Characterization of Fish Scale Regeneration: Environmental and Endocrine Control

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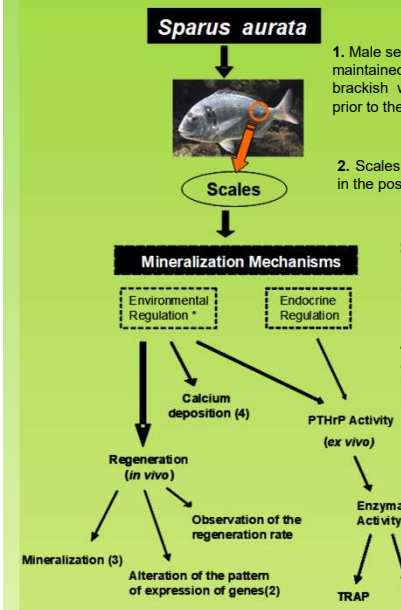
Introduction and Objective

Fish scales are calcified structures present in skin which have a protective function but which are also implicated in calcium homeostasis. The scale is organised into a basement plate containing cells, scleroblasts and scleroclasts which are equivalent to bone forming (osteoblasts) and resorbing (osteoclasts) cells, overlaid with calcifying and calcified matrix. Information about the molecular and cellular organisation of scales and the endocrine factors which regulate their turnover is scarce but calcaemic hormones such as parathyroid hormone (PTH), parathyroid hormone related protein (PTHrP) and calcitonin appear to be involved[1, 2].

The aim of the present study was to characterize the dynamics of scale regeneration and the possible regulation of this process by calcium availability and endocrine factors.

Experimental design



1. Male sea bream (n=10, ca 300g) were maintained in seawater (SW, 11mM Ca^{2+}) or brackish water (BW, 1.4mM) for 2 weeks prior to the experiment.

2. Scales were removed from a 1 cm^2 area in the posterior dorso-lateral region.

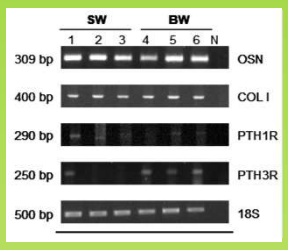
3. Regenerating scales were collected at 7, 13, 21, 30 and 45 days after removal.

4. Mineralisation mechanism assessed by: (1) PCR; (2) *in situ* hibridization; (3) histochemistry; (4) morphometric parameters.

* Salinity alterations (calcium availability);

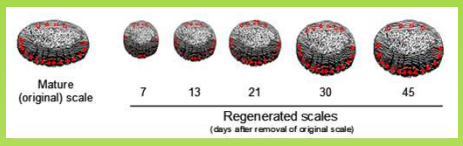
Ontogeny of gene expression

RT-PCR



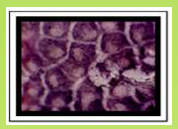
RT-PCR of osteonectin (OSN), collagen I (COL I), parathyroid hormone related peptide receptor 1 (PTH1R) and 3 (PTH3R) and 18S ribosomal. RNA in.mature scales from sea bream adapted to SW (1,2 and 3) and BW (4,5 and 6).

In situ hybridization



Localization and intensity of COL I gene expression in regenerated scales of the sea bream assessed by in situ hybridization. Higher expression occurs in anterior region of the scale and the intensity of expression increases with regeneration time.

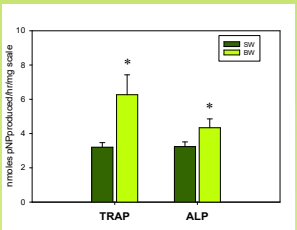
(● - very strong signal, • - strong signal, ○ - weak signal)



OSN expression in distinctive cells with a cuboid shape and a single nucleus (putative scleroblasts).

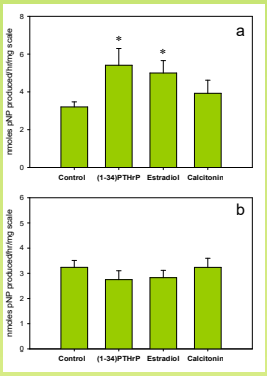
Enzyme activity

Effects of environmental salinity



Activity of TRAP and ALP in mature scales. In SW both enzymes have matched levels of activity. In BW, enzyme activity is significantly ($p< 0.05$) increased and TRAP activity is higher than ALP.

Effects of PTH-like peptides

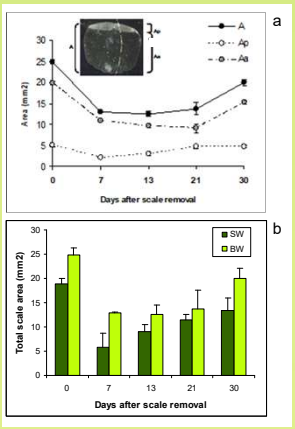


Effects on TRAP (**a**) and ALP activity (**b**) of mature scales (SW adaptation) incubated for 3 hours with the calciotropic hormones: Takifugu (1-34)PTHrP (10nM), E_2 (370 nM) and Salmon Calcitonin (30nM). (*$p< 0.05$)

Results

Chronology of regeneration

Ontogeny of scale regeneration in sea bream adapted to BW. In the initial stages little detail exists in the scale structure; 7 days after removal, the regenerated scale acquires the circula observed in the original scale; 30 days after removal the scale possesses a similar organisation to mature scales.



(a)Time course measurements of the total area (A) and area of the anterior (Aa) and posterior (Ap) region in regenerated scales.

(b)Comparison of total scale area in regenerating scales from fish adapted to seawater and brackish water.

Conclusions

✓ **COL I and OSN gene expression were observed in putative scleroblasts localised in the anterior region of regenerating scales.**

✓ **Regenerated scales become evident 5-7 days after removal in fish maintained in SW or BW.**

✓ **In BW, the activity of TRAP and ALP increased, indicating active remodelling.**

✓ **The hypercalcemic factors PTHrP and Estradiol evoked a significant increase in TRAP activity, suggesting endocrine regulation of calcium homeostasis occurs in scales.**

References: 1. Rotllant, J., et al. Calcium mobilization from fish scales is mediated by parathyroid hormone related protein via the parathyroid hormone type 1 receptor. Regul Pept, 2005. 132(1-3): 33-40. 2. Redruello, B et al, 2005. *Isolation and Characterization of Piscine Osteonectin and Downregulation of its expression by PTH-Related protein.* Journal of Bone and Mineral Research, 20(4):682-692.

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