

CALCIUM, THE BACKBONE OF FISH CULTURE: IMPORTANCE IN SKELETAL FORMATION, REPRODUCTION AND NORMAL PHYSIOLOGY

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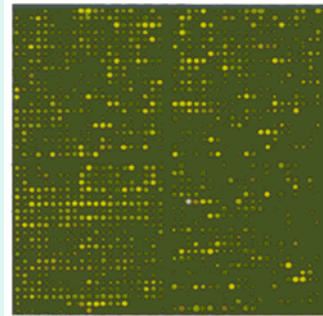
Objectives FISHCAL

- To identify the relative importance of dietary, endogenous and environmental sources of calcium during critical phases of development, growth and reproduction.
- Determine experimentally the roles of PTHrP in whole animal calcium homeostasis.
- Study the molecular mechanisms that underpin bone formation in fish and the way in which PTHrP regulates this process.
- Target species - sea bream (*Sparus auratus*).

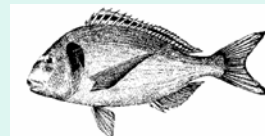
Benefits and Beneficiaries:

- Multitude of known roles for PTHrP in development, bone formation and immune system.
- Insight into the basis of skeletal development and how this can go wrong so as to provide some solutions for the problem of high incidence of developmental abnormalities at the early stages in sea bream.

Genomics input:

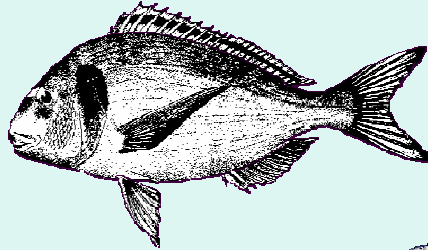


- Candidate gene approach.
- Identification of PTH/PTHrP promoters.
- Identification of PTHrP responsive genes.
- Gene expression profiles during endochondrial calcification.

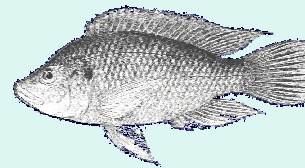


Candidate gene approach: Comparative analysis

Seabream
Sparus auratus

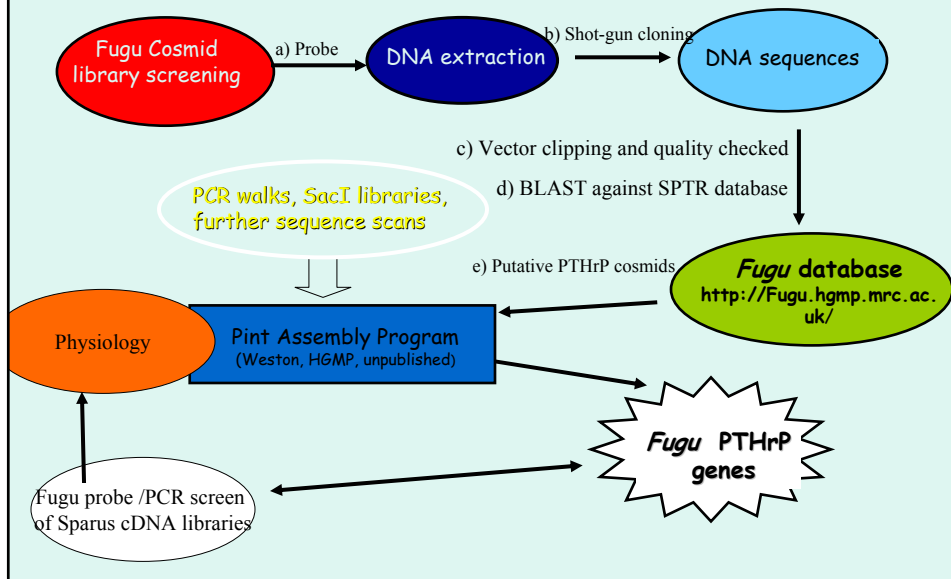


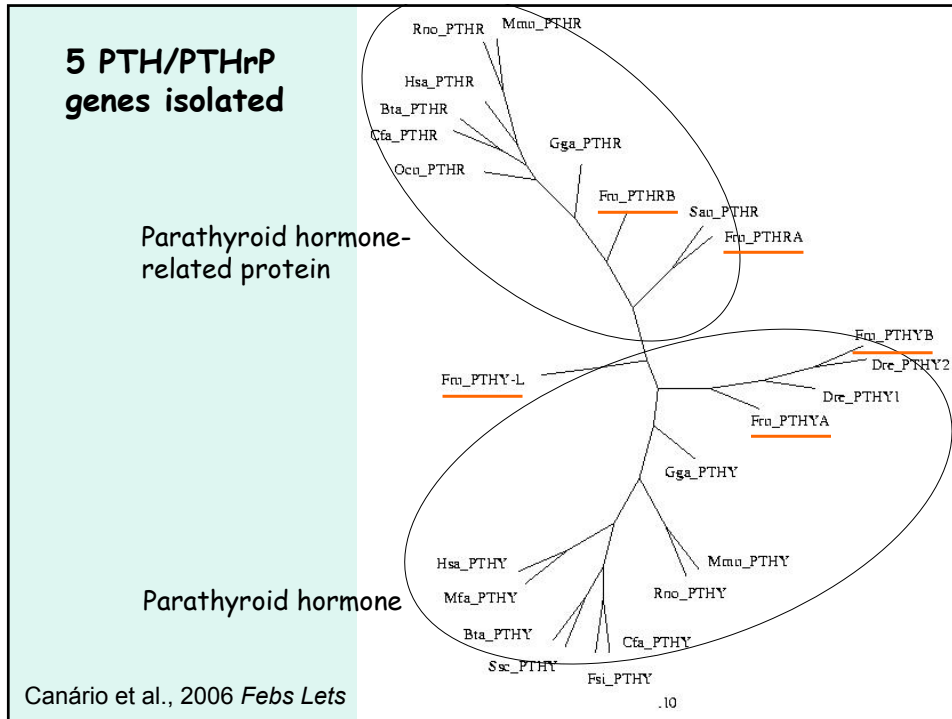
THE JAPANESE PUFFERFISH
(Fugu rubripes)



Tilapia
O. mossambicus

Example of approach - PTHrP





Microarray

1. Production of Differentially Enriched Library from PTHrP and Control larval heads and sequencing of clones

2. Production of Microarray

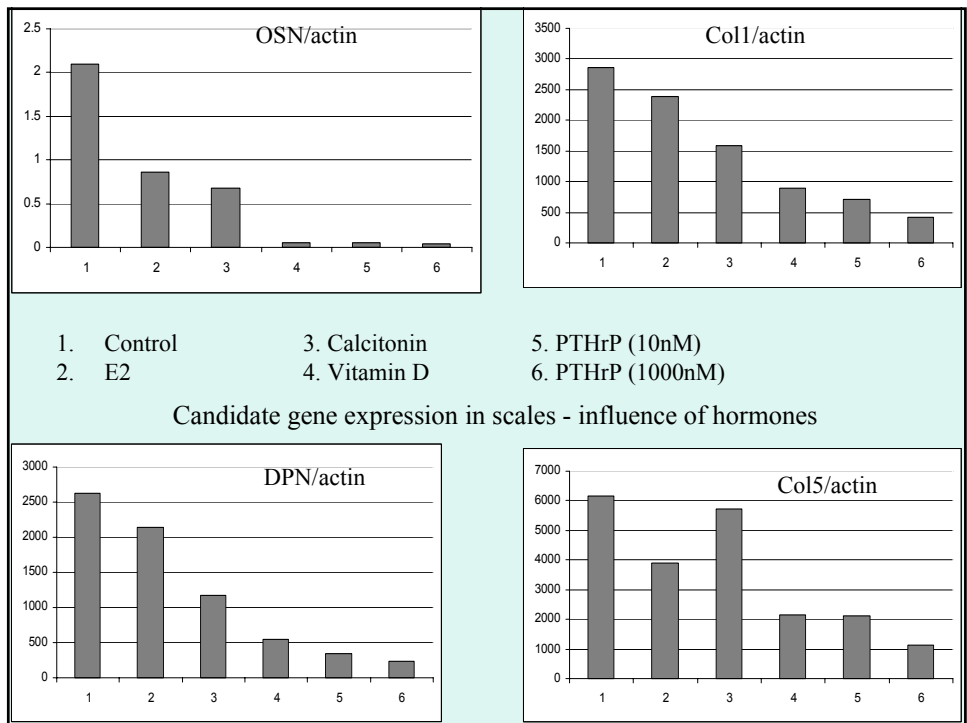
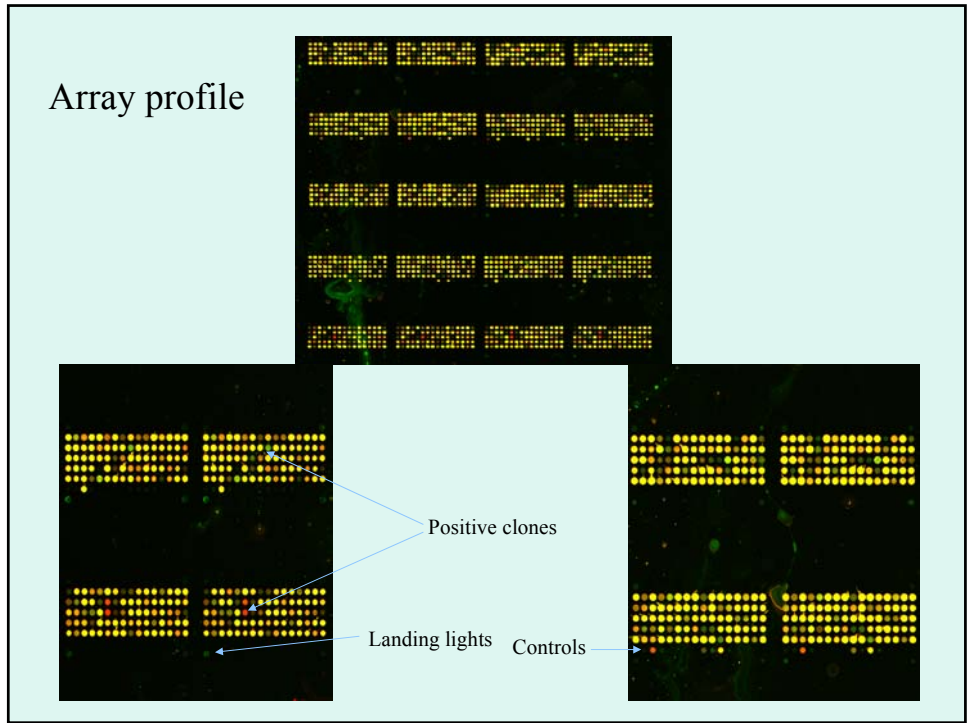
- 2000 clones from libraries selected for spotting on array slide.
- 100 known positive and negative clones spotted to allow verification of data.

3. Experiments

Larval tissue treated with PTHrP (dose/time course).
(quadruple screen, dye swap)

4. Analysis of Microarray

- Imagene 5.1 used outline and verify spots.
- Genespring used to analyse data and establish which clones are up or down regulated (4-fold cut-off).



Molecular Tools Generated

- Systems for production of recombinant sea bream proteins.
- Recombinant sea bream proteins and specific antisera.
- Quantitative RT-PCR for a range of endocrine hormones and receptors.
- Subtractive larval head cartilage cDNA library and a Microarray.
- Identification of candidate genes.
- Specific radioimmunoassays for parathyroid hormone (PTH) and parathyroid hormone related protein (PTHrP), thyroid hormones and steroids.
- Physiological studies and phenotype.

Publications

- Anjos, L., Rotllant, J., Guerreiro, P.M., Hang, X., Canario, A.V.M., Balment, R. and Power, D.M. (2005) Production and characterisation of recombinant sea bream (*Sparus aurata*) parathyroid hormone-related protein (PTHrP) *General and Comparative Endocrinology in press*
- Rotllant, J., Redruello, B., Guerreiro, P.M., Fernandes, H., Canario, A.V.M. and Power D.M. (2005) Calcium mobilization from fish scales is mediated by parathyroid hormone related protein via the parathyroid hormone type 1 receptor. *Regulatory Peptides in press*
- Rotllant, J., Guerreiro, P.M., Redruello, B., Fernandes, H., Apolónia, L., Anjos, L., Canario, A.V.M. and Power, D.M. (2005) Ligand binding and signalling pathways of PTH receptors in sea bream (*Sparus auratus*) enterocytes. *Cell and Tissue Research in press*
- Redruello, B., Estêvão, M.D., Rotllant, J., Guerreiro, P.M., Anjos, L.I., Canário A.V.M. and Power, D.M. (2005) Isolation and Characterization of Piscine Osteonectin and Downregulation of its expression by Parathyroid Hormone-Related Protein. *J. Bone and Mineral Research no prelo*
- Estêvão, M.D., Redruello, B., Canario A.V.M. and Power, D.M. (2005) Ontogeny of osteonectin expression in embryos and larvae of sea bream (*Sparus auratus*). *General and Comparative Endocrinology* 142, 155-162
- Rotllant, J., Guerreiro, P.M., Anjos, L., Redruello, B., Canario, A.V.M., and Power, D.M. (2005) Stimulation of cortisol release by the N-terminus of teleost parathyroid hormone-related protein: characterization of ligand determinants for receptor signalling in interrenal cells *in vitro*. *Endocrinology* 146, 71-76.
- Guerreiro, P.M., Fuentes, J., Flik, G., Rotllant, J., Power, D.M. and Canario, A.V.M. (2003) Water calcium concentration modifies whole-body calcium uptake in sea bream larvae during short-term adaptation to altered salinities. *Journal of Experimental Biology* 207, 645-653