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Programme: M.Sc., Biochemistry
Course Title : Cell biology
Course Code :BC105DCE

Unit-3
INTERNAL ORGANIZATION OF THE CELL

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Cytoskeleton

- All cells have to be correctly shaped, physically robust, properly structured internally
- They have to be able to rearrange their internal components as they grow, divide and adapt to changing circumstances
- These spatial and mechanical functions depends on a system of filaments called cytoskeleton

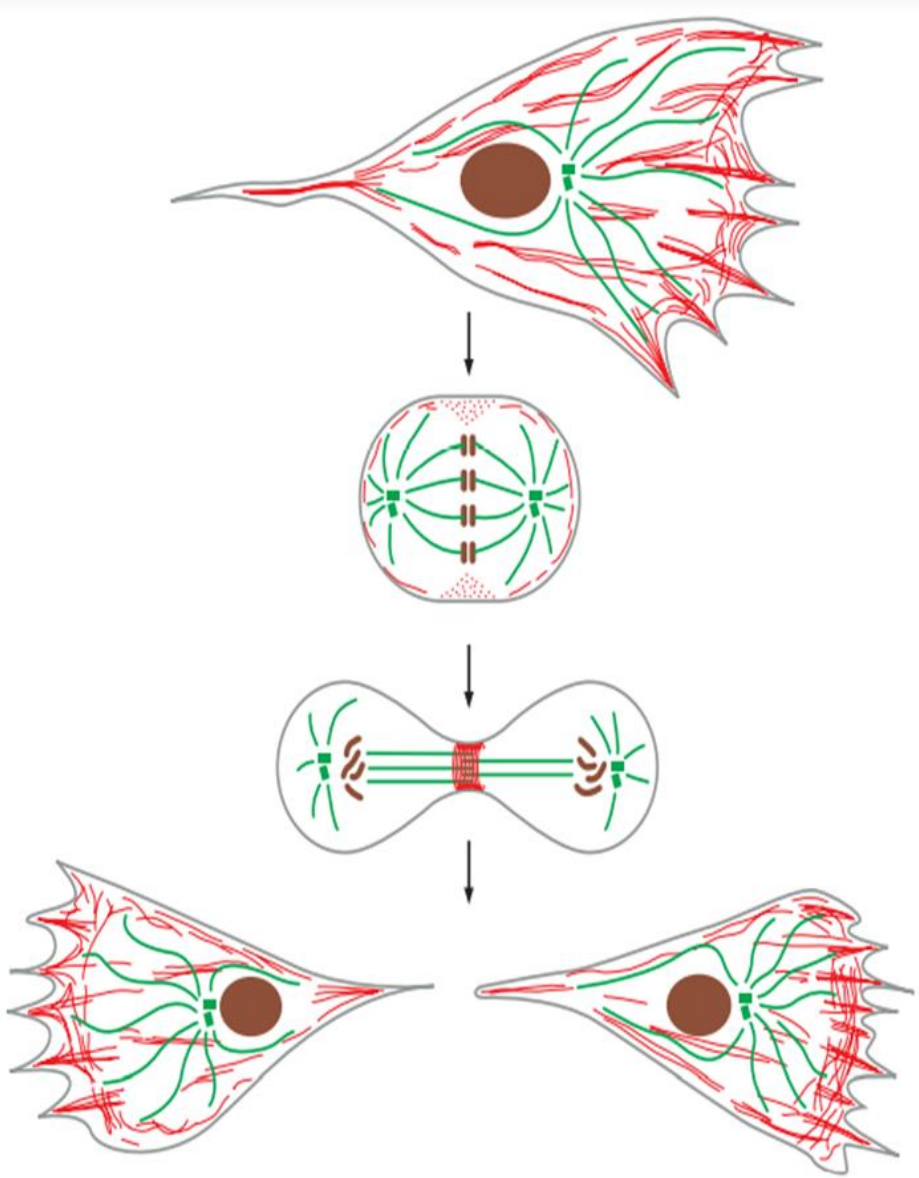
Three families of protein filaments

- Actin filament
- Microtubules
- Intermediate filaments

Cytoskeletal Filaments Adapt to Form Dynamic or Stable Structures

- a fibroblast growing in a tissue-culture dish
- After the chromosomes have replicated, the interphase microtubule array that spreads throughout the cytoplasm is reconfigured into the
- bipolar mitotic spindle, which segregates the two copies of each chromosome into daughter nuclei
- At the same time, the specialized actin structures that enable the fibroblast to crawl across the surface of the dish rearrange so that the cell stops moving and assumes a more spherical shape
- Actin and its associated motor protein myosin then form a belt around the middle of the cell, the contractile ring, which constricts like a tiny muscle to pinch the cell in two

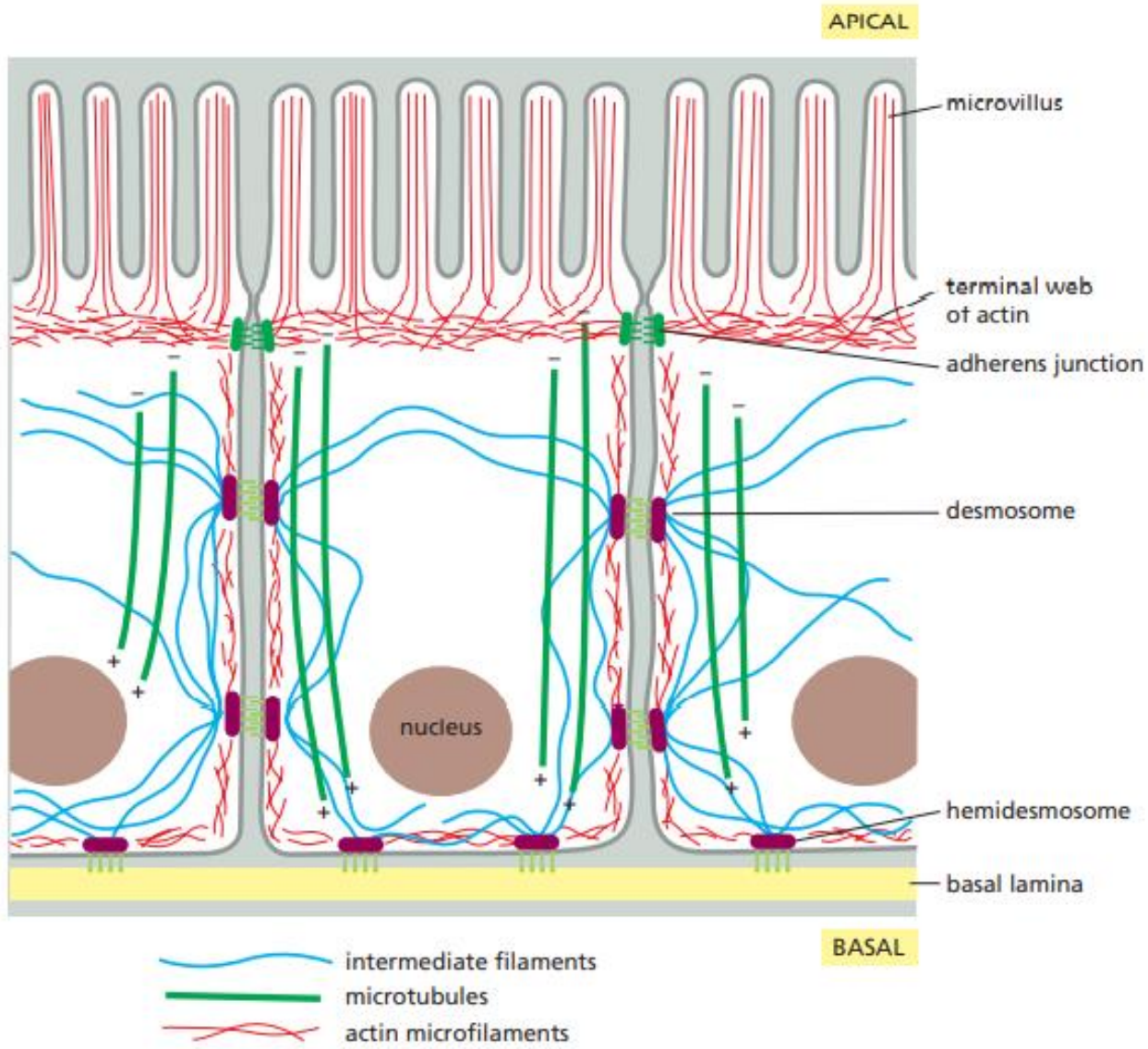
- When division is complete, the cytoskeletons of the two daughter fibroblasts reassemble into their interphase structures to convert the two rounded-up daughter cells into smaller versions of the flattened, crawling mother cell
- actin cytoskeleton – Red
- microtubule cytoskeleton – Green
- segregating the duplicated chromosomes – Brown
- Many cells require rapid cytoskeletal rearrangements for their normal functioning during interphase as well
- neutrophil, a type of white blood cell, chases and engulfs bacterial and fungal cells that accidentally gain access to the normally sterile parts of the body, as through a cut in the skin



- Like most crawling cells, neutrophils advance by extending a protrusive structure filled with newly polymerized actin filaments
- When the elusive bacterial prey moves in a different direction, the neutrophil is poised to reorganize its polarized protrusive structures within seconds
- On the intestine and the lung, cytoskeletal-based cell-surface protrusions including microvilli and cilia are able to maintain a constant location, length, and diameter over the entire lifetime of the cell
- Polarized epithelial cells use organized arrays of microtubules, actin filaments, and intermediate filaments to maintain the critical differences between the apical surface and the basolateral surface
- They also must maintain strong adhesive contacts with one another to enable this single layer of cells to serve as an effective physical barrier

Filaments Assemble from Protein Subunits That Impart Specific Physical and Dynamic Properties

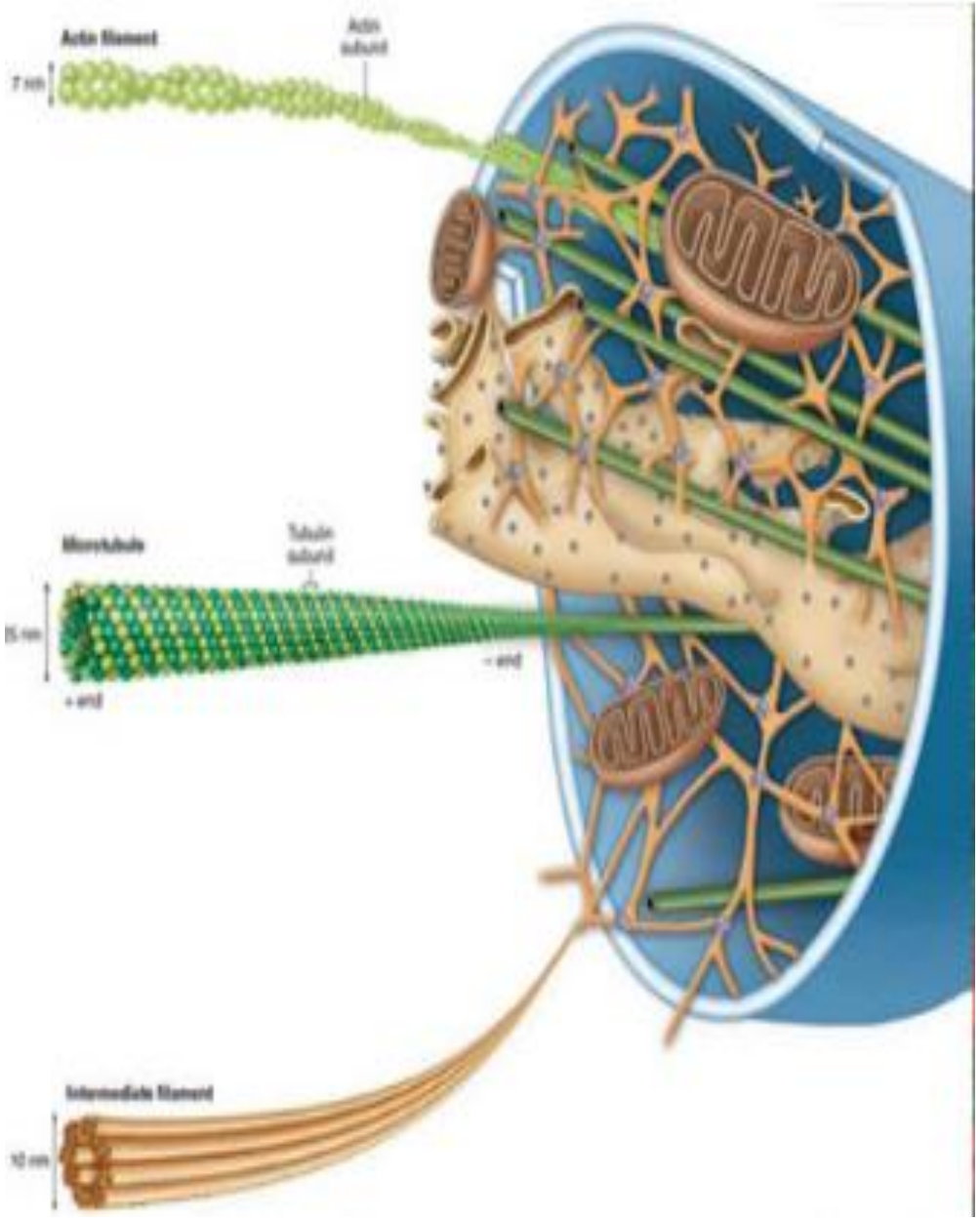
- Below the microvilli, a circumferential band of actin filaments is connected to cell–cell adherens junctions that anchor the cells to each other
- Intermediate filaments (blue)are anchored to other kinds of adhesive structures, including desmosomes and hemidesmosomes, that connect the epithelial cells into a sturdy sheet and attach them to the underlying extracellular matrix
- Microtubules (green) run vertically from the top of the cell to the bottom and provide a global coordinate system that enables the cell to direct newly synthesized components to their proper locations



- Actin filaments determine the shape of the cell’s surface and are necessary for whole-cell locomotion; they also drive the pinching of one cell into two
- Microtubules determine the positions of membrane-enclosed organelles, direct intracellular transport, and form the mitotic spindle that segregates chromosomes during cell division
- Intermediate filaments provide mechanical strength
- The accessory proteins are essential for the controlled assembly of the cytoskeletal filaments in particular locations, and they include the motor proteins
- molecular machines that convert the energy of ATP hydrolysis into mechanical force that can either move organelles along the filaments or move the filaments themselves

Actin Filament:

- The thinnest are the microfilaments (7 nm in diameter) which are solid and are principally made of two intertwined strands of a globular protein called actin. For this reason, microfilaments are also known as actin filaments
- Actin is powered by ATP to assemble its filamentous form, which serves as a track for the movement of a motor protein called myosin
- This enables actin to engage in cellular events requiring motion such as cell division in animal cells and cytoplasmic streaming, which is the circular movement of the cell cytoplasm in plant cells



Basic functions of Actin filaments

- Actin filaments underlie the plasma membrane of animal cells, providing strength and shape to its thin lipid bilayer
- They form many types of cell projections
- Dynamic structures such as *lamellipodia* and *filopodia* used for locomotion and to explore the territory
- More stable arrays allow cells to brace themselves against an underlying substratum and enable muscle to contract
- regular bundles of *stereocilia* on the surface of hair cells in the inner ear contain stable bundles of actin filaments that tilt as rigid rods in response to sound, and similarly organized microvilli on the surface of intestinal epithelial cells vastly increase the apical cell-surface area to enhance nutrient absorption
- In plants, they drive rapid streaming of plasma inside cells

Microtubules

- The thickest are the microtubules (20 nm in diameter) which consist primarily of the tubulin protein.
- Each tubulin subunit is made up of one alpha and one beta-tubulin that are attached to each other, so technically tubulin is a heterodimer, not a monomer. Since it looks like a tube, it is named as microtubule.
- In a microtubule structure, tubulin monomers are linked both at their ends and along their sides (laterally). This means that microtubules are quite stable along their lengths.
- Since the tubulin subunits are always linked in the same direction, microtubules have two distinct ends, called the plus (+) and minus (-) ends.
- On the minus end, alpha-tubulin is exposed, and on the plus end, beta-tubulin is exposed.
- Microtubules preferentially assemble and disassemble at their plus ends.

Intermediate Filament:

- The fibers of the middle-order are called the intermediate filaments (IFs) having a diameter of 10 nm.
- They are composed of a family of related proteins sharing common structural and sequence features.
- They having been classified according to their constituent protein such as desmin filaments, keratin filaments, neurofilaments, vimentin, and glial filaments

Microtrabecular Lattice

- Recently, cytoplasm has been found to be filled with a three-dimensional network of interlinked filaments of cytoskeletal fibers, called a micro-trabecular lattice
- Various cellular organelles such as ribosomes, lysosomes, etc., are found anchored to this lattice
- The micro-trabecular lattice being flexible changes its shape and results in the change of cell shape during cell movement.

Basic functions of Microtubules

- Microtubules, which are frequently found in a cytoplasmic array that extends to the cell periphery, can quickly rearrange themselves to form a bipolar mitotic spindle during cell division
- They also form cilia which act as motile whips or sensory devices on the surface of the cell
- Tightly align and serve as tracts for the transport of materials down long neuronal axons
- In plants, organized arrays of microtubules help to direct the pattern of cell wall synthesis
- In many protozoans they form the framework upon which the entire cell is built

Basic functions of Intermediate filament:

- Intermediate filaments line the inner face of the nuclear envelope, forming a protective cage for the cell’s DNA
- In cytosol, they are twisted into strong cables that can hold epithelial cell sheets together
- help nerve cells to extend long and robust axons
- they allow us to form tough appendages such as hair and fingernails

Intermediary filaments are symmetrical in nature and therefore donot form polarized filaments with two different ends.
Intermediate filaments donot catalyse the hydrolysis of nucleotides

Cell Junction

- Occur at points of cell-cell and cell-matrix contact in all tissues and they are particularly plentiful in epithelia
- Cell junctions are best visualized using either conventional or freeze-fracture electron microscopy
- reveals that the interacting plasma membranes are highly specialized in these regions

Cell junctions can be classified into three functional groups:

1.Occluding junctions seal cells together in an epithelium in a way that prevents even small molecules from leaking from one side of the sheet to the other

2.Anchoring junctions mechanically attach cells to their neighbors or to the extracellular matrix

3.Communicating junctions mediate the passage of chemical or electrical signals from one interacting cell to its partner

- cell junctions and the extracellular matrix is critical for every aspect of the organization, function, and dynamics of multicellular Structures
- Defects in this apparatus underlie an enormous variety of diseases
- two broad categories of tissues that are found in all animals:

1. Connective tissues -such as bone or tendon

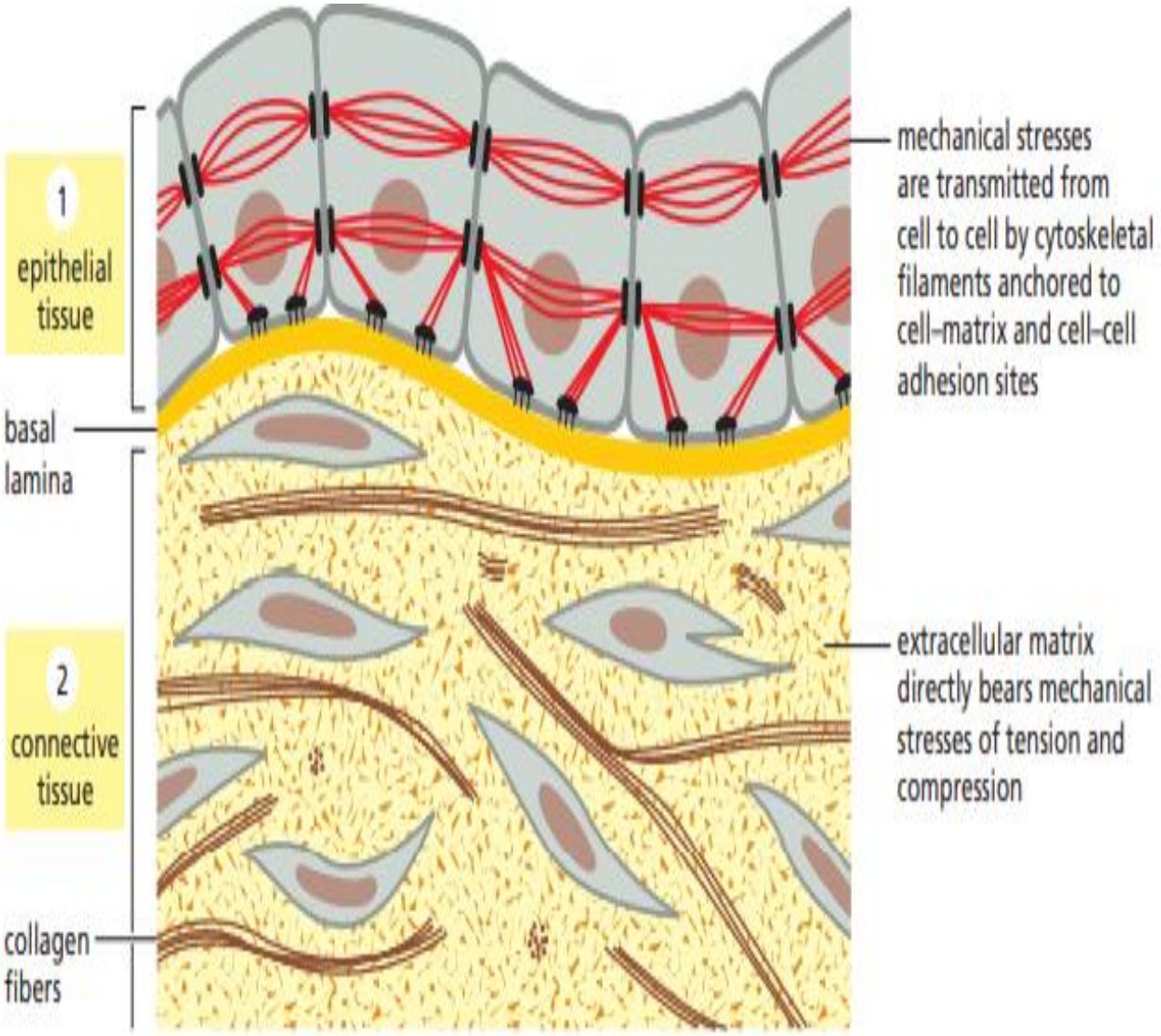
- It is formed from an extracellular matrix produced by cells distributed in the matrix
- cell–matrix junctions link the cytoskeleton to the matrix, allowing the cells to move through the matrix and monitor changes in its mechanical properties

2. epithelial tissues

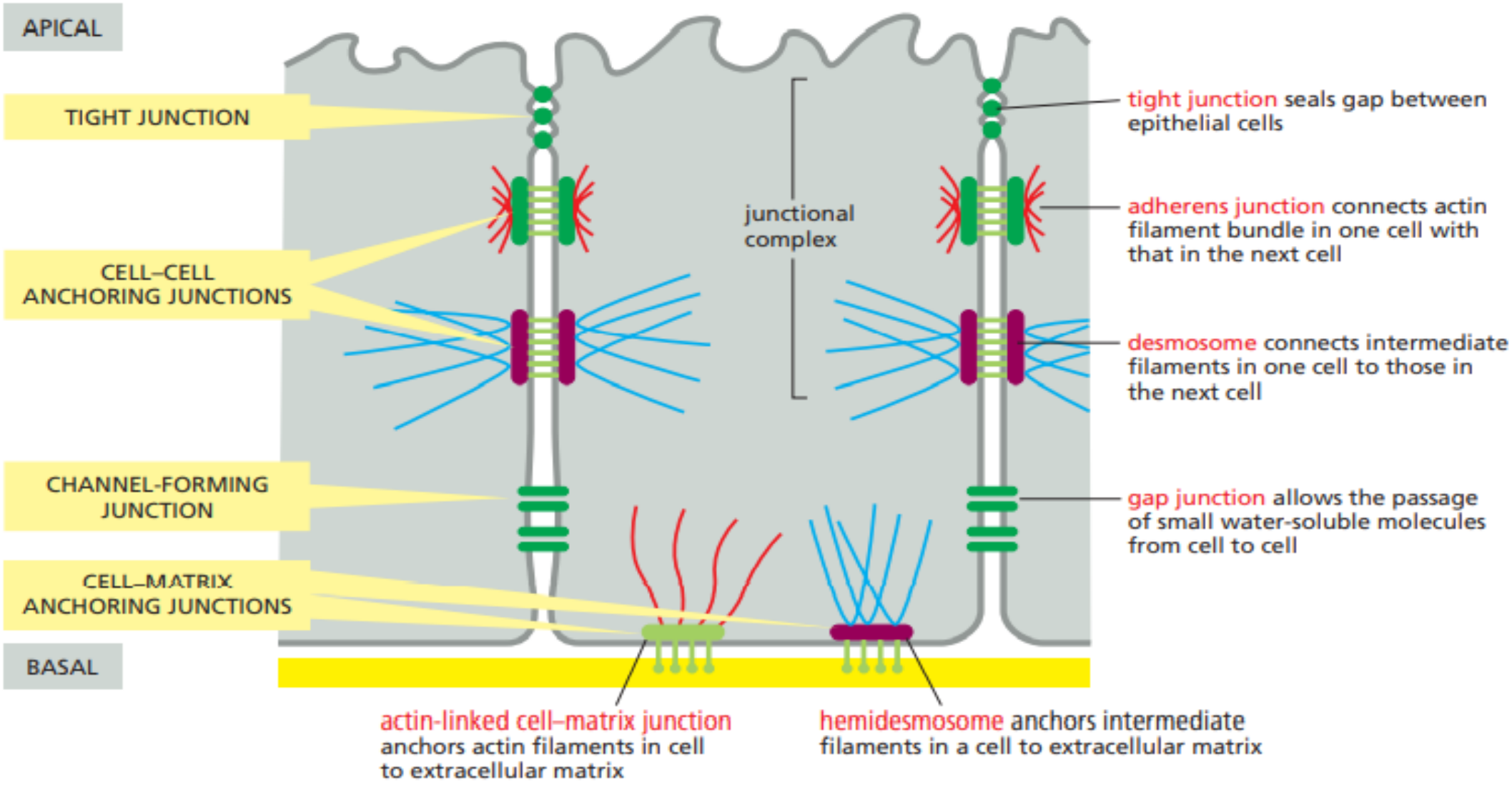
- cells are tightly bound together into sheets called epithelia

- the epithelium, cells are attached to each other directly by cell-cell junctions, where cytoskeletal filaments are anchored
- Two types of anchoring junctions link the cytoskeletons of adjacent cells: adherens junctions are anchorage sites for actin filaments; desmosomes are anchorage sites for intermediate filaments

Two main ways in which animal cells are bound together



cell junctions in a vertebrate epithelial cell, classified according to their primary functions



- four major anchoring junction types depends on transmembrane adhesion proteins that span the plasma membrane
- They fall into two protein superfamily based on the external attachment

1. Cadherin superfamilies mediates cell to cell attachment
2. Integrin superfamilies mediates cell to matrix attachment

- some cadherins link to actin and form adherens junctions
- while others link to intermediate filaments form desmosomes
- some integrins link to actin and form actin linked cell–matrix junctions
- while others link to intermediate filaments and form hemidesmosomes

Figure : Transmembrane adhesion proteins link the cytoskeleton to extracellular structures

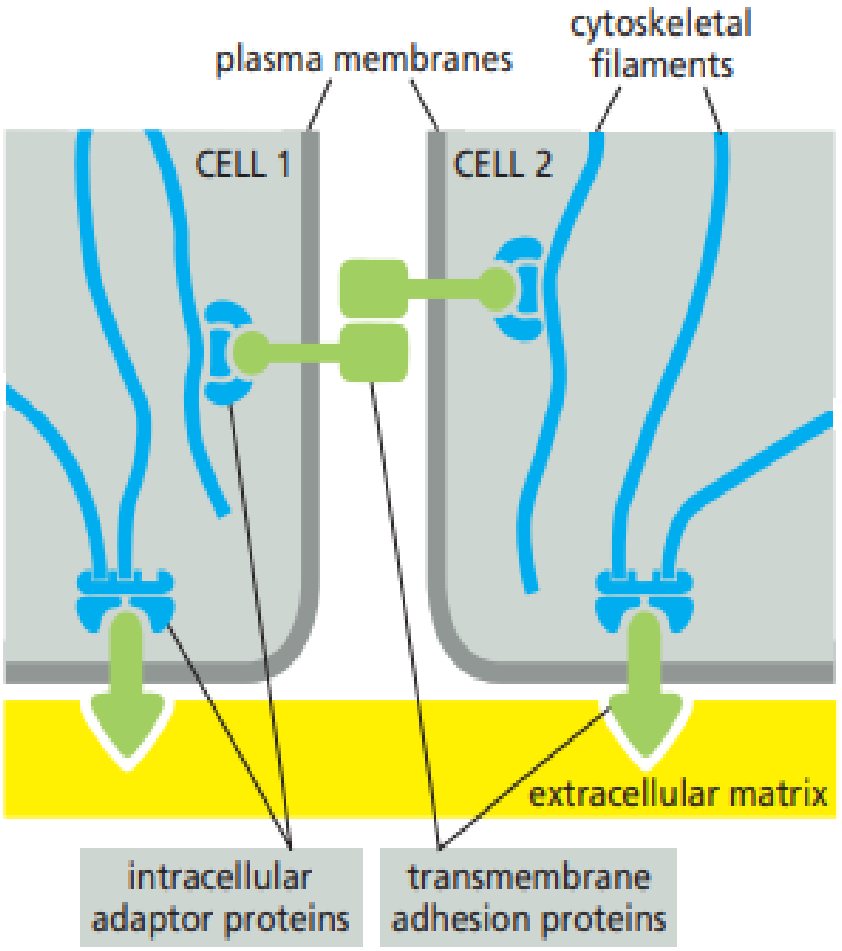


TABLE 19–1 Anchoring Junctions

Junction	Transmembrane adhesion protein	Extracellular ligand	Intracellular cytoskeletal attachment	Intracellular adaptor proteins
Cell–Cell				
Adherens junction	Classical cadherins	Classical cadherin on neighboring cell	Actin filaments	α-Catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin
Desmosome	Nonclassical cadherins (desmoglein, desmocollin)	Desmoglein and desmocollin on neighboring cell	Intermediate filaments	Plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
Actin-linked cell–matrix junction	Integrin	Extracellular matrix proteins	Actin filaments	Talin, kindlin, vinculin, paxillin, focal adhesion kinase (FAK), numerous others
Hemidesmosome	$\alpha_6\beta_4$ Integrin, type XVII collagen	Extracellular matrix proteins	Intermediate filaments	Plectin, BP230

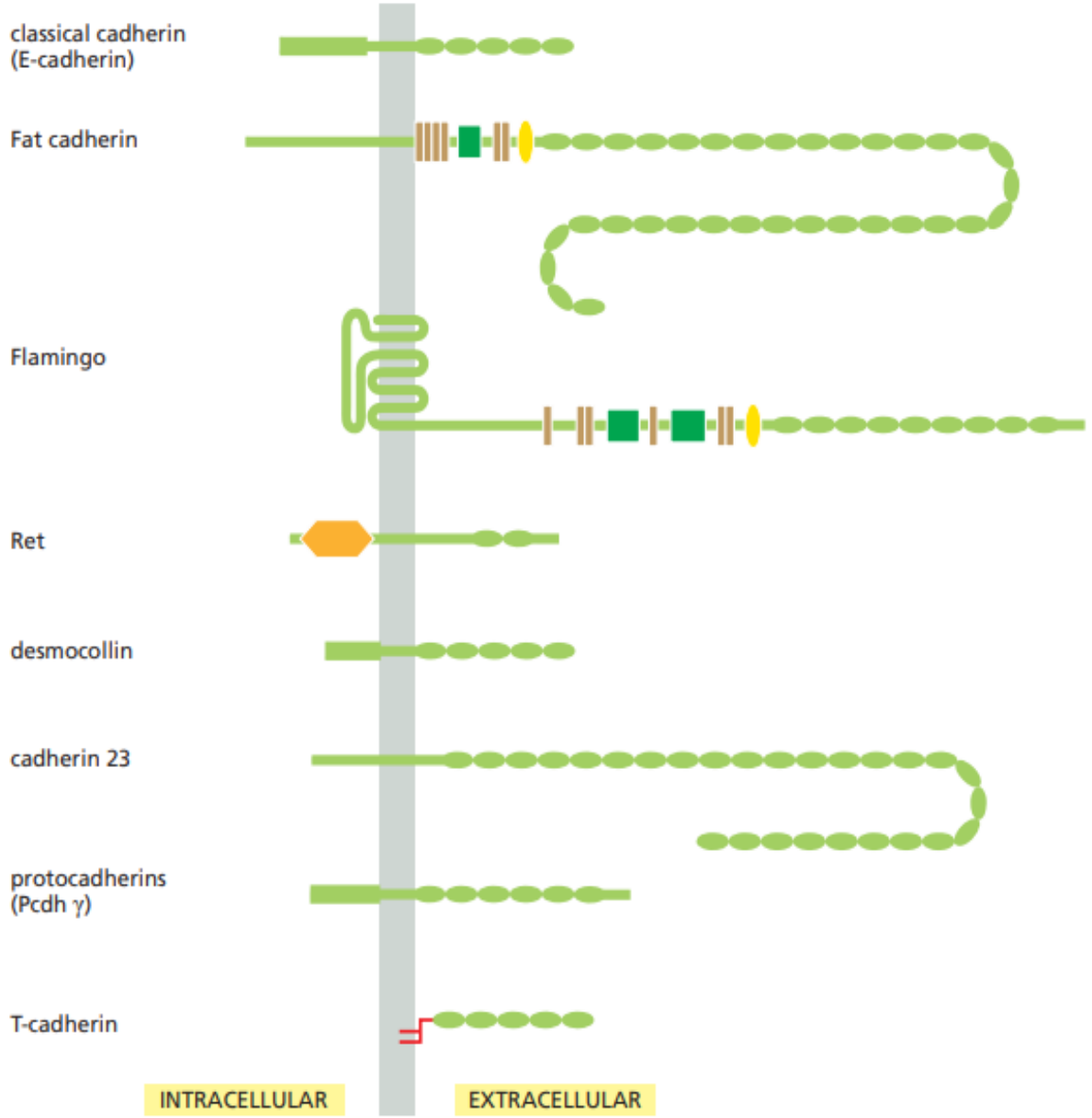
CELL - CELL JUNCTIONS

Cell - cell anchoring junctions - employ cadherins that link cytoskeleton of one cell to another

Its primary function is to resist the external forces that pull cells apart.

Cadherins Superfamilies

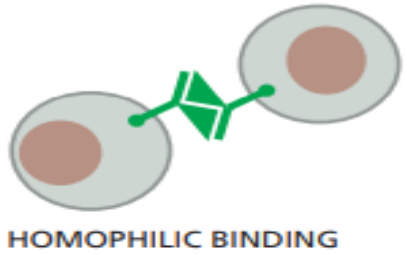
- The cadherins take their name from their dependence on Ca2+ ions: removing Ca2+ from the extracellular medium causes adhesions mediated by cadherins to come apart
- First three cadherins discovered earlier
- E-cadherin is present on many types of epithelial cells
- N-cadherin on nerve, muscle, and lens cells
- P-cadherin on cells in the placenta and epidermis
- These and other classical cadherins are closely related in sequence
- Non-classical cadherins are more distantly related in sequence, with more than 50 expressed in the brain

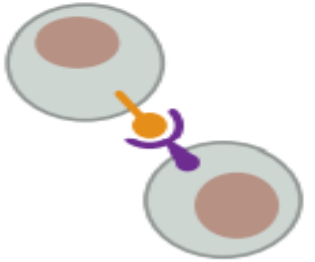


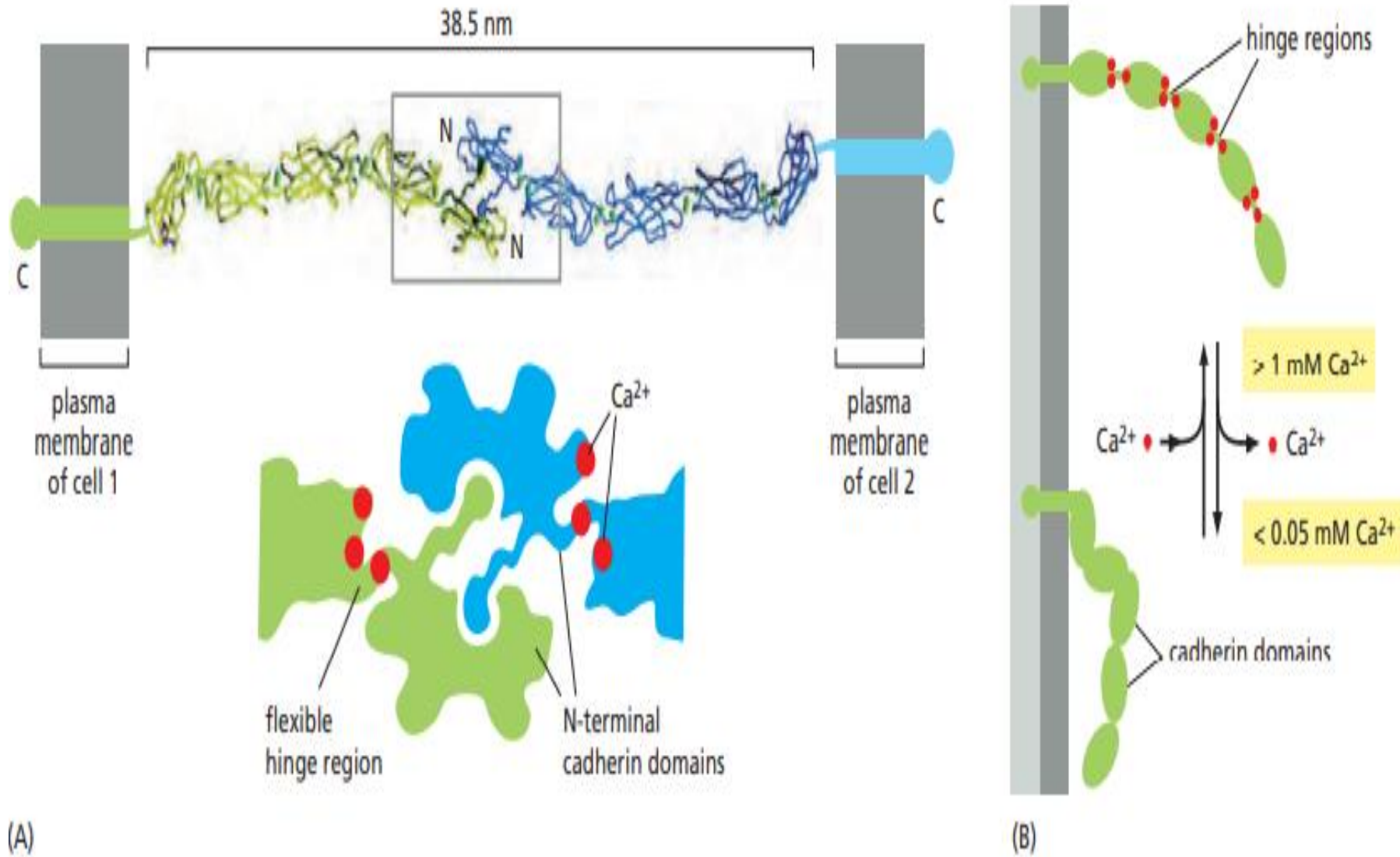
- These proteins all have extracellular portions containing multiple copies of the extracellular cadherin domain (green ovals)
- In the classical cadherins of vertebrates there are 5 of these domains, and in desmogleins and desmocollins there are 4 or 5, but some non-classical cadherins have more than 30
- The intracellular portions are more varied, reflecting interactions with a wide variety of intracellular ligands, including signaling molecules and adaptor proteins that connect the cadherin to the cytoskeleton
- In some cases, such as T-cadherin, a transmembrane domain is not present and the protein is attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor
- The differently colored motifs in Fat, Flamingo, and Ret represent conserved domains that are also found in other protein families

Cadherins Mediate Homophilic Adhesion

- the binding between cadherins is generally hemophilic
- It occurs at the N-terminal tips of the cadherin molecules—the cadherin domains that lie farthest from the membrane
- These terminal domains each form a knob and a nearby pocket, and the cadherin molecules protruding from opposite cell membranes bind by insertion of the knob of one domain into the other
- Cadherin domains joined to the next cadherin domain by a hinge (figure a)
- Ca2+ ions bind to sites near each hinge and prevent it from flexing, so that the whole string of cadherin domains behaves as a rigid and slightly curved rod
- When Ca2+ is removed, the hinges can flex, and the structure becomes floppy (figure b)



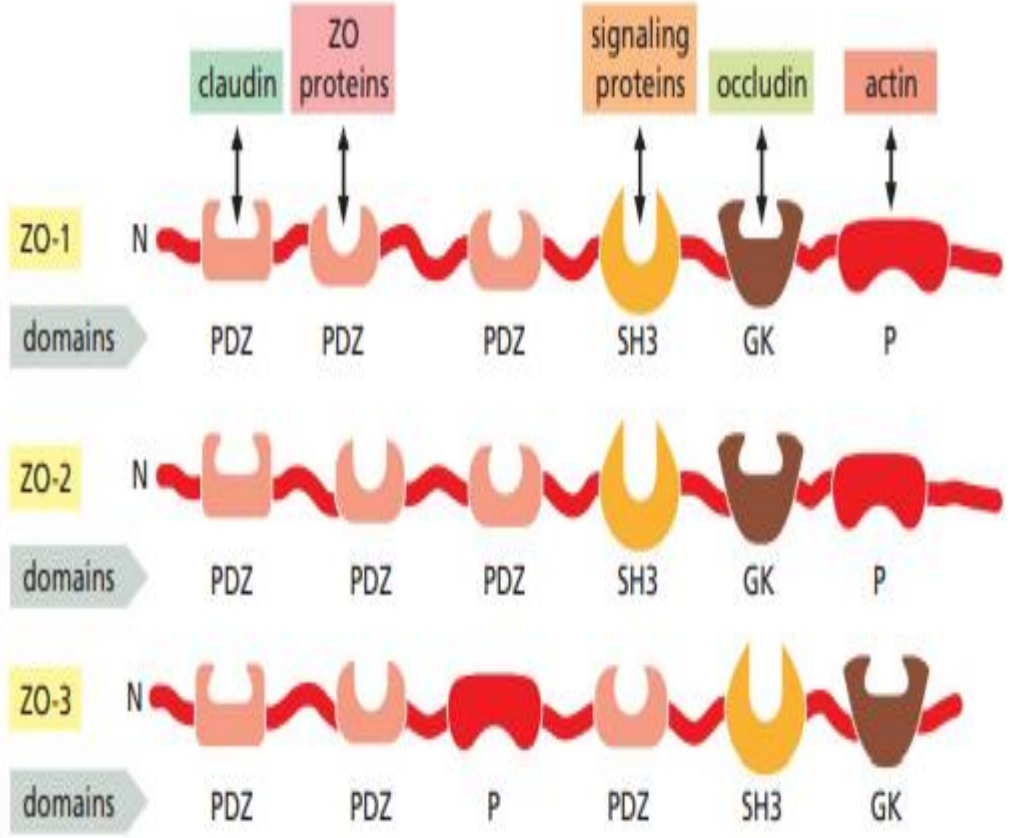



38.5 nm
N
N
C
C
plasma membrane of cell 1
plasma membrane of cell 2
Ca2+
flexible hinge region
N-terminal cadherin domains
(A)
hinge regions
> 1 mM Ca2+
Ca2+
Ca2+
< 0.05 mM Ca2+
cadherin domains
(B)

Scaffold Proteins Organize Junctional Protein Complexes

- In adherens junctions, the organization of adhesion proteins in a tight junction depends on additional proteins that bind the cytoplasmic side of the adhesion proteins
- key organizational proteins at tight junctions are the zonula occludens (ZO) protein
- Three major members of the ZO family—ZO-1, ZO-2, and ZO-3—are large scaffold proteins that provide a structural support on which the tight junction is built
- intracellular molecules consist of strings of protein-binding domains, typically including several PDZ domains—segments about 80 amino acids long that can recognize and bind the C-terminal tails of specific partner proteins
- One domain of these scaffold proteins can attach to a claudin protein, while others can attach to occludin or the actin cytoskeleton
- In this way, the cell can assemble a mat of intracellular proteins that organizes and positions the sealing strands of the tight junction

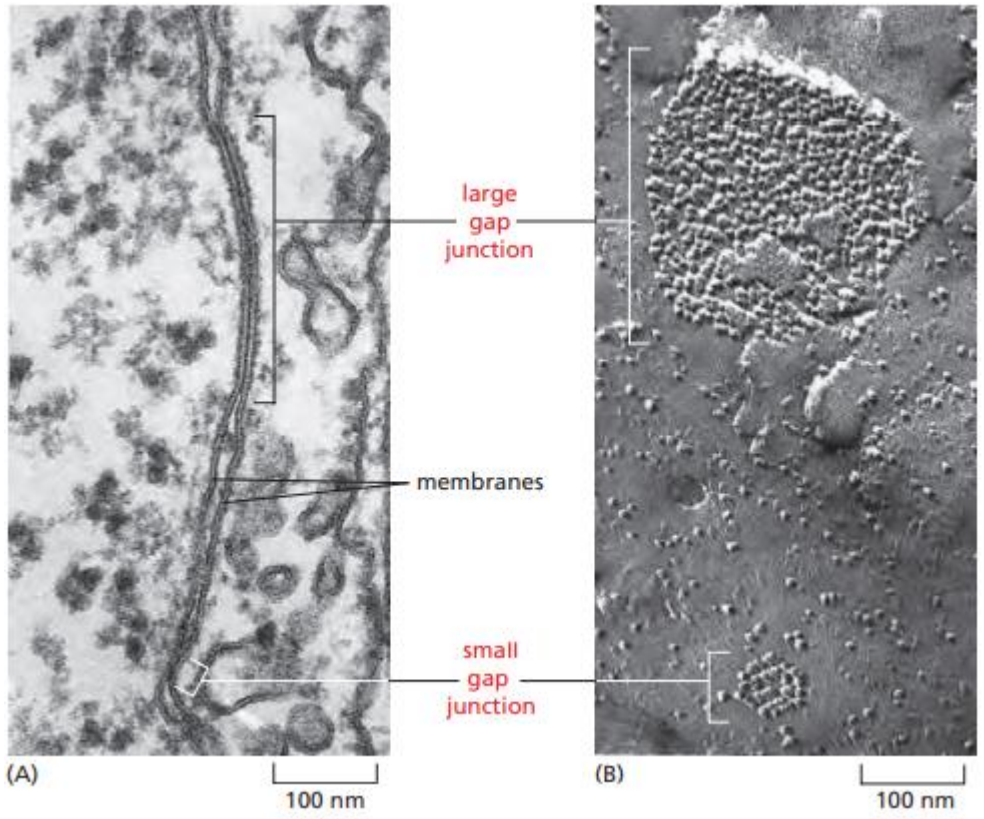
- The scaffold proteins ZO-1, ZO-2, and ZO-3 are concentrated beneath the plasma membrane at tight junctions.
- Each of the proteins contains multiple protein-binding domains, including three PDZ domains, an SH3 domain, and a GK domain, linked together like beads on a flexible string
- These domains enable the proteins to interact with each other and with numerous other partners, as indicated here, to generate a tightly woven protein network that organizes the sealing strands of the tight junction and links them to the actin cytoskeleton
- Scaffold proteins with similar structure help organize other *junctional complexes*, including those at neural synapses



Gap Junctions Couple Cells Both Electrically and Metabolically

junctional structure has a radically different function:

- it bridges gaps between adjacent cells so as to create direct channels from the cytoplasm of one to that of the other ,**Gap junctions**
- They are present in most animal tissues, including connective tissues as well as epithelia and heart muscle
- Each gap junction appears in conventional electron micrographs as a patch where the membranes of two adjacent cells are separated by a uniform narrow gap of about 2–4 nm



- Gap junctions also occur in many tissues whose cells are not electrically excitable
- The sharing of small metabolites and ions provides a mechanism for coordinating the activities of individual cells in such tissues and for smoothing out random fluctuations in small-molecule concentrations in different cells
- Figure: When fluorescent molecules of various sizes are

injected into one of two cells coupled by gap junctions,

molecules with a molecular weight (MW) of less than

about 1000 daltons can pass into the other cell,

but larger molecules cannot

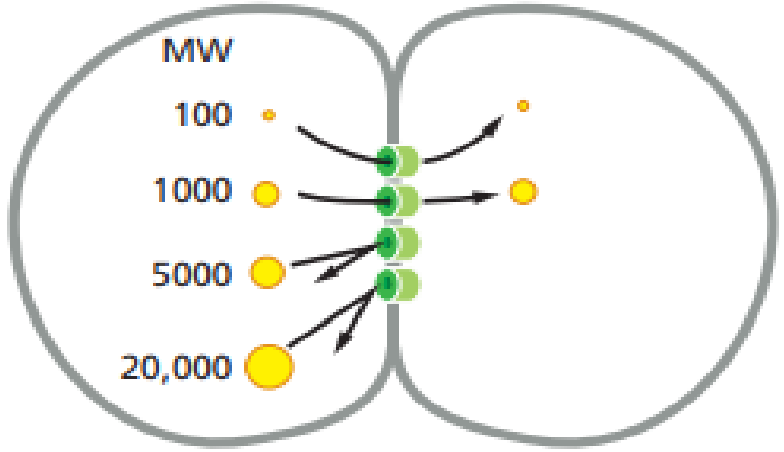


Figure: Determining the size of a gap-junction channel

GAP JUNCTIONS

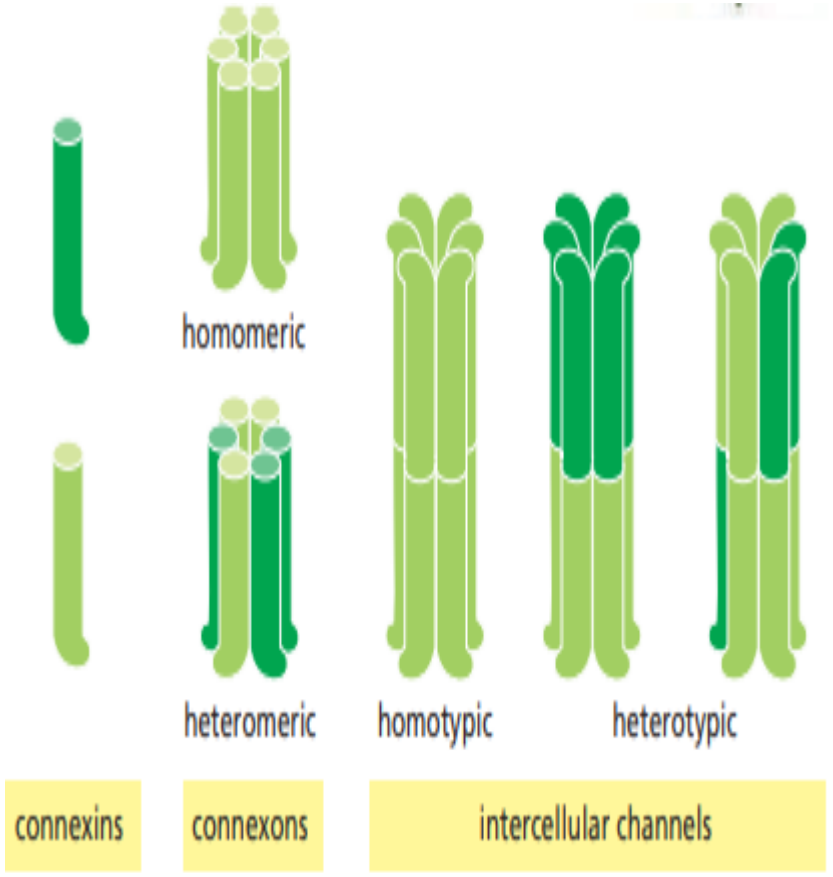
The gap is spanned by channel-forming proteins, of which there are two distinct families

1. connexins
2. Innexins

- Connexins are the predominant gap-junction proteins in vertebrates, with 21 isoforms in humans
- Innexins are found in the gap junctions of invertebrates
- Gap junctions have a pore size of about 1.4 nm, which allows the exchange of inorganic ions and other small water-soluble molecules, but not of macromolecules such as proteins or nucleic acids
- An electric current injected into one cell through a microelectrode causes an electrical disturbance in the neighboring cell, due to the flow of ions carrying electric charge through gap junctions
- electrical coupling through gap junctions synchronizes the contractions of heart muscle cells as well as those of the smooth muscle cells responsible for the peristaltic movements of the intestine

A Gap-Junction Connexon Is Made of Six Transmembrane Connexin Subunits

- Connexins are four-pass transmembrane proteins, six of which assemble to form a hemichannel, or connexon
- When the connexons in the plasma membranes of two cells in contact are aligned, they form a continuous aqueous channel that connects the two cell interiors
- A gap junction consists of many such connexon pairs in parallel, forming a sort of molecular sieve
- Not only does this sieve provide a communication channel between cells, but it also provides a form of cell–cell adhesion that supplements the cadherin- and claudin-mediated adhesions



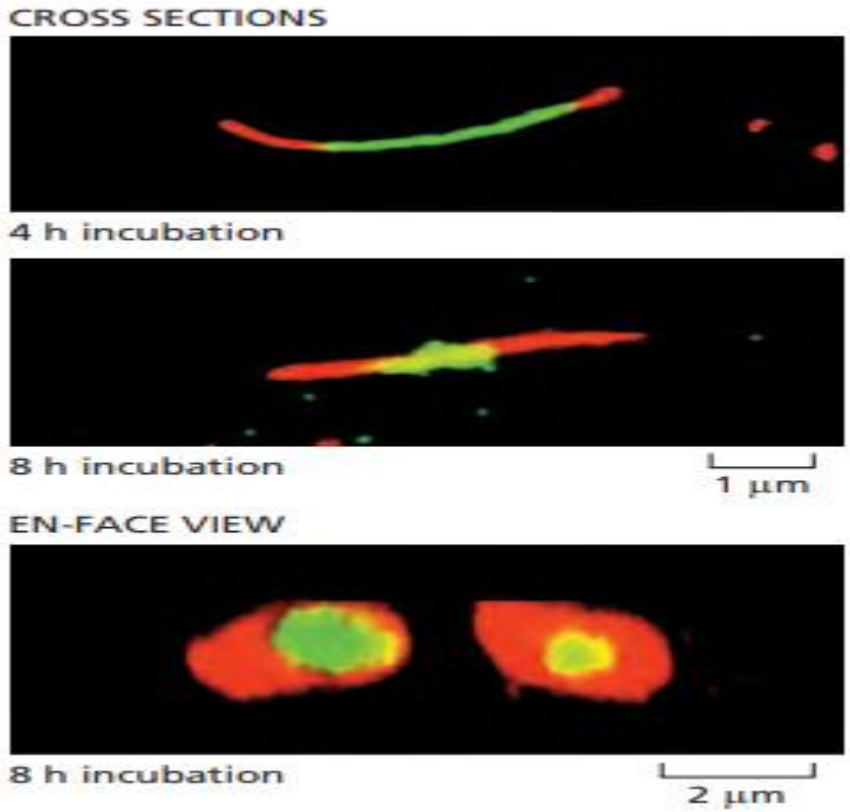
- Most cell types express more than one type of connexin, and two different connexin proteins can assemble into a heteromeric connexon, with its own distinct properties
- individual gap junction channels do not remain open all the time; instead, they flip between open and closed states
- These changes are triggered by a variety of stimuli including

1. the voltage difference between the two connected cells
2. the membrane potential of each cell
3. various chemical properties of the cytoplasm
4. including the pH and concentration of free Ca2+
5. Some subtypes of gap junctions can also be regulated by extracellular signals such as neurotransmitters

- Each gap-junctional plaque is a dynamic structure that can readily assemble, disassemble, or be remodeled, and it can contain a cluster of a few to many thousands of connexons
- Studies with fluorescently labeled connexins in living cells show that new connexons are continually added around the periphery of an existing junctional plaque, while old connexons are removed from the middle of it and destroyed
- This turnover is rapid: the connexin molecules have a half-life of only a few hours

Figure:

- coding for a connexin with a short amino acid tag containing four cysteines in the sequence Cys-Cys-X-XCys-Cys (where X denotes an arbitrary amino acid)
- The fluorescence images show gap junctions between pairs of cells treated in this way
- The central part of the gap-junction plaque is green, indicating that it consists of old connexin molecules
- the periphery is red, indicating that it consists of connexins synthesized during the previous 4 or 8 hours



In Plants, Plasmodesmata Perform Many of the Same Functions as Gap Junctions

- tissues of a plant are organized on different principles from those of an animal
- because plant cells are imprisoned within tough cell walls
- composed of an extracellular matrix rich in cellulose and other polysaccharides
- cell walls of adjacent cells are firmly cemented to those of their neighbors, which eliminates the need for anchoring junctions to hold the cells in place
- But a need for direct cell–cell communication remains
- In Plants,only one class of intercellular junctions present, plasmodesmata
- the cell wall between a typical pair of adjacent cells is at least 0.1 μm thick, and so a structure very different from a gap junction is required to mediate communication across it,Plasmodesmata helps to solve the problem

- plasma membrane of one cell is continuous with that of its neighbor at each plasmodesma, which connects the cytoplasms of the two cells by a roughly cylindrical channel with a diameter of 20–40 nm
- Running through the center of the channel in most plasmodesmata is a narrower cylindrical structure, the desmotubule, which is continuous with elements of the smooth endoplasmic reticulum (ER) in each of the connected cells
- Between the outside of the desmotubule and the inner face of the cylindrical channel formed by plasma membrane is an annulus of cytosol through which small molecules can pass from cell to cell
- passage of molecules with a molecular weight < 800

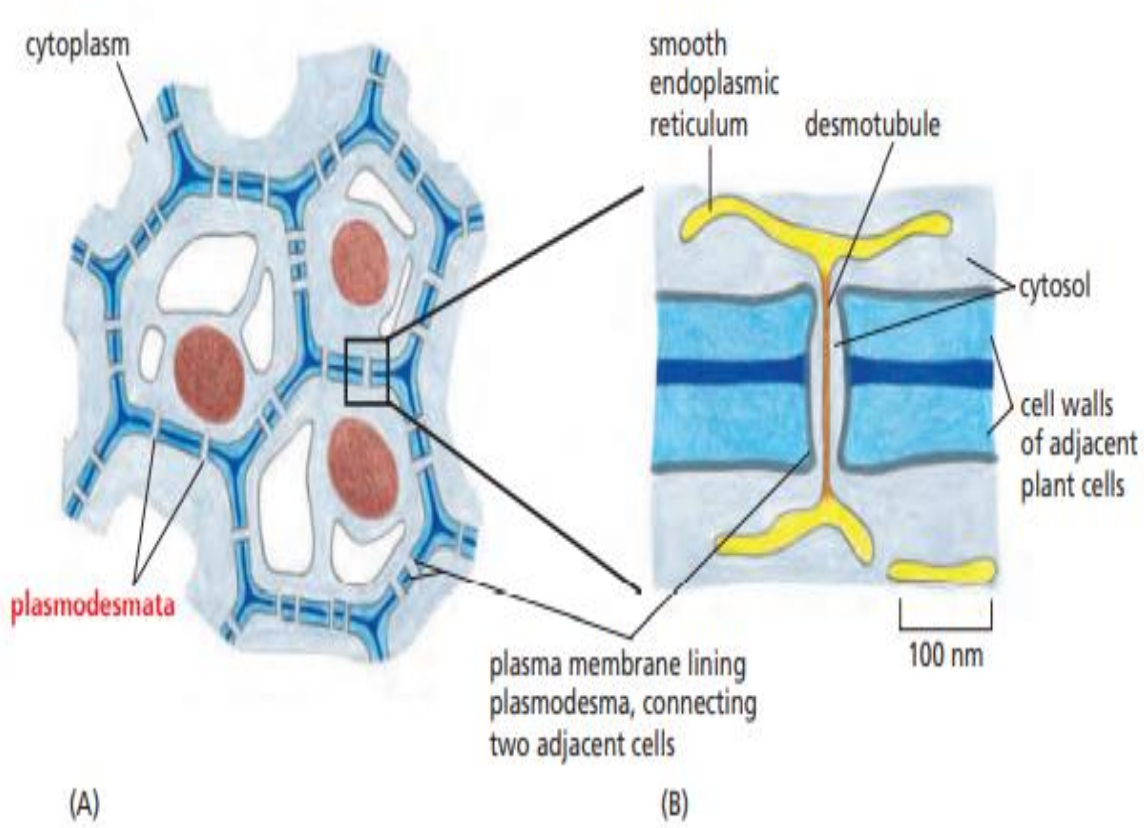


Figure: The cytoplasmic channels of plasmodesmata pierce the plant cell wall and connect cells in a plant together

(B) Each plasmodesma lines the two connected cells

Selectins Mediate Transient Cell–Cell Adhesions in the Bloodstream

- Cell cell adhesion protein families:
- integrins, selectins,and the adhesive immunoglobulin (Ig) superfamily members
- Selectins, like cadherins and integrins, require Ca2+ for their adhesive function; Ig superfamily members do not
- They are cell-surface carbohydrate-binding proteins (lectins) that mediate a variety of transient cell–cell adhesion interactions in the bloodstream
- main role, in vertebrates at least, is in governing the traffic of white blood cells into normal lymphoid organs and any inflamed tissues
- selectins control the binding of white blood cells to the endothelial cells lining blood vessels, thereby enabling the blood cells to migrate out of the bloodstream into a tissue

- They are transmembrane protein with a conserved lectin domain that binds to a specific oligosaccharide on another cell
- three types:
- L-selectin on white blood cells
- P-selectin on blood platelets and on endothelial cells that have been locally activated by an inflammatory response
- E-selectin on activated endothelial cells
- In Lymph node or the spleen, the endothelial cells express oligosaccharides that are recognized by L-selectin on lymphocytes, causing the lymphocytes to loiter and become trapped
- At sites of inflammation, the roles are reversed: the endothelial cells switch on expression of selectins that recognize the oligosaccharides on white blood cells and platelets, flagging the cells down to help deal with the local emergency

- adhesions mediated by both selectins and integrins are heterophilic
- selectins bind to specific oligosaccharides on glycoproteins and glycolipids, while integrins bind to specific Ig-family proteins
- The selectins mediate a weak adhesion because the binding of the lectin domain of the selectin to its carbohydrate ligand is of low affinity
- This allows the white blood cell to adhere weakly and reversibly to the endothelium, rolling along the surface of the blood vessel, propelled by the flow of blood
- The rolling continues until the blood cell activates its integrins
- these transmembrane molecules can be switched into an adhesive conformation that enables them to latch onto specific macromolecules external to the cell—in the present case, proteins on the surfaces of the endothelial cells
- Once it has attached in this way, the white blood cell escapes from the bloodstream into the tissue by crawling out of the blood vessel between adjacent endothelial cells.

The structure and function of selectins

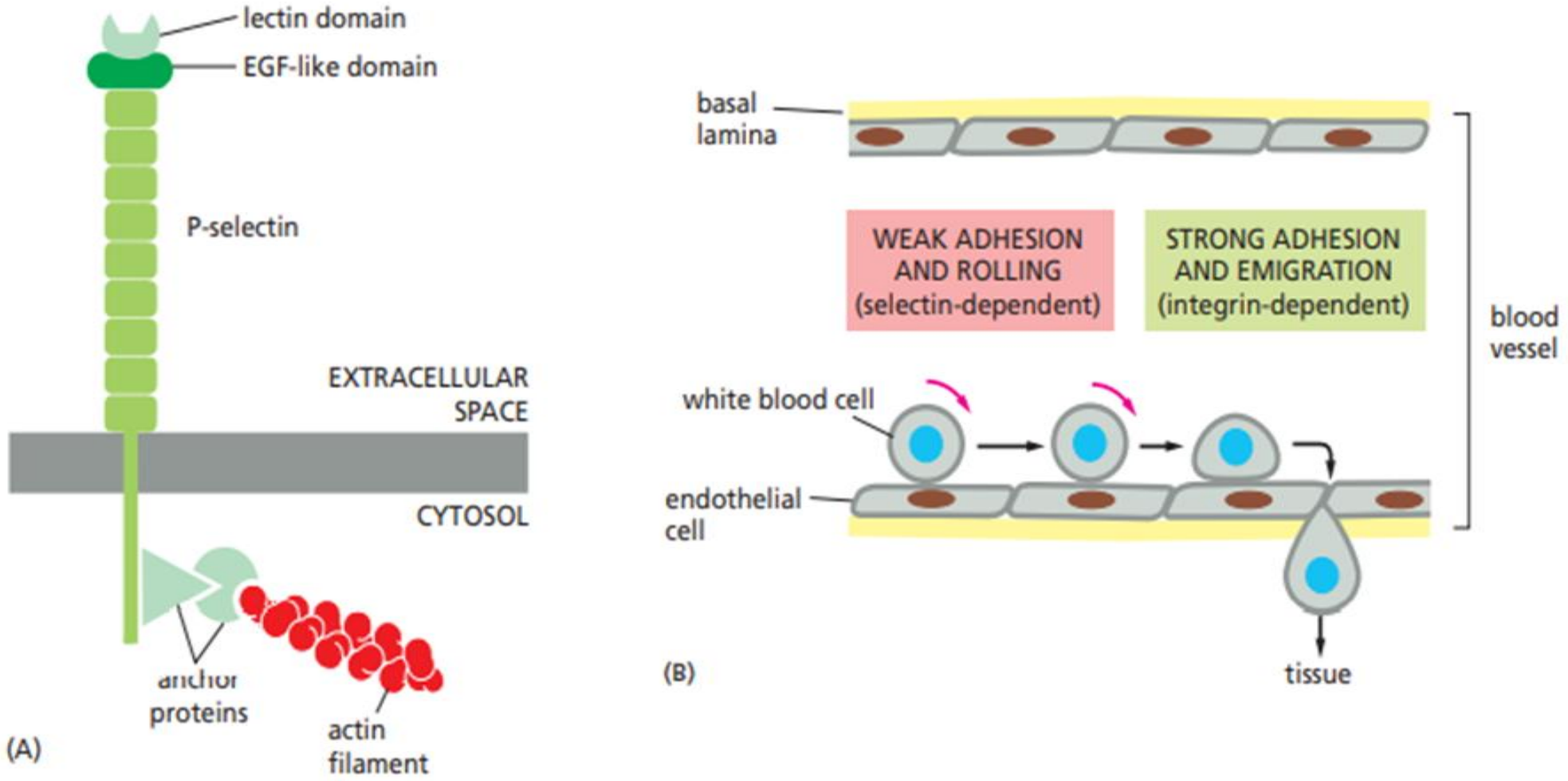


Figure: (A) The structure of P-selectin. The selectin attaches to the actin cytoskeleton through adaptor proteins that are still poorly characterized.

- (B) How selectins and integrins mediate the cell–cell adhesions required for a white blood cell to migrate out of the bloodstream into a tissue
- First, selectins on endothelial cells bind to oligosaccharides on the white blood cell, so that it becomes loosely attached and rolls along the vessel wall
- Then the white blood cell activates a cell surface integrin called LFA1, which binds to a protein called ICAM1 (belonging to the Ig superfamily) on the membrane of the endothelial cell
- The white blood cell adheres to the vessel wall and then crawls out of the vessel by a process that requires another immunoglobulin superfamily member called PECAM1 (or CD31). EGF, epidermal growth factor

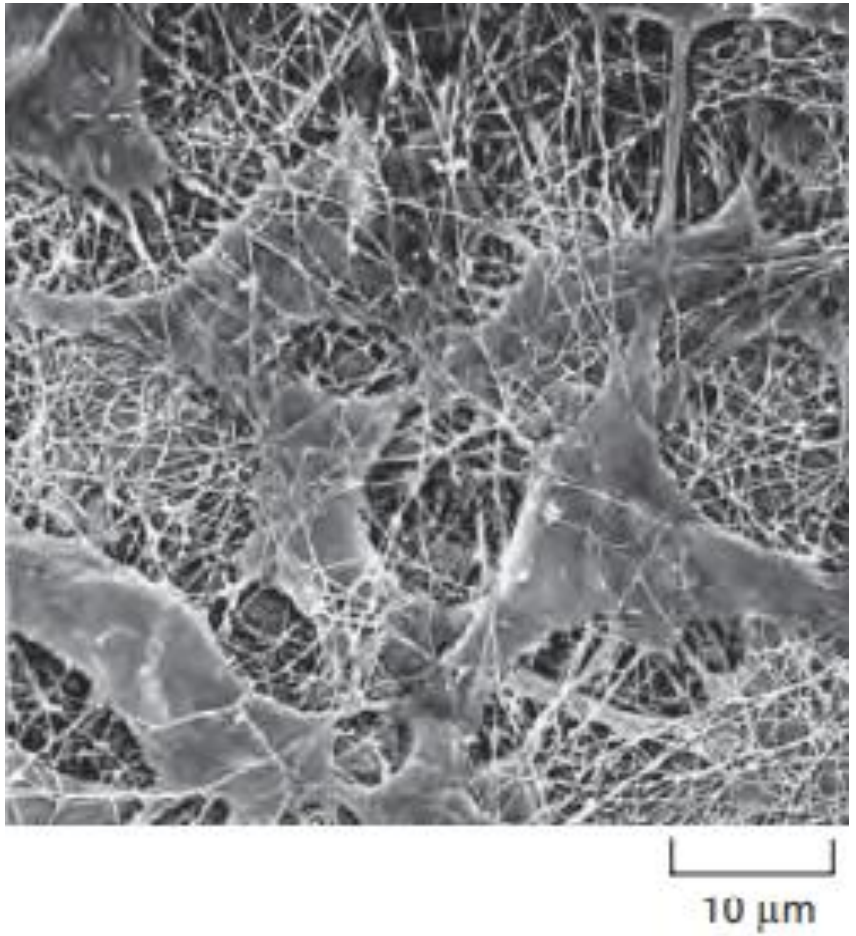
Extracellular Matrix of Animals

- extracellular matrix: Tissues are not only made up of cells, but it has complex and intricate network of macromolecules
- Matrix can become to either calcified form of rock hard structure (bone,teeth), or can form transparent subtances (cornea), or can adopt rope like structures (tendons)
- It is more than a passive scaffold to provide physical support
- It has an active and complex role in regulating the behavior of the cells and influencing their survival, development, migration, proliferation, shape, and function
- basal lamina, the thin layer of specialized extracellular matrix that lies beneath all epithelial cells

The Extracellular Matrix Is Made and Oriented by the Cells Within It

- the orientation of the cytoskeleton inside the cell can control the orientation of the matrix produced outside
- most connective tissues, the matrix macromolecules are secreted by cells called **fibroblasts**
- specialized types of connective tissues, such as chondroblasts, for example, form cartilage, and osteoblasts form bone
- The extracellular matrix is constructed from three major classes of macromolecules: (1) glycosaminoglycans (GAGs), which are large and highly charged polysaccharides that are usually covalently linked to protein in the form of proteoglycans

(2) fibrous proteins, which are primarily members of the collagen family



Fibroblasts in connective tissue

(3) a large class of non collagen glycoproteins, which carry conventional asparagine-linked oligosaccharides

- They are in different shapes and size
- Mammals will have 300 matrix proteins, including about 36 proteoglycans, 40 collagens, and 200 glycoproteins. They contain multiple sub domains self associate to form multimers
- matrix-associated proteins and enzymes that can modify matrix behavior by cross-linking, degradation, or other mechanisms
- Each tissue contains its own unique blend of matrix components, resulting in an extracellular matrix that is specialized for the needs of that tissue
- proteoglycan molecules in connective tissue typically form a highly hydrated, gel-like “ground substance” in which collagens and glycoproteins are embedded

- polysaccharide gel resists compressive forces on the matrix while permitting the rapid diffusion of nutrients, metabolites, and hormones between the blood and the tissue cells
- The collagen fibers strengthen and help organize the matrix, while other fibrous proteins, such as the rubberlike elastin, give it resilience
- many matrix glycoproteins help cells migrate, settle, and differentiate in the appropriate locations

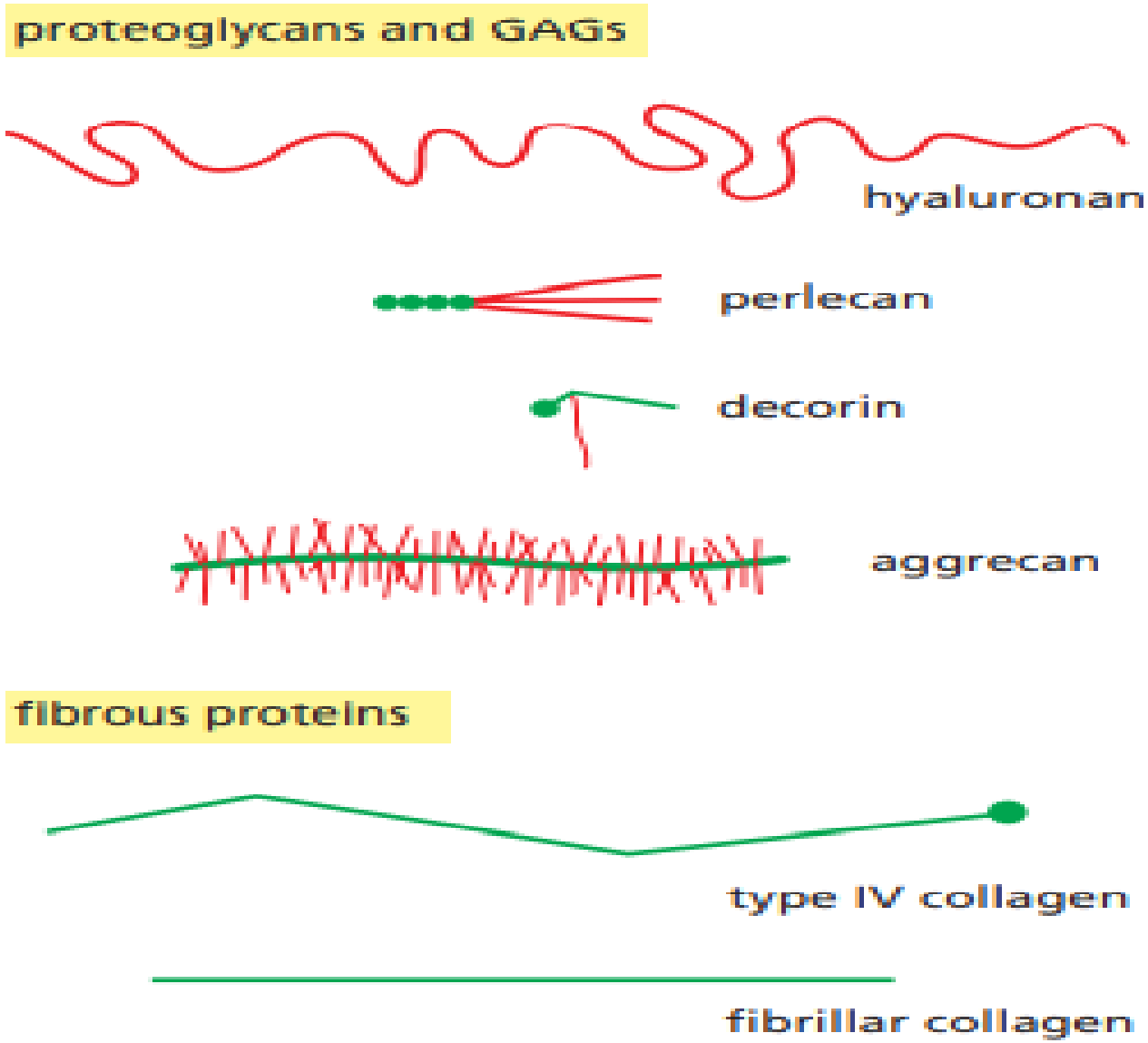
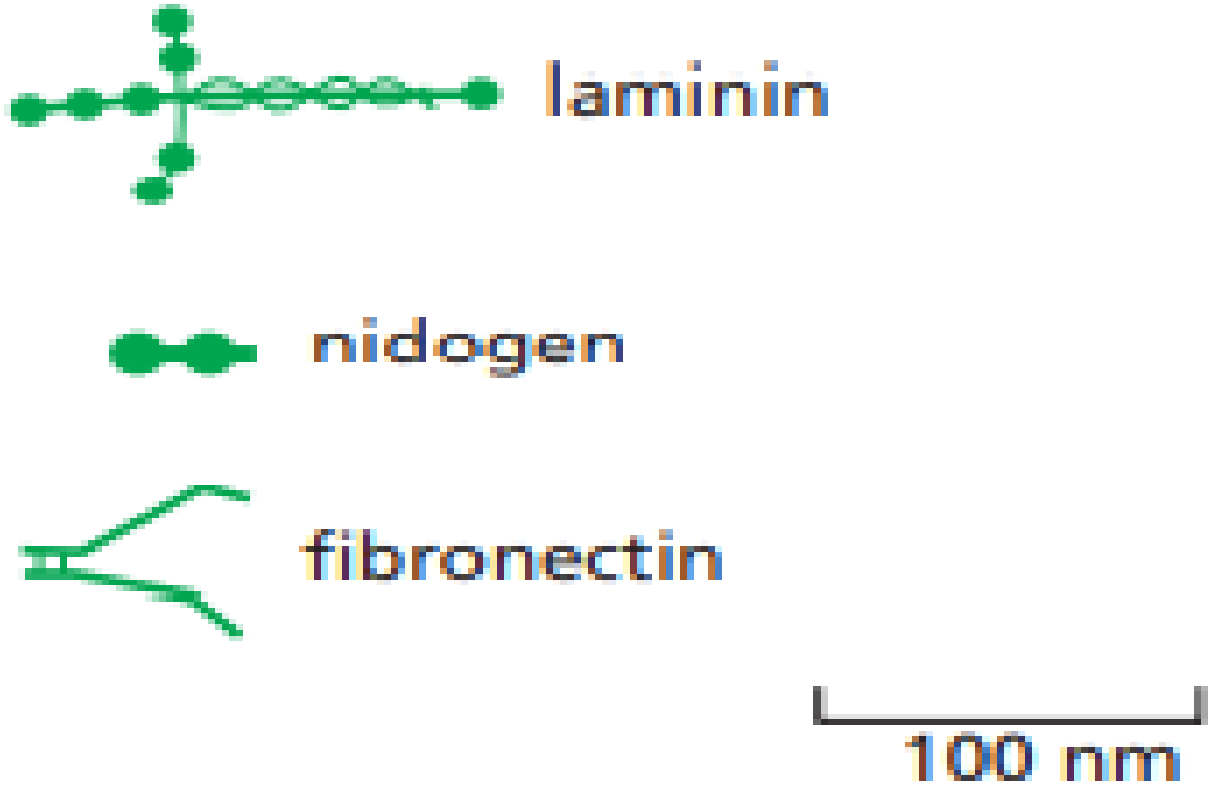


Fig:The comparative shapes and sizes of some of the major extracellular matrix macromolecules. Protein is shown in green, and glycosaminoglycan (GAG) in red

glycoproteins



Glycosaminoglycan (GAG) Chains Occupy Large Amounts of Space and Form Hydrated Gels

- **unbranched polysaccharide** chains composed of repeating disaccharide units (NAG-Uronic)
- Two sugars are N-acetylglucosamine or N-acetylgalactosamine –mostly sulphated, another sugar is uronic acid (glucuronic or iduronic)
- sulfate or carboxyl groups on most of their sugars, GAGs are highly negatively charged and therefore the most anionic molecules produced by animal cells
- Four main groups of GAGs are distinguished by their sugars, the type of linkage between the sugars, and the number and location of sulfate groups: (1) hyaluronan
- (2) chondroitin sulfate and dermatan sulfate
- (3) heparan sulfate
- (4) keratan sulfate

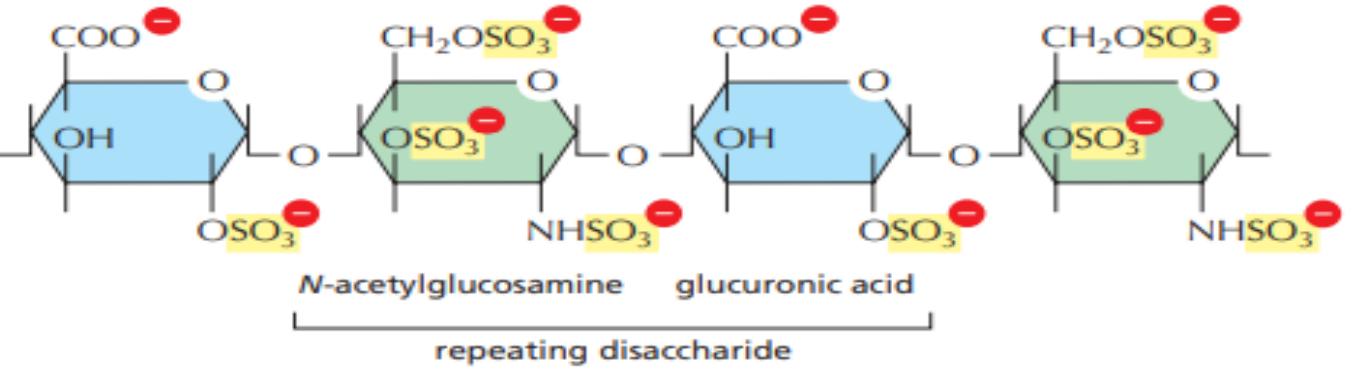


Fig : The repeating disaccharide sequence of a heparan sulfate glycosaminoglycan (GAG) chain

- Polysaccharide chains are too stiff to fold into compact globular structures, and strongly hydrophilic
- GAGs occupy a huge volume relative to their mass and they form hydrated gels even at very low concentrations
- Their high density of negative charges attracts a cloud of cations, especially Na+, that are osmotically active, causing large amounts of water to be sucked into the matrix
- This creates a swelling pressure that enables the matrix to withstand compressive forces (in contrast to collagen fibrils, which resist stretching forces)

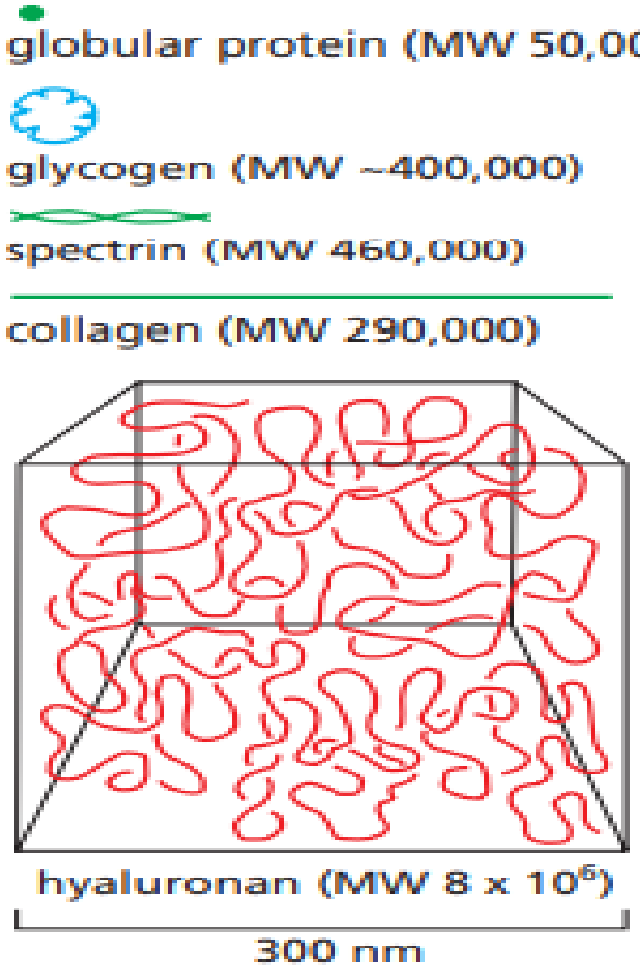


Fig. The relative dimensions and volumes occupied by various macromolecules

- The cartilage matrix that lines the knee joint, for example, can support pressures of hundreds of atmospheres in this way
- Defects in the production of GAGs can affect many different body systems
- for example, if there is a severe deficiency in the synthesis of dermatan sulfate disaccharide, the affected individuals have a short stature, a prematurely aged appearance, and generalized defects in their skin, joints, muscles, and bones

Hyaluronan Acts as a Space Filler During Tissue Morphogenesis and Repair

- simplest form of GAGs, no sulphonate group and have repeating sequence of up to 25,000 dissacharide units not linked covalently
- It has important role as a space filler during embryonic development,where it force a change in the shape of the structure
- Small quantity expands to occupy large volume
- Resisting compressive forces in tissues and joints
- Other GAGs synthesized inside the cell and released by exocytosis
- But Hyaluronan spun out directly from the cell surface by enzymes in the plasma membrane
- It will be synthesized from the basal side, can deform the epithelium by creating the free space beneath it, cells to migrate. After cell migration hyalurone will be degraded by hyaluronidase
- Eg. It forms valves and septa in heart. It will produced in large quantities during wound healing

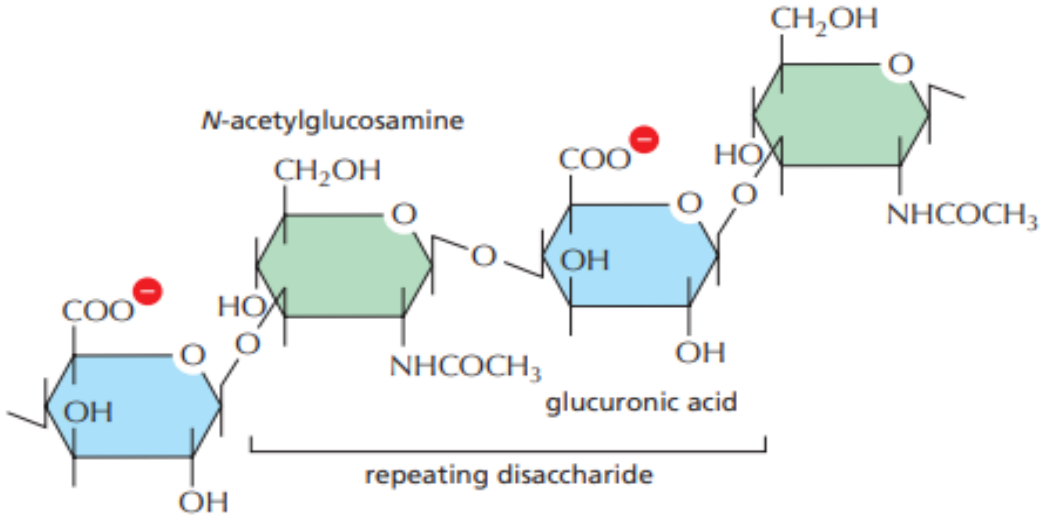


Fig. The repeating disaccharide sequence in hyaluronan, a relatively simple GAG

Proteoglycans Are Composed of GAG Chains Covalently Linked to a Core Protein

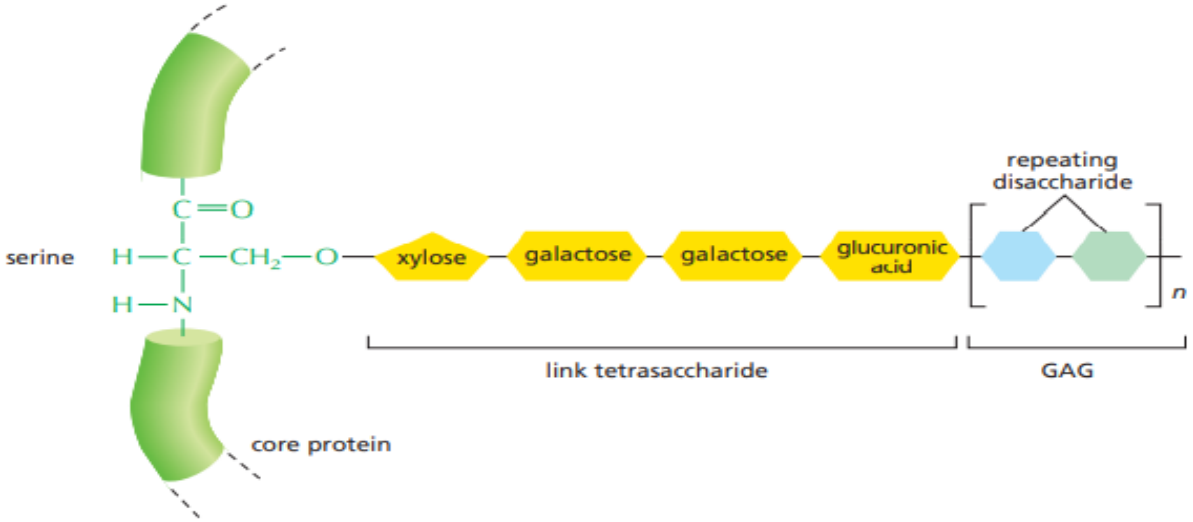


Fig. The linkage between a GAG chain and its core protein in a proteoglycan molecule.

- All GAGs are covalently attached to proteins as proteoglycans
- Ribosomes make the polypeptide chain or core protein of proteoglycan
- Then threaded into the lumen of the endoplasmic reticulum , polypeptide chain assembled in GA
- a special linkage tetrasaccharide is attached to a serine side chain on the core protein to serve as a primer for polysaccharide growth; then, one sugar at a time is added by specific glycosyl transfer
- many of the polymerized sugars are covalently modified by a sequential and coordinated series of reactions
- It can be ditinguished from other glycoproteins by the nature, quantity and arrangement of their sugar side chains, it must have atleast one GAG

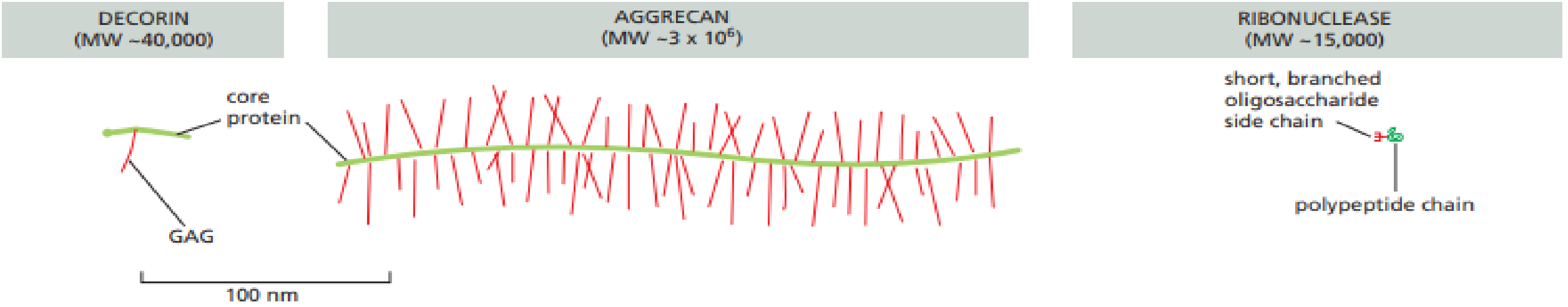
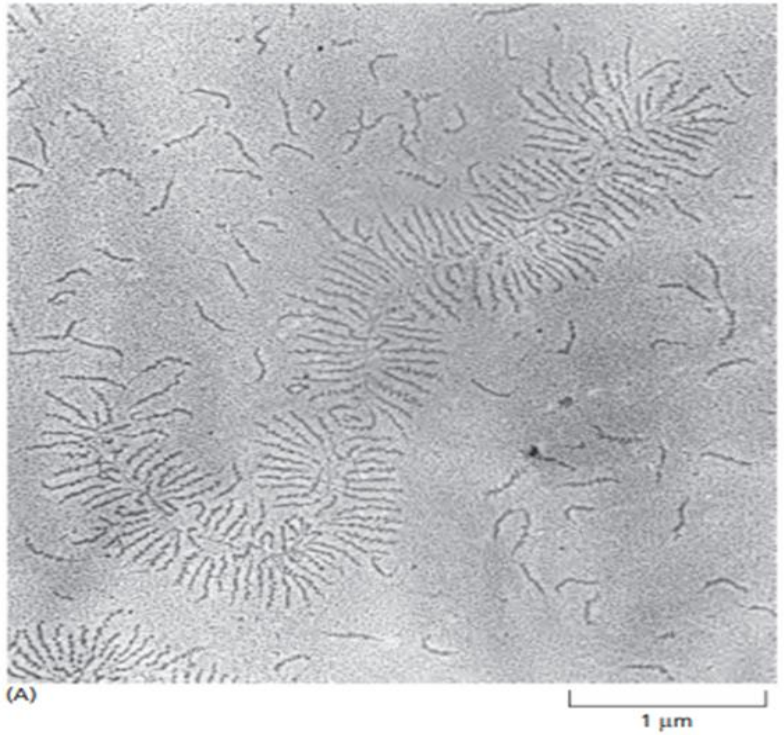


Fig. decorin and aggrecan

proteoglycan found in the extracellular matrix.

The figure compares these two proteoglycans with a typical secreted glycoprotein molecule, pancreatic ribonuclease B

- Aggrecan typically consists of about 100 chondroitin sulfate chains and about 30 keratan sulfate chains linked to a serine-rich core protein of almost 3000 amino acids
- Decorin “decorates” the surface of collagen fibrils



A) An electron micrograph of an aggrecan aggregate shadowed with platinum

- Many free aggrecan molecules are also visible.

(B) A drawing of the giant aggrecan aggregate shown in (A).

It consists of about 100 aggrecan monomers noncovalently

bound through the N-terminal domain of the core protein to a single hyaluronan Chain A link protein binds both to the core protein of the proteoglycan and to the hyaluronan chain, thereby stabilizing the aggregate

- The link proteins are members of a family of hyaluronan-binding proteins, some of which are cell-surface proteins
- The molecular mass of such a complex can be 108 daltons or more, and it occupies a volume equivalent to that of a bacterium, which is about 2 × 10–12 cm3

- Proteoglycans have the potential, limitless heterogeneity
- Many core protein share characteristic domain such as LINK domain , involved in binding to GAGs
- Aggrecan –huge proteoglycan
- It is a major component of cartilage, 3 × 106 daltons with over 100 GAG chains
- Decorin –small which have only 1-10 GAG chains
- Secreted by fibroblast and has single GAG
- Decorin binds to collagen fibrils and regulates fibril assembly and fibril diameter; mice that cannot make decorin have fragile skin that has reduced tensile strength
- GAGs and proteoglycans of these various types can associate to form even larger polymeric complexes in the extracellular matrix
- Molecules of aggrecan, for example, assemble with hyaluronan in cartilage matrix to form aggregates that are as big as a bacterium

- Besides associating with one another, GAGs and proteoglycans associate with fibrous matrix proteins such as collagen and with protein meshworks such as the basal lamina, creating extremely complex composites

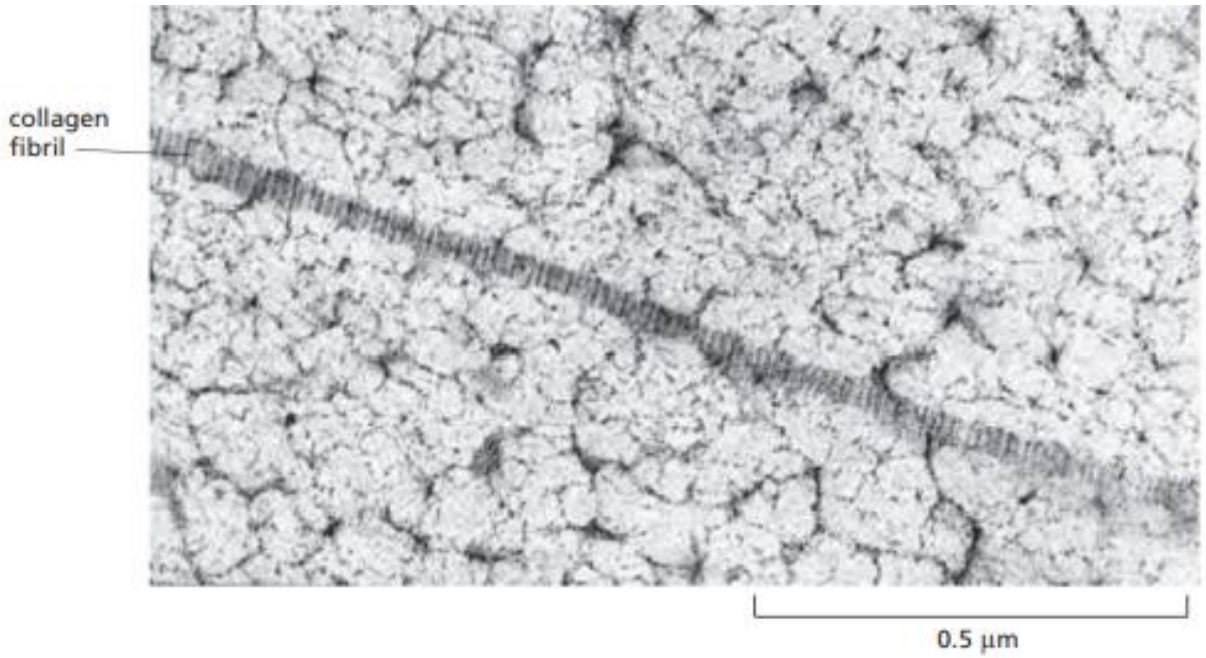


Fig. Proteoglycans in the extracellular matrix of rat cartilage

- Tissues was fixed using freeze substitution method
- the proteoglycan molecules are seen to form a fine filamentous network in which a single striated collagen fibril is embedded

- All proteoglycans are not secreted components of EM
- Their core protein either inserted across the lipid bilayer or attached to the lipid bilayer by a glycosylphosphatidylinositol (GPI) anchor
- Among the best-characterized plasma membrane proteoglycans are the syndecans, which have a membrane-spanning core protein whose intracellular domain is thought to interact with the actin cytoskeleton and with signaling molecules in the cell cortex

- syndecans can be found in cell–matrix adhesions where they modulate integrin function by interacting with fibronectin on the cell surface and with cytoskeletal and signaling proteins inside the cell
- syndecan and other proteoglycans also interact with soluble peptide growth factors, influencing their effects on cell growth and proliferation

Collagens Are the Major Proteins of the Extracellular Matrix

- The collagens are a family of fibrous proteins found in all multicellular animals.
- secreted in large quantities by connective-tissue cells, major component of skin and bone
- 25% of the total protein mass
- Primary features is long, stiff, triple stranded helix structure, in which three polypeptide chain are called as α chains
- Rich in Proline , Glycine which is important in helix formation

- 42 distinct genes codes for different collagen alpha chain
- Various combination using 42 α chains are formed - only a limited number of triple helical combinations are possible
- 40 types of collagens were found to exhibit various combinations based on the need
- Type I –most common , principal collegen of skin and bone ,belongs to fibrillar collagens or fibril-forming collagen
- After secreted into EM , they will assemble into higher order polymers called collagen fibrils- thin structure (10-300nm)
- They will aggregate into large cable lie structre which can be visible in light microscope as collagen fibres
- Collagen types IX and XII are called fibril-associated collagens, as they decorates the surface of the collegen fibrils

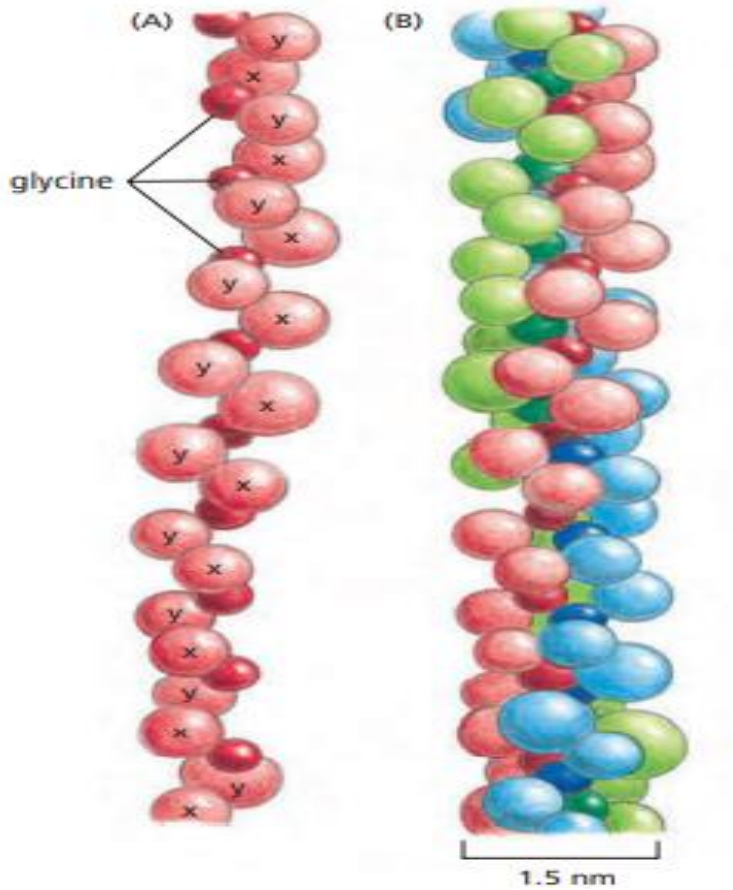


Fig. The structure of a typical collagen molecule

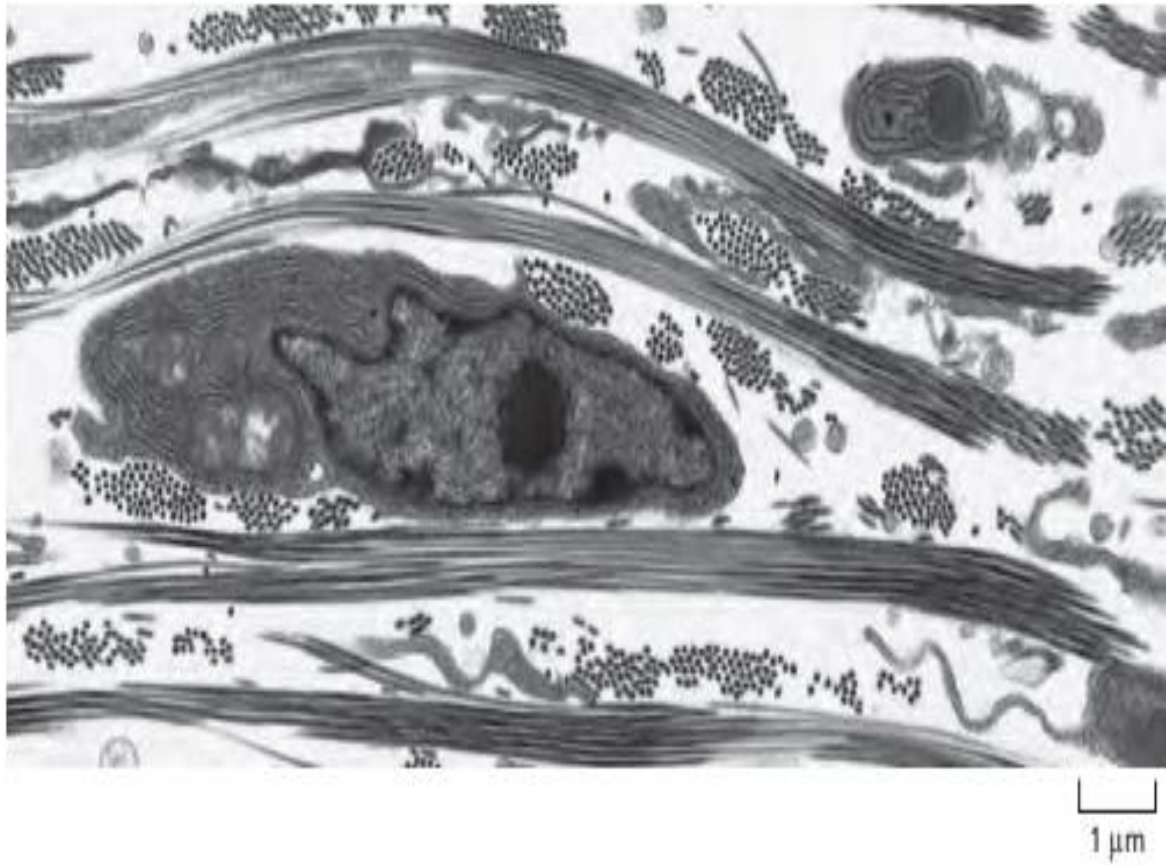


Fig. A fibroblast surrounded by collagen fibrils in the connective tissue of embryonic chick skin.

- They are thought to link these fibrils to one another and to other components in the extracellular matrix
- Type IV is a network-forming collagen, forming a major part of basal laminae
- type VII molecules form dimers that assemble into specialized structures called anchoring fibrils
- Anchoring fibrils help attach the basal lamina of multilayered epithelia to the underlying connective tissue and therefore are especially abundant in the skin
- There are also a number of “collagen-like” proteins containing short collagen-like segments. These include collagen type XVII, which has a transmembrane domain and is found in hemidesmosomes, and type XVIII, the core protein of a proteoglycan in basal laminae
- exons are 54, or multiples of 54, nucleotides long, suggesting that these collagens originated through multiple duplications of a primordial gene containing 54 nucleotides and encoding exactly six Gly-X-Y repeats

TABLE 19–2 Some Types of Collagen and Their Properties

	Type	Polymerized form	Tissue distribution	Mutant phenotype
Fibril-forming (fibrillar)	I	Fibril	Bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)	Severe bone defects, fractures (*osteogenesis imperfecta*)
	II	Fibril	Cartilage, intervertebral disc, notochord, vitreous humor of the eye	Cartilage deficiency, dwarfism (*chondrodysplasia*)
	III	Fibril	Skin, blood vessels, internal organs	Fragile skin, loose joints, blood vessels prone to rupture (*Ehlers–Danlos syndrome*)
	V	Fibril (with type I)	As for type I	Fragile skin, loose joints, blood vessels prone to rupture
	XI	Fibril (with type II)	As for type II	Myopia, blindness
Fibril-associated	IX	Lateral association with type II fibrils	Cartilage	Osteoarthritis
Network-forming	IV	Sheetlike network	Basal lamina	Kidney disease (glomerulonephritis), deafness
	VII	Anchoring fibrils	Beneath stratified squamous epithelia	Skin blistering
Transmembrane	XVII	Nonfibrillar	Hemidesmosomes	Skin blistering
Proteoglycan core protein	XVIII	Nonfibrillar	Basal lamina	Myopia, detached retina, hydrocephalus

Note that types I, IV, V, IX, and XI are each composed of two or three types of α chains (distinct, nonoverlapping sets in each case), whereas types II, III, VII, XVII, and XVIII are composed of only one type of α chain each.

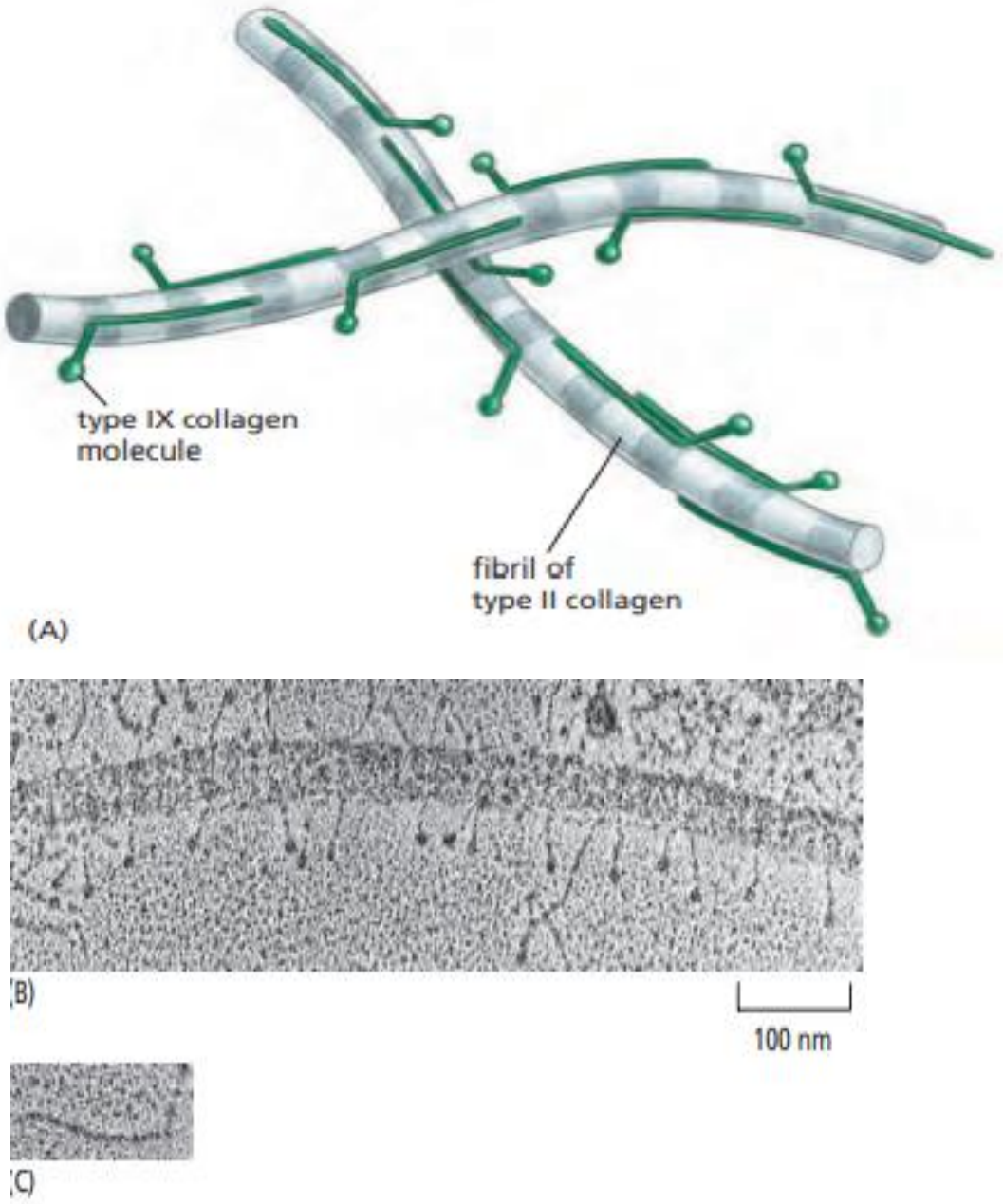


Figure. Type IX collagen.

(A) Type IX collagen molecules binding

in a periodic pattern to the surface of a fibril containing type II collagen

(B) Electron micrograph of a rotary-shadowed type-II-collagen-containing

fibril in cartilage, decorated by type IX collagen molecules

(C) An individual type IX collagen molecule.

Cells Help Organize the Collagen Fibrils They Secrete by Exerting Tension on the Matrix

- If two small pieces of embryonic tissue containing fibroblasts are placed far apart on a collagen gel, the intervening collagen becomes organized into a compact band of aligned fibers that connect the two explants
- The fibroblasts subsequently migrate out from the explants along the aligned collagen fibers. Thus, the fibroblasts influence the alignment of the collagen fibers, and the collagen fibers in turn affect the distribution of the fibroblasts

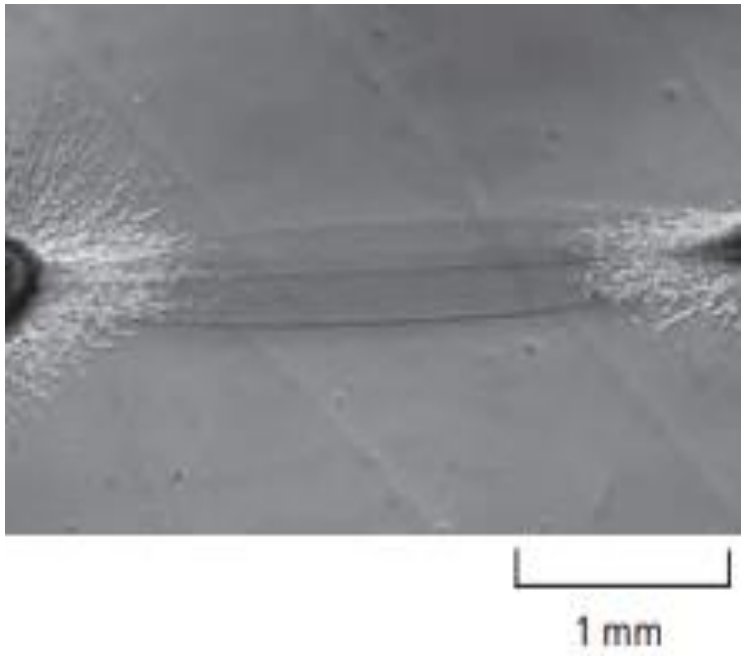


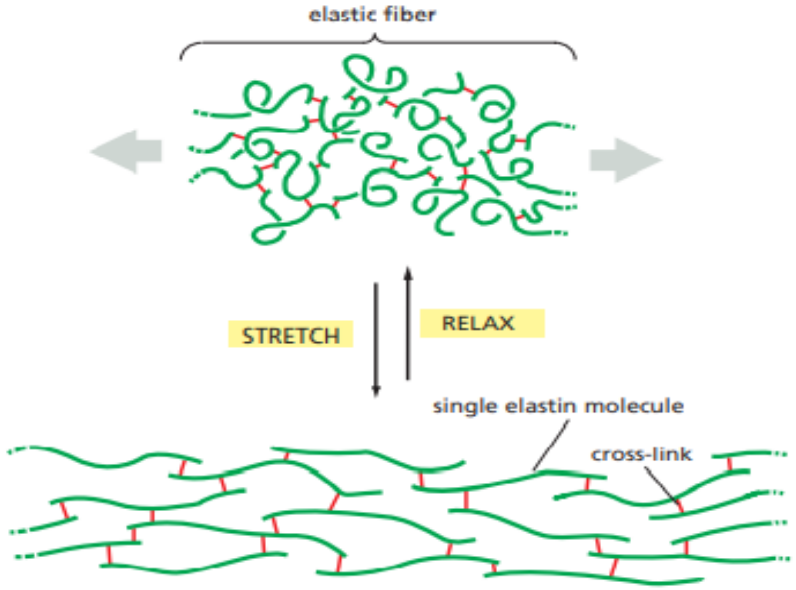
Fig. The shaping of the extracellular matrix by cells

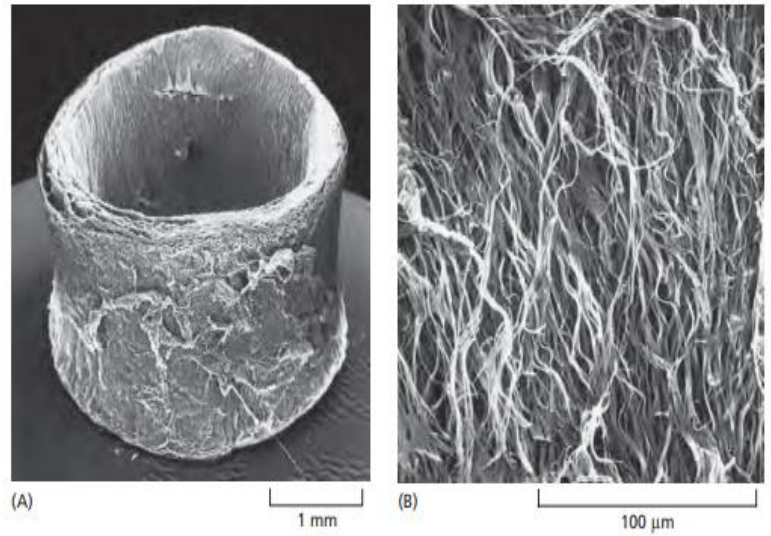
- Fibroblasts may have a similar role in organizing the extracellular matrix inside the body
- First they synthesize the collagen fibrils and deposit them in the correct orientation
- Then they work on the matrix they have secreted, crawling over it and tugging on it so as to create tendons and ligaments and the tough, dense layers of connective tissue that surround and bind together most organs
- All the other components have been digested away with enzymes and formic acid

Elastin Gives Tissues Their Elasticity

- In mammals, skin, blood vessels, and lungs, need to be both strong and elastic in order to function
- A network of elastic fibers in the extracellular matrix of these tissues gives them the resilience to recoil after transient stretch
- inelastic collagen fibrils are interwoven with the elastic fibers to limit the extent of stretching and prevent the tissue from tearing
- Major component is elastin, a highly hydrophobic protein about 750 amino acids long
- Soluble tropoelastin (the biosynthetic precursor of elastin) is secreted into the extracellular space and assembled into elastic fibers close to the plasma membrane
- After secretion, they will form highly cross linked to form network of elastin fibres and sheets
- Elastin protein have two short segment-hydrophobic segments, which are responsible for the elastic properties of the molecule

- Other one is alanine- and lysine-rich α-helical segments, which are cross-linked to adjacent molecules by covalent attachment of lysine residues
- Each segment is encoded by a separate exon
- Fig 1. parts of the elastin polypeptide chain, like the polymer chains in ordinary rubber, adopt a loose “random coil” conformation
- it is the random coil nature of the component molecules cross-linked into the elastic fiber network that allows the network to stretch and recoil like a rubber band
- Fig. 2 Elastin is the dominant extracellular matrix protein in arteries, comprising 50% of the dry weight of the largest artery—the aorta





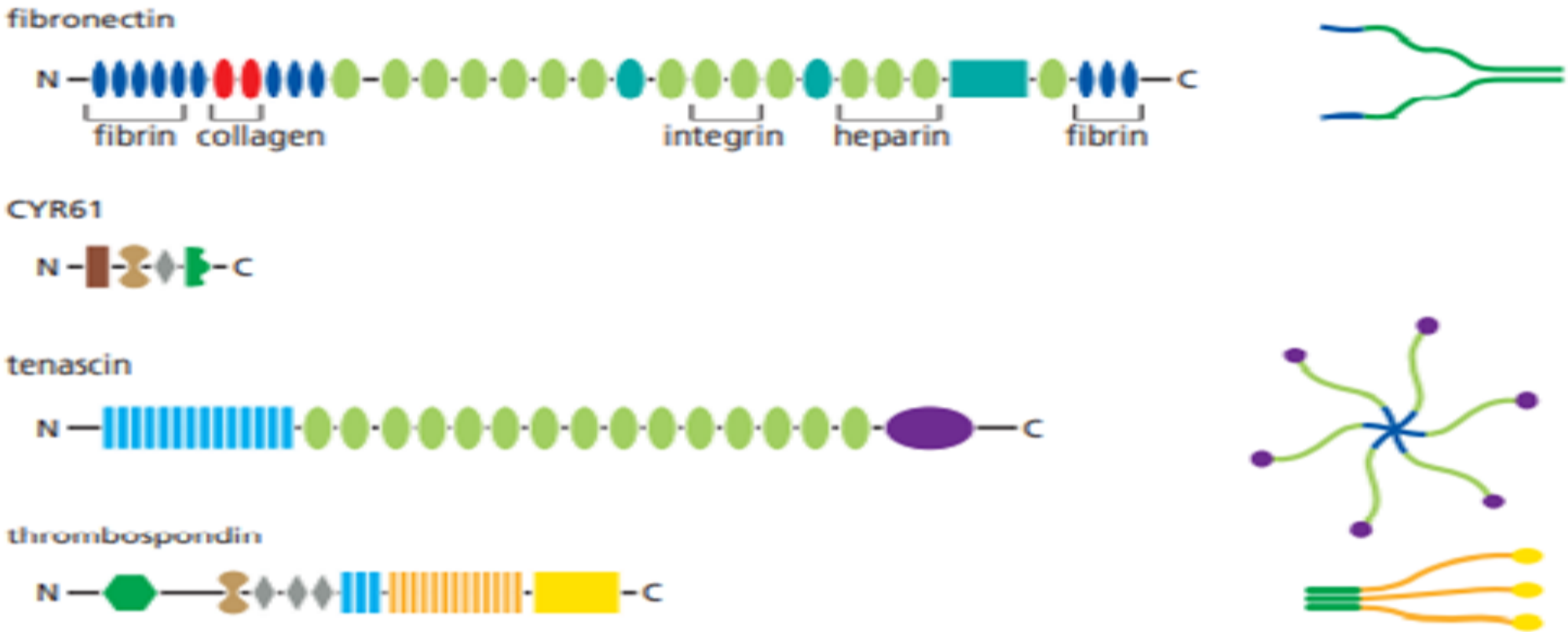
SEM image

- The elastin core is covered with a sheath of microfibrils, each of which has a diameter of about 10 nm
- microfibrils appear before elastin in developing tissues and seem to provide scaffolding to guide elastin deposition
- Arrays of microfibrils are elastic in their own right, and in some places they persist in the absence of elastin: they help to hold the lens in its place in the eye, for example
- 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
- Marfan’s syndrome, a relatively common human disorder
- The aorta is prone to rupture; other common effects include displacement of the lens and abnormalities of the skeleton and joints
- Affected individuals are often unusually tall and lanky: Abraham Lincoln is suspected to have had the condition

Fibronectin and Other Multidomain Glycoproteins Help Organize the Matrix

- glycoproteins that typically have multiple domains, with specific binding sites for other matrix macromolecules and for receptors on the surface of cells
- organizing the matrix and helping cells attach to it
- they also guide cell movements in developing tissues by serving tracks along which cells can migrate or as repellents that keep cells out of forbidden areas
- They can also bind and thereby influence the function of peptide growth factors and other small molecules produced by nearby cells
- fibronectin, a large glycoprotein found in all vertebrates and important for many cell– matrix interactions

- Mutant mice that are unable to make fibronectin die early in embryogenesis because their endothelial cells fail to form proper blood vessels
- The defect is thought to result from abnormalities in the interactions of these cells with the surrounding extracellular matrix, which normally contains fibronectin
- Fibronectin is a dimer composed of two very large subunits joined by disulfide bonds at their C-terminal ends
- Fibronectin is a dimer composed of two very large subunits joined by disulfide bonds at their C-terminal ends
- major repeat domain in fibronectin is called the type III fibronectin repeat, which is about 90 amino acids long and occurs at least 15 times in each subunit. This repeat is among the most common of all protein domains in vertebrates



Complex glycoproteins of the extracellular matrix.

•Each domain is folded into a discrete globular structure, and many such domains are arrayed along the protein like beads on a string

•This diagram shows four representative proteins among the roughly 200 matrix glycoproteins that are found in mammals

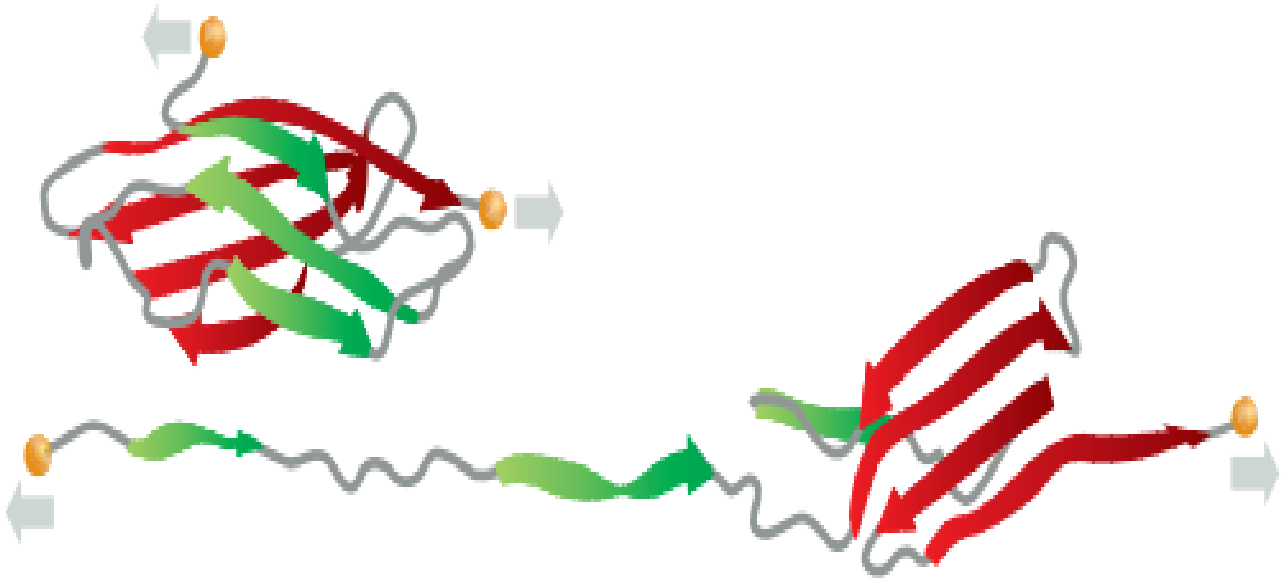
• •Two type III repeats near the C-terminus contain important binding sites for cell-surface integrins, whereas other FN repeats are involved in binding fibrin, collagen, and heparin, as indicated Other matrix proteins contain repeated sequences resembling those of epidermal growth factor (EGF), a major regulator of cell growth and proliferation; these repeats might serve a similar signaling function in matrix proteins.

TO ADD MORE STRUCTURAL DIVERSITY, MANY OF THESE PROTEINS ARE ENCODED BY RNA TRANSCRIPTS THAT CAN BE SPLICED IN DIFFRENT WAYS, ADDING OR REMOVING EXONS, SUCH AS THOSE IN FIBRONECTIN.

Fibronectin Binds to Integrins

- To analyze a complex multifunctional protein molecule such as fibronectin is to synthesize individual regions of the protein and test their ability to bind other proteins
- Other method is, one region of fibronectin binds to collagen, another to proteoglycans, and another to specific integrins on the surface of various types of cells
- binding depends on a specific tripeptide sequence (Arg-Gly-Asp, or RGD) that is found in one of the type III repeats
- very short peptides containing this RGD sequence can compete with fibronectin for the binding site on cells, thereby inhibiting the attachment of the cells to a fibronectin matrix
- Many of these proteins are components of the extracellular matrix, while others are involved in blood clotting

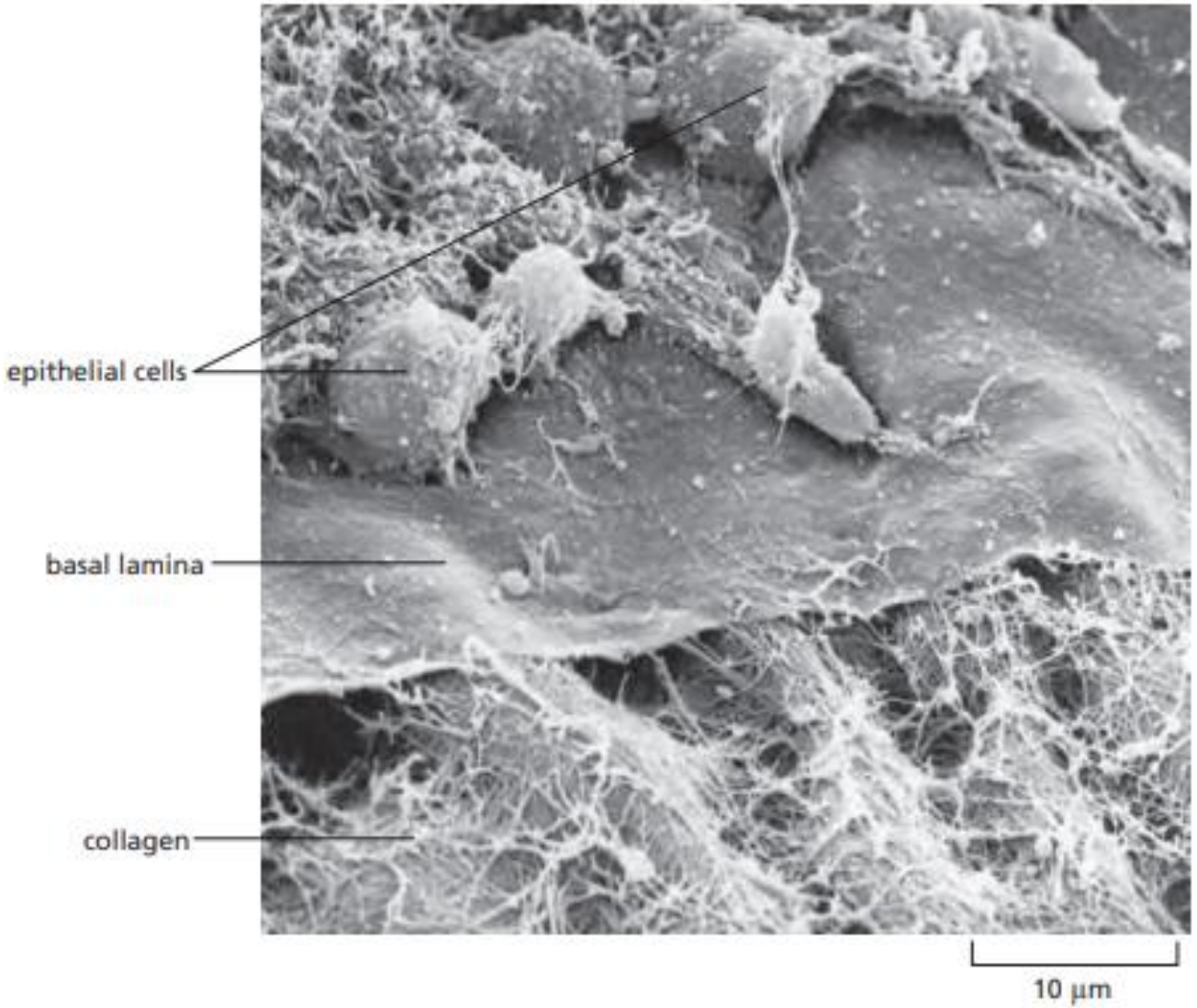
Tension Exerted by Cells Regulates the Assembly of Fibronectin Fibrils



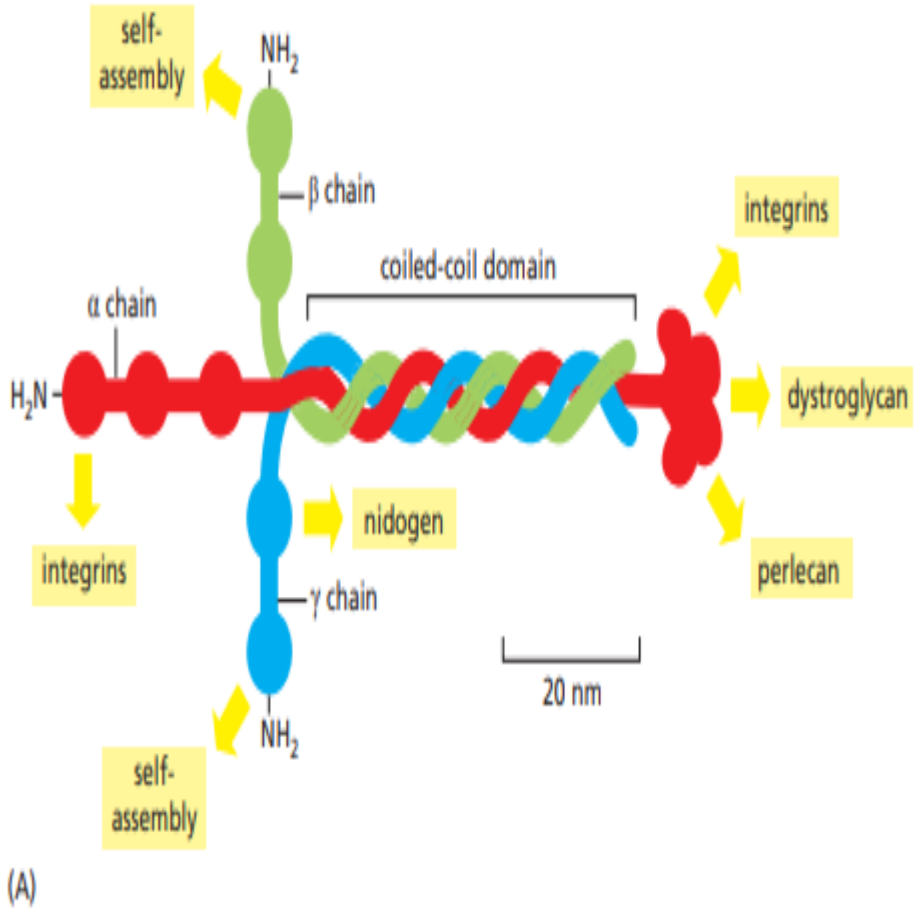
- Fibronectin can exist in soluble form, circulating in the blood and other body fluids
- Tension-sensing by fibronectin.
- Some type III fibronectin repeats are thought to unfold when fibronectin is stretched.
- The unfolding exposes cryptic binding sites that interact with other fibronectin molecules resulting in the formation of fibronectin filaments

The Basal Lamina Is a Specialized Form of Extracellular Matrix

- components are assembled into a specialized type of extracellular matrix called the basal lamina
- also known as the basement membrane
- This exceedingly thin, tough, flexible sheet of matrix molecules is an essential underpinning of all epithelia
- Like cadherin it is important in vertebrates in the construction
- major molecular components of the basal lamina are among the most ancient extracellular matrix macromolecules
- They are 40–120 nm thick
- A sheet of basal lamina not only lies beneath epithelial cells but also surrounds individual muscle cells, fat cells,and Schwann cells



SEM image of The basal lamina in the cornea of a chick embryo

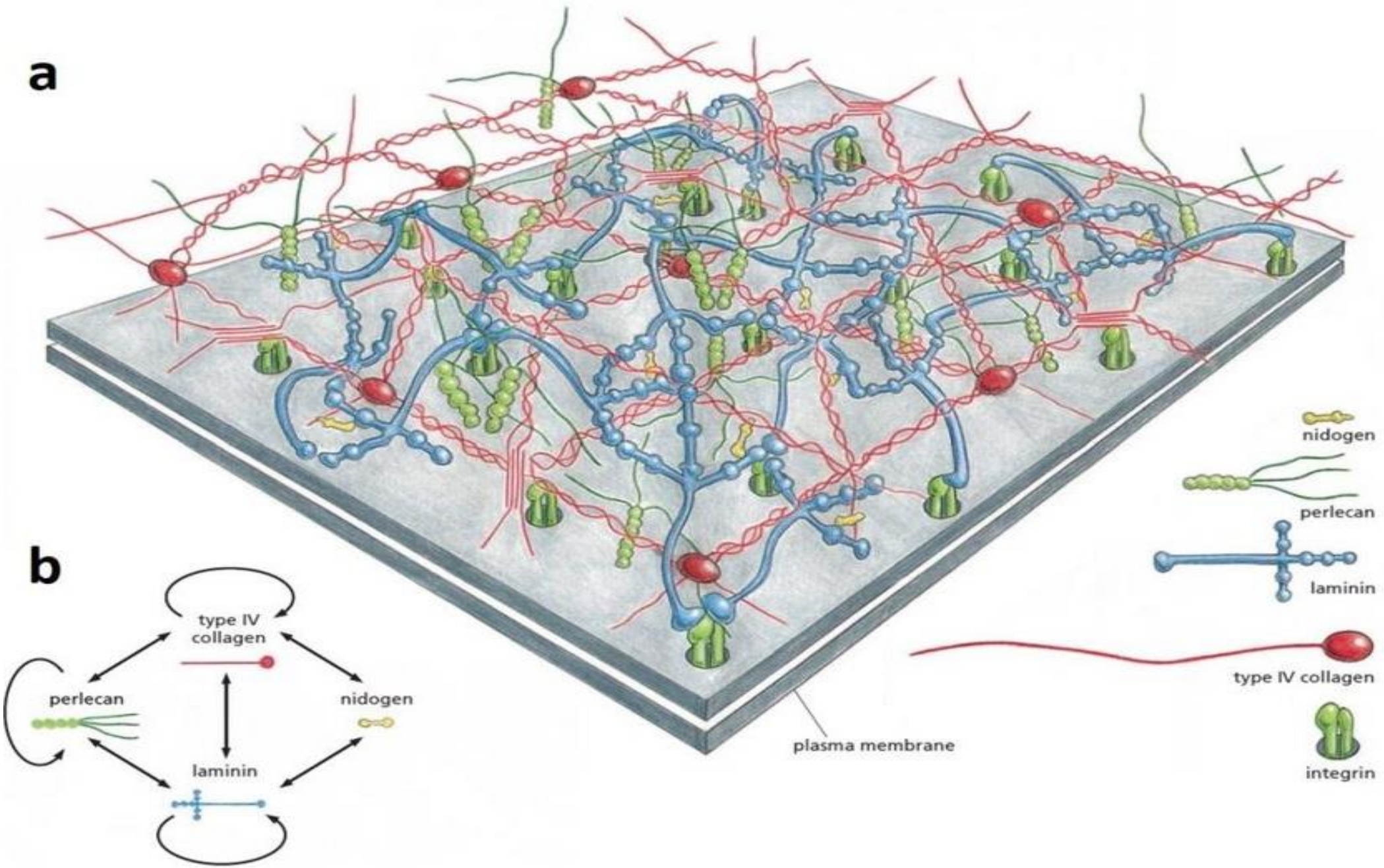


The structure of laminin

- Three ways in which basal laminae are organized. Basal laminae (yellow) surround certain cells (such as skeletal muscle cells), underlie epithelia, and are interposed between two cell sheets (as in the kidney glomerulus)
- Note that, in the kidney glomerulus, both cell sheets have gaps in them, and the basal lamina has a filtering as well as a supportive function, helping to determine which molecules will pass into the urine from the blood
- The filtration also depends on other protein-based structures, called slit diaphragms, that span the intercellular gaps in the epithelial sheet

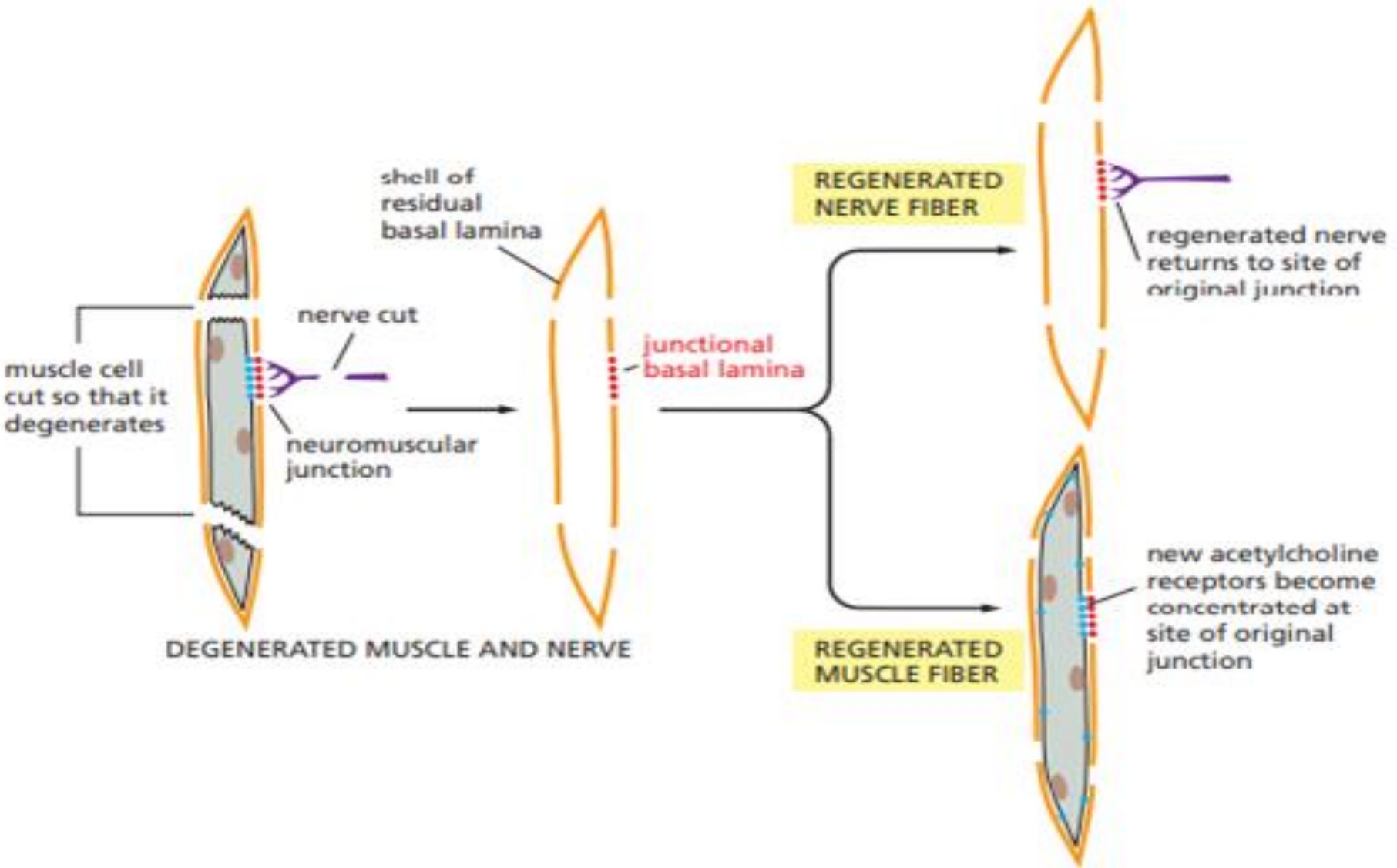
- They are able to determine cell polarity; influence cell metabolism; organize the proteins in adjacent plasma membranes; promote cell survival, proliferation, or differentiation; and serve as highways for cell migration
- The mechanical role is nevertheless essential. In the skin, for example, the epithelial outer layer—the epidermis—depends on the strength of the basal lamina to keep it attached to the underlying connective tissue—the dermis
- In people with genetic defects in certain basal lamina proteins or in a special type of collagen that anchors the basal lamina to the underlying connective tissue, the epidermis becomes detached from the dermis
- This causes a blistering disease called junctional epidermolysis bullosa, a severe and sometimes lethal condition

MODEL OF MOLECULAR STRUCTURE OF BASAL LAMINA



Laminin and Type IV Collagen Are Major Components of the Basal Lamina

- The basal lamina is synthesized by the cells on each side of it: the epithelial cells contribute one set of basal lamina components,while cells of the underlying bed of connective tissue
- most of them are glycoproteins laminin, type IV collagen, and nidogen, along with the proteoglycan perlecan
- Laminin is the primary organizer of the sheet structure, and, early in development, basal laminae consist mainly of laminin molecules
- laminin γ1 chain is, however, a component of most laminin heterotrimers; mice lacking it die during embryogenesis because they are unable to make basal laminae
- Type IV collagen is a second essential component of mature basal laminae
- Type IV collagen molecules interact via their terminal domains to assemble extracellularly into a flexible, feltlike network that gives the basal lamina tensile strength



Regeneration experiments in frog demonstrating the special character of the junctional basal lamina at a neuromuscular junction.

- example: role of the basal lamina in regeneration comes from studies of the neuromuscular junction, the site where the nerve terminals of a motor neuron form a chemical synapse with a skeletal muscle cell
- In vertebrates, the basal lamina that surrounds the muscle cell separates the nerve and muscle cell plasma membranes at the synapse, and the synaptic region of the lamina has a distinctive chemical character with special isoforms of type IV collagen and laminin and a proteoglycan called agrin
- After a nerve or muscle injury, the basal lamina at the synapse has a central role in reconstructing the synapse at the correct location
- Defects in components of the basal lamina at the synapse are responsible for some forms of muscular dystrophy, in which muscles develop normally but then degenerate later in life.

Reference

Albert Bruce 6th edition Molecular Biology of the cell