

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

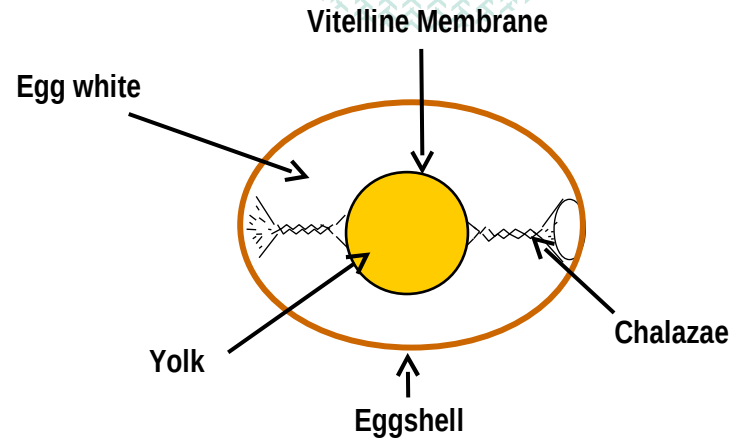
M.T. Hincke, Department of cellular and molecular medicine, University of Ottawa, K1H8M5, Canada



ALIMENTATION
AGRICULTURE
ENVIRONNEMENT



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

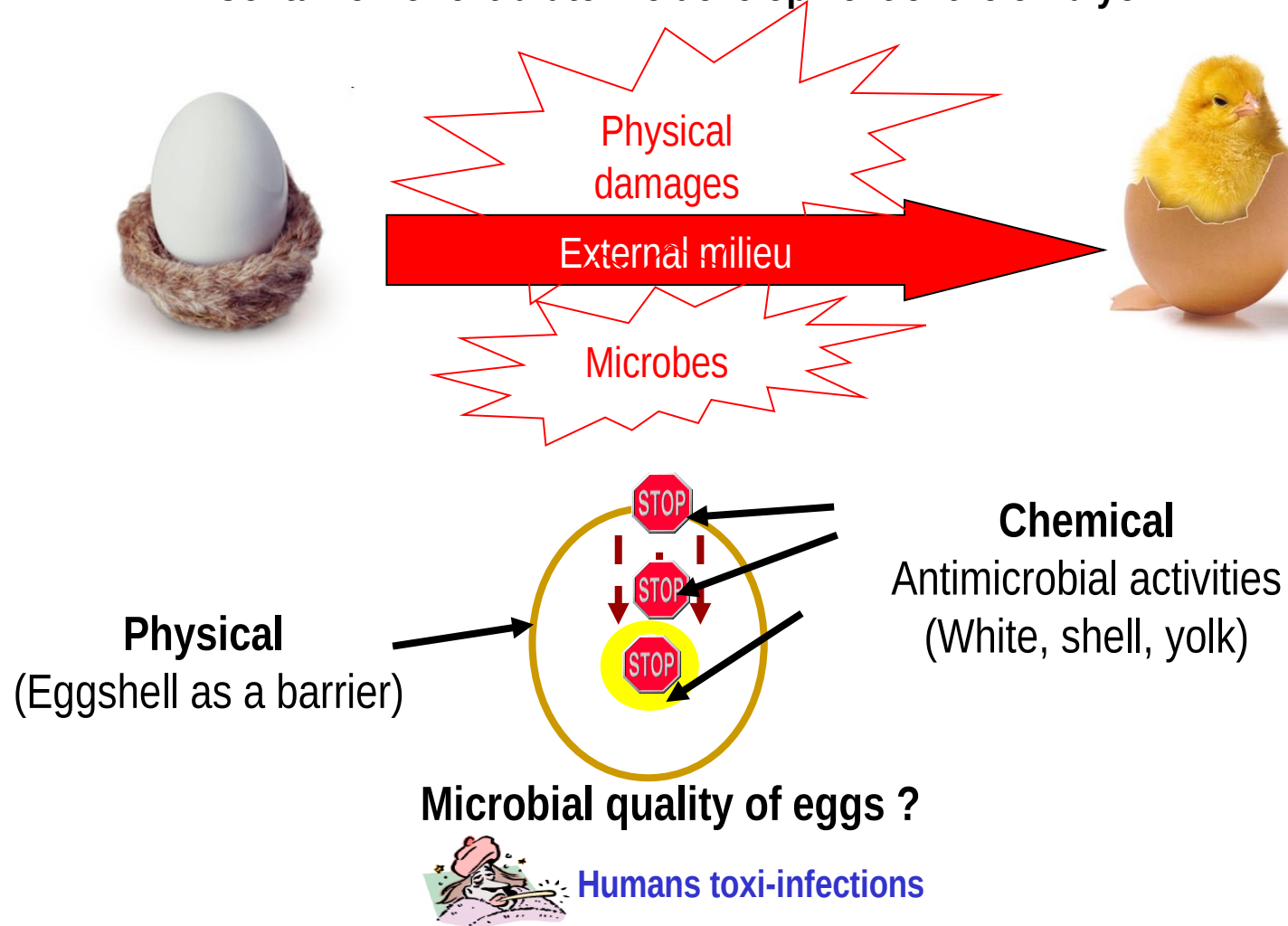
World egg market (2008)

- 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



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

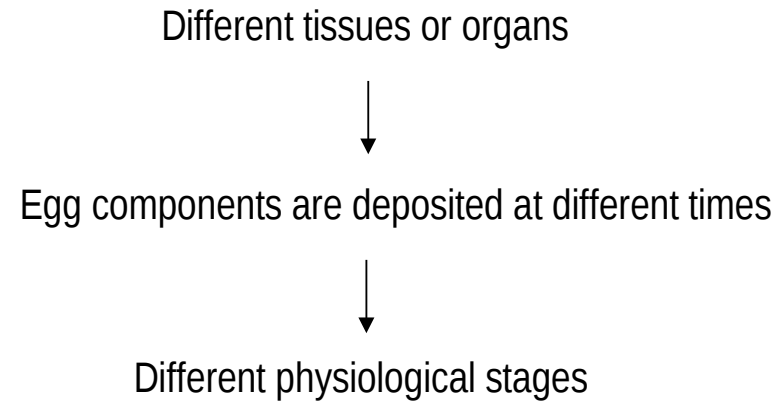
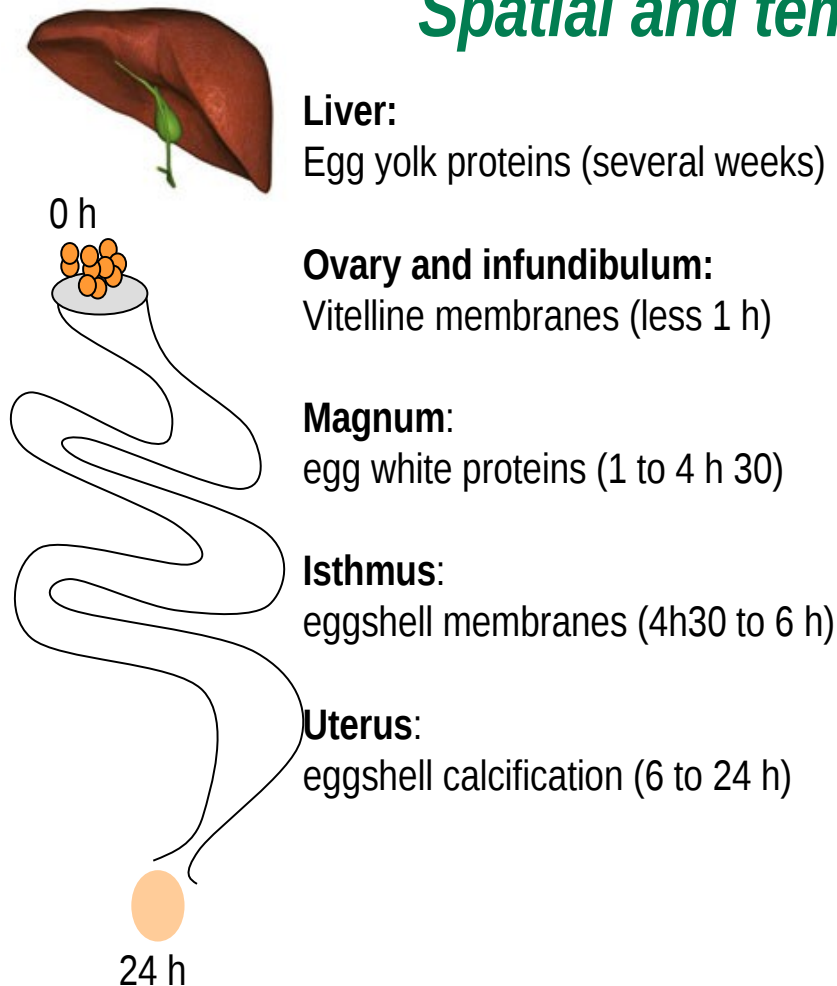
Understand and improve the egg's natural defences



Identify and characterize egg proteins

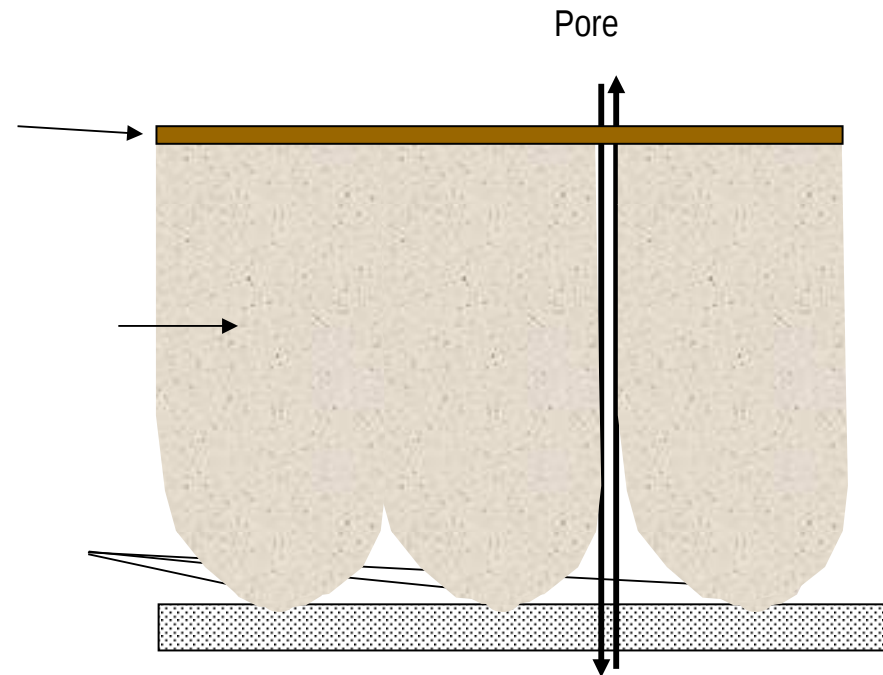
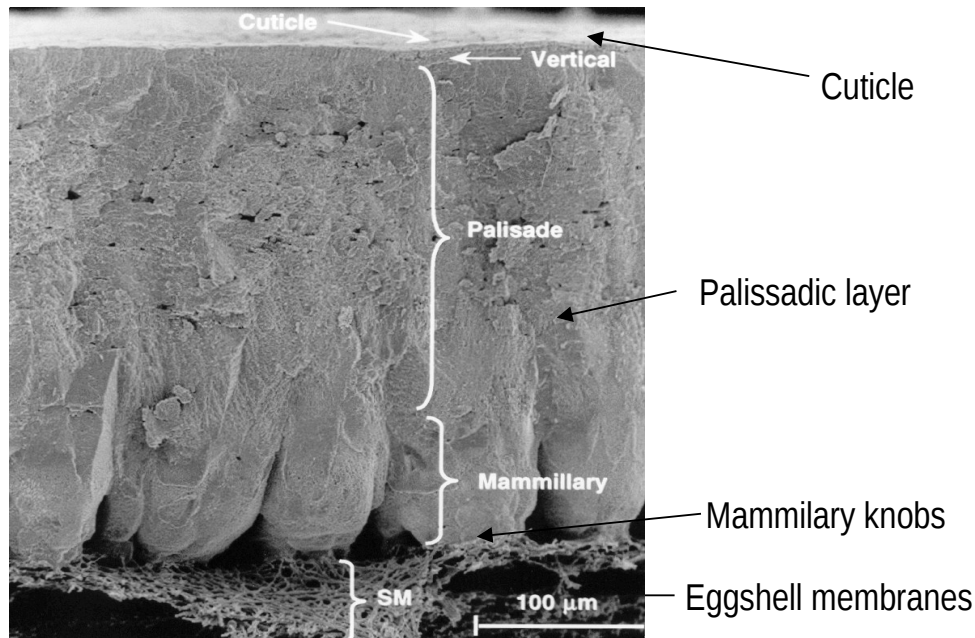
Egg formation

Spatial and temporal regulation



The eggshell (physical defence)

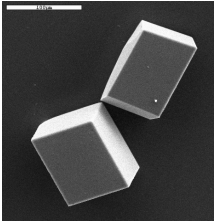
- Eggshell biomineralisation in uterus (one of the fastest on earth)
- Into the uterine fluid with the appropriate physico-chemical conditions
 - Hypersaturation of calcium and bicarbonates*



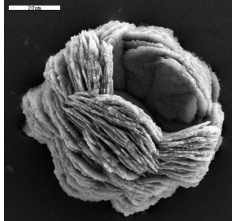
The eggshell (physical defence)

→ 95% of calcium carbonate on calcitic polymorph

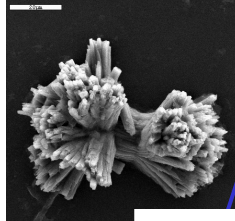
Calcite



Aragonite



Vaterite



Interaction

Quantity

Mechanical properties

- about 300 μm

- eggshell breaking strength (about 4 kg)

Control of the calcification
process

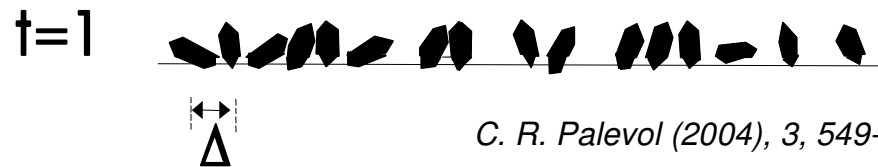
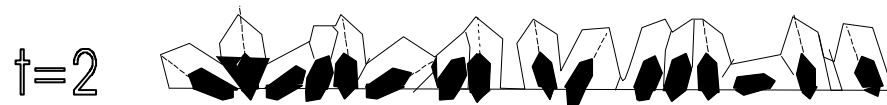
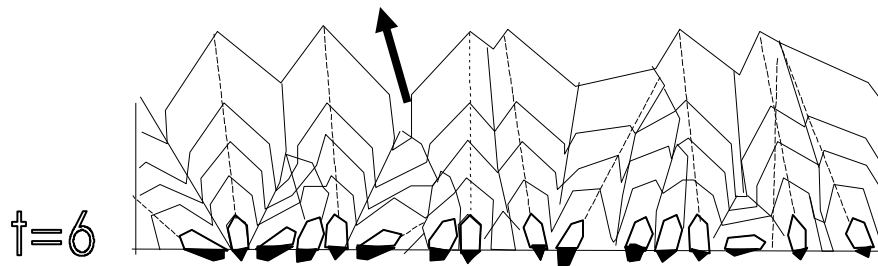
→ 3,5% of organic matter (organic matrix)

Proteins and proteoglycans

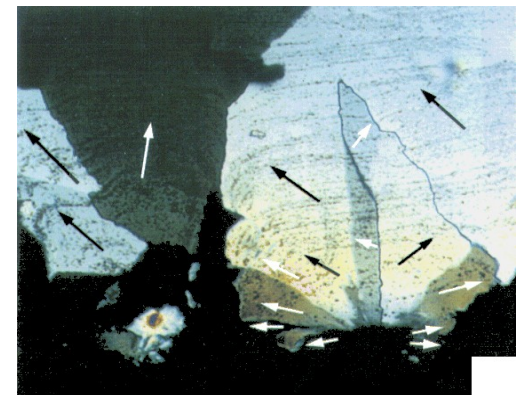
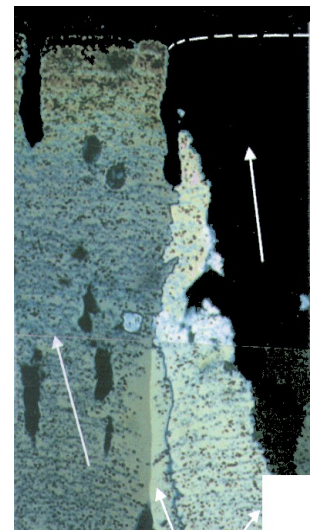
The eggshell (physical defence)

Temporal sequence of the nucleation, the growth

Oriented crystals $\rightarrow$ microstructure



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



$\rightarrow$ Identification and characterization of organic matrix proteins

Ovocalyxin-36

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 282, NO. 8, pp. 5273–5286, February 23, 2007
© 2007 by The American Society for Biochemistry and Molecular Biology, Inc. Printed in the U.S.A.

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.

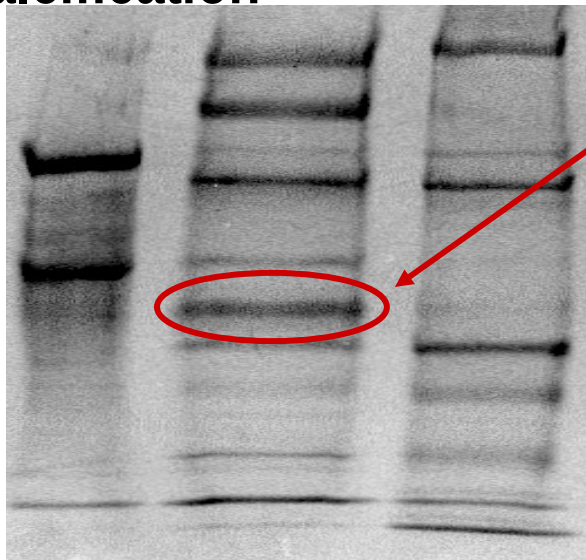
Joël Gautron^{†1}, Emi Murayama[§], Alain Vignal[¶], Mireille Morisson[¶], Marc D. McKee^{||}, Sophie Réhault[‡],
Valérie Labas^{**}, Maya Belghazi^{**}, Mary-Laure Vidal[‡], Yves Nys[‡], and Maxwell T. Hincke^{††}

- 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

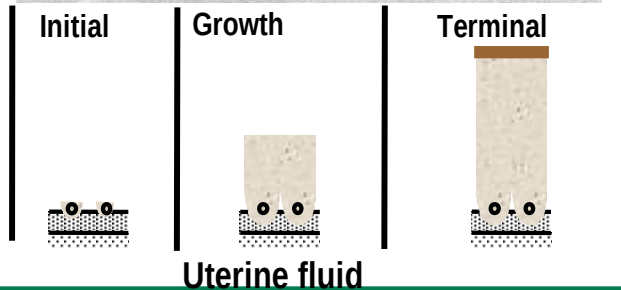


36 kDa Band

N-terminal Amino acid sequencing :
VLGSGLSCAISPAMQQVLSDAIQTGGL

No correspondences in databases

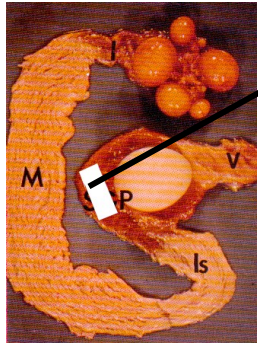
Production of specific antibodies



Uterine fluid

Ovocalyxin-36

→ Expression screening



RNA prepared from uterus
harvested during the shell
calcification

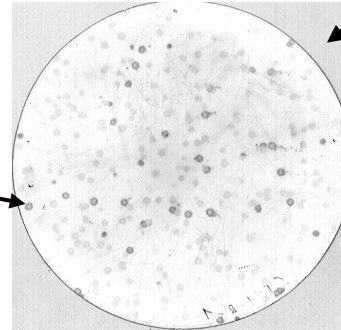
Preparation of a bacteriophage
cDNA expression library

OCX-36 specific antibodies



Expression screening of the
cDNA

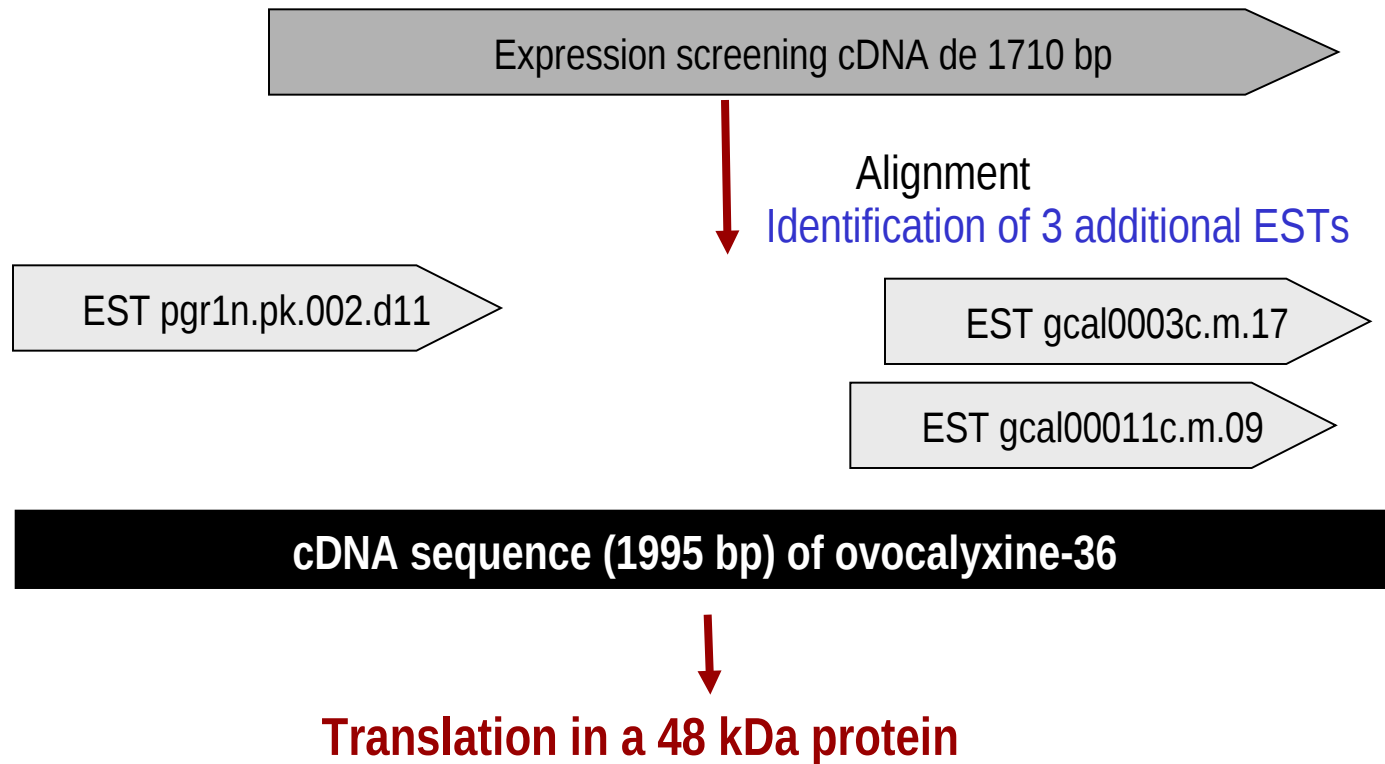
cDNA sequence
of positive clones



cDNA sequence of 1710 bp

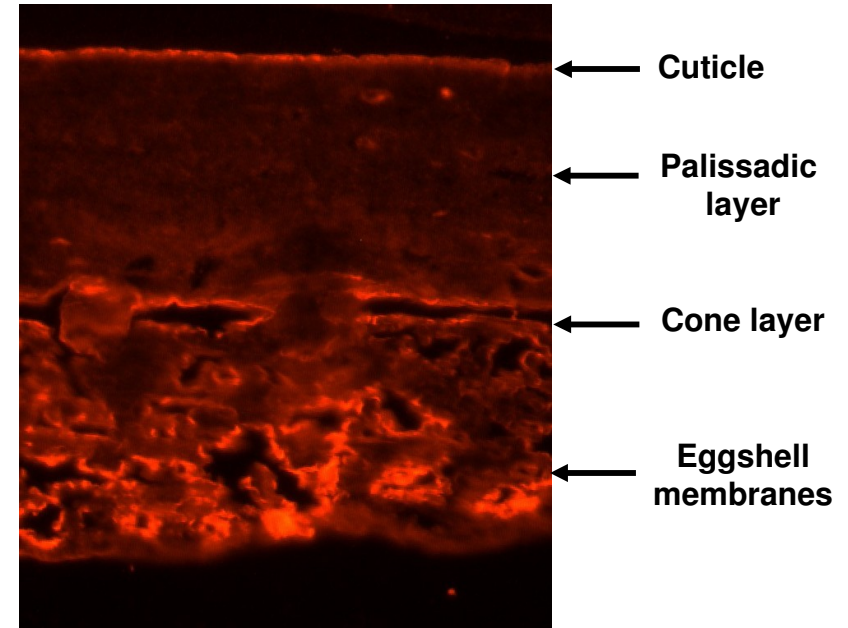
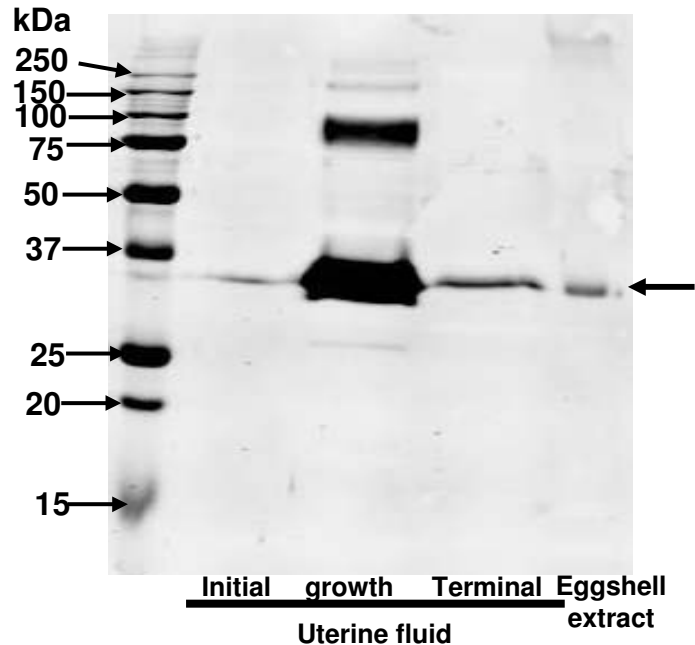
Ovocalyxin-36

- Expression screening
- Cloning and determination of the OCX-36 full-length cDNA



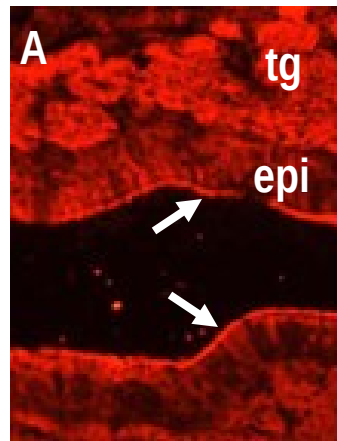
Ovocalyxin-36

→ Ovocalyxin-36 is an eggshell matrix protein

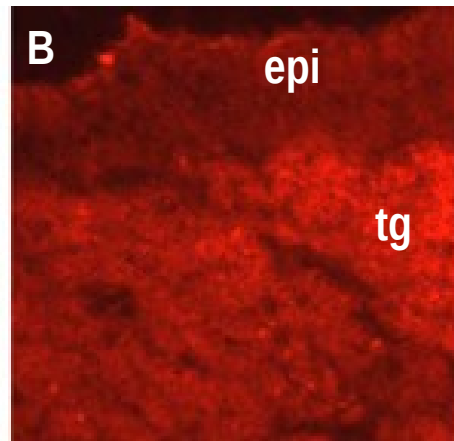


Ovocalyxin-36

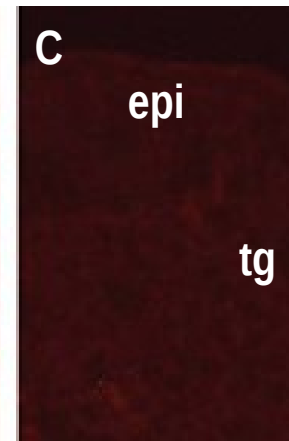
- Ovocalyxin-36 is an eggshell matrix protein
- Ovocalyxin-36 is synthesized in uterine cells



2204 (1/500)



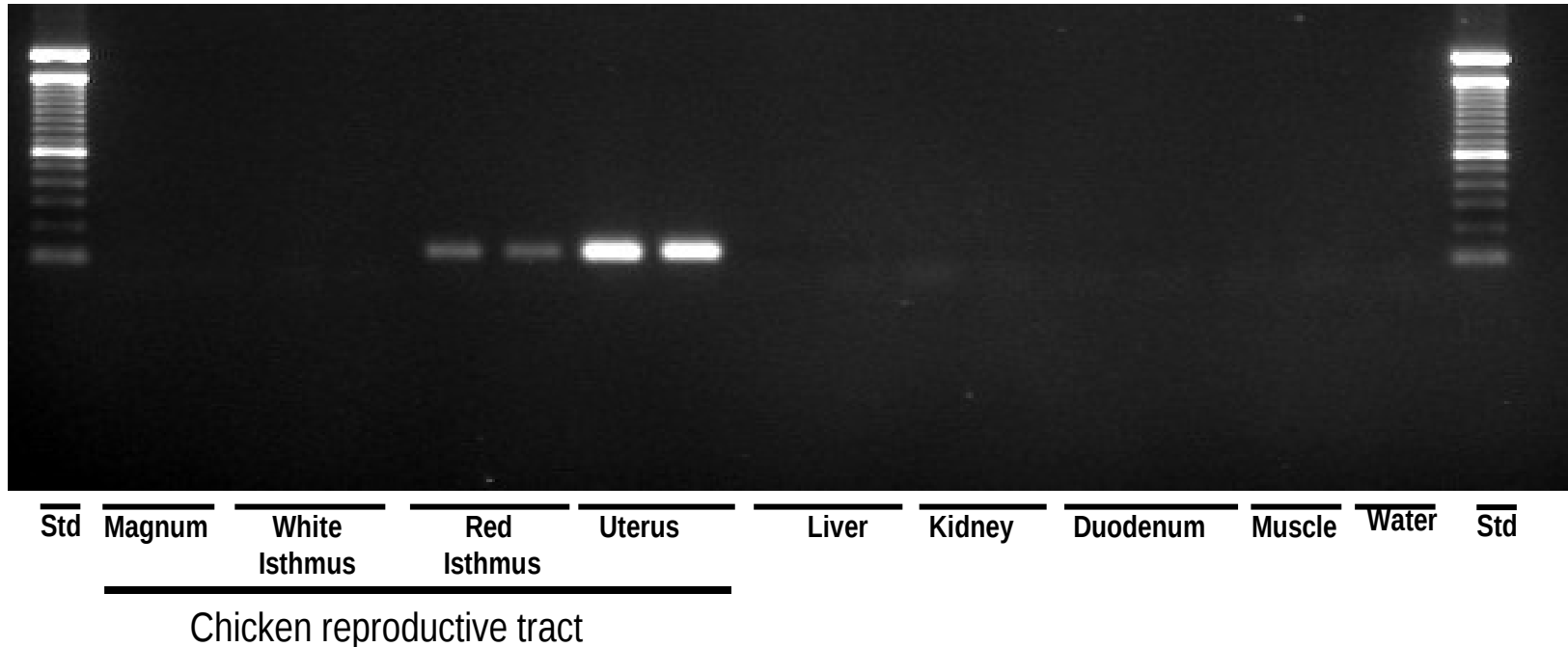
2205 (1/125)



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



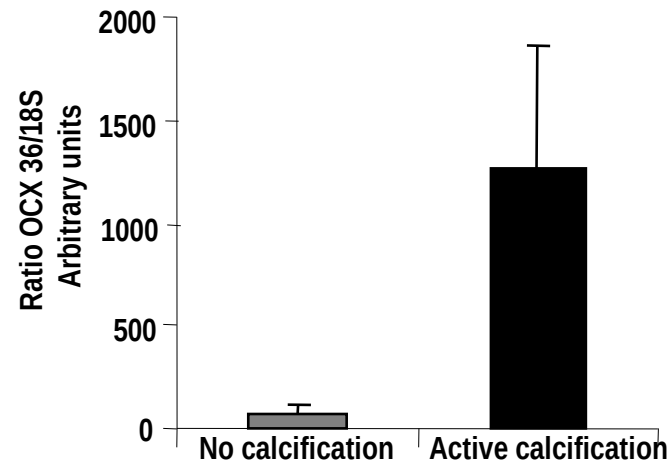
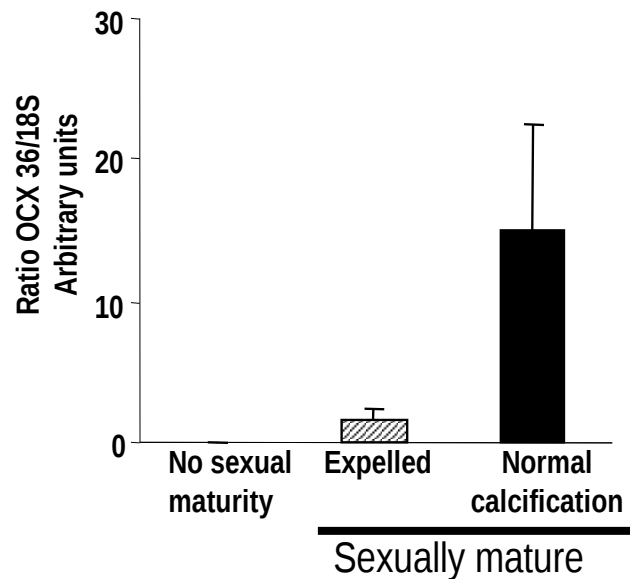
Novel cDNA sequence specific of the eggshell

→ 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



RESEARCH ARTICLE

Proteomic analysis of the chicken egg vitelline membrane

Karlheinz Mann

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

Accession	Accession		Protein name	Modifications	Mass	Charge	Score	Score	Score	Score
00589985.3	Q90835	+	Elongation factor 1- α 1	y,s,	364	4	13	11	6, 9	0.8
00575989.1	P02552	+	Similar to tubulin α 2 (~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



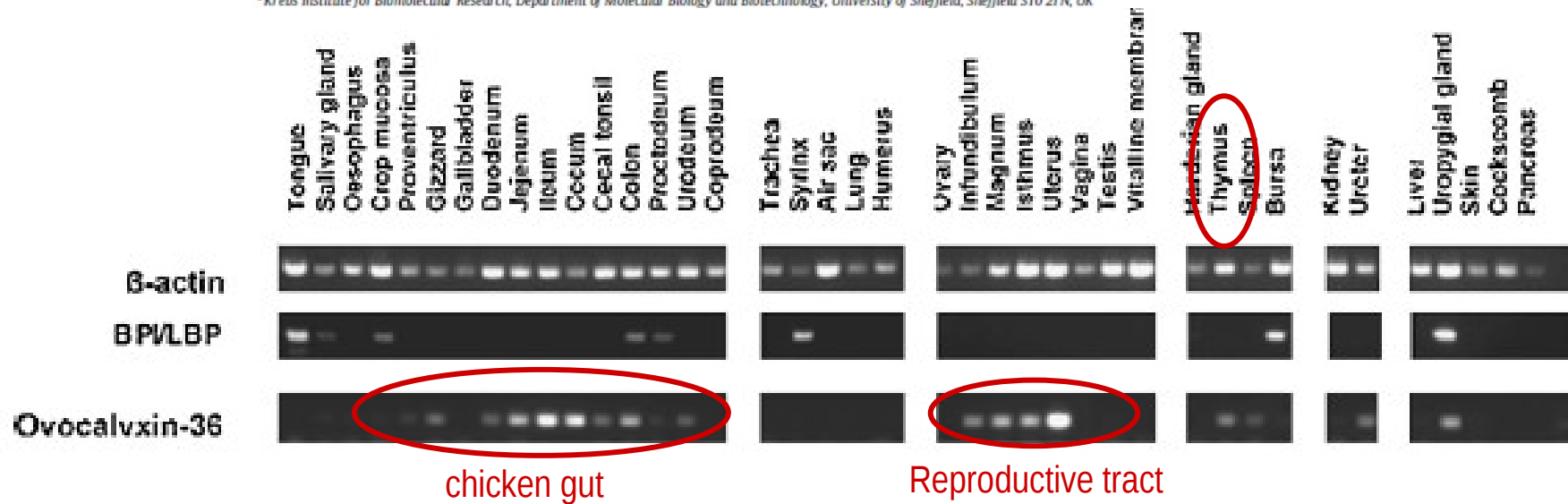
Identification and characterisation of the BPI/LBP/PLUNC-like gene repertoire in chickens reveals the absence of a LBP gene[☆]

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



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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 lipopolysaccharide (LPS) cell wall of the gram negative bacteria
→ Death of bacteria

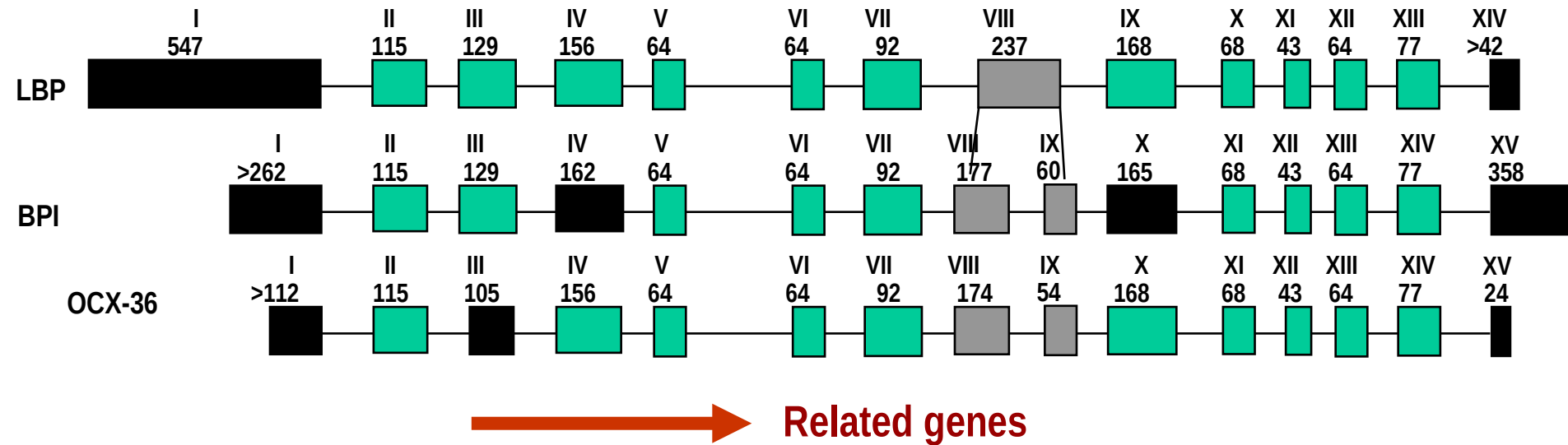
Early recognition of bacterial product

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 Genes¹

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

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[☆]

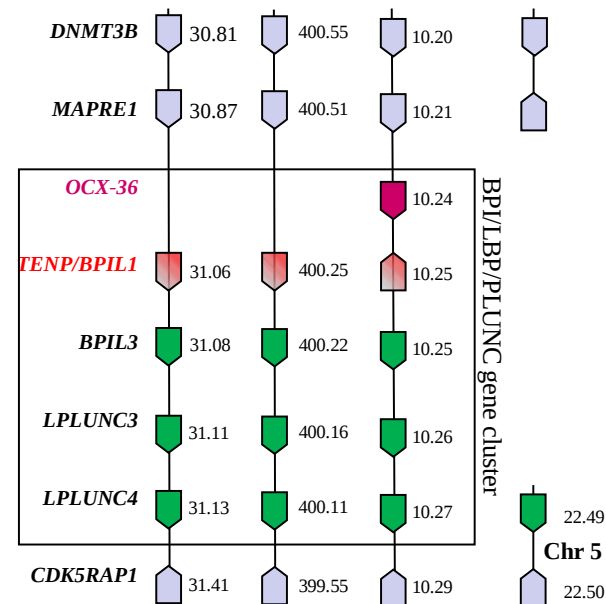
Shih-Chieh Chiang^{a,1}, Edwin J.A. Veldhuizen^b, Frances A. Barnes^a, C. Jeremy Craven^c,
Henk P. Haagsman^b, Colin D. Bingle^{a,*}

^a Academic Unit of Respiratory Medicine, Department of Infection and Immunity, University of Sheffield, Sheffield S10 2JF, UK

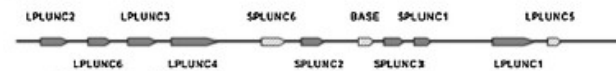
^b Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80165, 3508 TD Utrecht, The Netherlands

^c Krebs Institute for Biomolecular Research, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield S10 2TN, UK

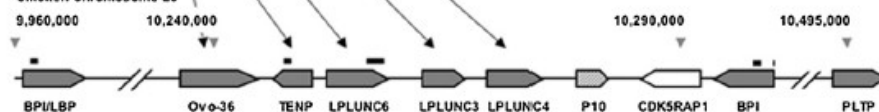
H. sapiens Chr 20 *M. domestica* Chr 1 *G. Gallus* Chr 20 *O. latipes* Ultra90



Human Chromosome 20



Chicken Chromosome 20



ALIMENTATION

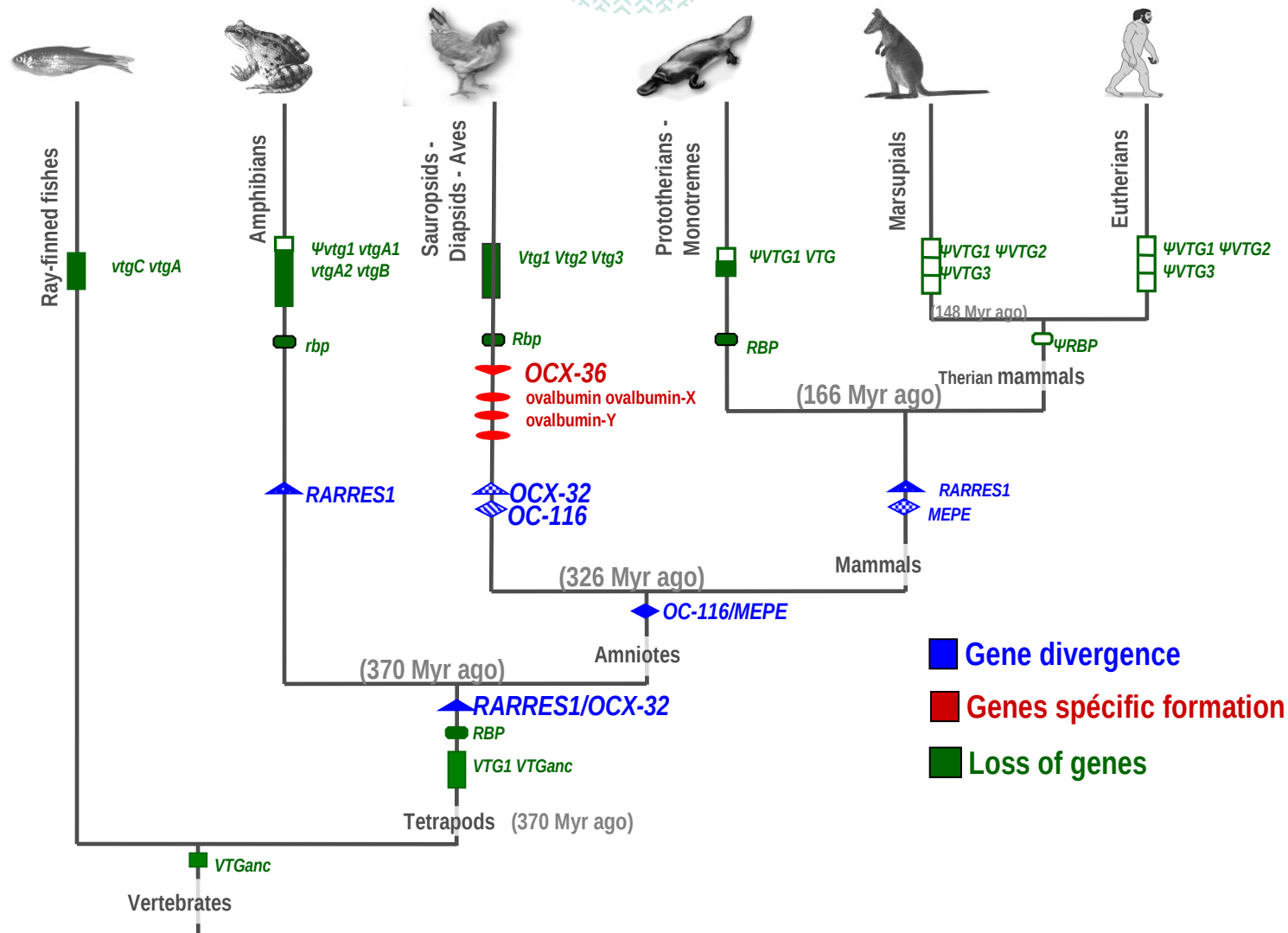
AGRICULTURE

ENVIRONNEMENT



Ovocalyxin-36

→ Evolutionary scenarios of some egg-specific genes along the vertebrate lineages (*Tian et 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*

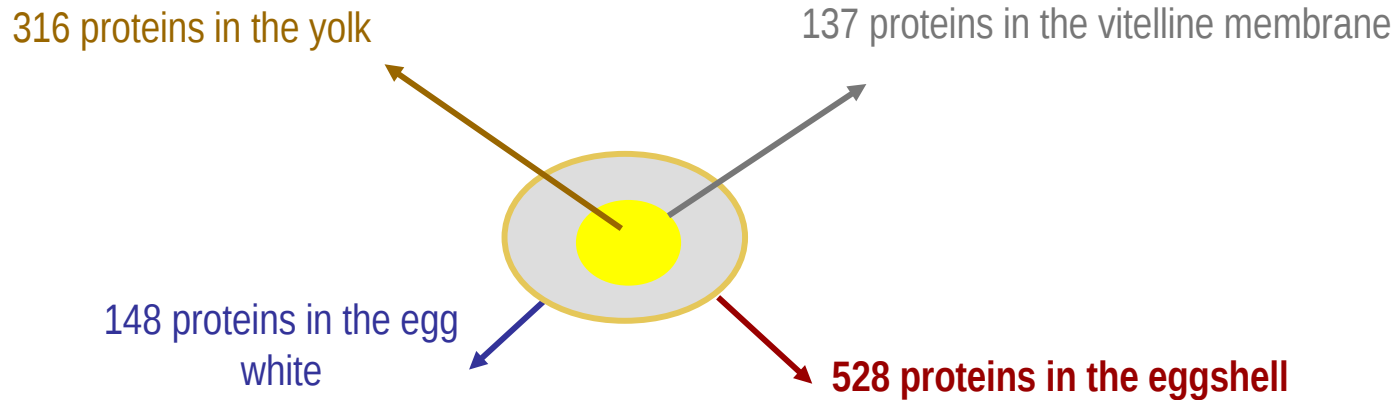
Recent developments

- 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

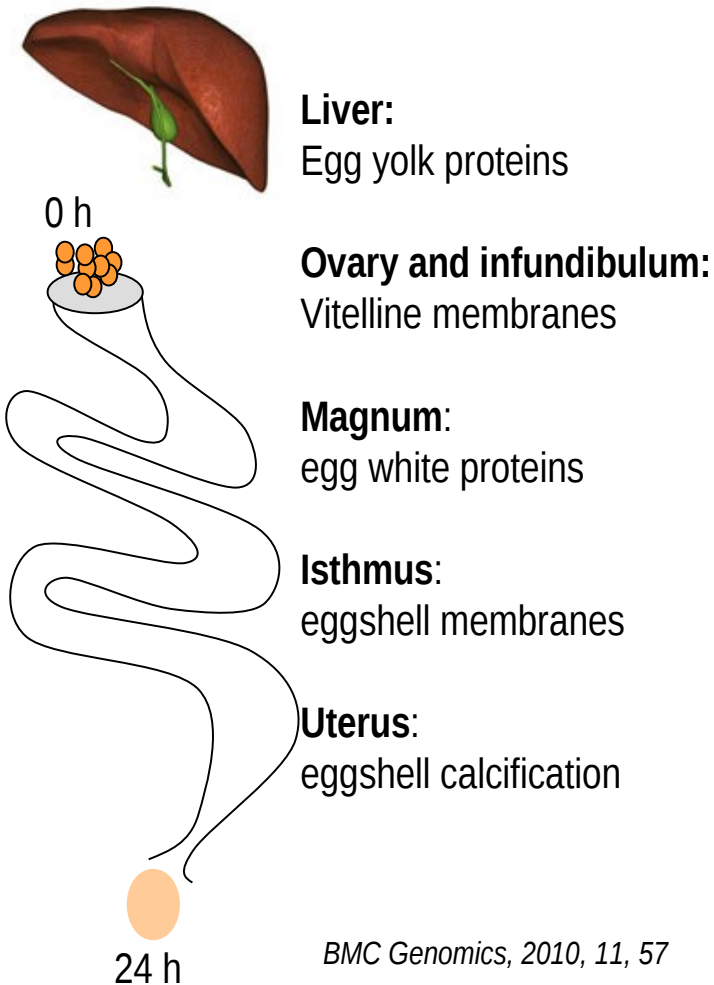
→ **2010, several hundred egg proteins are described**

→ Egg proteome



Recent developments

→ Egg transcriptome



BMC Genomics, 2010, 11, 57

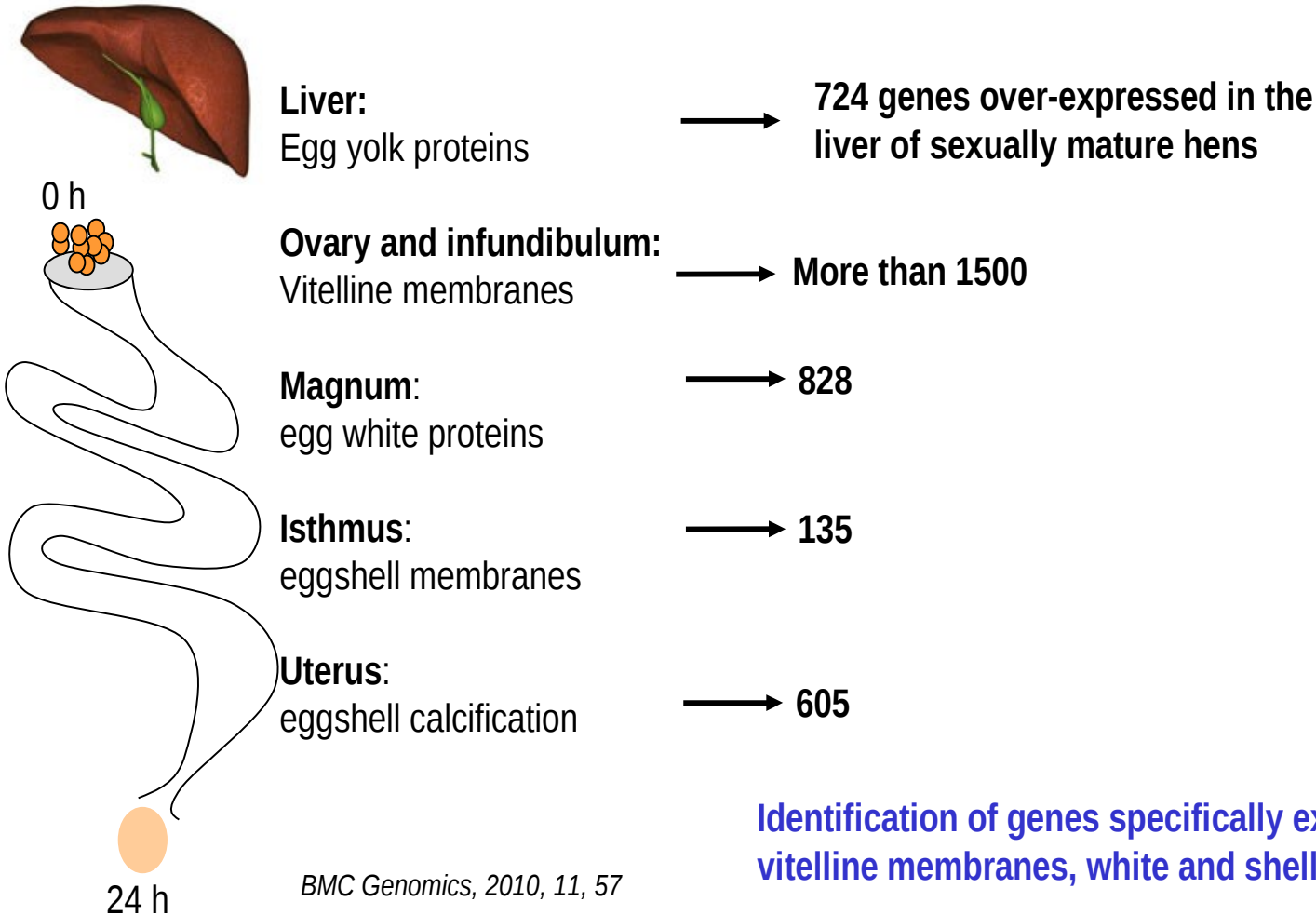
cDNA Microarrays



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

Recent developments

→ Egg transcriptome



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 3558

DOI 10.1002/pmic.200700397

Proteomics 2007, 7, 3558–3568

RESEARCH ARTICLE

The chicken egg white proteome

Proteomic Analysis of Hen Egg White

CATHERINE GUÉRIN-DUBIARD,^{*,†} MARYVONNE PASCO,[†] DANIEL MOLLÉ,[†]
COLETTE DÉSERT,[‡] THOMAS CROGUENNEC,[†] AND FRANÇOISE NAU[†]

Karlheinz Mann

research articles **Journal of
proteome
research**

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

JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY
ARTICLE

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^{*,*}

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

KENJI MAEHASHI,^{*,†} MAMI MATANO,[†] TOMOHIRO IRISAWA,[‡] MASATAKA UCHINO,[‡]
YASU HARU ITAGAKI,^{§,#} KATSUMI TAKANO,[‡] YUTAKA KASHIWAGI,[†] AND
TOSHIHIRO WATANABE[†]

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT

INRA

→ 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

2322

DOI 10.1002/pmic.200800032

Proteomics 2008, 8, 2322–2332

RESEARCH ARTICLE

The chicken egg white proteome

Karlheinz Mann

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

I.C. Dunn^{a,*}, P.W. Wilson^a, Z. Lu^a, M.M. Bain^b, C.L. Crossan^b, R.T. Talbot^a, D. Waddington^a

^a Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin, Midlothian, Scotland EH25 9PS, UK

^b University of Glasgow, Faculty of Veterinary Medicine, Glasgow, Scotland G61 1QH, UK

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Similar to BPI (XP_425484)

→ Similar to BPI in egg white

Journal of
research articles **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)



Reducing Egg Susceptibility to Contaminations in Alternative Production in Europe (2006-2009)

(Coordinator Y. Nys, INRA, UR 83 Recherches avicoles, F-37380 Nouzilly, France)



2006-2010

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

ALIMENTATION
AGRICULTURE
ENVIRONNEMENT



Researchers



Yves NYS
Director



Joël
GAUTRON



Sophie REHAULT
-GODBERT



Nicolas
GUYOT

PhD Students



Larbi
BEDRANI



Marie
BOURIN

Research technicians



Aurélien
BRIONNE



Maryse
MILLS



Jean-Claude
POIRIER



Magali
BERGES

SIGENAE (bioinformatic)



Vincent
JONCHERE



Cédric
CABAU