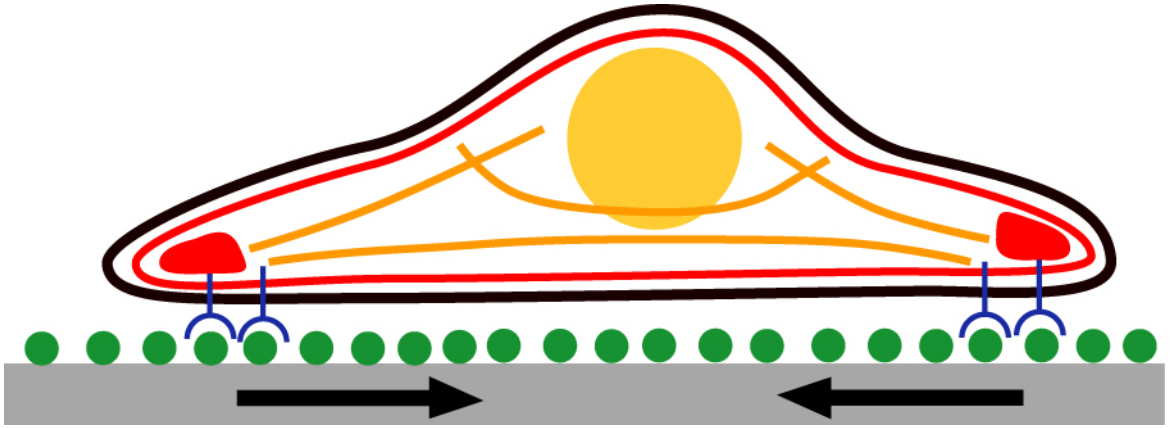
Summer term 2016
Nanobiophysics Module

Introduction to Molecular and Cellular Biology

LECTURES 25-26:

Cell junctions and adhesion



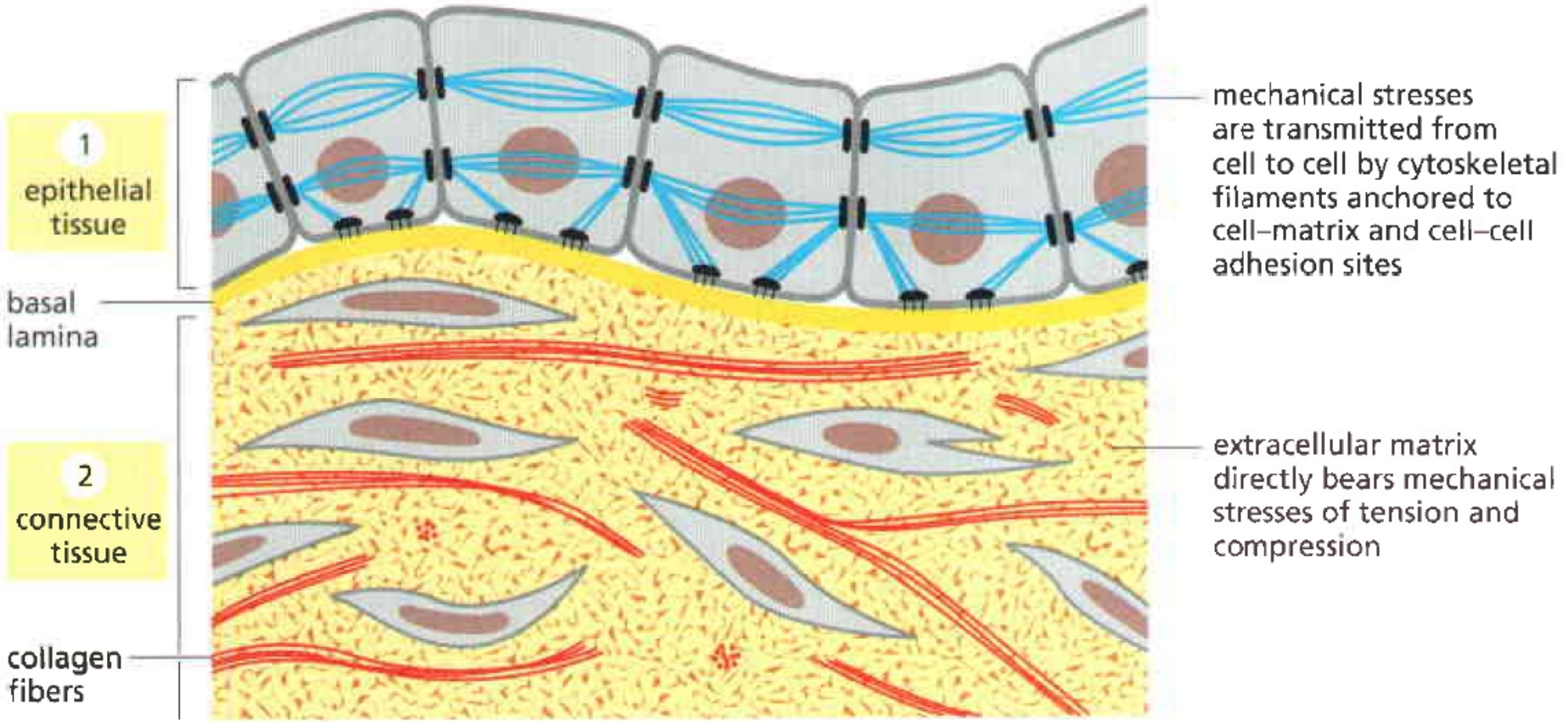
LECTURES 25-26: CELL JUNCTIONS AND ADHESION

- Cadherins in cell adhesion
  - adherens junctions
  - desmosomes
- Tight junctions
- Gap junctions and plasmodesmata
- Basal lamina
- Integrins in cell adhesion
- Extracellular matrix
- Plant cell wall

INTRODUCTION

Cells should be hold together in order to communicate

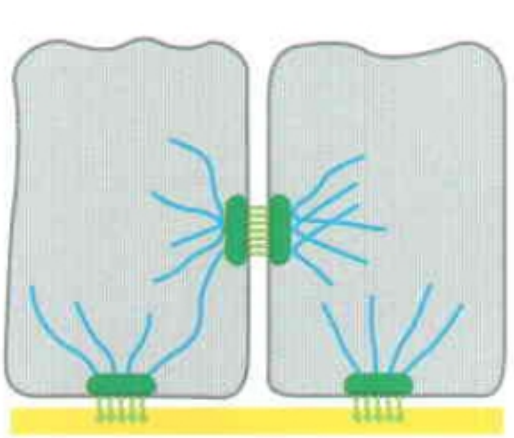
- **Cell-cell adhesion (epithelial tissue)**
- **Extracellular matrix (connective tissue)**



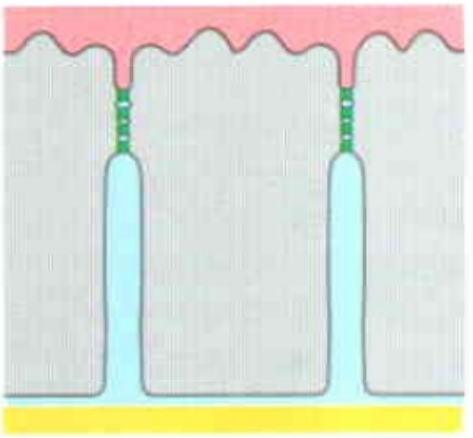
CELL JUNCTIONS

Multiprotein complexes providing contacts between the cells or cells and extracellular matix

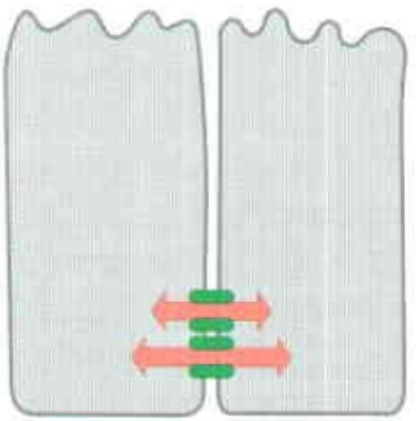
- **Types of junctions:**
  - **anchoring junctions (cell-cell, cell-matrix)**
  - **occluding junctions (gaps between epithelia)**
  - **channel-forming junctions**
  - **signal-relaying junctions**



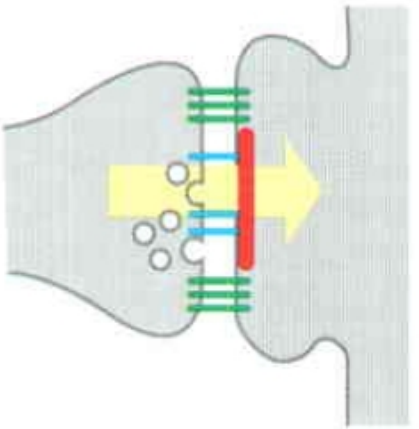
ANCHORING JUNCTIONS



OCCLUDING JUNCTIONS



CHANNEL-FORMING JUNCTIONS



SIGNAL-RELAYING JUNCTIONS

CELL JUNCTIONS

ANCHORING JUNCTIONS

Actin filament attachment sites

1. cell–cell junctions (adherens junctions)
2. cell–matrix junctions (actin-linked cell–matrix adhesions)

Intermediate filament attachment sites

1. cell–cell junctions (desmosomes)
2. cell–matrix junctions (hemidesmosomes)

OCCLUDING JUNCTIONS

1. tight junctions (in vertebrates)
2. septate junctions (in invertebrates)

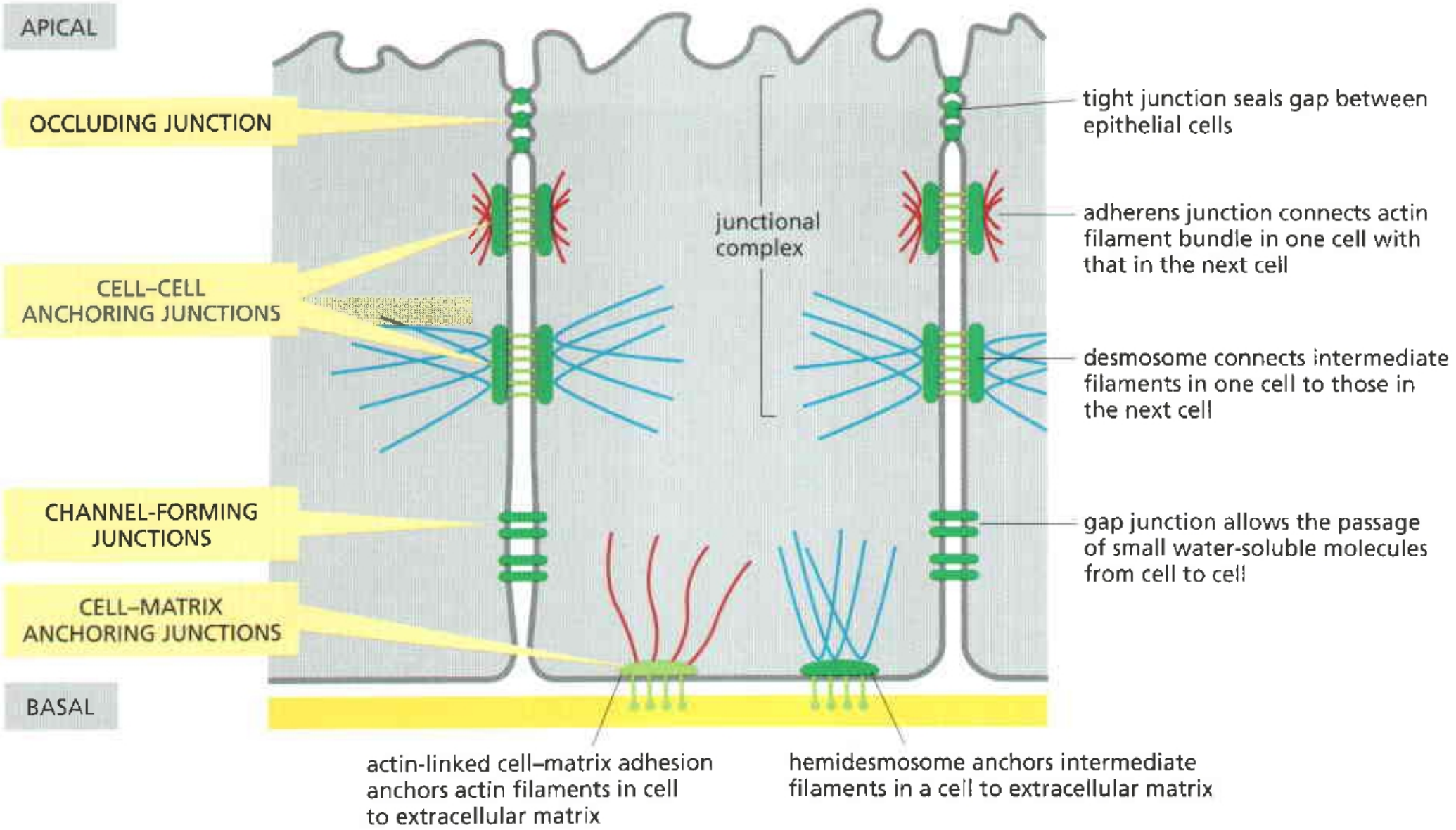
CHANNEL-FORMING JUNCTIONS

1. gap junctions (in animals)
2. plasmodesmata (in plants)

SIGNAL-RELAYING JUNCTIONS

1. chemical synapses (in the nervous system)
2. immunological synapses (in the immune system)
3. transmembrane ligand–receptor cell–cell signaling contacts (Delta-Notch, ephrin-Eph, etc.). Anchoring, occluding, and channel-forming junctions can all have signaling functions in addition to their structural roles

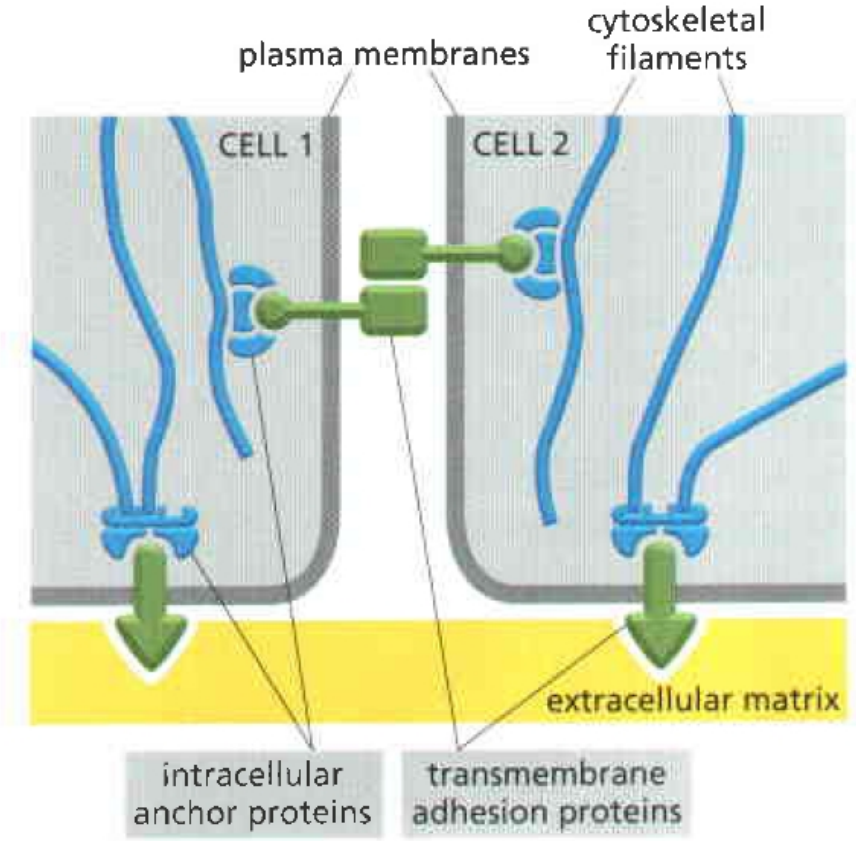
CELL JUNCTIONS IN SIMPLE COLUMNAR EPITHELIUM



TM ADHESION PROTEINS IN ANCHORING JUNCTIONS

Linking cytoskeleton and structures outside the cell

- **Cadherins: cell-cell**
- **Integrins: cell-matrix (with some exceptions)**



TM ADHESION PROTEINS IN ANCHORING JUNCTIONS

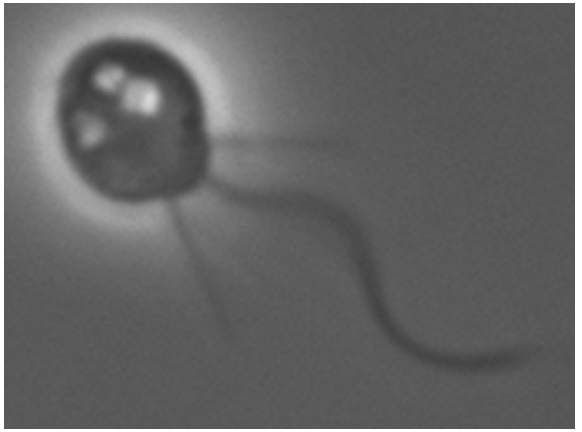
Linked to:

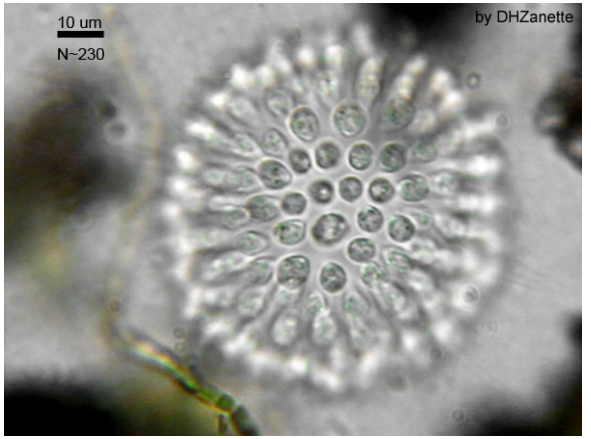
- **actin**
- **intermediate filaments (desmosomes/hemidesmosomes)**

JUNCTION	TRANSMEMBRANE ADHESION PROTEIN	EXTRACELLULAR LIGAND	INTRACELLULAR CYTOSKELETAL ATTACHMENT	INTRACELLULAR ANCHOR PROTEINS
Cell–Cell				
adherens junction	cadherin (classical cadherin)	cadherin in neighboring cell	actin filaments	α-catenin, β-catenin, plakoglobin (γ-catenin), p120-catenin, vinculin, α-actinin
desmosome	cadherin (desmoglein, desmocollin)	desmoglein and desmocollin in neighboring cell	intermediate filaments	plakoglobin (γ-catenin), plakophilin, desmoplakin
Cell–Matrix				
actin-linked cell–matrix adhesion	integrin	extracellular matrix proteins	actin filaments	talin, vinculin, α-actinin, filamin, paxillin, focal adhesion kinase (FAK)
hemidesmosome	integrin α6β4, type XVII collagen (BP180)	extracellular matrix proteins	intermediate filaments	plectin, dystonin (BP230)

CA^{2+}-DEPENDENT CADHERIN ADHESION

- **Choanoflagellate**



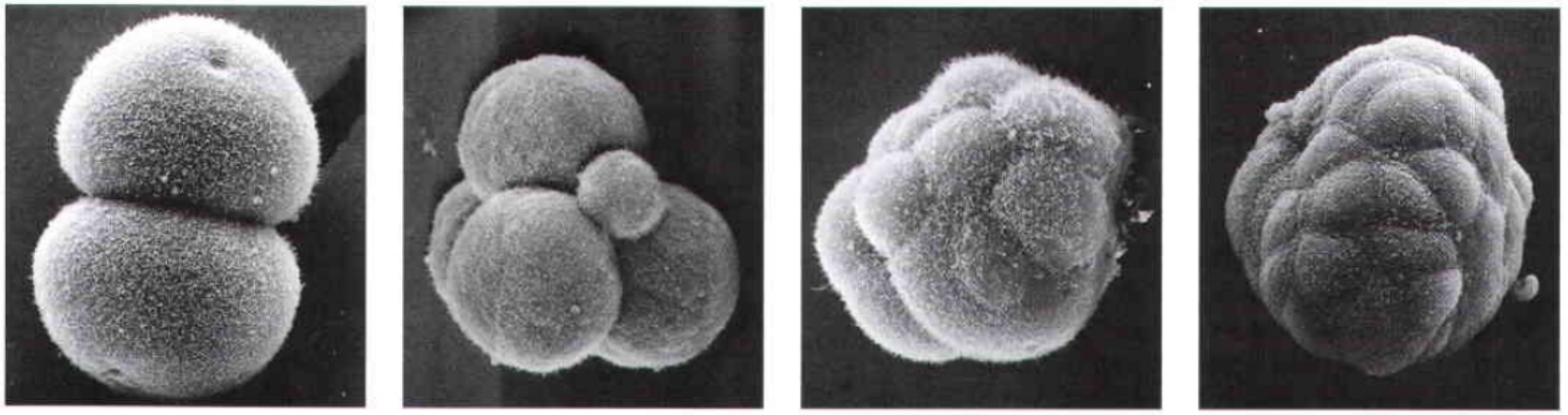


- **Removal of Ca^{2+}**
- **Trypsin in intercellular space**

→ **Junctions formation**

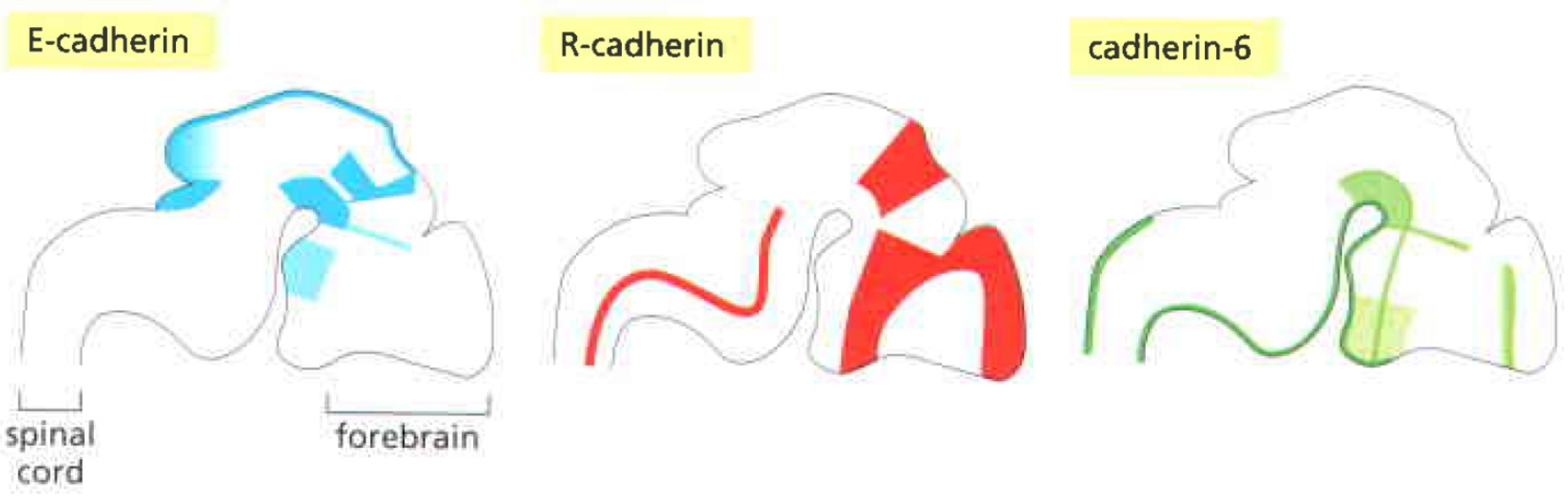
- **In embryonic development**

Mouse embryo



CADHERIN DIVERSITY

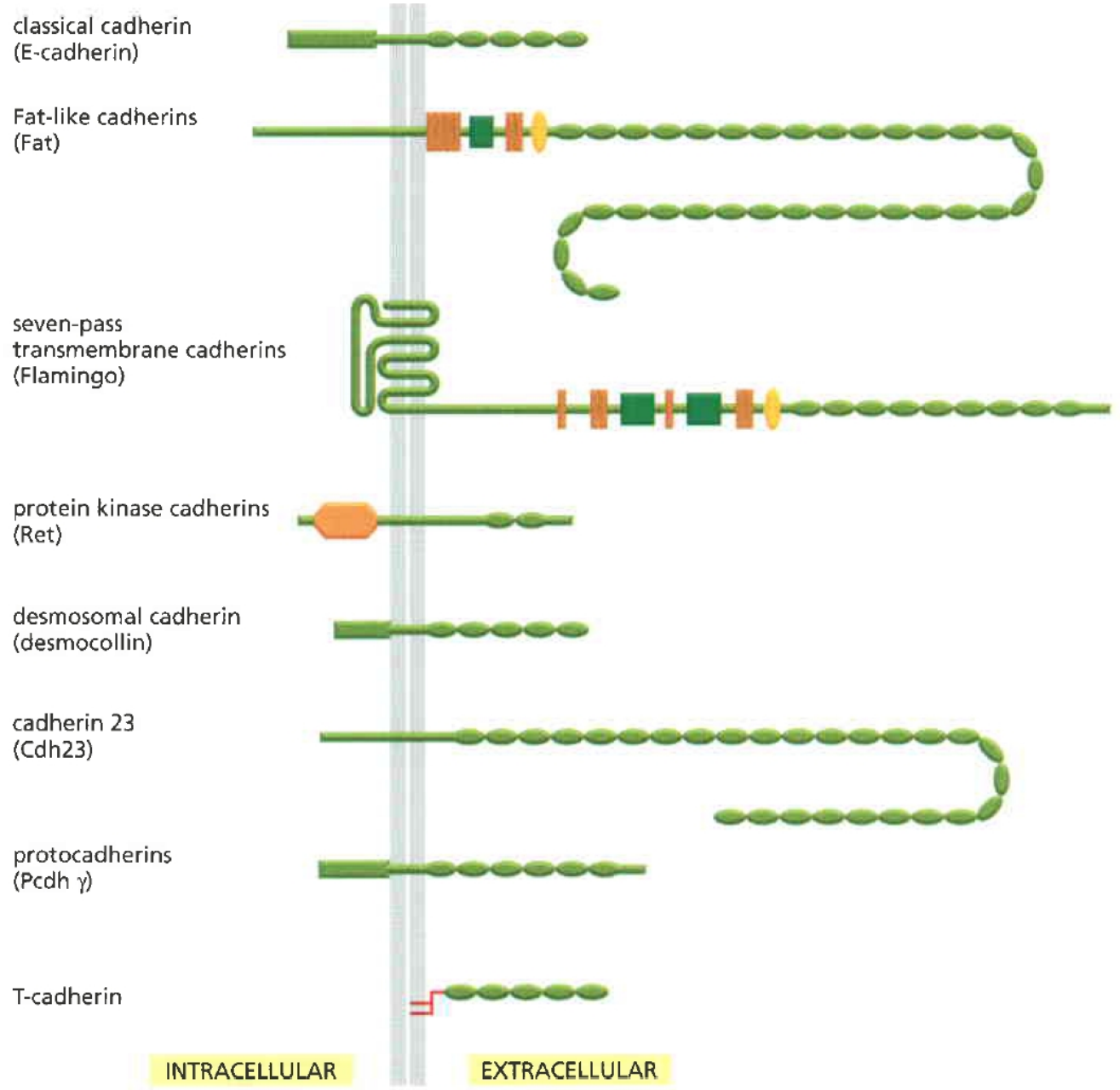
- Tissue-specific
- ~180 members in human:
  - classical (conserved extra- and intracellular domains)
  - non-classical (~50 only in brain)
- Membrane topology:
  - TM
  - GPI-anchored

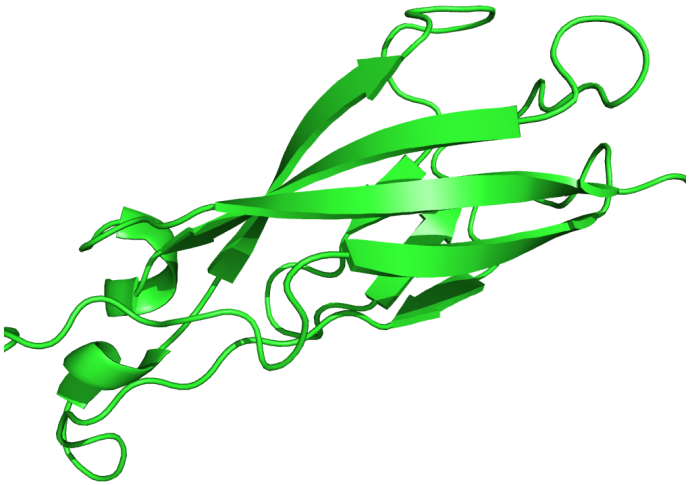


CADHERIN DIVERSITY

NAME	MAIN LOCATION	JUNCTION ASSOCIATION	PHENOTYPE WHEN INACTIVATED IN MICE
Classical cadherins			
E-cadherin	many epithelia	adherens junctions	death at blastocyst stage; embryos fail to undergo compaction
N-cadherin	neurons, heart, skeletal muscle, lens, and fibroblasts	adherens junctions and chemical synapses	embryos die from heart defects
P-cadherin	placenta, epidermis, breast epithelium	adherens junctions	abnormal mammary gland development
VE-cadherin	endothelial cells	adherens junctions	abnormal vascular development (apoptosis of endothelial cells)
Nonclassical cadherins			
Desmocollin	skin	desmosomes	blistering of skin
Desmoglein	skin	desmosomes	blistering skin disease due to loss of keratinocyte cell–cell adhesion
T-cadherin	neurons, muscle, heart	none	unknown
Cadherin 23	inner ear, other epithelia	links between stereocilia in sensory hair cells	deafness
Fat (in *Drosophila*)	epithelia and central nervous system	signal-relaying junction (planar cell polarity)	enlarged imaginal discs and tumors; disrupted planar cell polarity
Fat1 (in mammals)	various epithelia and central nervous system	slit diaphragm in kidney glomerulus and other cell junctions	loss of slit diaphragm; malformation of forebrain and eye
α, β, and γ-Protocadherins	neurons	chemical synapses and nonsynaptic membranes	neuronal degeneration
Flamingo	sensory and some other epithelia	cell–cell junctions	disrupted planar cell polarity; neural tube defects

CADHERIN SUPERFAMILY

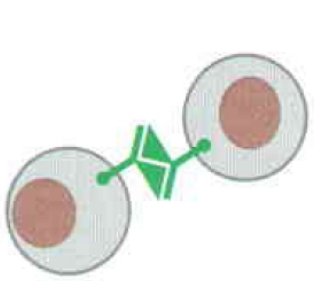




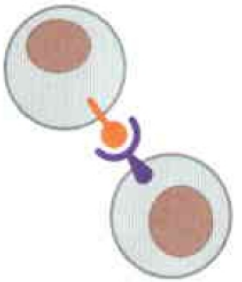
Cadherin repeating unit (E-cadherin)

CADHERIN MECHANISM OF HOMOPHILIC ADHESION

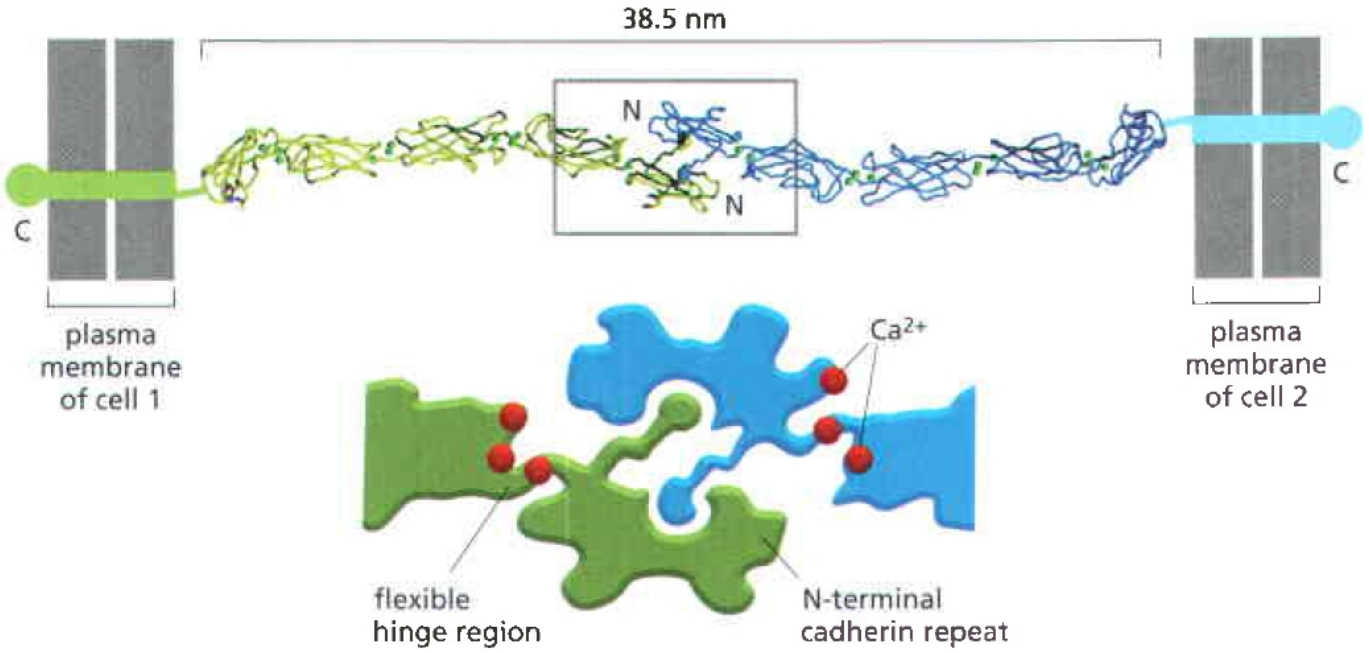
- **Homophilic adhesion:**
  - **same extracellular part**
  - **same intracellular part**
- **Cadherin domains are connected by a hinge**
- **Ca^{2+} in hinge => rigidity**
- **Rapid degradation without Ca^{2+}**

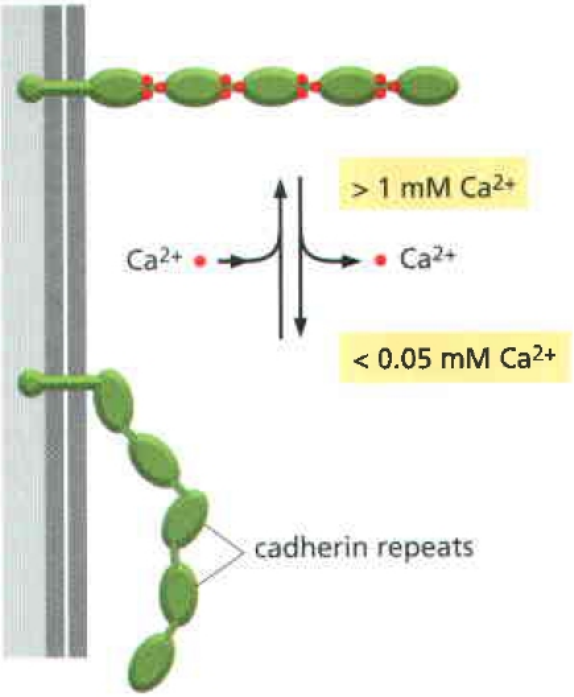


HOMOPHILIC BINDING



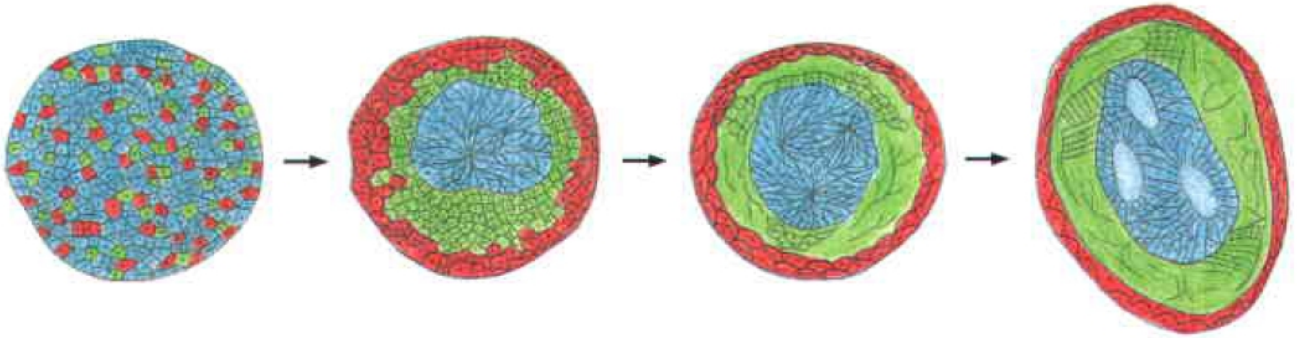
HETEROPHILIC BINDING



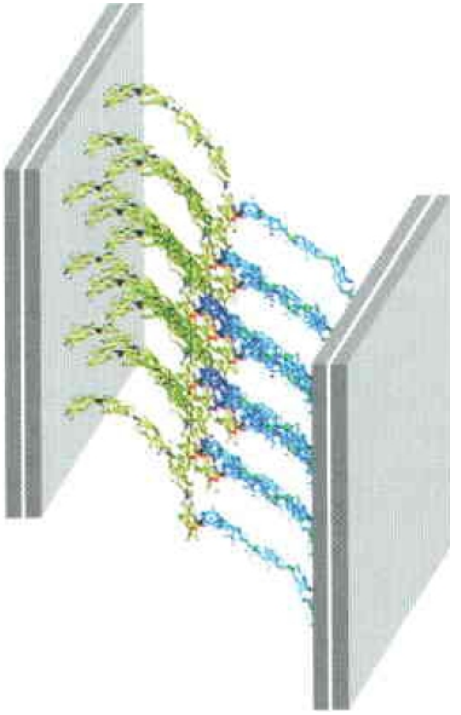


CADHERIN MECHANISM OF HOMOPHILIC ADHESION

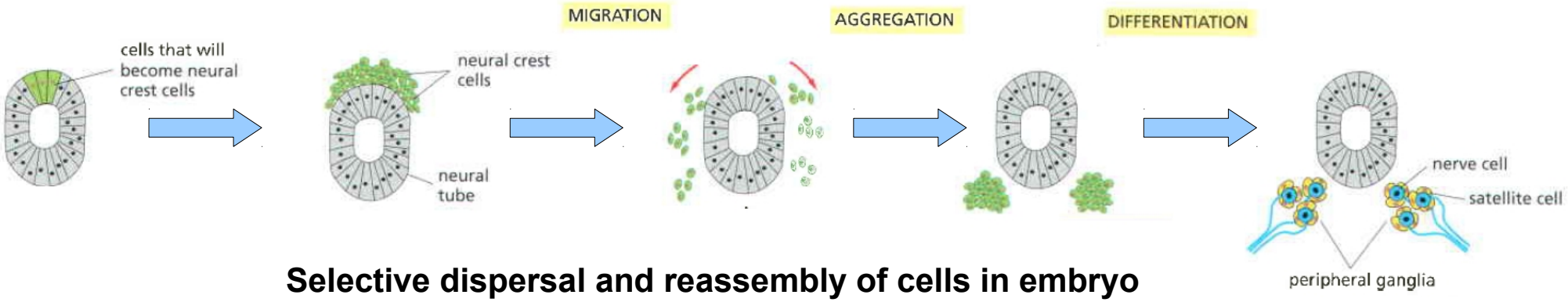
- Cadherin-cadherin: very weak interactions
- Amplification by multiplication
- Role in sorting cells and their proper aggregation



Embryonic cells reorganization



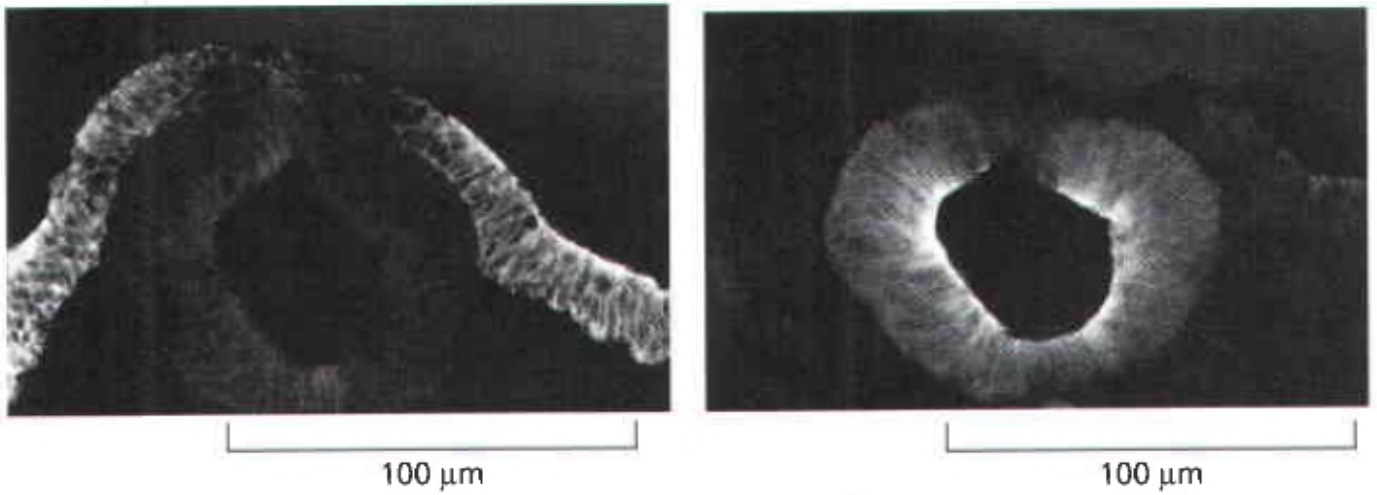
Multiple cadherin-cadherin complexes



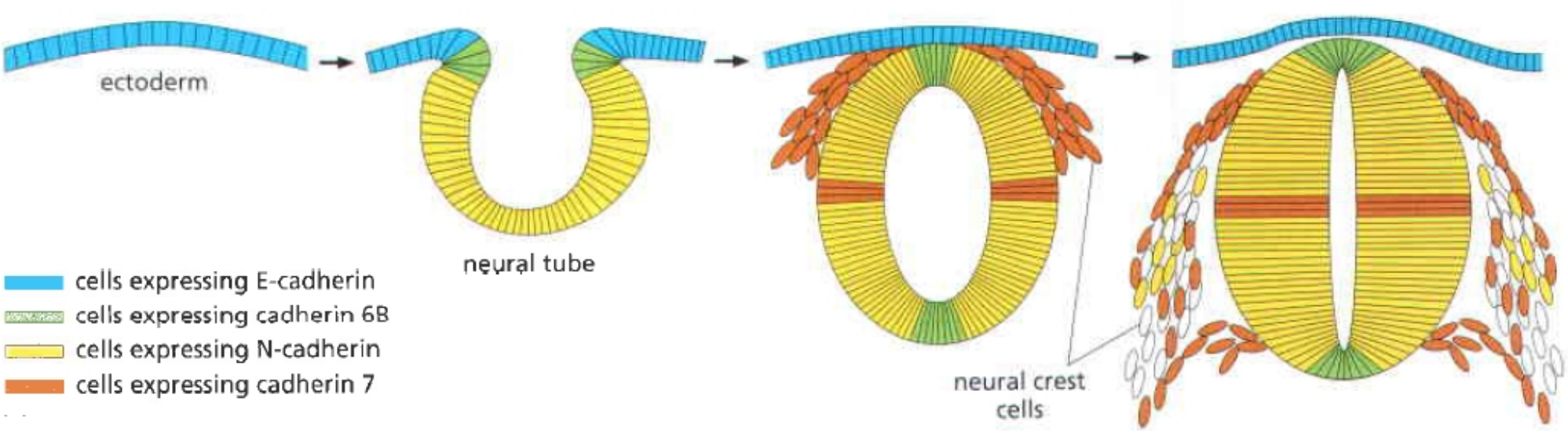
Selective dispersal and reassembly of cells in embryo

CADHERIN SPECIFICITY IN DEVELOPMENT

E-cadherin



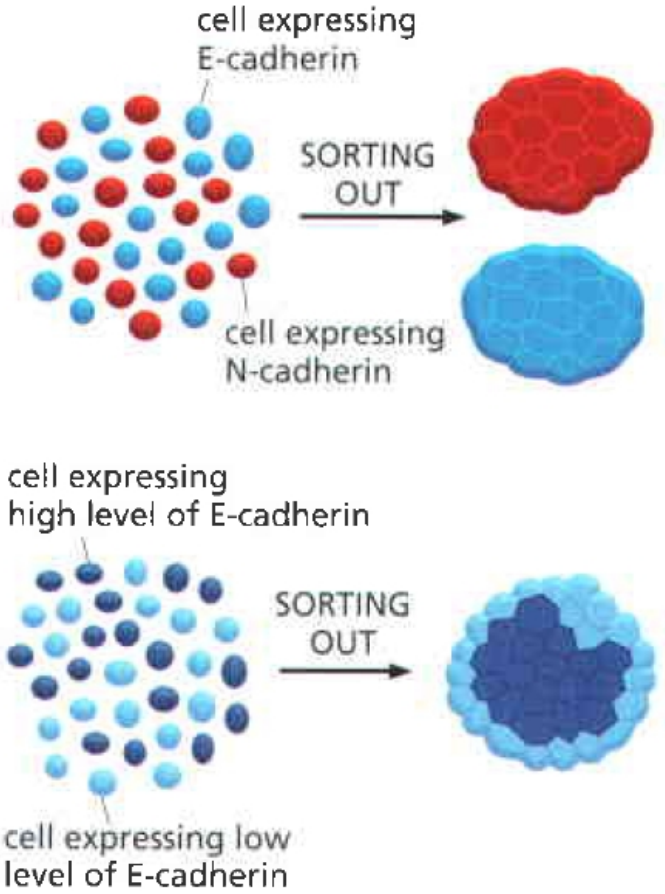
N-cadherin



CADHERIN SPECIFICITY IN DEVELOPMENT

Sorting of cells with different cadherins => proof of homophilic binding

- **Mesenchymal cells: dispersed unattached cells**
- **Epithelial-mesenchymal transitions: key in embryonic development**
- **Twist factor: inhibition of cadherins expression => involvement in cancer**



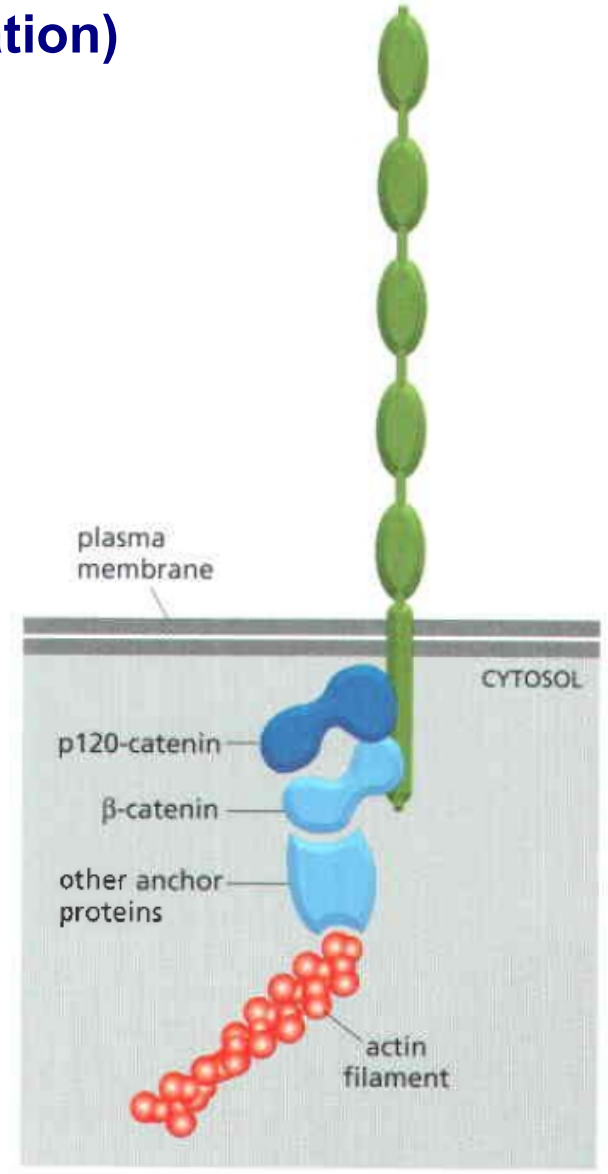
CADHERIN LINKAGE TO ACTIN FILAMENTS

- **Catenins:**

  - **p120-catenin (depletion => cadherins degradation)**

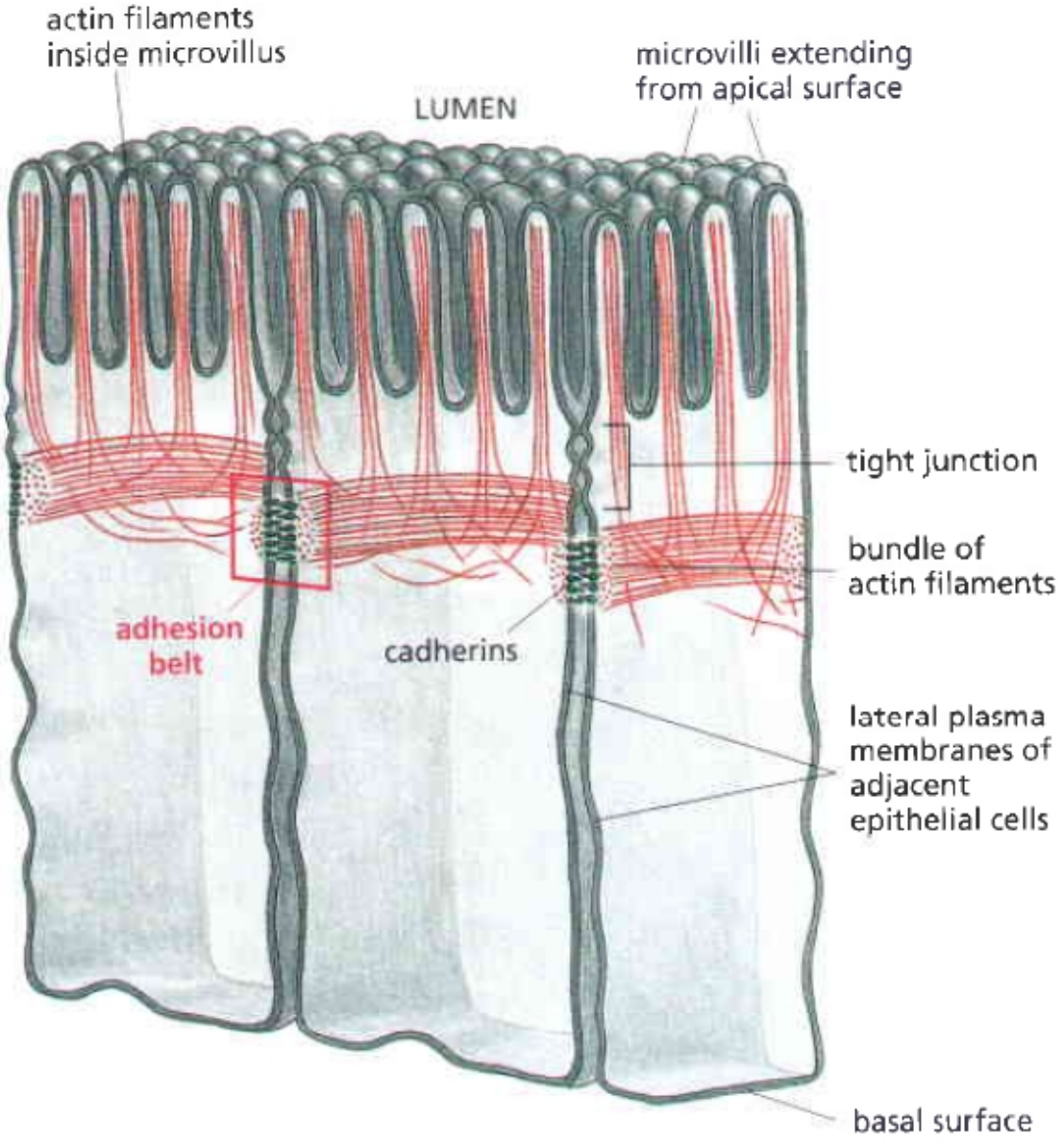
  - **β-catenin**

  - **γ-catenin**

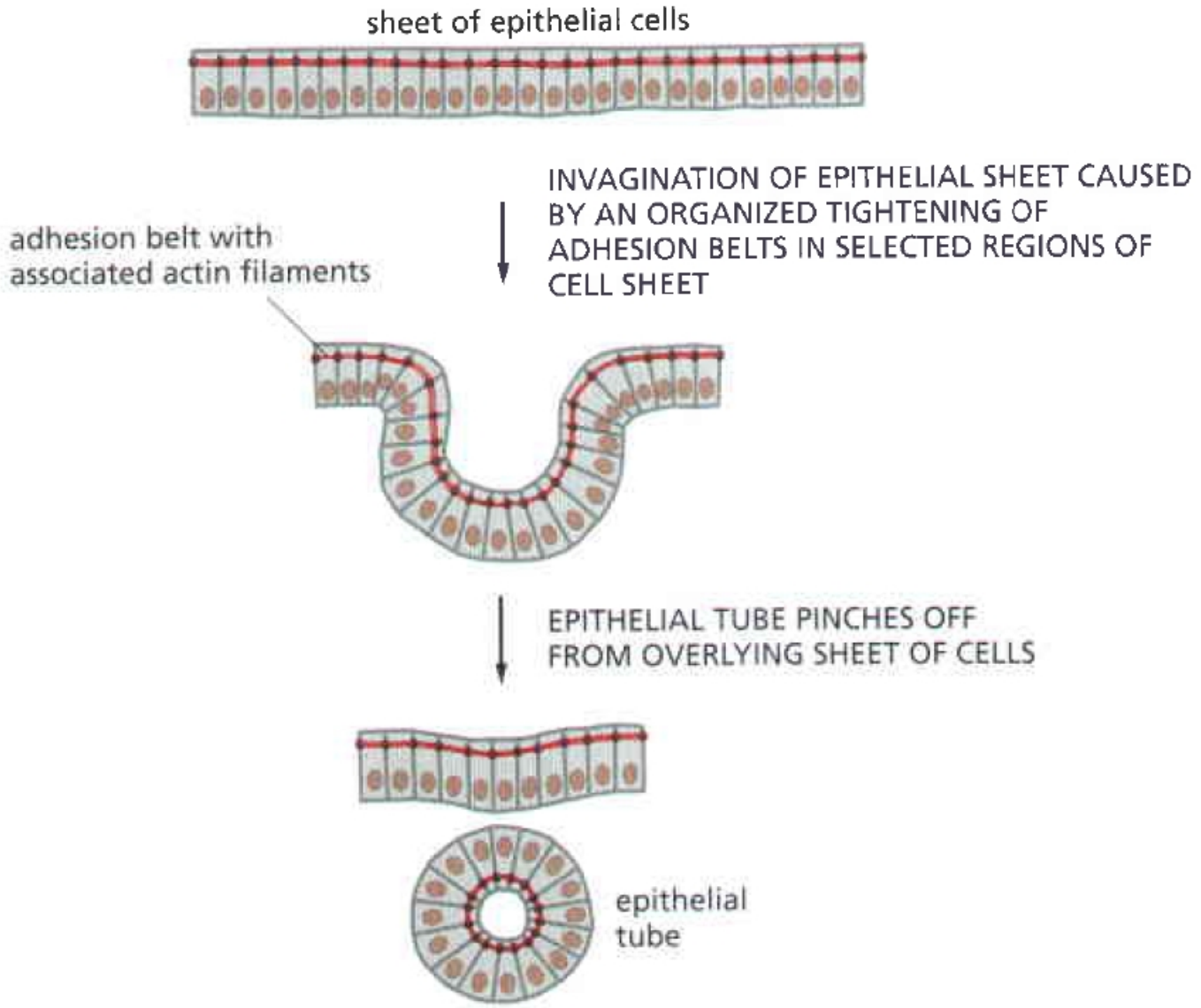


CADHERINS => COLLECTIVE CELL BEHAVIOUR

Adhesion belt contributes to the control of conserted cell behaviour through actin filaments organization



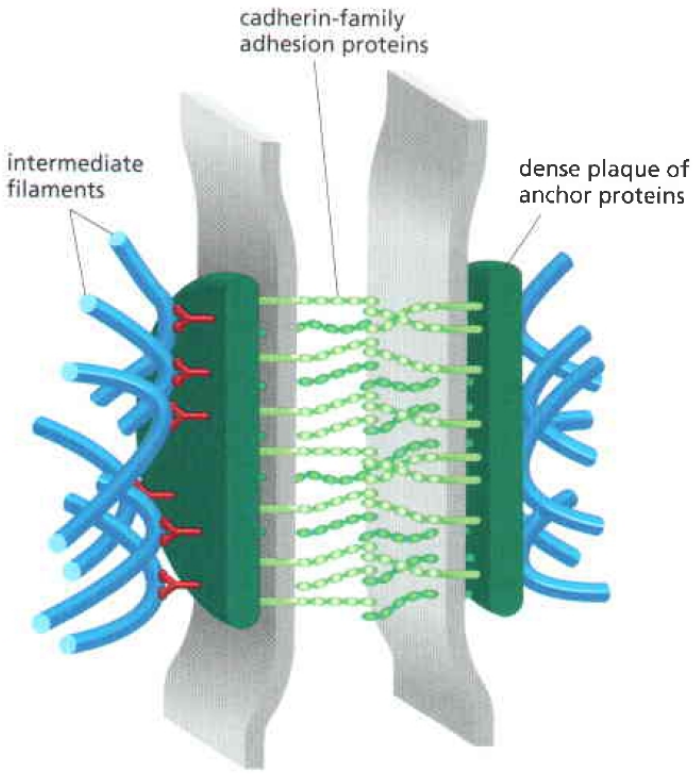
CADHERINS => COLLECTIVE CELL BEHAVIOUR

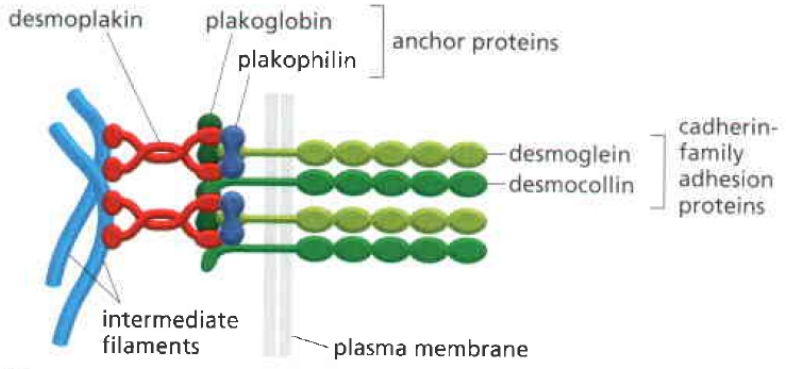


Neural tube formation

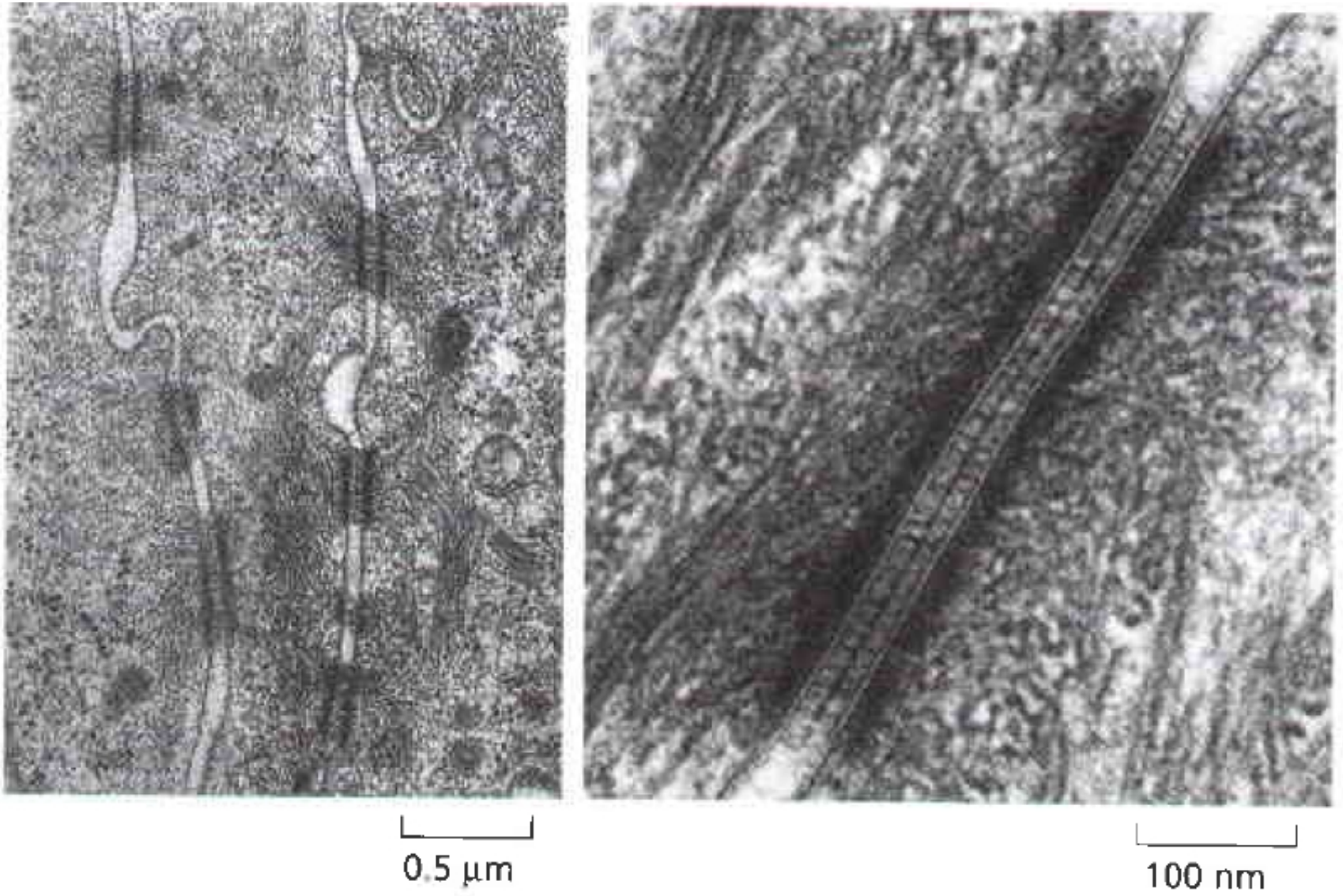
DESMOSOME JUNCTIONS

- Cell-cell
- Intermediate filaments
- Important in vertebrates
- Plentiful in epidermis
- Ca^{2+}-removal dependent



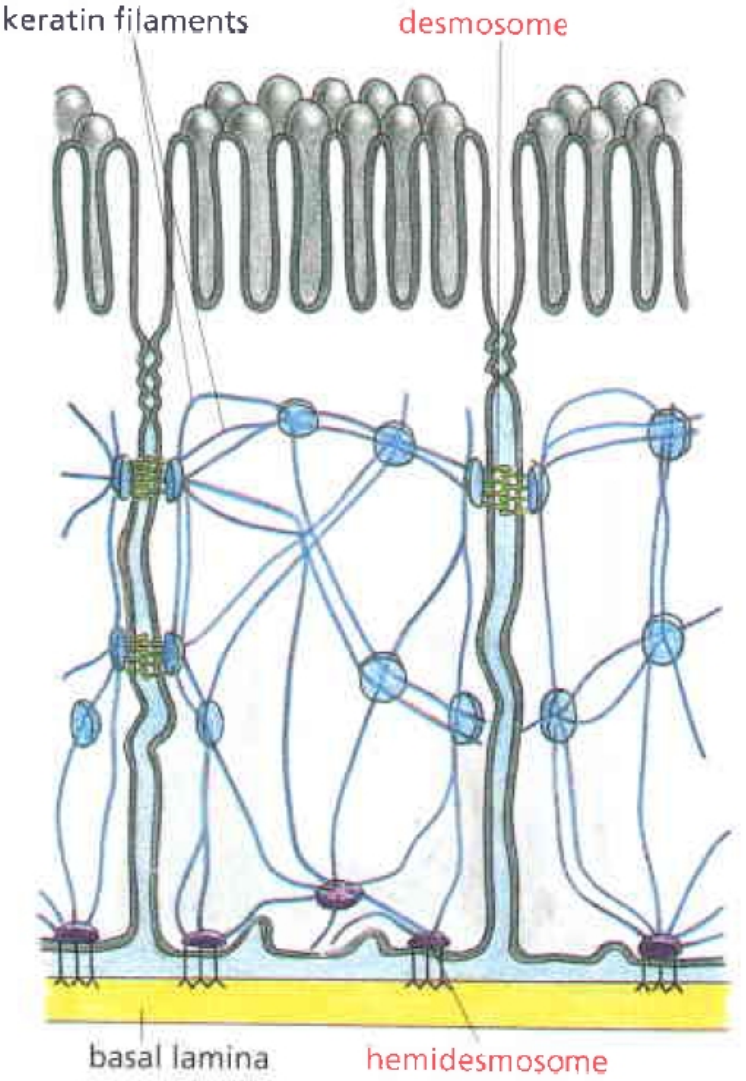


DESMOSOME JUNCTIONS



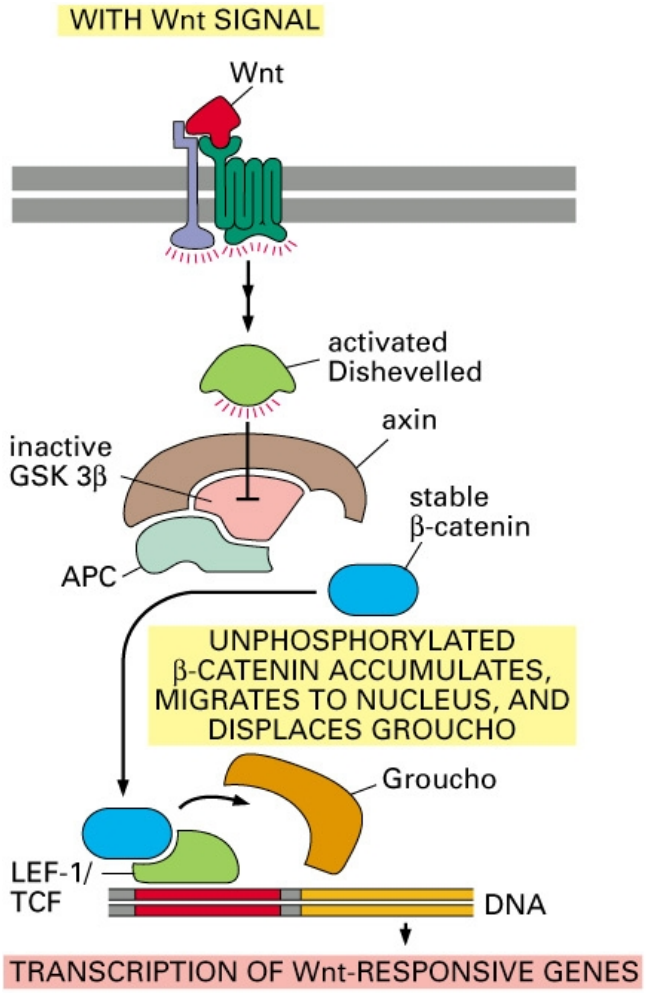
DESMOSOME JUNCTIONS

- **Intermediate filaments:**
  - **keratin filaments (epithelia)**
  - **desmin filaments (heart)**
- **Pemphigus (AB against own cadherin proteins)**



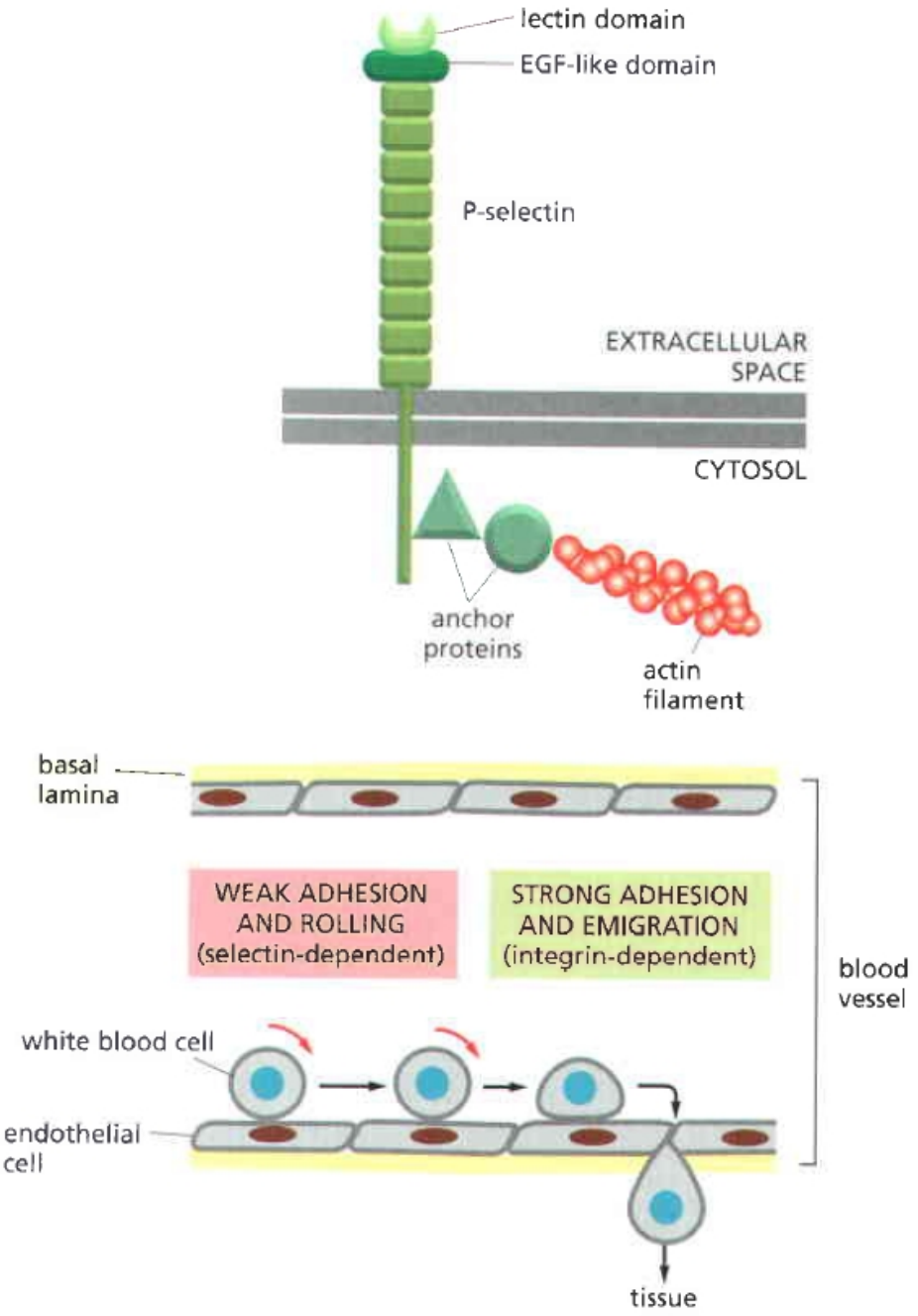
CADHERINS AND SIGNALLING

- β-catenin => Wnt-signaling
- Flamingo cadherins: 7 TMD => G-protein coupling receptors
- VE-cadherin is required for VEGF (vascular endothelial growth factor)



CELL-CELL ADHESION IN BLOODSTREAM

- Selectins: class of lectins
- Functions:
  - inflammatory responses
  - guidance of white blood cells: blood => tissue
- Carbohydrate specificity
- Cell specificity:
  - L-selectin (white blood cells)
  - P-selectin (platelets)
  - E-selectin (endothelial cells)
- Heterophilic binding
- Involvement of integrins

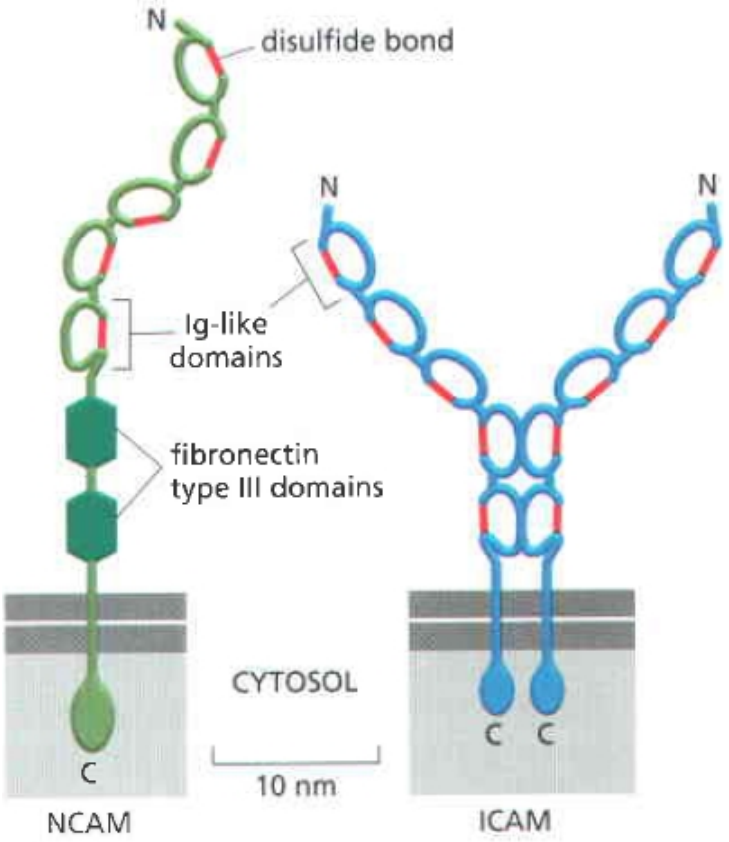


IMMUNOGLOBULINS

- White blood cells integrins recognize:
  - ICAMs (intercellular cell adhesion molecules)
  - VCAMs (vascular cell adhesion molecules)

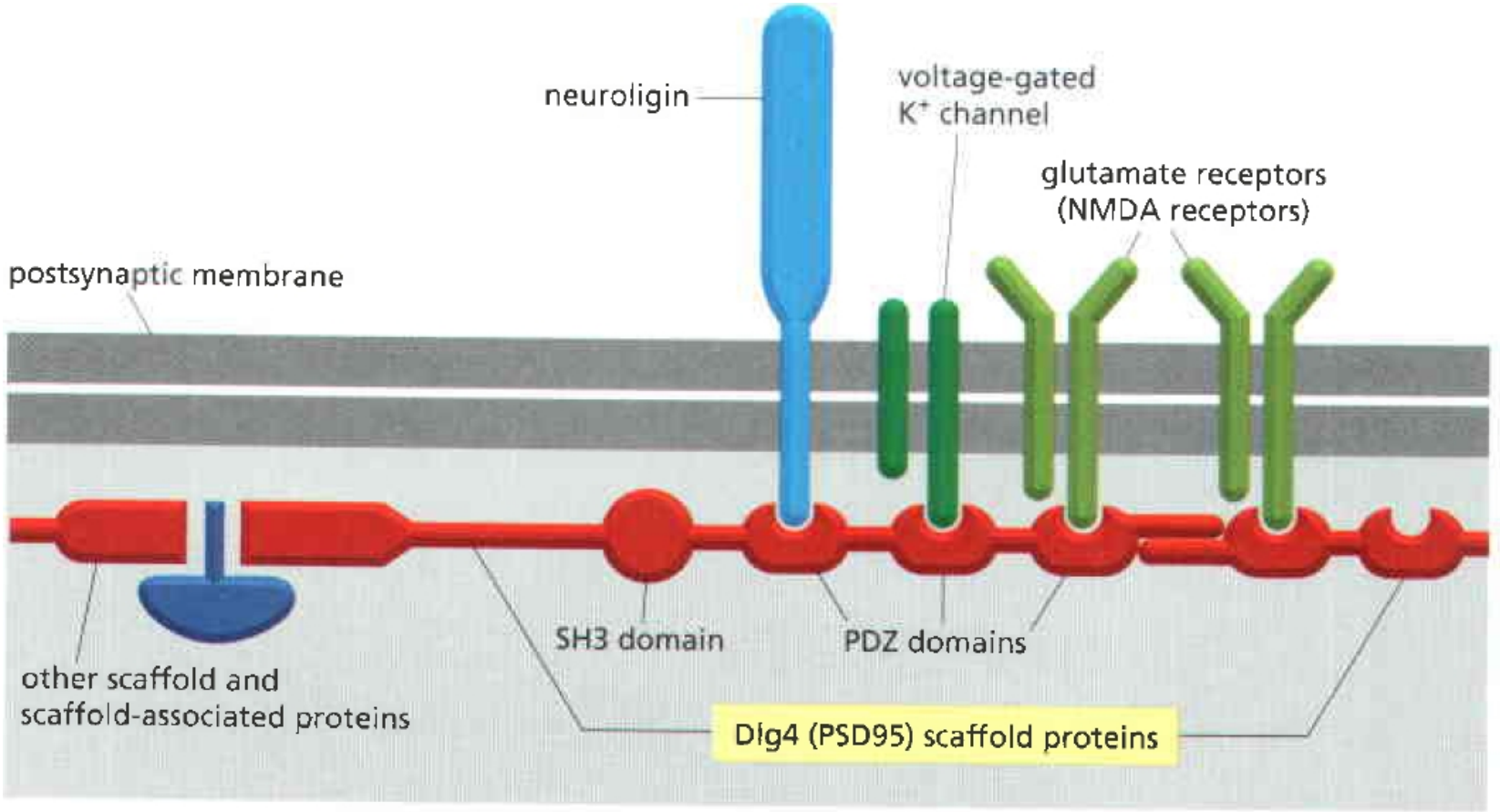
→ Immunoglobulin (Ig) superfamily

- Binding:
  - homophilic (f.i.: NCAM, neural cell adhesion molecule)
  - heterophilic (f.i.: ICAM, with integrins)
- NCAM: many sialic acids => adhesion fine-tuning through repulsion
- Many classes are involved in synapses

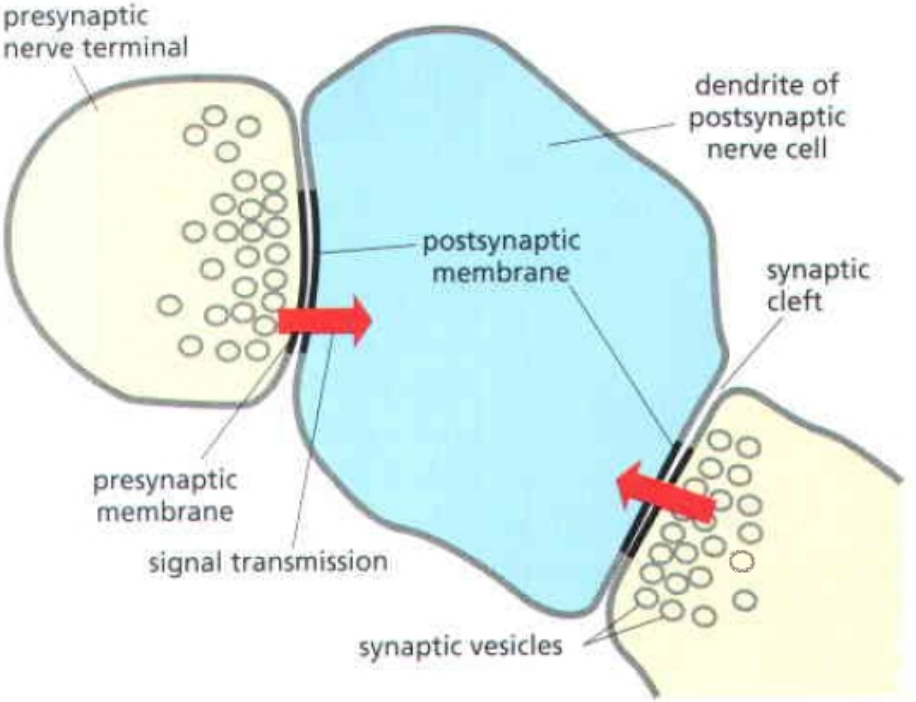


SCAFFOLD PROTEINS IN SYNAPTIC JUNCTIONS

- Vast variety of cadherins (~ 20) and IGs in synapses
- Scaffolds:
  - concentration
  - organization



SYNAPSE ORGANIZATION



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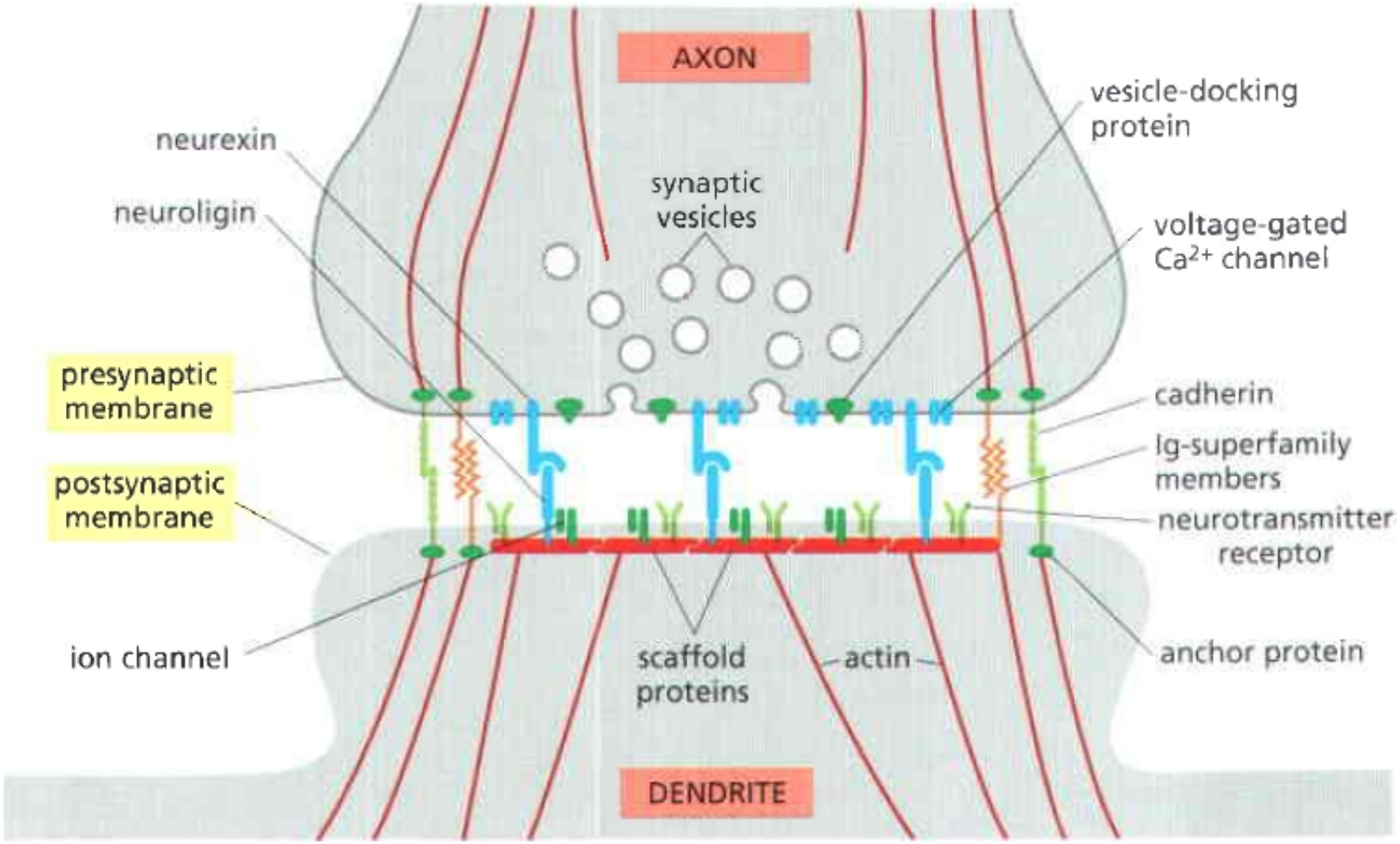
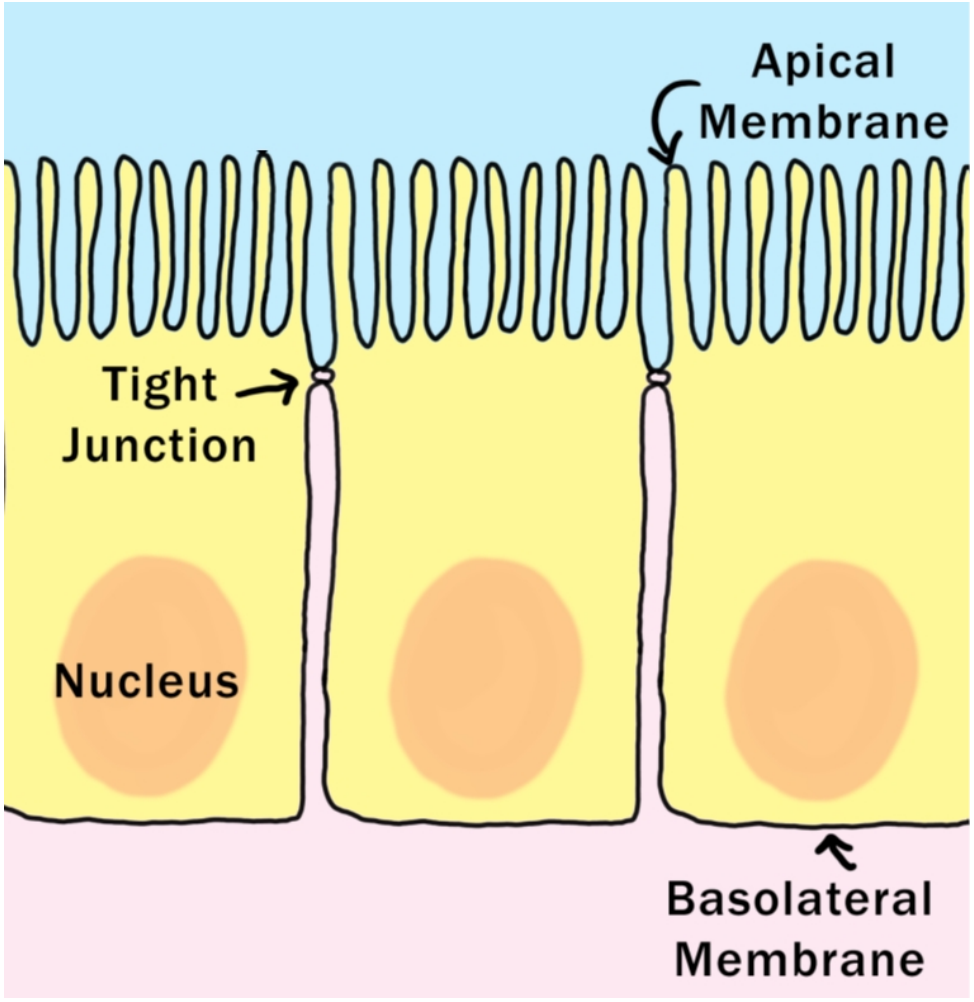


TIGHT JUNCTIONS IN EPITHELIA

- In vertebrates ~ 60% epithelia
- Polarity:
  - apical side
  - basal side
- Permeability barrier
- Occluding (tight in vertebrate epithelia) junctions



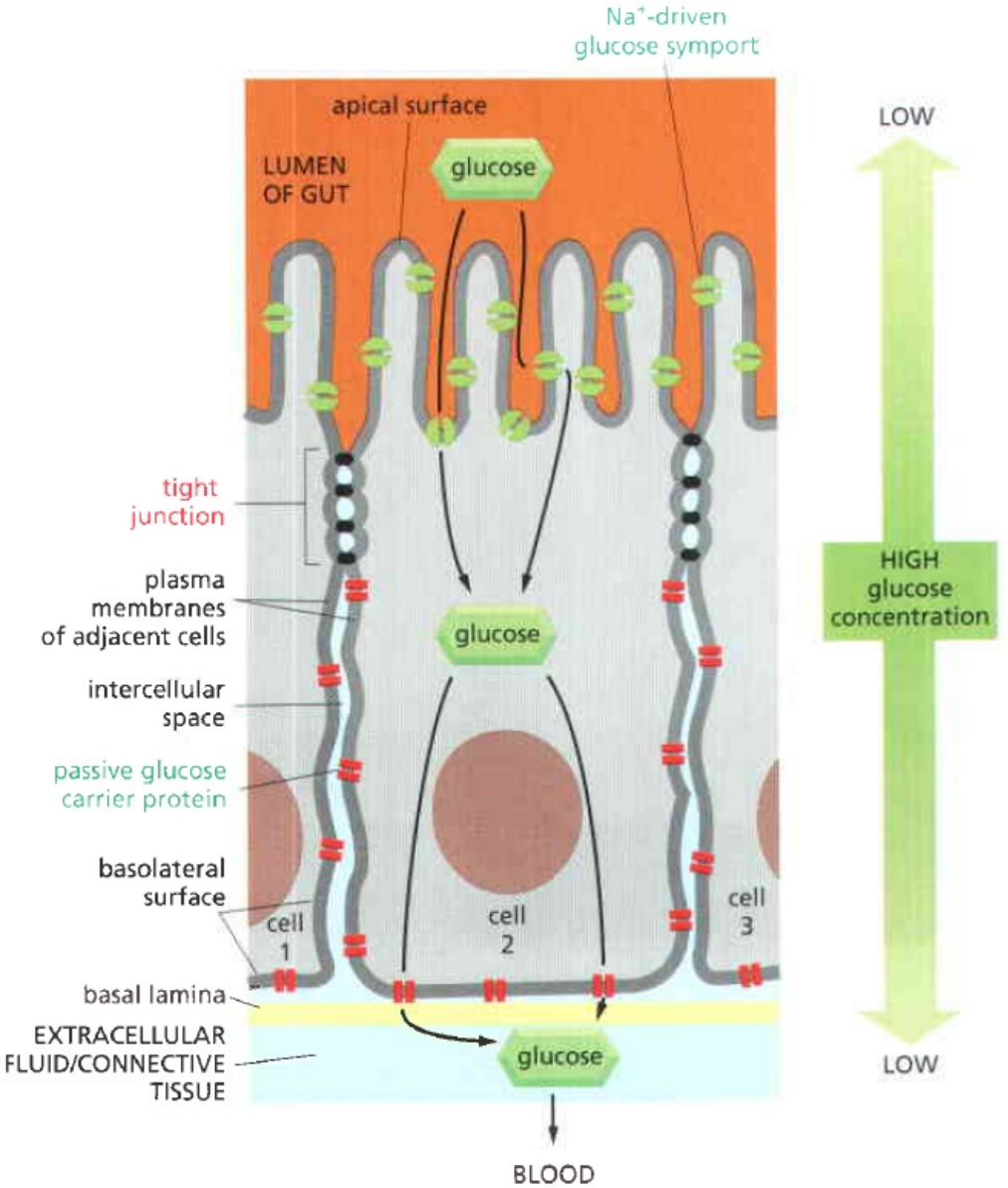
TIGHT JUNCTIONS: ROLE IN TRANSPORT

- Transport between cells = f (distance between their membranes)
- Polarity of the transport: two sets of proteins
- Paracellular transport:

  - amino acids, monosaccharides

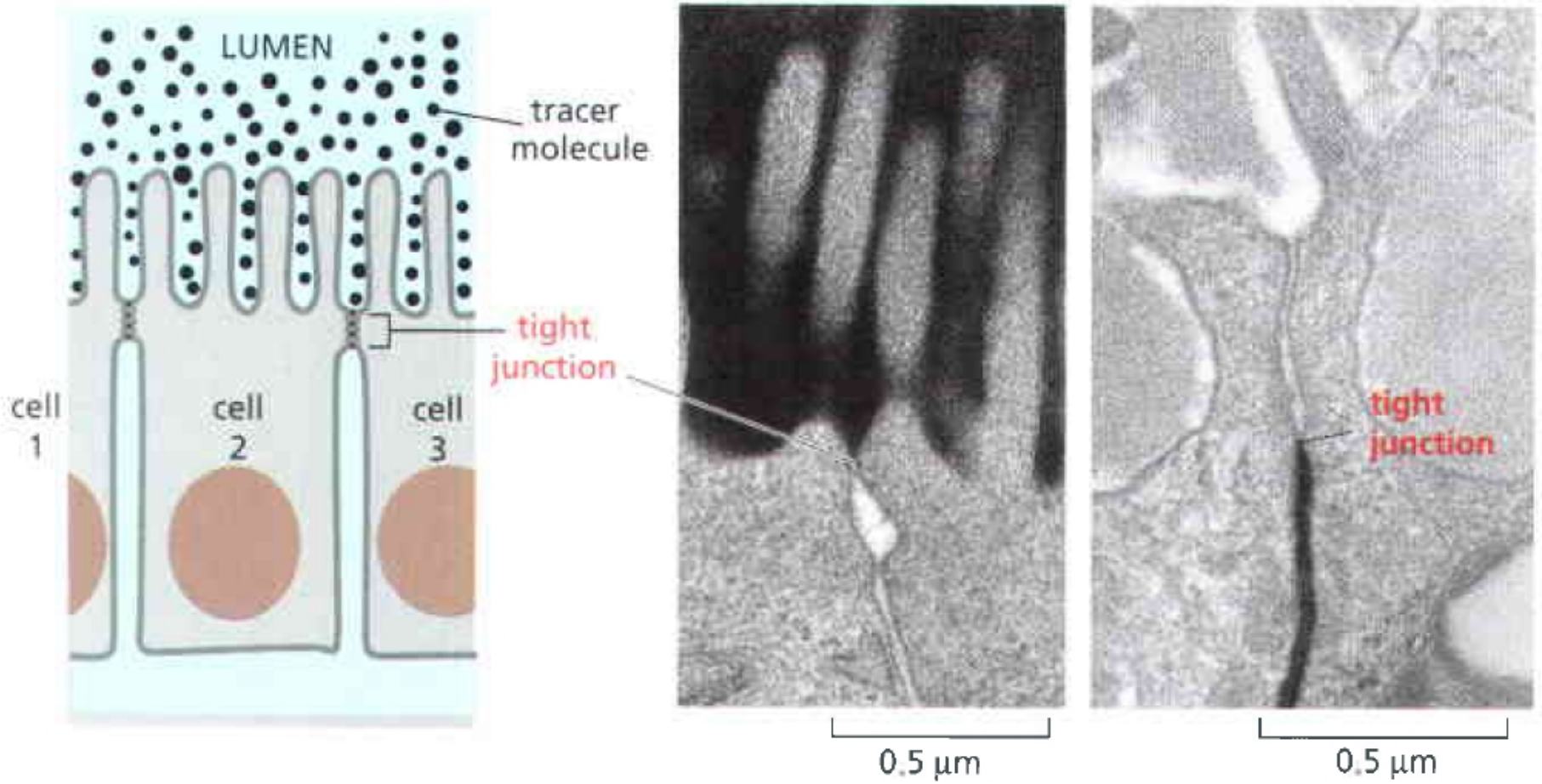
  - control of effective nutrient flow

  in the proper direction



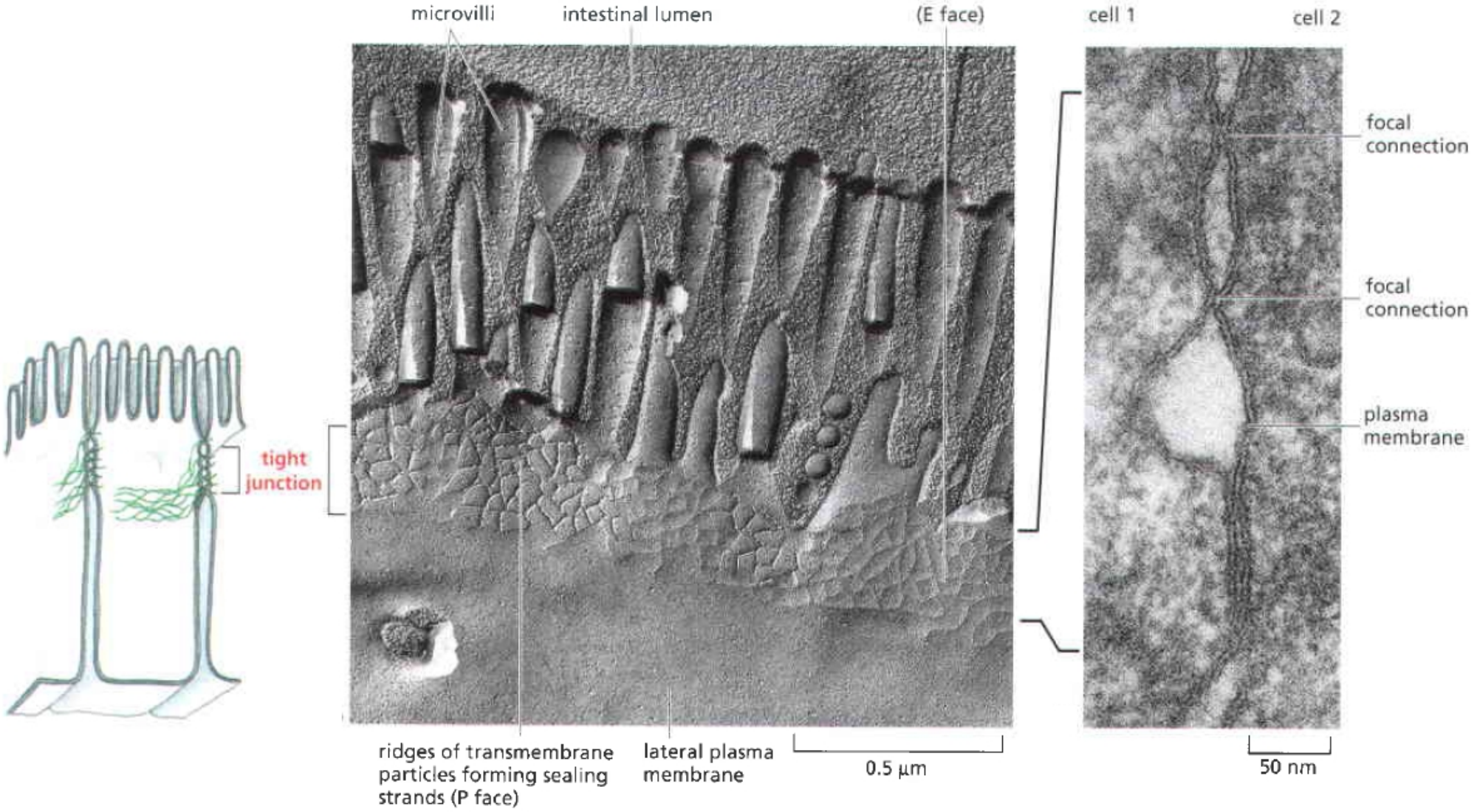
TIGHT JUNCTIONS: ROLE AS BARRIERS

- Apical and basolateral sides are separated by tight juctions
- High cell specificity: 10^4 permeability difference for Na^+ in intestine *vs.* bladder



STRUCTURE OF TIGHT JUNCTIONS

- **Sealing strands: abundant in the apical ends of the cell**



STRUCTURE OF TIGHT JUNCTIONS

- **Claudins (~24 in human):**

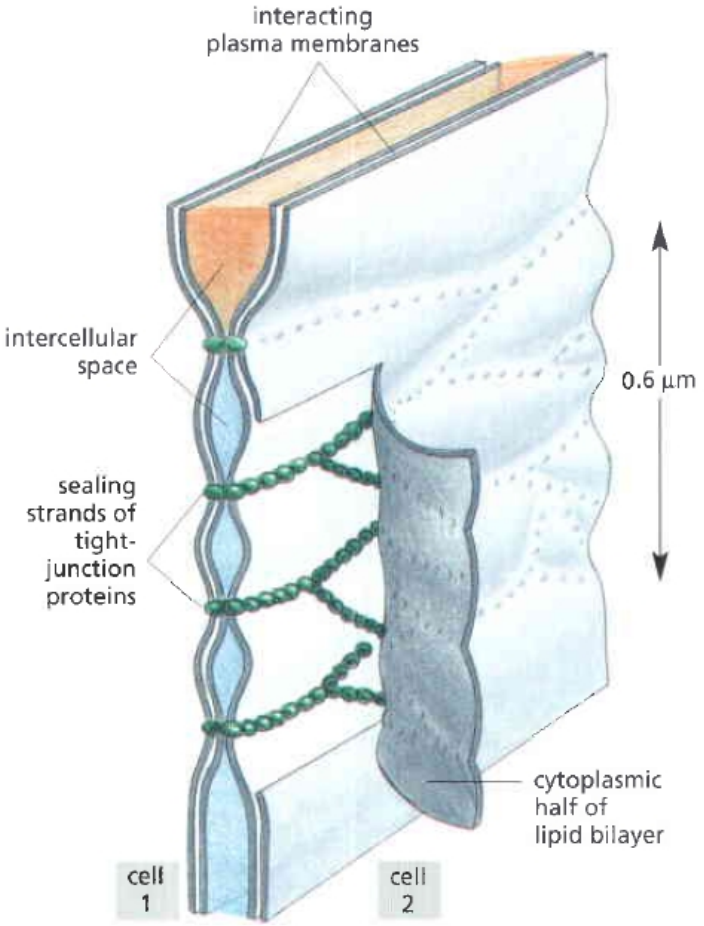
  - claudin-1 knock-out in mice => water lose

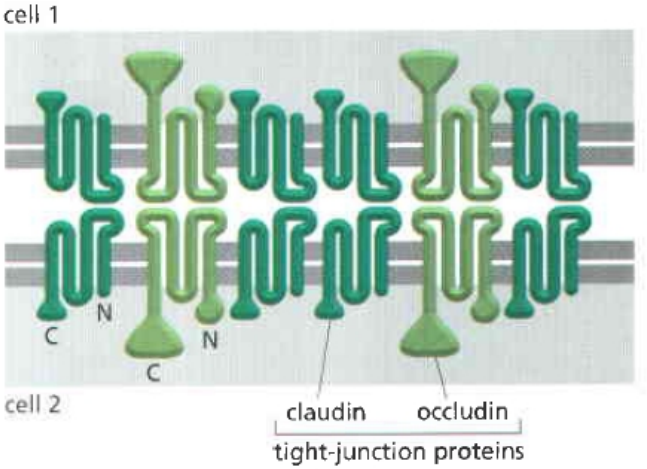
  - claudin-1 expression in fibroblasts => tight junctions

  - ion-specific paracellular pores

- **Occludin**

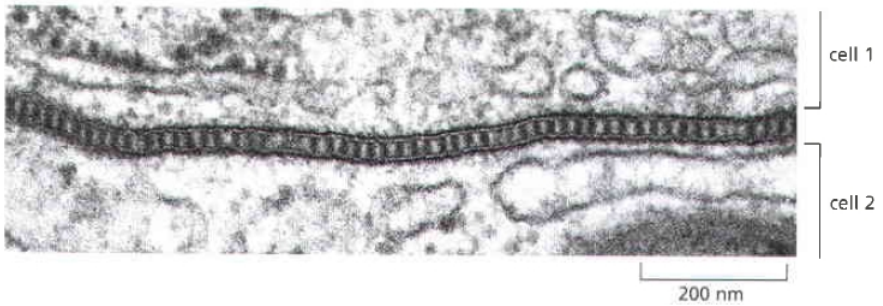
- **Tricellulin**



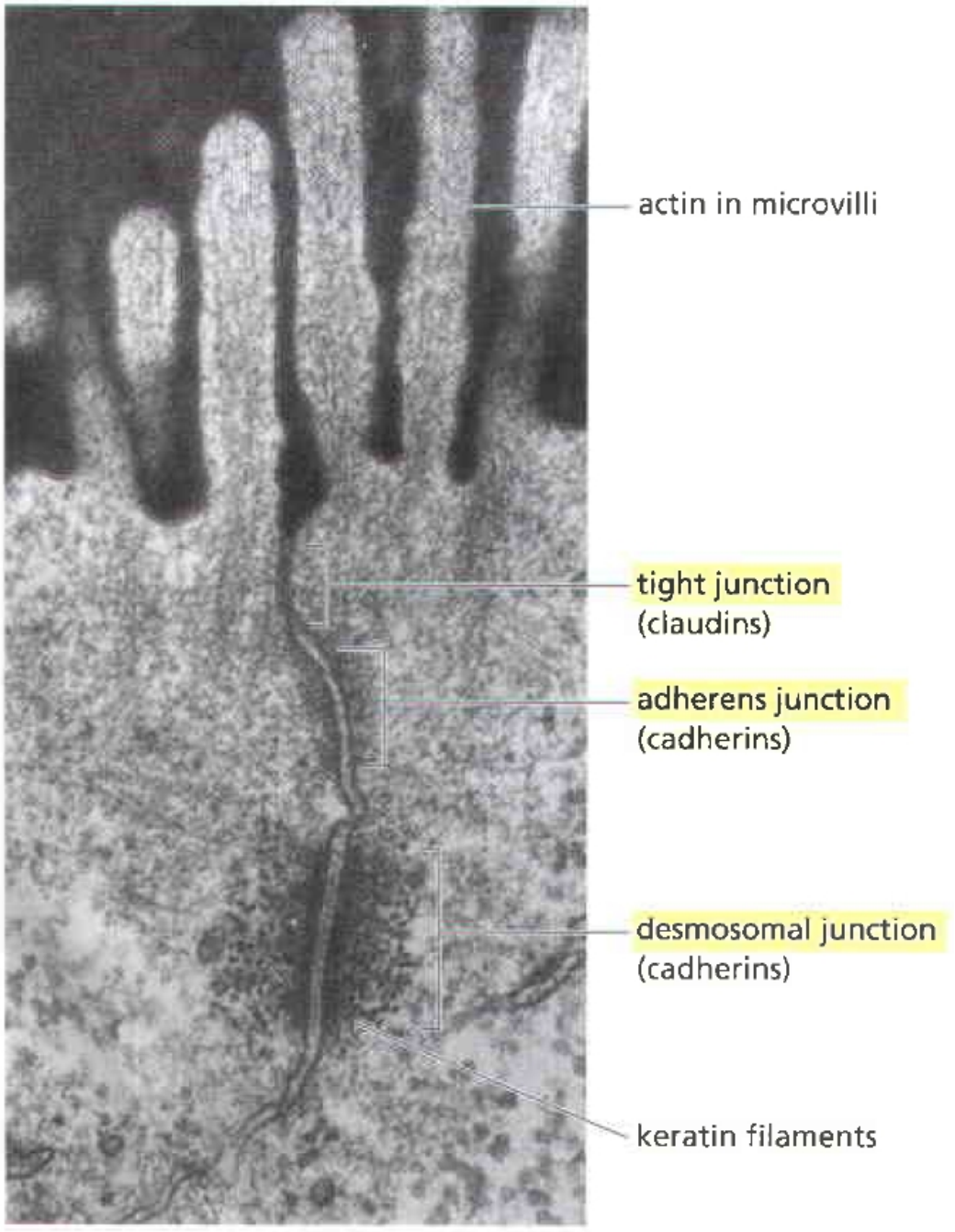


SCAFFOLD AND PROLIFERATION

- **Junctional complex:**
  - tight junction
  - adherens junction
  - desmosomal junction
- **Tight junction proteins => scaffold**
- **Septate junctions:**
  - regular structures
  - proteins similar to claudins
  - insects/mollusks
- **Mutants => tumours**

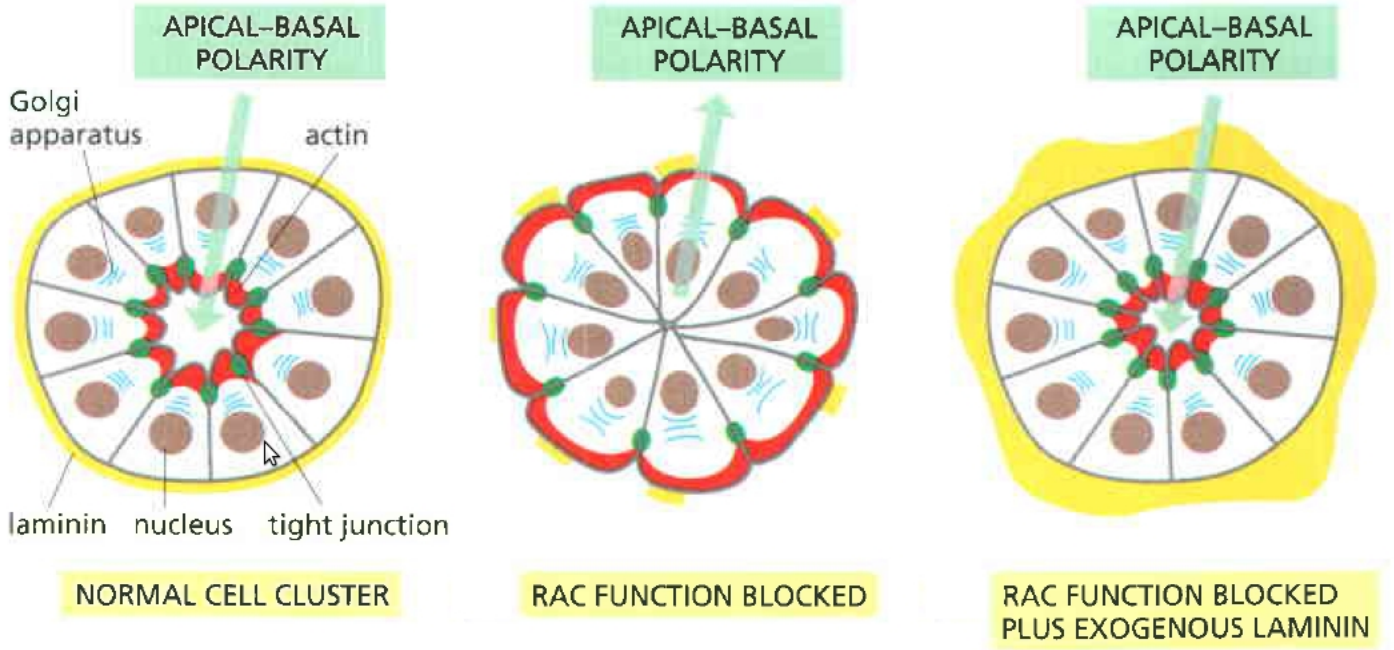


Septate junction between two mollusk epithelial cells



CELL JUNCTIONS AND POLARITY IN EPITHELIA

- **Polarity => function:**
  - **dendrite-axon**
  - **white blood cells**
  - **migrating fibroblasts**
  - **embryonic cells**
- **Polarity is in all animals**
- **MDCK cells (dog kidney epithelium): one was not polarized but many**

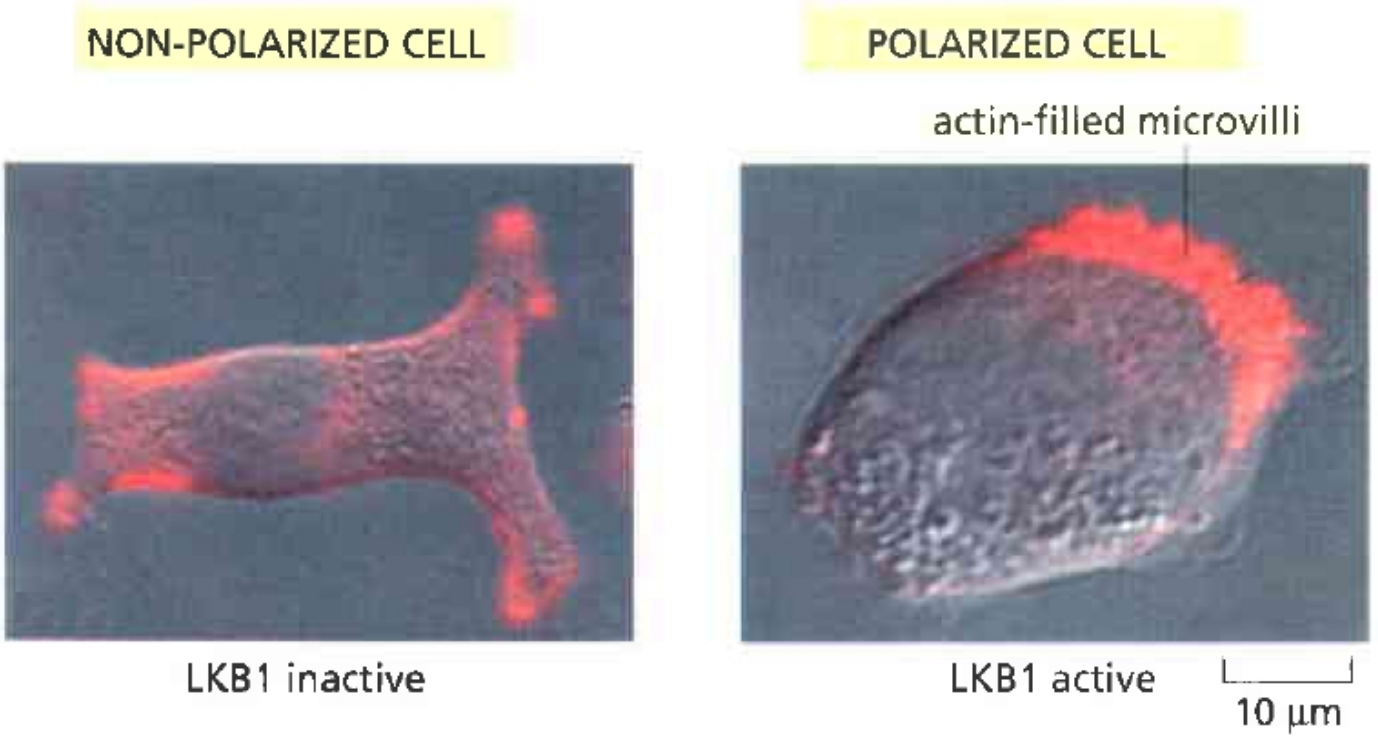


CELL JUNCTIONS AND POLARITY IN EPITHELIA

- Pars: partitioning defective genes
- Par4 (Ser/Thr-kinase): mutations disrupt polarity, cancer predisposition
- Two mechanisms:
  - autonomous cell polarity
  - adaptation to the environment

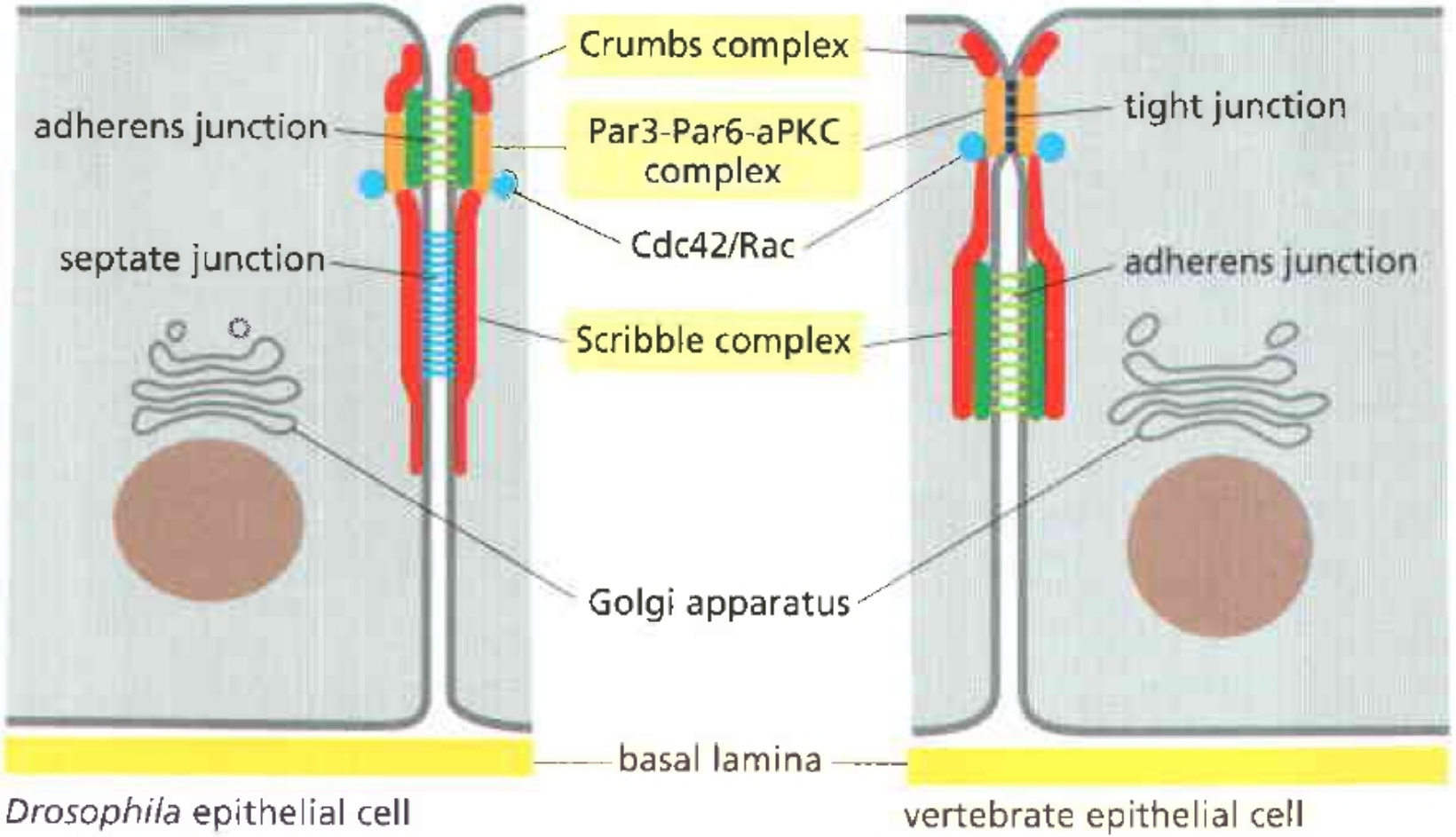
} Pars classification

- Cdc42, Rac



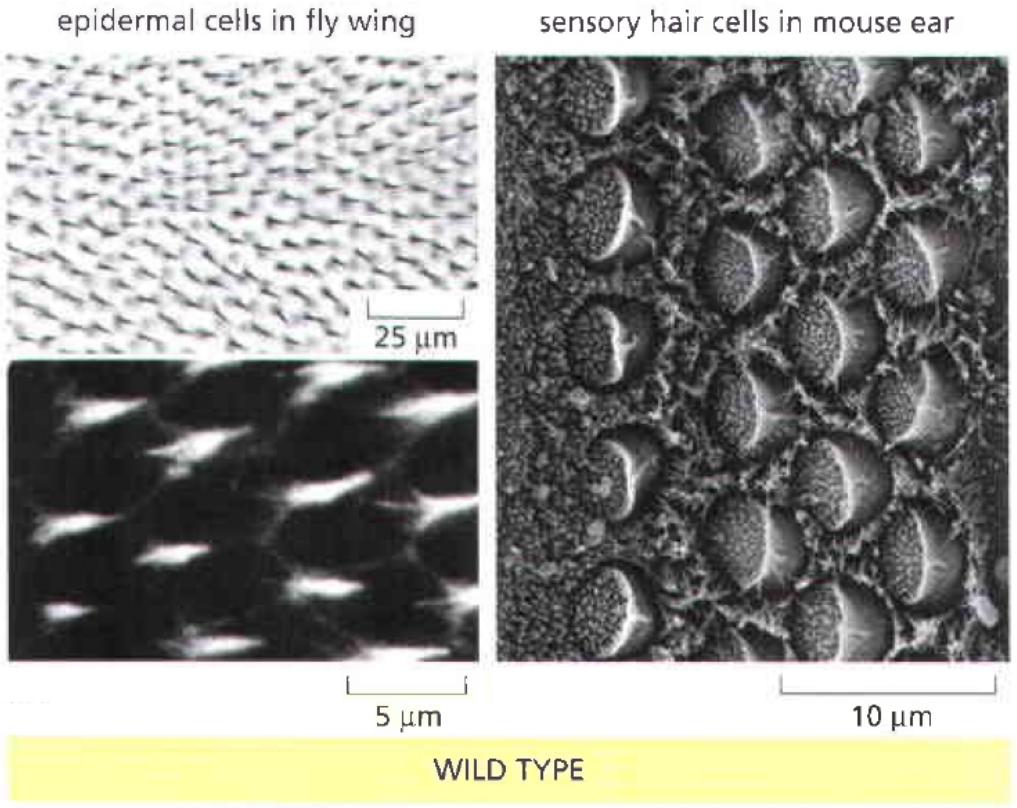
CELL JUNCTIONS AND POLARITY IN EPITHELIA

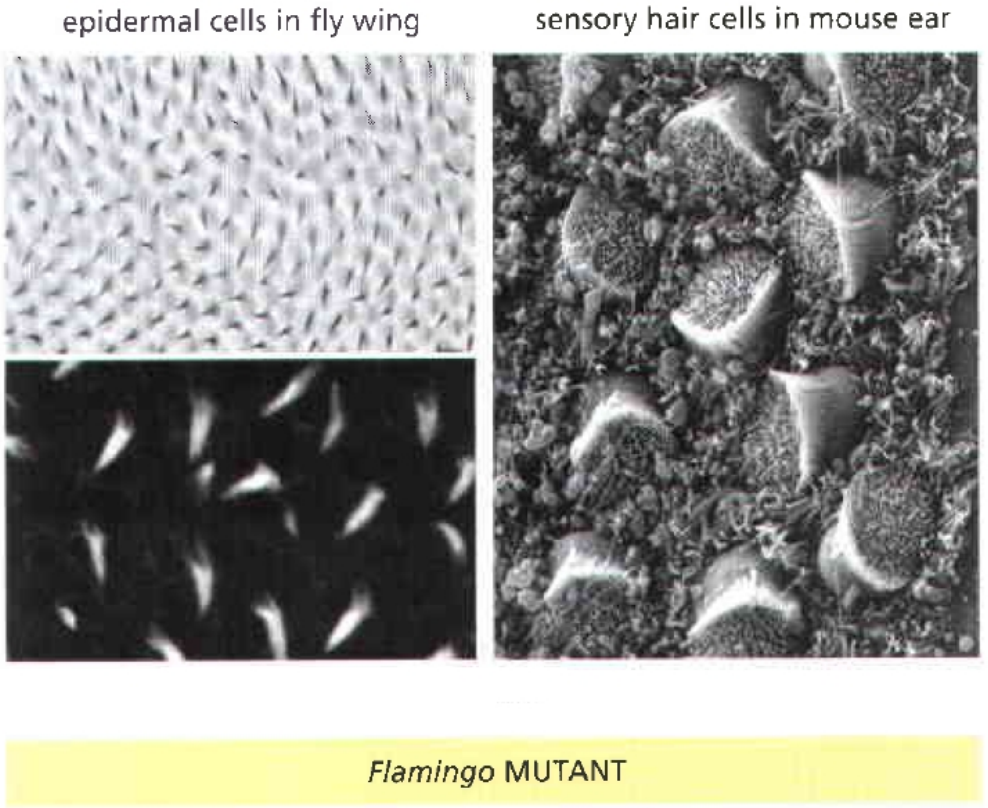
- **Par3-Par5-aPKC + Cdc42 + Rac => Crumbs complex**
- **PDZ-domains + Siscs-lost + Stardust => Scribble complex**
- **Rac influences the distribution of proper components of basal lamina to the opposite side of the cell**



CELL JUNCTIONS AND POLARITY IN EPITHELIA

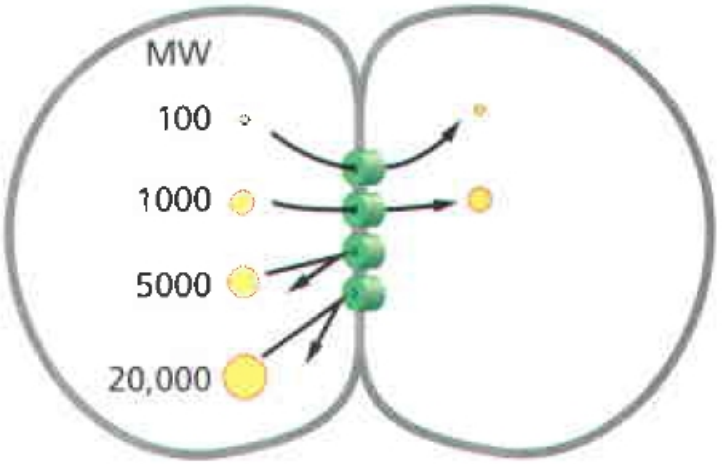
- **Planar cell polarity: specific direction of epithelium:**
  - **wing of fly**
  - **ear of vertebrate**
- **Ion channels open by tilting in one direction**





GAP JUNCTIONS

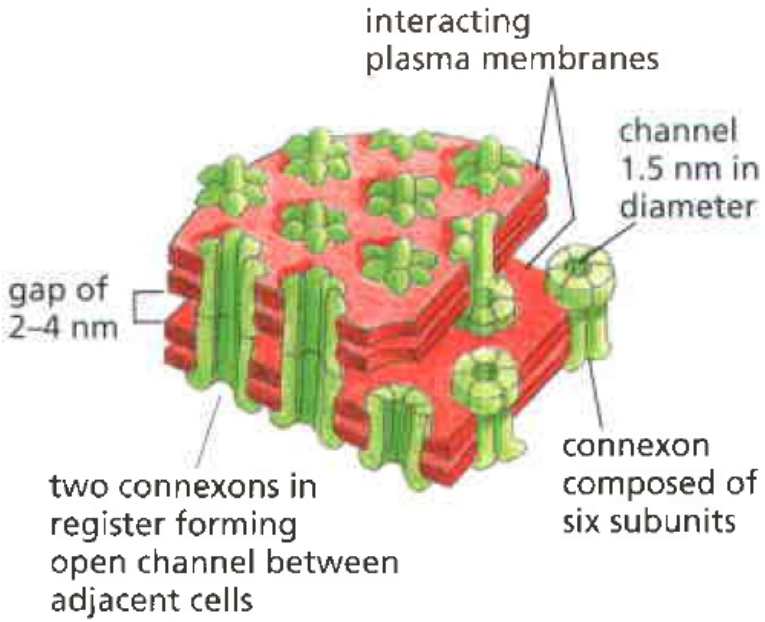
- Animals: gap junctions
- Plants: plasmodesmata
- Function:
  - connecting two adjacent cells
  - establishment of small molecules exhange
- Coupling cells:
  - electrically
  - metabolically
- Channels (~1.5 nm):
  - connexins
  - innexins

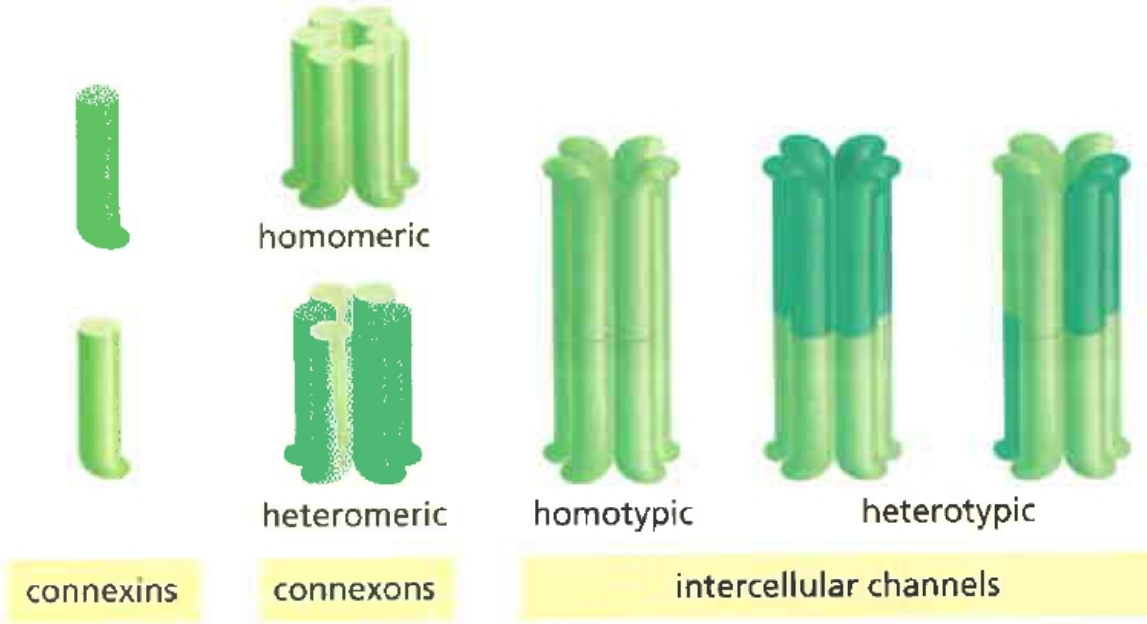


Transport is limited by size

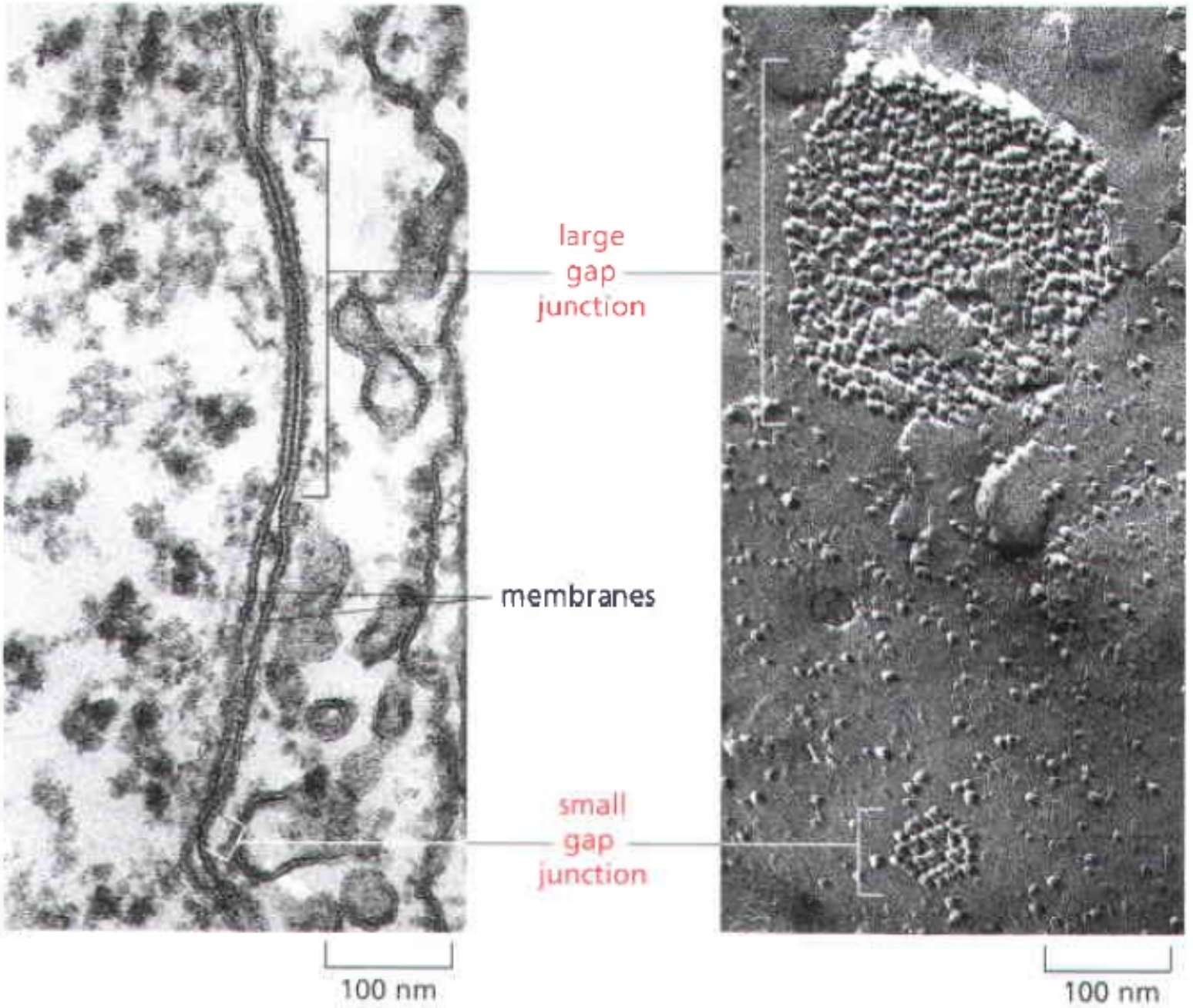
GAP JUNCTIONS STRUCTURE

- Connexin: 4 TMD
- 6 connexins => connexon
- Many connexon in gap junctions: aligned parallel
- Composition => specific permeability
- 'Unpaired'-hemichannels are closed



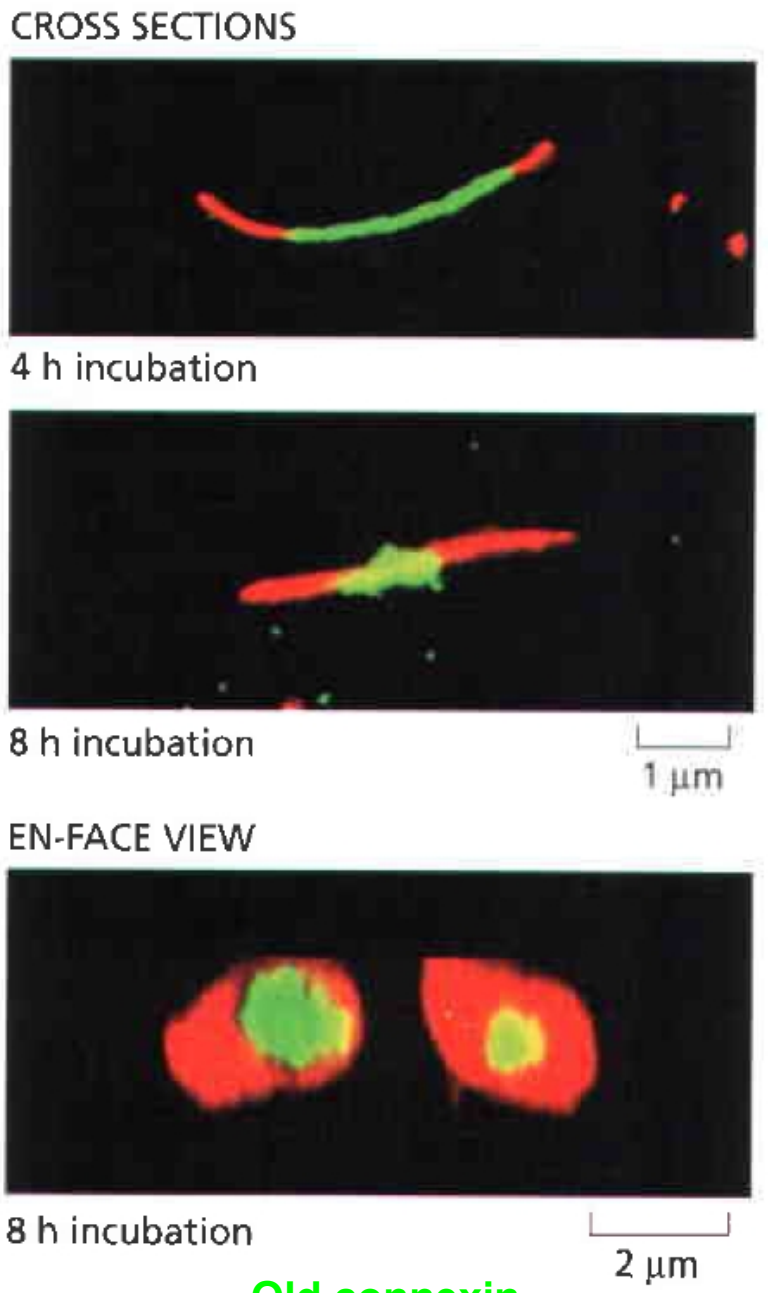


GAP JUNCTIONS STRUCTURE



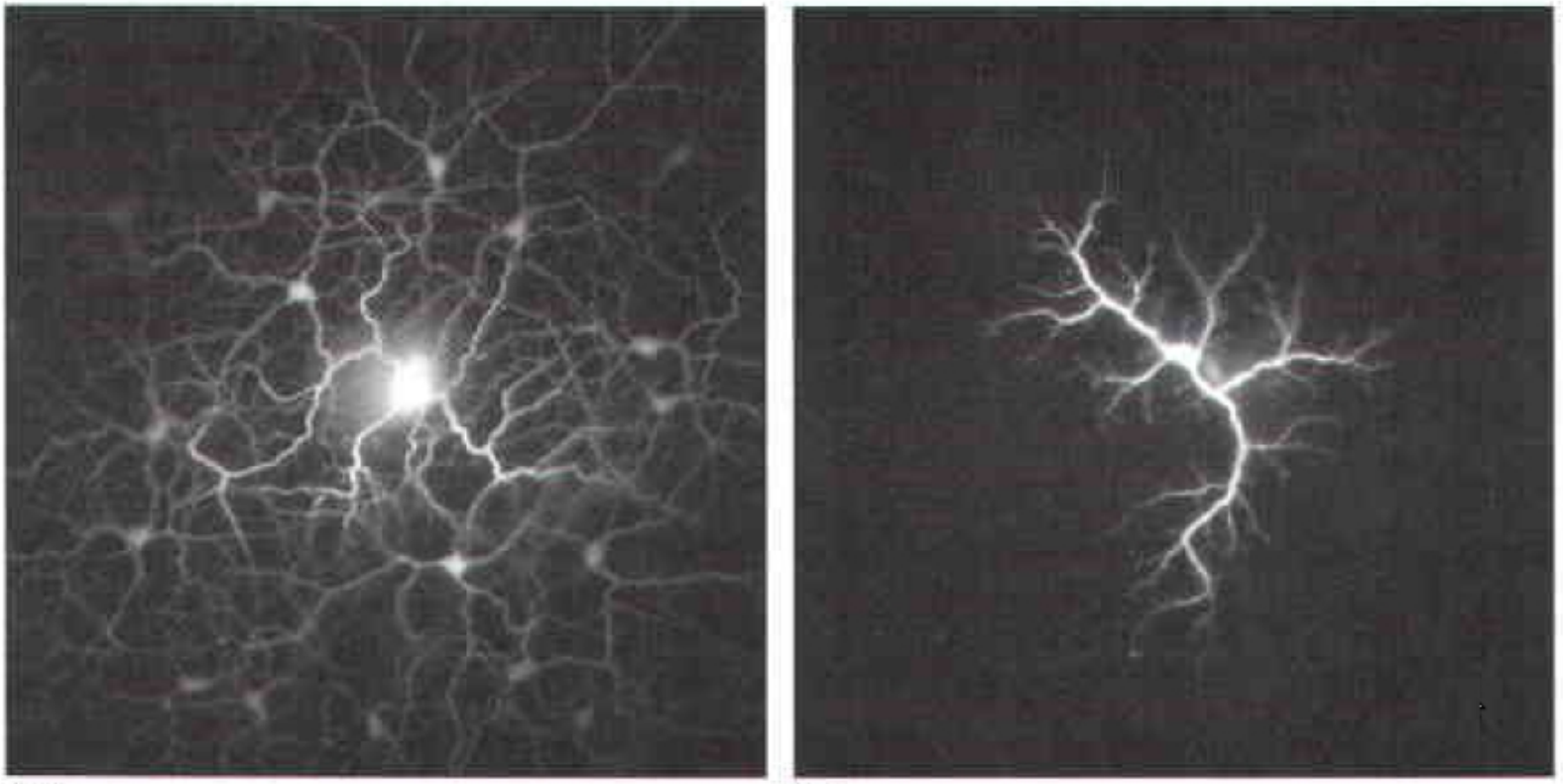
GAP JUNCTIONS DYNAMICS

- Highly dynamic structures ($t_{1/2}$ ~ hours)
- Delivery by exocytosis
- Cell-specific functions:
  - neurons (spreading action potential)
  - heart (electrical synchronization)
  - liver (smoothing concentrations)
  - corti organ (sound transduction)
  - embryonic cells (coordination of collective behaviour)



GAP JUNCTIONS PERMEABILITY REGULATION

- Flipping: open <=> closed states
- pH regulation (unclear)
- Ca^{2+} regulation: damage => leak => $[Ca^{2+}]_{in}$ increases => closing gaps
- Dopamine: switch rods => cones



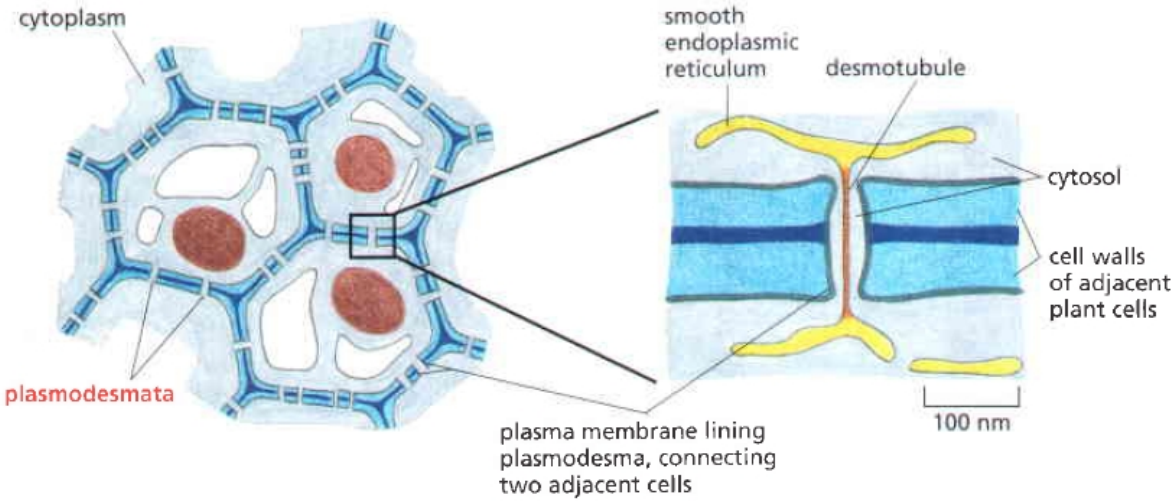
Luciferase in the retina

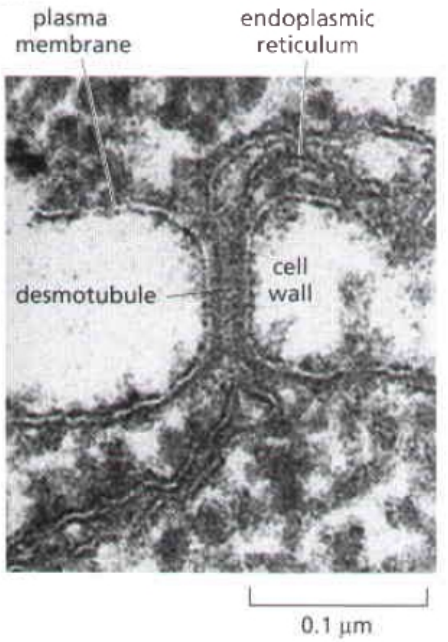
No dopamine

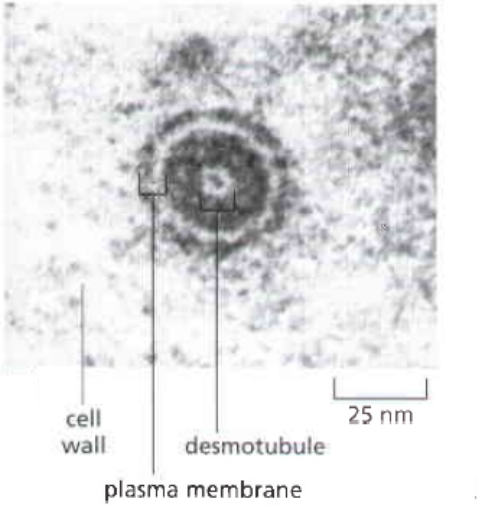
Dopamine

PLASMODESMATA

- Cell walls ~ 100 nm thick
- Cylindrical channels: 20-40 nm
- Desmotubule: elongation of ER
- Size restriction < 800 Da
- Viral products can pass

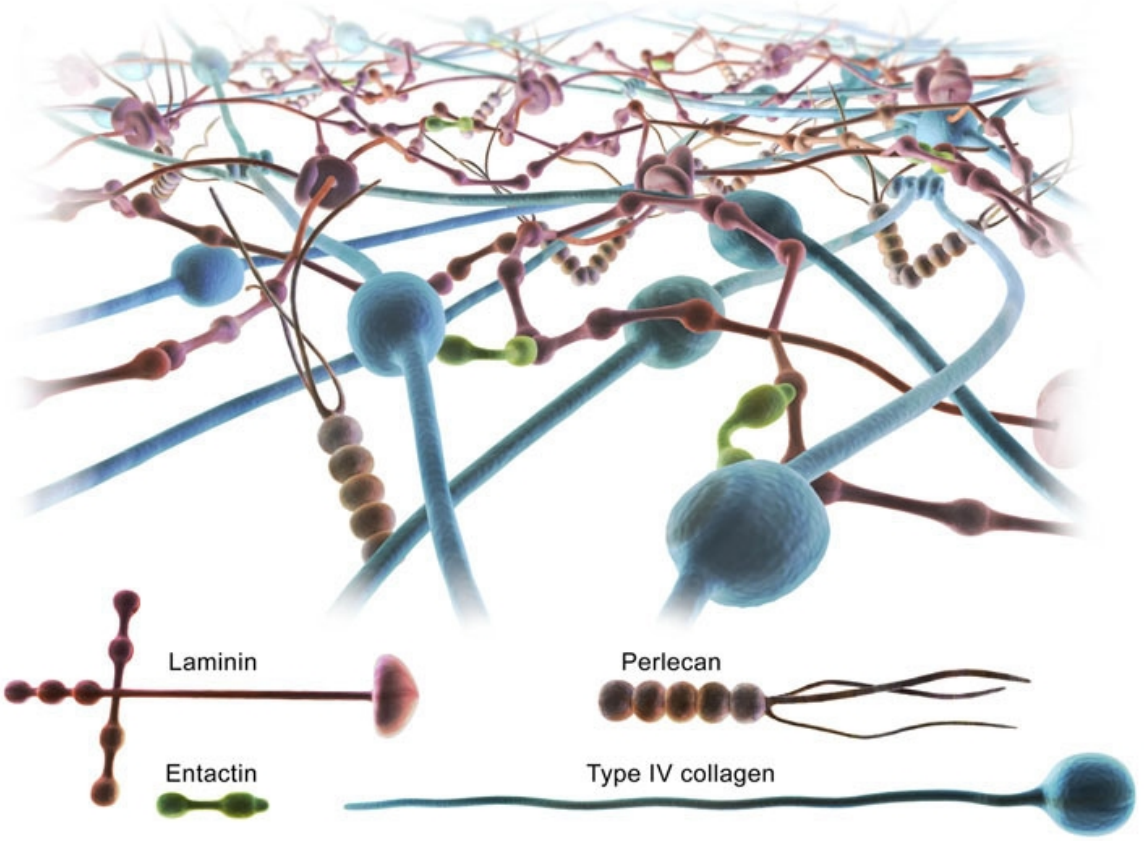






BASAL LAMINA AND ECM

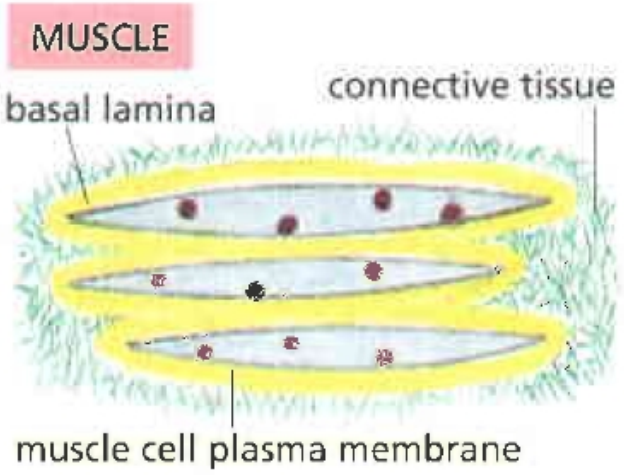
- **Extracellular matrix (ECM): extracellular space full of macromolecules networks:**
  - **proteins**
  - **polysaccharides**
- **Basal lamina: thin, tough, flexible sheet of matrix molecules adjacent to epithelia cells**

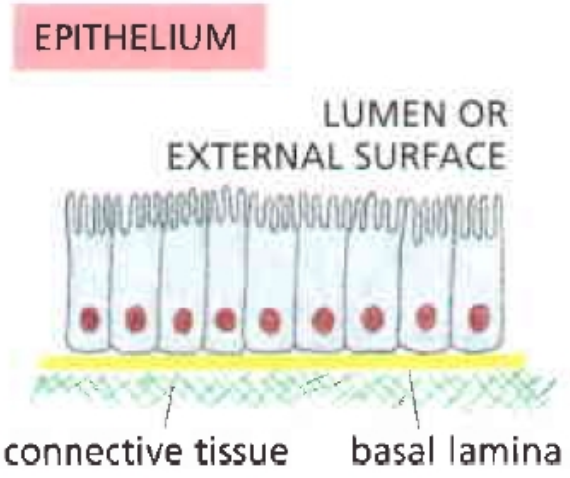


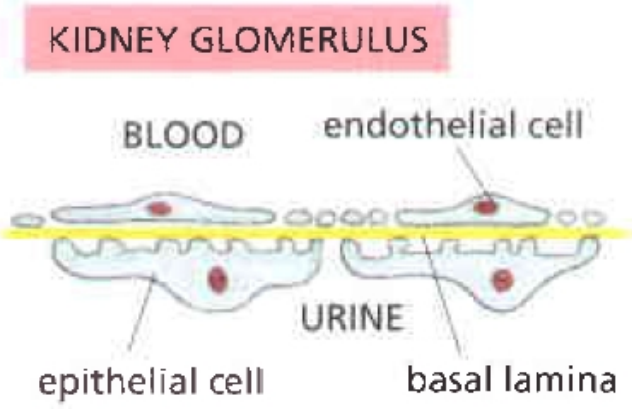
BASAL LAMINA

- 40-120 nm thick
- Types of organization:
  - surrounding cells
  - underlying cells
  - interposing between cells
- Disease: junctional epidermolysis bullosa

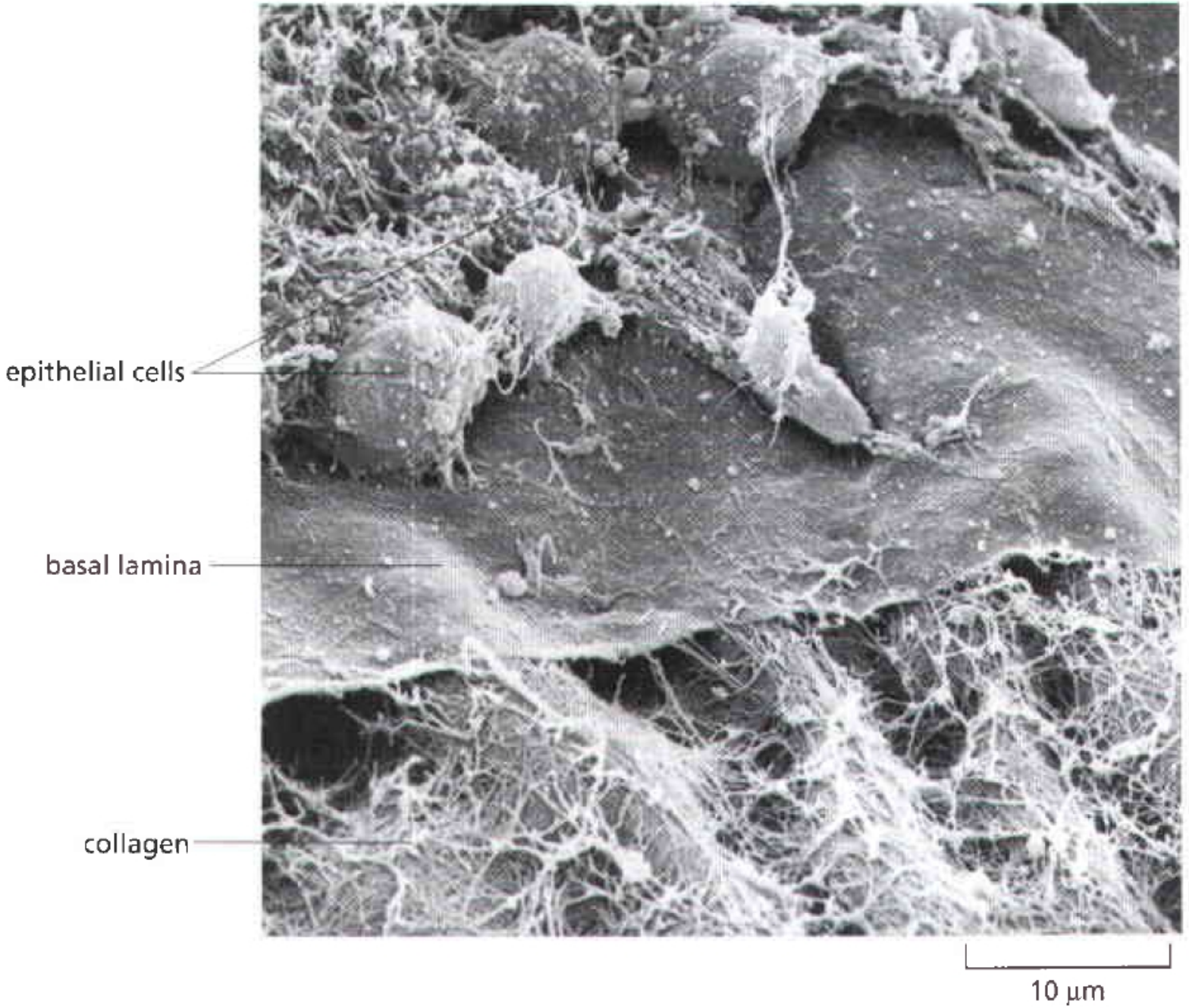
- Function:
  - cells separation
  - cells mechanical connections
  - metabolism
  - membrane proteins organization
  - survival
  - proliferation
  - migration





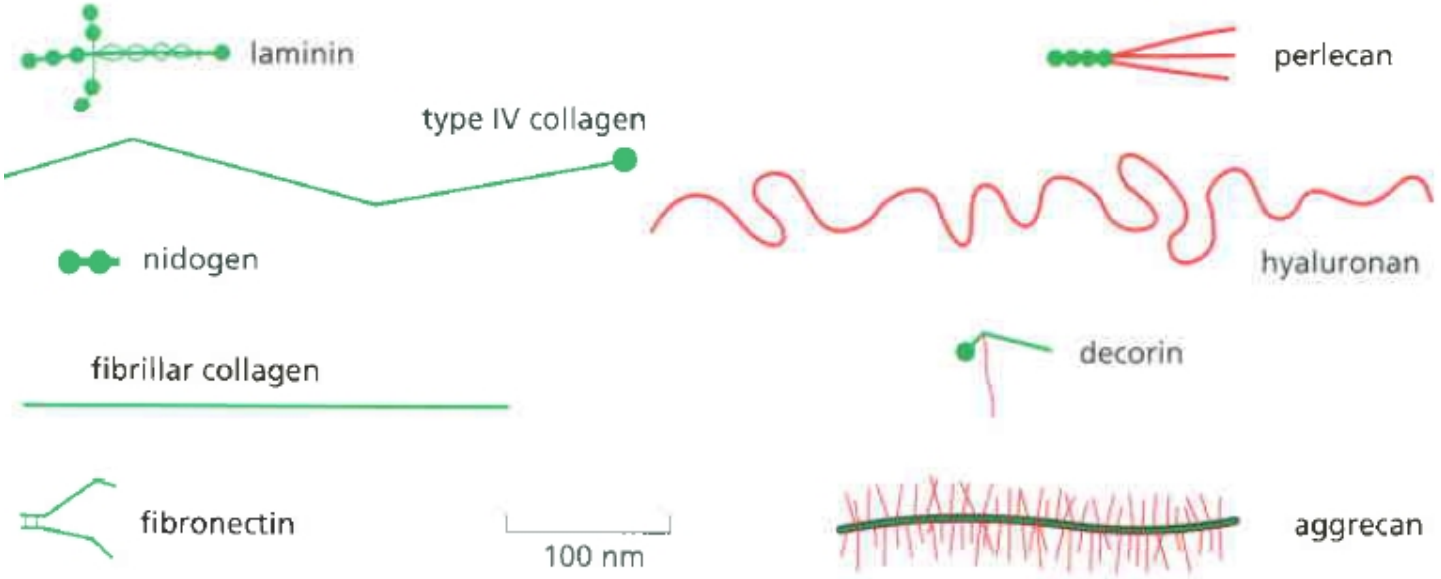


BASAL LAMINA



MAJOR MACROMOLECULES OF BASAL LAMINA

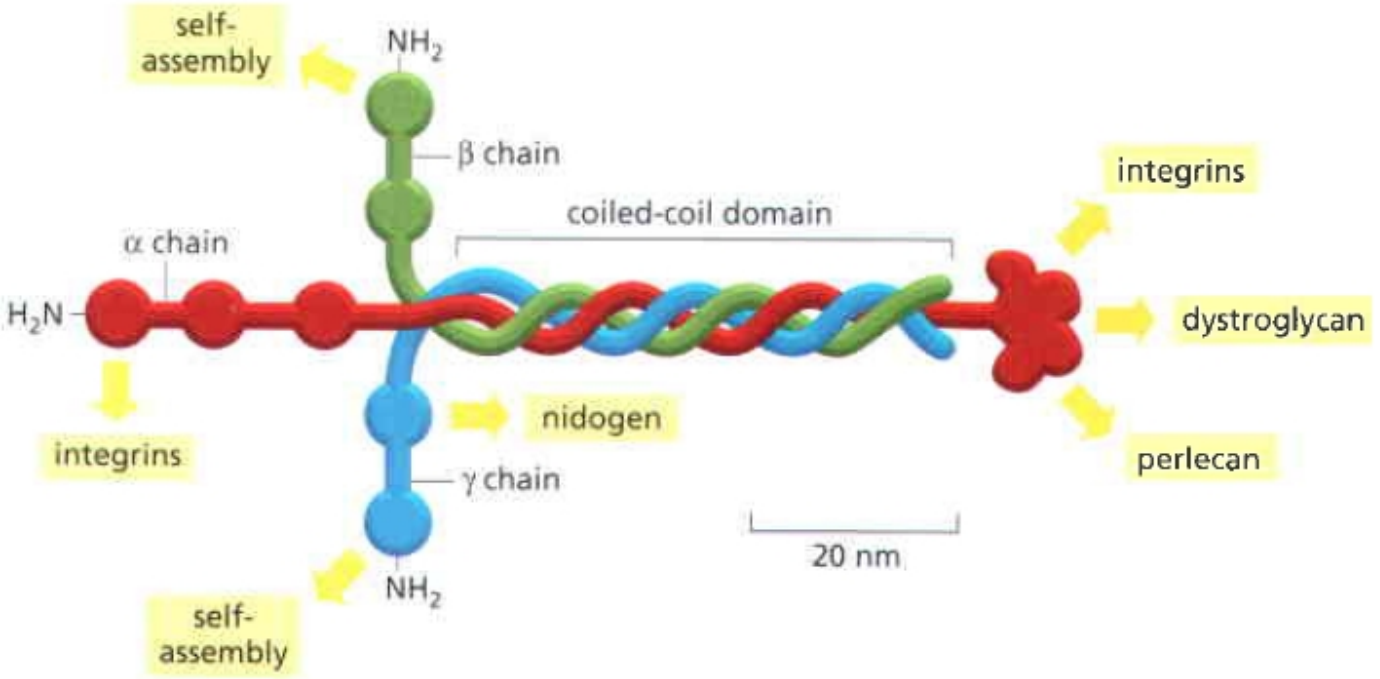
- **Glycoproteins:**
  - **laminin**
  - **type IV collagen**
  - **nidogen**
- **Proteoglycans:**
  - **perlecan**
  - **collagen XVIII**
- **Fibronectin**

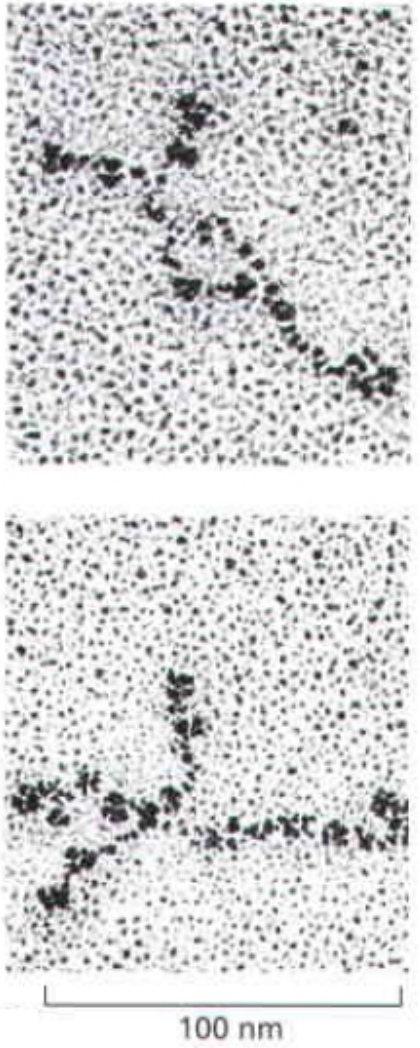


Macromolecules in ECM

LAMININ

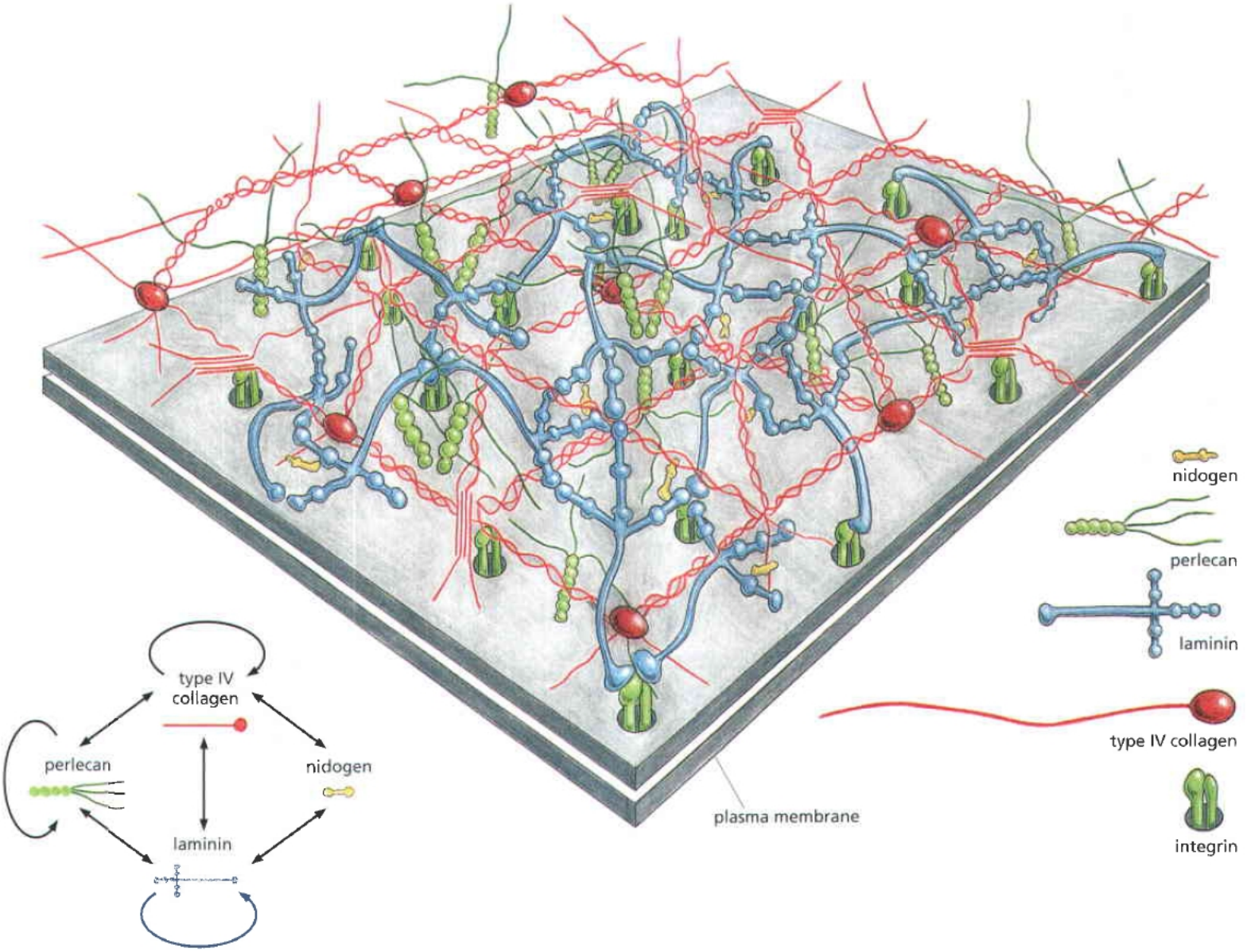
- Primary organizer of the sheet structures
- Three chains: α, β, γ
- 45 isoforms (5 x 3 x 3)
- Held by disulfide bridges





TYPE IV COLLAGEN

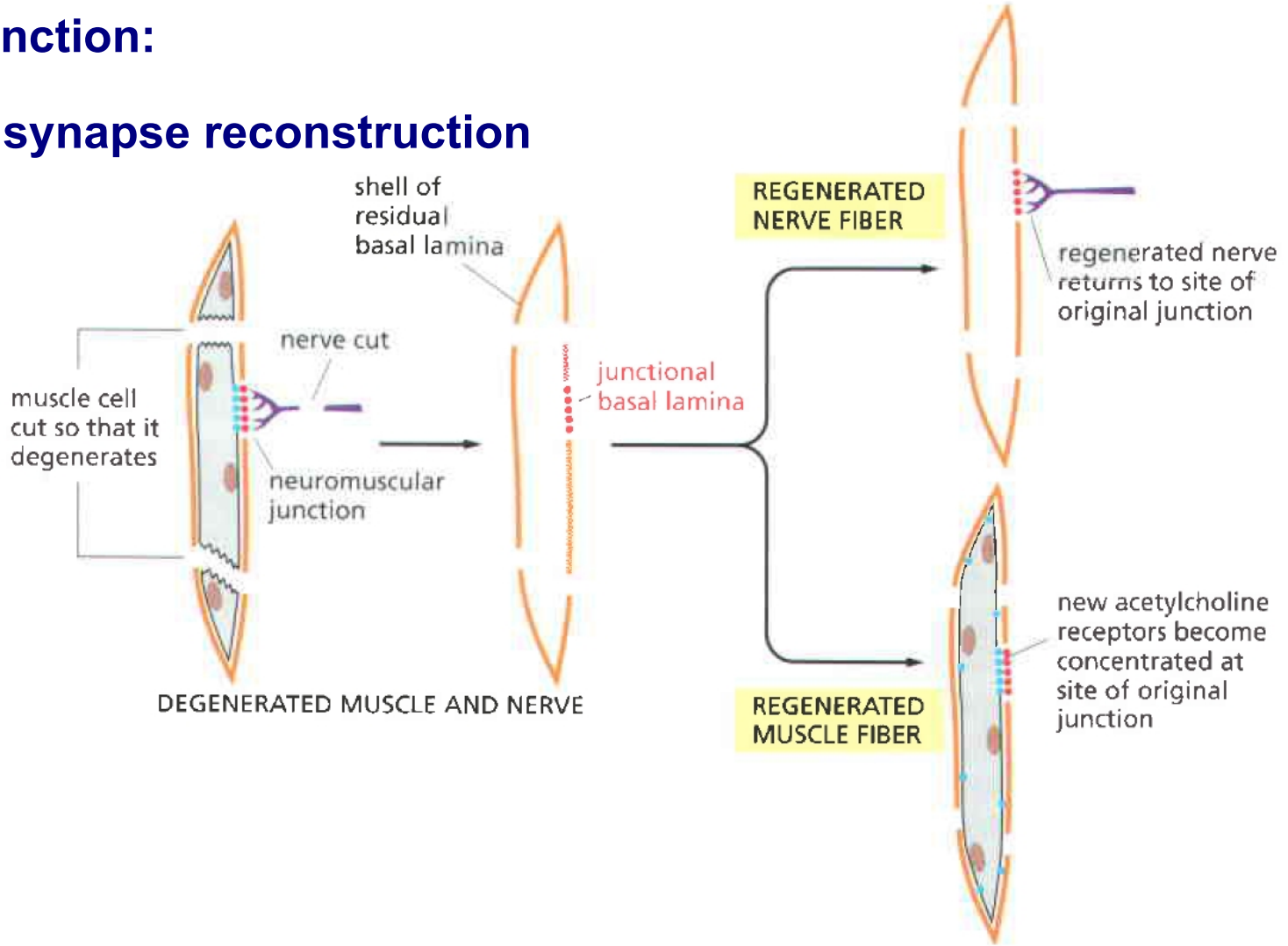
- Three-chained filaments
- Multiple bends



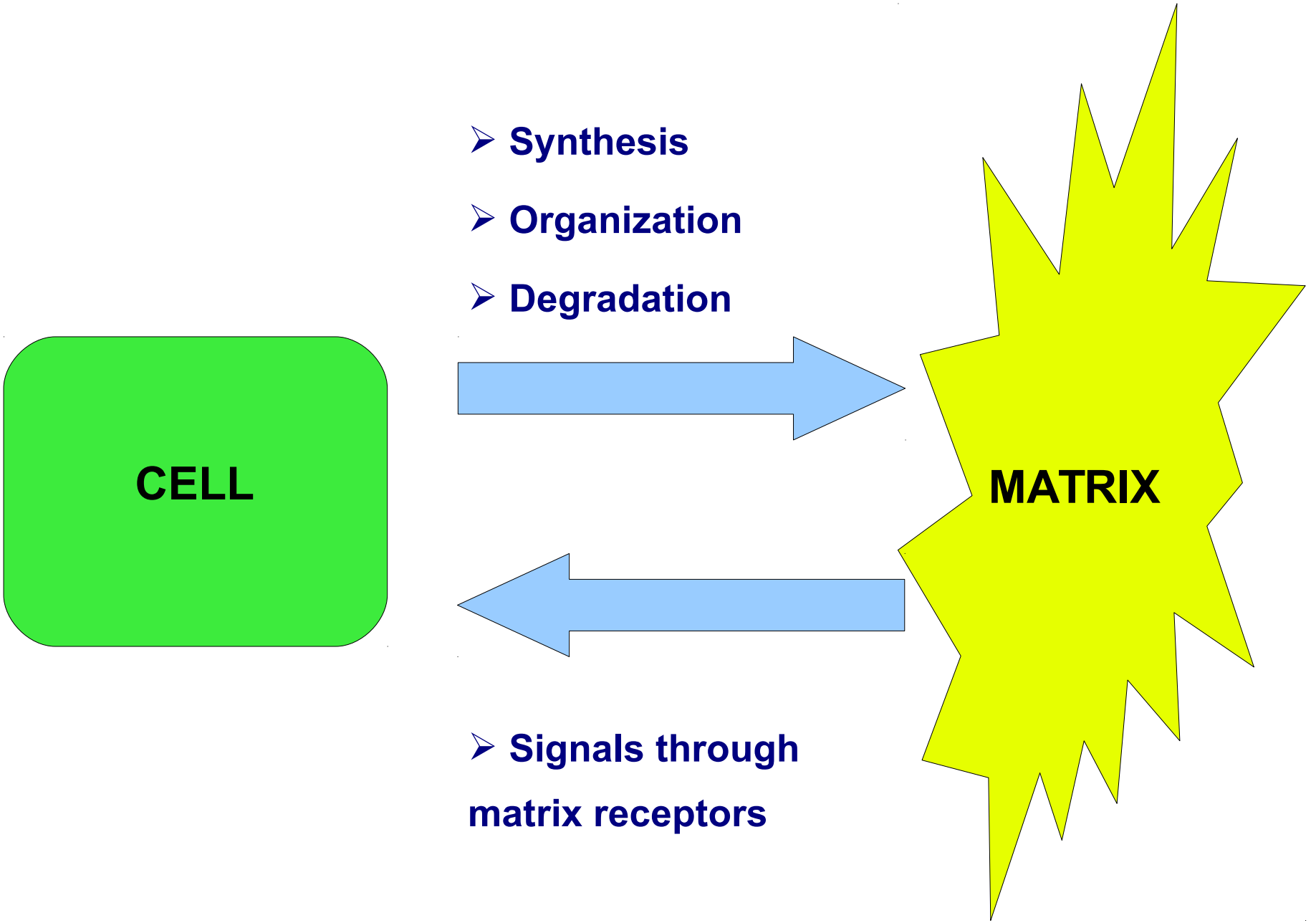
EXAMPLES OF BASAL LAMINA FUNCTIONS

- Bladder: holding blood from entering urine
- Kidney's filtering
- Filtering cells to contact epithelia: fibroblasts - “no”, macrophages - “yes”
- Neuromuscular junction:

lamina-dependent synapse reconstruction

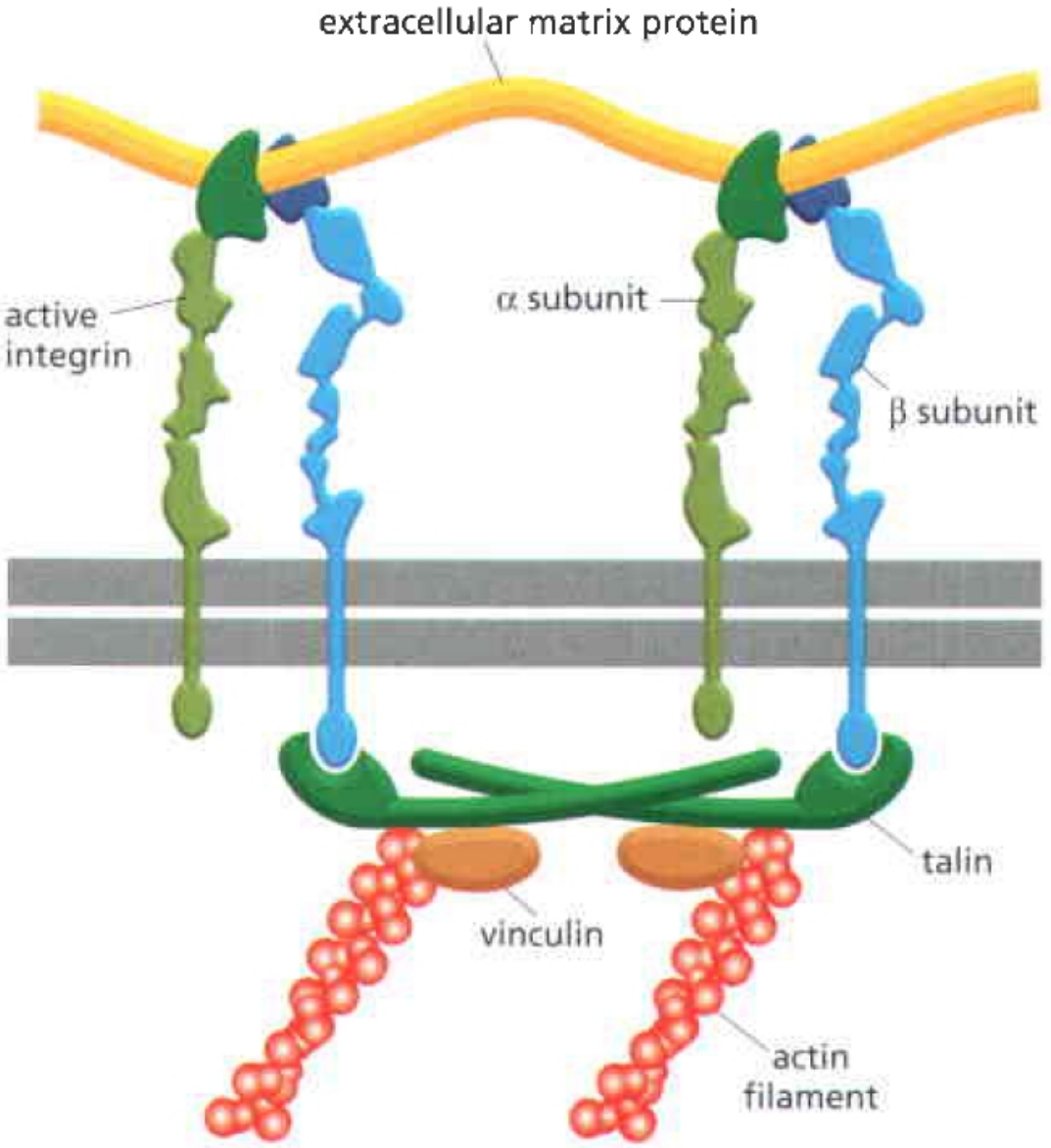


CELL-MATRIX ADHESION

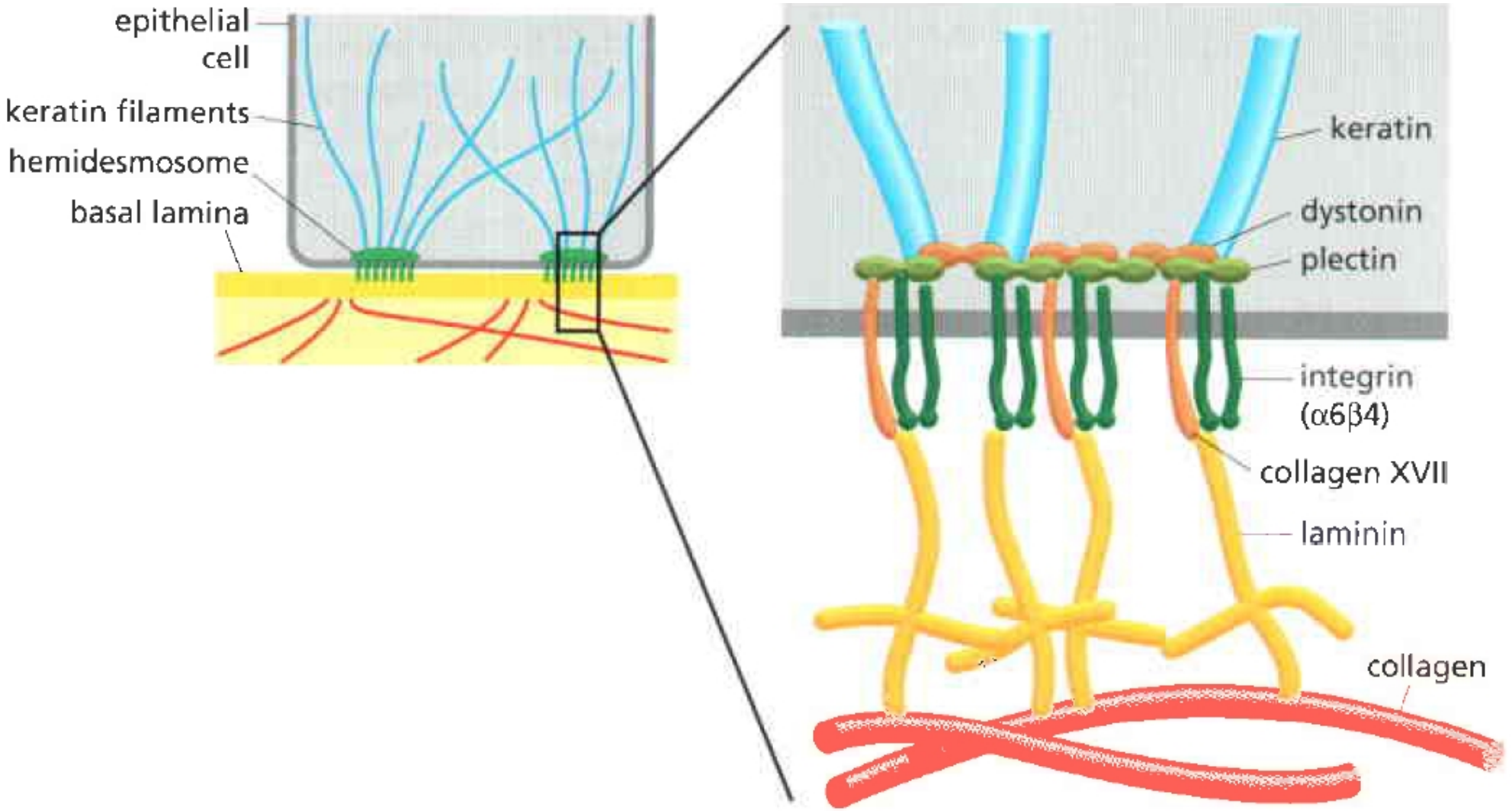


INTEGRINS

- **Function:**
  - **Homophilic interactions (both directions)**
  - **Mechanical signal**
  - **Molecular signal**
  - **Signal interconversion**
- **TM heterodimeric glycoprotein**
- **24 types => specificity:**
  - **N-terminal interactors**
  - **C-terminal interactors**



INTEGRINS IN HEMIDESMOSOMES

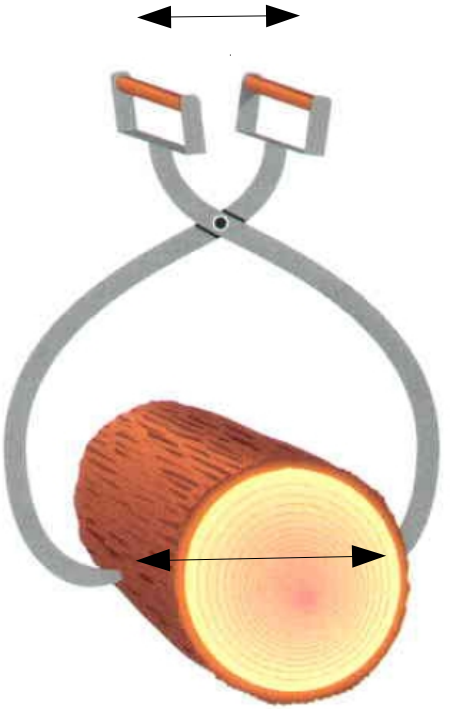


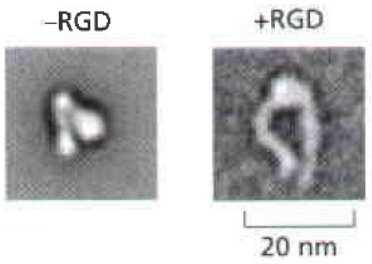
INTEGRINS IN ACTIVE AND INACTIVE STATES

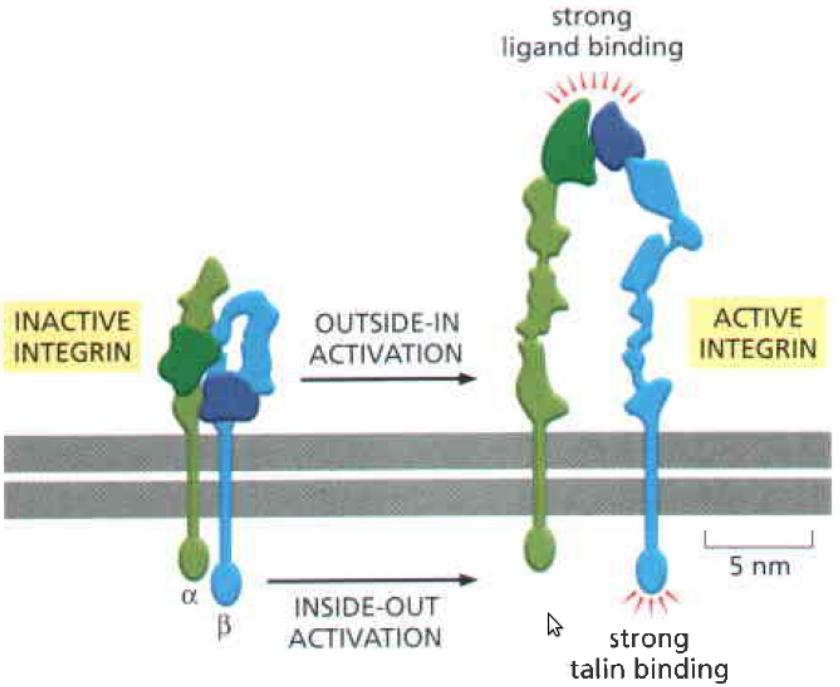
- Cells establish/break attachments to ECM

=> dynamic integrin

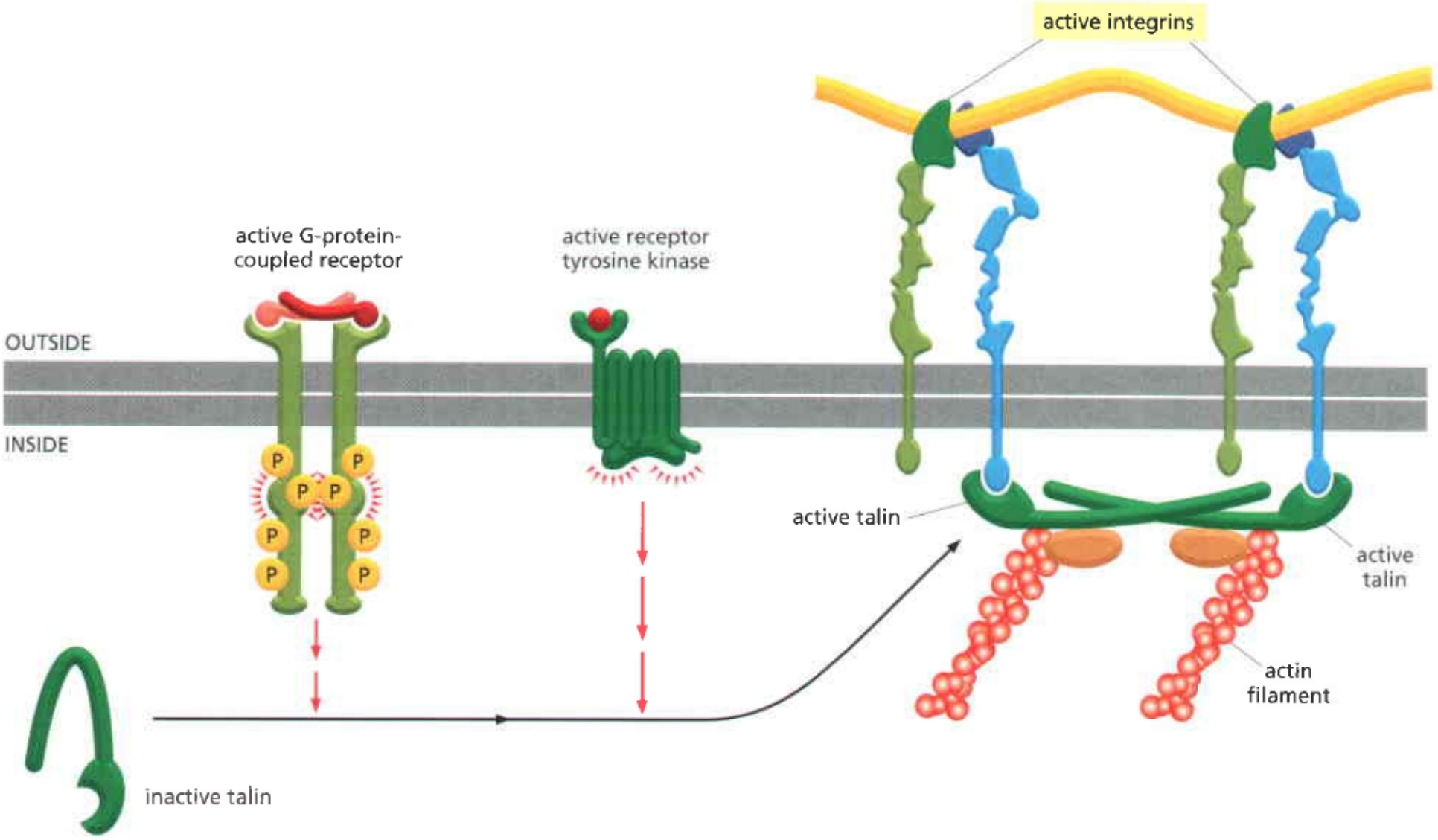
- Cytoskeleton assembles/disassembles in the cell
- Ligands binding by integrin => allosteric regulation
- RGD peptide as an extracellular ligand
- PIP_2 as an intracellular ligand







INTEGRINS AND SIGNALING PATHWAYS



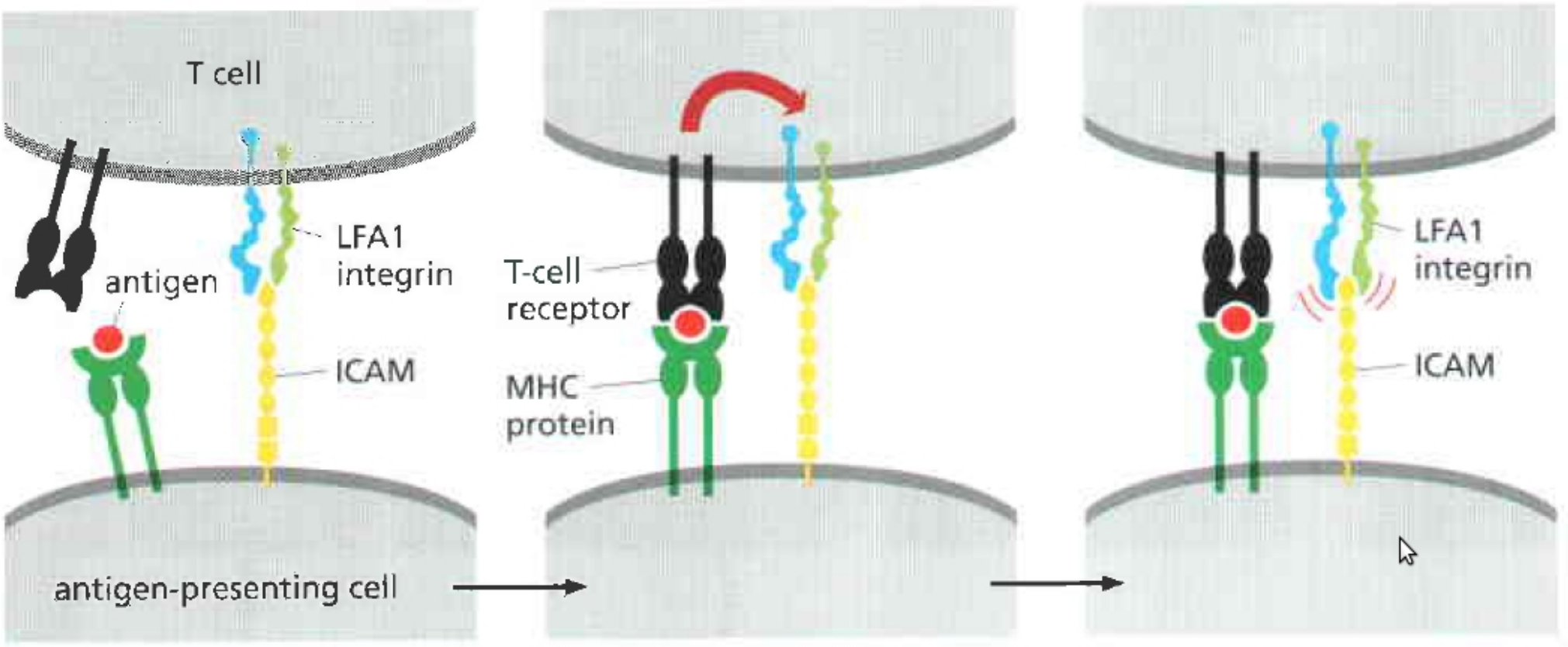
INTEGRIN TYPES => SPECIFICITY

- 24 types:
  - 18 α-gene
  - 8 β-genes
- Different ligands
- Ca^{2+} and Mg^{2+} binding domains

INTEGRIN	LIGAND	DISTRIBUTION	PHENOTYPE WHEN α SUBINUT IS MUTATED	PHENOTYPE WHEN β SUBUNIT IS MUTATED
α5β1	fibronectin	ubiquitous	death of embryo; defects in blood vessels, somites, neural crest	early death of embryo (at implantation)
α6β1	laminin	ubiquitous	severe skin blistering; defects in other epithelia also	early death of embryo (at implantation)
α7β1	laminin	muscle	muscular dystrophy; defective myotendinous junctions	early death of embryo (at implantation)
αLβ2 (LFA1)	Ig superfamily counterreceptors (ICAM)	white blood cells	impaired recruitment of leucocytes	leucocyte adhesion deficiency (LAD) impaired inflammatory responses; recurrent life-threatening infections
αIIbβ3	fibrinogen	platelets	bleeding; no platelet aggregation (Glanzmann's disease)	bleeding; no platelet aggregation (Glanzmann's disease); mild osteopetrosis
α6β4	laminin	hemidesmosomes in epithelia	severe skin blistering; defects in other epithelia also	severe skin blistering; defects in other epithelia also

INTEGRIN IN T-CELL MECHANISMS

- **Integrin-ICAM interaction stabilized the adhesion of the cells providing more effective T-cell receptor/MHC protein recognition**

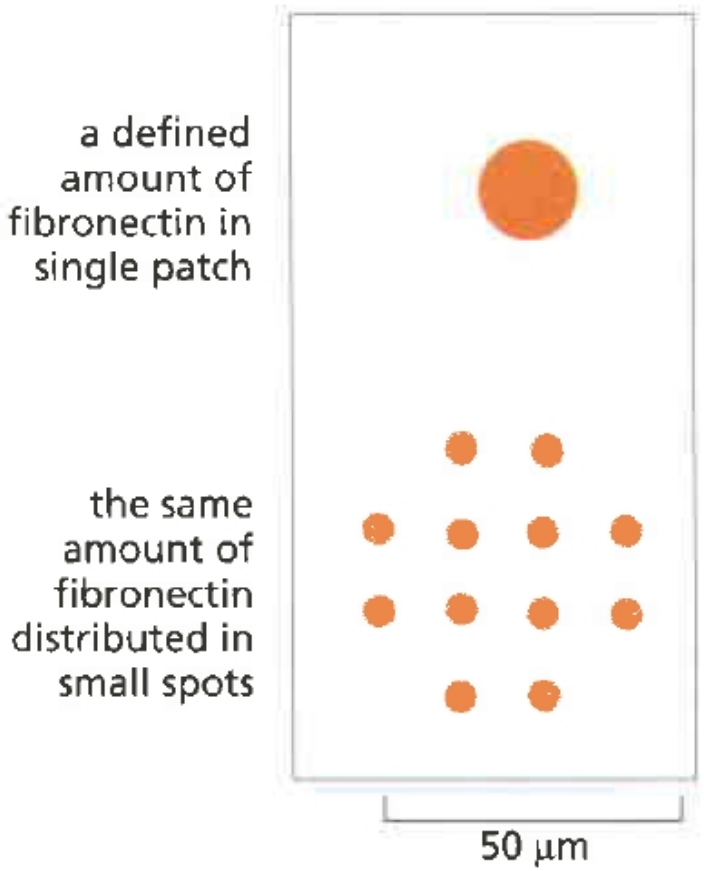


INTEGRIN ADHESION FEATURES

- **Amplification (~ 100 integrin contacts)**
- **Anchorage dependence:**
  - **cell proliferates in proper environment (f.i. Ras/MAP)**
  - **apoptosis in improper environment**

  } **integrin-mediated signaling**

- **Cells spreading**

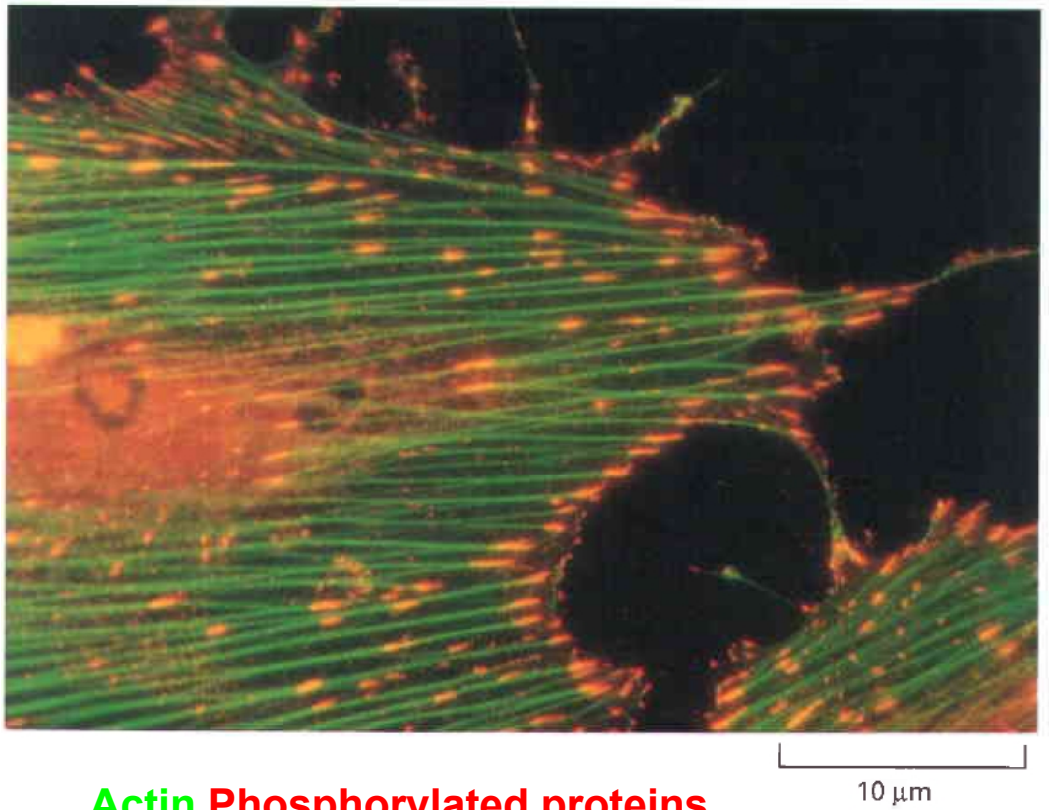


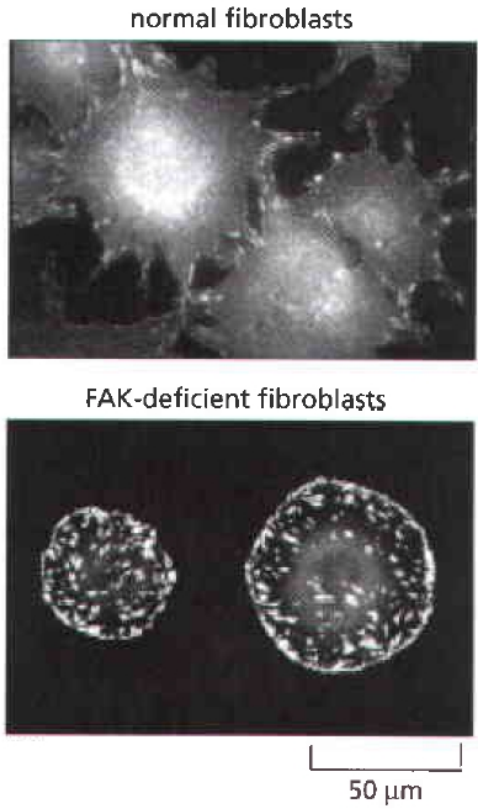
CELL DIES BY APOPTOSIS

CELL SPREADS, SURVIVES, AND GROWS

INTEGRIN IN SIGNALING

- **Focal adhesion kinase (FAK) assist disassembling focal adhesion:**
  - **Tyr-kinase**
  - **binds to talin (binds in turn to β-integrin subunit) or paxillin (α-integrin subunit)**
  - **cross-phosphorylation**
  - **docking of Src kinases**
  - **elevated expression in cancer**



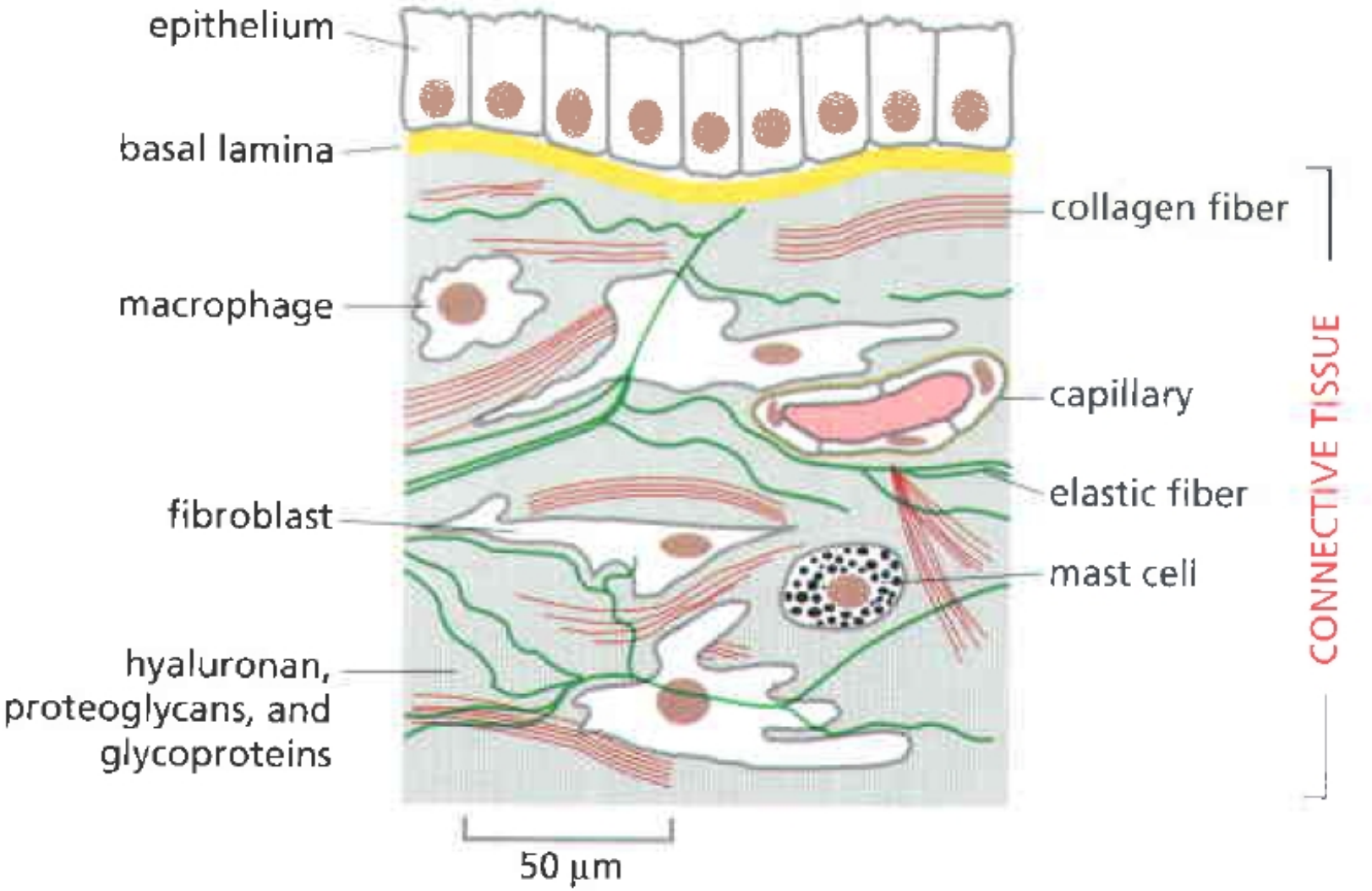


ADHESION MOLECULES: SUMMARY

	SOME FAMILY MEMBERS	Ca^{2+} OR Mg^{2+} DEPENDENCE	HOMOPHILIC OR HETEROPHILIC	CYTOSKELETON ASSOCIATIONS	CELL JUNCTION ASSOCIATIONS
Cell–Cell Adhesion					
Classical cadherins	E, N, P, VE	yes	homophilic	actin filaments (via catenins)	adherens junctions, synapses
Desmosomal cadherins	desmoglein, desmocollin	yes	homophilic	intermediate filaments (via desmoplakin, plakoglobin, and plakophilin)	desmosomes
Ig family members	N-CAM, ICAM	no	both	unknown	neuronal and immunological synapses
Selectins (blood cells and endothelial cells only)	L-, E-, and P-selectins	yes	heterophilic	actin filaments	(no prominent junctional structure)
Integrins on blood cells	αLβ2 (LFA1)	yes	heterophilic	actin filaments	immunological synapses
Cell–Matrix Adhesion					
Integrins	many types	yes	heterophilic	actin filaments (via talin, paxillin, filamin, α-actinin, and vinculin)	focal adhesions
	α6β4	yes	heterophilic	intermediate filaments (via plectin and dystonin)	hemidesmosomes
Transmembrane proteoglycans	syndecans	no	heterophilic	actin filaments	(no prominent junctional structure)

EXTRACELLULAR MATRIX IN CONNECTIVE TISSUES UNDERLYING EPITHELIUM

- **Mechanics: different stiffness (calcified vs. gels)**
- **Function: complex regulation**

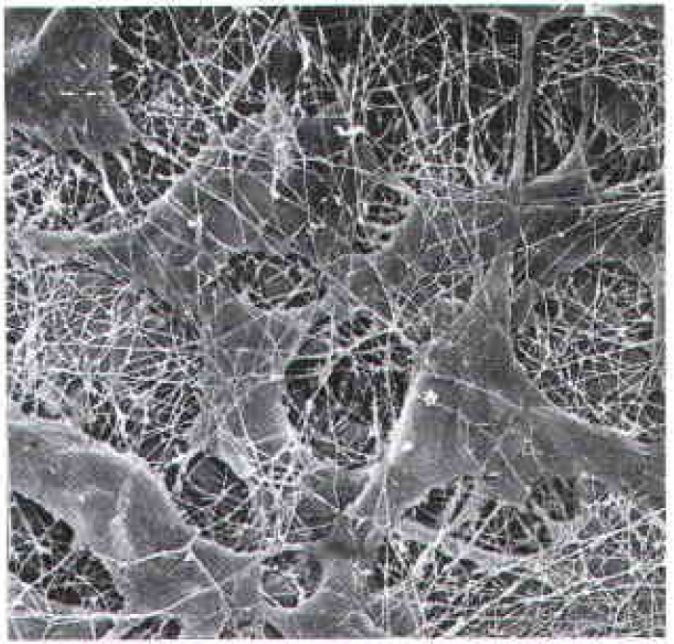


EXTRACELLULAR MATRIX MAJOR COMPONENTS

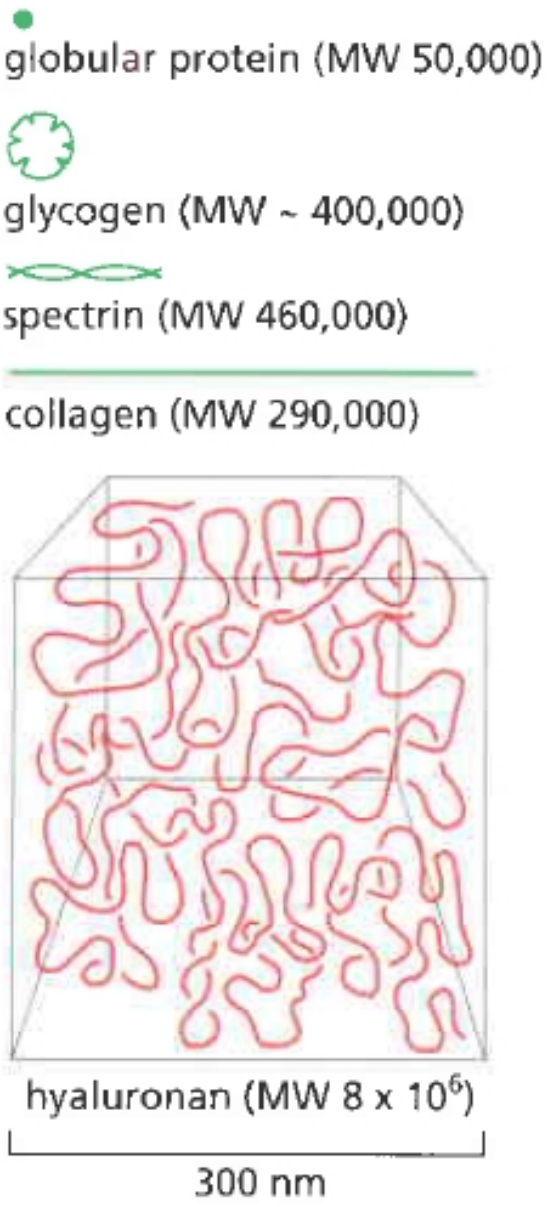
- **Fibroblasts (chondroblasts, osteoblasts)**
- **Non-cellular components:**

  - glycosaminoglycans

  - fibrous proteins (collagen, elastin)

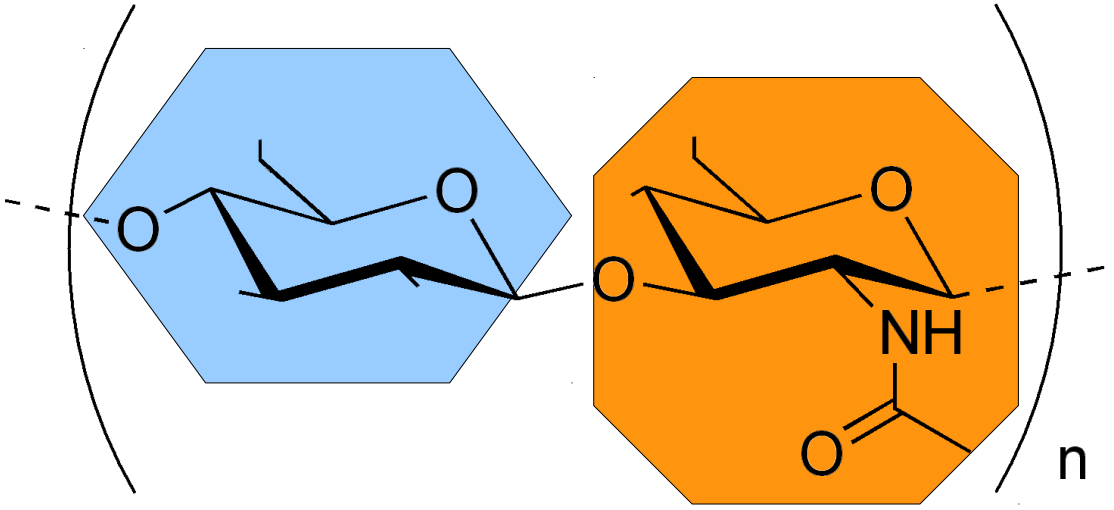


Fibroblasts and collagen in rat cornea



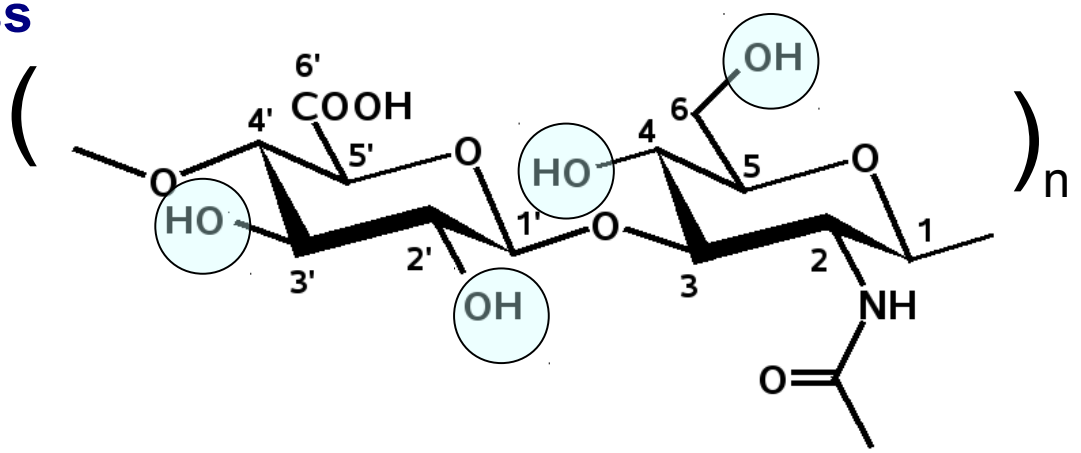
GLYCOSAMINOGLYCANS

- 10% of ECM
- Classes:
  - Hyaluronan (free)
  - Chondroitin sulfate
  - Dermatan sulfate
  - Keratan sulfate
  - Heparin
  - Heparan sulfate
- Na^+ bound => osmotic press



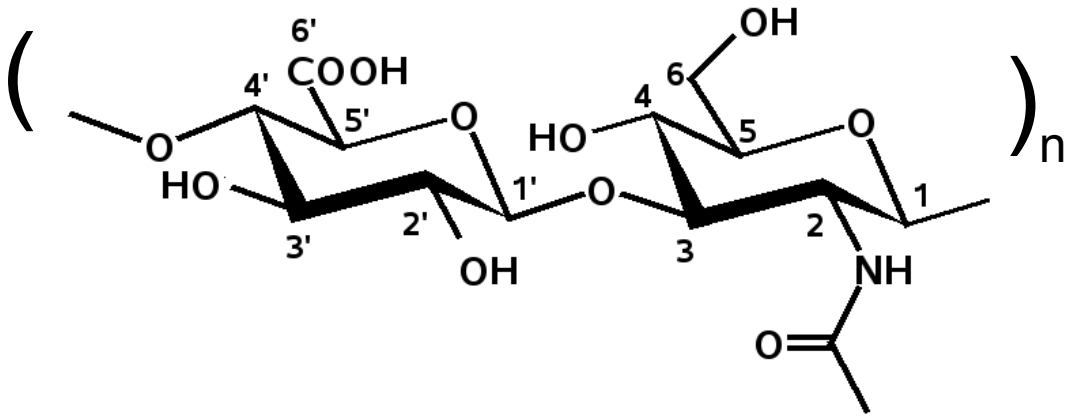
Hexose/Hexuronic acid:
- GlcU
- IdoU
- Gal
- Sulfated derivatives

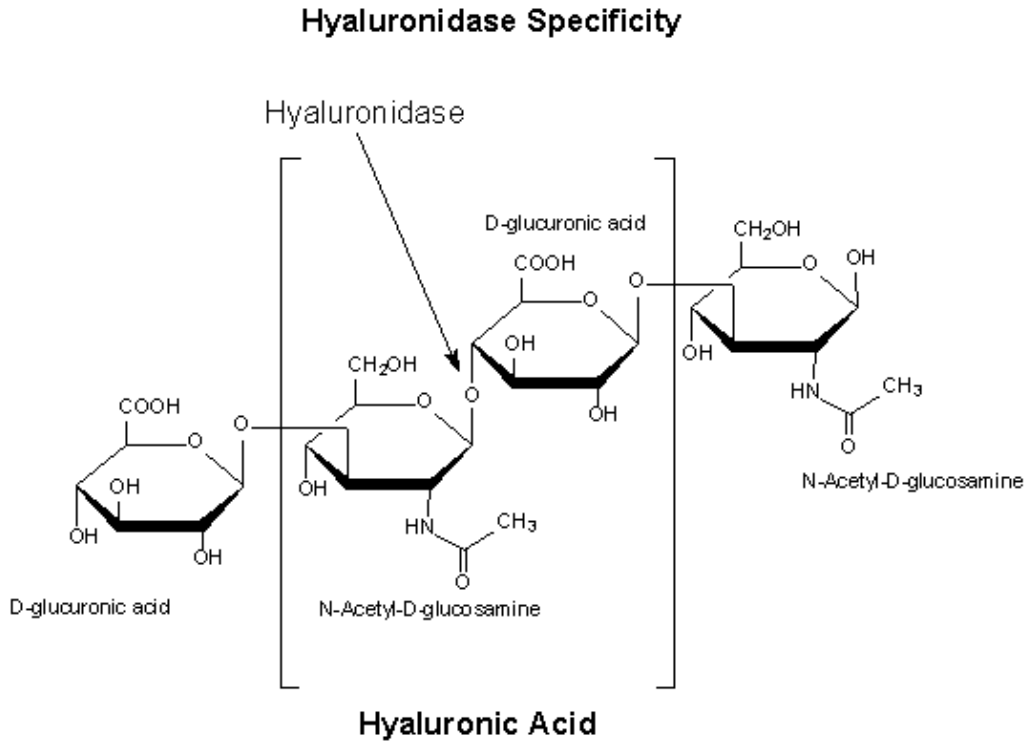
Hexosamine:
- GlcNAc
- GalNAc
- Sulfated derivatives



HYALURONAN

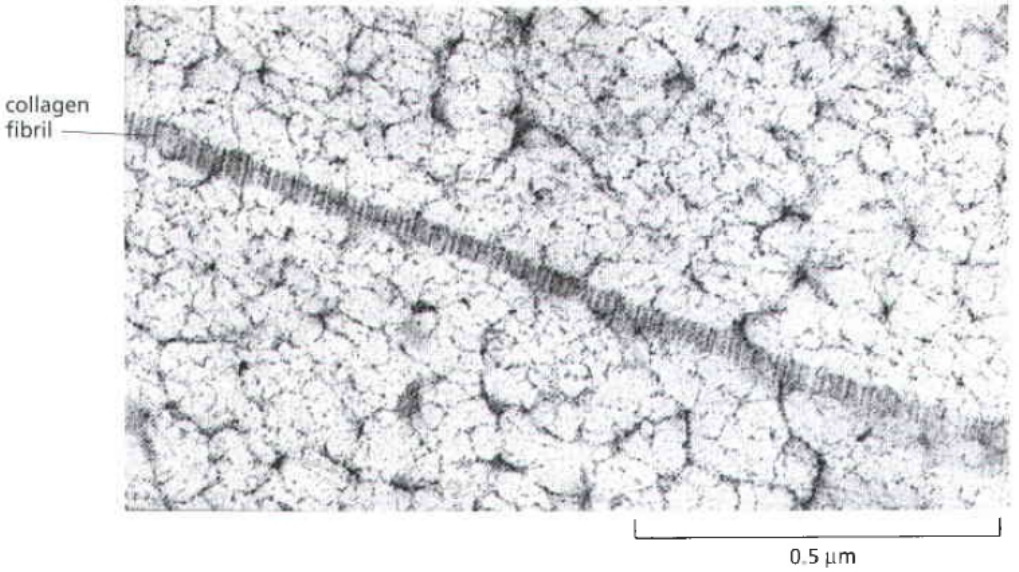
- $(GlcNAc\text{-}GlcU)_n$
- ~ 25 000 disaccharide units
- Ubiquitous
- Contains sulfated blocks
- Released from enzyme complex in plasma membrane
- Processed by hyuluronases
- Function:
  - joints
  - embryonic development
  - heart chambers

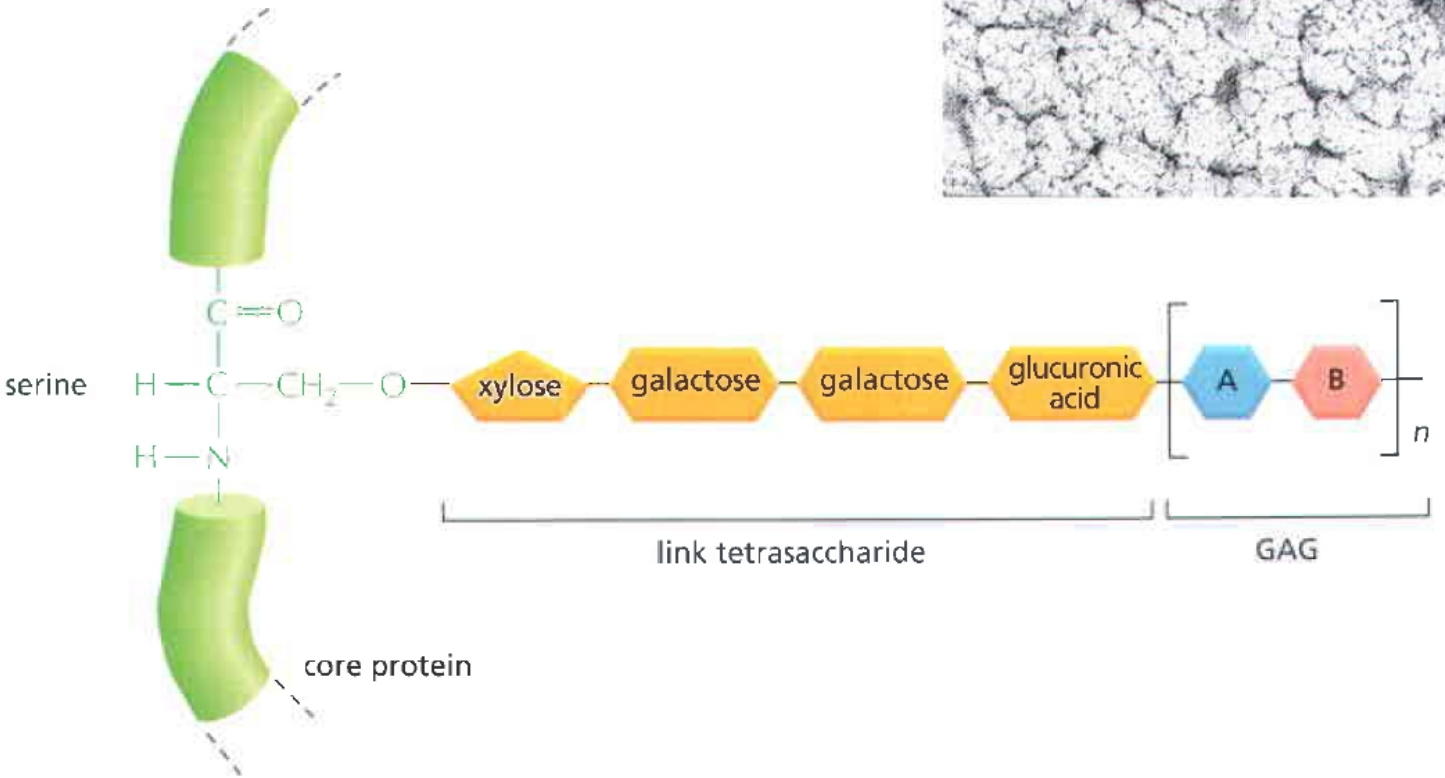




GAGS IN PROTOGLYCANS

- All except hyaluronan are parts of proteoglycans
- ER: core protein
- GA: Ser => linked tetrasaccharide, GAG
- GAG ~ 95% of weight
- ~ 40 disaccharide units



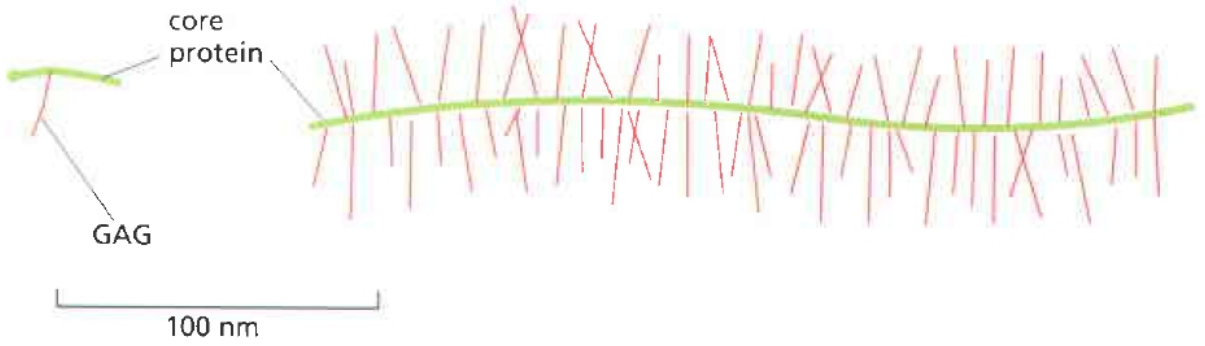


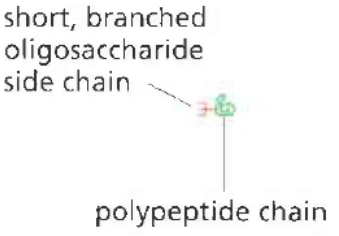
VARIETY OF PROTEOGLYCANS

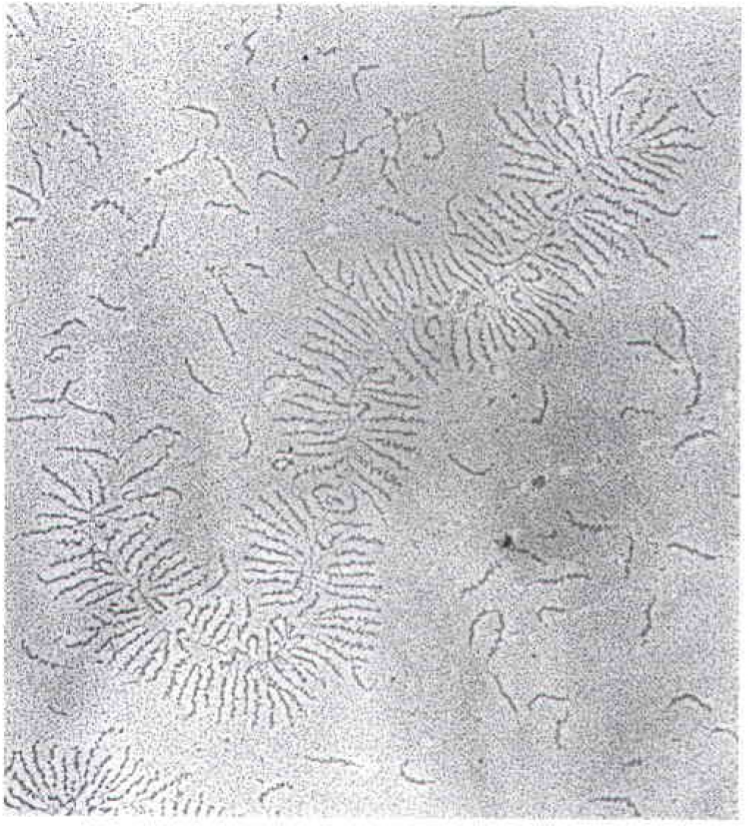
DECORIN
(MW ~ 40,000)

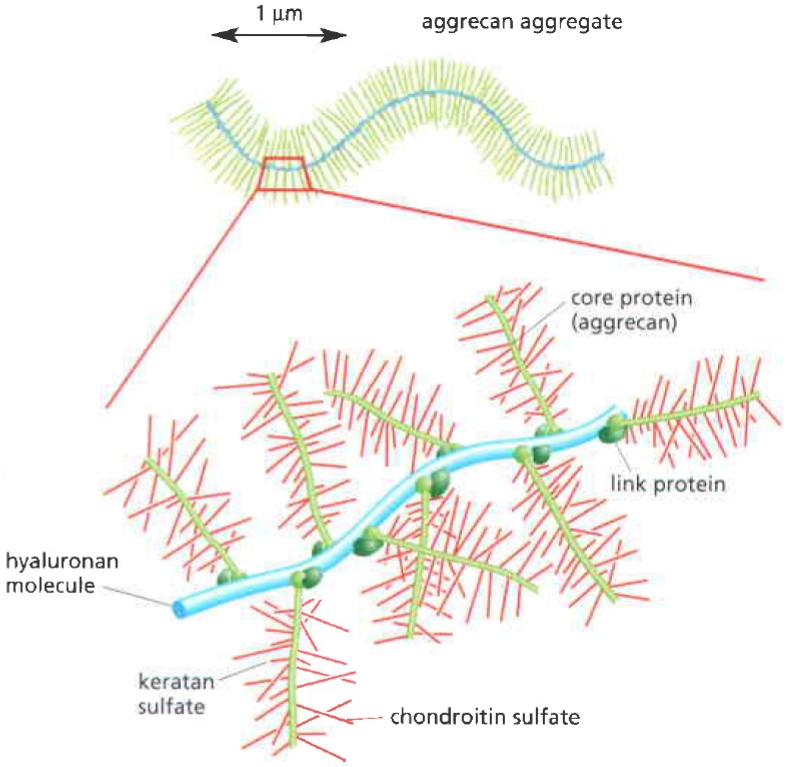
AGGRECAN
(MW ~ 3×10^6)

RIBONUCLEASE
(MW ~ 15,000)







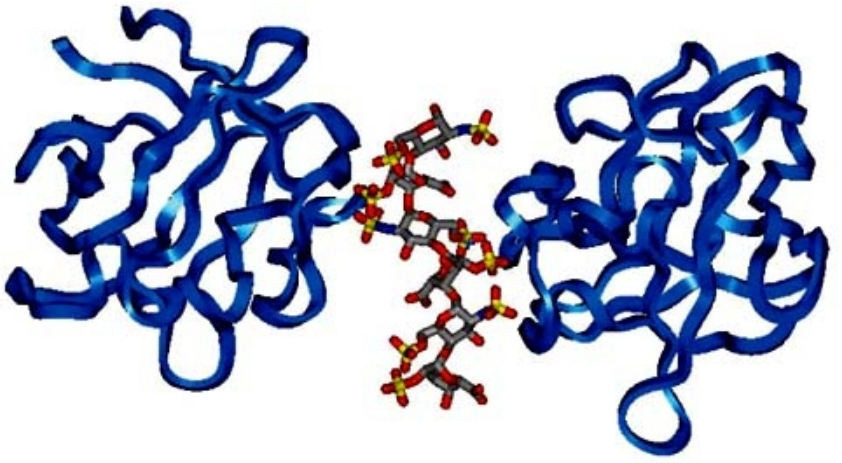


VARIETY OF PROTEOGLYCANS

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 *Drosophila*)	60,000	heparan sulfate	1–3	cell surface	co-receptor for Wingless and Decapentaplegic signaling proteins

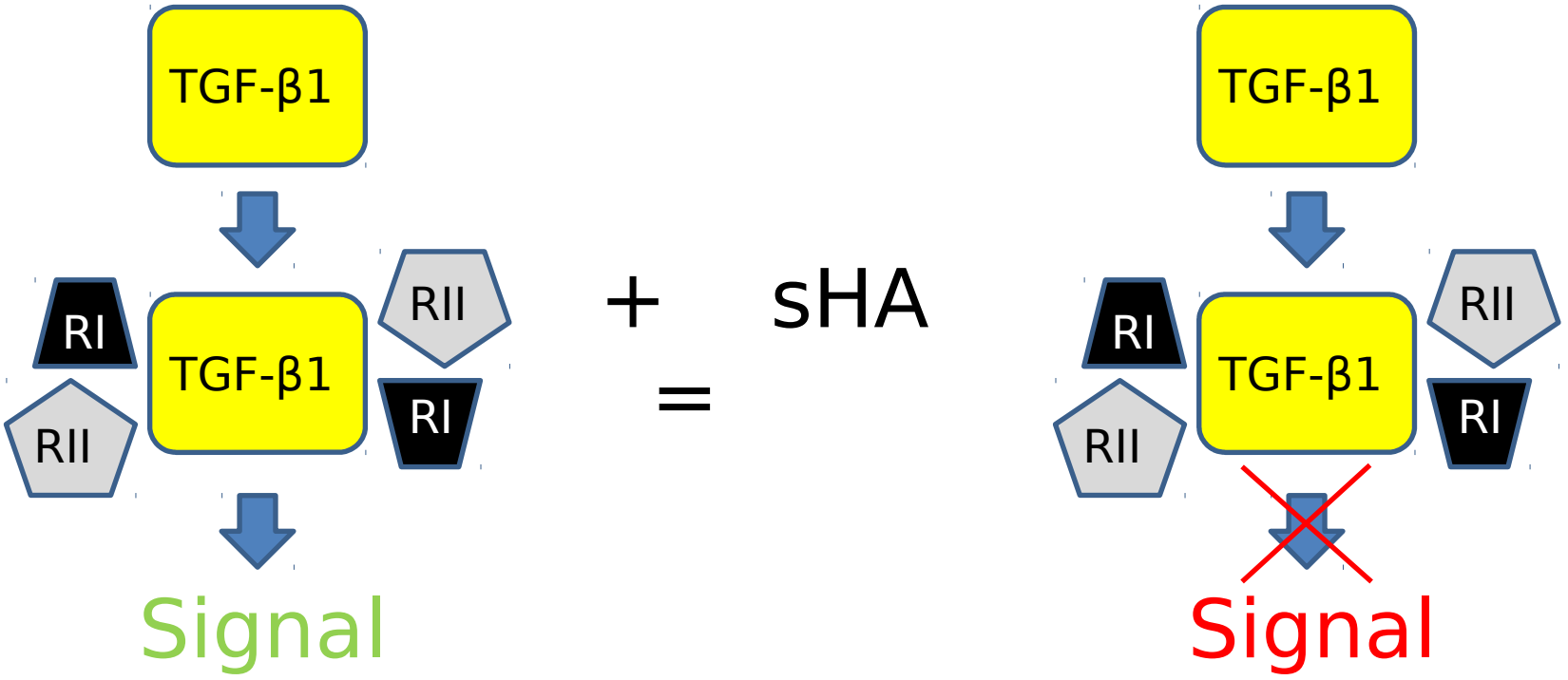
FUNCTIONS OF PROTEOGLYCANS

- **Regulation of secreted proteins trafficking:**
  - **size**
  - **charge**
- **Signaling:**
  - **growth factors binding**
  - **chemokines binding**
- **Co-receptors: FGF/FGFR complexes**
- **Interactions with cytoskeleton and cellular cortex (syndecans)**



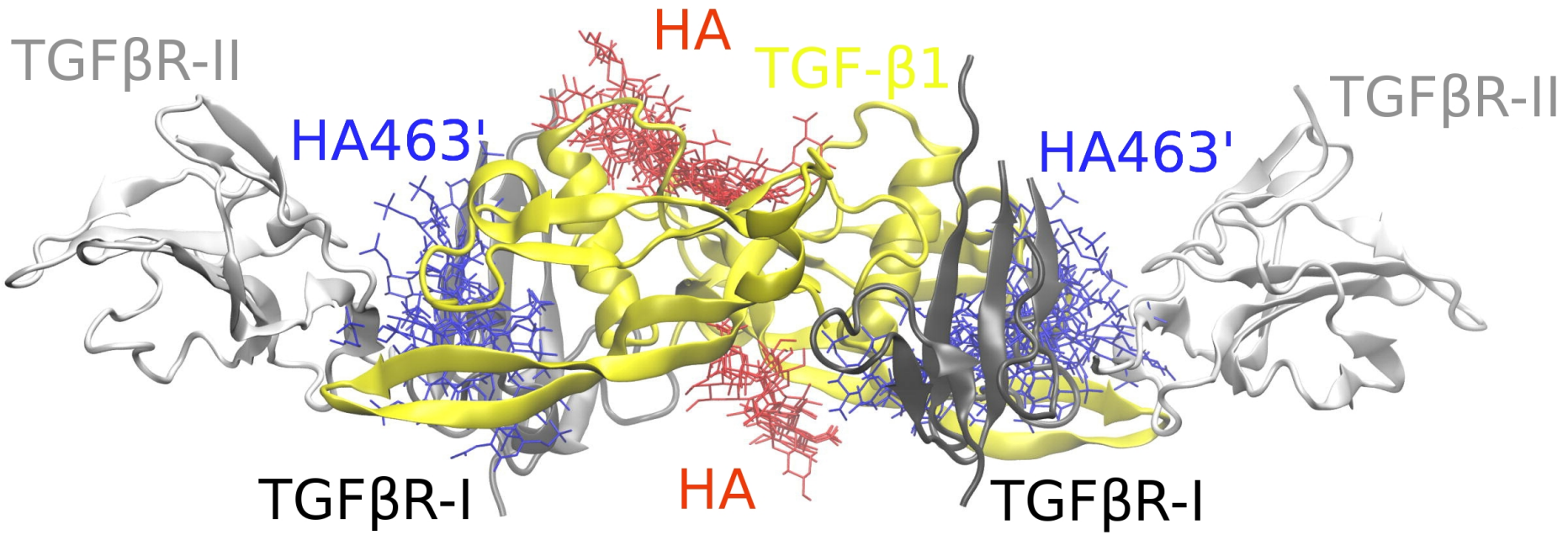
FGF/heparin sandwich

FUNCTIONS OF PROTEOGLYCANS: TGF-β EXAMPLE



sHA = sulfated hyaluronan

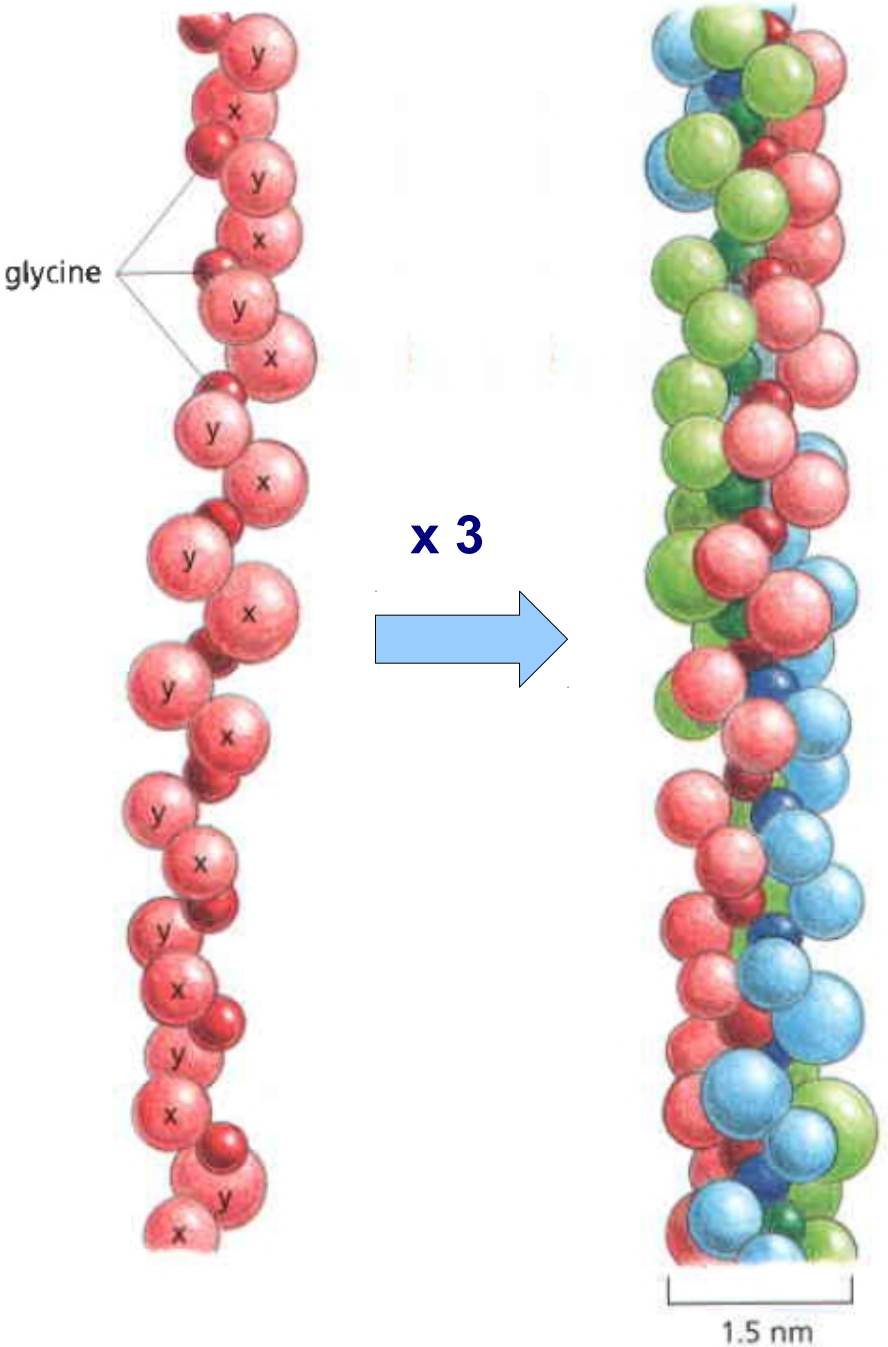
FUNCTIONS OF PROTEOGLYCANS: TGF-β EXAMPLE



Depending on sulfation, HA can block receptor binding sites on TGF-β.

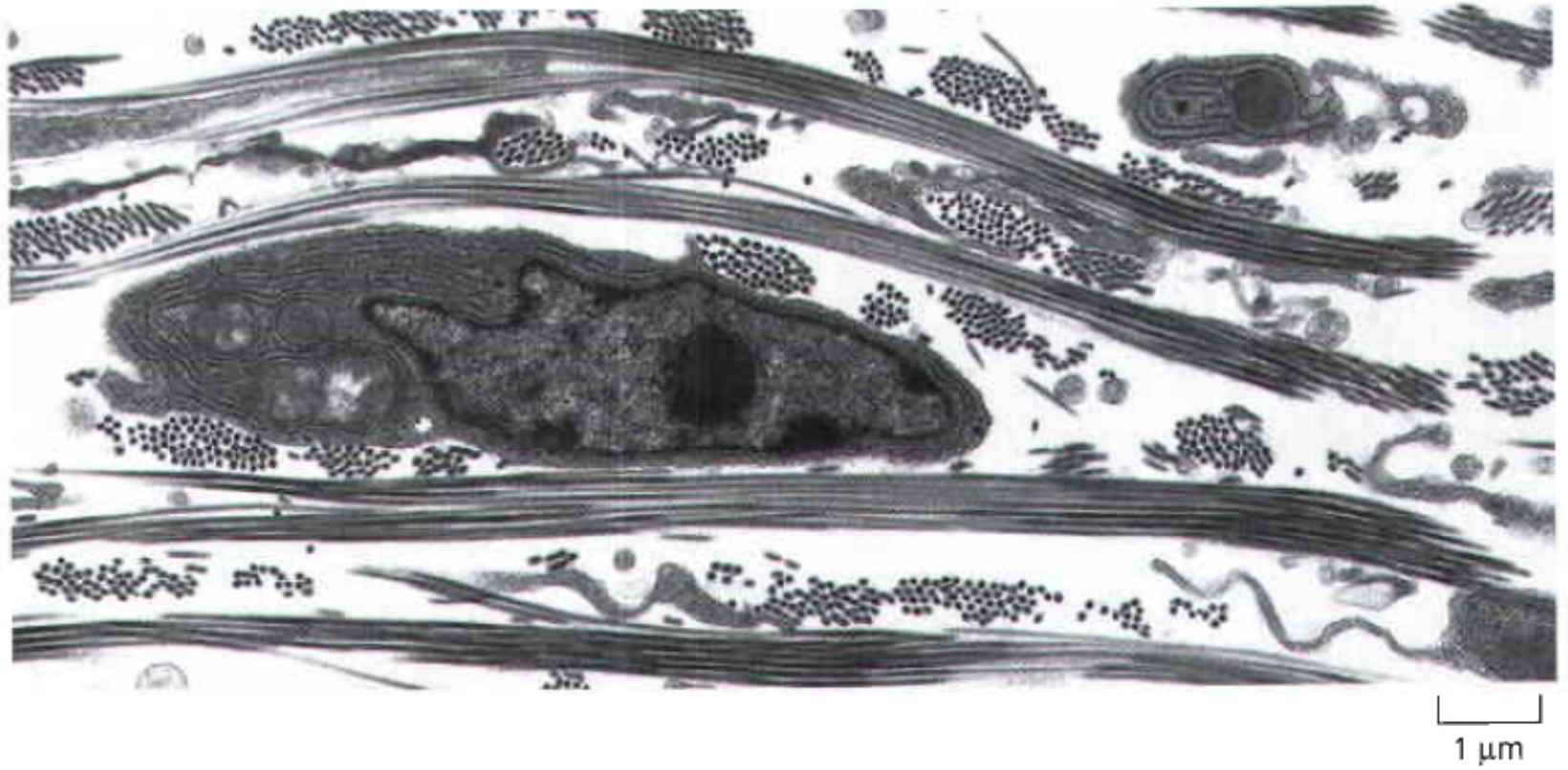
COLLAGEN

- Collagens: family of fibrous proteins
- ~ 25% in skin and bone
- Triple helix of left-handed α-helices
- (Gly-X-Y): most X are Pro, Y hydroxyPro
- Chain ~ 1000 aa
- 42 genes
- 40 types of collagen known



COLLAGEN

- Collagen fibrils => collagen fibers
- Types classification:
  - fibril-associated collagens: IX, XII
  - network dorming: IV
  - anchoring fibrils: VII

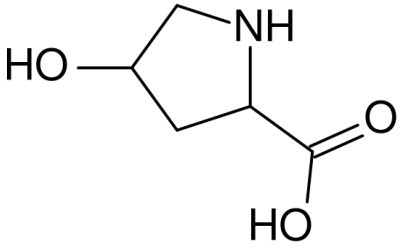


COLLAGEN TYPES

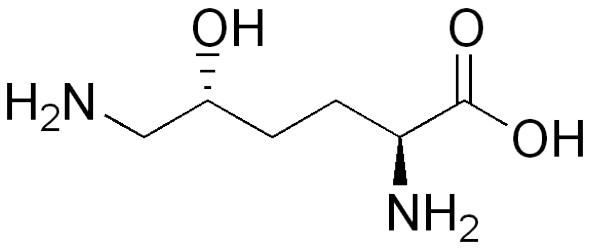
	TYPE	POLYMERIZED FORM	TISSUE DISTRIBUTION	MUTANT PHENOTYPE
Fibril-forming (fibrillar)	I	fibril	bone, skin, tendons, ligaments, cornea, internal organs (accounts for 90% of body collagen)	severe bone defects, fractures
	II	fibril	cartilage, invertebral disc, notochord, vitreous humor of the eye	cartilage deficiency, dwarfism
	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 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	non-fibrillar	hemidesmosomes	skin blistering
Proteoglycan core protein	XVIII	non-fibrillar	basal lamina	myopia, detached retina, hydrocephalus

COLLAGEN POSTTRANSLATIONAL MODIFICATIONS

- **Pro-α chains:**
  - **ER-signal**
  - **N- and C-propeptides**
- **ER: hydroxylation of Pro and Lys**
- **Glycosylation of some hydroxyLys**
- **Procollagen: triple helix**
- **Prosignals are cut after secretion:**
  - **localization signal**
  - **prevent fibrillization in the cell**
- **Formation of collagen fibrils from tropocollagen**

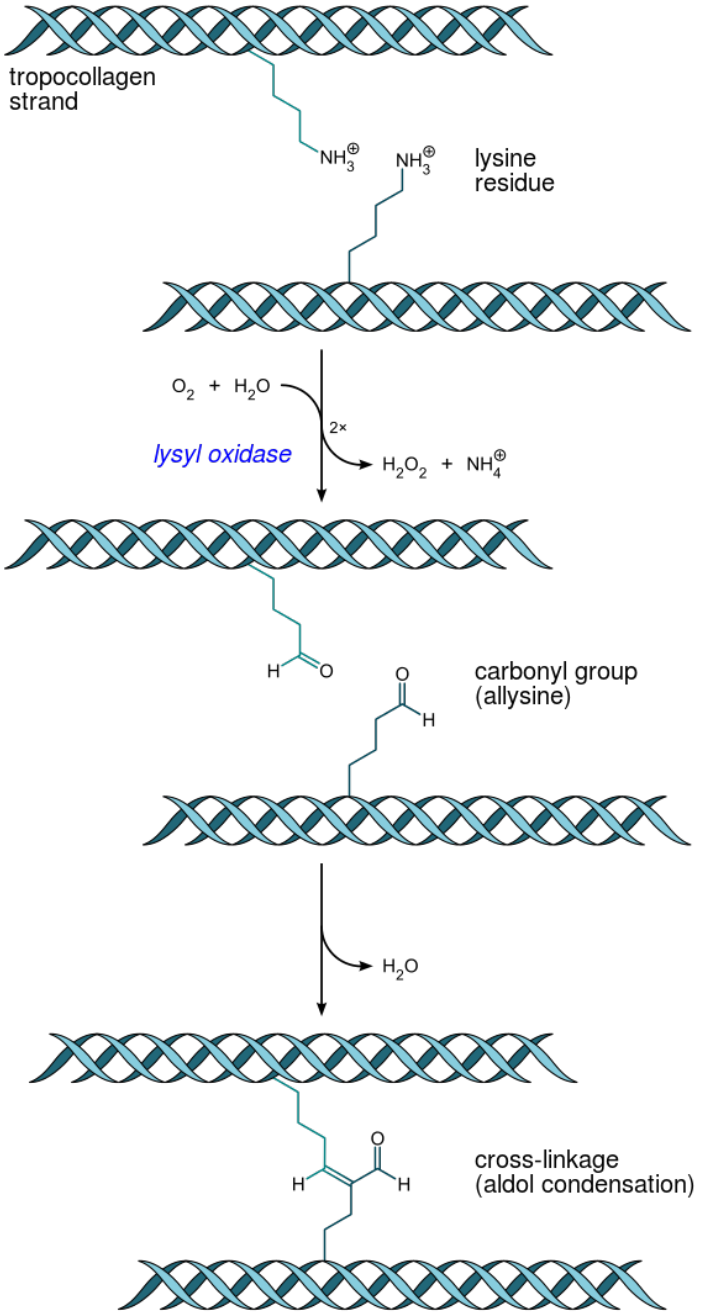


Hydroxyproline

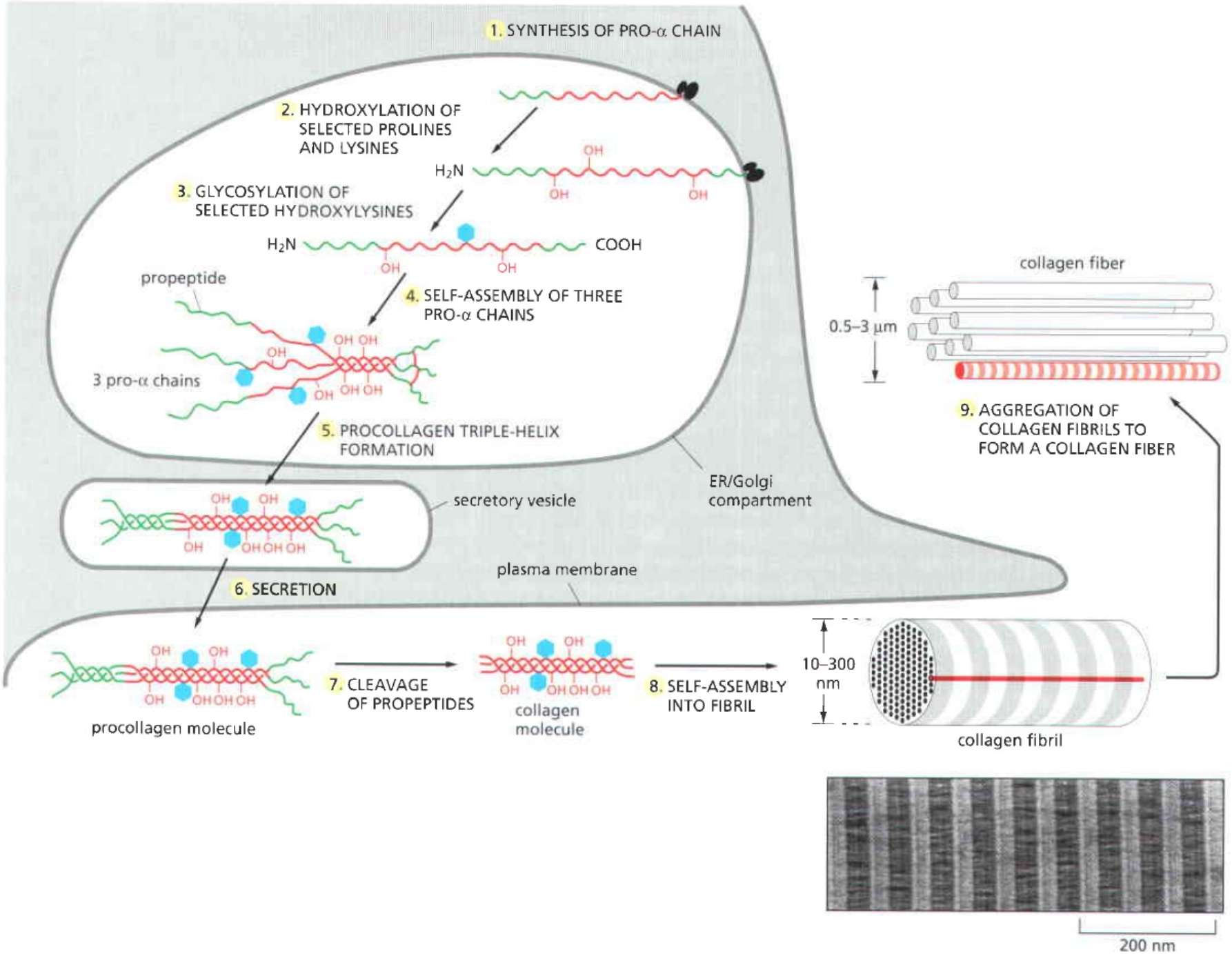


Hydroxylysine

LYSYL OXIDASE IN CROSS-LINKAGE

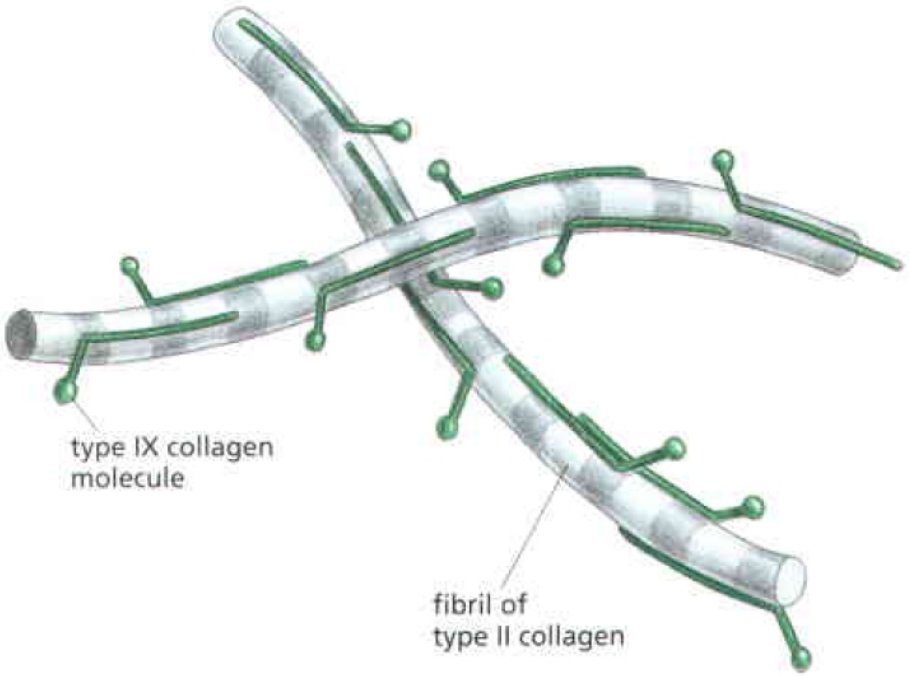


COLLAGEN FIBRIL FORMATION



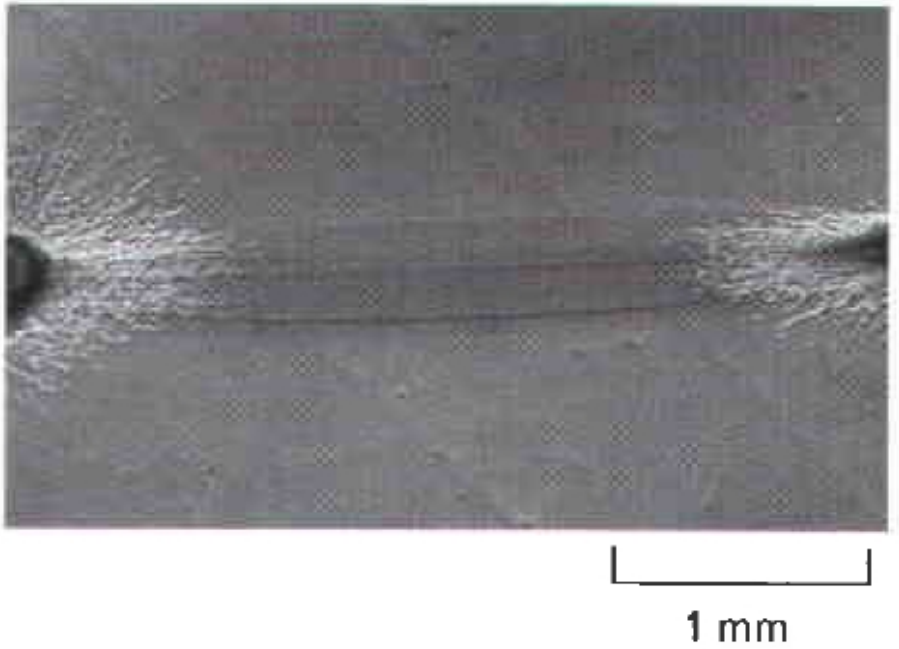
FIBRIL ASSOCIATED COLLAGENS

- Several non-helical domains => higher flexibility
- Propeptides are not cleaved
- No own fibril formation



FIBROBLASTS ORGANIZE COLLAGEN FIBRILS

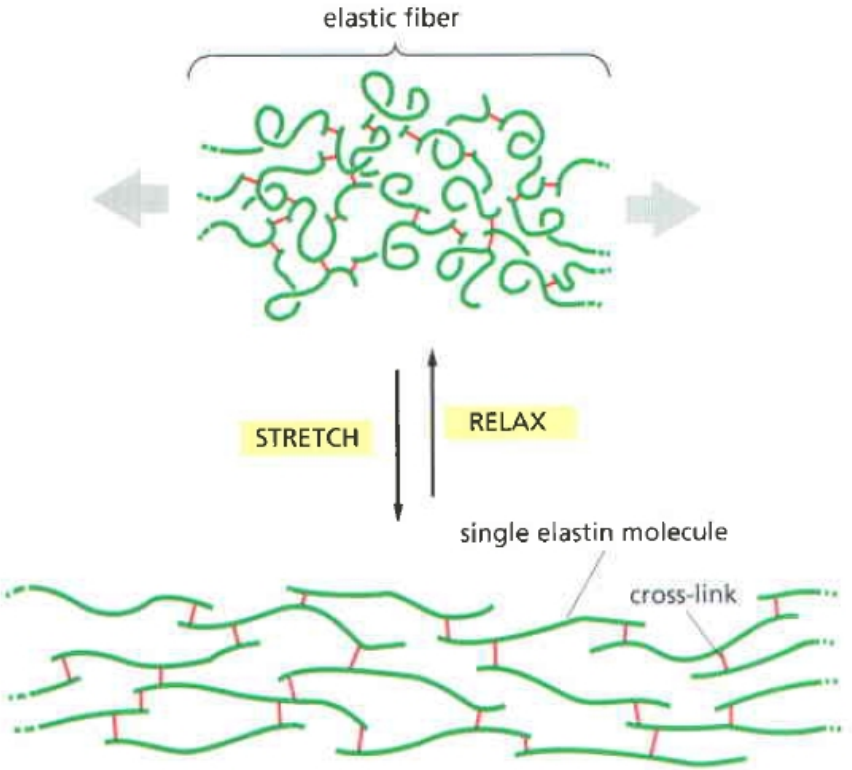
- Tugging collagen fibers
- Surrounding by a densely packed collagen fibers
- Two fibroblasts in collagen => two explants are connected
- Orientation of the connective tissue



Shaping collagen by fibroblasts

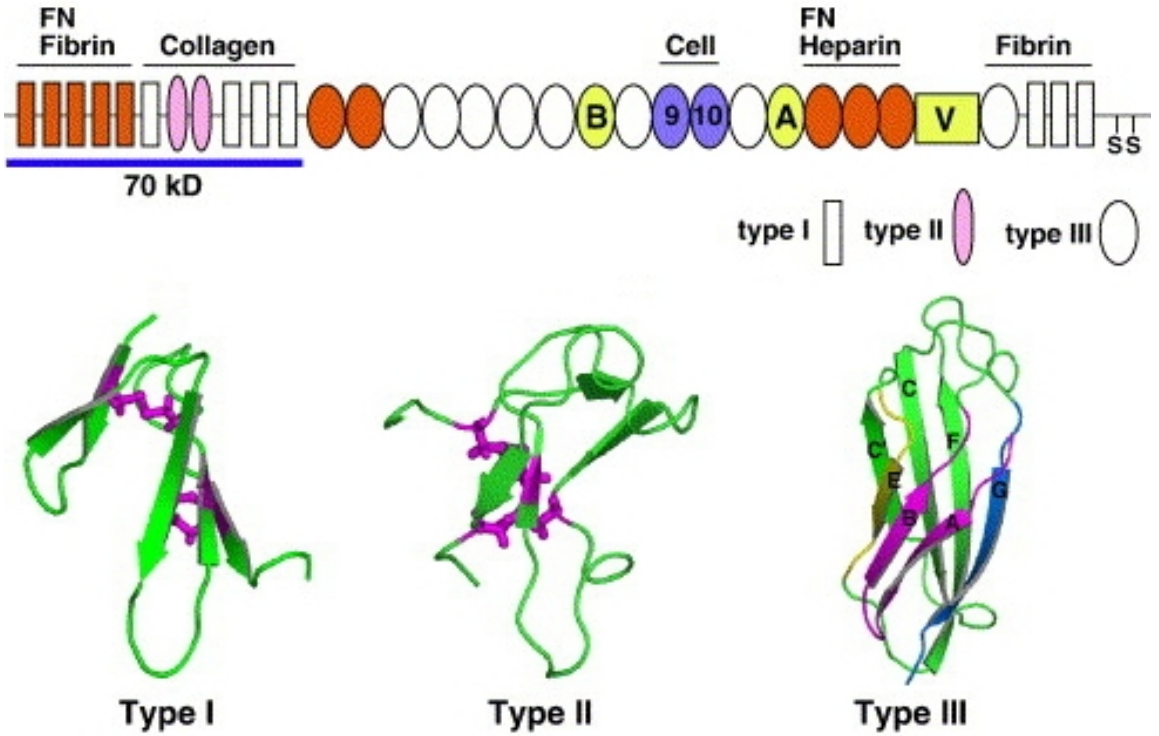
FIBERS ELASTICITY

- Requirement for skin, blood vessels, lungs etc.
- Function: stretching, prevention of tearing
- Elastin: 750 aa, highly hydrophobic, rich in Gly, Pro, not glycosylated
- Tropoelastin => secretion => cross linking to collagen via Lys
- Microfibrils: fibrillin (Marfan's syndrome)

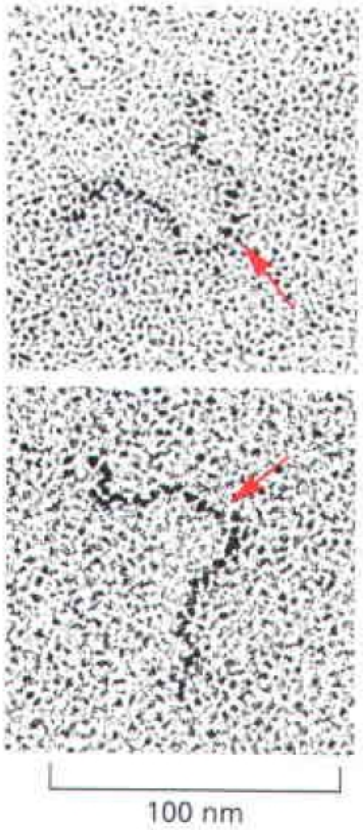


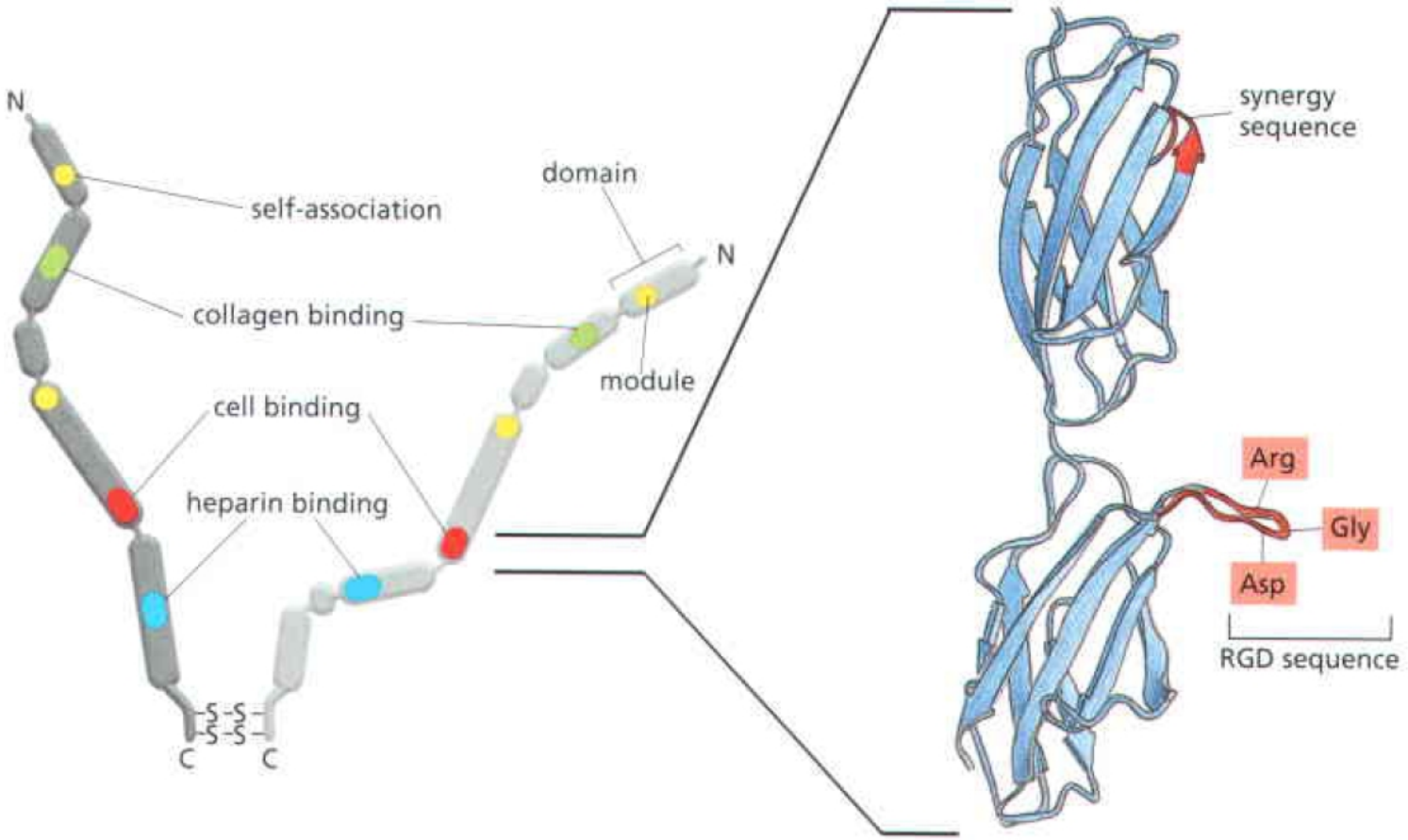
FIBRONECTIN

- Function: cells attachment to the matrix
- Two long (~2500 aa) multidomain subunits joined by disulfide bridges
- Modular global proteins: many repeats (f.i. type III repeat ~ 90 aa)
- Many isoforms



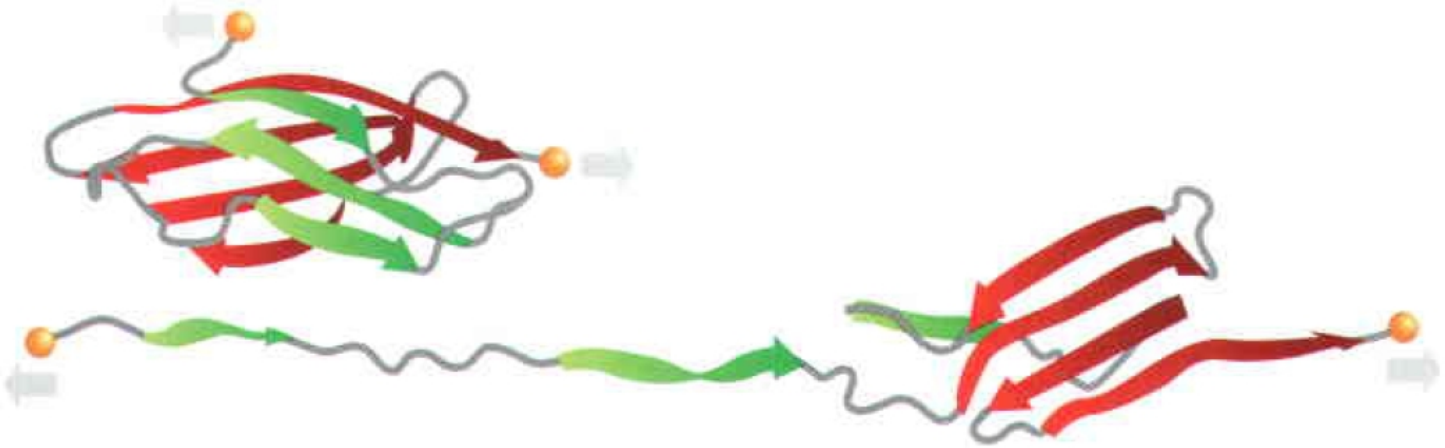
FIBRONECTIN STRUCTURE

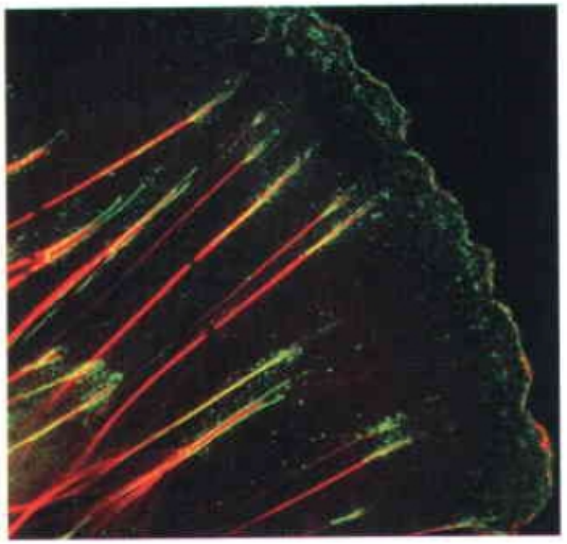




FIBRONECTIN FIBRILLIZATION

- Fibrillization only on the cell surface through integrins
- Tension => uncovering cryptic binding sites => polymerization



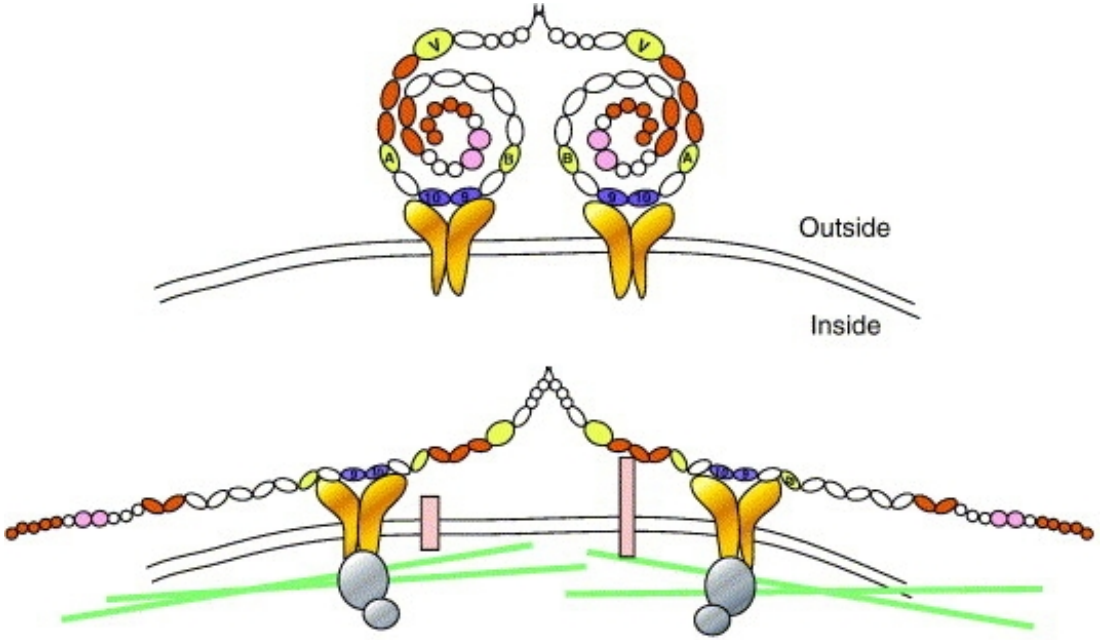


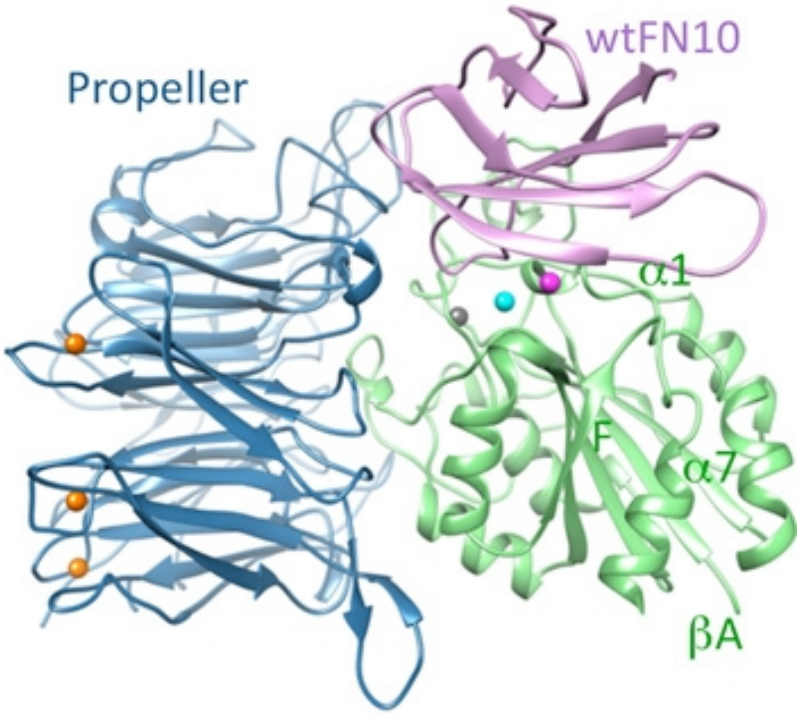
Fibronectin

Intracellular filament

FIBRONECTIN/INTEGRIN COMPLEX

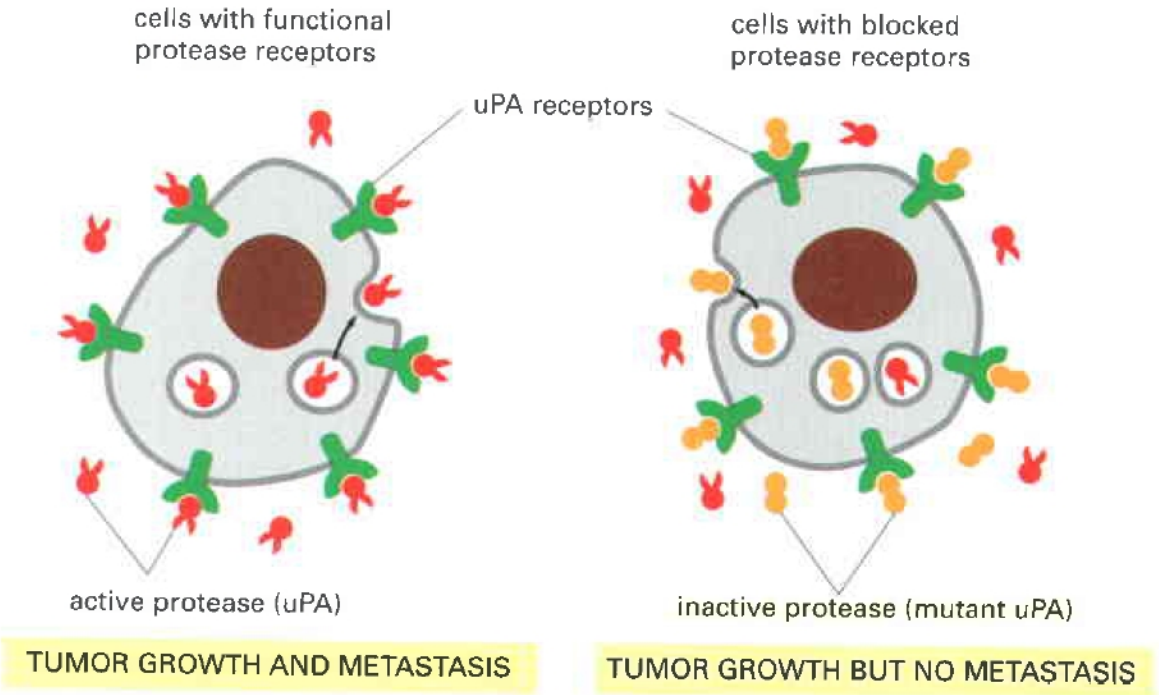
- RGD recognition motif in integrin
- Disintegrins from poison have RGD: anticlotting => bleeding
- Other motifs => specificity





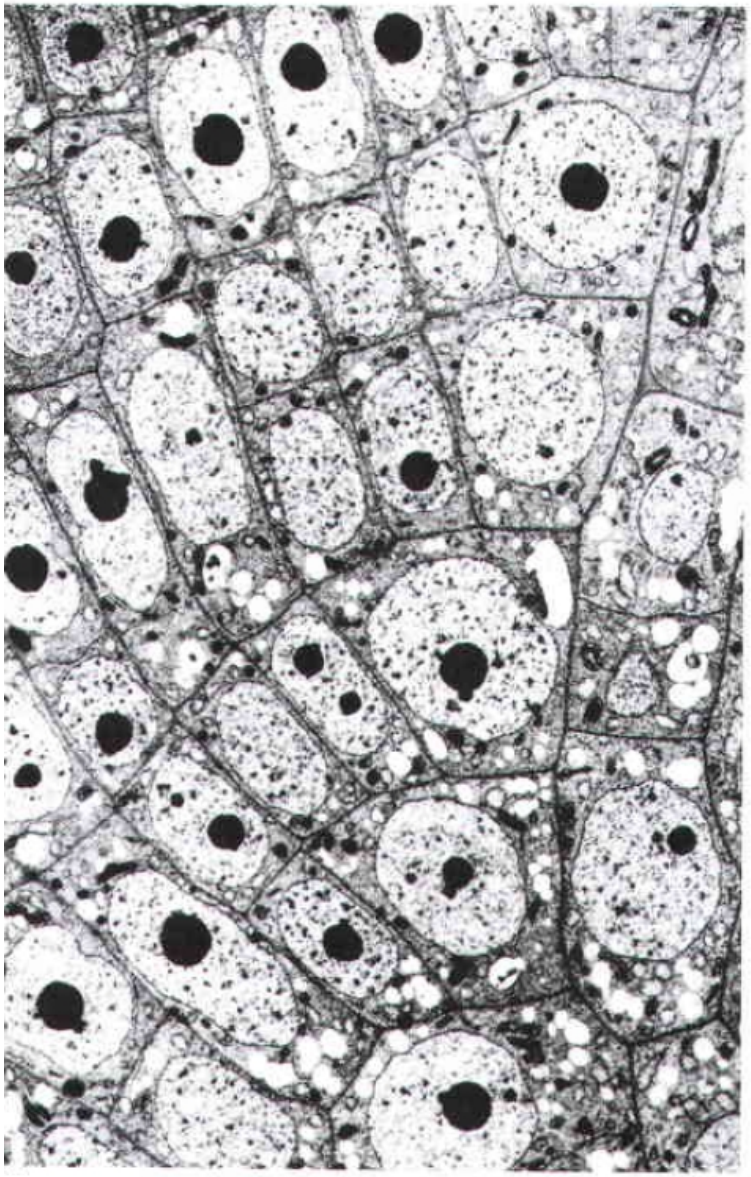
CELL AND ECM

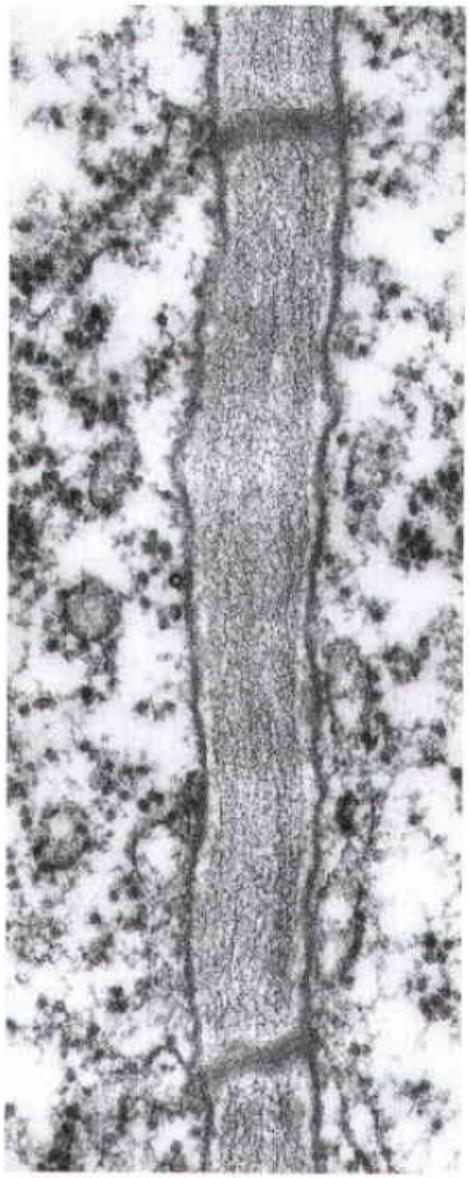
- Cells create and destroy ECM
- Function of cell-ECM interactions:
  - tissue repair
  - adaptation to the external stress
  - division
  - transport
  - ECM degradation
- Extracellular proteases:
  - matrix metalloproteases (Zn^{2+}-, Ca^{2+}-dependent)
  - Serine proteases
- Proteases control:
  - local activation (plasminogen)
  - cell-surface receptor (urokinase plasminogen activator)
  - secretion of inhibitors (TIMPs)



THE PLANT CELL WALL

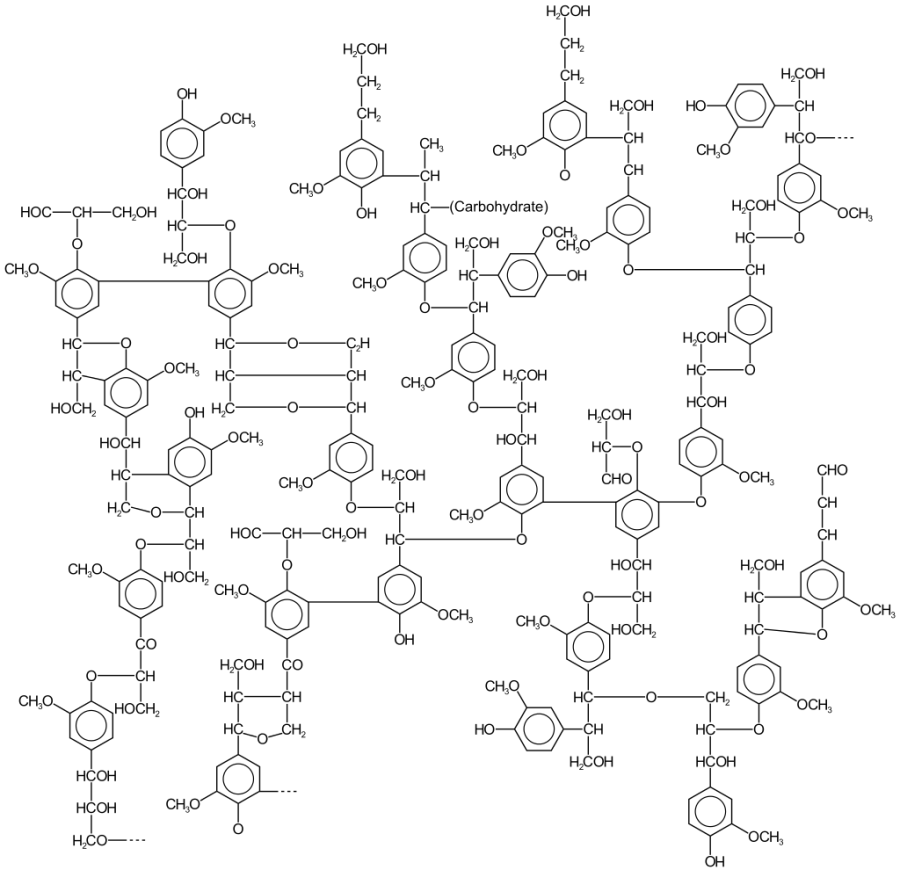
Structural layer of the plant cell outside the cell membrane





COMPOSITION OF THE CELL WALL

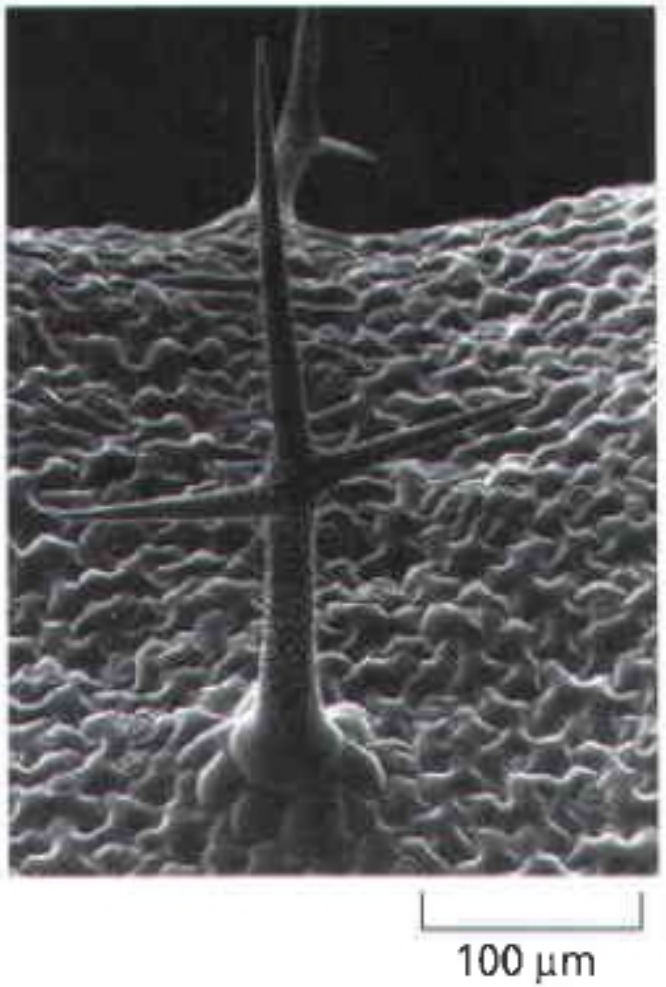
- Cell type specific
- Primary cell walls: formed first, thin, extensible, tough
- Secondary cell walls: deposits of the new layers in the old ones
- Lignin: widely spread polymer in the secondary cell walls
- Cellulose-rich
- Cross-linking glycans



Lignin

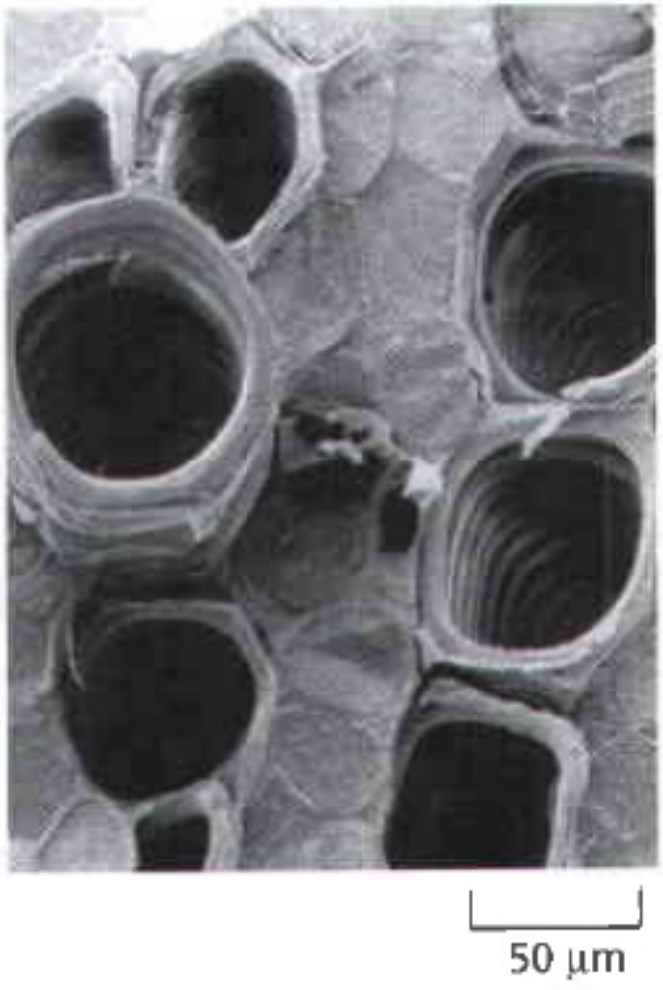
THE CELL WALL FUNCTION

- Skeleton of the plant
- Turgor pressure control (up to 10 atmospheres)



Arabidosis leaf surface

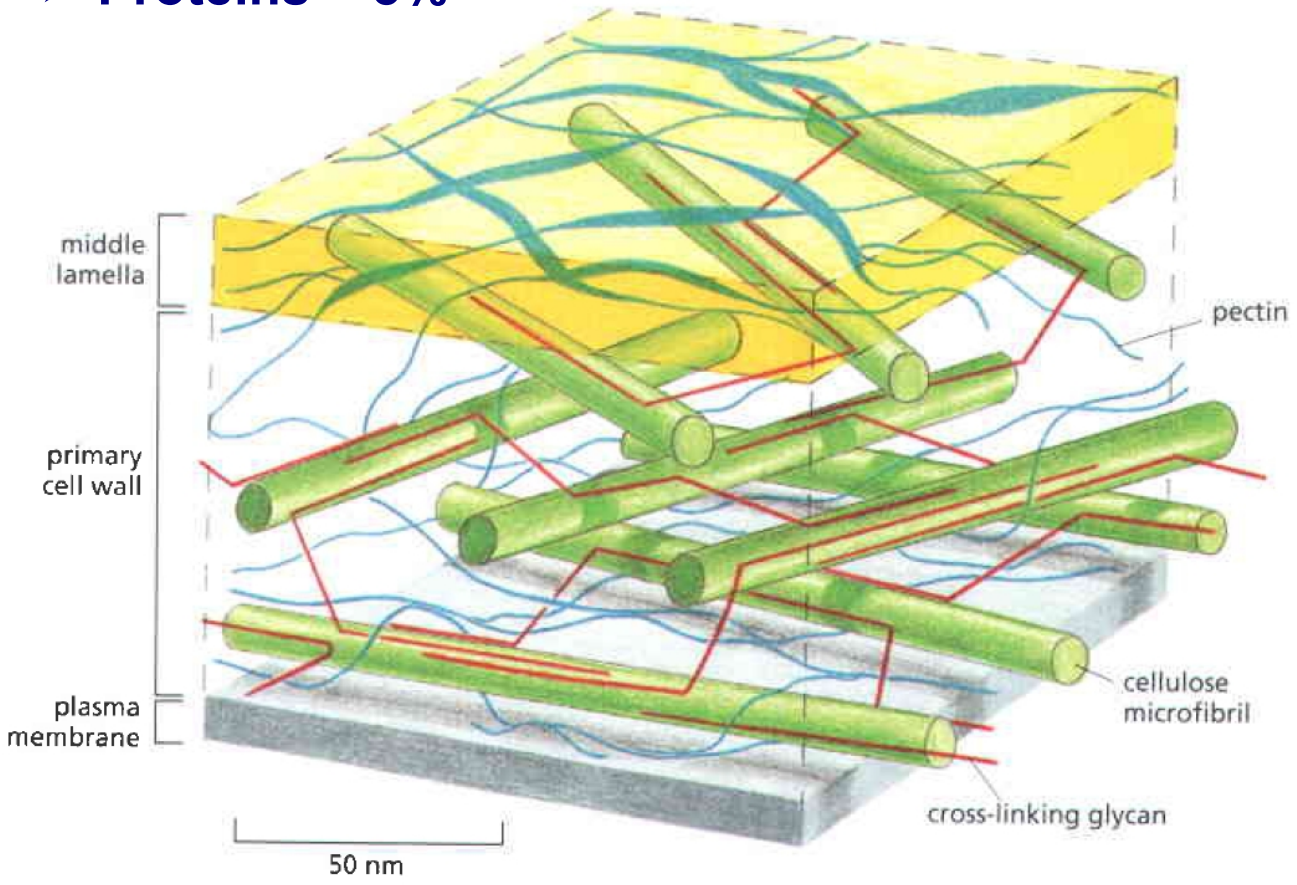
Tomato leaf epidermis

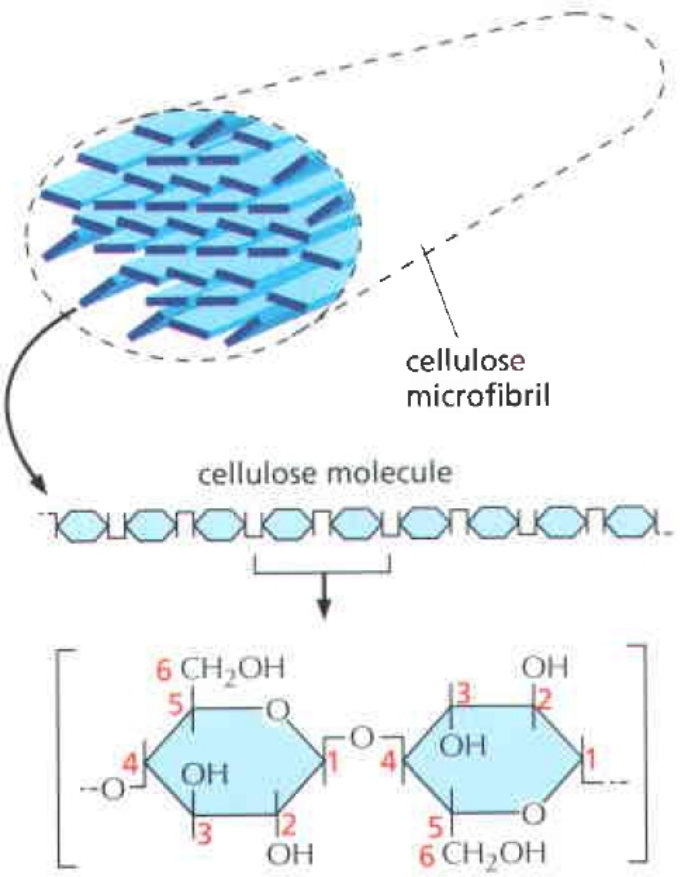


Young wood elements

PRIMARY CELL WALL STRUCTURE

- Cellulose: $(Glc\text{- }\beta1\Rightarrow4\text{- }Glc)_n$, n ~ 500
- ~ 40 chains => bundles => microfibrils
- Microfibrils are linked by long (20-40 nm) glycan
- Pectin
- Proteins ~ 5%





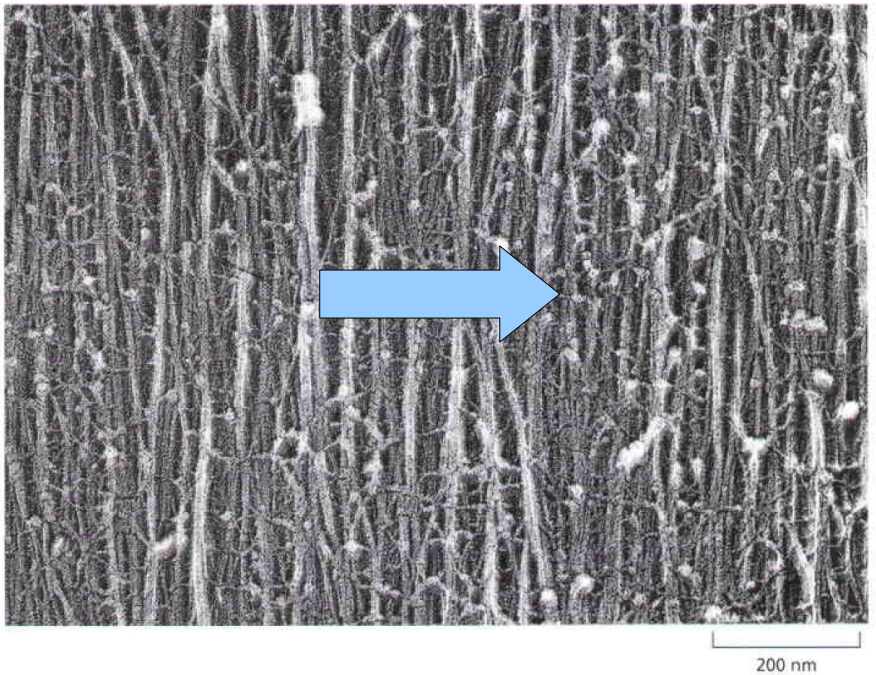
Cellulose

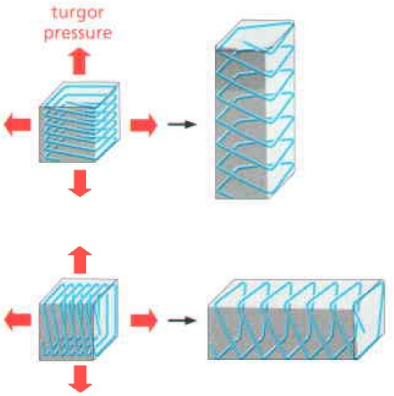
CELL WALL STRUCTURE

POLYMER	COMPOSITION	FUNCTIONS
Cellulose	linear polymer of glucose	fibrils confer tensile strength on all walls
Cross-linking glycans	xyloglucan, glucuronoarabinoxylan, and mannan	cross-link cellulose fibrils into robust network
Pectin	homogalacturonans and rhamnogalacturonans	forms negatively charged, hydrophilic network that gives compressive strength to primary walls; cell–cell adhesion
Lignin	cross-linked coumaryl, coniferyl, and sinapyl alcohols	forms strong waterproof polymer that reinforces secondary cell walls
Proteins and glycoproteins	enzymes, hydroxyproline-rich proteins	responsible for wall turnover and remodeling; help defend against pathogens

- **Direction of the growth:**

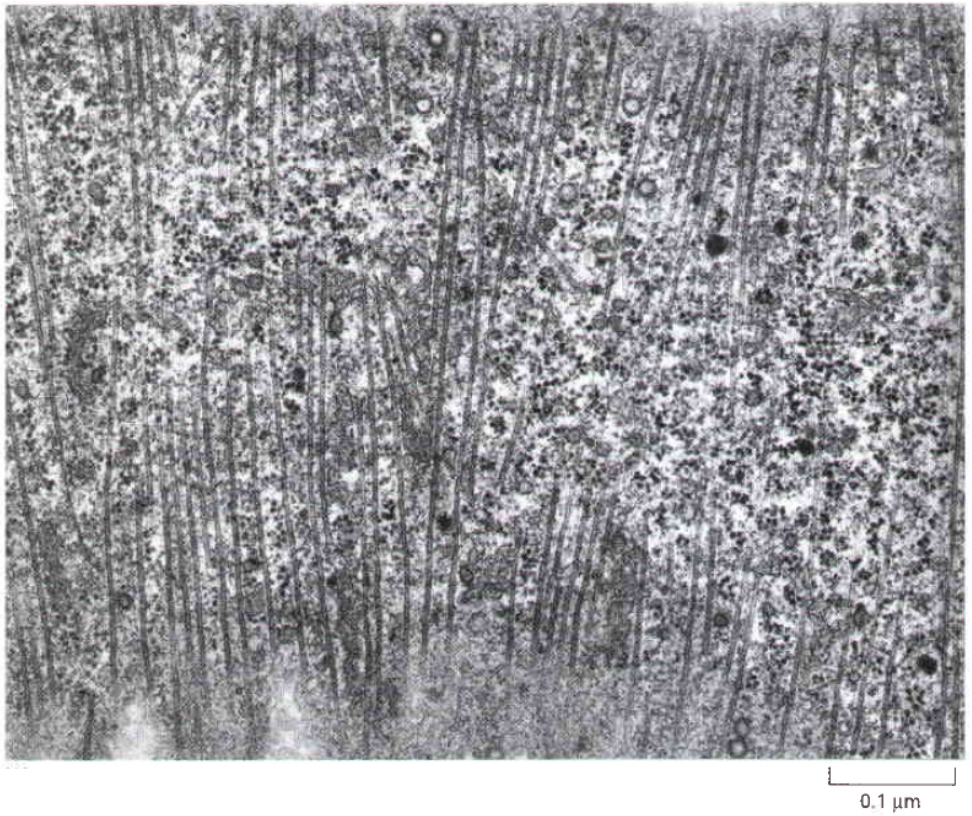
  - microfibrils structure



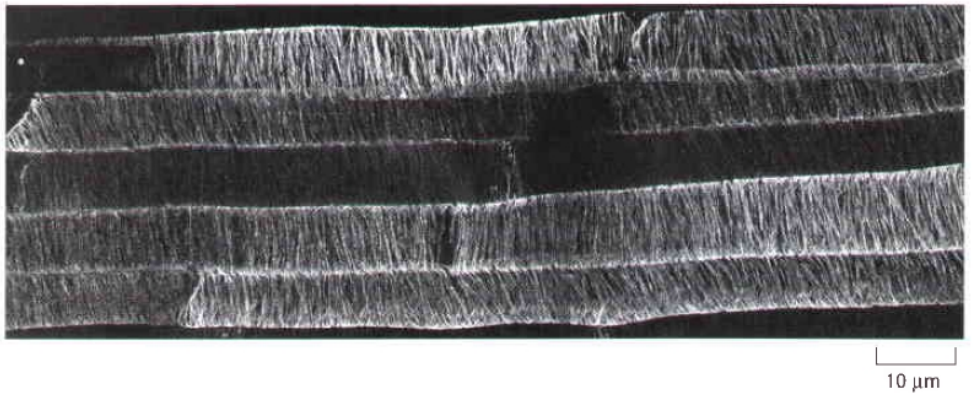


MICROTUBULES IN CELL GROWTH

- Microtubules are perpendicular to the direction of the cell growth
- Microfibrils deposition is dependent on microtubules orientation



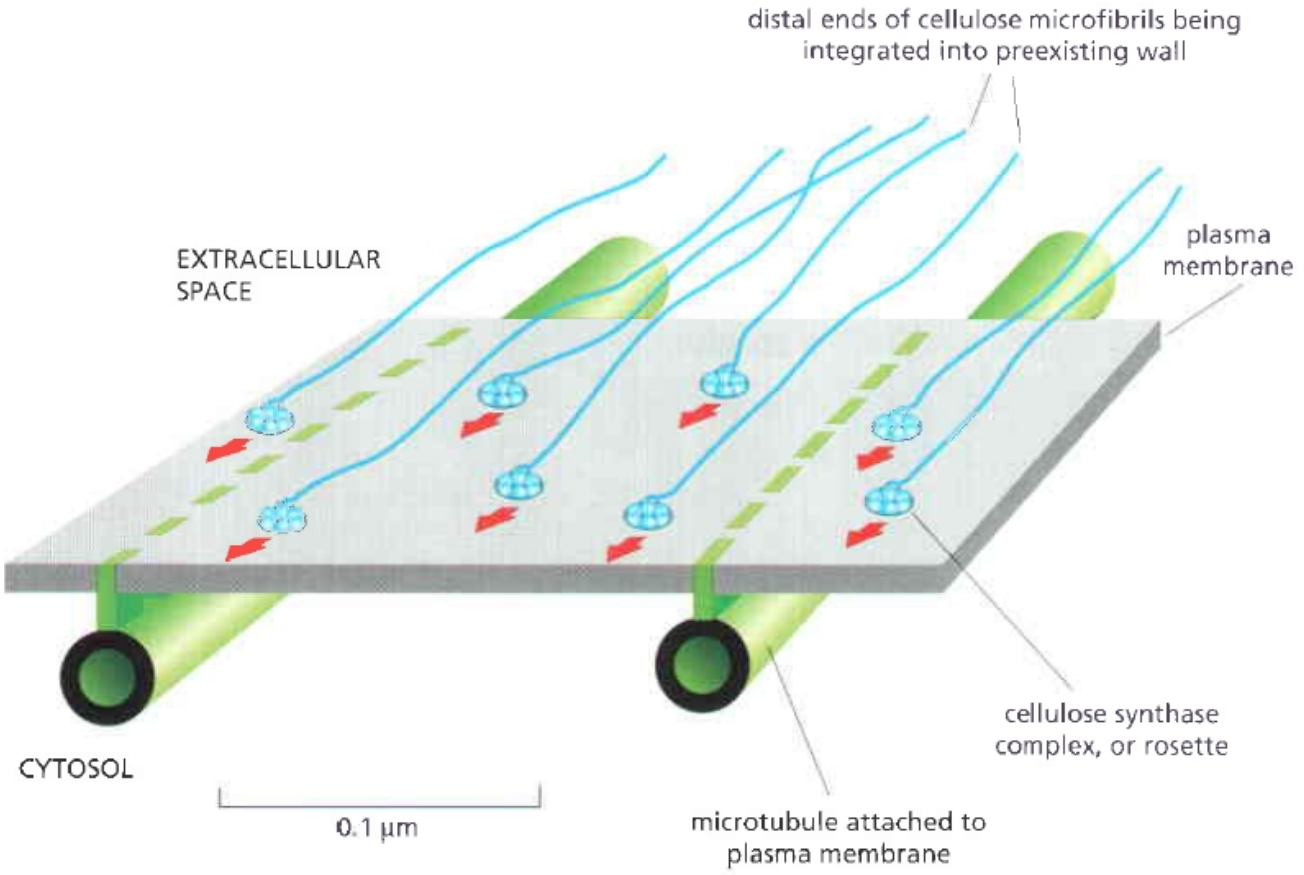
Microtubules below the plasma membrane

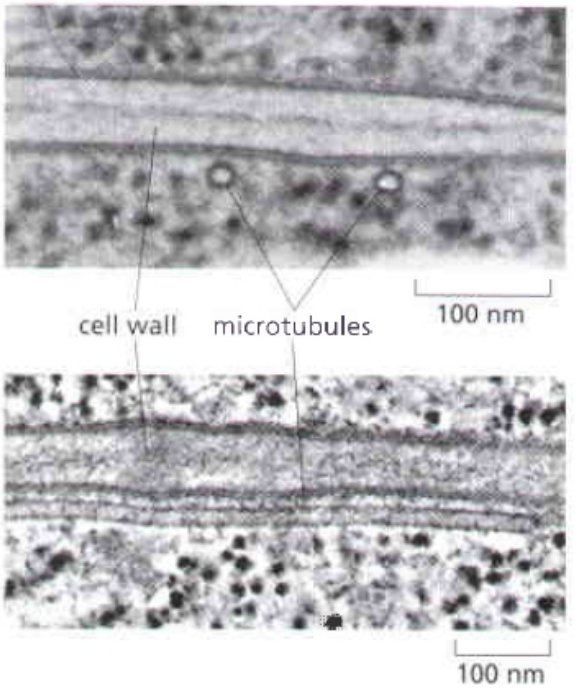


Microtubules in growing epidermal cells of Arabinosa

MICROTUBULES IN CELL GROWTH: MODEL

- Enzyme moves along the microtubule-organized membrane domain
- Other participants of cytoskeleton are also involved





LECTURES 25-26: CELL JUNCTIONS AND ADHESION

- Cadherins in cell-cell adhesion
  - adherens junctions
  - desmosomes
- Tight junctions
- Gap junctions and plasmodesmata
- Basal lamina
- Integrins in cell-matrix adhesion
- Extracellular matrix
- Plant cell wall