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Review

Matrix Gla protein and osteocalcin: From gene duplication to neofunctionalization

M. Leonor Cancela [a,b,*,1], Vincent Laizé [a,1], Natércia Conceição [a,1]

[a] Centre of Marine Sciences, University of Algarve, 8005-139 Faro, Portugal
[b] Department of Biomedical Sciences and Medicine, University of Algarve, 8005-139 Faro, Portugal

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ABSTRACT

Osteocalcin (OC or bone Gla protein, BGP) and matrix Gla protein (MGP) are two members of the growing family of vitamin K-dependent (VKD) proteins. They were the first VKD proteins found not to be involved in coagulation and synthesized outside the liver. Both proteins were isolated from bone although it is now known that only OC is synthesized by bone cells under normal physiological conditions, but since both proteins can bind calcium and hydroxyapatite, they can also accumulate in bone. Both OC and MGP share similar structural features, both in terms of protein domains and gene organization. OC gene is likely to have appeared from MGP through a tandem gene duplication that occurred concomitantly with the appearance of the bony vertebrates. Despite their relatively close relationship and the fact that both can bind calcium and affect mineralization, their functions are not redundant and they also have other unrelated functions. Interestingly, these two proteins appear to have followed quite different evolutionary strategies in order to acquire novel functionalities, with OC following a gene duplication strategy while MGP variability was obtained mostly by the use of multiple promoters and alternative splicing, leading to proteins with additional functional characteristics and alternative gene regulatory pathways.

Introduction

Osteocalcin[2] (OC or bone Gla protein, BGP) and matrix Gla protein (MGP) are two members of the growing family of vitamin K-dependent (VKD) proteins, and the first found not to be involved in coagulation and being synthesized outside the liver [33,78,73,74]. Indeed, shortly after the discovery of Gla as a novel amino acid residue derived from the γ-carboxylation of glutamate [88] and essential for the blood clotting capability of several coagulation factors, bone was found to contain high amounts of Gla suggesting the presence of Gla-containing proteins in this tissue. The follow up from this finding culminated with the purification of the bone Gla protein/osteocalcin from the mineralized matrix of bovine bone, which accounted for up to 2% of the total proteins of bone [73]. However, already in 1980 Price et al. predicted that OC was not the only Gla protein in bone since, when analyzing fetal bone, they could not extract its Gla content by demineralization, which suggested that it was not from osteocalcin and argued in favor of the presence of another Gla-containing protein in fetal bone but associated with the collagenous matrix [72]. This hypothesis proved to be correct and matrix Gla protein was later purified from the organic matrix of bone [78,77] and thought to account for the remaining Gla content of bone, since the presence of additional Gla proteins in bone was not anticipated at the time. OC and MGP share with the other members of this family their capability of binding calcium and calcified matrices through interaction with their Gla residues, which result from a γ-carboxylation of glutamate residues, a post translation modification dependent of vitamin K and catalyzed by the enzyme γ-glutamyl carboxylase, a ubiquitous protein found both in vertebrates and in invertebrates, and more recently also in bacteria [81] although its targets in the latter remain essentially unknown. Warfarin, a vitamin K antagonist discovered in the late 1950s [51], is capable of inhibiting this process resulting in the appearance of undercarboxylated Gla proteins, a process that negatively affects their calcium binding capabilities and thus their established functions [17,69].

Both proteins were found to accumulate in bone, although osteocalcin was later identified as being secreted under normal, non-pathological conditions, only by osteoblasts, odontoblasts

* Corresponding author at: Department of Biomedical Sciences and Medicine, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal. Fax: +351 289 800971.
E-mail address: lcancela@ualg.pt (M.L. Cancela).
[1] All authors contributed equally.
[2] *Abbreviations used:* OC, osteocalcin; BGP, bone Gla protein; MGP, matrix Gla protein; VKD, vitamin K-dependent; Gla, γ-carboxylated glutamate; OA, osteoarthritis; ucMGP, uncarboxylated MGP; cMGP, carboxylated MGP; AnaOC, Adriatic sturgeon osteocalcin.

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and cementoblasts [8,35,61], while MGP was found to be expressed mainly by chondrocytes and fibroblasts [11,28], smooth muscle cells [85] and more recently by tooth cementum [30] and trabecular meshwork cells from the eye [25,90].

Both OC and MGP share similar structural features, both in terms of protein domains and gene organization. However, previous work has demonstrated that the evolutionary appearance of MGP is likely to have preceded osteocalcin, both proteins sharing a common ancestor [12,47,80]. Furthermore, the earlier appearance of MGP is concomitant with the development of cartilage-like tissues in ancestral vertebrates, being still debatable if it is present in the jawless fish (Agnatha). In contrast, OC appears to have evolved together with the appearance of bone tissue in bony vertebrates (Osteichthyes; [47] and references therein). This article reviews the knowledge associated with the discovery of OC and MGP and their structural domains, and the landmark discoveries that culminated with the present understanding of their functions. Although both proteins have a common ancestor, our data suggests that each protein followed distinct evolutionary strategies to achieve the present diversification of proposed functions.

MGP and OC: protein structure and functional domains

MGP and osteocalcin share some common protein features but they also have their own individuality (Fig. 1). Both are small secreted proteins that localize in the extracellular matrix and thus both of their primary structures contain a signal peptide. In addition, the mature MGP contains in the N-terminal moiety a domain of phosphorylation (SxxSxxS) and a cleavage site identified by the consensus sequence ANxF, which appear to be conserved in all species analyzed [10,47,80]. The conserved γ-carboxylase recognition site is also included in this N-terminal moiety, a characteristic which is not found in all other known secreted VKD proteins in which that domain is included in the propeptide. In addition, MGP contains another cleavage site between either two conserved arginine residues (RR) or an arginine and a glycine (RG) located close to its carboxy-terminal end and previously suggested to be involved in its function [29]. Four (out of five in human) of the γ-carboxyglutamate residues and the two conserved cysteines, which form MGP characteristic disulfide bond, are located between ANxF and RR/RG cleavage sites. To date, and due to the low solubility of the protein in aqueous solutions [71], no tridimensional (3D) structure of MGP is available.

OC is synthesized as a pre-pro-protein. As for MGP, the signal peptide will target OC to the extracellular matrix, while the prodomain, which is cleaved by a furin-like proteolytic enzyme at RxxR site, only contains the γ-glutamyl carboxylase recognition site. The mature protein is small (49 amino acids in human) and contains the three glutamate residues, which once γ-carboxylated are responsible for its binding to calcium and hydroxyapatite, and the two conserved cysteine residues forming the intramolecular disulfide bond, which contributes to stabilize its 3D structure. NMR studies have previously suggested that the configuration of OC apoprotein form was in a disorganized state in the absence of calcium, likely acquiring its 3D structure upon binding of its γ-carboxylated glutamate (Gla) residues to calcium [19,31]. Analysis of the crystal structures obtained for OC from pig [38] and fish [22] confirmed that Gla residues are clustered at the surface of the protein, coordinating the Ca^{2+} ions present in each of those structures and leaving its carboxy-terminus accessible. This was indicative of the existence of a mechanism for attachment to the surface of the hydroxyapatite crystals and for promoting the adhesion to osteoblasts and osteoclasts during bone remodeling. Recently, the X-ray crystal structure of bovine 3Glu-osteocalcin (i.e. non-carboxylated OC) was described, and surprisingly its structure was found to be very similar to that of the 3Gla-osteocalcin (i.e. the fully carboxylated OC), contradicting the previous idea that, in the absence of Gla, osteocalcin would have a disorganized structure and suggesting that the helical structure of the uncarboxylated form adopted a structure similar to that of the carboxylated osteocalcin and thus folded in a calcium-independent way [54]. In light of these results, authors suggested that the 3Glu-OC, which does not appear to bind calcium, could be involved in interactions with its recently identified receptor [67] in a calcium poor environment, while the 3Gla-OC would bind the crystal structure and through these interactions affect bone. The binding of osteocalcin to GPRC6A receptor is however controversial after the recent publication of the study by Jacobsen et al. where agonistic activity of osteocalcin could not be detected [41].

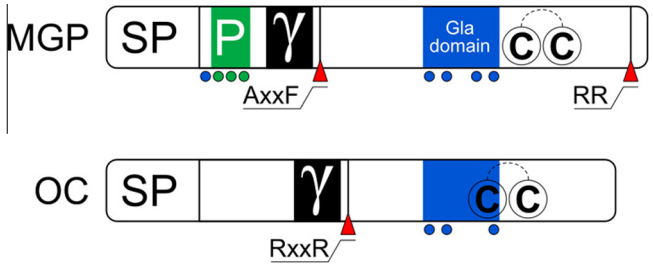

Fig. 1. Schematic representation of matrix Gla protein (MGP) and osteocalcin (OC) archetype protein structures. Gla residues are indicated by blue dots and Gla domain (Gla) is represented by a blue box; Phosphoserine residues are indicated by green dots and phosphorylated domain (P) is represented by a green box; red triangles indicate proteolytic cleavage sites AXXF and RR (MGP) and RXXR (OC); γ indicates the docking site for γ-glutamyl carboxylase; Circled C indicate conserved cysteine residues involved in intramolecular disulfide bond (dashed line); SP, signal peptide.

MGP and OC: established functions

The development of knockout mice [20,53] clearly showed the role of MGP and OC in the control of tissue mineralization, although acting through quite different mechanisms. Accordingly, expression of OC at sites of ectopic mineralization in MGP null mice cannot reverse the abnormal phenotype, in contrast with re-expression of MGP at those same sites. This indicates that despite their evolutionary proximity, OC and MGP functions are not redundant [58]. Previous works had already suggested the involvement of VKD proteins in the mineralization process. While characterizing the newly identified Gla protein of bone, Price et al. also identified Gla in calcified arteries and suggested that a Gla protein could be involved in soft tissue calcification [73]. Later, Hale et al. hypothesized that MGP inactivation in cartilage could be responsible for both (i) the appearance of foci of mineralization in the growth plate of infants affected by fetal warfarin syndrome, occurring when mothers received this anticoagulant drug and vitamin K antagonist during the first trimester of their pregnancy, and (ii) the abnormal growth plate mineralization observed in rats treated with warfarin. The authors hypothesized that these phenotypes could be caused by the functional inhibition of a protein responsible for actively preventing ectopic mineralization in cartilage, which they suggested could be MGP [28]. Indeed, this proved to be true and although its mode of action at the molecular level is still not completely elucidated, all available evidence points to a role for MGP in the control of tissue mineralization. In 1997 the publication of the phenotype developed by the mutant $MGP^{-/-}$ mouse, which included, in addition to cartilage calcifications, death by artery rupture within 8 weeks after birth due to calcifications of vessel walls, clearly showed that MGP was indeed a physiological inhibitor of calcification. The identification of a human pathology