

Lecture 9: the extracellular matrix and cell-cell interaction

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The extracellular matrix (ECM)

- ▶ The extracellular matrix fills the spaces between cells and binds cells and tissues together.
- ▶ Composed of different types of proteins and polysaccharides

- ▶ Types:

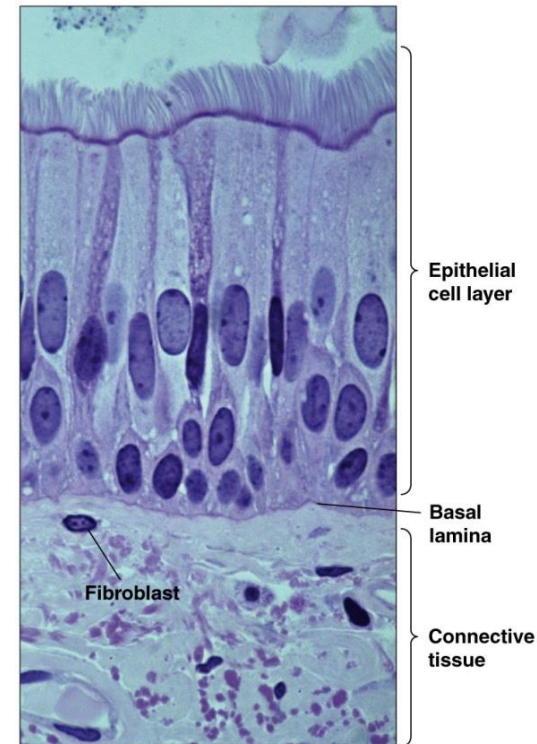
1. Basal laminae (basements membranes): thin, sheet-like, structure upon which layers of epithelial cells rest

- It supports sheets of epithelial cells

- It surrounds muscle cells, adipose cells, and peripheral nerves.

2. Connective tissues (more ECM)

- Loose network of proteins and carbohydrates underneath epithelial cell layers where fibroblasts are distributed.
- Others: bone, tendon, and cartilage.



Matrix structural proteins

► Components:

1. Tough **fibrous proteins** embedded in a gel-like **polysaccharide ground substance**.

2. Adhesion proteins that link components of the matrix both to one another and to attached cells.

► Differences in the type and amount of different components make different types of ECM

Different ECM composition

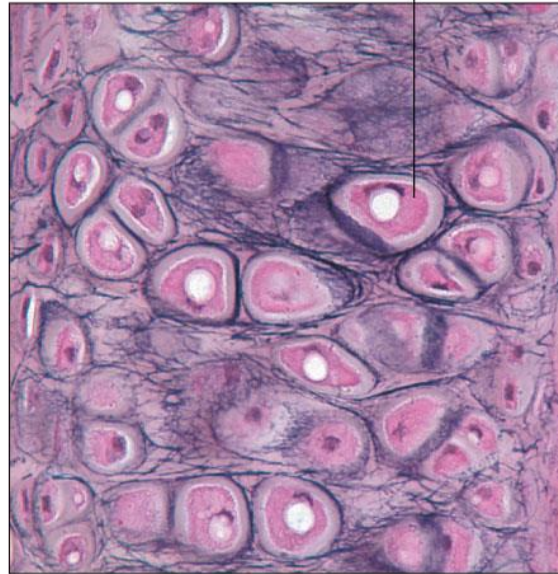


(a) Bone

20 μ m

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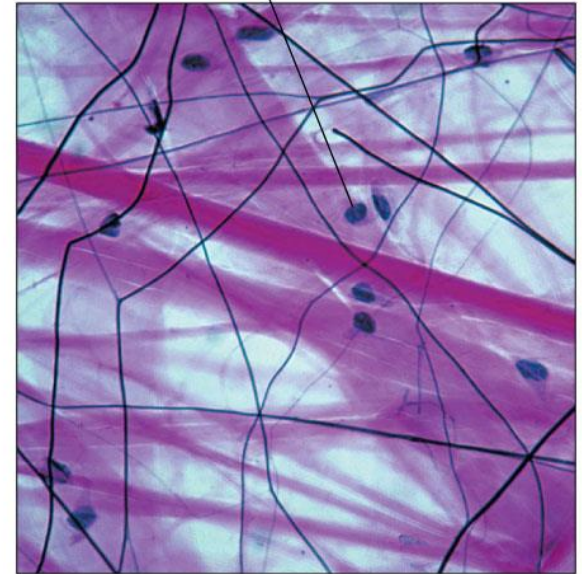
In bone, the extracellular matrix is hardened by deposition of calcium phosphate crystals.



(b) Cartilage

20 μ m

Cartilage contains a high concentration of polysaccharides that form a firm compression-resistant gel.



(c) Connective tissue

20 μ m

Tendons contain a high proportion of fibrous proteins.

Collagens: the major structural protein of the ECM

- ▶ The most abundant proteins in mammals (25% of the total protein mass).
- ▶ Long, stiff, triple-stranded helical structure made of three chains
- ▶ A basic unit of mature collagen is called tropocollagen.
- ▶ Rich in glycine (33%), proline (13%), and hydroxyproline (9%)
- ▶ Sequence is (Gly-Pro- hydroxypro)
- ▶ Hydroxyl groups stabilize the structure by hydrogen bond formation
- ▶ Contains hydroxylysine (attachment of polysaccharides)
- ▶ Crosslinking of chains via lysine and hydroxylysine

(A) Collagen triple helix

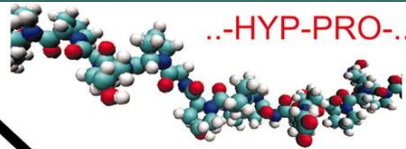


Types of collagens

- ▶ 30 collagen genes that form >20 different types of collagen that resist tissue stretching.
- ▶ **Types:**
 - ▶ **Fibril-forming (fibrillary) collagens** such as collagen I
 - ▶ **Fibril-associated collagens** (link collagen fibrils to each other and to ECM components, e.g. collagen types IX and XII link fibrils to one another and to other components in the ECM).
 - ▶ **Network-forming collagens**, flexible because they are interrupted by non helical short domains e.g. type IV is a constituent of the basal laminae.
 - ▶ **Anchoring fibrils** associate network-forming collagens to fibrillar collagens
 - ▶ **Transmembrane collagens** participate in cell matrix interactions

Assembly of fibrillar collagens

amino acids
~1 nm



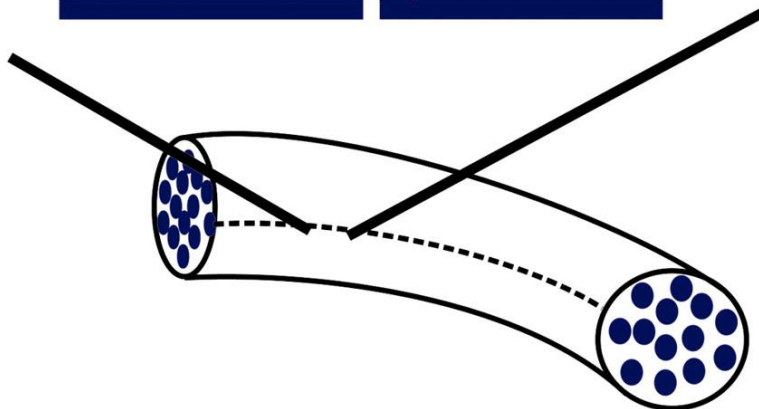
tropocollagen
~300 nm



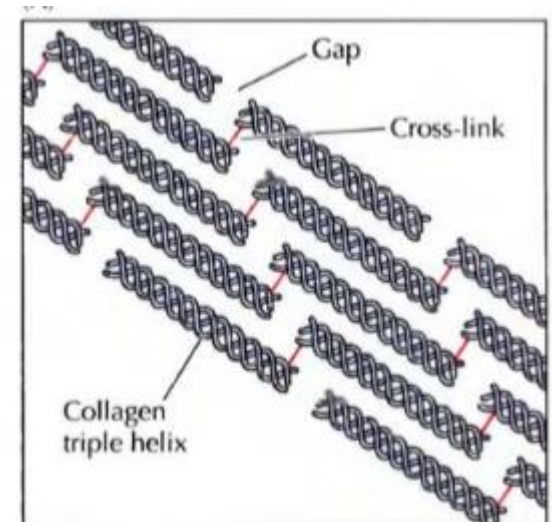
fibrils
~1 μm



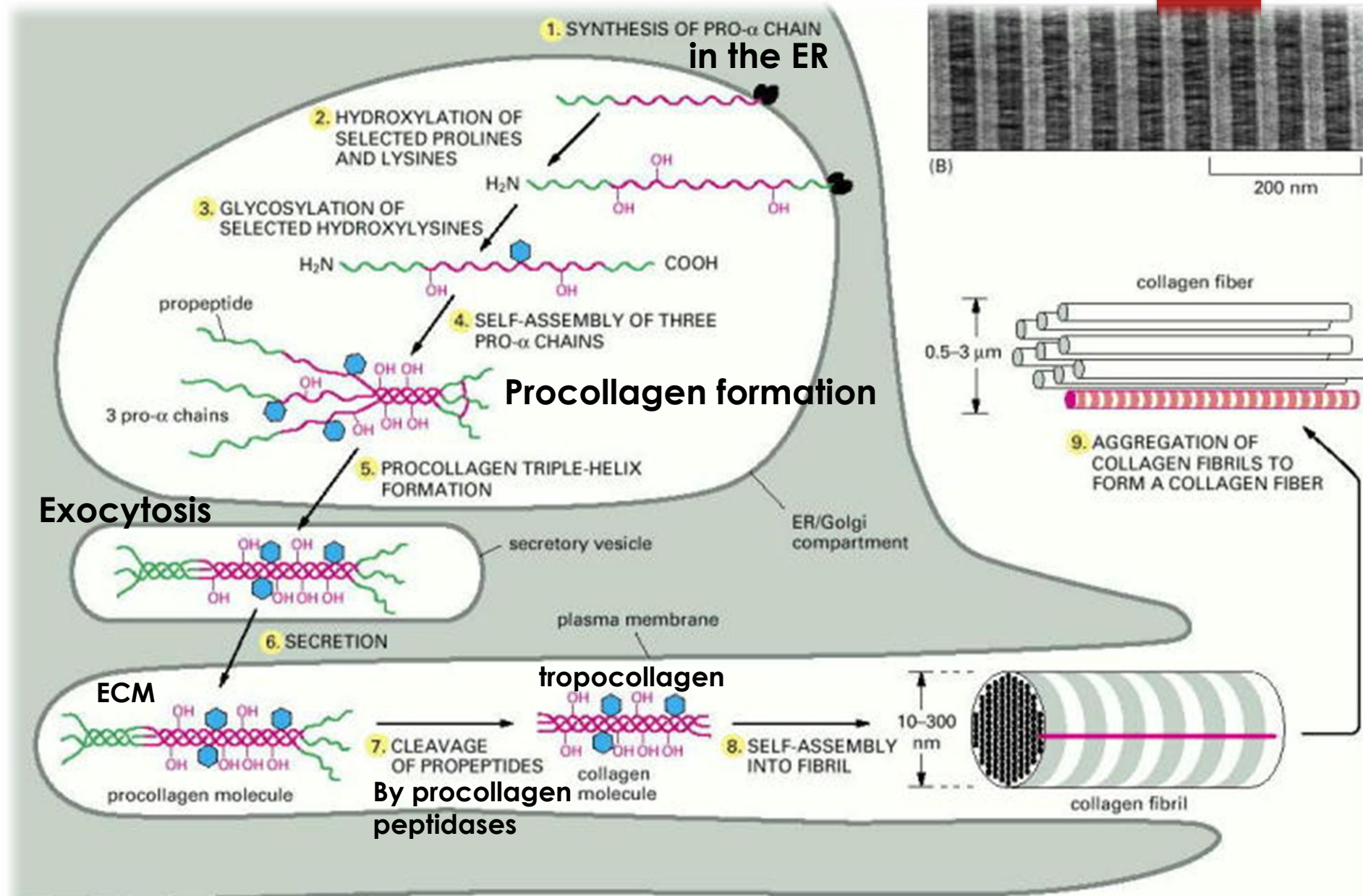
fibers
~10 μm



- ▶ After secretion, they assemble into collagen fibrils.
- ▶ Collagen fibrils aggregate into collagen fibers



Synthesis of collagen



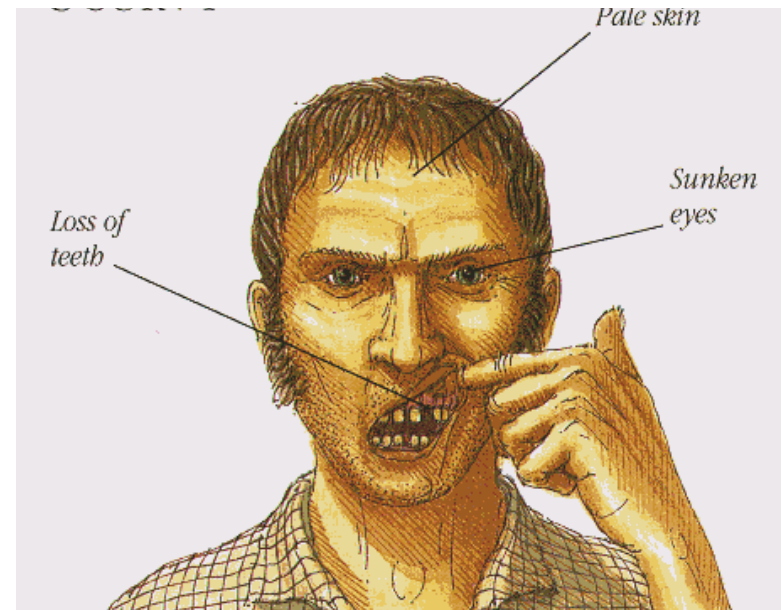
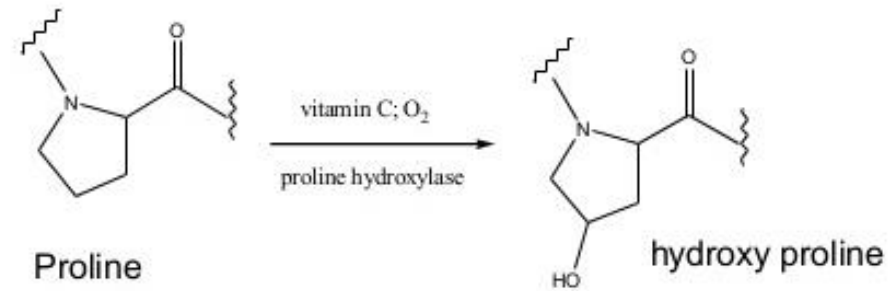
Synthesis of collagen

- ▶ Individual collagen polypeptide chains are synthesized into the endoplasmic reticulum (ER) as pro- α chains.
- ▶ In the ER, selected prolines and lysines are hydroxylated and some of the hydroxylysines are glycosylated.
- ▶ One α chain combines with two others forming procollagen.
- ▶ During or following exocytosis, extracellular enzymes, the procollagen peptidases, remove the N-terminal and C-terminal propeptides forming tropocollagen (or simply collagen).
- ▶ Excision of both propeptides allows the collagen molecules to polymerize into normal fibrils in the extracellular space.

Collagen-related diseases

Scurvy

- ▶ Deficiency of vitamin C results in **insufficient formation of hydroxyproline** and, hence, poor synthesis of collagen, formation of unstable triple helices.
- ▶ Non-hydroxylated procollagen chains are then degraded within the cell.
- ▶ Symptoms: skin and gum lesions and weak blood vessels



Collagen-related diseases

Osteogenesis imperfect (OI) (Brittle-bone disease)

- ▶ “Osteogenesis imperfecta” = imperfect bone formation
- ▶ A genetic disorder that cause fragile, soft, brittle, and easily broken bones due to mutations in COL1A1 and COL1A2 genes that interfere with the assembly of type I collagen.
- ▶ Four types of OI designated as type I through type IV
 - ▶ Type I: the mildest form of the condition
 - ▶ Type II: the most severe form that results in death in utero or shortly after birth
 - ▶ Milder forms generate a severe crippling disease



- ✓ **Autosomal dominant pattern of inheritance**
 - ✓ **One copy of the altered gene is sufficient to cause the condition**

Collagen-related diseases

Chondrodysplasias

- ▶ Mutations affecting type II collagen cause chondrodysplasias, characterized by abnormal cartilage, which leads to bone and joint deformities.



A



B



Collagen-related diseases

Ehlers-Danlos syndrome

- ▶ A heterogeneous group of disorders that affect the skin, bones, blood vessels, and other organs.
- ▶ The signs and symptoms vary from mild to life-threatening.
- ▶ All result from defects in collagen synthesis and/or processing.
 - ▶ Mutations in type I, III, or V collagen or in the synthesis of collagen processing enzymes like procollagen N-peptidase, or lysyl hydroxylase



Major manifestations:

Skin fragility and hyperextensibility

Joint hypermobility.



Collagen-related diseases

Type III EDS

- ▶ The most clinically important mutations are found in the gene of type III collagen.
- ▶ Since **type III collagen** is a major component of arteries, mutations affecting type III collagen result in fragile blood vessels.

Hypermobile joints

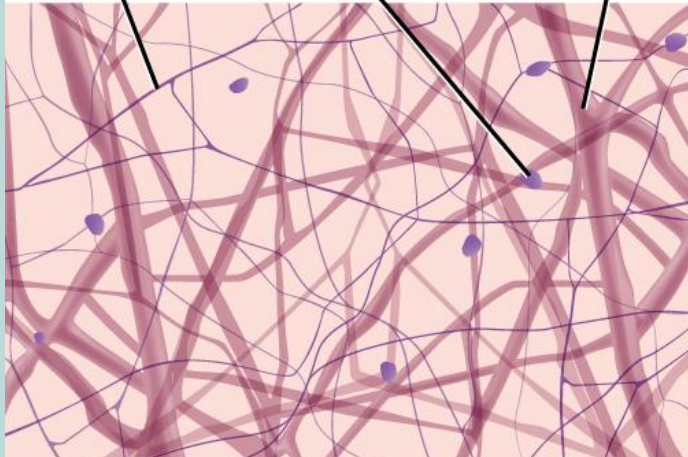


Symptoms

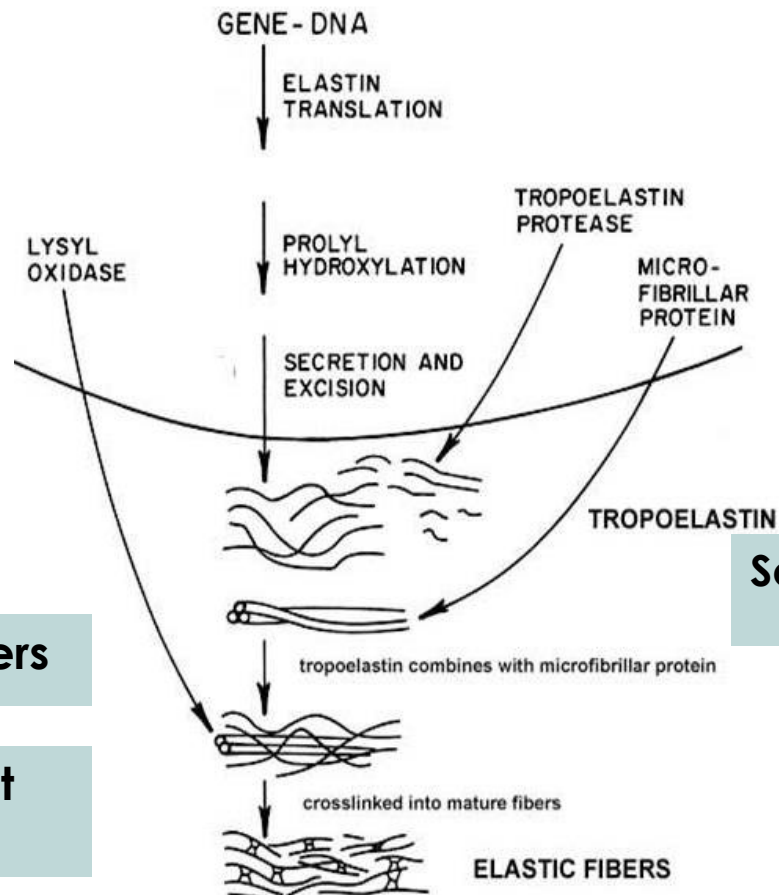
Stretchy skin



Elastic fibers structure

- ▶ Abundant in organs to allow them to stretch then return to the original shape, e.g. lungs
 - ▶ The main component of elastic fibers is elastin
 - ▶ Rich in proline and glycine.
 - ▶ Contains hydroxyproline, but no hydroxylysine
 - ▶ Not glycosylated
- 
- The image is a light micrograph of a tissue section stained with Masson's trichrome. It shows a dense network of fibers. Labels with leader lines point to specific structures: 'Elastin fiber' points to a thin, dark purple, branching fiber; 'Fibroblasts' points to small, dark purple, spindle-shaped cells; and 'Collagen fiber' points to a thick, wavy, pinkish-red fiber.
- ▶ Elastin has two types of short segments that alternate along the polypeptide chain
 1. Hydrophobic segments, which are responsible for the elastic properties of the molecule
 2. Alanine- and lysine-rich α -helical segments, which form cross-links between adjacent molecules

Formation of elastic networks



Assembly into elastic fibers

Crosslinking via covalent bonds between lysines

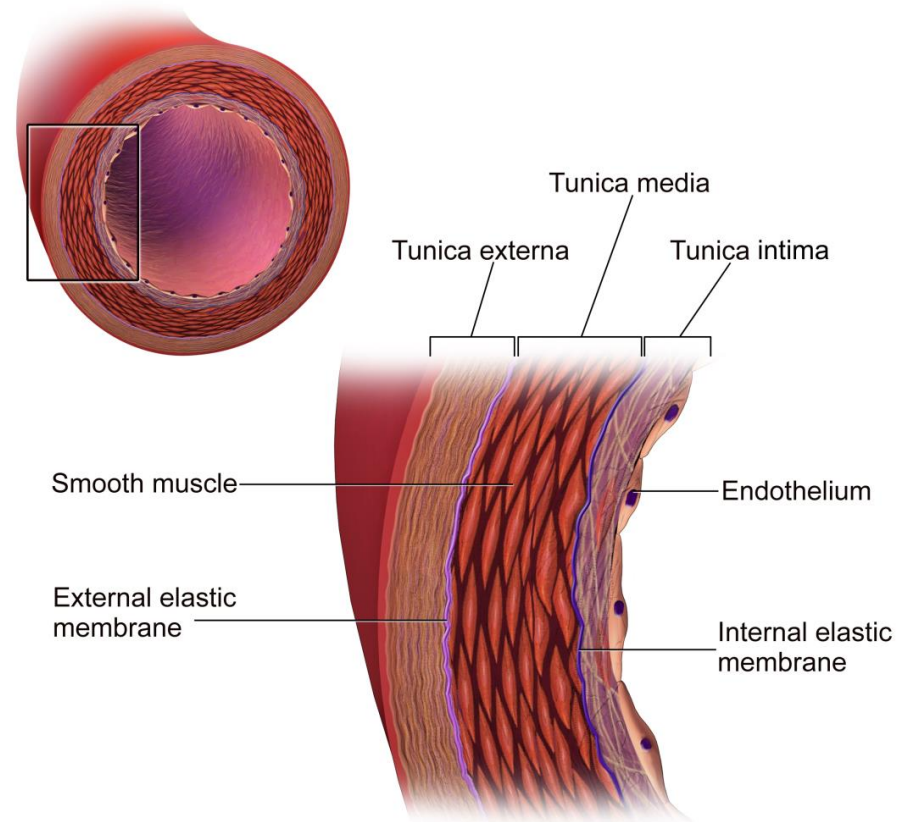
Secretion of tropoelastin

ELASTIC FIBERS

Elastic fiber in the arterial walls

- ▶ Elastin is the **dominant ECM protein in arteries**.
- ▶ The normal elasticity of an artery restrains the proliferation of smooth muscle cells.
- ▶ Abnormal or deficiency of the elastin results in **excessive proliferation of smooth muscle cells in the arterial wall and narrowing of the arteries**.

The Structure of an Artery Wall



Microfibrils

- ▶ An elastin core is covered with a sheath of microfibrils
- ▶ Microfibrils are composed of a number of distinct **glycoproteins**, including the large glycoprotein **fibrillin**, which binds to elastin and is essential for the integrity of elastic fibers.
- ▶ Present in hair and sperm tail.

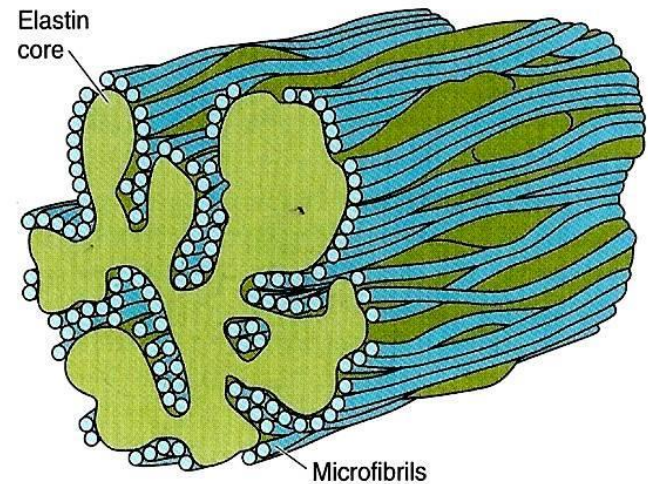


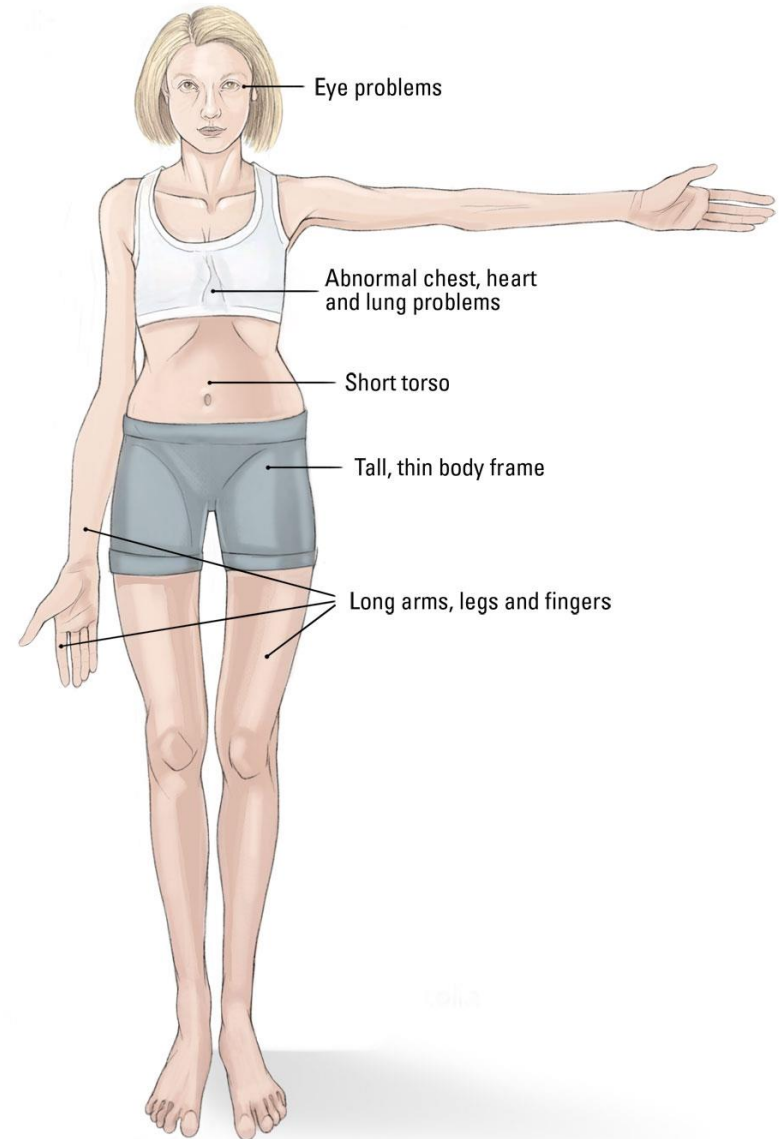
Figure 4-11 Schematic diagram of elastic fiber. Microfibrils surround the amorphous elastin.

Marfan's syndrome

- ▶ Due to mutated fibrillin
- ▶ Rupture of aorta.

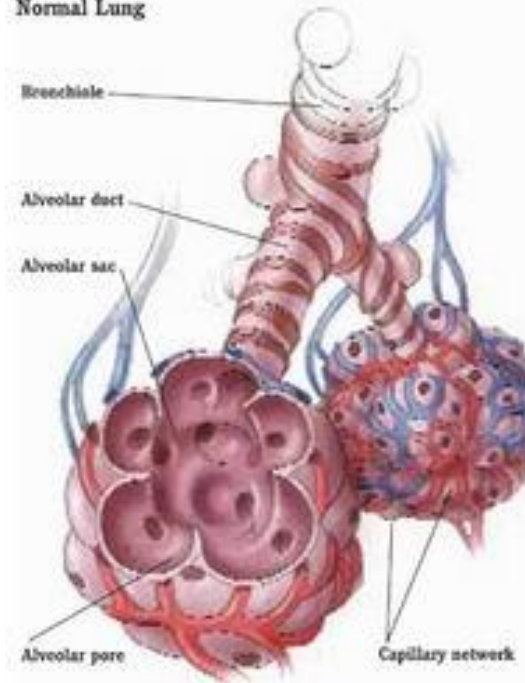
✓ **Signs and symptoms:**
A tall, thin build
Long arms, legs, fingers, and toes
Flexible joints
Scoliosis, or curvature of the spine
A chest that sinks in or sticks out
Crowded teeth
Flat feet

Marfan syndrome

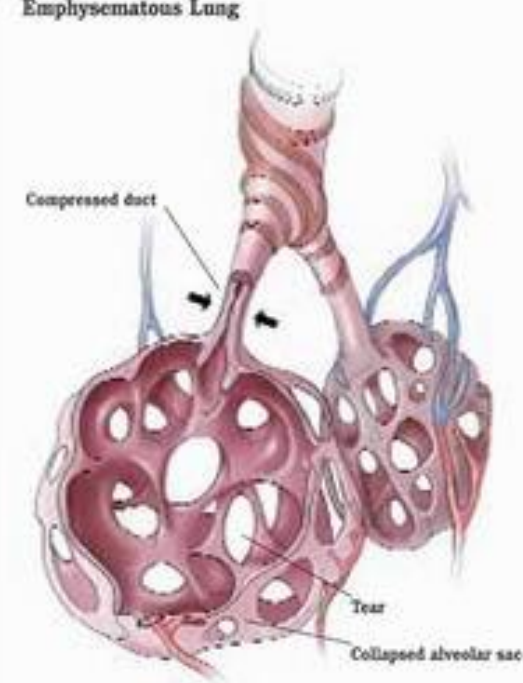


Emphysema (destructive lung disease)

Normal Lung



Emphysematous Lung



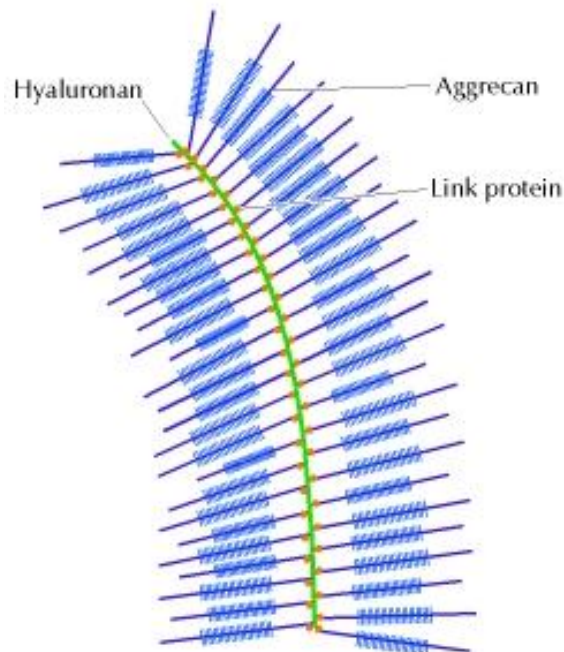
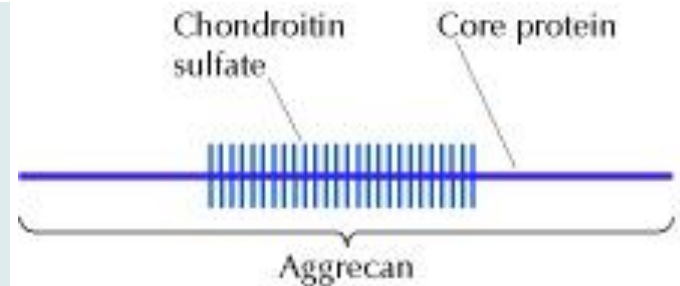
- ▶ Due to a dysfunctional alpha-1 antitrypsin leading to increased activity of elastase in lungs
- ▶ The lysine to-glutamate mutation causes protein misfolding, formation of an aggregate and block of ER export.
- ▶ Cigarette smoking also inactivates alpha 1-antitrypsin by oxidizing essential methionine residues, decreasing the enzyme activity by a factor of 2000.

Matrix polysaccharides

- ▶ Glycosaminoglycans (GAGs): Polysaccharides of **repeated disaccharides** in which fibrous proteins are embedded
 - ▶ One is either **N-acetylglucosamine** or **N-acetylgalactosamine**
 - ▶ The other is usually **acidic** (either glucuronic acid or iduronic acid) → highly negatively charged
 - ▶ Modified by **sulfate groups**, e.g. dermatan sulfate, chondroitin sulfate, keratan sulfate, heparin sulfate.
 - ▶ Exist as **proteoglycans**
 - ▶ Some are cell surface proteins with either **transmembrane domains** (syndecans) or **GPI anchors** (glycipans) interacting with integrins.
- ▶ Hyaluronan: is the only GAG with a single long polysaccharide chain.

Aggrecan and hyaluronan

- ▶ The major proteoglycan of cartilage
- ▶ A large proteoglycan consisting of more than 100 chondroitin sulfate chains joined to a core protein.

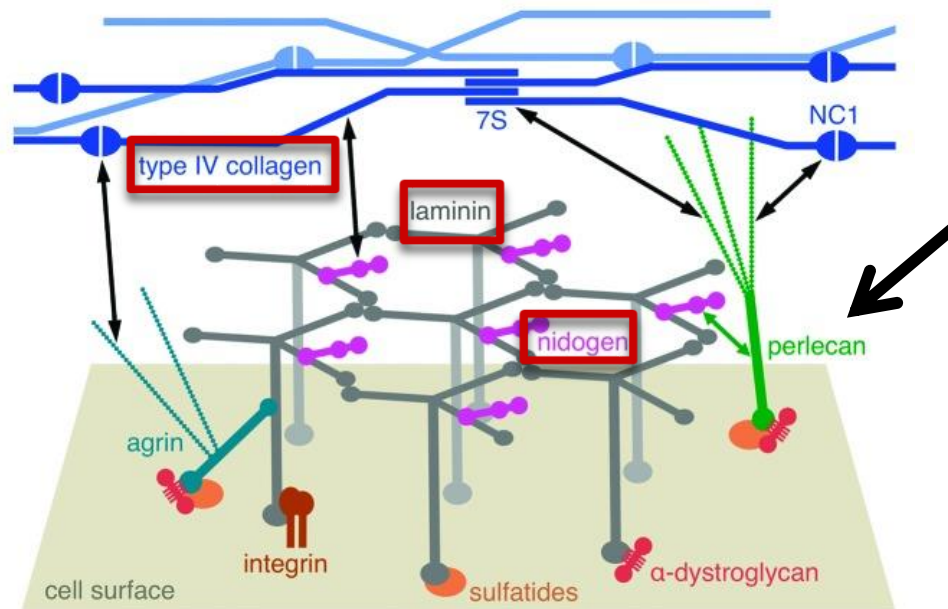


- ▶ Multiple aggrecan molecules bind to long chains of hyaluronan that become trapped in the collagen network, forming large complexes in the ECM of cartilage.

Perlecan

- Proteoglycans bind to matrix proteins forming gel-like networks

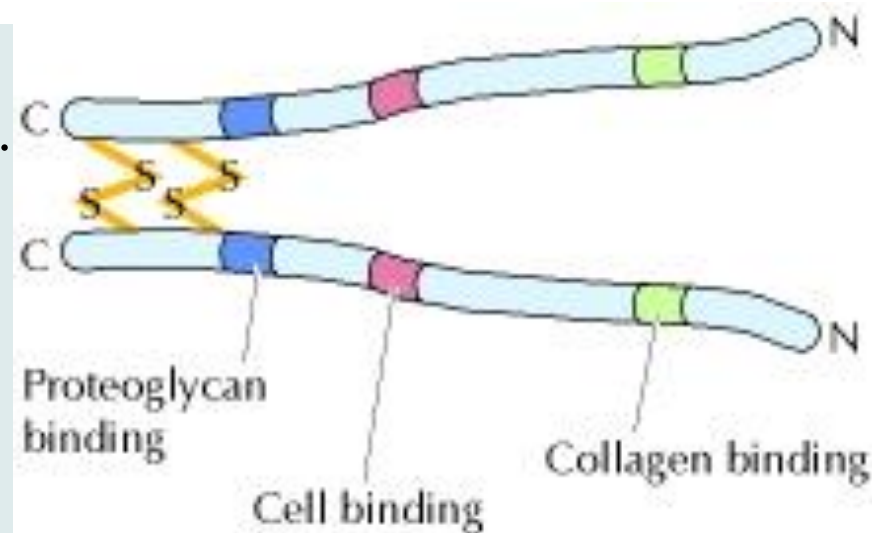
Heparan sulfate proteoglycan of basal lamina + collagen + laminin



Matrix adhesion proteins

Fibronectin

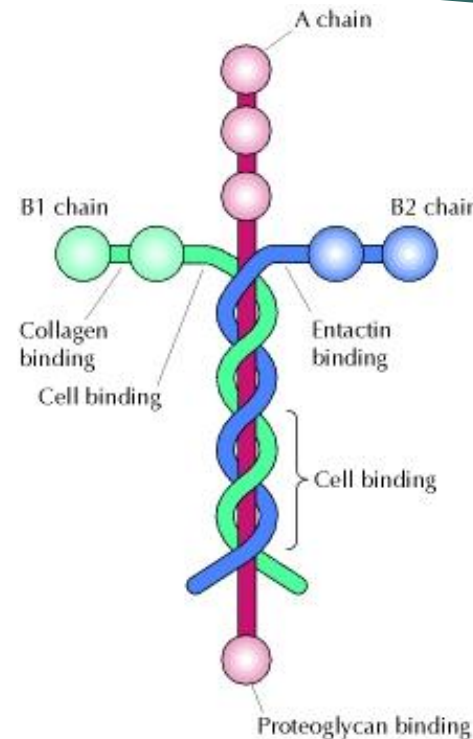
- ▶ They link matrix proteins with one another and to the surfaces of cells.
- ▶ They **interact with collagen and proteoglycans** and **specify matrix organization** and are **major binding sites for cell surface receptors** such as integrins



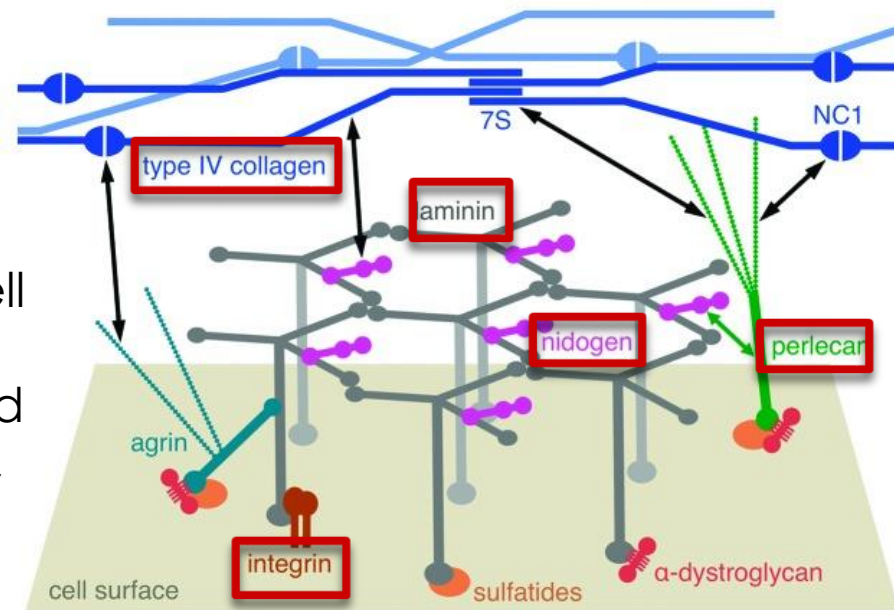
- ▶ **Fibronectin**: the principal adhesion protein of connective tissues.
 - ▶ A dimeric glycoprotein that is crosslinked into fibrils by s-s bonds.
 - ▶ Binds to collagen and GAGs
 - ▶ Binds to cell surface proteins like integrins linking cells to the ECM

Matrix adhesion proteins

Laminin



- Found in basal laminae
- T-shaped heterotrimers with binding sites for cell surface receptors (e.g. integrins), and ECM components, e.g. type IV collagen, and perlecan.

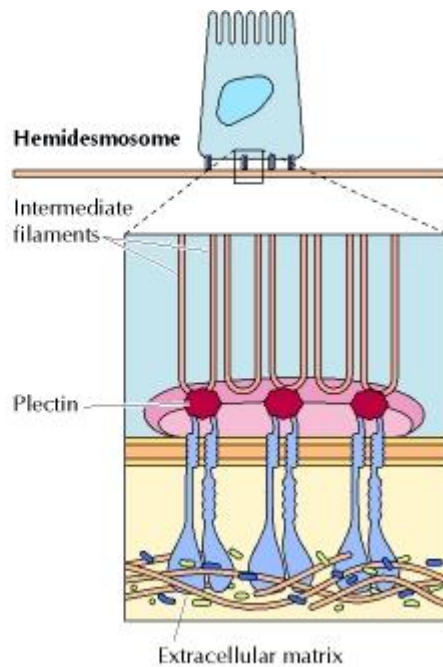
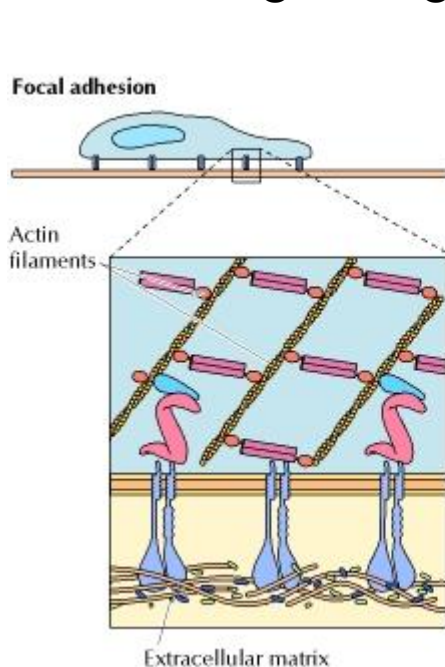


- Laminins are tightly associated with another adhesion protein, called nidogen, which also binds to type IV collagen, all of which form crosslinked networks in the basal lamina.

Cell-matrix interactions

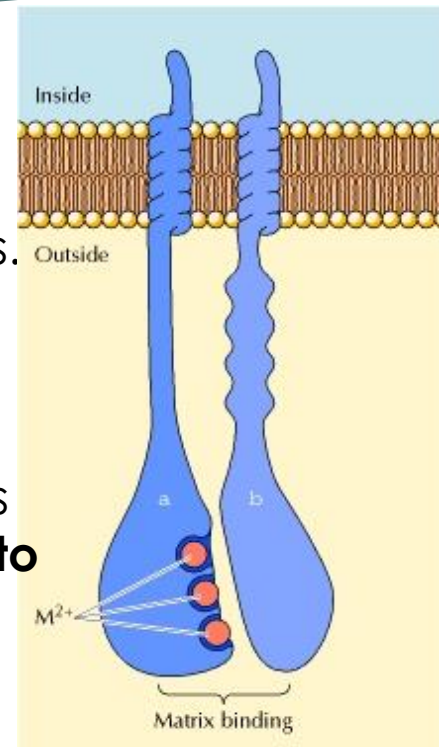
Role of integrins

- ▶ Integrins are a family of transmembrane heterodimers (α and β)
- ▶ They bind to short sequences present in ECM proteins including collagen, fibronectin, laminin and proteoglycans.



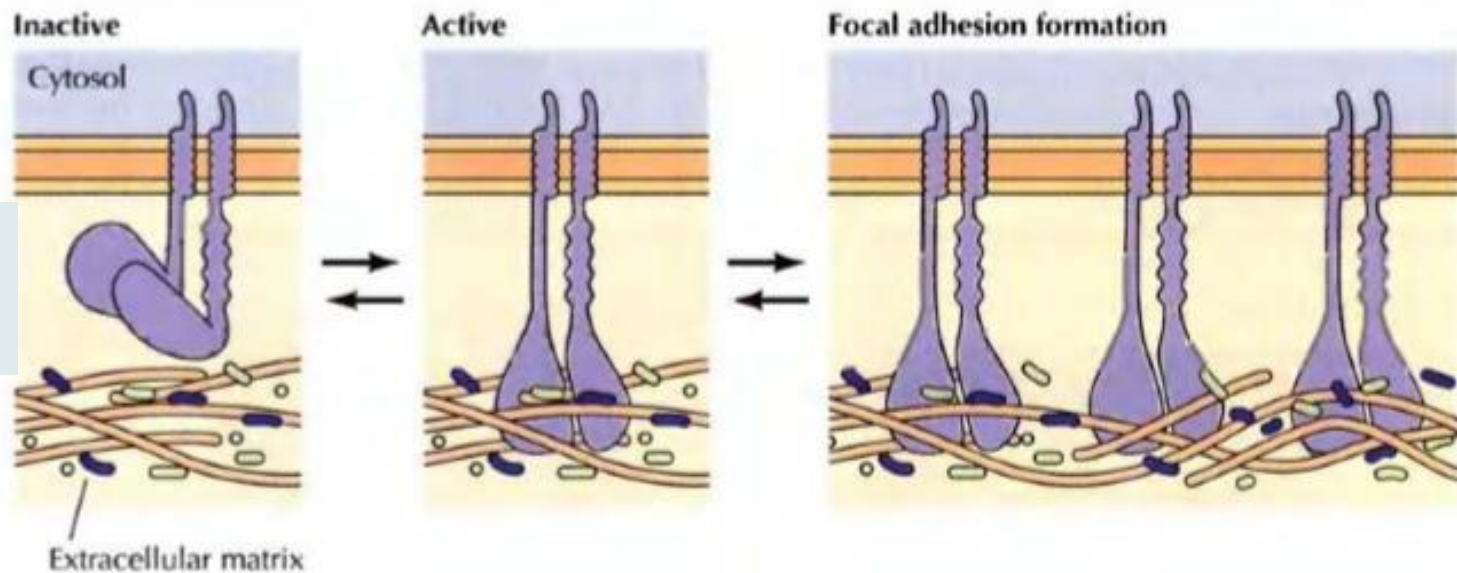
Functions of integrins:

1. The major cell surface receptors that **attach cells to ECM**
2. They **anchor the cytoskeleton** at focal adhesions and hemidesmosomes.



Assembly of focal adhesions

Inactive integrins can not bind the matrix.



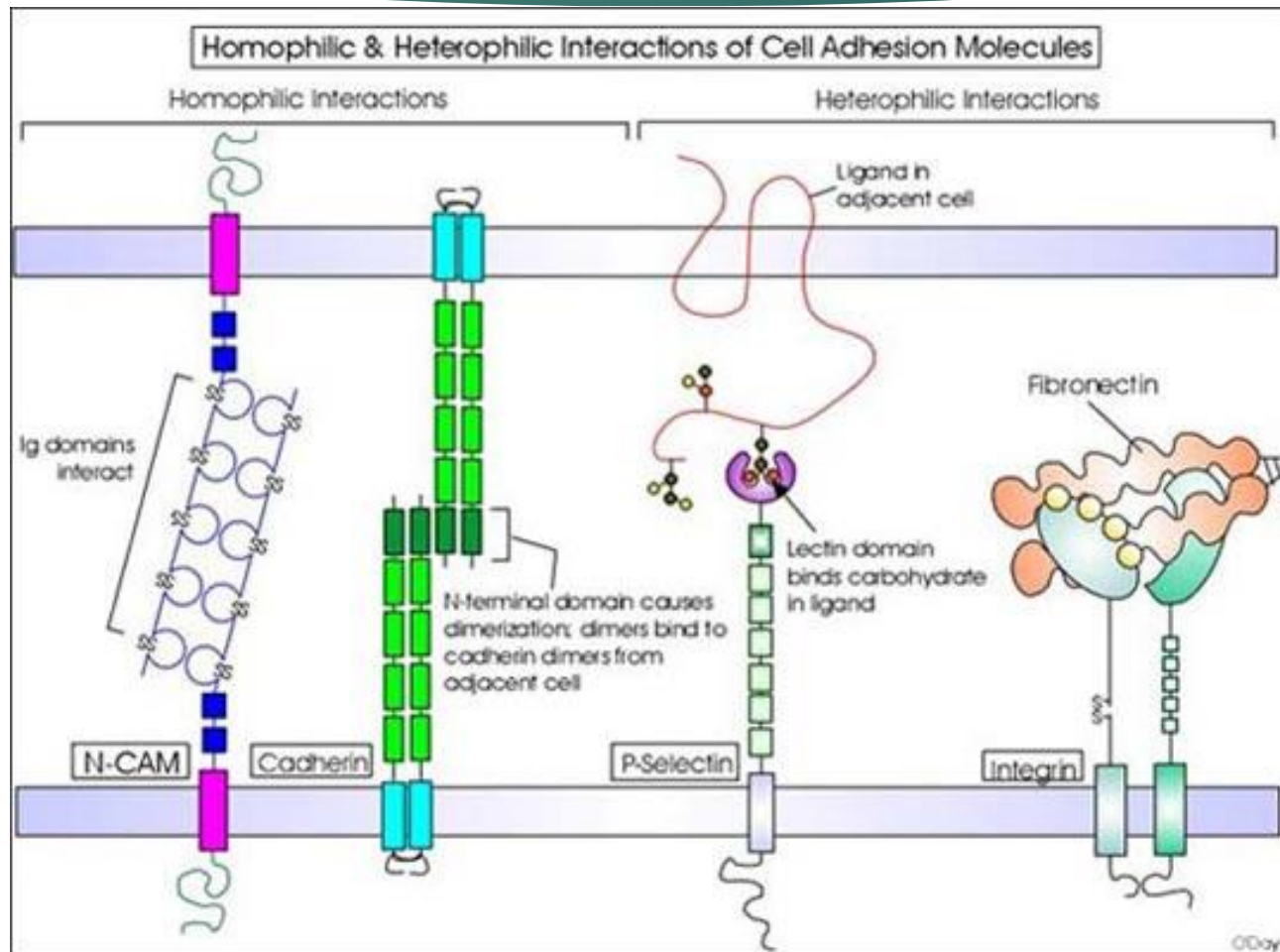
1. Activation of integrin and binding to ECM
2. Recruitment of additional integrins forming focal complex
3. Development of small integrin clusters called focal complexes
4. Development of focal adhesions by the recruitment of formin, talin, vinculin and α -actinin

Cell-cell adhesion is a selective process

Cell Adhesion Molecules

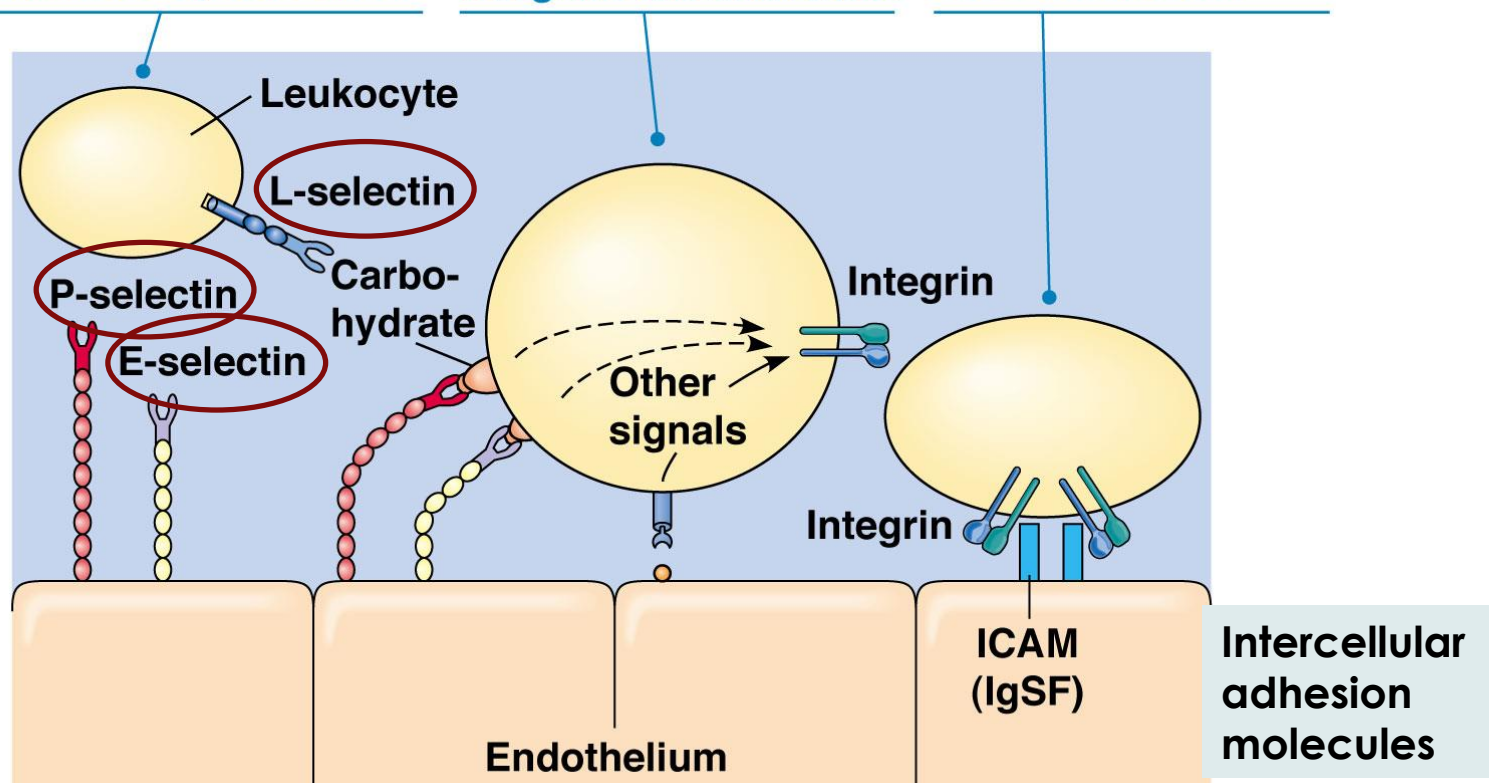
Family	Ligands recognized	Stable cell junctions
Selectins	Carbohydrates	No
Integrins	Extracellular matrix	Focal adhesions and hemidesmosomes
	Members of Ig superfamily	No
Ig superfamily	Integrins	No
	Homophilic interactions	No
Cadherins	Homophilic interactions	Adherens junctions and desmosomes

Homophilic vs. heterophilic interactions



Selectin-mediated interaction between leukocytes and endothelial cells

- ① Rolling of leukocytes is mediated by selectins.
- ② Prior to invading a blood vessel, integrins are activated.
- ③ Firm attachment allows invasion.



Cadherins adhesion molecules

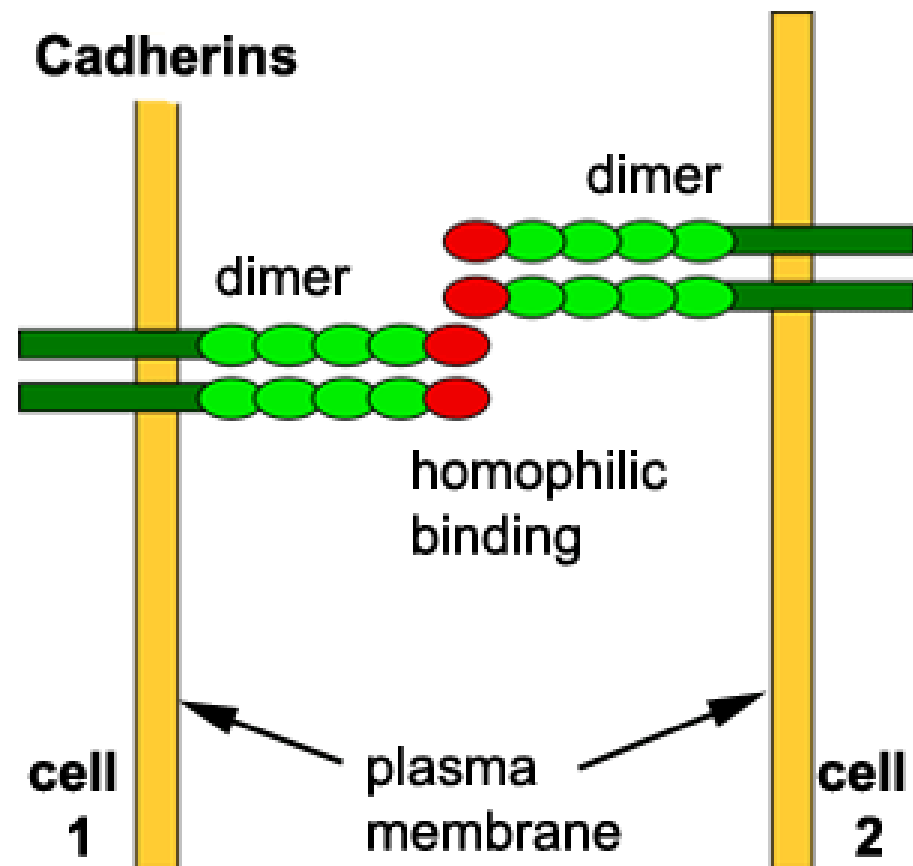
- ▶ **Selective** adhesion between embryonic cells and formation of stable junctions between cells in tissues.

- ▶ **Classic** cadherins

- ▶ E-cadherin: epithelial cells
- ▶ N-cadherin: neural cells
- ▶ P-cadherin: placental cells

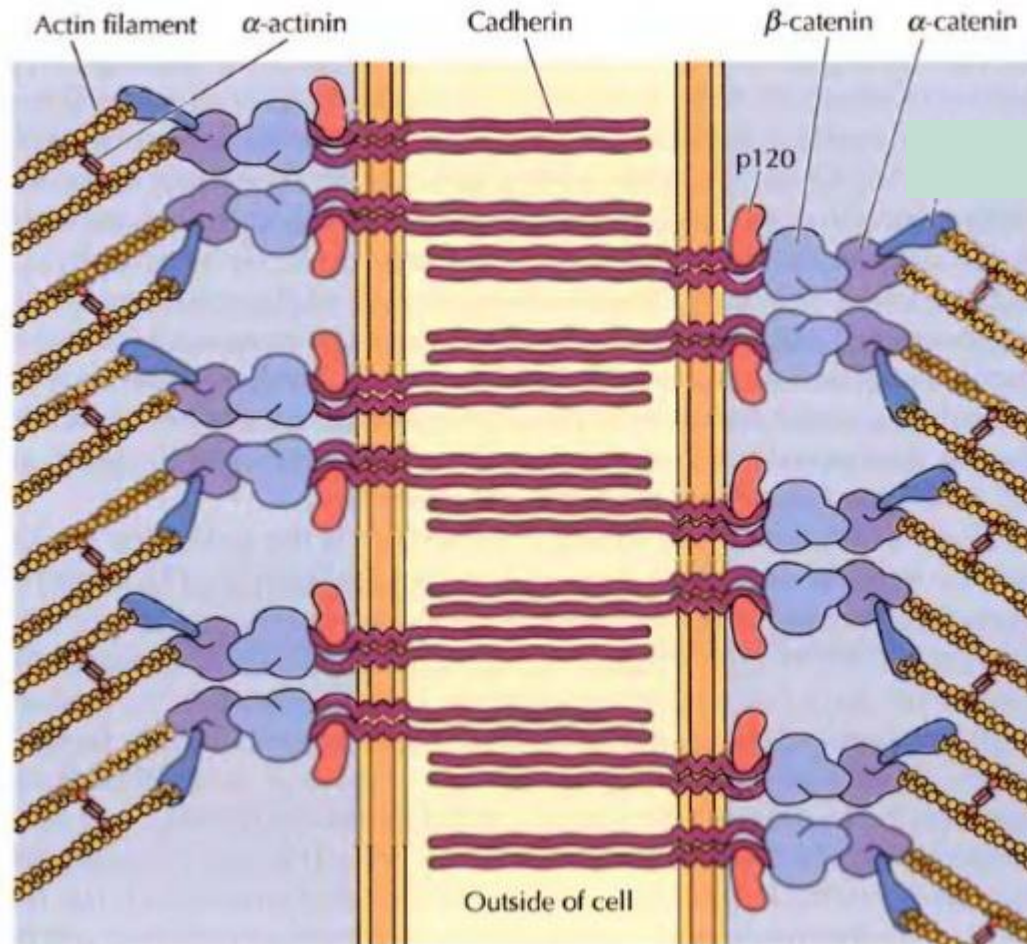
Several subfamilies

- ▶ Classic, Desmosomal, fat-like and 7-Transmembrane cadherins

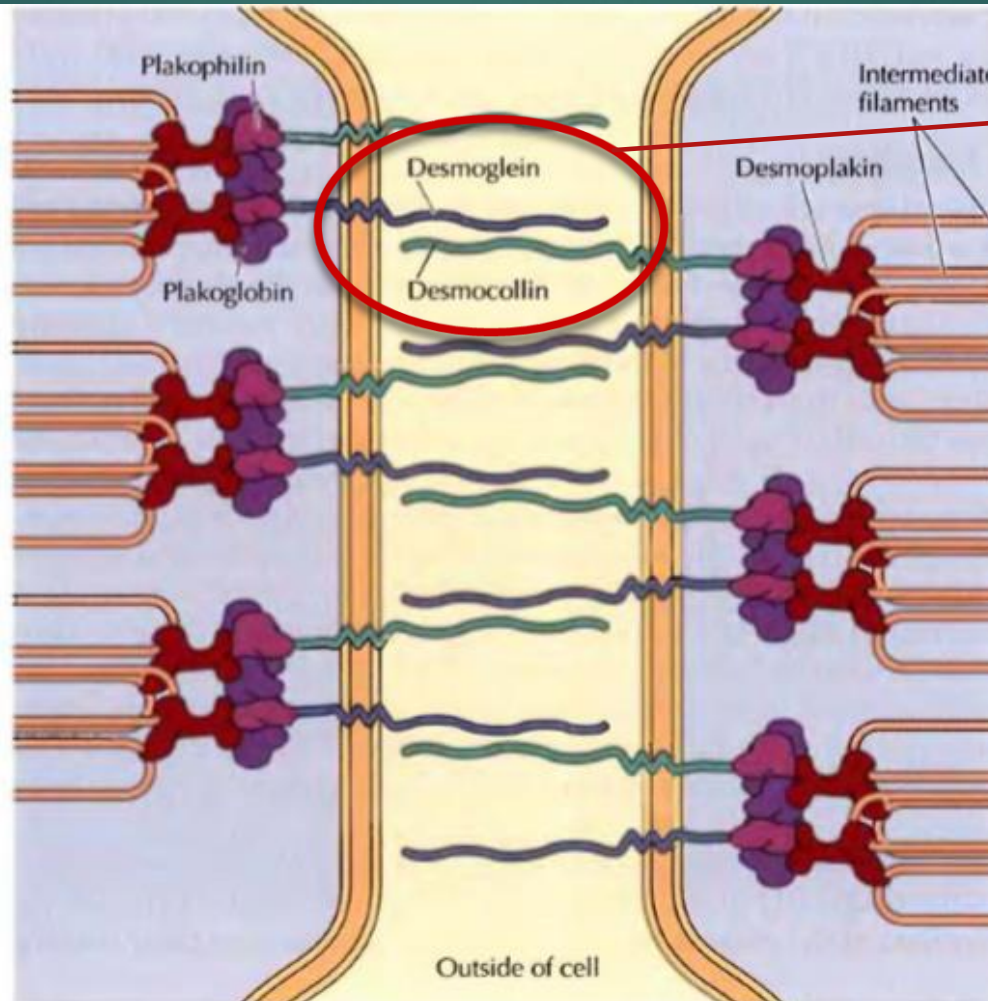


Adherens junctions

Classic
cadherins



Desmosomes

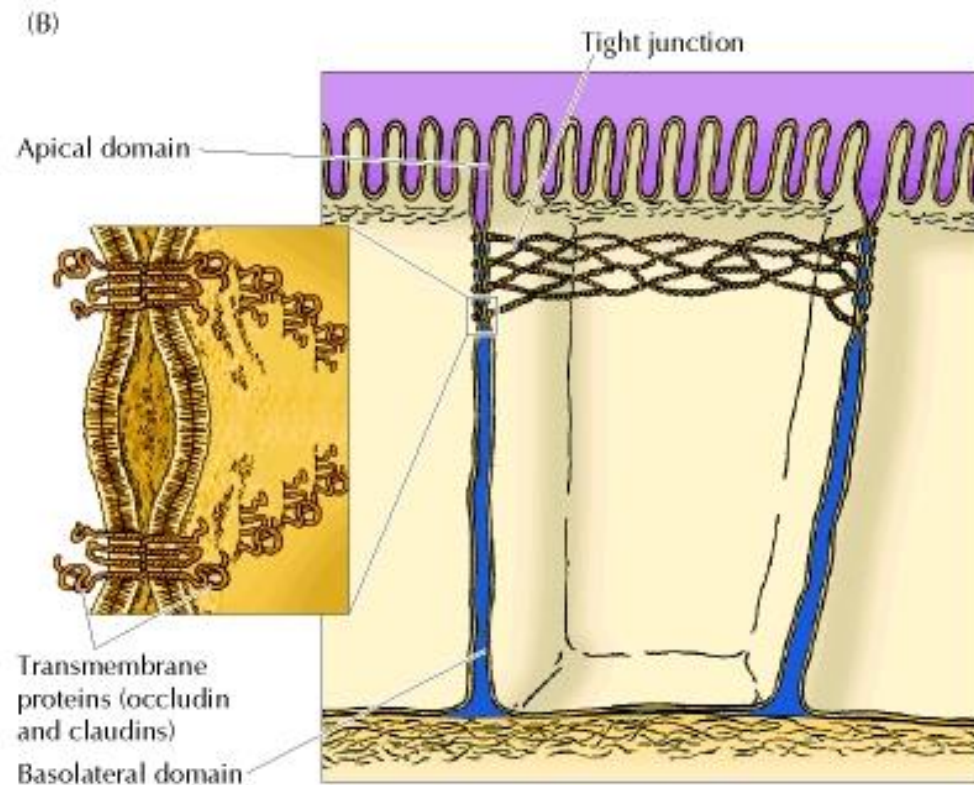


**Desmosomal
cadherins**

heterophilic interaction

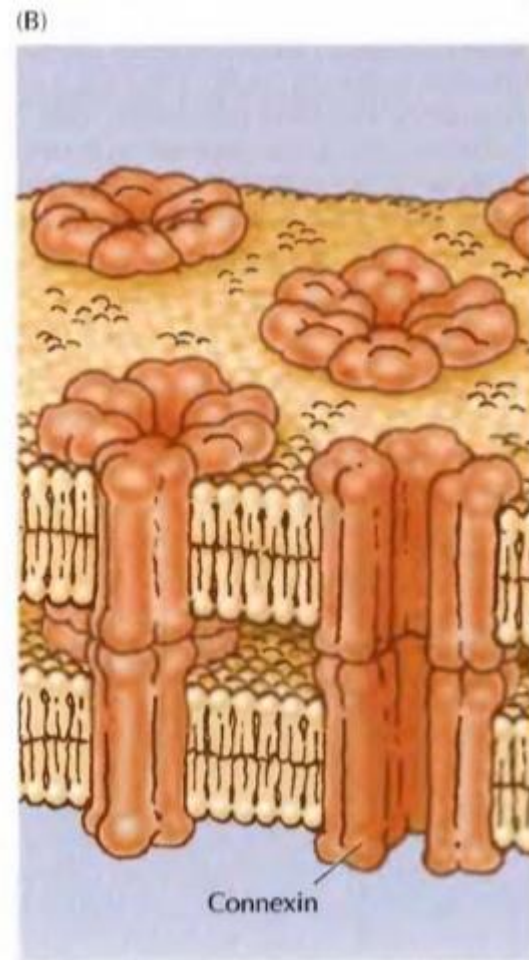
Molecular composition of tight junctions

- ▶ A network of protein strands that continues around the entire circumference of the cell
- ▶ Each strand in these networks is composed of transmembrane proteins (claudins, occludin, and JAMs) that bind to similar proteins on adjacent cells, thereby sealing the space between their plasma membranes
- ▶ These proteins interact with the actin cytoskeleton via zonula occludens protein.
- ▶ Separate the apical part from the basolateral part of membranes
- ▶ Prevent the passage of molecules (including ions)between epithelial cells



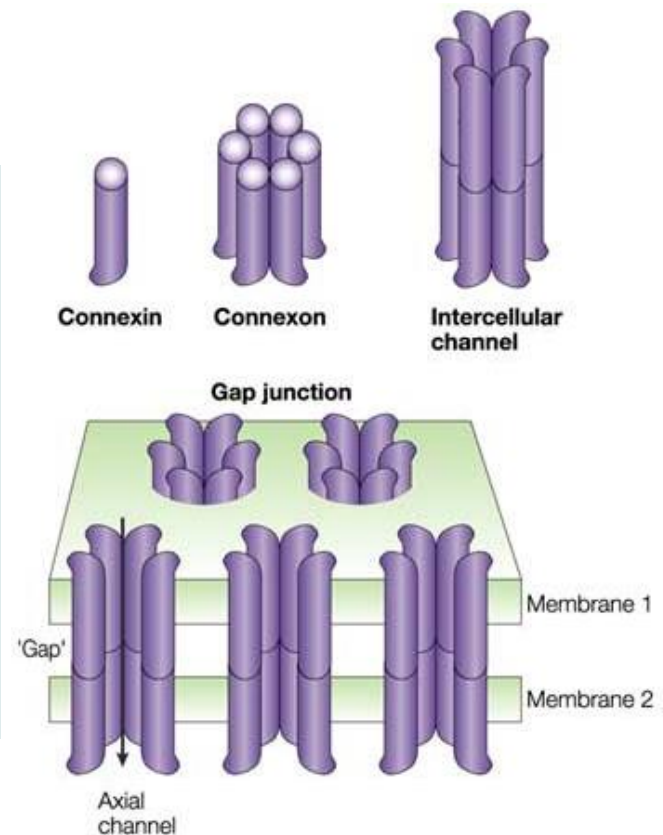
Gap junctions

- ▶ They provide **direct connections between the cytoplasm of adjacent cells** as open channels allowing **ions and small molecules (<1000 Da) including signaling molecules** to diffuse freely between neighboring cells, but **preventing the passage of proteins and nucleic acids**.
- ▶ Present in cells like epithelial cells, endothelial cells, cardiac cells and smooth muscle cells

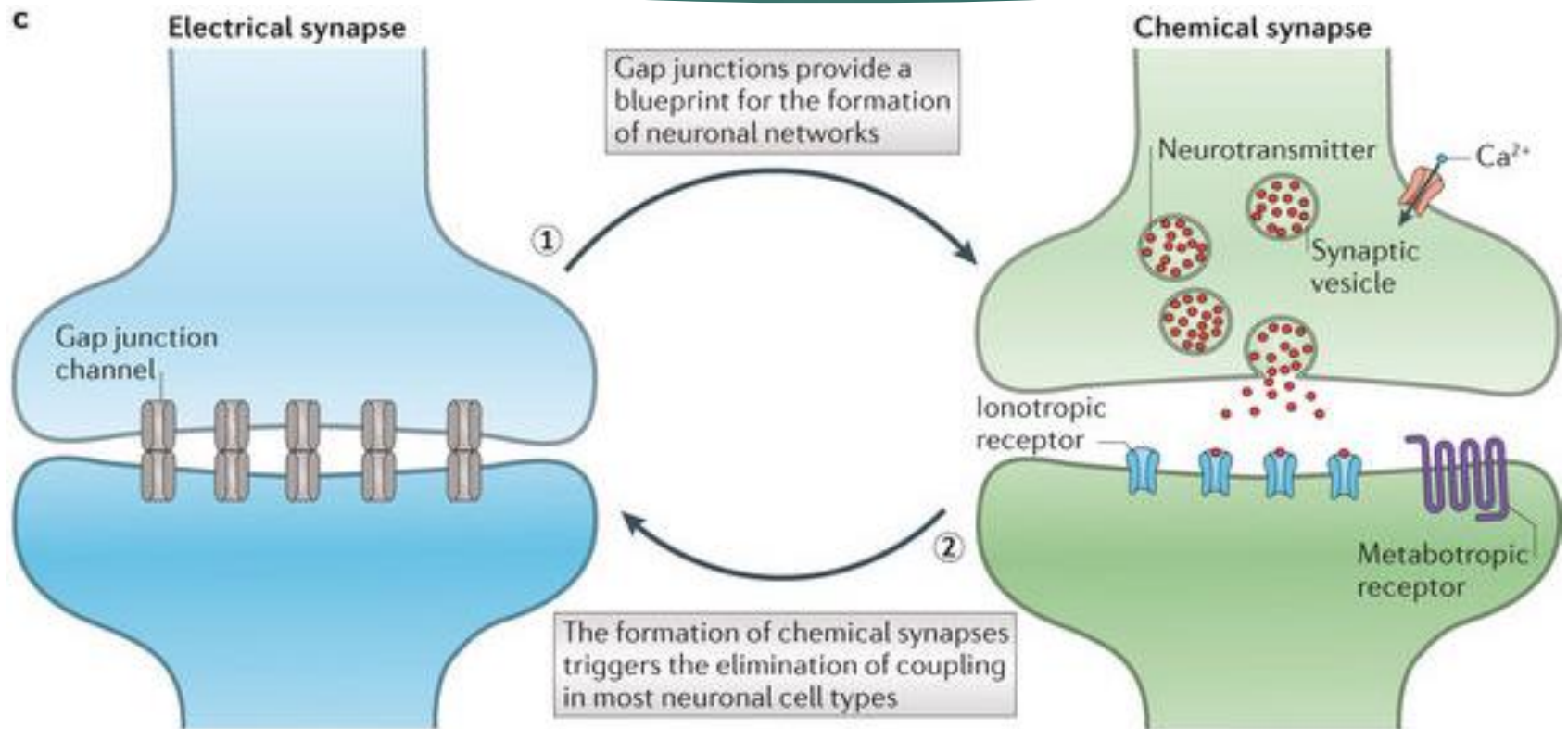


Molecular composition of gap junctions

- ▶ They are made of transmembrane proteins called **connexins**
- ▶ **Six connexins** assemble to form a **connexon** (a cylinder with an open aqueous pore in its center).
- ▶ **Two connexons** on adjacent cells make a **gap junction**



Gap junctions and electrical synapses



Specialized connexins in electrical synapse

Gap junction diseases

Mutations in different types of connexins result in many diseases such as:

- ▶ **Charcot-Marie-Tooth disease** (degeneration of peripheral myelinated nerves)
- ▶ **Deafness**: inability to rapidly exchange K^+
- ▶ **Cataracts**: inability to obtain nutrients from the lens epithelial cells
- ▶ **Skin disease**