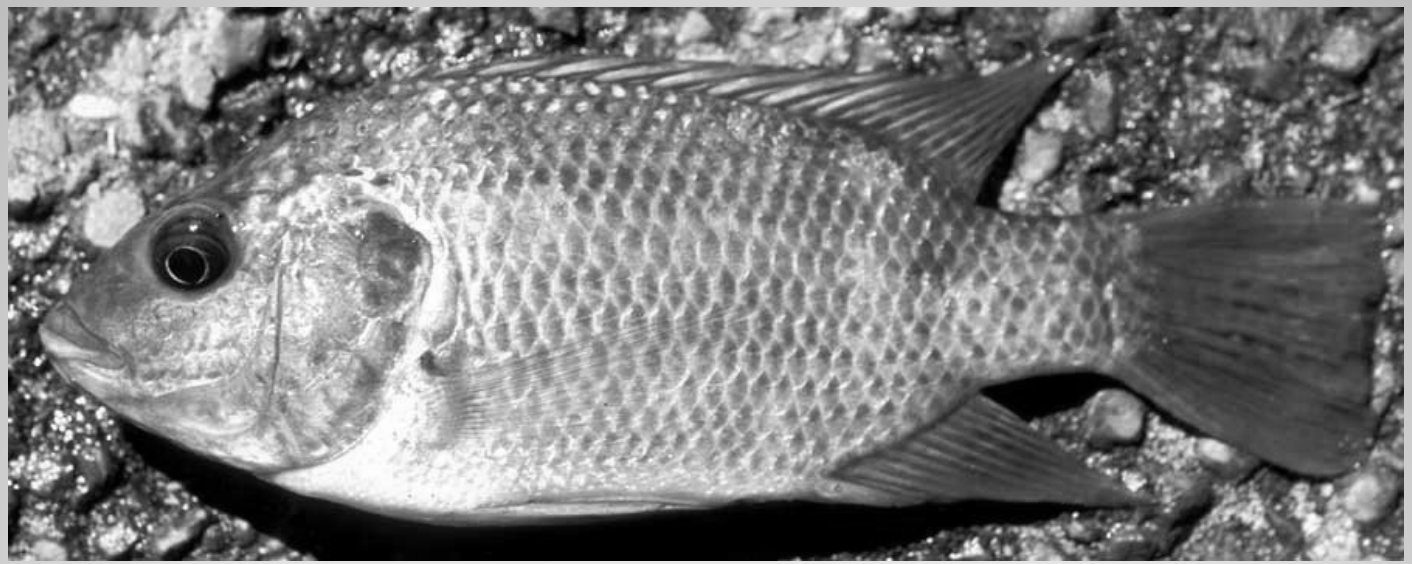
Faculdade de Ciências do
Mar e do Ambiente

GENE EXPRESSION DURING OOGENESIS IN THE MOZAMBIQUE TILAPIA (*O. MOSSAMBICUS*)



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Co-supervisor: Doctor Laurence Deloffre

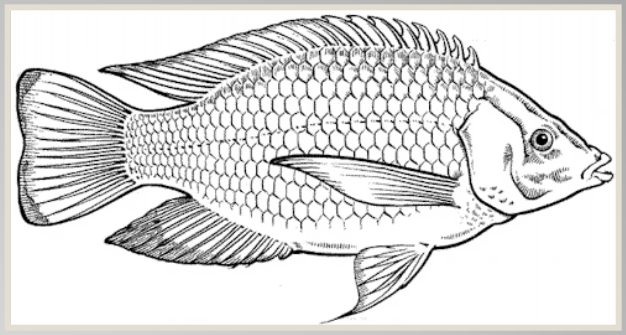
Nuno Manuel Cardoso Domingues

Faro, 26th October 2007

Introduction – Tilapia biology and value

TILAPIA:

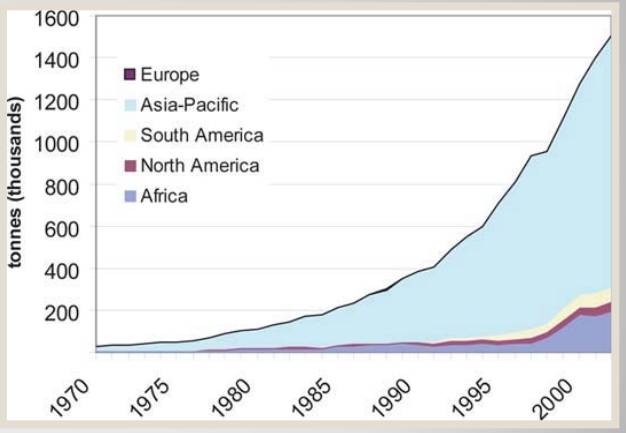
- **Ecological adaptations and tolerances;**
- **Tendency to overpopulate;**
- **Asynchronous ovaries.**



(Gunther Schmida in Mackenzie, et al. 2001)

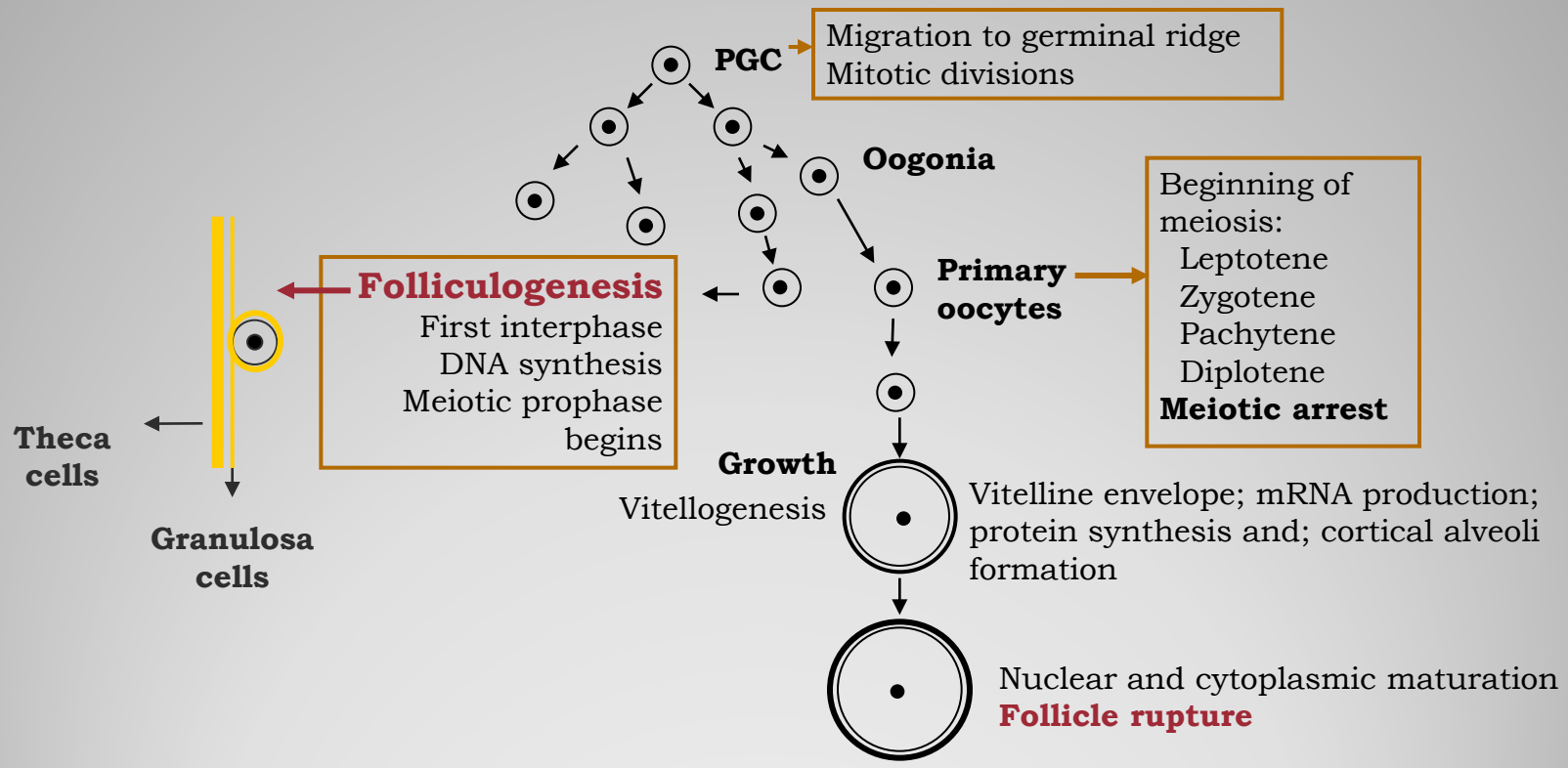
VALUE:

- **Interdisciplinar applications;**
- **Main area of interest aquaculture.**

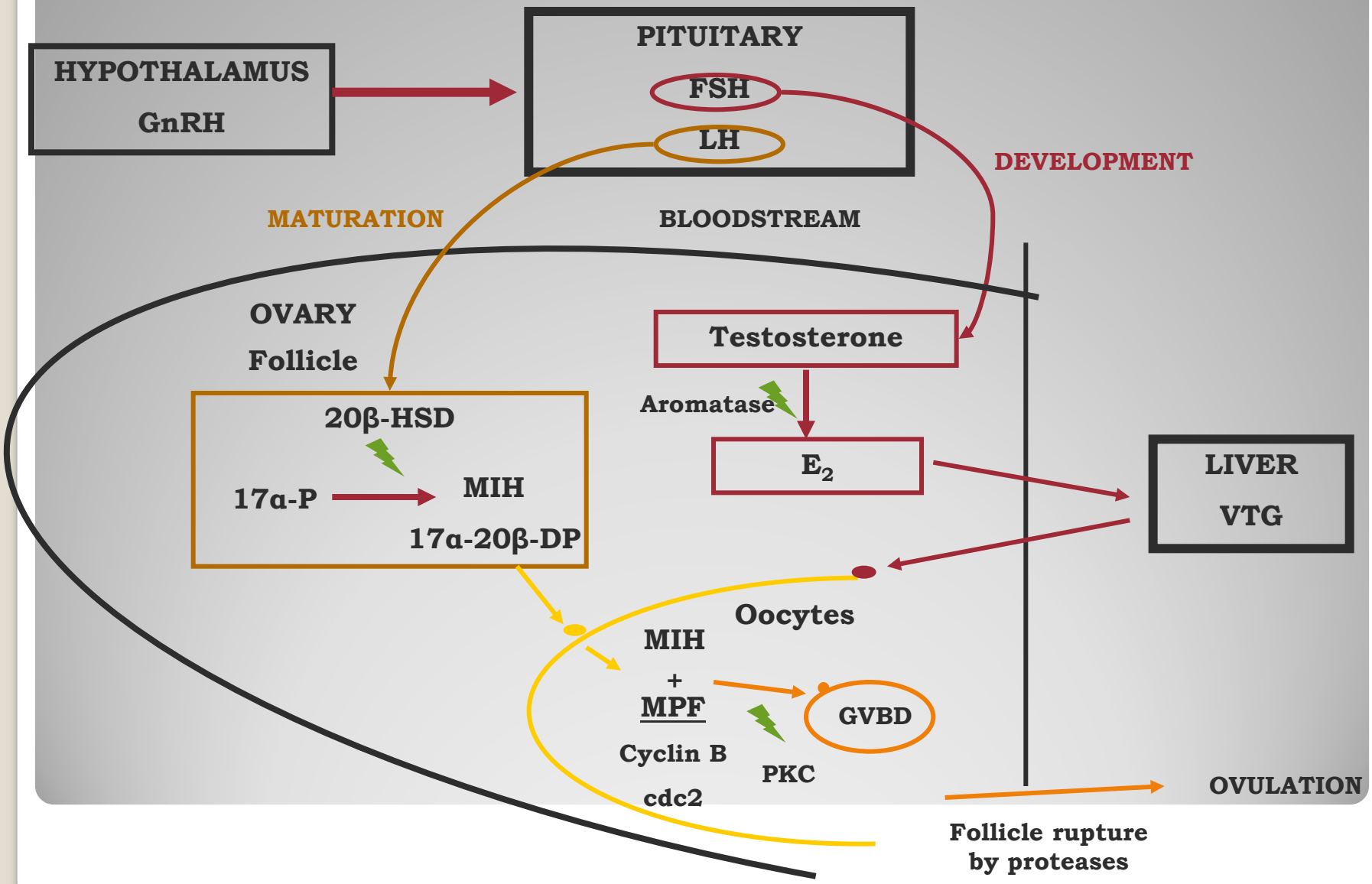


(De Silva et al. 2004).

Introduction – Ovary recrudescence in teleosts and oogenesis



Introduction – Hypothalamus-pituitary-gonadal axis in teleosts



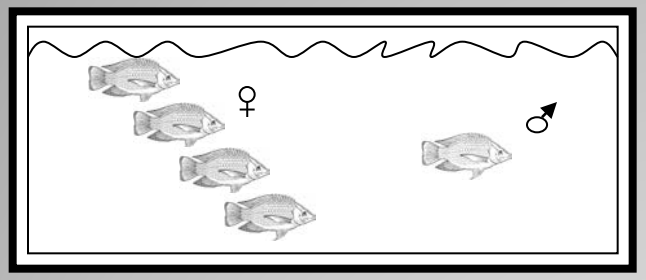
Objectives

➡ **mRNA expression analysis of putative factors regulating the development of the ovary of *Oreochromis mossambicus*;**

- **BMP-R IB, RBMX, Aly protein, CPI-17, SART, PPMP protein, XP2, Clone 26**

➡ **Full sequences of these genes would be obtained by means of RACE-PCR.**

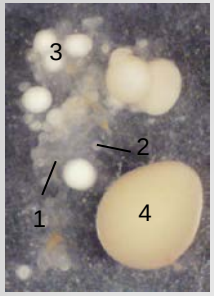
Material and methods – Biological material



- 200 L fresh water;
- 27ºC;
- 2 hour photoperiod;
- constant oxygenation;
- sand on the bottom;
- fed daily with commercial cichlid ration.

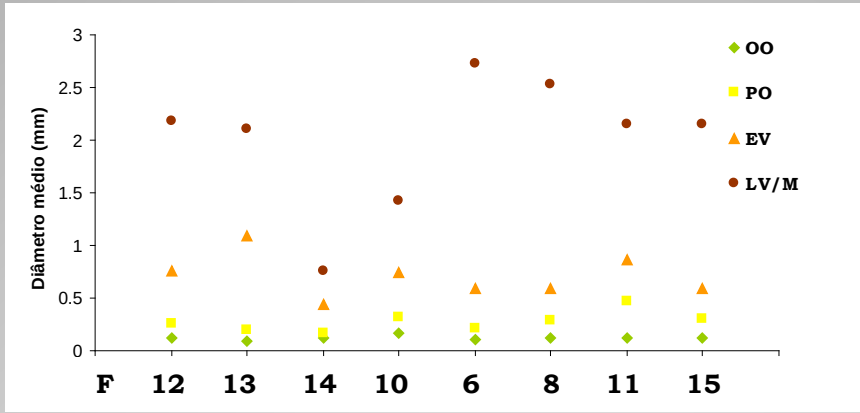
1) Lengths and weights registered (GSIs determined);

2) Oocytes separated under a binocular microscope in 0,9% NaCl:



1) oogonia (OO);
2) primary oocytes (PO);
3) early vitellogenic oocytes (EV);
4) late vitellogenic oocytes or mature oocytes (LV/M).

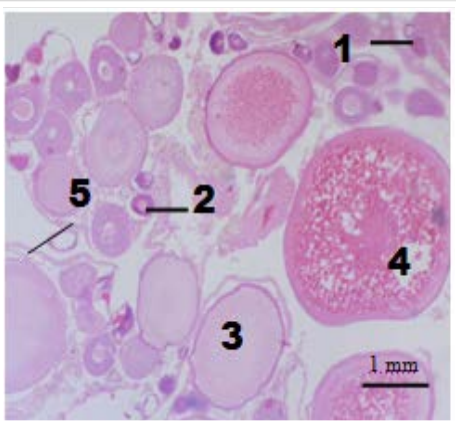
Material and Methods (continued)



Sizes significantly different from each others (P ≤ 0,001)

Histology

Tissues stained with haematoxylin-eosin:

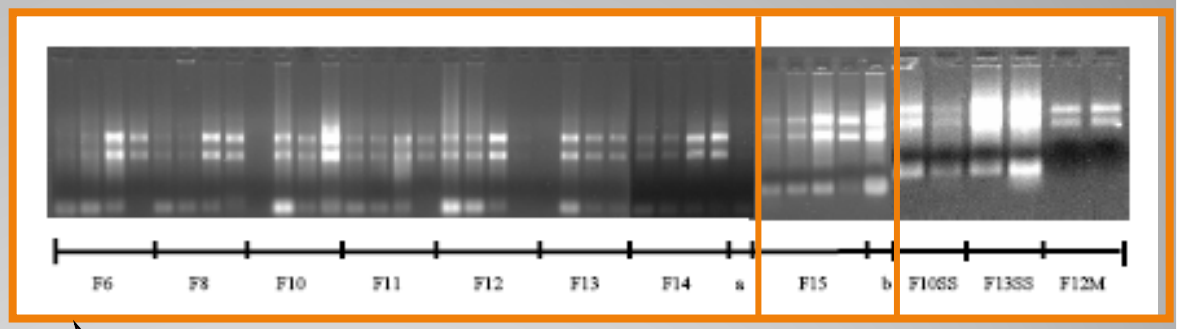


1) **Oogonia;**
2) **Primary oocytes;**
3) **Early vitellogenic oocytes;**
4) **Late-vitellogenic oocyte;**
5) **Ovarian follicle.**

Material and Methods – RNA extraction and cDNA synthesis

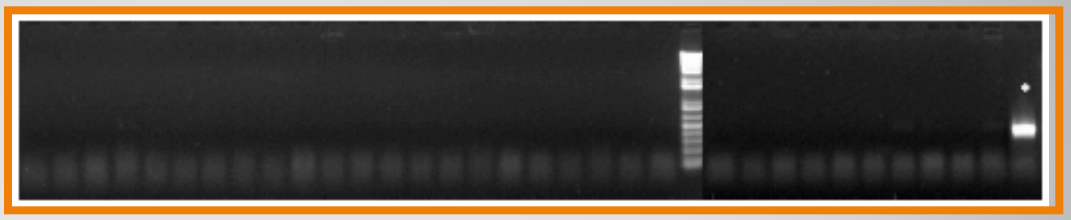
RNA extraction

(TRI Reagent)



cDNA synthesis with M-MLV RT

DNAse treatment



RT-PCR

Sexual dimorphic expression

Patterns through oocyte growth

Primers:

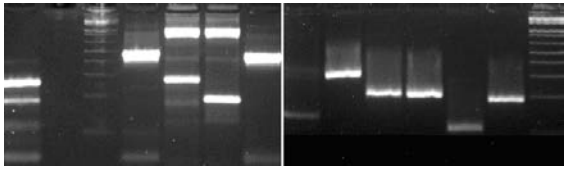
GC ≈ 50%

Annealing temperature FW-RV ≠ < 5ºC

No hairpin effect or primer dimmers.

Material and Methods – RT-PCR

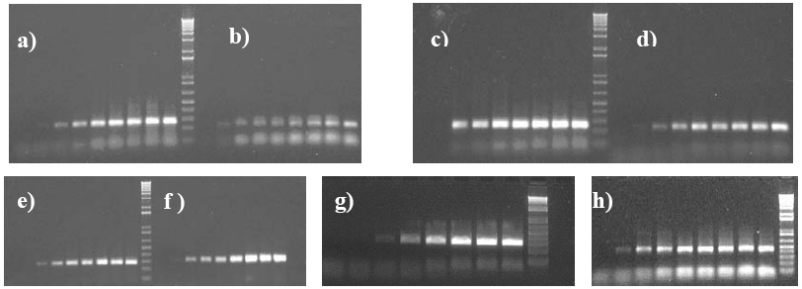
Optimization procedures



Temperature

$MgCl_2$

Nr of cycles



➡ **RT-PCR in BIO RAD myCycler**

94ºC 4 minutes;

Y times
- 94ºC 25 s;
- X ºC 25 s;
- 72ºC 35 s.

➡ 2 or 3% agarose gel by electrophoresis;

➡ Quantification (Biorad Quantity One);

1x Taq Buffer

$MgCl_2$ (3 mM)

Taq DNA polymerase (0,5 units)

dNTPs (0,2 mM each)

Primers (0,4 pmol/µl or 0,8 pmol/µl)

cDNA

H_2O (Sigma)

25 µl

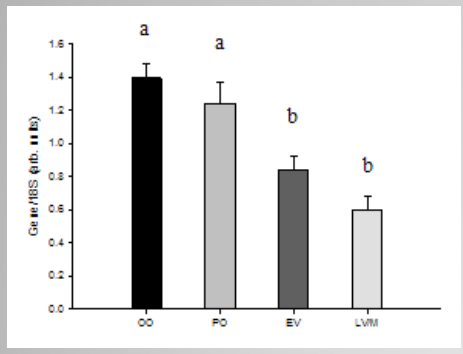
➡ Statistical analysis (ANOVA, ANOVA on ranks and Pearson correlation).

Results – Control genes

18S – reference gene

Accumulation in oocytes? → Vasa, FoxL2 and CYP19a tested.

Vasa



→ **PGC formation and migration to germinal ridge;**

→ **Decrease in vitellogenic stages.**

↘

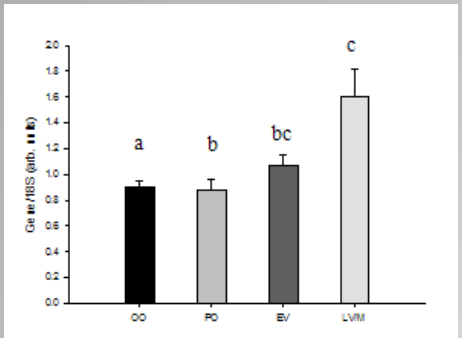
Dispersion through the cytoplasm?
(Kobayashi et al. 2000; Ye et al. 2007)

Observed in:

- **Nile tilapia** (Kobayashi et al. 2000);
- **Zebrafish** (Braat et al. 1999b);
- **Rainbow trout** (Ye et al. 2007).

Results – FoxL2 and CYP19a

FoxL2 (trancription factor)



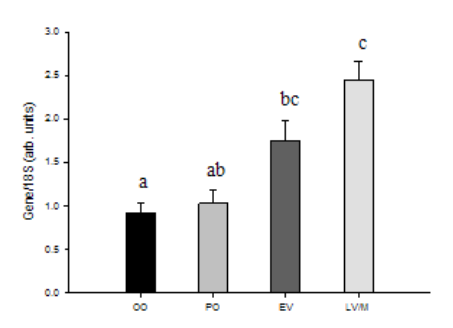
➡ **Ovarian development and function;**

➡ **Differentiation of PGC to oogonia.**

FoxL2 binds to CYP19a ACAAATA in promoter

Positively correlated

CYP19a (ovarian aromatase gene)



Activation of CYP19a

➡ **Higher in vittelogenic stages** ⟹ **E_2 synthesis.**

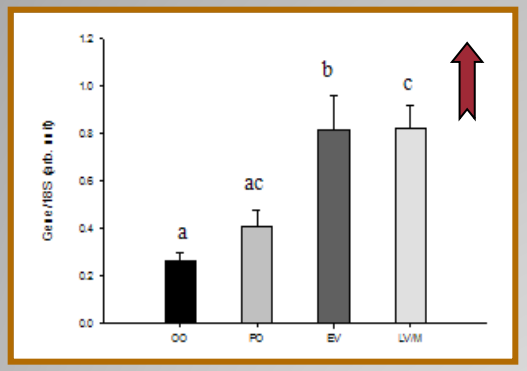
Results agree with:

- Wang et al. 2007;
- Yoshiura et al. 2003;
- Pannetier et al. 2006.

Results – Tested genes

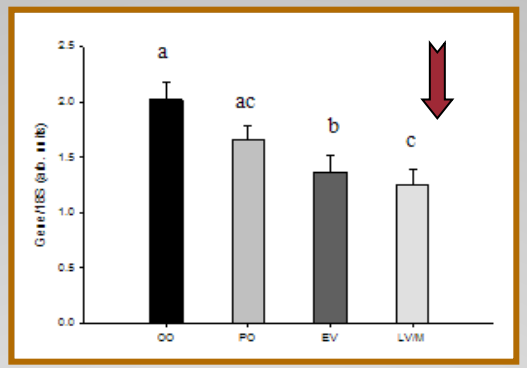
BMP-R IB

O T



RBMX

O T

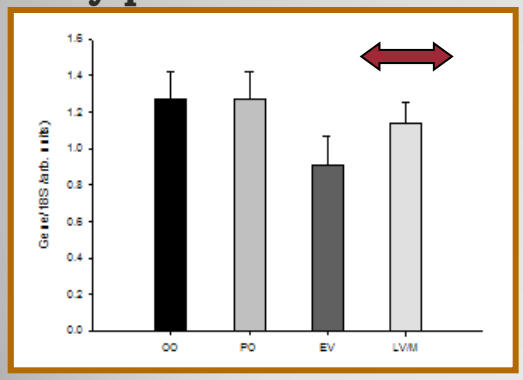


Major role in cellular differentiation

Major role in maturation

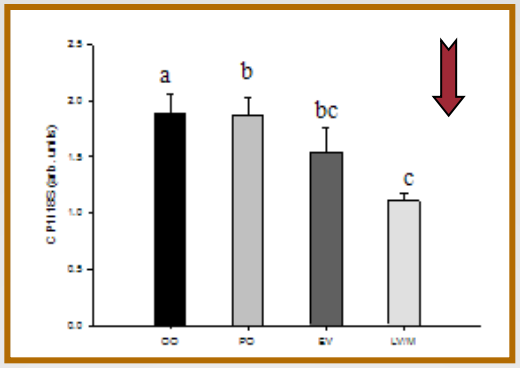
Aly protein

O T



CPI-17

O T



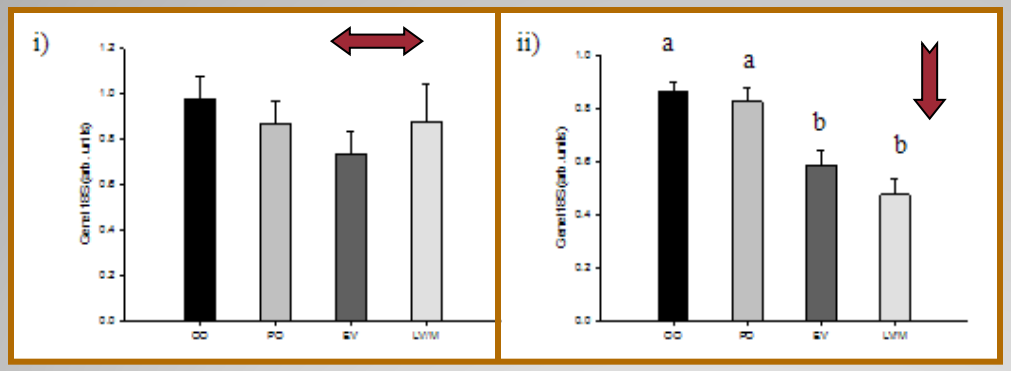
Basic cellular processes

18S

O T

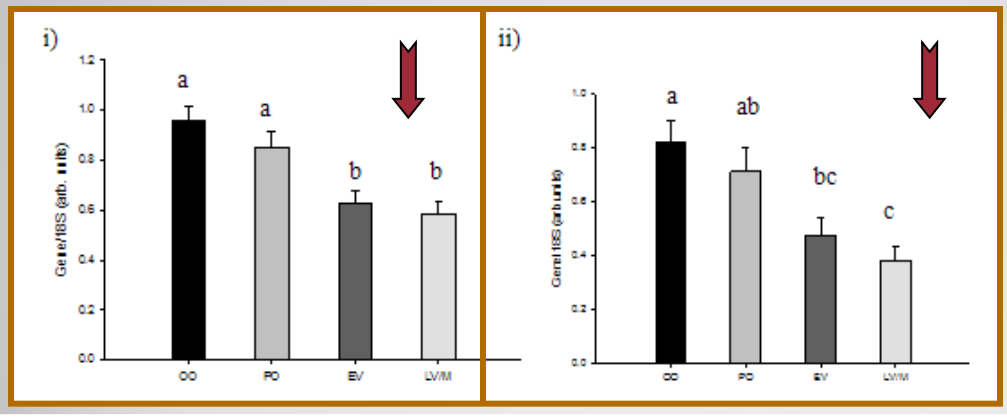
Results – Tested genes

SART



PPMP

XP2



Clone 26

Major role in cellular differentiation

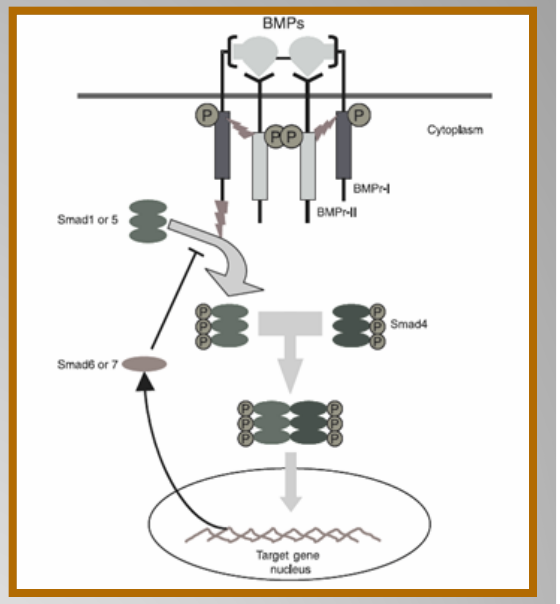
Major role in maturation

Basic cellular processes

18S

Discussion – BMP-R IB

Expressed in mouse granulosa cells, theca cells (higher) and oocytes



(Source: Granjeiro, et al. 2005).

FSH-BMP signalling in mammals

BMPs increase FSH signalling by Smads cascade

Potentiation of steroidogenic pathway

Supress LH receptor expression

At theca cells
BMP - 4
BMP - 7

At oocytes
BMP -6
BMP - 15

Autocrine / paracrine actions

Correlated positively with FoxL2 and CYP19a

Role in inhibition/delaying of luteinization and granulosa cell differentiation

Discussion – hnRNPGs

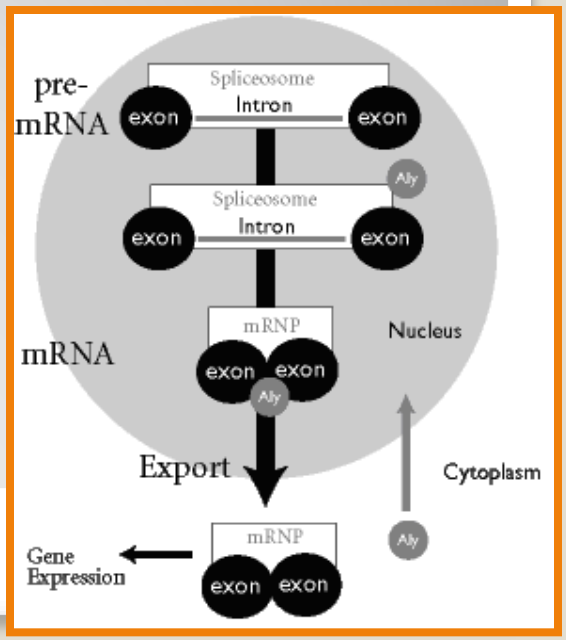
RBMX ↓

- **Expression suggests involvement in basic development processes (brain of *Danio rerio*);**
- **Decrease through follicle differentiation;**
- **Required for mRNA splicing.**

Positively correlated

Aly protein ↔

- **Exportation of mRNA from nucleus to cytoplasm and involved in splicing;**
- **Aly recycles to the nucleus (Zhou et al. 2000).**



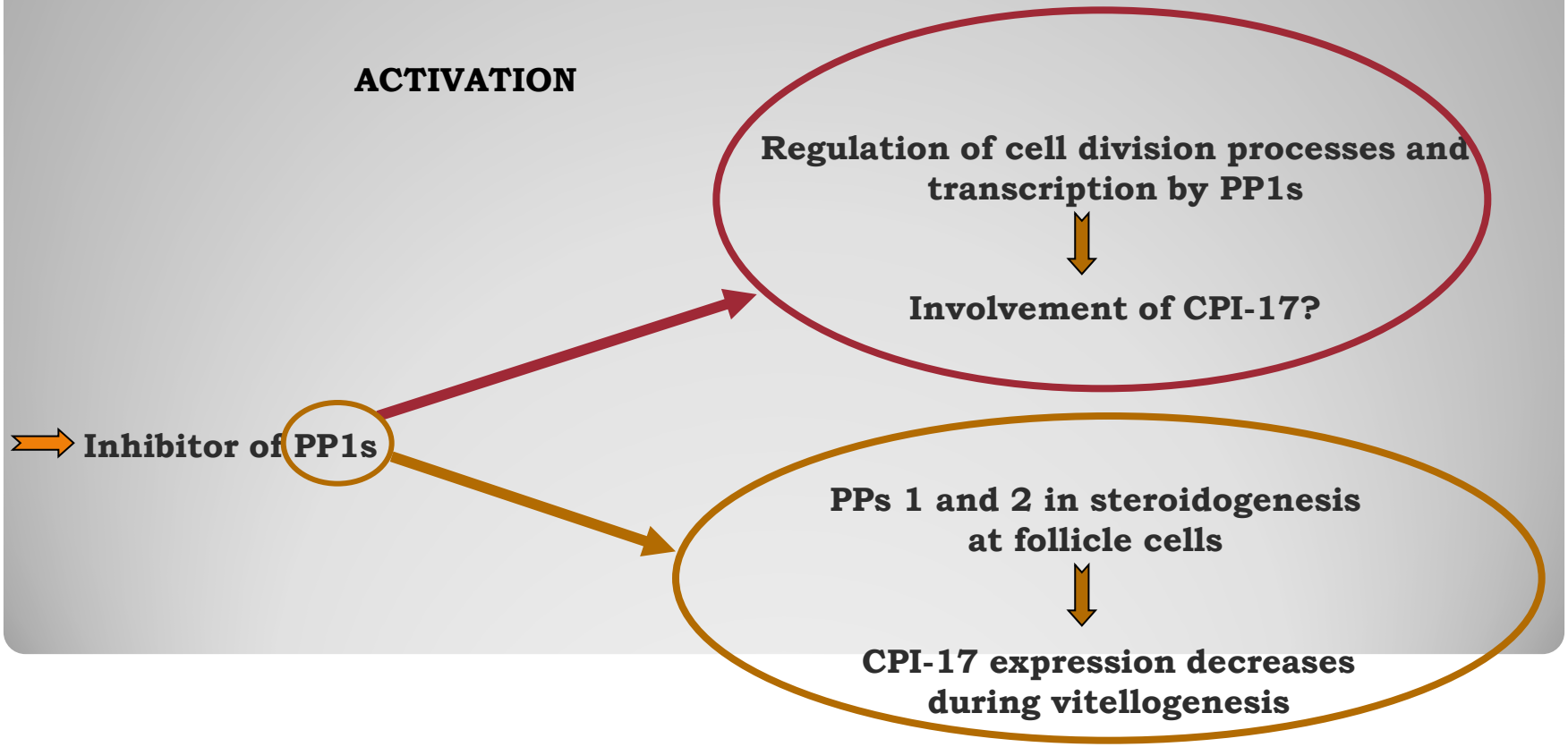
(Source: Zhou et al. 2000)

Discussion – CPI-17

Cysteine protease inhibitor – 17 KDa

Phosphorylated by PKC at Thr38

ACTIVATION



Discussion (SART, PPMP, XP2 and Clone 26)

↔ **SART-1** → **Squamous cell carcinoma-associated reactive antigen for cytotoxic T cells;**

↓ **PPMP protein** → **no reference to this gene.**

↓ **XP2** → **hypothetical new protein (also in *Danio rerio*);**

↓ **Clone 26** → **no homolog sequence (might be UTR).**

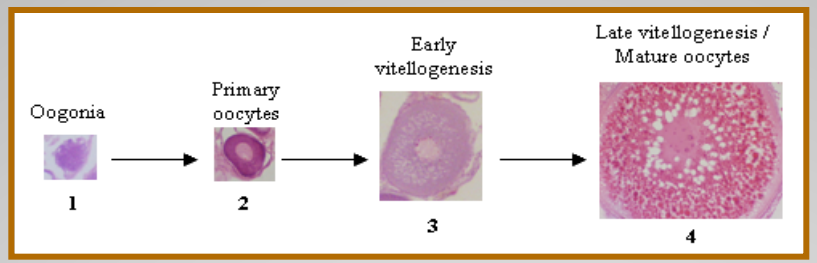
Further characterization required

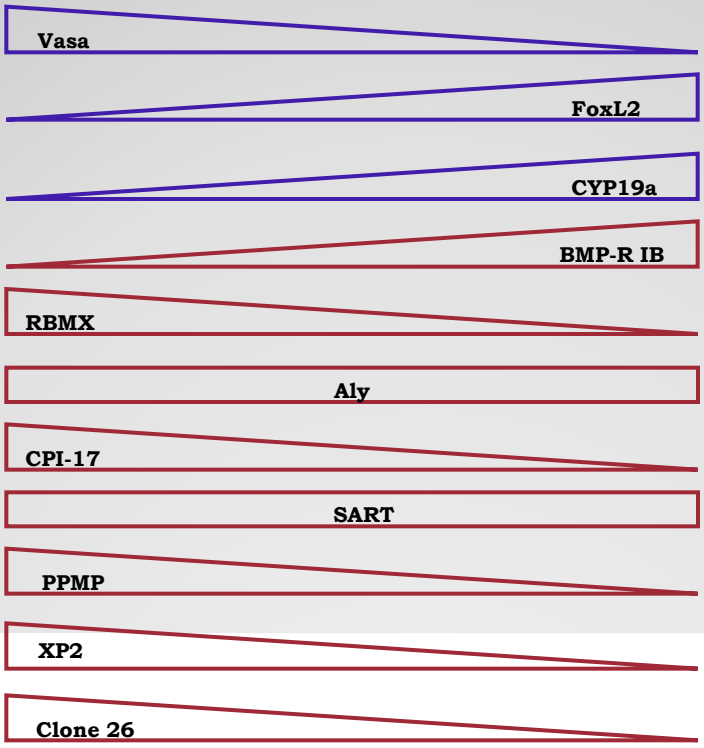
Conclusions and Further Work

- **18S is a good reference gene proven by Vasa, FoxL2 and CYP9a results;**

Conclusions and Further Work

- 18S is a good reference gene proven by Vasa, FoxL2 and CYP9a results;
-





To confirm reliability of rRNA 18S as reference

No proven identities or known functions in the ovary

Conclusions and Further Work

- **18S is a good reference gene proven by Vasa, FoxL2 and CYP9a results;**
- **Decreasing expression - role in cellular differentiation;**
  - **RBMX, PPMP, Clone 26, XP2 and CPI-17**
- **Increasing expression – important role in maturation;**
  - **BMP-R IB**
- **Constant expression – always necessary;**
  - **Aly protein and SART**
- **Further work required for characterization of unidentified genes.**

References

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De Silva SS, Subasinghe RP, Bartley DM & Lowther A 2004 Tilapias as alien aquatics in Asia and the Pacific: a review. In *FAO Fisheries Technical Paper*, p 65: FAO.

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Mackenzie R, Jackson P & Cotterell E 2001 An operational strategy for Queensland freshwaters. In *Control of Exotic Pest Fishes*. Brisbane: Department of Primary Industries.

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Acknowldgements

Professor Doutor Adelino Canário

Doutora Laurence Deloffre

Laboratório de Endocrinologia Molecular Comparada (FCMA)

Laboratório de Biologia Molecular (FERN)

Olinda, Nádia, Rita, Ângela, Rute, João, Isabel, Patrícia e os outros

Joana, Tiago, Alejandro, Andrea

Pai, Mãe, João.

THE END