NAME OF TEACHER	**Dr. ROLEE SHARMA**
MOBILE NUMBER	**8953595903**
EMAIL ID	**roleesharma@.ac.in, roleesh@gmail.com**
DESIGNATION	**Professor**
UNIVERSITY NAME	**C.S.J.M. University, Kanpur**
COLLEGE NAME	**C.S.J.M. University, Kanpur**
STREAM NAME	**BIOLOGY**
FACULTY NAME	**SCIENCE**
DEPARTMENT NAME	**BIOSCIENCES**
SUBJECT NAME	**CELL BIOLOGY**
COURSE	**M.Sc. BIOCHEMISTRY and LIFE SCIENCES**
COURSE DURATION	**2 Years**
SUBTOPIC NAME	**Cell - Cell interaction, matrix interactions**
CONTENT TYPE	**PDF**
SEARCH KEYWORD	**cell–cell, cell-matrix interaction, extracellular matrix**

Cell Junctions, Cell Adhesion, and the Extracellular Matrix

Objectives:

To acquaint the students about:

i) Occluding junctions and their types

ii) Anchoring junctions and their types

iii) Communicating junctions and their types

iv) Extracellular matrix proteins in connective tissue

v) Collagen: Structure and function

vi) Fibronectin: Structure and function

vii) Laminin: Structure and function

viii) Elastin: Structure and function

ix) Integrins: Structure and function

Cell Junctions

- Specialized cell junctions occur *at points of cell-cell and cell-matrix contact* in all tissues, and they are *particularly plentiful in epithelia*.
- best visualized using either conventional or freeze-fracture electron microscopy, which reveals that the interacting PM (and often the underlying cytoplasm and the intervening intercellular space as well) are highly specialized in these regions.

- Cell junctions can be classified into three functional groups:

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

2. **ANCHORING JUNCTIONS *mechanically attach cells (and their cytoskeletons) to*** their **neighbors or** to the ***extracellular matrix***.

3. **COMMUNICATING JUNCTIONS *mediate the passage of chemical or electrical*** signals from one cell to its partner.

1. **OCCLUDING JUNCTIONS**
    1. tight junctions (vertebrates only)
    2. septate junctions (invertebrates mainly)

2. **ANCHORING JUNCTIONS**
    - *Actin filament attachment sites*
        1. Cell-cell junctions (adherens junctions)
        2. Cell-matrix junctions (focal adhesions)
    - *Intermediate filament attachment sites*
        1. Cell-cell junctions (desmosomes)
        2. Cell-matrix junctions (hemi desmosomes)

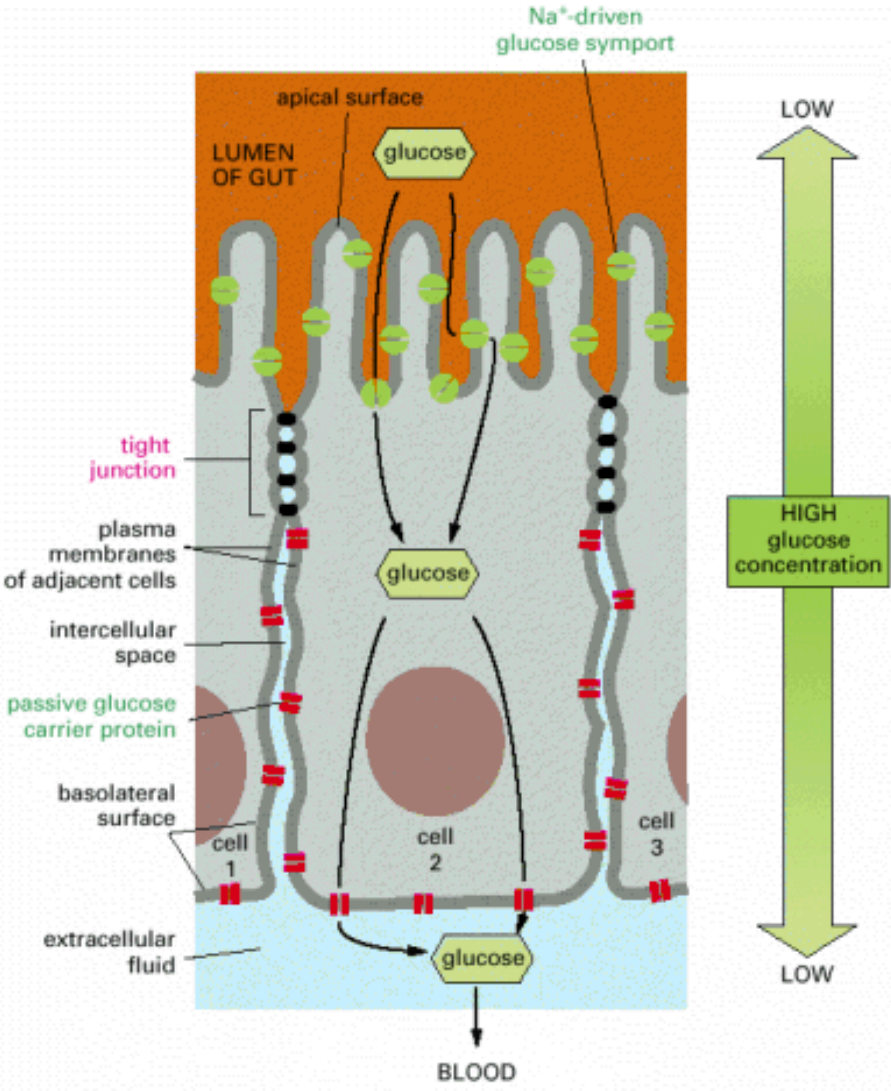
3. **COMMUNICATING JUNCTIONS**
    1. Gap junctions
    2. Chemical synapses
    3. Plasmodesmata (plants only)

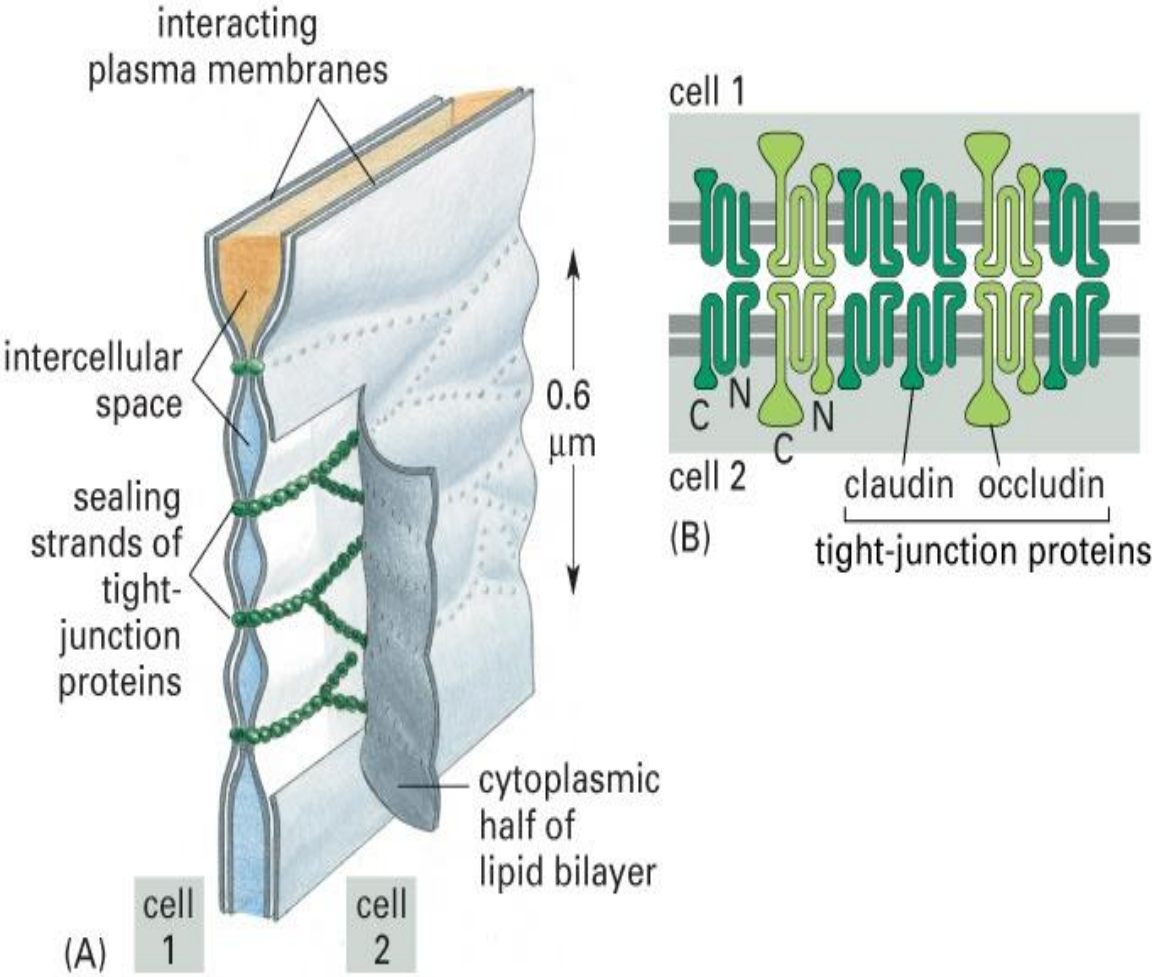
Occluding Junctions form selective-permeability barrier across epithelial cell sheets

In *vertebrates*, function as *barriers to diffusion* of some membrane protein and lipid.

All epithelia have at least one important function in common: they serve as selective permeability barriers, *separating fluids on either side that have a different chemical composition*. This function requires that the adjacent cells be sealed together by occluding junctions. Tight junctions have this barrier role in vertebrates, such as in the epithelium of the Mammalian small intestine/gut

Tight Junctions - confine the transport of proteins to their appropriate membrane domains by acting as diffusion barriers within the lipid bilayer of the PM and block the backflow of glucose from the basal side of the epithelium into the gut lumen.





•Tight junctions are composed of a branching network of **sealing strands** that completely encircle apical end of each cell in epithelial sheet.

•Ability to restrict passage of small molecule – dependent on number of strands.

•Major transmembrane protein in Tight junction = **claudins**

•Also **occludins.**

In **invertebrates- Septate junctions**

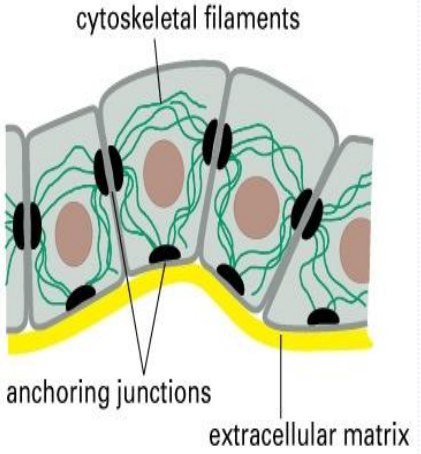
-Main occluding junction in invertebrates, form continuous band around each epithelial cell.

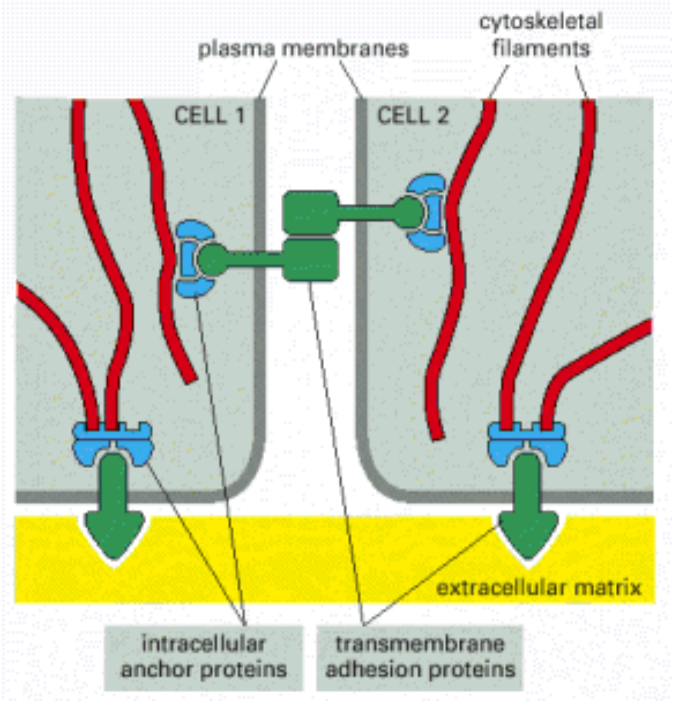
Model of a tight junction. Sealing strands hold adjacent PM together.

❖ **Anchoring Junctions** (AJ) - connect cytoskeleton of a cell to cytoskeleton of its neighboring cell or to extracellular matrix (ECM).

-Transmit large forces from cell →cell/ cell →ECM by forming a membrane spanning structure tethered inside cell to the tension bearing filaments of cytoskeleton.

-Most abundant in tissues subiected to stress as heart, muscle and epidermis.





2 main classes of pro:

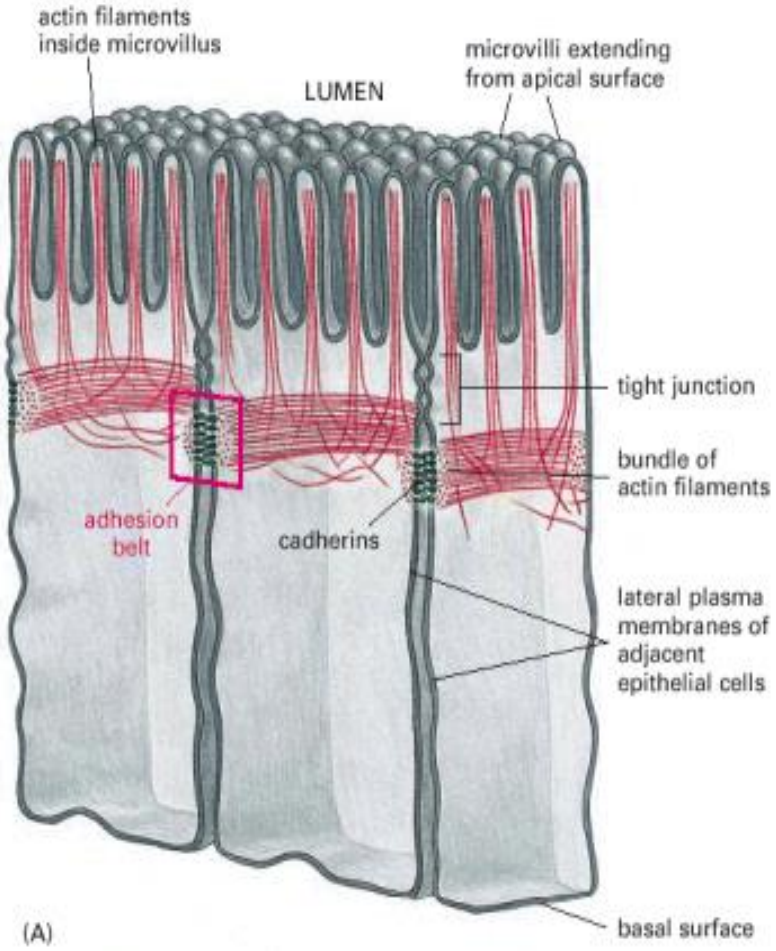
1. **Intracellular anchor proteins**:
 -form plaque on cytosolic face of PM,
- connect junctional complex to actin/ intermediate filaments

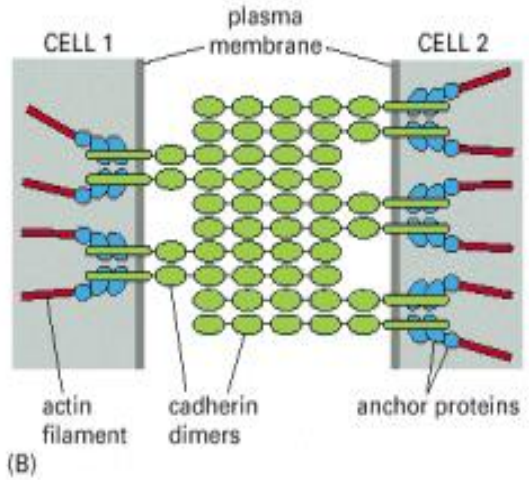
2. **T.M. adhesion proteins**:
-have cytoplasmic tail that binds to 1 or more intracellular anchor proteins and extracellular domain of specific TM adhesion protein of other cells.

Many A. J.s also have intracellular signaling proteins which enable junctions to signal to cell interior. A.Js occur in 2 forms:

1. **Adherens junctions & Desmosomes**: hold cells together.
 Formed by transmembrane adhesion proteins belonging to **cadherin family.**

2. Focal adhesions & hemi desmosomes: bind cells to ECM.
 Formed by adhesion proteins of **integrin family.**





Adherens junctions (form a continuous adhesion belt just below Tight Junctions); encircling each of interacting cells in the sheet; attach to actin.

Adjacent PMs held by cadherins

Adherens junctions. (A) Adherens junctions, in the form of adhesion belts, in epithelium. connect bundles of actin filaments from cell to cell. encircles the interacting cells. A contractile bundle of actin filaments running along the cytoplasmic surface of the junctional PM. (B) Some of the molecules that form an adherens junction. **Actin filaments are joined from cell to cell by transmembrane adhesion proteins called cadherins**. The cadherins form homodimers in PM of each interacting cell. The e.c. domain of one cadherin dimer binds to the e.c. domain of an identical cadherin dimer on the adjacent cell. The intracellular tails of the cadherins bind to anchor proteins that tie them to actin filaments. These anchor proteins include α-catenin, β-catenin, γ-catenin (also called plakoglobin), α -actinin, and vinculin.

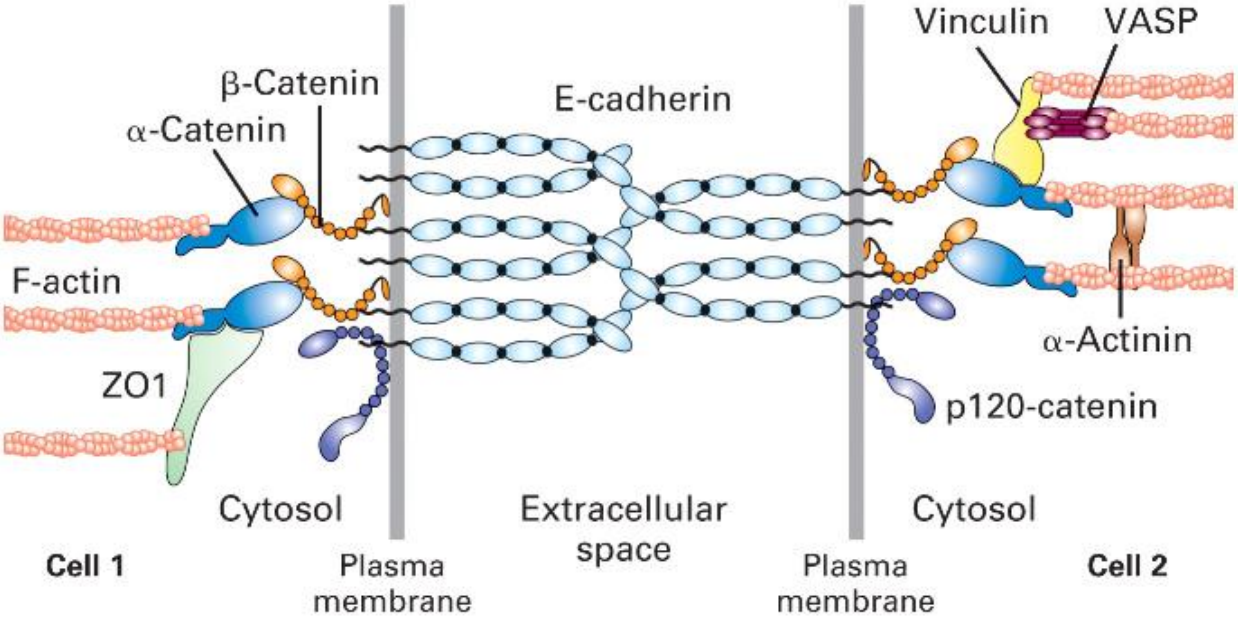
Adherens Junctions

- Composition: cadherins

- Function: can contract (with help of myosin)

- Folding of sheets into tubes during morphogenesis, other folding processes during morphogenesis

- Binding partners: catenins, and via catenins to cytoskeleton (actin)



Desmosomes

Desmosomes connect intermediate filaments from cell to cell.

[**Hemidesmosomes** – half desmosomes (connect basal surface of an epithelial cell to underlying basal lamina)]

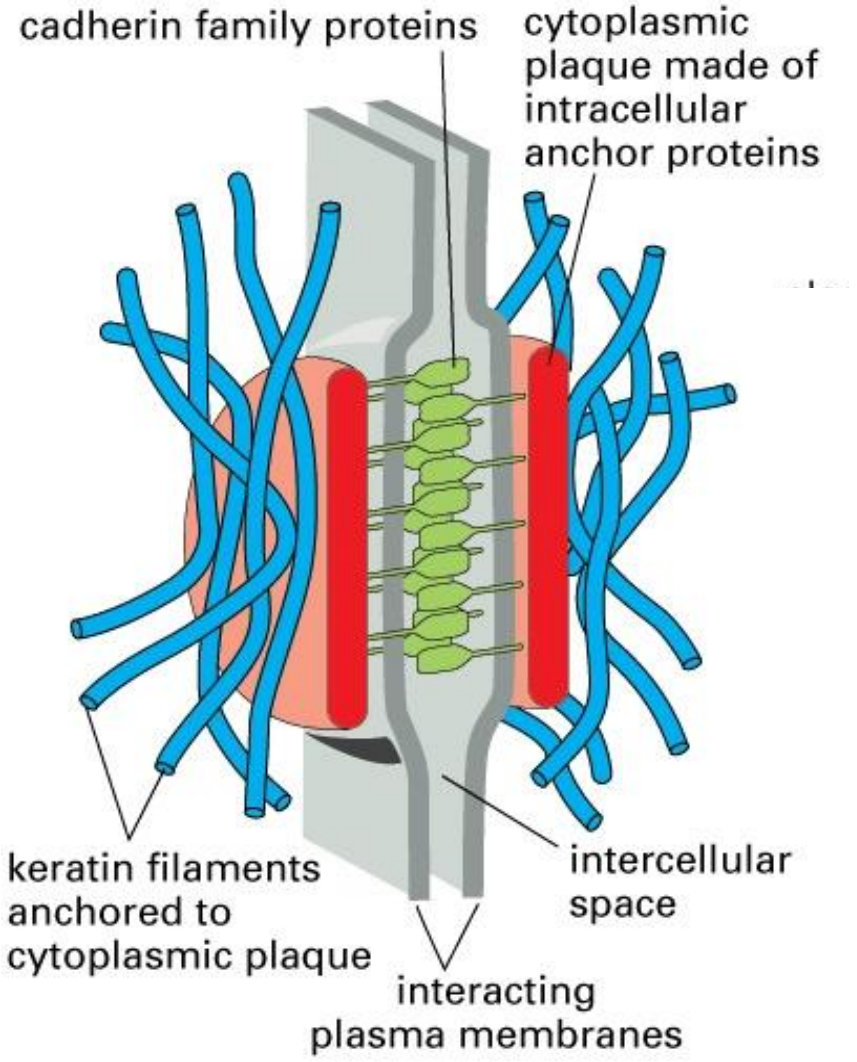
Desmosomes

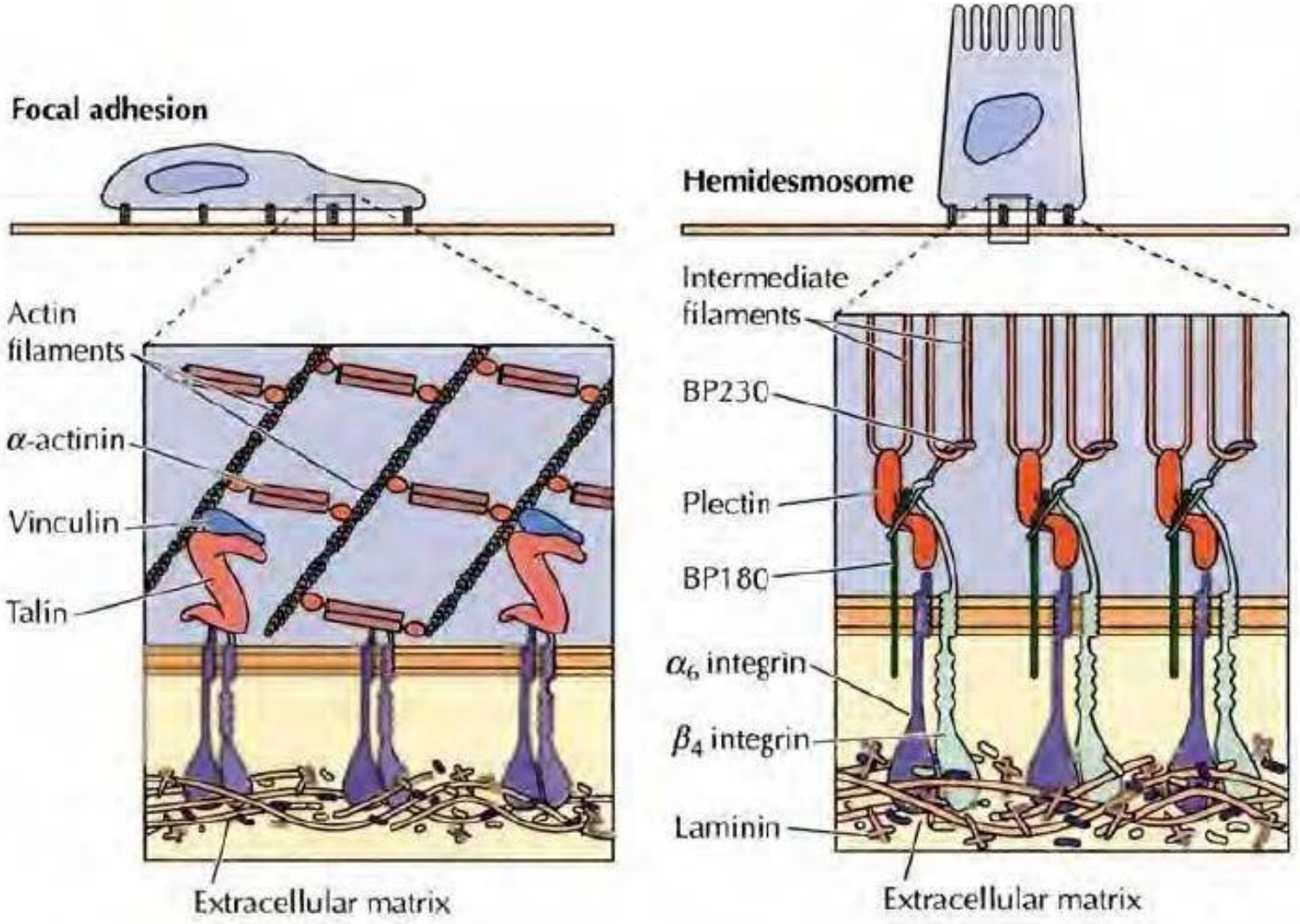
•Connect Intermediate filaments (IFs) from cell to cell.

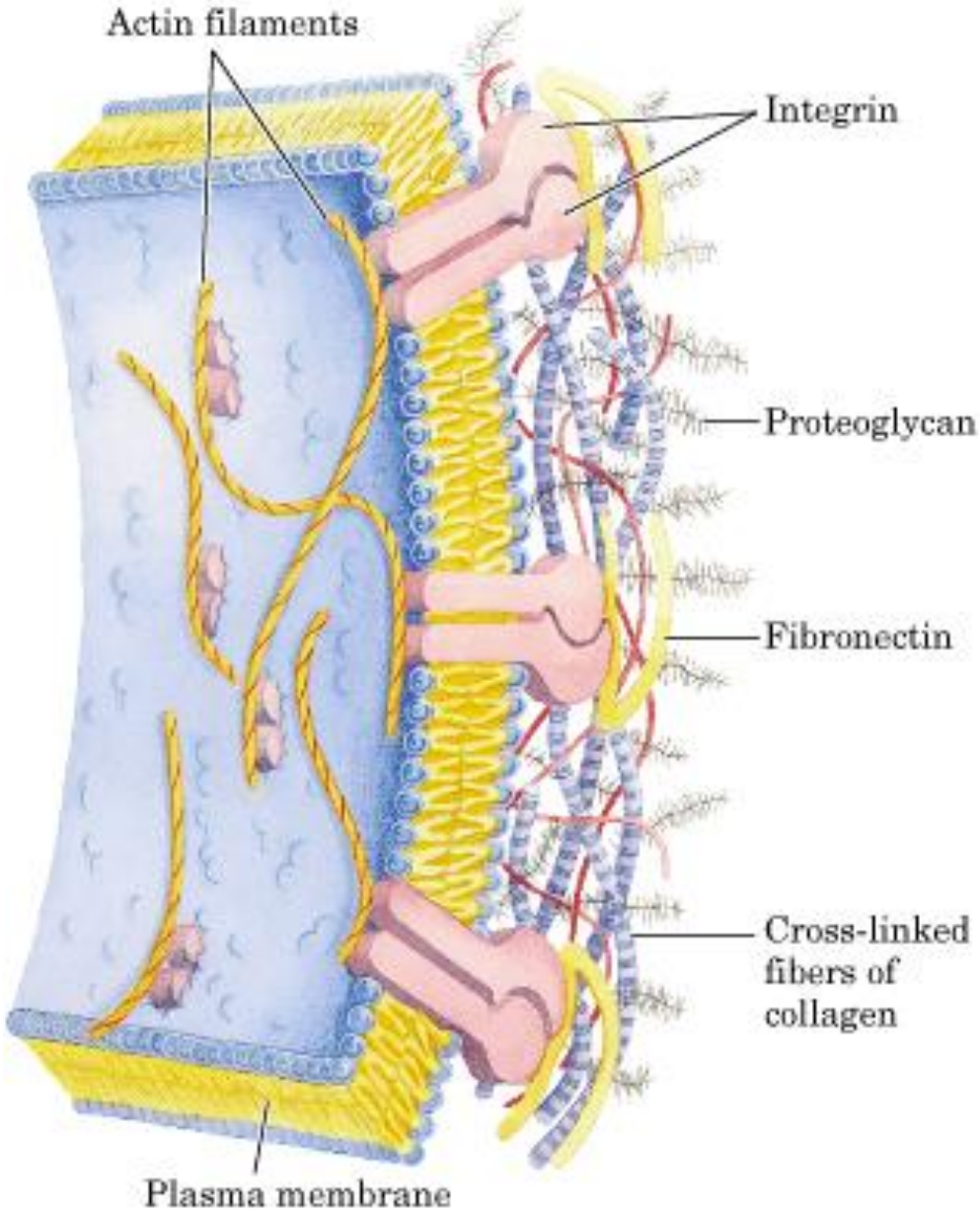
•These are button like points of intercellular contact.

• Anchoring sites for rope like IFs which form structural framework of great tensile strength.

• With the help of desmosomes, IFs of adjacent cells are linked into a net which extends throughout many cells of a tissue.




Focal adhesion
Actin filaments
α-actinin
Vinculin
Talin
Extracellular matrix
Hemidesmosome
Intermediate filaments
BP230
Plectin
BP180
α6 integrin
β4 integrin
Laminin
Extracellular matrix

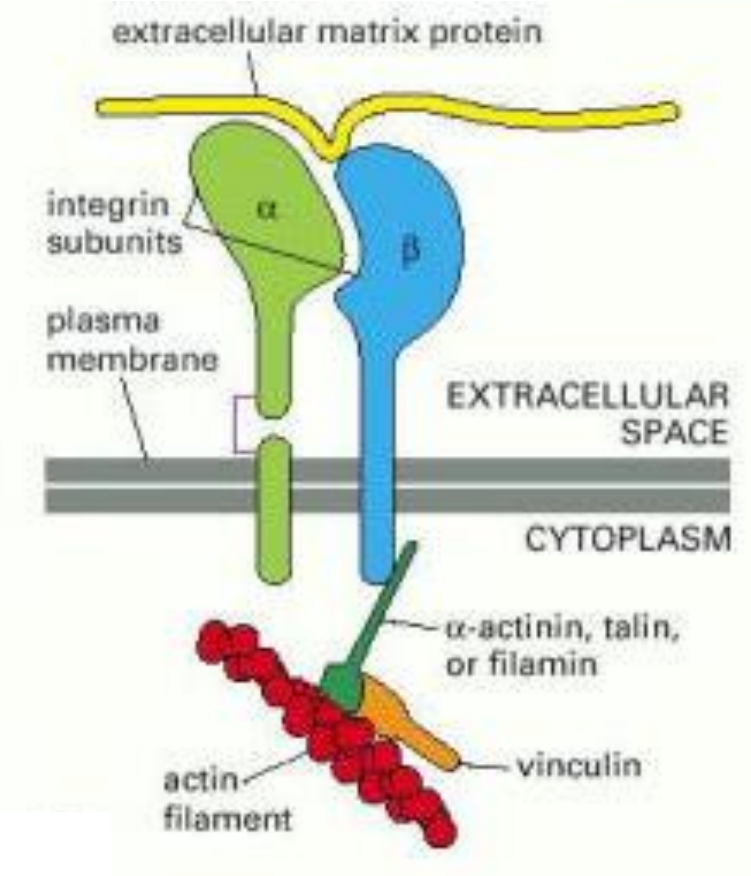


Cell matrix: All cells are surrounded by a 3 dimensional network of extracellular macromolecules, such as collagen, enzymes, and glycoproteins, that form the extracellular matrix (ECM) and provide structural and biochemical support to cells.

Anchoring Junctions also bind cells to ECM.

Adhesion protein in these cell-matrix junctions are <u>integrins</u>

Focal Adhesions enable cells to get hold on ECM through integrins which link intracellularly to actin filaments.

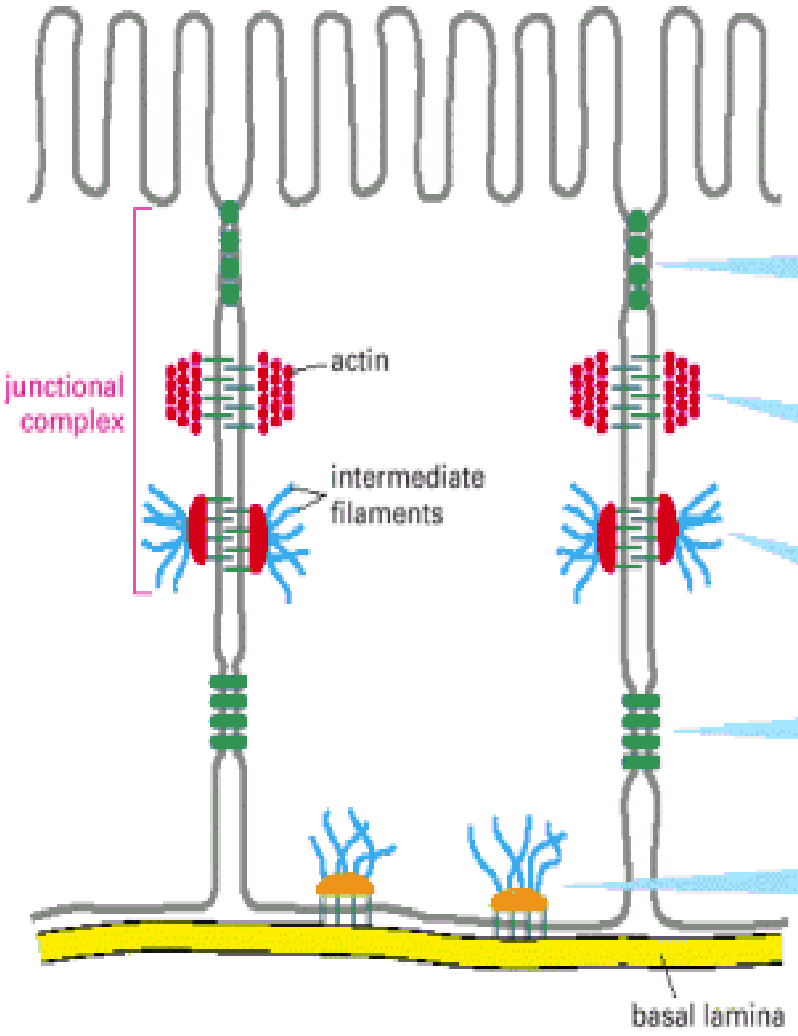


Examples: myotendinous junction (muscle cells attach to tendons).

At such adhesions-

• Extracellular domains of transmembrane integrin bind to a protein component of ECM.

• Intracellular domains – bind indirectly to actin filaments via anchor proteins - α-actinin, talin, filamin and vinculin

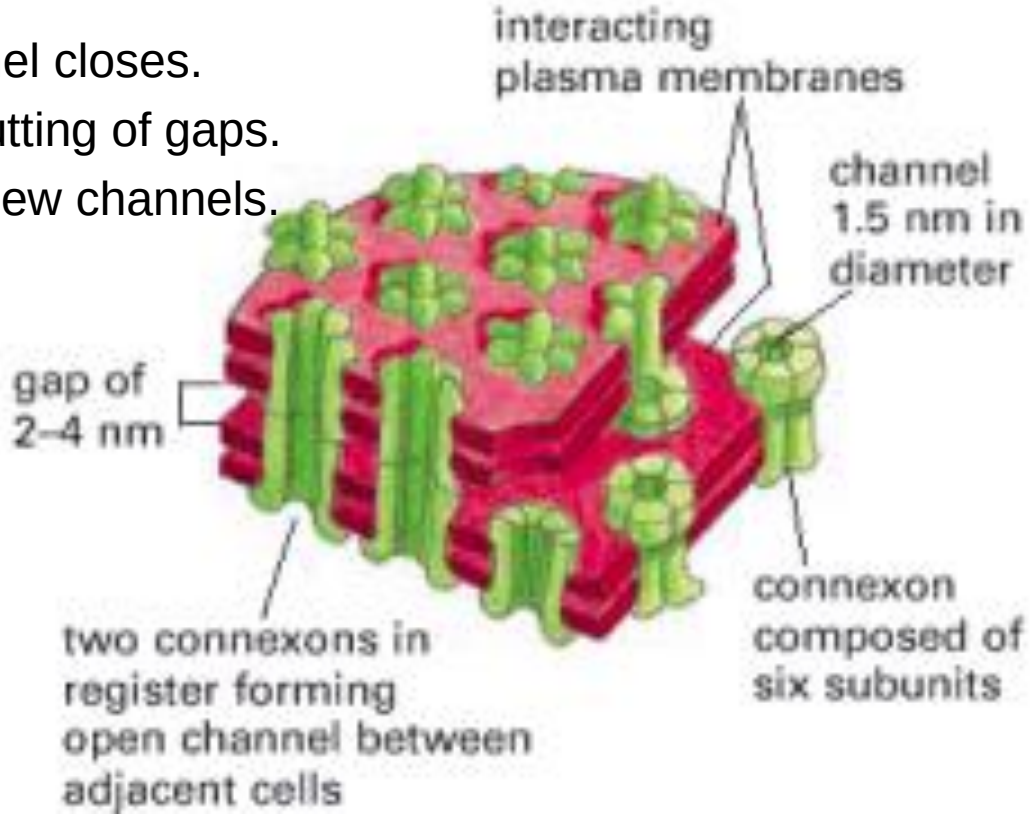


name	function
tight junction	seals neighboring cells together in an epithelial sheet to prevent leakage of molecules between them
adherens junction	joins an actin bundle in one cell to a similar bundle in a neighboring cell
desmosome	joins the intermediate filaments in one cell to those in a neighbor
gap junction	allows the passage of small water-soluble ions and molecules
hemidesmosome	anchors intermediate filaments in a cell to the basal lamina

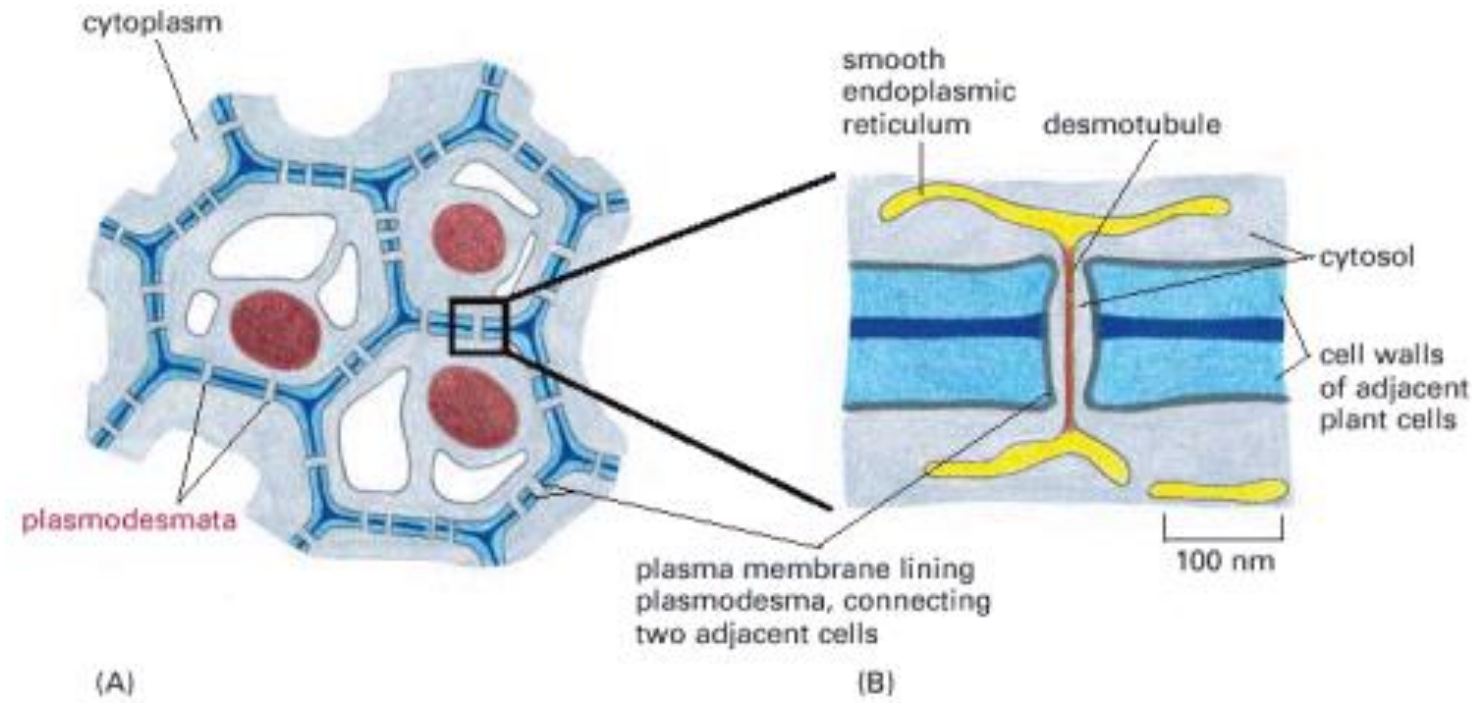
Pictorial representation of diverse cellular interactions

GAP JUNCTIONS

- Allow small molecules (such as metabolites, ions) to pass directly from cell to cell and thus co-ordinate activities in tissues.
- Membrane spanned by channel forming proteins (connexins) that form channel (connexon).
- A Gap Junction connexon channel – made up of 6 TM connexin protein subunits.
- In tissues containing electrically excitable cells, coupling via Gap Junctions allow action potential to spread rapidly from cell to cell.
- Permeability can be regulated.
- Ca^{++} elevation to ~$5x10^5$ M ⟶ channel closes.
- Depolarization with high K^+ causes shutting of gaps.
- cAMP increase - causes formation of new channels.

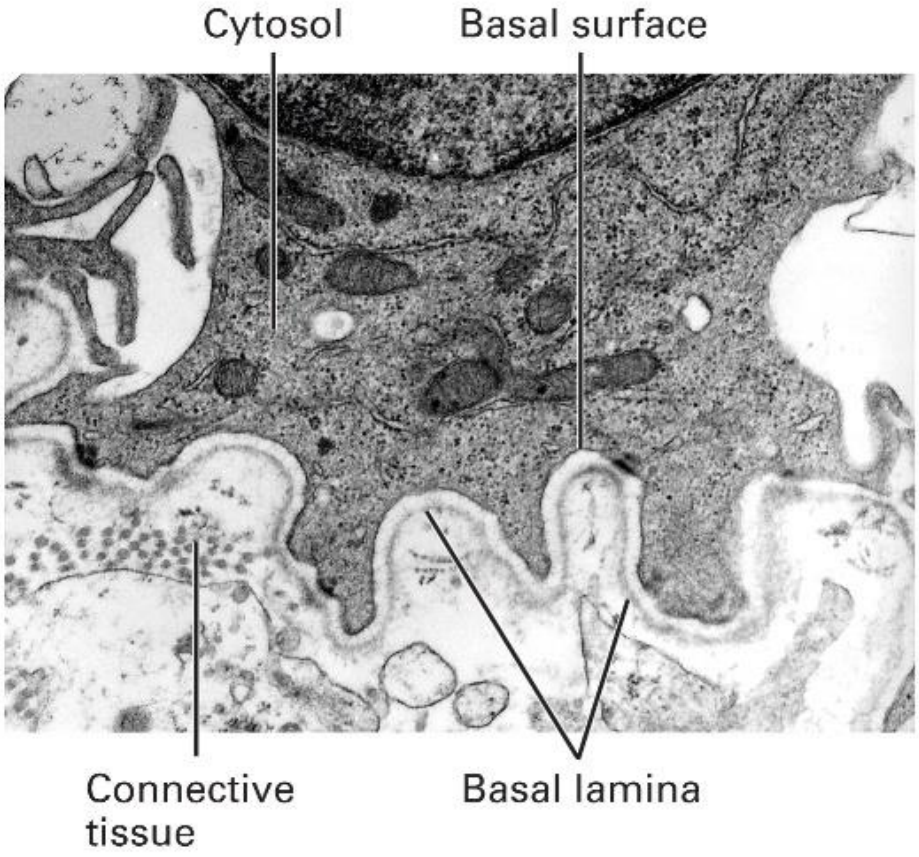


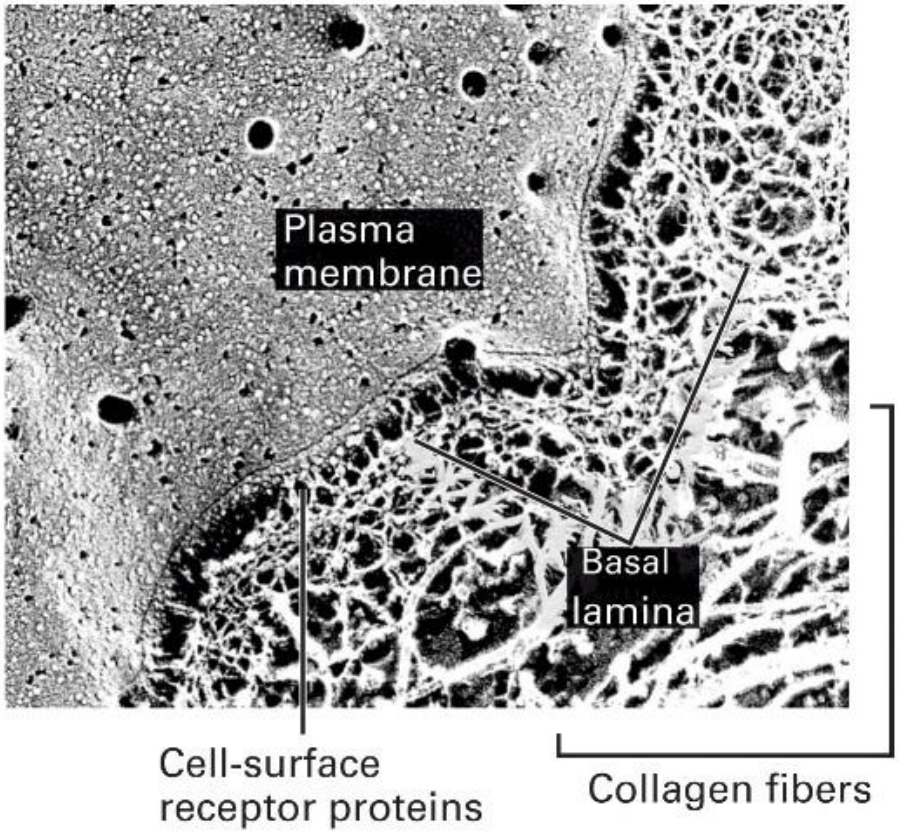
PLASMODESMATA



- Plasmodesmata are channels in cell walls of plant cells that connect cytoplasms of adjacent cells. (Channel diameter : 20-40 nm)
- Running through center of channel is a fine tubular structure, ***plasmotubule*** **,** that is derived from smooth ER.
- These allow passage of molecules with Molecular Weight<800.
- Some mRNA, plant viruses, infectious viral RNA can pass cell to cell through plasmodesmata.

Basal Lamina – extracellular matrix; a sheetlike meshwork underlying or surrounding groups of cells

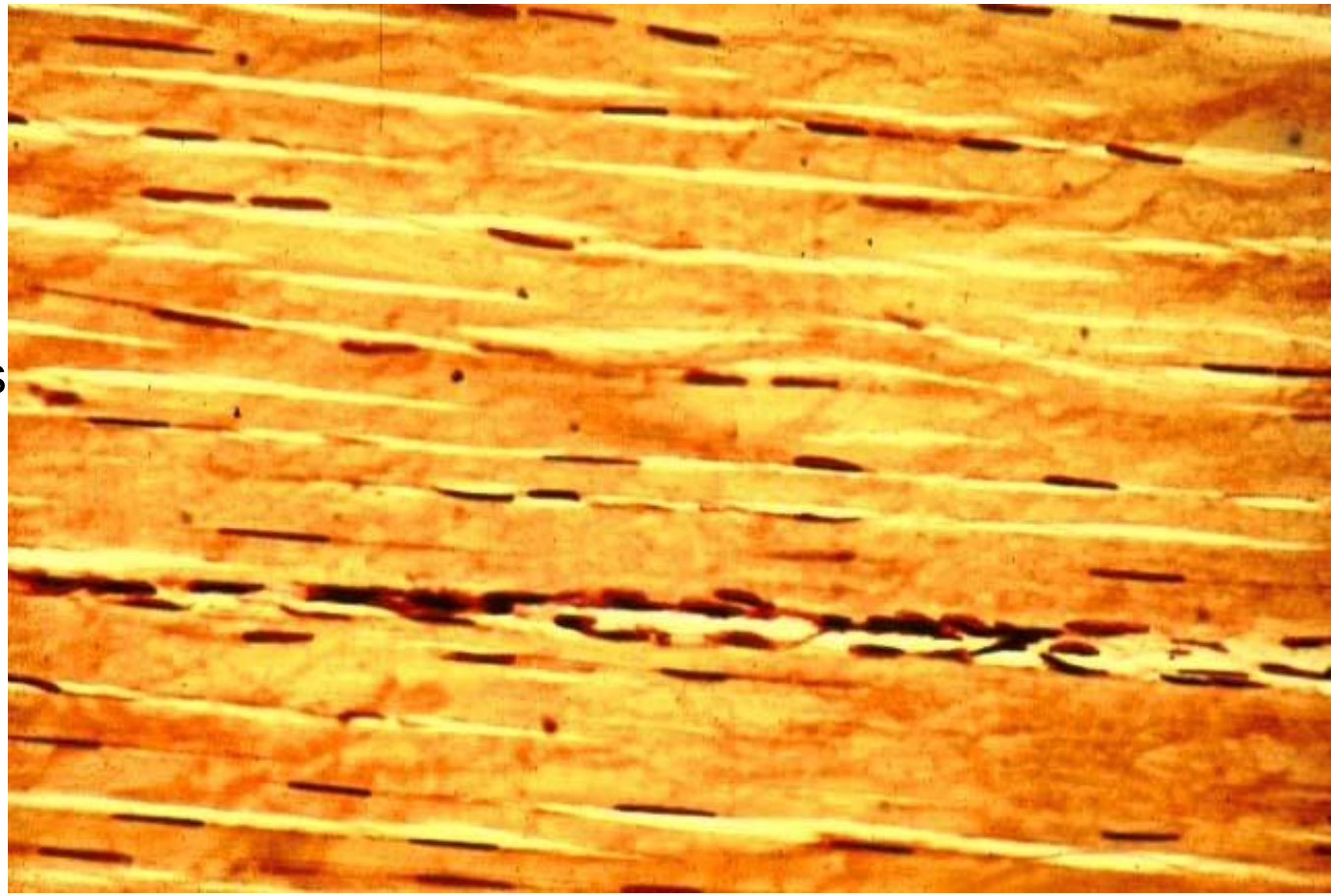




Function : to organize cells into tissues, works in tissue repair; provides a guide for migrating cells during tissue formation (i.e. neural cells)

ECM proteins in connective tissue

- Collagen
- Proteoglycans
- Adhesion proteins
- Hyaluronan
- Elastic fibers



Tendon – dense connective tissue

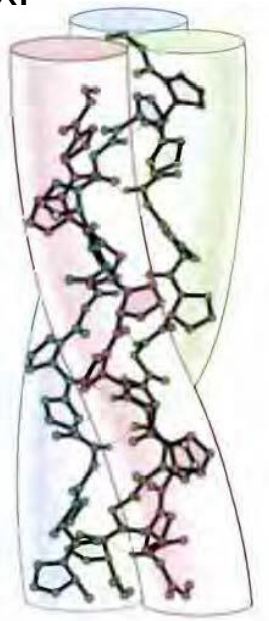
Collagen

Most abundant structural protein in the ECM that give structural support to cells.

- Main protein of connective tissue in animals, supports most tissues
- Long fibrous structural protein, secreted by connective tissue and other cells.
- Has great tensile strength, main component of fascia, skin, cartilage, ligaments, bones, tendons & teeth. Strengthens blood vessels; plays role in tissue development.
- Accounts for 90% of bone matrix protein content.
- Used in cosmetic surgery, burns surgery and construction of artificial skin substitutes.
- Sold commercially as joint mobility supplements.

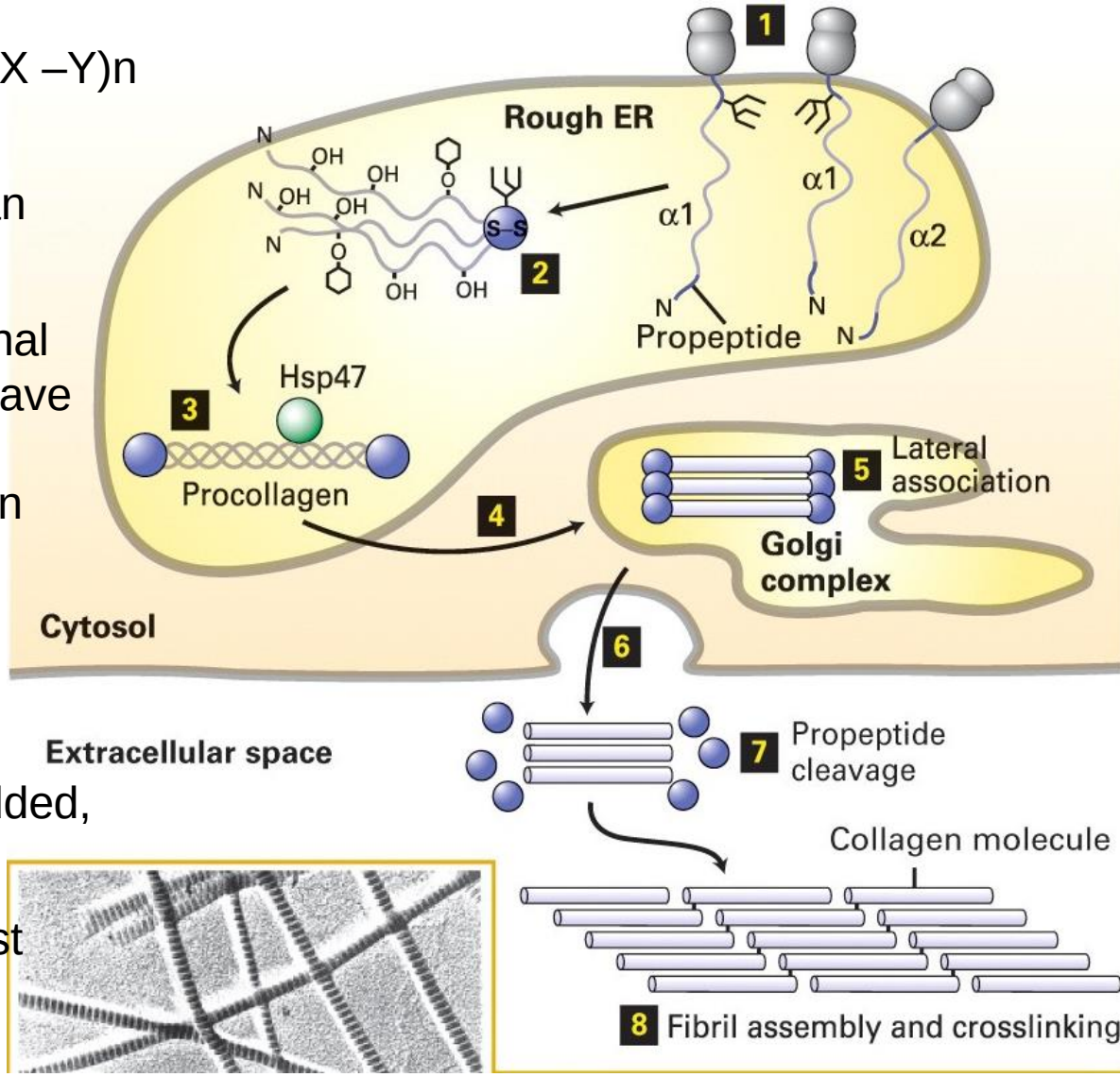
STRUCTURE:

- Collagen or tropocollagen = Trimeric rod shaped protein molecules (~300 nm x1.5 nm), made up of 3 polypeptide strands, each of which is a left handed helix.
- **These left handed helices – twisted together into right handed helix.**
- Tropocollagen subunits spontaneously assemble with staggered ends into large arrays.
- Also show covalent cross linking
- Organize into overlapping bundles=**collagen fibrils** (10-300 nm diameter) which pack into tough bundles k/a **collagen fibers** (several µm diameter)



Triple helices in which 3 polypeptide chains are wound tightly around one another in a rope-like structure. Different collagen polypeptides assemble into 42 different types of trimers.

- Repeating sequence Glycine – X –Y)n [X = proline, Y= hydroxyproline]
- Resulting molecule twists into an elongated, left handed helix.
- When synthesized, the N-terminal and C-terminal of polypeptide have globular domains, keeping the molecule soluble. (cleaved when secreted from cell - rendering it insoluble)
- As they pass ER and Golgi apparatus, the molecules are glycosylated, OH groups are added, S-S bonds link three chains.
- End result -three molecules twist together to form a **triple helix**.



•When the triple helix is secreted from cell (usually fibroblast), the globular ends are cleaved off. The resulting linear, insoluble molecules assemble into **collagen fibers**. They assemble in a staggered pattern that gives rise to the striations. (Type IV collagens are an exception; they form a meshwork rather than striated fibers.)

•In some collagens (e.g., Type II), the three molecules are identical (the product of a single gene). In other collagens (e.g., Type I), two polypeptides of one kind (gene product) assemble with a second, quite similar, polypeptide, that is the product of a second gene.

•Collagens Are Secreted with a Nonhelical Extension at Each End.
•Individual collagen polypeptide chains are synthesized on membrane-bound ribosomes and injected into the ER lumen as larger precursors, called ***pro-α chains***.

•In the lumen of the ER, selected prolines and lysines are hydroxylated to form hydroxyproline and hydroxylysine, respectively, and some of the hydroxylysines are glycosylated.

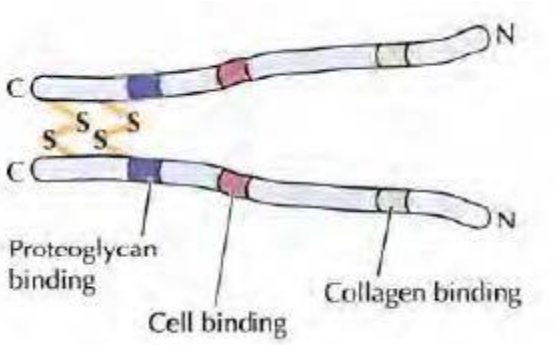
• Each pro- ***α*** chain then combines with two others to form a hydrogen-bonded, triplestranded, helical molecule known as ***procollagen***.

Fibronectin

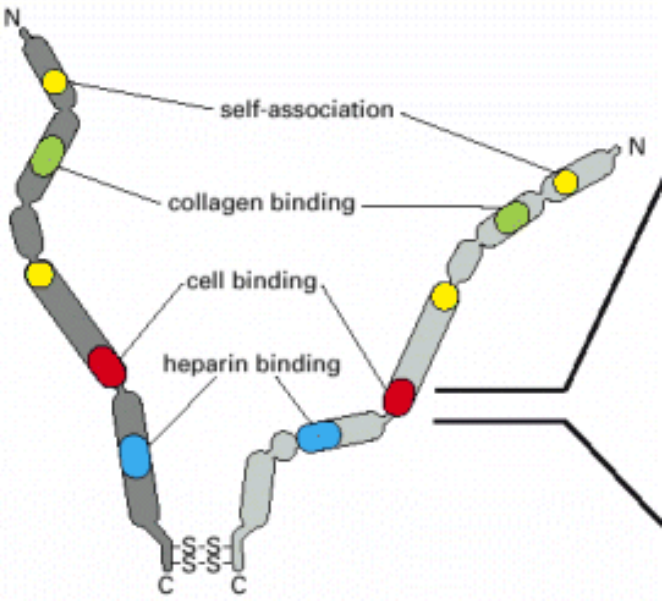
Extracellular protein that helps cells attach to matrix.

• Principal adhesion protein of connective tissues, consisting of 2 polypeptide chains, each 250 KDa. Each chain is 60-70 nm long, 2-3 nm thick.

•Large dimeric glycoprotein (in vertebrates) with multiple domains, each with specific binding sites for other matrix macromolecules. (as collagen, fibrin and heparin) and for cell surface receptors as integrins.



•Thus, ***contribute to organizing matrix***, helping cells attach to it (***cell adhesion to matrix***) and ***guiding cell migrations in vertebrate embryos.***



•Connect cells with collagen fibers in the ECM, allowing cells to move through the ECM.

•In ECM, fibronectin (FN) is often cross-linked into fibrils.

• FN has binding sites for both collagen and heparin, so it cross-links these matrix components.

•**FN bind collagen and cell surface integrins, causing a reorganization of the cell's cytoskeleton and facilitating cell movement**.

•FN are secreted by cells in an unfolded, inactive form.

•Binding to integrins folds fibronectin molecules, allowing them to form dimers so that they can function properly.

Fibronectins – exist in soluble and fibrillar forms (In blood plasma - soluble form of fibronectin)

Multiple isoforms of FN-

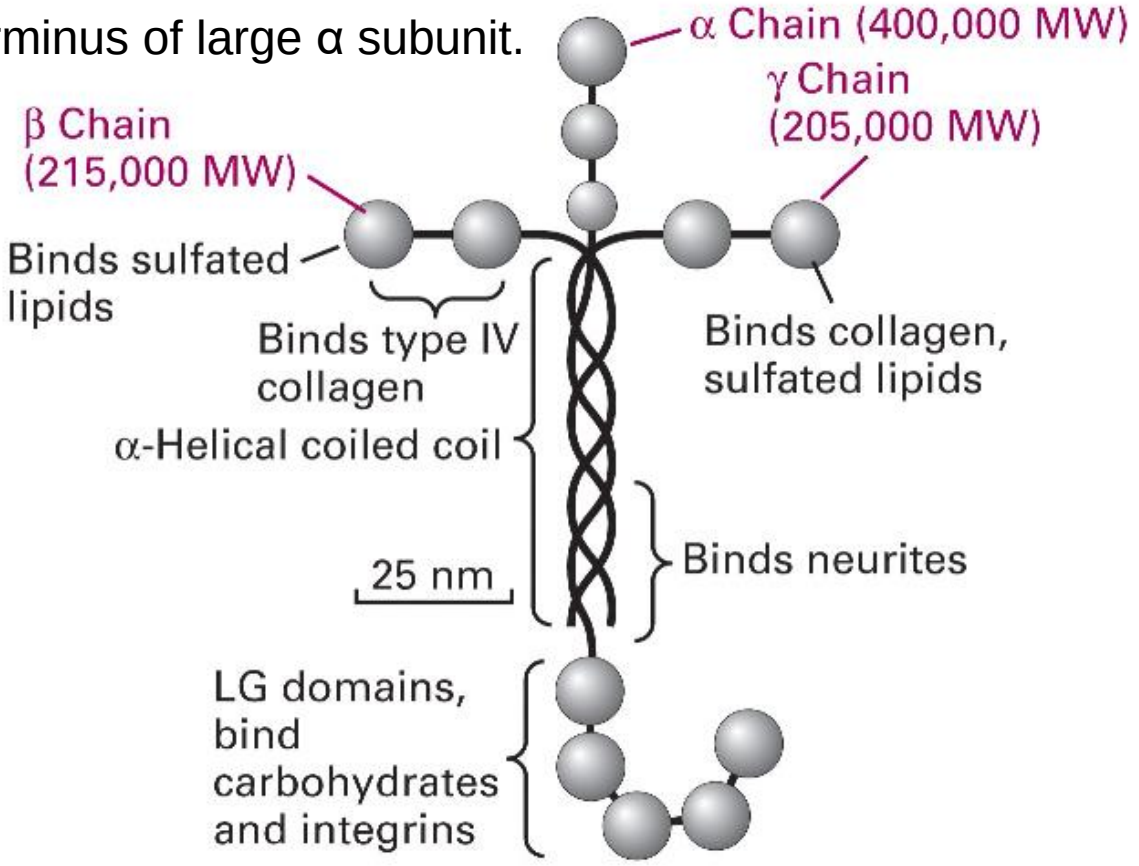
- Plasma FN- soluble, circulates in blood and body fluids, enhances blood clotting, wound healing and phagocytosis.
- All other forms assemble on cell surfaces, deposited in ECM as highly insoluble FN fibrils.

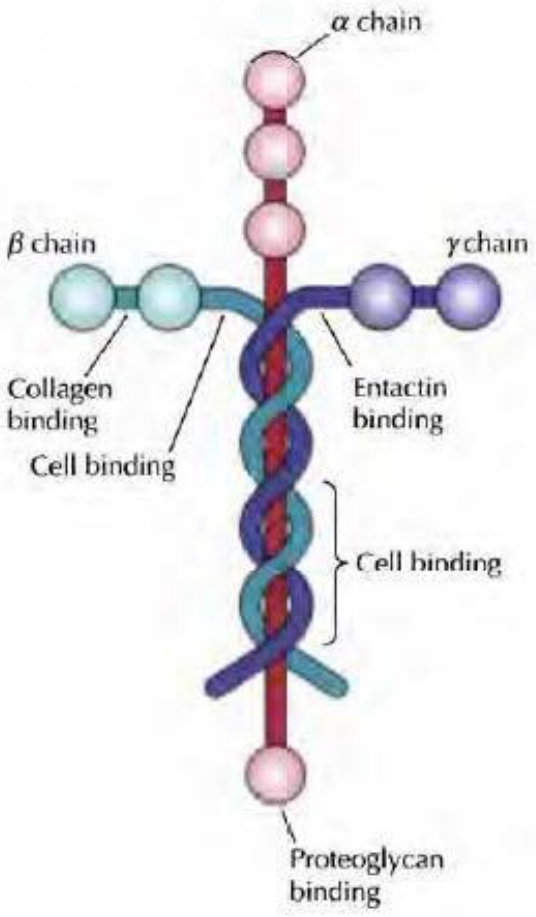
Fibronectins bind to ECM macromolecules and facilitate their binding to transmembrane integrins.

Laminins are multiadhesive matrix proteins

Function: **organization of basement membrane; have binding sites for integrin receptors (important in embryonic development and tissue remodeling)**

- Heterotrimeric, cross shaped proteins (M.W. 820 kDa), self-assemble into meshlike polymers (laminin networks).
- Have binding sites for cell surface receptors such as integrins, type IV collagen, and heparan sulfate proteoglycan, perlecan.
- Has globular LG domains at C terminus of large α subunit.
- Laminins are tightly associated with **entactin** or **nidogen** a 150-kD sulfated glycoprotein, which also binds to type IV collagen. As a result of these multiple interactions, laminin, entactin, type IV collagen, and perlacan form crosslinked networks in the basal lamina.





•Laminins are extracellular matrix proteins which consist of α, β and γ chains with molecular masses of 140-400 kDa.
•Chain association occurs through a large triple alpha-helical coiled-coil domain towards the C-terminus of each chain.

•8 genetically distinct laminin chains (α1, α2, α3, β1, β2, β3, γ1, γ2) and seven different assembly forms (laminins-1 to -7) are known so far.

•The most extensively characterized laminin-1 (α1, β1, γ1) shows **Ca-dependent self assembly** and heterotypic binding to perlecan, nidogen, fibulin-1 and other matrix components. This binding indicates a crucial role in the supramolecular organization of basement membranes. Laminins also possess ***binding sites for at least six different integrin receptors*** and are thus involved in many cell-matrix interactions. Such interactions have been shown to be important during embryonic development and for tissue homeostasis and remodelling.

•The globular LG domains at the C terminus mediate Ca^{2+} dependent binding to specific carbohydrates on certain cell-surface molecules such as syndecan and dystroglycan. LG domains can mediate binding to steroids and proteins also.

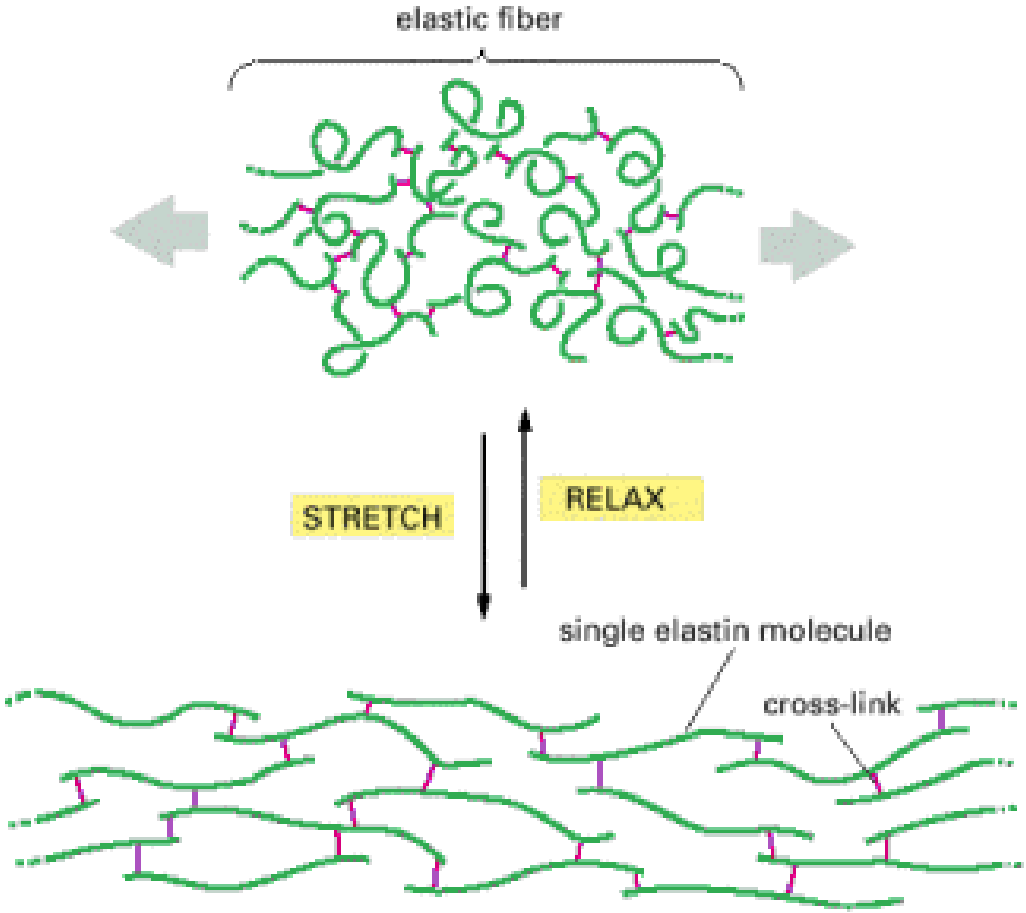
Elastin

- A protein in connective tissue, allows many tissues to resume their shape after stretching/ contracting. Elastins give elasticity to tissues, allowing them to stretch when needed and then return to their original state. This is useful in blood vessels, the lungs, in skin, and the ligaments. A patch of skin-poked or pinched soon returns to normal state due to the presence of a network of elastin fibres in the extracellular matrix.

- *Elastin* protein is highly hydrophobic, and is rich in proline and glycine, (not glycosylated) and also some hydroxyproline. Cross links are formed between lysine; Alanine and lysine rich α-helical segments-crosslink adjacent molecules – to form coils.

- Elastins are **synthesized by fibroblasts and smooth muscle cells**. Elastin is secreted as soluble tropoelastin, which is then deaminated to become incorporated into the elastin strand and is assembled into insoluble mature elastic fibres close to plasma membrane.

- Diseases such as **cutis laxa and Williams syndrome** are associated with deficiency or absence of elastin fibers in the ECM.



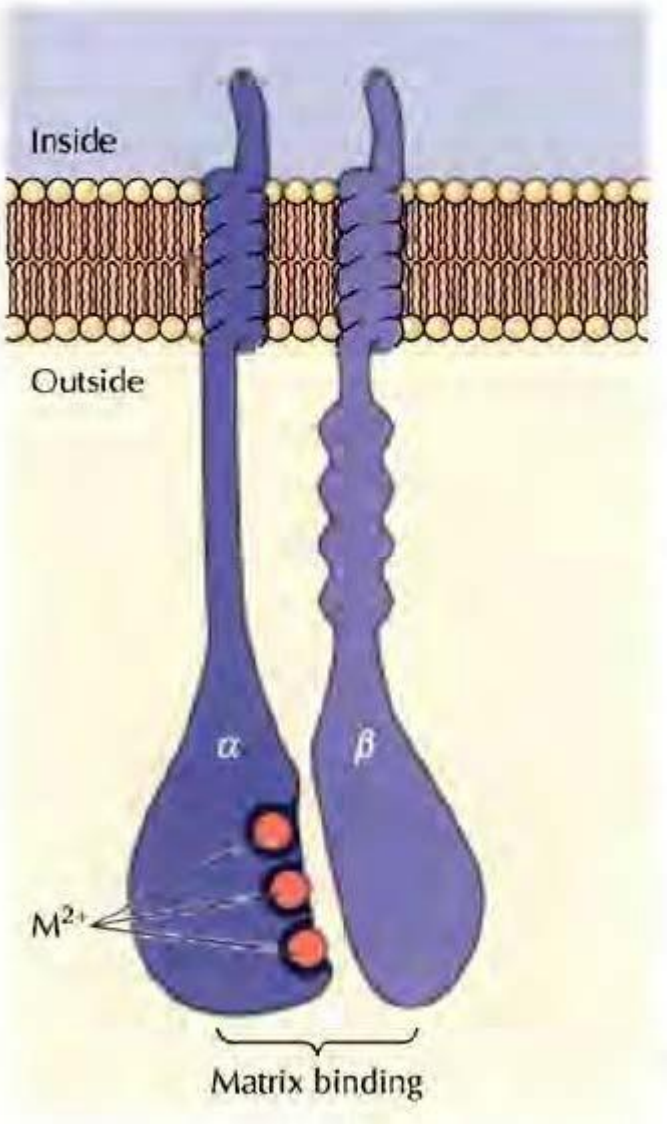
Stretching a network of elastin molecules. The molecules are joined together by covalent bonds *(red) to generate a cross-linked network. In* this model, each elastin molecule in the network can expand and contract as a random coil, so that the entire assembly can stretch and recoil like a rubber band.

Cell adhesion to the ECM: Many cells bind to components of the ECM.

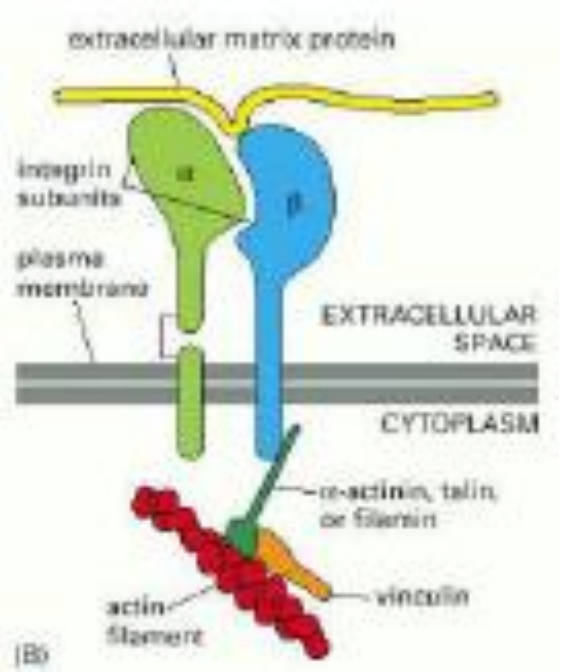
Cell adhesion can occur in two ways by
- *By focal adhesions*, connecting ECM to actin filaments of the cell, and
-***By hemi-desmosomes:*** connecting the ECM to intermediate filaments such as keratin.

This **cell-to-ECM adhesion is regulated by specific cell surface cellular adhesion molecules (CAM) known as integrins**. The integrins are a family of transmembrane proteins consisting of two subunits, designated α and β.

Integrins are cell surface proteins that bind cells to ECM structures such as fibronectin and laminin, and also to integrin proteins on the surface of other cells



Integrins



- Integrin is a family of proteins that
  -attach a cell to its surroundings and also
  -mediate signaling between cell interior and extracellular matrix, thereby communicating to the cell the character of ECM that is bound.

- The transmembrane adhesion protein is an integrin heterodimer, composed of an α and β subunit.

- Its extracellular domains bind to components of the extracellular matrix, while the cytoplasmic tail of the β subunit binds indirectly to actin filaments via several intracellular anchor proteins.

Selected Vertebrate Integrins

Subunit Composition	Primary Cellular Distribution	Ligands
α1β1	Many types	Mainly collagens; also laminins
α2β1	Many types	Mainly collagens; also laminins
α4β1	Hematopoietic cells	Fibronectin; VCAM-1
α5β1	Fibroblasts	Fibronectin
αLβ2	T lymphocytes	ICAM-1, ICAM-2
αMβ2	Monocytes	Serum proteins (e.g., C3b, fibrinogen, factor X); ICAM-1
αIIbβ3	Platelets	Serum proteins (e.g., fibrinogen, von Willebrand factor, vitronectin); fibronectin
α6β4	Epithelial cells	Laminin

*The integrins are grouped into subfamilies having a common β subunit. Ligands shown in red are CAMs; all others are ECM or serum proteins. Some subunits can have multiply spliced isoforms with different cytosolic domains.

SOURCE: R. O. Hynes, 1992, *Cell* **69**:11.

SUMMARY

CELL–CELL INTERACTION

Homotypic:

Contact-mediated (CAMs, Gap junctions)

Diffusible - homocrine factors

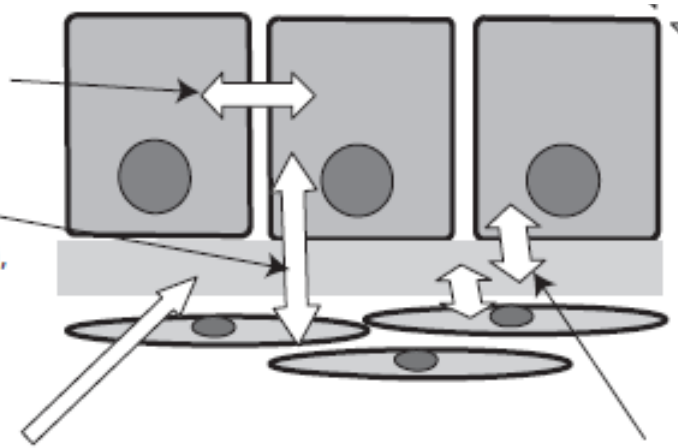
Heterotypic:

Diffusible - paracrine factors

Extracellular matrix

In vitro simulation: High-density (histotypic), recombinant cultures (organotypic).

Soluble paracrine factors (e.g., IL-6, KGF)



SYSTEMIC OR EXOGENOUS FACTORS

Hormones, circulating cytokines and growth factors. Vitamins, Ca^{2+}

In vitro simulation: Medium additives (hormones, etc.) planar-polar compounds, signal transduction and chromatin modifiers, such as PMA and valproic acid

MATRIX INTERACTION

Extracellular matrix

Collagen, laminin, proteoglycans, intergrin signaling

Binding and translocation of cytokines

In vitro simulation: matrix products, Matrigel, extracellular matrix (ECM)

(Note: All the original contributors of the concept and findings published elsewhere are gratefully acknowledged while preparing the E-content for the purpose of student reading material in convenient form for biochemistry and allied discipline).

References

- Alberts, Bruce, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. New York: Garland Science, 2002.

- Lodish, Harvey F. *Molecular Cell Biology.* 5th ed. New York: W.H. Freeman, 2003.

- Cooper, Geoffrey M, Hausman, Robert E. *The Cell. A Molecular Approach*. 4th ed. 2007.