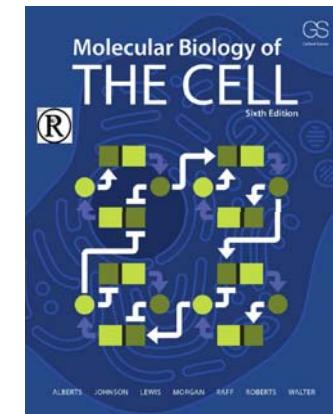


Lecture 19: Integrating cells into tissues II

Outline

- I. Occluding junctions
- II. Channel-forming Junctions
- III. Basal Lamina
- IV. The extracellular matrix (ECM)

Peter Pimpl
Dept. Biology
Institute of Plant and Food Science
RB1, R307, pimpl@sustc.edu.cn



CHAPTER 19

I. Occluding junctions: *tight junctions (zonula occludens)*

Where do we find tight junctions?

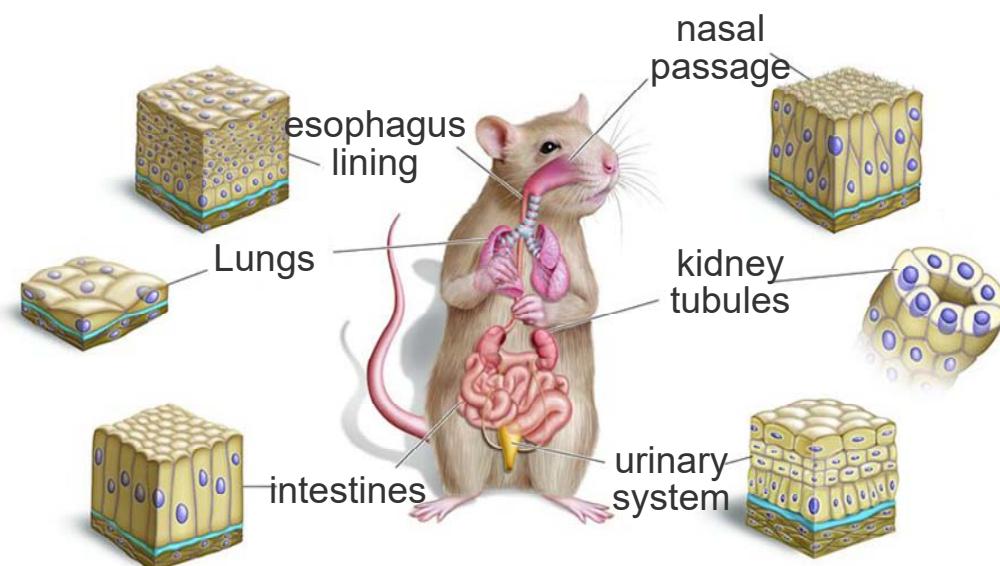
Epithelial tissue

- Lines all surfaces & cavities
- Serves as permeability barrier

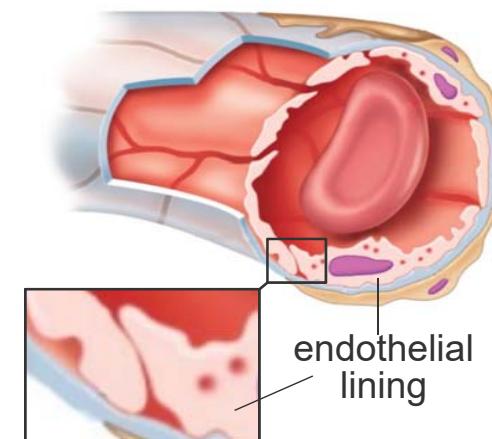
Endothelial tissue

- Lines capillaries
- Permeability barrier

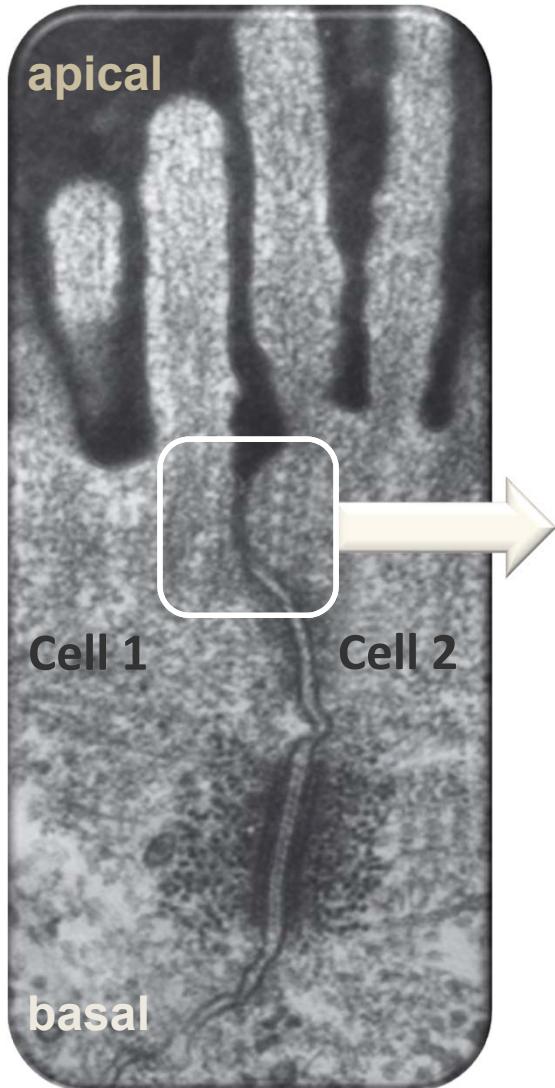
epithelial tissues



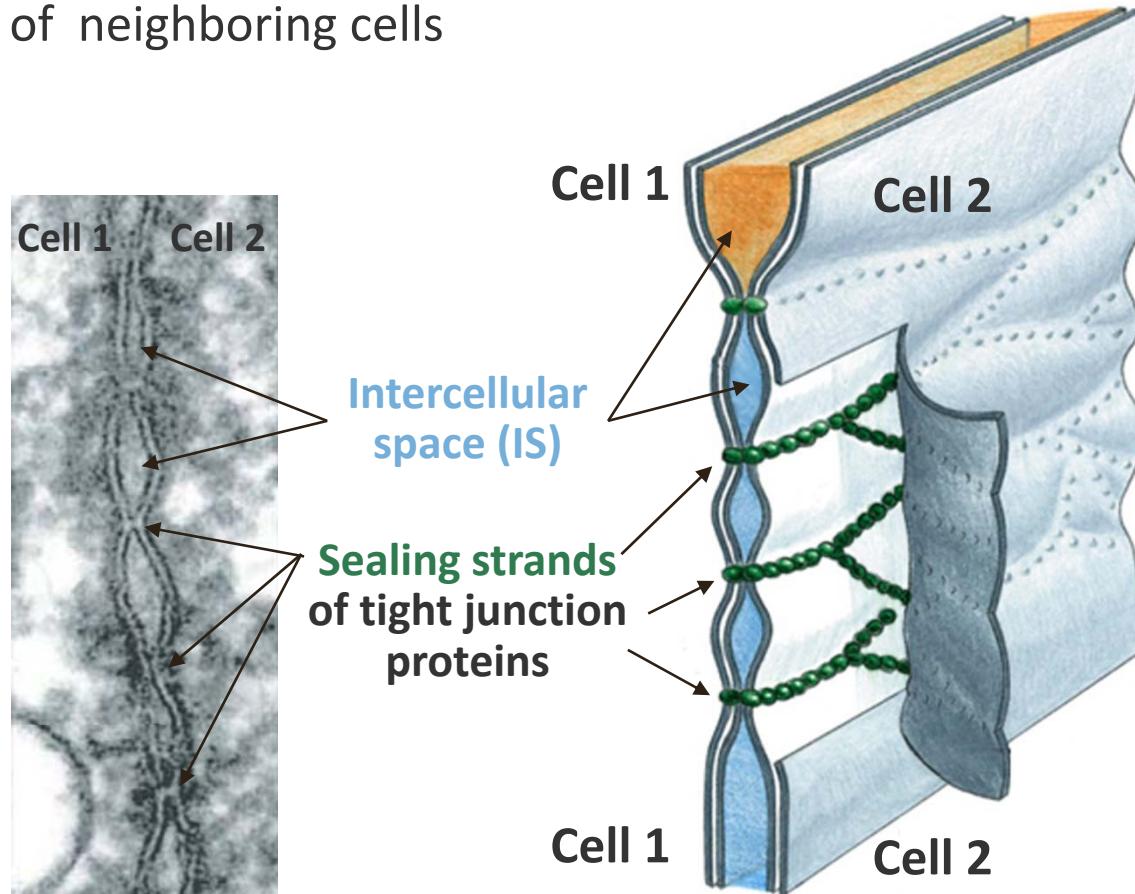
endothelial tissues



Organization of tight junctions



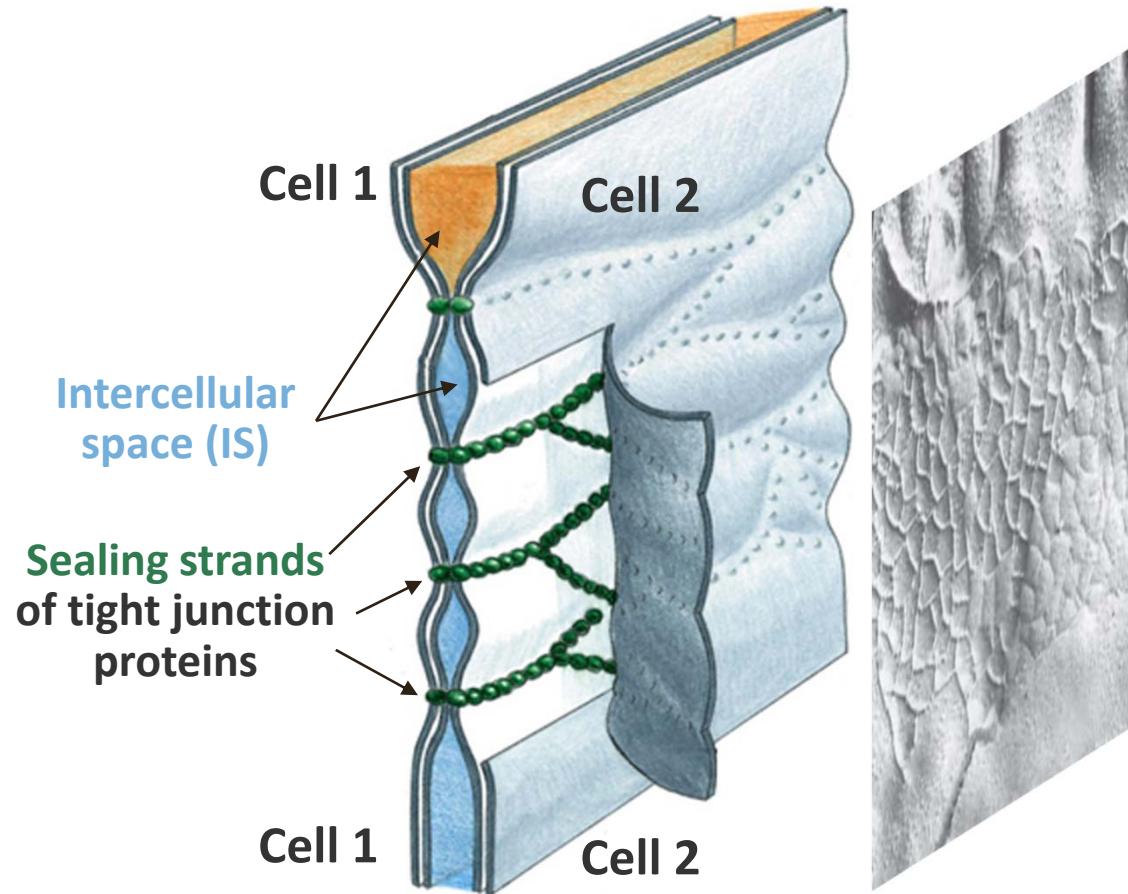
Tight junctions spot-weld the plasma membranes of neighboring cells



Organization of tight junctions

Freeze-fracture electron microscopy reveals the sealing strands

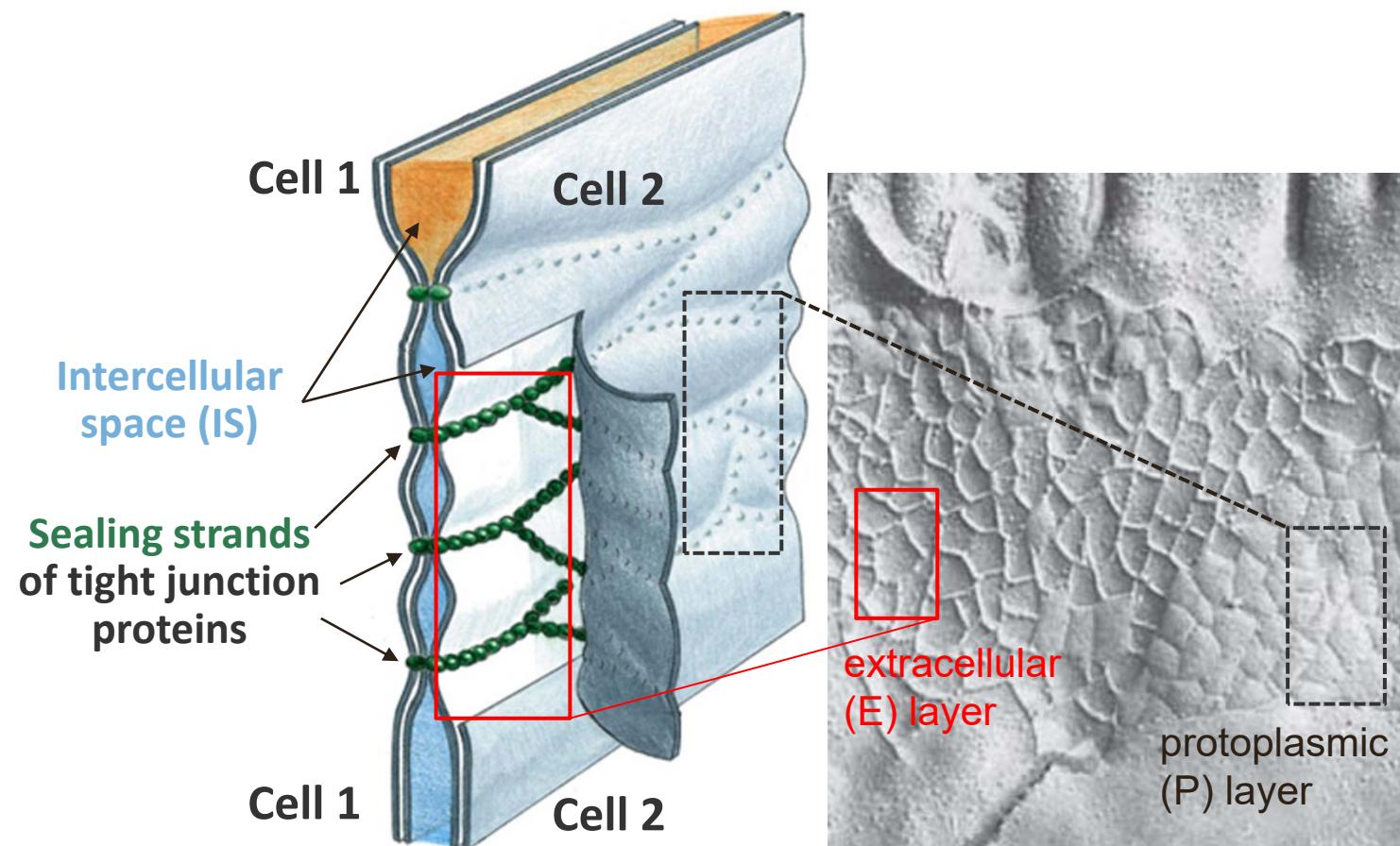
“Peeling-off” by “freeze-fracture” preparation for EM



Organization of tight junctions

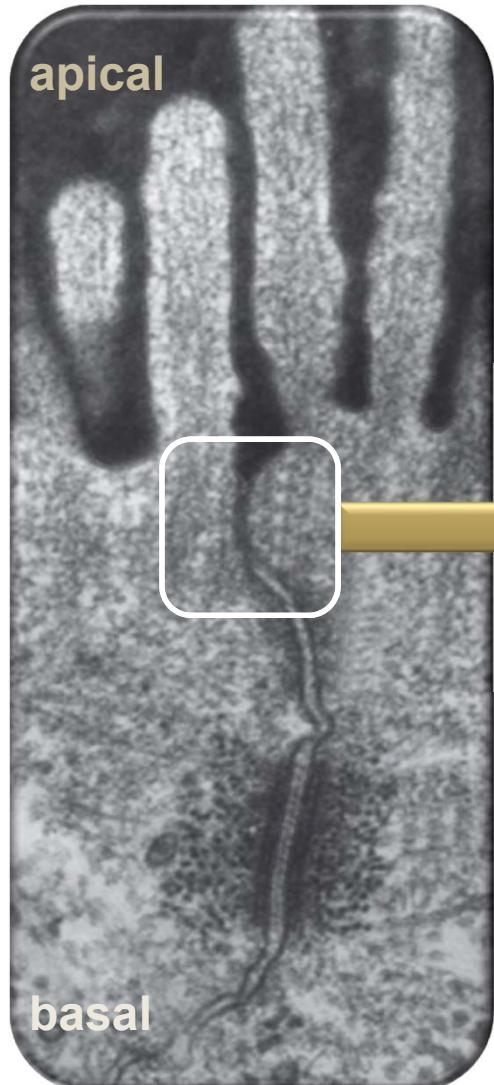
Freeze-fracture electron microscopy reveals the sealing strands

The sealing strands surround all cells



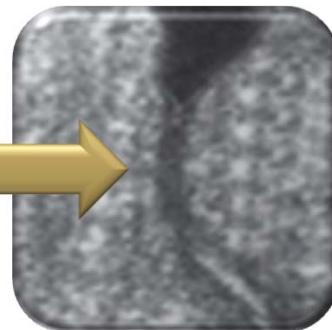
Tight junctions seal the intracellular space (IS)

What are the functions of tight junctions?



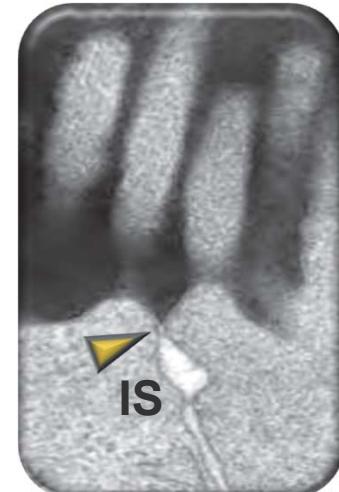
Occluding junctions (*zonula occludens*) seal the intercellular space between adjacent cells

- *tight junctions* in vertebrates
- *septate junctions* in invertebrates



Electron-dense tracer cannot pass through the intracellular space (IS)

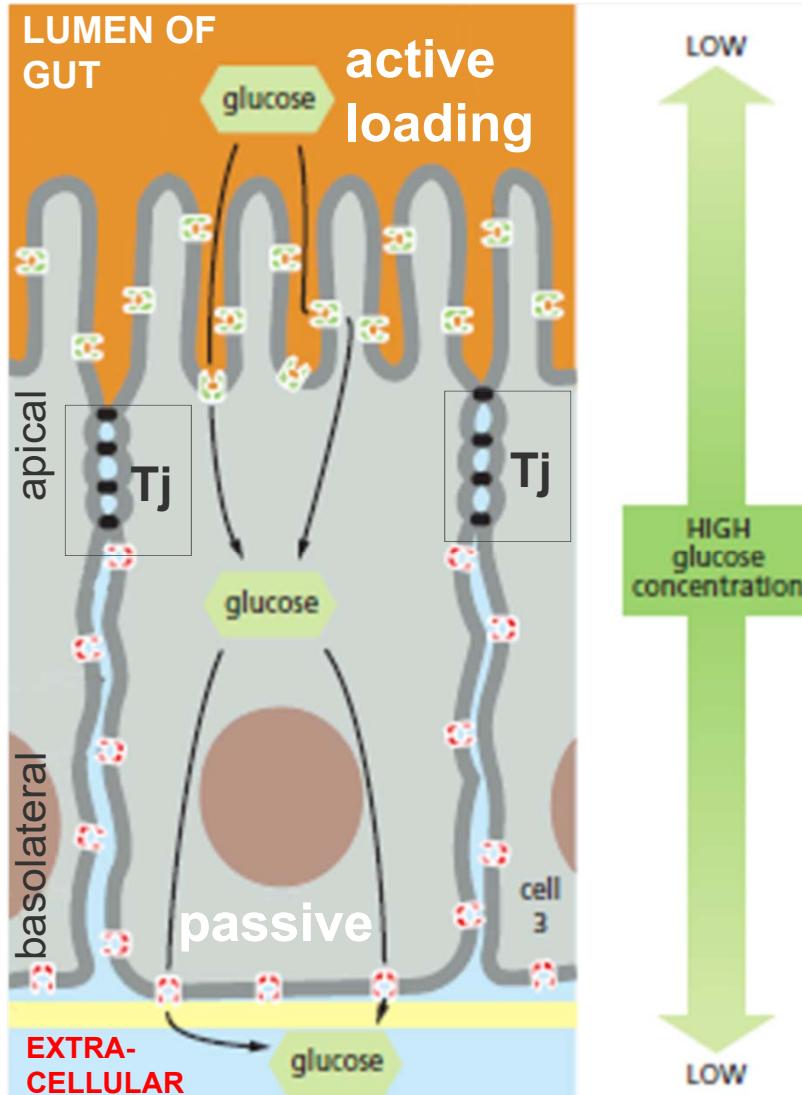
added at
apical side



added at
basolateral side



Tight junctions maintain cell polarity: fencing function



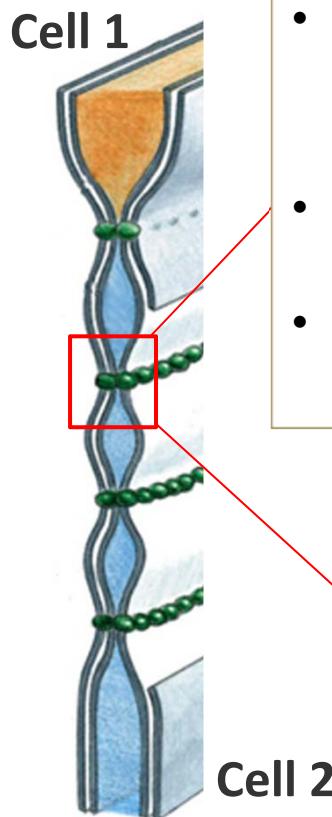
This segregation permits a vectorial transfer of nutrients across the epithelium from the gut lumen to the blood.

Function of tight junctions:

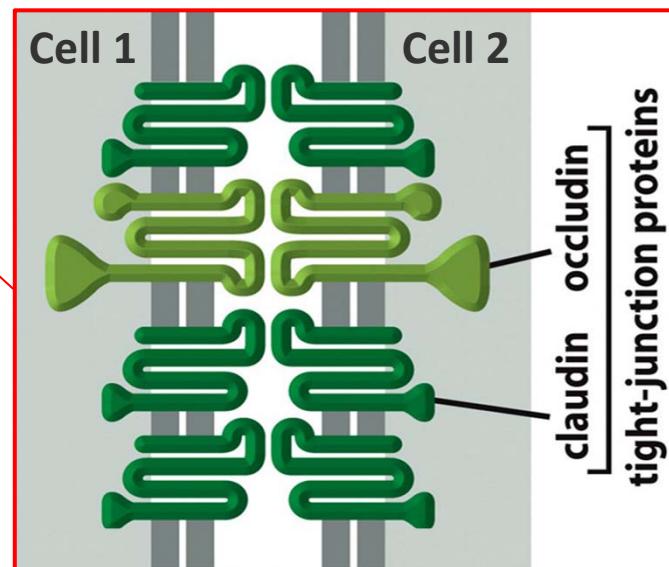
1. They **seal** the intercellular space to form a barrier
2. Serve as “**fences**” to **separate domains** within the PM
3. Tjs **Maintain cellular polarity**
4. Tjs are **selective for ions**, but do not allow macromolecules to pass.

Molecular organization of tight junctions

Three key proteins form tight junctions: claudin, occludin & tricellulin

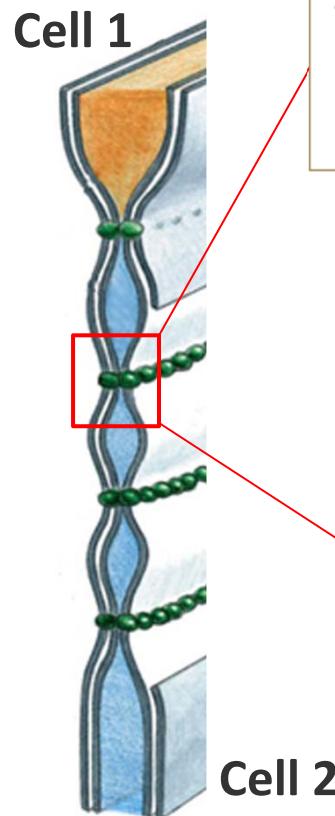


- **Claudin:**
 - **Loss** causes dehydration and death in mice
 - **Overexpression** causes tight junction formation in fibroblasts
- **Occludin:**
 - Detailed function unknown
- **Tricellulin:**
 - Required to seal membrane

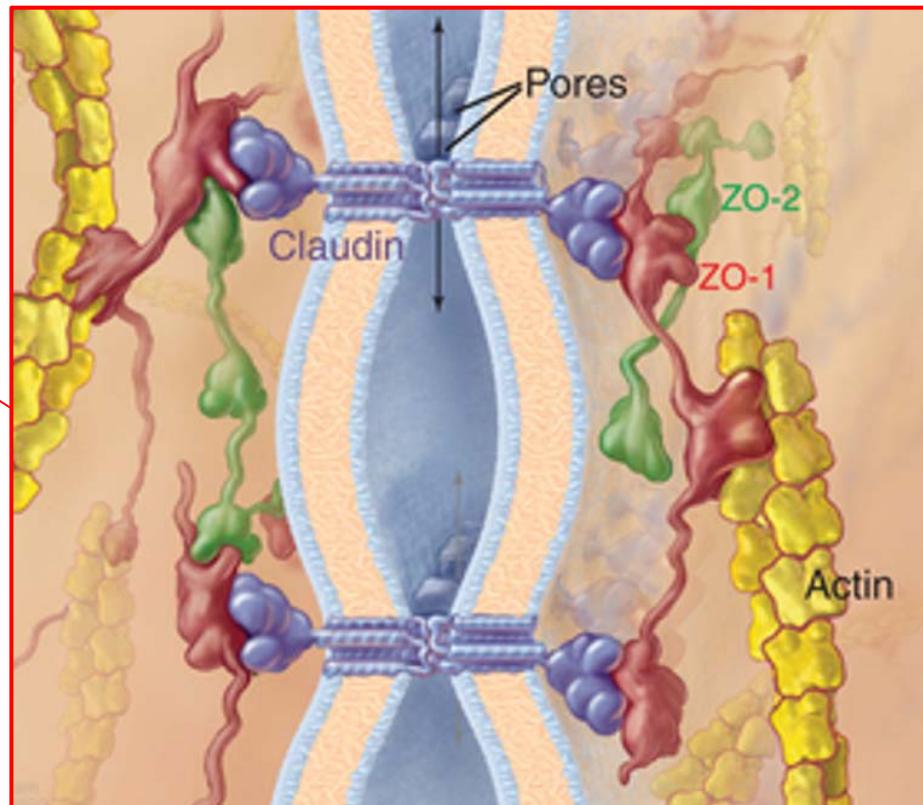


Molecular organization of tight junctions

Scaffold proteins (**ZO/Tjp**) link claudins to the actin-cytoskeleton



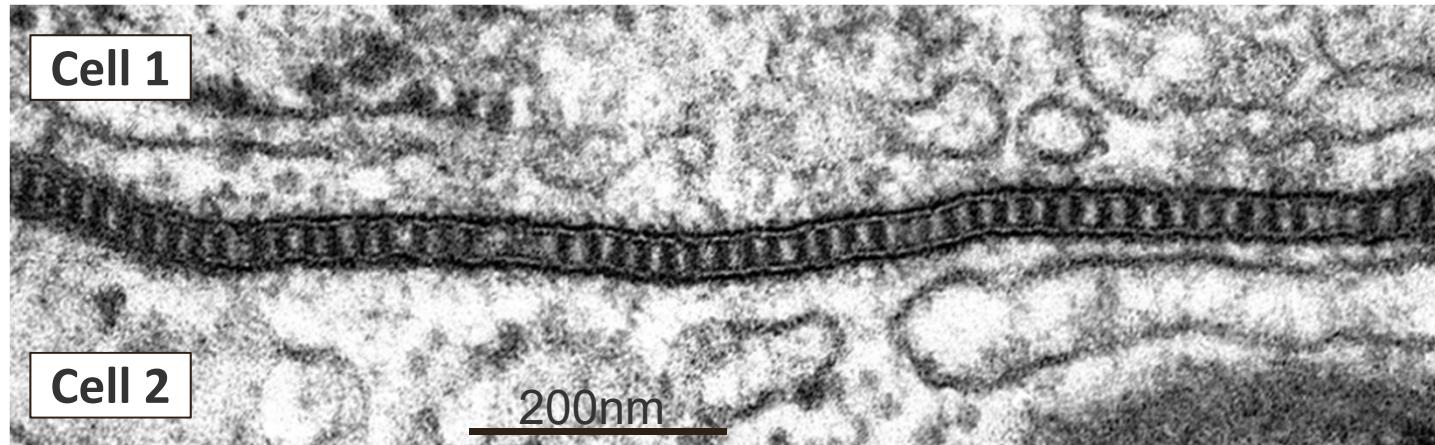
- **ZO** (zonula occludens) proteins (**ZO-1/-2**)
also called **Tjp** (tight junction proteins)
are anchor sites for tight-junctional strands



Invertebrates possess septate junctions, instead

Septate junctions:

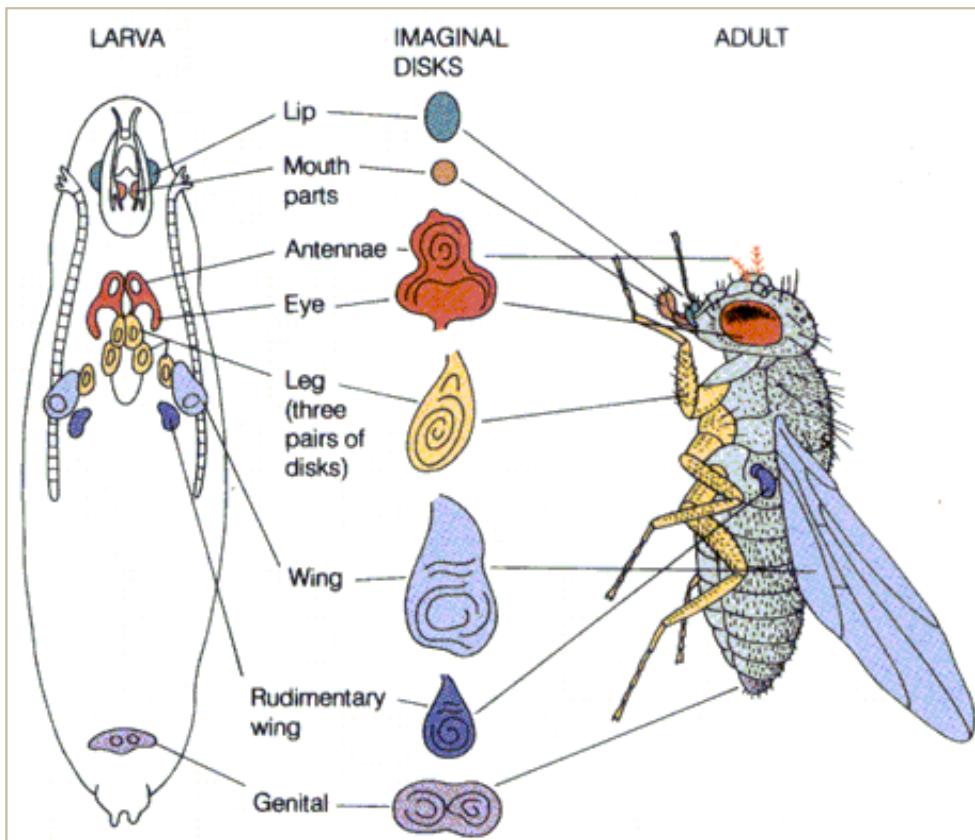
- Structure of septate junctions is more regular, “ladder-like” in parallel rows
- Septate junctions are formed by claudin homologs and scaffold proteins



Cell adhesion is important to regulate cell proliferation

Example:

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



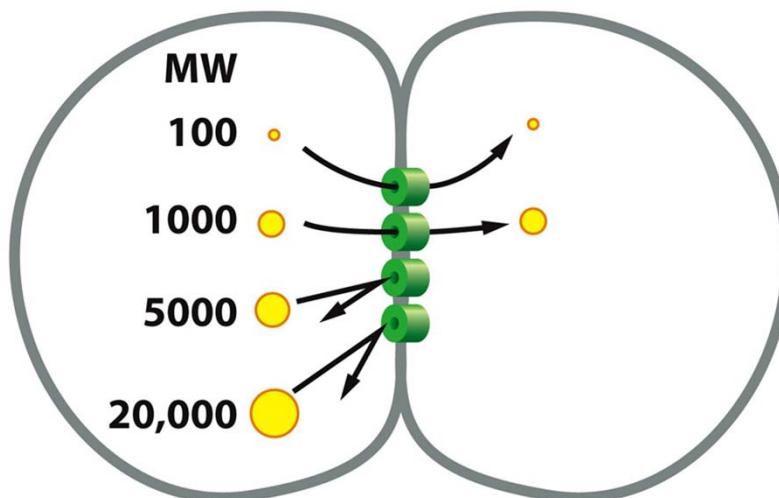
Reason?

Imaginal discs of *Drosophila* :

- Model system to study growth control
- Discs form in the embryo from epidermal placodes (6-40 cells)
- Placodes invaginate as “hollow sacs” and form a single-layered epithelium

II. Channel-forming junctions

- Gap junctions (in animals)
- Plasmodesmata (in plants)
- Allow direct **exchange of small molecules and inorganic ions** from **cytosol to cytosol**, but very few macromolecules in rare exceptions.
- Occur in epithelia and connective tissue



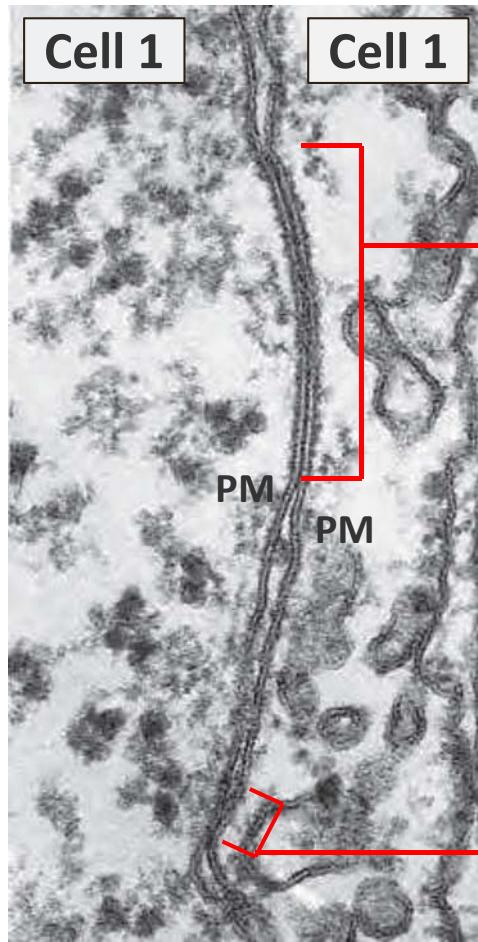
Molecules with molecular weight of up to 1,000 Dalton (cut-off) can pass the gap-junctions

Gap junction proteins

- Two protein families: **connexins** and **innexins**.
different in sequence but **similar** in shape and function.
- Vertebrates possess predominantly **connexins**
- *Drosophila* and *C. elegans* possess only **innexins**
- **Each protein family contains multiple members** in a species:
 - 21 connexins (human),
 - 15 innexins (fly)
 - 25 innexins (worm)Differences confer **different permeability** for molecules in gap junctions.

The organization of gap junctions

Ultrastructural analysis



Thin-section EM

large gap junction

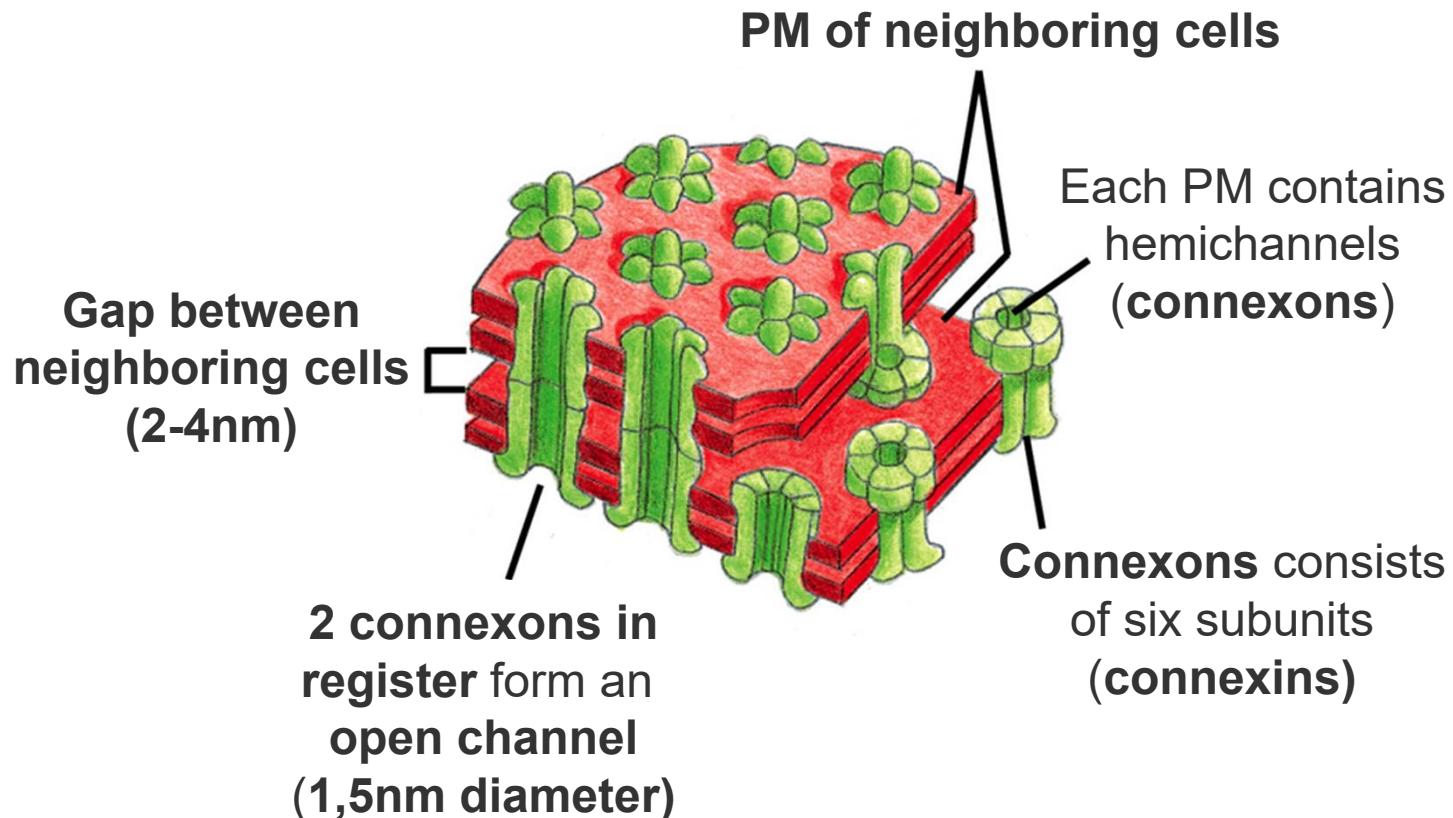
small gap junction



Freeze-fracture EM

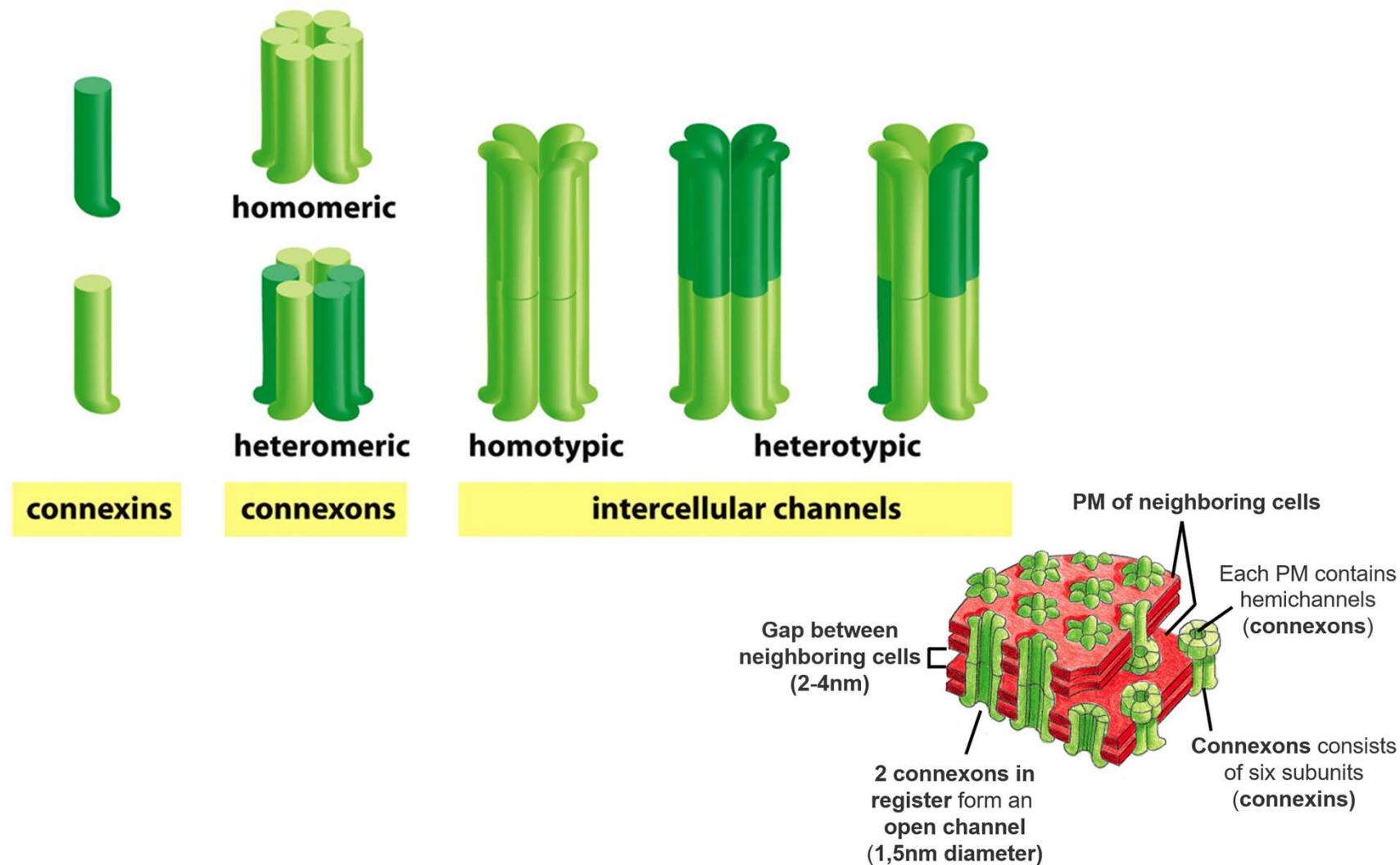
Structure of Gap junctions

Six connexins form one connexon and two connexons build one channel



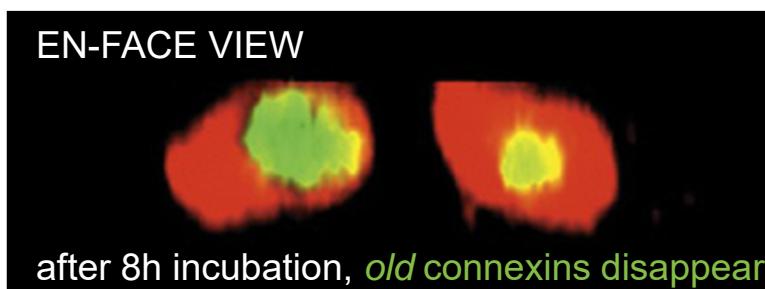
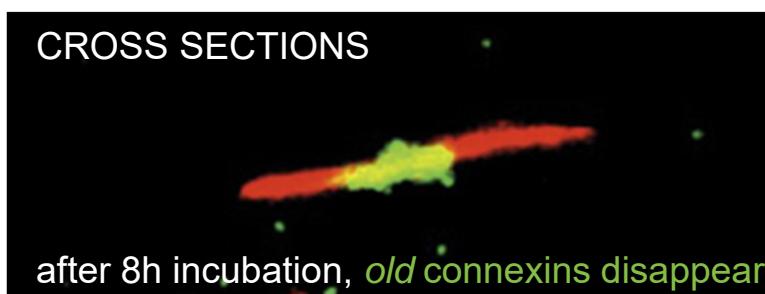
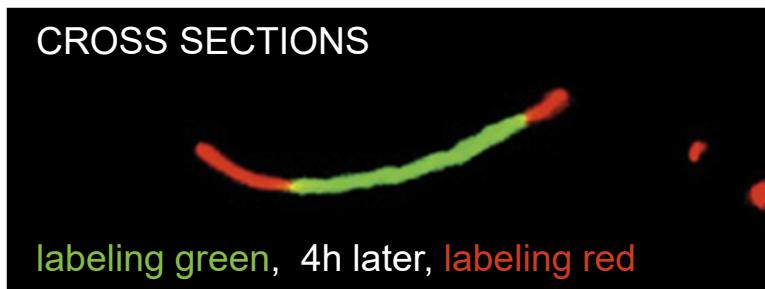
Structure of Gap junctions

Connexons can be homomeric or heteromeric



Gap junctions are dynamic structures

Assembly and remodeling of gap junctions over 20 hours



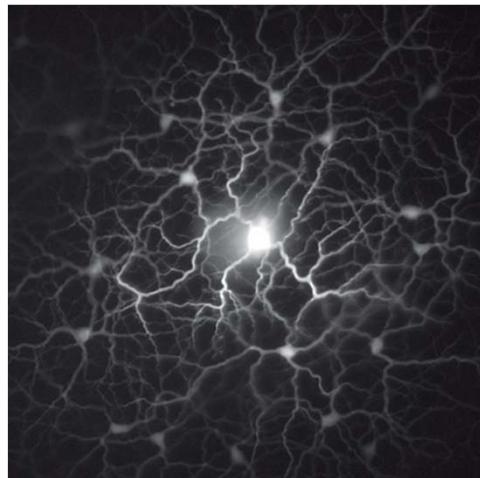
Experiment:

- Cells expressing tetra-cysteine-tagged connexin (connexin-4C) were used to label gap junctions.
- The tag is detected via fluorescent dyes: first with the green dye FlAsH and 4 hours later with the red dye ReAsH.
- After 8h incubation, most of the “**old green label** disappeared **and was replaced by newer red connexins**.”

Regulation of gap-junction communication

- Gap junctions have **open** and **closed** states
- Closure is triggered by lower pH or an increase in Ca^{2+} levels
- Gap junctions can be regulated by extracellular signals.

Gap junction coupling of neurons is reduced by a neurotransmitter



Lucifer yellow
injection in the
control neuron

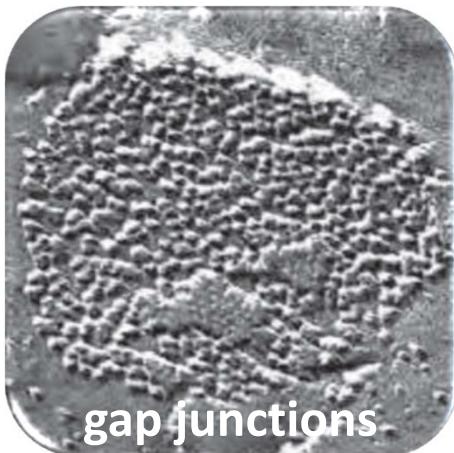


Lucifer yellow
injection
after dopamine

Experiment:

- **One neuron** was injected with a fluorescent dye (lucifer yellow).
- The dye diffuses via gap junctions, **revealing all connected cells (left)**.
- After dopamine treatment, permeability/connecitity between neurons is greatly reduced (**right**).

Functions of gap junctions



Electrically excitable cells:

- **rapid spreading** of action potentials (no delay by synapses)
- **synchronization** of a set of neurons
- **electrical coupling** through gap junctions allows:
 - **synchronization of heart muscle cells**
 - **synchronization of smooth muscle cells** (peristaltic movement of intestine)

Other cells:

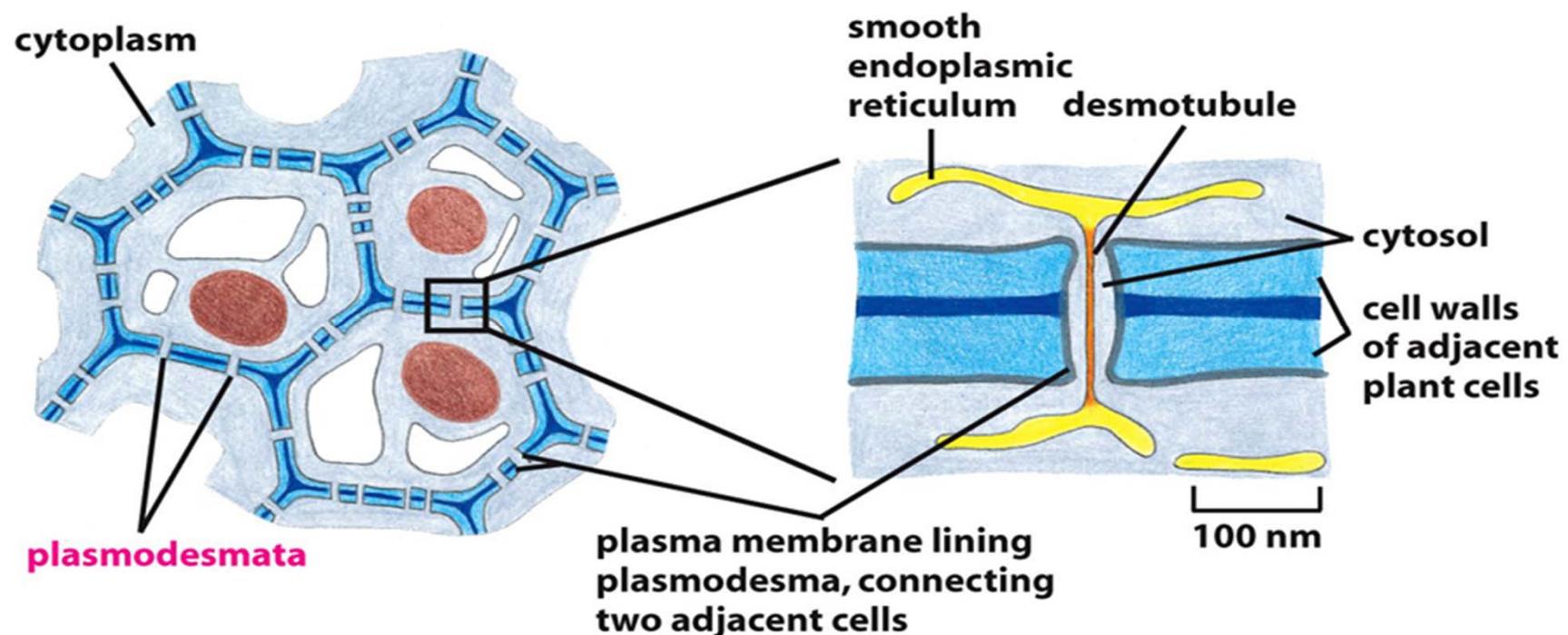
- **sharing** of metabolites
- **smoothing-out random fluctuations** of metabolites and small molecules
- **coordination of cells** in tissues / organs (e.g. liver)

Mutations in connexins are severe:

- deafness (connexin 26)
- cataracts of lenses
- neurodegenerative disease, etc.

Plasmodesmata in plants: a type of gap junction

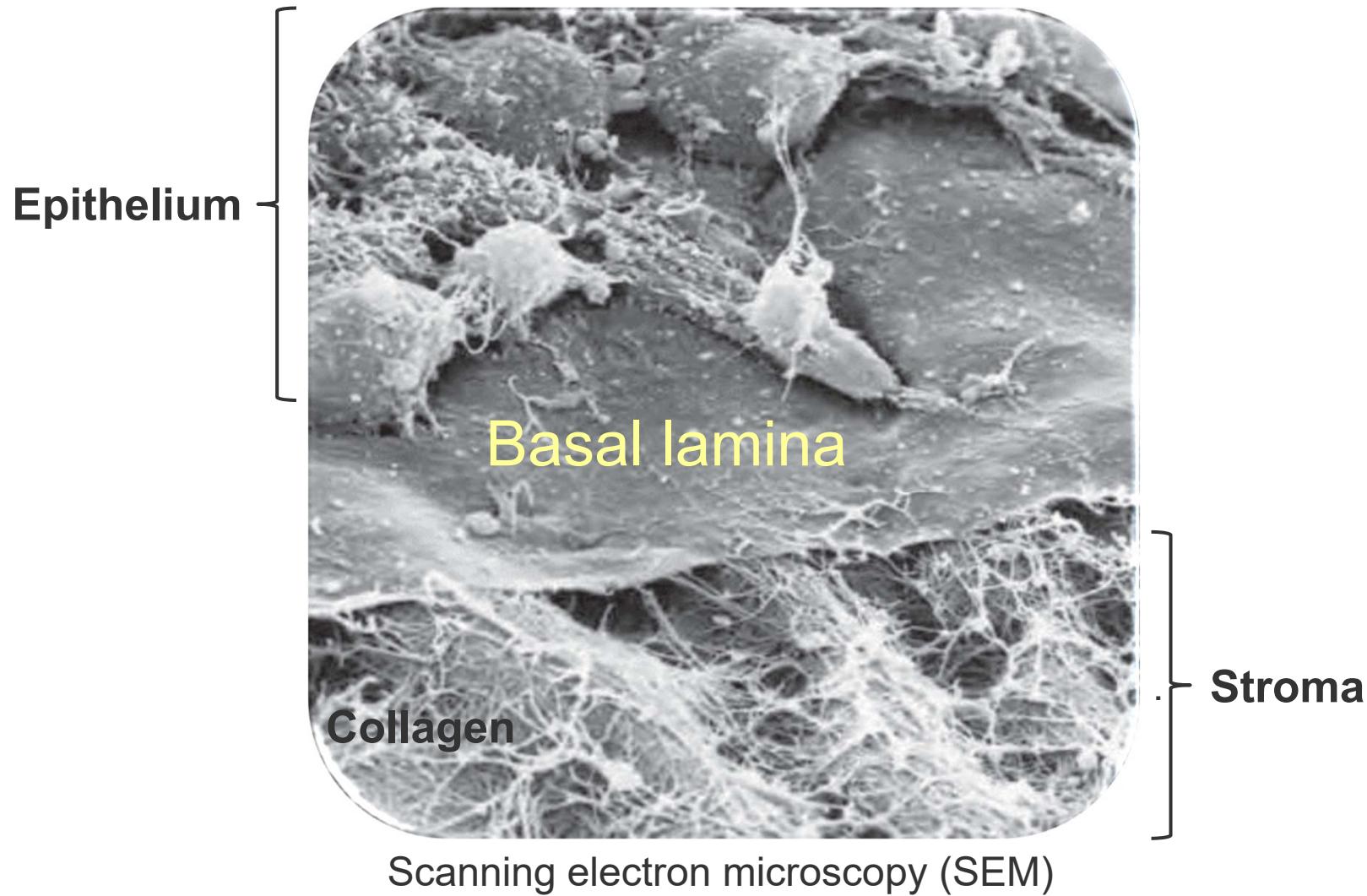
- Connect the cytoplasm of the two adjacent cells, cut-off 800 Dalton by a rough and cylindrical channel with a diameter of 20-40nm.
- Connected by desmotubule which is the continuation of the smooth endoplasmic reticulum (ER).
- certain virus movement proteins spread via plasmodesmata



Stone cell from pears have very long plasmodesmata



III. The basal lamina



III. The basal lamina

What is the basal lamina?



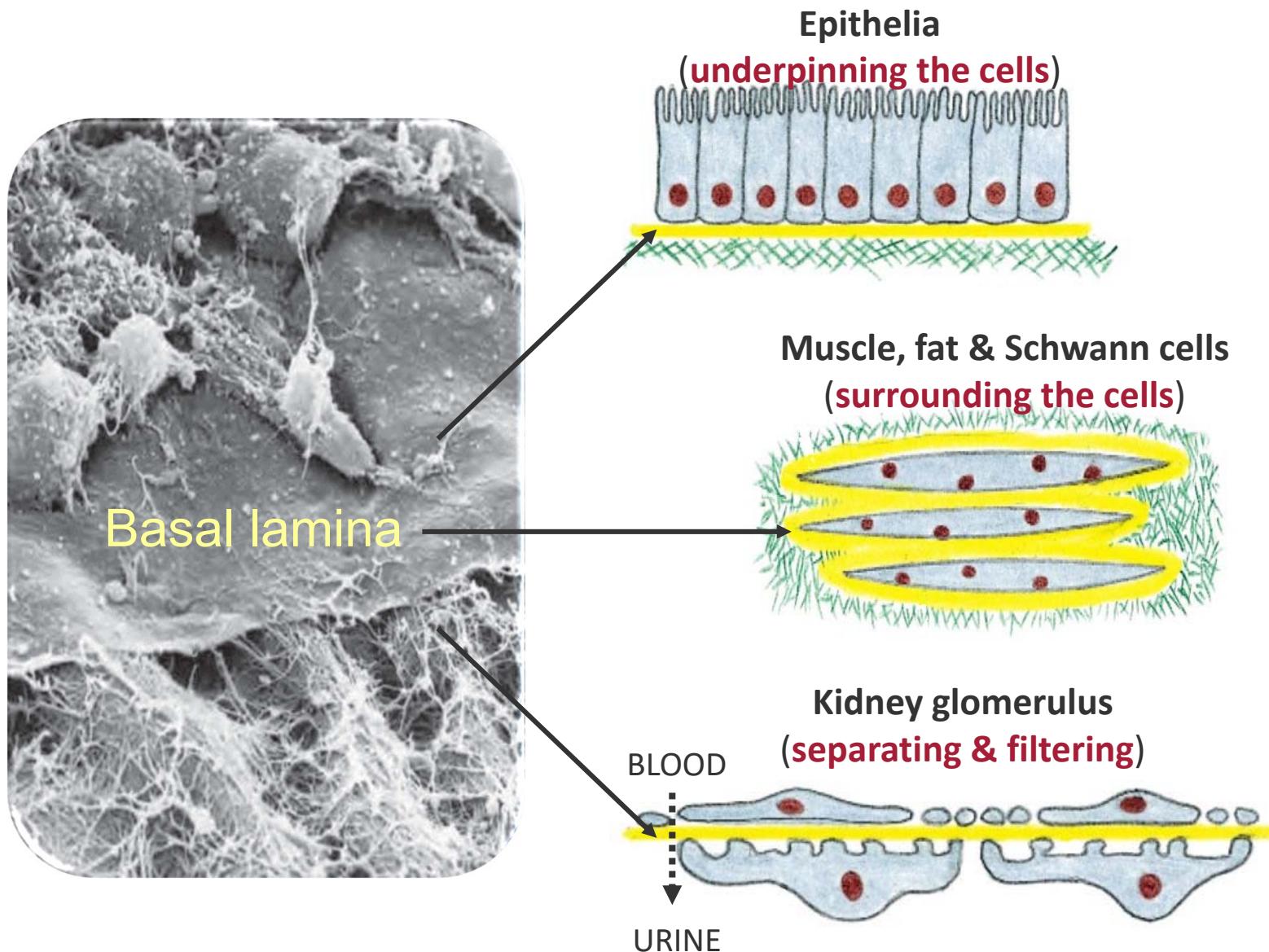
Basal lamina is a structure
of the ECM
(extracellular matrix)

Produced by both:
epithelia & the stroma,

40-120 nm thick

Plays important mechanical role
(strength of the epidermis)

The basal lamina comes in different configurations:

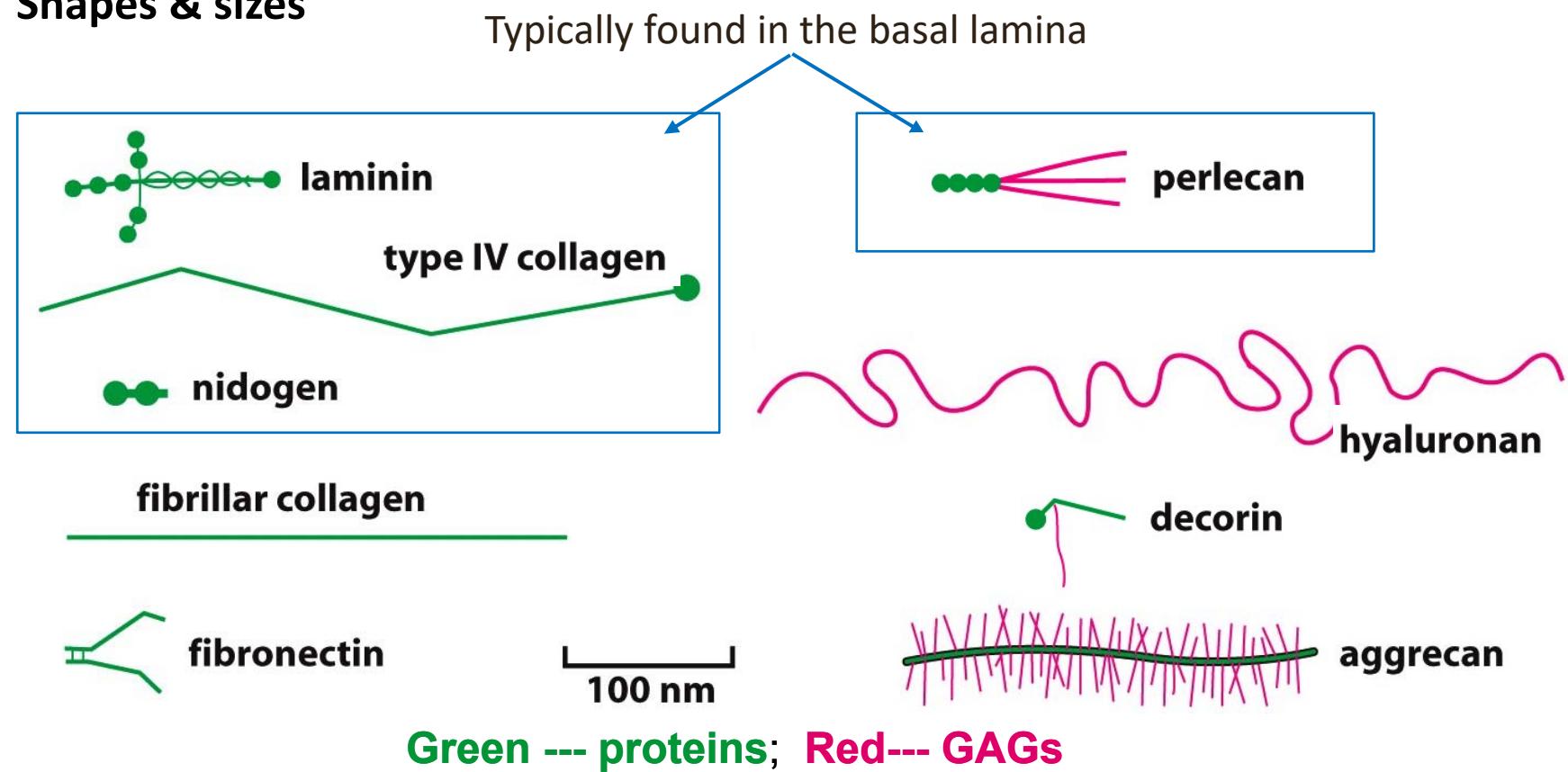


Composition of the basal lamina

The basal lamina consists of 2 main classes of secreted macromolecules:

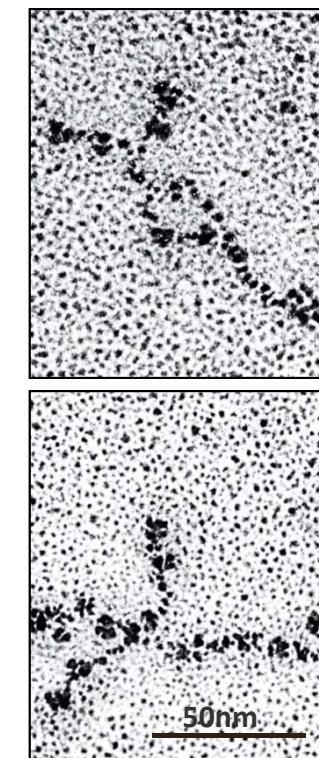
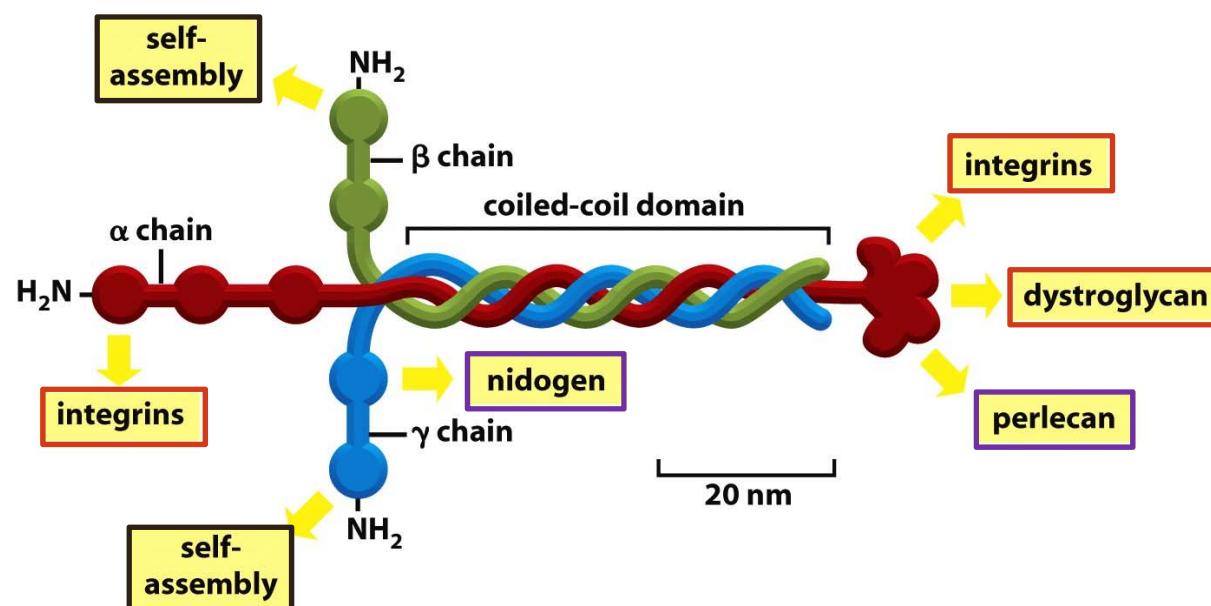
- **Fibrous proteins** (with short oligosaccharide side chains)
- **Proteoglycans** (proteins with polysaccharides, glycosaminoglycans, **GAGs**)

Shapes & sizes



Composition of the basal lamina: laminin

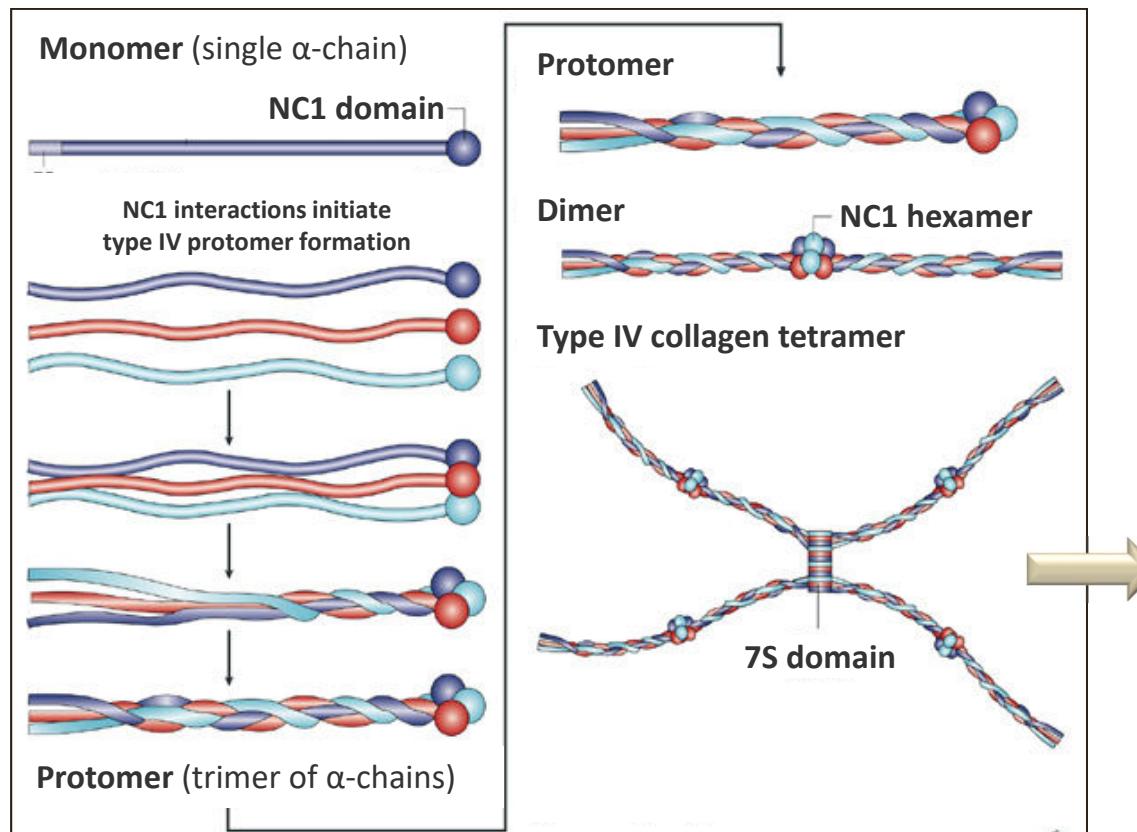
- Laminin is the primary organizer for the basal lamina structure
- Large heterotrimeric complex, consisting of α -, β -, γ -chains (about 3000 amino acids each subunit), which are held together by disulfide bonds.
- Can self-assemble via their head domains into a network *in vitro*



Composition of the basal lamina: type IV collagen

Second essential component in the basal lamina

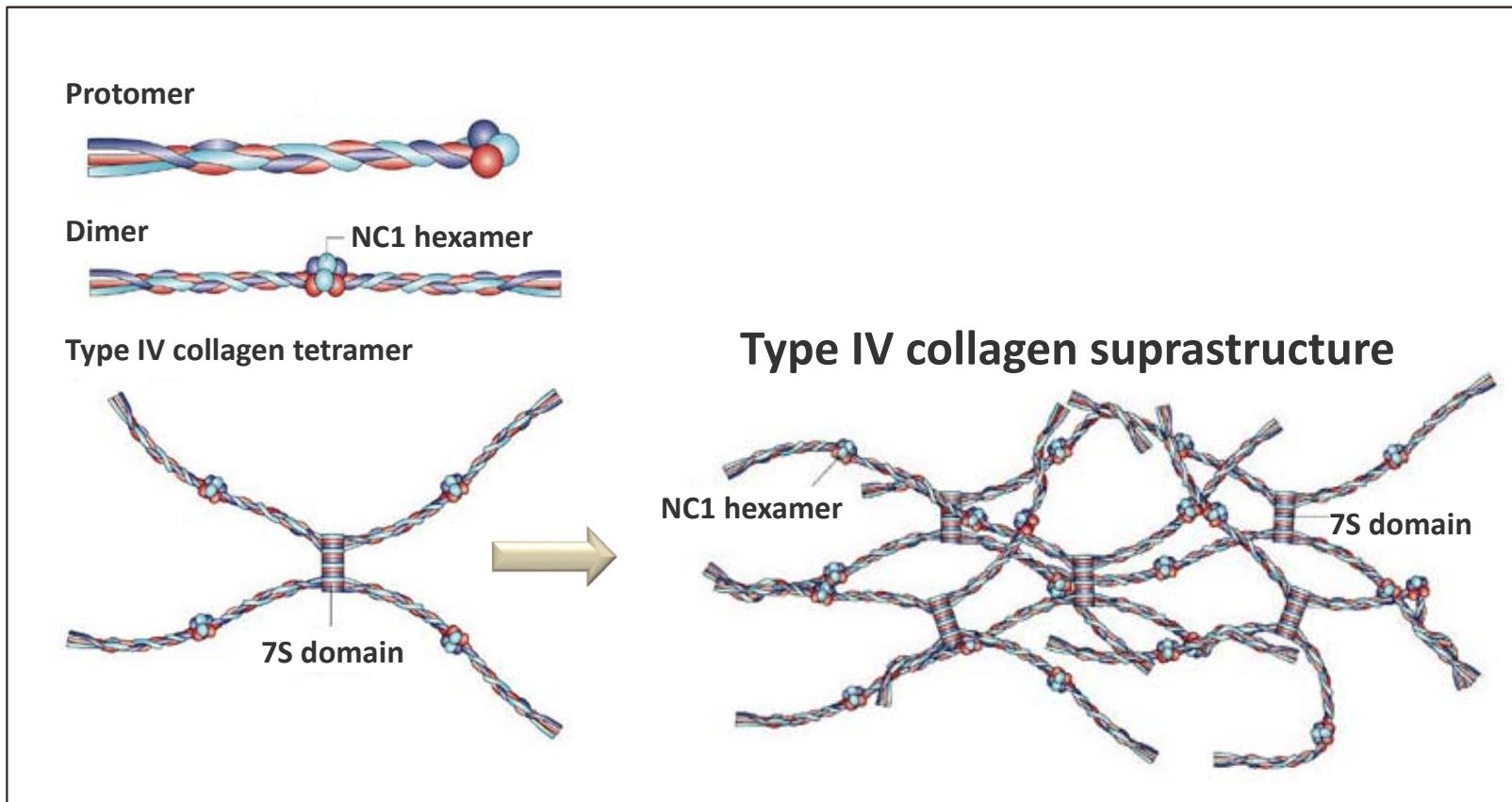
- Three separate chains twist together to form rope-like superhelix, with multiple bends.
- Interact with other basal lamina proteins via their terminal domains.



Composition of the basal lamina: type IV collagen

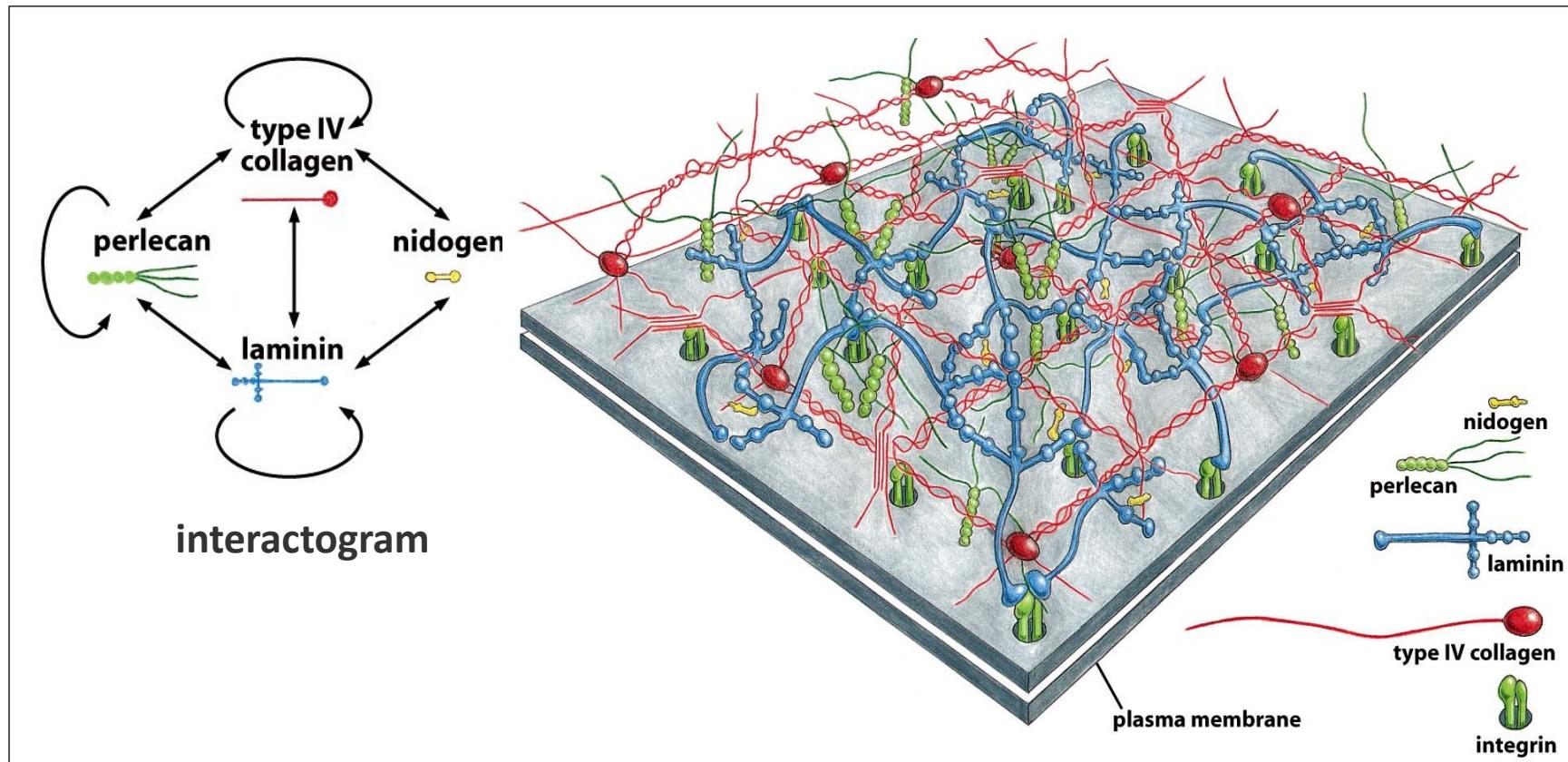
Second essential component in the basal lamina

- 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 the formation of the basal lamina

- Laminin and type IV collagen form a meshwork
- Nidogen and perlecan act 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.



III. The basal lamina

What we have learned last lecture:



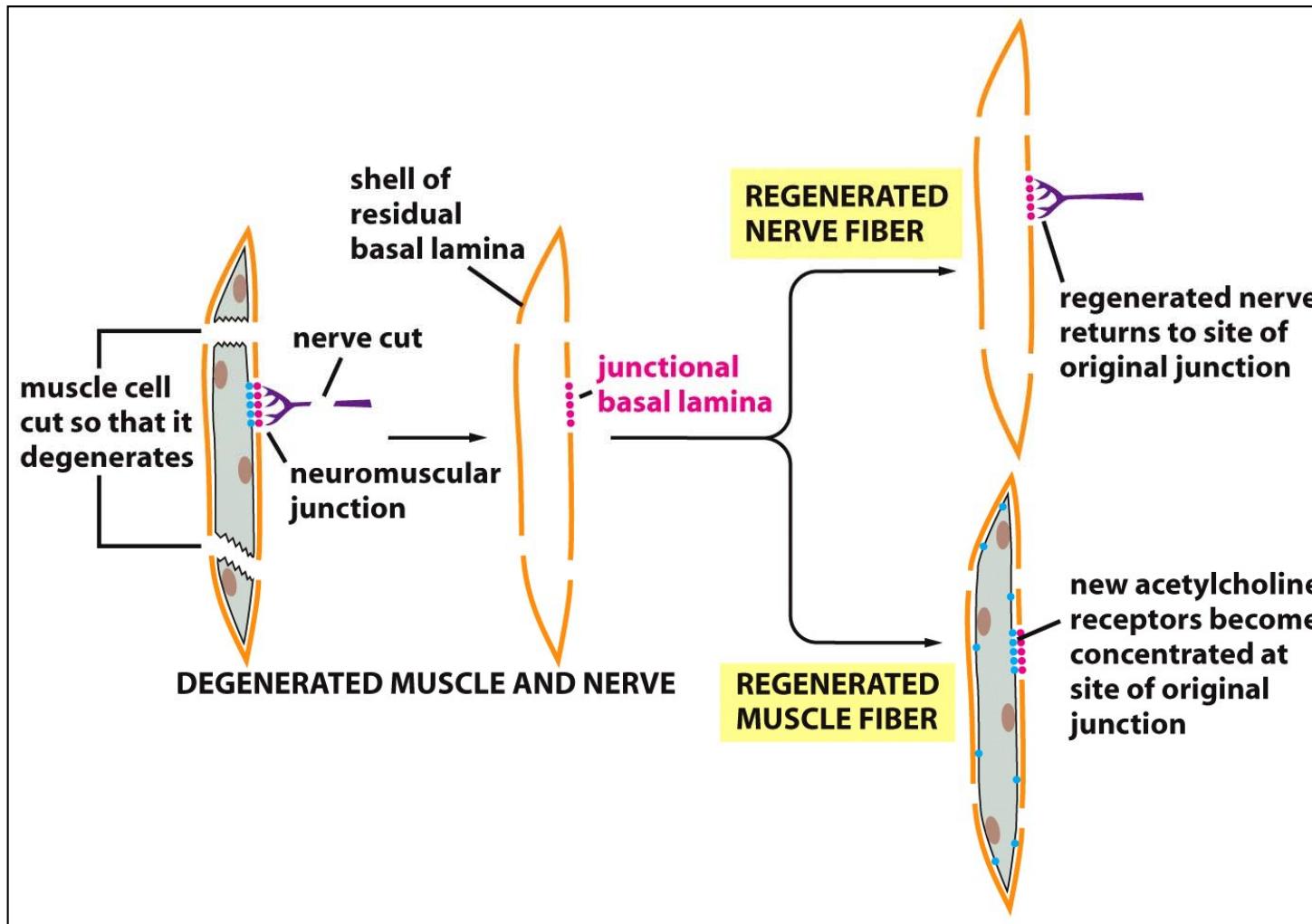
Basal lamina is a structure
of the ECM
(extracellular matrix)

Produced by both:
epithelia & the stroma,

40-120 nm thick

Plays important mechanical role
(strength of the epidermis)

The basal lamina guides tissue regeneration



III. The basal lamina

Summary



Provides mechanical support.

Upon cell damage, basal lamina often survives and guides tissue regeneration

Acts as barriers to keep cells in place

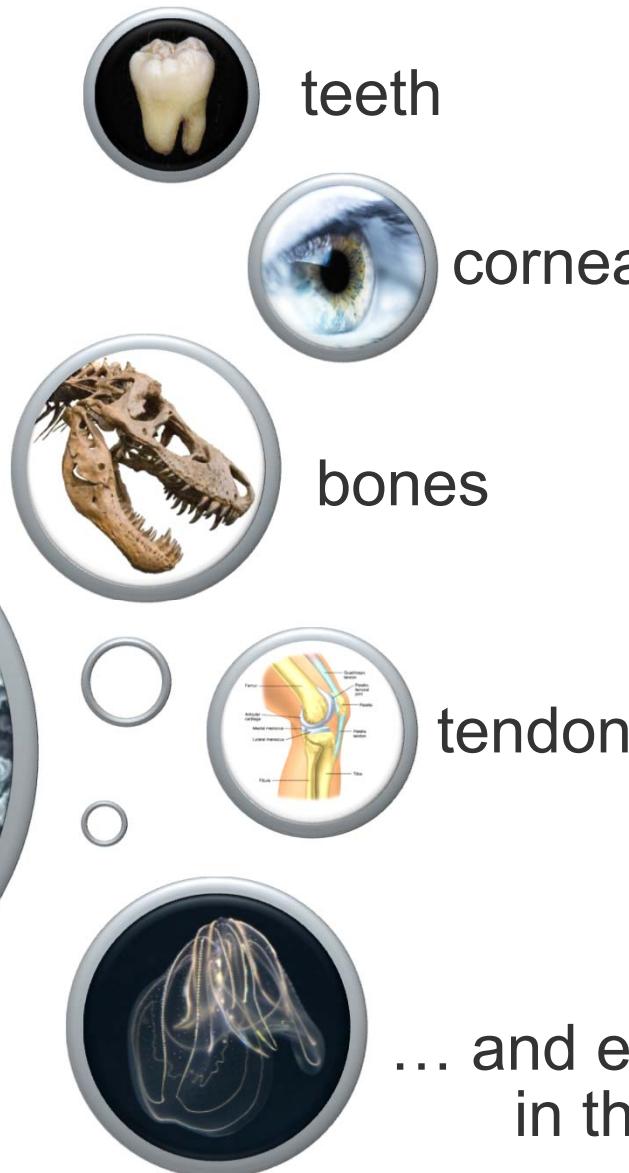
Serves as filters in kidney.

Influence cell polarity, differentiation and migration

Serve as “highways” for cell migration

IV. The extracellular matrix, ECM

The ECM is most diverse:
in structure & in function



teeth

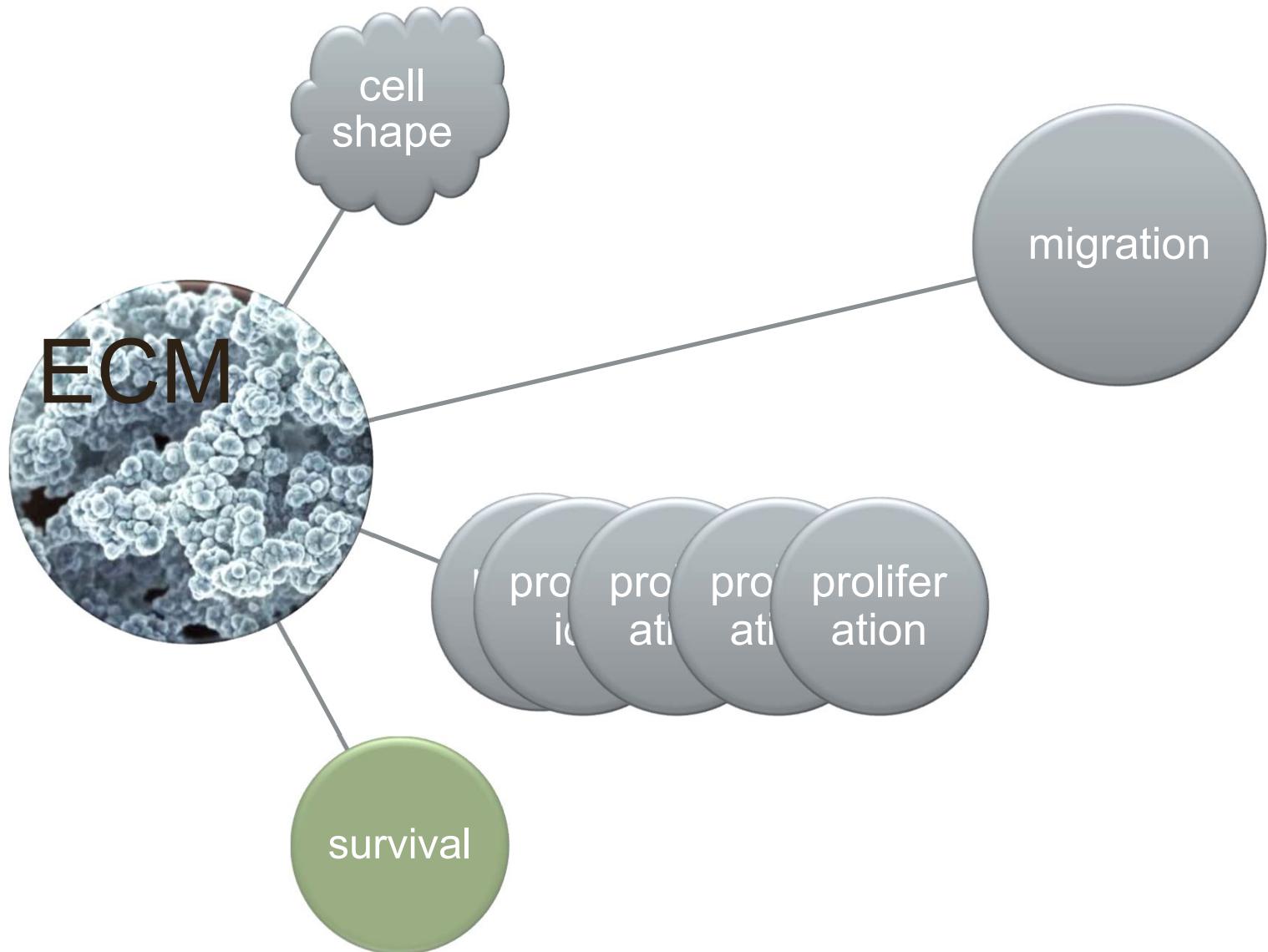
cornea

bones

tendon

... and even the jelly
in the jellyfish

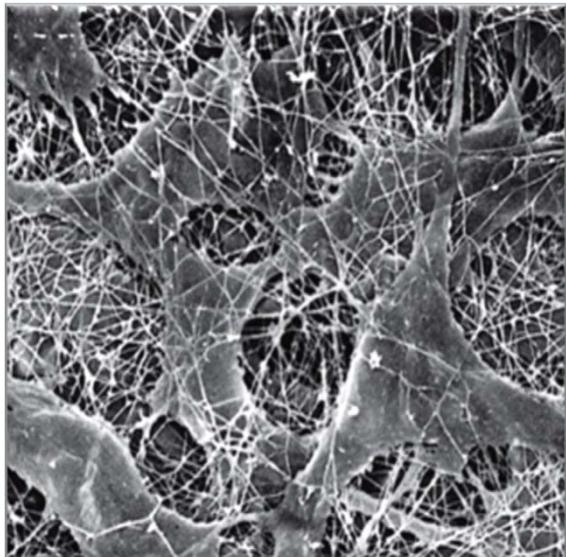
The ECM regulates and influences development & behavior



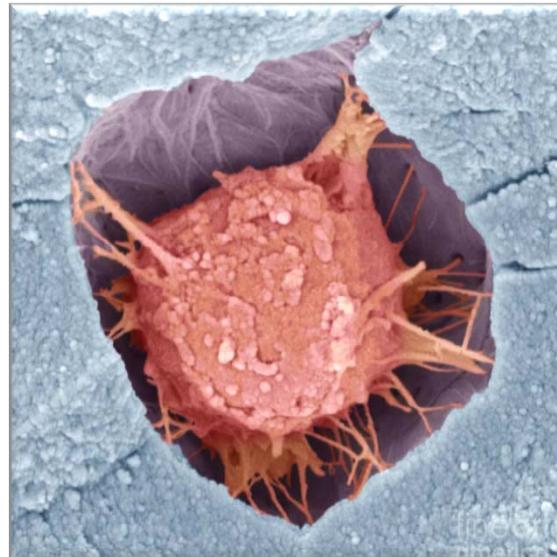
Different cells produce different ECMs

- The ECM is material that is **secreted by cells**
- Most of the ECM is connective tissue, produced by fibroblasts

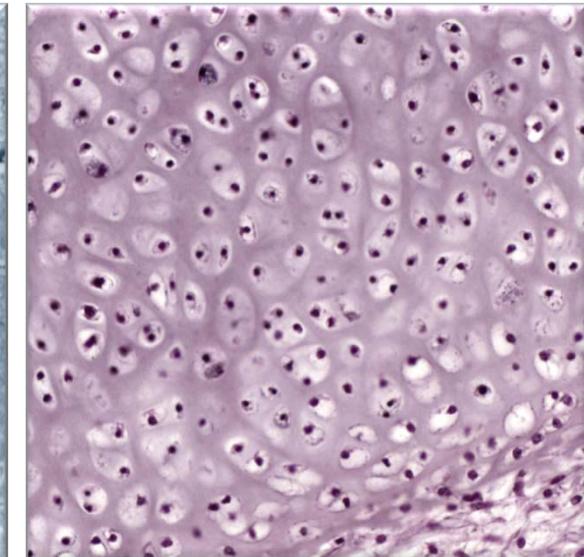
Fibroblasts
(connective tissue)



Osteoblast
(bone tissue)



Chondroblasts
(cartilage tissue)



The ECM consists of proteoglycans & fibrous proteins

ECM: substances & organization:

Proteoglycans

- proteins with polysaccharides (**GAGs**)

Fibrous proteins

- proteins with short oligosaccharide side chains

The **proteoglycans** form a hydrated **gel-like matrix**, in which the **fibrous proteins** are **embedded**

ECM consistency is the result of the composition

Biochemistry ahead ...

The Scream by Edvard Munch
everydayweirdness.com

The background image is a reproduction of Edvard Munch's painting "The Scream". It depicts a figure with a pale, distorted face screaming in anguish, set against a dark, swirling landscape with orange and yellow streaks suggesting a setting or rising sun.

Don't panic!

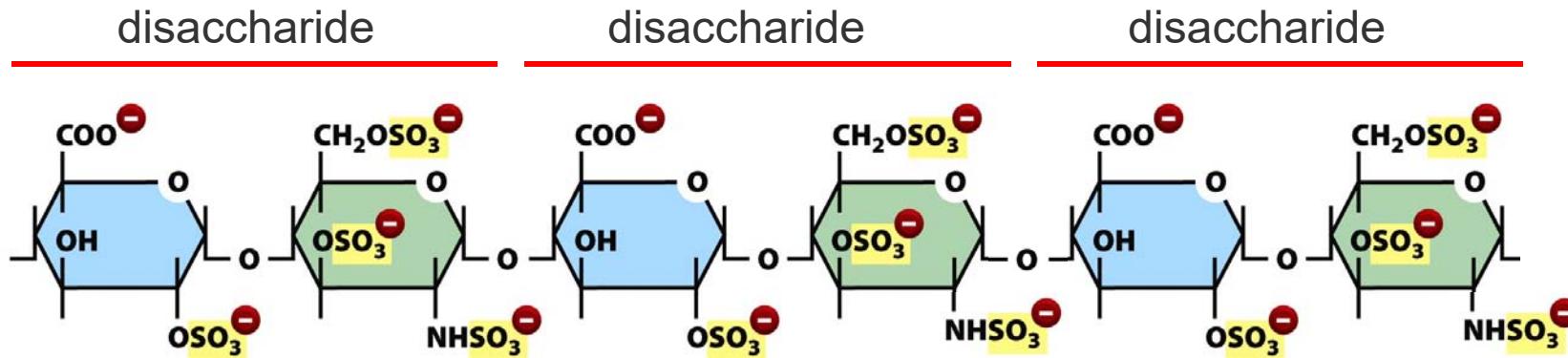
Rehearse

Biochemistry I - Carbohydrates: Sugars, Saccharides, Glycans

Glycosaminoglycans (GAGs)

What are GAGs?

- GAGs are **unbranched** chains of **repeating disaccharides** (up to 200) **Hyaluronan** as special case contains **up to 25,000** disaccharide repeats
- high density of **negative charges** along the chain due to carboxyl (COO^-) and sulfate groups (SO_3^-)

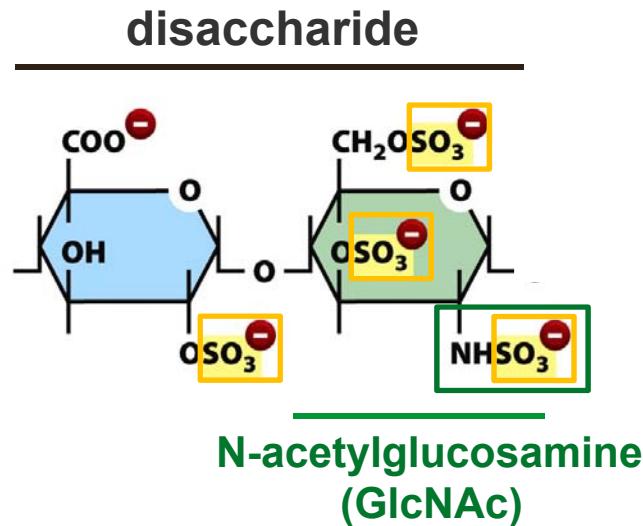


GAGs are the most anionic molecules that are produced by cells

Glycosaminoglycans (GAGs)

Why are they called glycosaminoglycans?

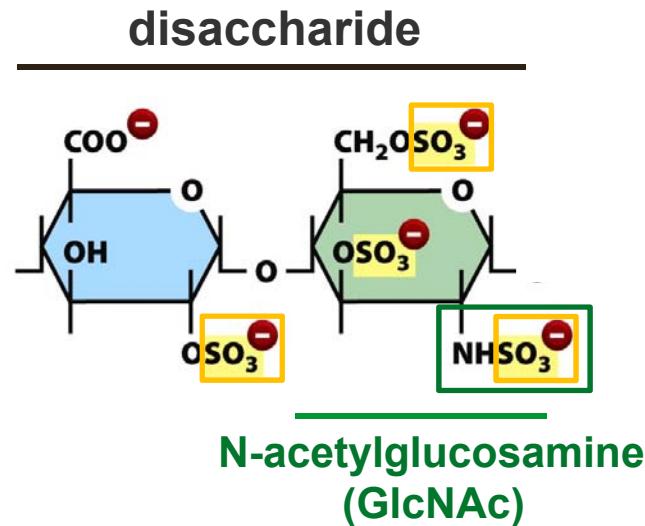
- One of the two sugars is always an **amino** sugar, frequently **sulfated**:
N-acetylglucosamine (GlcNAc) (based on **glucose**) or
N-acetylgalactosamine (GalNAc) (based on **galactose**)



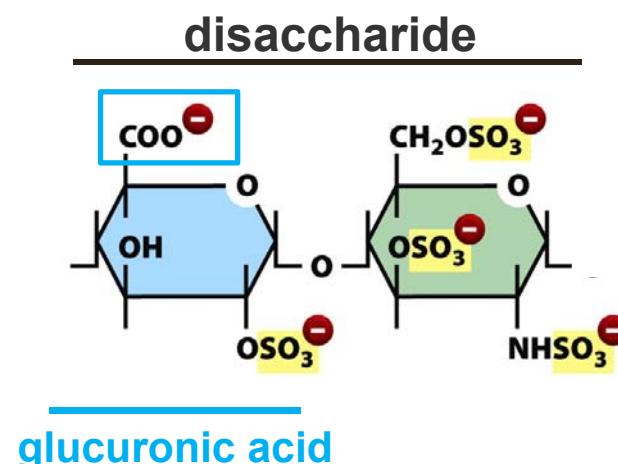
Glycosaminoglycans (GAGs)

Why are they called glycosaminoglycans?

- One of the two sugars is always an **amino** sugar, frequently **sulfated**:
N-acetylglucosamine (GlcNAc) (based on **glucose**) or
N-acetylgalactosamine (GalNAc) (based on **galactose**)



- The second sugar is a **uronic acid** (oxidation \rightarrow carboxyl group, COO^-). Here, **glucuronic acid** (based on glucose), also **iduronic acid** (based on **idose**)

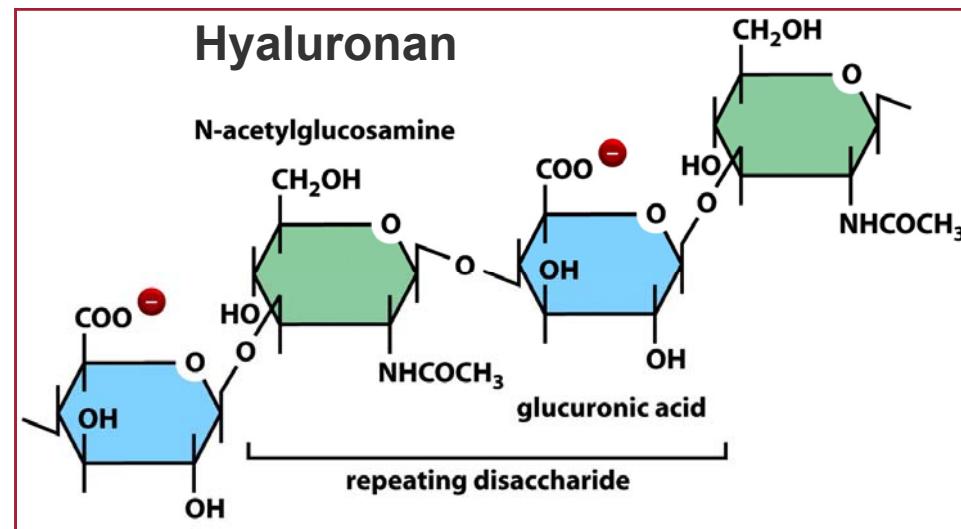


Glycosaminoglycans (GAGs)

Four different groups of GAGs are classified according to:

- the type of sugar
- type of linkage between the sugars
- number and location of sulfate groups

1. Hyaluronan
2. Chondroitin sulfate
3. Heparin sulfate
4. Keratan sulfate

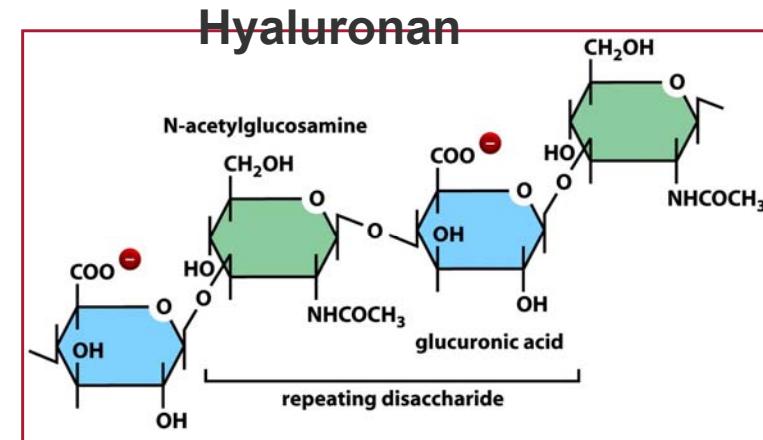


Glycosaminoglycans (GAGs)

Hyaluronan:

...actually, it is not a typical GAG:

- doesn't contain sulfated sugars
- only GlcNAc & glucuronic acid dimers
- not linked to proteins at all
- not secreted by cells, it
- is synthesized by enzymes **on the cell surface**, instead



Major functions:

- **guides cell migration** during tissue morphogenesis and repair
- it occupies a large volume compared to its mass:
it **provides** the space and it **fills** the **space** up
- it is degraded by **hyaluronidase**

Proteoglycans: almost limitless heterogeneity

Core proteins:

- diverse group of core proteins
- shared feature: the “link” domain

GAG side chains:

- diverse in composition and combination of sugars
- variable in modifications (sulfatation)
- a single core protein can carry a
highly variable number of different types of GAG side chains
 - sometimes more than 100 GAGs on a core protein (e.g. aggrecan)
 - sometimes only 1-10 (e.g. decorin)

Assembly of polymeric complexes:

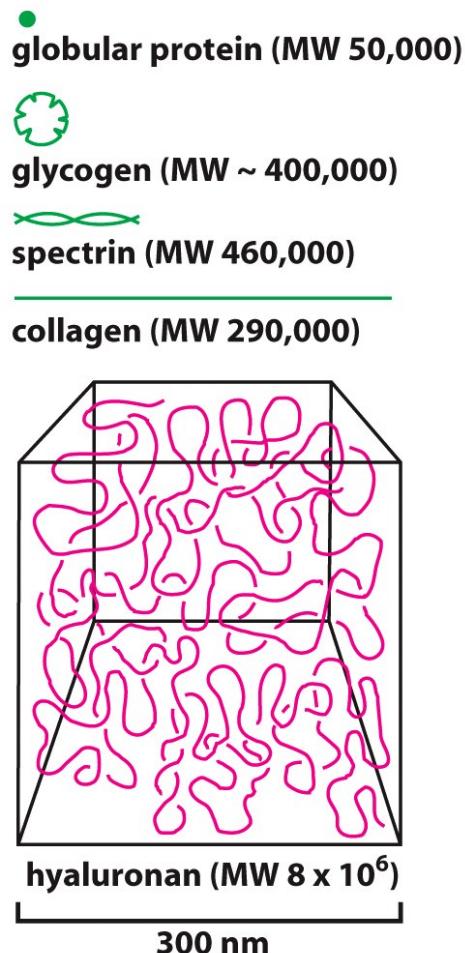
- GAGs & proteoglycans can associate to form **Polymeric complexes**:
 - aggrecan and hyaluronan form aggregates in cartilage matrix
the **size of a bacterium!**
 - GAGs & proteoglycans can associate with **fibrous proteins** like **collagen** and with **protein meshworks (basal lamina)** to extremely complex structures

General functions of proteoglycans

- Form gels (matrices) of varying pore size and charge density
- Serve as a selective sieves to regulate traffic of molecules and cells
- Regulate signaling through **binding** e.g. FGF (**fibroblast growth factor**), trTGF β (**transforming growth factor**) and chemical attractants (chemokines)
This allows control of growth and movement of cells
- Regulate activities of other proteins in the matrix e.g. proteolytic enzymes
- Some cell surface bound proteoglycan such as **syndecans** acts as co-receptors for growth factors to participate in cell signaling

GAGs create and occupy the space for and of ECM

GAGs adopt highly extended structure, allowing occupation of a large volume relative to their mass



Due to **strong negative charge**:

- GAGs attract Na^+ ions on their surface, **water is absorbed** due to **osmosis**, causing swelling & turgor
- GAGs swelling allows therefore withstanding of compressive forces.

That's the reason **why** they are so interesting for the **cosmetic industry**

Hyaluronic Acid...

Get the full effect of the needle-free plumping treatments with the **Hyaluronic Acid Bonus Pack**

that gives your skin a visibly more youthful appearance without invasive procedures.

Containing a unique blend of **six Hyaluronic Acids** that plump up the skin naturally, these **age-defying beauty treatments** target the areas that require a gentle boost of hydration for a smoother, firmer look.

295 US\$



Proteoglycans are not simple glycoproteins

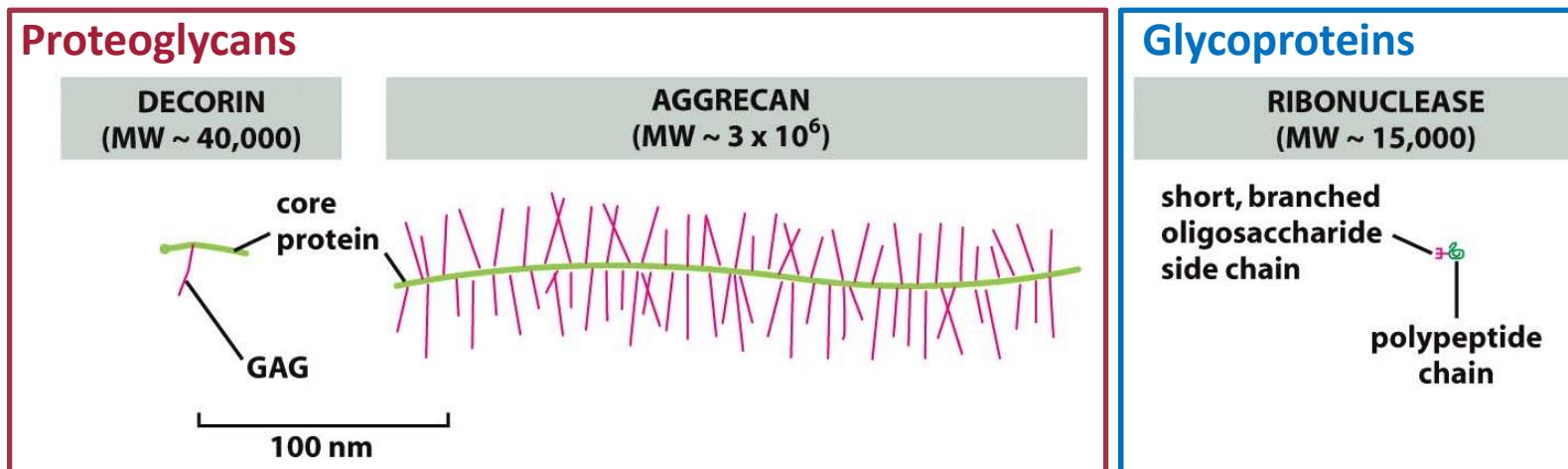
Comparison between proteoglycan and glycoproteins:

Glycoproteins:

- sugar content usually only (1-60%) but usually only few percent in many short & branched sugar chains
- usually low molecular weight proteins (only few hundred kDa)

Proteoglycans:

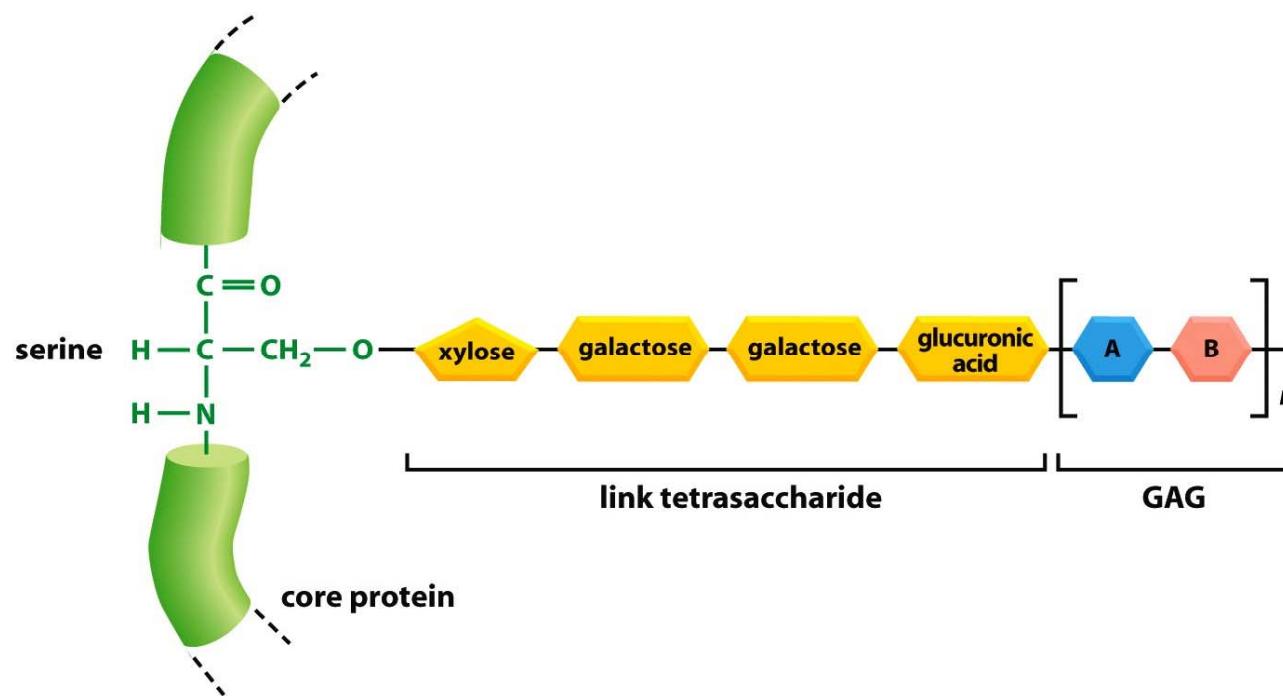
- **sugar content up to 95%**
- contain at least one GAG - a long (up to 25,000), unbranched sugar chains
- usually very high in molecular weight, up to 3000 kDa.



The linkage of GAGs to the core protein

The core protein is synthesized and folded in the ER,
linkage and modification occurs later in the Golgi apparatus:

1. Attachment of the “**link tetrasaccharide**” to a **serine side chain**
(O-linked glycosylation)
2. One by one sugar group addition by glycosyltransferases
(modifications occur later)



A list of most common proteoglycans

... for further reading

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

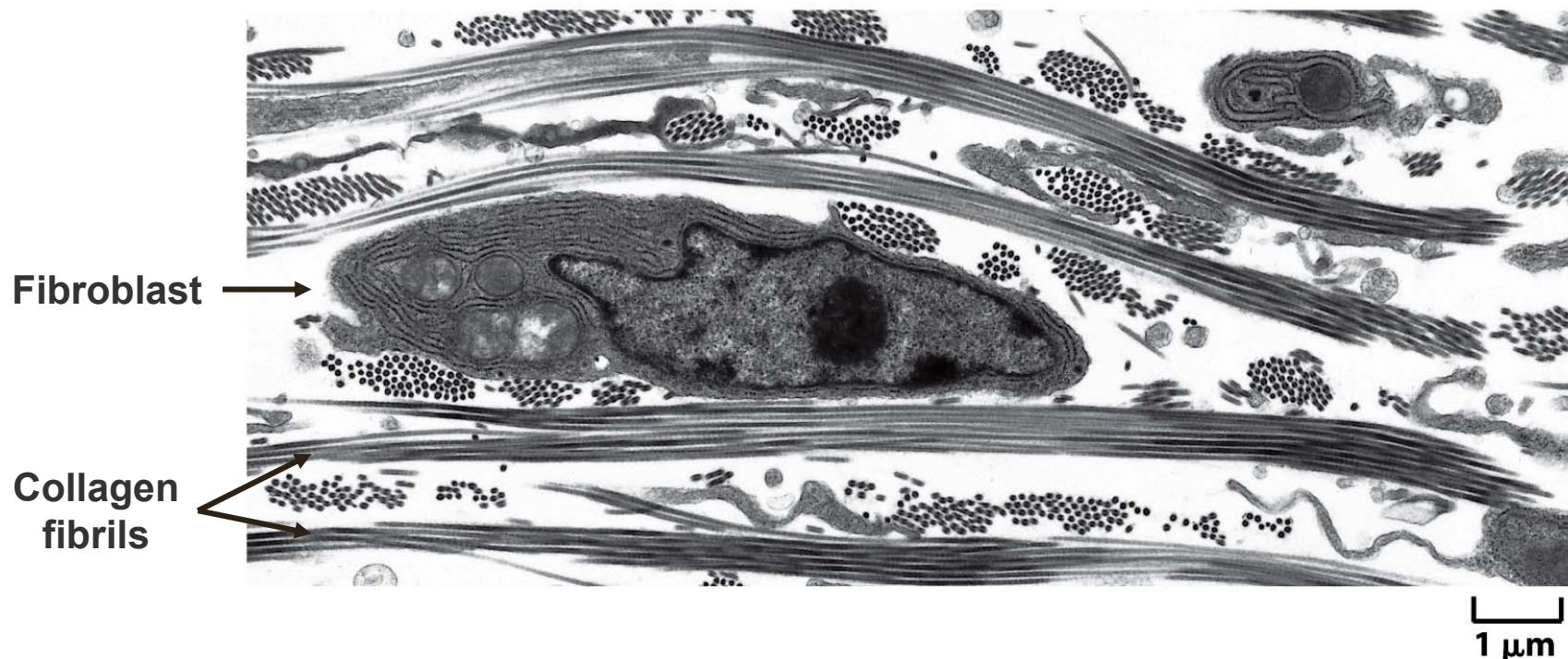
Fibrous proteins: Collagens

What are collagens:

- Major fibrous proteins of ECM in skin and bone
- The most abundant protein in mammals
~25% of total protein mass.
- 42 genes for distinct collagen α chains
- Multiple types of collagen and they have different properties
- Two main collagen classes:
 - Fibrillar collagens
(form fibrils)
 - Fibril-associated collagens
(associate with fibrils)

Fibrous proteins: Collagens

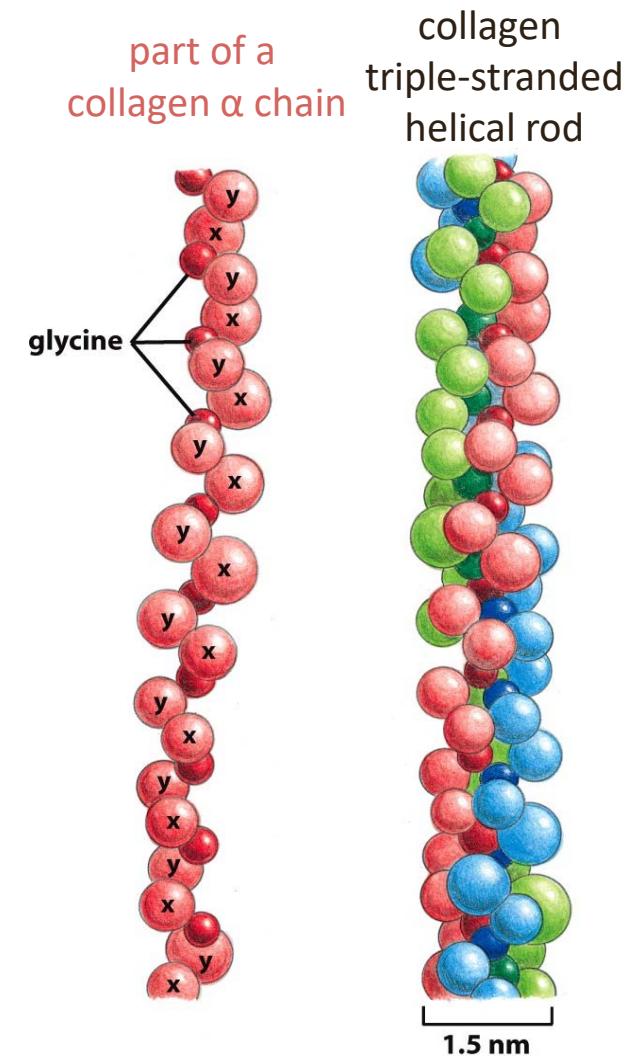
Connecting tissue:



Fibrous proteins: Collagens

What are collagens:

- Typical collagen molecule is long (~1,000aa), stiff and triple stranded helical structure
- 3aa per turn: **Gly-X-Y**
- Primary amino acid sequence is **rich in proline and glycine**.
- Large molecules:
The entire molecule is up to **300nm long**



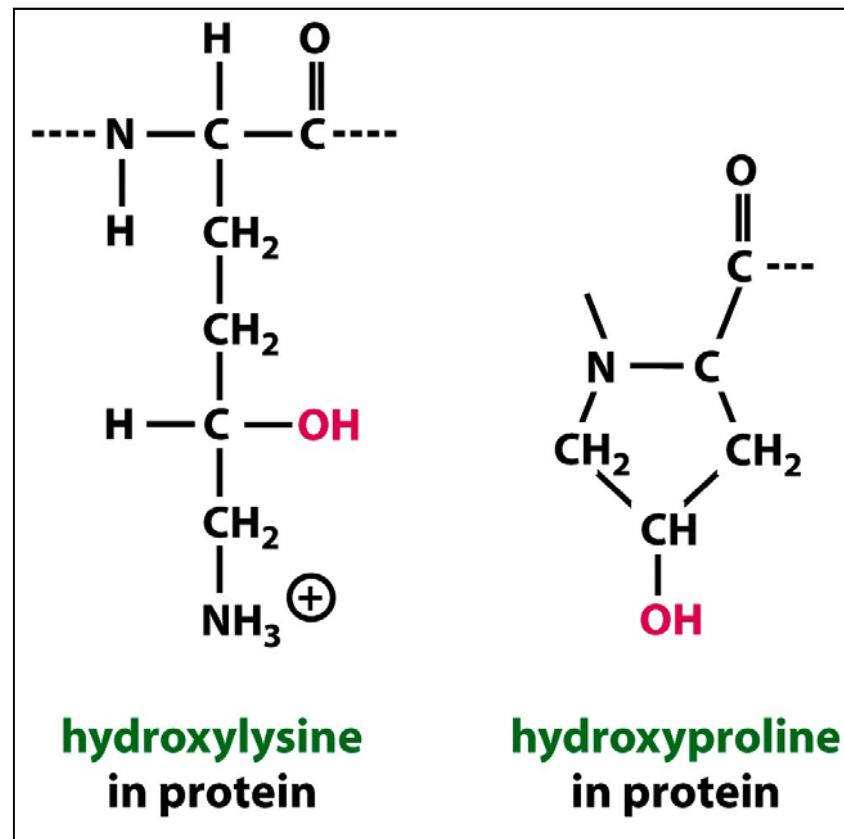
Some types of collagen and their properties

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 (<i>osteogenesis imperfecta</i>)
	II	Fibril	Cartilage, intervertebral disc, notochord, vitreous humor of the eye	Cartilage deficiency, dwarfism (<i>chondrodysplasia</i>)
	III	Fibril	Skin, blood vessels, internal organs	Fragile skin, loose joints, blood vessels prone to rupture (<i>Ehlers–Danlos syndrome</i>)
	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.				

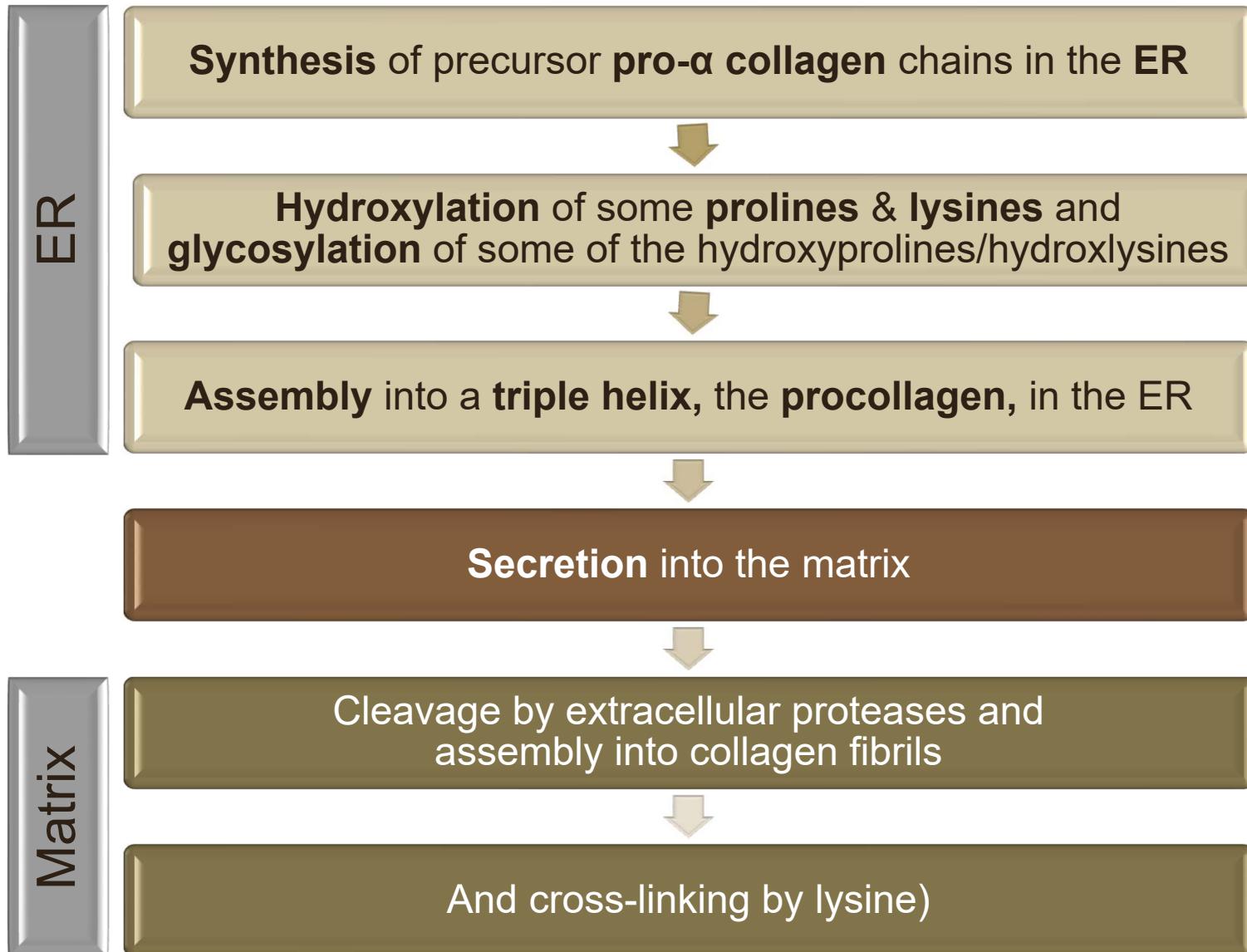
Hydroxylation allows formation of interchain hydrogen bonds

Hydroxyl groups help forming hydrogen bonds that stabilize the triple-stranded helices.

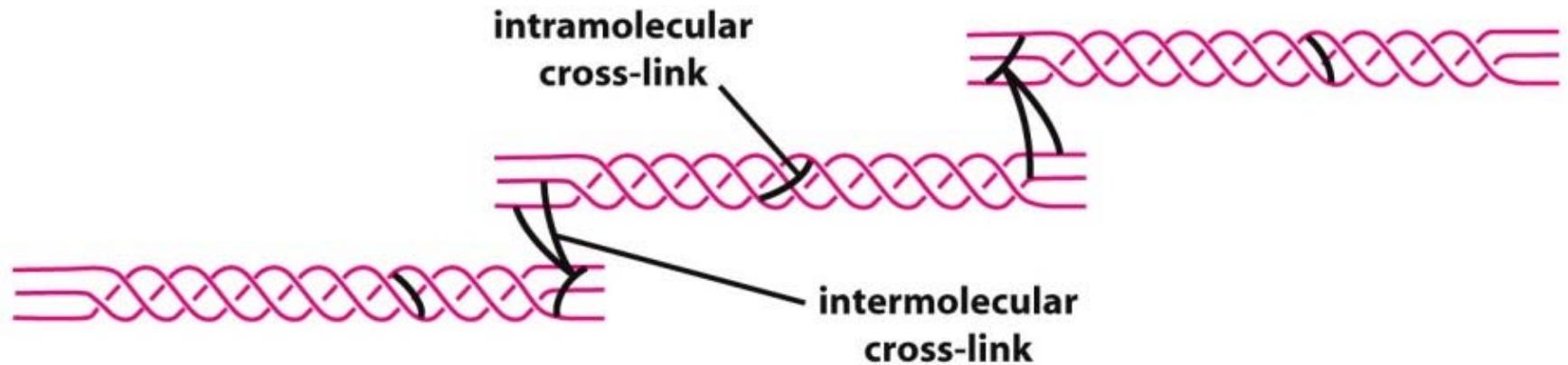


- Deficiency in Vitamin C (ascorbic acid) causes defects in proline hydroxylation.
- This results in failure to assemble stable triple helices
- This causes blood vessels to be fragile, teeth to be loosen & wounds cease to heal (\rightarrow scurvy)

Biogenesis of collagen fibers

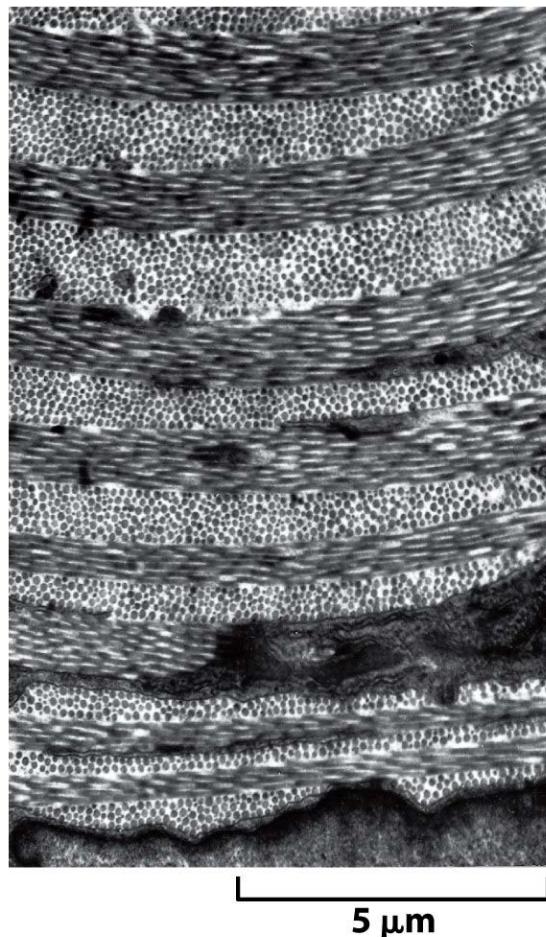


Biogenesis of collagen fibers



Fibril-associated collagens help to organize the fibrils

Collagen fibrils are arranged in orderly layers



Tadpole skin collagen fibrils

Fibril-associated collagens vs. fibrillar collagens

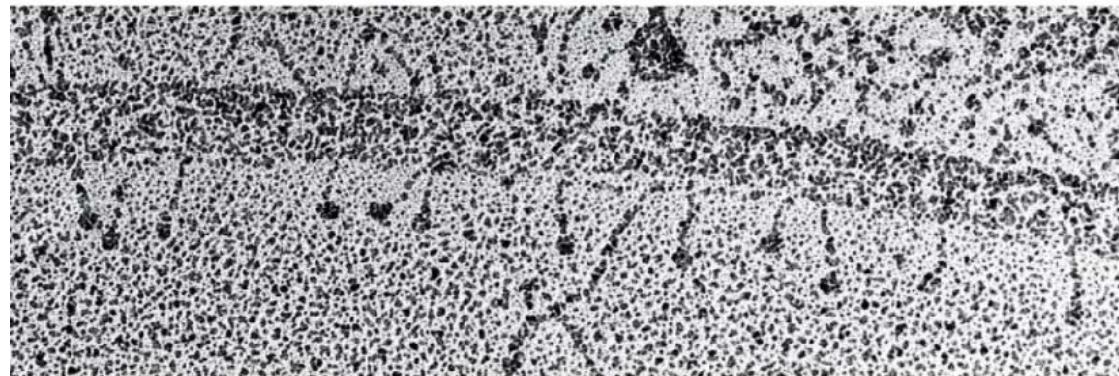
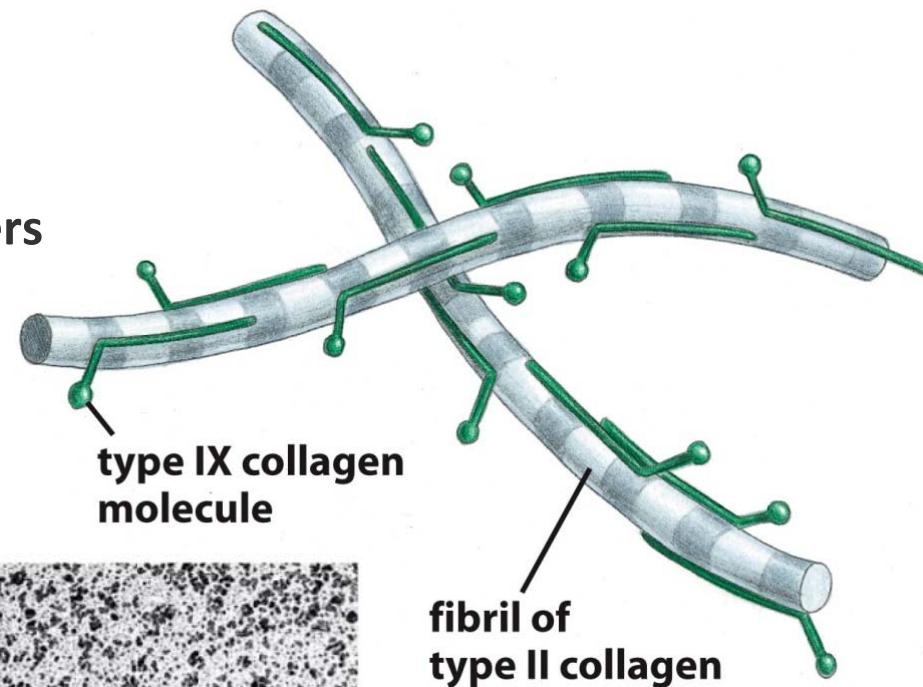
Fibril-associated type IX and XII collagens differ from fibrillar collagens:

- 1) Their triple stranded helix is interrupted by short non-helical domains, this results in a more flexible structure
- 2) No proteolytic cleavage/processing after secretion
- 3) No aggregation & formation of fibrils, but they bind to fibrillar collagens:
Type IX: binds to type II collagen-containing fibrils
(in cartilage, cornea & vitreous of the eye)
Type XII: binds to type I collagen-containing fibrils
(in tendon and other tissues)
- 4) They mediate the interaction between the fibrils of fibrillar collagens
➤ They help to determine the organization of the fibrils in the matrix!

Fibril-associated collagens: Type IX collagen

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

- Type XII collagen binds to type I-collagen-containing fibers e.g. in tendon



binding of type XII collagens to a fibril

100 nm



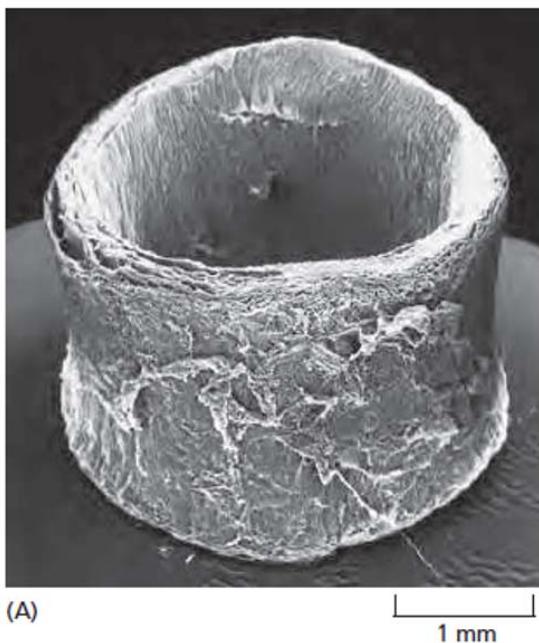
1 single type XII collagen

Elastin gives elasticity to blood vessels and lungs

Elastins are the primary constituent of the ECM in arteries

- Elastic fibers are >5X more elastic than rubber bands with the same cross-section area
- Elastic fibers mainly consist of **elastin**, but also contain some microfibrils which are composed of glycoproteins, including fibrillin.

aorta



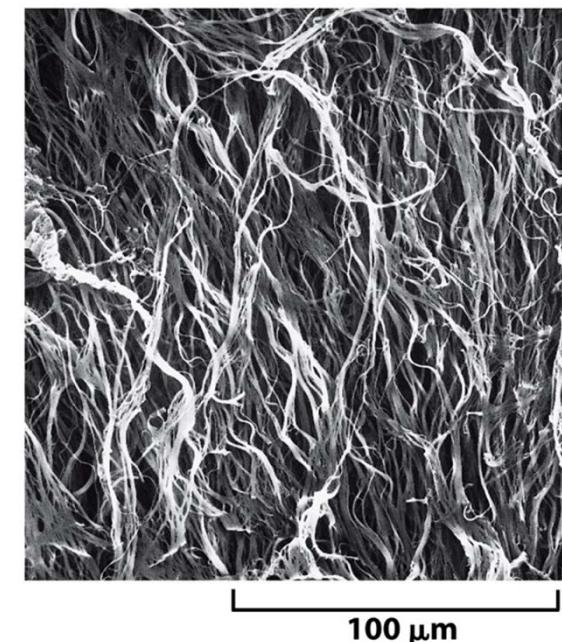
longitudinally oriented fibers



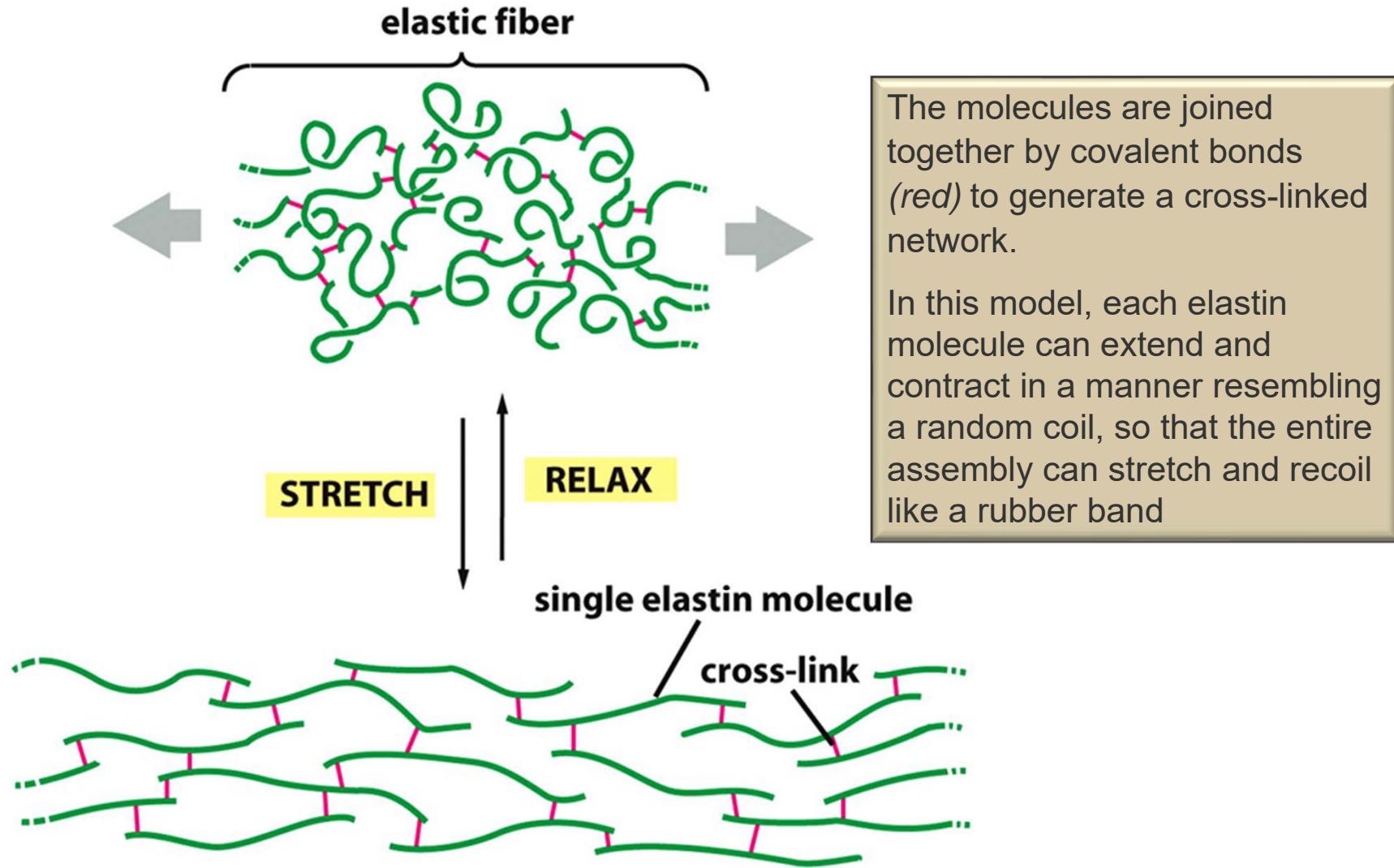
Elastin gives elasticity to blood vessels and lungs

Composition and properties

- Elastin is **proline and glycine rich protein (750aa)** and it contains **hydroxyproline** but **doesn't contain hydroxylsine** and it is **not glycosylated**
- Elastin is **synthesized in the ER as the precursor termed “tropoelastin”**
- After secretion/exocytosis, tropoelastin is highly crosslinks into elastin fibers and sheets
- Elastin contains **alternating two domains**:
 - one domain is **highly hydrophobic** and **is rich in proline and glycine**
 - the other domain is rich in **alanine and lysine** which are important for crosslinking between elastins.



Stretching a network of elastin molecules



Genetic diseases from defects in elastin fiber

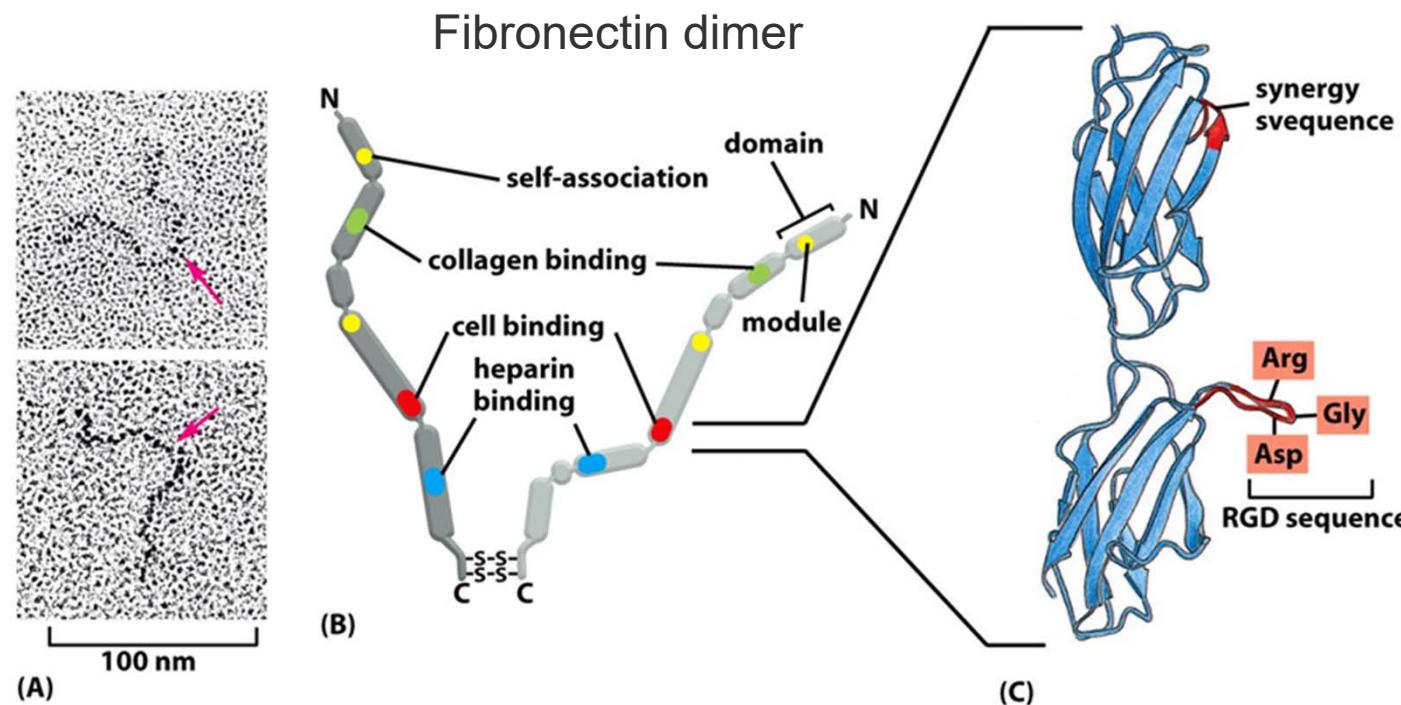
- Mutation in elastin:
 - thinning of arteries and excessive proliferation of smooth muscle cells lining the arteries.
- Marfan's syndrome:
 - mutation from fibrillin, easy rupturing aortas, displacement of the lens and abnormalities of skeleton and joints

Fibronectin

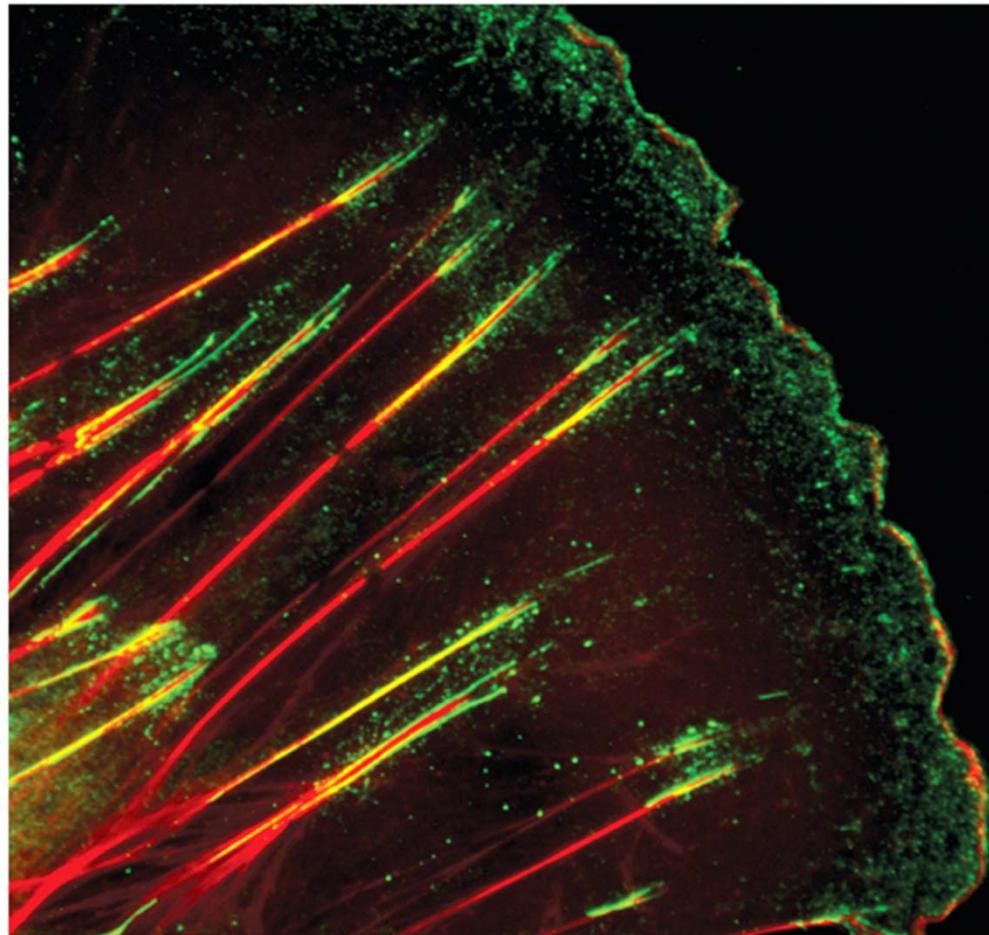
a large glycoprotein dimer joined by disulfide bond;

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 (Arg-Gly-Asp) peptide can competitively inhibit fibronectin binding to integrins
- One extracellular protein with RGD motif can lead to blood clotting
- Some snakes secret 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), Ca^{2+} or Zn^{2+} dependent)**
2. **Serine protease**

Three ways to activate & regulate these proteases:

1. **Local activation:**

The protease is transported as an **inactive precursor**.

It's activation occurs upon transport at it's destination.

Example:

The **protease plasmin**, which helps to break down blood clots in the capillaries, is **secreted as an inactive precursor, called plasminogen**.

Activation occurs by the plasmin-activating proteases in the blood vessel

Degradation of extracellular matrix

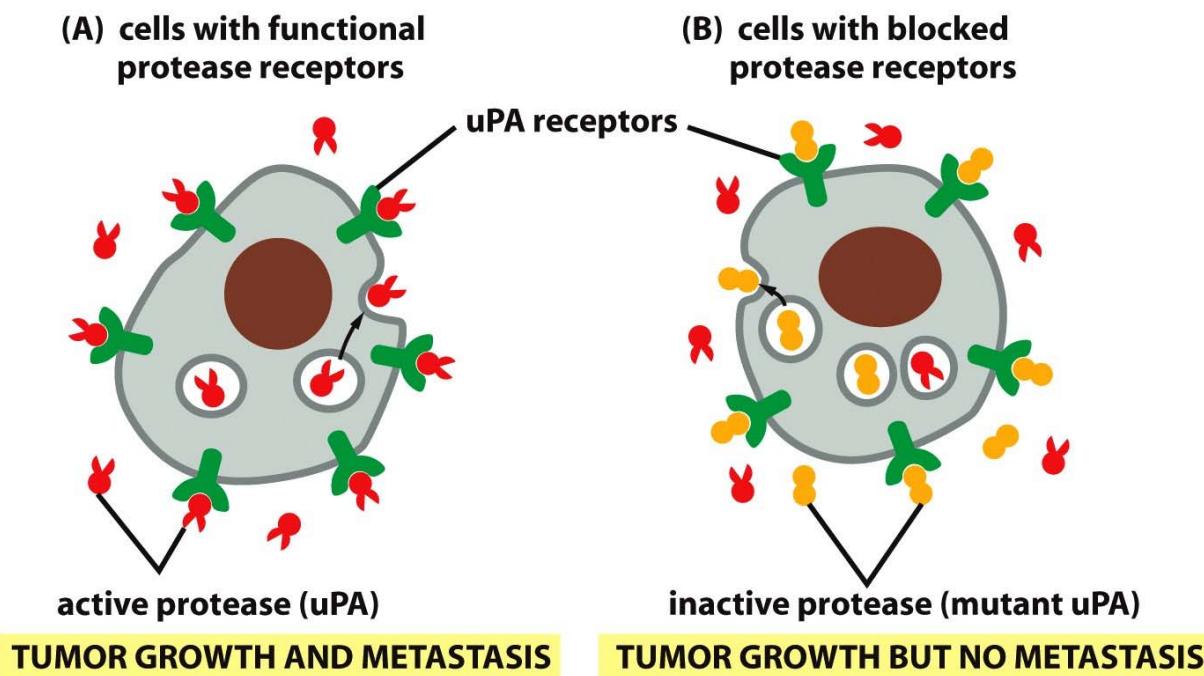
Three ways to activate & regulate these proteases

2. Confined to membrane surface:

Proteases are bound at the cell surface and are only active in the vicinity of the cell, to clear a pathway during cell migration

(Membrane bound matrix metalloproteases (MMPs))

Urokinase-type plasminogen activator)



Degradation of extracellular matrix

Three ways to activate & regulate these proteases

3. Secretion of inhibitors

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