

Lecture 19 Integrating cells into tissues II

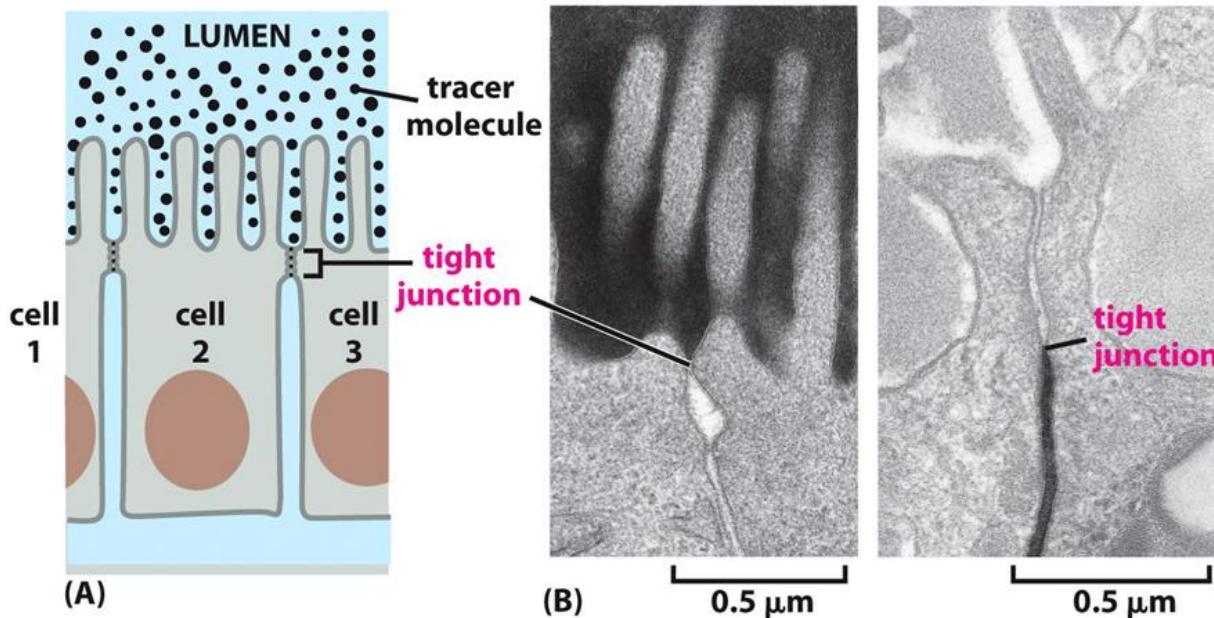
Outline

- I. Occluding junctions
- II. Channel-forming Junctions
- III. Basal Lamina
- IV. Extracellular Matrix

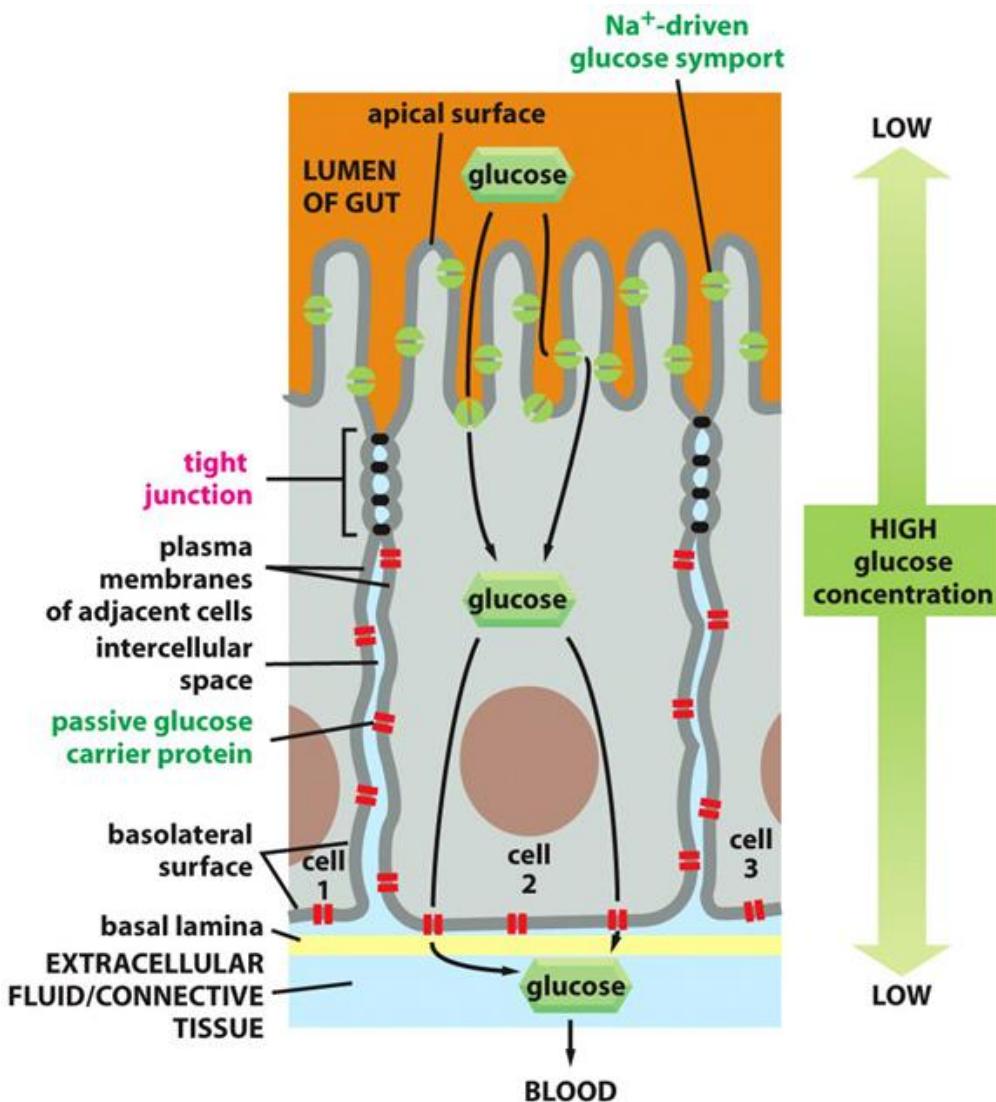
I. Tight junctions

About epithelia cells:

- Epithelia take ~60% of the cell types in the vertebrate body.
- Epithelia enclose and partition the animal body, serve as a **selective** permeability barrier.
- All Epithelia cells are **polarized**, have apical site and basal side.
- In between epithelia, **occluding junctions** (**tight junctions** in vertebrates **septate junction** in invertebrates) seal the adjacent epithelial cells



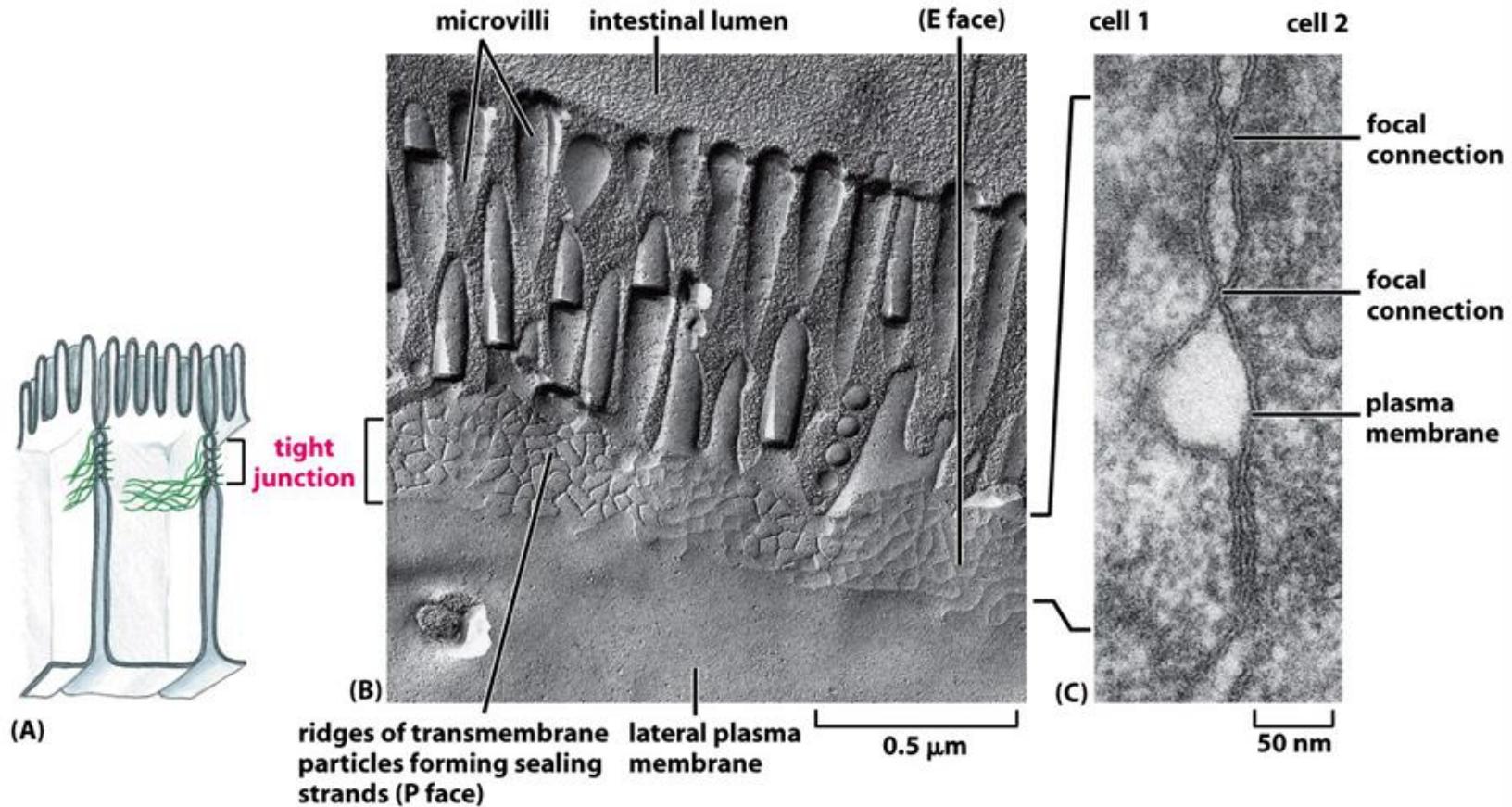
Overview: the role of tight junctions in transcellular transport



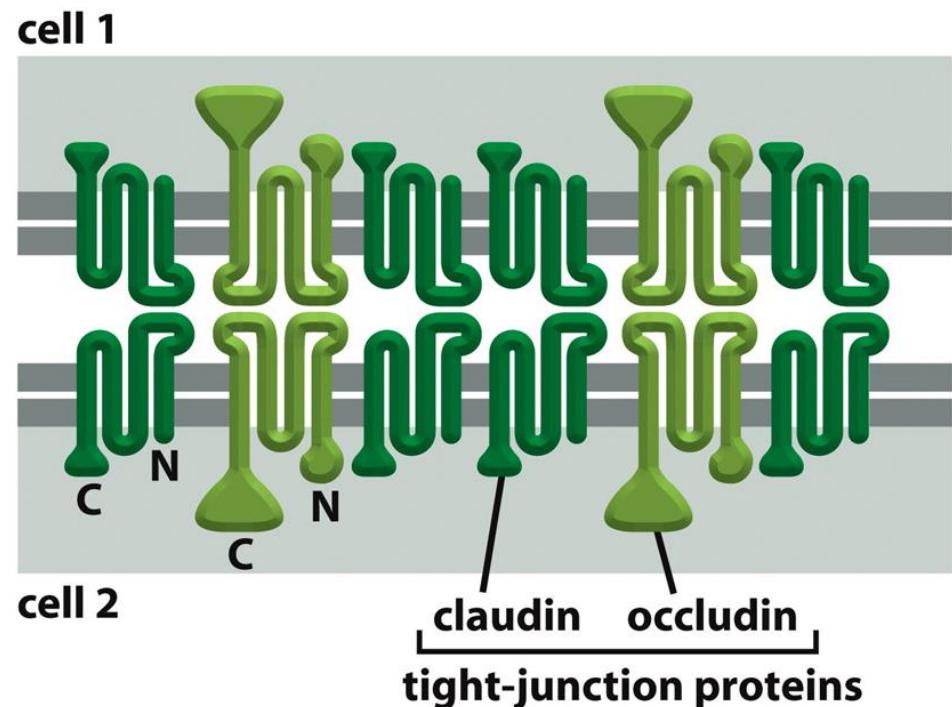
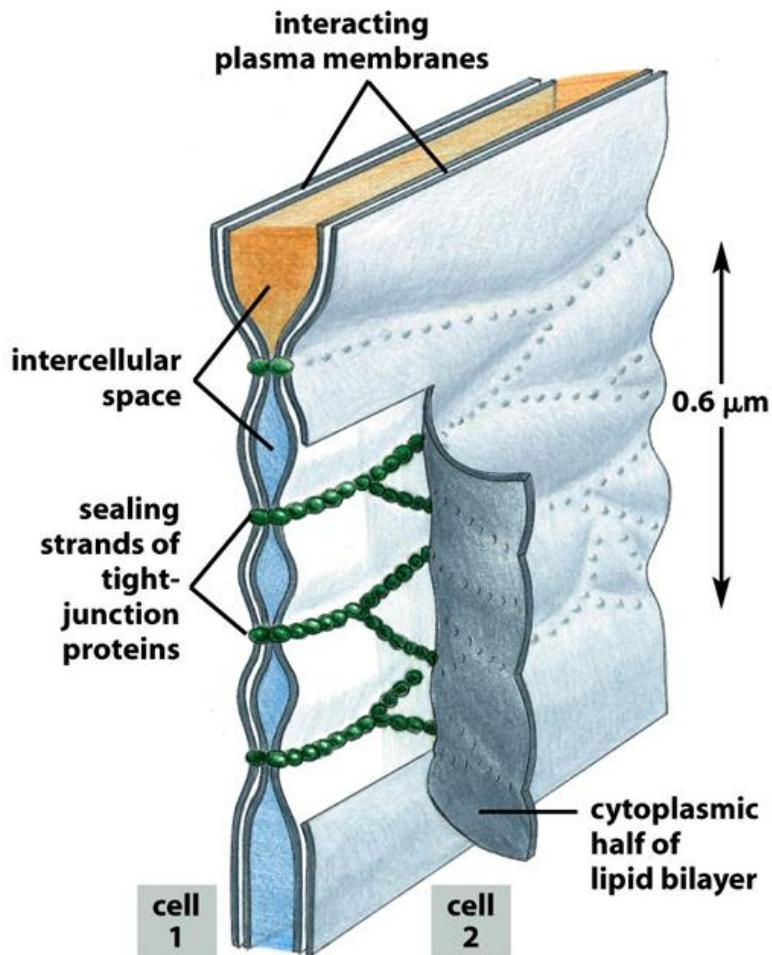
Function of tight junctions:

1. Serves as “fences” to separate domains within the plasma membrane
2. Seal cells to form a barrier
3. Tight junctions **selectively** let certain ions pass, depending on the their protein, but not for allow macromolecules to pass.

Organization of tight junctions



Model of the tight junction



Proteins for tight junction

- Claudin: ~24 members in human

Its loss leads to dehydration and death of mice

Its overexpression leads to fibroblast to form tight junctions

- Occludin:

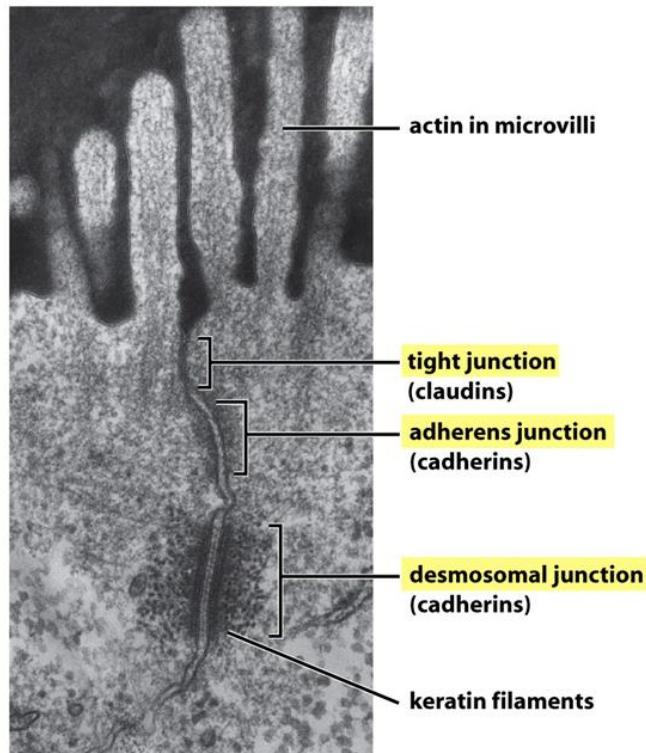
detailed function unknown.

- Tricellulin:

required to seal membrane together

Formation of tight junction is dependent on scaffold protein (Tjp)

- **Tjp**, tight junction protein , also called *ZO* protein, anchor site for tight-junctional strands.
- Links tight junction to other cell junctions, e.g., actin-cytoskeleton



Depletion of cadherins will block adherens junction,
In turn will block tight junction formation

Septate junctions: tight junctions for invertebrates

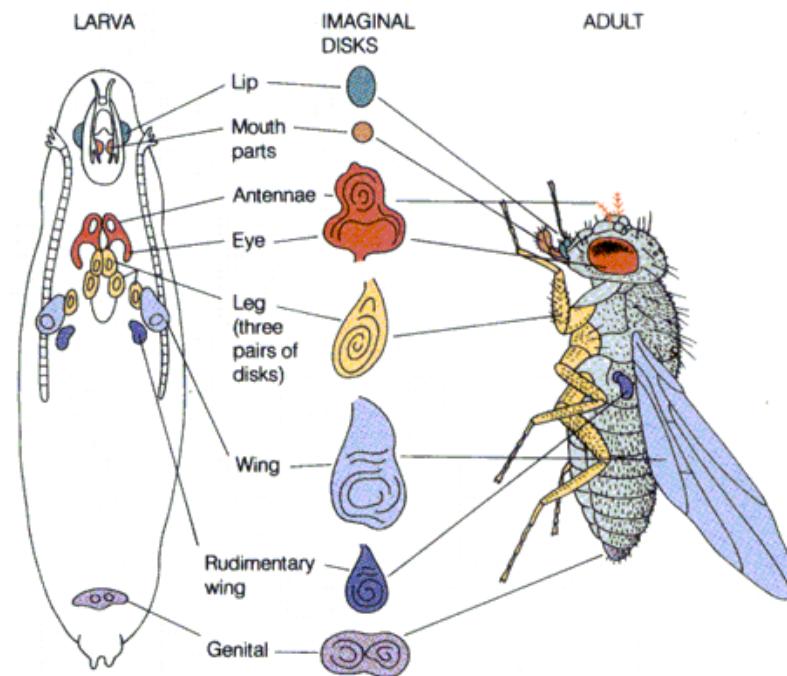
- Structure is more regular, parallel rows
- claudin homologs and scaffold proteins.



Cell adhesion is important to regulate cell proliferation

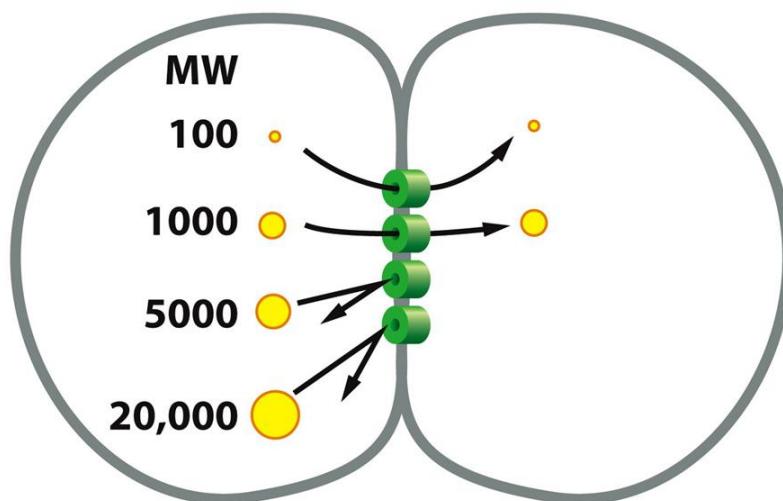
- Example: defective scaffold protein (Discs-large protein) in fly leads to defective septate junction, but also leads to overgrowth of imaginal discs

Reason?



II. Channel-forming junctions

- In animals: Gap junctions
- In plants: plasmodesmata
- Allow direct exchange of small molecules and inorganic ions in the cytoplasm, but very few macromolecules in rare exceptions.
- Occur for both epithelia and connective tissue

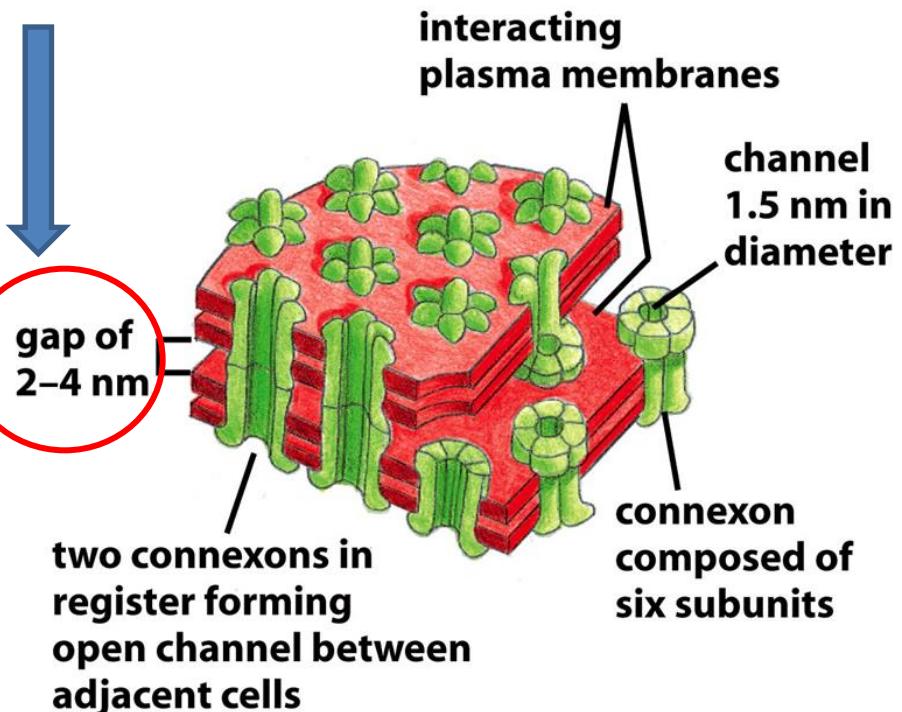


Molecules with molecular weight of 1000 Dalton (cut-off) can pass the gap-junctions

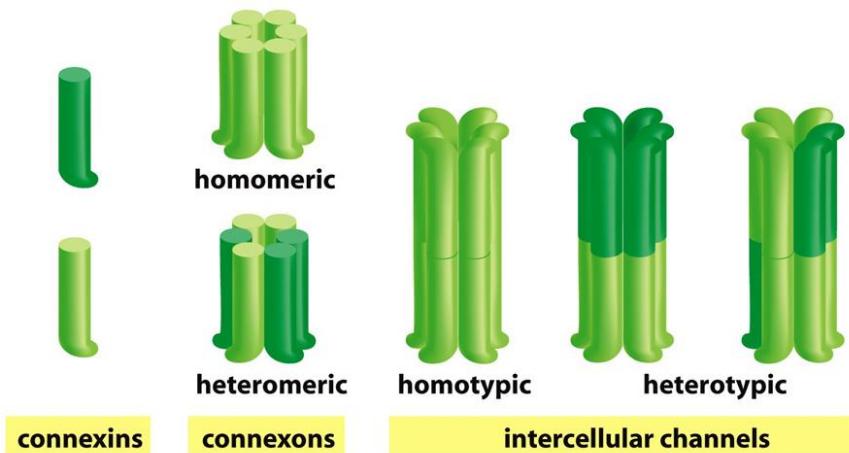
Gap junction proteins

- Two families: connexins and innexins, which are different in sequence but similar in shape and function.
- Vertebrates: connexins predominates
- Drosophila and C.elegans: only innexins
- Each of them has multiple members in a species, e.g., human has 21 connexins, fly has 15 innexins and worm has 25 innexins, these difference confer different permeability of molecules in gap junctions.

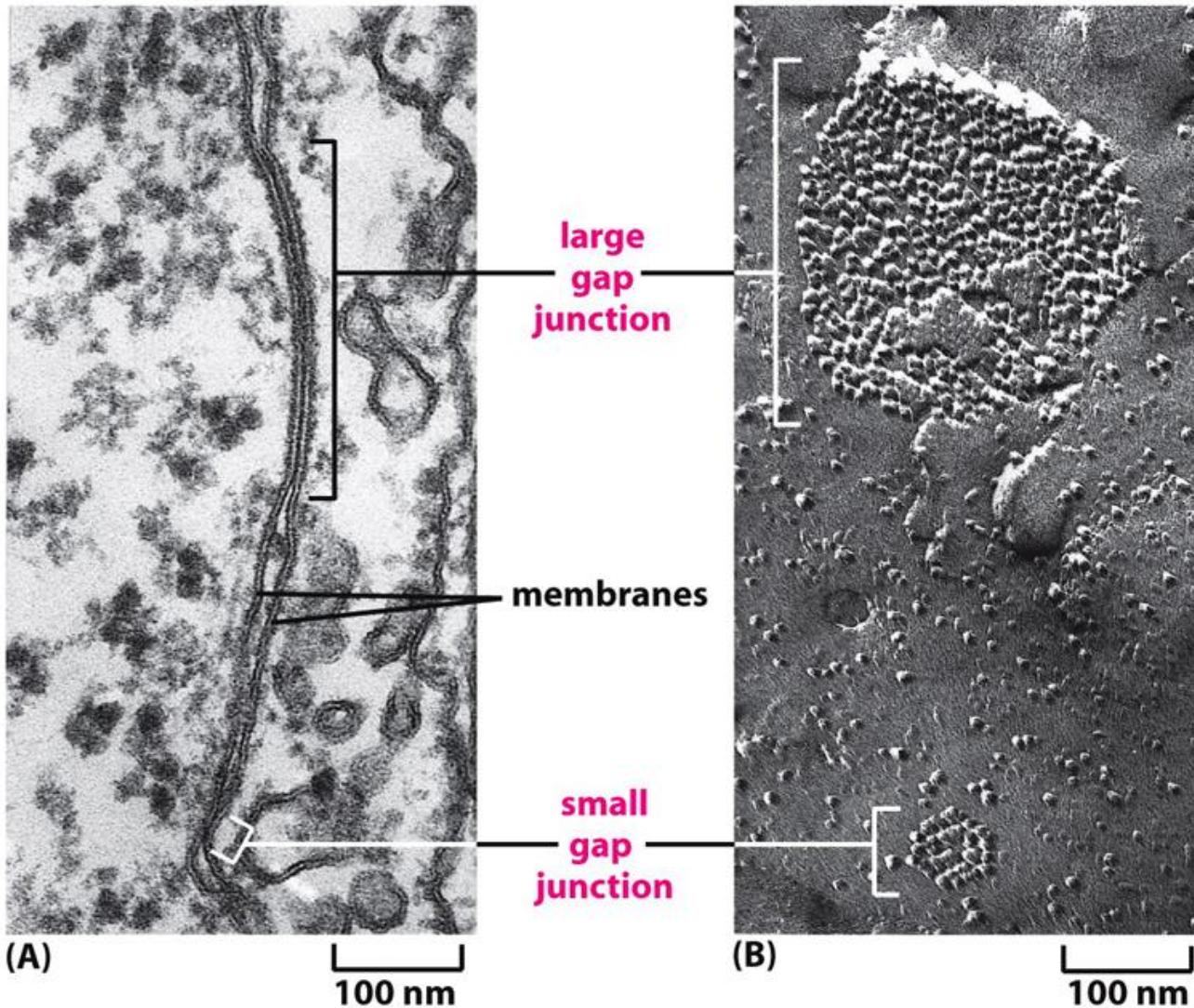
Structure of Gap junctions



Either homomeric or heteromeric Connexons.



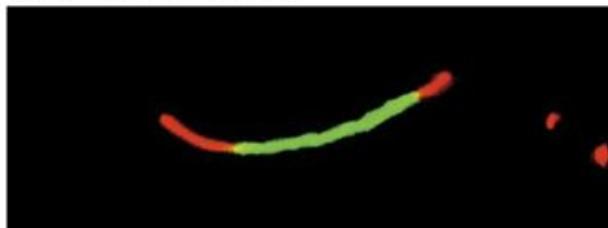
Gap junctions as seen by EM



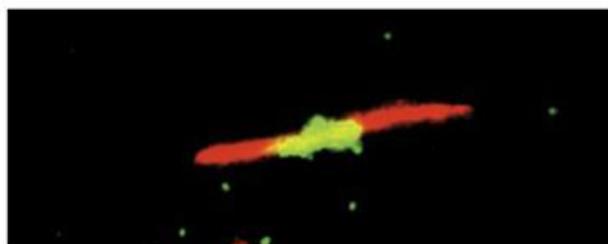
Dynamic structure of gap junctions

- Rapidly assemble, disassemble and remodeled

CROSS SECTIONS



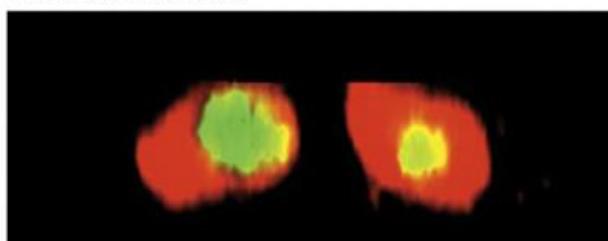
4 h incubation



8 h incubation

1 μm

EN-FACE VIEW



8 h incubation

2 μm

Green : old gap junctions

Red: new gap junctions

Cells were transfected with connexin with engineered four Cys tag, which can bind to fluorescence dye.

As to how the middle gap junctions are Removed, answer not available.

Functions of gap junctions

- Electrically excitable cells: synchronized action and faster reaction:

heart muscle contraction

intestine smooth muscle contraction

synchronized neurons

- Non electrically excitable cells:

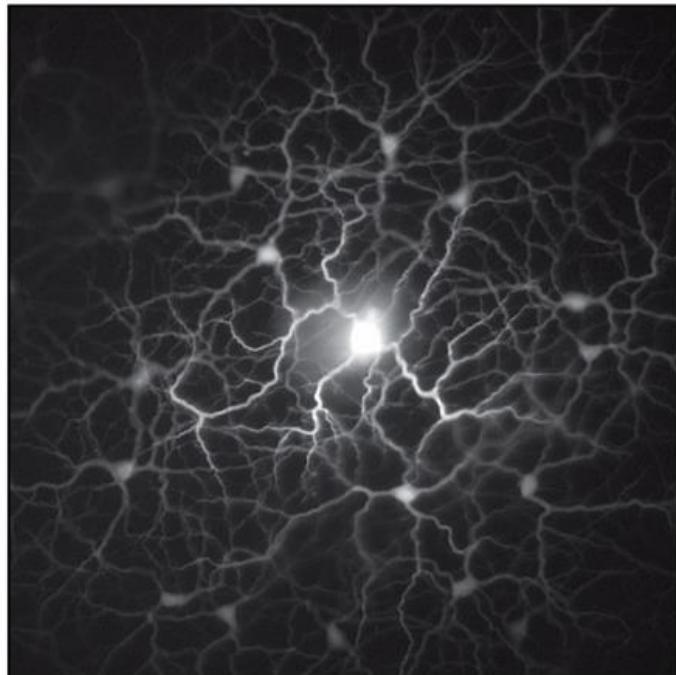
liver cells , etc.

Mutation of connexins is associated with diseases

- Connexin 26---deafness
- Cataracts
- Neurodegenerative disease, etc.

Regulation of gap-junctions

- Gap junctions have open and closed states, e.g., lower pH or increase in Ca^{2+} decrease the permeability of gap junctions.
- Gap junctions can be regulated by extracellular signals.



(A)

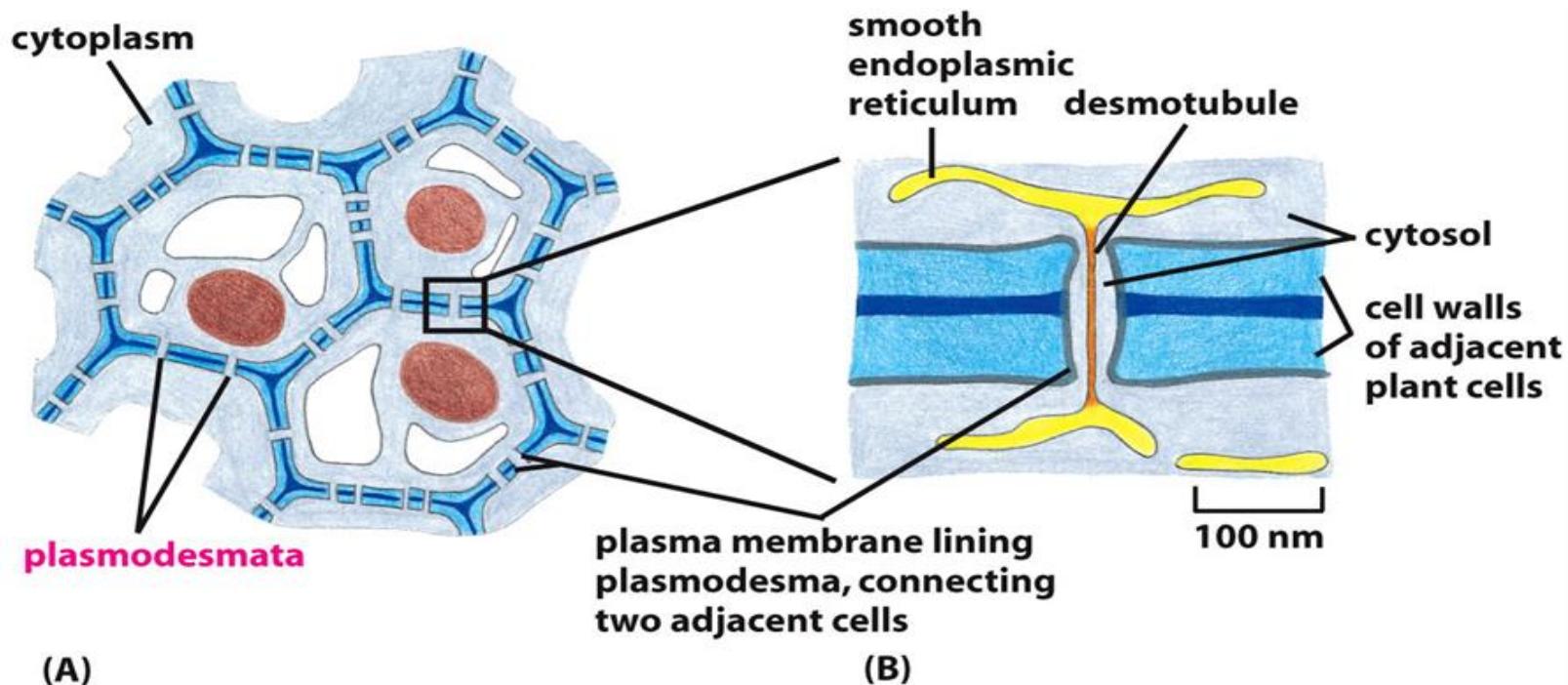


(B)

Cells were injected with lucifer yellow dye, the dye will diffuse to other neurons by gap junctions (A). But with dopamine treatment, the permeability of gap junctions greatly decreased (B).

Plasmodesmata---plant gap-junctions

- Connects the cytoplasms of the two cells by a rough and cylindrical channel with a diameter of 20-40nm.
- Connected by desmotubule which is the continuation of the smooth endoplasmic reticulum.
- molecular cut-off ~ 800 Dalton

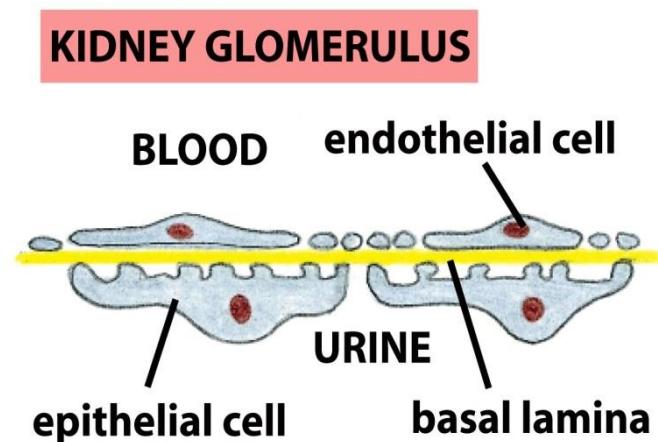
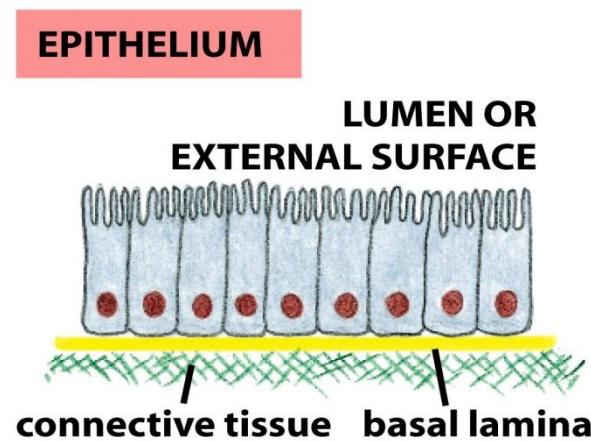
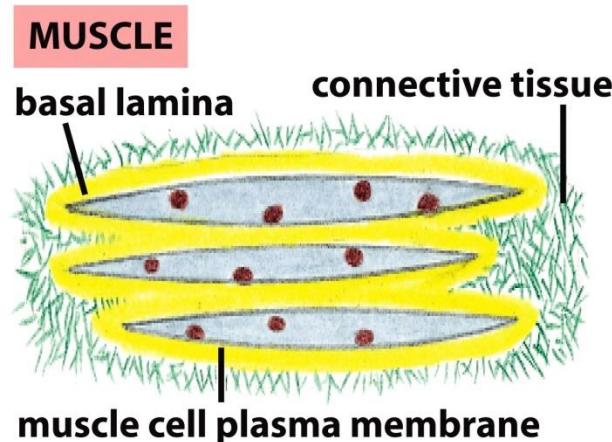


III. Basal lamina

- 1. overview of basal lamina
- 2. Composition of basal lamina
- 3. Functions of basal lamina

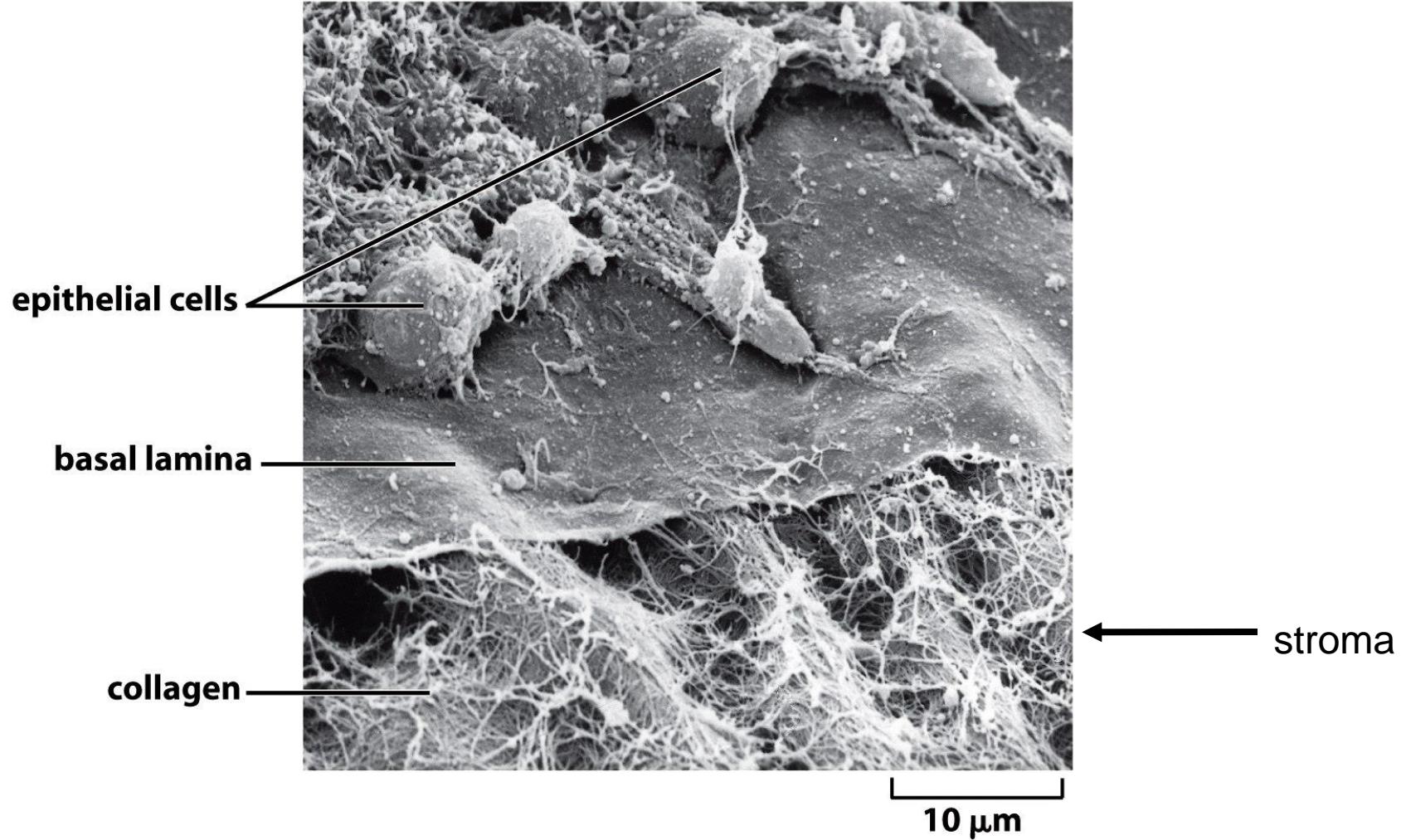
1. Overview of basal lamina

- 40-120nm in thickness
- It has mechanical role.
- It determines cell polarity, influence cell metabolism, cell survival, proliferation, migration and differentiation



Or surrounding **fat cells**
and **Schwann cells**

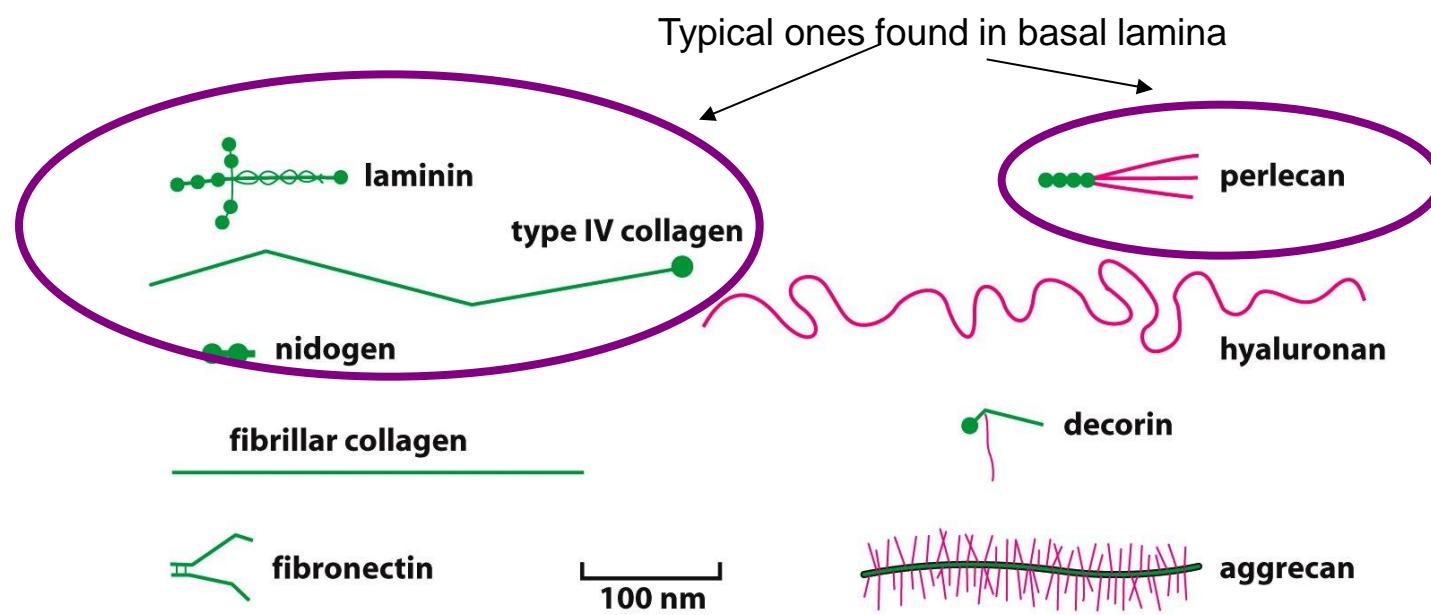
SEM image of a basal lamina



2. Composition of basal lamina

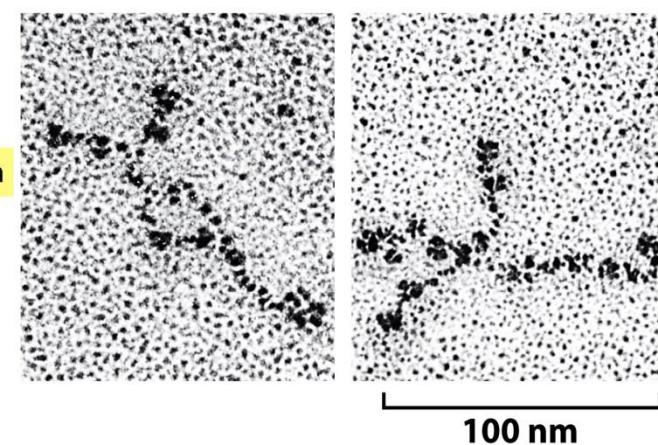
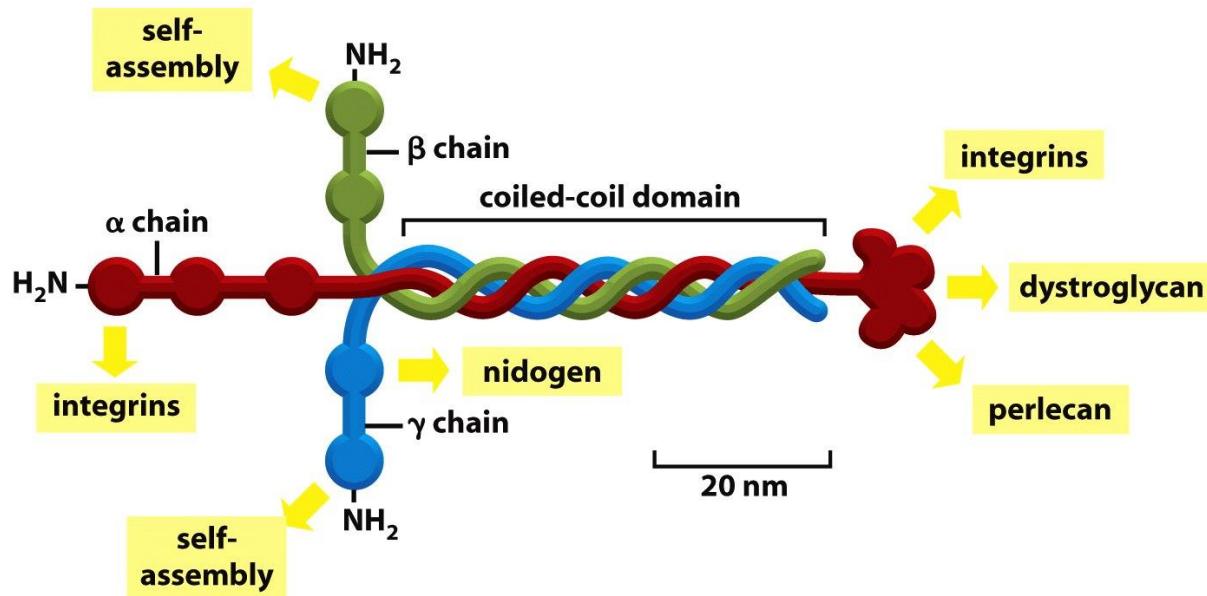
Both epithelia and stroma contribute to the composition of basal lamina:

- Fibrous proteins (with short oligosaccharide side chains)
- Proteoglycans (proteins with polysaccharides, glycosaminoglycans---GAGs)

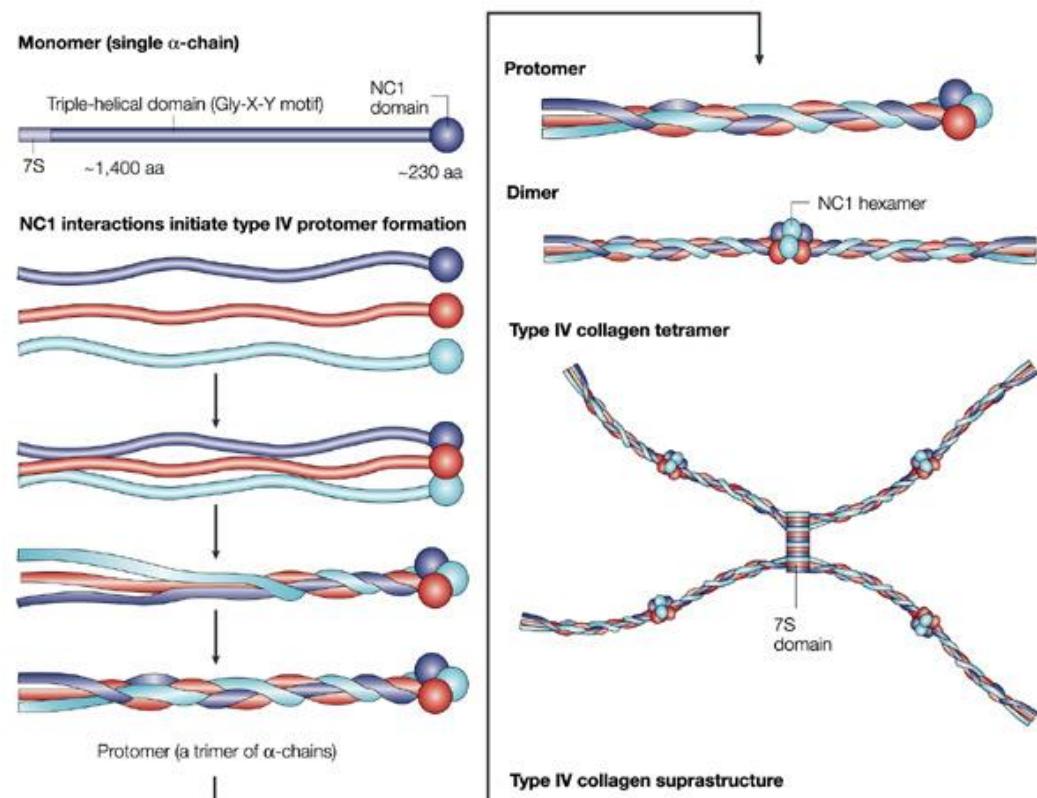


2.1 laminin

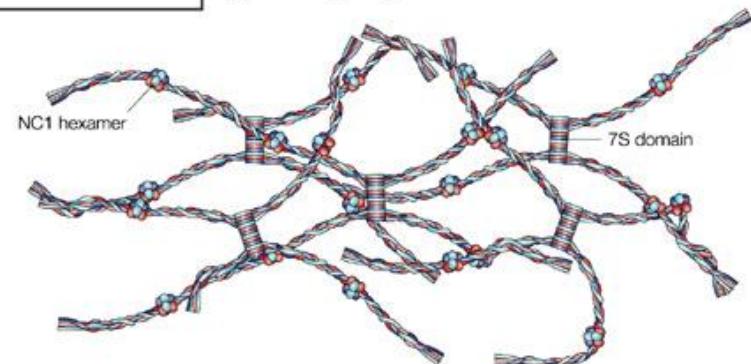
- Primary organizer for basal lamina
- Heterotrimer consisting of α, β, γ chains, which are held together by disulfide bonds.
- Can self-assemble through their head domains into a network *in vitro*



2.2 Type IV collagen

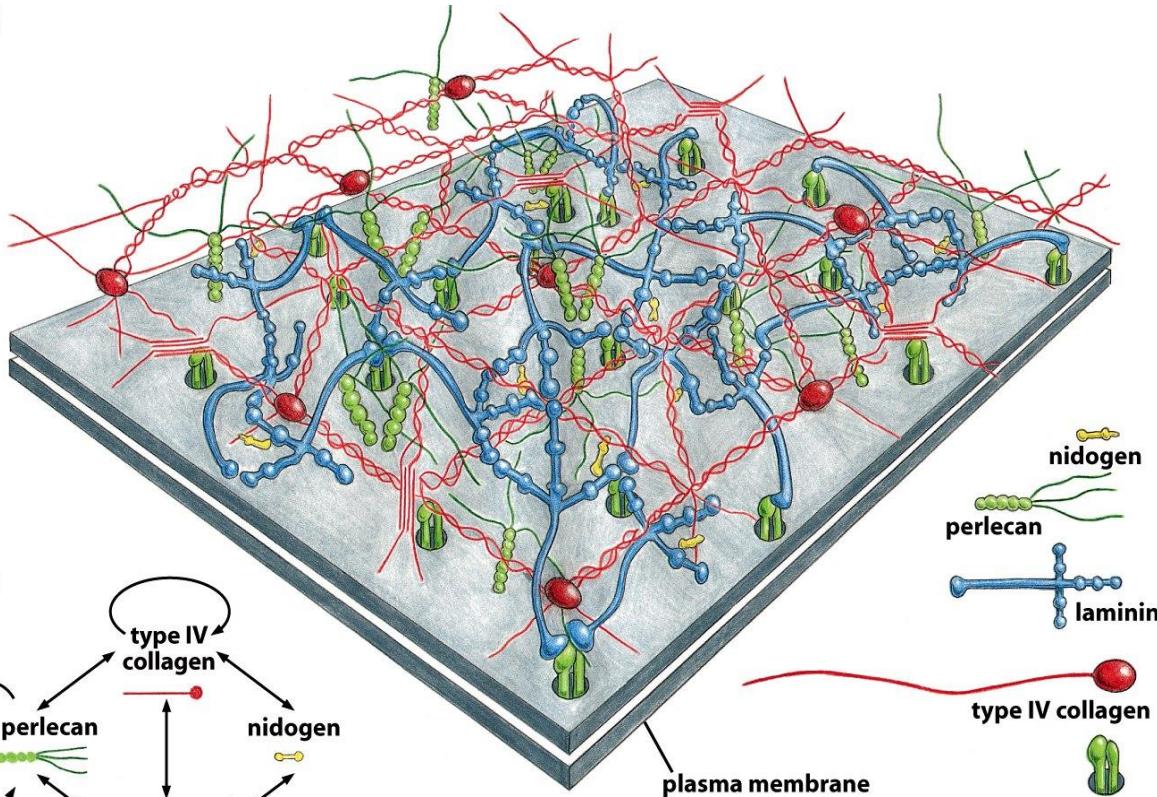


- Second essential component in basal lamina
- Three separate chains twist together to form rope-like superhelix, with multiple bends.
- Interact with other basal lamina proteins via their terminal domains.

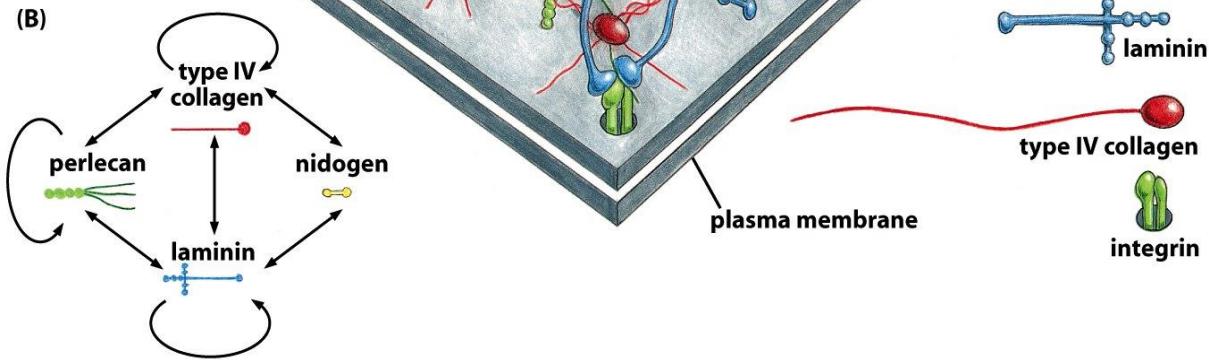


A model for how basal lamina is formed

(A)



(B)



Laminin and Type IV collagen can form network;

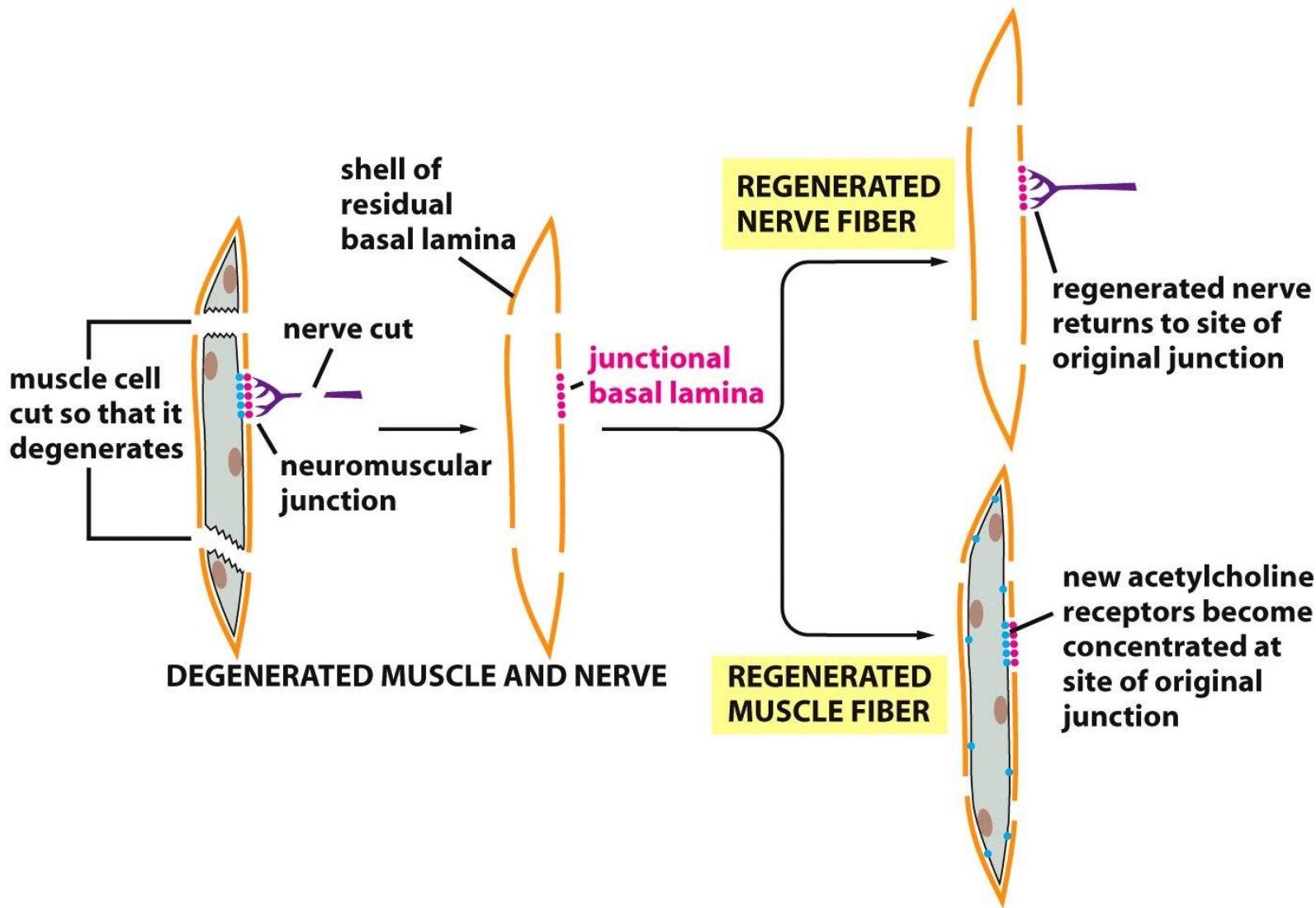
Nidogen and perlecan as linkers, they have binding sites for both laminin and collagen.

Laminin and type IV collagen Have binding sites for cell surface receptors such as Integrin.

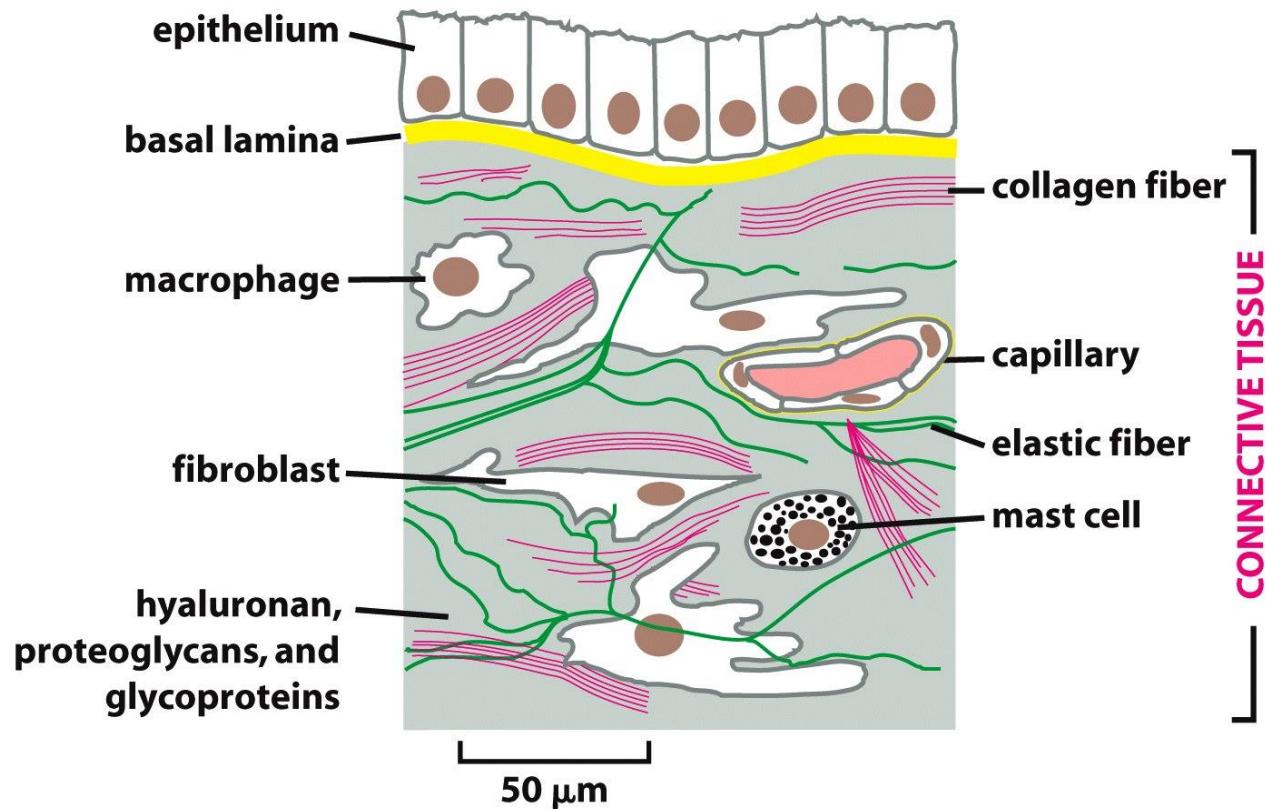
3. Functions of basal lamina

- Provide mechanical support.
- When cells are damaged, basal lamina often survive and help guide tissue regeneration
- Acts as barriers to keep cells in place.
- Serves as filters in kidney.
- Influence cell polarity, differentiation and migration.

Tissue regeneration guided by basal lamina



IV. Extracellular matrix



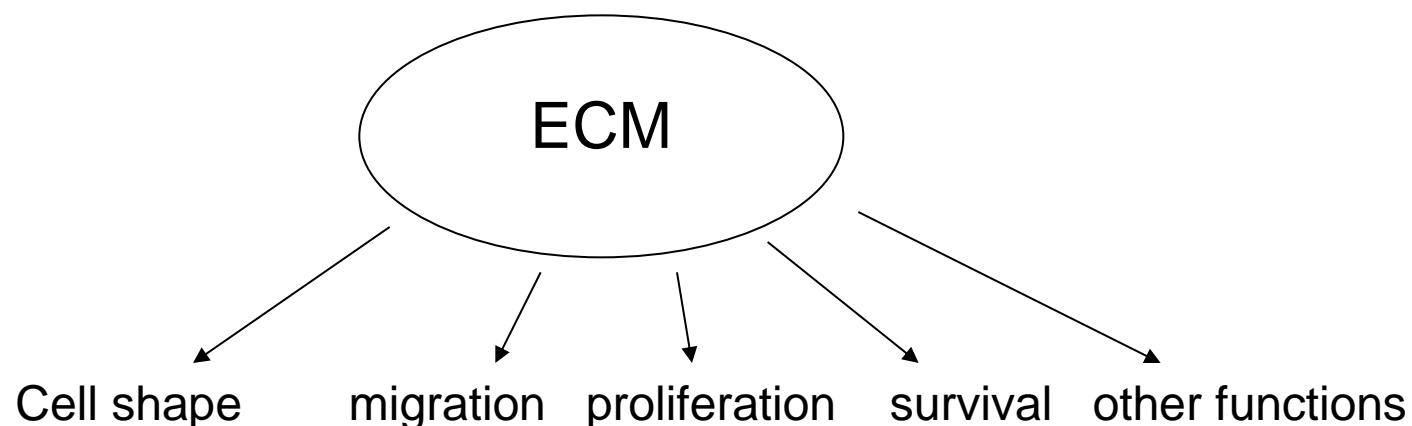
Outline for this part

- 1. Overview of ECM
- 2. The making and composition of ECM
 - 2.1. GAGs
 - 2.2. Hyaluronan
 - 2.3. proteoglycans
 - 2.4. collagens
 - 2.5. elastin
 - 2.6. fibronectin
- 3. The degradation of ECM

1. Overview of ECM

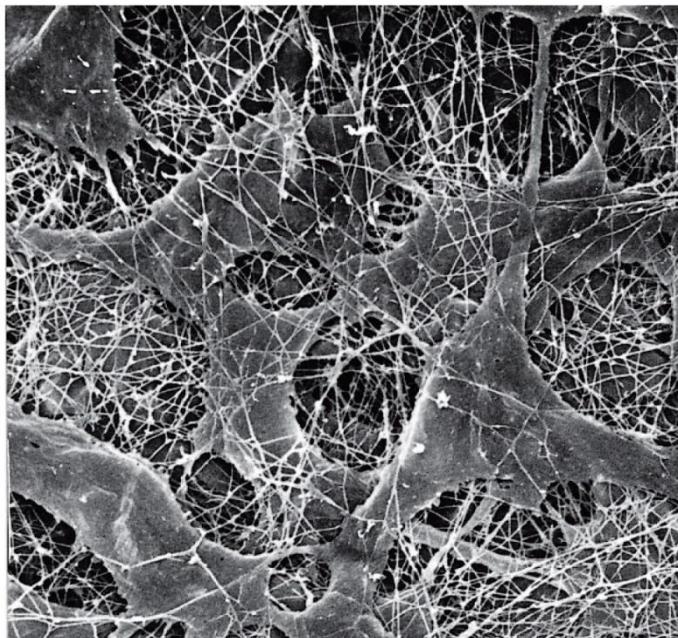
Like connective tissues have multiple forms, ECM is a diversified material:

- constitute bone and teeth
- Constitute tendon
- Constitute cornea
- Jelly in jelly fish, etc.



The making of the ECM

- Performed mainly by fibroblast
- In bone, by osteoblast
- In cartilage, by chondroblast



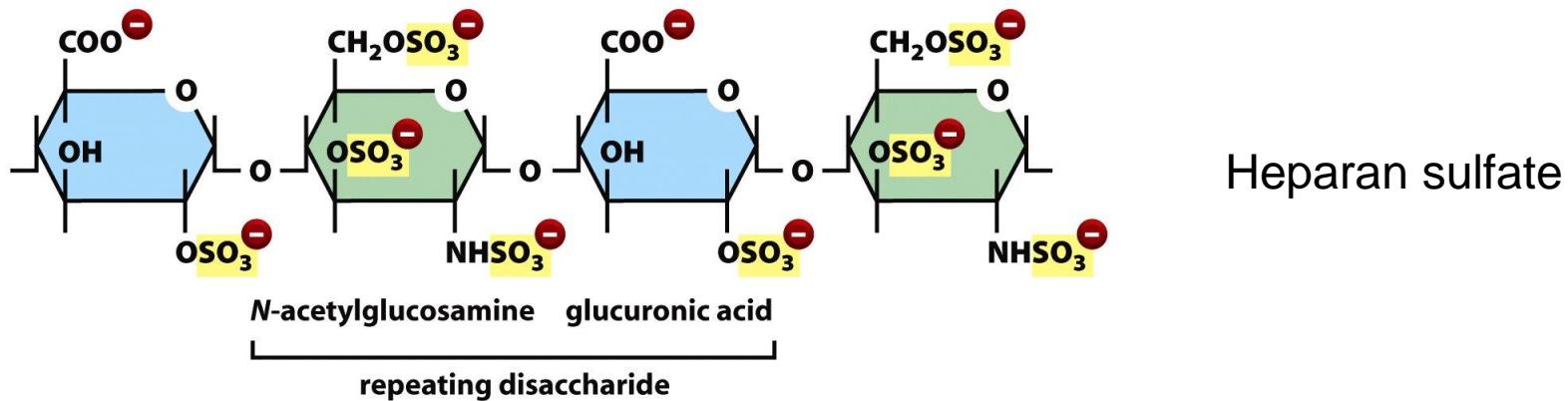
10 μm

Fibroblasts in connective tissue

- Like basal lamina, ECM mainly consists of two classes of macromolecules
- Fibrous proteins
- Protoproteins (GAGs covalently linked to proteins)

2.1. glycosaminoglycans (GAGs)

- Unbranched repeating disaccharides: sulfated N-acetylglucosamine or N-acetylgalactosamine followed by a uronic acid) glucuronic/iduronic



- Highly anionic molecules, extended and form gel-like structure in cells
- Four main groups: **hyaluronan, chondroitin sulfate and dermatan sulfate, heparan sulfate and keratan sulfate**

GAGs takes up a major volumes in ECM

● globular protein (MW 50,000)

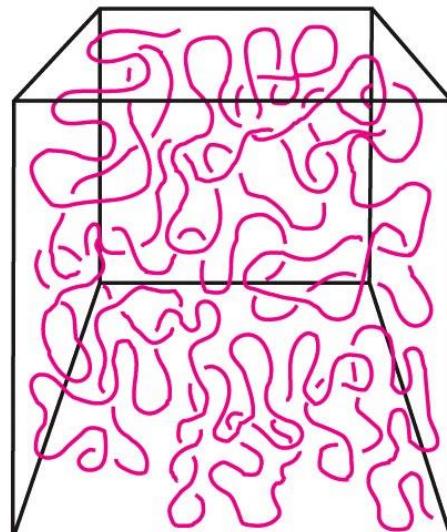


glycogen (MW ~ 400,000)



spectrin (MW 460,000)

collagen (MW 290,000)



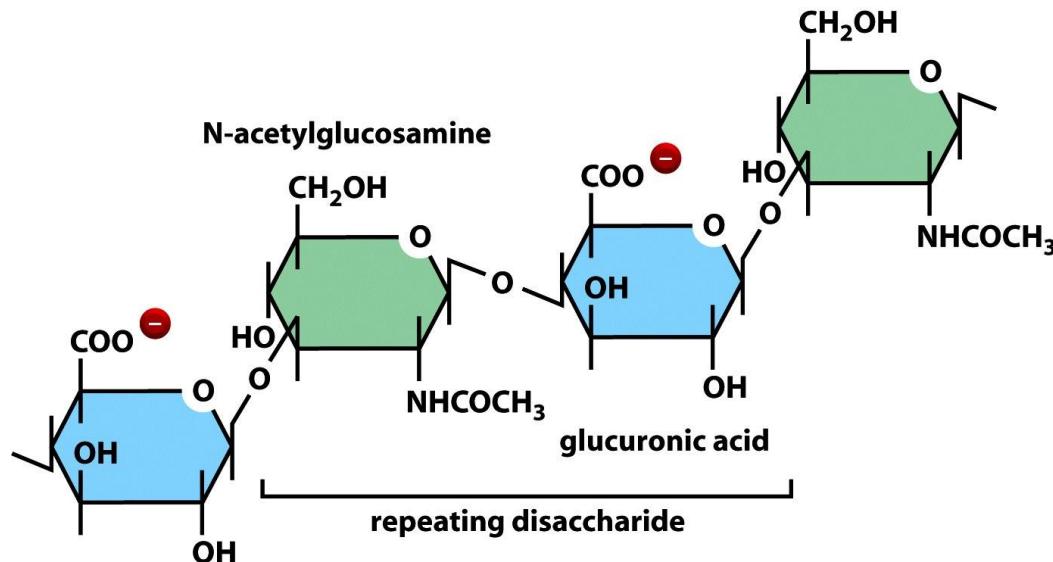
hyaluronan (MW 8×10^6)

300 nm

Due to strong negative charge, GAGs attract Na⁺ ions on their surface, water are absorbed To them, too. GAGs swell and can withstand Compressive forces.

2.2 hyaluronan

- the simplest of GAGs, enormous length of up to 25000 repeats of disaccharide units.
- No sulfate group, generally not linked to protein
- Synthesized directly from cell surface enzymes as opposed to other GAGs by exocytosis.
- Can guide cell migration during tissue morphogenesis and repair; space-filler
- Degraded by hyaluronidase

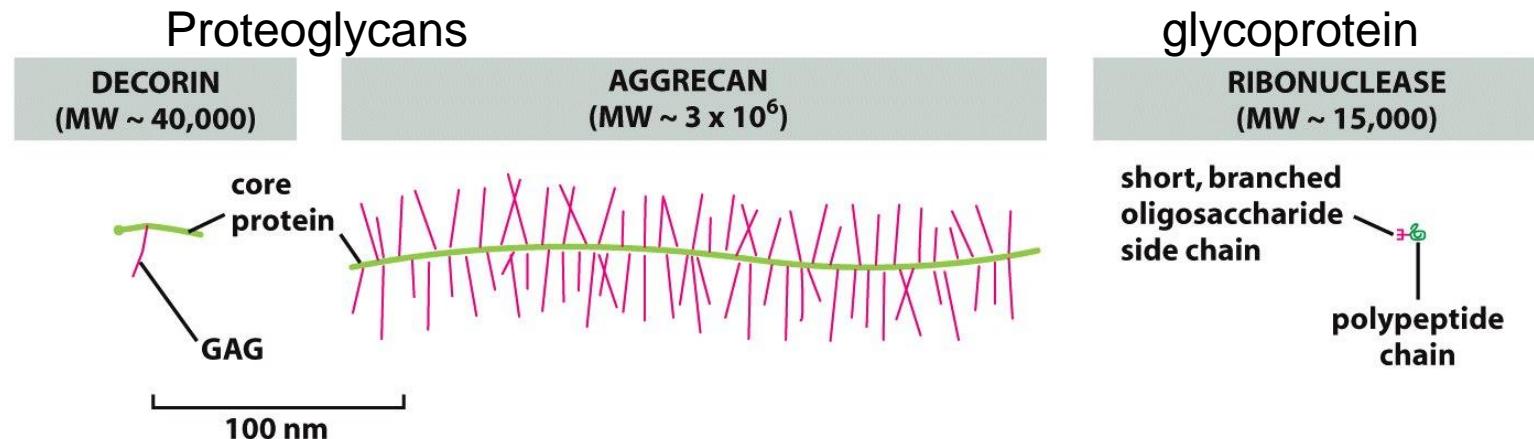


2.3. Proteoglycans

- Comparison between proteoglycan and glycoproteins:

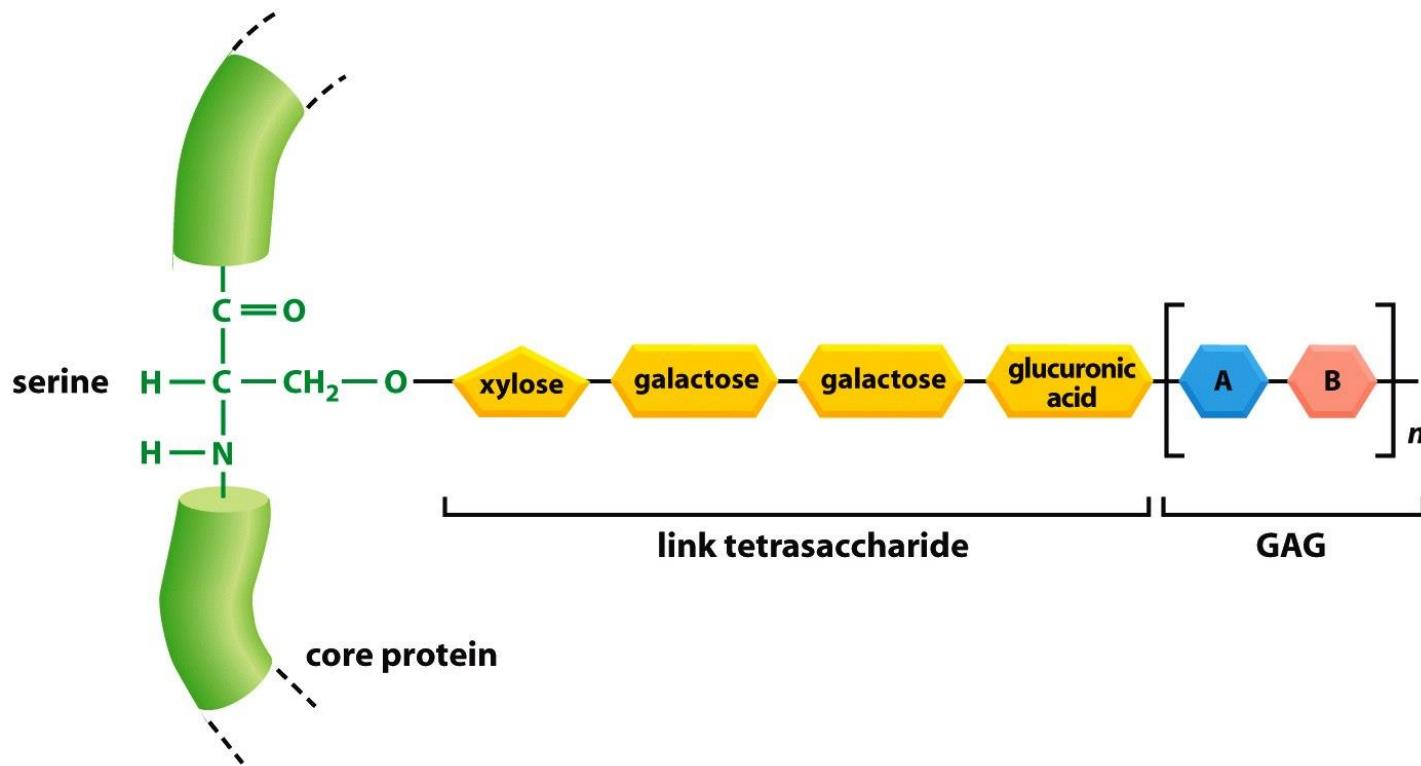
Glycoproteins (sugar 1-60%, usually only a few percent), small, branched sugar chains, small molecular weight

Proteoglycans (sugar up to 95%), all contain at least one GAGs, long, unbranched sugar chains, molecular weight up to 3000 kD.



The linkage of GAGs with core protein

- Glycosylation occurs in Golgi apparatus, O-linked tetrasaccharide, followed by one by one sugar group addition, modification occurs later.



Functions of proteoglycans

- Forms gels of varying pore size and charge density, serves as a selective sieves to regulate traffic of molecules
- Regulate signaling through binding of growth factors such as FGF, TGF β and chemokines.
- Some cell surface bound proteoglycan such as *syndecans* acts as co-receptors for growth factors to participate in cell signaling

Some common proteoglycans

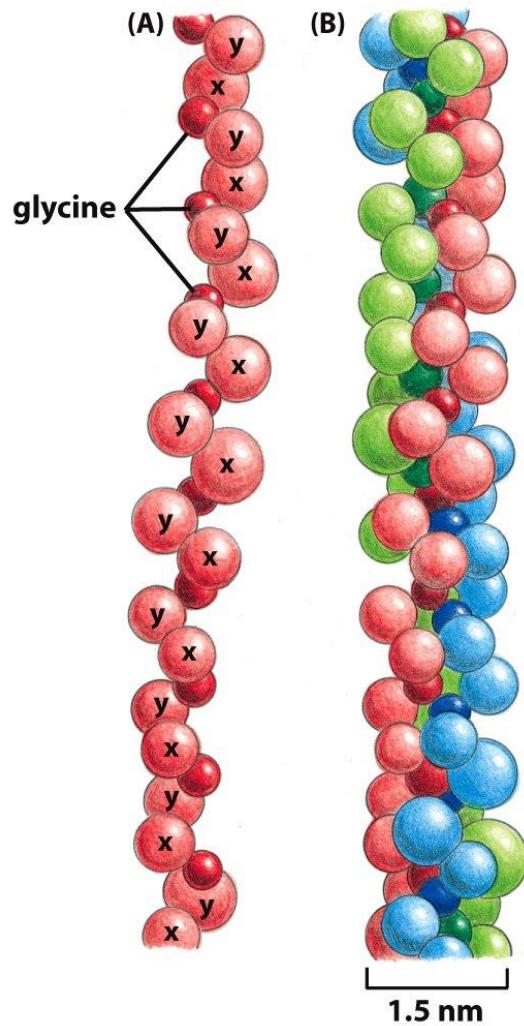
Table 19–6 Some Common Proteoglycans

PROTEOGLYCAN	APPROXIMATE MOLECULAR WEIGHT OF CORE PROTEIN	TYPE OF GAG CHAINS	NUMBER OF GAG CHAINS	LOCATION	FUNCTIONS
Aggrecan	210,000	chondroitin sulfate + keratan sulfate (in separate chains)	~130	cartilage	mechanical support; forms large aggregates with hyaluronan
Betaglycan	36,000	chondroitin sulfate/dermatan sulfate	1	cell surface and matrix	binds TGFβ
Decorin	40,000	chondroitin sulfate/dermatan sulfate	1	widespread in connective tissues	binds to type I collagen fibrils and TGFβ
Perlecan	600,000	heparan sulfate	2–15	basal laminae	structural and filtering function in basal lamina
Syndecan-1	32,000	chondroitin sulfate + heparan sulfate (in separate chains)	1–3	cell surface	cell adhesion; binds FGF and other growth factors
Dally (in <i>Drosophila</i>)	60,000	heparan sulfate	1–3	cell surface	co-receptor for Wingless and Decapentaplegic signaling proteins

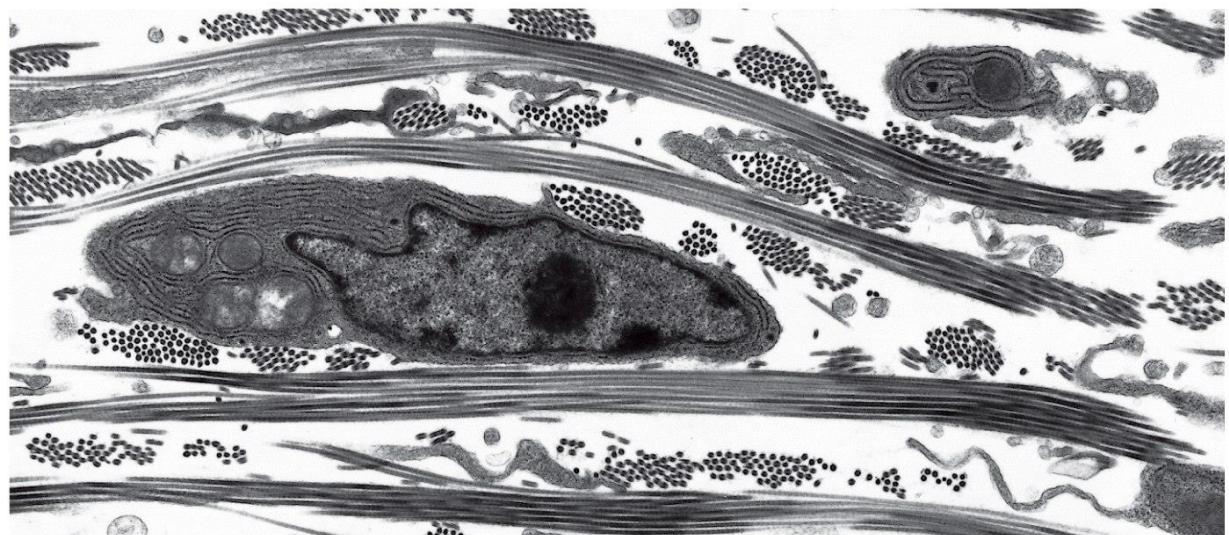
2.4 Collagens

- Major fibrous protein for ECM, the most abundant protein in mammals, ~25% of total protein mass.
- Multiple types of collagen and they have different properties
- Typical collagen molecule is long, stiff and triple stranded helixal structure
- Primary amino acid sequence is rich in proline and glycine.

The structure of a typical collagen molecule



Collagen fiber in connective tissue



1 μm

Some types of collagen and their properties

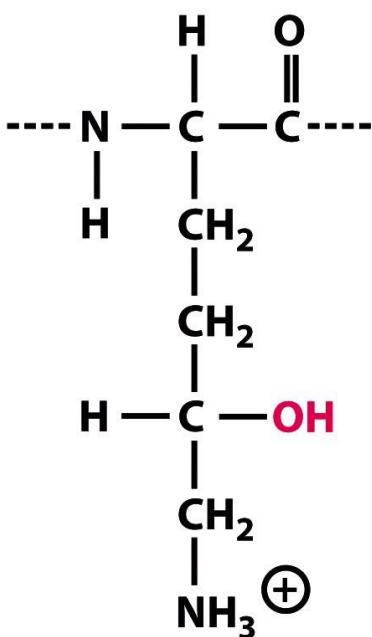
Table 19–7 Some Types of Collagen and Their Properties

	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
	II	fibril	cartilage, invertebral disc, notochord,	cartilage deficiency, dwarfism vitreous humor of the eye
	III	fibril	skin, blood vessels, internal organs	fragile skin, loose joints, blood vessels prone to rupture
	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	cartilage with type II fibrils	osteoarthritis
Network-forming	IV	sheetlike network	basal lamina	kidney disease (glomerulonephritis), deafness
Transmembrane	VII	anchoring fibrils	beneath stratified squamous epithelia	skin blistering
	XVII	non-fibrillar	hemidesmosomes	skin blistering
Proteoglycan core protein	XVIII	non-fibrillar	basal lamina	myopia, detached retina, hydrocephalus

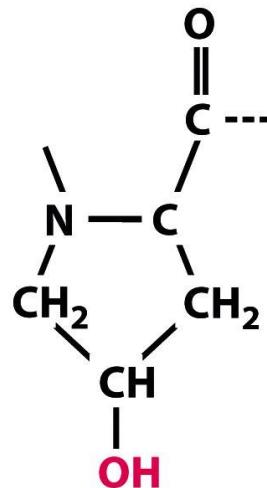
Note that types I, IV, V, IX, and XI are each composed of two or three types of α chains (distinct, nonoverlapping sets in each case), whereas types II, III, VII, XII, XVII, and XVIII are composed of only one type of α chain each. Only 10 types of collagen are shown, but about 27 types of collagen and 42 types of α chains have been identified in humans.

- Hydroxylysine and hydroxyproline are common modification in collagen

The Hydroxyl group help to form Hydrogen bond to stabilize triple-stranded helix.



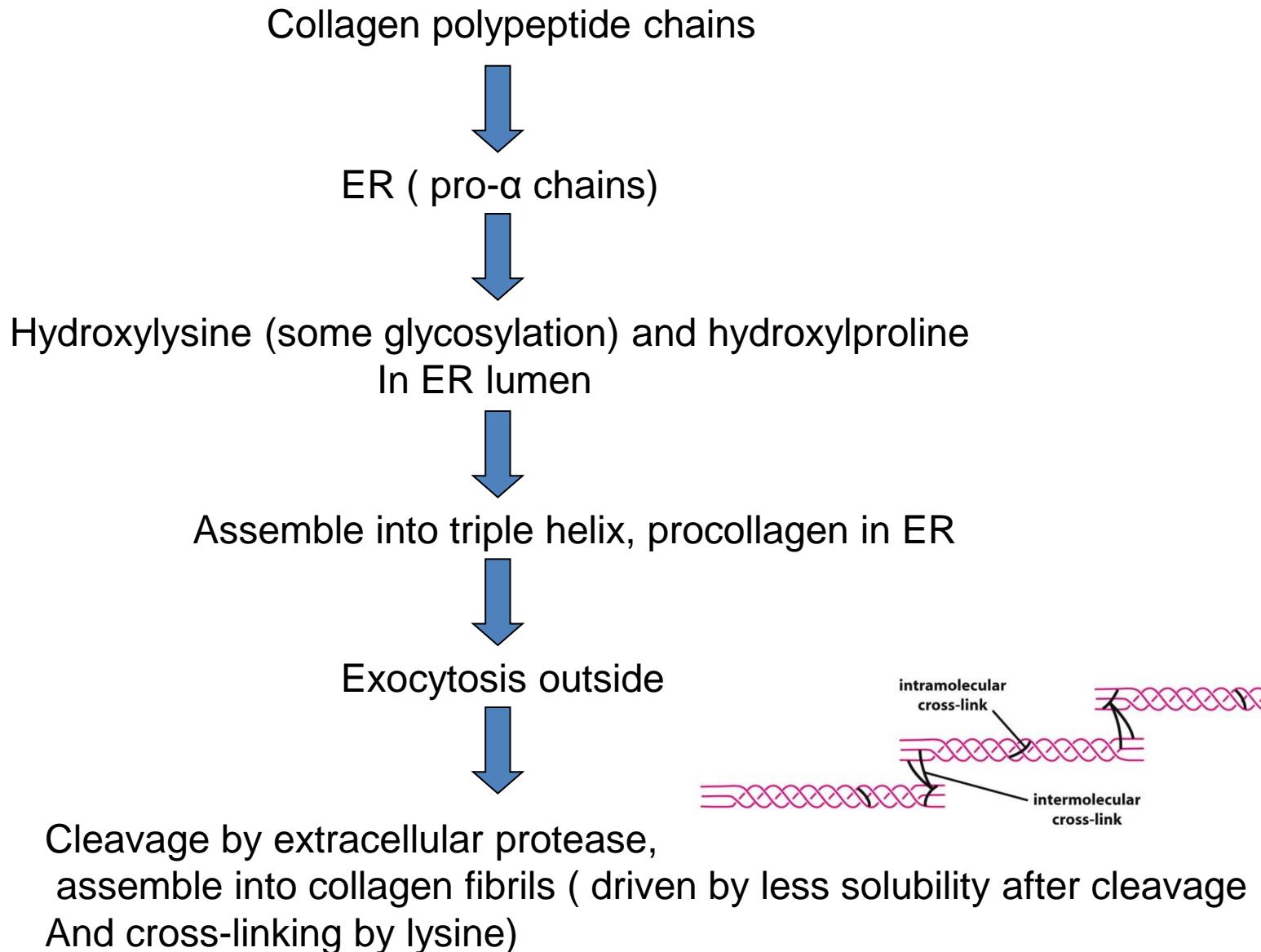
**hydroxylysine
in protein**



**hydroxyproline
in protein**

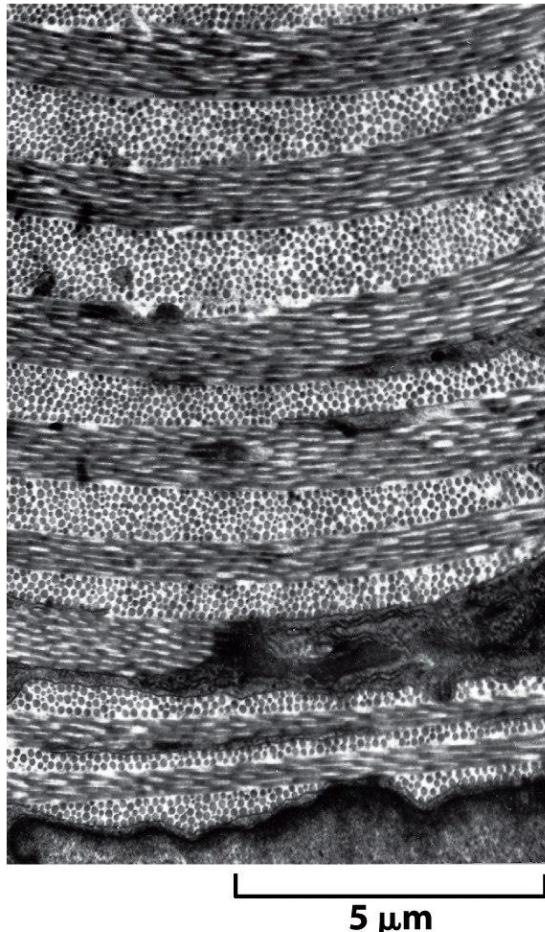
Deficiency in Vitamin C (ascorbic acid) cause defects in proline hydroxylation, Resulting in failure to assemble stable triple helix, this will cause blood vessel to be fragile and teeth to be loosen, Wounds cease to heal.

Biogenesis of collagen fibers



Fibril-associated collagens help organize the fibrils

- Collagen fibrils are arranged in orderly layers



Tadpole skin collagen fibrils

Fibril-associated collagens

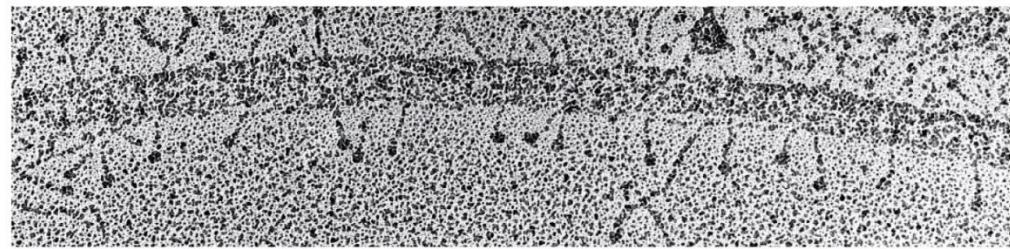
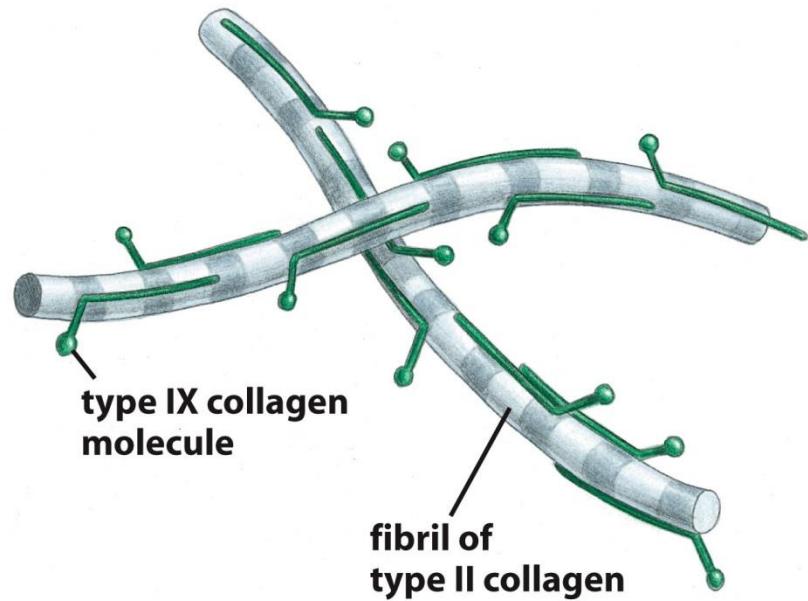
- Type IX and XII collagens:
 - A. triple stranded helix has disruption of short non-helical domains, more flexible
 - B. no cleavage after secretion
 - C. no aggregation, but bind to different types of fibrillar collagen:

Type IX: bind to type II collagen

Type XII: bind to type I collagen

Type IX collagen

Type IX collagen binds to the surface of collagen fibers in a periodic pattern



(B)



(C)

100 nm

2.5 Elastin

Elastic fibers are >5X more elastic than rubber band with the same cross-section area;

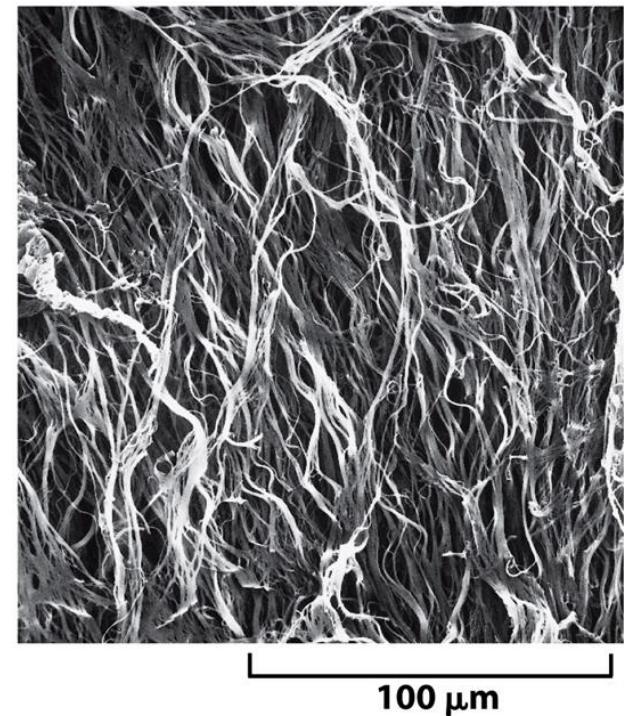
Elastic fibers mainly consist of **elastin**, some microfibrils which are composed of glycoproteins Including fibrillin.

Elastin is Proline and glycine rich, no glycosylation, with hydroxyproline but no hydroxylsine;

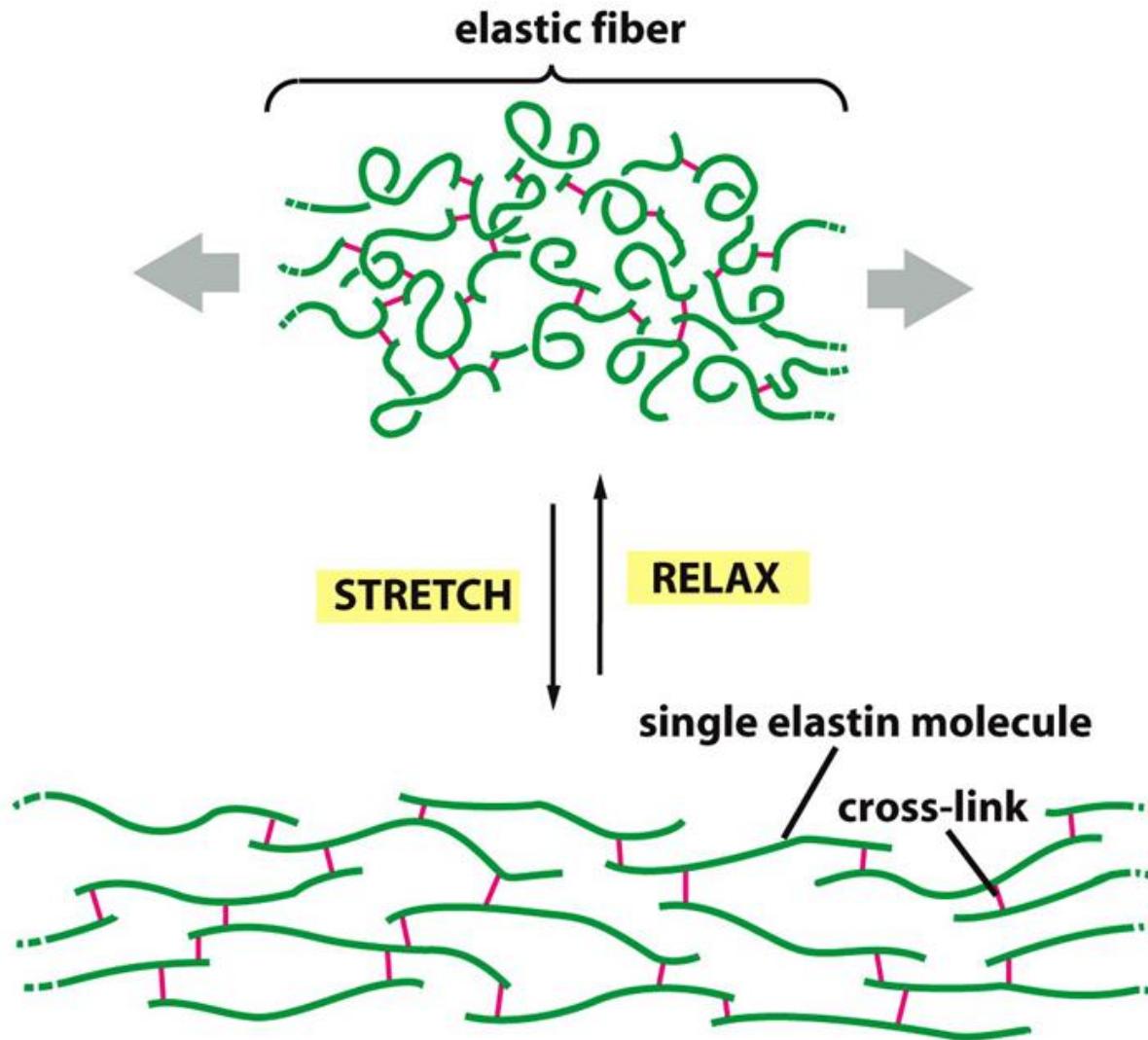
Precursor is tropoelastin, after exocytosis, highly crosslinks into elastin fibers and sheet;

Elastin contains alternative two different domains, one highly hydrophobic With rich Proline and Glycine, the other with rich Alanine and Lysine which are important for crosslinking between elastins.

Primary constituent for ECM in arteries.



Stretching a network of elastin molecules



Genetic diseases from defects in elastin fiber

Mutation in elastin----thinning of arteries and excessive proliferation of smooth muscle cells lining the arteries.

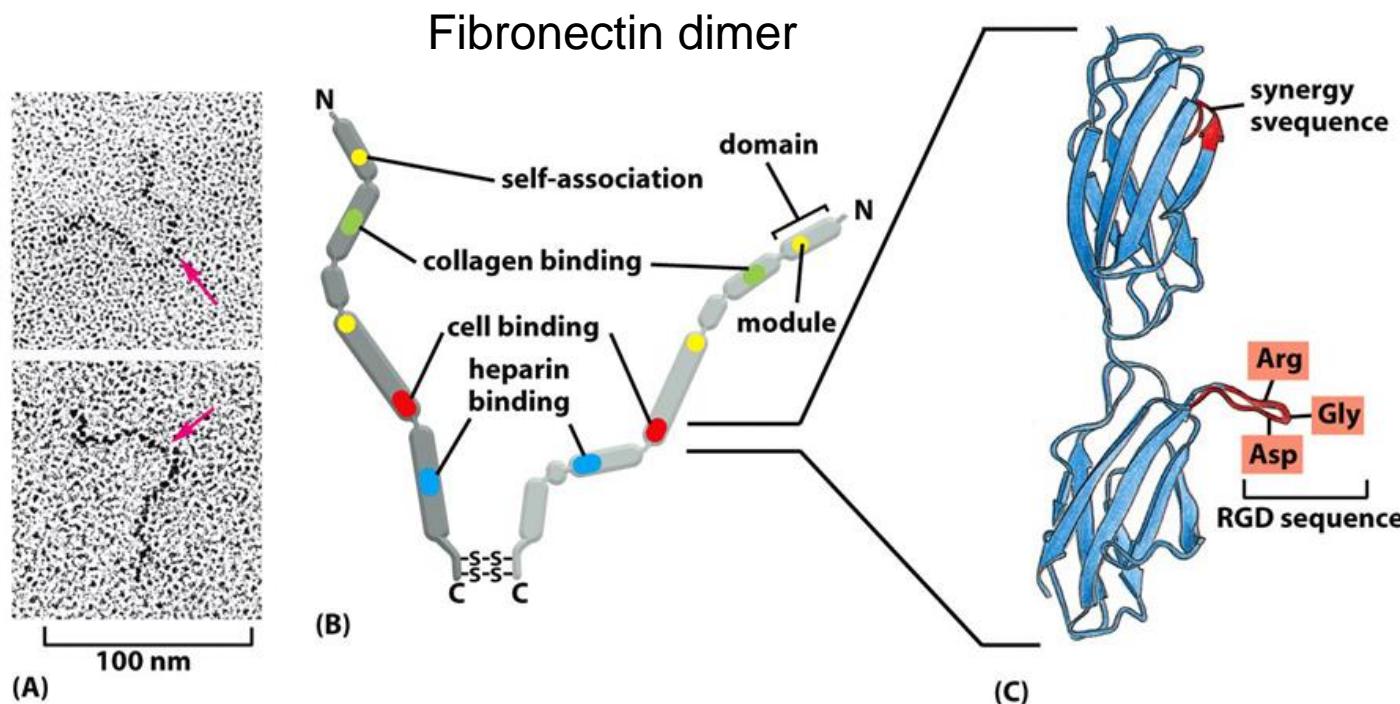
Marfan's syndrome---mutation from fibrillin, easy rupturing aorta, displacement of the lens and abnormalities of skeleton and joints

2.6 Fibronectin

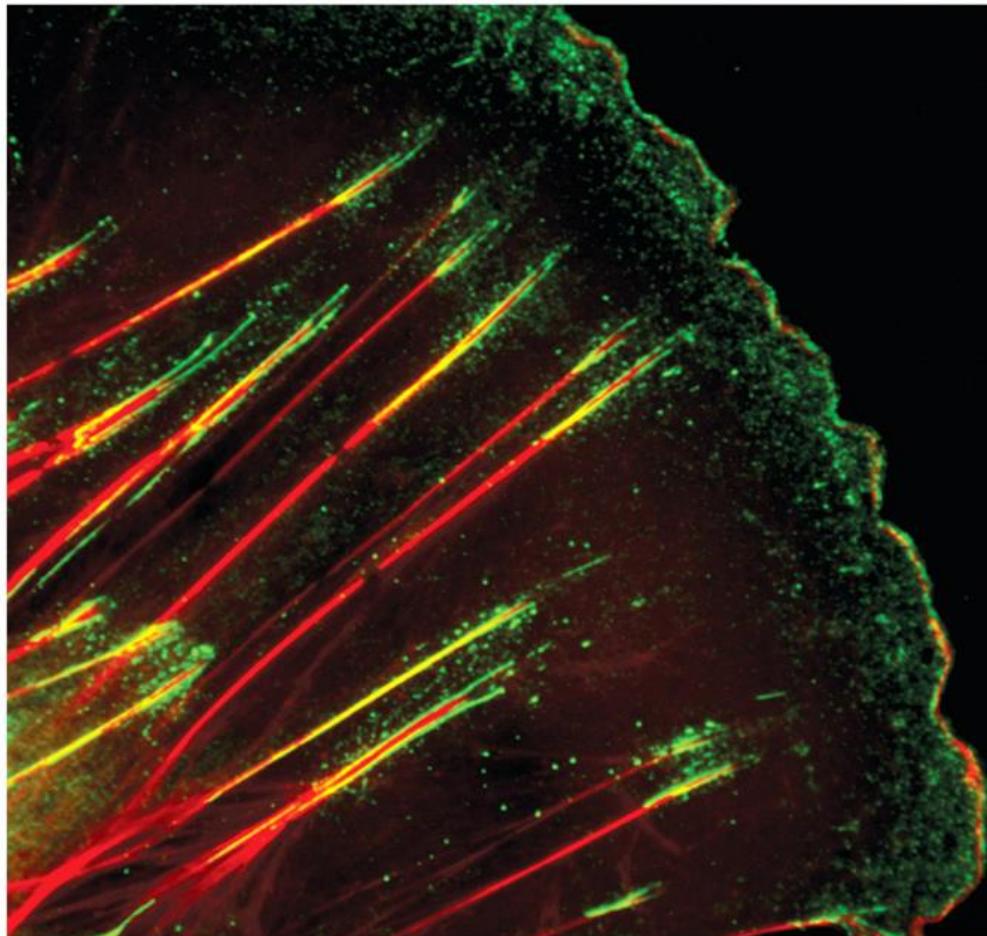
a large glycoprotein dimer joined by disulfide bond;

Exists both in soluble or insoluble fibers;

They don't self-assemble until sensing tension and cell surface receptors.



Experiment: organization of fibronectin into fibrils on cell surface



Red: actin
Green : fibronectin

In sensing tensions
During migration,
fibronectin assembles
Into fibers, in parallel
with actin fibers.

Migrating mouse fibroblast

- RGD motif on fibronectin mediates binding between fibronectin and integrin

RGD peptide can competitively inhibit fibronectin binding to integrins

One extracellular protein with RGD motif can lead to blood clotting.
Some snakes secrete disintegrin which contains the RGD motif in its sequence and cause victims to bleed.

Degradation of extracellular matrix

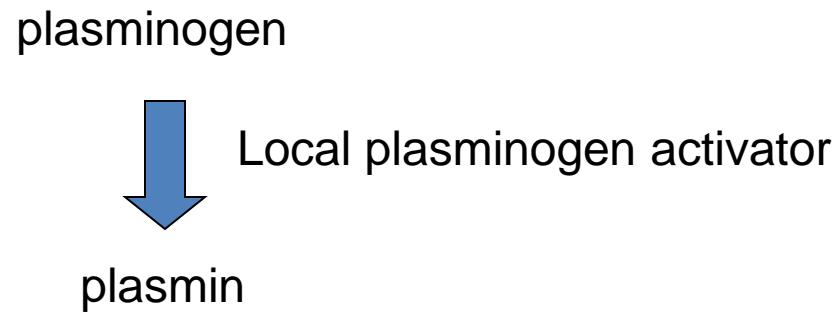
- Two different classes of proteases:
 1. Matrix metalloprotease(MMP,Ca²⁺ or Zn²⁺ dependent)
 2. Serine protease

Ways to activate these proteases

- Local activation
- Confined to membrane surface
- Secretion of inhibitors

Local activation

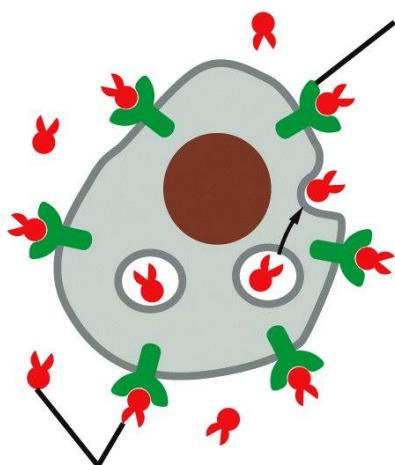
- Example: plasmin who helps to break up blood clots



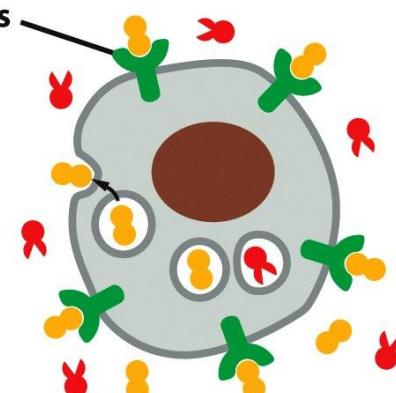
Membrane confinement

- Membrane bound MMPs
- Urokinase-type plasminogen activator

(A) cells with functional protease receptors



(B) cells with blocked protease receptors



active protease (uPA)

inactive protease (mutant uPA)

TUMOR GROWTH AND METASTASIS

TUMOR GROWTH BUT NO METASTASIS

Secretion of inhibitors

- Tissue inhibitors of metalloproteases (TIMPs)
- Serine protease inhibitors--- serpins