HSS2305A: MOLECULAR MECHANISMS OF DISEASE

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Credits:	3
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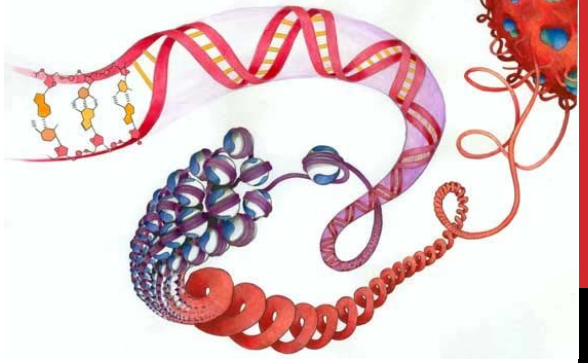
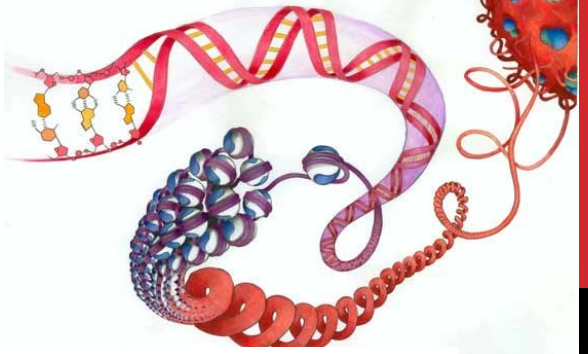
CELLS AND THEIR ENVIRONMENT– CHAPTER 7

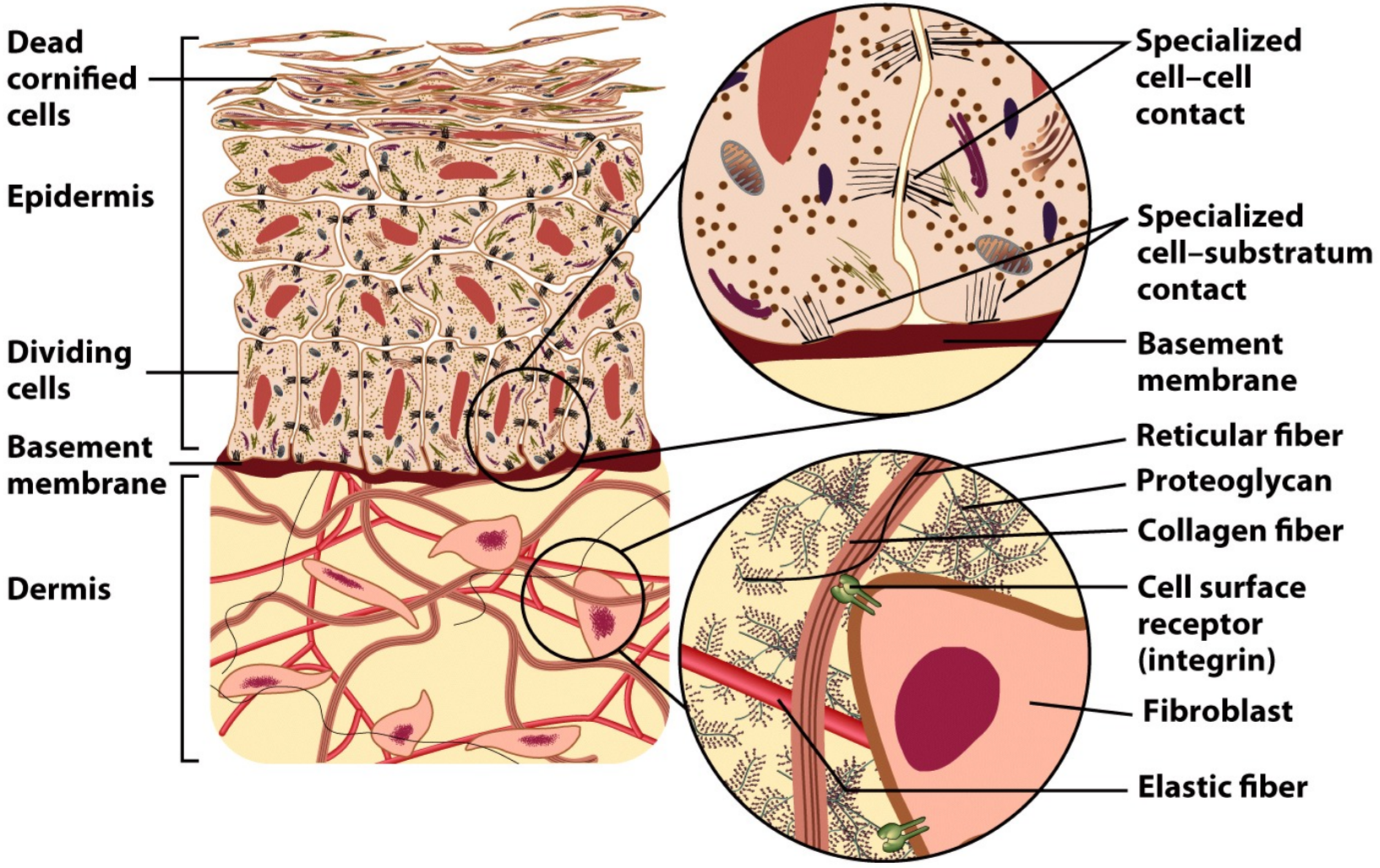
LEARNING OBJECTIVES



- ✓ Define the general structure and function of the extracellular matrix
- ✓ Describe the function and structure of basement membranes
- ✓ Describe the structures of the components of the extracellular matrix (collagen, fibronectin, laminin, proteoglycans) and differentiate between them
- ✓ Compare the different mechanisms through which cells adhere to a non cellular surface
- ✓ Describe the membrane proteins involved in the adhesion of cells to non cellular surfaces
- ✓ Describe the structures and functions of the different cell junctions
- ✓ Describe the membrane proteins involved in cell-cell adhesion

TISSUE ORGANIZATION





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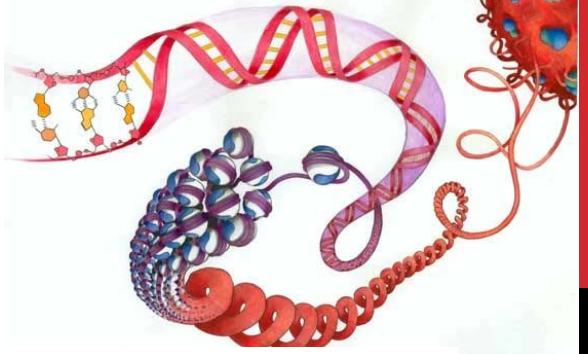

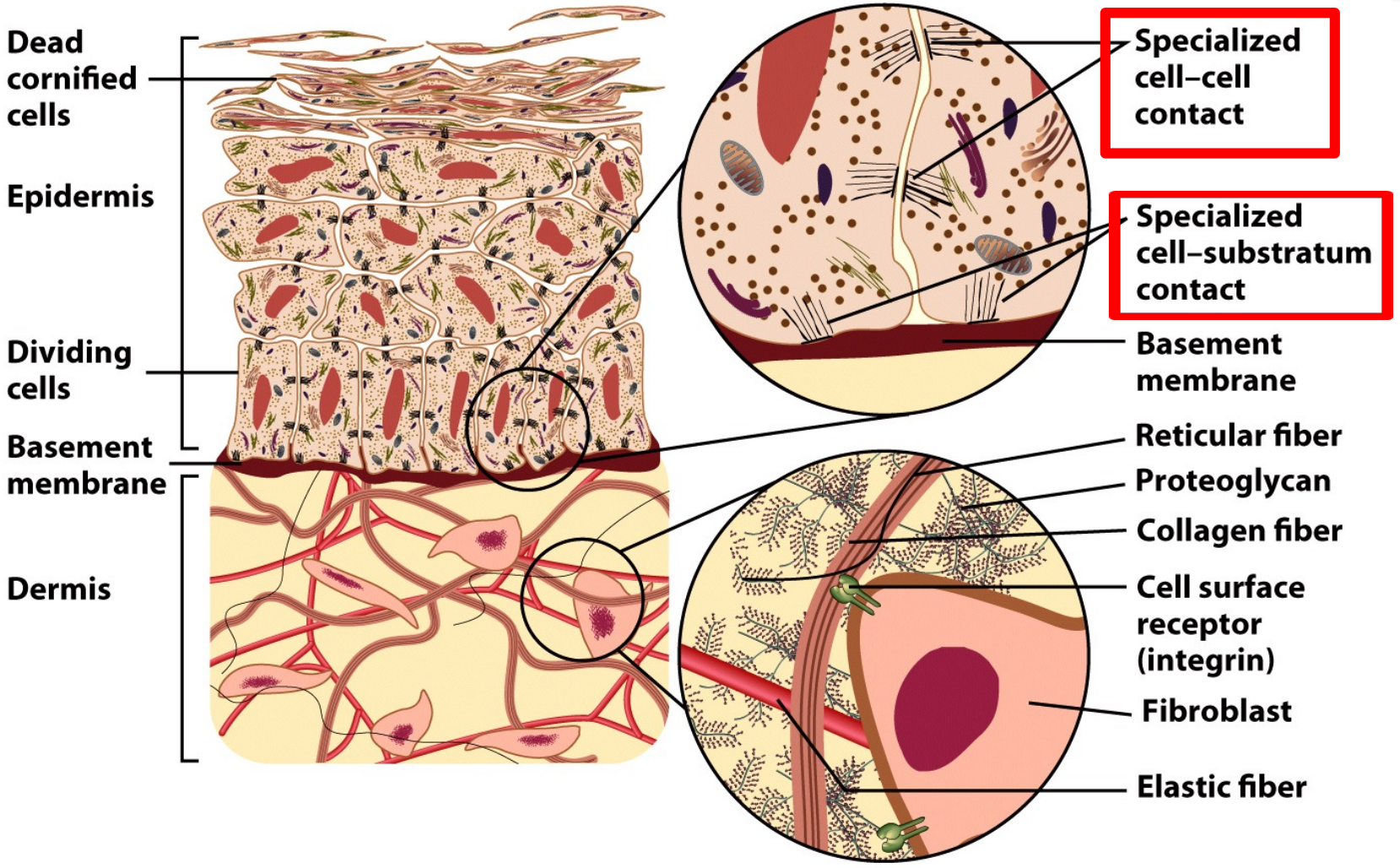

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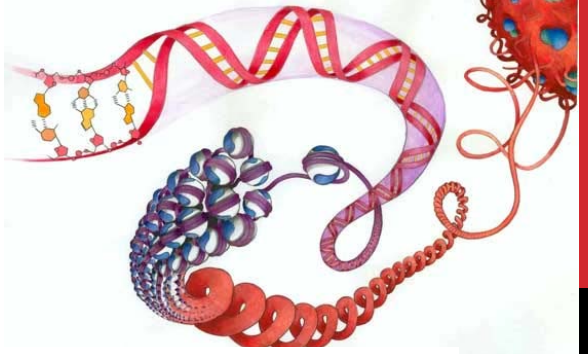

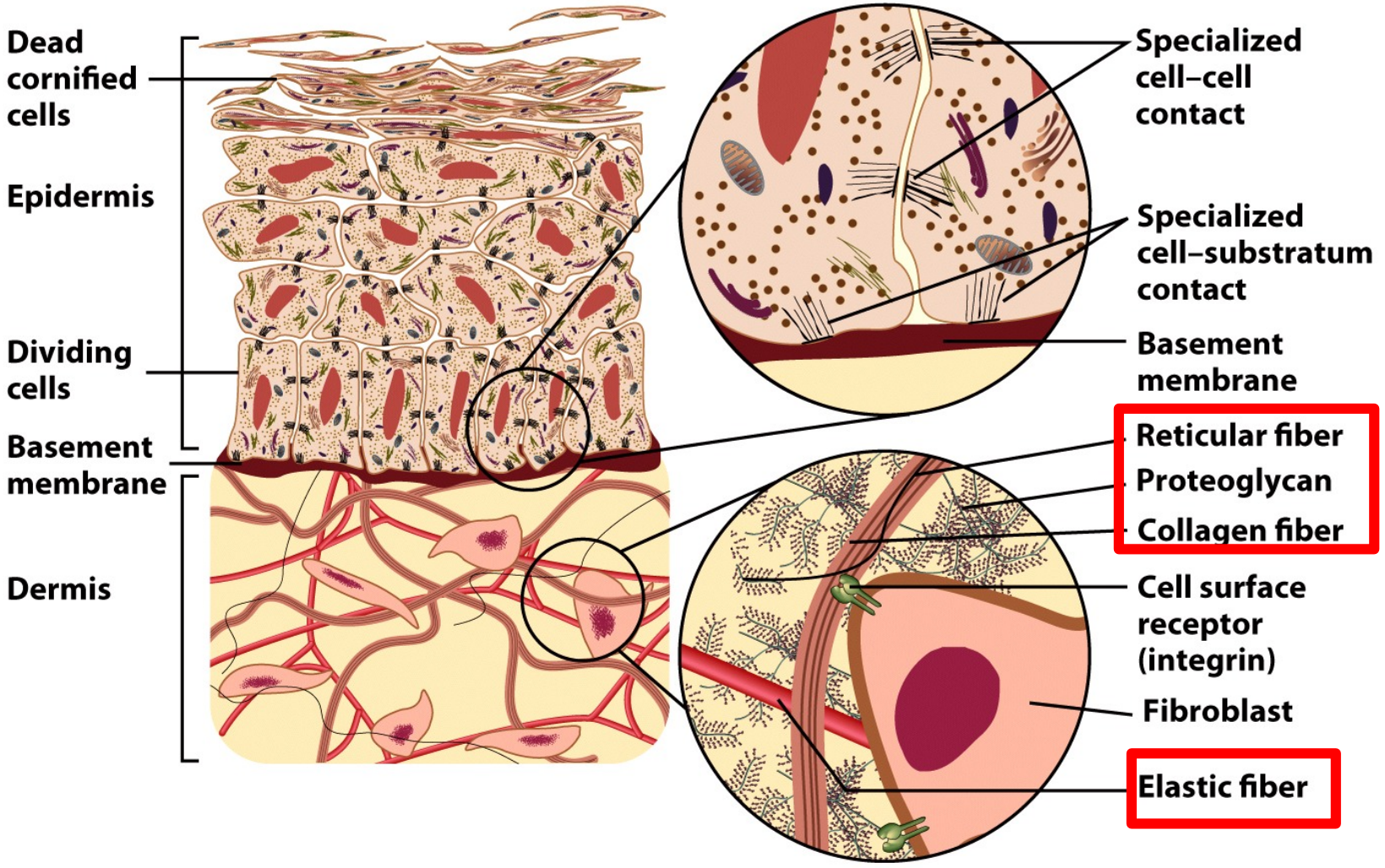

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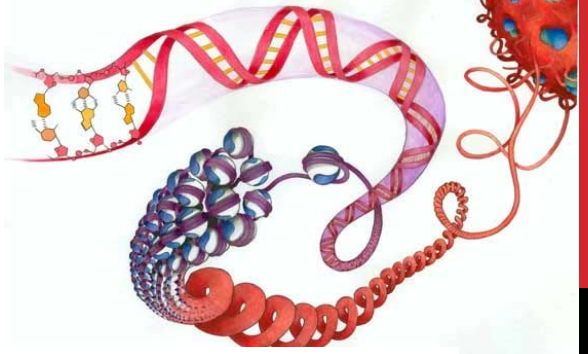
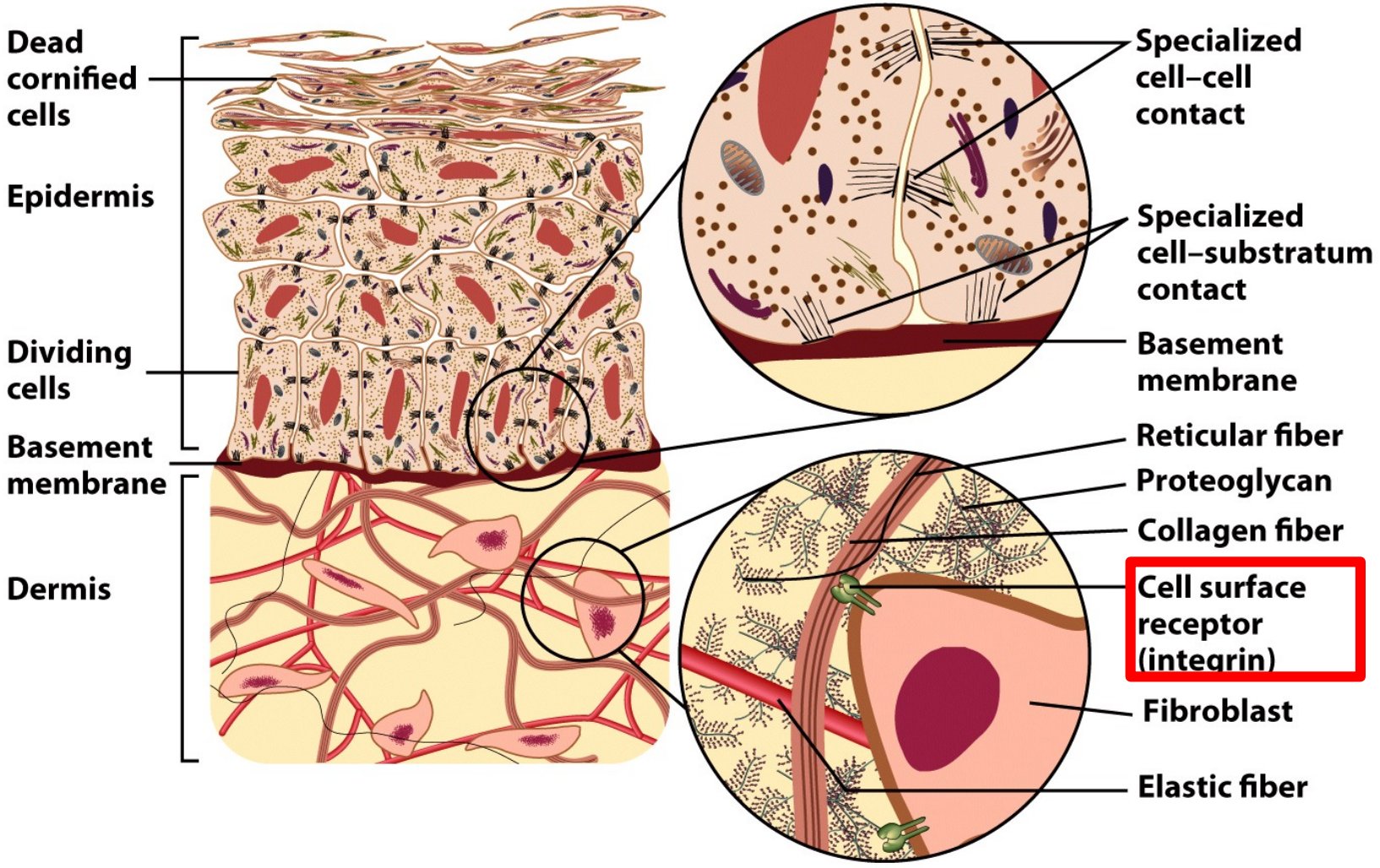
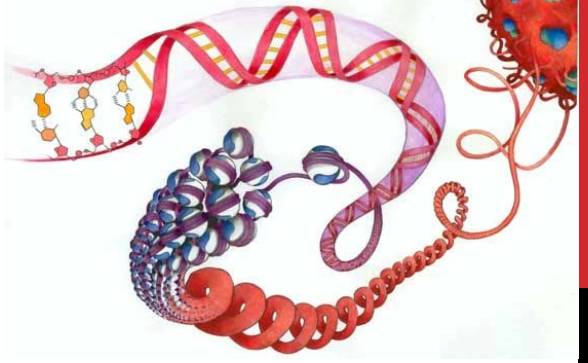
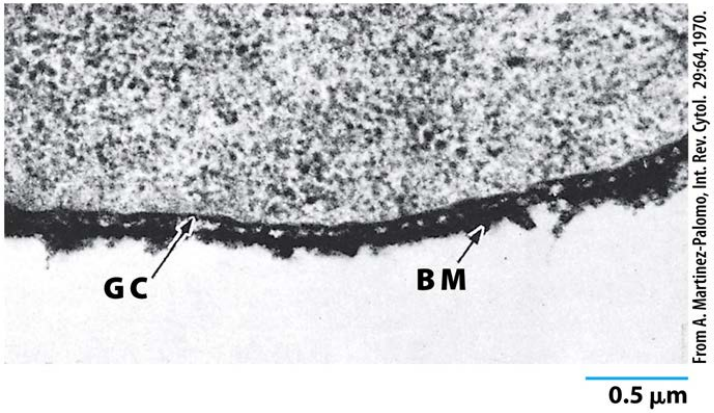




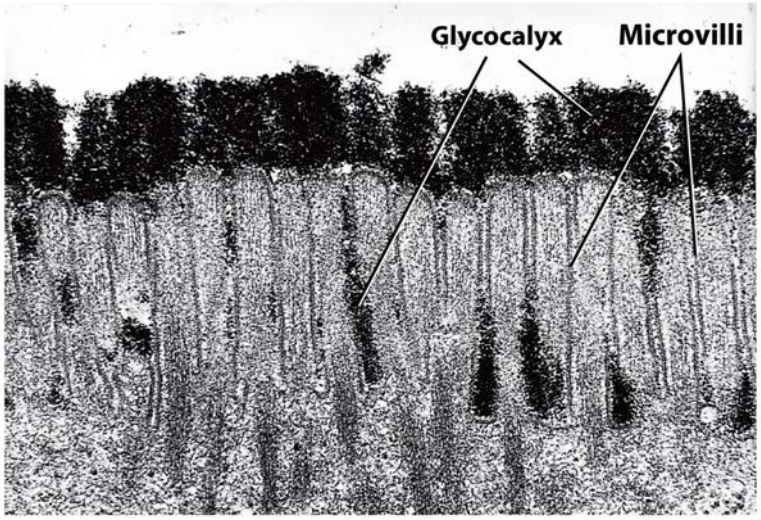
THE EXTRACELLULAR SPACE



- All space and any element outside of plasma membrane
- Portions of integral membrane proteins and membrane lipids project into the extracellular space

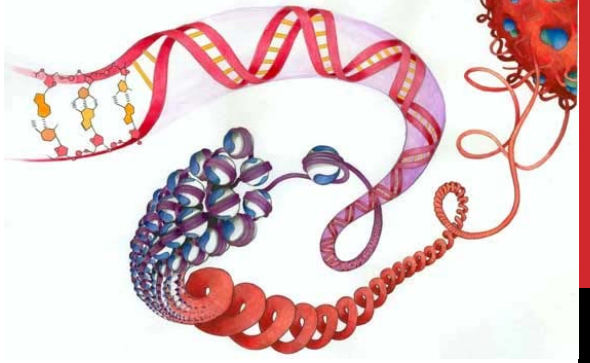


- **Glycocalyx** =cell coat formed from carbohydrate projections
  - mediate cell-cell and cell-substratum interactions
  - provide mechanical protection
  - serve as a barrier
  - bind regulatory factors

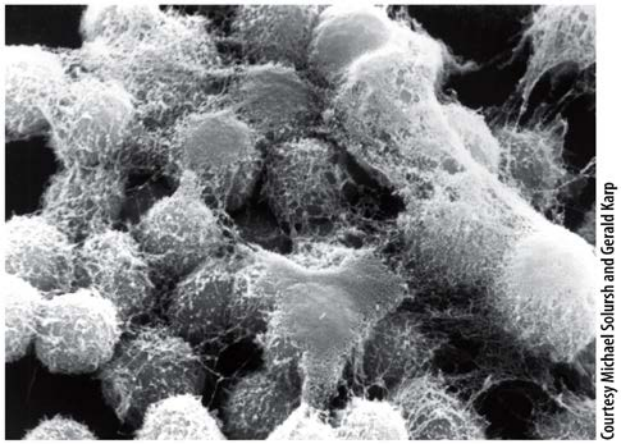


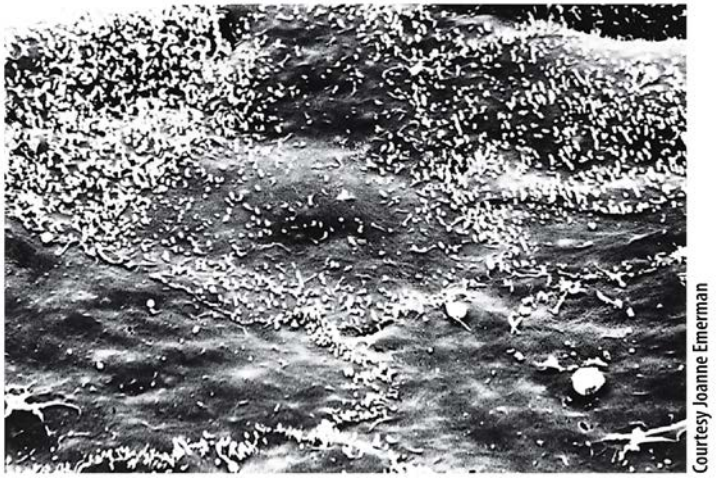
EXTRACELLULAR SPACE

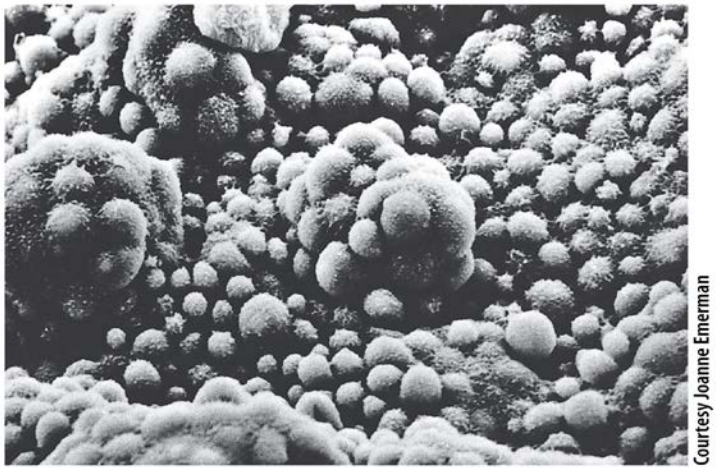
EXTRACELLULAR MATRIX



- **The extracellular matrix (ECM)** = organized network of extracellular material
- Provides physical and biochemical signals that regulate shape and activities of the cell

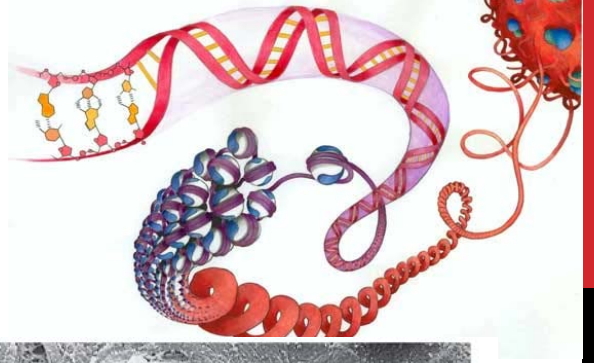




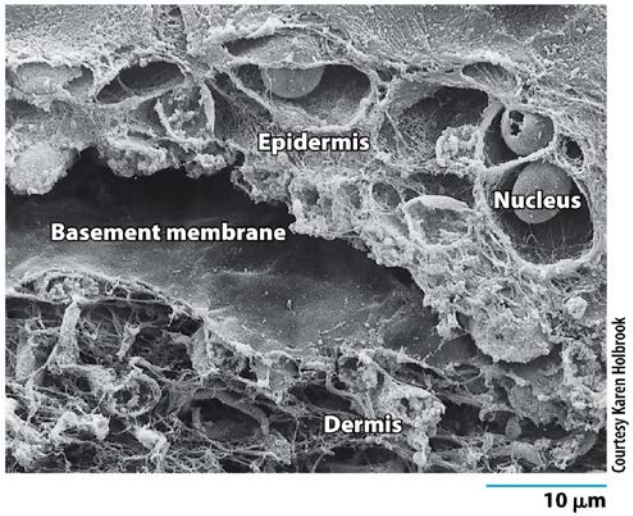


Mammary gland epithelial cells

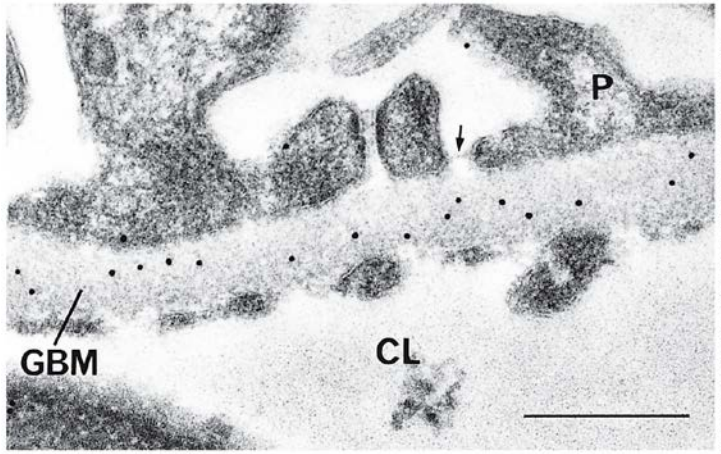
THE EXTRACELLULAR SPACE
EXTRACELLULAR MATRIX



- **Basement membrane**:
  - Highly defined extracellular matrices
  - 50-200 nm
  - underlies epithelial tissue and surrounds blood vessels
  - provides mechanical support
  - generates signals to maintain cell survival
  - serves as substratum for cell migration
  - separates adjacent tissues within an organ
  - barrier to macromolecules
    - i.e. keeps proteins in blood

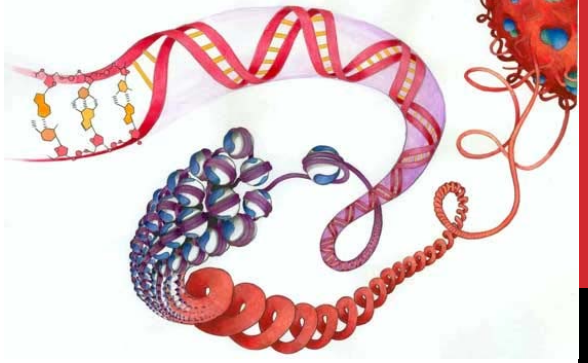


Courtesy Karen Holbrook

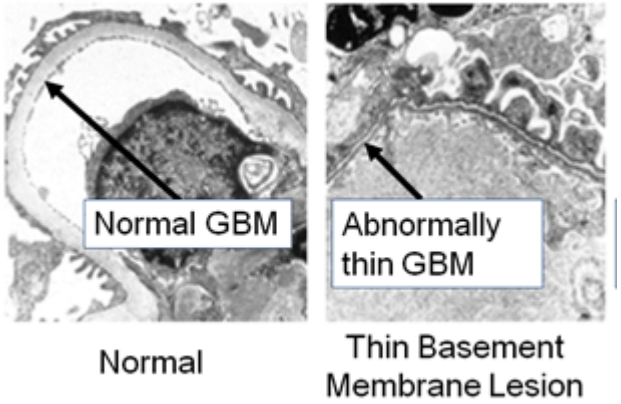


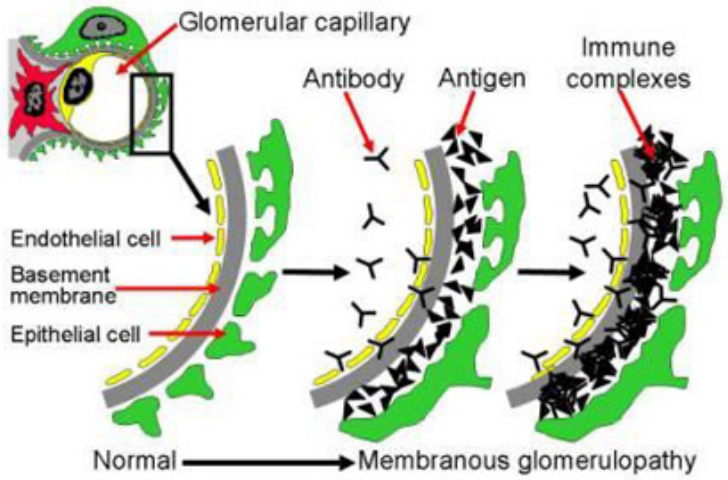
From Michael Desjardins and M. Bendayan, J. Cell Biol. 113:695, 1991, Figure 5. © 1991 Rockefeller University Press

THE EXTRACELLULAR SPACE
EXTRACELLULAR MATRIX



- **Basement membrane**:





Thin Basement Membrane Disease:

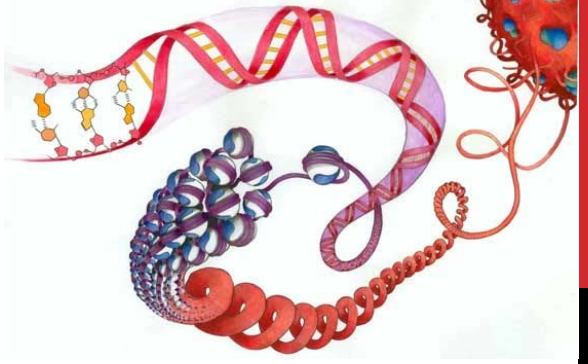
- Genetically inherited →collagen
- Blood and protein in urine

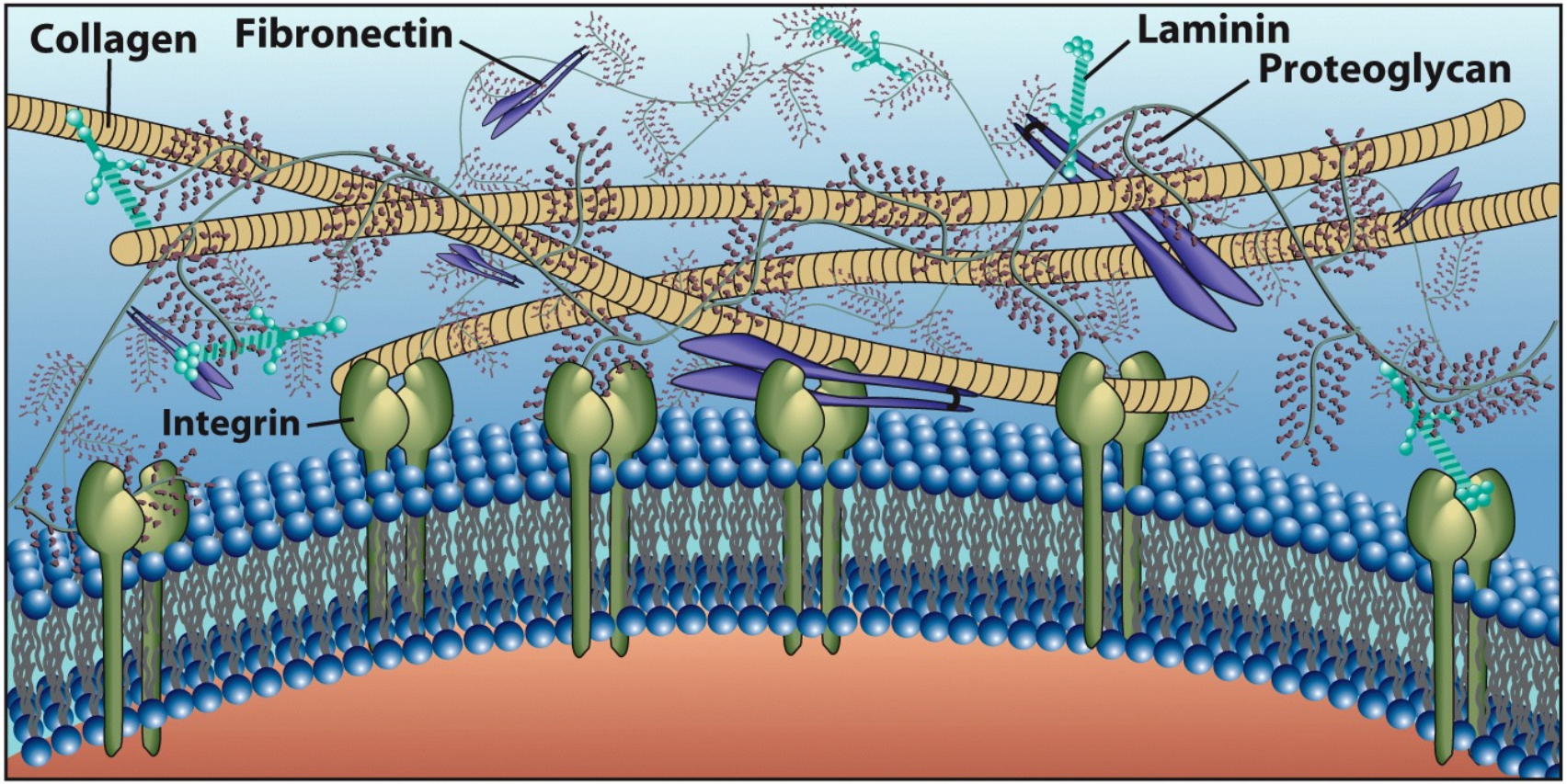
Anti–glomerular basement membrane (Goodpasture's Syndrome):

- Autoimmune disease →Ab against collagen
- Kidneys and lungs

EXTRACELLULAR SPACE

EXTRACELLULAR MATRIX





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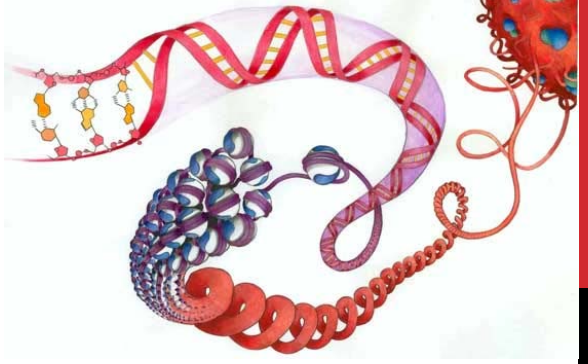
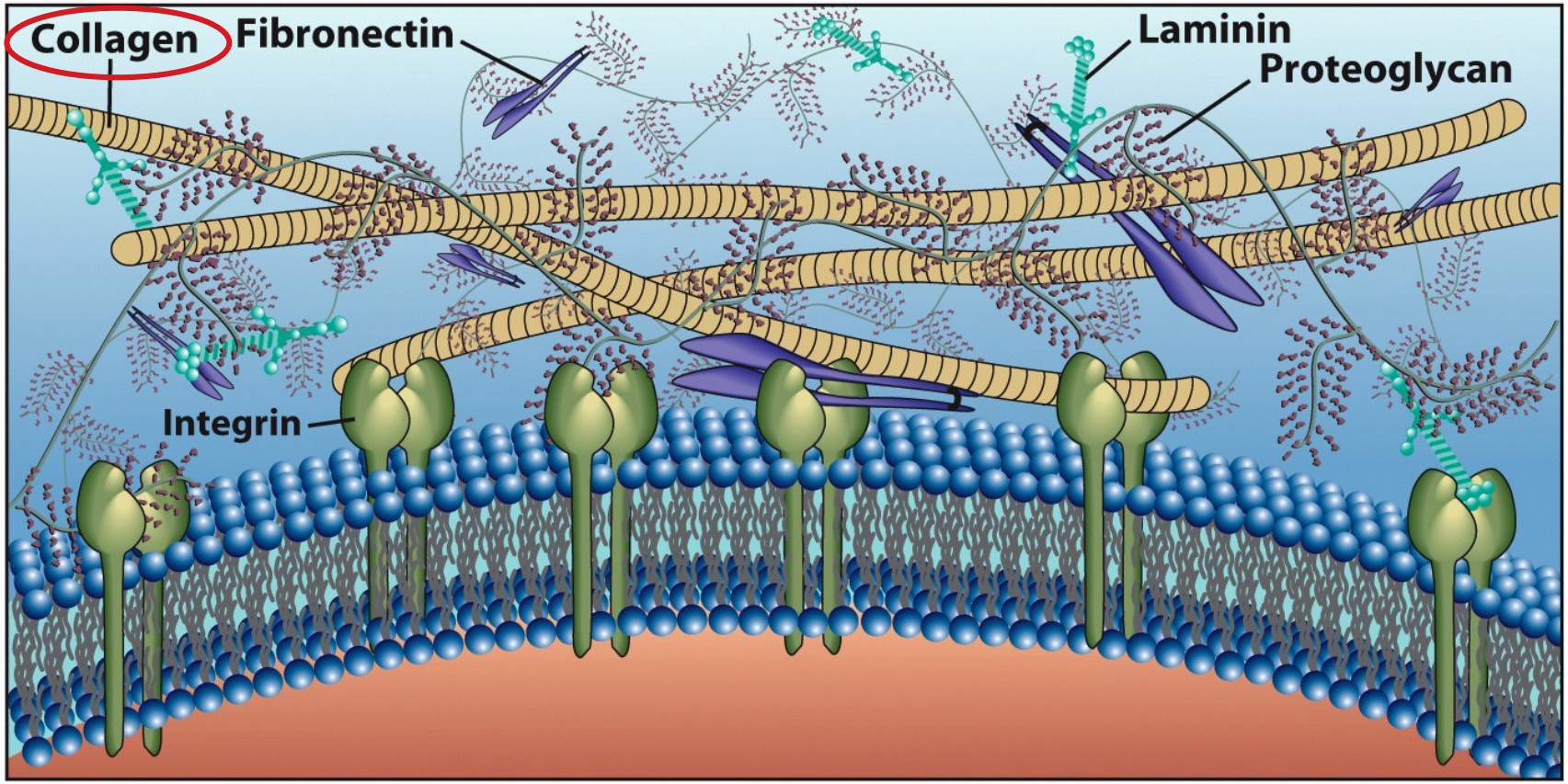
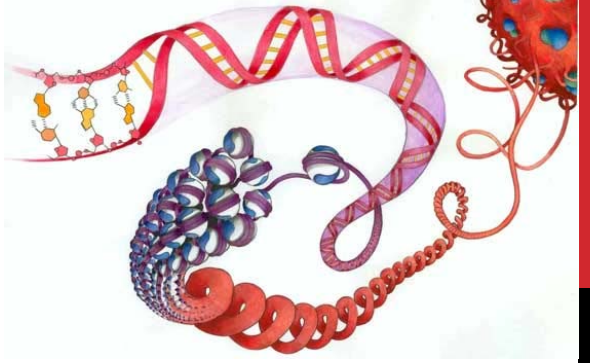
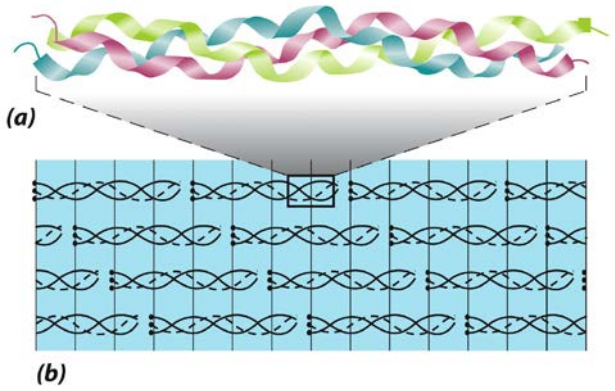


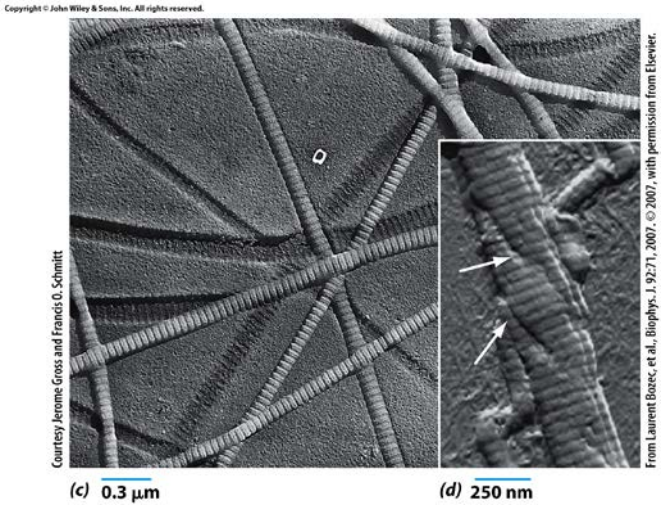


EXTRACELLULAR SPACE
COLLAGEN



- fibrous glycoproteins found only in the ECM
- most abundant protein in the human body (>25%)
- high tensile strength
  - fiber of 1 mm diameter can suspend ~ 22lbs
- produced by fibroblasts, SMC, epithelial cells
- 27 types of collagen fibers
  - restricted to particular locations
- trimer of polypeptide chains (α chains) wound around each other in a helix





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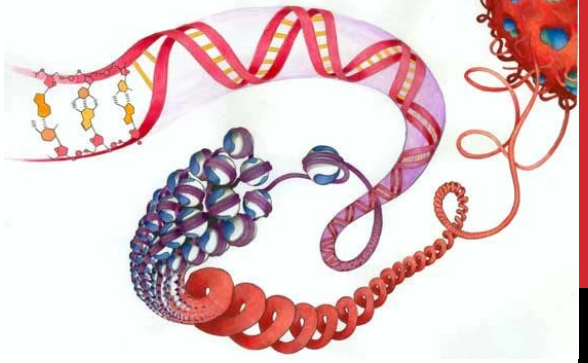

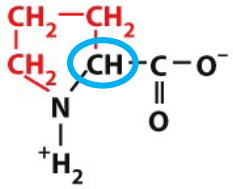

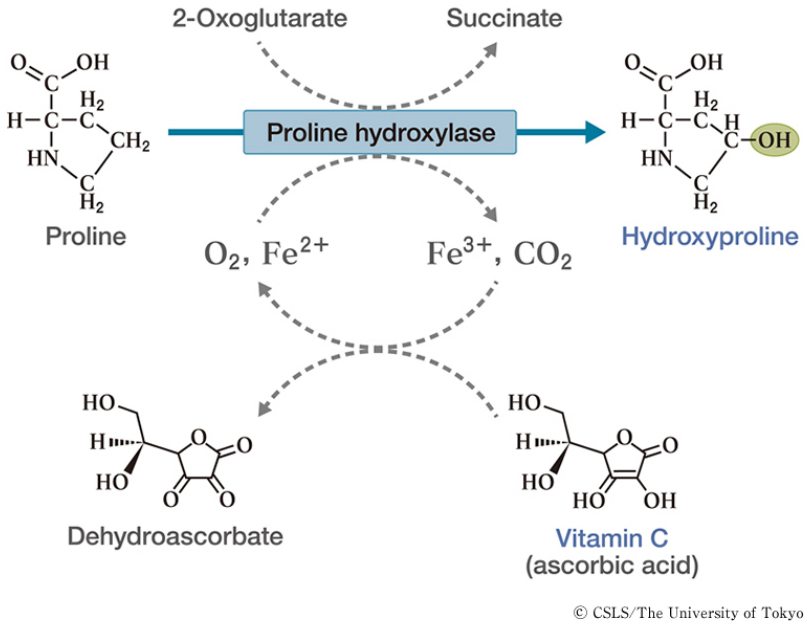

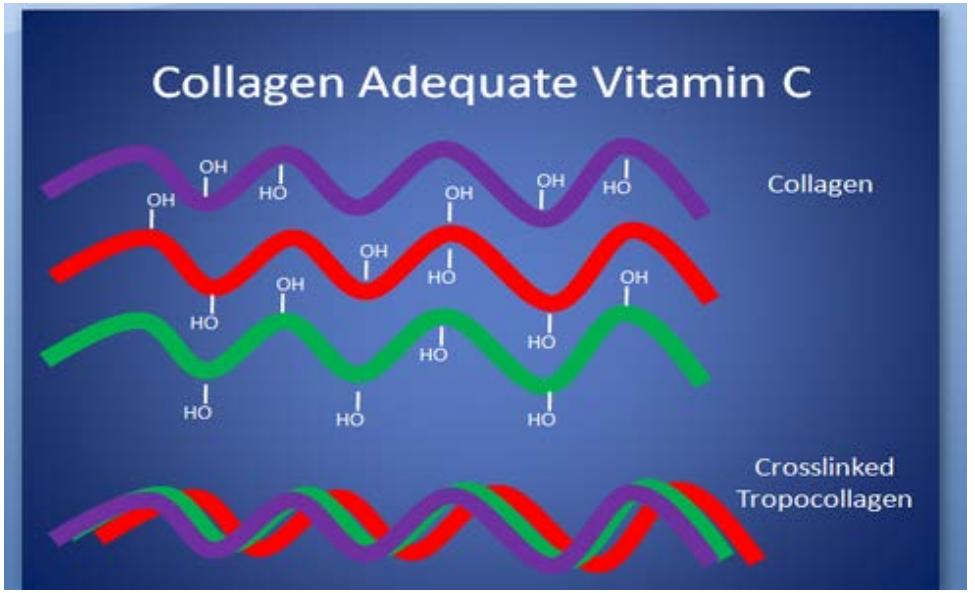

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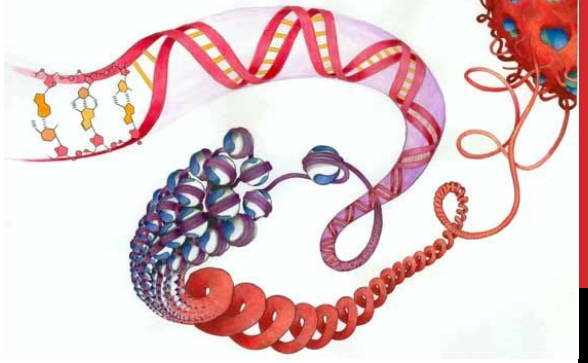
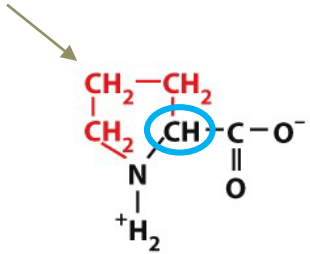
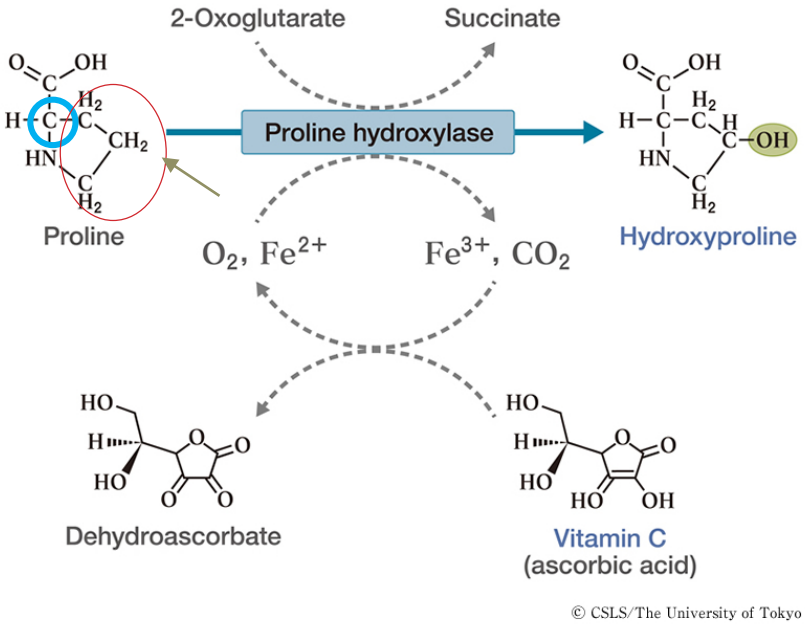
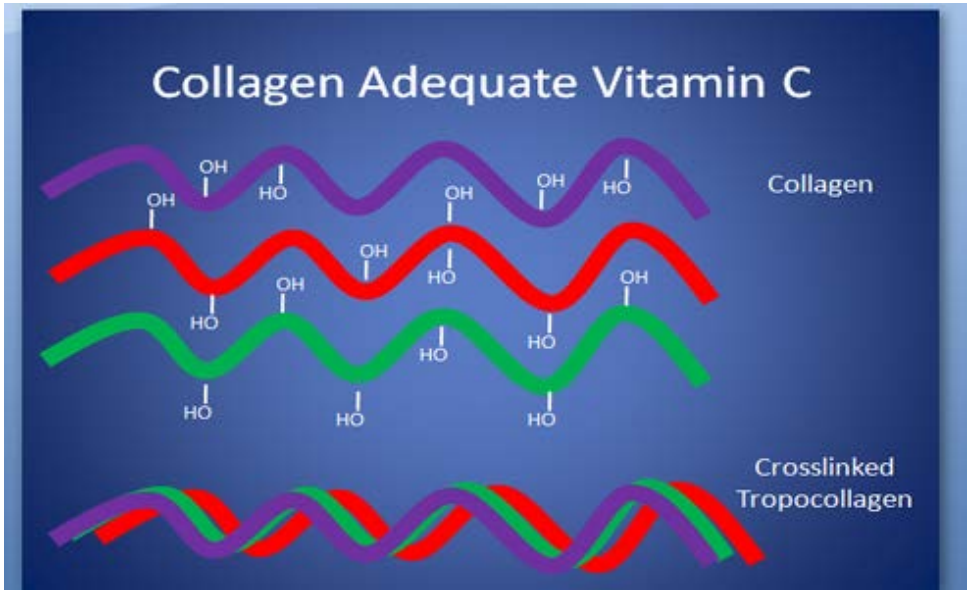




What kind of bonds?





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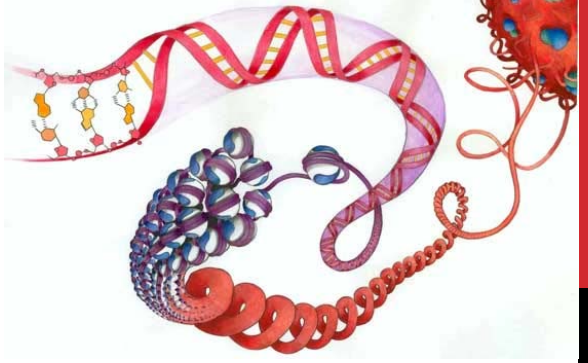
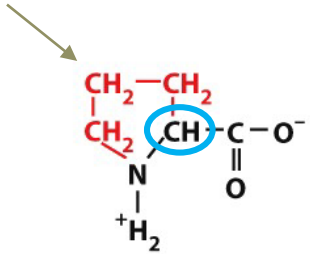
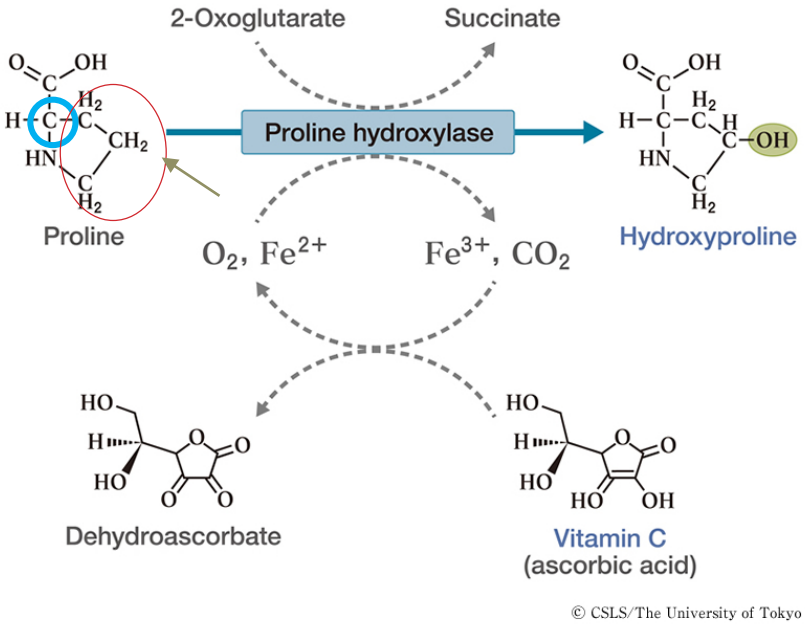
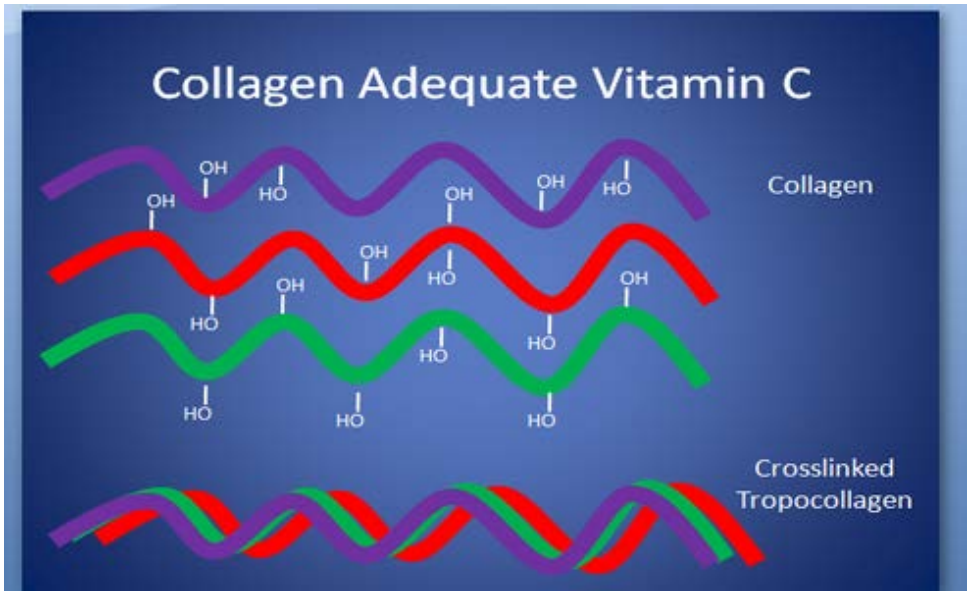


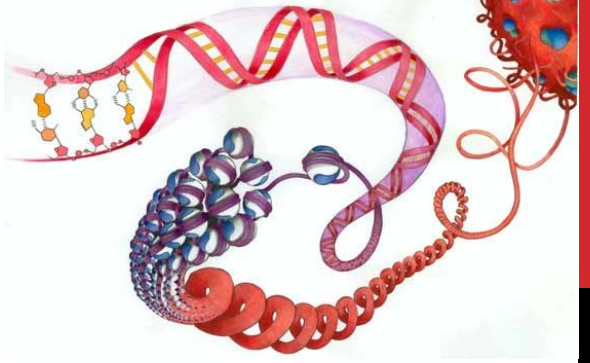




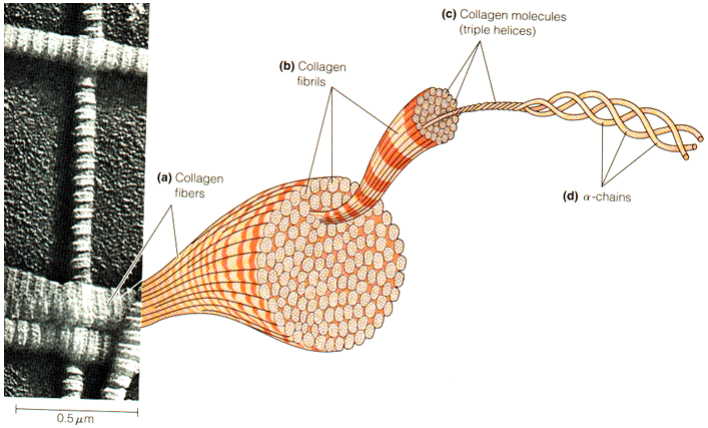


Vitamin C deficiency → Scurvy

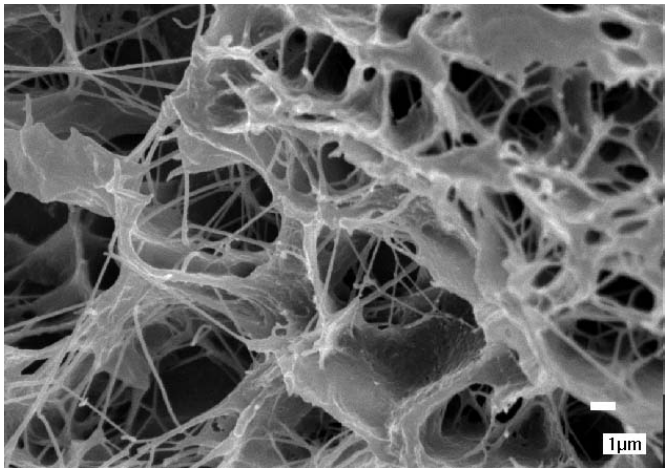
EXTRACELLULAR SPACE
COLLAGEN



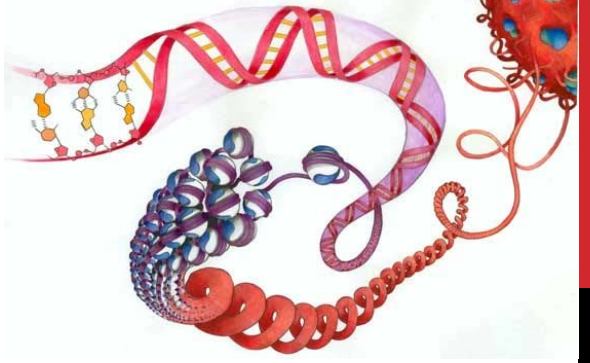
- **Fibrillar collagens** = assemble into rigid, cable-like fibrils which get packaged into thicker fibers
  - Col I, II, III
  - Mechanical framework
  - Tendons, cornea



- **Non-Fibrillar collagens** = non-helical lattice arrangement of collagens with globular domains
  - found in basement membranes
  - Col IV
  - Mechanical support with flexibility

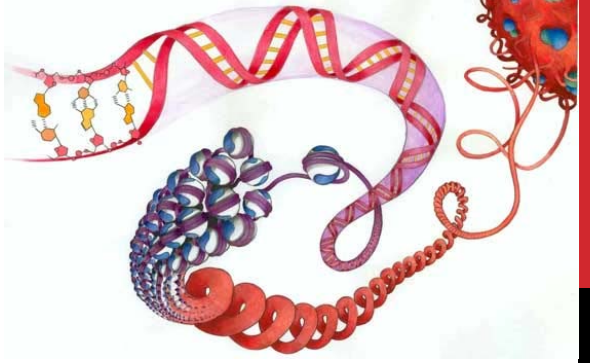


EXTRACELLULAR SPACE
COLLAGEN



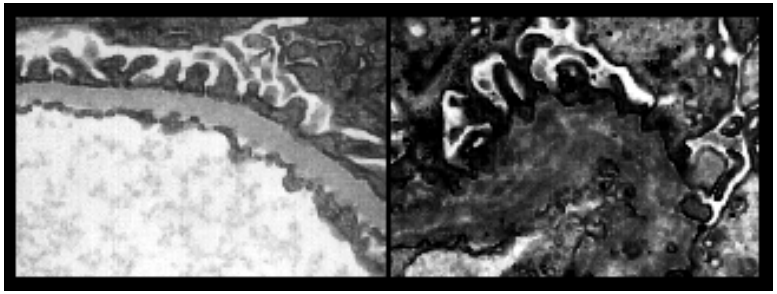
Collagen type		Genetic mutations
Type I	• most abundant • found in tendons, skin, vessel walls, fibrocartilage, bones, teeth • present in scar tissue	Osteogenesis Imperfecta
Type II	• hyaline cartilage, makes up 50% of all cartilage protein • vitreous humour of the eye	Dwarfism
Type III	• wound healing, often replaced by type I • vessel walls, skin, intestines and the uterus	Ehlers-Danlos Syndrome
Type IV	• primary collagen of basement membrane in glomerulus, capillaries, eye lens	Alport Syndrome

EXTRACELLULAR SPACE
COLLAGEN



Case Study:

- 30 year old male presents at his GP with blood in his urine. A urinalysis demonstrates elevated levels of protein (proteinurea). The GP also notes elevated blood pressure.
- The patient also complains of gradual bilateral hearing loss, particularly at high tones.
- A routine medical history uncovers a family history of end-stage kidney disease (maternal grandfather).
- The GP orders an ultrasound, no major structural abnormalities or blockages are detected. Urinary tract and kidney infections are ruled out.
- Upon completion of a kidney biopsy profound thickening and splitting of the glomerular basement membrane is apparent.



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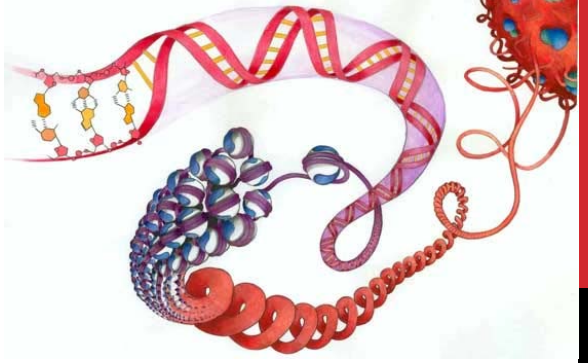
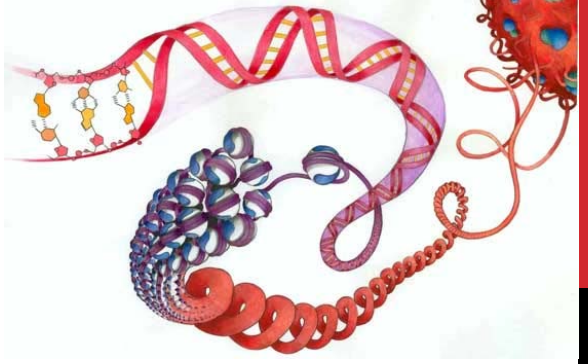


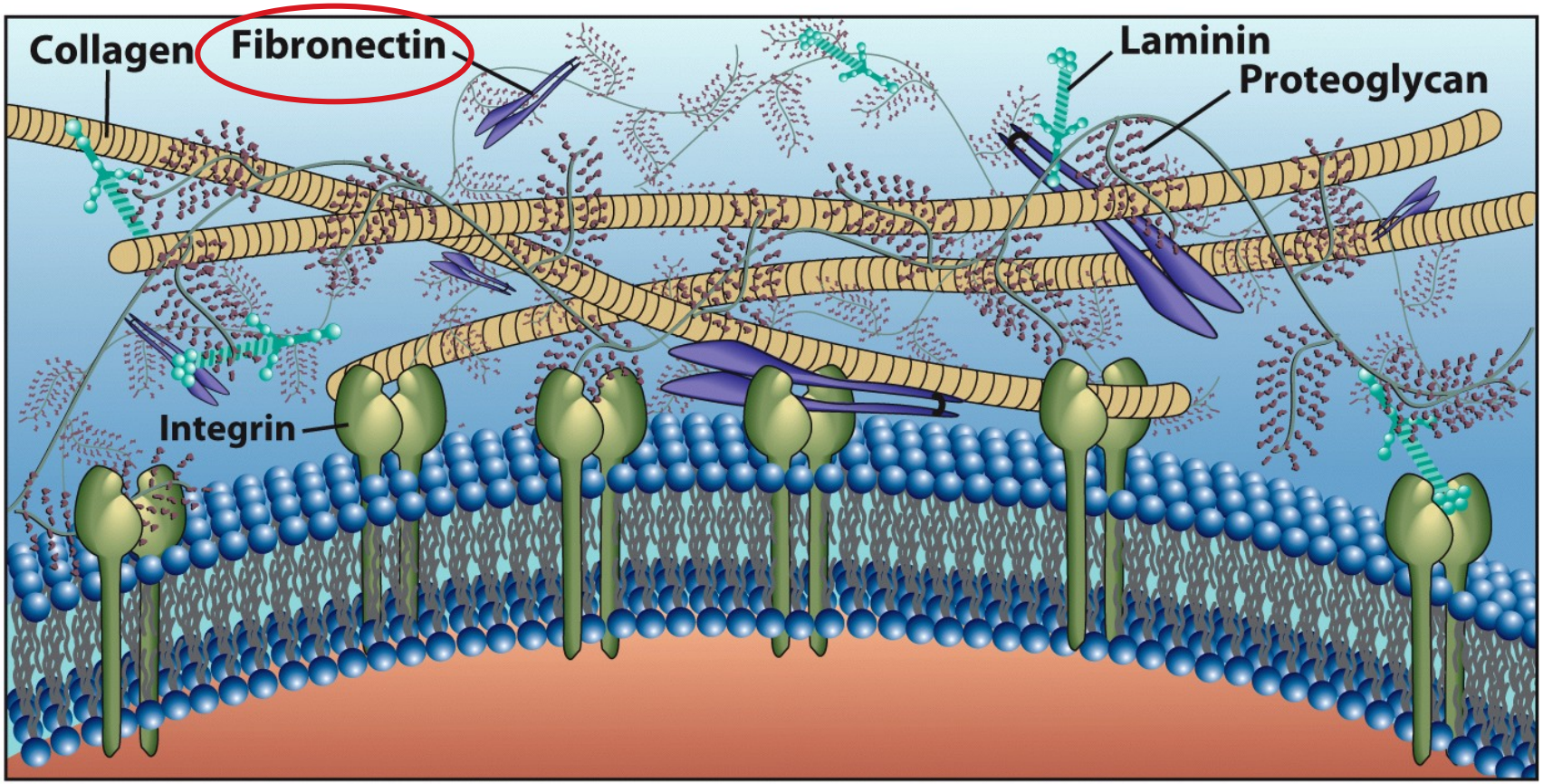
Case Study - Alport Syndrome

- characterized by glomerulonephritis, end-stage kidney disease, and hearing loss and in some instances can affect the eyes. The presence of blood in the urine (hematuria) is almost always found in this condition.
- mutations in collagen type IV biosynthesis genes
  - prevents proper production or assembly of the type IV collagen network →important structural component of basement membranes in the kidney, inner ear, and eye
- X-linked, predominantly affects males.
- Treatment – dialysis, kidney transplant, hearing aids

EXTRACELLULAR SPACE

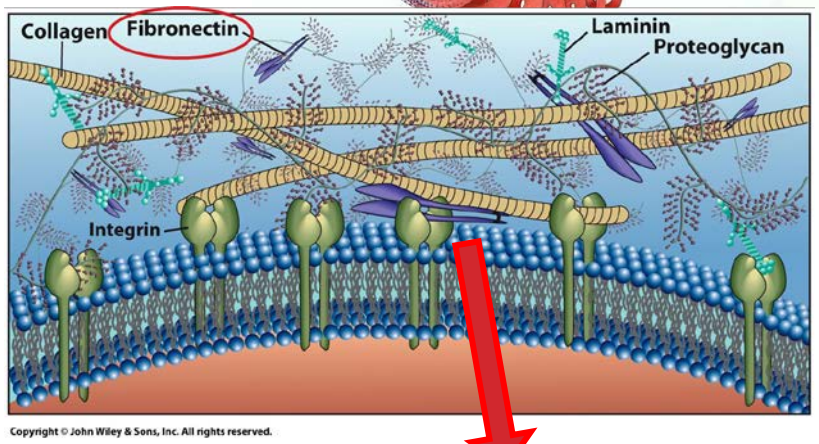
EXTRACELLULAR MATRIX



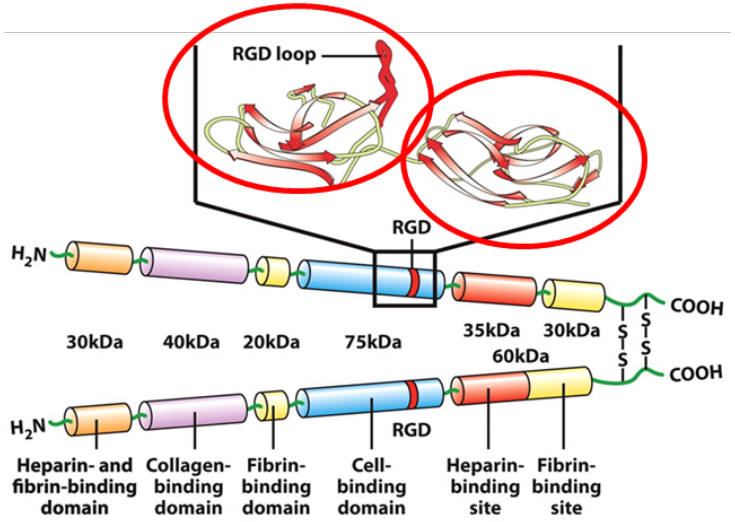


EXTRACELLULAR SPACE
FIBRONECTIN

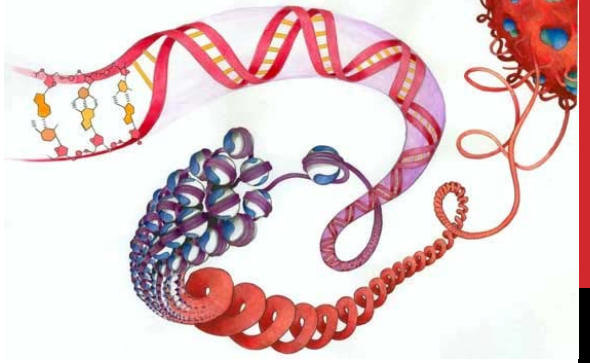
- **Fibronectin** = glycoprotein of ECM that binds to membrane-spanning receptor proteins (integrins), collagen, fibrin, and proteoglycans
- exists as a protein dimer, 2 nearly identical monomers linked by a pair of disulfide bonds
- each polypeptide monomer is composed of distinct modules (Fn), organized into several functional units



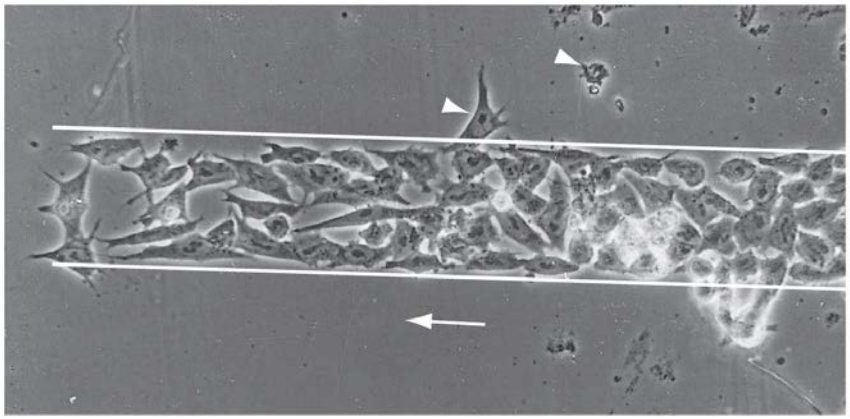
Arg-Gly-Asp



EXTRACELLULAR SPACE
FIBRONECTIN



- secreted primarily by fibroblasts as a soluble protein dimer
- assembled into insoluble matrix in a cell-mediated process
- plays a major role in cell adhesion, growth, migration, and differentiation
- very important in wound healing and embryonic development
  - waves of cell migration guided by fibronectin

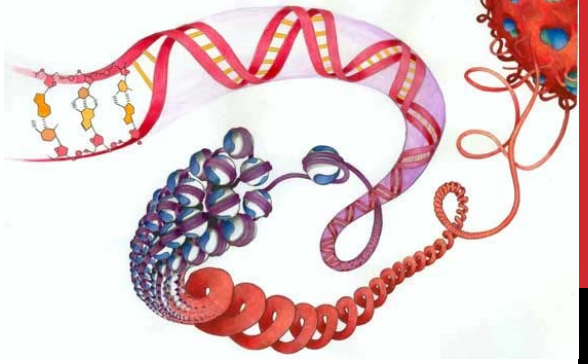


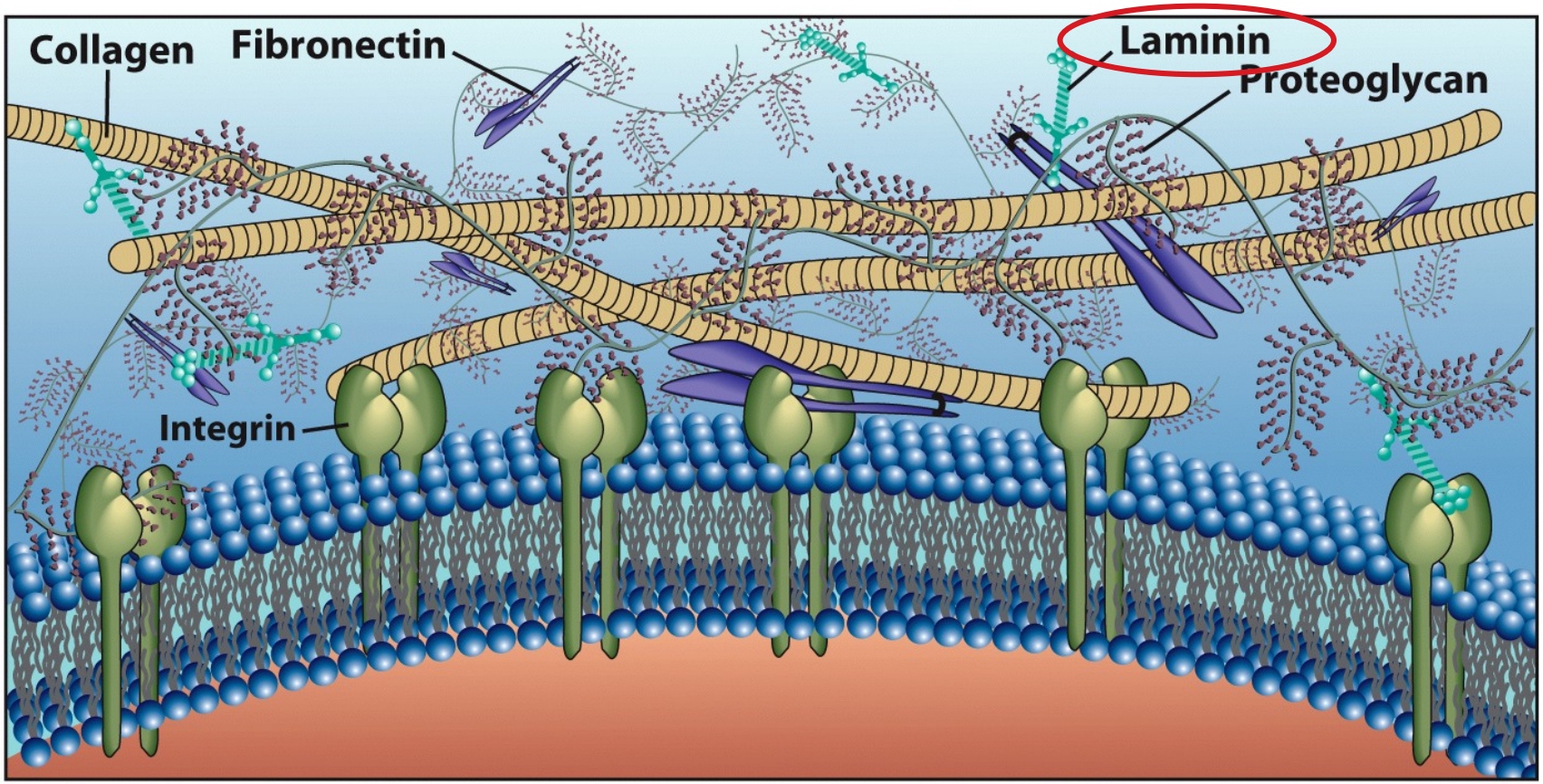
From Giovanni Levi, Jean Loup Duband and Jean Paul Thiery, Int. Rev. Cytol. 123: 213, 1990. © 1990, with permission from Elsevier.

https://youtu.be/XFjIaTfJpaw

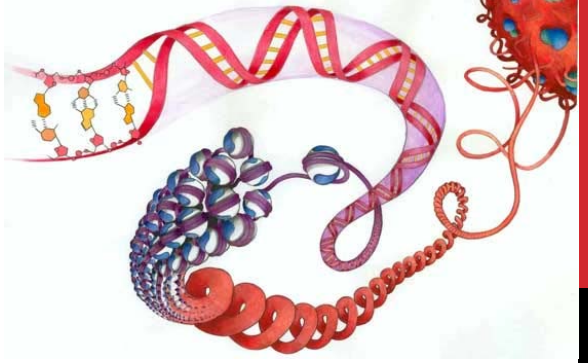
EXTRACELLULAR SPACE

EXTRACELLULAR MATRIX

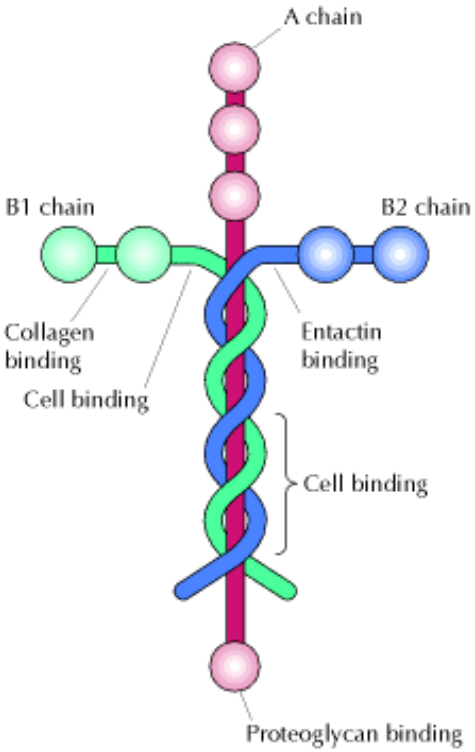


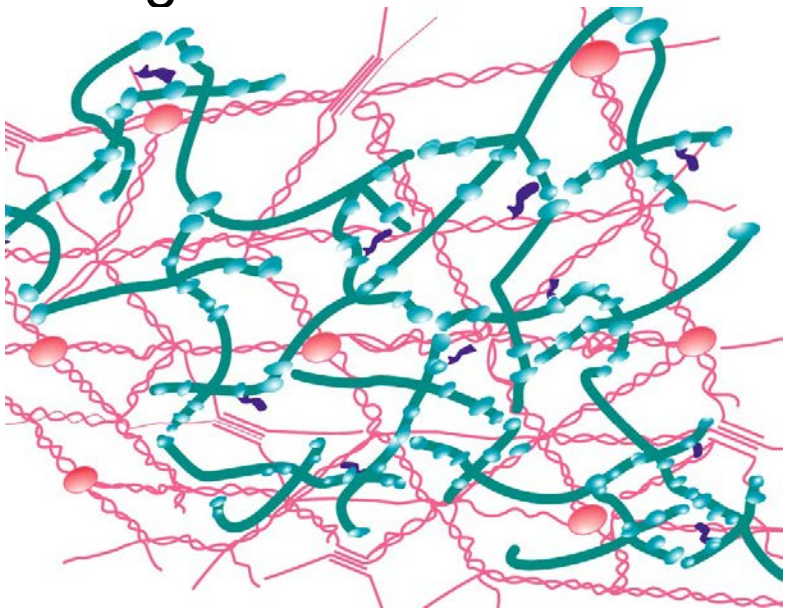


EXTRACELLULAR SPACE
LAMININ



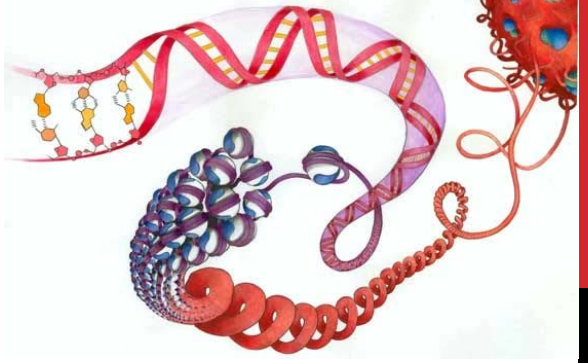
- **Laminin** = family of glycoproteins (>15) that consist of 3 polypeptide chains linked by di-sulfide bonds organized into molecule resembling a cross
- bind tightly to cell-surface receptors, laminin, proteoglycans (heparin) and collagen of basement membrane



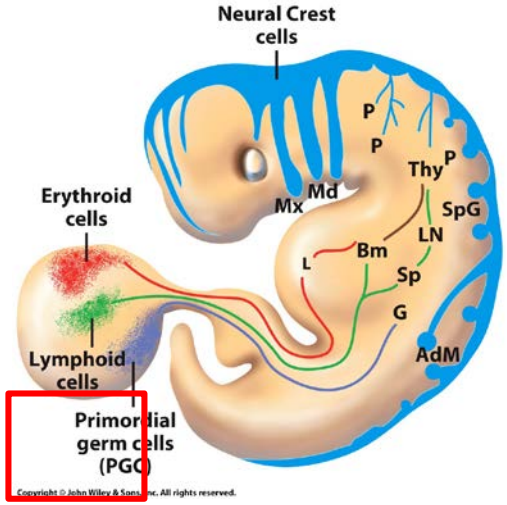


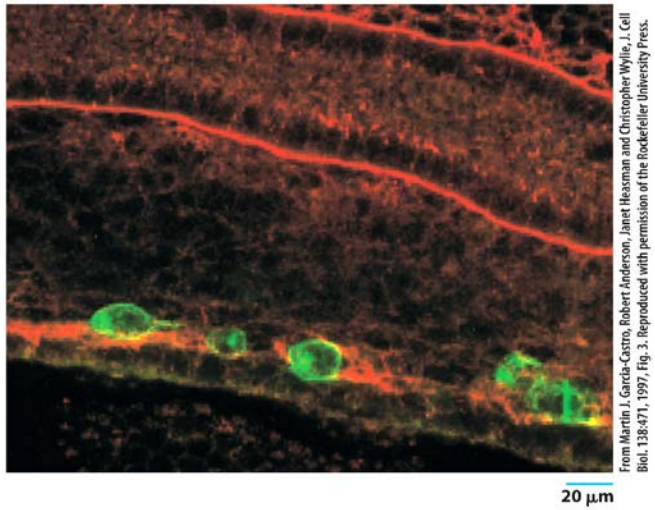
Collagen IV
Laminin
Entactin molecules
(BM)

EXTRACELLULAR SPACE
LAMININ



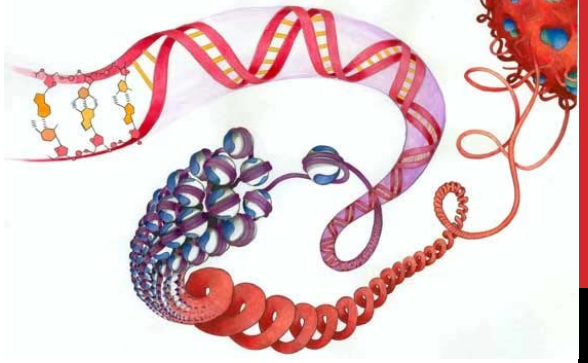
- greatly influence a cell's potential for migration, growth and differentiation
  - pivotal role in the migration of primordial germ cells

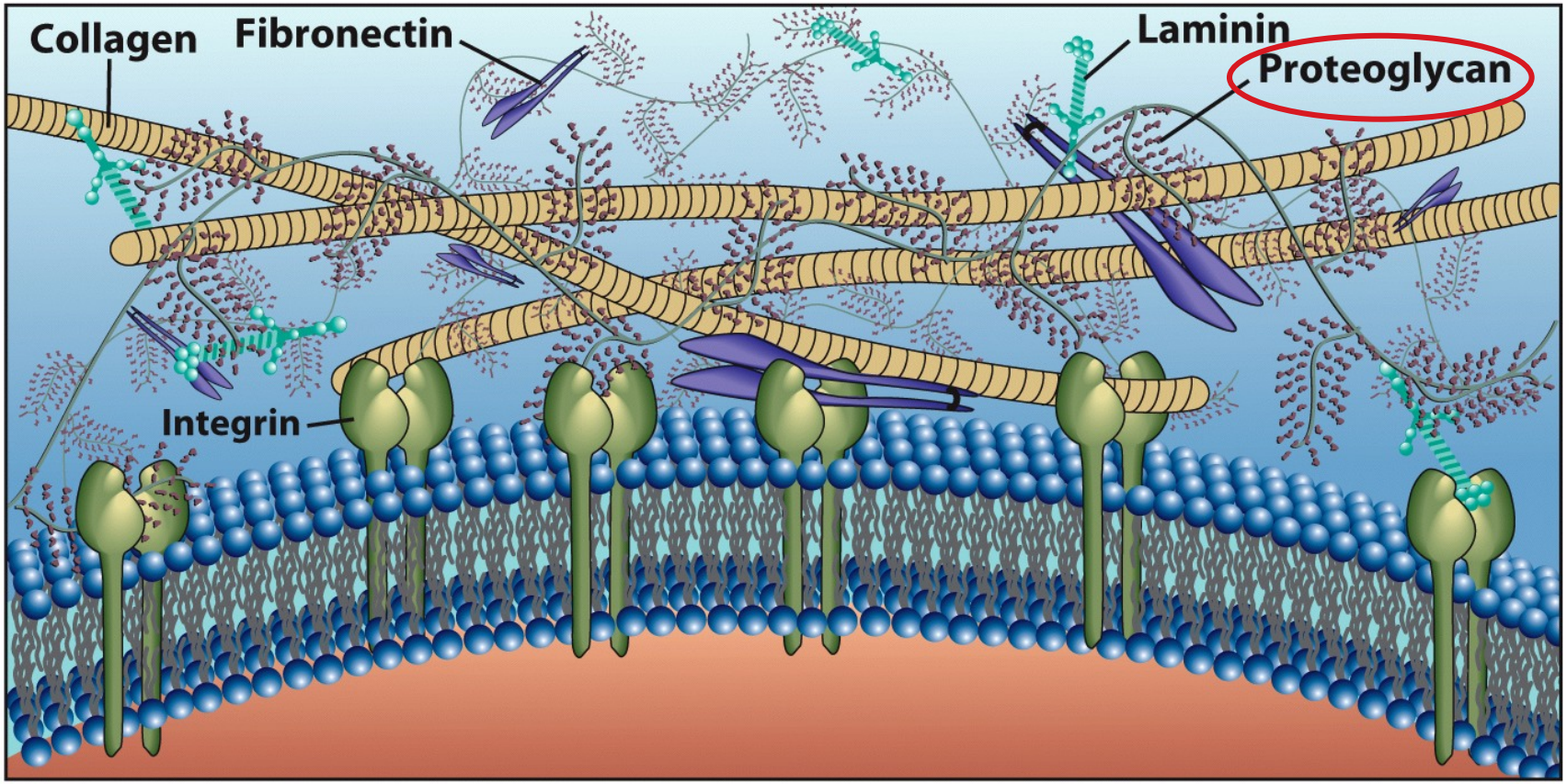




primordial germ cells migrating along a tract of **laminin** from the dorsal mesentery to the developing gonad

EXTRACELLULAR SPACE
PROTEOGLYCANS



- 

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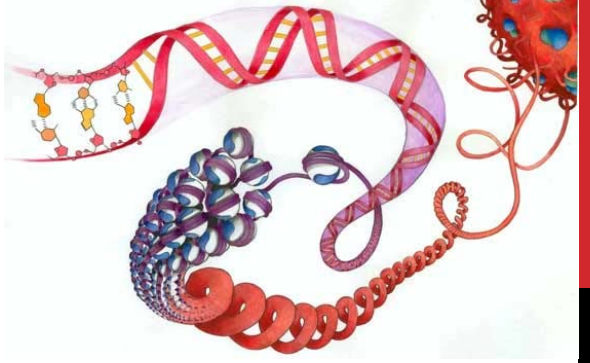
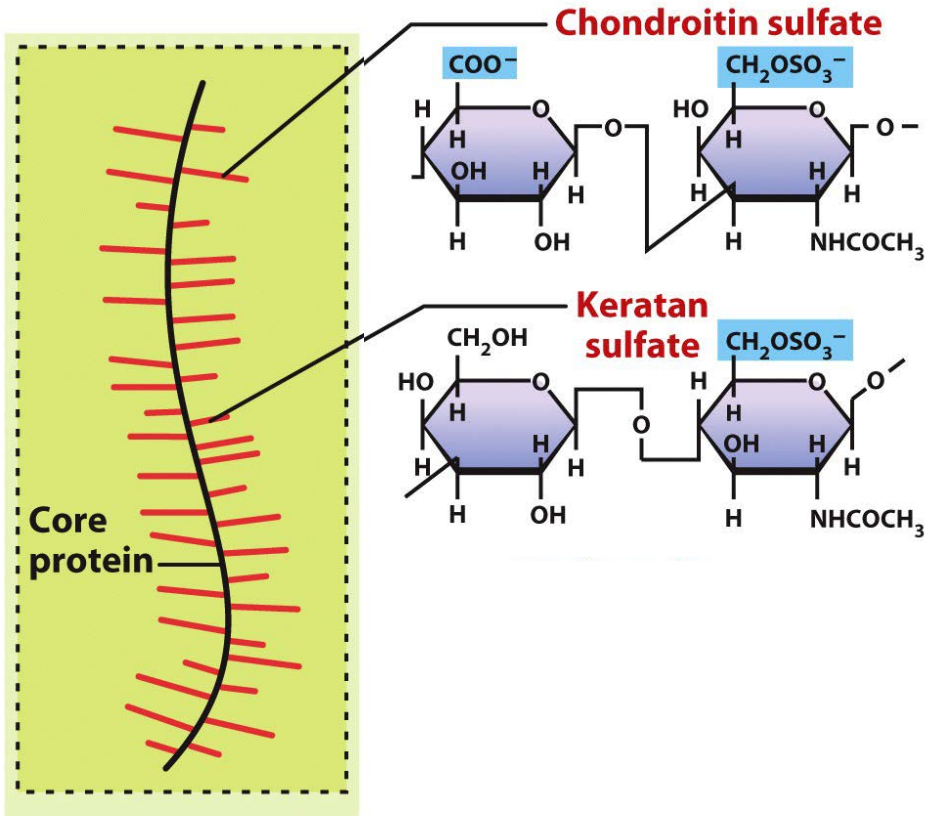
PROTEOGLYCANS



- **Proteoglycans** = ECM protein-polysaccharide complex
  - core protein molecule to which glycosaminoglycans (GAGs) are covalently attached
- may assemble into gigantic complexes by linkage of their core proteins to a molecule of hyaluronic acid (nonsulfated GAG)



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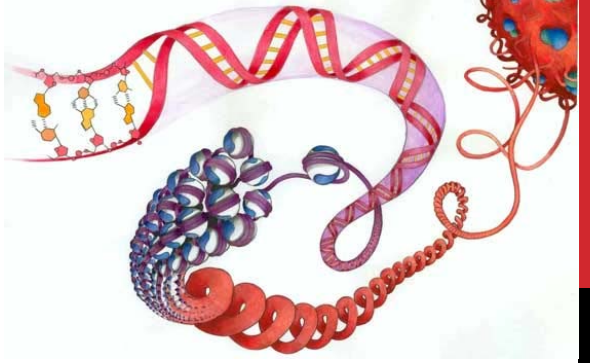
PROTEOGLYCANS



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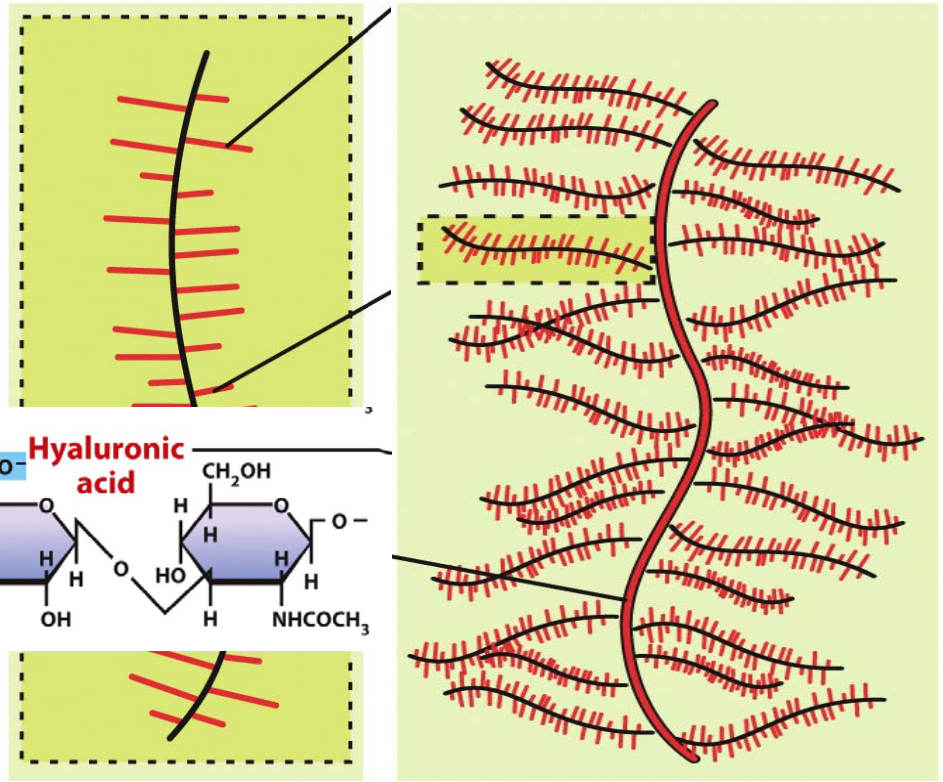
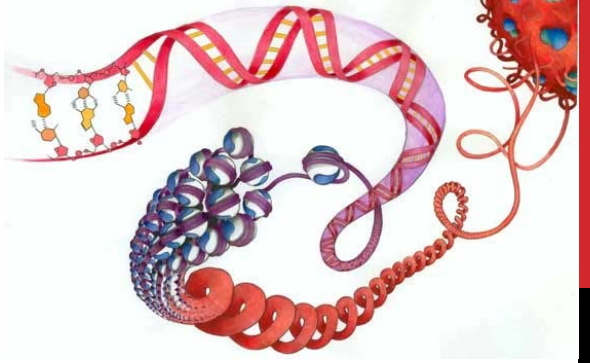


EXTRACELLULAR SPACE
PROTEOGLYCANS



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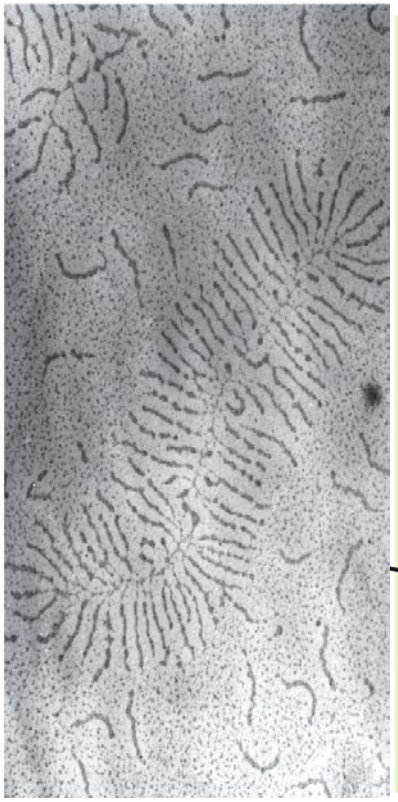
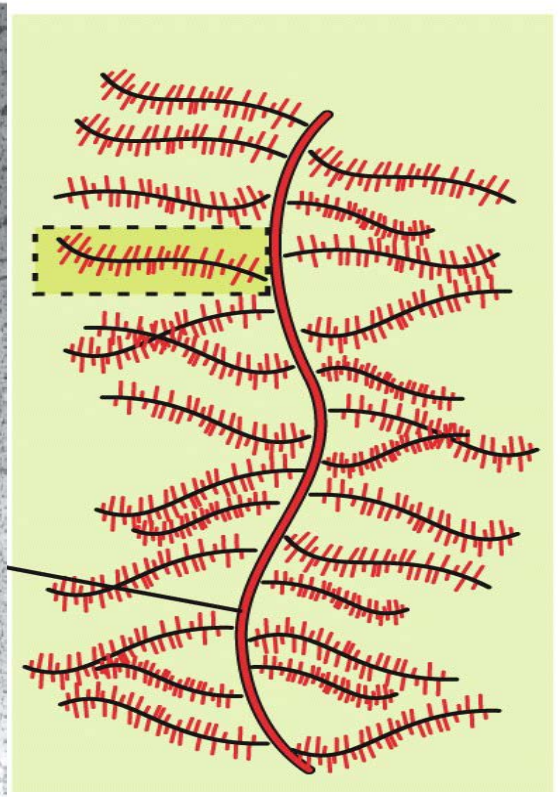
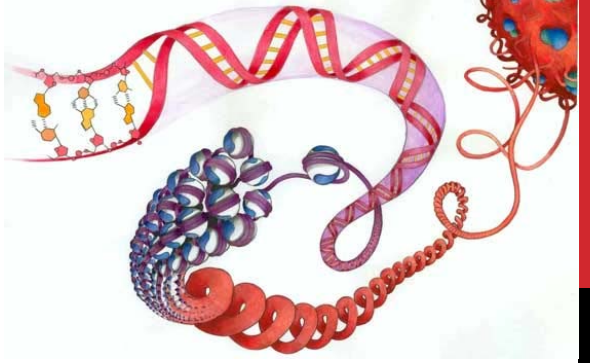
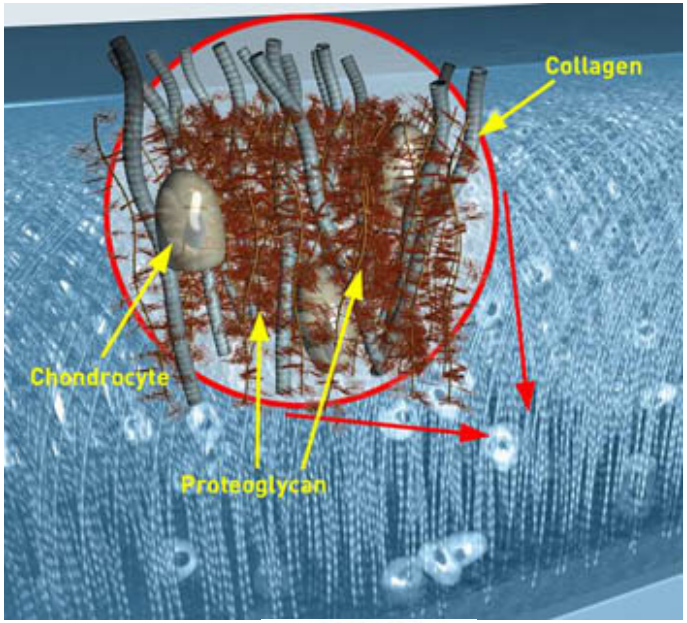


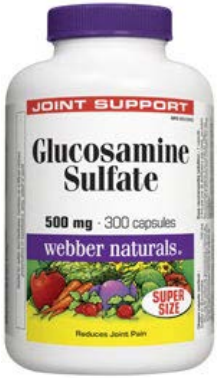


EXTRACELLULAR SPACE
PROTEOGLYCANS



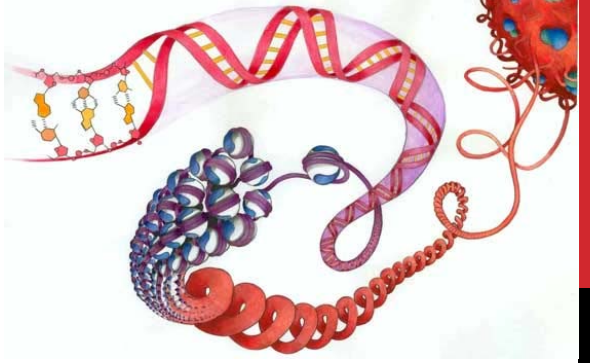
- bind large number of cations, which in turn bind H_2O
- Thereby forming porous, hydrated gel → packing material that resist crushing forces
- Along with collagens give cartilage and other ECM strength and resistance to deformation
- Sulfated and nonsulfated GAGs taken as health supplements to improve skin and joints



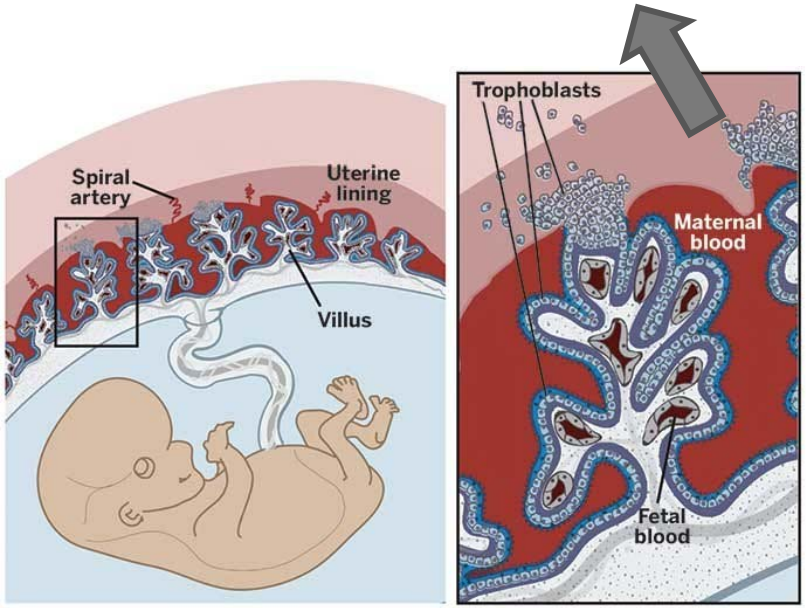


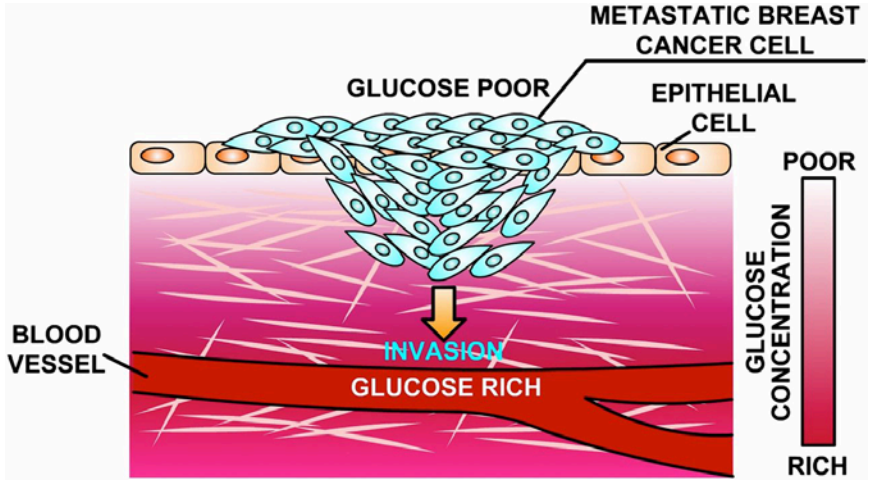
EXTRACELLULAR SPACE

EXTRACELLULAR MATRIX

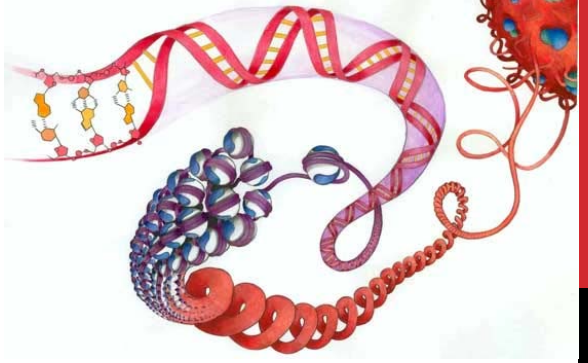


- Dynamic, components are continuously subject to degradation and reconstruction
- Renew the matrix and allow for remodelling
  - Embryonic development
  - Wound healing

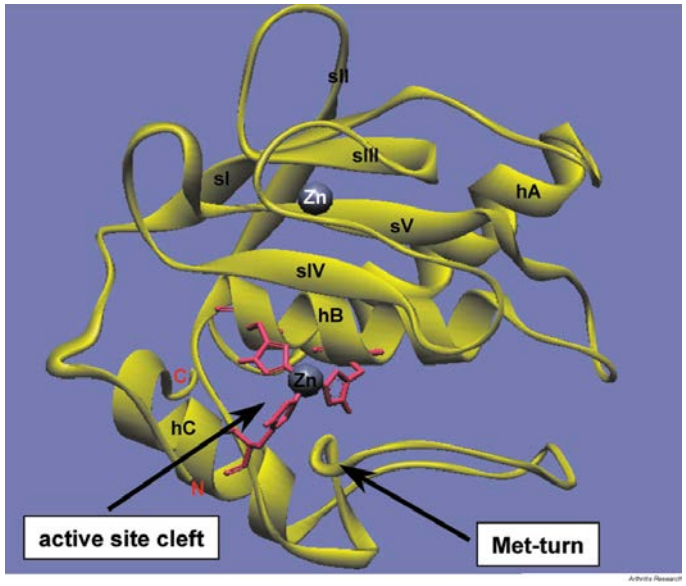




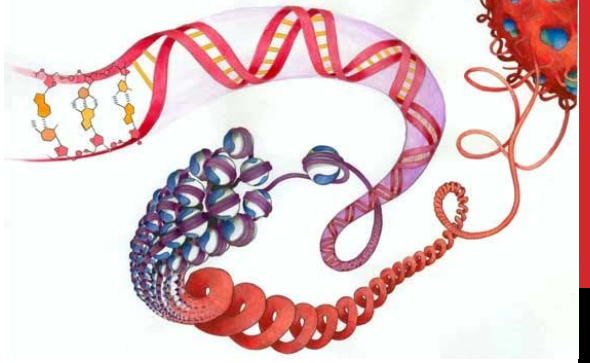
EXTRACELLULAR SPACE
EXTRACELLULAR MATRIX



- **Matrix metalloproteinases (MMPs):**
  - family of zinc containing enzymes
  - secreted into the ECM or anchored to plasma membranes
  - digest nearly all ECM components
  - actively involved in tissue remodeling, cell migration/invasion, wound healing and neovasculogenesis



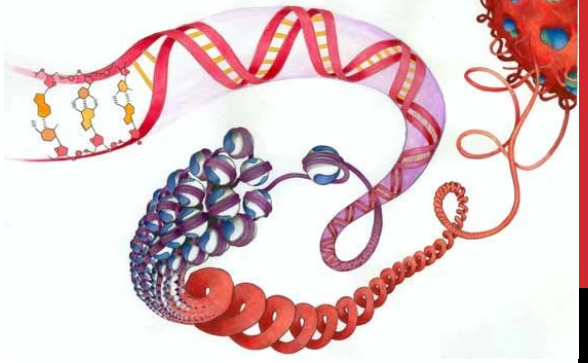
EXTRACELLULAR SPACE
EXTRACELLULAR MATRIX

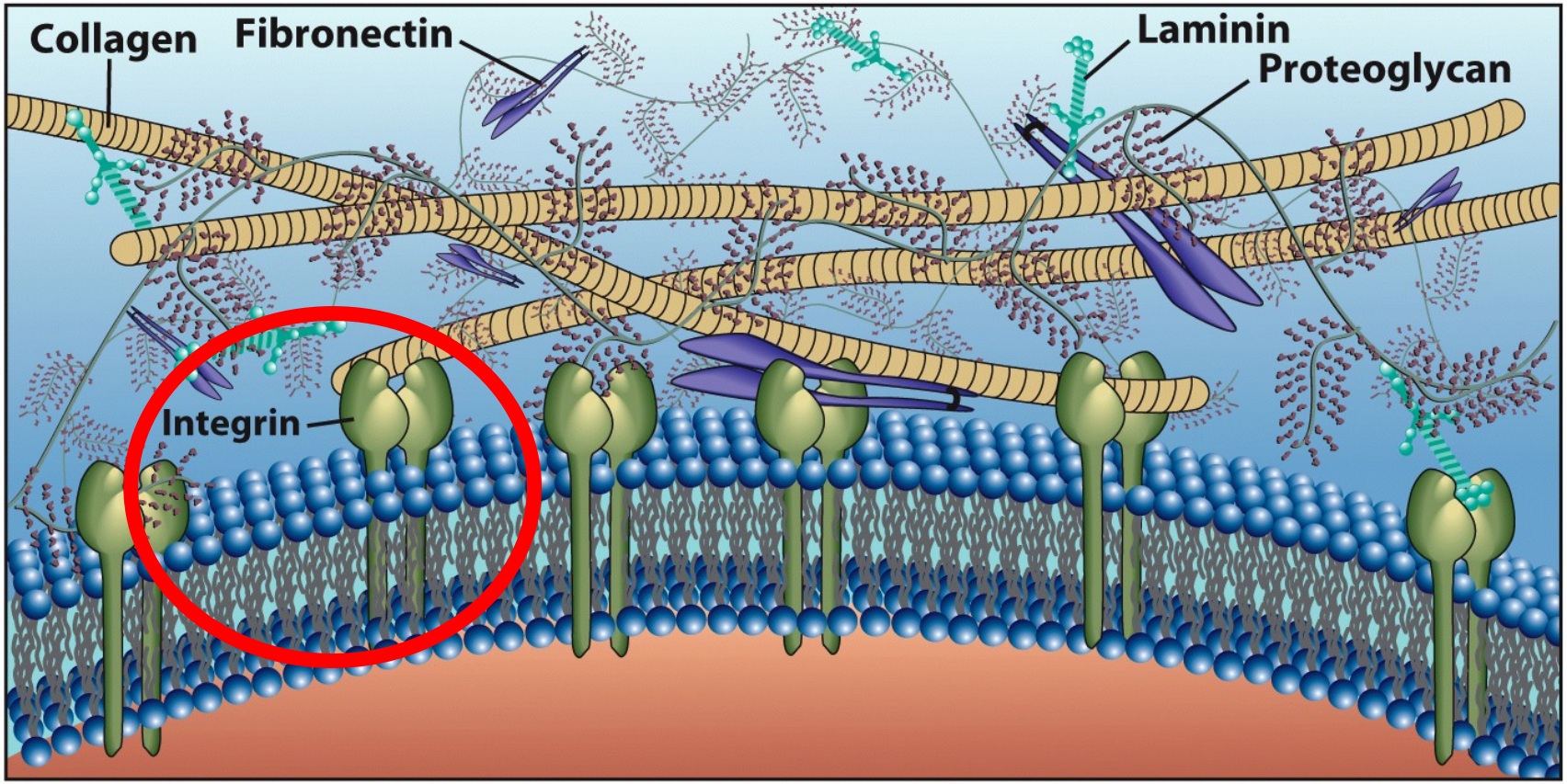


- **Matrix metalloproteinases (MMPs):**

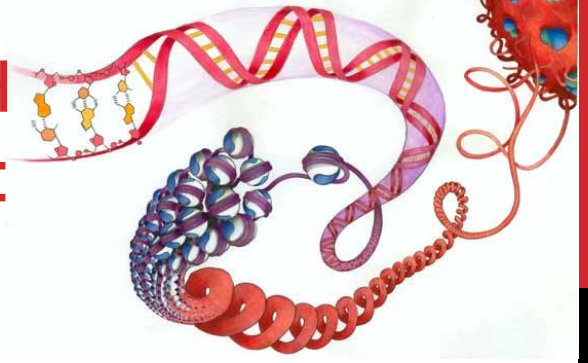
Enzyme	Other names	Preferred substrates
MMP-1	Collagenase-1, interstitial collagenase	Collagens I, II, III, VII, X, gelatins
MMP-2	Gelatinase A, 72kDa gelatinase	Gelatins, collagens IV, V, VII, X, elastin, fibronectin; activates pro-MMP-13
MMP-3	Stromelysin-1	Proteoglycans, laminin, fibronectin, gelatins.
MMP-7	Pump, Matrilysin	Proteoglycans, laminin, fibronectin, gelatins, collagen IV, elastin, activates pro-MMP-1 and -2 .
MMP-8	Collagenase-2, neutrophil collagenase	Collagens I, II, III
MMP-9	Gelatinase B, 92 kDa gelatinase	Gelatins, collagens IV, V, elastin
MMP-12	Macrophage metalloelastase	Elastin, collagen IV, fibronectin, activates pro-MMP-2 & 3.
MMP-13	Collagenase-3	Collagens I, II, III, gelatins
MMP-14	MT-MMP-1	Activates pro-MMP-2 & 13, gelatins
MMP-15	MT-MMP-2	unknown
MMP-16	MT-MMP-3	Activates pro-MMP-2
MMP-17	MT-MMP-4	unknown

INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS

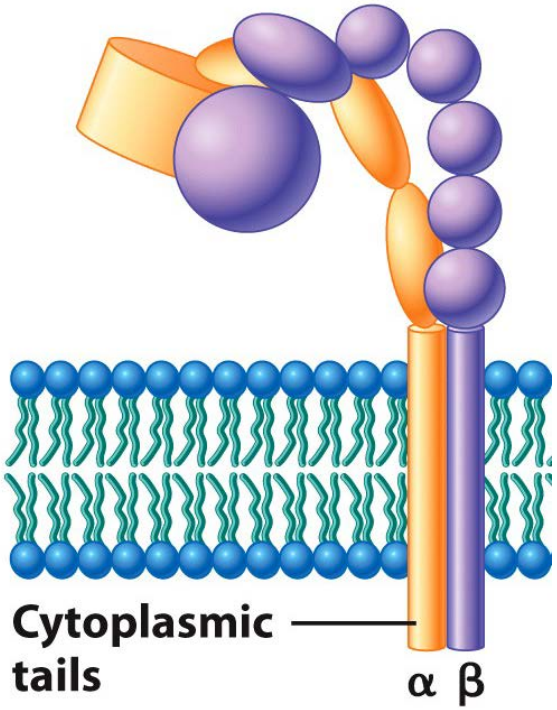




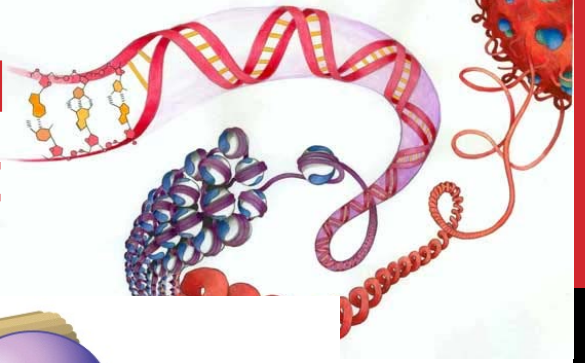
INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: INTEGRINS

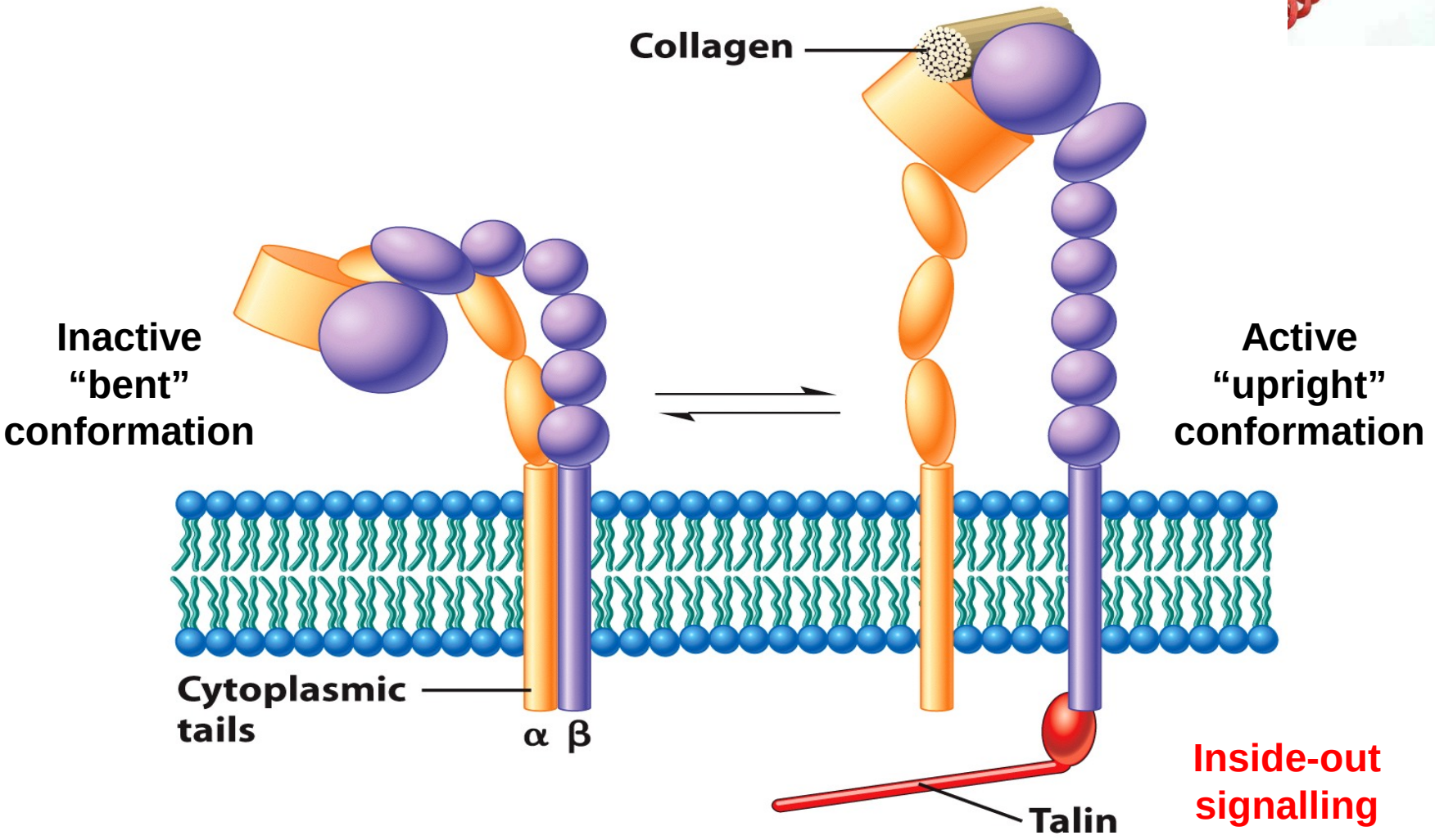


- **Integrin =** family of membrane proteins play a key role in integrating extracellular and intracellular environments
- Integrins bind fibronectin, laminin, proteoglycans, collagen
  - Specific amino acid sequence → Arg-Gly-Asp (RGD)
- 2 membrane-spanning polypeptide chains (α and ß) that are non-covalently linked
- 18 different α subunits and 8 different ß subunits → >100 different pairings (~ 24 identified)

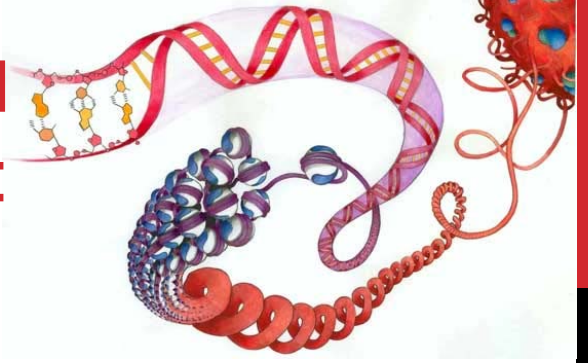


INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: INTEGRINS

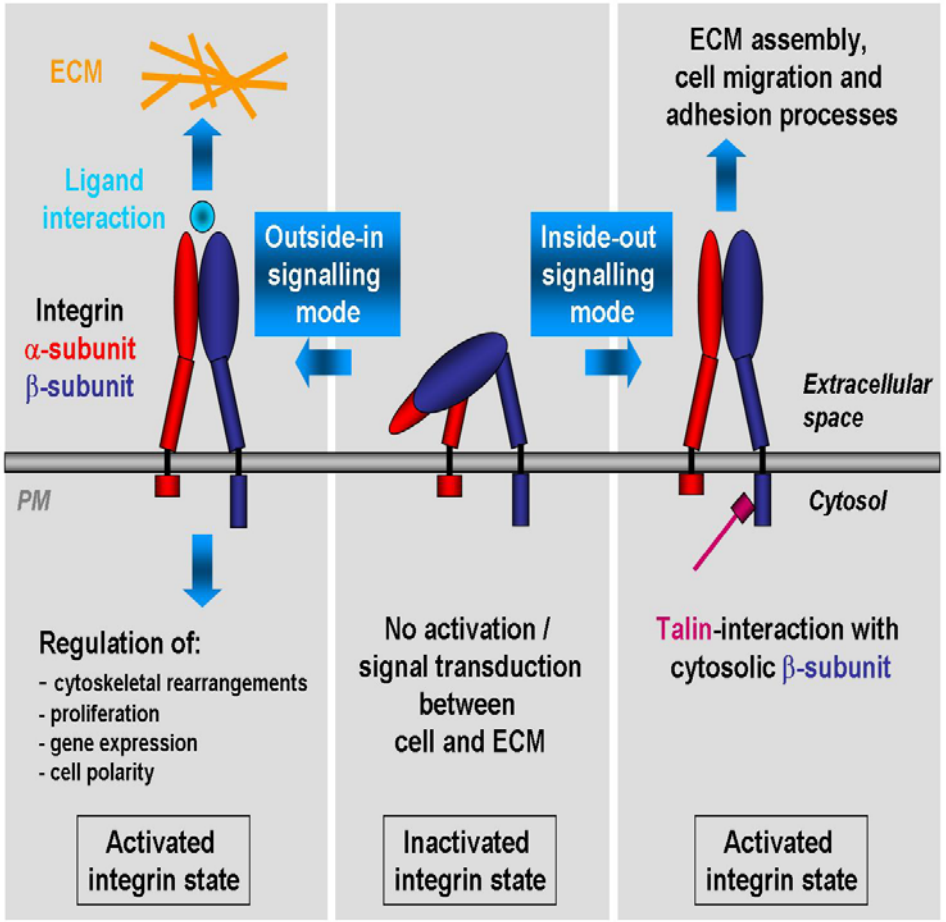




INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: INTEGRINS



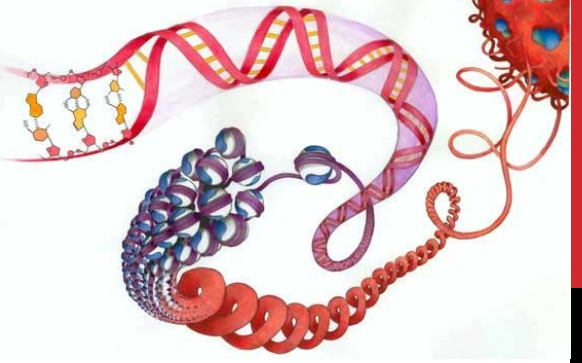
- Functions:
  1. adhesion of cells to their substratum → "Inside-Out signalling"
  2. transmission of signals between external environment and cell interior → "Outside-in signalling"
     - differentiation, motility, growth, survival

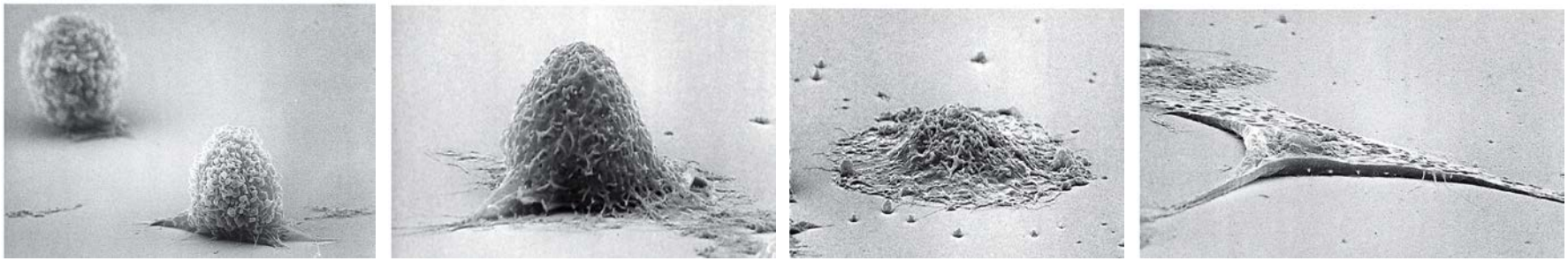


Normal vs. malignant cells

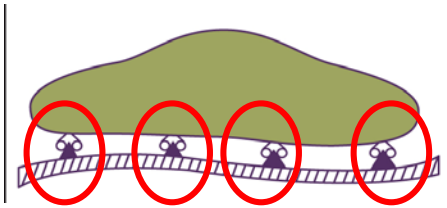
Blood clotting → fibrinogen

INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: FOCAL ADHESIONS



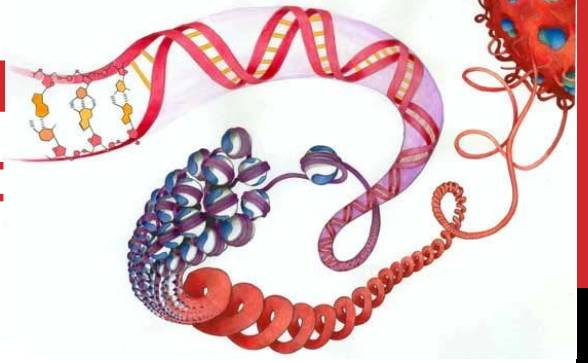


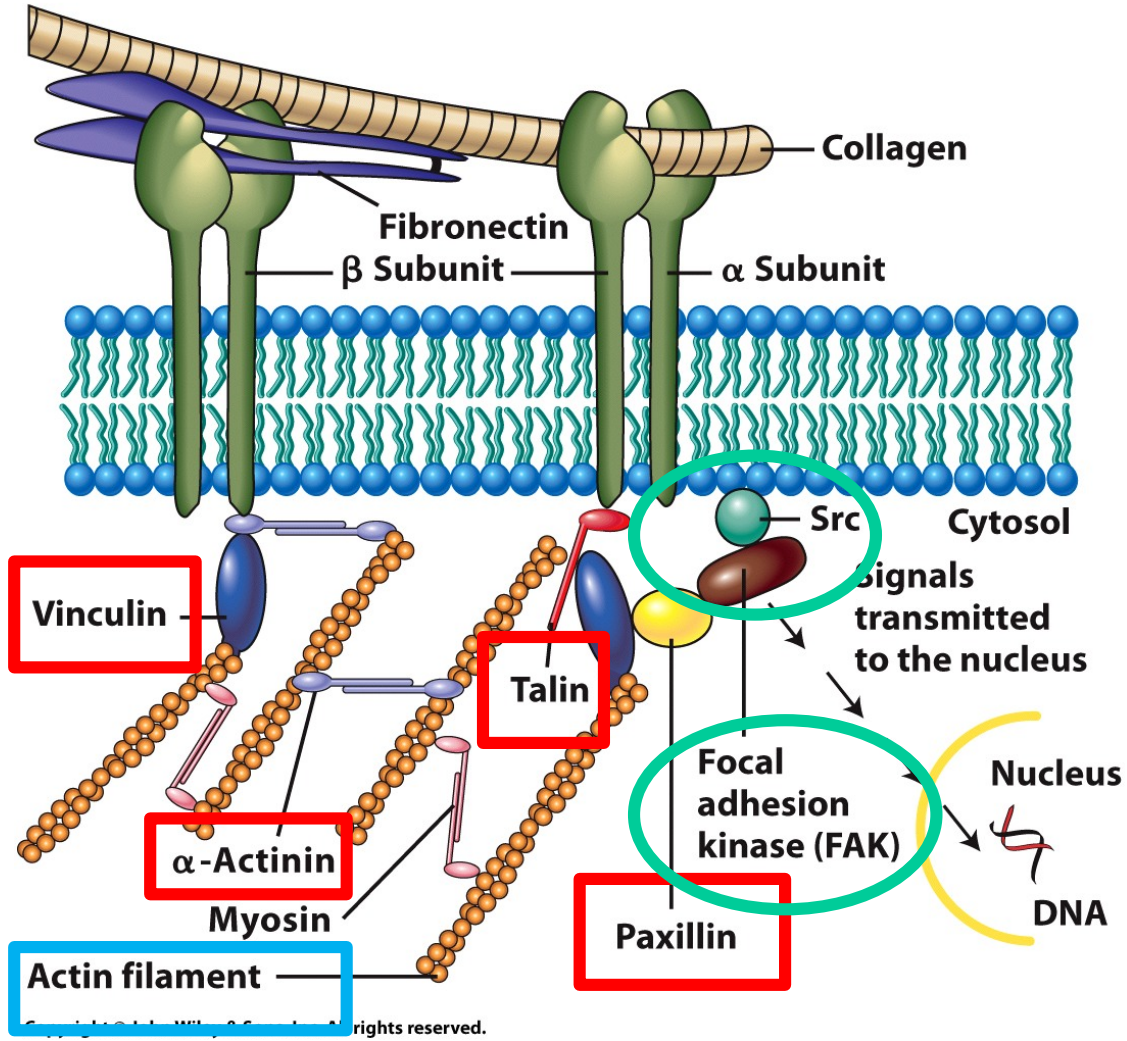
- **Focal adhesions** = dynamic structures that anchor a cell to a substratum, large clusters of integrins
- can be disassembled for movement or cell replication
- sensory structure, information about physical and chemical properties of extracellular environment
  - Cell adhesion, proliferation, survival, locomotion



Focal adhesions

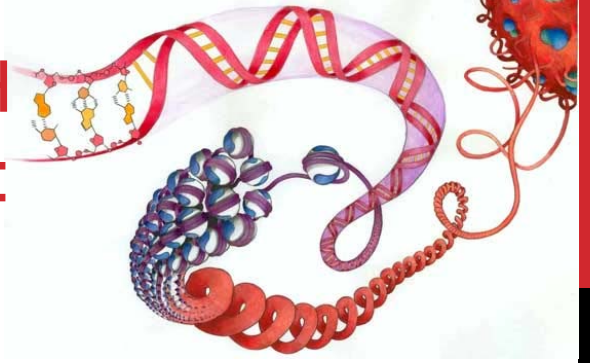
INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: FOCAL ADHESIONS



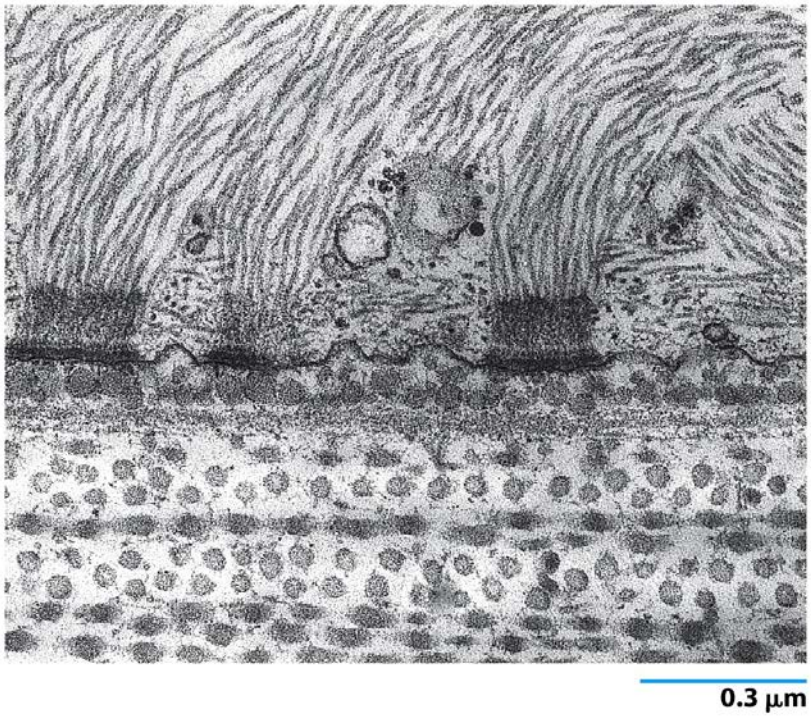


- common in cells grown in vitro
- muscle, tendon

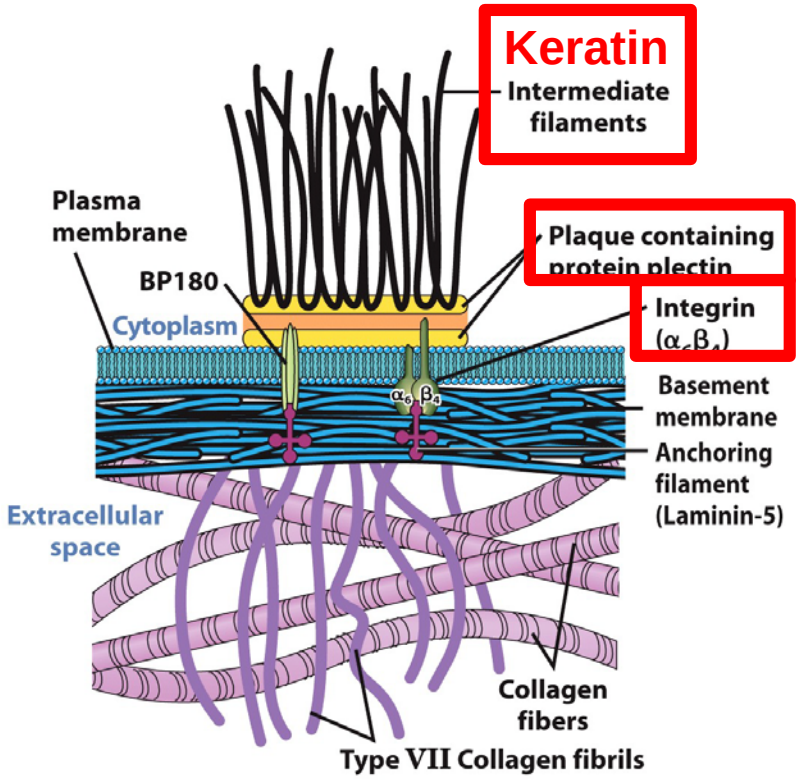
INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: HEMIDESMESOMES



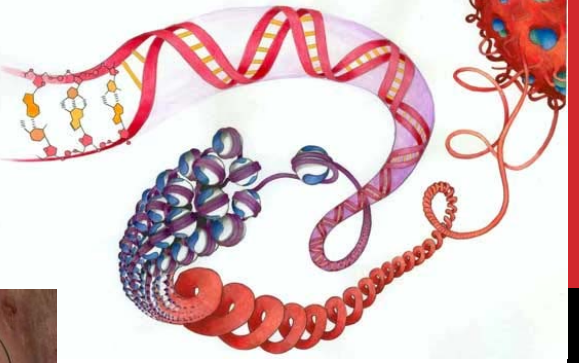
- Hemidesmosome = specialized integrin adhesive structure in epithelial cells in vivo



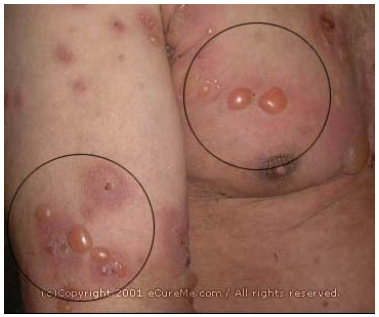
From Douglas E. Kelly, J. Cell Biol. 28:61 (bottom figure), Fig. 11, 1966; Reproduced with permission of the Rockefeller University Press.

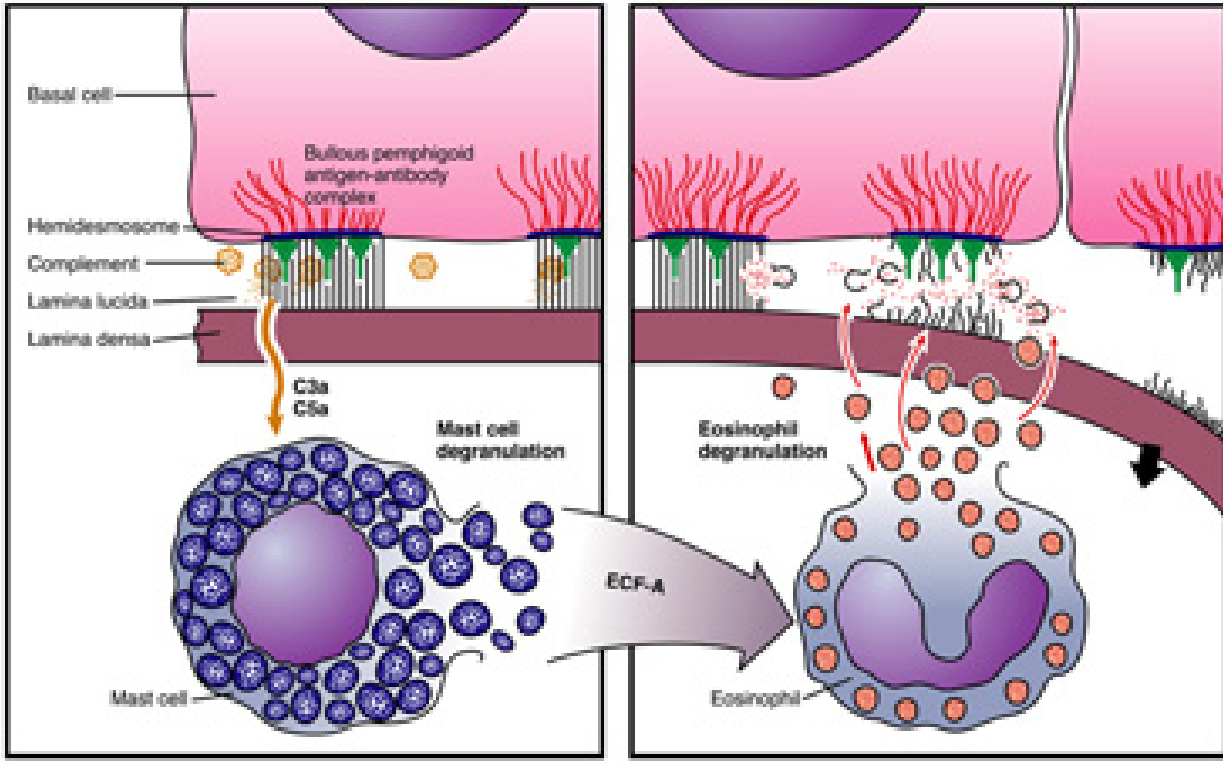


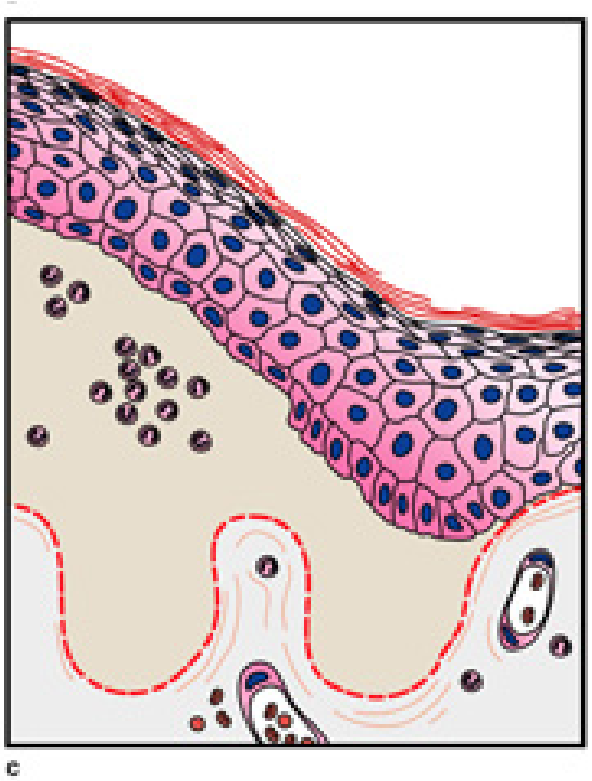
INTERACTION OF CELLS WITH EXTRACELLULAR MATERIALS: HEMIDESMESOMES



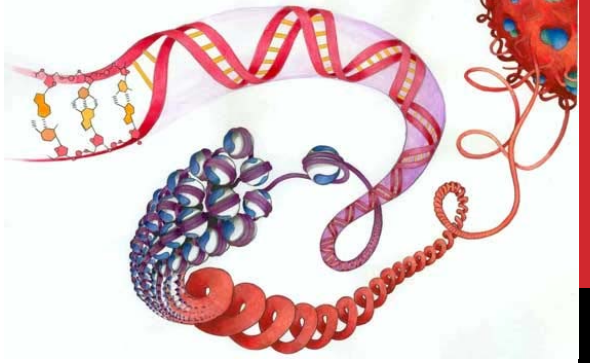
- Bullous pemphigoid antigen



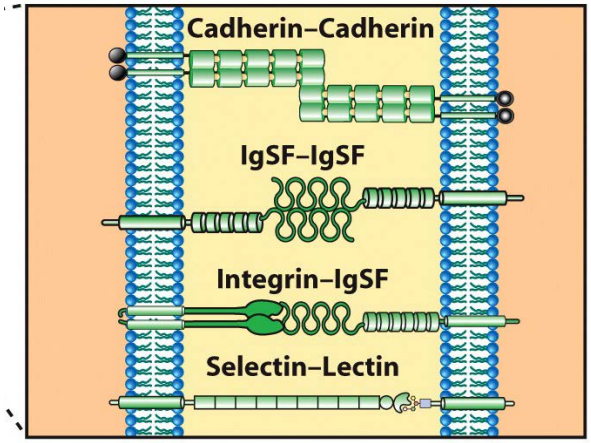


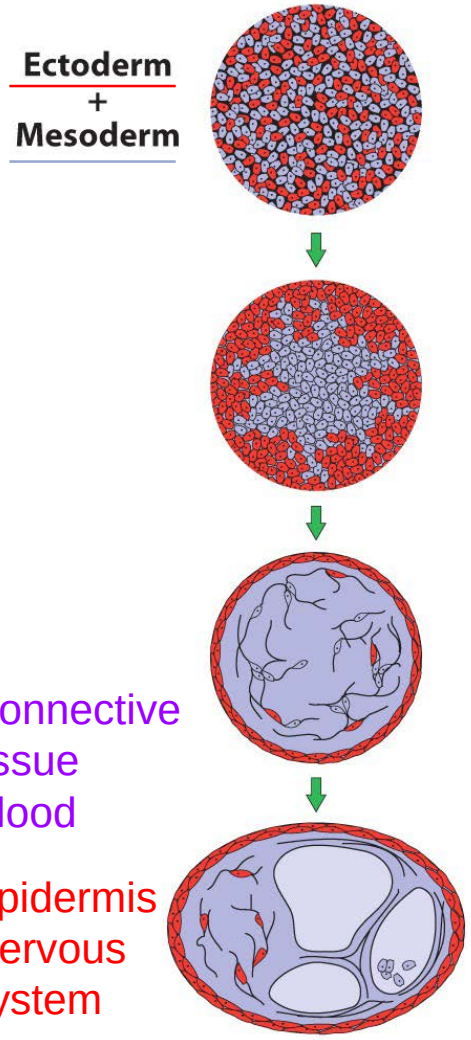


INTERACTION OF CELLS WITH OTHER CELLS



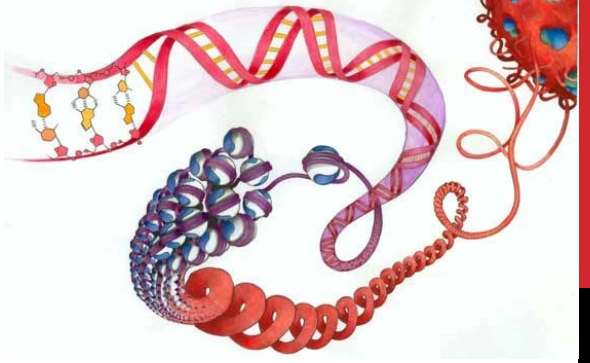
- 3-D arrangement of cells within an organ
- selective interaction between cells → recognize surfaces of cells and choose which cells to interact with
- distinct integral membrane proteins mediate cell-cell adhesion
  - selectins
  - IgSF
  - integrins
  - cadherins



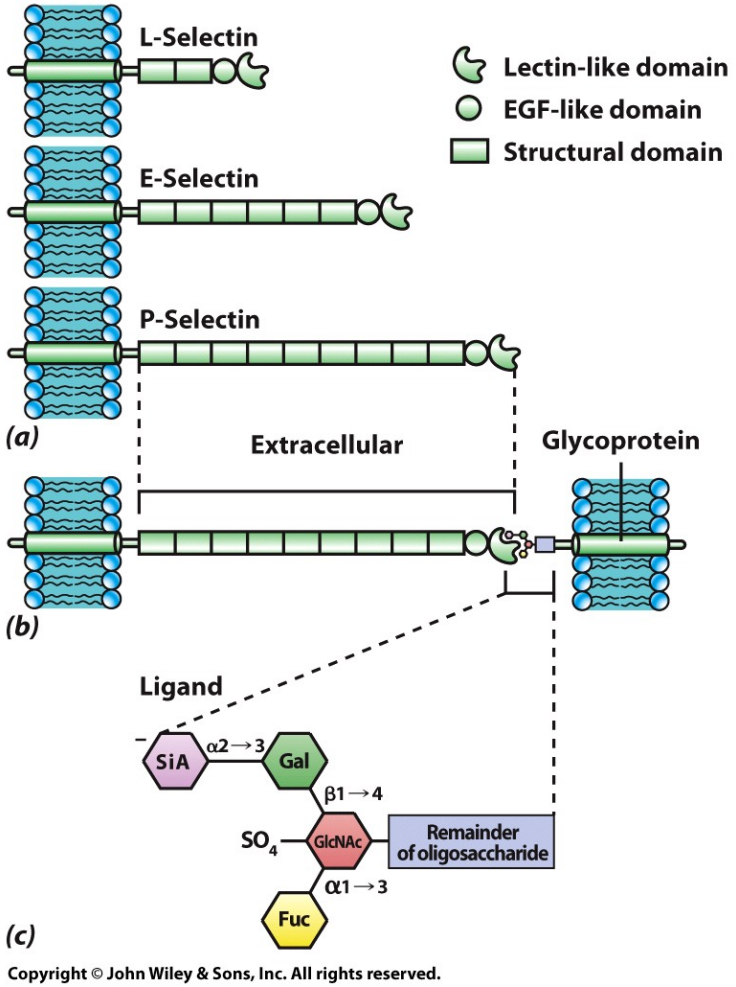


- Connective tissue
- Blood
- Epidermis
- Nervous system

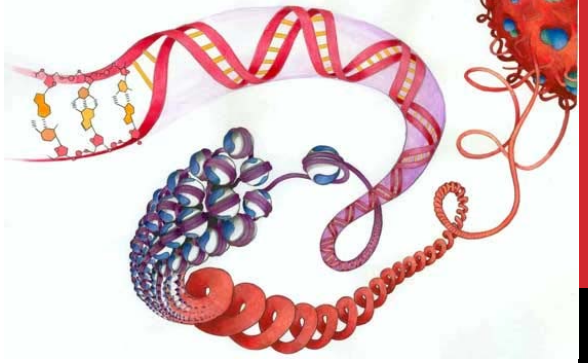
INTERACTION OF CELLS WITH OTHER CELLS: SELECTINS

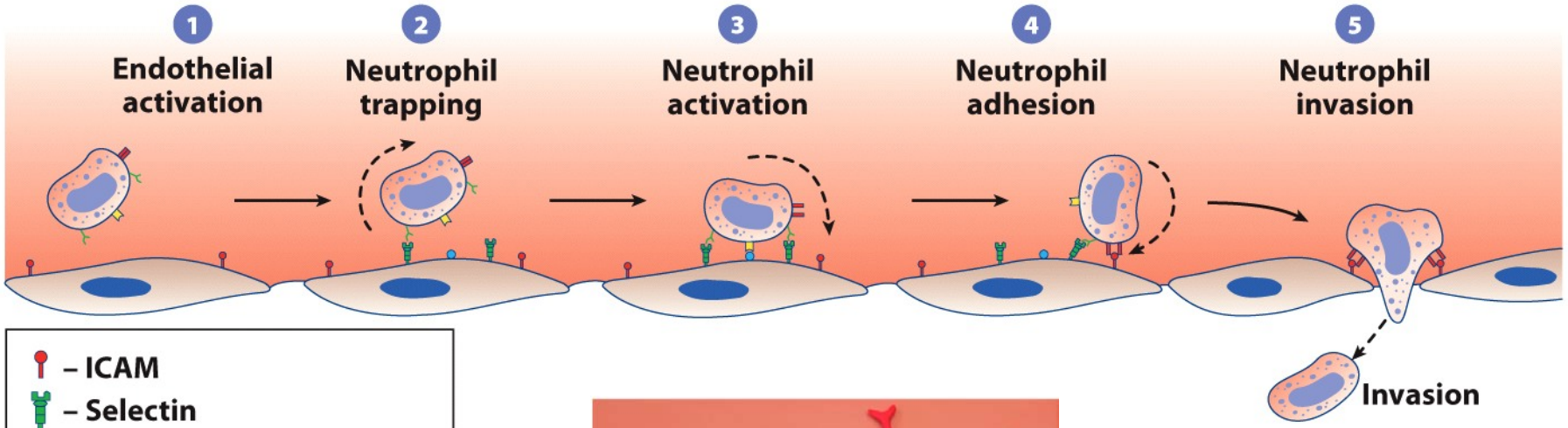


- **Selectins =** integral membrane glycoproteins that recognize and bind sugars in oligosaccharides that project from the surfaces of other cells
- Calcium dependent
- Mediates transient interactions between circulating leukocytes and vessel walls (inflammation and clotting)
  - L-selectin → leukocytes (WBC)
  - E-selectin → endothelial cells
  - P-selectin → platelets and endothelial cells



INTERACTION OF CELLS WITH OTHER CELLS: SELECTINS

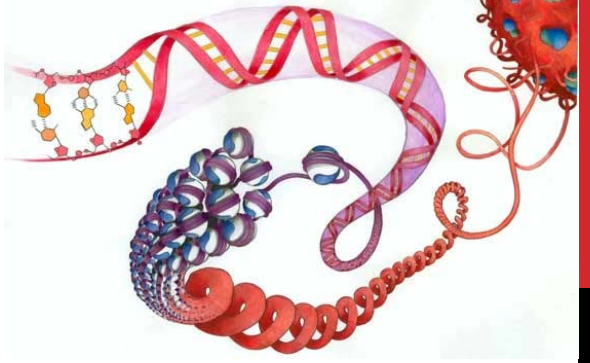




Leukocyte: Tumbling to Adhering

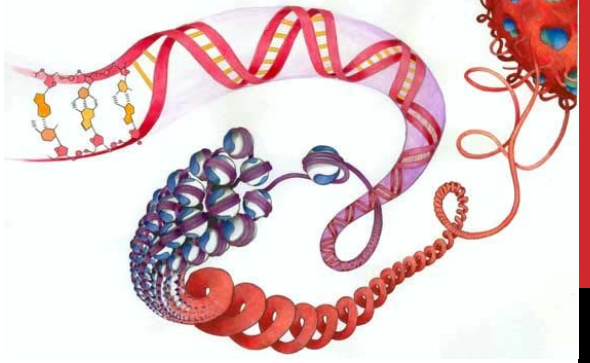
http://youtu.be/9wkJx2elQQ0

INTERACTION OF CELLS WITH OTHER CELLS: IMMUNOGLOBULINS

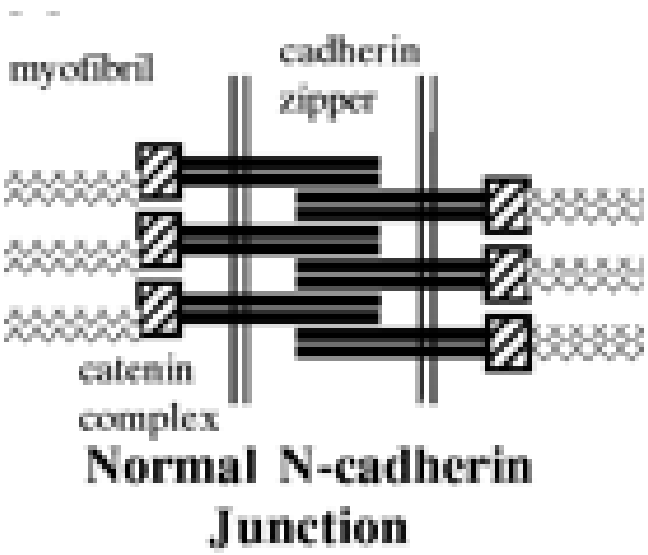


- **Immunoglobulins** = polypeptide chains organized into a folded structure
- entire group → immunoglobulin superfamily (IgSF)
  - most involved in aspects of immune function
  - others mediate calcium independent cell-cell adhesion
- most mediate interactions between lymphocytes and cells required for immune response (macrophages, lymphocytes)
- VCAM/ICAM → leukocyte-endothelial cell adhesion and signaling
- NCMA and L1 → nerve outgrowth, synapse formation, development of nervous system
  - L1 mutations → hydrocephalus, neuromuscular defects

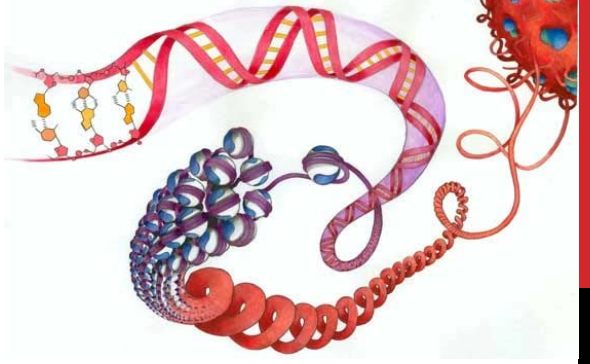
INTERACTION OF CELLS WITH OTHER CELLS: CADHERINS



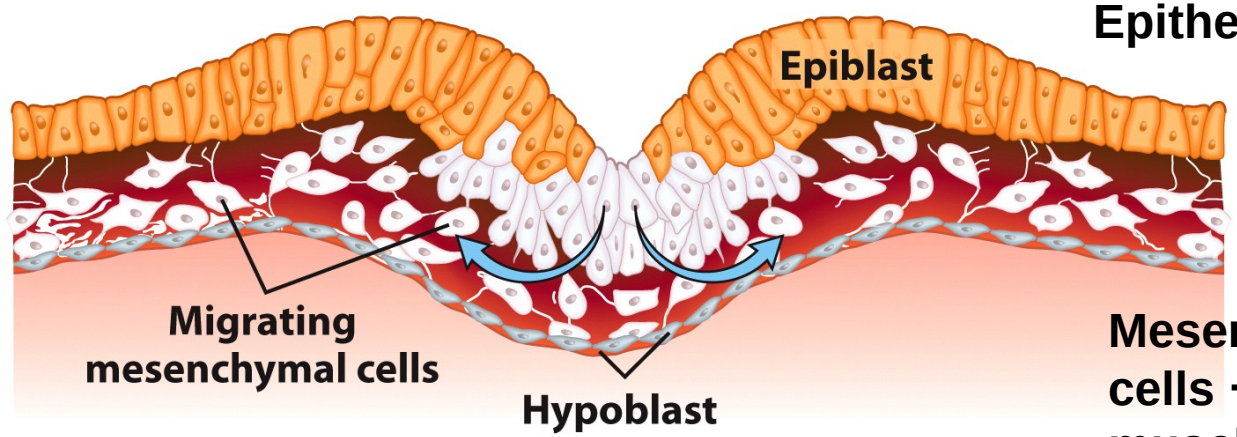
- **Cadherins** = family of glycoproteins that mediate calcium-dependent cell-cell adhesion
- typically join cells of similar type → same cadherin on neighboring cell
  - E-cadherins – epithelial
  - N-cadherins – neural
  - P-cadherins – placental
- cell adhesion zipper, velcro
- catenin – cytosolic protein
  - tether to cytoskeleton
  - transmit signals



INTERACTION OF CELLS WITH OTHER CELLS: CADHERINS

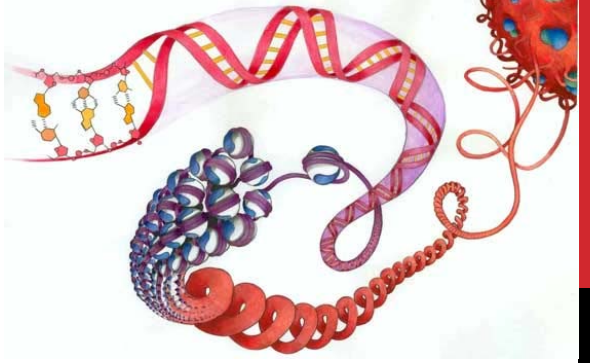


- play pivitol role in embryonic development → morphogenesis
- epithelial-mesenchymal transition (EMT)
  - epithelial – tightly adherent, apical-basal polarized
  - mesenchyme – solitary, nonadhesive, migratory

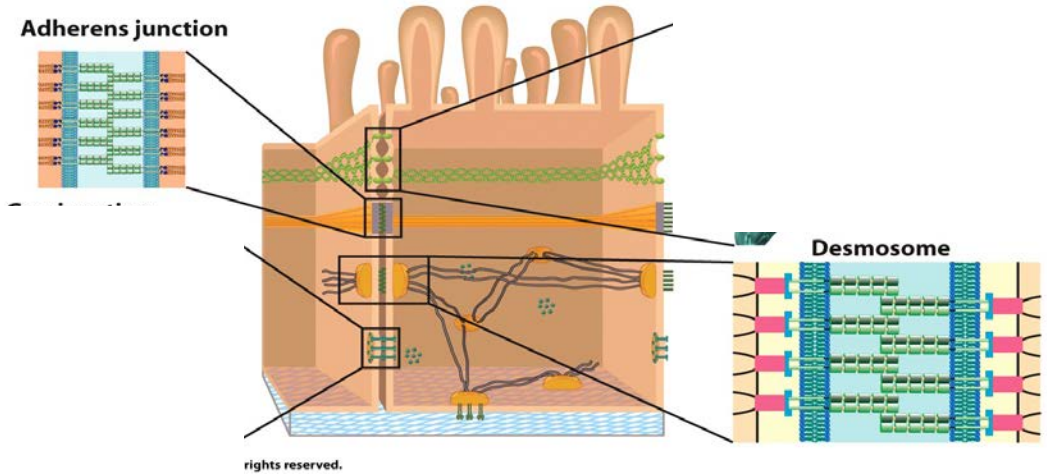


E-cadherin

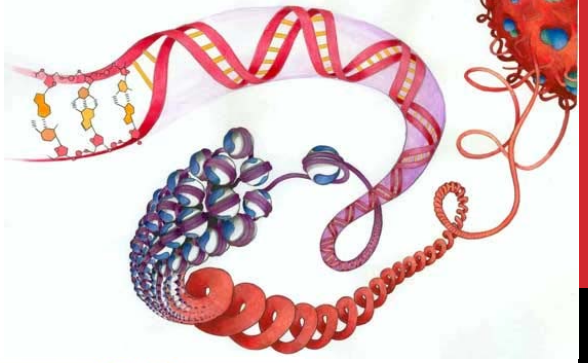
INTERACTION OF CELLS WITH OTHER CELLS: ANCHORING JUNCTIONS

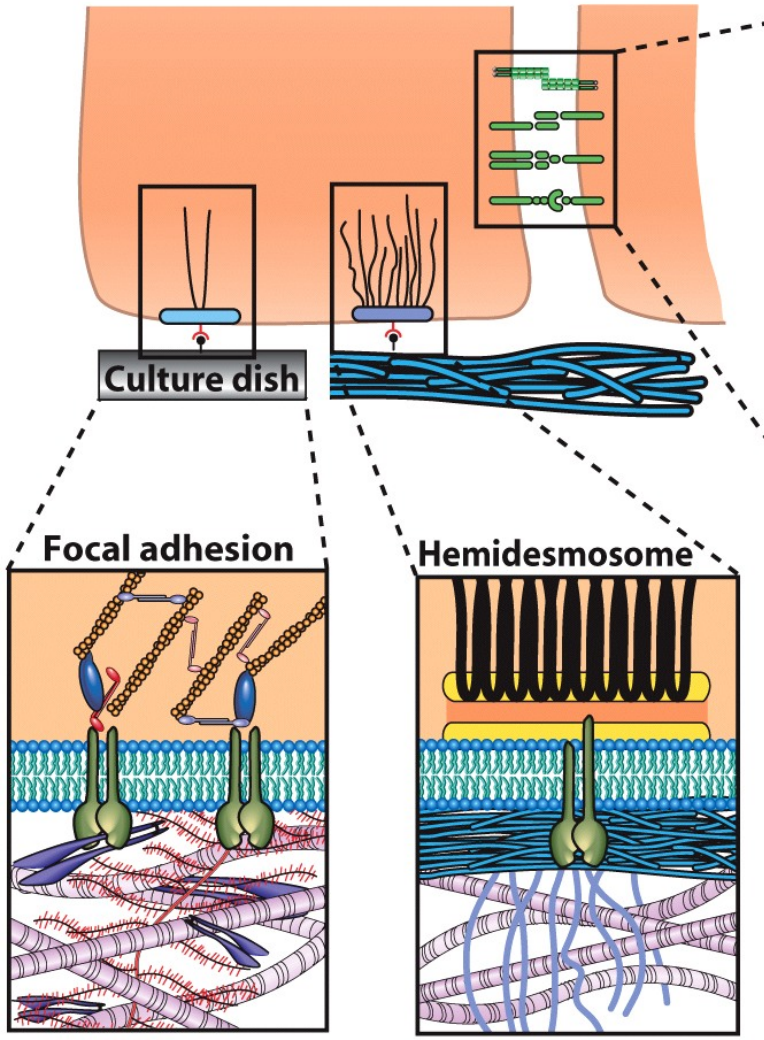


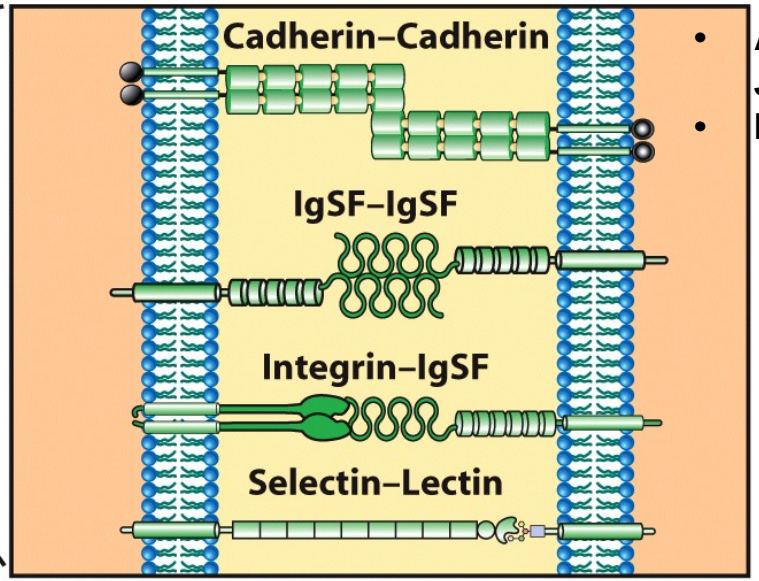
- **Adherens junctions** = cells held together by calcium dependent cadherin clusters. Common in epithelia. Catenin-actin intracellular interactions and signaling.
- **Desmesomes** = disk shaped adhesive junctions (~1 um), in tissues with mechanical stress. Contain non-classical cadherins → desmogleins, desmocollins. Plaque-keratin intracellular interactions and signaling.



INTERACTION OF CELLS WITH OTHER CELLS: CELL-ADHESION RECEPTORS







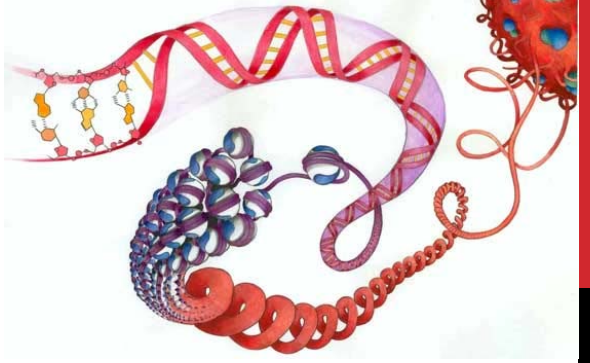
- **Adherens Junctions**
- **Desmisomes**

All cell-ECM and cell-cell interactions can take part in transmembrane signaling

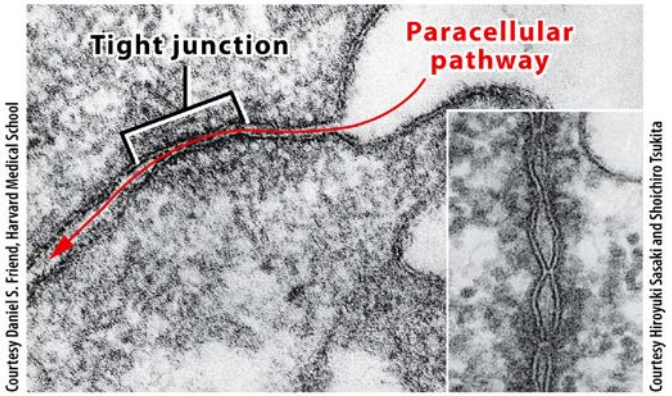
- protein kinases → phosphorylation
- G proteins → physical interaction

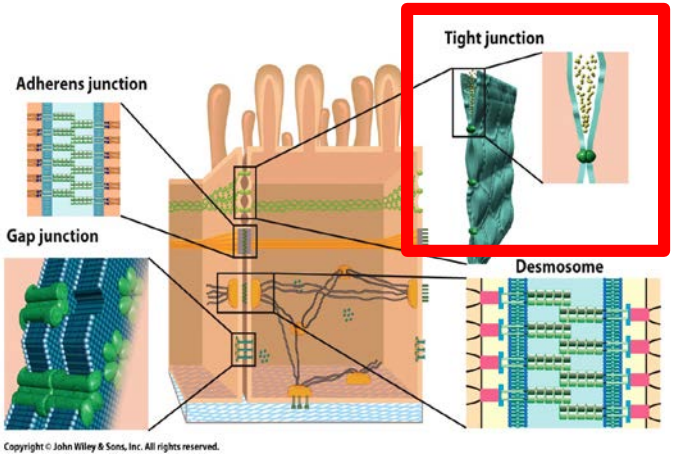
➢ pH, Ca^{2+}, protein phosphorylation, **gene expression**

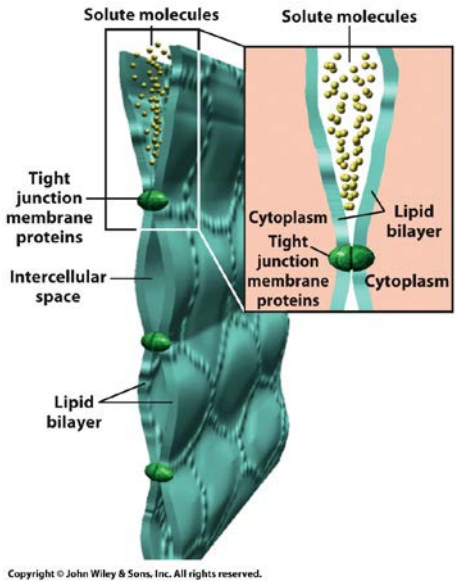
TIGHT JUNCTIONS



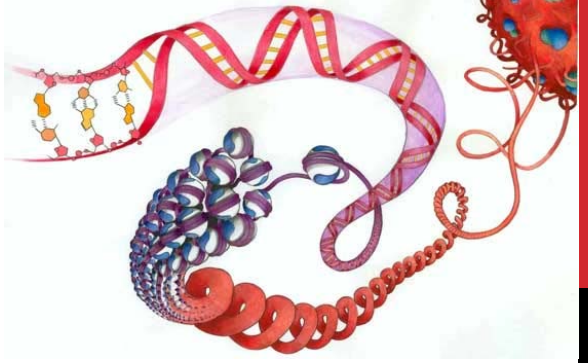
- **Tight junctions** = specialized contacts between epithelial cells
- Located at very apical end of the junctional complex between adjacent cells
- Serve as barrier to free diffusion of water and solutes from the extracellular compartment
- Some are permeable to *specific* ions or solutes



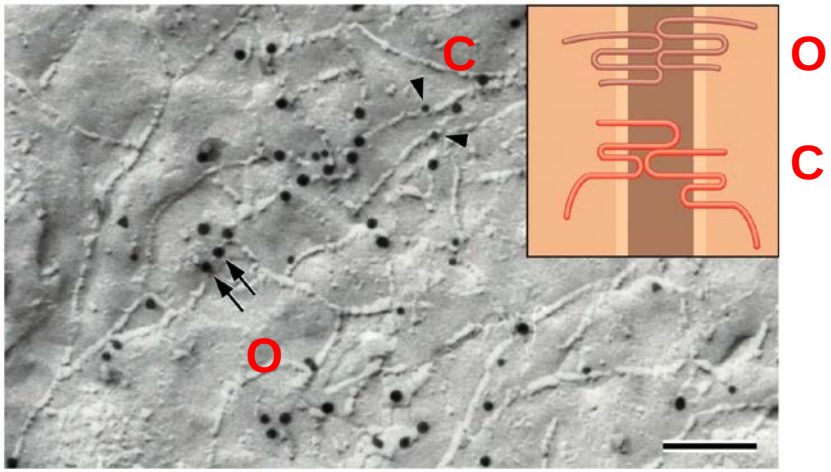




TIGHT JUNCTIONS

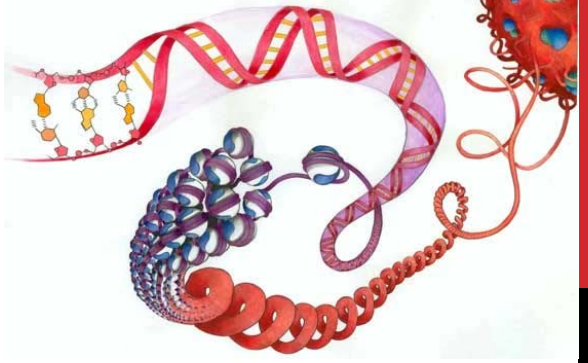


- **Occludins and Claudins** = major structural proteins of TJs, account for selective differences in TJ permeability
- Claudin-16: expressed in kidney tubule; abnormal claudin-16 makes tubule impermeable to Mg+2; excreted not reabsorbed
- Claudin-1: KO mice die from dehydration from uncontrolled water loss. Cannot form watertight epidermal tight junctions.

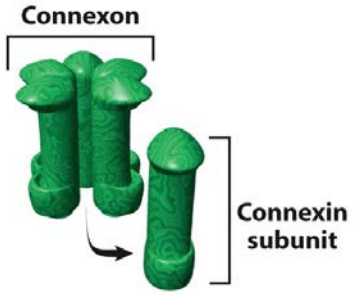


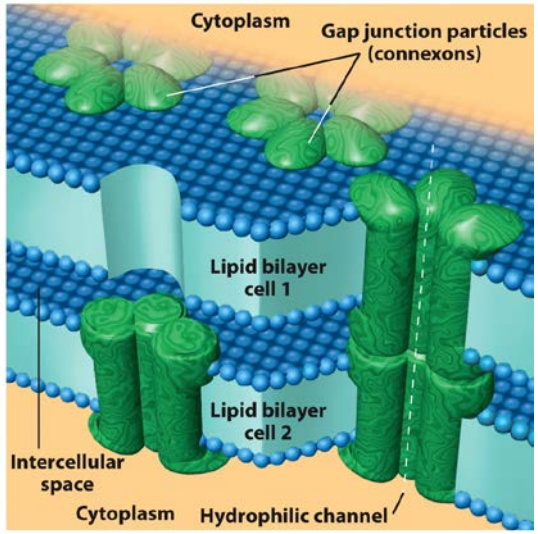
From Mikio Furuse, Hiroyuki Sasaki, Kazushi Fujimoto, and Shoichiro Tsukita, J. Cell Biol. 143:398, 1998, Fig. 6; Reproduced with permission of the Rockefeller University Press.

GAP JUNCTIONS

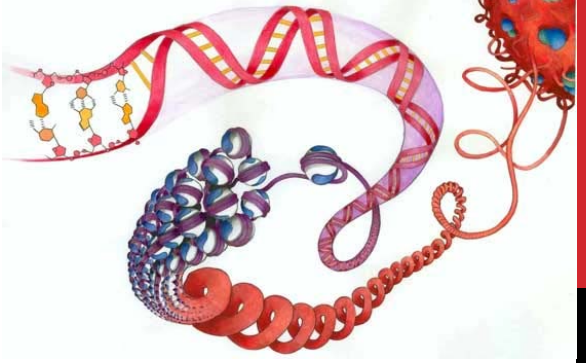


- **Gap Junctions** = specialized sites between cells that allow for intercellular communication
- Molecular pipelines that pass through adjoining plasma membranes and open into cytoplasm of adjoining cells
- Composed of 6 connexin subunits surrounding a central opening (annulus) → connexon
- Gap-junction plaques = clusters of connexons

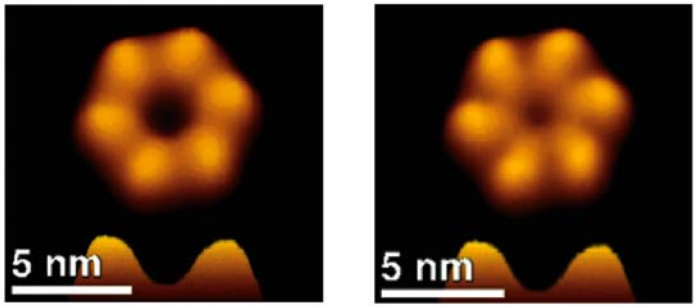




GAP JUNCTIONS



- Allow for nonselective passage of ionic currents or low-molecular weight molecules
- Sharing of key metabolites between cells (i.e. ATP)
- Channels can be closed via phophorylation , changes in voltage across junction, abnormally high Ca^{2+}
- Electrical impulse in cardiomycoytes
  - Electrical impulse in SA node → spreads a current of ions through Cx43 of neighbouring cardiomyocytes → synchronized contraction



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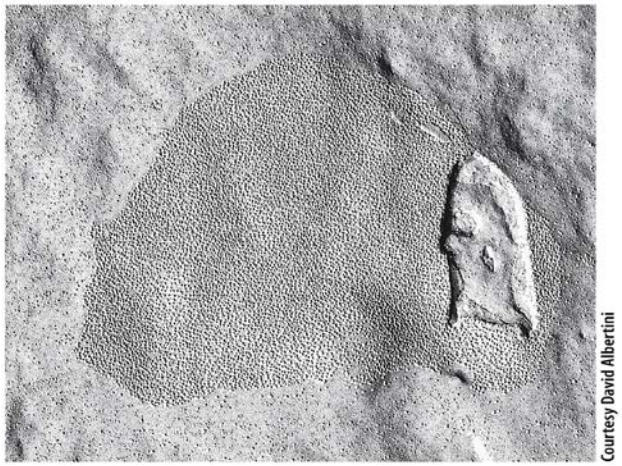
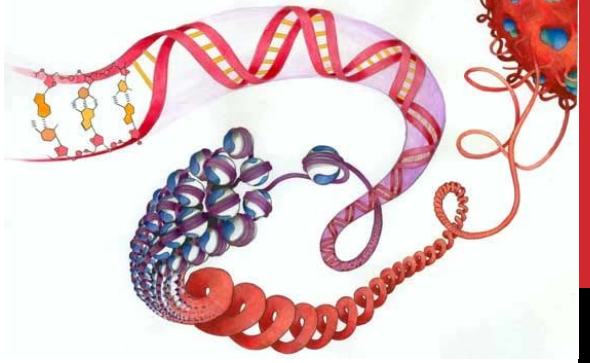
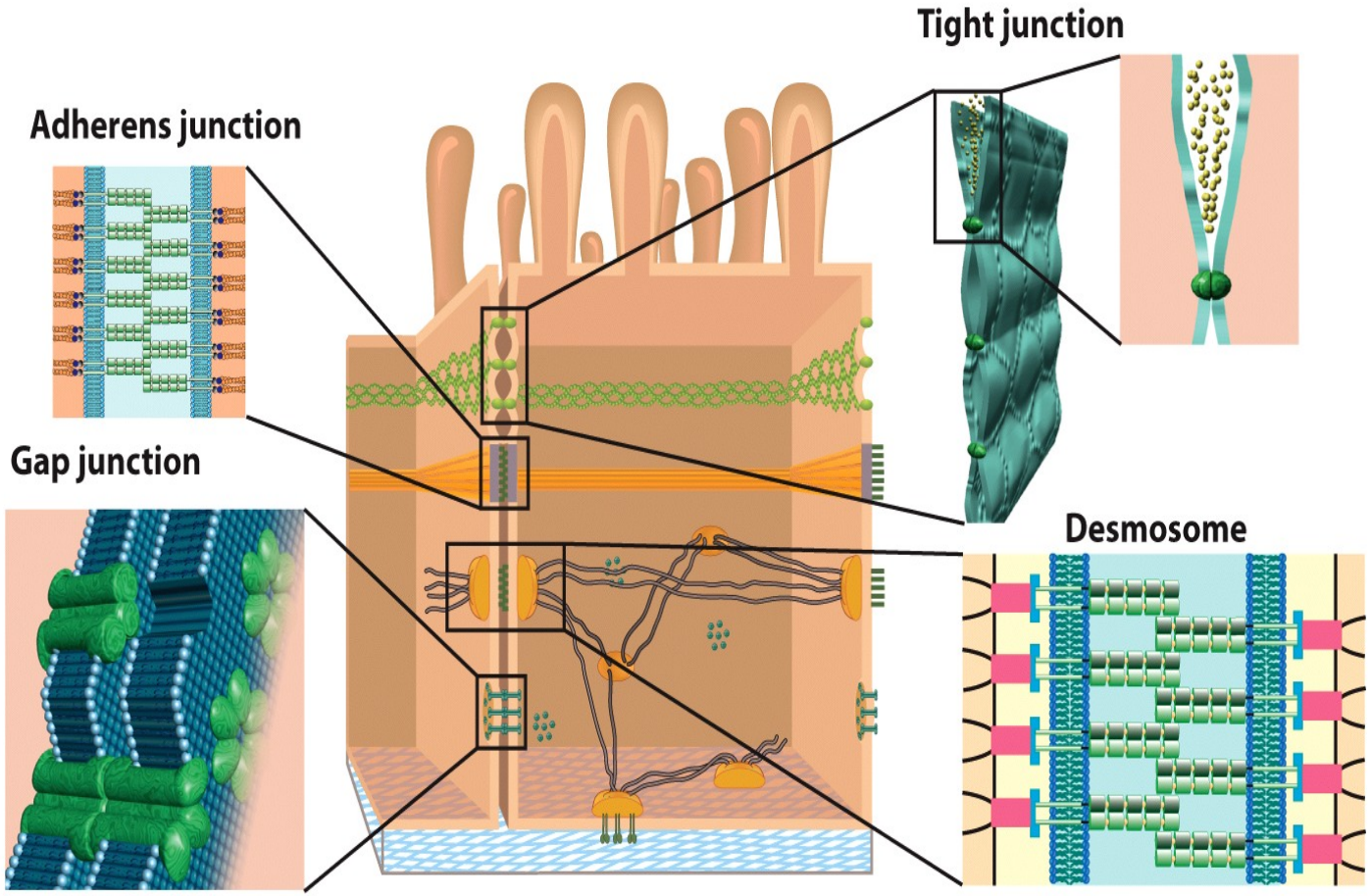


Courtesy David Albertini

CELL-CELL INTERACTIONS REVIEW





QUESTIONS?