IMPORTANCE OF SELECTED PROTEINS OF COMPACT BONE TISSUE IN POULTRY

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ABSTRACT

Within modern poultry lines, the integrity of the skeleton is subjected to increasing genetic and production stress, which in many cases leads to different health problems of the skeletal system, including problems with osteoporosis and the development of fractures. The role of genetics in bone integrity has been demonstrated by several studies, while the knowledge gained from the targeted study of genes, i.e., proteins that play an important role in bone metabolism, is of great value both for skeletal health and may provide new clues to the biological processes underlying diseases leading to the weakening of the bones. In addition to summarizing basic knowledge about bone metabolism, this review provides insight into the structure and function of proteins that are part of compact bone tissue, focusing on non-collagenous proteins and proteins that are encoded by genes involved in signaling pathways that play an important role in bone metabolism.

Keywords: bone, bone metabolism, protein, gene, signaling pathway

INTRODUCTION

Birds are adapted morphologically and physiologically very well to locomotion, when their typical movement is flight. The whole range of these adaptations makes them a very specific and quite diverse group of vertebrates (Serrano et al., 2020). Therefore, the skeleton of birds must be lightweight to minimize the metabolic expenditure of flight and strong enough to withstand the forces with which it comes into contact. The evolution of skeletal structures is governed by these principles, where both the shape of bones and the material properties of bone tissue make bones strong and rigid, but also fragile. There was gradual reduction, loss, and fusion of many skeletal elements and expansion of pneumatized spaces in some bones (Dumont, 2010).

BONE STRUCTURE

Bone, as a very dynamic organ, has several functions within the skeleton. In addition to fulfilling the function of structural support of the body, where it is exposed to various loadings of movement, it is furthermore the interface between muscles and protection of internal organs. It is a reservoir of calcium and phosphorus as well, with almost 99 % of the body's total calcium and 80 % of phosphorus found in the skeleton, which is very important in the terms of reproduction (Conti et al., 2023; Wu et al., 2021). Approximately one-third of bone is made up of organic compounds, while two-thirds is made up of the inorganic part, i.e., the mineral matrix. Collagen (primary type I) makes up approximately 85 to 90 % of the organic component of bone. It gives bones resistance against tensile forces when resistance against compressive forces is provided by proteoglycans. Another organic components are non-collagenous proteins such as osteocalcin and osteonectin and glycoproteins such as osteopontin. Mineral matrix consists of small crystals of hydroxyapatite, calcium phosphate and mineral calcium salts, which give the bones the right mechanical properties and stiffness. Up to 70 % of inorganic salts are arranged in the hydroxyapatite lattice structure, within cortical bone. The bone structure, thus the necessary components of the bone, must be stored in such a form that it can be easily removed and replaced by cellular control depending on the organism's requirements, while the mineral component must remain insoluble. Bone can respond to load, including stress, by remodeling, so its internal organization can vary considerably, even if the general shape is controlled and given genetically (Anatomy and radiography, 2022; Henry & Bordoni, 2022; Johnson et al., 2015; Sobezek et al., 2009).

TYPES OF BONE TISSUE

Cortical and cancellous bone, i.e., trabecular, or spongy, which are synonyms for cancellous bone, are classifications of two types of bone that can be distinguished at the macroscopic level. Strength, stiffness, and movement are ensured by compact cortical bone tissue. Trabecular bone tissue is soft, flexible, while providing a reduction in total bone mass and cortical bone support (Koju et al., 2022). The microscopic structural unit of compact bone is called an osteon or Haversian system. Each osteon is composed of concentric rings of calcified matrix called lamellae. A central canal or Haversian canal runs through the center of each osteon, which contains blood vessels, nerves, and lymphatic vessels. The structure of trabecular bone makes its surface area per unit volume larger, but more labile, while the rate of metabolic activity is also higher, compared to cortical bone, which has a higher true volumetric density. Cancellous bone is found throughout the bird’s skeleton as thin, intersecting lamellae within cortical bone. In adults, it is filled with fatty bone marrow, or forms air-filled cavities, while in young, growing individuals, it is filled with red bone marrow (Anatomy and radiography, 2022; Caon, 2018; Prince & Draper, 2000). Another type of special endosteal tissue is non-structural medullary bone, which is naturally formed under the influence of estrogenic and androgenic hormones in the hematopoietic medullary cavities of bones only in female birds during egg-laying, i.e., in the reproductive phase, and serves as a calcium reservoir that can be quickly mobilized for the formation of eggshells (Prondvai, 2017). This highly vascularized bone tissue closely resembles cancellous, or embryonic bone, while lacking the Haversian system. It is formed as a trabecular meshwork, when individual spicules grow from the endosteal surface of long bones, which are connected to each other. Also due to its function, it is characterized by containing less collagen compared to cortical and trabecular bone (Canoville et al., 2020).

BONE REMODELING AND CELLS INVOLVED IN THIS PROCESS

Bone, as a living tissue, is not inert and undergoes constant change during life. This process of change, which continues with varying intensity throughout life, is known as remodeling. It includes the bone formation and destruction, i.e., the resorption of old or damaged bone, all depending on the degree of mechanical load, which leads either to the strengthening of the bone architecture or to the weakening of the bone layers, while anabolic and catabolic processes are usually balanced. It therefore protects the structural integrity of the bone system, while also helping to maintain the balance of calcium and phosphorus. In the case of adults, there is a loss of bone mineral density due to the predominance of destructive processes of the skeleton. In remodeling, two primary cells are mainly important, which are...
osteoblasts and osteocytes, but osteocytes are also significantly involved (Rowe et al., 2022; Wawrzyniak & Balawender, 2022; Zhou et al., 2008). These cells originate from the mesenchymal and hematopoietic lineage of stem cells, while this fact emphasizes both the very close relationship between bone and the immune system and the unique regulation of bone homeostasis (Bordoni et al., 2013). Two types of ossification take place during the bone formation process. Within both types, mesenchymal progenitors condense and initiate evolutionary schemes, including chondrogenesis and osteoblastogenesis. The first type is endochondral ossification, where the cartilage growth plate is gradually replaced by bone. This cartilage growth plate was formed by chondrocytes that differentiated from mesenchymal cells. The second type is intramembranous ossification, which lacks the cartilage intermediate and in which bone organization occurs by direct differentiation of mesenchymal stem cells into osteoblasts. The balance between osteoblasts, which are responsible for bone formation, and osteoclasts, which resorb bone, maintains bone integrity and function (Shahi et al., 2017; Chen et al., 2012; Zhang, 2010).

In the case of physiological conditions, the basic and primary role of osteoclasts is the formation of new bone, both in the developing skeleton and in the remodeling process by synthesizing and then depositing organic bone matrix proteins, which further mineralize. These proteins, e.g., collagen type I, osteocalcin, bone sialoprotein and osteopontin. Osteoblast precursors are two lineages of embryonic mesenchymal stem cell populations. These initially differentiate into osteoprogenitor cells in a process that requires the action of transcription factors. One such that has been identified as essential for their differentiation is Runx-related transcription factor 2 (Runx2) of the Runx family of transcription factors, osteocalcin (OC), osteopontin (OPN), collagen type I, and osteoprotegerin (OPG). This makes osteocytes cells that are involved in many prosthetic-related processes. They are also a significant source of receptor activator of nuclear factor-xB ligand (RANKL), osteoprotegerin (OPG) and macrophage colony-stimulating factor (M-CSF), which regulate the differentiation of osteoclasts (Henry & Bordoni, 2022; Maeda et al., 2022; Ottewell, 2016; Soltanoff et al., 2009). Up to 95% of bone tissue is made up of a cell type that is derived from the osteoblast cell line, osteocytes, where the mechanisms that cause only some osteoblasts to differentiate into osteocytes are not fully understood. From the bodies of these cells, which are located in an ellipsoidal space called lacuna, distinct dendritic processes emerge and connect with each other, as well as with other cells on the surface of the bone and with nearby blood vessels. Dendritic processes reside in canaliculi, which are small cylindrical canals. According to some authors, osteocytes play a key role in the regulation of the dynamic nature of bone. Osteocytes are also the source of the RANKL, when it was proven through experiments in mice that those lacking the RANKL gene had significantly increased bone mass. They also produce a number of other proteins such as dentin matrix protein 1, phosphate-regulating neutral endopeptidase on chromosome X (PHEX), matrix metalloproteinase-2, small integrin-binding ligands, N-linked glycoproteins, proteoglycans, and γ-carboxyglutamate-containing proteins. These are the four groups into which NPCs can be divided (Lin et al., 2020). The distribution of NPCs may vary among different authors, for example Carvalho et al. (2021) divides these proteins into two large groups, namely glycoproteins and γ-carboxyglutamate-containing proteins. Therefore, the inclusion of individual proteins within individual types may overlap across authors.

Non-collagenous proteins

Effect on bone modeling and bone geometry, bone matrix mineralization and significant structural roles in bones. All of this is a list of important properties that NPCs possess. By regulating the activity of bone cells, NPCs influence the geometry of bones, but also their microstructure. NPCs are necessary for bone strength, i.e., resistance to fractures, when by removing these proteins from the bone matrix causes changes in cortical and trabecular bone that affect both bone diameter and thickness. Various conceptual models also assume that the arrangement of NPCs in the bone matrix makes these proteins into structural elements that act at the collagen-mineral interface, as a kind of intermediate member to increase toughness, thereby determining the mechanical properties of bone. NPCs also control cell-matrix interactions, the formation of collagen fibrils, and hydroxyapatite crystallites (Bekgame et al., 2019; Morgan et al., 2015).

TYPES OF NON-COLLAGENOUS PROTEINS

Small integrin-binding ligands N-linked glycoproteins (SIBLINGs), glycoproteins, proteoglycans, and γ-carboxyglutamate-containing proteins. These are the four groups into which NPCs can be divided (Lin et al., 2020). The distribution of NPCs may vary among different authors, for example Carvalho et al. (2021) divides these proteins into two large groups, namely glycoproteins and γ-carboxyglutamate-containing proteins. Therefore, the inclusion of individual proteins within individual types may overlap across authors.

Small integrin-binding ligands N-linked glycoproteins

The family of SIBLINGs includes five important proteins. They are osteopontin (OPN), bone sialoprotein (BSP), dentin sialophosphoprotein (DSP), dentin matrix protein 1 (DMP1) and matrix extracellular phosphoglycoprotein (MEPE). The designation SIBLING do not refer to their identical functional activity, but to affect the strength and flexibility of the bones, thereby increasing the risk of fractures. It is a family of five identically oriented tandem genes located on chromosome 4 in poultry. They appear to be poorly conserved, by comparison with each other themselves at the amino acid level. Functionally, they play an important role in mineralization by controlling hydroxyapatite mineralization and crystal growth. These proteins are secreted and soluble, containing integrin-binding ligands, primarily located in bone and dentin. They can also be modulators of cell adhesion (Dab et al., 2022; Staines et al., 2012; Bellahcène et al., 2008; Gallas gullus Ensembl genome browser, n.d.).

The phosphoprotein OPN was first described in 1979 by Songer and later precisely named. It was identified in bone matrix. It is a high abundance protein in all body fluids and is produced by various types of cells
and tissues, accounts for about 2% of non-collagenous bone in the bone marrow. It has approximately 300 amino acid residues and is encoded by the SPP1 gene. In the central part of this protein is an integrin-binding glycome-arginine-glycome-aspartic acid (RGD) sequence that is highly conserved in all vertebrates. It is the binding site and the two heparin binding domains. Through these membrane surface receptors that are highly correlated with many physiological and pathological processes are CD44 (hyaluronic acid receptor) and integrins (αβ1, αβ3, αβ5, αβ6, αβ11, β1β7, αβ51, αβ91). These processes are various immune reactions, biomineralization, inflammation, wound healing, fibrosis, cell migration and metastasis. The signal transduction of osteoblasts is essential in the development of many bone diseases, such as osteoporosis, rheumatoid arthritis, and osteosarcoma (Kitamura, 2021; Si et al., 2020; Mazzali et al., 2002; O’Rengan & Berman, 2000).

Another protein from the SIBLINGs family that is highly expressed by osteoblasts, osteosarcoma, and hypertrophic chondrocytes in the growth plate is BSP. In poultry, the gene encoding this protein (IBSP) is located between the DMP1 and MEPE genes and compared to OPN, its gene expression is more limited. Beyond the skeleton, it can also be expressed in tooth odontoblasts, cementoblasts, placental trophoblasts and strongly upregulated in many malignant tumors. Its exact role is not entirely clear, however, in in vitro experiments, it stimulates the formation of hydroxyapatite and enables interactions between cells through an integrin binding site. BSP may be a potential marker of bone turnover due to the fact, that small amounts of it are found in the circulation. This could help in the early detection of various bone disorders, as well as bone metastases (Boutet et al., 2015; McKee & Cole, 2012; Creemers et al., 2008).

DMP1 is a member of the TRAP family, which is encoded by a large mRNA transcript, where dentin sialoprotein (DSP) and dentin phosphophorytin (DPP) are expressed as that one a single mRNA transcript. These proteins are not unique to teeth, but DSPP has recently been found to be present in osteoblasts and bone, and when comparing teeth versus bone, there are very different regulatory mechanisms controlling DMP1 and DPP (Lu et al., 2004). A specific highly phosphorylated protein, originally identified from bone dentin, is DMP1, which is essential for both the proper biomineralization of cementum, dentin, and enamel, as well as bone and cartilage. As part of various chemical analyzes of proteins, it was found that DMP1, as a precursor, was cleaved into two fragments of its length, namely C-terminal and N-terminal fragments. It is also a molecule that, by transcription in the nucleus, initiates the differentiation of osteoblasts and, in the later stages of their maturation, extracellularly organizes the formation of a mineralized matrix. Also, the research of various mutations within human medicine led to the discovery of a new disease: autosomal recessive hyperphosphatemic osteomalacia (OHIO) (Lu et al., 2004; Nampei et al., 2003).

MEPE, as one of the phosphoglycoproteins involved in bone mineralization, is primarily expressed in osteocytes of adult bone, and in osteoblasts in vitro experiments during mineralization. In rodent experiments where this gene has been knocked out, MEPE can be considered a regulator of bone metabolism, primarily inhibiting bone formation. Originally, this gene was identified for high expression in tumors that cause oncogenic hydroxyphosphatemic osteomalacia (OHIO) (Qi et al., 2007; Kim et al., 2006; Narayanam et al., 2003).

Glycoproteins

Another group of NCs includes glycoproteins, which on the protein chain contain covalently attached carbohydrate molecules in various combinations and positions. This includes, for example, alkaline phosphatase (ALP), osteonectin, thrombospondins (TSPs) and fibronectin (FN), which are formed at various stages of osteoblast mineralization. They are immediately involved in several processes, which are cell-matrix interaction, cell proliferation, hydroxyapatite deposition (Lin et al., 2020; Robey, 2002).

To a group of glycoproteins that bind to the plasma membrane, specifically the surface of the cell membrane of osteoblasts by glycosylphosphatidylinositol includes the ectoenzyme ALP. In part, bone ALP is released into the circulation, in several isoforms that show the same enzymatic activity, only the content of carbohydrates and asac charid acid is different. The isoform found in normal bone is B1, while in bone expression (Qin et al., 2003; Zhang et al., 2003), B2, have different enzymatic activity within different types of bone tissue, with cancellous bone having higher B1 activity, while the total activity (B1 and B2) is lower, and the opposite within trabecular bone, with higher B2 activity bone ALP. Through the hydrolysis of inorganic pyrophosphate, which plays the role of a natural inhibitor of bone mineralization, biological functions of these proteins (SLRP) are important. In order for these secreted extracellular proteins to regulate normal as well as pathological cell behavior, these secreted extracellular proteins interact with cell surface receptors and cytokines. Important bone proteoglycans include, for example, biglycan (BGN), decorin (DCN), keratocan (KTN), osteoadherin (OSAD), osteolin/mincan (OGN), fibromodulin (FMOD), osteonectin (OCN), osteopontin (OPN), osteoactivin (OAR), and anitumor A minilucan (LUM). If there was a loss of SLRP due to unregulated proteolysis, or even a change in their expression profile, a whole range of bone diseases could
occur due to their involvement in bone morphogenesis and homeostasis (Svorina et al., 2023; Carvalho et al., 2021; Chen et al., 2021; Cohonous-Thomas et al., 2015).

DCN and BGN are very similar SLRPs, which are similarly located extracellularly and fall into class I. Their GAG chain is made of chondroitin sulfate, or dermatan sulfate, where DCN has one and BGN has two side chains. Early bone matrix deposition initiates DCN expression, while during cell proliferation and mineralization BGN expression occurs and is paused during bone matrix deposition. Both proteoglycans share a common binding site for collagen type I and DCN binds collagen type II with much higher affinity for collagen type I. However, in case DCN is absent, BGN functionally compensates for its overexpression. Furthermore, DCN binds collagen type II and III. It is not entirely clear whether, in the case of changes in the mechanical properties and organization of collagen, it is an overexpression of BGN, the absence of DCN, or certain conditions that lead to the switching pattern of BGN and DCN is encoded by the DCN gene located on chromosome 1 in poultry, while the BGN gene for BGN is located on chromosome 13 (Appunni et al., 2019; Robinson et al., 2017; Zanotti et al., 2005; Gallus gallus Ensemble genome browser, n.d.).

**Gamma-carboxyglutamate-containing proteins**

An important group of NCPs present in serum, dentin, bone matrix and many calcified tissues are γ-carboxyglutamic acid (Gla)-containing proteins. It is an acid product of a specific vitamin K-dependent carboxylation of glutamic acid residues (Ecarboxylation), while its affinity for bone is reduced. Some of these proteins are known in several species that differ in the intensity of carboxylation, one of them is K, while the proteins that contain Gla in bone are osteocalcin (OCN), matrix Gla protein (MGP) and perinastin (POSTN) (Lin et al., 2020).

The bone γ-carboxyglutamic acid protein (BGLAP), OCN, is a factor that is expressed and secreted by osteoblasts. As soon as OCN, as a mature peptide, undergoes several splicing events and subsequent γ-carboxylation on three residues, the result is a peptide that has a high affinity for bone and the extracellular matrix, but then decarboxylation occurs again due to the low pH inside the osteoclast resorption compartments. In this way, uncarboxylated OCN enters the circulation, while its affinity for bone is reduced. Previous studies looking at various osteoblastic cell lines have shown that this bone metabolism, in terms of mineralization, total bone density and as an inhibitor of bone mass, is less than expected, or no effect at all. It has also been tested as a factor that improves glucose metabolism, maintains muscle mass, and indicates testosterone synthesis in the testes, while more recent studies have not confirmed these roles and its effect on the crystallographic orientation of the c-axis of biological apatite (B/αP) has been demonstrated. This axis is normally parallel to the collagen fibrils, but in the case of the OCN knockout rodents, this axis was severely disrupted, leading to compromised bone strength (Komori, 2020; Morishti et al., 2020; Moser & van der Eerden, 2019). From the point of view of protein domains, or even the organization of genes, OCN is structurally very similar to MGP, from which it most likely arose by duplication of a tandem gene. Despite this similarity, both proteins followed different evolutionary strategies to gain different functions. MGP synthesis occurs in both bone and many mesenchymal cells. It is also highly represented by chondrocytes and vascular smooth muscle cells. This calcification inhibitor is known in several species that differ in the intensity of carboxylation and phosphorylation. OCN is encoded by the BGLAP gene, also known as OC, BGP, or OCN, and is located on chromosome 25 in poultry. MGP is encoded by the MGP gene and is located on chromosome 1 (Bjorklund et al., 2020; Cancela et al., 2014; Gallus gallus Ensemble genome browser, n.d.).

POSTN is another osteoblast-specific protein that is expressed in bone, but also in other collagen-rich tissues, such as heart valves, tendons, and some tumors, and plays a role in the regulation of bone formation (Naylor & Eastell, 2015).

**WNT AND RANK/RANKL/OPG SIGNALING PATHWAYS**

The importance of the Wnt and RANK/RANKL/OPG signaling pathways in bone metabolism has already been demonstrated several times. Disregulation of these pathways leads to many bone disorders due to their important role in bone cell differentiation and the processes involved (Zhu et al., 2021; Wang et al., 2020). Wnt signaling pathway and selected proteins involved in Wnt signaling

In the 1980s, one of the evolutionarily highly conserved pathways was discovered, which plays an important role in many biological processes, regulates many aspects of cell fate, and is critical in adult tissue homeostasis and many functions during embryonic development, including bone and cartilage formation. It is a Wnt signal pathway, which includes a family of proteins, while the main signaling branches downstream of the Frizzled receptor (FZD) have so far been discovered, which includes canonical and non-canonical signaling. The canonical pathway, or the Wnt/β-catenin dependent pathway, is mediated by β-catenin, which in the absence of Wnt stimulation is phosphorylated, then ubiquitinated and rapidly degraded through the proteasomal system to prevent cytoplasmic accumulation. Conversely, when Wnt is stimulated, cytoplasmic β-catenin accumulates. Expression of target genes occurs after the translocation of accumulated β-catenin into the nucleus. The non-canonical signaling pathway, which is independent of β-catenin, is further divided into a pathway regulating planar cell polarity (Wnt/PCP) and a Wnt/Ca++ pathway influencing the level of Ca++ in the cytoplasm. Activation of the canonical or non-canonical pathways occurs through specific interactions with co-receptors. In the case of activation of the canonical pathway, it is a combination of Wnt1 and Wnt3a ligands, the FZD and the co-receptors low density lipoprotein-related receptors 5 and 6 (LRP5/6). Inhibition of the pathways is ensured by binding to a specific region of the coreceptor, which is mediated by proteins of the Frizzled related family of proteins (FRFs, DKKs). This family was first described by Zhang et al. (2019; Houchy et al., 2019; Kim et al., 2013; Kobayashi et al., 2008; Komiyi & Habas, 2008). The Wnt family can be divided into two large groups. The first group, Wnt1, includes Wnt1, Wnt2, Wnt2b, Wnt3, Wnt3a, Wnt7a, Wnt8b, Wnt10a. These participate in the canonical signaling pathway. The non-canonical signaling pathway is activated by the Wnt5 category, which includes Wnt4, Wnt5a and Wnt11 (He et al., 2015).

Within the components of the Wnt signaling pathway, it was found, that the protein WISP-1 (WNT1-inducible-signaling pathway 1), encoded by the WISP1 gene, also known as CCN4, located on chromosome 2 in poultry, is a new target for modulating osteogenesis and improving bone strength (Ferrand et al., 2017; Gallus gallus Ensemble genome browser, n.d.). The structure of this protein consists of an amino-terminal secretory signal peptide that is followed by four structural domains. Absences of different domains, in certain variants of CCN proteins, play distinct biological roles, while also being involved in various pathologies. The protein is expressed in many places in the body, be it the lungs, heart, brain, kidneys, or muscle, in immature osteoblasts and cells of the perichondral mesenchyme during embryonic development. Cell death of osteoblasts or their precursors, impaired bone repair, blockage of cell proliferation, progressive spinal cord injury and many others can be caused by loss of Wnt1 signaling (Gurbuz & Chiquet-Ehrismann, 2015; Maiase, 2014; Ono et al., 2011; French, 2004). Various genetic experiments in mice show that after knocking out this gene, the test subjects have lower total bone volume and cortical bone thickness than wild-type mice. On the other hand, increased mineral density, total bone volume and cortical bone thickness were observed in test subjects with overexpressed WISP1 (Wang et al., 2018).

The high homologous protein Wnt10a plays a key role in canonical Wnt signaling as coreceptors. They are involved in skeletal remodeling, so mutations in the genes encoding these proteins are associated with a number of diseases, such as osteoporosis, but also cancer and metabolic disorders. In poultry, the genes encoding these proteins are located on different chromosomes, in the case of LRP5 on chromosome 5 and LRP6 on chromosome 1. Structurally, they contain large extracellular domains including four β-propeller motifs. These are followed by three low-density lipoprotein (LDL) type 1 ligand binding domains (Kang & Robling, 2015; Joiner et al., 2013; MacDonald et al., 2011; Gallus gallus Ensemble genome browser, n.d.).

One of the modulators of the endogenous secreted pathway, which is part of the canonical Wnt signaling pathway, is DKK1. They inhibit the canonical pathway by binding to the LRP5 and LRP6, which also regulates bone mass (Ueland et al., 2017). DKKs are a family of soluble LRP5/6 antagonists, where four DKK genes (1-4) have been described in human studies, with DKK1 being the most studied. The family of these proteins shows little sequence similarity, with only two domains highly conserved among individual members. The first is an N-terminal cysteine-rich (Cys1) domain that modulates the interactions of the second domain. That is the C-terminal cysteine-rich domain (Cys2), and this domain inhibits Wnt through its bindings (Giral et al., 2021). When DKK1 is forced to be overexpressed in osteoblasts, the result is osteopenia. Also, activation of DKK1 in osteoblasts can be the cause of osteoporosis, while DKK1 inhibits fracture repair and participates in erosive arthritis, so strengthening Wnt/β-catenin signaling or neutralizing DKK1 could help in the treatment of bone pathologies (Pinzone et al., 2009). The SOST gene, located in poultry on chromosome 27, expresses a small protein that is exclusively found in osteocytes within bone cells. It is a SOST that is a potent inhibitor of bone formation and was originally included in the TN family of BMP antagonists but was later shown to bind LRP5/6 with high affinity. After binding to osteoblast receptors, the intracellular signaling cascade is activated, with the final result being the inhibition of osteoblastic bone formation. In human studies, mutations in the SOST gene locus are associated with rare skeletal disorders characterized by bone overgrowth, osteosclerosis, and Van Buchem disease. Structure of SOST forms a cysteine knot with three cysteines that are flanked by highly flexible N- and C-terminal domains (Kim et al., 2022; Sebastian & Loots, 2017; Lewiecki, 2014; Robling et al., 2008).

The evolutionarily conserved protein GPR177 (G protein-coupled receptor 177/Wntless) is necessary for the secretion of Wnt ligands, which is encoded by the GPR177 (GPR177) gene located on chromosome 8 and is involved in osteoblastic stem cell family of membrane receptors that can activate heterotrimeric G proteins, which consist of α, β, and γ subunits, control cell behavior. Also, most of these proteins
are N-glycosylated, while it is not completely known whether this glycosylation is necessary for the interaction within Wnt signaling. GPR177 is involved in a variety of processes such as mammalian gland morphogenesis. Also, deletion of GPR177 within mature osteoblasts completely disrupted postnatal bone homeostasis (Du et al., 2019; Zhang et al., 2015; Das et al., 2012; Jin et al., 2010; Gallus gallas Ensembl genome browser, n.d.). Through the Wnt/β-catenin signaling pathway, osteogenic differentiation is activated, with the help of the important regulatory factor Runx2. This member of the Runt domain family of transcription factors controls osteoblast development and differentiation into osteocytes by regulating the transcription of many genes. Although it plays an important role in bone cell development and gene transcription, its role in gene expression and new bone formation is generally insufficient, whereas changes in expression levels are associated with skeletal disease. Many bone cancers are caused by its overexpression, while cleucdyran C-terminal oligosaccharides of this protein are not fully explored and understood, which can also be said about the genetic architecture of important bone features and the roles of some proteins within bone metabolism, while their targeted study may reveal new knowledge that can help to understand the processes that lead to skeletal disease. The result of this review is a summary of the knowledge of selected important proteins that are part of compact bone or are involved in signaling pathways that relate to bone metabolism.

**REFERENCES**


