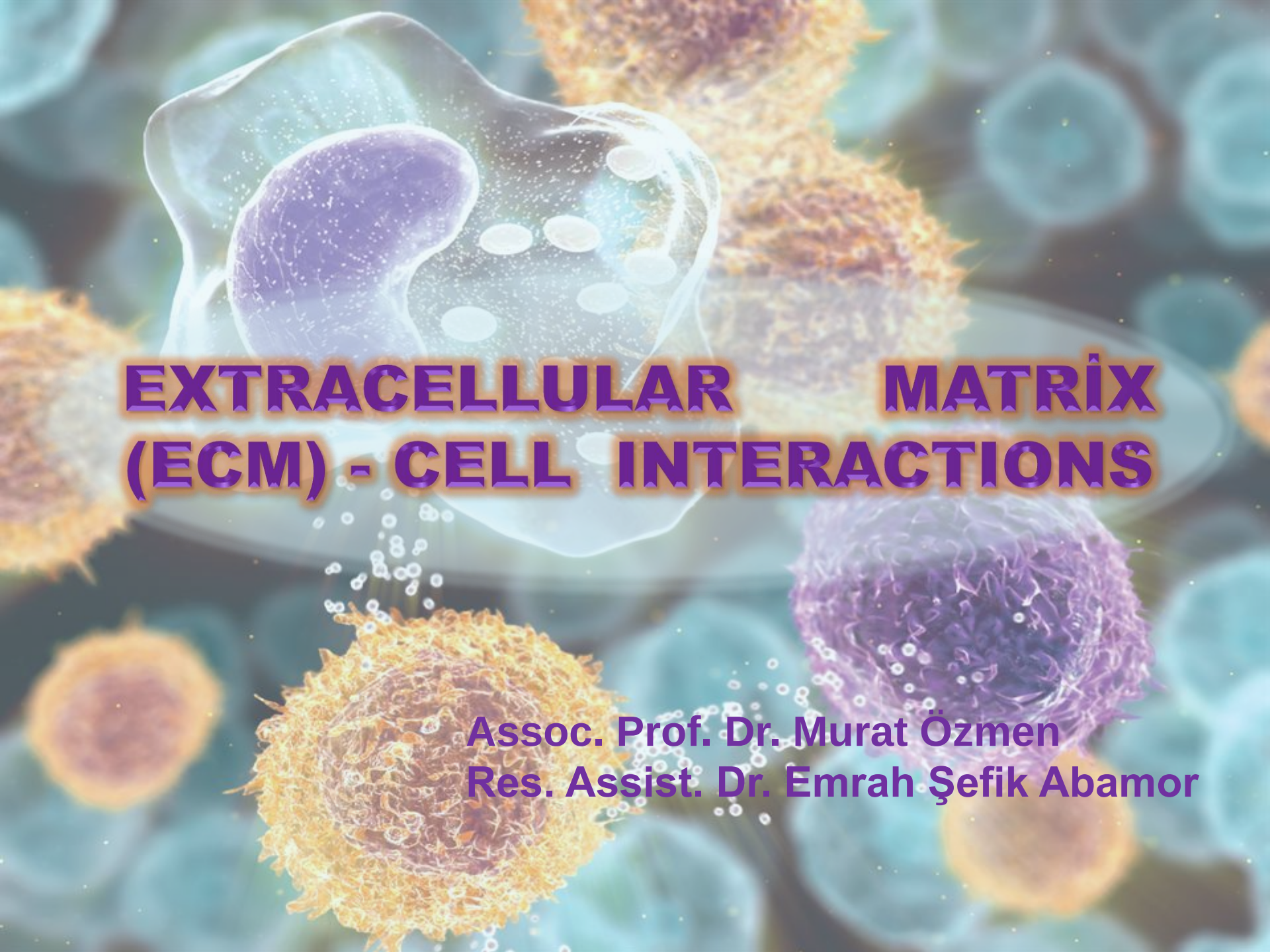
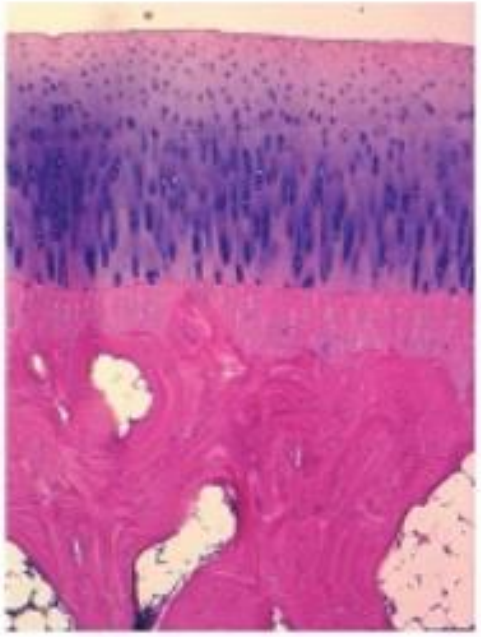


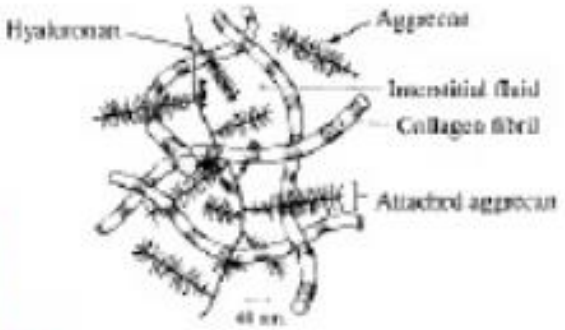
EXTRACELLULAR MATRİX (ECM) - CELL INTERACTIONS

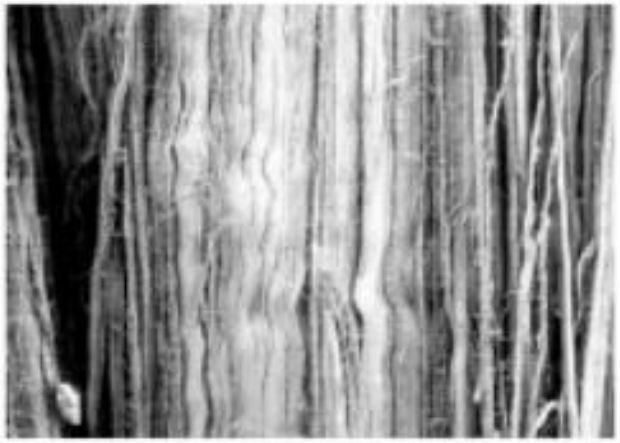
Assoc. Prof. Dr. Murat Özmen
Res. Assist. Dr. Emrah Şefik Abamor

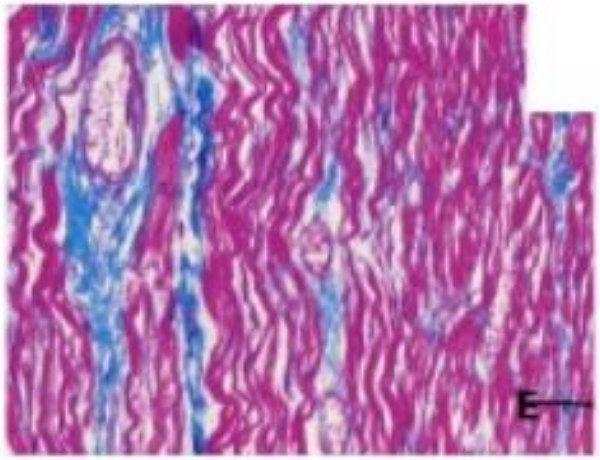
Outline:

- Overview of structural and signaling roles of ECM
- Two main classes of ECM molecules
  - Glycosaminoglycan polysaccharide chains
    - Structure and function
  - Fibrous proteins
    - Structure and function
- Basal lamina (basement membrane)
- Integrins



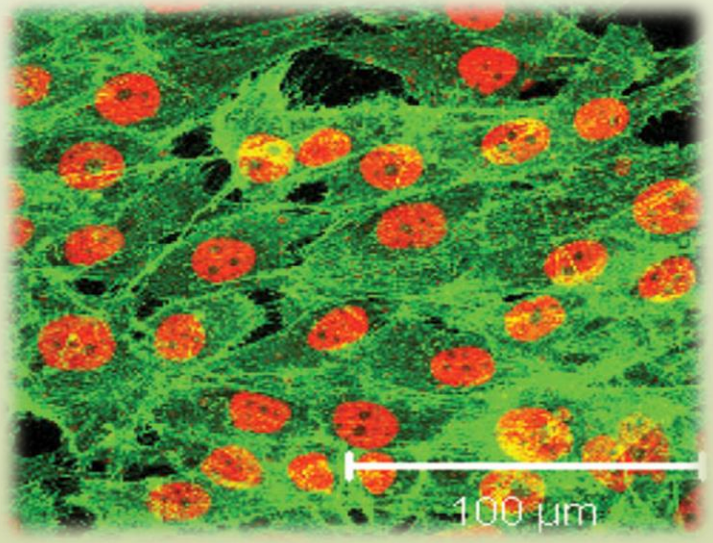






Extracellular Matrix

The extracellular matrix is a network of proteins and carbohydrates that binds cells together or divides one tissue from another.



The extracellular matrix is the product principally of **connective tissue**, one of the four fundamental tissue types, but may also be produced by other cell types, including those in **epithelial tissues**.

The Extra Cellular Matrix: ECM

- Extra Cellular: outside the cell
  Matrix: structure made from a network of interacting components

The ECM is composed of an interlocking mesh of
fibrous proteins and glycosaminoglycans (GAGs).

Components of the ECM are produced intracellularly by resident cells, and secreted into the ECM via exocytosis.

Connective Tissue underlying an epithelium
Fibroblast, primary cell secreting ECM

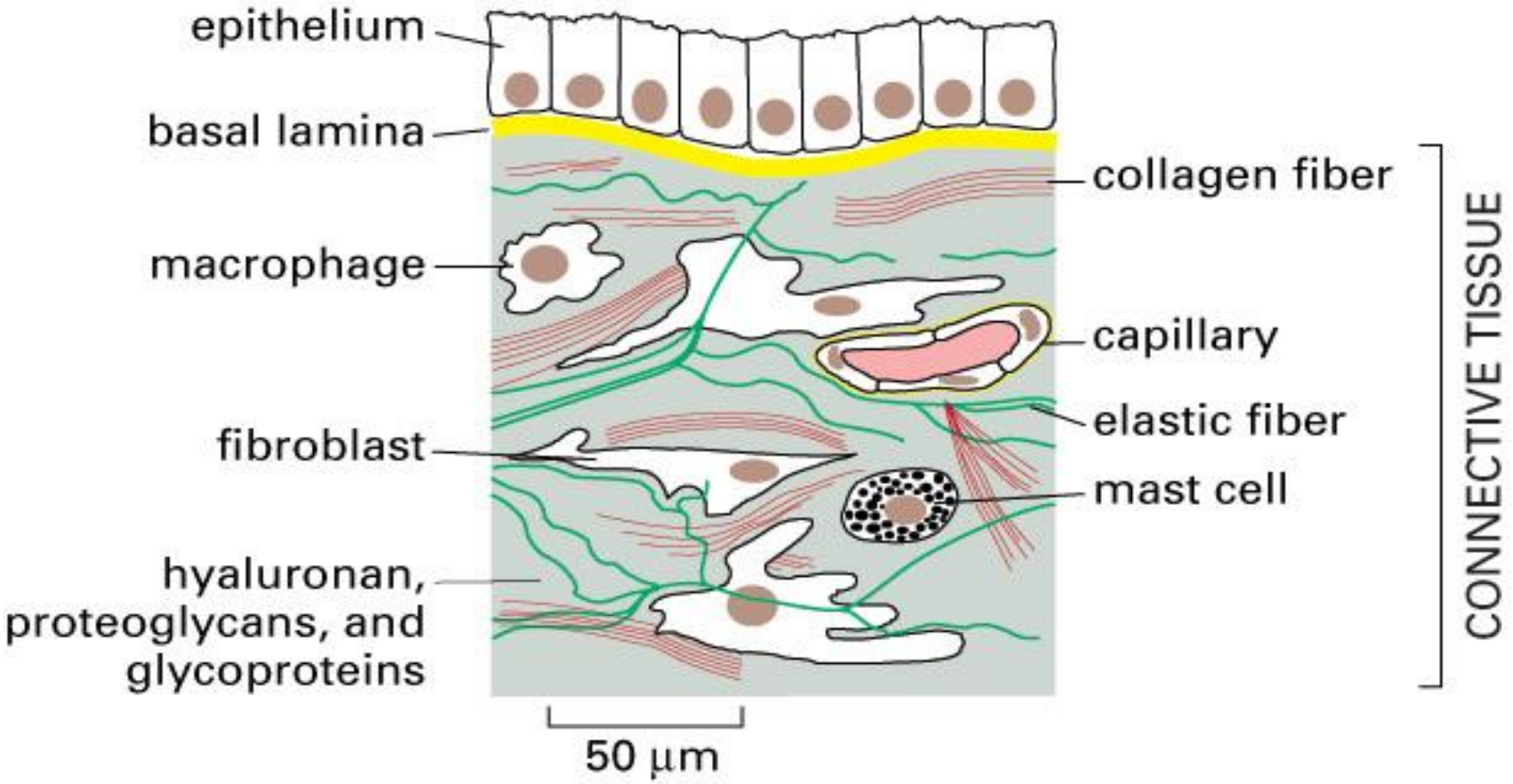


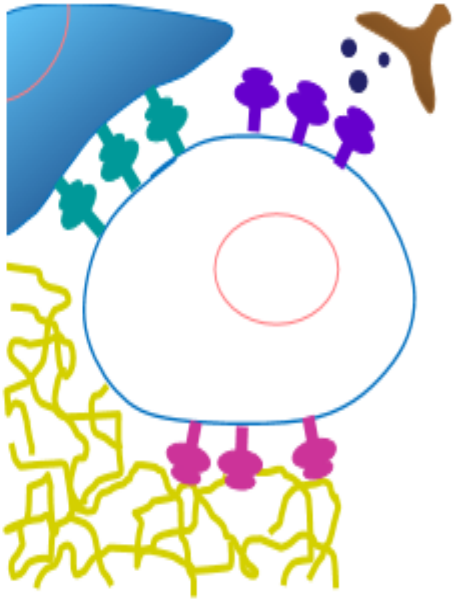
Figure 19–34. Molecular Biology of the Cell, 4th Edition.

ECM Function

- Structural support for cells
- Pattern of ECM regulates
  - Cell division
  - Adhesion
  - Motility
- Development
  - Migration
  - Differentiation
- Growth Factors

Cell-cell interactions
-Cadherins
-Cell adhesion molecules
-Notch ligands…

Biophysical aspects
-Substrate elasticity
-Physical forces
-Spatial arrangements …



ECM adhesion
-Fibronectins
-Laminins
-Collagens
-GAGs
-Vitronectin…

Soluble signals
-Growth factors
-Cytokines
-Chemokines
-Metabolic cues…

modified from Lutolf and Blau (2009)
Adv. Mater. 21, 3255-68

Functions of ECM

1-Role in establishing and maintaining cell shape, migration, mechanical support

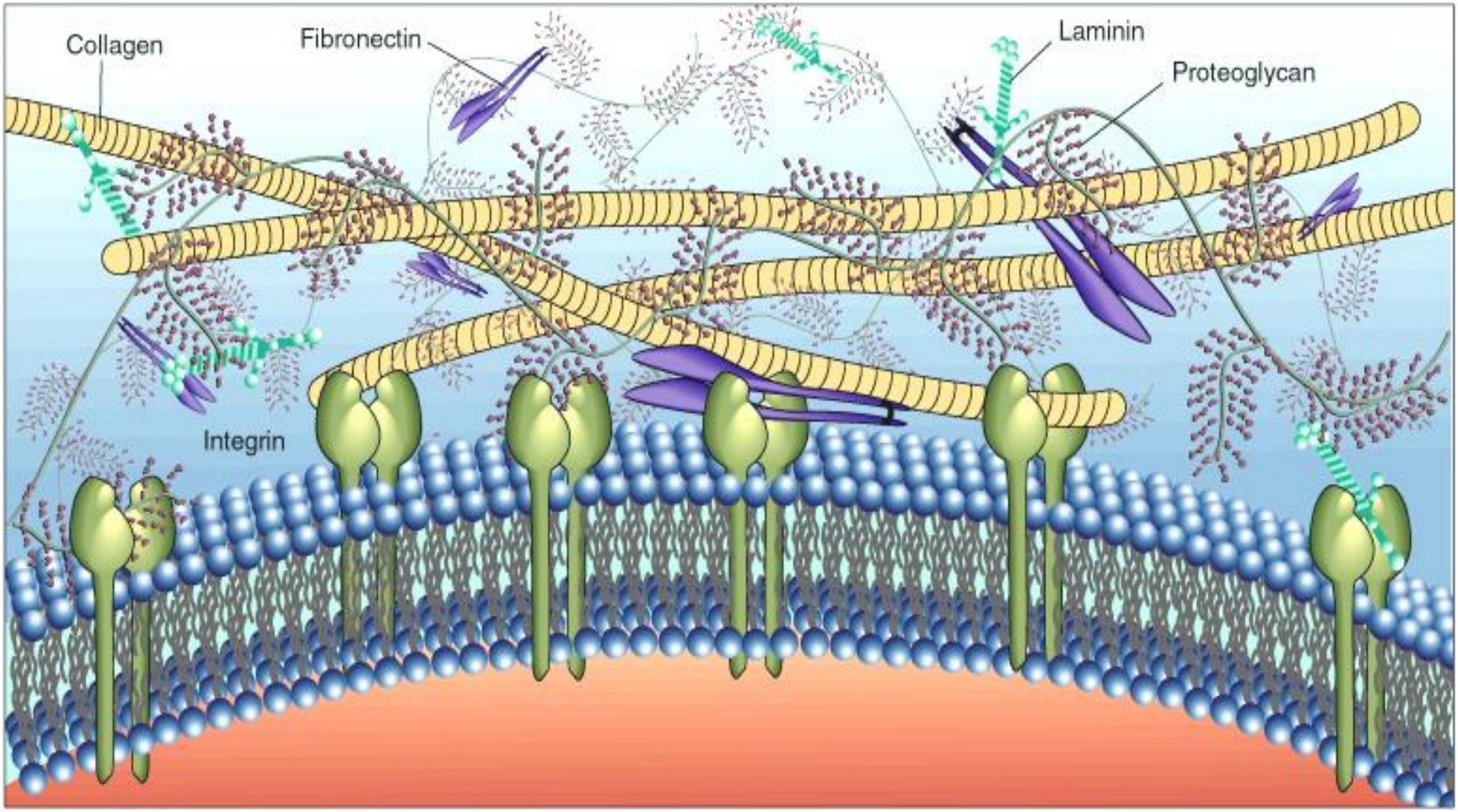
2-Anchorage for cells, segregating tissues from one another, and regulating intercellular communication

3-Sequesters a wide range of cellular growth factors, and acts as a local depot for them

4-Essential for processes like growth, wound healing etc

What are the major proteins of the ECM?

Collagens, Proteoglycans, Elastin, Fibronectin, Laminin, Tenascin.



Functions of ECM

Functions as adhesive substrate

- *tracks to direct migratory cells*
- *concentration gradients for haptotactic migration*

Provides structure

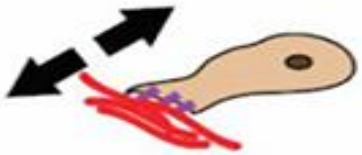
- *defines tissue boundaries*
- *provides integrity and elasticity to developing organs*
- *degraded by invasive cells during development and disease*

Presents growth factors to their receptors

- *controls spatial distribution of ECM-bound surface molecules*
- *facilitates crosstalk between growth factor receptors and ECM receptors*

Sequesters and stores growth factors

- *allows for spatio-temporal regulation of factor release*
- *organizes morphogen gradients*
- *mediates release of factors in the presence of appropriate cell-mediated forces or proteolytic degradation*



Senses and transduces mechanical signals

- *defines mechanical properties permissive/ instructive to cell differentiation*
- *activates intracellular signaling through interaction with cell-surface receptors*
- *engages cytoskeletal machinery and synergizes with growth factor signaling*

ECM consists of two main classes of molecules:

- Glycosaminoglycan polysaccharide chains
  - Form hydrated gels
- Fibrous proteins
  - Organize and strengthen

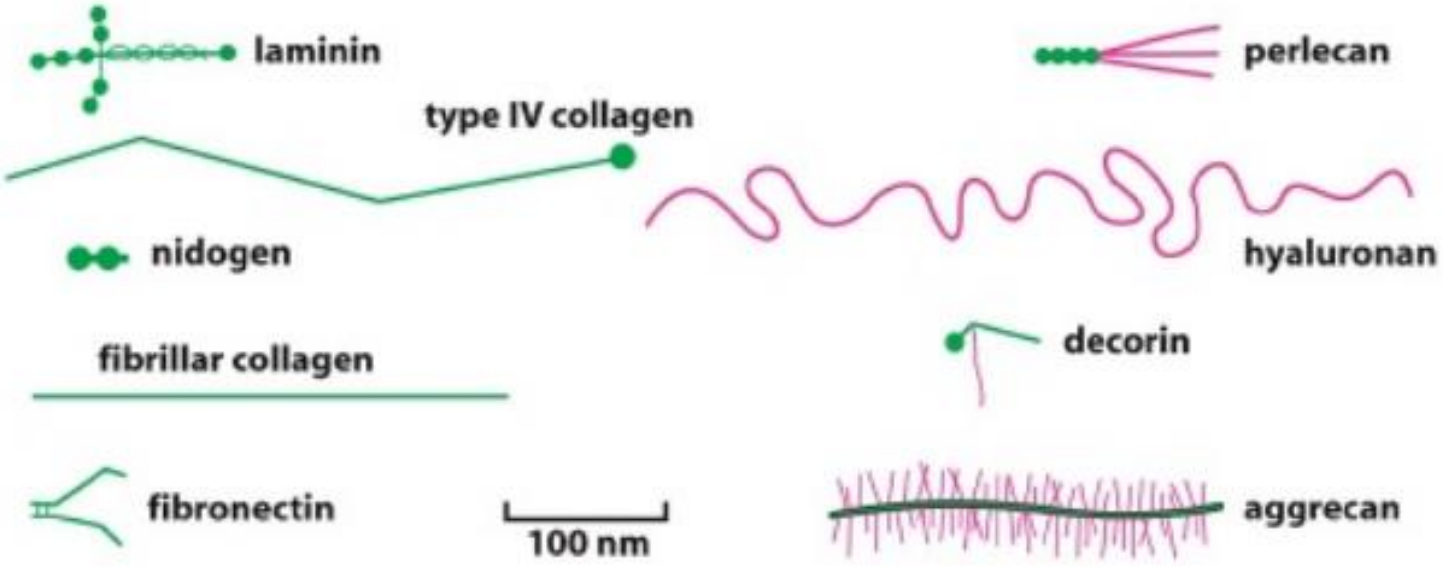


Figure 19-41 *Molecular Biology of the Cell* (© Garland Science 2008)

ECM Molecules

Glycosaminoglycans (GAG)

- Hyaluronic Acid
- Chondroitin Sulphate
- Heparan Sulphate
- Dermatan Sulphate
- Keratan Sulphate

Fibrous Proteins

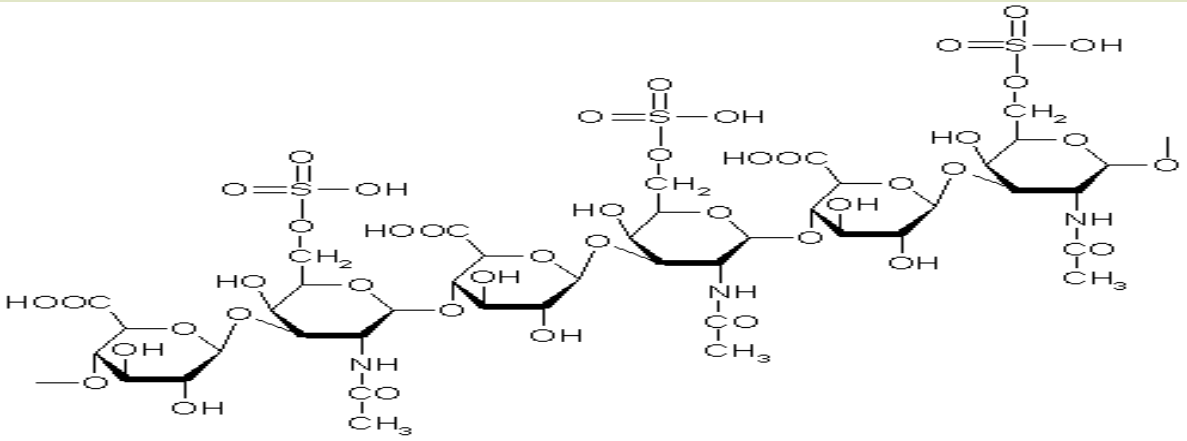
- Collagen
- Elastin
- Fibronectin
- Laminin
- Integrins

The ECM also contains molecules such as growth factors, cytokines, matrix degrading enzymes and their inhibitors.

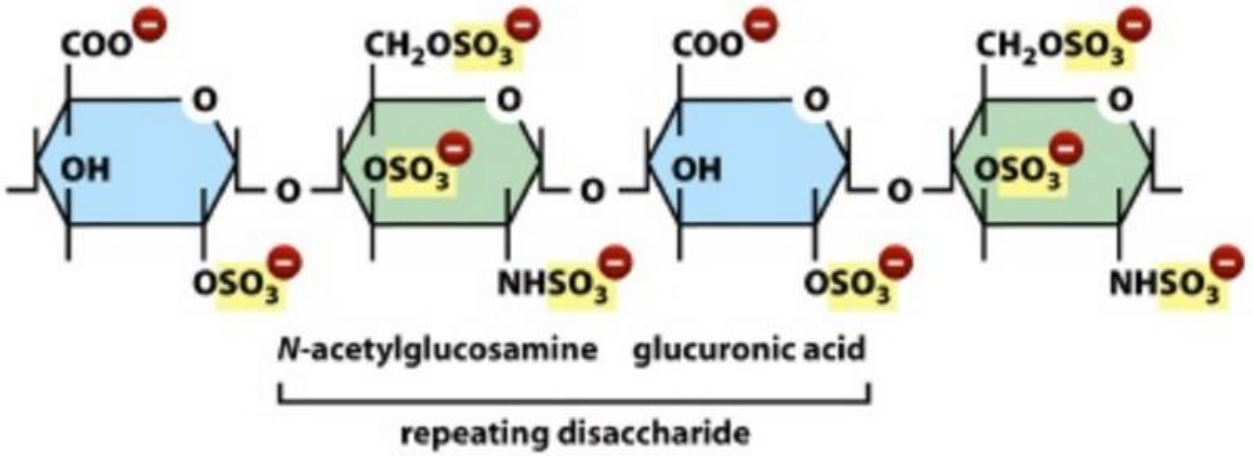
ECM Molecules

Glycosaminoglycans

- Glycosaminoglycans (GAGs) are unbranched polysaccharide chains that are composed of repeating disaccharide units.
- Glycosaminoglycans are always negatively charged because of the presence of sulfate or carboxyl groups on them.



Glycosaminoglycans (GAGs) are unbranched polysaccharide chains:



- Composed of repeating disaccharide units
- Too stiff to fold into compact globular shape
- Highly negatively charged due to sulfate or carboxyl groups on the sugars
- Highly hydrophilic
  - High density of negative charge attracts osmotically active cations

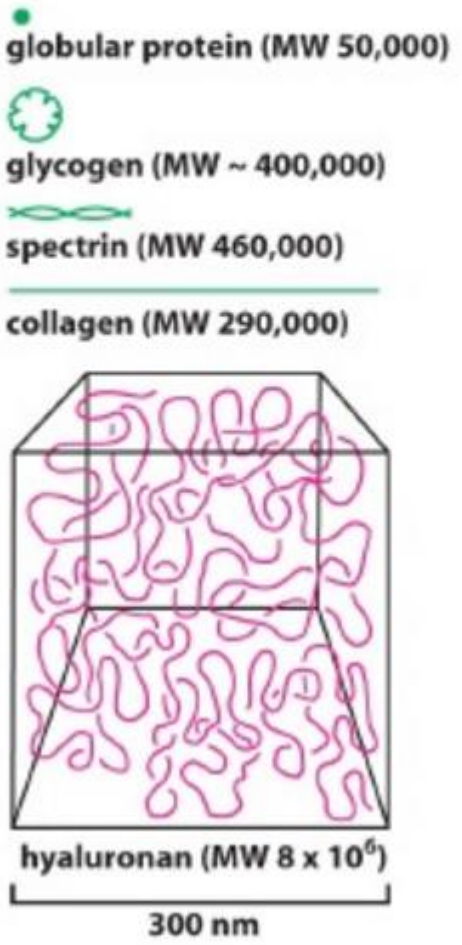
Gels of Polysaccharide and Protein Fill Spaces and Resist Compression

Dense, compact connective tissues (tendon, bone)
→ proportion of GAGs is small → very little water → matrix consists almost entirely of collagen
Other extreme = jelly-like substance in interior of eye → mainly one type of GAG → mostly water, → very little collagen.

GAGs in general;
strongly hydrophilic
adopt highly extended conformations
huge volume relative to their mass.
form gels at very low concentrations
multiple -ve charges attract cations → osmotically active → large amounts of water adsorbed into matrix

Create swelling pressure that is counterbalanced by tension in the collagen fibres and interwoven with the PGs.

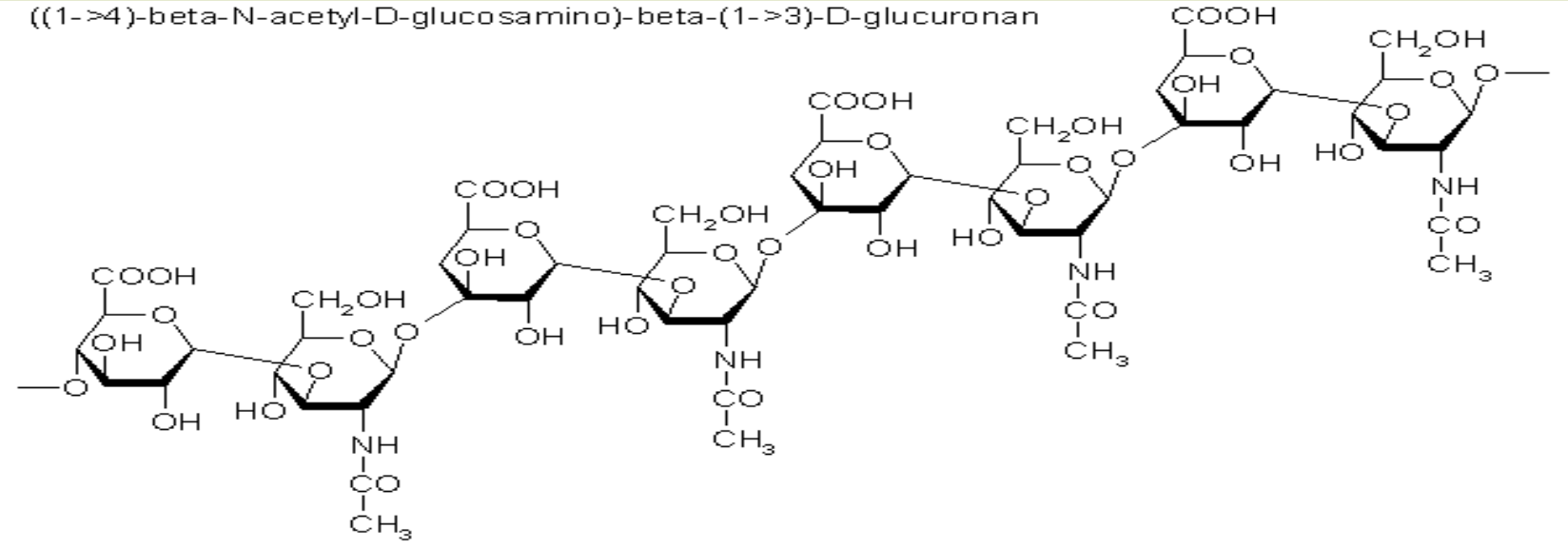
GAG chains occupy large amounts of space and form hydrated gels:



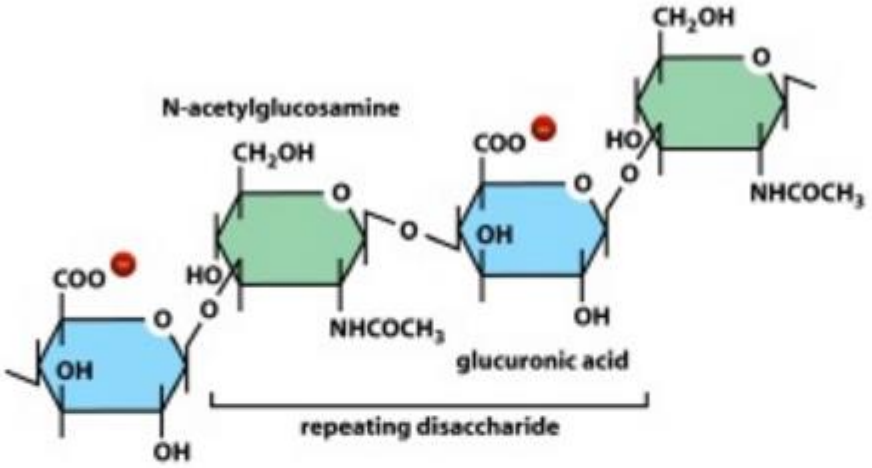
- Turgor
  - Withstand compressive forces
- Allow rapid diffusion of nutrients, metabolites, hormones between blood and tissues

Hyaluronan (hyaluronate)

- Hyaluronates are the simplest type of glycosaminoglycans and have not been sulphated, resulting in a regular repetition of more than 25,000 disaccharides.



Hyaluronan is a major polysaccharide component of the ECM:



- Present in all tissues and body fluids
- Simplest GAG
  - Regular repeating sequence of up to 25,000 disaccharide units
  - No sulfated sugars
  - Not linked to a core protein

- Hyaluronan is a glycosaminoglycan enriched in connective tissues
- Hyaluronan is highly negatively charged.
  - It binds to cations and water in the extracellular space.
    - This increases the stiffness of the extracellular matrix .
    - This provides a water barrier between cells that absorbs compressive forces.

Functions of hyaluronan:

- Resists compressive forces in joints and tissues
  - Important constituent of joint fluid
- In osteoarthritis, decreased concentration and decreased molecular weight of intra-articular HA

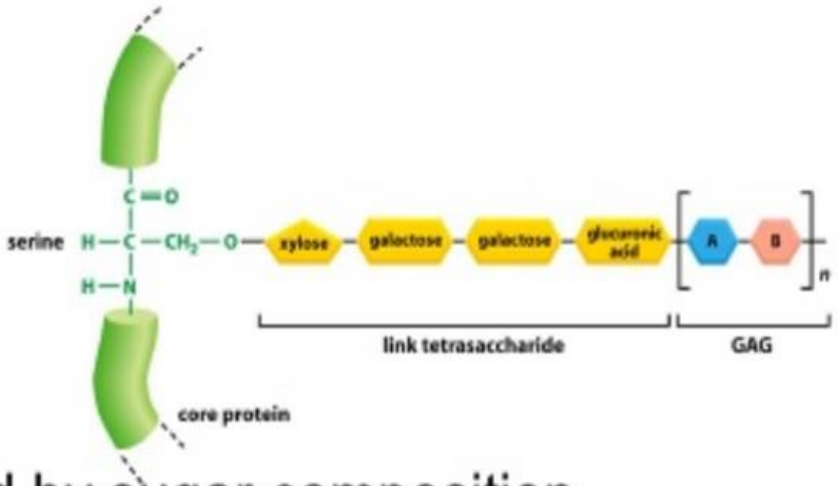
Role of HA in morphogenesis:

- Acts as space filler and facilitator of cell migration
  - If synthesized on the basal side of an epithelium, creates a cell-free space into which other cells can migrate

- Unlike other glycosaminoglycans, hyaluronans chains are:
  - synthesized on the cytosolic surface of the plasma membrane
  - translocated out of the cell

- Cells bind to hyaluronan via a family of receptors known as hyladherins.
  - Hyladherins initiate signaling pathways that control:
    - cell migration
    - assembly of the cytoskeleton

Proteoglycans are composed of GAG chains covalently linked to a core protein:



- Classified by sugar composition
  - Keratan sulfate, chondroitin sulfate, dermatan sulfate, heparan sulfate
- Modification of sugar residues allows for enormous diversity
- Associate with each other and with other ECM components to make complex meshworks

Function of Proteoglycans

- organize water molecules
  - resistent to compression
  - return to original shape
  - repel negative molecules
- occupy space between cells and collagen
- high viscosity
  - lubricating fluid in the joints
- specific binding to other macromolecules
- link to collagen fibers
  - form network
  - in bone combine with calcium salts (calcium carbonate, hydroxyapatite)
- cell migration and adhesion
  - passageways between cells
- anchoring cells to matrix fibers

Heparan sulfate

- Cell surface heparan sulfate proteoglycans
  - assist in the internalization of some proteins
  - act as co-receptors for:
    - soluble proteins such as growth factors
    - insoluble proteins such as extracellular matrix proteins
- Genetic studies in fruit flies show that heparan sulfate proteoglycans function in:
  - growth factor signaling
  - development

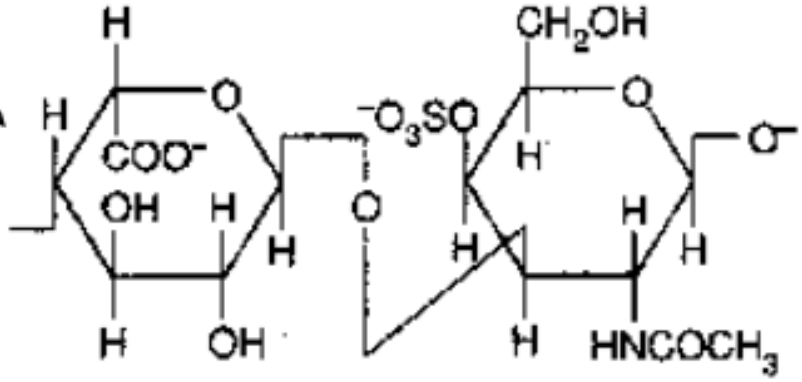
Glycosaminoglycans

GAG	Localization
Hyaluronate	synovial fluid, vitreous humor, ECM of loose connective tissue
Chondroitin sulfate	cartilage, bone, heart valves
Heparan sulfate	basement membranes, components of cell surfaces
Heparin	mast cells lining the arteries of the lungs, liver and skin
Dermatan sulfate	skin, blood vessels, heart valves
Keratan sulfate	cornea, bone, cartilage aggregated with chondroitin sulfates

Dermatan Sulphate:

absent in cartilage

identified in meniscus, tendon, skin and joint capsule



ECM Molecules

Fibrous Proteins

There are two functionally different types.

a) Structural proteins: For example: Collagen and elastin.

b) Adhesive proteins: For example: Fibronectin and laminin.

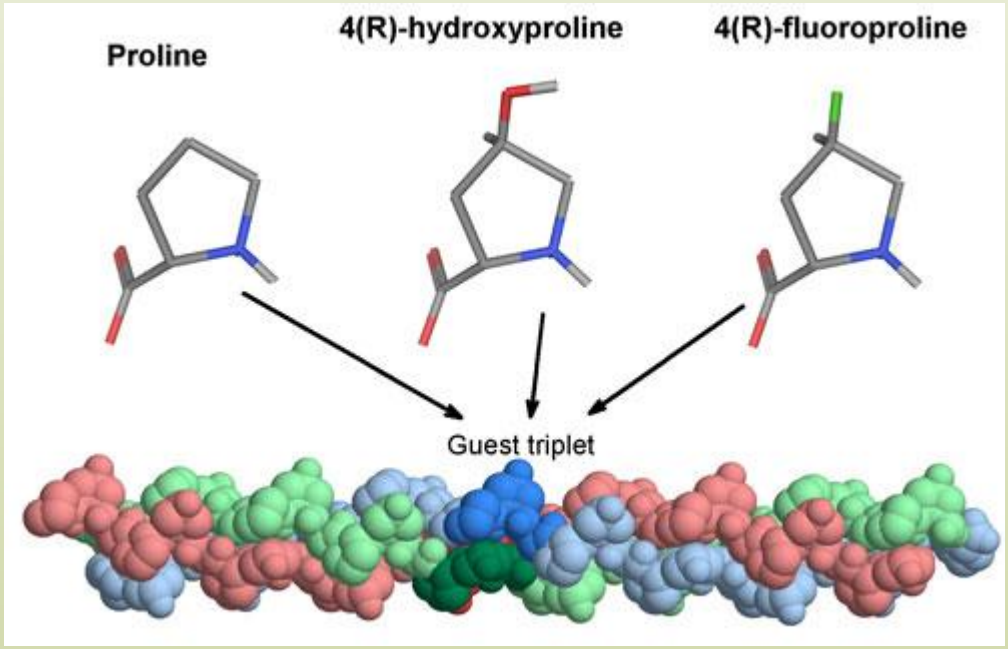
a) Structural proteins

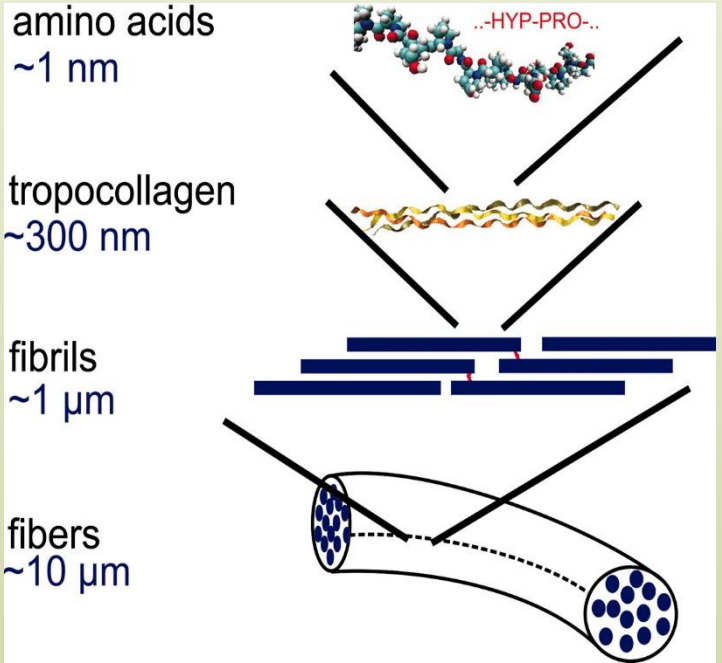
Collagen

- Collagen is the most abundant protein in the body.
- The most important structural protein in extracellular matrix.
- Collagen is the tissue that forms bone, cartilage, fibers and joints.
- 19 different types have been defined and are named type I, type II.
- Tropocollagen is the main molecule of collagen.

COLLAGEN

- Triple helical domain
- Repeated Gly - X - Y amino acid sequence, where X is often proline and Y hydroxyproline
- Collagen synthesis occurs in **fibroblasts** and is then secreted in the extracellular matrix.





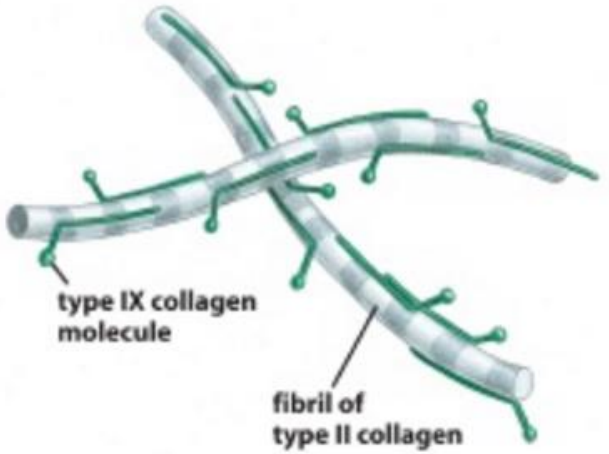
Collagen provides structural support to tissues

- The principal function of collagens is to provide structural support to tissues.

- Collagens are a family of over 20 different extracellular matrix proteins.
  - Together they are the most abundant proteins in the animal kingdom.

Different classes of collagen are organized in different ways:

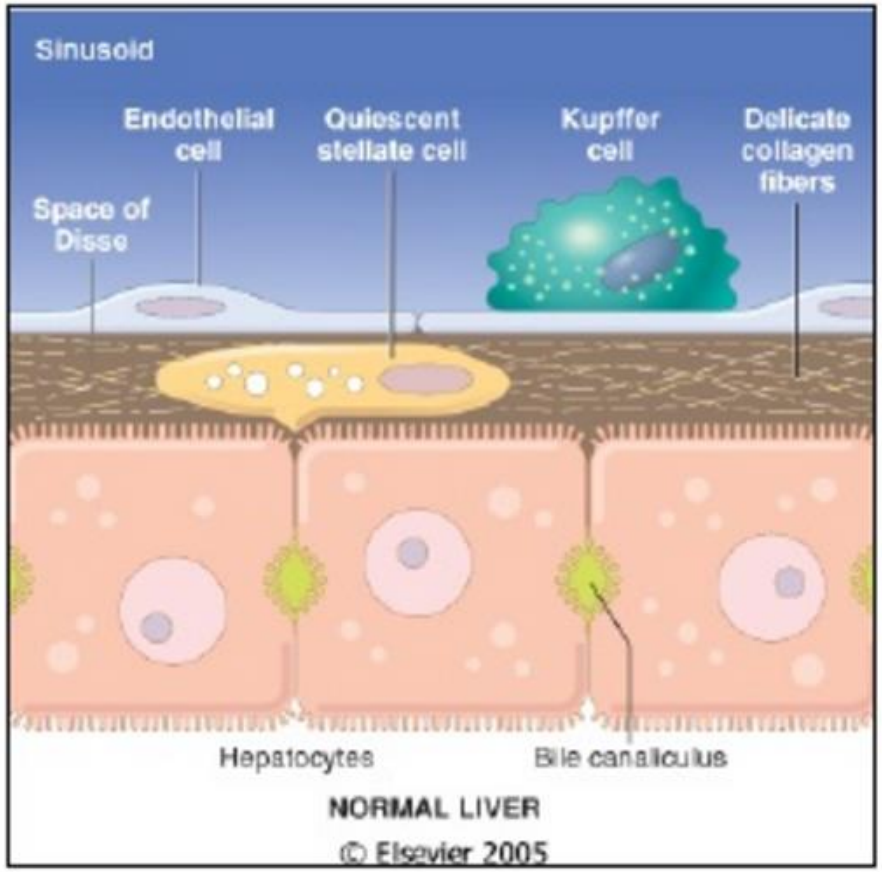
- Secreted fibril-associated collagens help organize the fibrils
- Fibrillar (fibril-forming)
  - Types I, II, III, V
  - Long, rope-like structures
  - principal collagen of bone and skin
- Fibril-associated
  - Type IX, Type XII
  - link fibrils to one another and to other components of ECM
- Network-forming
  - Type IV
  - major component of basal lamina
- Anchoring
  - Type VI
- Resistance to tensile forces



- **Type I**. The chief component of tendons, ligaments, and bones.
- **Type II**. Represents more than 50% of the protein in **cartilage**. It is also used to build the notochord of vertebrate embryos.
- **Type III**. Strengthens the walls of hollow structures like arteries, the intestine, and the uterus.
- **Type IV**. Forms the **basal lamina** of epithelia. (The basal lamina is often called the basement membrane, but is not related to lipid bilayer membranes.) A meshwork of Type IV collagens provides the filter for the blood **capillaries** and the glomeruli of the kidneys.

Collagen type IV forms a fine meshwork:

- More flexible structure than fibrillar collagens
- Interruptions of triple-helical structure
- Not cleaved after secretion
- Interact via uncleaved terminal domains to assemble into a flexible network



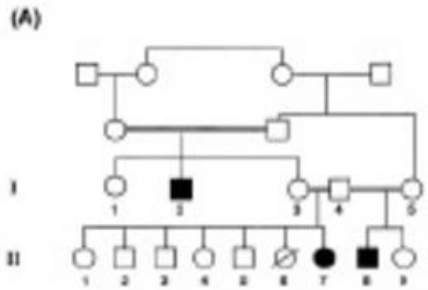
- Mutations of collagen genes can lead to a wide range of diseases, from mild wrinkling to brittle bones to fatal blistering of the skin.

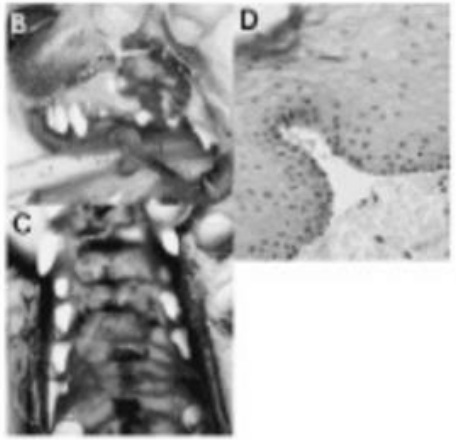
Osteogenesis Imperfecta

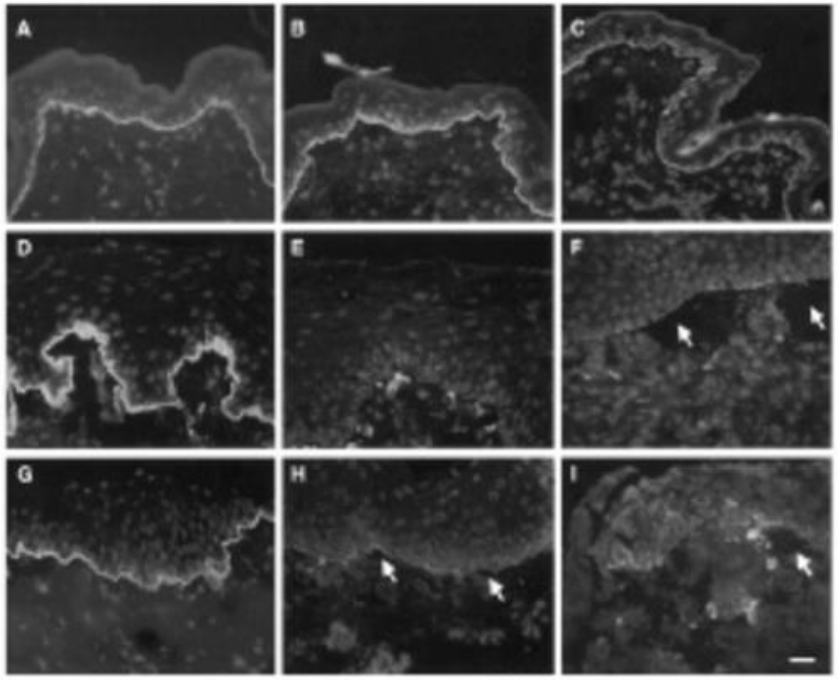
Type I	Type I	Type IV	Type IV	Type III	Type III
Female	Female	Male Age,	Female	Female	Male Age,
Age, 38 yr	Age, 63 yr	40 yr	Age, 35 yr	Age, 27 yr	40 yr
Height, 171 cm	Height, 137 cm	Height, 90 cm	Height, 124 cm	Height, 94 cm	Height, 84 cm

The deficiencies of some types of collagen lead to Osteogenesis Imperfecta which is resulted in easily crash of the bones within the body

Collagen VII defects cause blistering skin diseases:



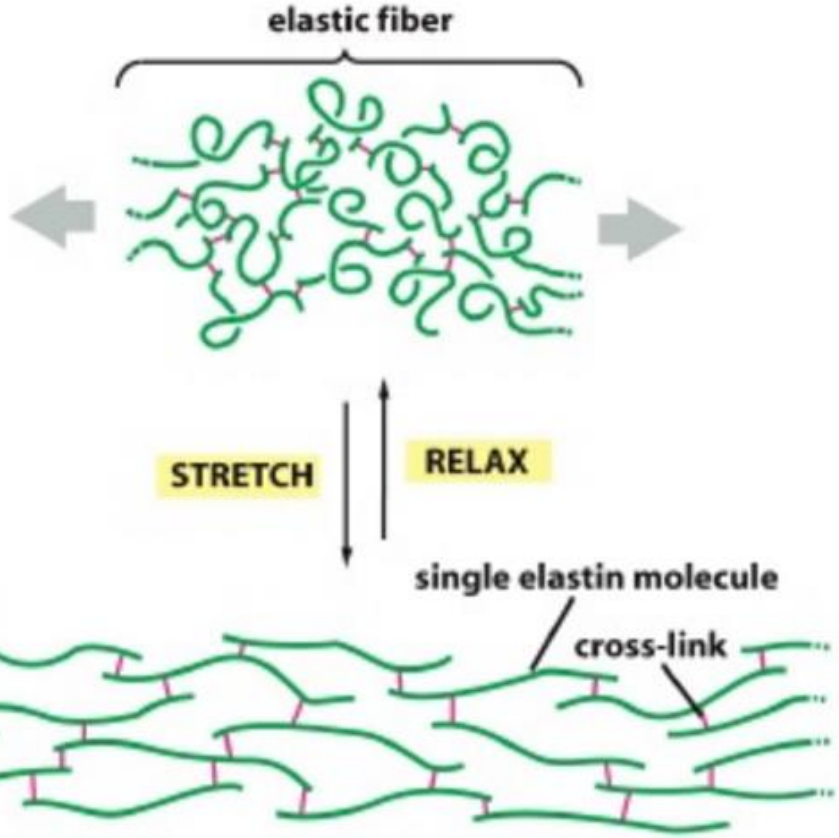


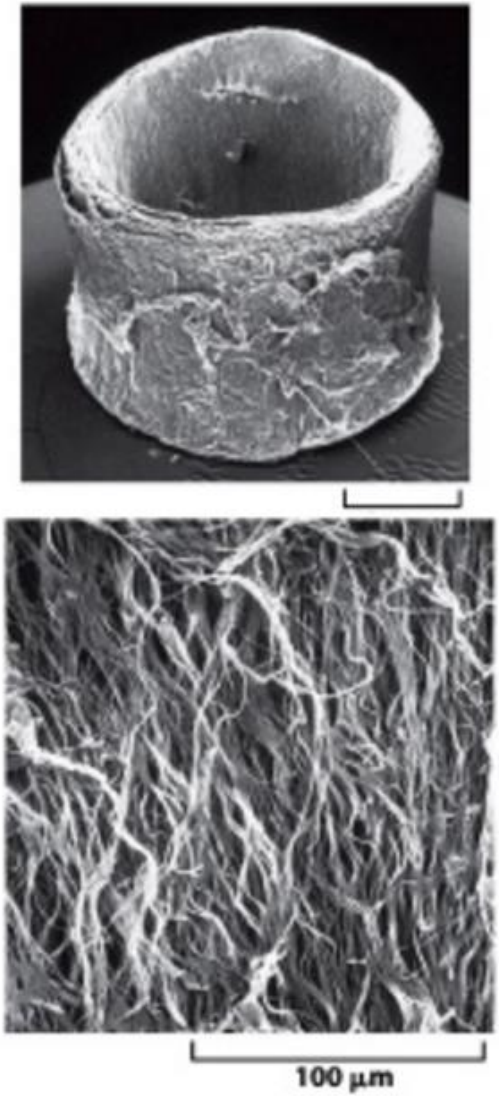


Elastin

- Elastin is the main element of elastic fibrils. Elastin is a highly hydrophobic protein, approximately 750 amino acids long, rich in proline and glycine, such as collagen.
- Elastin molecules are secreted into the extracellular space and are usually combined on the cell surface into elastin fibers that are very close to the cell membrane.

Elastin gives tissues their elasticity:





Elastic fibers impart flexibility to tissues

- The principal function of elastin is to impart elasticity to tissues.
- The strength of elastic fibers arises from covalent crosslinks formed between lysine side chains in adjacent elastin monomers.
- The elasticity of elastic fibers arises from the hydrophobic regions, which:
  - are stretched out by tensile forces
  - spontaneously reaggregate when the force is released

b) Adhesive Proteins

Fibronectin

- Fibronectin is one of the best known of the binding proteins.
- Fibronectin is a glycoprotein found in all vertebrates.
- These proteins have several binding sites for cell surface receptors and other matrix macromolecules.
- Thus, these proteins contribute to the organization of the matrix and to the maturation of the cells.

Fibronectin (crucial for angiogenesis)
A glycoprotein dimer connected by disulfite bonds at one end
Can exist in soluble or fibrillar forms

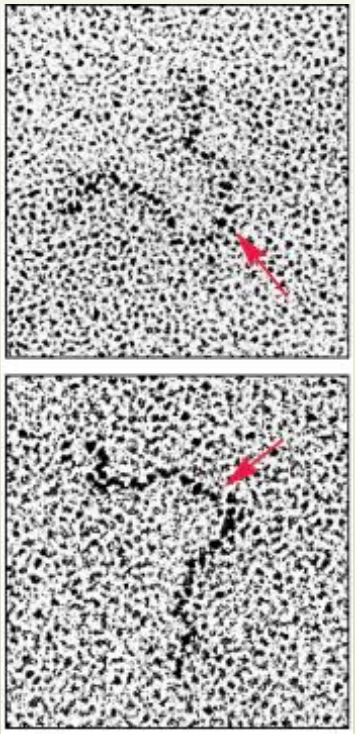


Figure 19–53 part 1 of

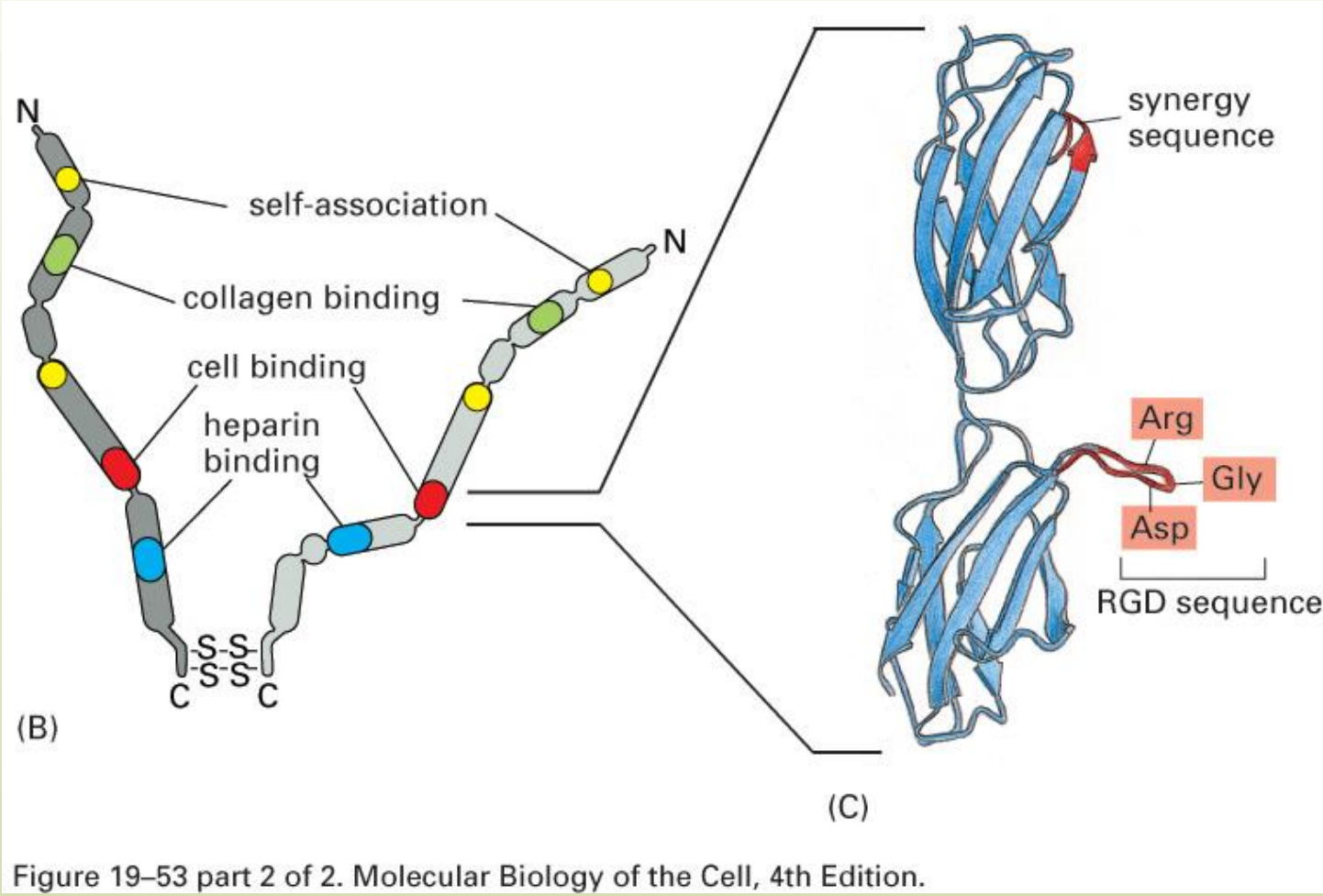
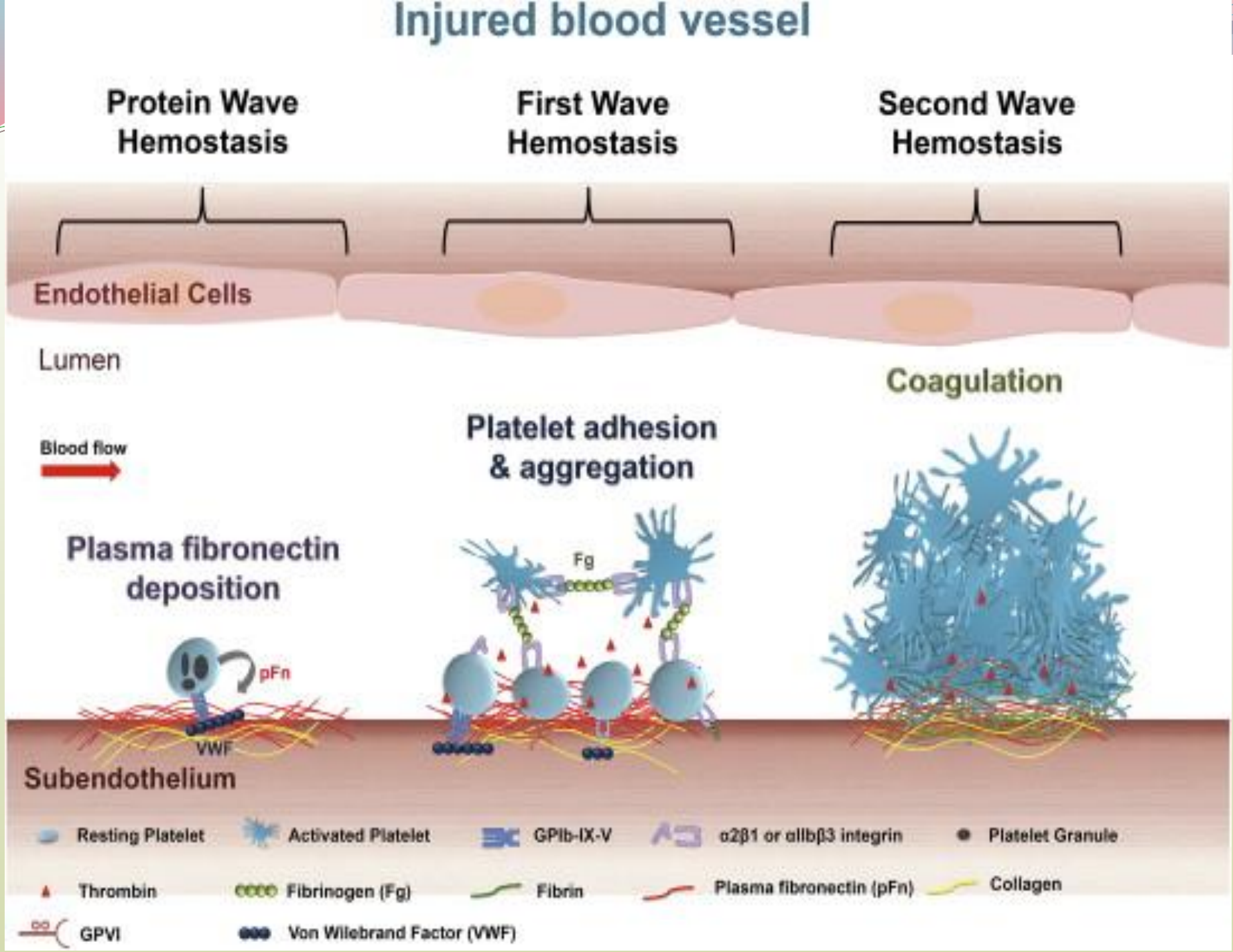


Figure 19–53 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Various isoforms of fibronectin are available.

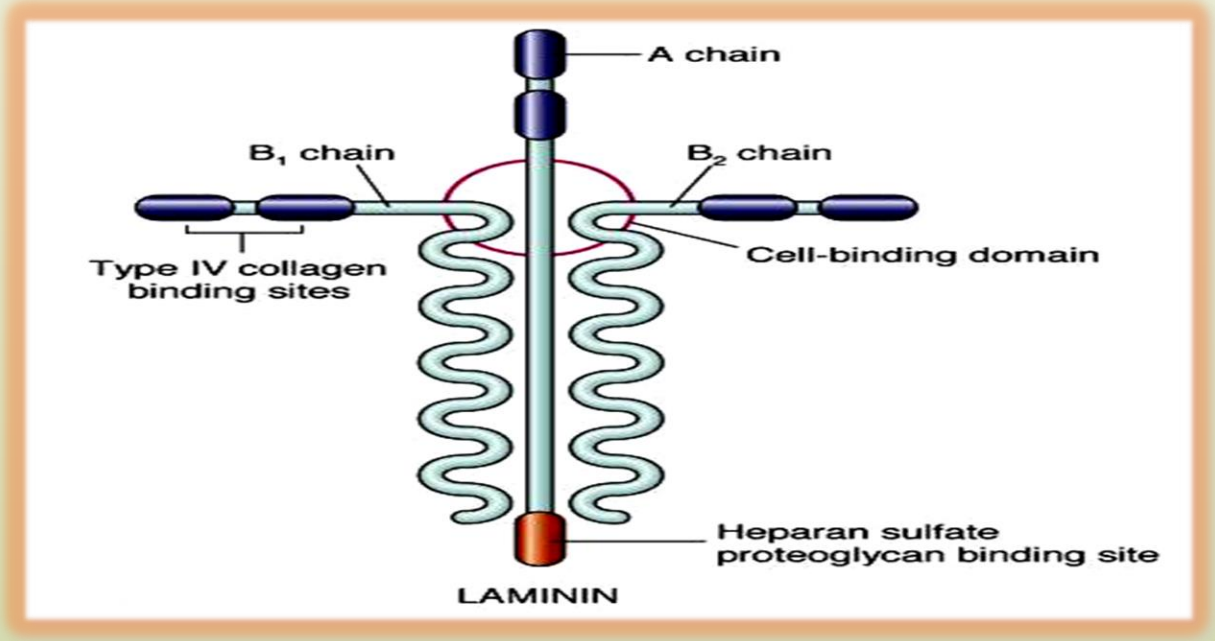
One of them is plasma fibronectin, which travels around blood and other body fluids due to soluble character. Plasma fibronectin is thought to increase blood clotting, wound healing and phagocytosis.

Other forms of fibronectin bind to the cell surface and are present as fibronectin filaments that are not soluble in the extracellular matrix.


Injured blood vessel
Protein Wave Hemostasis
First Wave Hemostasis
Second Wave Hemostasis
Endothelial Cells
Lumen
Coagulation
Blood flow
Platelet adhesion & aggregation
Plasma fibronectin deposition
Fg
pFn
VWF
Subendothelium
Resting Platelet
Activated Platelet
GPIb-IX-V
α2β1 or αIIbβ3 integrin
Platelet Granule
Thrombin
Fibrinogen (Fg)
Fibrin
Plasma fibronectin (pFn)
Collagen
GPVI
Von Willebrand Factor (VWF)

Laminin

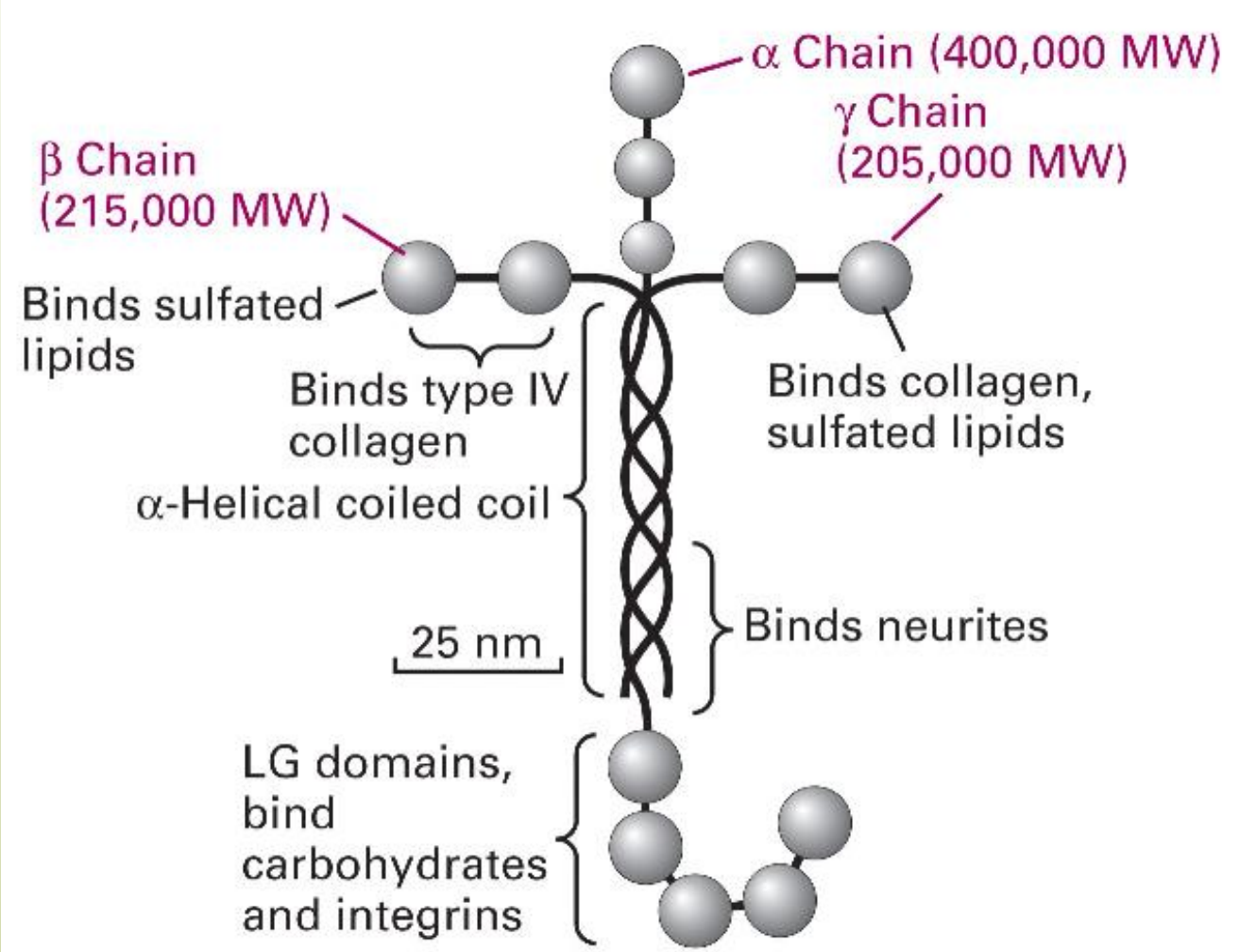
Laminin is a glycoprotein secreted by the epithelial cells. Laminin binds to cell surface receptors and other matrix proteins. This glycoprotein plays an important role in **cell adhesion**, **differentiation** and **motility**



Laminins – **multiadhesive matrix proteins**

Function: **organization of basement membrane; have binding sites for integrin receptors (important in embryonic development and tissue remodeling)**

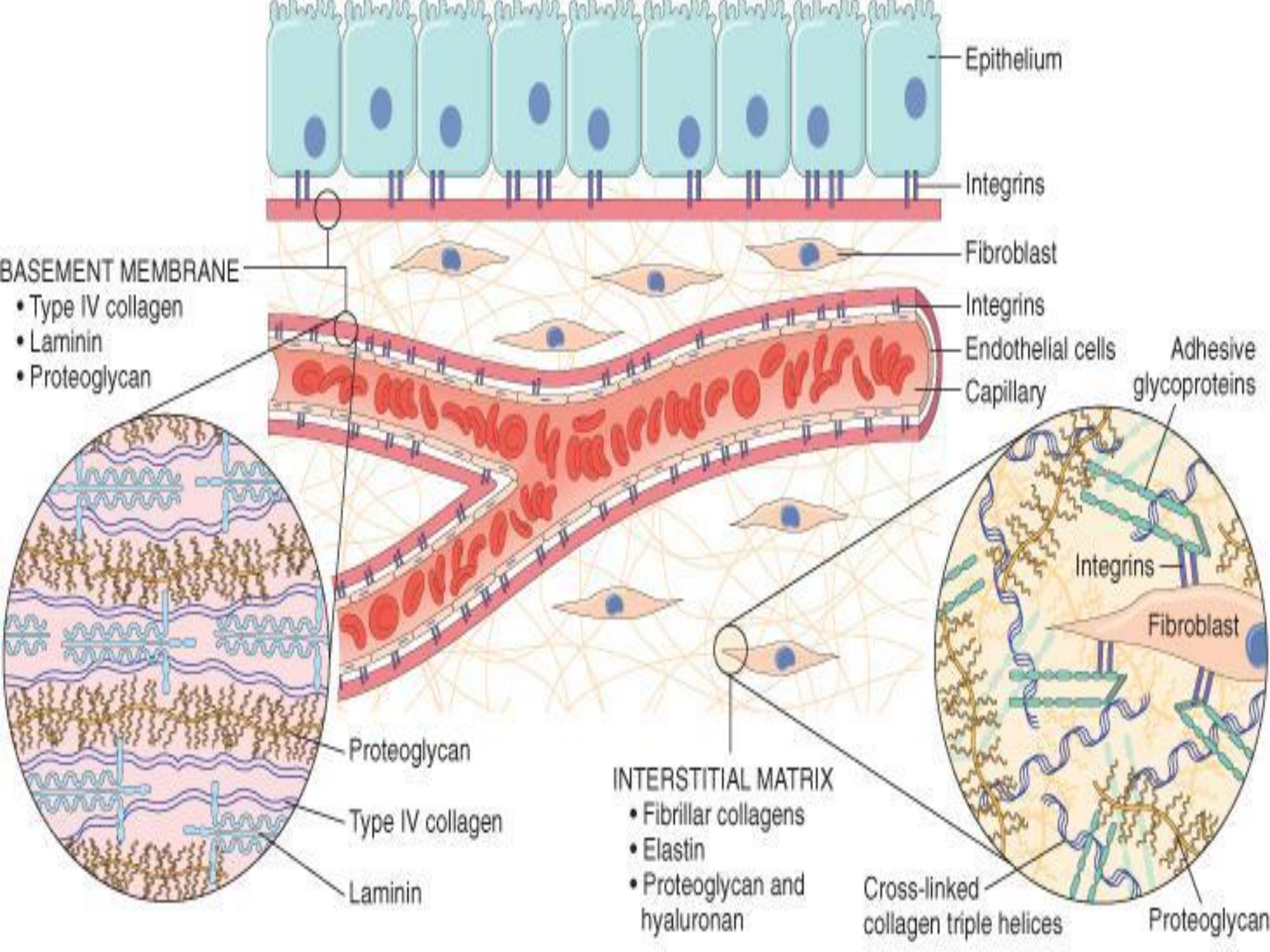
•Laminins are tightly associated with **entactin or nidogen** a 150-kD sulfated glycoprotein, which also binds to type IV collagen. As a result of these multiple interactions, laminin, entactin, type IV collagen, and perlacan form crosslinked networks in the basal lamina.



Basal Lamina

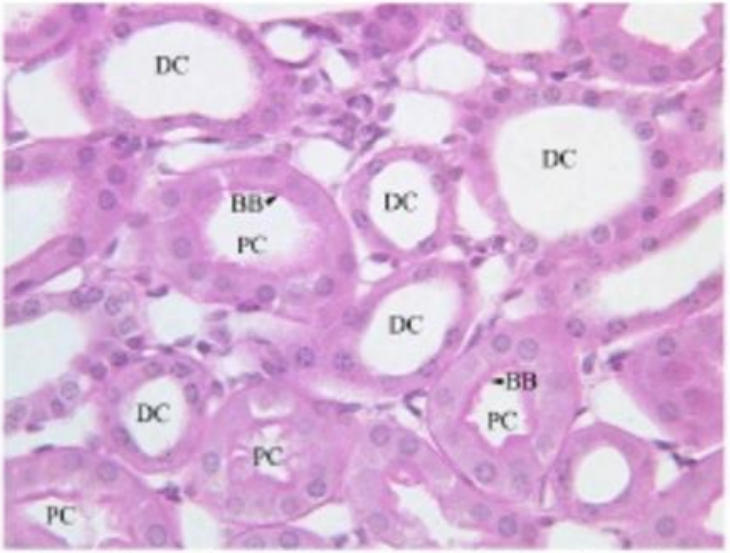
- The basal lamina is a flexible, thin (40-120 nm), specialized extracellular construct extending beneath all epithelial cell layers.
- **The basal lamina is synthesized by the adjacent tissues (epithelium and connective tissue).**

- Basal lamina *separate two tissues from each other*,
- Bind each tissue to other one
- serves as a diffusion barrier, support for cell division and migration.
- The substance exchange between the two tissues occurs via the basement membrane

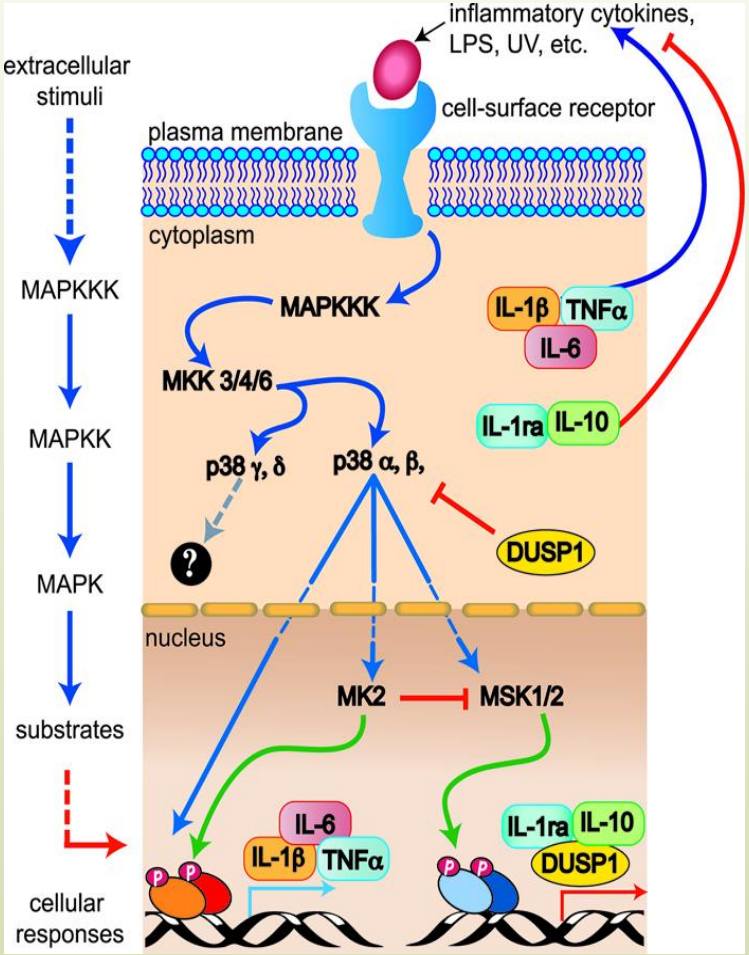

Epithelium
Integrins
Fibroblast
BASEMENT MEMBRANE
• Type IV collagen
• Laminin
• Proteoglycan
Integrins
Endothelial cells
Capillary
Adhesive glycoproteins
Integrins
Fibroblast
Proteoglycan
Type IV collagen
Laminin
INTERSTITIAL MATRIX
• Fibrillar collagens
• Elastin
• Proteoglycan and hyaluronan
Cross-linked collagen triple helices
Proteoglycan

Basal laminae have diverse functions:

- Structural
  - Critical role in the architecture of an organ
  - Mechanical connection between epithelia and underlying connective tissue
- Scaffold for tissue regeneration



Cytokines



•Cytokines are polypeptides produced and secreted by various cell types.

•Cytokines regulate immune and inflammatory events, including systemic responses to cell growth, healing and injury.

• The effects of cytokines start with binding to specific membrane receptors in target cells.

• The cellular response to cytokines is mediated by gene expression changes in target cells. Thus, target cells can develop new functions or proliferate.

Growth factors in ECM that plays active roles in wound healing

Growth Factor	Source	Functions
Platelet Derived Growth Factor (PDGF)	Thrombocytes, macrophages, endothelial cells, fibroblasts	Fibroblast proliferation, macrophage chemotaxis
Transforming Growth Factor Beta (TGF)	Thrombocytes, neutrophils, lymphocytes, macrophages	Fibroblast proliferation, Angiogenesis
Epidermal Growth Factor (EGF)	Thrombocytes, plasma, saliva, urine	Epithelial cells and fibroblast proliferation, stimulation of granulation
IL-2	lymphocytes, macrophages	Fibroblast proliferation, neutrophil chemotaxis

- Growth factors and cytokines interact with the ECM in a variety of ways.

- The ECM can function as a reservoir by binding growth factors and cytokines and protect them from degradation by delivering them more efficiently to their receptors or by affecting their synthesis.

- The ECM may affect the biological activity and local concentration of these factors.

- For example, neutrophils produce high levels of tumor necrosis factor (TNF) when bound to fibronectin.

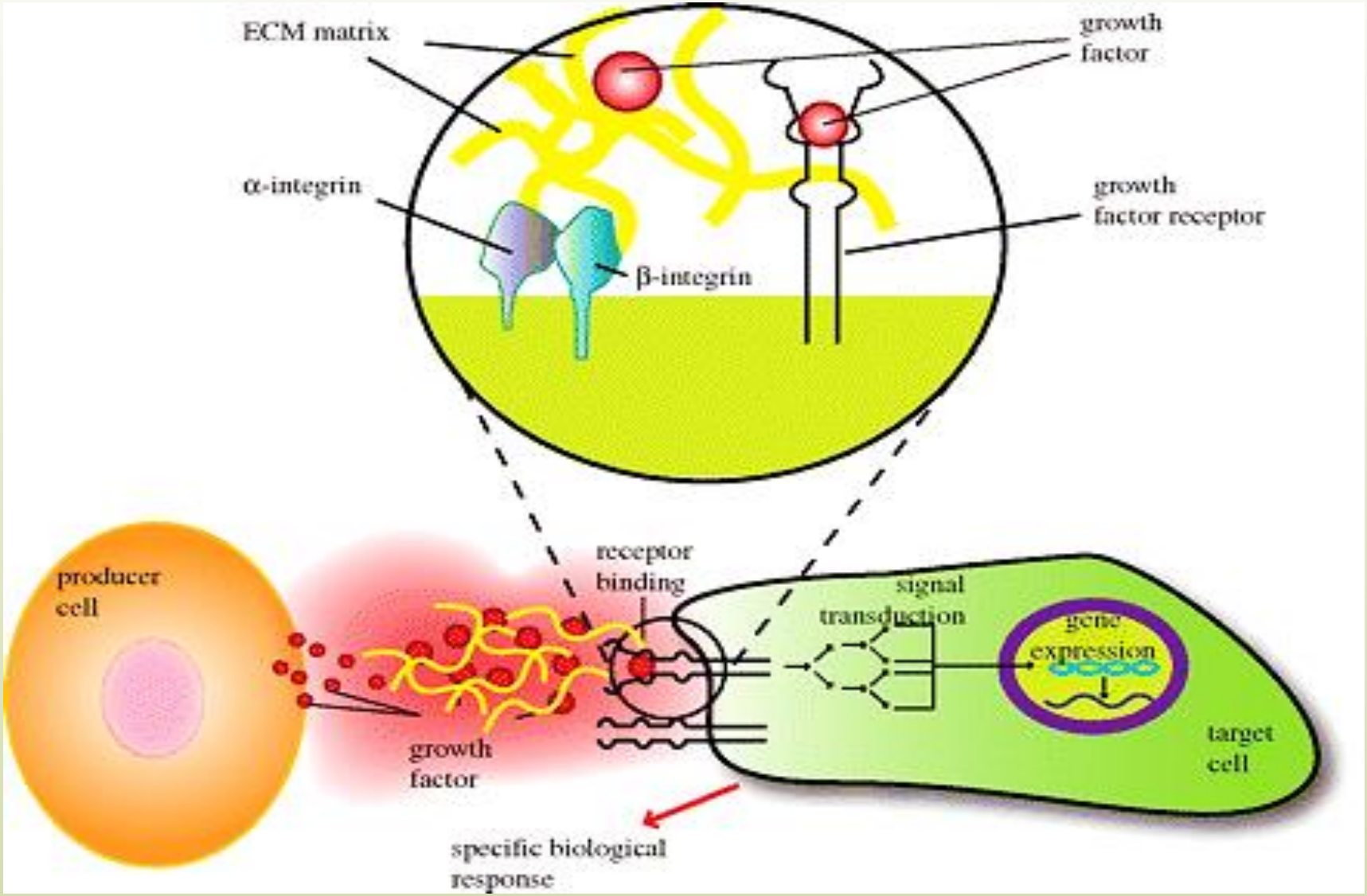
- Conversely, growth factors and cytokines may stimulate the cells to alter the production of ECM molecules, their inhibitors and / or their receptors.

- For example, growth hormone beta (TGF-beta) regulates expression of matrix molecules and enzyme inhibitors that reduce ECM molecules.

Interactions between cells and the ECM:

- Cells synthesize, organize, and degrade ECM
- Matrix influences cellular behavior
  - Tissue architecture
- Cells interact with ECM via matrix receptors
  - Integrins
  - Transmembrane proteoglycans

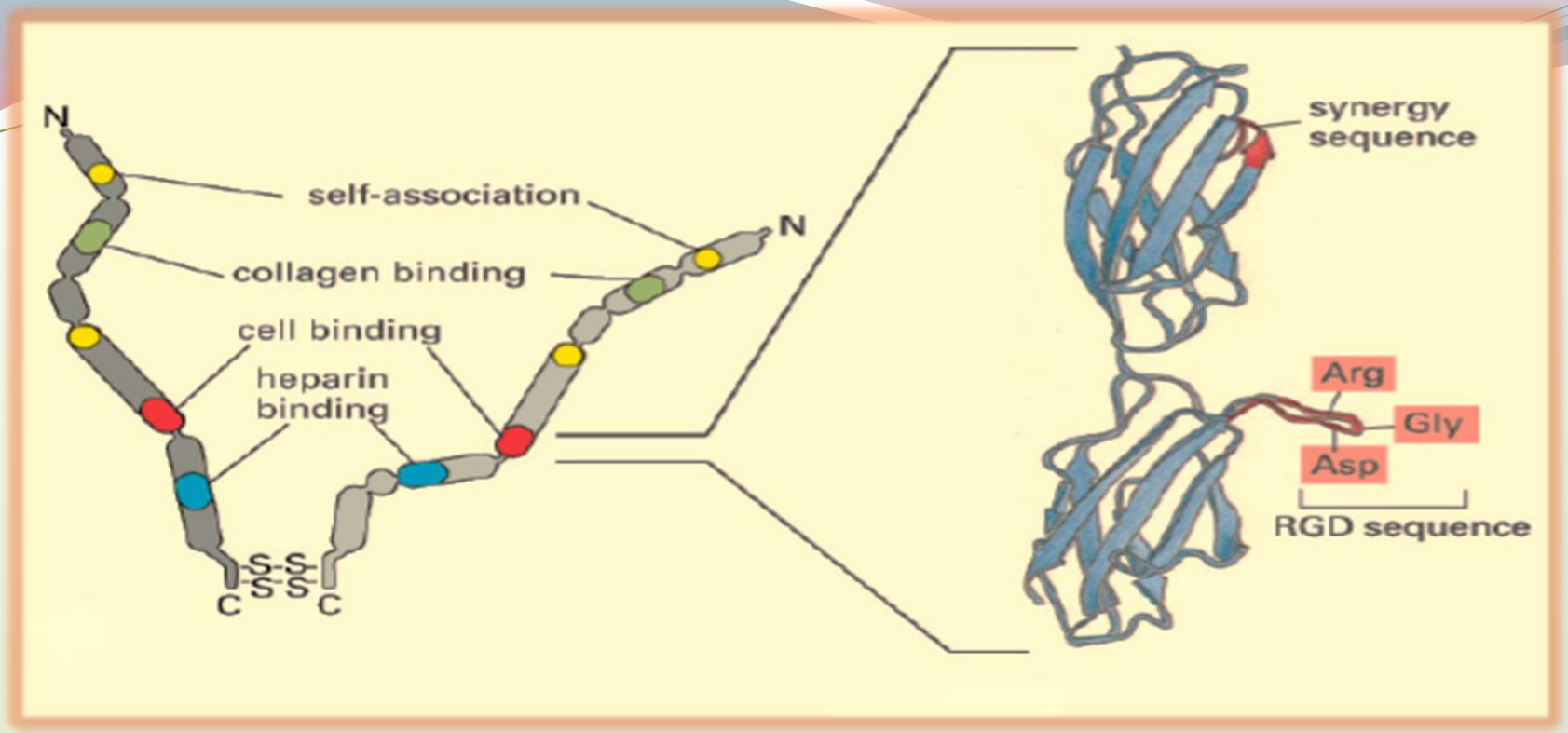
Receptors for ECM Molecules


ECM matrix
growth factor
α-integrin
growth factor receptor
β-integrin
producer cell
receptor binding
signal transduction
gene expression
target cell
growth factor
specific biological response

- For an effective ECM-cell interaction, receptor-ligand harmony is very crucial.

- Using biochemical and molecular biological approaches, it has been shown that various ECM molecules contain specific amino acid motifs for direct binding to cell surface receptors.

- The best characterized motif is the tripeptide RGD (Arginine-Glycine-Aspartic Acid) first found in fibronectin.



- Peptides containing these amino acid sequences increase cell adhesion
- These and other adhesive amino acid motifs are found in laminin, entactin, thrombin, tenascin, fibrinogen, vitronectin, type I and VI collagen, bone sialoprotein and osteopontin.

•*Integrins*

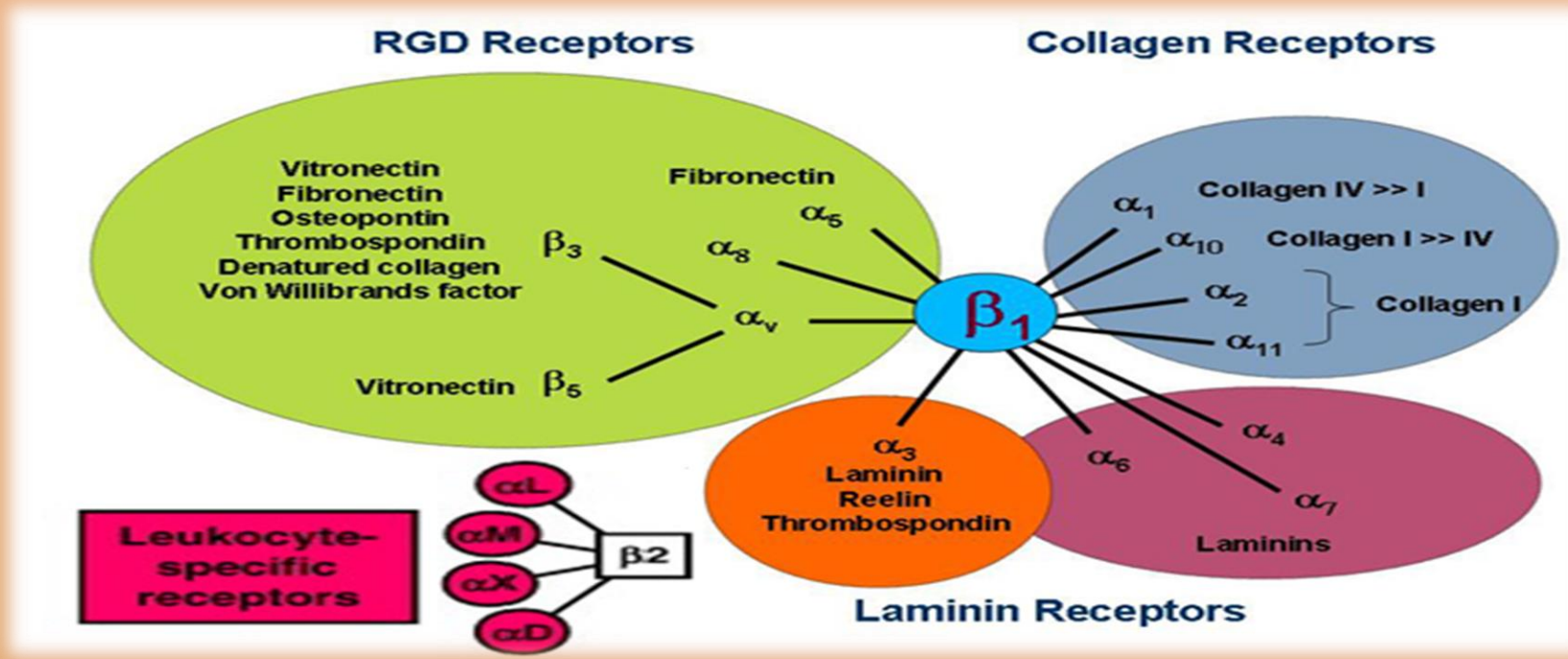
Integrins are the first ECM receptors to be detected (Hynes, 1987). These receptors are heterodimeric transmembrane proteins consisting of α and β subunits.

Up to now, at least 15 α and 8 β subunits have been defined. They form pairs in various combinations with each other. This leads to the formation of a large family that recognizes specific sequences on ECM molecules.

While some integrin receptors are very specific, some may bind several different epitopes on the same or different ECM molecules.

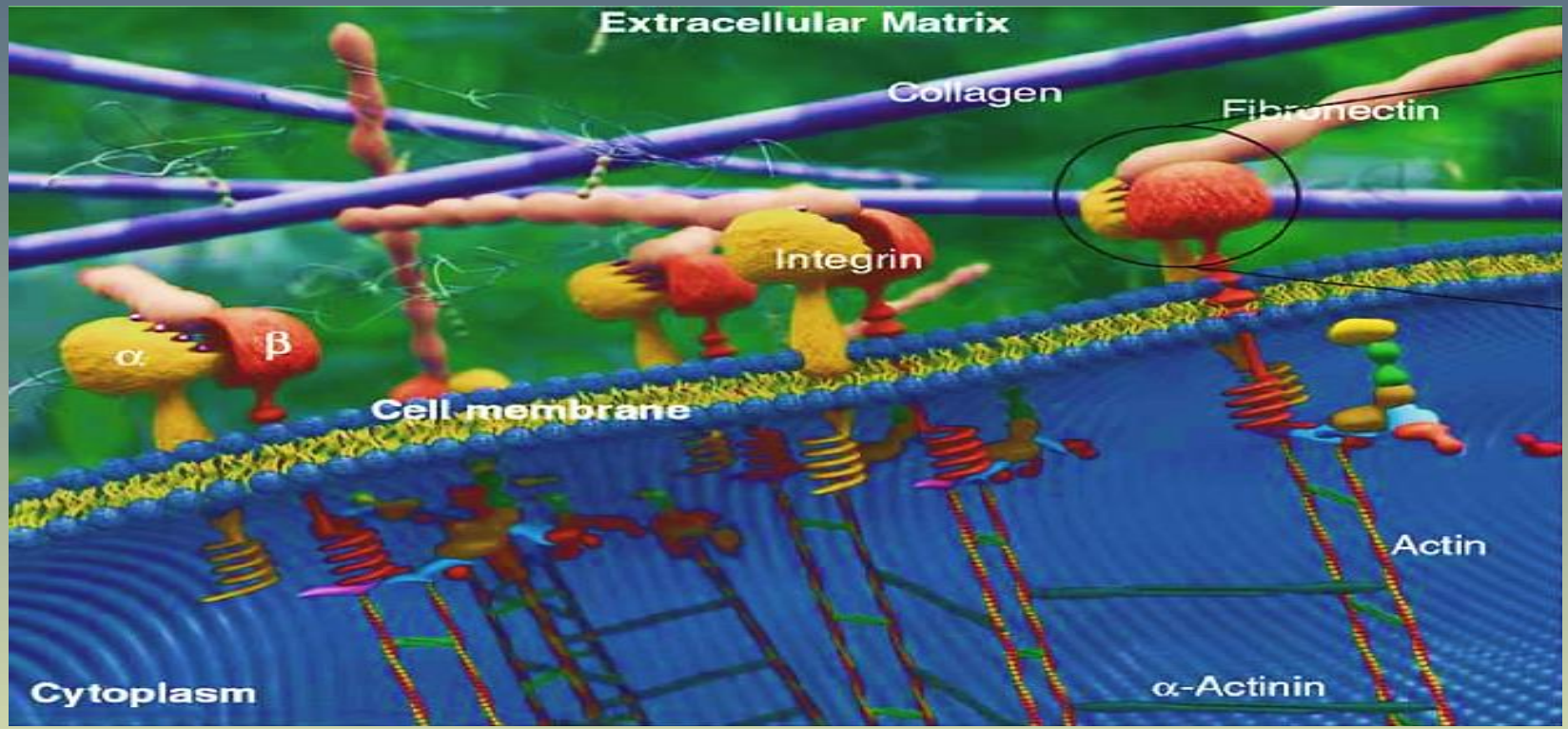
- ***The β1 and β3 classes mediate cell-matrix adhesion while the β2 class mediate cell-cell adhesion.***

- ***In addition, β3 class binds to vascular ligands such as fibrinogen, vitronectin, thrombospondin in general, whereas β1 class binds to connective tissue macromolecules such as fibronectin, laminin and collagen in general.***



- With ligand binding, integrins directly initiate biochemical signals within the cell.

- ECM signals are transmitted through the cytoskeletal elements throughout the integrins and induce cell morphology that causes growth and / or differentiation.



- ***Transmembrane proteoglicans***

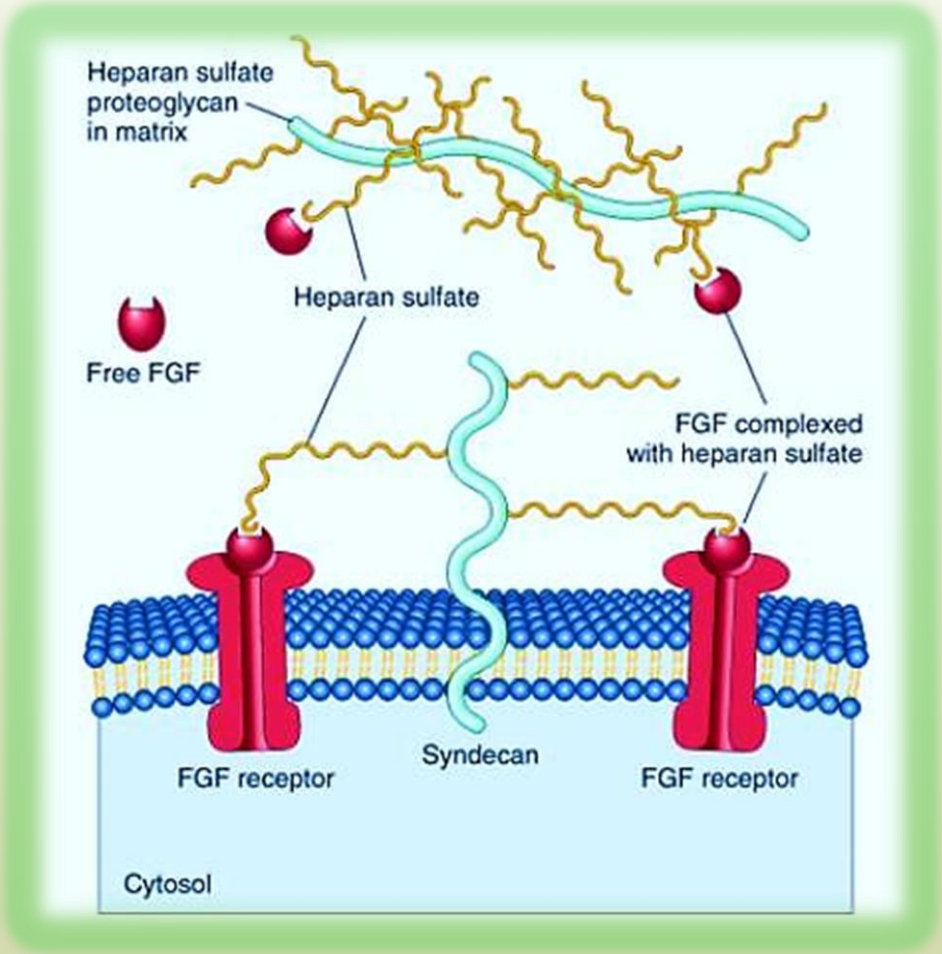
Proteoglycans have also been found to be receptors for ECM molecules.

At least four types of proteoglycan receptors that bind to ECM molecules have been isolated and characterized. **These are ;**

1. Syndecan,
2. CD44,
3. RHAMM and
4. trombomodulin.

Syndecan binds cells to extra cellular matrix via chondroitin sulphate and heparan sulphate glycosaminoglycans.

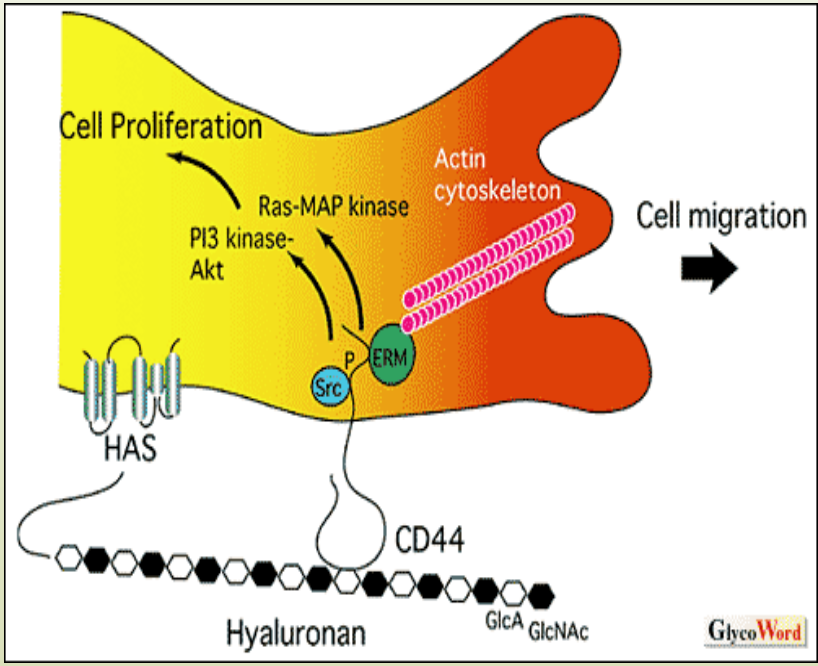
It is also associated with cystic cytoskeleton. That is, it plays an important role in signal transduction along the cell surface receptor.



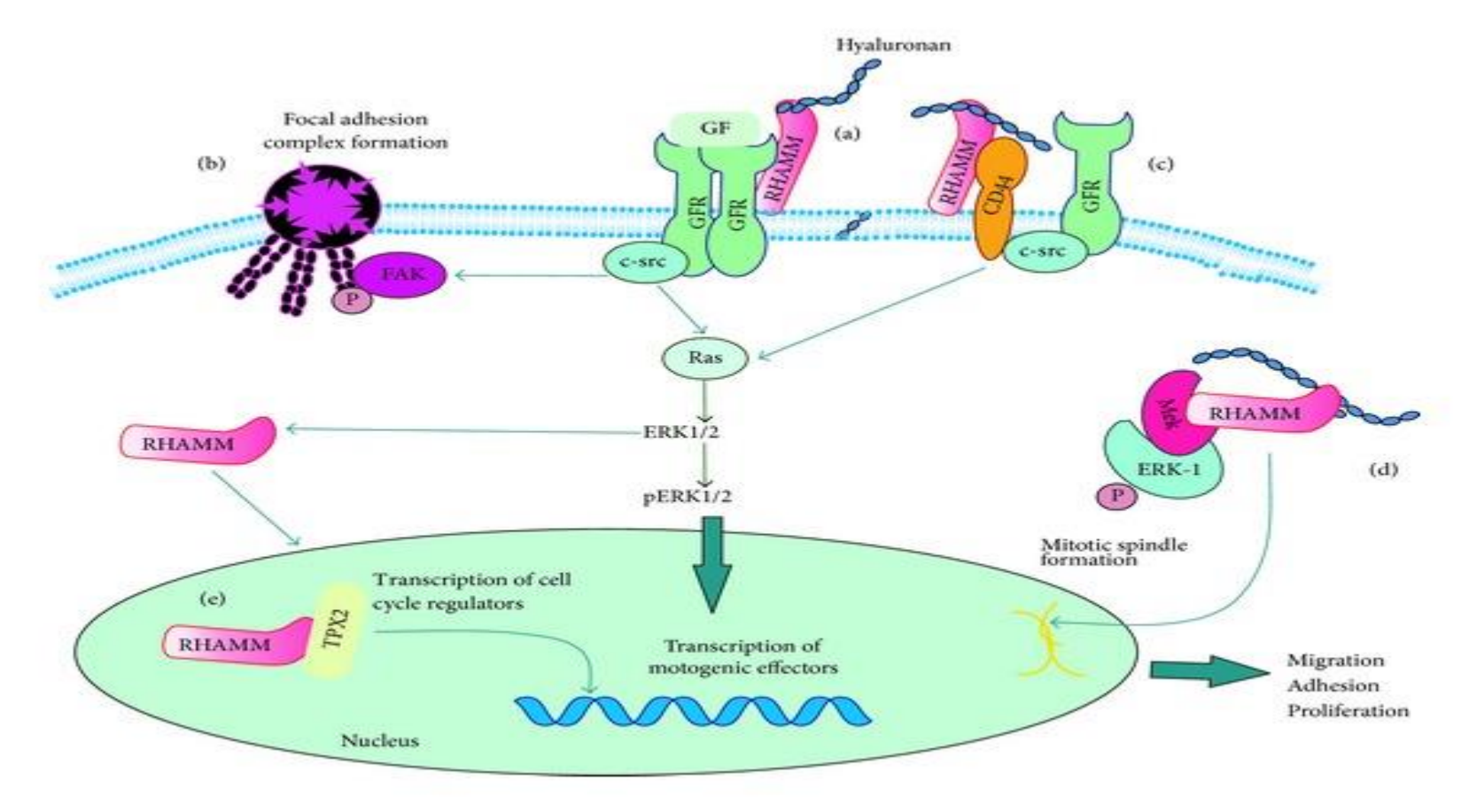
CD44 receptor can directly interact with **hyaluronan molecules**

Hyaluronan, which is synthesized by hyaluronan synthases, induces the activation of intracellular signaling molecules and cytoskeletal rearrangement via hyaluronan receptors such as CD44

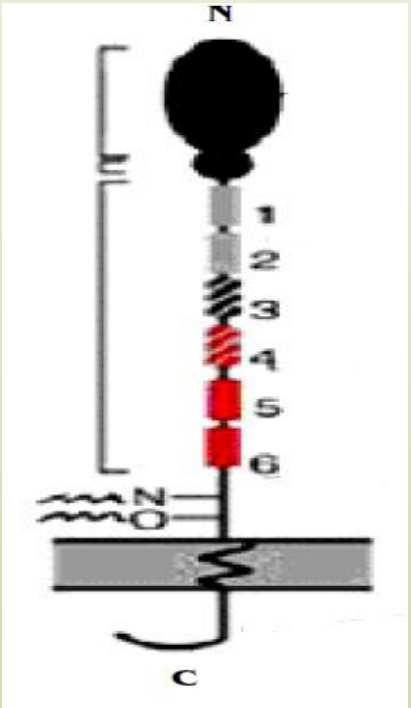
This lead to active cell migration

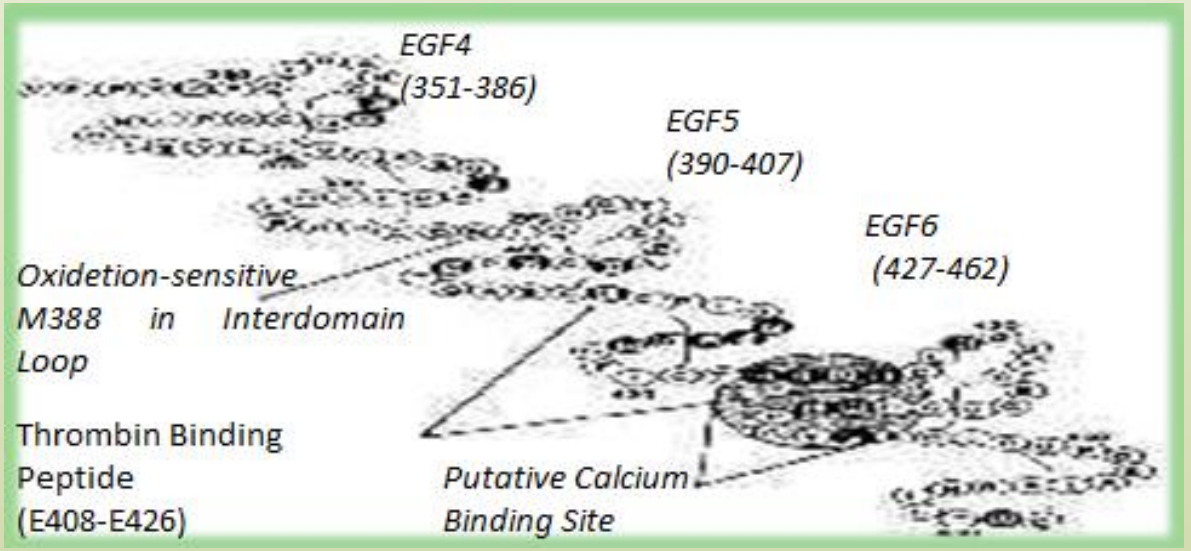


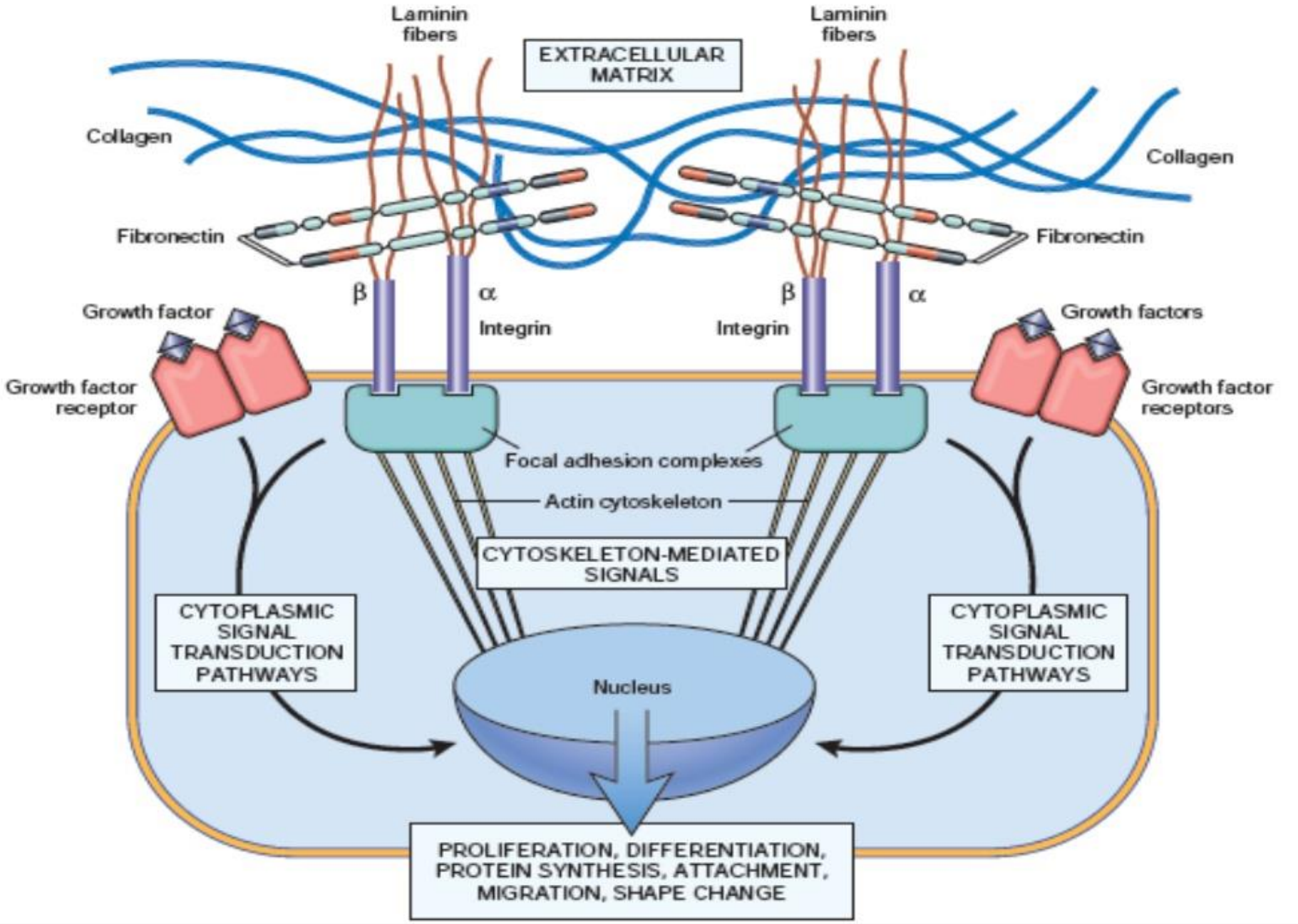
RHAMM is another important receptor for ECM molecules. It can also bind hyaluronan and play an active role in motility and cell migration



Trombomodulin is a membrane protein found on the surface of the endothelium and has an anticoagulant property that prevents blood from coagulation in the vein. The biological activity can be regulated either by proteins or by glycosaminoglycan components.






Laminin fibers
Laminin fibers
EXTRACELLULAR MATRIX
Collagen
Collagen
Fibronectin
Fibronectin
β
α
β
α
Integrin
Integrin
Growth factor
Growth factors
Growth factor receptor
Growth factor receptors
Focal adhesion complexes
Actin cytoskeleton
CYTOSKELETON-MEDIATED SIGNALS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
CYTOPLASMIC SIGNAL TRANSDUCTION PATHWAYS
Nucleus
PROLIFERATION, DIFFERENTIATION, PROTEIN SYNTHESIS, ATTACHMENT, MIGRATION, SHAPE CHANGE

CELL-ECM INTERACTIONS

- Interactions of cells with extracellular matrix molecules play an important role during
- tissue development and wound healing.
- The continuous interaction between the cells and the matrix surrounding the cells contributes to the process of clot formation during wound healing, inflammation, development of granulation tissue, and remodeling.

Basic Cellular Mechanisms;

- ✓ *Cell adhesion*
- ✓ *Migration*
- ✓ *Proliferation*
- ✓ *Differentiation*
- ✓ *Programmed cell death (apoptosis)*

TISSUE DEVELOPMENT

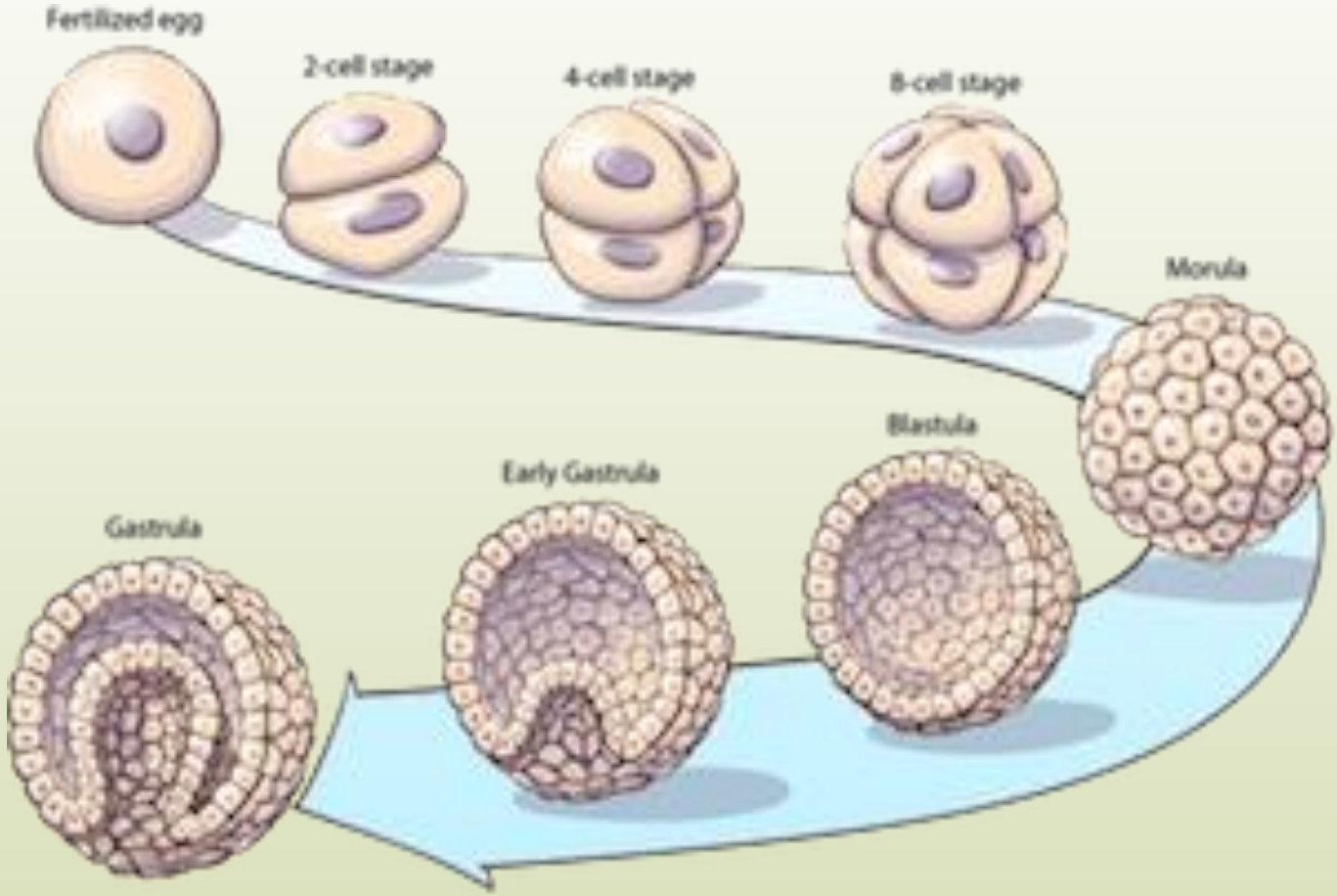
Adhesion and Migration

- Gastrulation stage of embryonic development
- Neural crest cells' (NCCs) migration
- Angiogenesis
- Epithelium formation in tissues

At these stages, ECM molecules directly contribute to cell adhesion and migration.

⊙ Gastrulation

- In animals, morula, blastula, and gastrula stages are seen during zygote development.
- Gastrulation is a phenomenon seen in almost all of the multiple cells after the blastula stage.
- In this phase, ectoderm and endoderm occur. When the ectoderm is forming body-covering structures, endoderm compose the intestines and the structures related to it.



Cell Cleavage

Process by which the number of cells in a developing embryo is multiplied through cell division.

- *Fibronectin-cell interactions are important during gastrulation.*

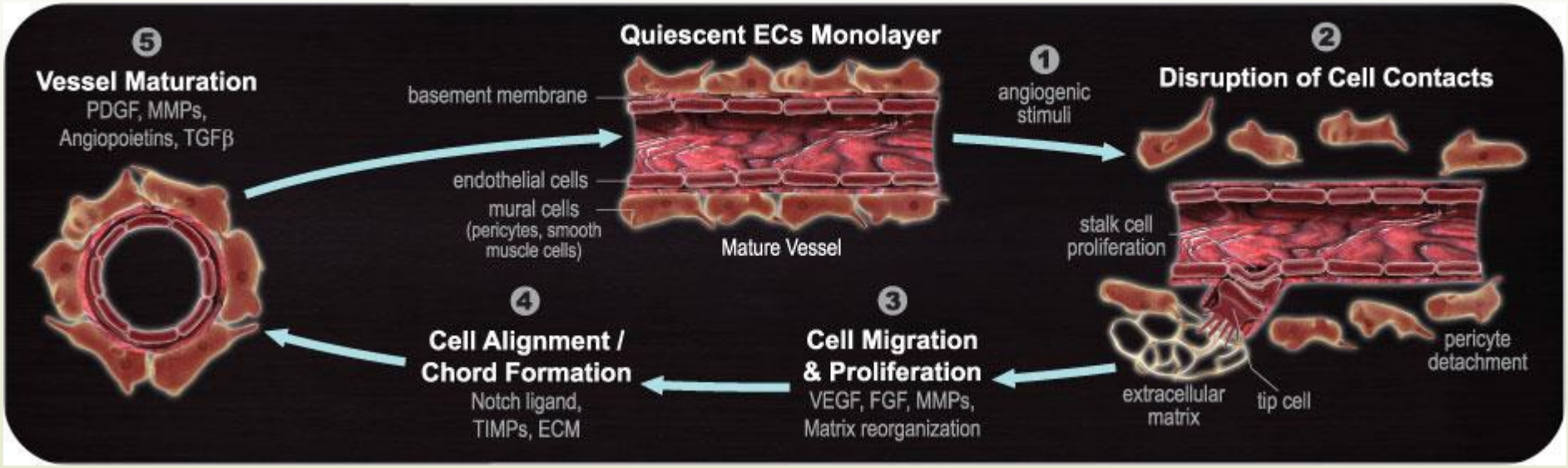
- The fibronectin-specific antibody microinjection in the blastosol clearance of Xenopus embryos disrupts normal cell movements

- Similarly, after microinjection of RGD (arginine-glycine-aspartate) -containing peptides and / or antibodies, these constructs bind to the β1 subunit of the integrin fibronectin receptor. Thus, the binding of fibronectin is inhibited and the gastrulation of the embryos of Pleurodeles salamanders is impaired.

⊙***Angiogenesis*** is the formation of new capillaries from already existing vessels.

During angiogenesis, the interaction of endothelial cells with ECM molecules, matrix type and conformation play an important role in cell migration and vascular development.

ECM binding to integrins provides critical signaling support for endothelial cell proliferation, survival, and migration. ECM also signals the endothelial cytoskeleton to initiate blood vessel morphogenesis.



ECM molecules such as laminin, fibronectin and collagen type 1 plays significant role in angiogenesis

Programmed Cellular Death (Apoptosis)