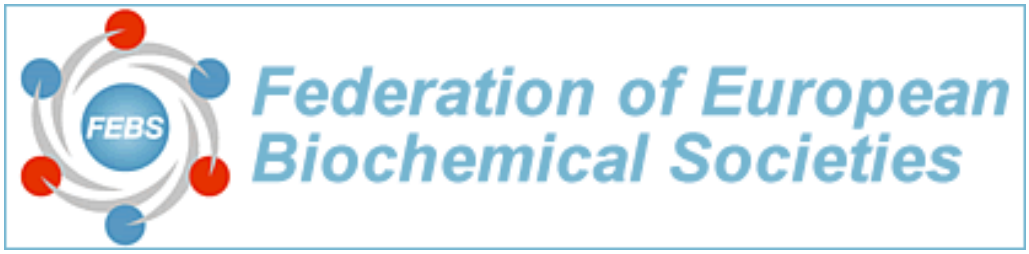

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Abstract Title: *Localization of osteocalcin (BGP) during fish (Sparus aurata) development by in situ hybridization and immunohistochemistry: comparison between gene expression/protein distribution and skeletal mineralization*

Author(s): J Pinto, P Gavaia, D Simes, R Kelsh and L Cancela

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Abstract:
Osteocalcin (Bone Gla protein, BGP) is a small noncollagenous protein which is synthesized by osteoblasts and odontoblasts and is found exlusively in mineralized bony tissues. Although isolated for the first time in 1978, only recently has a function for this protein been suggested, specifically in controlling hydroxyapatite crystal growth. Appearance of osteocalcin could be linked to the presence of an hydroxyapatite-containing bony skeleton, since the protein was never found in cartilaginous fishes. Furthermore, within its primary sequence the amino acid residues known to be essential for its function are present in fish as well as in mammals, suggesting that function has been conserved over 400 million years of evolution. Taken totgether, these findings prompted us to study in detail the localization of osteocalcin gene expression in fish.

We have recently cloned the *Sparus osteocalcin* gene and analysed its tissue distribution and developmental appearance in this species (Pinto *et al*., Gene 2001). Specific antibodies against *Sparus* BGP were also developed and used to analyse the presence of the protein in sections of *Sparus*, from nonmineralized to fully mineralized stages of development. These results were correlated with skeleton mineralization (assessed by alcian blue/alizarin red histological techniques), and sites of osteocalcin gene expression determined by *in situ* hybridization. Based on these data, we address the question of the evolution of function of this protein throughout vertebrate history.