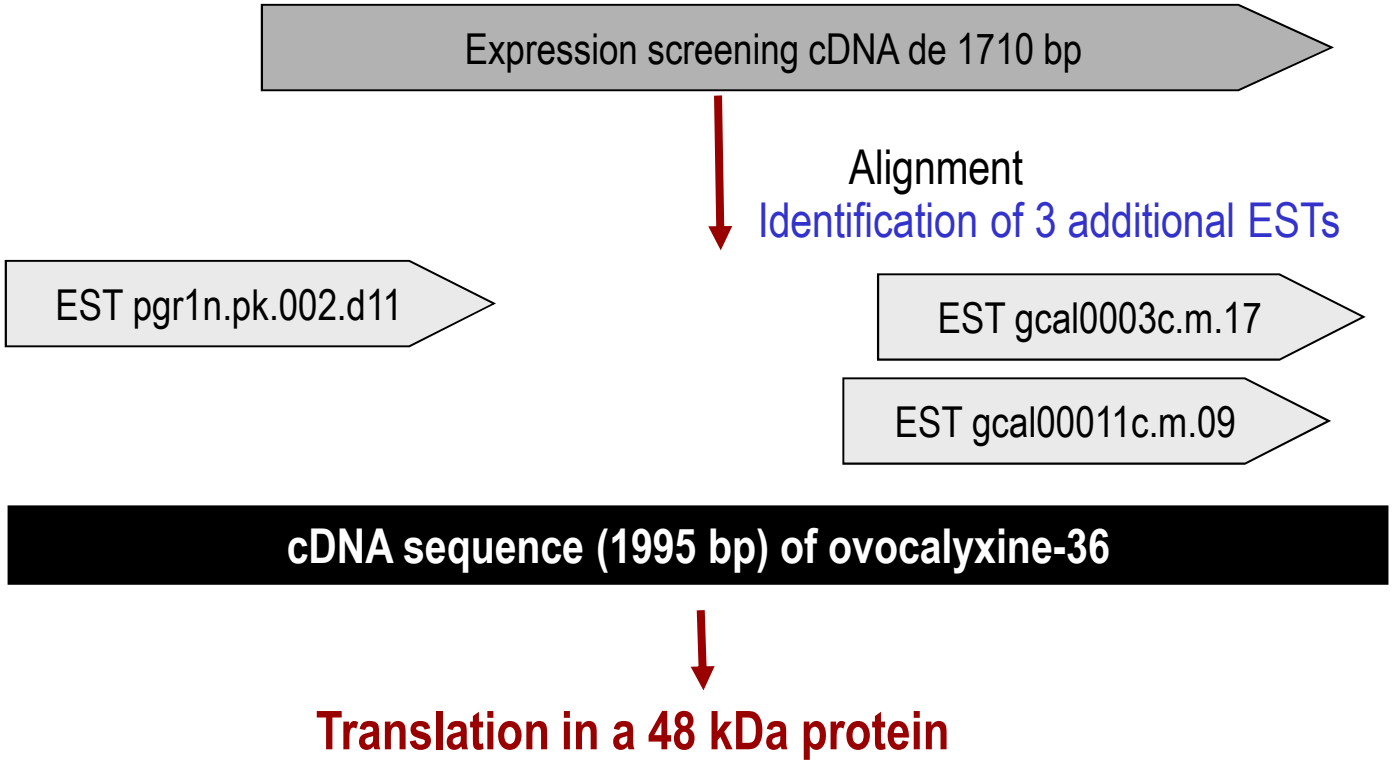
cDNA sequence of positive clones

cDNA sequence of 1710 bp

Ovocalyxin-36

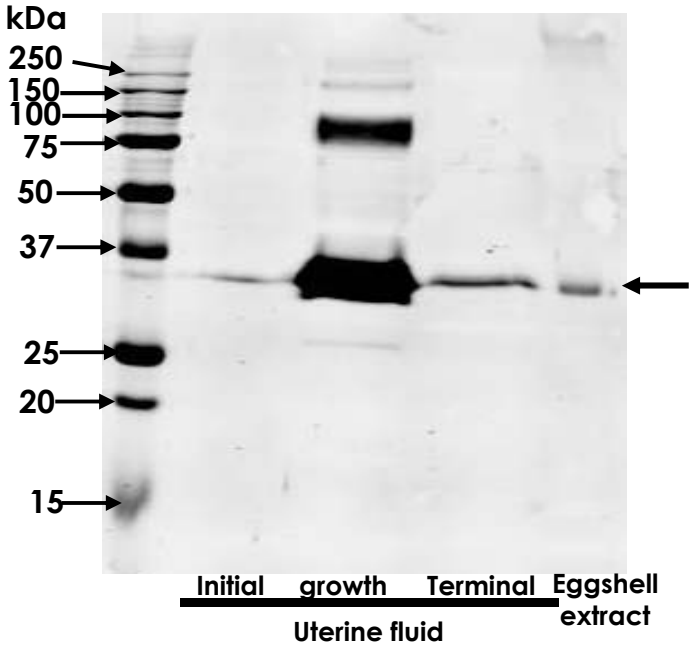
→ Expression screening

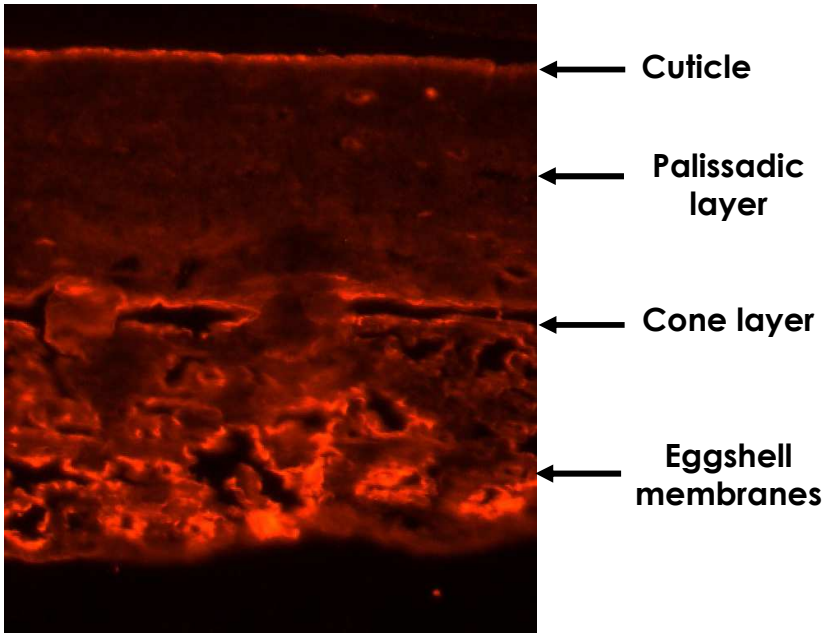
→ Cloning and determination of the OCX-36 full-length cDNA



Ovocalyxin-36

→ Ovocalyxin-36 is an eggshell matrix protein

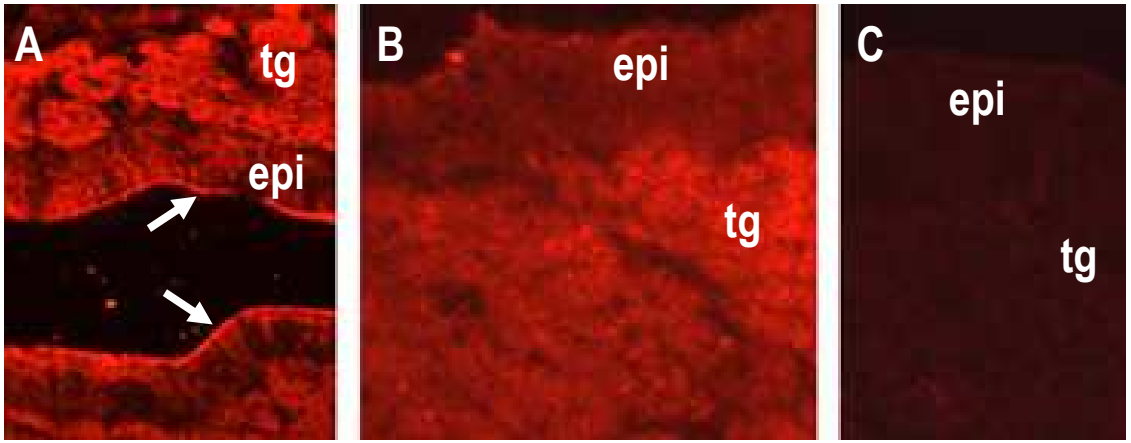




Ovocalyxin-36

→ Ovocalyxin-36 is an eggshell matrix protein

→ Ovocalyxin-36 is synthesized in uterine cells

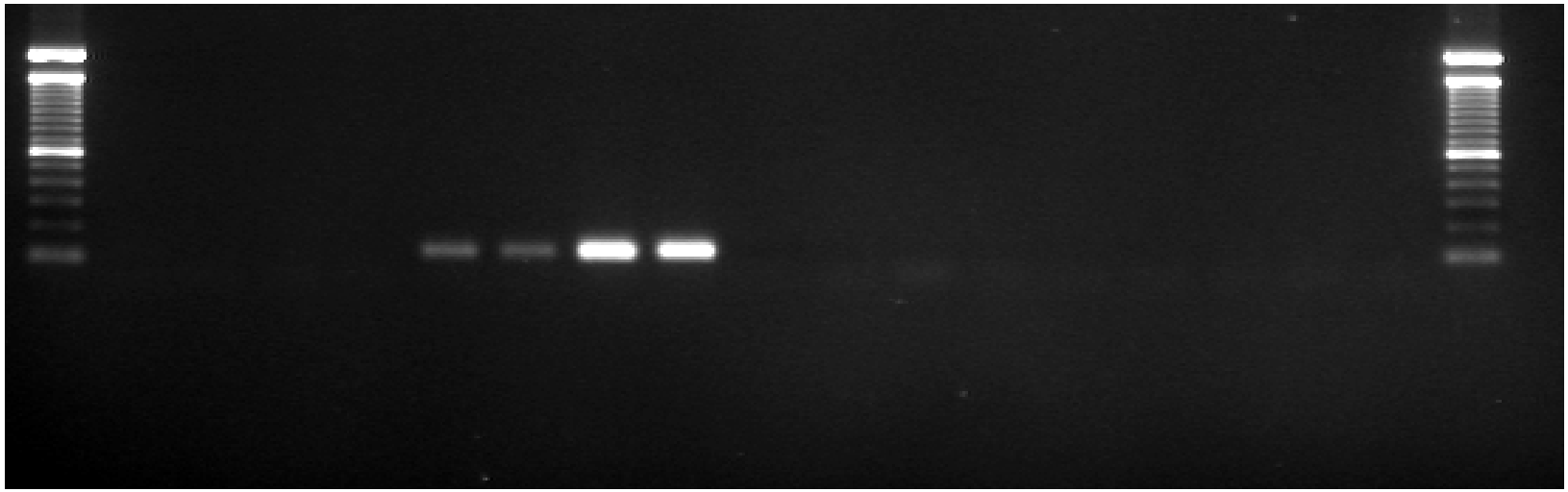


2204 (1/500) 2205 (1/125) pre-immune (1/125)

Ovocalyxin-36

→ Ovocalyxin-36 is an eggshell matrix protein

→ Expressed in the chicken reproductive tract and more specifically in tissue where calcification takes place



Novel cDNA sequence specific of the eggshell

→ Ovocalyxin-36 (*Ovum* for egg, *calyx* for shell and 36 for its apparent MW)

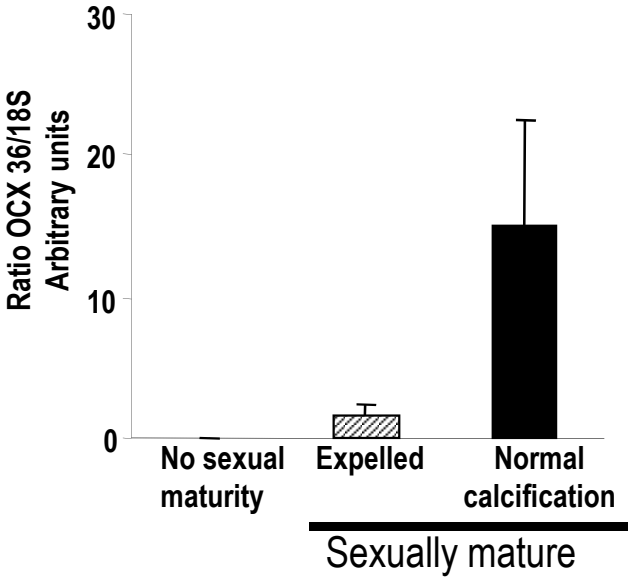
Ovocalyxin-36

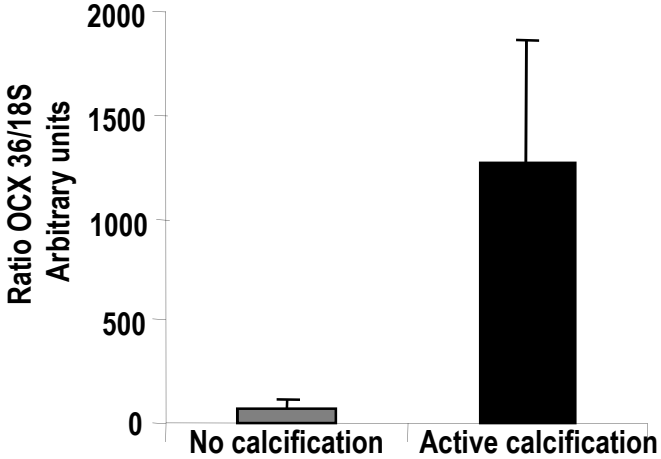
→ Ovocalyxin-36 is an eggshell matrix protein

→ Expressed in the chicken reproductive tract and more specifically in tissue where calcification takes place

→ Overexpressed in uterus during eggshell formation

Regulation of Uterine OCX-36 expression at different physiological stages





Ovocalyxin-36

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

RESEARCH ARTICLE

Proteomic analysis of the chicken egg vitelline membrane

Karlheinz Mann

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

[illegible]	[illegible]	+	Hypothetical protein/ADP-ribosylation factor 1	w,s	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
00589985.3	Q90835	+	Elongation factor 1-α1	y,s,	364	4	13	11	6, 9	0.8
00575989.1	P02552	+	Similar to tubulin α2 (~aa1–500)	s	676	6	15	16	6	0.8
00580626.1	P09206	+	Tubulin β-3	s	370	4	6	9	6	0.7
00577039.1	P17785	+	Annexin A2	s	321	5	7	15	8	0.7
00580765.1	P08250	+	Apolipoprotein A-I	y,w,s	390	4	7	18	10	0.7
00573506.2	Q53HW8	+	Ovocalyxin-36	s	207	2	4	8	9	0.7

Ovocalyxin-36

Developmental and Comparative Immunology xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

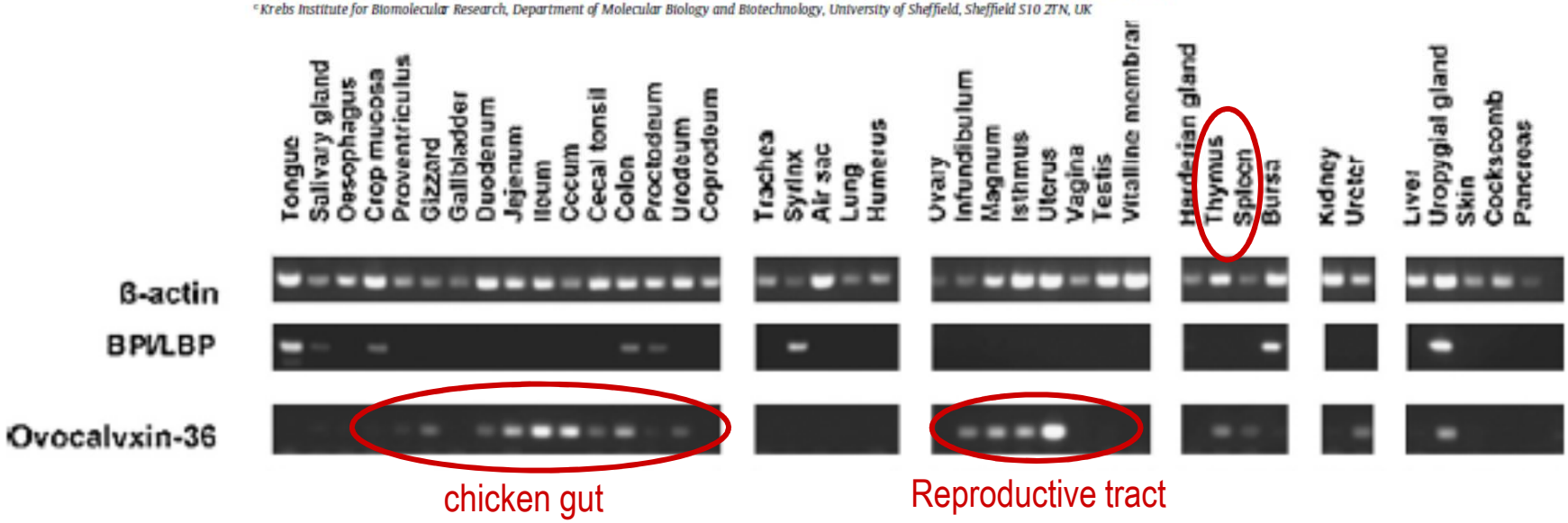
Developmental and Comparative Immunology

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

Identification and characterisation of the BPI/LBP/PLUNC-like gene repertoire in chickens reveals the absence of a LBP gene☆

Shih-Chieh Chiang[a,1], Edwin J.A. Veldhuizen[b], Frances A. Barnes[a], C. Jeremy Craven[c], Henk P. Haagsman[b], Colin D. Bingle[a,*]

[a] Academic Unit of Respiratory Medicine, Department of Infection and Immunity, University of Sheffield, Sheffield S10 2JF, UK
[b] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands
[c] Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK



Ovocalyxin-36

→ Characterisation of the protein using bioinformatics tools

Ovocalyxine-36 cDNA sequence

No homology in databases

Ovocalyxine-36 protein sequence

Limited identity and similarity with "lipopolysaccharide binding proteins (LBP)", "bactericidal permeability increasing protein (BPI)" and "Plunc families proteins"

Binds to the lipolysaccharide (LPS) cell wall of the gram negative bacteria

→ Death of bacteria

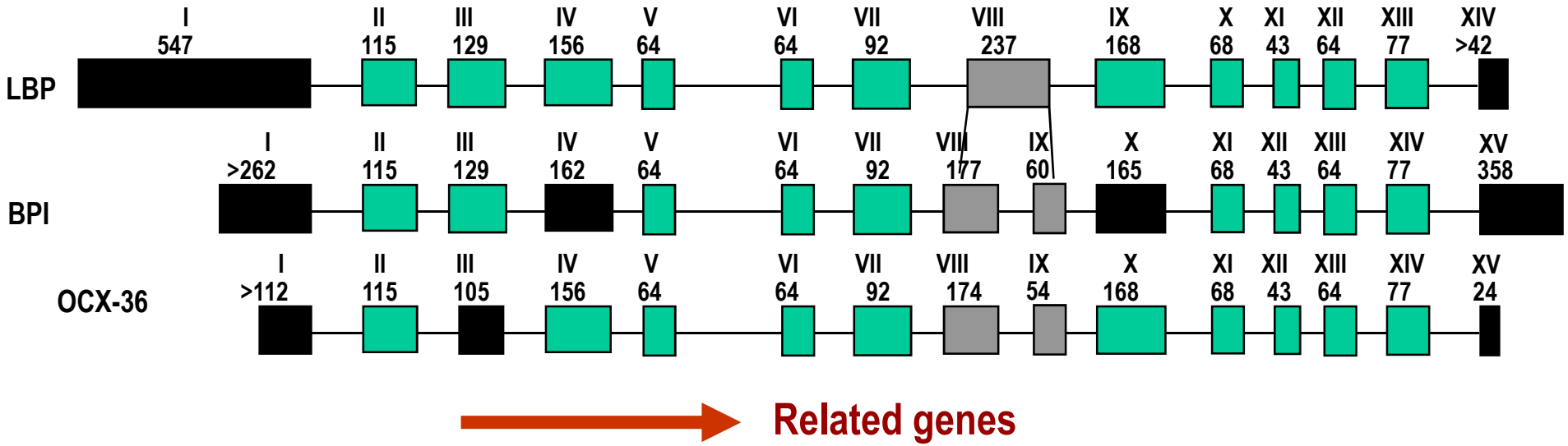
Early recognition of bacterial product

Ovocalyxin-36

→ Characterisation of the protein using bioinformatics tools

Ovocalyxine-36 protein is related to LBP/BPI and Plunc proteins

Gene architecture



Ovocalyxin-36

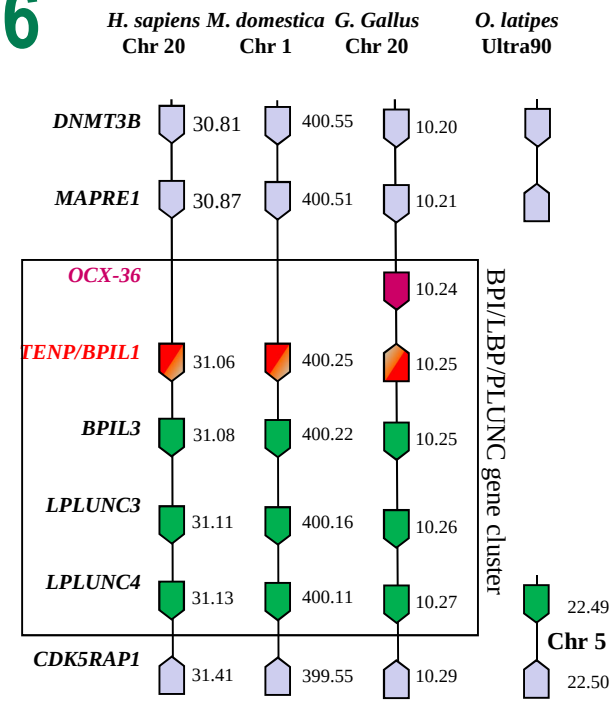
BIOLOGY OF REPRODUCTION 83, 893–900 (2010)
Published online before print 11 August 2010.
DOI 10.1095/biolreprod.110.085019

Minireview

What Makes an Egg Unique? Clues from Evolutionary Scenarios of Egg-Specific Genes[1]

Xin Tian,[3,4,5,6] Joel Gautron,[7] Philippe Monget,[3,4,5,6] and Géraldine Pascal[2,3,4,5,6]

UMR85,[3] Physiologie de la Reproduction et des Comportements, INRA, Nouzilly, France
UMR6175,[4] CNRS, Nouzilly, France
Université François Rabelais de Tours,[5] Tours, France
Haras Nationaux,[6] Nouzilly, France
UR83 Recherches Avicoles,[7] INRA, Nouzilly, France



Developmental and Comparative Immunology xxx (2010) xxx–xxx
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Developmental and Comparative Immunology
journal homepage: www.elsevier.com/locate/dci

Identification and characterisation of the BPI/LBP/PLUNC-like gene repertoire in chickens reveals the absence of a LBP gene☆

Shih-Chieh Chiang[a,1], Edwin J.A. Veldhuizen[b], Frances A. Barnes[a], C. Jeremy Craven[c], Henk P. Haagsman[b], Colin D. Bingle[a,*]

[a] Academic Unit of Respiratory Medicine, Department of Infection and Immunity, University of Sheffield, Sheffield S10 2JF, UK
[b] Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80.165, 3508 TD Utrecht, The Netherlands
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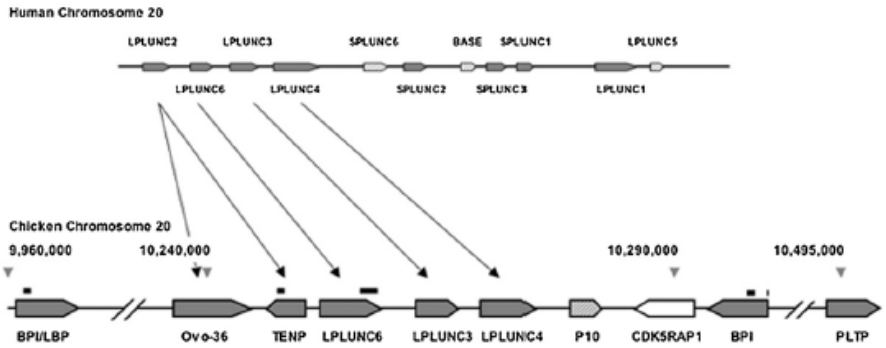
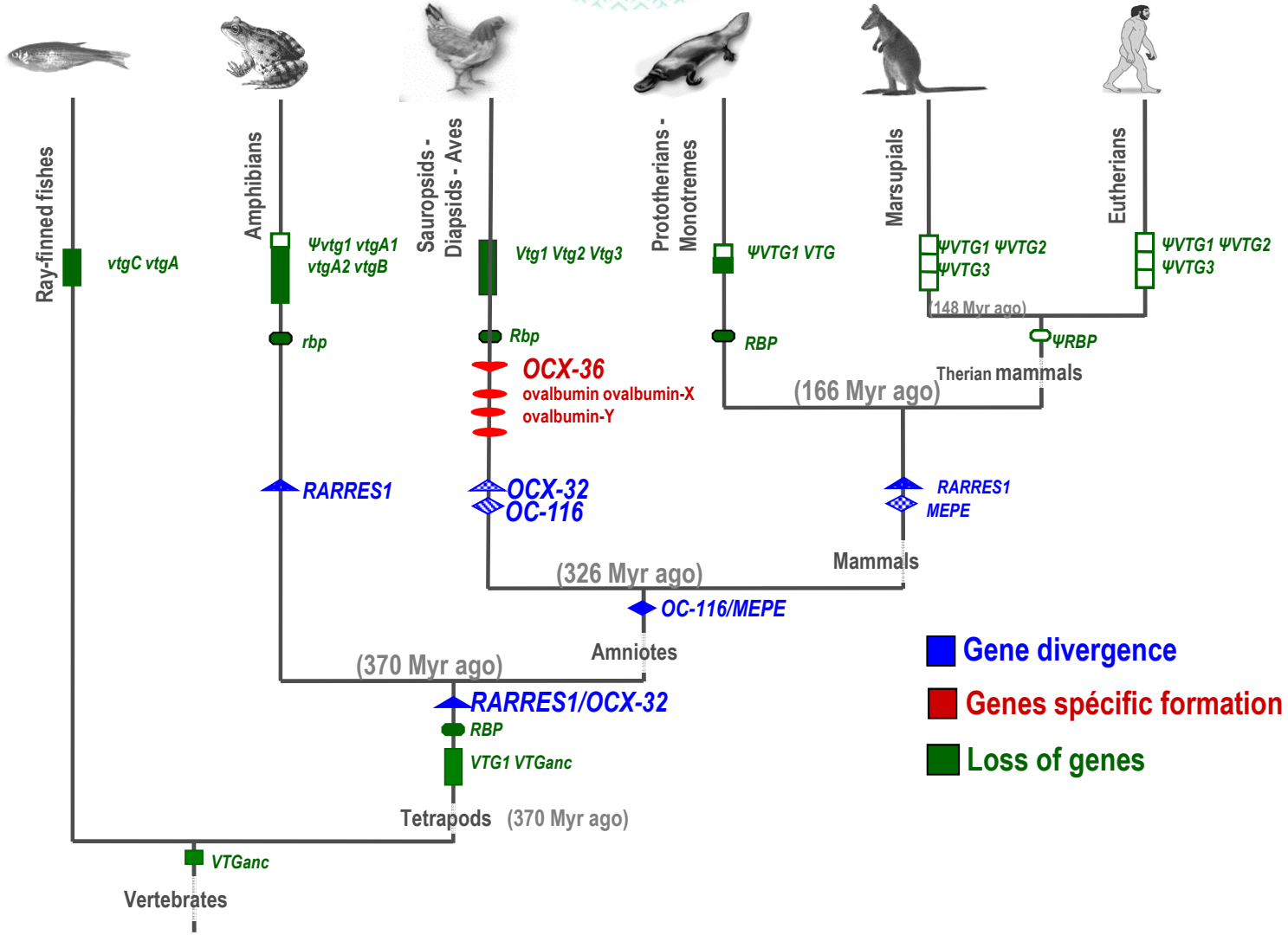


Ovocalyxin-36

→ Evolutionary scenarios of some egg-specific genes along the vertebrate lineages *(Tian el al., 2010)*



Ovocalyxin-36

Purification of a Potential Antimicrobial Protein, Ovocalyxin-36 (OCX-36), from Eggshell Membranes.

C. Cordeiro, H. Esmaili, M. Hincke

Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, Canada

European Poultry conference, Tours, 2010

→**Development of a method to extract and purify OCX-36 from eggshell membranes**

→ **The purified OCX-36 binds to *E. Coli* LPS**

→ **Modestly inhibit the bacterial growth of**

- ***Bacillus subtilis***
- ***Staphylococcus aureus***
- ***Escherischia Coli***
- ***Pseudomonas aeruginosa***

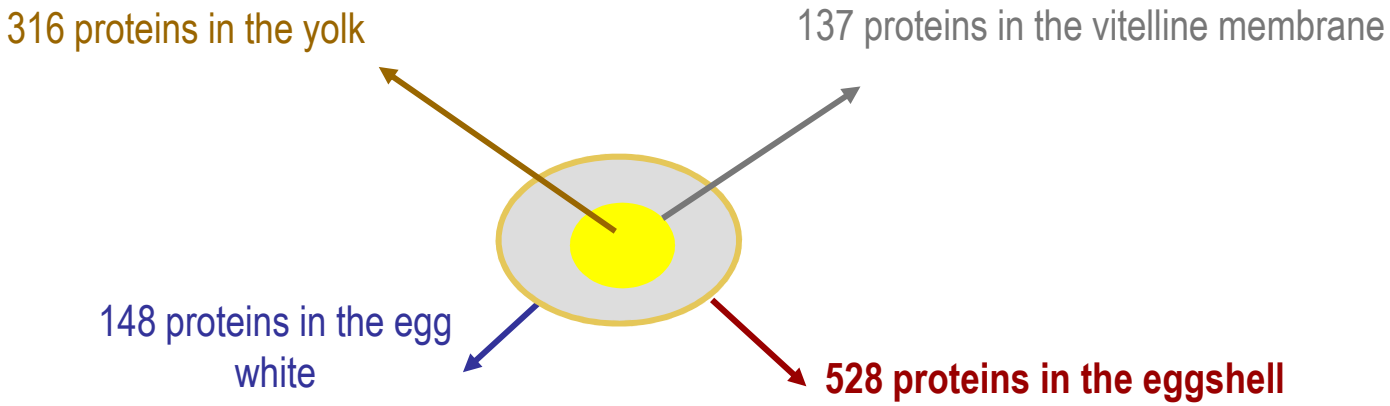
Recent developments

- 2004, Publication of the chicken genome sequence (*Gallus gallus*)
- 2009, cDNA and ESTs libraries (Identification of 630 000 functional genes in chickens)
- « omics » high-throughput techniques and data mining

→ 2006, about 50 proteins were identified in eggs

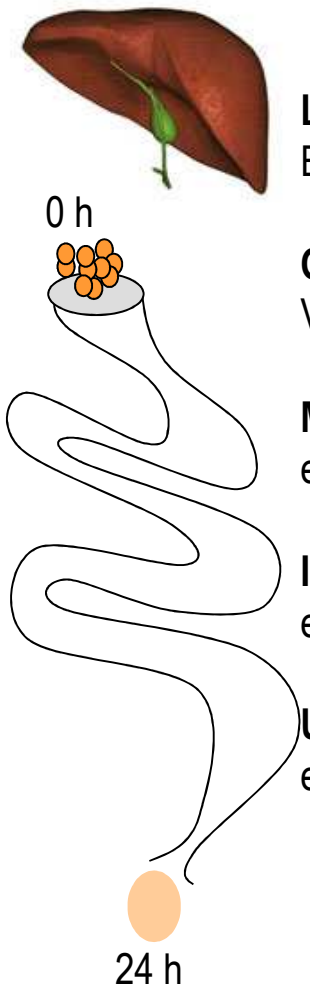
→ 2010, several hundred egg proteins are described

→ Egg proteome



Recent developments

→ **Egg transcriptome**



Liver:
Egg yolk proteins

Ovary and infundibulum:
Vitelline membranes

Magnum:
egg white proteins

Isthmus:
eggshell membranes

Uterus:
eggshell calcification

BMC Genomics, 2010, 11, 57

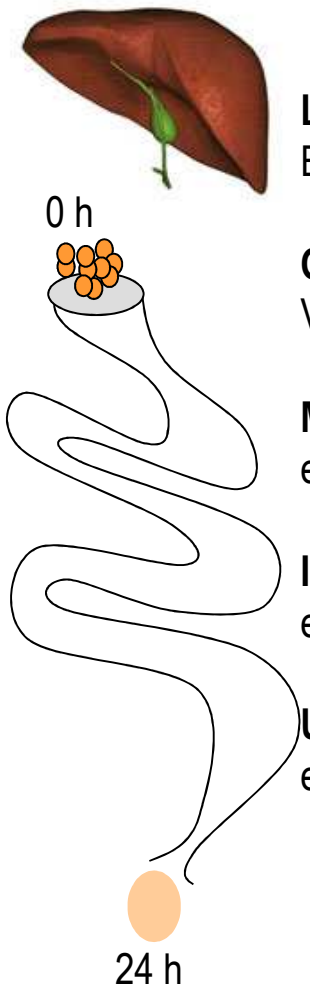
cDNA Microarrays

↓

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

Recent developments

→ **Egg transcriptome**



Liver:
Egg yolk proteins → **724 genes over-expressed in the liver of sexually mature hens**

Ovary and infundibulum:
Vitelline membranes → **More than 1500**

Magnum:
egg white proteins → **828**

Isthmus:
eggshell membranes → **135**

Uterus:
eggshell calcification → **605**

BMC Genomics, 2010, 11, 57

Identification of genes specifically expressed in the yolk, vitelline membranes, white and shell synthesis

Recent developments

Functional genomics reveals numerous novel egg proteins

↓

New egg components with bioactive properties, high potential for industry or/and involved in natural egg defenses

→ 3 new LBP/BPI/Plunc in the egg

Tenp

→ **Tenp is an egg white protein**

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. 2006, *54*, 3901–3910

Proteomic Analysis of Hen Egg White

Catherine Guérin-Dubiard,*,† Maryvonne Pasco,† Daniel Mollé,† Colette Désert,‡ Thomas Croguennec,† and Françoise Nau†

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

Research Article

The chicken egg white proteome

Karlheinz Mann

research articles Journal of proteome research

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*[,§]

l. Agric. Food Chem. **2010**, *58*, 12530–12536
DOI:10.1021/jf103239v

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY ARTICLE

Primary Structure of Potential Allergenic Proteins in Emu (*Dromaius novaehollandiae*) Egg White

Kenji Maehashi,*,† Mami Matano,† Tomohiro Irisawa,‡ Masataka Uchino,‡ Yasuharu Itagaki,§,# Katsumi Takano,‡ Yutaka Kashiwagi,† and Toshihiro Watanabe⊥

Tenp

→ Tenp is an eggshell protein

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

Karlheinz Mann, Boris Maček and Jesper V. Olsen

→ Tenp is a vitelline membrane and egg yolk protein

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

RESEARCH ARTICLE

Proteomic analysis of the chicken egg vitelline membrane

Karlheinz Mann

Journal of Chromatography A, 1216 (2009) 1241–1252

Contents lists available at ScienceDirect

Journal of Chromatography A

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

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,*]

BPIL2

→ **BPIL2 is an egg white and vitelline membrane protein**

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

RESEARCH ARTICLE

The chicken egg white proteome

Karlheinz Mann

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

RESEARCH ARTICLE

Proteomic analysis of the chicken egg vitelline membrane

Karlheinz Mann

→ **BPIL2 is strongly upregulated in sexually matures hens compared to juveniles**

General and Comparative Endocrinology 163 (2009) 225–232

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

New hypotheses on the function of the avian shell gland derived from microarray analysis comparing tissue from juvenile and sexually mature hens

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Similar to BPI (XP_425484)

→ **Similar to BPI in egg white**

research articles Journal of proteome research

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*[,§]

Conclusions

The chicken egg is a close chamber which protective systems

LBP/BPI/Plunc related proteins are present in the egg

→ OCX-36 is well characterized

→Tenp, BPIL2 and similar to BPI are less characterized

Function of these molecules are subject of intense researches

→Keep the egg free of pathogens

- Soluble in the egg white
- Soluble from the eggshell by calcium mobilization during embryonic development
- Soluble in the uterine fluid during eggshell assembly

Egg natural defences can be reinforced by MAS or by controlling environment

Reduce the risk of food-borne outbreaks for egg consumers

Granted actions

EggDefence

Improving quality and safety of hen eggs in new production system by reinforcing the antimicrobial natural defence and by developing tools for grading eggs **(2001-2004)**
(Coordinator Y. Nys, INRA, UR 83 Recherches avicoles, F-37380 Nouzilly, France)

Rescape

Reducing **E**gg **S**usceptibility to **C**ontaminations in **A**lternative **P**roduction in **E**urope **(2006-2009)**
(Coordinator Y. Nys, INRA, UR 83 Recherches avicoles, F-37380 Nouzilly, France)

SABRE CUTTING EDGE GENOMICS FOR SUSTAINABLE ANIMAL BREEDING

2006-2010
(Coordinator Chris Warkup, Genesis Faraday, Roslin BioCentre, Roslin, EH25 9PS, United Kingdom)

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