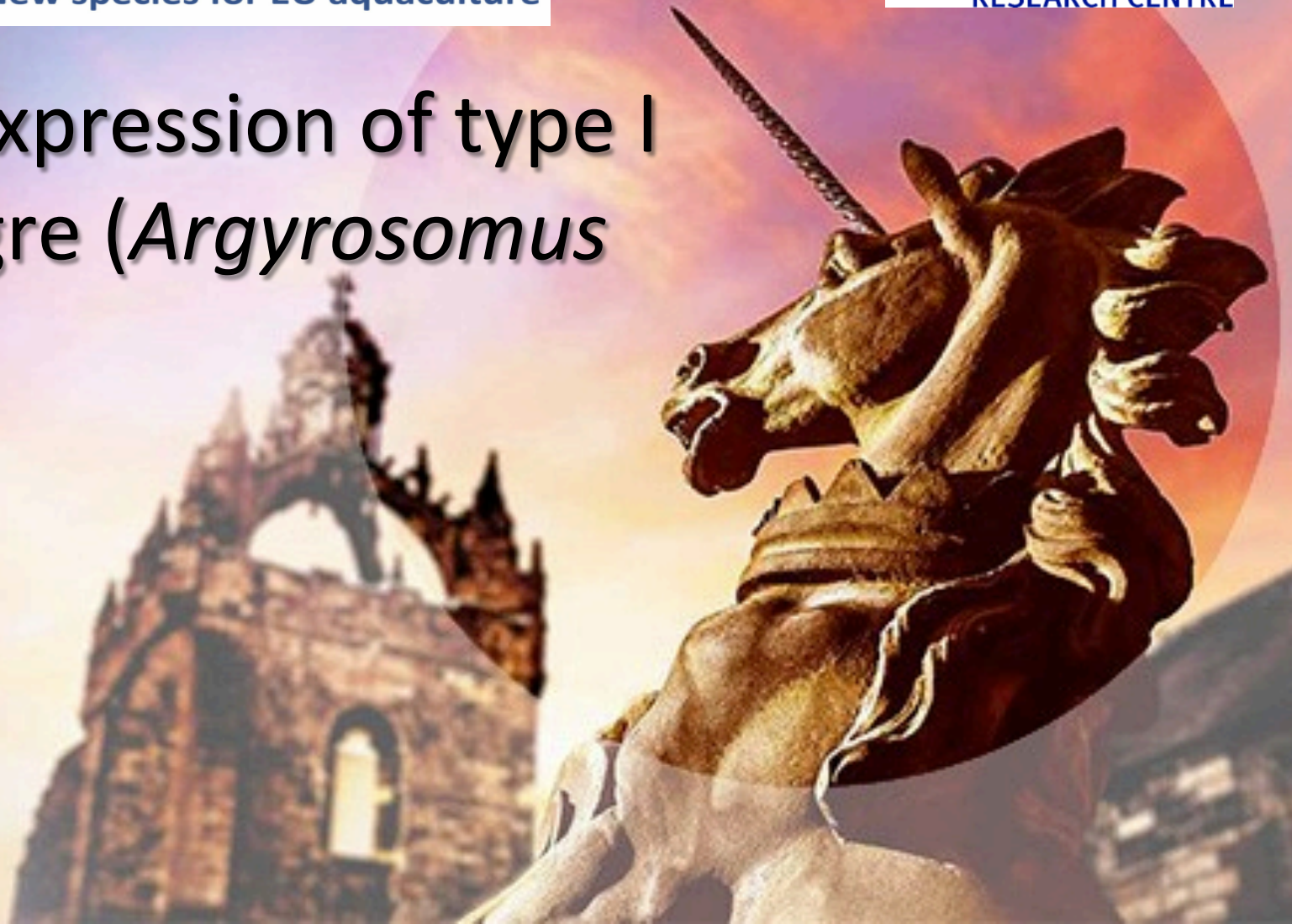


# Identification and expression of type I interferons in Meagre (*Argyrosomus regius*)

Chris  
Secombes



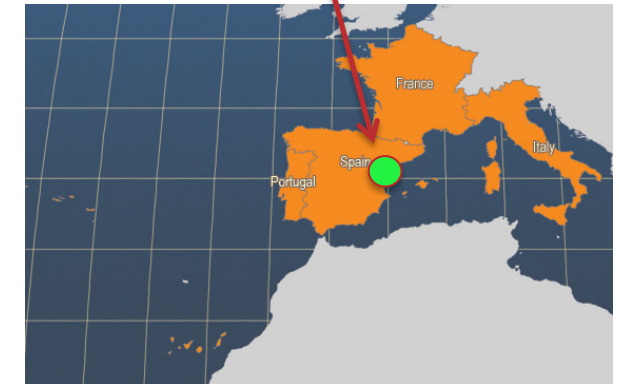
# Acknowledgements

University of Aberdeen, UK

Douglas J. Milne  
Jun Zou



Karl Andree  
Cindy Campoverde  
IRTA, Spain



Xinhua Chen, Inst Oceanography, Xiamen, China

## Viral diseases: a threat to aquaculture

**Infection can lead to high mortality**

As seen in infectious salmon anaemia (ISA) outbreaks in Scotland in 1998-9 and in Chile in 2007-8 which led to large losses of farmed salmon.

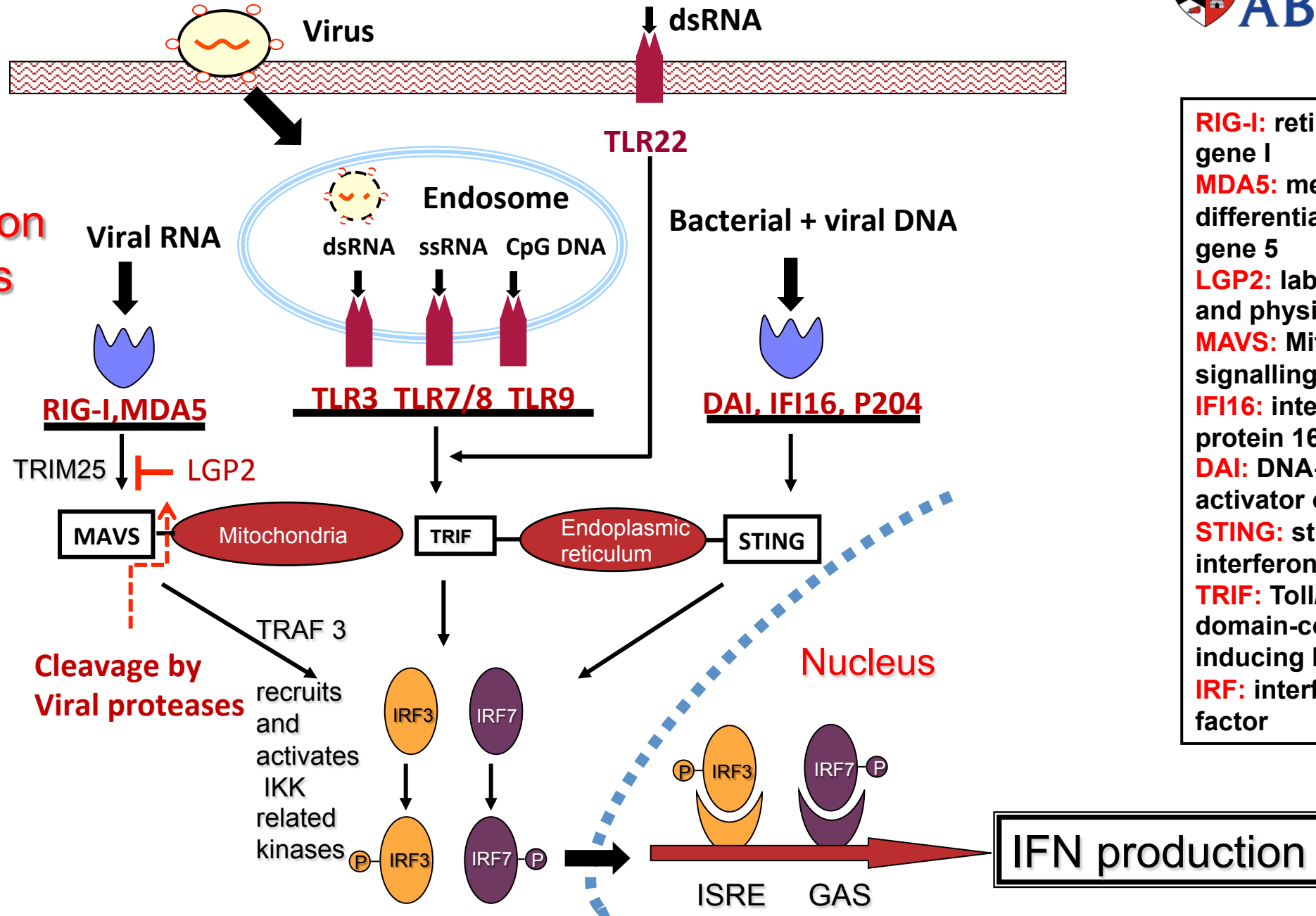
**Infection can lead to poor growth**

Salmonid alpha virus (SAV) causes chronic infection.

Virus	Abbreviation	Genome type	Taxonomic classification	Aquaculture host	Geographic distribution
Infectious salmon anaemia virus	ISAV	ssRNA (-)	Orthomyxoviridae	Salmonids	Europe, North and South America
Viral haemorrhagic septicemia virus	VHSV	ssRNA (-)	Rhabdoviridae	Samonids, Flatfish	Europe, North America, Asia
Viral nervous necrosis virus	VNNV	ssRNA (+)	Nodaviridae	Grouper, Turbot	Europe, North America, Asia
Salmon alpha virus	SAV	ssRNA (+)	Togaviridae	Salmonids	Europe
Infectious pancreatic necrosis virus	IPNV	dsRNA (-)	Birnaviridae	Salmonids	Europe, North America, Asia
Singapore grouper iridovirus	SGIV	dsDNA	Iridoviridae	Grouper	Europe, Asia, North America, Africa
Koi herpesvirus	KHV	dsDNA	Alloherpesviridae	Cyprinids	Europe, Asia, North America, Africa

# Virus detection and activation of antiviral pathways

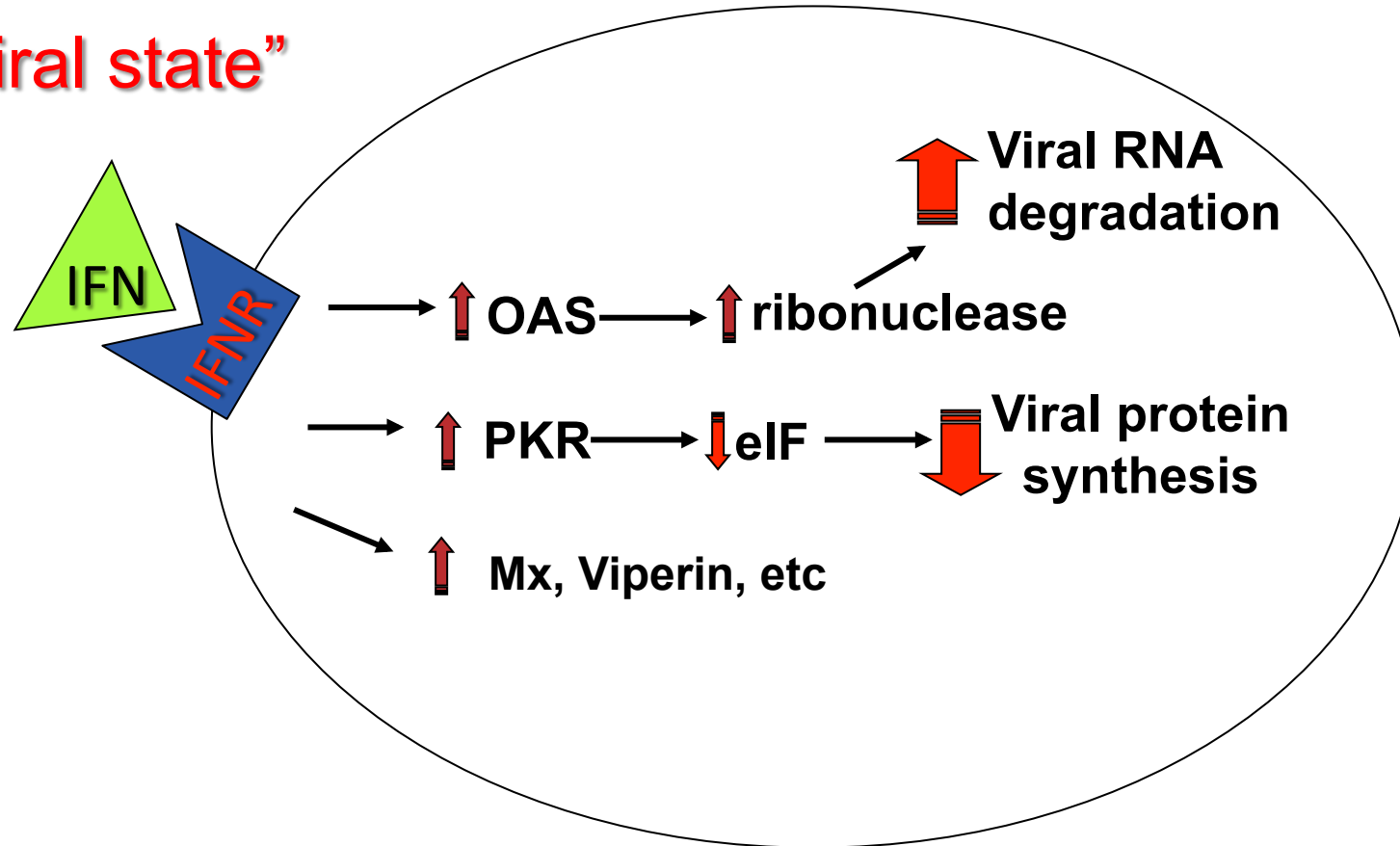
## Pattern Recognition Receptors (PRRs)



**RIG-I:** retinoic acid inducible gene I  
**MDA5:** melanoma differentiation-associated gene 5  
**LGP2:** laboratory of genetics and physiology 2  
**MAVS:** Mitochondria antiviral signalling protein  
**IFI16:** interferon inducible protein 16  
**DAI:** DNA-dependent activator of IFN  
**STING:** stimulator of interferon genes  
**TRIF:** Toll/IL-1 receptor domain-containing adaptor inducing IFN- $\beta$   
**IRF:** interferon regulatory factor

# Anti-viral defences in vertebrates

## The “antiviral state”



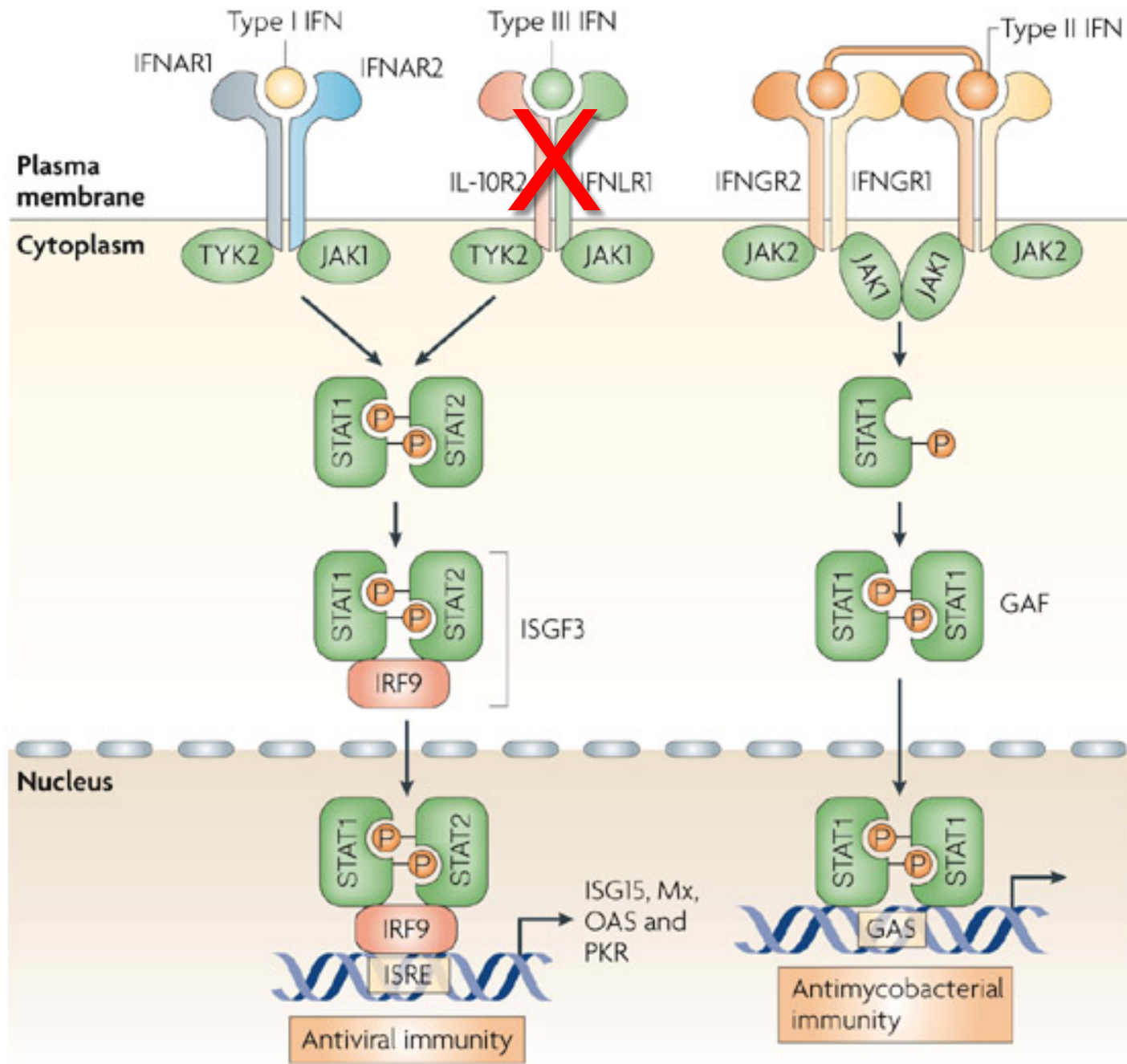
OAS = 2', 5' oligoadenylate synthetase

PKR = protein kinase R – phosphorylates eIF, which becomes inactive

eIF = eucaryotic translation initiation factor

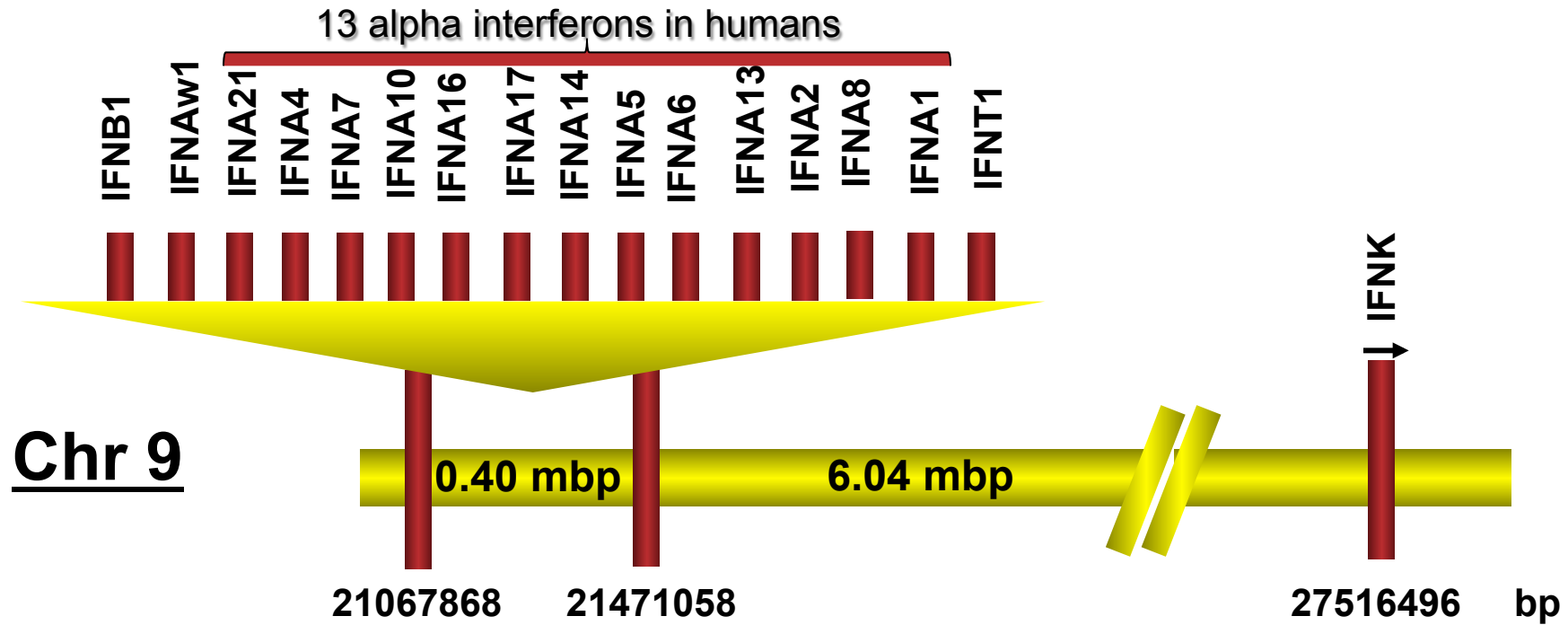
Mx = GTPase, able to block the traffic of viral proteins

Viperin = virus inhibitory protein, down-regulates viral structural proteins needed for virus assembly



In mammals type I IFNs occur as multiple genes and multiple subgroups -  $\alpha$ ,  $\beta$ ,  $\kappa$ ,  $\varepsilon$ ,  $\omega$ ,  $\tau$ ,  $\delta$ ,  $\zeta$

Eg Human type I IFN $\alpha$  genes are clustered, on chr 9





# Major events in fish interferon research

## 2003

JOURNAL OF VIROLOGY, Feb. 2003, p. 1992–2002  
0022-538X/03/\$08.00+0 DOI: 10.1128/JVI.77.3.1992-2002.2003  
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Vol. 77, No. 3

### Molecular and Functional Analysis of an Interferon Gene from the Zebrafish, *Danio rerio*†

Stephen M. Altmann, Mark T. Mellon, Daniel L. Distel, and Carol H. Kim\*

## 1975

INFECTION AND IMMUNITY, Apr. 1975, p. 815–822  
Copyright © 1975 American Society for Microbiology

Vol. 11, No. 4  
Printed in U.S.A.

### Partial Purification and Characterization of RTG-2 Fish Cell Interferon

JOHN DE SENA<sup>1\*</sup> AND GUIDO J. RIO

## 2009

The Journal of Immunology

### The Two Groups of Zebrafish Virus-Induced Interferons Signal via Distinct Receptors with Specific and Shared Chains<sup>1</sup>

Dina Aggad,<sup>\*,†</sup> Martine Mazel,<sup>\*,†</sup> Pierre Boudinot,<sup>‡</sup> Knud Erik Mogensen,<sup>\*,†</sup> Ole Jensen Hamming,<sup>§</sup> Rune Hartmann,<sup>§</sup> Sergei Kotenko,<sup>¶</sup> Philippe Herbomel,<sup>||#</sup> Georges Lutfalla,<sup>2\*,†</sup> and Jean-Pierre Levrud<sup>2||#</sup>

## 2011

JOURNAL OF VIROLOGY, Aug. 2011, p. 8181–8187  
0022-538X/11/\$12.00 doi:10.1128/JVI.00521-11  
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Vol. 85, No. 16

### Crystal Structure of Zebrafish Interferons I and II Reveals Conservation of Type I Interferon Structure in Vertebrates<sup>∇,†</sup>

Ole Jensen Hamming,<sup>1</sup> Georges Lutfalla,<sup>2,3</sup> Jean-Pierre Levrud,<sup>4,5\*</sup> and Rune Hartmann<sup>1\*</sup>

## 2007

The Journal of Immunology

### Identification of a Second Group of Type I IFNs in Fish Sheds Light on IFN Evolution in Vertebrates<sup>1</sup>

Jun Zou,<sup>2\*</sup> Carolina Tafalla,<sup>†</sup> Jonathan Truckle,<sup>\*</sup> and Chris J. Secombes\*

## 2009

### Original Paper

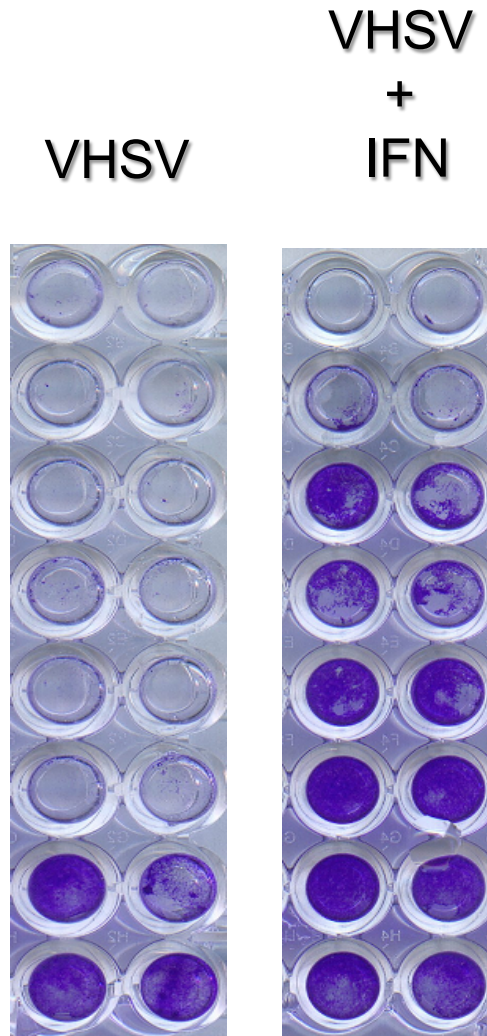
Identification of an additional two-cysteine containing type I interferon in rainbow trout *Oncorhynchus mykiss* provides evidence of a major gene duplication event within this gene family in teleosts

Mingxian Chang<sup>1, 2</sup>, Pin Nie<sup>2</sup>, Bertrand Collet<sup>3</sup>, Christopher J. Secombes<sup>1</sup> and Jun Zou<sup>1</sup>

Immunogenetics (2009) 61:315–325

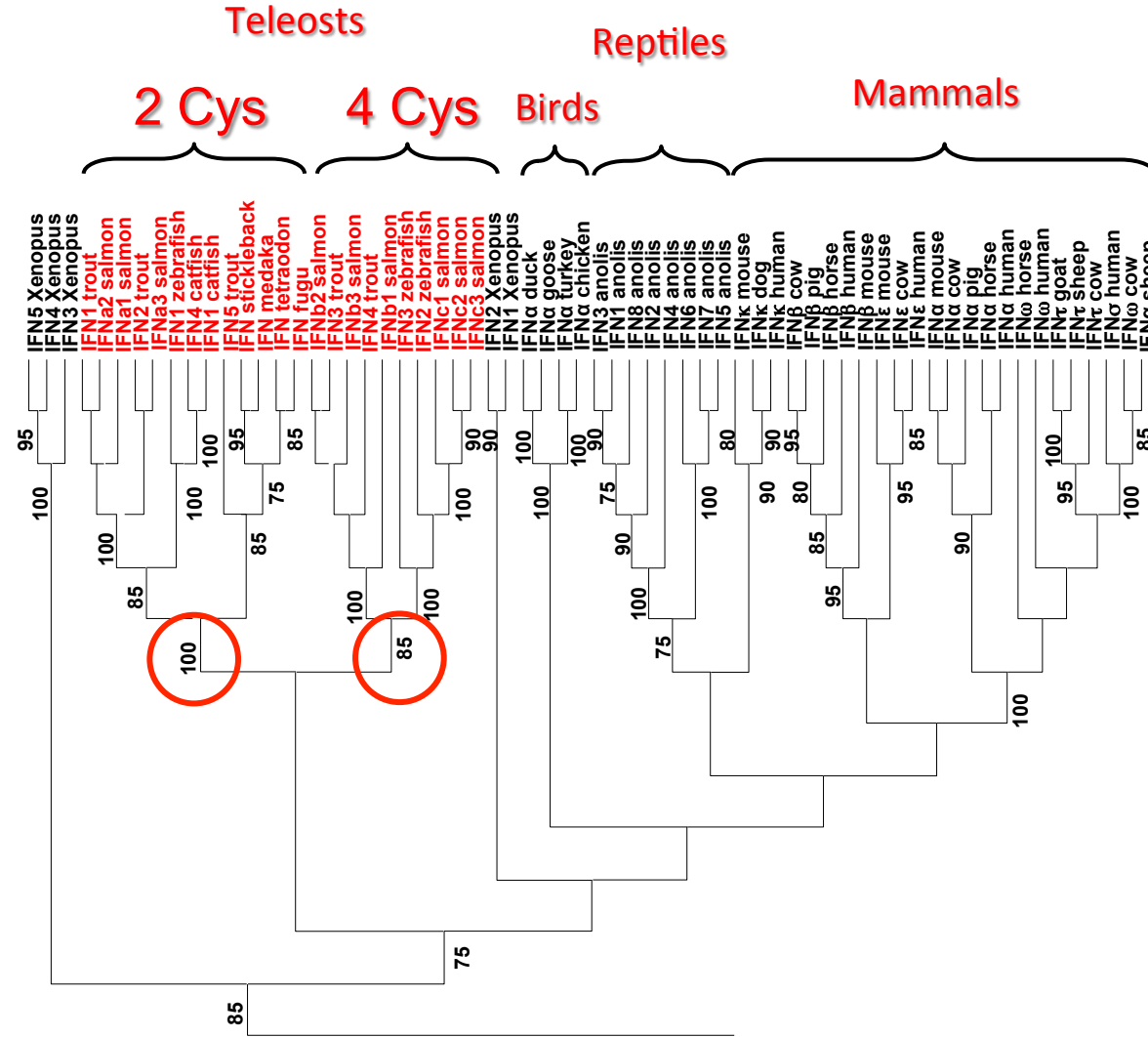
# Antiviral functions of fish IFNs are conserved

Trout type I IFNs  
enhance host resistance  
against viral infection

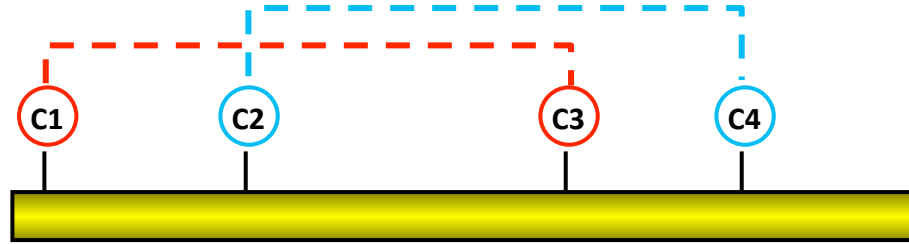
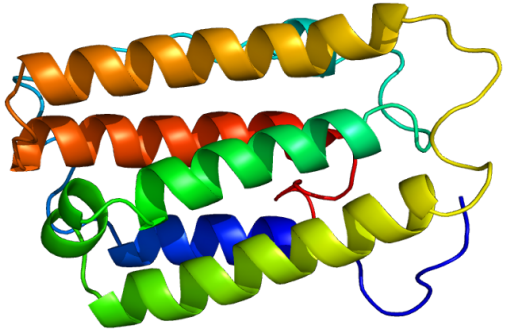


Trout RTG-2 cells were incubated  
with rIFN for 4 h prior to virus challenge.

# Phylogenetic tree of type I IFNs



# Vertebrates have three groups of type I IFNs



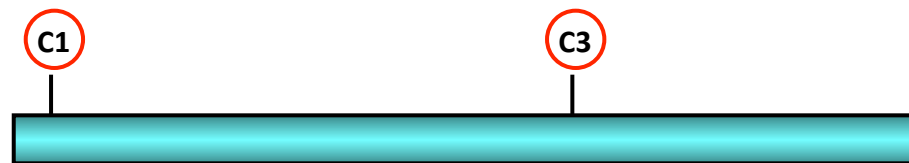
Tetrapod 4 Cys  
group



Mammalian 2 Cys  
group (IFN- $\beta$  and  $\epsilon$ )



Fish 4 Cys  
group



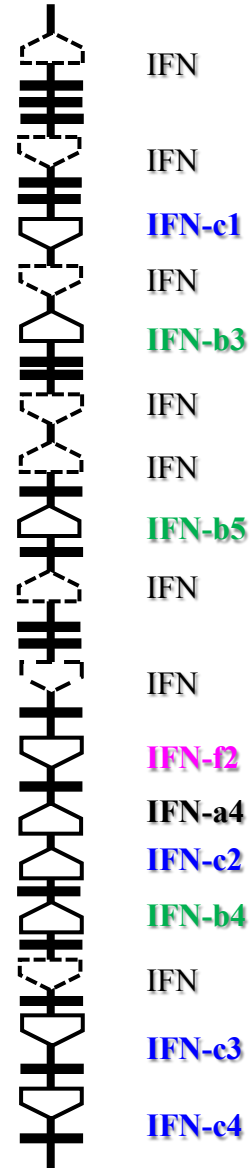
Fish 2 Cys  
group

# Analysis of 3 trout BAC clones

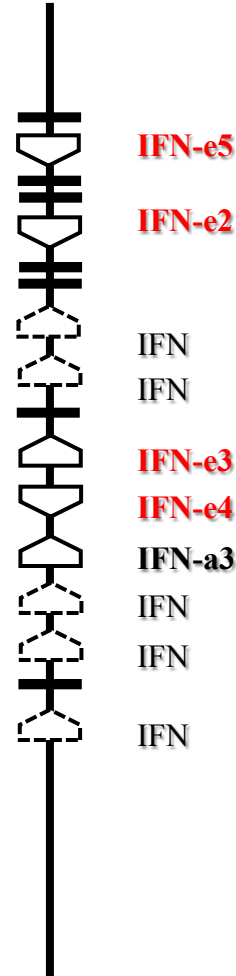
At least 44 copies  
of IFN genes and  
pseudo genes are  
found in trout



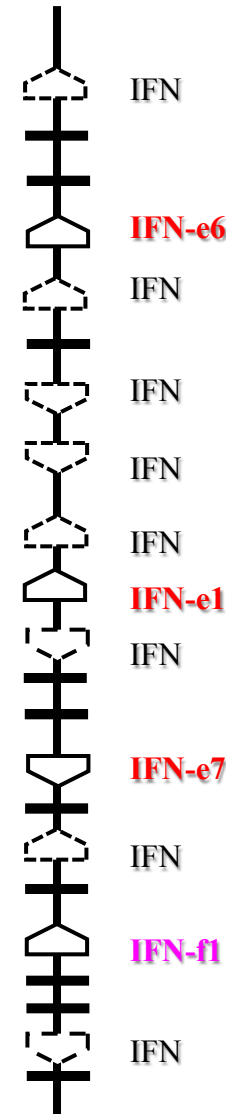
770C03  
149654 bp

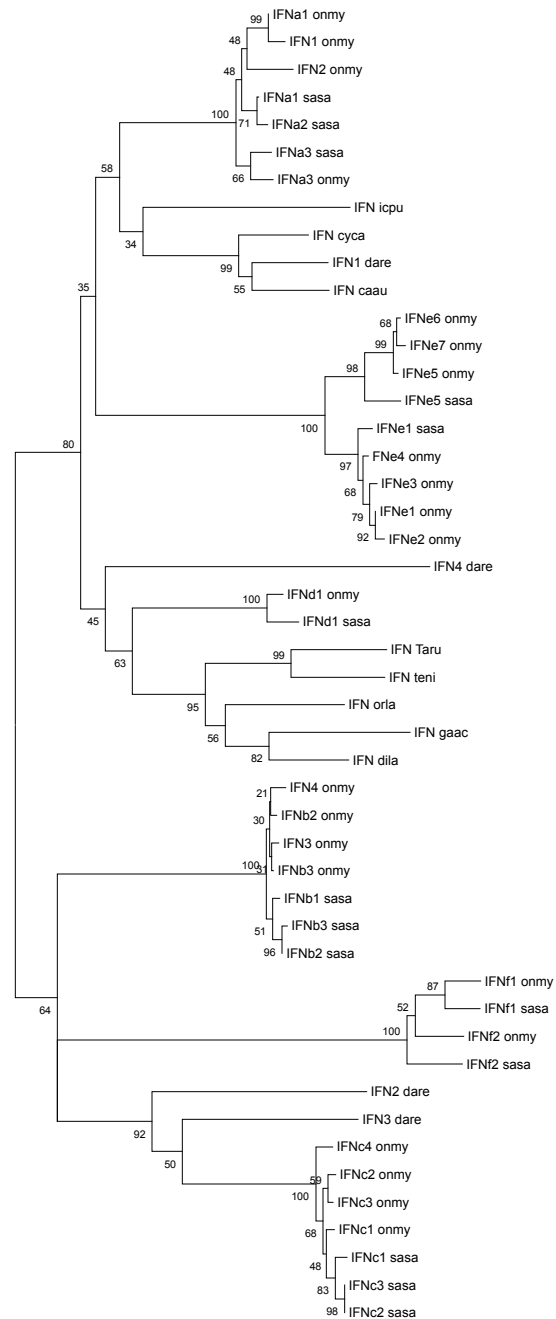


732E08  
116360 bp



816C01  
142050 bp





IFN-a

IFN-e

IFN-d

IFN-b

IFN-f

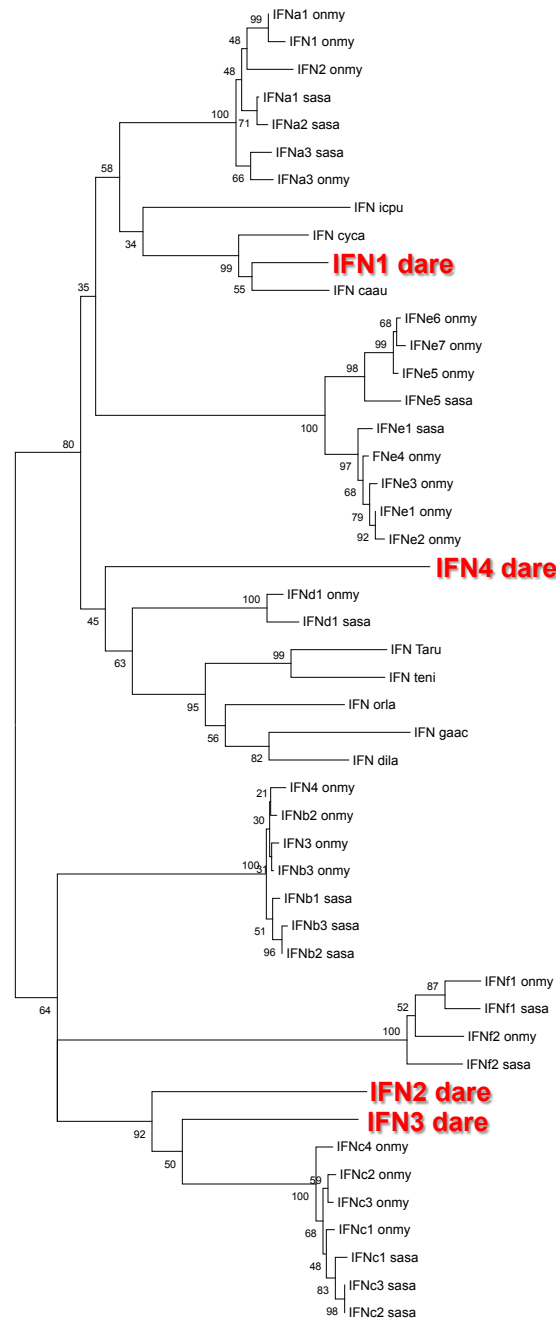
IFN-c

**Group I  
(2 Cys) IFNs**

**Group II  
(4 Cys) IFNs**

18-33% sequence identity  
between subgroups

>81% sequence identity  
within a subgroup



IFN-a

IFN-e

IFN-d

IFN-b

IFN-f

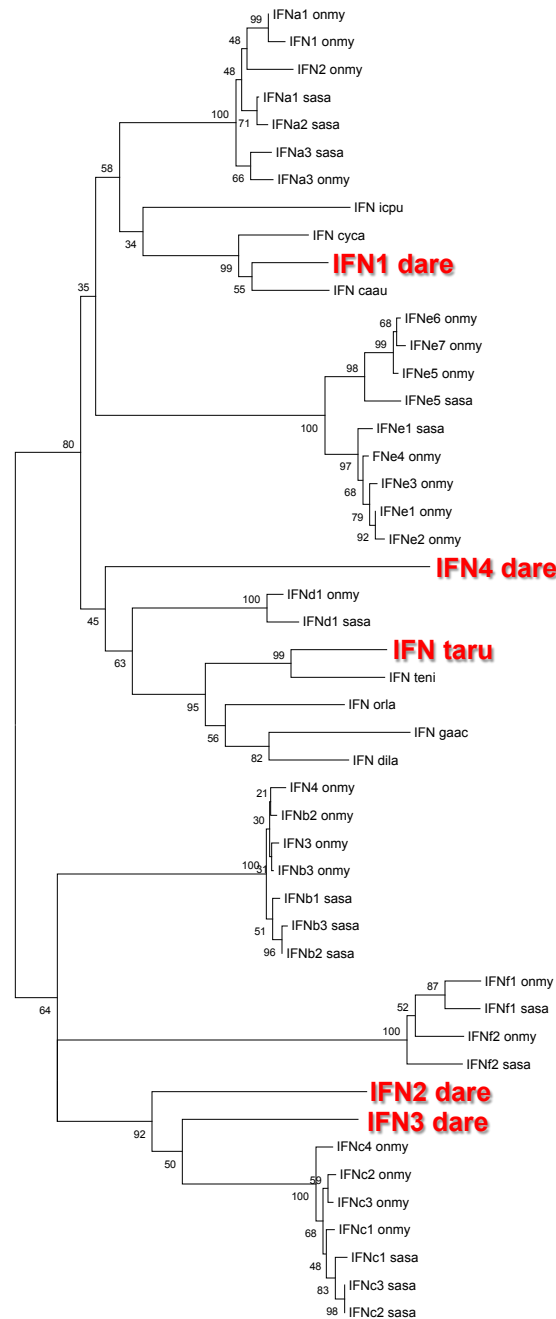
IFN-c

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IFN-a

IFN-e

IFN-d

IFN-b

IFN-f

IFN-c

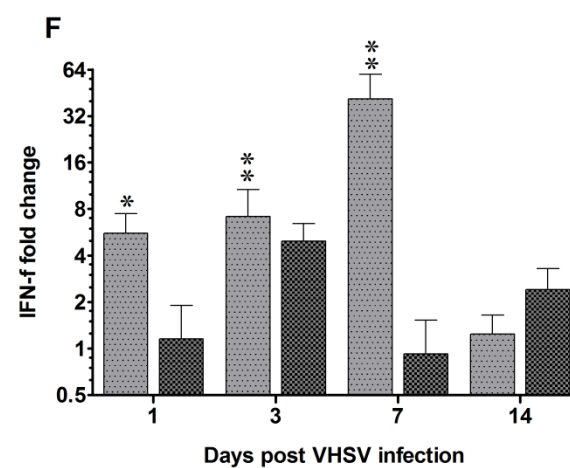
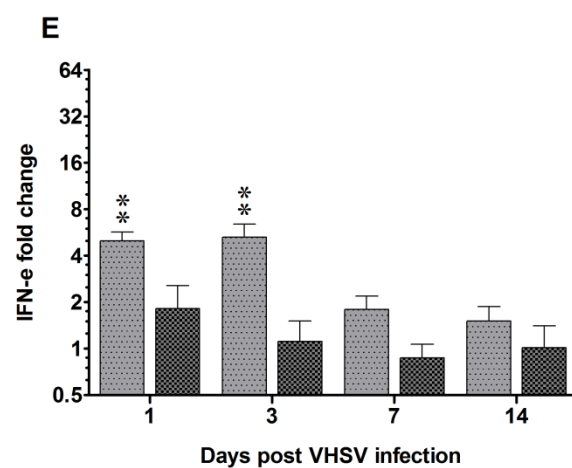
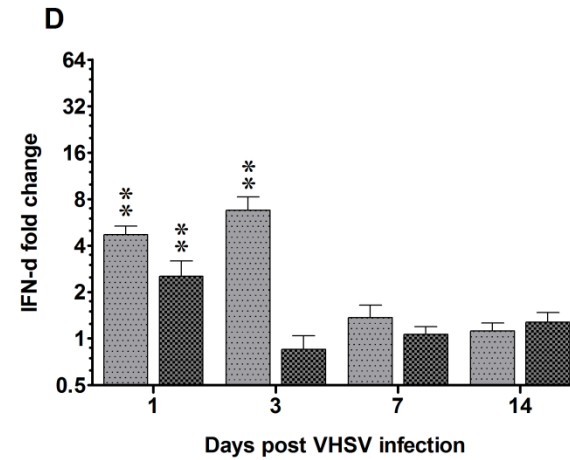
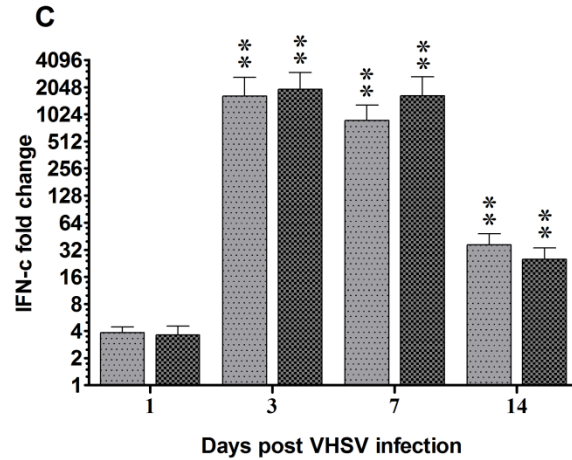
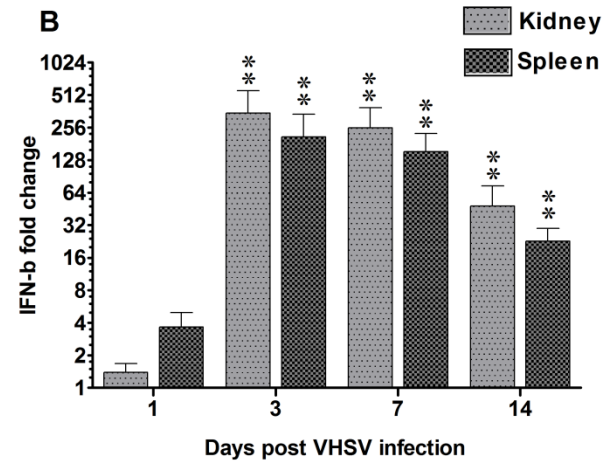
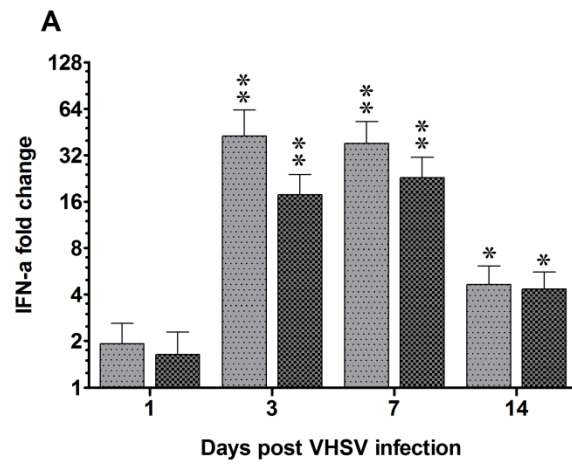
**Group I  
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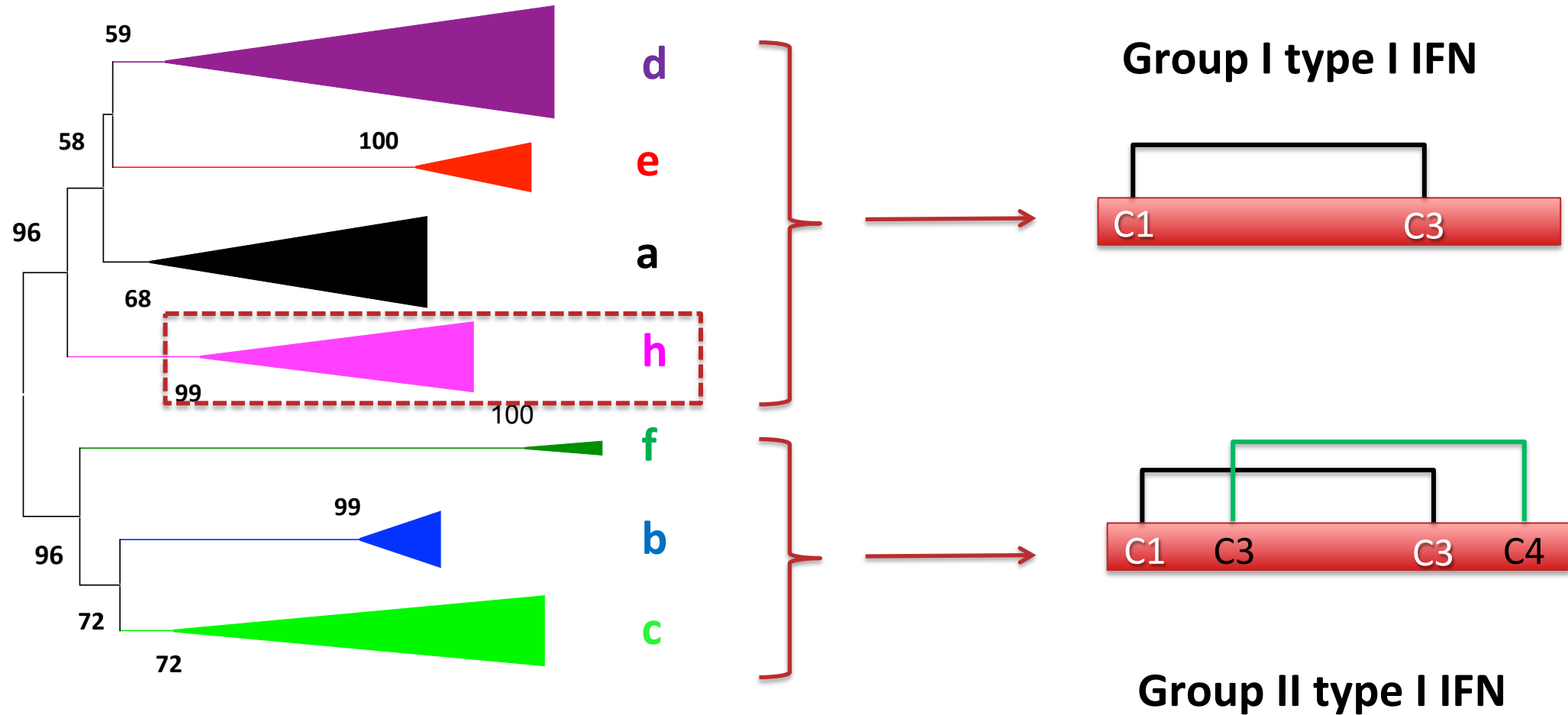
>81% sequence identity  
within a subgroup



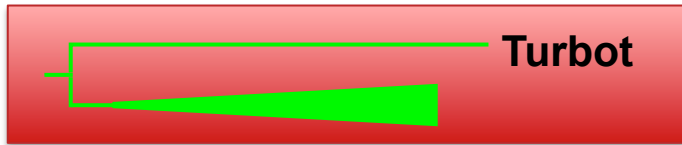
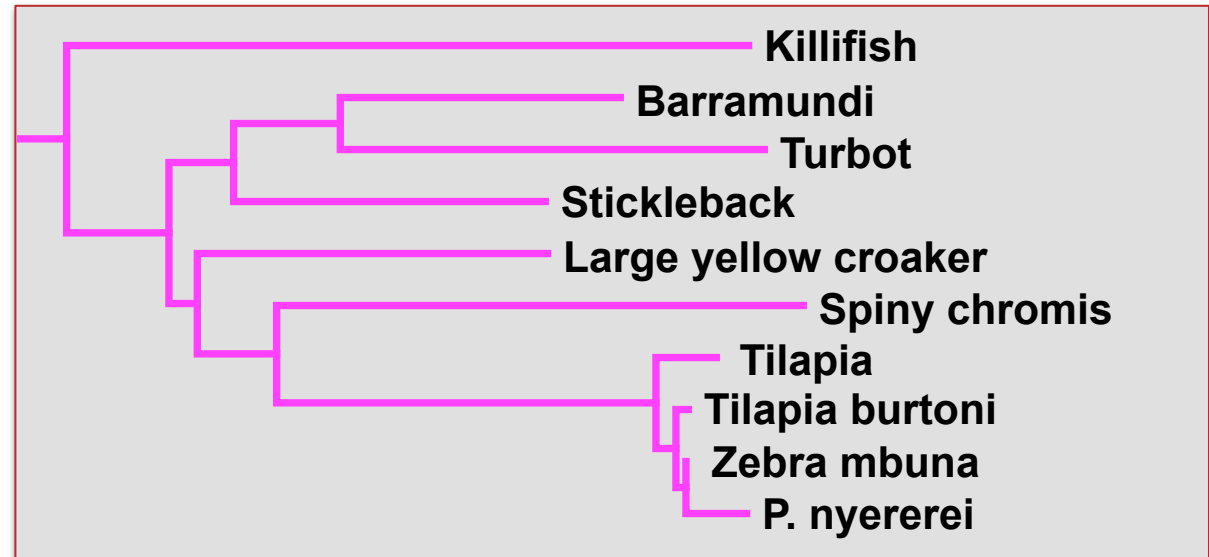
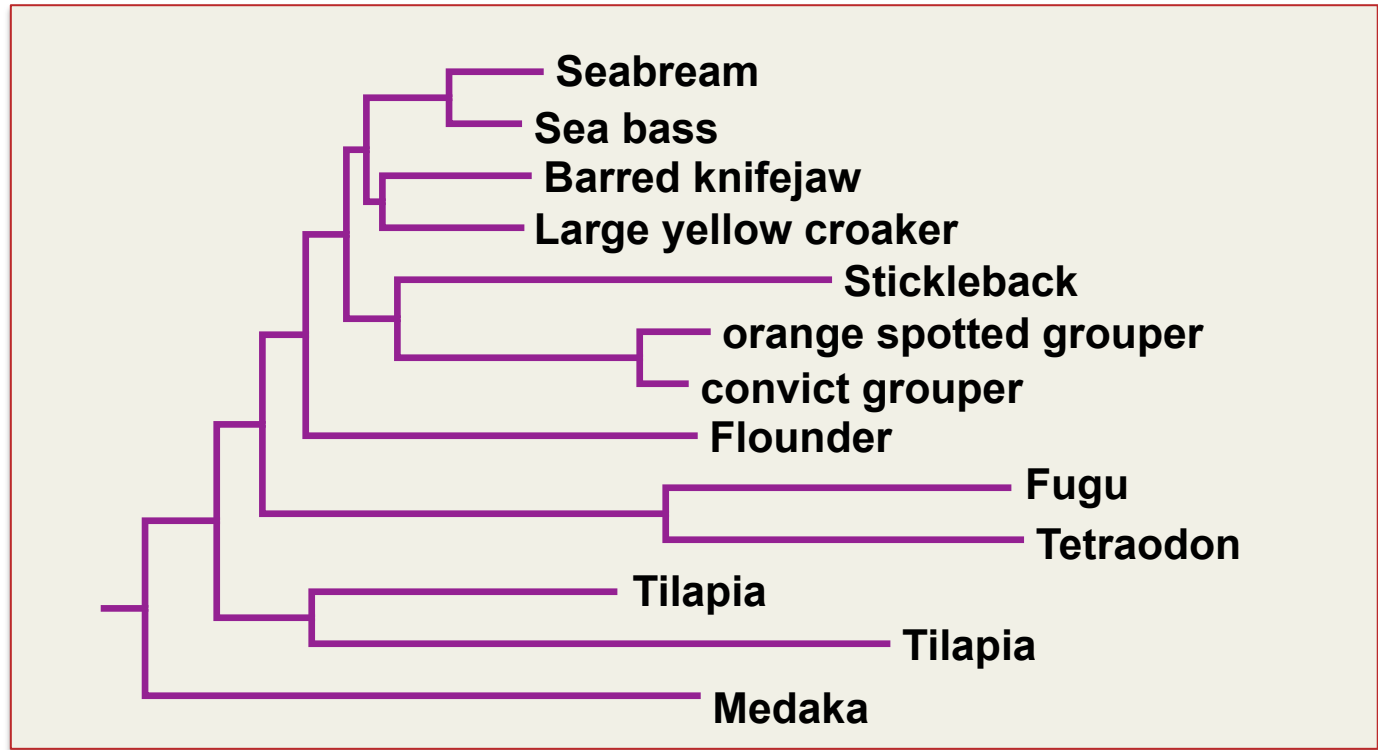
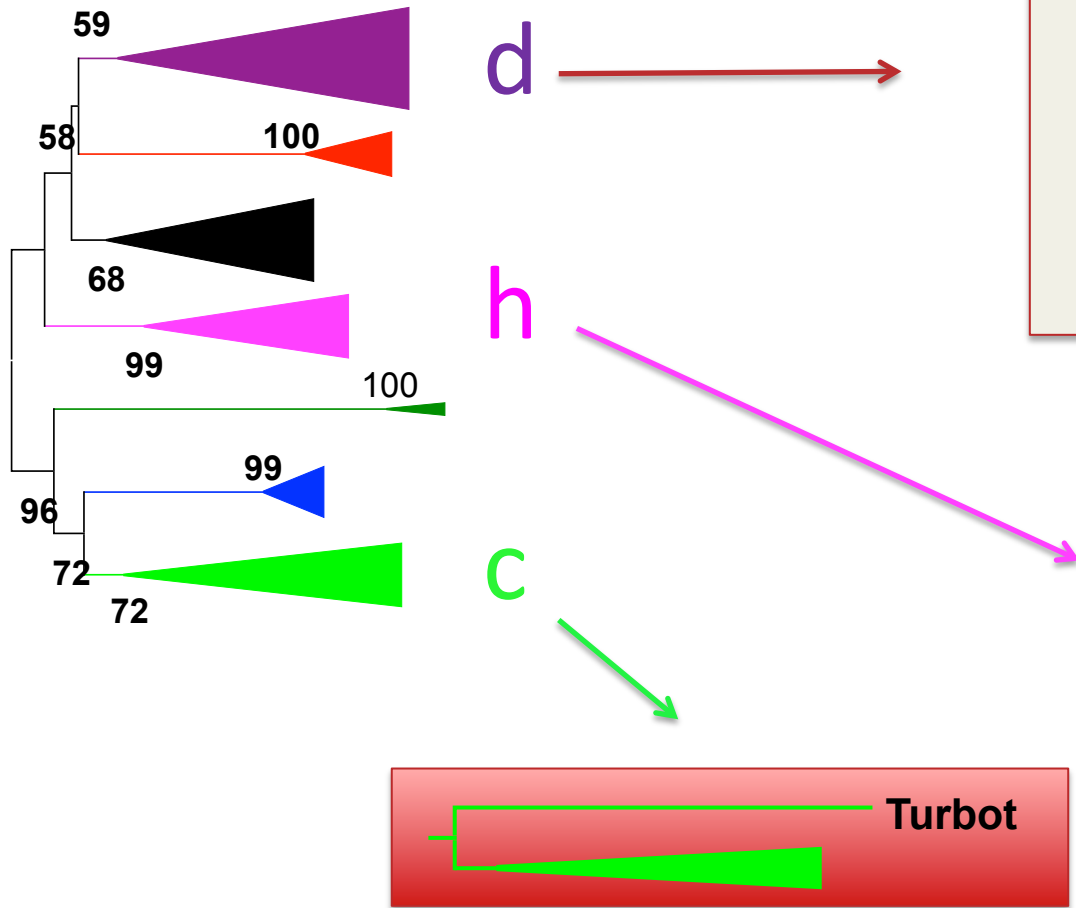


Interferon expression in kidney and spleen of trout following viral challenge (with VHSV).

# Diversifications of type I interferon subfamilies in teleosts



# Type I IFN repertoire of Acanthopterygian fish



# IFN gene cloning strategy

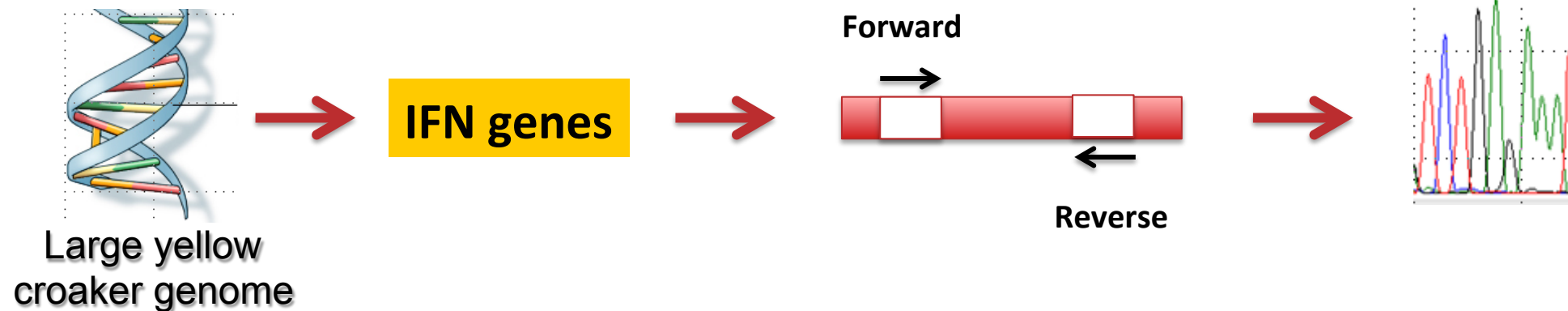
RESEARCH ARTICLE

## Genome Sequencing of the Perciform Fish *Larimichthys crocea* Provides Insights into Molecular and Genetic Mechanisms of Stress Adaptation

Jingqun Ao<sup>1</sup>, Yinnan Mu<sup>1</sup>, Li-Xin Xiang<sup>2</sup>, DingDing Fan<sup>3</sup>, MingJi Feng<sup>3</sup>, et al.



Vol 11 (4), 2015, e1005118





# 3 IFN genes have been cloned in meagre

## IFN1

```

ATGacacttcagtcctcttcagtcctcctgtcctcctcagtgctacagcctcaagttg
M T L Q S S S V L L V L L Q V Y S L K L
atggtggctgccatgccgacctgtcagctggaaggagacctggtccagtcggccaccac
M V A A M P T C Q L E G D L V Q S A H H
ctgctcagagacctggggcggtttcctgtccactgctgocgtacaacgccaacgctc
L L R D L G A A F P V H C L P Y N A N V
tcctttccaagctccgcttcctgctgccacagccaatcatcctcagtgccgtaaaagca
S F P S S A F P A A T A N H P Q C R K A
ttatgggtggtgatgaatccctgctgggaggggggtaaatattccaggacaatgacata
L W V V H E S L R E A G L I F Q D N D I
cctgtcggagagggcggtcacctggaacgacagaaactcgaagactccagaacttg
P V G E G G V T W N D Q K L E D F Q N I
cagtcaccgactggaggaggaggagctgtctgtccggtgcaatgggtcaggtgtttg
Q Y R L V E E G S C L S R V N G S G V L
tcgtctactcagtaacgtgacggcagttctcaagagcaggacagtgctgctgctggt
S S Y F S N V T A V L Q E Q D S A A C G
tgatggctotgaggagagatctgctctgggtcctaaagtctgccctgcagaaacaccac
W M A L R R D L L W V L K S A L Q K H H
aacctgcttacctggagaggtgtccgcTAA
T C F T W R G V R -
  
```

4 Cysteines

## IFN2

```

ATGctcagcaggatcttgtttgtgtgctgtctcagtcgtacagtgaggctcctcg
M L S R I L F V C L S L S L Y S A G S S
ctaagctgcagatggatggatcataaattcagacagtcagtgaaaattctttggatcta
L S C R W M D H K F R Q Y S E N S L D L
ctcagtcagatggctaaataactcactaacaccactgaggatgctgaagtgaggagcact
L S T M A N N S T N T T E D A E V E D T
gtggccttccctaatgatctgtacagccaggcgtccaaagcatcagctgaggataaactt
V A F P N D L Y S Q A S K A S A E D K L
catttcacagttcaggttctggaggagggcgtgocctgtttgaggaggatccagcaat
H F T V Q V L E E A A A L F E E D H S N
gcttcatgggaggagaacacagtgagaaactttgtcaatggtgtaaacagcaggctgac
A S W E E N T V E N F V N V V N Q Q A D
ggccttcgctcctgtactgggagtcacggccaagaagaagaacaagaagctgcacatg
G L R S C T G S H G H K K K N K K L H M
tatttcaagagactgtcgagtcaggtcctgaaagaaatgagccacagtgctgaagcctgg
Y F K R L S S H V L K K M S H S A E A W
gagctgatcaggaaggaatcaggaccatctgatgagagcagacagctggtttcatct
E L I R K E I R T H L M R A D Q L V S S
ctactcaacaccaacTAA
L L N T N -
  
```

2 Cysteines

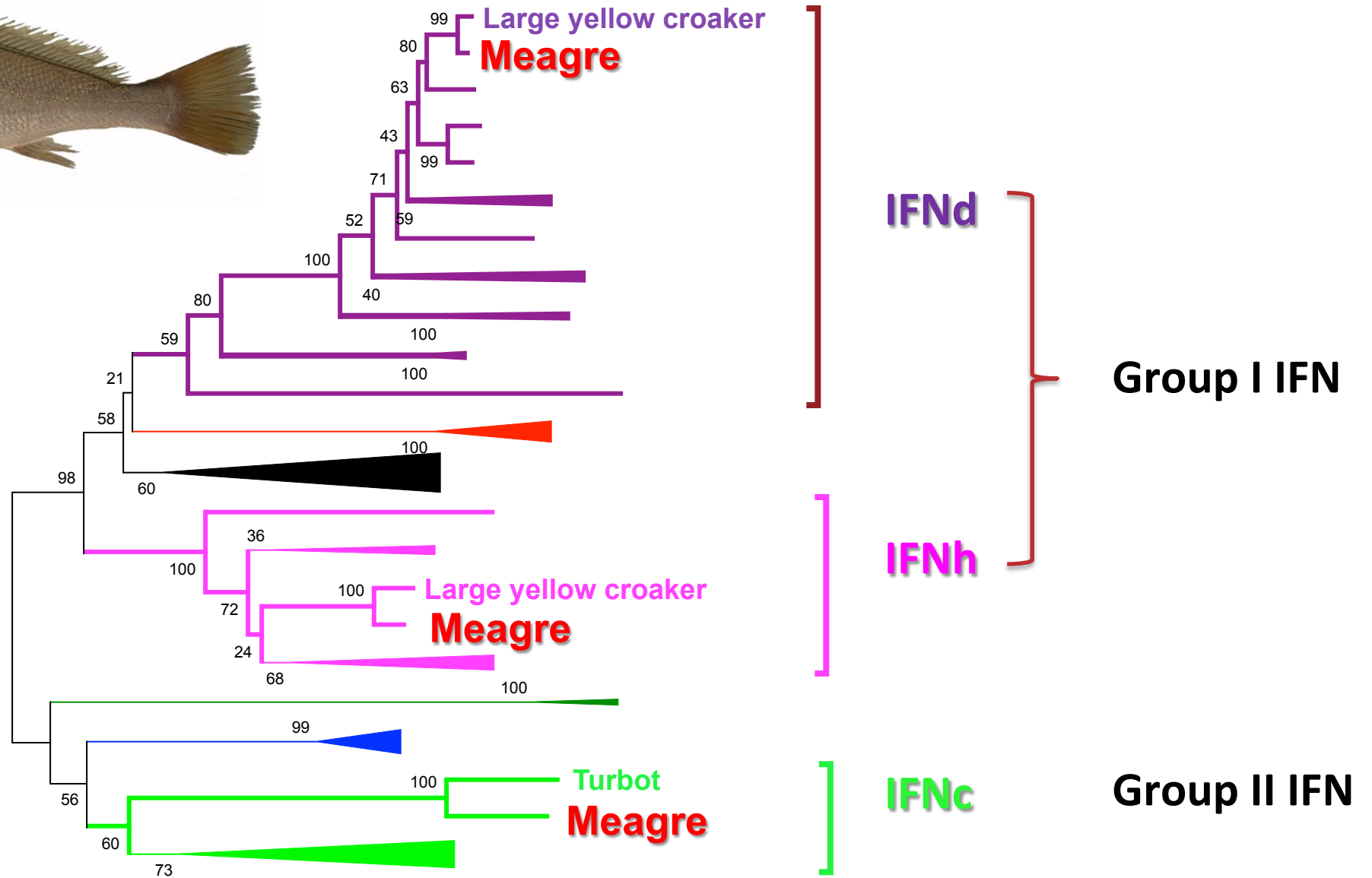
## IFN3

```

ATGgtaactggaccggcgtgctcttctcctctgtggggcctcctgaotcctgactc
M V N W T G V L F V L C G A L L T P A L
ttctgtgattggctcagatactacggtcaactgagcaacaactccttgactcctccag
F C D W L R Y Y G Q L S N N S L T L L Q
cagatggggcgtcagttcactgaacaggagtggtccagttogctttccaacaagaatctac
Q M G G Q F T E Q E C P V R F P T R I Y
agagacataataaggctgaggtgagctcagttggttttatcagagacagctcgaat
R D I Y K A E V Q S Q L V F I R D S L N
ctgatttctggtctctatcgccatgacaacctcacctctgtttcctgggacaccgtcaag
L I S G L Y R H D N L T S V S W D T V K
accgaacacttctgataaacatccaacagacagacaagaactcaacacctgtgtgtcg
T E H F L I N I H R Q T E E L N T C V S
acgaacaaagactccaacagcagctgacaaaactacacaggagactggccaaaagtact
T N K T S N S L T K Y Y R R L A K S T
ctgcaccgactggggcagtcctgctgctgggagctgatcaggaacagacaaaactg
L H R T G G S P A S W E L I R K Q T K L
cacctggatcagttggacctgctggtggagtgcatcaagagttcatctgccgcttgacgg
H L D Q L D L L V E C I K S S S A A C R
aggcgtctgcagcagtgagcggcagcacTGA
R R S A A S G R Q H -
  
```

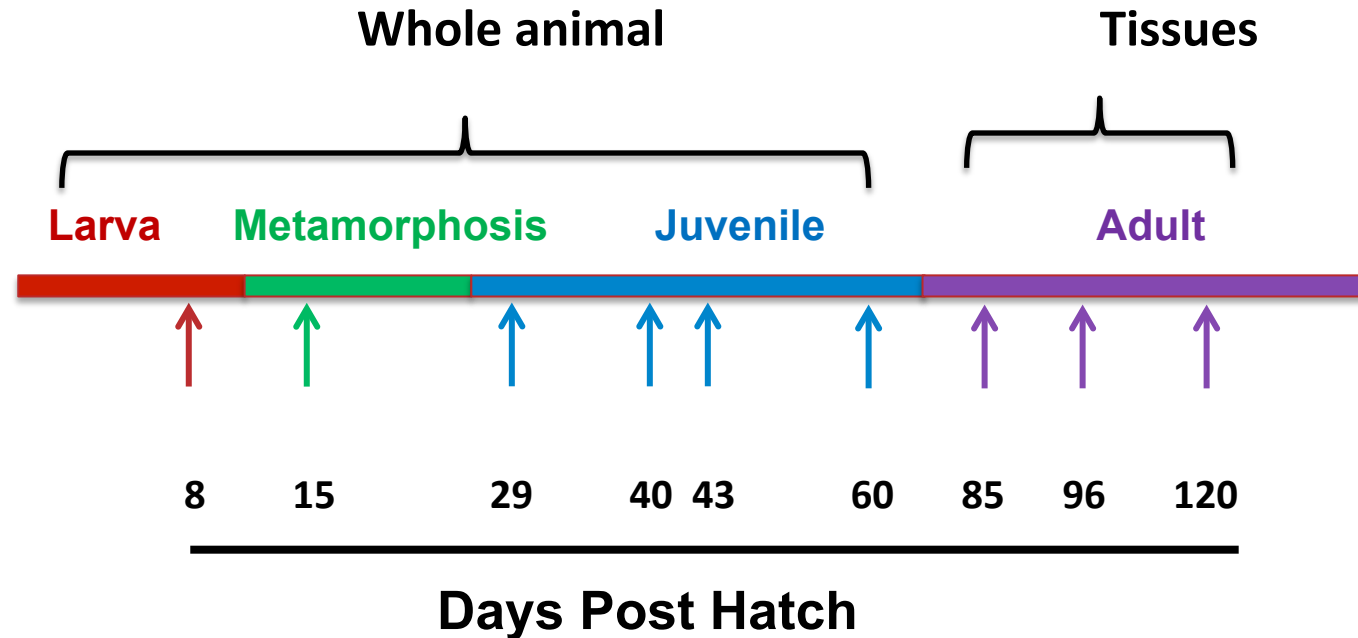
2 Cysteines

# Meagre type I IFN genes



# Interferon expression during development

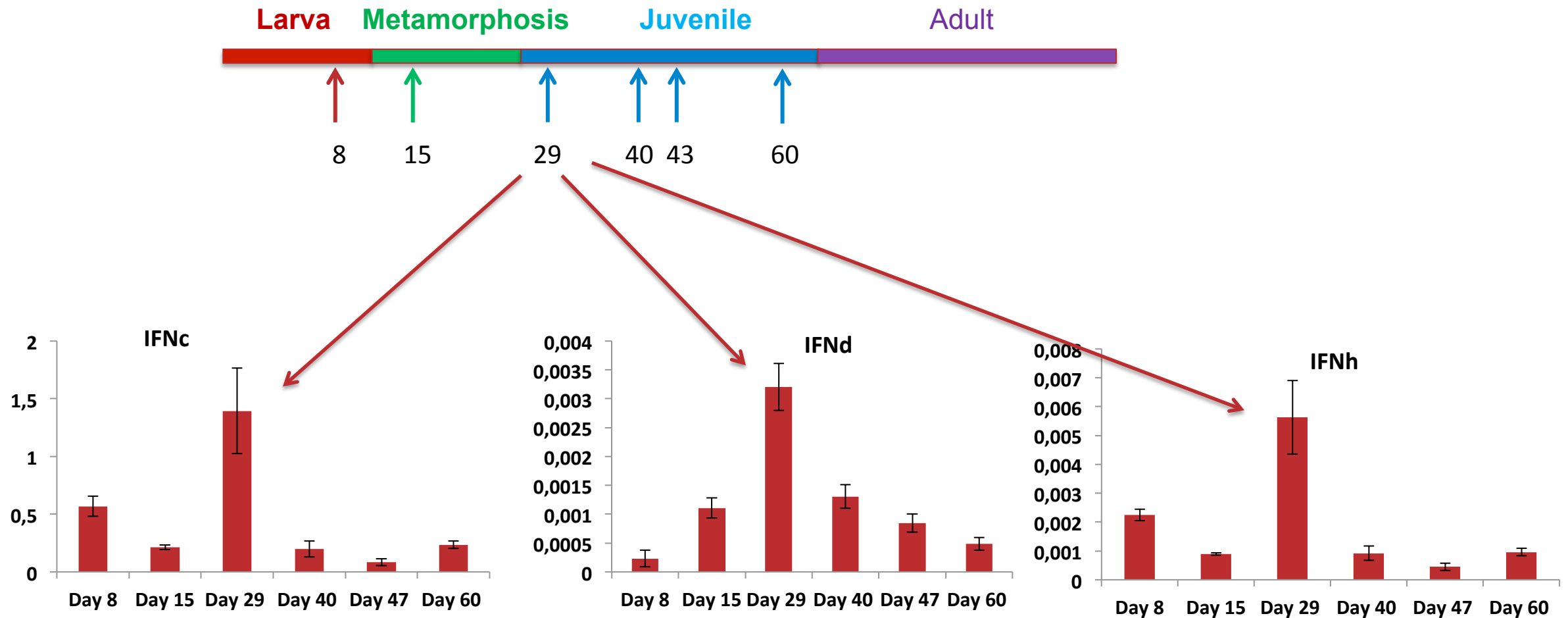
## Sampling



## Feeding regime

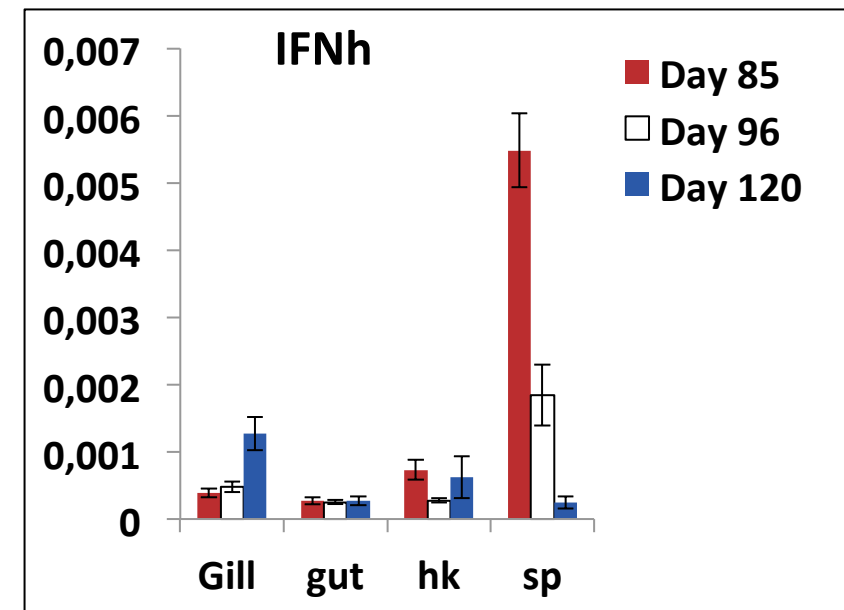
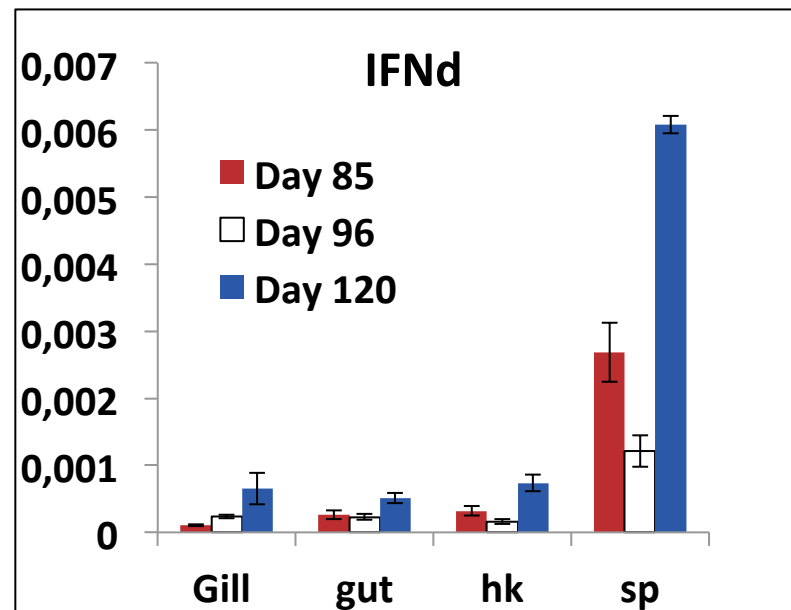
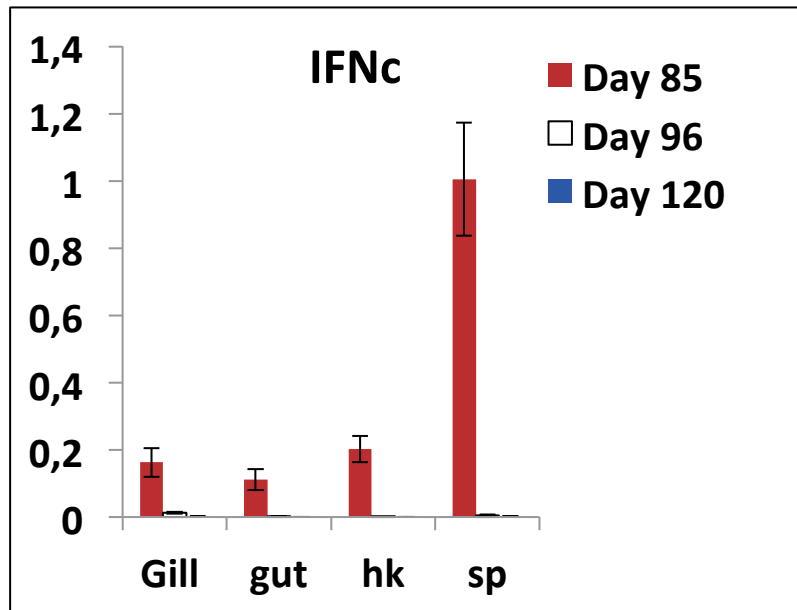


# Interferon expression during development





# Interferon expression during development



High expression of all 3 in spleen

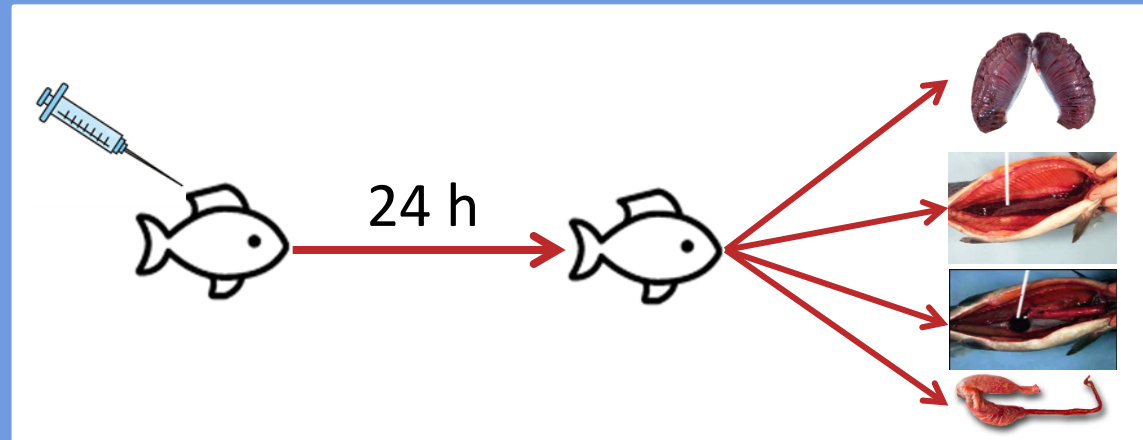
IFNc expression is the highest

# Meagre tissue stimulation setup

## In Vivo

Ip Injection of fish

- PBS
- Poly I:C
- LPS
- $\beta$ -glucan

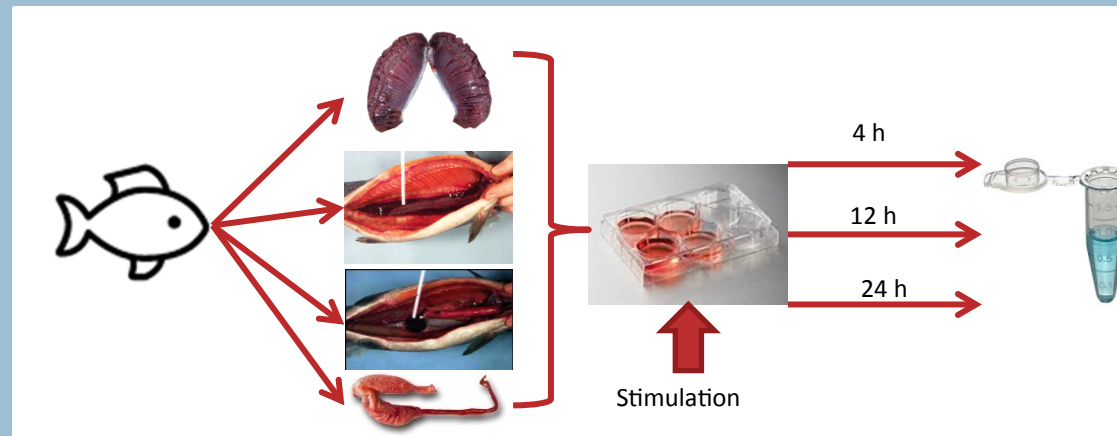


## In Vitro

Primary cell culture stimulated by:

- PBS
- Poly I:C
- LPS
- $\beta$ -glucan

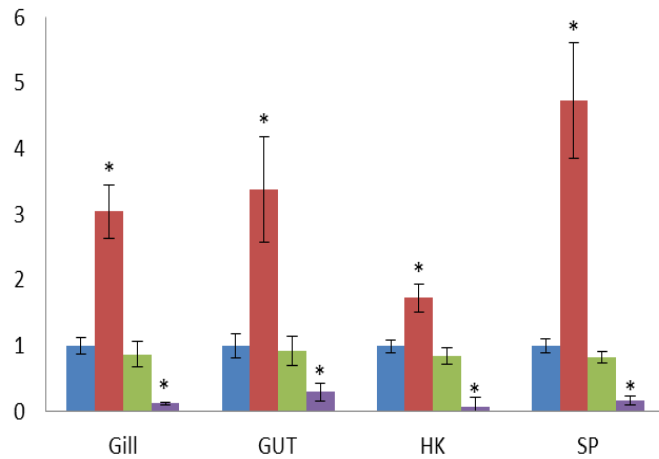
Samples taken after  
4, 12 and 24 h



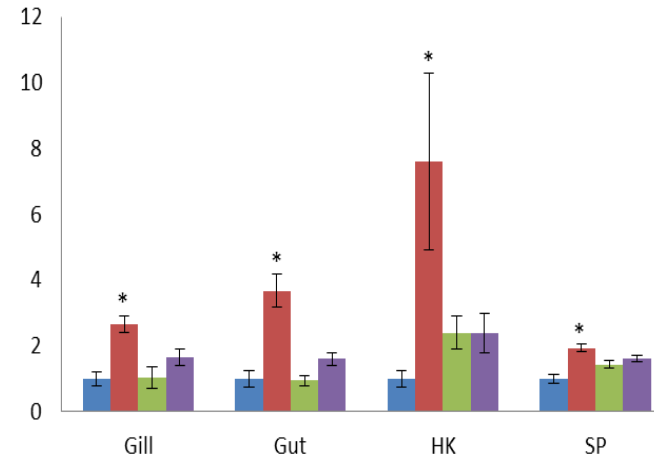
# Interferon expression in different tissues following PAMP stimulation in vivo



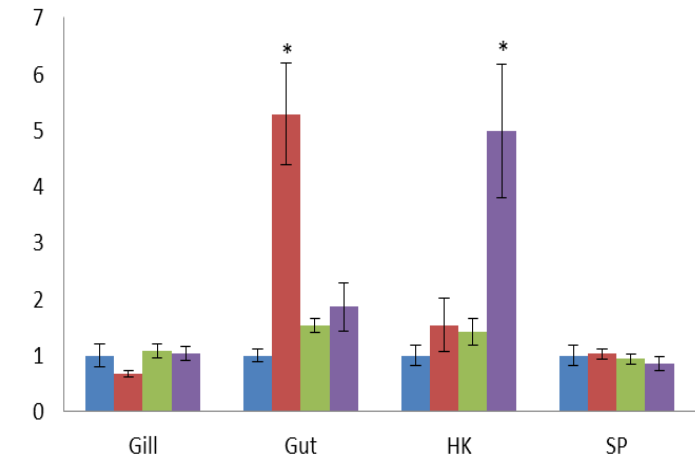
**IFNc**



**IFNd**



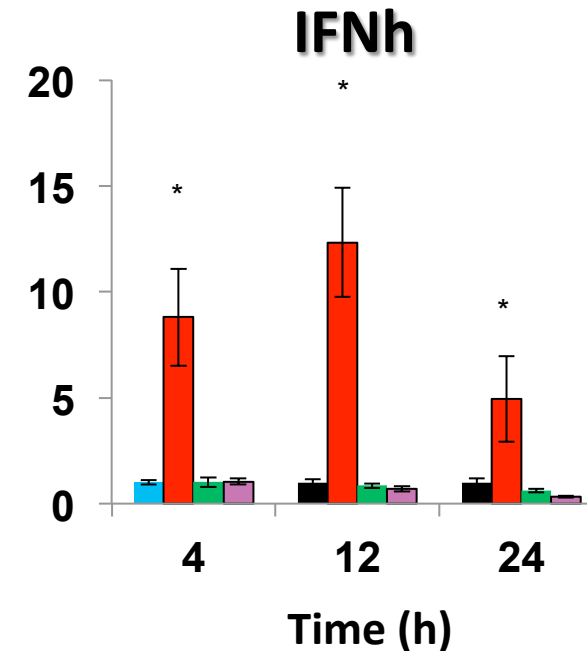
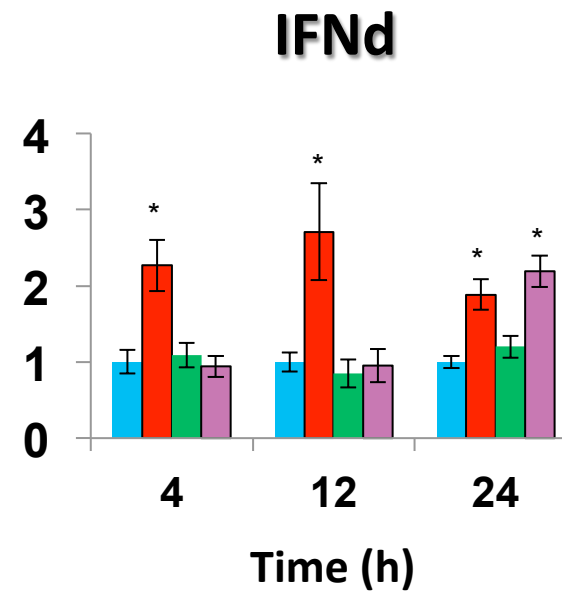
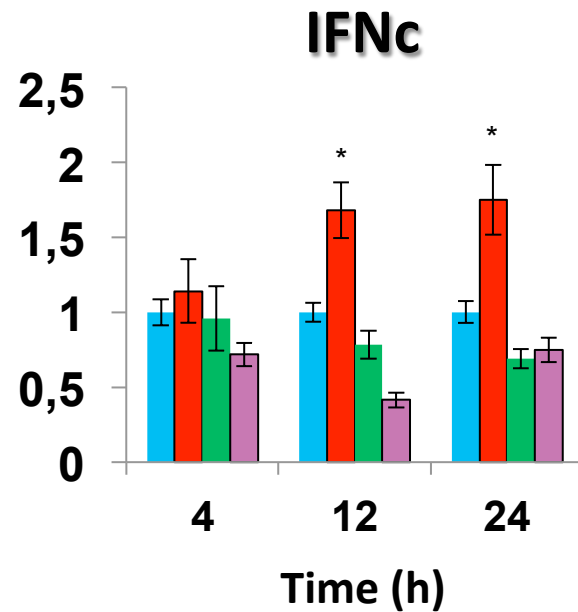
**IFNh**



HK = head kidney; SP = spleen

# Interferon expression in cultured splenocytes

- PBS
- Poly I:C: 100 µg/mL
- LPS: 50 µg/mL
- β-glucan: 50 µg/mL



N/B Similar results with HK cells

# Conclusions

- Meagre have a complex IFN system, with multiple IFN subgroups present
- Meagre IFNs are transiently up-regulated when fed a commercial diet
- Meagre IFNc is relatively highly expressed in embryos and spleen
- Meagre IFNs are induced by poly I:C in vivo and in vitro, indicating an involvement in antiviral defence
- In cultured meagre splenocyte IFNh is the most highly induced IFN
- Submitted to Dev. Comp. Immunol. – and the reviews are in!!

**King's College:**  
founded in 1495.



**Thanks for  
your attention.**