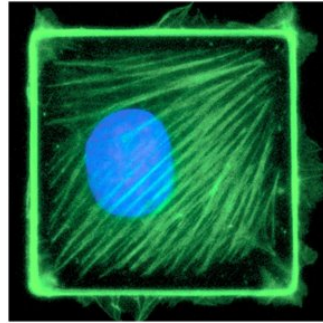


Week 10

Cell Interactions: The extracellular matrix



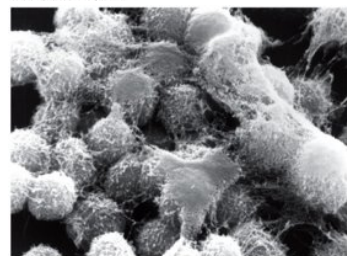
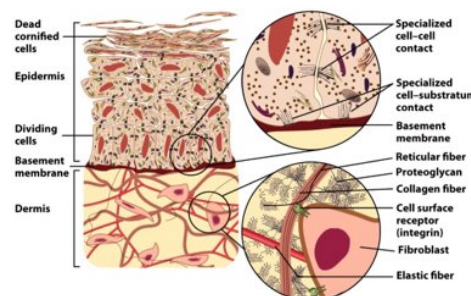
Why is this endothelial cell square?

This cell is grown in culture over a set of proteins called fibronectin arranged in a square. The fibronectin signaled where the cell should spread out to.

Suggested readings: Karp 9th ed. Chapter 7

Cells are in contact with each other and their immediate surroundings

- In multicellular organisms, cells make contact with other cells and/or an extracellular matrix (ECM)
- In some tissues, the ECM is a thin layer between tightly packed cells – often used to hold cells close to each other
- In some tissues, the ECM is a thick layer of material to cushion or protect cells
- ECM can serve variety of functions



Importance of the ECM in multicellular organisms

- The appearance of multicellular organisms allows specialization of cells and formation of organs
- Vertebrates have more than 100 specialized cell types (plants have more than 15)
- A special matrix, the extracellular matrix, ECM, fills out the space between cells
- ECM also binds cells together, acts as reservoir for growth factors and hormones, and creates an environment in which molecules and cells can migrate

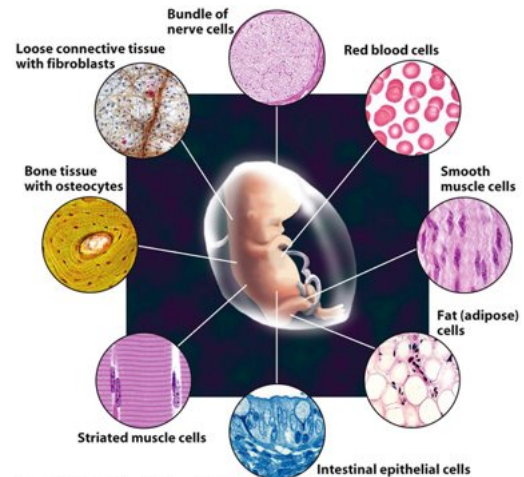
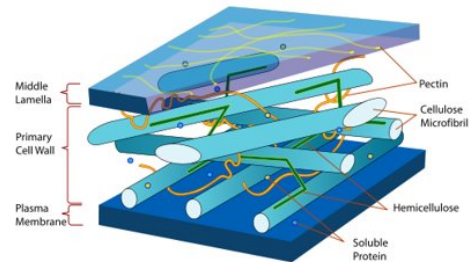


Figure 1-17 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Extracellular matrix – what organisms use it?

- Tissues in animals are not just made of cells: they also contain significant quantities of extracellular space, into which an extensive extracellular matrix may have been secreted.
- Plants and fungi (and bacteria) also have an ECM, only we call them 'walls'.
- Chitin in arthropods, is the main component of the ECM of an arthropod's epidermis – it forms the exoskeleton
- chitin and cellulose are by far the most abundant biopolymers on earth.



The plant cell wall is mostly made of carbohydrates. And that is all we'll speak of plant cell walls.



The extracellular matrix – what is it?

The main macromolecular components of the ECM in animals are:

- **glycosaminoglycans** (GAGs, acidic polysaccharide derivatives);
- **proteins** such as collagen, elastin, fibronectin and laminin;
- **proteoglycans**, which are proteins with GAGs attached to them covalently.

- The animal ECM is composed of a mixture of water, proteins and carbohydrates.

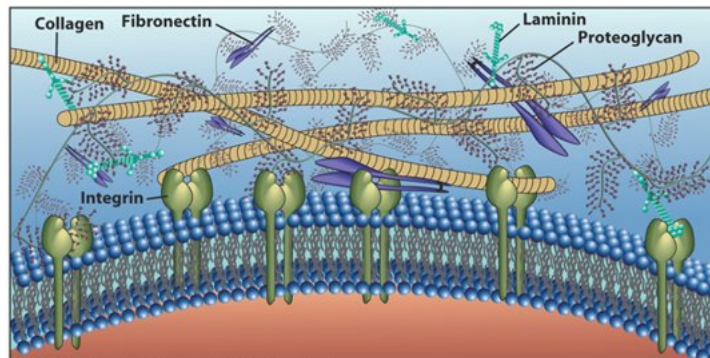
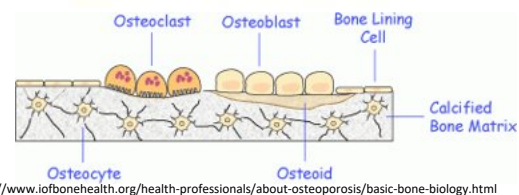
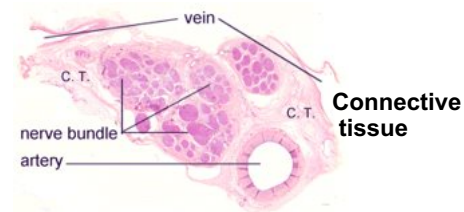


Figure 7-5 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

The ECM – where does it come from?

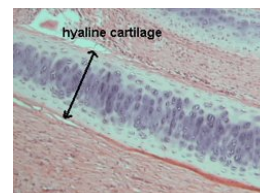
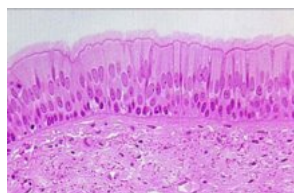
All cells can make extracellular matrix but certain specialist cells produce a specific type of ECM:

- Fibroblast cells secrete connective tissue ECM
- Osteoblast cells secrete bone-forming ECM
- Chondroblast cells secrete cartilage-forming ECM.
- Fibroblasts and epithelial cells together make basement membrane ECM



<http://www.iofbonehealth.org/health-professionals/about-osteoporosis/basic-bone-biology.html>

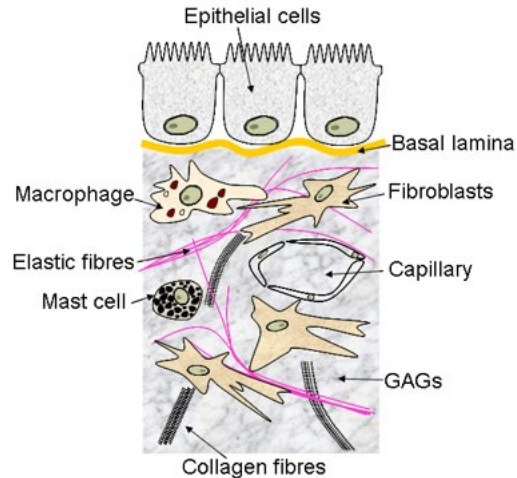
Basement membrane →



Function of ECM (with respect to cells)

For cells, the ECM provides:

- mechanical support
- a biochemical barrier
- a medium for:
 - extracellular communication that is assisted by CAMs (cell adhesion molecules)
 - the stable positioning of cells in tissues through cell matrix adhesion
 - the repositioning of cells by cell migration during cell development and wound repair



Function of the ECM (with respect to tissues)

ECM provides:

- tensile strength for tendons
- compressive strength for cartilage
- hydraulic protection for many types of cells
- elasticity to the walls of blood vessels

ECM can be calcified to form:

- bones and teeth
- the cell wall of bacteria
- the shells of molluscs and diatoms
- Chitinized (instead of calcified) to form the exoskeleton of arthropods



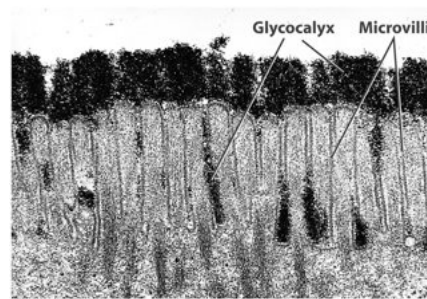
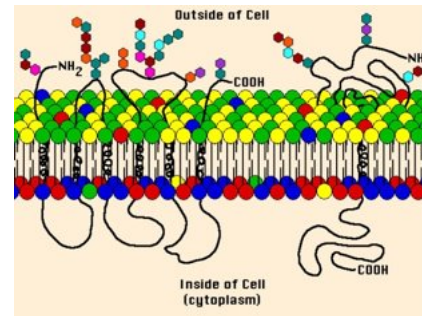
The glycocalyx

The carbohydrates attached to cell membrane proteins and lipids forms the cell coat called the glycocalyx

(glycolipids and glycoproteins from the plasma membrane lecture)

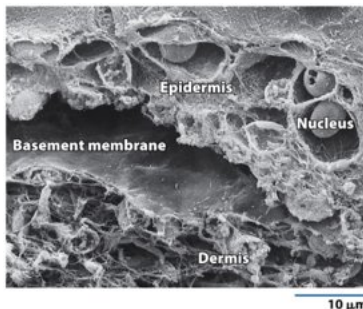
The glycocalyx is involved in:

- Mediating cell-cell interactions
- Mediating cell-substrate interactions
- Mechanical protection of cell
- Binding regulatory factors on cell surface



Thick glycocalyx protruding from intestine apical epithelial cell

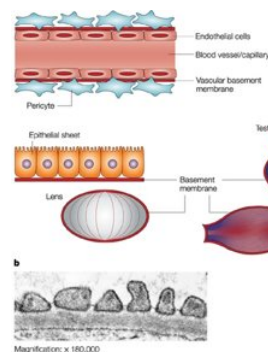
Basement membrane (= basal lamina)



Basement membrane functions include:

- providing mechanical support for attached cells,
- generating signals that maintain cell survival,
- separating adjacent tissues,
- acting as barrier to some macromolecules
- establishes cell polarity

- Basement membrane is a continuous sheet of ECM located:
 - around nerves, muscle and fat cells
 - under epithelial tissues (skin, lining of gut)
 - under endothelial lining of blood vessels



Nature Reviews | Cancer

The components of the extracellular matrix

Many of the ECM components interact with each other, and all can interact (bind) to cells.

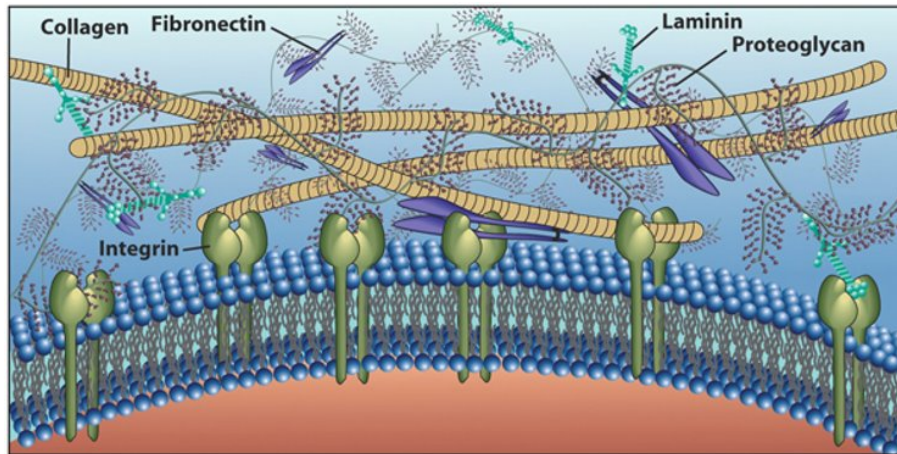
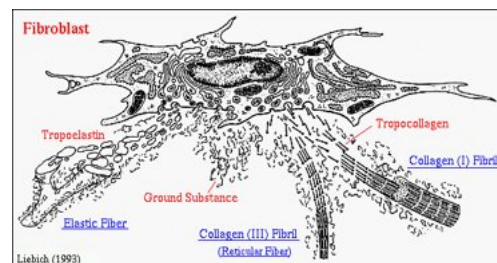


Figure 7-5 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Collagen: 25% of all your proteins is collagen!

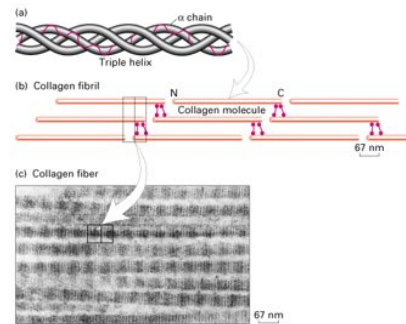
- Fibrous glycoproteins with high tensile strength (1 mm can hold 10 kg weight)
- Produced mostly by fibroblasts (cells of connective tissue), smooth muscle, epithelial cells
- 27 different types of collagen, with only a few types found in each tissue type. Combinations of the collagen protein types can yield fibers with different properties.



Lieblich (1993)

Collagen structure

- Collagen is a trimer protein – made of 3 alpha chains that intertwine to form a triple helix
- Proline and lysine amino acids get hydroxylated
- Collagen proteins assemble into fibrils for increased strength
- Fibrils are cross-linked to each other at hydroxylated proline residues
- Need vitamin C to assist in hydroxylation
- Lack of vitamin C results in scurvy – bruising of skin due to lack of collagen
- English sailors called limeys because they treated scurvy with citrus fruits such as limes



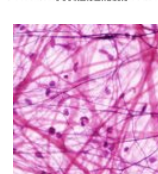
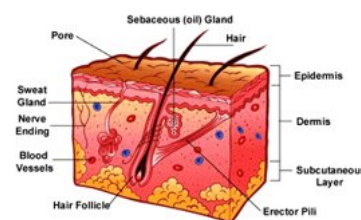
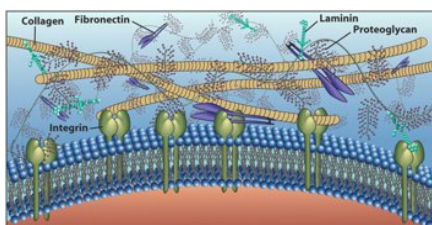
'lime suckers' would have been a better name

Importance of collagen

- Collagen is the fibrous protein constituent of skin, cartilage, bone, and other connective tissue.

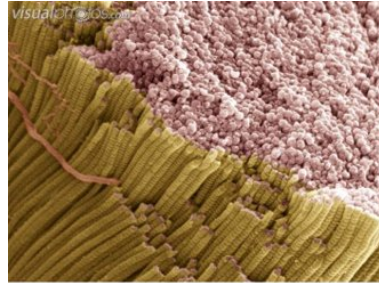
Collagen:

- acts as a scaffolding for our bodies.
- controls cell shape and differentiation
- is remodeled (broken down and rebuilt) to help broken bones regenerate, wounds heal, and directs blood v

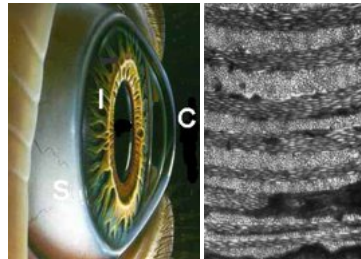


Collagen structure differs in tissues

- In addition to different types of collagens in different tissues, the way the fibers are arranged differs in different tissues:
- Tendons – all collagen fibers are parallel, in line with direction of the tensions exerted
- Eye's cornea – layers of collagen are perfectly arranged perpendicularly to other layers – gives strength while still allowing light to pass



Colorized SEM of tendon collagen fibres



collagen fibers

Images from Wikipedia

More fun with collagen

- Increased cross-linking occurs with age, causing decreased elasticity of skin
- Mutations in type I collagen results in osteogenesis imperfecta – thin skin, fragile bones
- Mutations in another collagen gene results in hyperflexible joints and extensible skin



Images from Wikipedia



This kind of stuff does *not* work!



Can be injected for that 'duckface' look

Perhaps most importantly, partially hydrolyzed collagen is used to make gelatin.



Although cherry flavor is gross.

Proteoglycans

- Proteoglycans are glycoproteins, but with far more carbohydrate than protein – usually a protein backbone covered with extensive network of carbohydrates
- The carbohydrates are *glycosaminoglycans* (GAGs), and are negatively charged
- The -ive charge attracts cations, especially Na^+ , which osmotically **attracts water** and causes the molecule to puff up into a gel. This allows GAGs, which only comprise 10% of the ECM mass, to take up 90% of the ECM volume
- The result is a spongy texture, good for cushioning and providing **compressive strength**
- Most abundant in cartilage
- Bone extracellular matrix also rich in collagen and proteoglycans, but hardened by impregnation of calcium phosphate

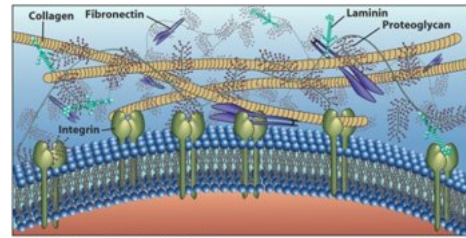


Figure 7-5 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Proteoglycans make a space-filling gel in the ECM



TEM of a proteoglycan complex in the cartilage matrix. Branches are the GAG emanating from the core protein.

Fibronectin

- dimer, with subunits with many different domains, each of which can bind to different components of ECM (e.g. collagen, fibrin, etc) and to cell receptors
- Different fibronectins bind ECM proteins and cell surface proteins differently... lots of different functions!
- Helps guide cells as they move through body
- During embryogenesis, fibronectin guides movements of many cells, including:
- Neural crest cells move out to form nervous system
- Primordial germ cells migrate from yolk sack to site of gonads

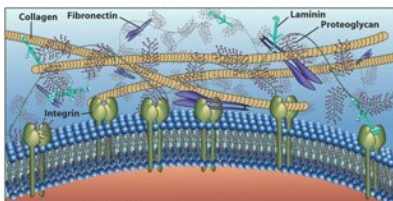


Figure 7-5 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

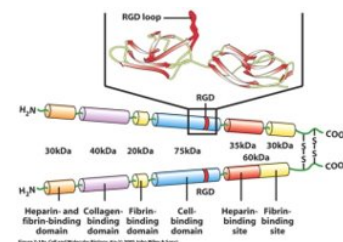


Figure 7-10a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Schematic of the domain structure of fibronectin. Note that they're all part of a single polypeptide!

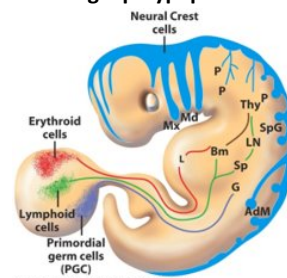
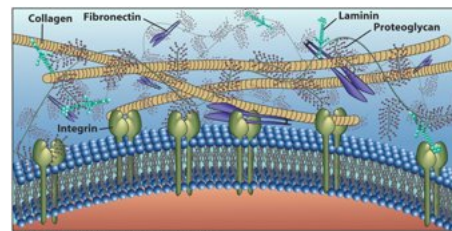
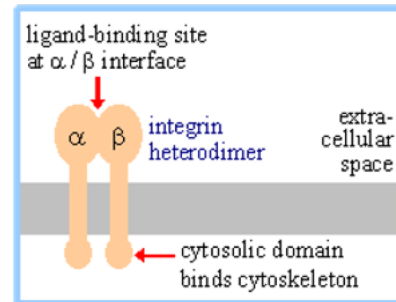


Figure 7-11a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Cells move around during development. Fibronectin tracts helps guide their movement as they pass through the ECM.

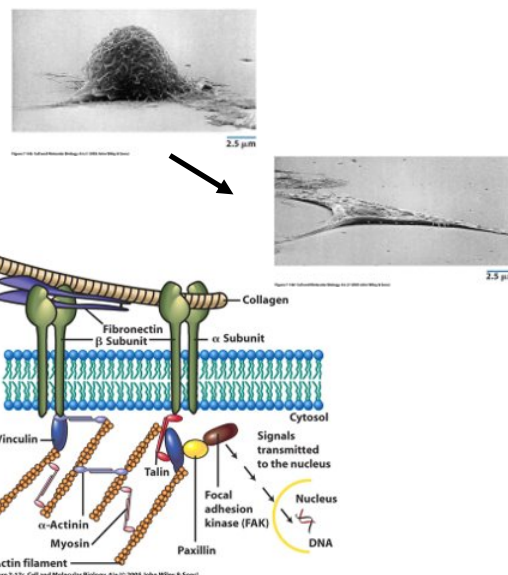
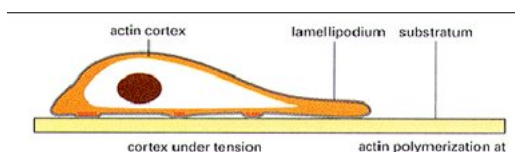
Cell-Matrix Adhesion is mediated by receptors

- Collagen, fibronectin, proteoglycans bind to cell receptors
- Most important of these cell receptors is **integrin**
- As name implies, integrin is an integral membrane protein
- has two polypeptides – α and β chains
- Different genes encode different α and β chains. Mix and match to bind different things!
- **cytosolic domains** of integrins bind to adaptor proteins (e.g., α -actinin, talin, filamin) that link integrins to elements of the cytoskeleton such as actin filaments.
- **extracellular domains** bind ligands in the ECM (e.g. fibronectin, collagen, etc).



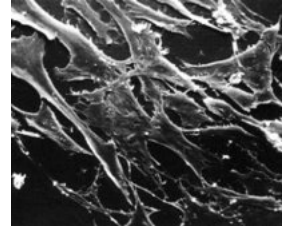
Anchoring cells with focal adhesions

- When a cell is in culture, it anchors itself to the substrate at discrete sites called focal adhesions
- The plasma membrane at the focal adhesion is rich in integrin proteins,
- At the site of a focal adhesion, the integrins are anchored to the cell's intracellular skeleton of actin microfilaments, using a number of adapter proteins, such as vinculin and talin
- The binding of the adapter proteins to integrin results in activation of **focal adhesion kinase (FAK)**
- FAK sends signal to the nucleus to prepare for shape change, movement, or change in cell cycle
- Cells in culture give insights into cell anchoring, but are they really what is seen in a multicellular organism?

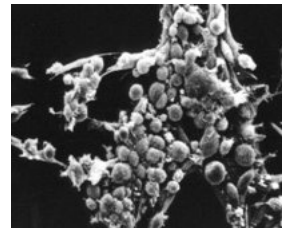


Integrins promote anchoring to ECM

- When integrins bind matrix fibers, the cytoplasmic domain of integrin changes, starting a signal cascade that can tell the cell to anchor
- Most normal vertebrate cells cannot survive unless they are anchored to the extracellular matrix. This **anchorage dependence** is often lost when a cell turns cancerous.
- normal cells use the signal from integrin to regulate normal cell survival activities; cancer cells do not rely on signal from integrin



Normal cells in culture anchor and flatten onto substrate

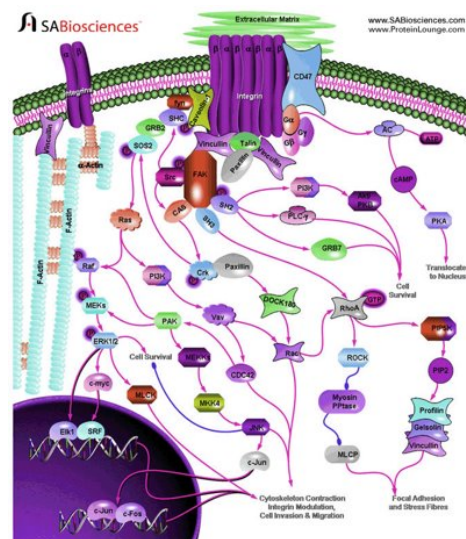


Cancerous cells round up and leave substrate

Images from: <http://www.cellimagelibrary.org>

How does the cell determine which choice to make following a signal from FAK (focal adhesion kinase)?

- What a cell decides to do is going to depend on what other signals have been received, and what other pathways have been activated.
- As you can see from this diagram, many options are available, and signal cross-talk will determine what the cell should do.



Don't memorize this, obviously.

What you should know after this section

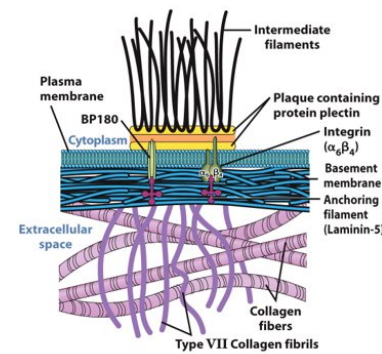
- What is the extracellular matrix and what are its functions?
- What types of ECM do fibroblasts, osteoblasts, and chondroblasts produce?
- What is the glycocalyx and what are its functions?
- Distinguish between glycosaminoglycans and proteoglycans and describe how they provide cushioning to tissues.
- What is collagen and how is it structured? What health-related issues can arise with improperly-structured collagen?
- What are the functions of fibronectin and where is it found?
- What are the functions of integrin and where is it found?
- What is a focal adhesion and how does it connect to the cytoskeleton?

Hemidesmosomes link the cell to the ECM

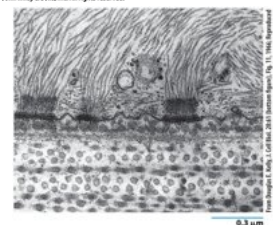
- hemidesmosomes provide tighter attachment to the ECM *in vivo* compared to focal adhesion (*in vitro*)
- Abundant where epithelial cells attach to the basement membrane
- composed of a dense plaque of plectin, to which intermediate filaments made of keratin are embedded inside the cell
- Intermediate filaments provide structural support on the inside of the cell
- The hemidesmosome is linked to outside of cell by **integrin**, which binds to ECM proteins and provides signaling to the nucleus



Defect in the integrin can result in loss of hemidesmosomes, which is most evident where the lower layer of the epidermis fails to attach to basement membrane, and chronic blistering is seen – disease is known as epidermolysis bullosa



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Cells attach to ECMs, but also to each other

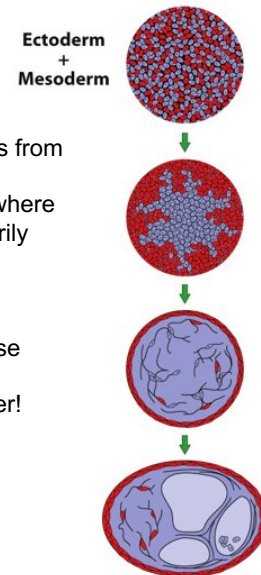
- Each human being begins life as a single cell - a cell that divides repeatedly to give rise to all **20-40 trillion** cells of which we are comprised.
- What keeps all of them where they belong?
- Cells recognize each other by means of cell adhesion molecules, CAMs

Check this out.

If you jumble all the cells from an early embryo...
They can all figure out where they should be and merrily proceed along with development.

They can do this because they can communicate and adhere to each other!

(cadherins do this)

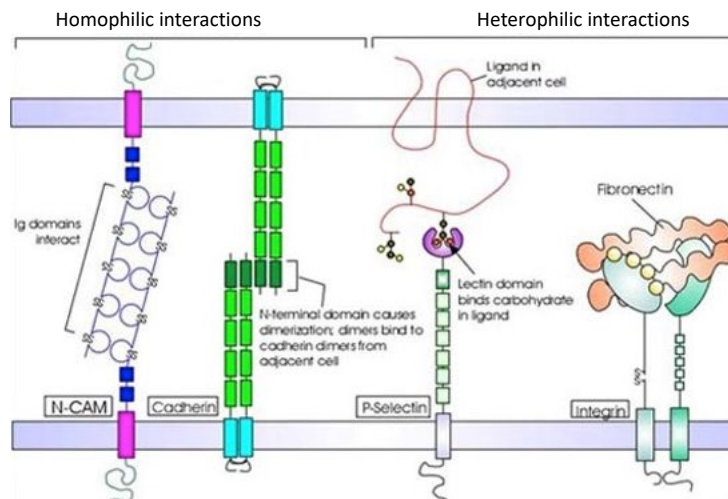


P.L. TOWNES, J. HOLTGRETER, JOURNAL OF EXPERIMENTAL ZOOLOGY 28:53, 1955

CAMs involved in cell-cell adhesions include...

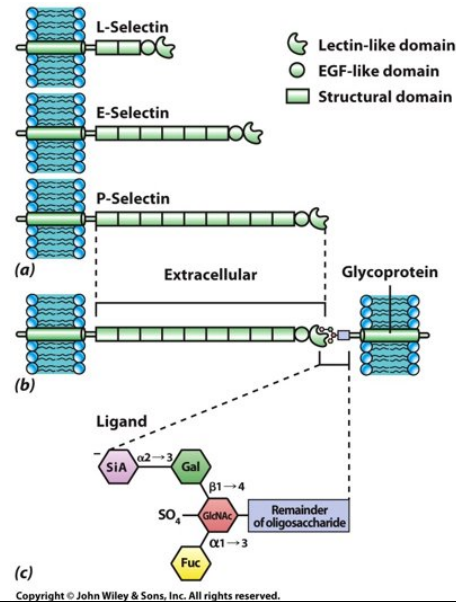
- Immunoglobulin superfamily
- Cadherins
- Selectins
- Integrins
- Connexins

Homophilic & Heterophilic Interactions of CAMs



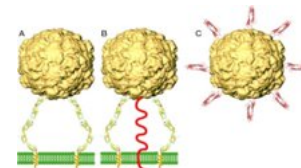
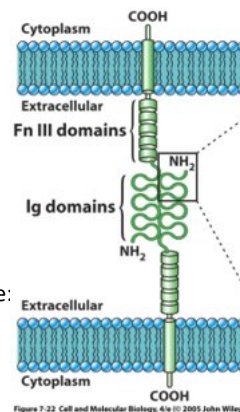
Selectins attach to adjacent glycoproteins

- Family of integral glycoproteins
- Extracellular domain binds specific carbohydrates on glycoproteins on adjacent cells (heterophilic interaction)
- L-selectins found on leukocytes (white blood cells (WBC)), E-selectins found on endothelial cells (cells lining blood and lymph vessels), P-selectins found on platelets
- Play a role in stopping leukocytes in blood flow at sites of inflammation and infection (later slide)

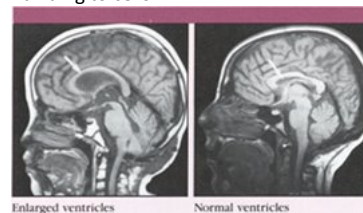


Immunoglobulins attach to each other (usually)

- Antibodies (immune system proteins that recognize foreign molecules) are a type of immunoglobulin (Ig), which recognize and bind other proteins and antigens
-
- Cell-bound integral Igs (not antibodies, but have an Ig domain) are referred to as Ig-Cell Adhesion Molecules (CAMs)
-
- Ig-CAMs belong to a superfamily of related molecules (765 of them!); some members include:
- ICAM (intercellular CAM)
- VCAM (vascular CAM)
- NCAM (neural CAM)
-
- Ig-CAMs bind similar molecules on other cells = homophilic interactions
-
- mediate cell-adhesion of lymphocytes (T-, B-, and natural killer WBCs) as well as vascular and neural cells (specificity is due to various Ig domains)



Rhinovirus attaches to target cells by binding to ICAMs, then delivers RNA; one therapy is to provide ICAM fragments to prevent virus from binding to cells



One Ig cell adhesion protein known as L1, assists in growth of nerve cells – mutants can have severe neural problems: mental retardation, hydrocephaly (fluid accumulation in ventricles of brain - arrows)

Cadherins bind to each other to form 'cellular velcro'

- integral glycoproteins
- typically have (5) tandem domains
- Ca^{2+} ions help separate the 5 domains
- Holds similar cells together to form tissues by binding to the same cadherin on a neighboring cell like Velcro (homophilic): the greater the number of **cadherins**, the greater the strength of adhesion
- Cadherins are anchored to the cytosolic actin network through adaptor proteins such as catenin

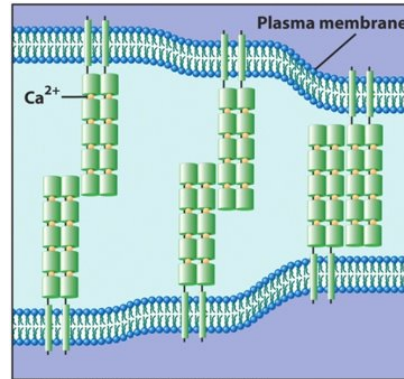
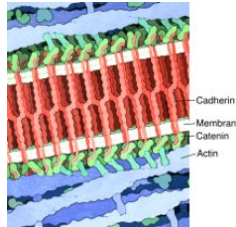


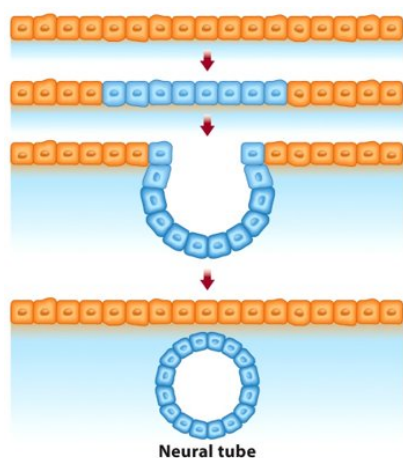
Figure 7-23 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

We're actually not sure which one of these types of interactions occurs.

Lots of cadherins together form strong connections called adherens junctions (later slide)

Cadherins play a role in development

During development, cells can move from one tissue and become other cell types. At the very beginning of the development of the nervous system, cells from the epithelium break away and begin to develop into what will become neural cells:



1. All the epithelial cells are expressing E-cadherin (epithelial cadherin), keeping them together

2. Some cells stop expressing E-cadherin and begin expressing N-cadherin instead (neural cadherin)

3. The N-expressing cells can't adhere to the E-cadherin expressing cells, so they pinch off and begin the development of the neural tube

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These *all* play a role in inflammation

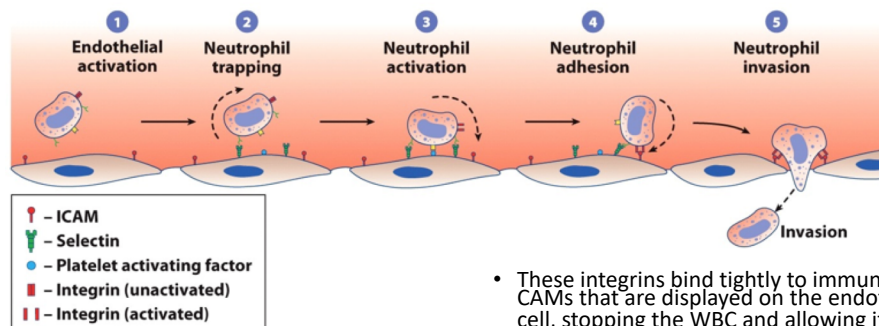
When bacteria is infecting a tissue, signals are sent out to attract a phagocytic white blood cells (neutrophils) to the site. But these WBCs are found in the bloodstream... so how to they get to the infection site??

But first... an awesome movie of a neutrophil chasing a bacterium:

<https://www.youtube.com/watch?v=Kb-m1uDoWfU>

Many CAMs play a role in inflammation

- When bacteria is infecting a tissue, signals are sent out to attract a phagocytic white blood cells (neutrophils) to the site. But these WBCs are found in the bloodstream... so how to they get to the infection site??
- The endothelial cells close to the site start displaying E- and P-type selectins.
 - These interact with carbohydrates on the WBC surface and slow it down to a roll.
 - Another protein on the endothelial cells (PAF) binds with a WBC receptor that activates integrins on the WBC surface.



- These integrins bind tightly to immunoglobulin-CAMs that are displayed on the endothelial cell, stopping the WBC and allowing it to penetrate the endothelial layer to eat bacteria. Nom nom nom.

Some cell-cell interactions are *super tight*

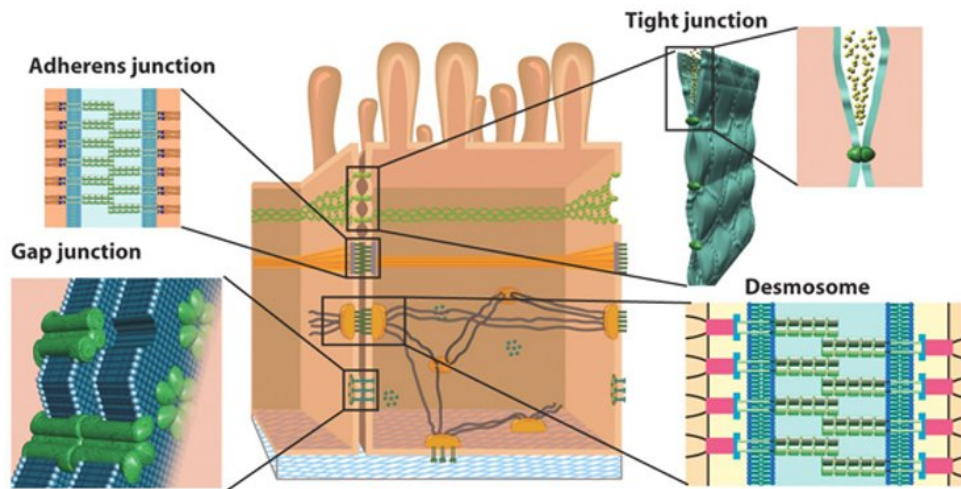
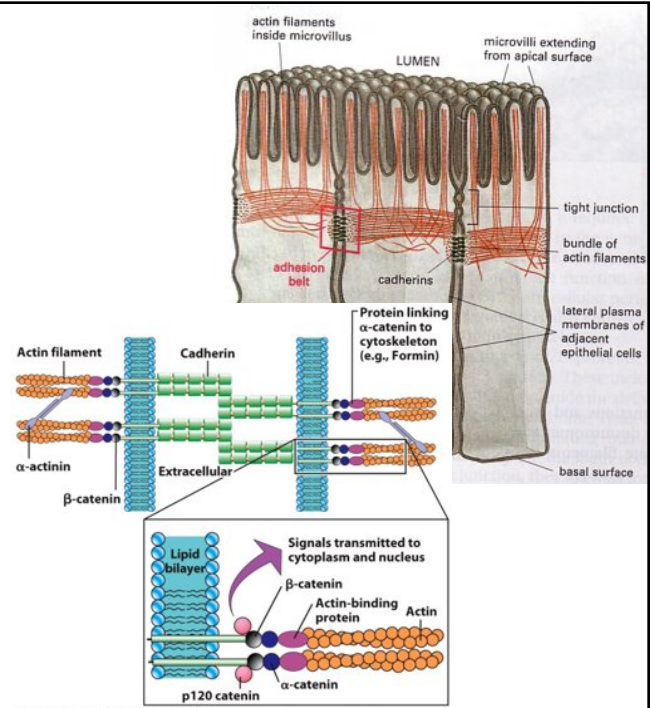


Figure 7-25a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

These types of junctions don't *always* occur in the same cell, as depicted in this cartoon!

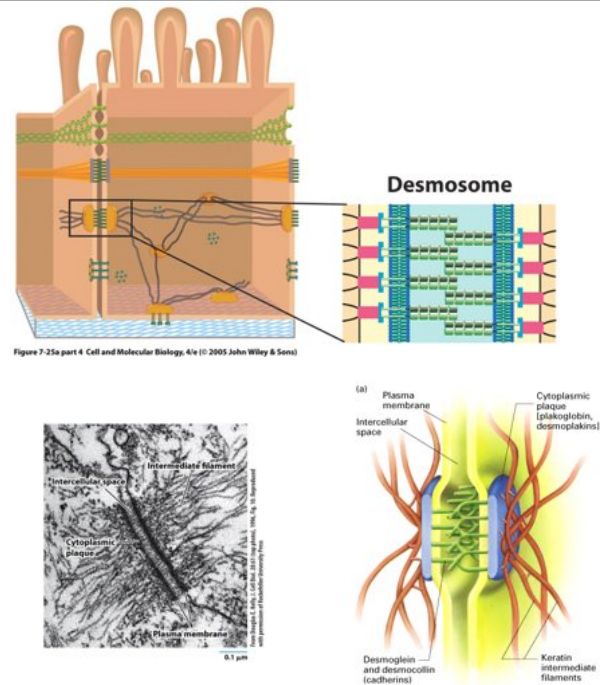
1. Adherens junctions

- Junctions between cells using **cadherin** molecules (just like we saw before)
- Found in many cells, but especially common around epithelial cells, where they form a belt around the apical ends of cells – maintains tissue integrity at tissue surface
- Like we saw with integrins, adherin junctions can signal to the nucleus via catenin to maintain cell survival
- Loss of functioning adherens junctions may lead to tumor **metastasis**.



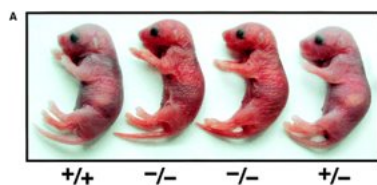
2. Desmosomes

- Disc-shaped adhesions that are present on cells subjected to mechanical stresses (e.g. muscles, epithelia, cervix)
- Also use cadherins on extracellular side, but they use 2 special types, which are arranged differently, providing more protein contacts between cells (note that desmosomes use cadherins, while hemidesmosomes use integrins)
- Cadherins are anchored intracellularly by some “plaque” proteins, which form thick plates of protein
- Plaques are further anchored to intermediate filaments of cytoskeleton
- An autoimmune disorder, known as pemphigus vulgaris, attacks the cadherin proteins in desmosomes, resulting in blistering.

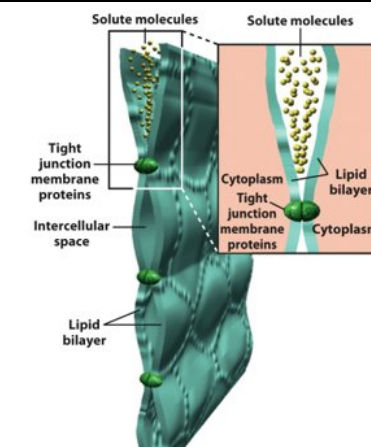


3. Tight junctions

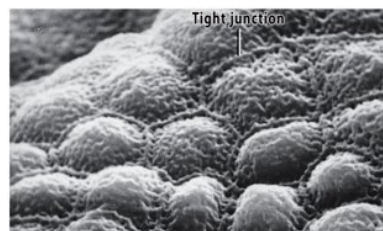
- Found at the apical end of cells, tight junctions prevent easy flow of liquid and solutes between cells
- Adjoining cells produce two classes of proteins: occludins and claudins, which form a line of “spot welds” on both cells’ membranes
- Different claudins can be permeable to just certain ions – allowing for controlled “leakage”
- Found in all epithelia
- Mutations in claudins result in tissue failure: e.g. mutation in claudin-1 means that skin epidermis leaks water and animals die of dehydration



claudin- mutant mice are dehydrated, wrinkled, and die after 1 day

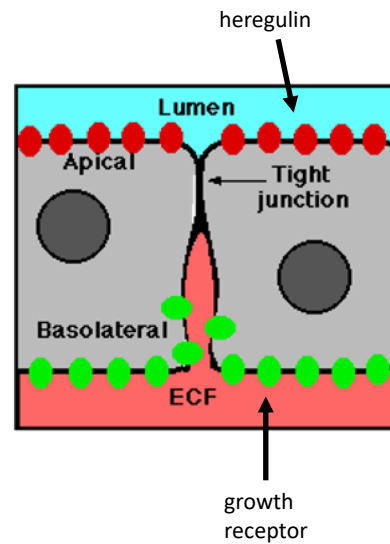


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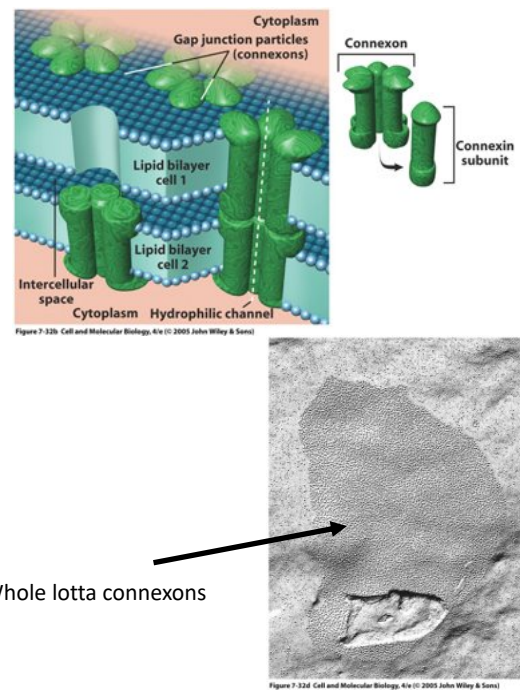
The epithelia of the human lung: an example of tight junctions

- The epithelial cells of the human lung express a growth stimulant, called **heregulin**, on their **apical** surface (red spots in diagram)
- Its receptors are on the **basolateral** surface.
- As long as the sheet of cells is intact, there is no stimulation of its receptors by heregulin thanks to the seal provided by tight junctions.
- However, if the sheet of cells becomes broken, heregulin can reach its receptors. The result is an autocrine stimulation of mitosis leading to healing of the wound.



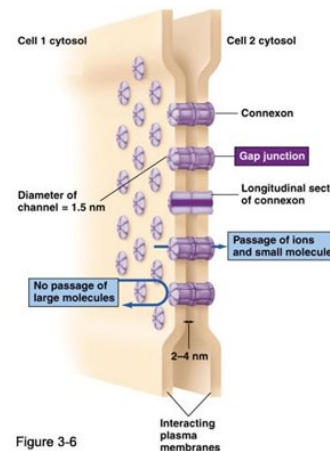
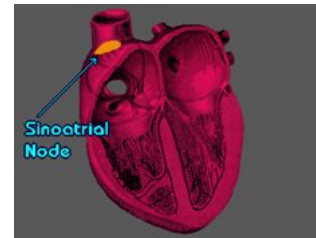
Gap junctions

- Protein “pipelines” allowing direct contact between adjacent cells’ cytoplasms
- Integral protein that lines the channel is called connexin
- Ring of 6 connexins comprise a “connexon”
- 2 connexons, one from each cell, must pair and bind to form channel
- Permits flow of small molecules – ions, simple sugars
- Flow is passive, but channel can be gated
- Flow can be regulated by number of connexons



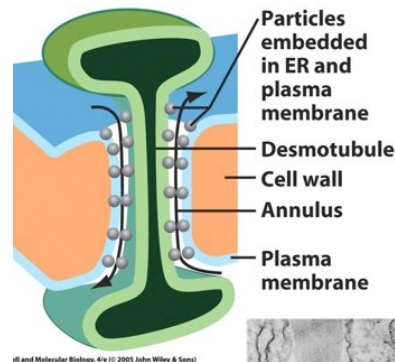
Gap junction functions

- Why allow adjacent cells to be intimately connected?
- Gap junction can permit fast signaling for cells that need to act in synchrony e.g. heart sinoatrial node – pacemaker – electrical impulse from this node causes heart to beat - gap junctions between cells allow for rapid ion flow, which provides electric current
- cAMP and inositol triphosphate (IP3) can be delivered to adjacent cells when hormones trigger a single cell – tissue acts in harmony – e.g. movement of cilia, peristalsis of gut
- ~20 different connexin proteins – certain cells produce different connexins – allows adjacent cells to connect to only those with the right connexon

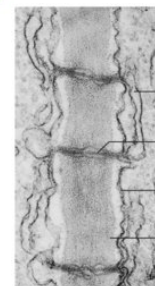


Plant cells are all interconnected by plasmodesmata

- Plant cells don't have the cell adhesion molecules because they are surrounded by the plant cell wall
- Plant cells connected to each other by channels known as **plasmodesmata**
- A typical plant cell may have between 103 and 105 plasmodesmata connecting it with adjacent cells
- Plasmodesma lined with cell membrane (usually also contains a desmotubule – derived from SER)
- Pore allows larger molecules to pass
- Pores can be controlled by dilation using contractile proteins (actin and myosin)
- Some viruses can modify the plasmodesmata proteins to open wider to allow passage of the virus



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What you should know after this lecture

- What is a hemidesmosome, what are the key molecules that comprise it, and what happens if it malfunctions?
- Name the five groups of cell adhesion molecules and know whether they are homo- or heterodimeric.
- Where do you commonly find selectins?
- Where do you commonly find immunoglobulin CAMs?
- How do cadherins attach to each other and what determines the strength of the bond with adjacent cells?
- How do cadherins facilitate embryo development?
- How do different cell adhesion molecules stop a neutrophil?
- Name the four types of cell-cell junctions, know which cell adhesion molecules are associated with each, and identify the function of each cell-cell junction.
- How do plasmodesmata differ from gap junctions?