



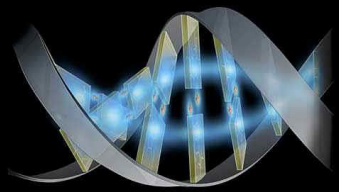
ISGA 2015



APPLICATION OF RNA-SEQ IN INVESTIGATING A MAJOR PARASITIC DISEASE OF TURBOT (*Scophthalmus maximus*), ENTEROMYXOSIS

**P. Ronza, D. Robledo, A. P. Losada, R. Bermúdez,
B. G. Pardo, P. Martínez and M. I. Quiroga**

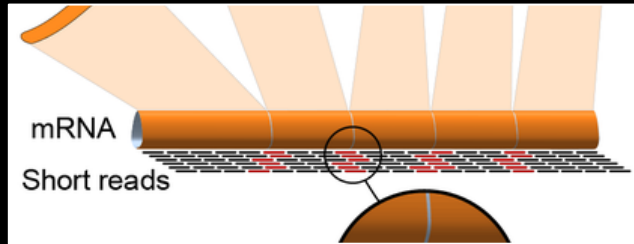
*International Symposium on Genetics in Aquaculture XII
21st-27th June 2015, Santiago de Compostela*



TRANSCRIPTOME ANALYSIS

•Microarrays

**Next Generation Sequencing
(NGS)**



•RNA-Seq

cDNA sequencing

Mapping to a reference genome

ILLUMINA HISEQ 2000



Huge amount of data
Complex analysis

***Interdisciplinary
approach***

↑ Specificity
Sensitivity
Reproducibility
Sequence variations
Whole transcriptome analysis



RNA-Seq and pathology

- SAMPLES CHARACTERIZATION
- CONTEXTUAL INTERPRETATION

**INTERPRETATION OF
PATHOLOGICALLY
RELEVANT SIGNALS**

OMICS A Journal of Integrative Biology
Volume 18, Number 2, 2014
© Mary Ann Liebert, Inc.
DOI: 10.1089/omi.2013.0110

RNA-Seq Technology and Its Application in Fish Transcriptomics

Xi Qian,¹ Yi Ba,² Qianfeng Zhuang,³ and Guofang Zhong⁴

DNA RESEARCH **20**, 449–460, (2013)
Advance Access publication on 10 June 2013

doi:10.1093/dnares/dst022

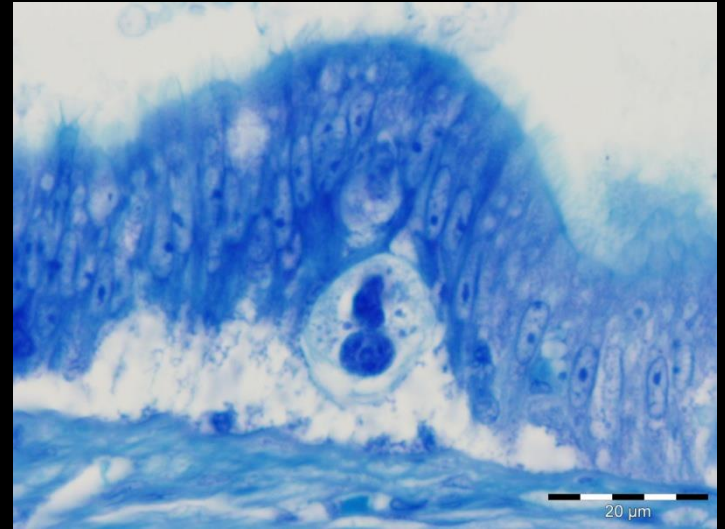
Analysis of Stress-Responsive Transcriptome in the Intestine of Asian Seabass (*Lates calcarifer*) using RNA-Seq

JUN HONG Xia¹, PENG Liu¹, FENG Liu¹, GRACE Lin¹, FEI SUN¹, RONGJIAN Tu¹, and GEN HUA Yue^{1,2,*}

Molecular Population Genetics Group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, Singapore 117604, Singapore¹ and Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore²

Enteromyxosis

- Intestinal parasitosis
- Cachectic syndrome
- No therapeutic options
- Economic losses

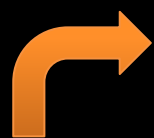


Enteromyxum leei



Enteromyxum scophthalmi

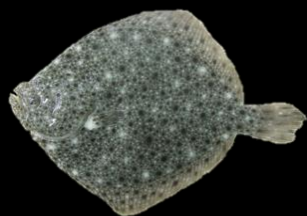
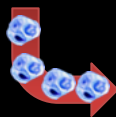




55 RCPT

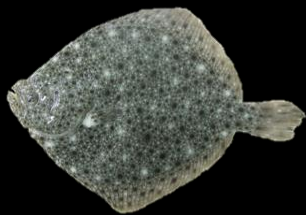
1 ml solution

Instestinal scrapings



65 CTRL

1 ml saline solution



Light microscopy



Sampling points



15 x RCPT

15 x CTRL

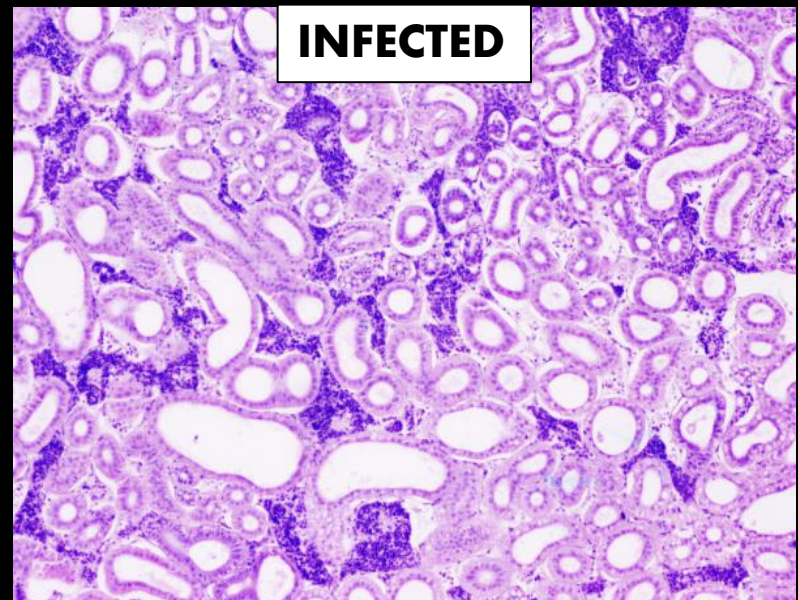
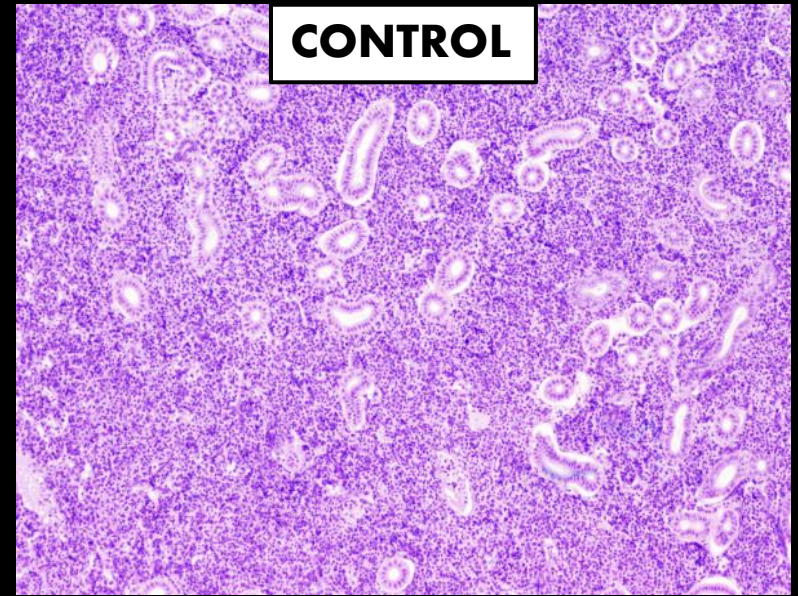
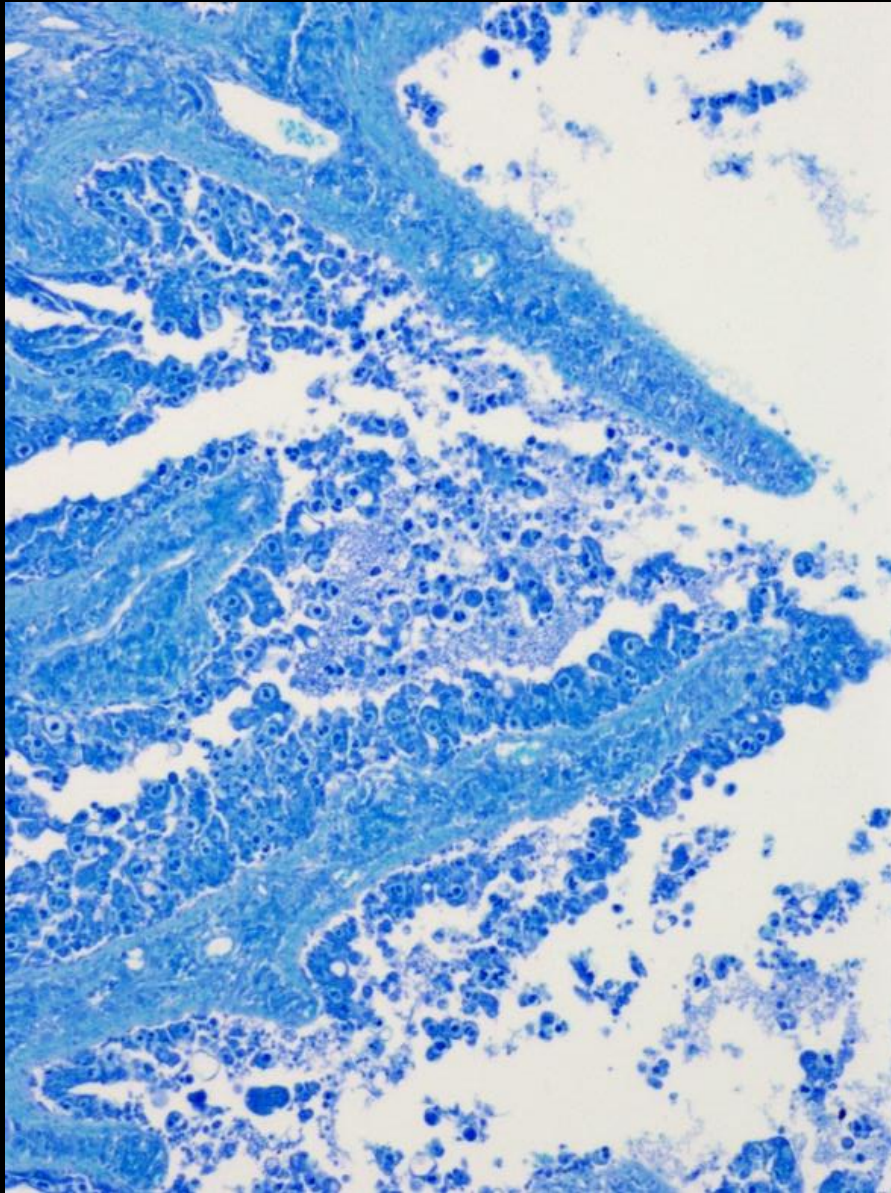
Bouin's fluid

RNAlater

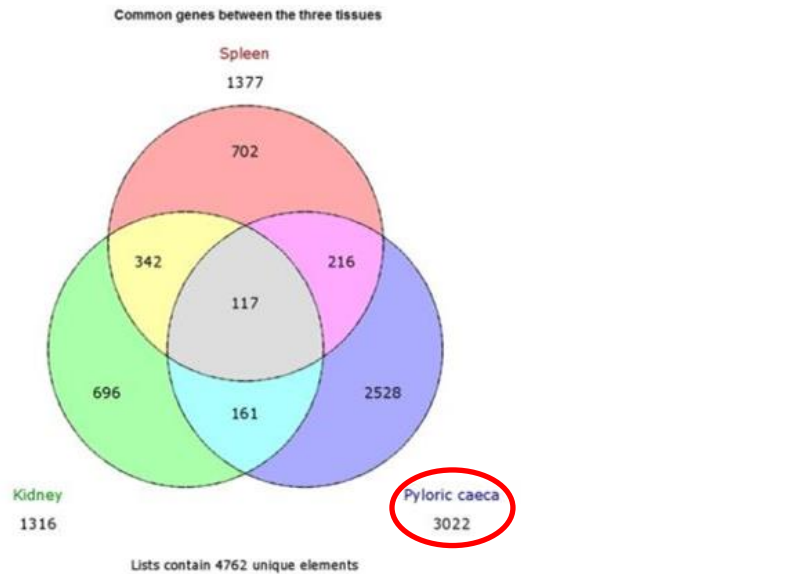
	Oesophagus
	Stomach
	Pyloric caeca
	Anterior intestine
	Medium intestine
Posterior intestine	
	Kidney
	Spleen
	Thymus
	Liver

Histopathological evaluation

SEVERE ENTEROMYXOSIS



SEVERE ENTEROMYXOSIS

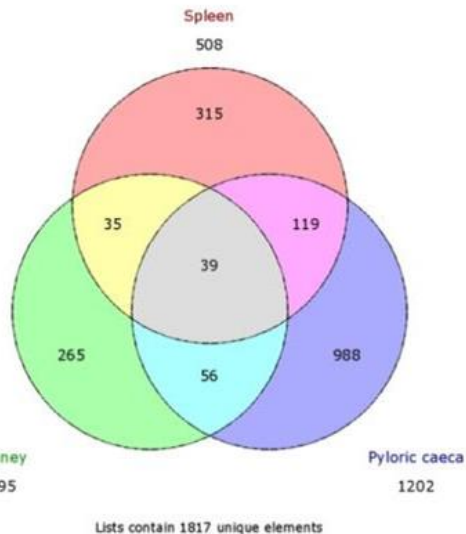


❖ >23000 identified genes

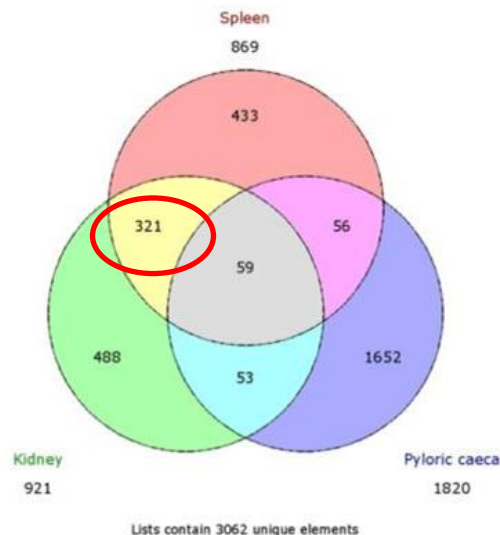
❖ 4762 differentially expressed between control and infected fish

❖ >3000 in pyloric caeca

Common up-regulated genes between the three tissues



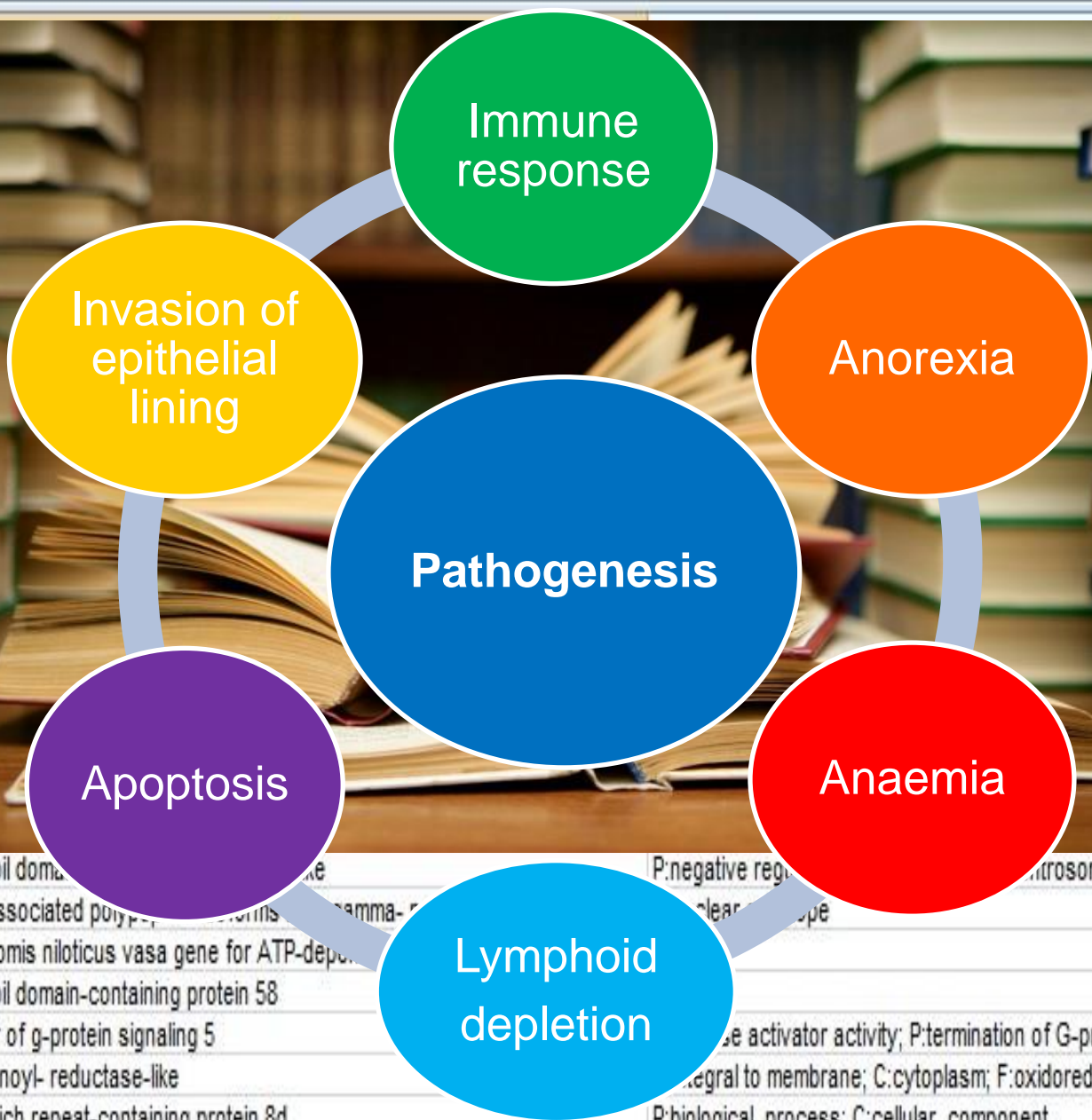
Common down-regulated genes between the three tissues



❖ 50% more downregulated than upregulated genes

❖ 321 common downregulated genes between spleen and kidney

F	G		
Media	kidneycontro		
2,29	0,00		
2,20	0,00		
1,49	0,00		
1,00	0,00		
0,43	0,00		
0,44	0,00		
0,59	0,00		
0,71	0,00		
18,04	0,23		
36,53	0,69		
16,42	0,58		
3,68	0,13		
17,41	0,74		
9,80	0,46		
3,86	0,20		
0,99	0,09		
1,77	0,09		
0,71	0,10		
0,57	0,03	coiled-coil doma	P:negative reg
0,87	0,05	lamina-associated poly	gamma- r
0,40	0,02	Oreochromis niloticus vasa gene for ATP-dep	clear
3,38	0,21	coiled-coil domain-containing protein 58	
6,76	0,42	regulator of g-protein signaling 5	se activator activity; P:termination of G-protein coupled receptor signaling
24,46	1,54	trans--enoyl- reductase-like	egral to membrane; C:cytoplasm; F:oxidoreductase activity, acting on the CH
0,45	0,03	leucine-rich repeat-containing protein 8d	P:biological process; C:cellular component



metabolic process
molecular_function; P:biolo
circadian sleep/wake cyc
n; P:transposition, DNA-m
oxidation-reduction proces
ense response to bacteri
ongation; P:GTP catabolic
ation of Wnt receptor sign
ed receptor signaling path
um ion transport; P:muscl
binding

RESEARCH ARTICLE

Open Access

RNA-seq analysis reveals significant transcriptome changes in turbot (*Scophthalmus maximus*) suffering severe enteromyxosis

Diego Robledo^{1†}, Paolo Ronza^{2†}, Peter W Harrison³, Ana Paula Losada², Roberto Bermúdez⁴, Belén G Pardo⁵, María José Redondo⁶, Ariadna Sitjà-Bobadilla⁶, María Isabel Quiroga^{2*} and Paulino Martínez^{3,5}

FIVE CATEGORIES

- 1) Immune and defence response
- 2) Apoptosis and cell proliferation
- 3) Iron metabolism and erythropoiesis
- 4) Cytoskeleton and extracellular matrix
- 5) Metabolism and digestive function

SEVERE ENTEROMYXOSIS

Iron metabolism and erythropoiesis

↓
**SPLEEN
KIDNEY**

ANAEMIA

Metabolism and digestive function

↓
**PYLORIC
CAECA**

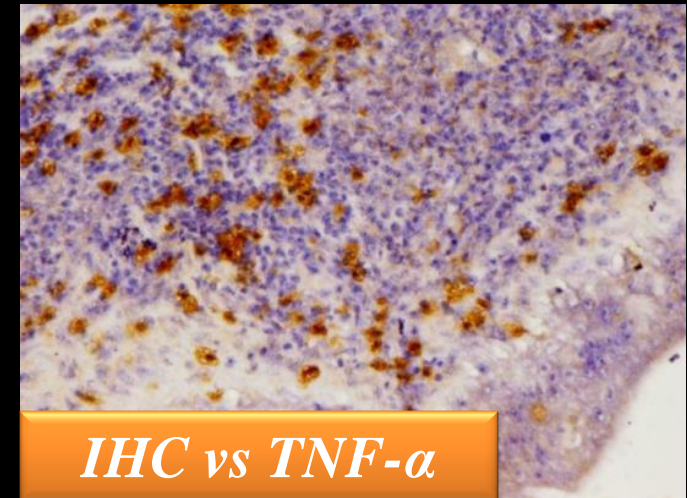
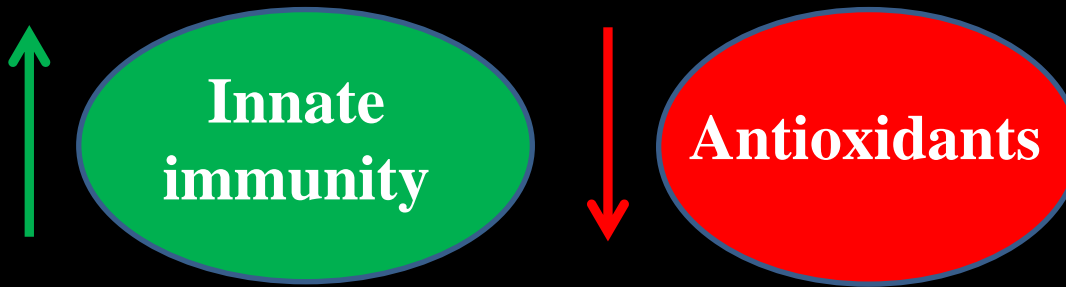
ANOREXIA

Cytoskeleton and extracellular matrix

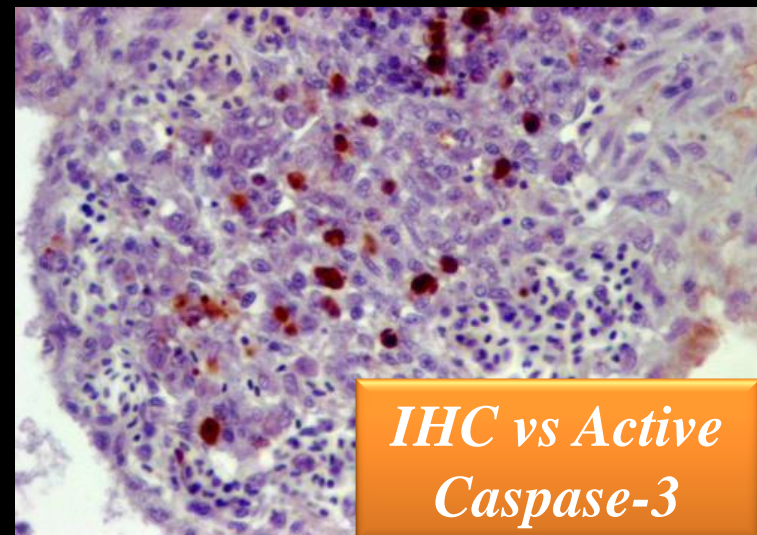
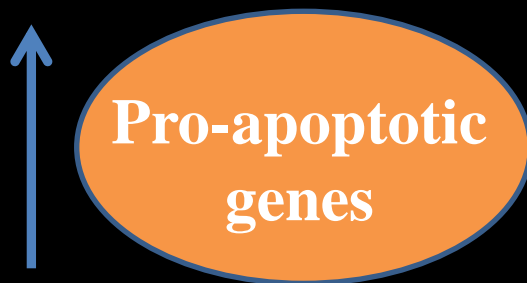
↓
**PYLORIC
CAECA
SPLEEN
KIDNEY**

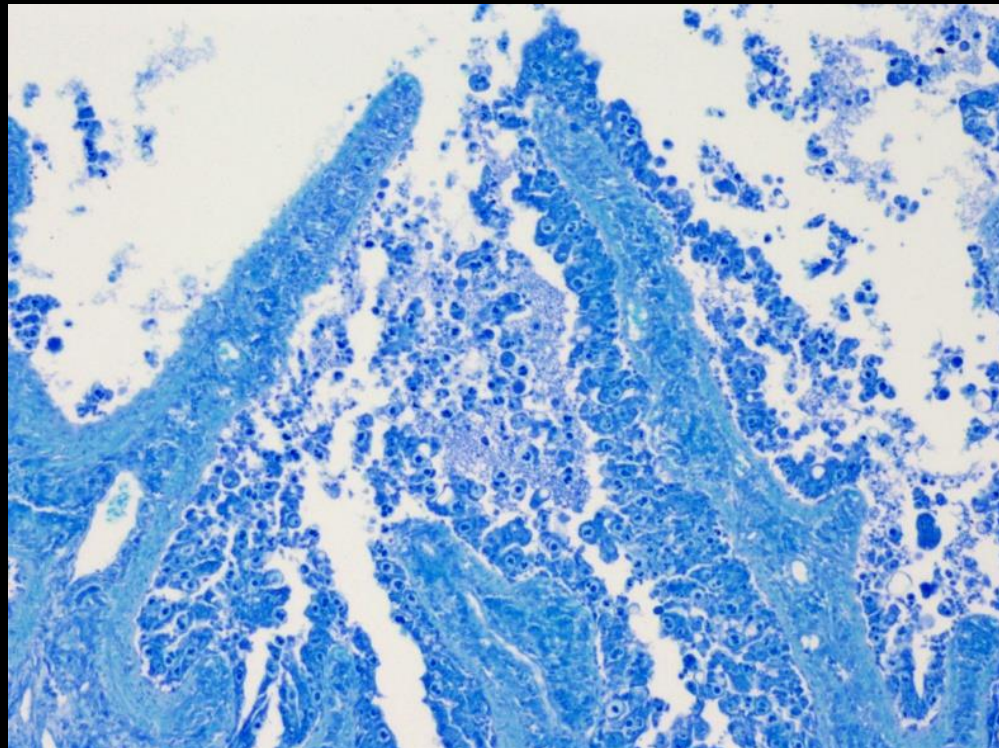
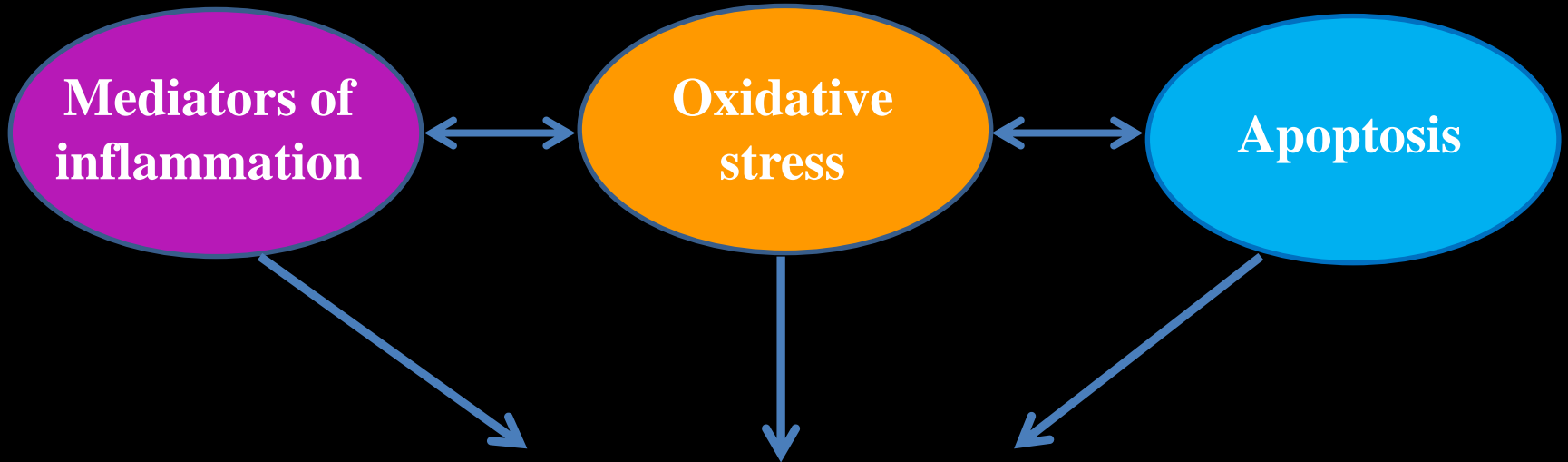
CACHEXIA

Immune and defence response

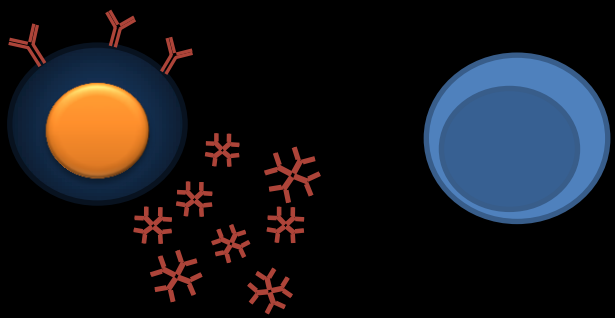


Apoptosis and cell proliferation





B - AND T-CELLS



FAILURE IN THE CONNECTION INNATE-ADAPTIVE IMMUNITY?

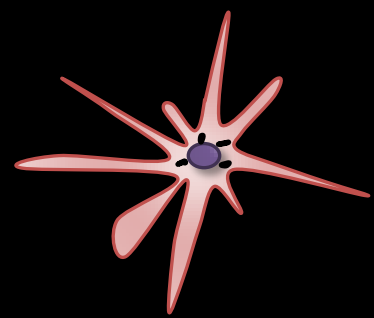
INTERFERONS

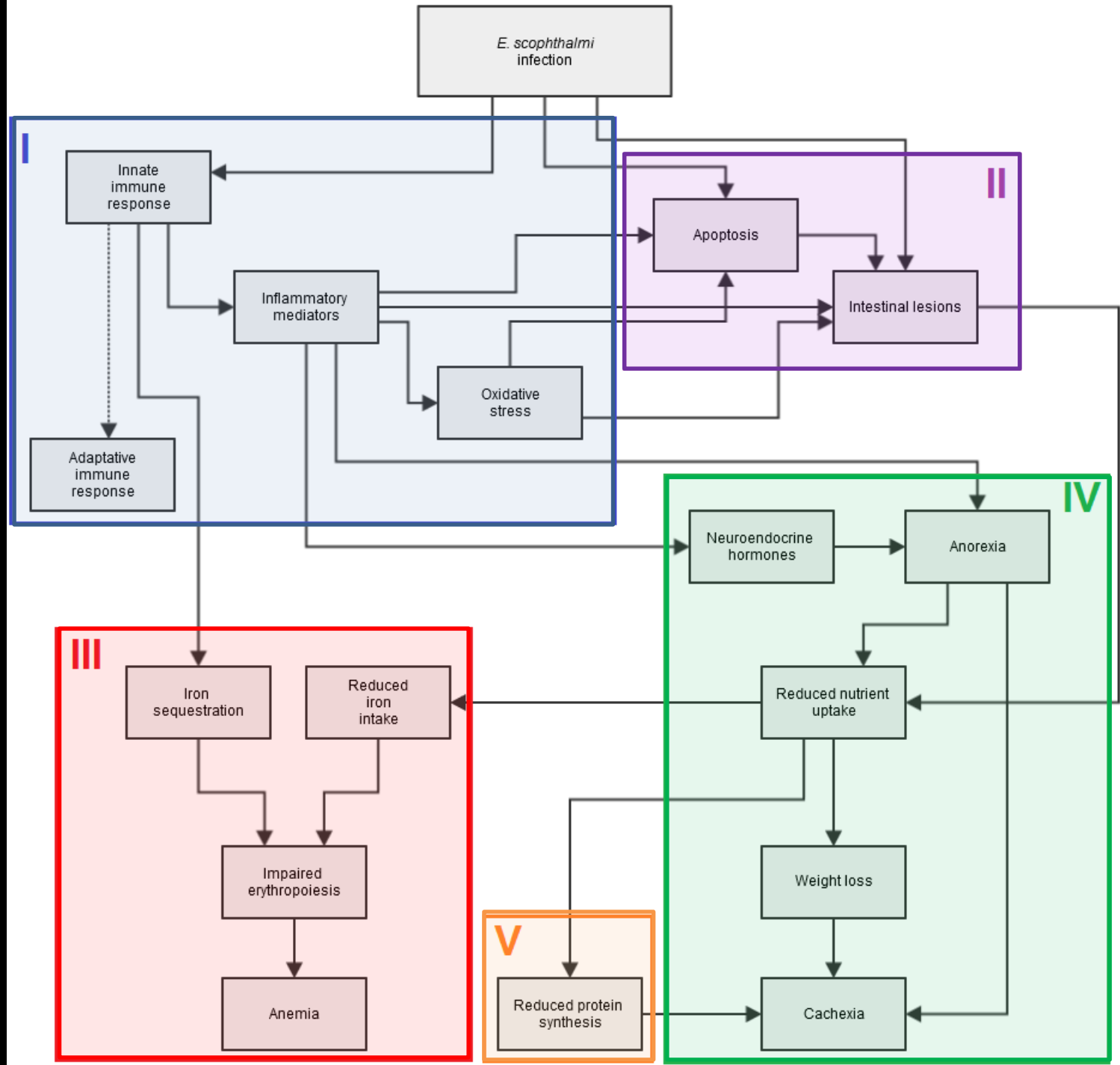


T HELPER 17 CELLS



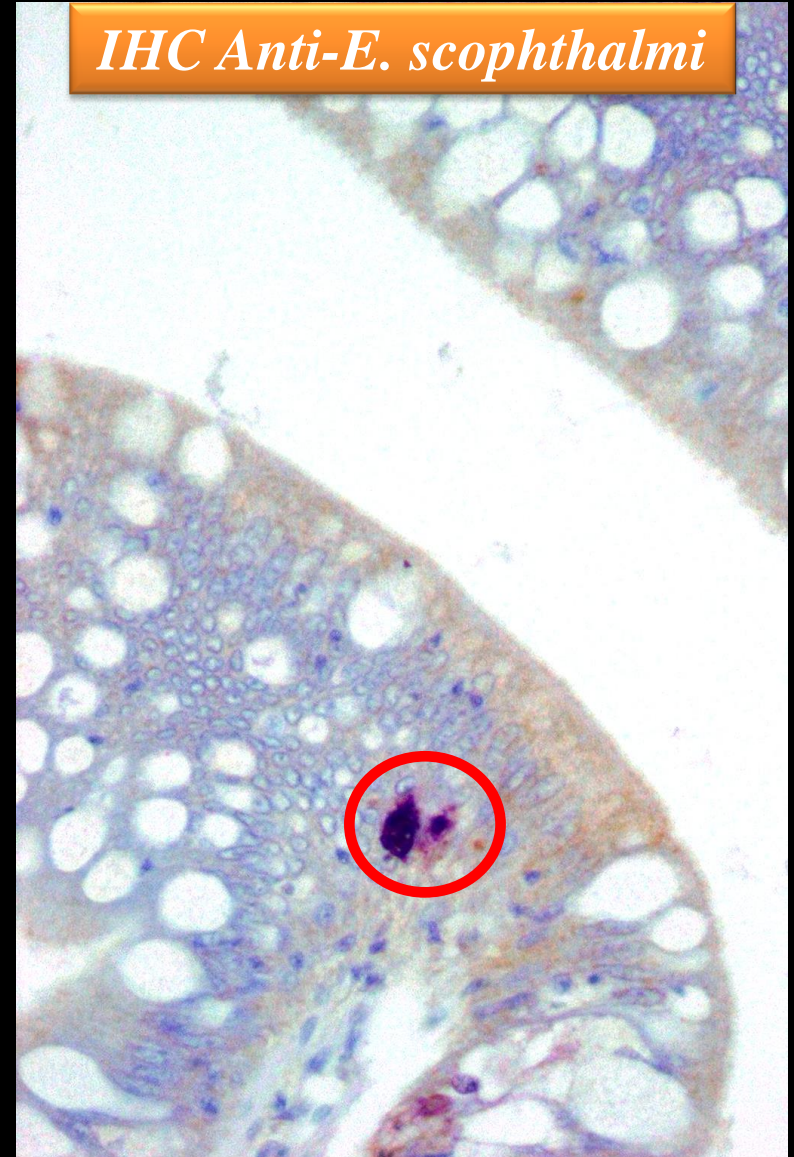
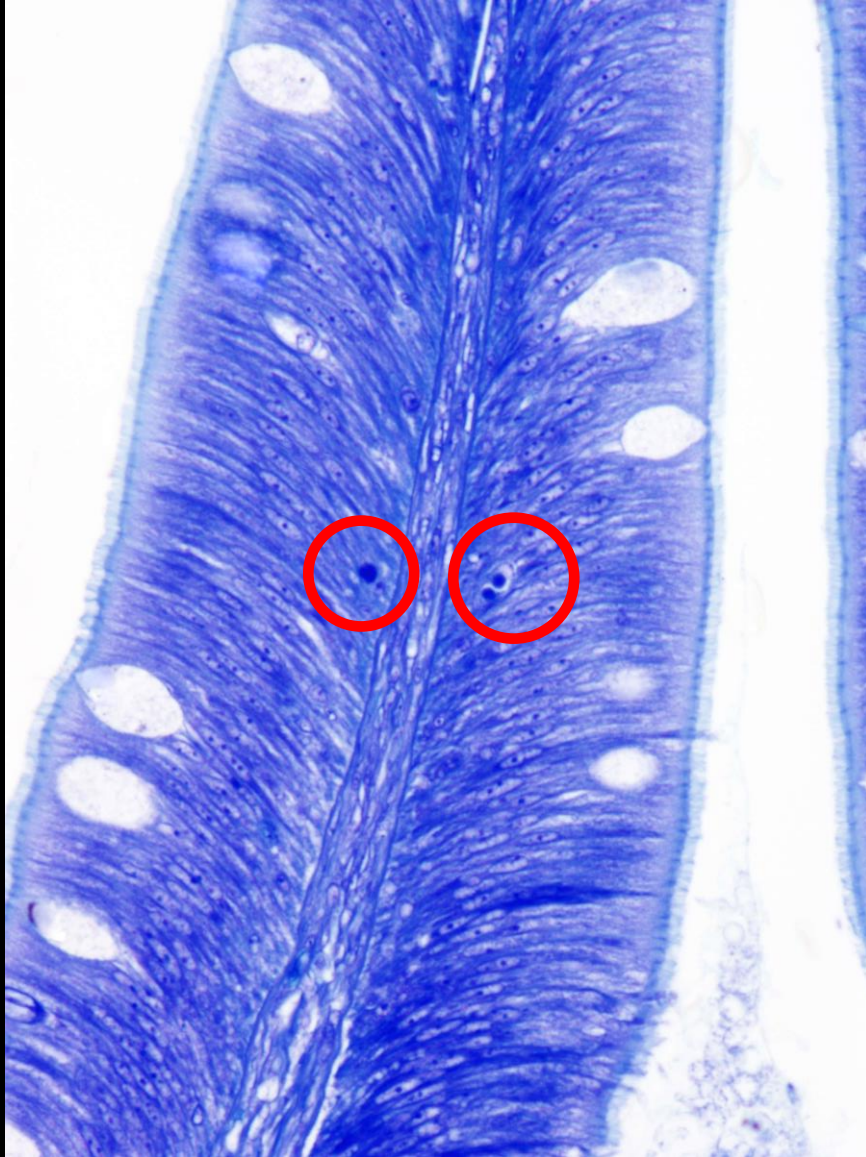
DENDRITIC CELLS








Histopathological evaluation

SLIGHT/INCIPIENT ENTEROMYXOSIS



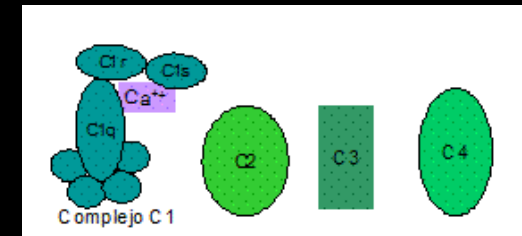
INCIPIENT ENTEROMYXOSIS

<i>ORGAN</i>	<i>D.E. GENES</i>	
	<i>SLIGHT</i>	<i>SEVERE</i>
KIDNEY 	287	1316
SPLEEN 	211	1377
PYLORIC CAECA 	187	3022

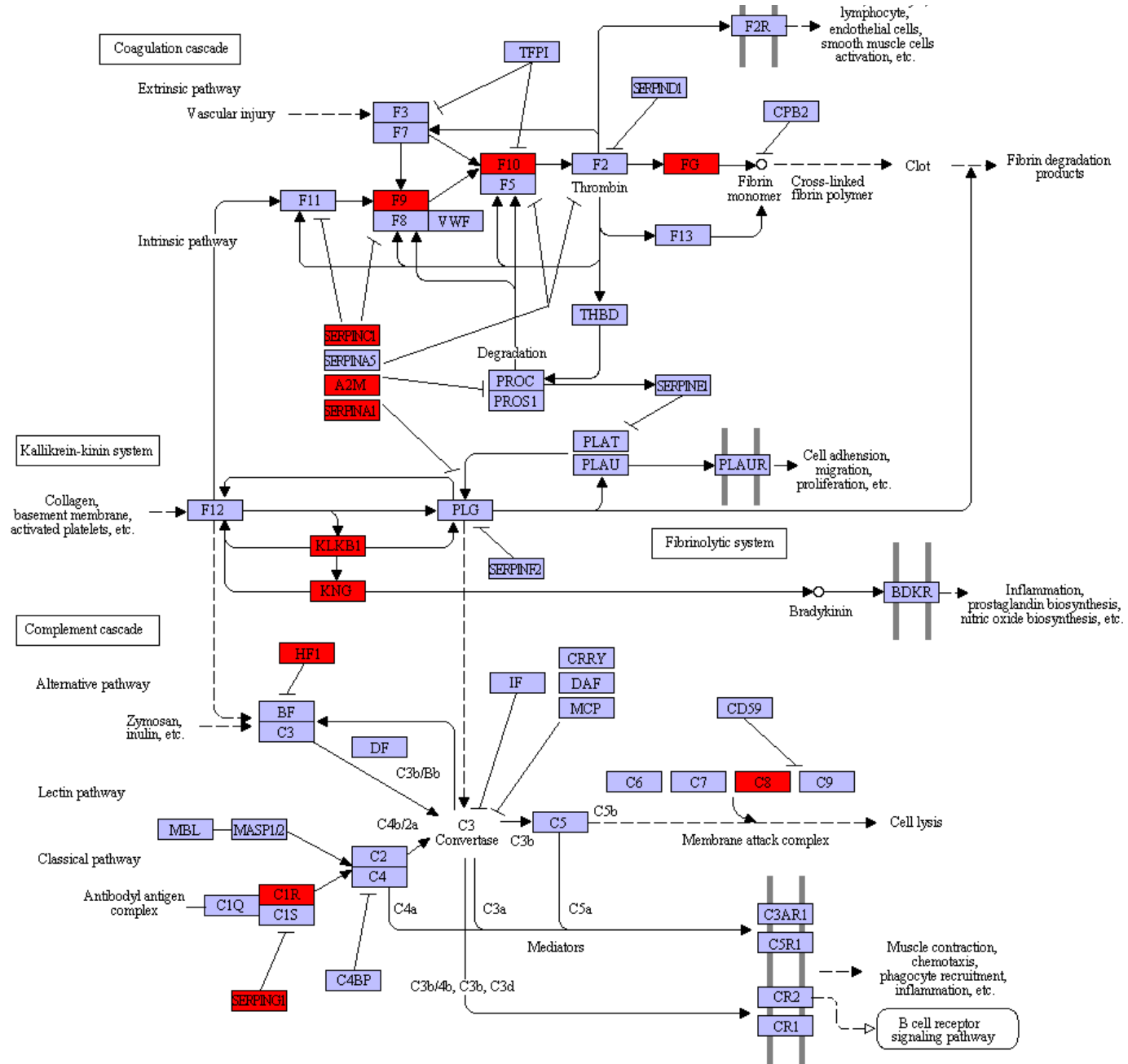
INTERFERONS



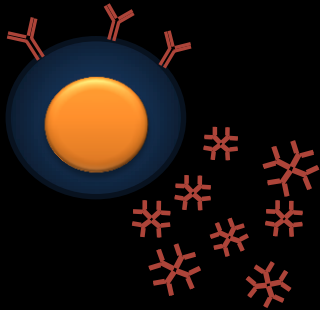
COMPLEMENT



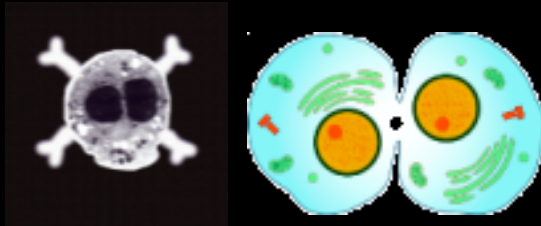
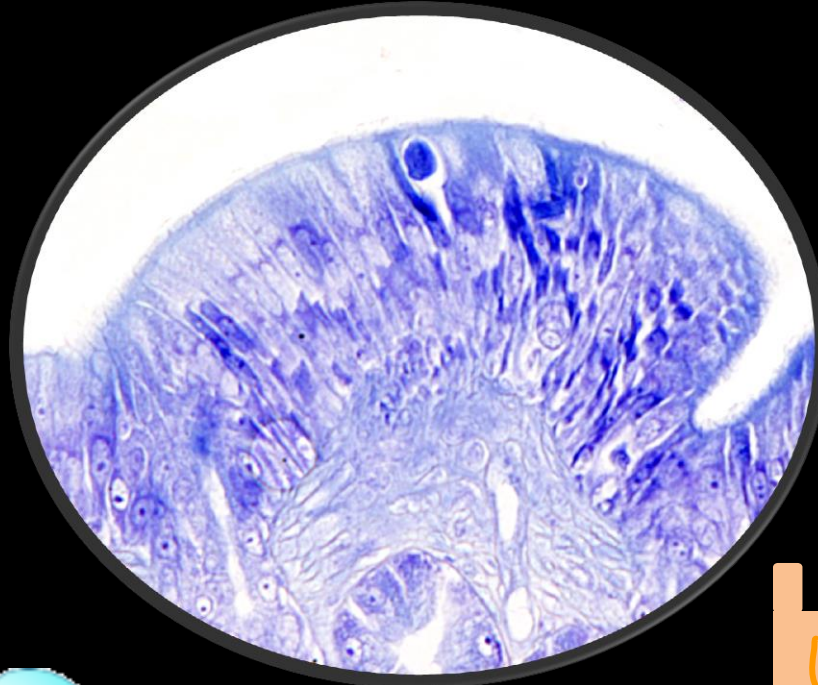
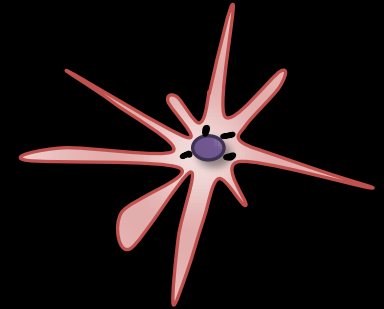
COMPLEMENT AND COAGULATION CASCADES



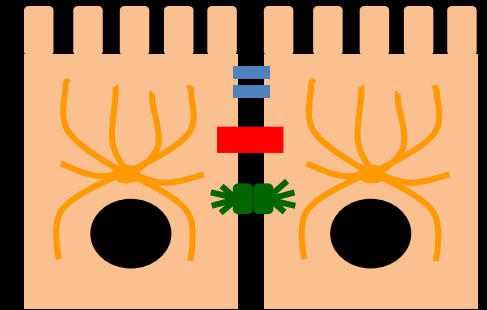
B-Cells
Ig production



CD209
C-type lectin



Apoptosis & cell proliferation



Structural proteins

- ↓
- CASPASE-3
 - CELL PROLIFERATION
 - CELL DIFFERENTIATION

- ↓
- CYTOSKELETON
- ↑
- CELL-CELL UNIONS
 - EXTRACELLULAR MATRIX

Conclusions

- Genetic basis underlying the physiopathological features of enteromyxosis
- Corroboration of histopathological observations
- New insights into disease's pathogenesis
- RNA-Seq + contextual interpretation of changes in gene expression
 - ✓ Identification of candidate genes: diagnosis, resistance, therapeutic options



**INMUNOGENOM
RESEARCH NETWORK**
Fish and shellfish
immunogenomics



ACUIGEN

GAPAVET

