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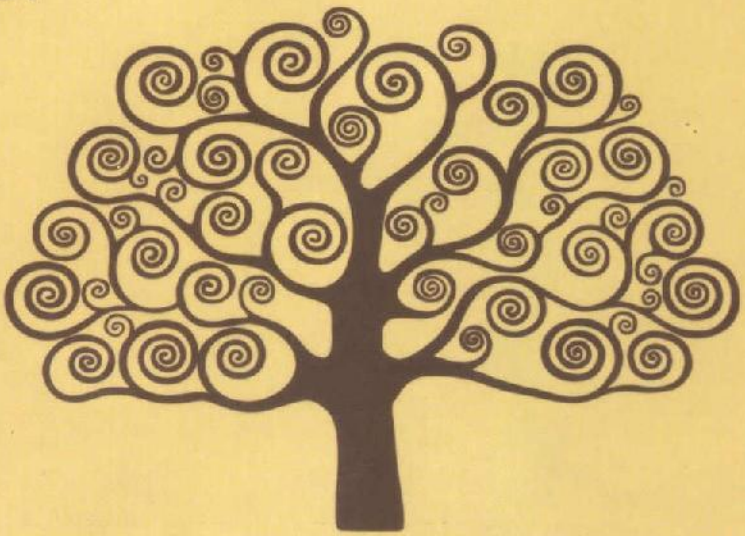

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EVIDENCES FOR NEW OSTEOCALCIN ISOFORMS IN FISH AND TETRAPODS

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Investigating the evolution of proteins involved in tissue calcification is essential to elucidate how vertebrate calcified tissues have evolved. Osteocalcin (OC) is a small calcium-binding protein accumulated exclusively in bone and teeth, whose function, although essential for tissue calcification, remains unclear. Until recently, only a single osteocalcin isoform had been described (OC1). The cloning of new osteocalcin sequences and in-depth analysis of sequence databases has uncovered two additional isoforms: OC2, which is specific for a unique fish taxonomic group, the Osteichthyes, and OC3, found only in tetrapods. All isoforms share key features of osteocalcin but differ in several aspects. OC2 exhibits a large and acidic propeptide which is likely to be phosphorylated and has no counter part in the OC1 propeptide. Both isoforms co-exist in 3 fish species where they are encoded by two distinct genes. OC3 has been uncovered so far only in few species and is highly similar to OC1. Both isoforms co-exist in 2 species where they are encoded by distinct genes localized onto different chromosomes. Interestingly, OC3 and matrix Gla protein (MGP) genes co-localize on the same chromosome in both species. We have recently proposed osteocalcin to be a duplicate of MGP (Laizé et al, 2005). In light of our new data, we now propose that OC3 first originated from MGP before jawed fish evolved, while OC1 originated more recently from OC3 through a gene duplication event that probably occurred in the tetrapod lineage, soon after divergence from teleost fish. Finally OC2 originated from OC3 through a second gene duplication that probably occurred only in the teleost fish lineage, soon after divergence from tetrapods. These new data raise important questions on the function, tissue distribution and gene regulation of each isoform. To answer these questions, available sequence data will need to be complemented with biological data.