The Role of the Extracellular Matrix in Skeletal Development

S. G. Velleman

Department of Animal Sciences, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio 44691

ABSTRACT The extracellular matrix of cartilage and bone is composed mostly of collagen with lesser amounts of other constituents such as proteoglycans. The focus of this brief review will be on the dynamic expression of collagens and proteoglycans in the cartilage and bone extracellular matrices. Recent research has shown the presence of different collagen types and proteoglycans that are differentially expressed in cartilage, in the transition from cartilage to bone, and in the bone extracellular matrices. These findings suggest the complexity of the skeletal extracellular matrix as well as its dynamic expression. Although the composition of both the cartilage and bone extracellular matrices are largely known, the function of each of the macromolecules composing these matrices and their developmental regulation is not well understood. Defects that modify the extracellular matrix, like the chicken chondrodysplasia, nanomelia, and tibial dyschondroplasia, have profound effects on skeletal structure. The poultry industry is currently confronting a high percentage of skeletal deformities due to selection for increased growth rate and needs to consider the effect of extracellular matrix modifications and how to maintain extracellular matrix integrity.

(Key words: collagen, proteoglycans, aggrecan, cartilage, bone)

INTRODUCTION

The importance of the extracellular matrix in skeletal development has been overlooked until the recent development of improved extraction procedures and recombinant DNA technology. Prior to these methodological advances, the extracellular matrix was described as a structural scaffold containing a proteinaceous fiber component (collagen) and an amorphous ground substance (proteoglycan). This definition implied that the extracellular matrix was a static structure with limited ability to influence tissue structure, function, development, or gene expression. It is now known that the extracellular matrix is not a static structure but a dynamic network of molecules secreted by cells. Different cell types will secrete unique matrices that change with developmental age. In addition to forming a complex architecture around the cell, extracellular matrix molecules regulate cell behavior by modulating cell proliferation and differentiation, response to growth factors, and signal transduction pathways.

The development of bone is linked to cartilage formation in that bone is formed from a cartilage template or anlage. The extracellular matrices of cartilage and bone are unique, which is a reflection of the distinct functional requirements of the tissues. Although their matrices are different, both have a predominant fibril-forming collagen matrix. Unlike bone, cartilage contains a large proteoglycan, aggrecan, the function of which is to distribute load and allow for the resilience of cartilage. The bone matrix consists of a high degree of mineralization that is necessary for the formation of a rigid functional structure. This review focuses on the proteoglycan and collagen extracellular matrix components.

THE CARTILAGE EXTRACELLULAR MATRIX PROTEOGLYCAN COMPONENT

Proteoglycans are macromolecules composed of a central core protein with covalently attached carbohydrates called glycosaminoglycans. Figure 1 represents a generalized schematic of a proteoglycan macromolecule. The core protein varies greatly in size from approximately 40,000 to greater than 350,000 Da (Iozzo and Murdoch, 1996). The glycosaminoglycans are polymers of disaccharide repeats that are highly sulfated and are, therefore, negative in charge. The negative charge permits ionic interactions with molecules like water. This property is of particular importance in cartilage formation, structure,
and function. Typical glycosaminoglycans attached to the proteoglycan central core protein include: chondroitin/dermatan sulfate, heparan sulfate, and keratan sulfate. Chondroitin sulfate is composed of repeats of glucuronic acid and N-acetylglucosamine with sulfate groups in the 4- or 6-position of the amino sugar. Heparan sulfate consists of glucuronic acid and N-acetylglucosamine. Keratan sulfate contains disaccharide repeats of galactose and N-acetylglucosamine with the sulfate at the 6-position of the amino sugar. Hyaluronic acid is an unsulfated glycosaminoglycan of glucuronic acid and N-acetylglucosamine that ionically interacts with only certain proteoglycan core proteins (for review see Carrino et al., 1999).

Because of the diversity of the extracellular matrix proteoglycan family, proteoglycans may be divided into two major classes: aggregating and nonaggregating interstitial proteoglycans. In cartilage, the predominant proteoglycan is the aggregating proteoglycan, aggrecan. Aggregating proteoglycans, such as aggrecan, have a specialized hyaluronic acid binding domain at the N-terminus of the molecule that ionically interacts with hyaluronic acid. The interaction between the hyaluronic acid binding-region and hyaluronic acid is stabilized by a glycoprotein called link protein (Wight et al., 1991). The proteoglycan aggregate structure, composed of the proteoglycan core protein, hyaluronic acid, and link protein, is extremely stable (Wight et al., 1991). This aggregate-forming proteoglycan structure binds numerous proteoglycans to hyaluronic acid and in cartilage provides a stable source of attracting water molecules to hydrate the cartilage matrix.

Aggrecan contains chondroitin and keratan sulfate glycosaminoglycan side chains as well as asparagine-linked oligosaccharides and O-linked oligosaccharides. The aggrecan core protein has a molecular weight of over 200,000 Da and when aggregated with hyaluronic acid has a total molecular weight of greater than 2 × 10^6 Da. The high charge density of the aggrecan chondroitin and keratan sulfate chains is directly responsible for the hydration of the cartilage extracellular matrix and provides mechanical strength to the cartilage. Therefore, if the structure of the aggrecan proteoglycan is modified, the functional integrity of the cartilage extracellular matrix is altered.
FIGURE 2. Autoradiograph of a denaturing gradient gel blot of genomic DNA isolated from a nanomelia family. The genomic DNA was digested with HaeIII and was loaded as follows: Lane 1, dam; Lane 2, sire; Lane 3, nanomelia; Lane 4, nanomelia; Lane 5, nanomelia; Lane 6, normal; Lane 7, normal; and Lane 8, normal. (Reprinted from Velleman and Clark, 1992, Matrix 12:66–72, with permission from Elsevier Science).

ing core protein biosynthesis. Li et al. (1993) showed that the nanomelia mutation is a one base change from a G to T transversion, which converts the codon GAA for glutamate at amino acid 1513 to TAA, a stop codon. The shortened core protein transcript is mostly degraded, which results in a 94% decrease in detectable aggrecan core protein levels (Stirpe et al., 1987).

THE EXTRACELLULAR MATRIX COLLAGEN COMPONENT

Collagens are the major constituent of all extracellular matrices. Currently, 19 different vertebrate collagens, Types I through XIX, have been described. These collagens have tissue-specific distributions, developmentally defined temporal and spatial distributions, and unique functional properties. The collagens can be subdivided into the following classes based on function or size: fibrillar, fibril associated, network forming, filamentous, short chain, and long chain (van der Rest and Garrone, 1991). Cartilage contains fibrillar Type II collagen, whereas bone is composed of fibrillar Type I collagen. The fibrillar collagens, such as Types I and II, are found in tissues subject to compression and tensile stress. These collagens form a fibrillar network in the extracellular matrix, imparting strength to the tissue to withstand compressive and tensile stresses.

All collagens are characterized by a triple helix of α-chains that vary from 600 to 3,000 amino acids (for review see Brown and Timpl, 1995). The α-chains contain repeats of the amino acid sequence Gly-X-Y, where X and Y can be any amino acid. Frequently, X is proline and Y is hydroxyproline. Appropriate folding of the collagen triple helix requires a glycine at every third amino acid residue. The α-chains often have nontriple helical regions that interpace the triple helical domains, which increases the functional diversity of the collagen family of macromolecules.

After the collagen molecules are synthesized, they are secreted from the cell into the extracellular matrix and align into a quarter-stagger array. At this point, the collagen forms a fibrillar network that is stabilized by crosslinks between collagen fibrils. Once collagen crosslinking is initiated between individual fibrils, larger diameter fibrils will form. Collagen crosslinking continues with development, and collagen fiber size increases (Reiser et al., 1992).

The process of crosslinking involves the oxidative deamination of specific lysine and hydroxylysine residues by the enzyme lysyl oxidase, creating lysine or hydroxylysine aldehydes. Covalent collagen crosslinks can also arise from the nonenzymatic condensation of specific lysine and hydroxylysine residues. For example, the hydroxylysylpyridinoline crosslink is a mature, nonreducible, trivalent crosslink that results from the condensation of two reducible, divalent, keto-imine crosslinks (Reiser et al., 1992). Crosslinking between and among collagen molecules is a major element in stabilizing fibrillar collagens and modulating the tensile strength properties of the collagen fibril network.

THE TRANSITION FROM A CARTILAGE TO BONE EXTRACELLULAR MATRIX

The cartilage extracellular matrix is mainly composed of proteoglycan aggrecan and Type II collagen. However, other extracellular matrix macromolecules, such as the leucine-rich proteoglycans, fibromodulin, fibronectin, and cartilage matrix protein, are located in cartilage. Many of the functional properties of the cartilage matrix are attributed to the interaction of Type II collagen with aggrecan. The proteoglycan matrix is mechanically weak but has a high negative charge density that leads to an ionic interaction with water, resulting in an osmotic swelling of the tissue. The swelling of the proteoglycan component is restricted by the Type II collagen fiber network. The Type II collagen fibers resist the proteoglycan-mediated swelling that results in increased collagen fiber tension and enables the cartilage to support load-bearing
stress. Bone requires a mineralized rigid matrix that is able to support weight, unlike the cartilage matrix that largely cushions compressive force. Therefore, a developmental transition from a cartilage matrix to one specific for bone function must occur. During this transition from a cartilage to a bone matrix, the chondrocytes in the central portion of the developing bone enlarge and begin to produce a unique noncartilaginous extracellular matrix. The cells are now termed hypertrophic chondrocytes and secrete a matrix containing Type X collagen, osteopontin, and alkaline phosphatase (Schmid and Linsenmeyer, 1985; Chen and Linsenmeyer, 1993; Knopov et al., 1995). During hypertrophy, there is an increase in mineral deposition and the formation of vascular channels. Eventually the cartilage matrix and hypertrophic chondrocytes are completely degraded and replaced by bone.

As the center of the cartilage anlage is converted to bone, the ossification front spreads in both directions from the center. To permit continued growth of the long bones, the chondrocytes near the ossification front proliferate prior to undergoing hypertrophy. This process provides new cartilage for additional bone growth. The cartilaginous region at the end of the long bones is termed the epiphyseal growth plate, which contains a region of proliferating chondrocytes, differentiated chondrocytes, and hypertrophying chondrocytes. The transition from proliferating chondrocytes to hypertrophying chondrocytes requires the precise regulated expression of genes specific to each stage. Therefore, if this developmental progression is modified, the formation of bone will be impaired.

**INvolvement of the Extracellular Matrix in Tibial Dyschondroplasia**

Tibial dyschondroplasia (TD) is a skeletal disease common to rapidly growing commercial poultry stocks. The disease is caused by the formation of an avascular cartilaginous lesion extending from the epiphyseal growth plate into the bone metaphysis (Leach and Nesheim, 1965). Birds afflicted with TD exhibit lameness and deformed bones. Furthermore, birds with advanced lesions are prone to fractures during handling at the processing plant.

The TD lesion is characterized by prehypertrophic chondrocytes that do not mature into hypertrophic chondrocytes. Because the cellular transition of chondrocytes is modified in TD, one needs to consider the impact on the expression of extracellular matrix macromolecules and architecture. Type X collagen is exclusively expressed by hypertrophic chondrocytes and is an integral extracellular matrix macromolecule in the process of mineralization (Schmid and Linsenmeyer, 1987). In the TD lesion, Type X collagen is significantly reduced (Bashey et al., 1989). Although protein levels of Type X collagen are decreased, TD chondrocytes synthesize elevated levels of Type X collagen mRNA (Reginato et al., 1998). Interestingly, the synthesized Type X collagen is primarily intracellular and is not secreted into the extracellular space (Tselepis et al., 1996). The expression of Types I and II collagen are also modified in TD chondrocytes (Wardale and Duance, 1996).

Although the amount of collagen present is important in considering extracellular matrix architecture, the organization of homotypic and heterotypic collagen fibrils must also be evaluated. Many of the functional properties of collagen can be attributed to the amount of crosslinking within a fibril and between fibrils and the types of collagen composing the fibril network. Levels of the mature collagen crosslink, hydroxylysylpyridinoline, are low in the normal growth plate, whereas in TD levels are six times higher (Wardale and Duance, 1996). Orth et al. (1996) found in TD cartilage high levels of the lysylpyridinoline crosslink in the Type X collagen, whereas Type II collagen contained primarily the hydroxylysylpyridinoline crosslink. Increased collagen crosslinking would likely decrease the resilience of the growth plate cartilage.

The data on collagen spatial localization and crosslinking suggest a different collagen fibril network organization in the TD cartilage. In normal hypertrophic cartilage, collagen Type X likely associates with the preexisting collagen fibril network and forms a heterotypic collagen fibril predominantly composed of Types II and X collagen that is stabilized by covalent collagen crosslinking. In TD, the Type X collagen is maintained intracellularly and, therefore, would not be able to interact with the existing extracellular Type II collagen network. This change in extracellular matrix architecture may result in a matrix that does not have a permissive structure to support vascularization that is necessary for bone formation.

The mechanical properties of the growth plate are further influenced by the proteoglycan aggrecan. Tselepis et al. (1996) have reported a reduction in the expression and deposition of aggrecan in TD cartilage. A decrease in aggrecan in the growth cartilage could alter the spacing of chondrocytes and the organization of the extracellular matrix. As in the example of nanomelia, this alteration would impact on the mechanical properties of cartilage, which may also be important in the progression of TD in which deformation of the long bone is apparent. The cartilage anlage is a template for the future bone. If the cartilage structure is altered, then it is likely that the defect will be further manifested in the bone.

**Why Should the Poultry Industry Be Concerned About the Extracellular Matrix?**

Proper development of the skeletal structure requires a precisely regulated expression of cellular and extracellular matrix genes. The poultry breeding industry has selected birds reared for meat production based on growth rate and related traits. Tibial dyschondroplasia, for example, is a disease of rapidly growing birds and occurs when birds are growing at their maximum rate. Each year approximately 27% of market-weight birds suffer from TD. Skeletal problems like TD were not a health or economic risk 30 yr ago.
In understanding the etiology of skeletal disorders, the extracellular matrix must be considered. The extracellular matrix modulates tissue tensile strength and cell growth properties. Both of these properties are essential for the development of a mechanically strong skeletal structure. The extracellular matrix and its role in skeletal growth has largely been ignored by the poultry industry. The poultry industry needs to develop a comprehensive understanding of the cartilage and bone extracellular matrices and breeding stock selections should encompass the impact on the expression of these macromolecules.

**REFERENCES**


