Cell Junctions

Lecture 8

Objectives

- Three classes of cell junctions
- Four specific examples of cell junctions and their components and roles/functions

1. desmosomes
2. adherens junction
3. tight junctions
4. gap junctions

- Compare & contrast the cell junction types

Cell Junctions

Adhering or Anchoring Junctions

- Desmosomes
- Adherens Junction ("belt desmosome")
- Hemidesmosomes

Occluding / Barrier Junctions

- Tight Junctions

Communicating or Connecting Junctions

- Gap Junctions

Desmosomes

- Usually used by types of cells who tissues are subjected to extreme mechanical stress
- These are anchoring junctions used as couplings to prevent cell separation
- They link *between* and *through* cells
- Desmosomes are commonly found in epidermal (skin) cells and cardiomyocytes, these types of cells stressed greatly

Desmosome Features

Plaque

button-like thickening of the membranes of the two cells

Cadherins

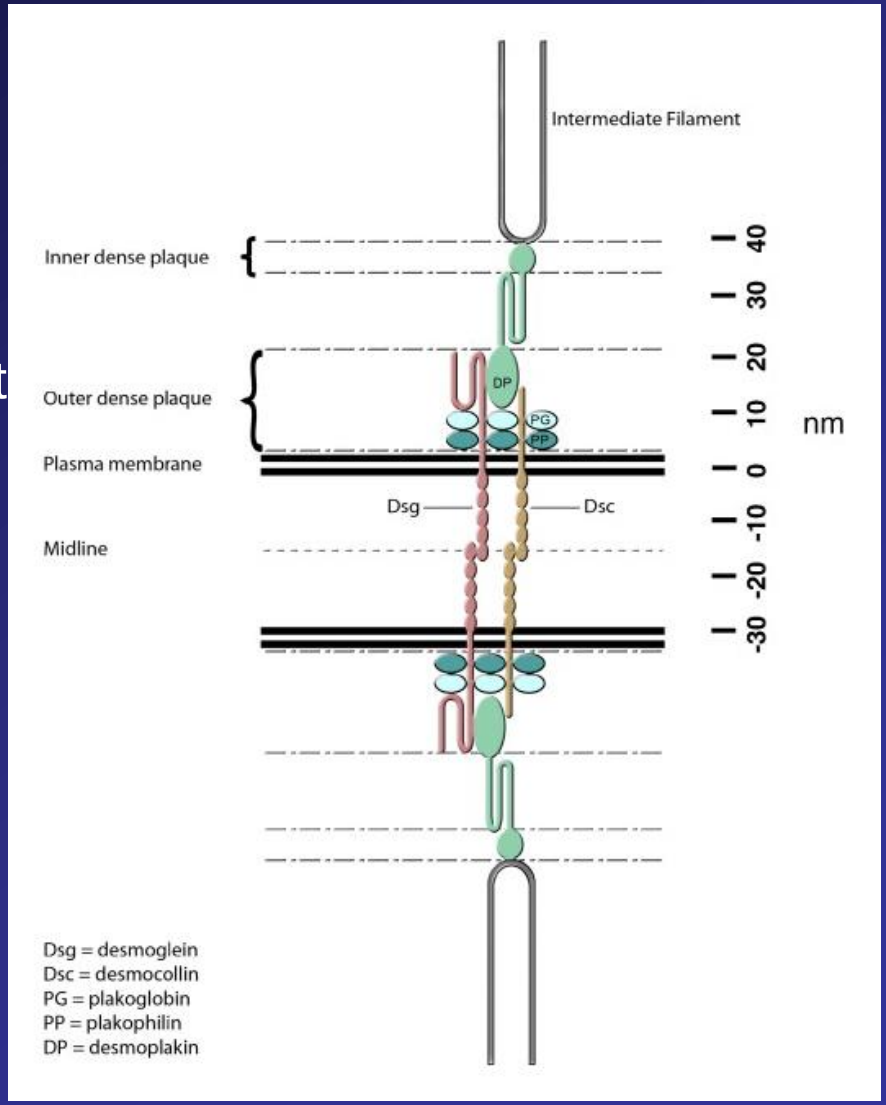
- protein filaments extend from plaques, fit like teeth of a zipper in intercellular space
- This is the link between cells

Intermediate filaments

- These form the link through a cell that extend from a plaque on one side of cell to a plaque on the other side of the cell
- This is the link through a cell

Desmosome Structure

- The intercellular space shows the connections by cadherin family of proteins called desmoglein (Dsg) & desmocolin (Dsc)
- These cadherins extend through the plasma membranes and connect with the proteins that make up the plaque. These plaque proteins are plakophilin and plakoglobin
- Desmoplakin (DP) is a protein that connects plakoglobin (PG)
- The complex of plaque proteins (PP, PG, DP) connect the cadherins to the intermediate filaments



Anchoring junction (desmosome)

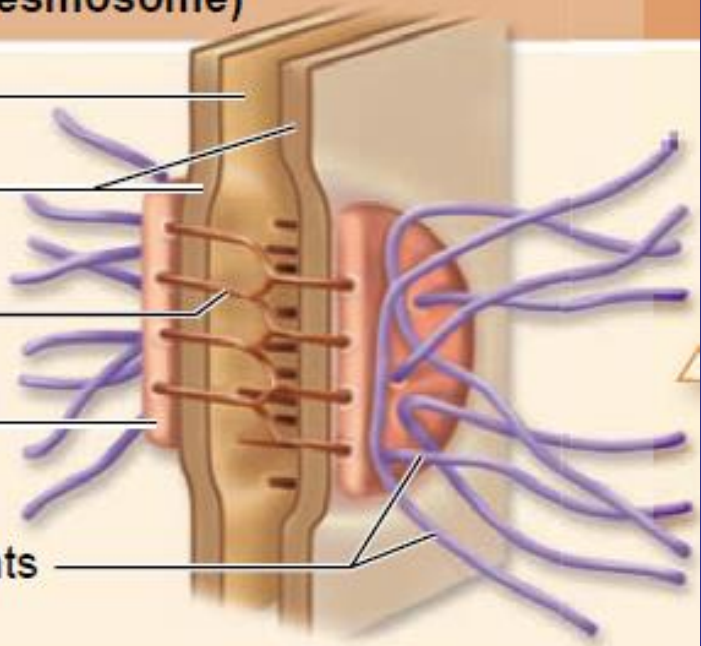
Intercellular space

Adjacent plasma membranes

Cadherin

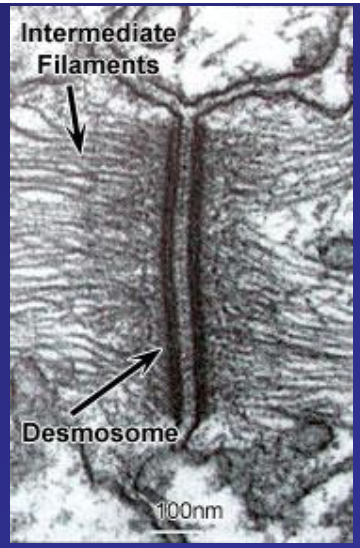
Cytoplasmic protein plaque

Cytoskeletal filaments anchored to plaque



b. 0.1 µm

http://www.derm101.com/wp-content/uploads/ac01g020-150x150.jpg



Desmosome Dysfunction

Epidermis bullosa simplex

- defects in genes for keratins
- skins blisters easily from rubbing, scratching
- severe cases: mouth or intestinal blistering

Pemphigus

- autoimmunity to cadherin family proteins **desmoglein** that connect cells
- several types of pemphigus with different appearances

Lethal acantholytic epidermolysis bullosa

- partial deletion of the desmoplakin gene
- this presents usually after birth and is lethal in infancy

Skin fragility syndrome (ectodermal dysplasia)

- defect in the plakophilin gene

Adherens Junction

- This junction is similar to desmosomes at least in function
- The important difference with desmosomes is that the through-cell filaments are not intermediate filaments, but rather actin filaments (F-actin)
- Actin is a protein that interacts with myosin in muscle contraction

Adherens Junction Features

Plaque (adhesion plaques)

like desmosomes, a dense collection of proteins forms on the intracellular side that looks like a plaque in microscopy

Cadherins

- these are the molecules that connect the two cells together
- a different set of cadherins from those in desmosomes do the actual linking of the cells between the two membranes

Linking proteins

- These form a ring around the cells, connecting to other plaques on the cell

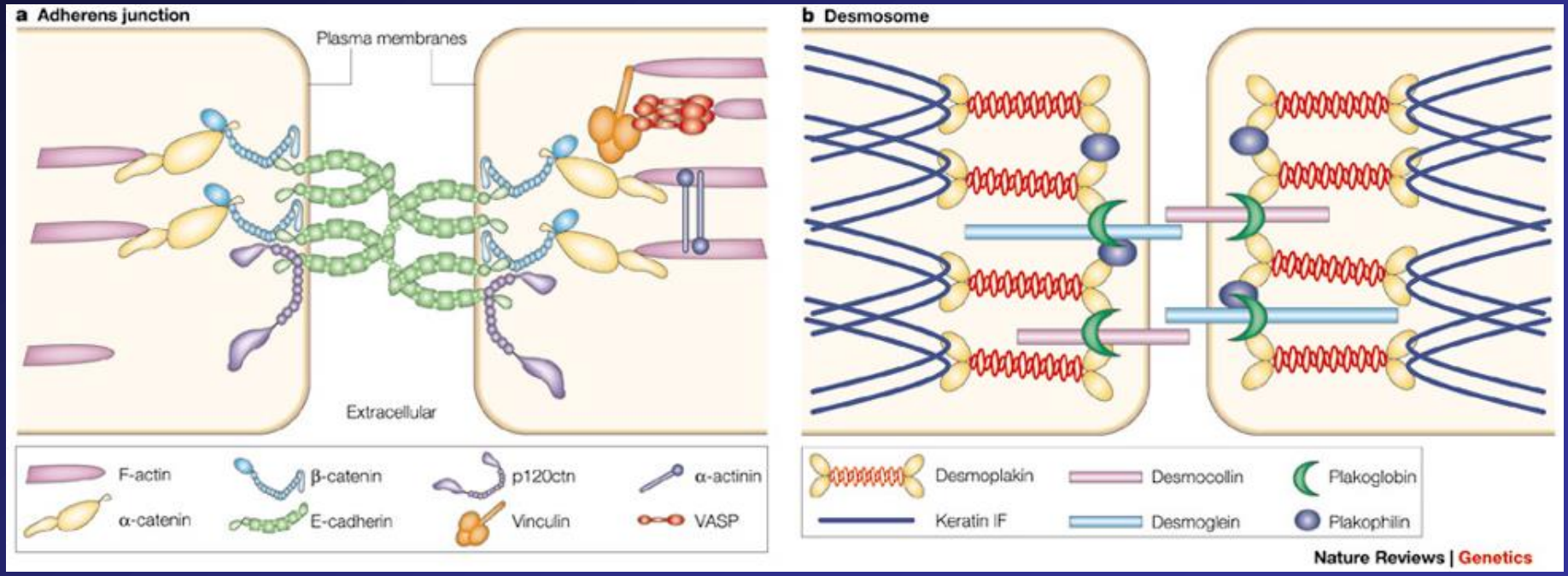
Actin filaments

- These form a ring around the cells, connecting to other plaques on the cell

Comparing Adherens Junctions to Desmosomes

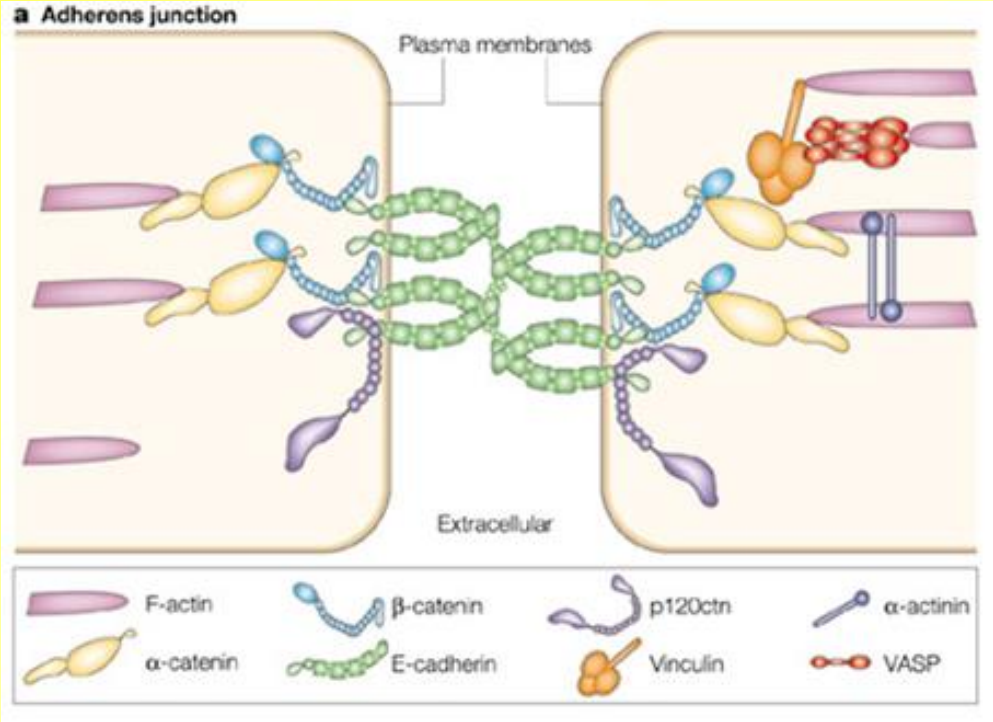
Although the actual proteins differ, the protein types are similar between the adherens junctions and the desmosomes

- cadherins are the proteins between the cells linking them
- and the proteins that connect to the cadherins (via linking proteins) are filaments that connect the membranes through a cytoskeleton



Molecular Details

- The green molecules in between cells are E-cadherins, which are homodimers that make the binding between cells
- The dark blue-colored molecule is called p120, an "armadillo protein" appears to stabilize the E-cadherin
- The light blue-colored beta-catenin is a chain link between E-cadherin and yellow-colored alpha-catenin



- Alpha-catenin connects the complex to filamentous actin (F-actin)
- Plakoglobin in desmosomes is also called gamma-catenin, and has a sequence quite similar to beta-catenin
- Vinculin and VASP are proteins commonly found linking actin filaments to membrane proteins involved in cell anchoring

Tight Junctions

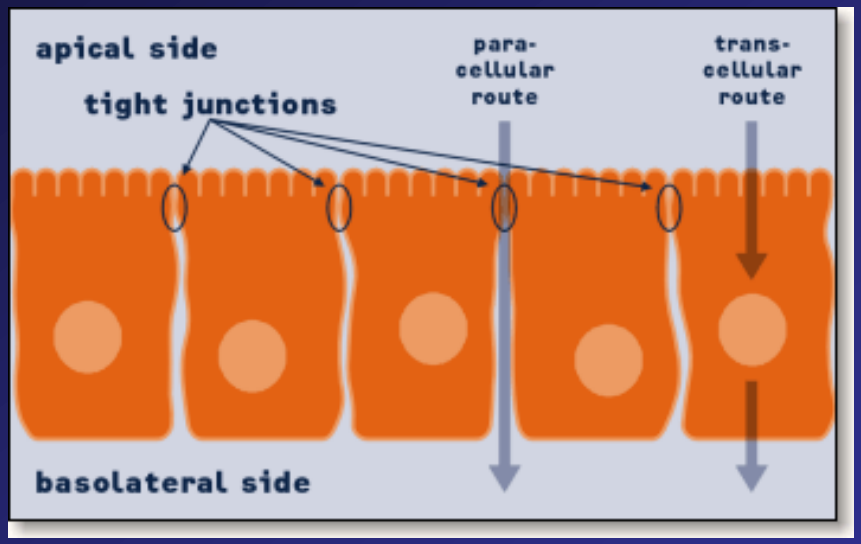
Tight junctions are occluding junctions

- Regulate paracellular transport pathway
  - This means they guard the space between cells (the "alleyways")
  - They are not impermeable to everything, but they can open a gate to molecules or ions or close it
- Like desmosomes, they also contribute to holding cells together as a layer

- Most likely to form with an epithelial (one cell-thick) cell layer

Transcellular & Paracellular Paths

- Molecules & ions of all kinds have two ways of being transported from the relative outside (apical side) of the body to the inside (basolateral side) of the body: *through* cells or *around* them
- Tight junctions hold the gate around—down the alleyways—of barrier cell layers.
- TJs are are generally found on:



- intestinal mucosa epithelium (near the brush border)
- distal convulted tubules and collecting ducts in kidney nephrons
- bile ducts within liver

Tight Junction Features

Tight barrier

Permeability for hydropholic nonelectrolytes is up to 0.4 nm

diameter of H_2O: 0.3 nm

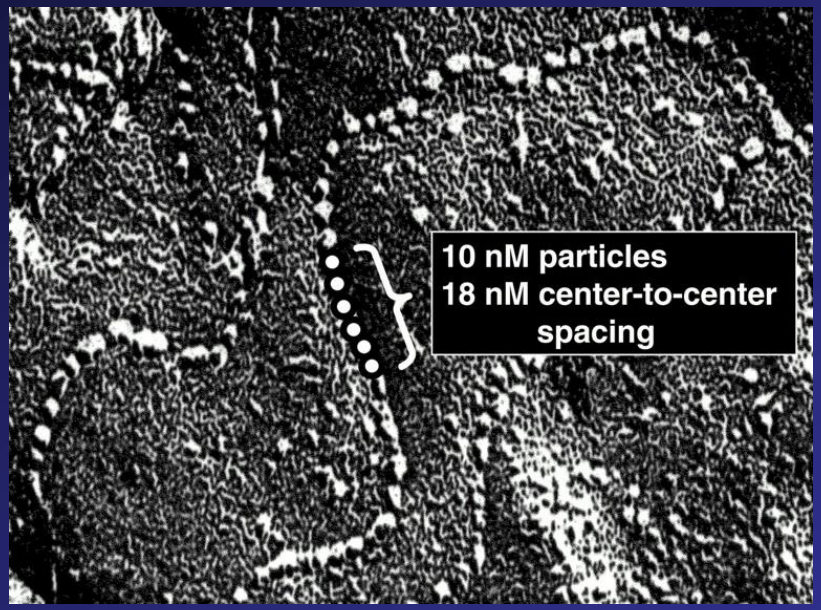
10 nm particles

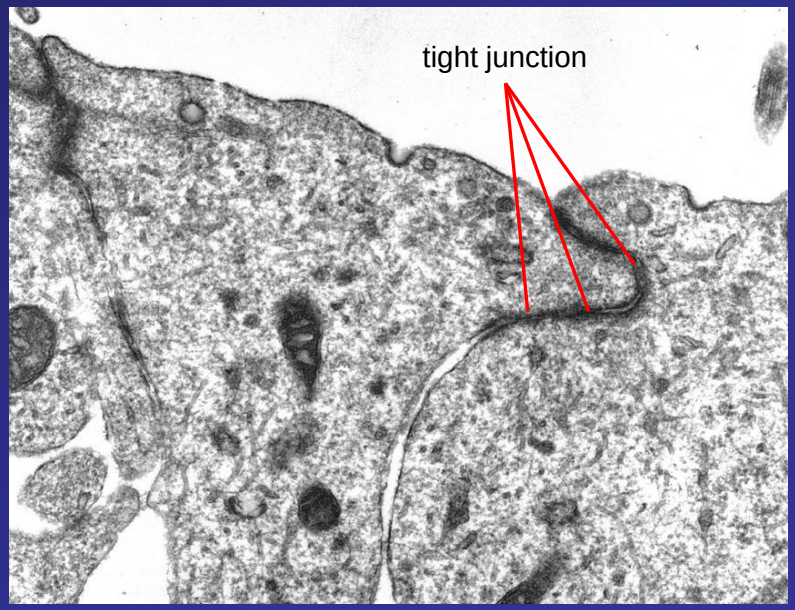
These appear as rows of interweaving strands, each particle space 18 nm center-to-center from each other

Claudin proteins & the occludin protein

These are the proteins that connect between the cells and are believed to form the tight barrier to diffusion to all but the smallest ions or molecules

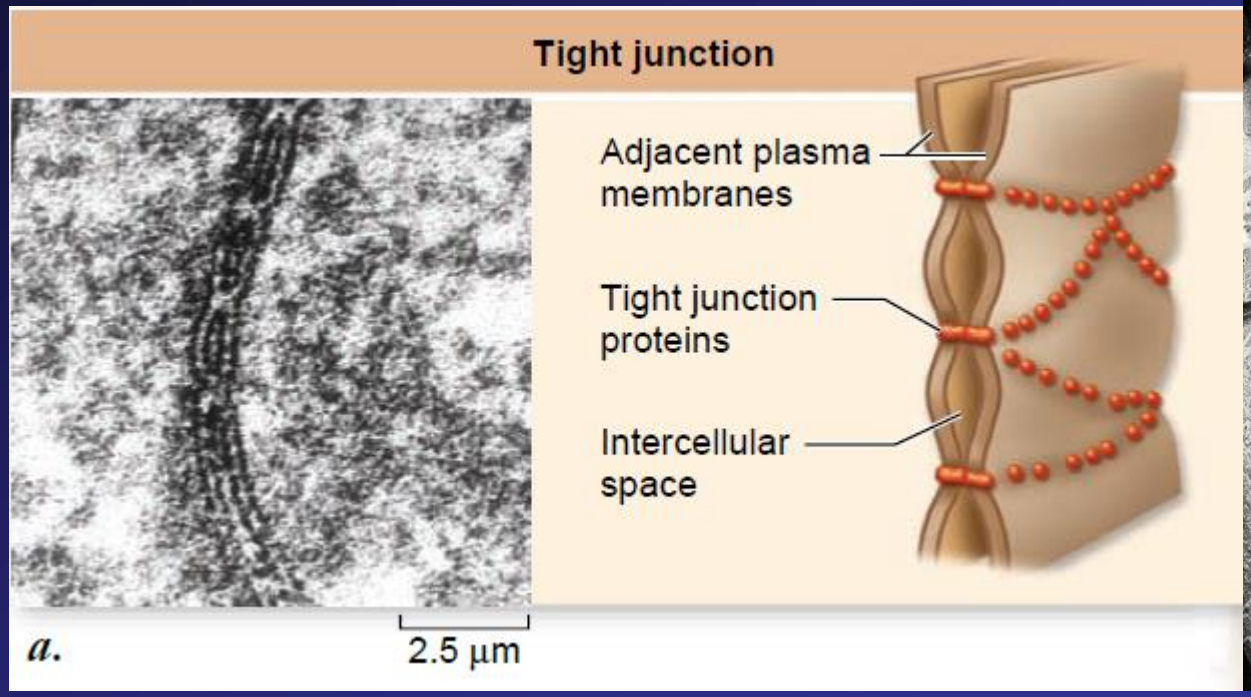
- Freeze fracture electron microscopy (lower left) divides the lipid bilayer of the membrane to reveal the 10 nm particles
- The transmission electron micrograph (lower right) shows the tight junction





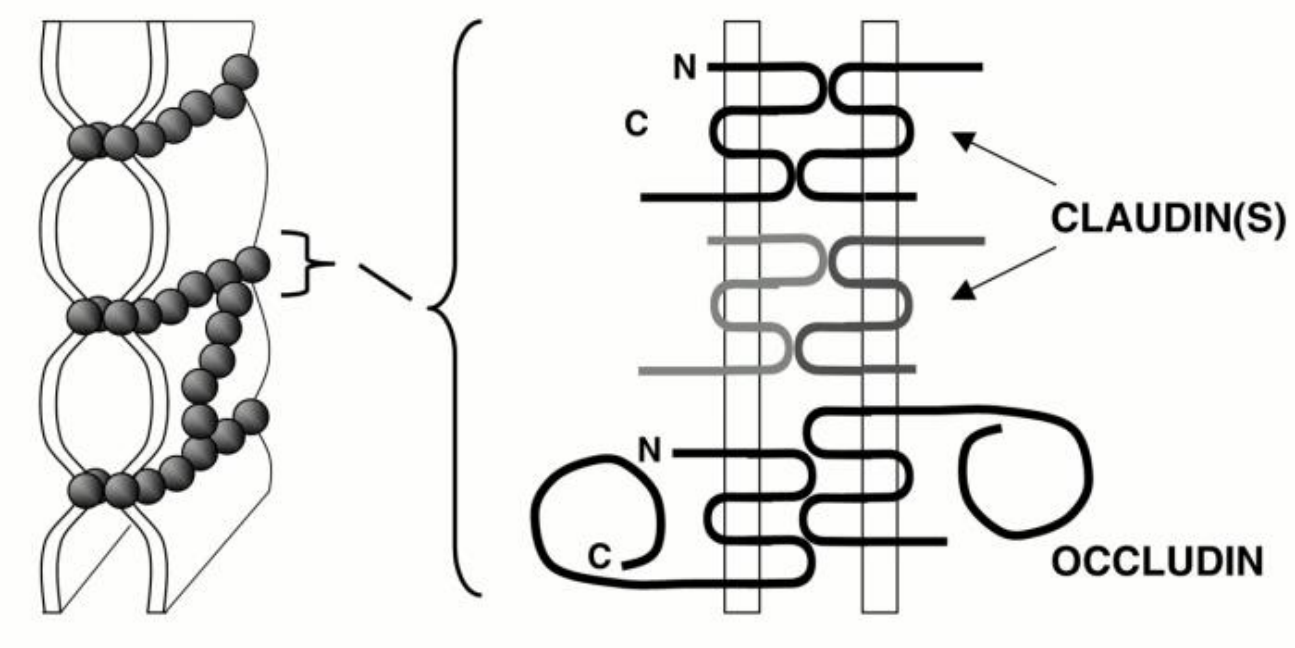
http://physiologyonline.physiology.org/content/16/3/126

- Another closer look at TJs with TEM (lower left) shows oval openings, which are interpreted in the illustration
- Another freeze fracture EM (lower right) shows another view of how the weaving pattern can occur as it circles the cell



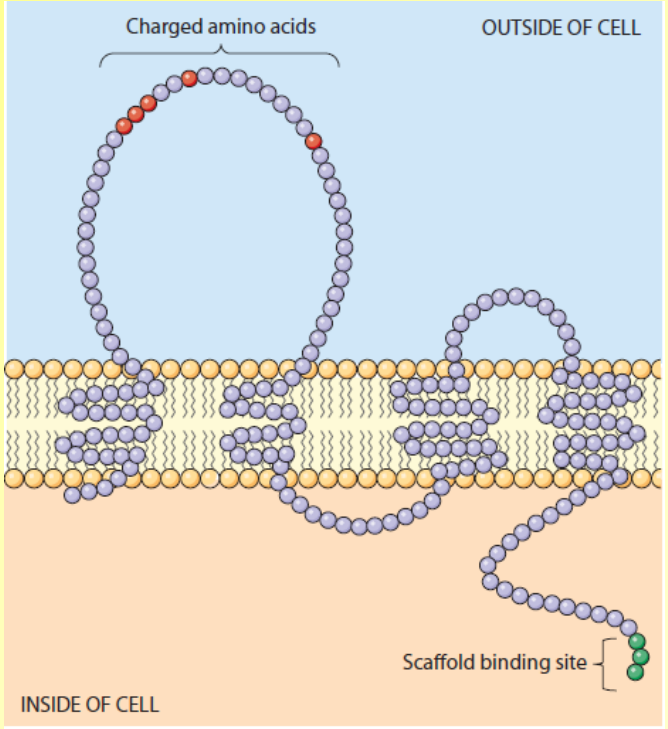
The Main Components of TJs

- The 10 nm particles are made of a cluster of proteins, but the main proteins involved in connecting with each other are the claudin protein family and the protein occludin



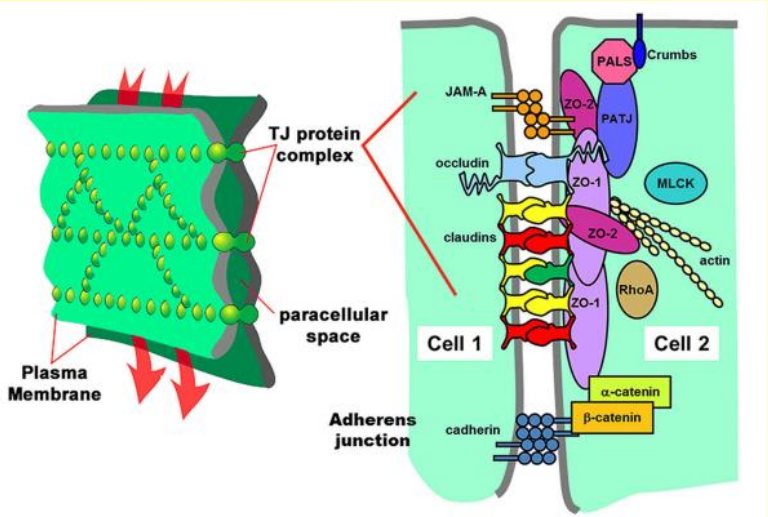
The Biomolecules of Tight Junctions

- Many proteins make TJs: on this and the next slide, you can see the proteins identified, some described in great detail
- claudins: these membrane proteins (4 transmembrane segments) form the net (semipermeable seal). Some claudins (there are at least 20 of them having different properties) make the seal tighter (impermeable) while other are weaved into promote paracellular transport. One large extracellular loop of claudins has many charged amino acids and this may be a barrier to ions passing (or specific ones being allowed)



The Biomolecules of Tight Junctions

- occludin: similar to claudin in structure, but function unknown
- JAM-A: (JAM=junctional adhesion molecule) is a member of the immunoglobulin superfamily
- cingulin
- 7H6:
- ZO-1, -2, -3



Tight Junction Dysfunction

- Familial hypomagnesia with hypercalcuria and nephrocalcinosis (FHHNC) is a severe imbalance of Mg^{2+} and Ca^{2+} and is connected to mutations in claudin-16
- Crohn's disease may be a failure of tight junction function
- Ulcerative colitis may result in TJ dysfunction secondary to inflammatory processes

Gap Junctions

- Gap junctions cause adjoining cells to form a "partial union of their cytoplasm" (syncytium)
- shared cytosol: metabolic coupling of cells
- Permeability is for substances which are up to 1200 Da, which covers many of the small organic and inorganic molecules
  - metabolites: ATP
  - ions: Ca^{2+} (important in signaling!)
  - antioxidants: glutathione
  - signaling molecules: cyclic AMP, inositol trisphosphate (IP_3)
- Polymeric (large) molecules would be impermeable

Gap Junction Features

Connexons

This is the special name for the cylindrical channels that connect the cytosol between two cells

Connexins

Connexins are the single polypeptide functional proteins that form connexons by oligomerizing with 5 other connexins to form a hexagonal connexon hemichannel

Plaques

These are formed by clusterings of connexons A single gap junction can have from a few to thousands of connexons clustered in the junction

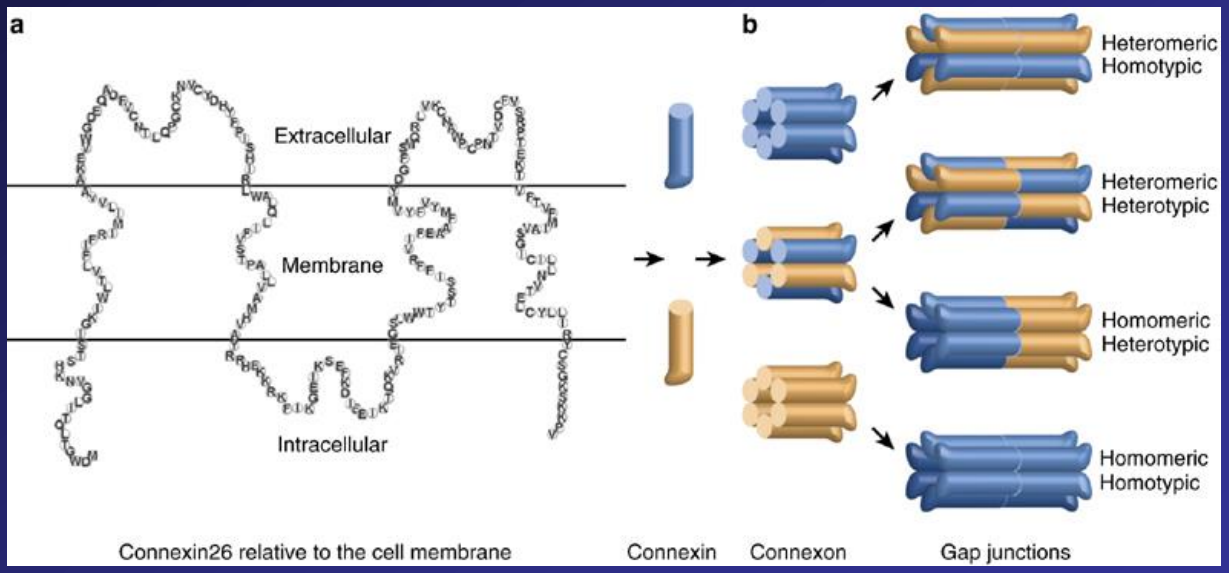
Gap junction equivalent in plants: plasmodesmata

Connexon Structure

- Each cell will make connexin proteins

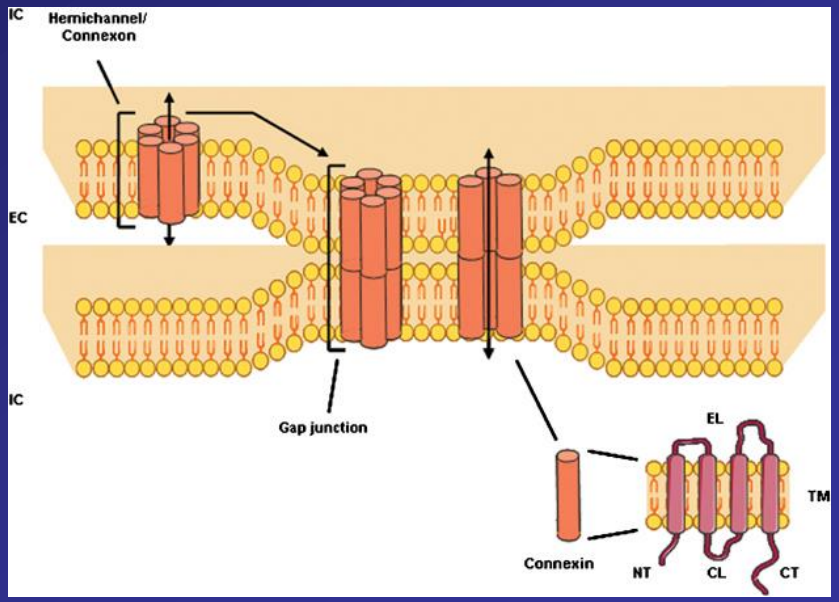
  The connexin protein (a single polypeptide) is made as a transmembrane protein that crosses the membrane four times, and the N-terminal and C-terminal tails will be on the inside of the cell

- Six (6) of those proteins will assemble into a hemichannel with the cells before they have even reached the cell surface



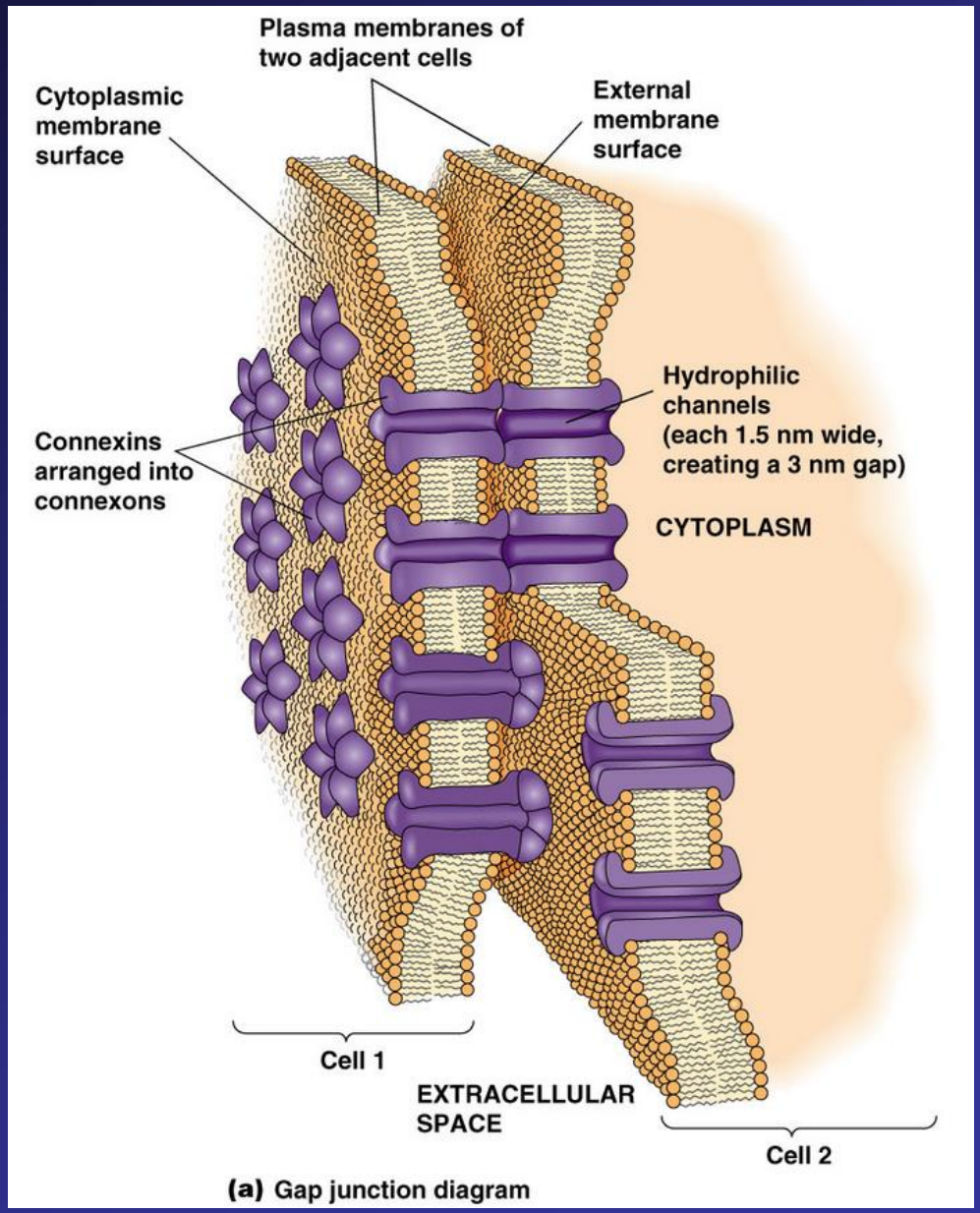
Gap Junction Building

- When hemichannels have reached the cell surface, they look for hemichannels from the other cell in order to make a connection for the full channel, and the fully functioning connexon
- More connexons (complete) will form in a cluster around previously formed connexons, and the gap junction plaque forms
- Gap junctions exist for only a few hours: connexons will disperse and hemichannels removed from the membrane surface

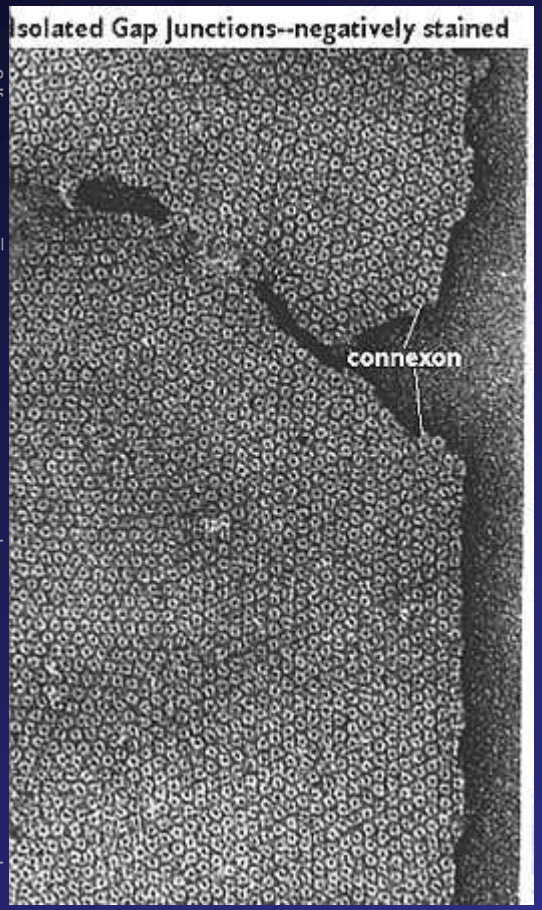


A nice illustration again in Becker's book

Note that 6-connexin subunit hemichannels form in the membrane of one cell, and they try to align with the hemichannels of the other cell, the alignment creating the porous channel



Experimental evidence, namely electron micrographs (leftmost) and filtered EM images (two rightmost) of connexons as seen looking down the axis of the channels. The leftmost image shows how connexons are clustered to form the gap junction plaque.



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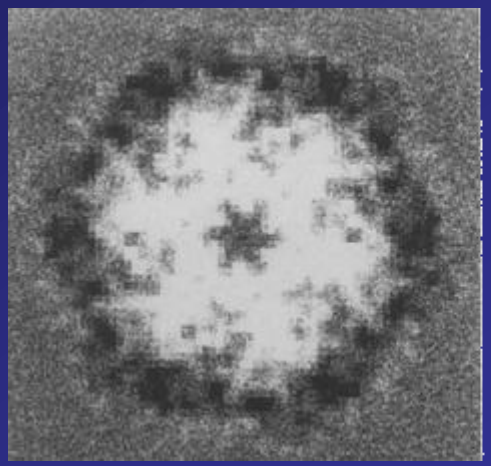
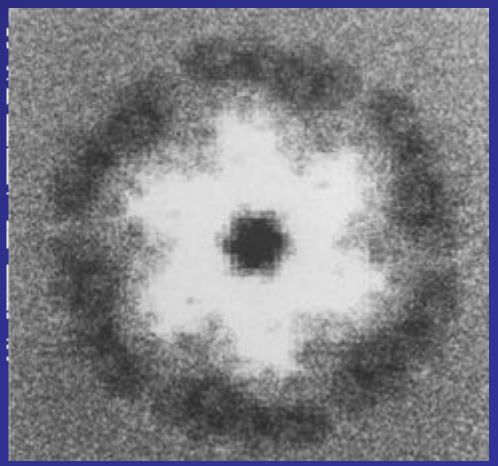
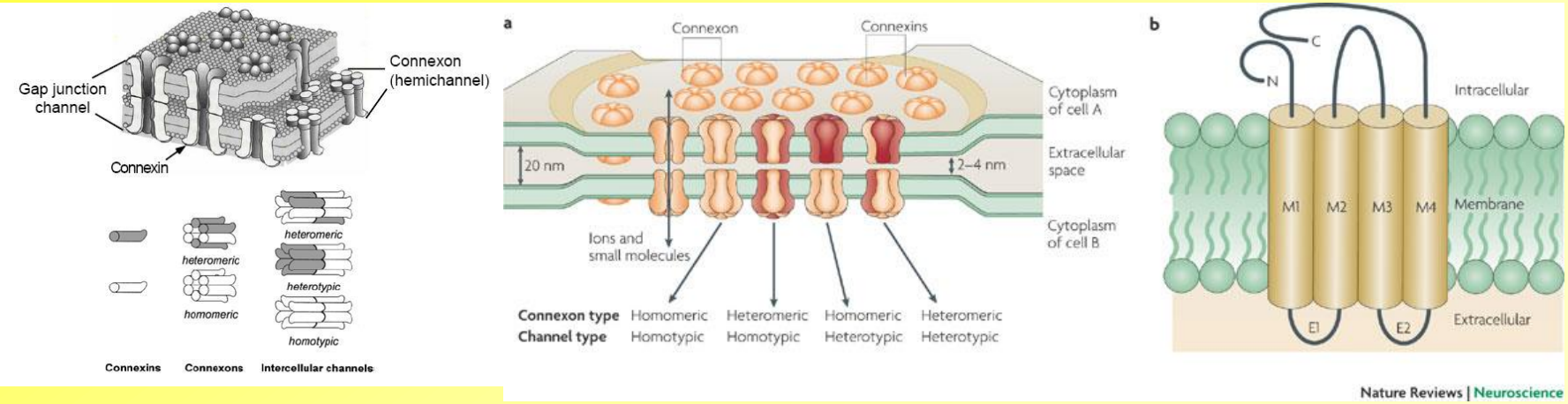




http://php.med.unsw.edu.au/cellbiology/images/6/66/Electron_Micrograph_Filtered_Image_of_Two_Connexons.jpg

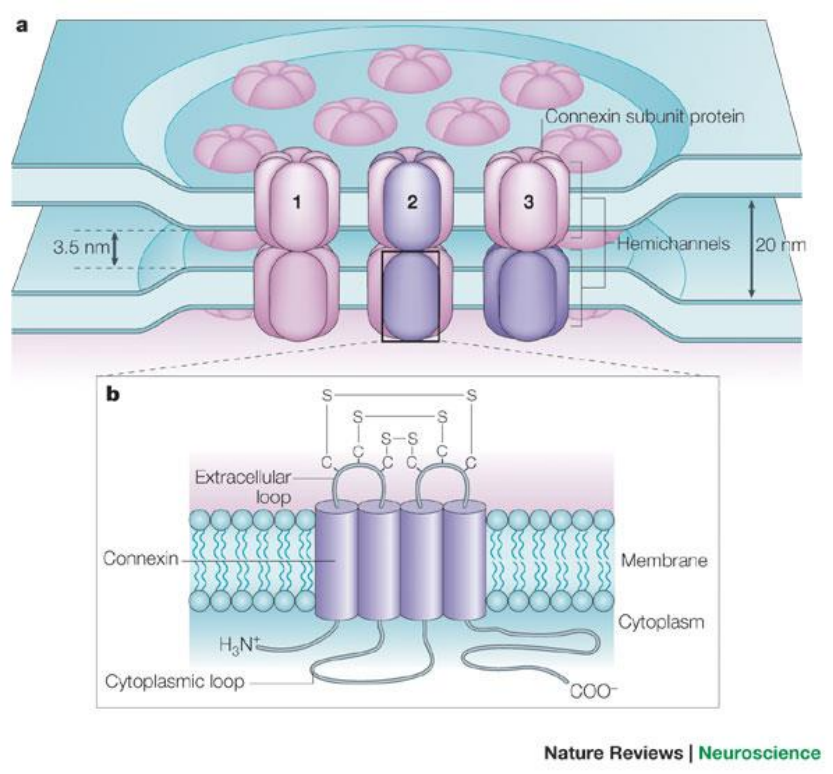
These figures and their legends provide slightly more information that may be informative or enlightening to you. You are not required to know the extra detail given in the figures or the legends though: only the basics already presented



a | Gap junctions are formed between the opposing membranes of neighbouring cells. Hemichannels on each side dock to one another to form conductive channels between the two cells. An extended field of these channels forms a gap junctional plaque. Each hemichannel, or connexon, is comprised of 6 connexin protein subunits that are oriented perpendicular to the cells' membranes to form a central pore. This central pore serves as a conduit for ions and low-molecular-mass molecules of up to 1,000 Da. Connexons can contain only one type of connexin subunit (homomeric connexons) or a mixture of different connexins (heteromeric connexons). Gap junctional channels can consist of two of the same connexon (homotypic channels) or of connexons with different subunit compositions (heterotypic channels). **b** | Connexin subunits are proteins that have four transmembrane domains, two extracellular loops (E1 and E2) and one intracellular loop, as well as carboxyl and amino termini in the cytoplasm. Although the four transmembrane domains (M1–M4) share a conservative sequence that is important for docking in the cellular membrane, their cytoplasmic domains vary in length and amino acid sequence. Regulation of the three-dimensional connexin structure, which underlies the opening and closing of gap junction channels, is mediated at the cytoplasmic regions.

Again, another set of figures and their legends providing hopefully enlightening, certainly more informative detail

Consider the possibility you might be interested in delving into this detail in molecular structure, function or roles in disease and disorders <u>for your oral presentations</u>

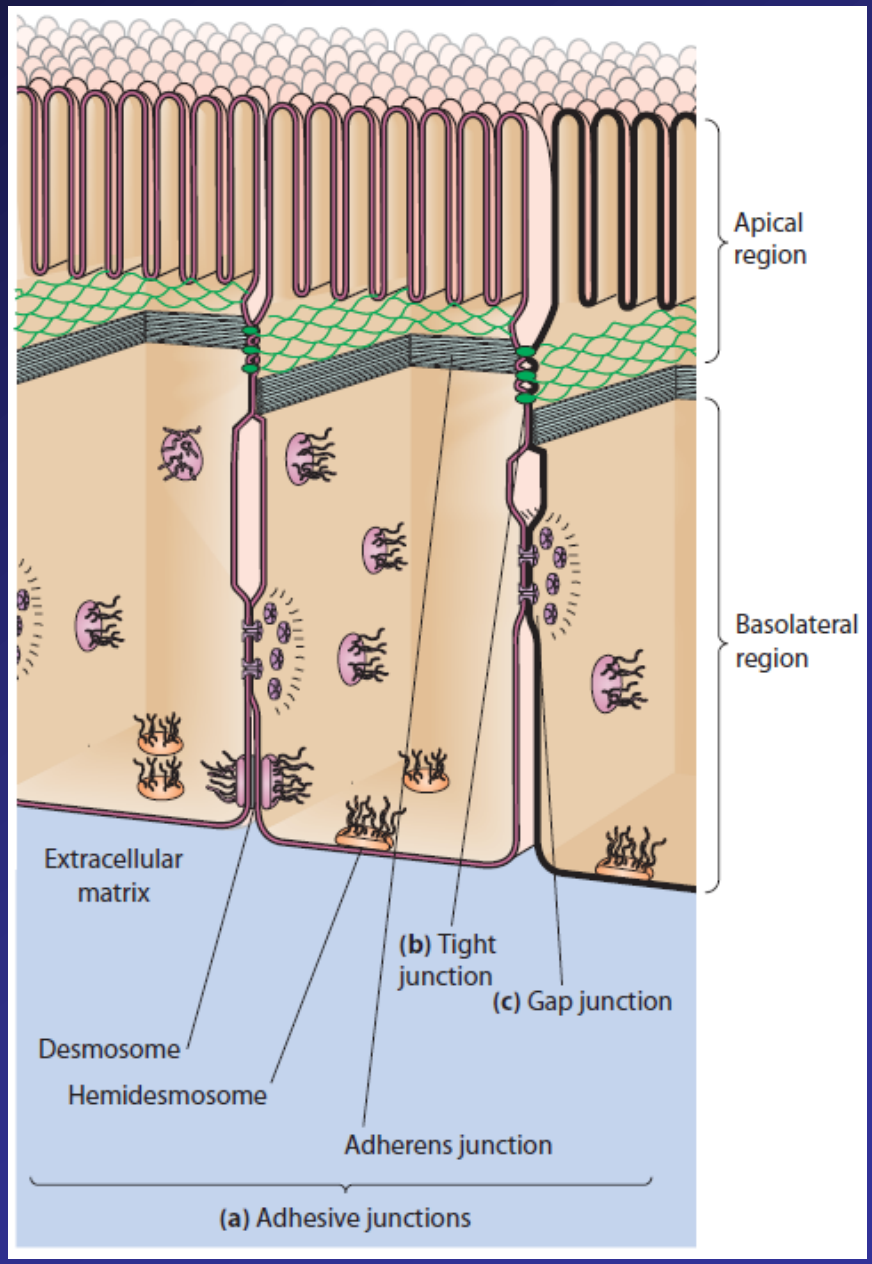


Hemichannels in apposed plasma membranes of neighbouring cells can dock to each other and form gap junction channels. Three different types of gap junction have been reported — homomeric/homotypic (1), heteromeric (2) and heterotypic (3) — depending on their molecular composition. Homotypic or heterotypic gap junctions comprise two identical or two different types of hemichannel, respectively. Homomeric or heteromeric hemichannels are composed of one or more connexin (or possibly pannexin) isoforms, respectively. Each hemichannel represents an assembly of six connexin protein subunits. **b** | Connexin protein subunits are tetra-spanning membrane proteins that share three conserved extracellular cysteine residues, which are crucial for docking. The subunits vary mainly in their cytoplasmic loop and carboxy-terminal region. S–S represents conserved disulphide bonds in the extracellular domains of connexins. Pannexin proteins are sequence-related to the invertebrate innexin family and can also form intercellular gap junction channels.

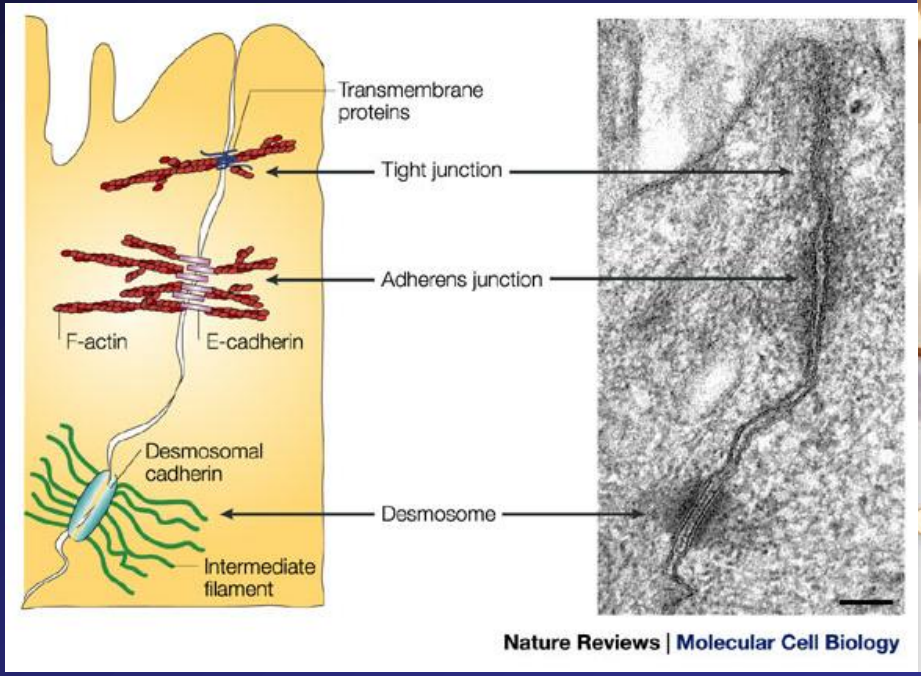
Hemidesmosomes

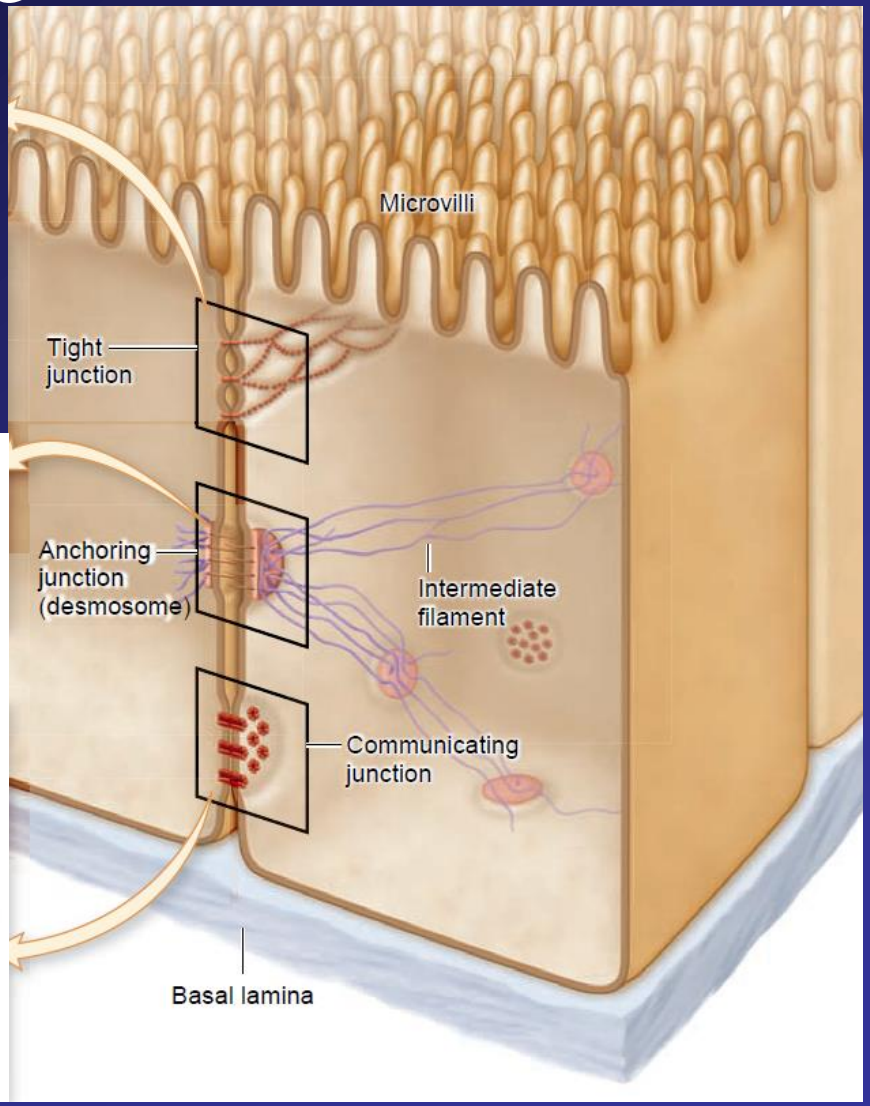
- These help the cell to adhere to an extracellular matrix (basal lamina) instead of to another cell
- Their description is beyond the scope of the course and knowledge of structure and function will not be assessed in the student

- This impressive illustration from Becker's book shows a nice perspective of the cell connections considered adhesive as well as the occlusive (tight junctions) and communicating (gap junction)
- Note the circumferential ring created by adherens junctions (the green-colored net)



These additional illustrations offer other views of the cell junctions presented here





Reading (Sources)

- Becker's WotC: Chapter 17: 481-487
- Raven: Chap 4.8
- Marieb: Chapter 3: p 67