

49 The Extracellular Matrix and Connective Tissue

Many of the cells in tissues are embedded in an **extracellular matrix** that fills the spaces between cells and binds cells and tissue together. In so doing, the extracellular matrix aids in determining the shape of tissues as well as the nature of the partitioning between tissue types. In the skin, loose connective tissue beneath epithelial cell layers consists of an extracellular matrix in which fibroblasts, blood vessels, and other components are distributed (Fig 49.1). Other types of connective tissue, such as tendon and cartilage, consist largely of extracellular matrix, which is principally responsible for their structure and function. This matrix also forms the sheetlike **basal laminae**, or basement membranes, on which layers of epithelial cells rest, and which act as supportive tissue for muscle cells, adipose cells, and peripheral nerves.

Basic components of the extracellular matrix include fibrous structural proteins, such as **collagens**, **proteoglycans** containing long glycosaminoglycan chains attached to a protein backbone, and **adhesion proteins** linking components of the matrix to each other and to cells.

These **fibrous structural proteins** are composed of repeating elements that form a linear structure. **Collagens**, **elastin**, and **laminin** are the principal structural proteins of connective tissue.

Proteoglycans consist of a core protein covalently attached to many long, linear chains of **glycosaminoglycans**, which contain repeating disaccharide units. The repeating disaccharides usually contain a **hexosamine** and a **uronic acid**, and these sugars are frequently **sulfated**. Synthesis of the proteoglycans starts with the attachment of a sugar to a serine, threonine, or asparagine residue of the protein. Additional sugars, donated by **UDP-sugar precursors**, add sequentially to the nonreducing end of the molecule.

Proteoglycans, such as glycoproteins and glycolipids, are **synthesized in the endoplasmic reticulum (ER) and the Golgi complex**. The glycosaminoglycan chains of proteoglycans are **degraded by lysosomal enzymes** that cleave one sugar at a time from the nonreducing end of the chain. An inability to degrade proteoglycans leads to a set of diseases known as the **mucopolysaccharidoses**.

Adhesion proteins, such as fibronectin and laminin, are extracellular glycoproteins that contain separate distinct binding domains for proteoglycans, collagen, and fibrin. These domains allow these adhesion proteins to bind the various components of the extracellular matrix. They also contain specific binding domains for specific cell surface receptors known as the **integrins**. These integrins bind to **fibronectin** on the external surface, span the plasma membrane of cells, and adhere to proteins, which, in turn, bind to the intracellular **actin** filaments of the **cytoskeleton**. Integrins also provide a mechanism for signaling between cells via both internal signals as well as through signals generated via the extracellular matrix.

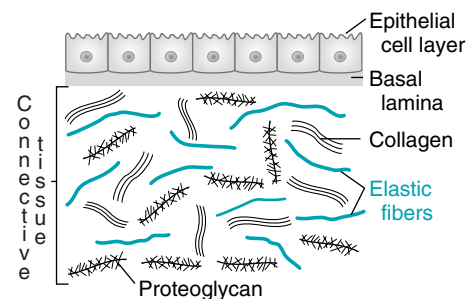


Fig. 49.1. An overview of connective tissue extracellular matrix. Supporting the epithelial cell layer is a basal lamina, beneath which are collagen, elastic fibers, and proteoglycans. The cell types present in connective tissue, such as fibroblasts and macrophages, have been removed from the diagram for clarity.

Cell movement within the extracellular matrix requires remodeling of the various components of the matrix. This is accomplished by a variety of **matrix metalloproteinases (MMPs)** and regulators of the MMPs, **tissue inhibitors of matrix metalloproteinases (TIMPs)**. Dysregulation of this delicate balance of the regulators of cell movement allows cancer cells to travel to other parts of the body (**metastasize**) as well as to spread locally to contiguous tissues.



THE WAITING ROOM



Sis Lupus (first introduced in Chapter 14) noted a moderate reduction in pain and swelling in the joints of her fingers when she was taking a 6-week course of high-dose prednisone, an anti-inflammatory steroid. As the dose of this drug was tapered to minimize its long-term side effects, however, the pain in the joints of her fingers returned, and, for the first time, her left knee became painful, swollen, and warm to the touch. Her rheumatologist described to her the underlying inflammatory tissue changes that her systemic lupus erythematosus (SLE) was causing in the joint tissues.



Ann Sulin complained of a declining appetite for food as well as severe weakness and fatigue. The reduction in her kidneys' ability to maintain normal daily total urinary net acid excretion contributed to her worsening metabolic acidosis. This plus her declining ability to excrete nitrogenous waste products, such as creatinine and urea, into her urine ("azotemia") are responsible for many of her symptoms. Her serum creatinine level was rising steadily. As it approached a level of 5 mg/dL, she developed a litany of complaints caused by the multisystem dysfunction associated with her worsening metabolic acidosis, retention of nitrogenous waste products, and so forth ("uremia"). Her physicians discussed with Ann the need to consider peritoneal dialysis or hemodialysis.

I. COMPOSITION OF THE EXTRACELLULAR MATRIX

A. Fibrous Proteins

1. COLLAGEN

Collagen, a family of fibrous proteins, is produced by a variety of cell types but principally by fibroblasts (cells found in interstitial connective tissue), muscle cells, and epithelial cells. Type I collagen [collagen(I)], the most abundant protein in mammals, is a fibrous protein that is the major component of connective tissue. It is found in the extracellular matrix (ECM) of loose connective tissue, bone, tendons, skin, blood vessels, and the cornea of the eye. Collagen(I) contains approximately 33% glycine and 21% proline and hydroxyproline. Hydroxyproline is an amino acid produced by posttranslational modification of peptidyl proline residues (see Chapter 7, section V.C., for an earlier introduction to collagen).

Procollagen(I), the precursor of collagen(I), is a triple helix composed of three polypeptide (pro- α) chains that are twisted around each other, forming a rope-like structure. Polymerization of collagen(I) molecules forms collagen fibrils, which provide great tensile strength to connective tissues (Fig. 49.2). The individual polypeptide chains each contain approximately 1,000 amino acid residues. The three polypeptide chains of the triple helix are linked by interchain hydrogen bonds.

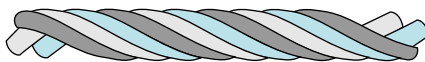


Fig. 49.2. The triple helix of collagen.

Each turn of the triple helix contains three amino acid residues, such that every third amino acid is in close contact with the other two strands in the center of the structure. Only glycine, which lacks a side chain, can fit in this position, and indeed, every third amino acid residue of collagen is glycine. Thus, collagen is a polymer of (Gly-X-Y) repeats, where Y is frequently proline or hydroxyproline, and X is any other amino acid found in collagen.

Procollagen(I) is an example of a protein that undergoes extensive posttranslational modifications. Hydroxylation reactions produce hydroxyproline residues from proline residues and hydroxylysine from lysine residues. These reactions occur after the protein has been synthesized (Fig. 49.3) and require vitamin C (ascorbic acid) as a cofactor of the enzymes, for example, prolyl hydroxylases and lysyl hydroxylase. Hydroxyproline residues are involved in hydrogen bond formation that helps to stabilize the triple helix, whereas hydroxylysine residues are the sites of attachment of disaccharide moieties (galactose-glucose).

The side chains of lysine residues also may be oxidized to form the aldehyde, allysine. These aldehyde residues produce covalent cross-links between collagen molecules (Fig. 49.4). An allysine residue on one collagen molecule reacts with the amino group of a lysine residue on another molecule, forming a covalent Schiff base that is converted to more stable covalent cross-links. Aldol condensation also may occur between two allysine residues, which forms the structure lysinonorleucine.



The role of carbohydrates in collagen structure is still controversial. The hydroxyproline residues in collagen are required for stabilization of the triple helix by hydrogen bond formation. In the absence of vitamin C (scurvy), the melting temperature of collagen can drop from 42°C to 24°C, because of the loss of inter-strand hydrogen bond formation from the lack of hydroxyproline residues.

i. Types of Collagen

At least 19 different types of collagen have been characterized (Table 49.1). Although each type of collagen is found only in particular locations in the body, more than one type may be present in the ECM at a given location. The various types of collagen can be classified as fibril-forming (types I, II, III, V, and XI), network-forming (types IV, VIII and X), those that associate with fibril surfaces (types IX, XII, and XIV), those that are transmembrane proteins (types XIII and XVII), endostatin-forming (types XV and XVIII), and those that form periodic beaded filaments (type VI).

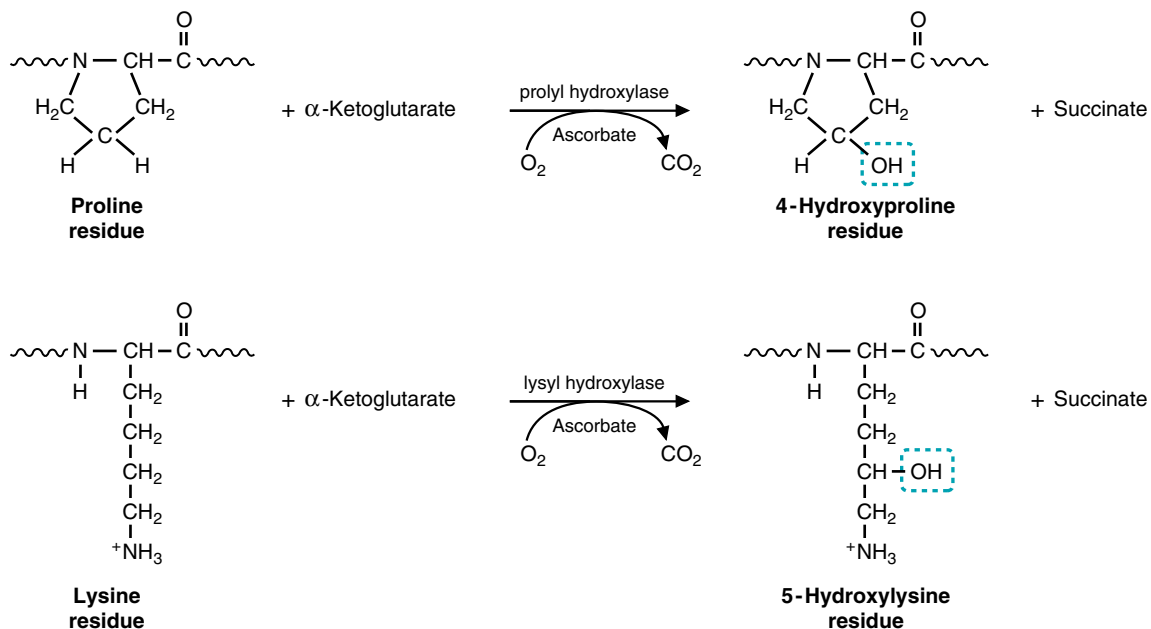


Fig. 49.3. Hydroxylation of proline and lysine residues in collagen. Proline and lysine residues within the collagen chains are hydroxylated by reactions that require vitamin C.

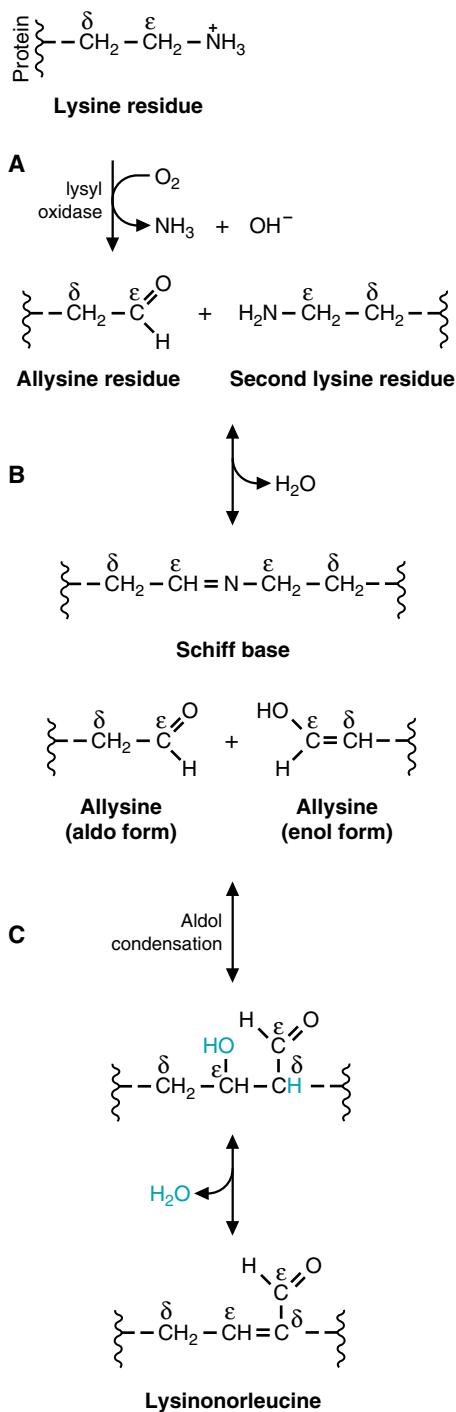


Fig. 49.4. Formation of cross-links in collagen. A. Lysine residues are oxidized to allysine (an aldehyde). Allysine may react with an unmodified lysine residue to form a Schiff base (B), or two allysine residues may undergo an aldol condensation (C).

Table 49.1. Types of Collagen

Collagen Type	Gene	Structural Details	Localization
I	Col1A1-Col1A2	Fibrils	Skin, tendon, bone, cornea
II	Col2A1	Fibrils	Cartilage, vitreous humour
III	Col3A1	Fibrils	Skin, muscle, associates with type I collagen
IV	Col4A1-Col4A6	Nonfibrillar, mesh collagen	All basal laminae (basement membranes)
V	Col5A1-Col5A3	Small fibers, N-terminal globular domains	Associates with type I collagen in most interstitial tissues
VI	Col6A1-Col6A3	Microfibrils, with both N and C-terminal globular domains	Associates with type I collagen in most interstitial tissues
VII	Col7A1	An anchoring collagen	Epithelial cells; dermal-epidermal junction
VIII	Col8A1-Col8A2	Nonfibrillar, mesh collagen	Cornea, some endothelial cells
IX	Col9A1-Col9A3	Fibril-associated collagens with interrupted triple helices (FACIT); N-terminal globular domain	Associates with type II collagen in cartilage and vitreous humour
X	Col10A1	Nonfibrillar, mesh collagen, with C-terminal globular domain	Growth plate, hypertrophic and mineralizing cartilage
XI	Col11A1-Col11A3	Small fibers	Cartilage, vitreous humor
XII	Col12A1	FACIT	Interacts with types I and II collagen in soft tissues
XIII	Col13A1	Transmembrane collagen	Cell surfaces, epithelial cells
XIV	Col14A1	FACIT	Soft tissue
XV	Col15A1	Endostatin-forming collagen	Endothelial cells
XVI	Col16A1	Other	Ubiquitous
XVII	Col17A1	Transmembrane collagen	Epidermal cell surface
XVIII	Col18A1	Endostatin-forming	Endothelial cells
XIX	Col19A1	Other	Ubiquitous

See the text for descriptions of the differences in types of collagen.

All collagens contain three polypeptide chains with at least one stretch of triple helix. The non-triple helical domains can be short (such as in the fibril-forming collagens) or can be rather large, such that the triple helix is actually a minor component of the overall structure (examples are collagen types XII and XIV). The FACIT (fibril-associated collagens with interrupted triple helices, collagen types IX, XII, and XIV) collagen types associate with fibrillar collagens, without themselves forming fibers. The endostatin-forming collagens are cleaved at their C-terminus to form endostatin, an inhibitor of angiogenesis. The network-forming collagens (type IV) form a mesh-like structure, because of large (approximately 230 amino acids) non-collagenous domains at the carboxy-terminal (Fig. 49.5). And finally, a number of collagen types are actually transmembrane proteins (XIII and XVII) found on epithelial or epidermal cell surfaces, which play a role in a number of cellular processes, including adhesion of components of the ECM to cells embedded within it.

Types I, II, and III collagens form fibrils that assemble into large insoluble fibers. The fibrils (see below) are strengthened through covalent cross-links between lysine residues on adjacent fibrils. The arrangement of the fibrils gives individual tissues their distinct characteristics. Tendons, which attach muscles to bones, contain collagen



Endostatins block angiogenesis (new blood vessel formation) by inhibiting endothelial cell migration. Because endothelial cell migration and proliferation are required to form new blood vessels, inhibiting this action blocks angiogenesis. Tumor growth is dependent on a blood supply; inhibiting angiogenesis can reduce tumor cell proliferation.

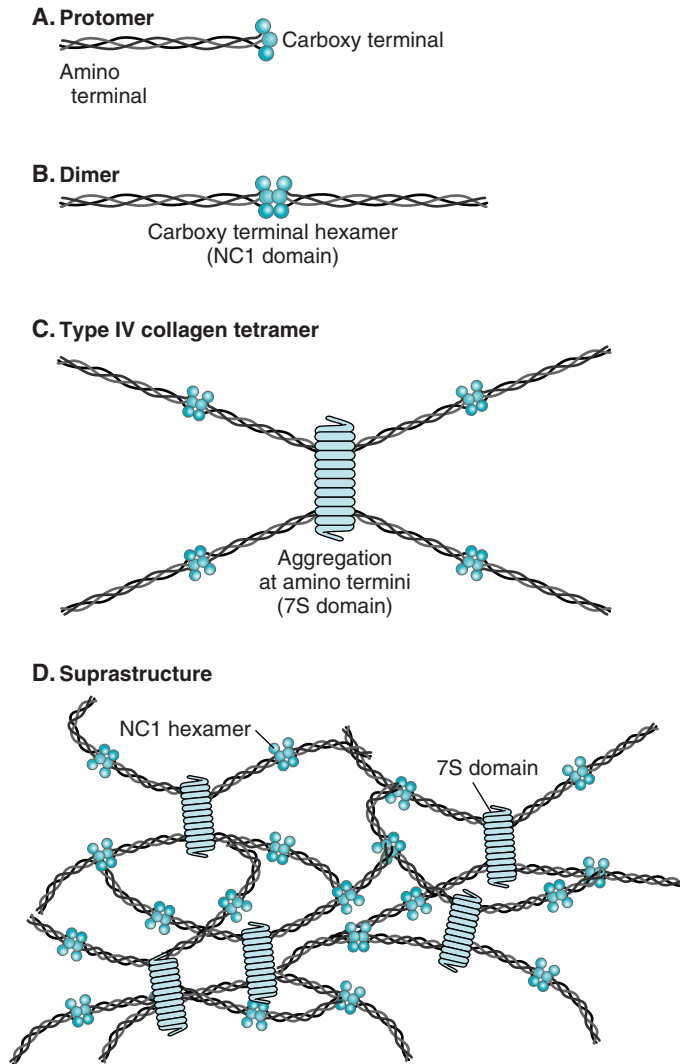


Fig. 49.5. Type IV collagen contains a globular carboxy-terminal domain (A), which forms tropocollagen dimers (hexamers of collagen, B). Four dimers associate at the amino-terminal domains to form a 7S domain (C), and the tetramers form a lattice (D), which provides structural support to the basal lamina.

fibrils aligned parallel to the long axis of the tendon, thus giving the tendon tremendous tensile strength.

The types of collagen that do not form fibrils perform a series of distinct roles. Fibril-associated collagens bind to the surface of collagen fibrils and link them to other matrix-forming components. The transmembrane collagens form anchoring fibrils that link components of the extracellular matrix to underlying connective tissue. The network-forming collagens (type IV) form a flexible collagen that is part of the basement membrane and basal lamina that surround many cells.

ii. Synthesis and Secretion of Collagen

Collagen is synthesized within the endoplasmic reticulum as a precursor known as procollagen. The presequence acts as the signal sequence for the protein and is cleaved, forming procollagen within the endoplasmic reticulum. From there it is transported to the Golgi apparatus (Table 49.2). Three procollagen molecules associate through formation of intrastrand disulfide bonds at the carboxy-terminus; once



One type of osteogenesis imperfecta (OI) is caused by a mutation in a gene that codes for collagen. The phenotype of affected individuals varies greatly, depending on the location and type of mutation. See the Biochemical Comments for more information concerning this type of OI.

Table 49.2. Steps Involved in Collagen Biosynthesis

Location	Process
Rough endoplasmic reticulum	Synthesis of procollagen; insertion of the procollagen molecule into the lumen of the ER
Lumen of the ER	Hydroxylation of proline and lysine residues; glycosylation of selected hydroxylysine residues
Lumen of ER and Golgi apparatus	Self-assembly of the tropocollagen molecule, initiated by disulfide bond formation in the carboxy-terminal extensions; triple helix formation
Secretory vesicle	Procollagen prepared for secretion from cell
Extracellular	Cleavage of the propeptides, removing the amino- and carboxy-terminal extensions, and self-assembly of the collagen molecules into fibrils, and then fibers

these disulfides are formed, the three molecules can align properly to initiate formation of the triple helix. The triple helix forms from the carboxy-end toward the amino-end, forming tropocollagen. The tropocollagen contains a triple helical segment between two globular ends, the amino- and carboxy-terminal extensions. The tropocollagen is secreted from the cell, the extensions are removed using extracellular proteases, and the mature collagen takes its place within the ECM. The individual fibrils of collagen line up in a highly ordered fashion to form the collagen fiber.

2. ELASTIN

Elastin is the major protein found in elastic fibers, which are located in the ECM of connective tissue of smooth muscle cells, endothelial and microvascular cells, chondrocytes, and fibroblasts. Elastic fibers allow tissues to expand and contract; this is of particular importance to blood vessels, which must deform and reform repeatedly in response to the changes in intravascular pressure that occur with the contraction of the left ventricle of the heart. It is also important for the lungs, which stretch each time a breath is inhaled and return to their original shape with each exhalation. In addition to elastin, the elastic fibers contain microfibrils, which are composed of a number of acidic glycoproteins, the major ones being fibrillin-1 and fibrillin-2.

i. Tropoelastin

Elastin has a highly cross-linked, insoluble, amorphous structure. Its precursor, tropoelastin, is a molecule of high solubility, which is synthesized on the rough endoplasmic reticulum (RER) for eventual secretion. Tropoelastin contains two types of alternating domains. The first domain consists of a hydrophilic sequence rich in lysine and alanine residues. The second domain consists of a hydrophobic sequence rich in valine, proline, and glycine, which frequently occur in repeats of VPGVG or VGGVG. The protein contains approximately 16 regions of each domain, alternating throughout the protein (Fig. 49.6).

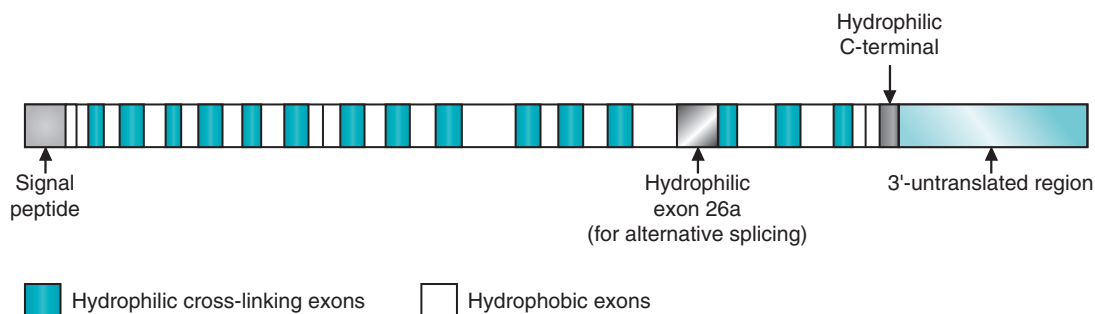


Fig. 49.6. The cDNA structure of elastin, indicating the repeating cross-linking and hydrophobic domains.

On secretion from the cell, the tropoelastin is aligned with the microfibrils, and lysyl oxidase initiates the reactions that cross-link elastin molecules, using lysine residues within the hydrophilic alternating domains in the proteins. This cross-linking reaction is the same as that which occurs in collagen. In this reaction, 2, 3, or 4 lysine residues are cross-linked to form a stable structure. The net result of the cross-linking is the generation of a fibrous mesh that encircles the cells.

ii. Elastic Properties of Elastin

Elastic fibers have the ability to stretch and then to reform without requiring an obvious energy source to do so. The mechanism by which this stretching and relaxing actively occurs is still controversial but does relate to the basic principles of protein folding described in Chapter 7. When the elastic fibers are stretched (such as when a breath is taken in and the lung fills up with air), the amorphous elastin structure is stretched. This stretching exposes the repeating hydrophobic regions of the molecule to the aqueous environment. This, in turn, leads to a decrease in the entropy of water, because the water molecules need to rearrange to form cages about each hydrophobic domain. When this stretching force within the lung is removed (e.g., when the subject exhales), the elastin takes on its original structure because of the increase in entropy that occurs because the water no longer needs to form cages about hydrophobic domains. Thus, the hydrophobic effect is the primary force that allows this stretched structure to reform. Elastin is inherently stable, with a half-life of up to 70 years.

3. LAMININ

After type IV collagen, laminin is the most abundant protein in basal laminae. Laminin provides additional structural support for the tissues through its ability to bind to type IV collagen, to other molecules present in the ECM, and to cell surface-associated proteins (the integrins, see section D).

i. Laminin Structure

Laminin is a heterotrimeric protein shaped, for the most part, like a cross (Fig. 49.7). The trimer is composed of α , β , and γ subunits. There are five possible α proteins (designated $\alpha 1$ – $\alpha 5$), three different versions of the β subunit ($\beta 1$ – $\beta 3$), and three different γ forms ($\gamma 1$ – $\gamma 3$). Thus, there is a potential for the formation of as many as 45 different combinations of these three subunits. However, only 12 have been discovered (designated laminins 1–12). Laminin 1, composed of $\alpha 1\beta 1\gamma 1$, is typical of this class of proteins. The major feature of the laminin structure is a coiled α -helix, which joins the three subunits together and forms a rigid rod. All three chains have extensions at the amino-terminal end. Only the α chain has a significant carboxy-terminal extension past the rod-like structure. It is the laminin extensions that allow laminin to bind to other components within the ECM and to provide stability for the structure. Components of the ECM that are bound by laminin include collagen, sulfated lipids, and proteoglycans.

ii. Laminin Biosynthesis

Like other secreted proteins, laminin is synthesized with a leader sequence targeting the three chains to the endoplasmic reticulum. Chain association occurs within the Golgi apparatus before secretion from the cell. After laminin is secreted by the cell, the amino terminal extensions promote self-association, as well as the binding to other ECM components. Disulfide linkages are formed to stabilize the trimer, but there is much less posttranslational processing of laminin than there is of collagen and elastin.



Supravalvular aortic stenosis (SVAS) results from an insufficiency of elastin in the vessel wall, leading to a narrowing of the large elastic arteries. Current theory suggests that the levels of elastin in the vessel walls may regulate the number of smooth muscle cell rings that encircle the vessel. If the levels of elastin are low, smooth muscle hypertrophy results, leading to a narrowing and stenosis of the artery.

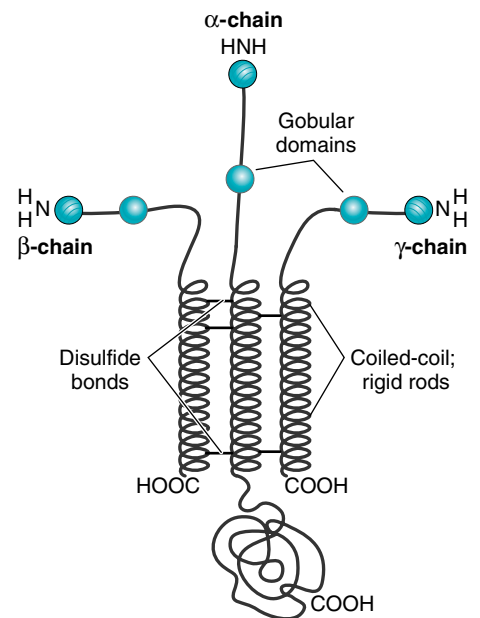


Fig. 49.7. The structure of laminin.



Defects in the structures of laminin 5 or laminin 6 (proteins that contribute to the cohesion of the dermis and epidermis) lead to the disorder referred to as junctional epidermolysis bullosa (JEB). In this disorder, there can be severe spontaneous blistering of the skin and mucous membranes. A severe form of the disease, JEB gravis, is often fatal early in life. Death occurs as a result of epithelial blistering of the respiratory, digestive, and genitourinary systems.

Congenital muscular dystrophy (CMD) results from a defect in laminin 2, which is a component of the bridge that links the muscle cell cytoskeleton to the extracellular matrix. Lack of this bridge triggers muscle cell apoptosis, which results in weakened muscles.



The ECM is not simply a glue that holds cells together; it also serves to keep cells from moving to other locations and to prevent large molecules and other particles, such as microorganisms, from reaching contiguous and distant cells. This confining property of the matrix is medically important. For example, infections spread, in part, because the infectious agent alters the “containing” capacity of the ECM. Cancer cells that metastasize (migrate to other tissues) can do so only by altering the integrity of the matrix. Diseases such as rheumatoid arthritis (an autoimmune destruction of articular and periarticular tissues) and osteoarthritis (degenerative joint disease often associated with aging) involve damage to the functional capacity of the matrix. Alterations in the structural characteristics of the matrix of the renal glomerulus may allow proteins to be excreted into the urine, an indication of inexorable decline in renal function. Genetic defects may cause components of the matrix to be structurally and functionally abnormal, resulting in connective tissue disorders such as the Ehlers-Danlos syndrome (caused by a number of mutations that affect specific collagen genes) and Marfan’s syndrome (a defect in the protein, fibrillin, in which over 330 different mutations, many of which give rise to different phenotypes, have been identified). Deficiencies of lysosomal enzymes involved in normal degradation of molecules of the matrix result in diseases such as the mucopolysaccharidoses.



The principal components of the matrix of cartilage are collagen and proteoglycans, both of which are produced and degraded by the chondrocytes that are embedded in this matrix. An autoimmune attack on articular proteins alters the balance between cartilage degradation and formation. The resulting loss of cartilage organization accompanied by an inflammatory response is responsible for the symptoms experienced by **Sis Lupus**.

The collagen component forms a network of fine fibrils that give shape to the cartilage. The proteoglycans embedded in the cartilage are responsible for its compressibility and its deformability.

B. Proteoglycans

The fibrous structural proteins of the ECM are embedded in gels formed from proteoglycans. Proteoglycans consist of polysaccharides called glycosaminoglycans (GAG) linked to a core protein. The GAGs are composed of repeating units of disaccharides. One sugar of the disaccharide is either N-acetylglucosamine or N-acetylgalactosamine, and the second is usually acidic (either glucuronic acid or iduronic acid). These sugars are modified by the addition of sulfate groups to the parent sugar. A proteoglycan may contain more than 100 GAG chains and consist of up to 95% oligosaccharide by weight.

The negatively charged carboxylate and sulfate groups on the proteoglycan bind positively charged ions and form hydrogen bonds with trapped water molecules, thereby creating a hydrated gel. The gel provides a flexible mechanical support to the ECM. The gel also acts as a filter that allows the diffusion of ions (e.g., Ca^{2+}), H_2O , and other small molecules, but slows diffusion of proteins and movement of cells. Hyaluronan is the only GAG that occurs as a single long polysaccharide chain and is the only GAG that is not sulfated.

1. STRUCTURE AND FUNCTION OF THE PROTEOGLYCANS

Proteoglycans are found in interstitial connective tissues, for example, the synovial fluid of joints, the vitreous humor of the eye, arterial walls, bone, cartilage, and cornea. They are major components of the ECM in these tissues. The proteoglycans interact with a variety of proteins in the matrix, such as collagen and elastin, fibronectin (which is involved in cell adhesion and migration), and laminin.

Proteoglycans are proteins that contain many chains of GAGs (formerly called mucopolysaccharides). Glycosaminoglycans are long, unbranched polysaccharides composed of repeating disaccharide units (Fig. 49.8). The repeating disaccharides usually contain an iduronic or uronic acid and a hexosamine and are frequently sulfated. Consequently, they carry a negative charge, are hydrated, and act as lubricants. After synthesis, proteoglycans are secreted from cells; thus, they function extracellularly. Because the long, negatively charged glycosaminoglycan chains repel each other, the proteoglycans occupy a very large space and act as “molecular sieves,” determining which substances enter or leave cells (Table 49.3). Their properties also give resilience and a degree of flexibility to substances such as cartilage, permitting compression and reexpansion of the molecule to occur.

At least seven types of glycosaminoglycans exist, which differ in the monosaccharides present in their repeating disaccharide units—chondroitin sulfate, dermatan sulfate, heparin, heparin sulfate, hyaluronic acid, and keratan sulfates I and II. Except for hyaluronic acid, the glycosaminoglycans are linked to proteins, usually attached covalently to serine or threonine residues (Fig. 49.9). Keratan sulfate I is attached to asparagine.

2. SYNTHESIS OF THE PROTEOGLYCANS

The protein component of the proteoglycans is synthesized on the ER. It enters the lumen of this organelle, where the initial glycosylations occur. UDP-sugars serve as



The long polysaccharide side chains of the proteoglycans in cartilage contain many anionic groups. This high concentration of negative charges attracts cations that create a high osmotic pressure within cartilage, drawing water into this specialized connective tissue and placing the collagen network under tension. At equilibrium, the resulting tension balances the swelling pressure caused by the proteoglycans. The complementary roles of this macromolecular organization give cartilage its resilience. Cartilage can thus withstand the compressive load of weight bearing and then reexpand to its previous dimensions when that load is relieved.

Table 49.3. Some Specific Functions of the Glycosaminoglycans and Proteoglycans

Glycosaminoglycan	Function
Hyaluronic acid	Cell migration in: Embryogenesis Morphogenesis Wound healing
Chondroitin sulfate proteoglycans	Formation of bone, cartilage, cornea
Keratan sulfate proteoglycans	Transparency of cornea
Dermatan sulfate proteoglycans	Transparency of cornea Binds LDL to plasma walls
Heparin	Anticoagulant (binds antithrombin III) Causes release of lipoprotein lipase from capillary walls
Heparan sulfate (syndecan)	Component of skin fibroblasts and aortic wall; commonly found on cell surfaces

the precursors that add sugar units, one at a time, first to the protein and then to the nonreducing end of the growing carbohydrate chain (Fig. 49.10). Glycosylation occurs initially in the lumen of the ER and subsequently in the Golgi complex. Glycosyltransferases, the enzymes that add sugars to the chain, are specific for the sugar being added, the type of linkage that is formed, and the sugars already present in the chain. Once the initial sugars are attached to the protein, the alternating action of two glycosyltransferases adds the sugars of the repeating disaccharide to the growing glycosaminoglycan chain. Sulfation occurs after addition of the sugar. 3'-Phosphoadenosine 5'-phosphosulfate (PAPS), also called active sulfate, provides the sulfate groups (see Fig. 33.34). An epimerase converts glucuronic acid residues to iduronic acid residues.

After synthesis, the proteoglycan is secreted from the cell. Its structure resembles a bottle brush, with many glycosaminoglycan chains extending from the core protein (Fig. 49.11). The proteoglycans may form large aggregates, noncovalently attached by a "link" protein to hyaluronic acid (Fig. 49.12). The proteoglycans interact with the adhesion protein, fibronectin, which is attached to the cell membrane protein integrin. Cross-linked fibers of collagen also associate with this complex, forming the ECM (Fig. 49.13).



The functional properties of a normal joint depend, in part, on the presence of a soft, well-lubricated, deformable, and compressible layer of cartilaginous tissue covering the ends of the long bones that constitute the joint.

In **Sis Lupus'** case, the pathologic process that characterizes SLE disrupted the structural and functional integrity of her articular (joint) cartilage.

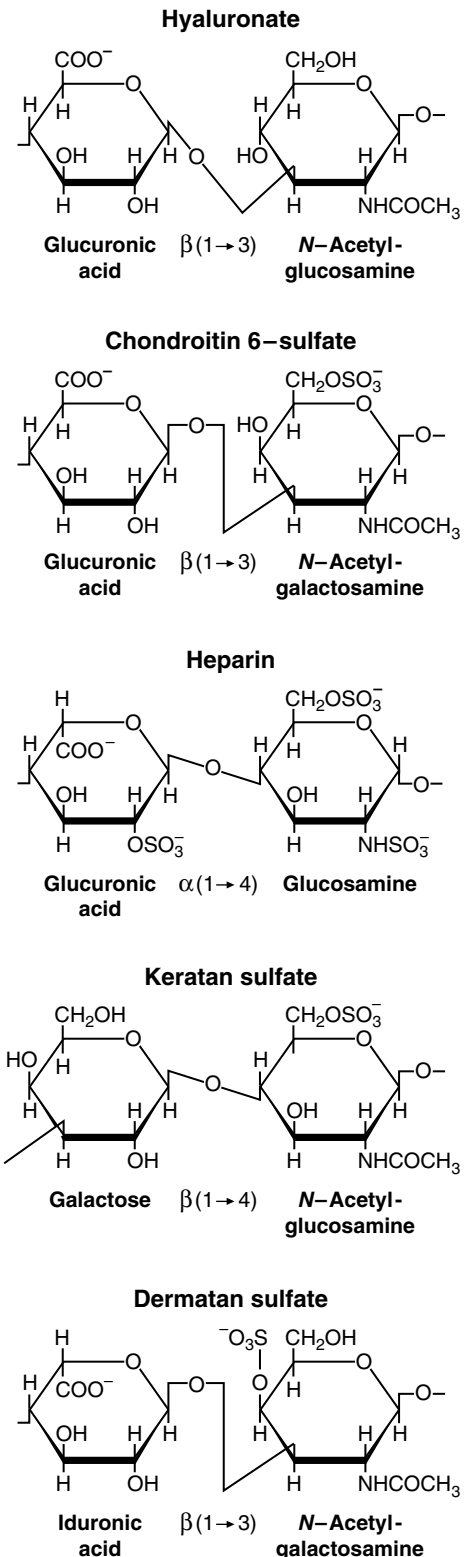
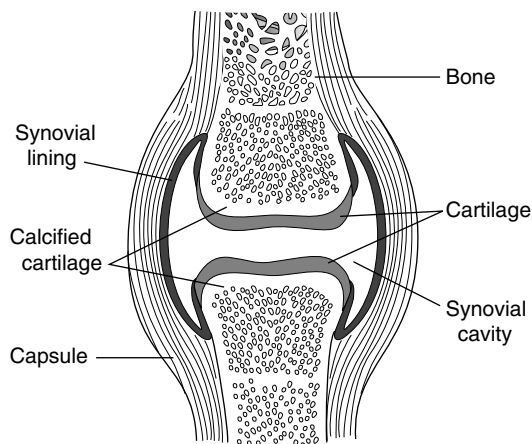


Fig. 49.8. Repeating disaccharides of some glycosaminoglycans. These repeating disaccharides usually contain an *N*-acetylated sugar and a uronic acid, which usually is glucuronic acid or iduronic acid. Sulfate groups are often present but are not included in the sugar names in this figure.

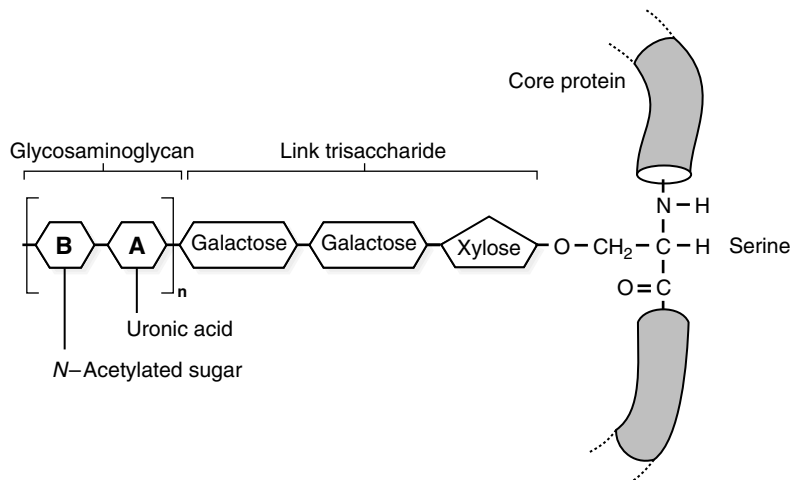


Fig. 49.9. Attachment of glycosaminoglycans to proteins. The sugars are linked to a serine or threonine residue of the protein. A and B represent the sugars of the repeating disaccharide.

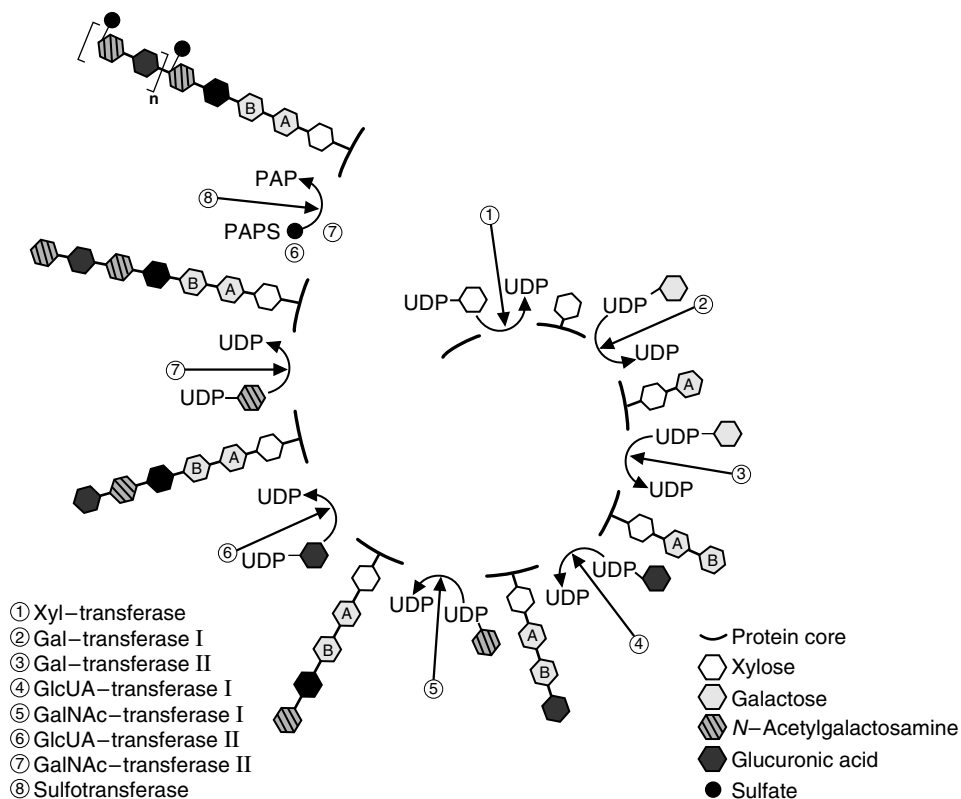


Fig. 49.10. Synthesis of chondroitin sulfate. Sugars are added to the protein one at a time, with UDP-sugars serving as the precursors. Initially a xylose residue is added to a serine in the protein. Then two galactose residues are added, followed by a glucuronic acid (GlcUA) and an N-acetylglucosamine (GalNAc). Subsequent additions occur by the alternating action of two enzymes that produce the repeating disaccharide units. One enzyme (6) adds GlcUA residues, and the other (7) adds GalNAc. As the chain grows, sulfate groups are added by phosphoadenosine phosphosulfate (PAPS). Modified from Roden L. In: Fishman WH, ed. *Metabolic Conjugation and Metabolic Hydrolysis*, vol II. Orlando, FL: Academic Press, 1970:401.

Table 49.4. Defective Enzymes in the Mucopolysaccharidoses

Disease	Enzyme Deficiency	Accumulated Products
Hunter	Iduronate sulfatase	Heparan sulfate, Dermatan sulfate
Hurler + Scheie	α -L-Iduronidase	Heparan sulfate, Dermatan sulfate
Maroteaux-Lamy	<i>N</i> -Acetylgalactosamine sulfatase	Dermatan sulfate
Mucopolidosis VII	β -Glucuronidase	Heparan sulfate, Dermatan sulfate
Sanfilippo A	Heparan sulfamidase	Heparan sulfate
Sanfilippo B	<i>N</i> -Acetylglucosaminidase	Heparan sulfate
Sanfilippo D	<i>N</i> -Acetylglucosamine 6-sulfatase	Heparin sulfate

These disorders share many clinical features, although there are significant variations between disorders, and even within a single disorder, based on the amount of residual activity remaining. In most cases, multiple organ systems are affected (with bone and cartilage being a primary target). For some disorders, there is significant neuronal involvement, leading to mental retardation.

3. DEGRADATION OF PROTEOGLYCANS

Lysosomal enzymes degrade proteoglycans, glycoproteins, and glycolipids, which are brought into the cell by the process of endocytosis. Lysosomes fuse with the endocytic vesicles, and lysosomal proteases digest the protein component. The carbohydrate component is degraded by lysosomal glycosidases.

Lysosomes contain both endoglycosidases and exoglycosidases. The endoglycosidases cleave the chains into shorter oligosaccharides. Then exoglycosidases, specific for each type of linkage, remove the sugar residues, one at a time, from the nonreducing ends.

Deficiencies of lysosomal glycosidases cause partially degraded carbohydrates from proteoglycans, glycoproteins, and glycolipids to accumulate within membrane-enclosed vesicles inside cells. These “residual bodies” can cause marked enlargement of the organ with impairment of its function.

In the clinical disorder known as the mucopolysaccharidoses (caused by accumulation of partially degraded glycosaminoglycans), deformities of the skeleton may occur (Table 49.4). Mental retardation often accompanies these skeletal changes.

II. INTEGRINS

Integrins are the major cellular receptors for ECM proteins and provide a link between the internal cytoskeleton of cells (primarily the actin microfilament system) and extracellular proteins, such as fibronectin, collagen, and laminin. Integrins

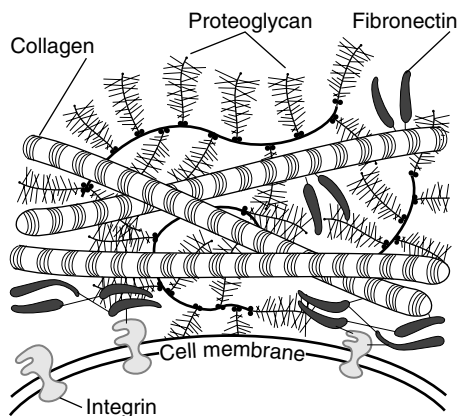


Fig. 49.13. Interactions between the cell membrane and the components of the extracellular matrix.

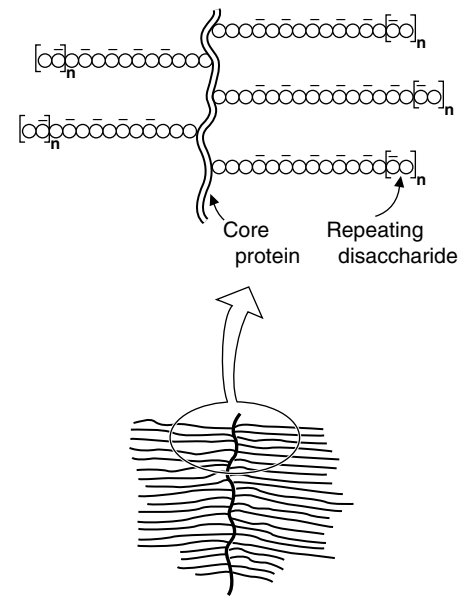


Fig. 49.11. “Bottle-brush” structure of a proteoglycan, with a magnified segment.

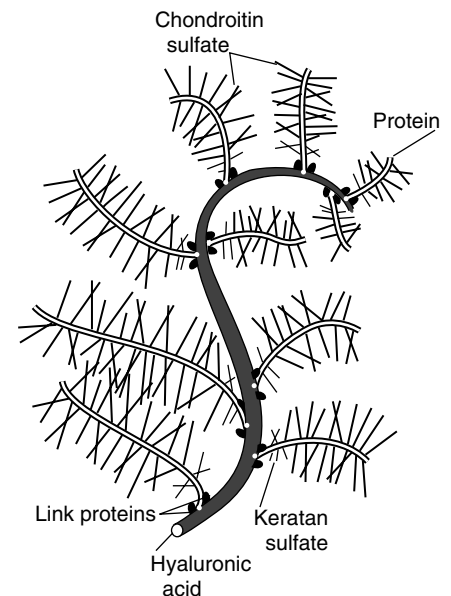


Fig. 49.12. Proteoglycan aggregate.

consist of an α and a β subunit. There are 18 distinct α and eight distinct β gene products. Twenty-four unique α/β dimers have been discovered. Mice have been genetically engineered to be unable to express many of the integrin genes (one gene at a time), and the phenotypes of these knockout mice vary from embryonic lethality (the $\alpha 5$ gene is an example) to virtually no observable defects (as exemplified by the $\alpha 1$ gene). In addition to anchoring the cell's cytoskeleton to the ECM, thereby providing a stable environment in which the cell can reside, the integrins are also involved in a wide variety of cell signaling options.

Certain integrins, such as those associated with white blood cells, are normally inactive because the white cell must circulate freely in the bloodstream. However, if an infection occurs, cells located in the area of the infection release cytokines, which activate the integrins on the white blood cells, allowing them to bind to vascular endothelial cells (leukocyte adhesion) at the site of infection. Leukocyte adhesion deficiency (LAD) is a genetic disorder that results from mutations in the $\beta 2$ integrin such that leukocytes cannot be recruited to the sites of infection. Conversely, drugs are now being developed to block either the $\beta 2$ or $\alpha 4$ integrins (on lymphocytes) to treat inflammatory and autoimmune disorders by interfering with the normal white cell response to cytokines.

Integrins can be activated by “inside-out” mechanisms, whereby intracellular signaling events activate the molecule, or “outside-in” mechanisms, in which a binding event with the extracellular portion of the molecule initiates intracellular signaling events. For those integrins that bind cells to ECM components, activation of specific integrins can result in migration of the affected cell through the ECM. This mechanism is operative during growth, during cellular differentiation, and in the process of metastasis of malignant cells to neighboring tissues.

III. ADHESION PROTEINS

Adhesion proteins are found in the ECM and link integrins to ECM components. Adhesion proteins, of which fibronectin is a prime example, are large multidomain proteins that allow binding to many different components simultaneously. In addition to integrin binding sites, fibronectin contains binding sites for collagen and glycosaminoglycans. As the integrin molecule is bound to intracellular cytoskeletal proteins, the adhesion proteins provide a bridge between the actin cytoskeleton of the cell and the cells' position within the ECM. Loss of adhesion protein capability can lead to either physiologic or abnormal cell movement. Alternative splicing of fibronectin allows many different forms of this adhesion protein to be expressed, including a soluble form (versus cell-associated forms), which is found in the plasma. The metabolic significance of these products remains to be determined.

IV. MATRIX METALLOPROTEINASES

The ECM contains a series of proteases known as the matrix metalloproteinases, or MMPs. These are zinc-containing proteases that use the zinc to appropriately position water to participate in the proteolytic reaction. At least 23 different types of human MMPs exist, and they cleave all proteins found in the ECM, including collagen and laminin.

A propeptide is present in newly synthesized MMPs that contains a critical cysteine residue. The cysteine residue in the propeptide binds to the zinc atom at the active site of the protease and prevents the propeptide from exhibiting proteolytic activity. Removal of the propeptide is required to activate the MMPs. Once activated, certain MMPs can activate other forms of MMP.

Regulation of MMP activity is quite complex. These regulatory processes include transcriptional regulation, proteolytic activation, inhibition by the circulating protein $\alpha 2$ -macroglobulin, and regulation by a class of inhibitors known as



Fibronectin was first discovered as a large, external transformation-sensitive protein (LETS), which was lost when fibroblasts were transformed into tumor cells. Many tumor cells secrete less than normal amounts of adhesion protein material, which allows for more movement within the extracellular milieu. This, in turn, increases the potential for the tumor cells to leave their original location and take root at another location within the body (metastasis).



Because MMPs degrade extracellular matrix (ECM) components, their expression is important to allow cell migration and tissue remodeling during growth and differentiation. In addition, many growth factors bind to ECM components and, as a bound component, do not exhibit their normal growth-promoting activity. Destruction of the ECM by the MMPs releases these growth factors, thereby allowing them to bind to cell surface receptors to initiate growth of tissues. Thus, coordinated expression of the MMPs is required for appropriate cell movement and growth. Cancer cells that metastasize require extensive ECM remodeling and usually use MMP activity to spread throughout the body.

tissue inhibitors of metalloproteinases, or TIMPs. It is important that the synthesis of TIMPs and MMPs be coordinately regulated, because dissociation of their expression can facilitate various clinical disorders, such as certain forms of cancer and atherosclerosis.

CLINICAL COMMENTS



Articular cartilage is a living tissue with a turnover time determined by a balance between the rate of its synthesis and that of its degradation (Fig. 49.14). The chondrocytes that are embedded in the matrix of intra-articular cartilage participate in both its synthesis and its enzymatic degradation. The latter occurs as a result of cleavage of proteoglycan aggregates by enzymes produced and secreted by the chondrocytes.

In SLE, the condition that affects **Sis Lupus**, this delicate balance is disrupted in favor of enzymatic degradation, leading to dissolution of articular cartilage and, with it, the loss of its critical cushioning functions. The underlying mechanisms responsible for this process in SLE include the production of antibodies directed against specific cellular proteins in cartilage as well as in other intra-articular tissues. The cellular proteins thus serve as the “antigens” to which these antibodies react. In this sense, SLE is an “autoimmune” disease because antibodies are produced by the host that attack “self” proteins. This process excites the local release of cytokines such as interleukin-1 (IL-1), which increases the proteolytic activity of the chondrocytes, causing further loss of articular proteins such as the proteoglycans. The associated inflammatory cascade is responsible for **Sis Lupus**’ joint pain.

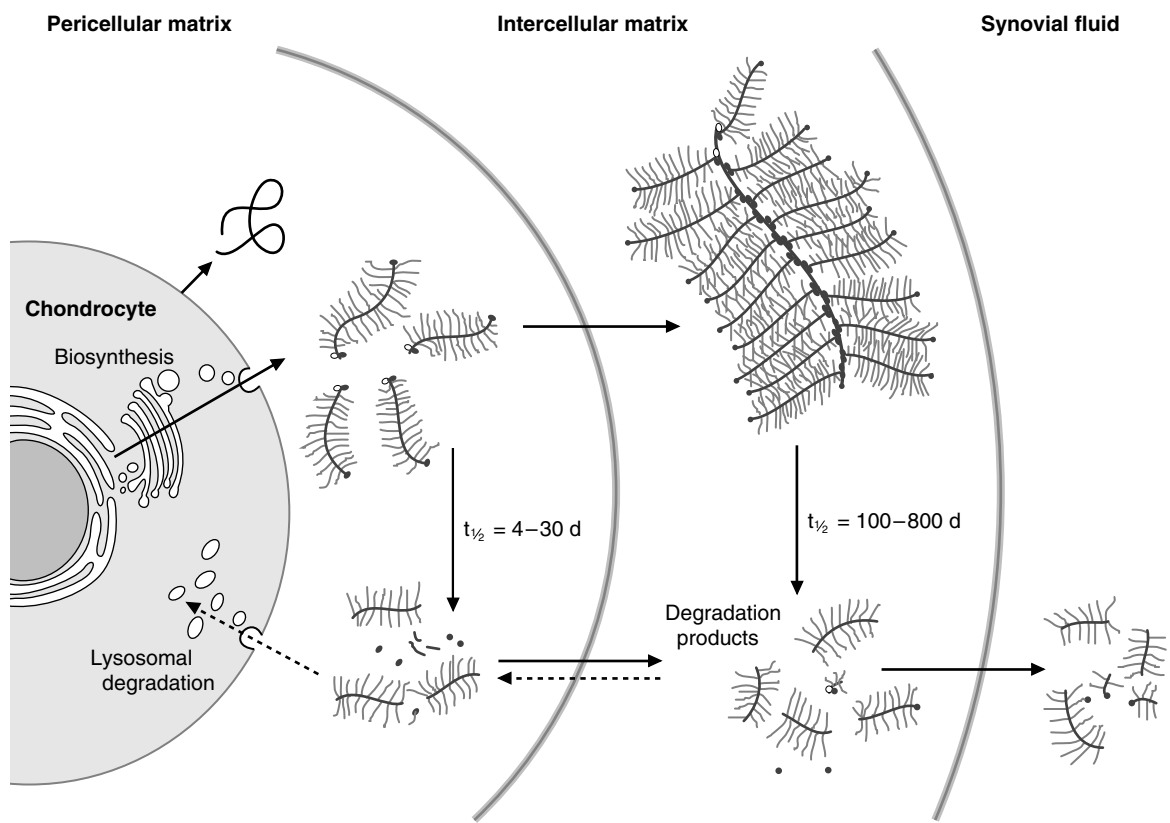


Fig. 49.14. Synthesis and degradation of proteoglycans by chondrocytes. From Cohen RD, et al. *The Metabolic Basis of Acquired Disease*, vol 2. London: Bailliere Tindall, 1990:1859.



The microvascular complications of both type 1 and type 2 diabetes mellitus involve the small vessels of the retina (diabetic retinopathy), the renal glomerular capillaries (diabetic nephropathy), and the vessels supplying blood to the peripheral nerves (autonomic neuropathy). The lack of adequate control of **Ann Sulin's** diabetic state over many years caused a progressive loss of the filtering function of the approximately one-and-one-half million glomerular capillary–mesangial units that are present in her kidneys.

Chronic hyperglycemia is postulated to be a major metabolic initiator or inducer of diabetic microvascular disease, including those renal glomerular changes that often lead to end-stage renal disease (“glucose toxicity”).

For a comprehensive review of the four postulated molecular mechanisms by which chronic hyperglycemia causes these vascular derangements, the reader is referred to an excellent review by Sheetz and King (see suggested references).

Regardless of which of the postulated mechanisms (increased flux through the aldose reductase or polyol pathway [see Chapter 30], the generation of advanced glycosylation end products [AGEs], the generation of reactive oxygen intermediates [see Chapter 24], or excessive activation of protein kinase C [see Chapter 18]) will eventually be shown to be the predominant causative mechanism, each can lead to the production of critical intracellular and extracellular signaling molecules (e.g., cytokines). These, in turn, can cause pathologic changes within the glomerular filtration apparatus that reduce renal function. These changes include: (1) increased synthesis of collagen, type IV, fibronectin, and some of the proteoglycans, causing the glomerular basement membrane (GBM; Fig. 49.15) to become diffusely thickened throughout the glomerular capillary network. This membrane thickening alters certain specific filtration properties of the GBM, preventing some of the metabolites that normally enter the urine from the glomerular capillary blood (via the fenestrated capillary endothelium) from doing so (a decline in glomerular filtration rate or GFR). As a result, these potentially toxic substances accumulate in the blood and contribute to the overall clinical presentation of advancing uremia. In spite of the

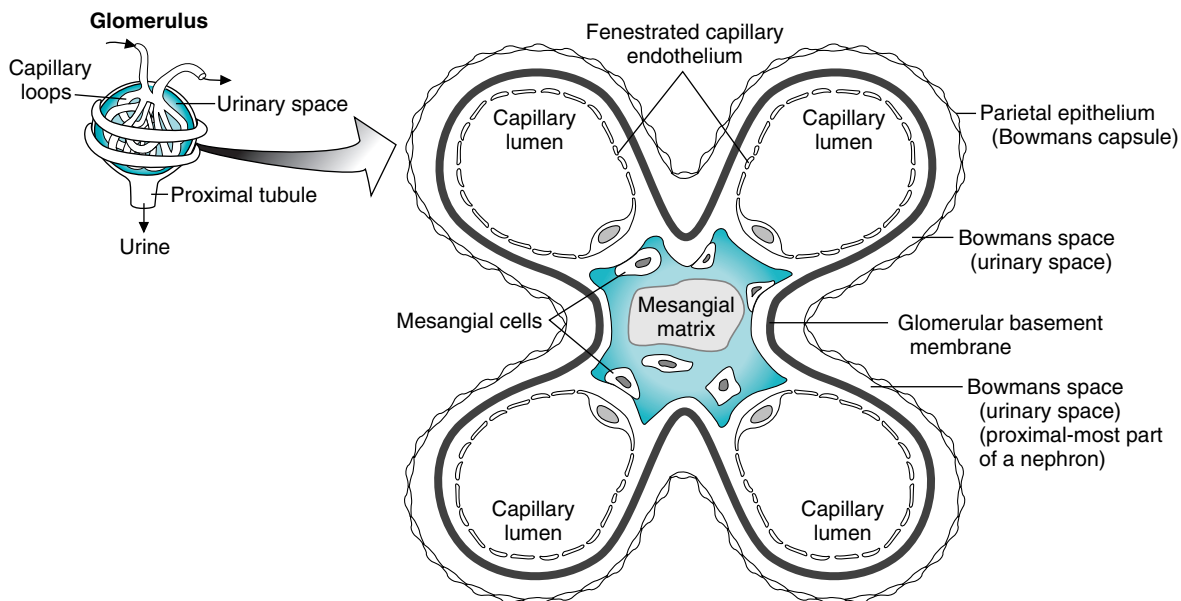


Fig. 49.15. A cross-section of a normal renal glomerulus showing four capillary tufts delivering blood to the glomerulus for filtration across the fenestrated capillary endothelium then through the glomerular basement membrane into the Bowman's space to form urine. The urine then enters the proximal tubule of the nephron. This filtration removes potentially toxic metabolic end products from the blood. The mesangium, by contracting and expanding, controls the efficiency of these filtering and excretory functions by regulating the hydraulic filtration pressures within the glomerulus. An intact basement membrane must be present to maintain the integrity of the filtering process.

thickening of the GBM, this membrane becomes “leaky” for some macromolecules (e.g., albumin) that normally do not enter the urine from the glomerular capillaries (microalbuminuria). Suggested mechanisms for this increased permeability or leakiness include reduced synthesis of the specific proteoglycan, heparan sulphate, as well as increased basement membrane production of vascular endothelium growth factor (VEGF), a known angiogenic and permeability factor; and expansion of the extracellular matrix in the mesangium. The mesangium consists of specialized tissue containing collagen, proteoglycans, and other macromolecules that surround the glomerular capillaries and that, through its gel-like and sieving properties, determine, in part, the glomerular capillary hydraulic filtration pressure as well as the functional status of the capillary endothelium–mesangial glomerular basement membrane filtration apparatus (see Fig. 49.15). As the mesangial tissue expands, the efficiency of glomerular filtration diminishes proportionately. The cause of these mesangial changes is, in part, the consequence of increased expression of certain growth factors, especially transforming growth factor β (TGF- β) and connective tissue growth factor (CTGF). Current therapeutic approaches in patients with early diabetic nephropathy include the use of antibodies that neutralize TGF- β .

BIOCHEMICAL COMMENTS



Osteogenesis imperfecta (OI) is a heterogenous group of diseases that have in common a defect in collagen production. This defect can be either of two types: The first type is associated with a reduction in the synthesis of normal collagen (due to a gene deletion or splice-site mutation). The second type is associated with the synthesis of a mutated form of collagen. Most of the mutations have a dominant-negative effect, leading to an autosomal dominant mode of transmission.

In the second type of OI, many of the known mutations involve substitutions of another amino acid for glycine. This results in an unstable collagen molecule, because glycine is the only amino acid that can fit between the other two chains within the triple helix of collagen. If the mutation is near the carboxy-terminal of the molecule, the phenotype of the disease is usually more severe than if the mutation is near the amino-terminal end (recall that triple helix formation proceeds from the carboxy- to the amino-terminal end of the molecule). Of interest are mutations that replace glycine with either serine or cysteine. Such mutations are more stable than expected, because of the hydrogen-bonding capabilities of serine and the ability of cysteine to form disulfide bonds. Both would aid in preventing the strands of the triple helix from unwinding.

Children with OI can be treated with a class of compounds known as bisphosphonates, which consist of two phosphates linked by a carbon or nitrogen bridge (thus, they are analogs of pyrophosphate, in which the two phosphates are linked by oxygen). Normal bone remodeling is the result of a coordinated “coupling” between osteoclast activity (cells that resorb bone) and osteoblast activity (cells that form bone). In OI, bone resorption outpaces bone formation because osteoclast activity is enhanced (perhaps because of the reduced levels of normal collagen present to act as nucleating sites for bone formation). This leads to a net loss of bone mass and fragility of the skeleton. Bisphosphonates inhibit osteoclast action with the potential to increase bone mass and its tensile strength.

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REVIEW QUESTIONS—CHAPTER 49

- Individuals who develop scurvy suffer from sore and bleeding gums and loss of teeth. This is due, in part, to the synthesis of a defective collagen molecule. The step that is affected in collagen biosynthesis attributable to scurvy is which of the following?
 - The formation of disulfide bonds, which initiates tropocollagen formation
 - The formation of lysyl cross-links between collagen molecules
 - Secretion of tropocollagen into the extracellular matrix
 - The formation of collagen fibrils
 - The hydroxylation of proline residues, which stabilizes the collagen structure
- The underlying mechanism that allows elastin to exhibit elastic properties (expand and contract) is which of the following?
 - Proteolysis during expansion, and resynthesis during contraction
 - Breaking of disulfide bonds during expansion, reformation of these bonds during contraction
 - A decrease in entropy during expansion, and an increase in entropy during contraction
 - The breaking of salt bridges during expansion, and reformation of the salt bridges during contraction
 - Hydroxylation of elastin during expansion, and decarboxylation of elastin during contraction
- The underlying mechanism by which glycosaminoglycans allow for the formation of a gel-like substance in the extracellular matrix in which of the following?
 - Charge attraction between glycosaminoglycan chains
 - Charge repulsion between glycosaminoglycan chains
 - Hydrogen bonding between glycosaminoglycan chains
 - Covalent cross-linking between glycosaminoglycan chains
 - Hydroxylation of adjacent glycosaminoglycan chains
- The movement of tumor cells from their site of origin to other locations within the body requires the activity of which of the following proteins?
 - Collagen
 - Laminin
 - Proteoglycans
 - Elastin
 - Matrix metalloproteinases
- Fibronectin is frequently absent in malignant fibroblast cells. One of the major functions of fibronectin is which of the following?
 - To inhibit the action of matrix metalloproteinases
 - To coordinate collagen deposition within the extracellular matrix
 - To fix the position of cells within the extracellular matrix
 - To regulate glycosaminoglycan production
 - To extend glycosaminoglycan chains using nucleotide sugars