



CHARACTERIZATION OF THE STRUCTURE AND MOLECULAR COMPOSITION OF SCALES IN TELEOST FISH

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Introduction

Calcium is an essential ion for a range of cellular and tissue functions and is strictly controlled. The scales represent an important source of calcium and are responsive to a number of hormones, such as estrogen and parathyroid hormone related protein (PTHrP). To better understand if and how calcium mobilization occurs from scales is essential to understand the structure and organization of this specialized tissue. In the present study we establish the structural organization and extracellular matrix composition (ECM) protein composition of this mineralized tissue.

Scale morphology

1) Experimental models

Sea bream (*Sparus auratus*), marine teleost haemphrodite
Tilapia (*Oreochromis mossambicus*), euryhaline teleost.

2) General histological methods

Haematoxylin-eosin
Toluidine blue
Alcian blue-alizarin red double staining (cartilage and bone)

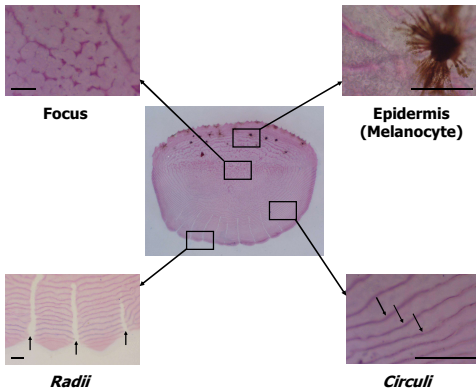
3) *In situ* hybridization (ISH)

Temperature (pre-hyb and hyb): 65°C
Riboprobe concentration: 3 µL/mL HM
Riboprobe detection: Anti-digoxigenin (AP Fragments)
Colour development: NBT/BCIP, 37°C

4) Riboprobes utilized

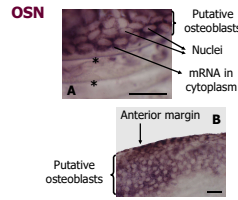
Type I collagen, α1 (Col1A1)	} Structural proteins
Type V collagen, α2 (Col5A2)	
Fibronectin (FN)	} Regulatory proteins
Osteonectin (OSN)	
Tartrate-resistant acid phosphatase (TRACP)	} Cell markers
Acidic secreted protein in cartilage ASPIC	

Sea bream elasmoid scale

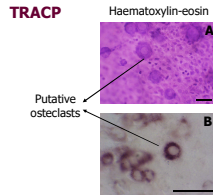
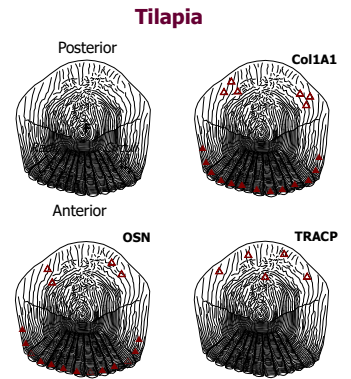


Sea bream scale stained with alcian blue and alizarin red. The characteristic features of an elasmoid scale such as the *radii*, the *circuli* and the focus are identified. Melanocytes are present in the epidermal layer that covers the posterior scale region.

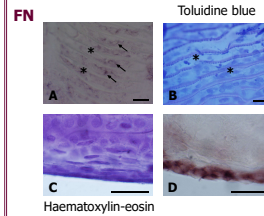
General distribution of target genes



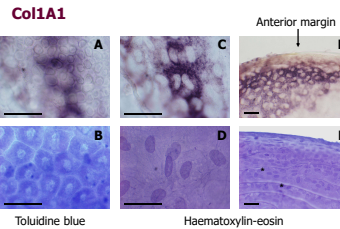
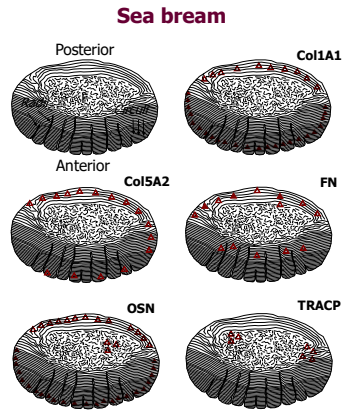
OSN expression was identified in the scale anterior region in the cytoplasm of flattened cells (B) and in more rounded cells, associated with the basal layer (A), the putative osteoblasts.



Expression of TRACP was detected by ISH in the cytoplasm of big round cells in the scale posterior region, which were identified as putative osteoclasts.



Marginal flattened cells in the scale posterior margin express FN (C, D). Osteoblast-like cells, with large nuclei, located between the *circuli* (*) express low levels of FN (A, B).



Col1A1 signal was detected by ISH in large rounded cells with big nuclei (C, D) and in more flattened cells near the scale margin (E, F). Large polyedric cells forming the epidermal layer (B) do not express Col1A1 (A).

(▲) represents an intense signal and (△) represents a weak signal.

Conclusions

- ✓ Col1A1 and OSN are highly expressed in both species. TRACP is also expressed in both species but with low intensity.
- ✓ Col5A2 and FN were only detected in sea bream. ASPIC is not expressed in neither species.
- ✓ The general distribution of the studied genes and the histological staining methods used allowed the identification of putative osteoblasts and osteoclasts in sea bream and tilapia scales.

Acknowledgements

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