ECM and Adhesion :

Cell-Matrix and Cell-Cell Adhesion

Using this book: This book is designed to be used in both introductory and advanced cell biology courses. The primary text is generally on the left side of the vertical divider, and printed in black. Details that are usually left to an advanced course are printed in blue and found on the right side of the divider. Finally, additional biomedically relevant information can be found in red print on either side of the divider.

Interactions between a cell and its environment or with other cells are governed by cell-surface proteins. This chapter examines a subset of those interactions: direct cell contact with either other cells or extracellular matrix (ECM). Extracellular matrix is a general term for the extremely large proteins and polysaccharides that are secreted by some cells in a multicellular organism, and which acts as connective material to hold cells in a defined space. Cell density can vary greatly between different tissues of an animal, from tightly-packed muscle cells with many direct cell-to-cell contacts to liver tissue, in which some of the cells are only loosely organized, suspended in a web of extracellular matrix.

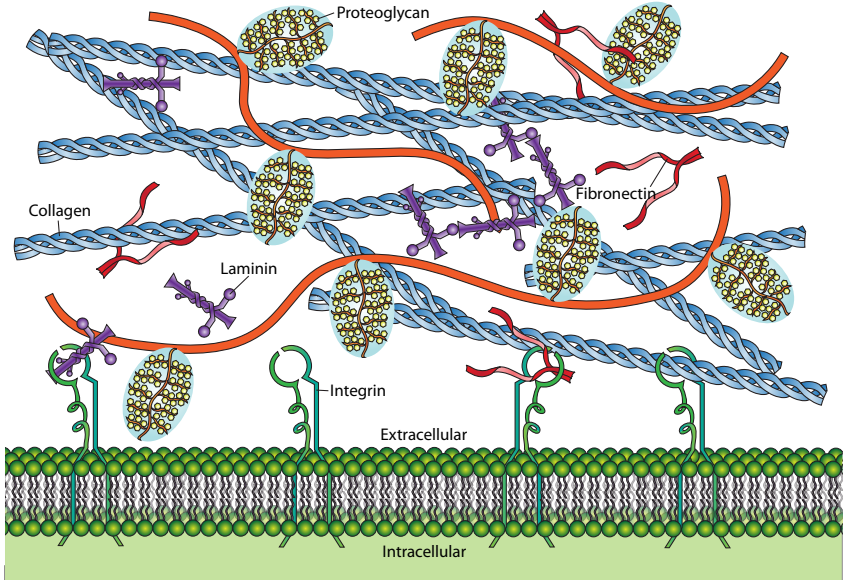


Figure 1. Extracellular matrix (ECM). Typical components include collagen, proteoglycans (with hydration shell depicted around sugars), fibronectin, and laminin. The cellular receptors for a number of these ECM components are integrins, although the exact integrin αβ pair may differ.

ECM is a generic term encompassing mixtures of polysaccharides and proteins, including collagens, fibronectins, laminins, and proteoglycans, all secreted by the cell. The proportions of these components can vary greatly depending on tissue type. Two, quite different, examples of ECM are the basement membrane underlying the epidermis of the skin, a thin, almost two-dimensional layer that helps to organize the skin cells into a nearly-impenetrable barrier to most simple biological insults, and the massive three-dimensional matrix surrounding each chondrocyte in cartilaginous tissue. The ability of the cartilage in your knee to withstand the repeated shock of your footsteps is due to the ECM proteins in which the cells are embedded, not to the cells that are actually rather few in number and sparsely distributed. Although both types of ECM share some components in common, they are clearly distinguishable not just in function or appearance, but in the proportions and identitiy of the constituent molecules.

Collagen

The largest and most prominent of the extracellular matrix proteins, constituting a quarter of the dry mass of the human body, are the members of the collagen family. Collagens are polymers that can be categorized into fibrillar (e.g. collagens I, II, III) and nonfibrillar (e.g. collagen IV) types. The fibrillar collagens are made up of triple helical monomers of either identical (homotrimer) or different (heterotrimer) subunits. These monomers are then associated in an offset parallel interaction with other collagen monomers, leading to the formation of long fibers. Electron microscopic examination of these long fibers shows a banding pattern, which is indicative of the slight gap between monomers along the same parallel.

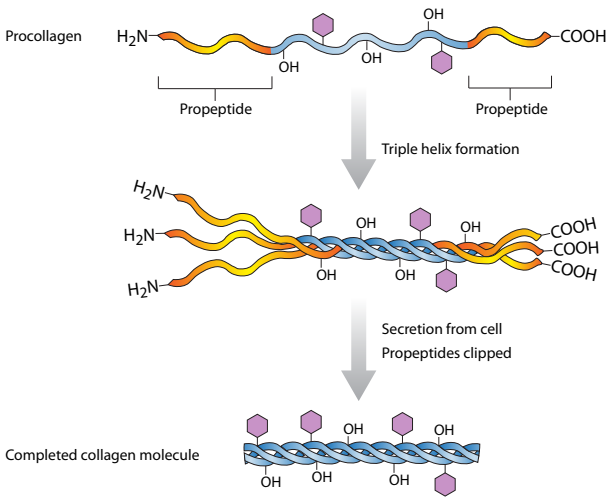


Figure 2. Collagen is a triple-helical protein consisting of three fibrillar subunits. Some of the amino acids are hydroxylated (see fig. 3), and the protein is also glycosylated (represented by purple hexagons).

The "basal lamina" and "basement membrane" are frequently confused by students and professionals alike. The basement membrane was discovered first as a very thin layer of connective proteins just beneath an epithelial cell layer. The basal lamina was not discovered until later because it is not visible by light microscopy (normally only ~50 nm thick). Technically, the basal lamina, which consists of multiple layers itself, is a layer of ECM proteins secreted by the epithelial layer. The basal lamina and a thick reticular lamina (ECM secreted by other cell types) together form what is considered the basement membrane.

The basal lamina around glomerular blood vessels in the kidneys is twice as thick (up to 100 nm) as usual, accomplishing part of the kidneys' physiological role in blood filtration.

Like all secreted proteins, collagen I is processed in the ER (fig. 2), but not completely assembled there: the three pro-α-chains are assembled into a procollagen triple helix, which is secreted. Extracellularly, they must then be cleaved at both termini to form the active collagen protein, which is completely fibrillar. Other collagen types do not have the same cleavage, and may have globular domains at the ends of the fibrils. Collagens are also interesting for their unusual amino acid makeup. They contain a high proportion of hydroxylated amino acids, mostly prolines and lysines (fig. 3). This hydroxylation is necessary for the extensive hydrogen bonding that occurs between subunits and between monomers. The fibrils are associated with high tensile strength. An example of this would be the long collagen fibers that run parallel to the long axis of tendons and ligaments. These high-stress-bearing structures (connecting bone to muscle, and bone to bone, respectively) require the resilience that collagen fibers can provide.

Conversely, conditions that adversely affect collagen formation can lead to serious disease conditions. In fact, a form of epidermolysis bullosa (the heritable skin blistering disease introduced in the previous chapter) is caused by mutation in collagen VII which is primarily produced by epidermal keratinocytes and secreted into the dermal-epidermal basement membrane layer. A variety of chondrodysplasias as well as bone malformations such as osteogenesis imperfecta (which can be perinatally lethal) have been linked to mutations in various collagen genes. Finally, several symptoms of scurvy are due to malformation of collagen in the ECM: weak blood vessel walls, bleeding gums and loose teeth, and fragile bones. Scurvy is a disease of ascorbic acid (vitamin C) deficiency, and the effect on ECM is due to the need for ascorbic acid as a cofactor for enzymes that hydroxylate the prolines and lysines of collagen.

Collagen is a major component of the basement membrane and basal lamina. The basal lamina is strong and flexible, able to serve as structural support for the epithelial sheets attached to it, as well as providing a semi-permeable matrix/filter that allows the passage of water and smaller molecules, but excludes larger macromolecules. The two major protein components to the basal lamina are collagen IV and laminin. Collagen IV has both long fibrillar, alpha-helical domains as well as globular domains that can interact in different orientations to form the meshwork that sets up the basement membrane. The laminin network is connected to the collagen network through entactin (nidogen) linker proteins.

An interesting application of collagen fibrils is in the cornea, the protective clear covering of the eye. The cornea is the primary protection against eye injury, and must be tough. The central layer (stroma, or substantia propria) is composed of approximately 200 layers of tightly packed, regularly spaced parallel collagen fibrils, with adjacent lay-

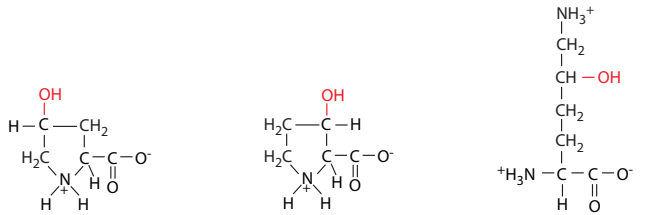


Figure 3. Collagens have a high proportion of hydroxylated prolines and lysines.

ers arranged so that the collagen fibrils lie perpendicularly from one layer to the next. This kind of laminar structure is used in a variety of man-made construction materials (including the ubiquitous building material, plywood) and provides great strength in a relatively small mass. Somewhat amazingly, and quite unlike plywood, the cornea is transparent. That property is thought to come from the regularity of the collagen lattice, which allows for cancellation of scattered light from one fibril by destructive interference from the scattered light of another fibril. Somewhat counterintuitively, it actually gets cloudy (due to refraction) when it absorbs fluid from the aqueous humor, and has active mechanisms to pump any such fluid back out of the cornea. This is why the cornea thickens and becomes translucent after death – the pump mechanism no longer has energy to run, and the aqueous humor diffuses into the cornea.

Proteoglycans

The protein component of proteoglycans are not as large as fibrillar collagens in general, but they often fill a massive volume because of heavy glycosylation. The sugars, many of which are sulfated or carboxylated, are hygroscopic to begin with, but being negatively charged, attract positive ions, which in turn brings in more water. Sugars attached to the core proteins are usually repeating disaccharide units such as chondroitin (D-Glucuronic acid and GalNAc), chondroitin sulfate, heparin (D-Glucuronic acid and GlcNAc by α/β1-4 bond), heparan sulfate, keratan sulfate (Galactose and GlcNAc), or hyaluronan (also called hyaluronic acid, composed of D-Glucuronic acid linked by β1-3 bond to GlcNAc). As with all glycoproteins, assembly of the GAGs occurs in the Golgi,

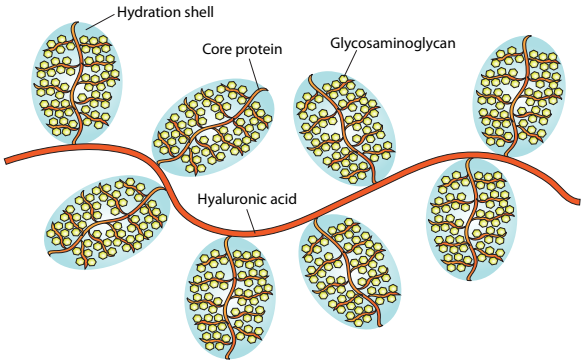


Figure 4. Proteoglycans are composed of multiple glycosaminoglycans attached to a core protein. These core proteins are sometimes attached to a hyaluronic acid molecule. Negatively charged sugars, like chondroitin sulfate or heparan sulfate, are depicted in yellow. They attract positive ions and water, forming a hydration shell around the proteoglycan. This figure depicts aggrecan, a cartilaginous aggregate of proteoglycans assembled on a hyaluronic acid core.

but beyond that, mechanisms for control of the extent and length of the disaccharide polymer addition is unknown. Unlike collagens and most other ECM components, proteoglycans can either be secreted or membrane bound. In fact, of the membrane

The reasoning behind the use of glucosamine and chondroitin sulfate supplements by people with joint problems is that they are two of the sugars found in proteoglycans of cartilaginous tissue such as the meniscus of the knee, and in other joints. Chondroitin sulfate in particular is the major sugar in articular cartilage proteoglycans. Both are thought to stimulate GAG synthesis, and limite documentation of protease inhibitory and collagen synthesis effects have been noted. Data from rabbit models (but potential conflict of interest, Lippiello et al, 2000) suggests a therapeutic benefit from such supplements. However, human studies have so far shown no significant improvement in patients already suffering from moderate to severe arthritis and other joint-related ailments (Clegg et al, 2006). A secondary survey analysis suggested that there was some promise with regard to effects on mild to moderate cases, but the data was not significant.

Heparin, a hypersulfated form of heparan sulfate, is also used medically as an anticlotting drug. It does so not by preventing clots directly, but by activating antithrombin III, which inhibits clotting.

bound proteoglycans, some are actually transmembrane proteins (these are designated syndecans), while other are bound to the cell surface via glycosylphosphatidylinositol (GPI) anchor (glypicans). In addition to these three basic varieties of core proteins, proteoglycans exhibit extraordinary diversity in glycosylation, ranging from the addition of only a few sugars, to well over a hundred. Interestingly, the core protein for chondroitin sulfate proteoglycans in basal lamina of muscle can be a collagen (Type XV)!

One of the paradoxes of proteoglycans is that they can function either as a substrate for cells to attach to, or due to the hydration shell, they can be very effective barriers to other cells as well. This is useful during development when there is a great deal of cell migration, and there needs to be ways to segregate cells both by attracting them and repelling them. Unfortunately, this can have deleterious consequences in some situations. For example, when the brain or spinal cord is injured, a glial scar is formed, and that scar contains a chondroitin sulfate proteoglycan. Unfortunately, this proteoglycan is an inhibitor of neural growth, which contributes to the prevention of neural regeneration, and for the unlucky patient, likely paralysis or worse depending on location and severity of the lesion.

On the other hand, there is also evidence (Rolls et al, *PLoS Med.* 5: e171, 2008) that the CSPG may be needed to activate microglia and macrophages to promote healing.

Fibronectins

Fibronectin and laminin are significantly smaller than either collagens or proteoglycans, and play different roles in the extracellular matrix. Fibronectin is formed by the joining of two similar polypeptide subunits via a pair of disulfide bonds near the C-terminal of each (fig. 5). Each subunit is arranged as a linear sequence of 30 functional domains (varies slightly by species). Within each subunit, each domain acts as a semi-independent unit with respect to secondary and even tertiary structure. Structurally, there are three major types of domains (Fig. 6) that can be distinguished not only by sequence, but by the binding sites they form. The figure above shows binding sites for other fibronectins, fibrin, collagen, heparin, and syndecan. Fibronectin is therefore an excellent linkage protein between these different molecules to stabilize and strengthen the ECM.

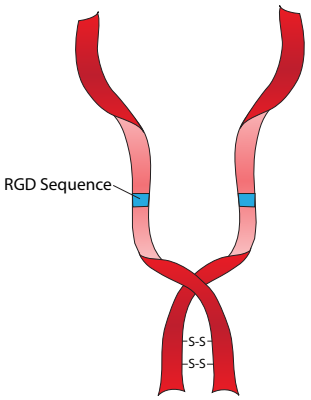


Figure 5. Fibronectin. The C-terminus of each subunit is at the bottom of the figure. The RGD sequence is a binding site for integrin receptors.

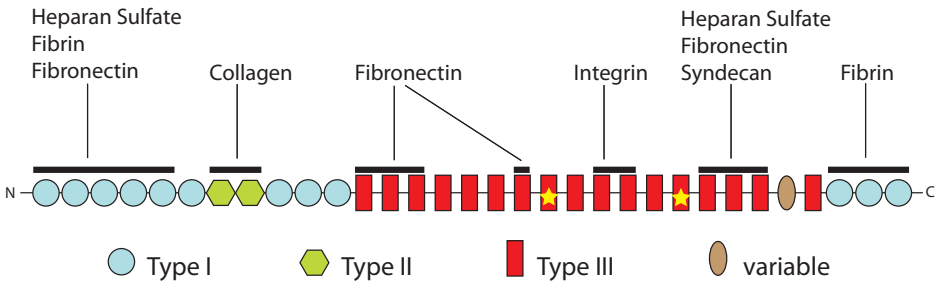


Figure 6. Each fibronectin subunit is composed of about 30 modular domains, of which there are three major structural types. Binding sites for other molecules are labeled. The two Type III domains that are marked with a star are alternatively spliced domains not found in fibronectin that circulates in the bloodstream, where it helps to promote clotting.

Importantly, in addition to linking a variety of extracellular matrix proteins together, fibronectin also has a site that binds to integrin receptors on cells. Whereas collagen for the most part acts as a passive substrate that cells are willing to attach to or crawl on, fibronectin can *actively* induce cell migration by activation of the integrins. Fibronectin expression along very specific pathways are crucial for the migration of neural crest and other cell types in development. *In vitro* experiments with fibroblasts and other cell types show a marked preference for areas coated with fibronectin over areas coated with collagen. This is also true *in vivo*: an upregulation of fibronectin in response to injury promotes migration of fibroblasts and cells associated with wound healing into the lesioned area.

The integrin binding site is charactized by the presence of an arginine-glycine-aspartic acid (RGD) sequence. If this site is abolished or mutated, the mutant fibronectin does not bind to cells. Similarly, if cells are treated with high concentrations of short peptides that contain the RGD sequence, those peptides bind to the integrins, and the cells ignore fibronectin. Finally, in addition to serving as a linker between other ECM proteins, or even to cells, fibronectin can form fibrils through interaction with other fibronectins.

Laminins

Although there are many other less abundant proteins in the extracellular matrix, laminin is the final ECM molecule to be discussed in this chapter. Laminins are a family of secreted glycoproteins that are found in many ECM formations, and like fibronectin, bind to cells via integrin receptors. The laminin protein is composed of three subunits (α, β, γ) arranged in a cruciform shape. There are multiple isoforms of each subunit

One of the interesting aspects of fibronectin fibril formation is that the self-association site is generally hidden, but is revealed when a cell binds to the integrin-binding site. Thus cell-binding seems to nucleate the formation of fibrils, perhaps helping to form strong anchors in certain situations in which the cell is not migrating, but establishing itself permanently.

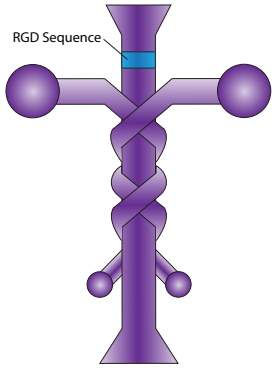


Figure 7. The cruciform structure of laminin is composed of three subunits. The arms bind collagens and sulfolipids, while the foot contains LG domains that bind integrins and carbohydrates.

yielding the variety (15) of laminin proteins catalogued to date. Although laminin contains an RGD sequence like fibronectin, its role in cell adhesion is not universal. Although some cell types have been demonstrated to bind to the RGD site, others clearly bind to other domains of laminin, primarily located on the opposite end of the protein.

Laminin plays a crucial role in neural development, where it acts as a guiding path along which certain axons extend to find their eventual synaptic targets. One prominent example is the retinotectal pathway that leads retinal ganglion cell axons from the eye to the brain. Another example of the role of laminin in development is the guidance of primordial germ cells (PGC). These are cells that eventually become the gametes, but need to migrate from the yolk sac, which is outside the embryo proper, to the site of gonad formation. Laminin is found along this pathway. Interestingly, as the PGCs reached a stretch of laminin very close to the final destination, the adhesion to laminin increased, and this adhesion was found to involve not integrin receptors, but an interaction with a cell surface heparan-sulfate proteoglycan. This and other evidence suggests that migration on laminin may be mediated by integrin receptors, whose adhesivity can be regulated intracellularly, while more static interactions with laminin may be mediated by other types of binding proteins. Finally, as already discussed, laminin is an impotant component of basal lamina, able to form fibrils and networks itself, as well as with collagen IV.

At present, there are 5 α-chain genes, 4 β-chains, and 3 γ-chains known. The α2/β1/γ1 combination is known as laminin-2 or merosin, and is found primarily in the basal lamina of striated muscle. Mutations that affect the function of this laminin cause a form of congenital muscular dystrophy.

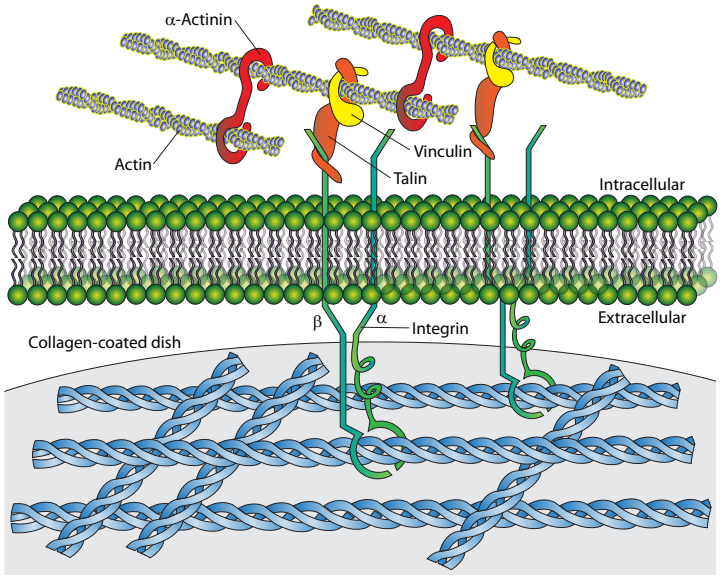
Integrins

The integrins have thus far been introduced as receptors for fibronectin and laminin, but it is a large family with a wide variety of substrates. For example, the focal adhesion (fig. 8) shows an an integrin receptor bound to collagen. As already discussed in the previous chapter, focal adhesions are usually transient, and seen as points of contact as fibroblasts or other migratory cells crawl on a culture dish or slide coated with ECM proteins. In addition to collagen, fibronectins and laminins are also potential binding partners for integrins. As table 1 shows, the diversity of subunits and combinations means that integrins are involved in a wide array of cellular processes, and can bind cell surface proteins as well as ECM. With this variety, it is not surprising that not all integrins bind RGD sequences, although most do. For example, α2β1 integrins prefer

Subunits	Ligand	Distribution
α1β1	mostly collagens, also laminin	widespread
α2β1	mostly collagens, also laminin	widespread
α4β1	fibronectin, VCAM-1	hematopoietic cells
α5β1	fibronectin	fibroblasts
α6β1	laminin	widespread
α6β4	laminin	epithelial cells
αLβ2	ICAM-1, ICAM-2	T-lymphocytes
αIIbβ3	fibronectin, fibrinogen	platelets

Table 1. Integrin receptors, their ligands, and distribution.

Figure 8. A focal adhesion is a dynamic point of contact formed by a cell growing on a collagen-coated dish.



YYGDLR or FYFDLR sequences, and αIIbβ3 binds both the RGD and a KQAGDV sequence strongly. Integrin activation has been shown to initiate signaling pathways, beginning focal adhesion kinase (FAK) or a few other central kinases, which control activities from cytoskeletal rearrangement to cell survival.

Both α and β subunits are transmembrane proteins that pass through the membrane just once. Evolutionarily, they are found only in metazoan species, but they are also found in *all* metazoan species. All integrins but one, α6β4, connect to the actin microfilament cytoskeleton through the β subunit cytoplasmic domain. The α6β4 integrin links to the intermediate filament cytoskeleton, in part because the β4 cytoplasmic domain is very large and extends further into the cytoplasm. On the extracellular side, there is a metal ion coordination site usually occupied by Mg^{++}, that is necessary for ligand binding. There are also several other divalent ion binding sites, The receptor can be found in either an inactive (somewhat bent over towards the membrane) or an active state (straightened up). In the inactive state, the α subunit binds the β subunit closely preventing interaction with the cytoskeleton. However, once a cytoskeletal element such as talin attaches to the β

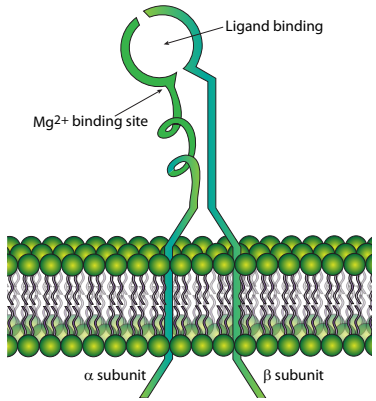


Figure 9. Integrin receptors are composed of two polypeptides that each pass through the membrane once.

subunit cytoplasmic domain, it displaces the α subunit, causing a slight separation of the two subunits and leading to activation of the receptor. In fact, integrins demonstrate what is known as "inside-out" signaling, in which a cellular signal (for example, from the signaling cascade of a growth factor) leads to alterations to the cytoplasmic domain and which shifts the conformation of the extracellular domain to an active straightened-up state in which it can more readily bind to ligands. This is why integrins are so well suited to focal adhesions and other "in motion" adhesions that must adhere and release quickly. Though recycling of receptors also happens, turning them on or off by inside-out signaling is an effective mechanism for fast movement.

As one might expect from an actin-linked structure, focal adhesions and their in vivo equivalents are transient, dynamic points of contact between the cell and the substrate it is crawling over. However, there are many situations in which a cell is not only stationary, it needs to be firmly attached to its substrate in order to gird itself for whatever stressors might come to test its resolve. In these cases, the actin cytoskeleton is too ephemeral for the task.

Hemidesmosomes

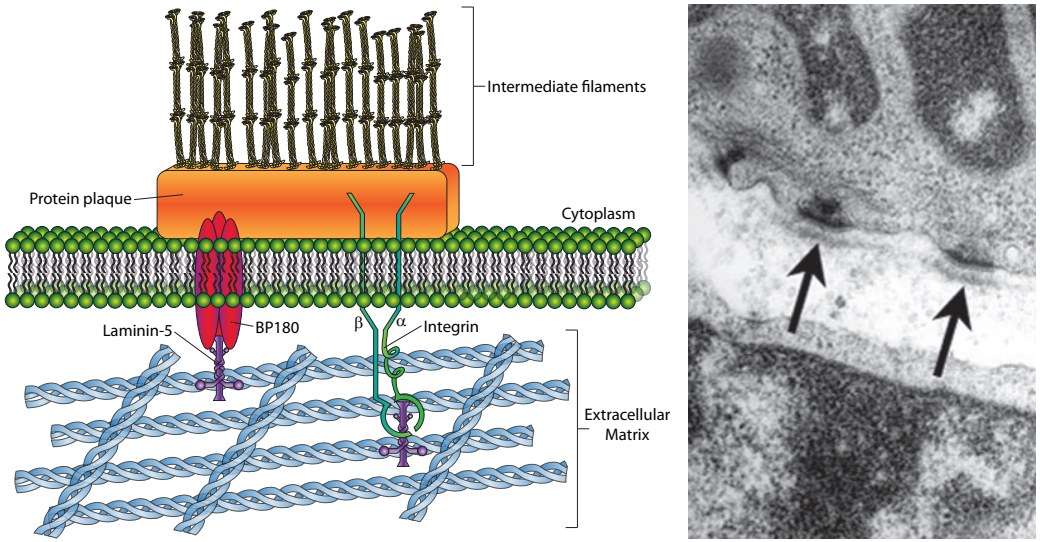


Figure 10. Hemidesmosomes. (Left) Diagram depicting involvement of intermediate filaments and a dense protein plaque reinforcing the membrane at the point of contact. (Right) This shows up as the electron dense areas pointed out by the arrows in this electron micrograph of epithelial cells in mouse trachea. Micrograph released with creative commons attribution license by Nguyen et al, Respiratory Research 7:28 (2006).

Hemidesmosomes, particularly those attaching epithelial cells to their basement membrane, are the tightest adhesive interactions in an animal body. This close contact, and the reinforced structure of these contacts, is crucial for the protective resilience of epithelial layers. Remember the $\alpha 6\beta 4$ integrin? That would be the one that links with intermediate filaments instead of f-actin. Intermediate filaments, as we've already noted, are not dynamic, but about as stable as a cellular component can be. They are also very strong and are used to buttress cellular integrity. So, it is no surprise to see intermediate filaments and the $\alpha 6\beta 4$ integrin playing roles in hemidesmosomes. The distinguishing characteristic of hemidesmosomes though, is the electron-dense plaque. It can be thought of as reinforcement so that when the epithelium is stretched, the cell does not just pop loose leaving behind part of its membrane. The plaque contains several proteins, but the primary component are plectins, the linker proteins that help to bundle intermediate filaments, and connect them to each other as well as other cytoskeletal elements. Another major element of the plaques is BP230, which connects the plaque to keratin. On the extracellular side, in addition to the integrin already mentioned, there is also a transmembrane glycoprotein called BP180, which also binds to laminin elements of the basement membrane.

BP230 and BP180 are named for bullous pemphigoid, the subepidermal bullous disorder characterized by chronic blistering of the skin. It is an autoimmune disorder and in which the aberrant antibody response is to these two hemidesmosomal proteins.

Dystrophin Glycoprotein Complex

Another type of cell-ECM connection is the dystrophin glycoprotein complex (DGC) of skeletal muscle cells. Similar complexes are found in smooth muscle and in some non-muscle tissues. Muscle cells, of course are subject to frequent mechanical stress, and connectivity to the ECM is important in supporting the cell integrity. The DGC uses the large transmembrane glycoprotein, dystroglycan, as its primary binding partner to basal lamina laminin. A sarcoglycan complex and sarcospan are other major transmembrane components of the DGC, but their roles do not appear to include direct interaction with basal lamina. The sarcoglycan complex (consisting of 4 sarcoglycans) is postulated to act as structural reinforcement for the membrane at these contact points. The role of sarcospan, a 4-pass transmembrane protein, has not been demonstrated within the DGC, but homologous proteins in other cells are found in adhesive com-

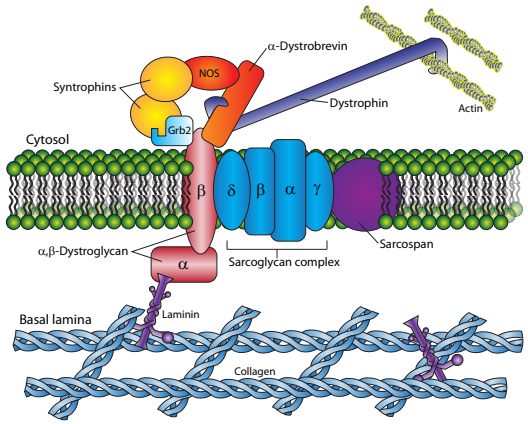


Figure 11. Dystrophin Glycoprotein Complex.

Mutations leading to loss of sarcospan have not been linked to any muscular dystrophies. However, sarcospan is not found in muscular dystrophy patients who have mutations in the sarcoglycans. It thus appears that the tetrameric sarcoglycan complex is required for normal membrane localization of sarcospan.

plexes with integrin receptors, suggesting the same function here. Although the DGCs are long-lived adhesive contact that have more in common with hemidesmosomes than focal contacts, the cytoskeletal component attached to the DGC is actin (via dystrophin), not intermediate filaments. However, it is important to note that the actin cytoskeleton of muscle cells has very different functions from its counterpart in a fibroblast. Mutations to the sarcoglycans, dystroglycan, and dystrophin have all been shown to cause muscular dystrophies.

Desmosomes

Cells will form adhesive interactions with other cells as well as with ECM. Most of these interactions utilize a different set of proteins, although integrins have been found to interact with some cell adhesion proteins. An example of a cell-cell interaction with many similarities to a cell-ECM interaction, but using different adhesion molecules, is the desmosome. Like its basal-lamina-attached couterpart, the hemidesmosome, the desmosome is found in epithelial sheets, and its purpose is to link cells together so that

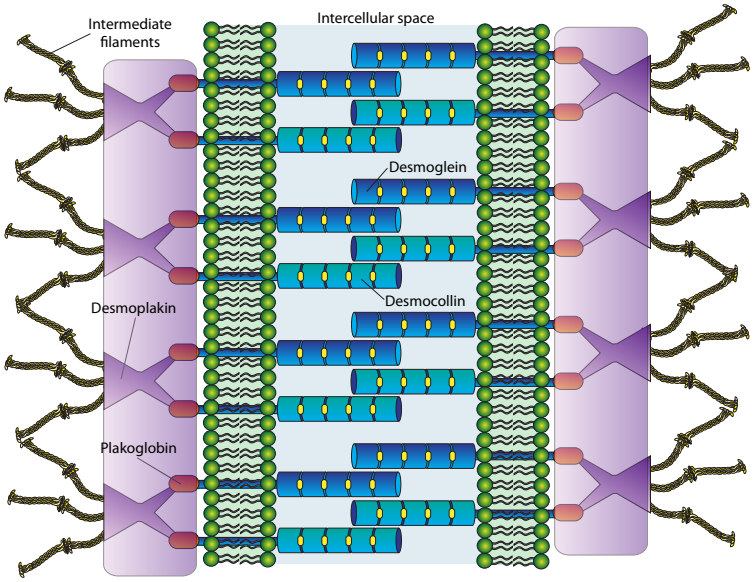


Figure 12. Desmosomes connect the intermediate filaments of adjacent cells across adhesion molecules strengthened by a protein plaque. Desmoglein and desmocollin, the adhesion molecules are members of the cadherin family, and the reinforcing plaque contains plakoglobin, which connects the adhesion molecules to the IF linker, desmoplakin.

pressure is spread across many cells rather than concentrated on one or a few. Desmosomes are necessary for the structural integrity of epithelial layers, and are the most common cell-cell junction in such tissues. The primary structural characteristic of the hemidesmosome, the dense plaque reinforcing the intracellular side of the adhesion, is also found in desmosomes, although it is composed of different proteins. In desmosomes, the plaque is composed primarily of plakoglobins and desmoplakins. The plakoglobins connect the adhesion molecules to the desmoplakins, and the desmoplakins link to intermediate filaments such as keratin.

Another similarity to hemidesmosomes, and one predicted by the involvement of keratin, is the permanence of desmosomes. On the other hand, a key difference between the two types of adhesions is the adhesion proteins involved. The major proteins of the desmosome are desmoglein and desmocollin, both of which are members of the cadherin superfamily of Ca^{++}-dependent adhesion molecules.

Mutations in desmoplakin (on chromosome 6p24) are linked to Carvajal syndrome (also known as dilated cardiomyopathy with woolly hair and keratoderma). Patients are born with woolly hair, and palmoplantar keratoderma appears within the first year. Dilation of the left ventricle and attending weakness in contractility may lead to death from heart failure in teenage years.

Pemphigus vulgaris is another rare disease involving dysfunction of desmosomes. It is an autoimmune disease targeted to the patient's own desmoglein proteins. The reduced epithelial adhesion leads to blistering of skin and mucous membranes.

Cadherins

The cadherin superfamily is comprised of the desmogleins (of which 4 have been identified in humans) and desmocollins (3 in humans), the cadherins (>20), and the protocadherins (~20) as well as other related proteins. They share structural similarity and a dependence on Ca^{++} for adhesive activity, and they can be found in most tissues, and for that matter, most metazoan species. Cadherins are single-transmembrane modular proteins. On the outside of the cell, the cadherin has five domains of similar but not identical structure. It was originally thought that Ca^{++} was used between cadherins to mediate adhesion, but it is now clear that Ca^{++} is bound in between each extracellular domain, apparently coordinating them into a more rigid structure. Cadherins can also act in *cis*, i.e. cadherins from the same cell can form dimers. This property allows a patch of cadherin adhesions such as a desmosome to "zipper" together into very strong clusters.

The cytoplasmic domain of cadherins characteristically binds to a family of proteins called the catenins, and this binding can be regulated by phosphorylation of the cadherin. The most common catenins are α and β, usually with the β-catenin acting as intermediary between cadherin and α-catenin, and the α-catenin linking them to the actin microfilaments. This kind of arrangement is found in both cells that are motile, crawling over other cells that are expressing cadherin, as well as stationary cells. Although this is not the arrangement in desmosomes, the desmosomal plaque protein plakoglobin is a member of the catenin family.

The primary binding site for cadherins appears to be the N-terminal domain (most distal extracellular), although there is evidence that as many as three domains can be involved. Cadherins mostly bind homophilically (E-cadherin binds E-cadherin on another cell, but not P-cadherin), although some cadherins can bind heterophilically (e.g. N-cadherin can bind to either N-cadherin or E-cadherin). Incidentally, these three, E-cadherin (epithelial), N-cadherin (neural), and P-cadherin (placental) are the best-studied cadherins. Both E- and P-cadherins are important in early embryonic development, while N-cadherin has been studied in the context of axon guidance in the developing nervous system. E-cadherin is also a target of scrutiny because it is also important in the metastasis of cancer. In order for a cancer cell to break from the initial tumor, it must downregulate its adhesion to neighboring cells before migrating elsewhere. This is known as the epithelial-mesenchymal transition and is accompanied by decreased E-cadherin expression.

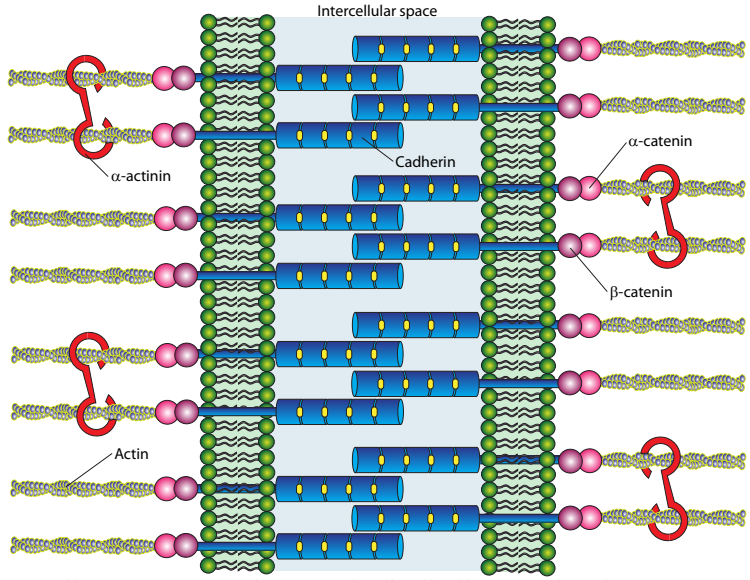


Figure 13. Adherens Junction. This type of cell-cell adhesion is based on interaction of cadherins, which are connected intracellularly to the actin cytoskeleton through the linker proteins α- and β- catenin. Also depicted here is the actin bundling protein α-actinin.

With the patch of cadherin interactions, the adherens junction (fig. 13) looks very similar to the desmosome (fig. 12). Adherens junctions serve some of the same purposes as desmosomes: providing connectivity to neighboring cells, and reinforcing and shaping the cells. However, adherens junctions are mostly localized near the apical surface of epithelial cells, and instead of intermediate filaments, they are connected to actin microfilaments that form a circumferential belt that produce tension and shaping forces in conjunction with myosins that associate with it.

Tight Junctions

Sometimes, holding cells together, even with great strength, is not enough. In epithelia especially, a layer of cells may need to not only hold together but form a complete seal to separate whatever is in contact with the apical side from whatever is in contact with the basal side. That would be a job for The Tight Junction! Well, more accurately, for many tight junctions in an array near the apical surface. Perhaps the best example of the utility of tight junctions is in the digestive tract. The tight junctions that form between cells of the epithelial lining of the gut separate the food and its digestion products from the body at large, forcing macromolecule nutrients to be transported through the epithelial cell by endocytosis/transcytosis to the bloodstream where they can be most efficiently distributed. The tight junctions also form in blood vessels to prevent leakage of blood, and in a variety of organs where liquids must be contained.

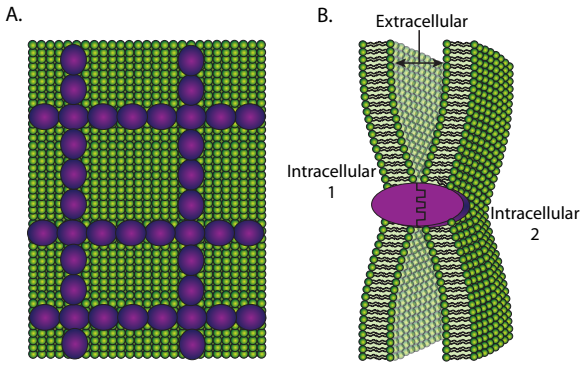


Figure 14. Tight Junctions. (A) Tight junctions are usually present in arrays that seal off one side of an epithelial layer from the other multiple times. (B) Each tight junction is formed by very small transmembrane proteins, claudins and occludins, so that the membranes of opposing cells can come into extremely close contact .

An individual tight junction is formed by the interaction of claudins and occludins. They are each 4-pass transmembrane proteins with both N- and C-termini on the cytoplasmic side; the extracellular side has a very low profile, consisting of one (claudin) or two (occludin) small loops. Because of their small size, when they interact, the membranes are brought together very closely. In order to actually form a seal between cells though, tight junctions must be lined up in close order all the way around the cell, and in fact, usually there are multiple lines, which one could think of as "backup" in case one line develops a leak. Claudin molecules have relatively small cytoplasmic domains and it is not clear whether there are significant interactions with other proteins. However, occludin has a large C-terminal cytoplasmic domain that contains a PDZ-binding domain. PDZ is a protein interaction motif of approximately 80-90 amino acids found in a number of signaling proteins, most often in use to hold signaling complexes near the membrane by interacting with a transmembrane protein, as would be the case here with occludin. These PDZ-containing proteins both have signaling functions and can

act as adapters to the cytoskeleton, primarily the actin filaments. Finally, although an exact mechanism is unclear, elevated levels of Ca^{++}, either extracellularly or perimembranously, is associated with tight junction assembly.

Ig Superfamily CAMs

In addition to occludin and claudins, junction adhesion molecules (JAMs) have recently been found in tight junctions. These molecules are members of a gigantic superfamily of cell adhesion molecules known as the Ig (immunoglobulin domain) superfamily because all of these proteins contain an immunoglobulin loop domain that plays an important part in the adhesion mechanism. The purpose of immunoglobulins (antibodies) is to recognize and adhere to other molecules, so it makes sense that such a structural motif would also be used for other kinds of adhesion. Ig loops fall into two primary categories, again based on the loops of an immunoglobulin molecule. These are the variable (V) loop and the constant (C1) loop. Some IgSF molecules contain a C2 loop, which has amino acid homologhy to the V loop, but structural/size similarity to the C1 loop. The size of IgSF molecules ranges greatly, and the number of Ig loop domains and other domains (e.g. fibronectin Type III domains) can also vary significantly.

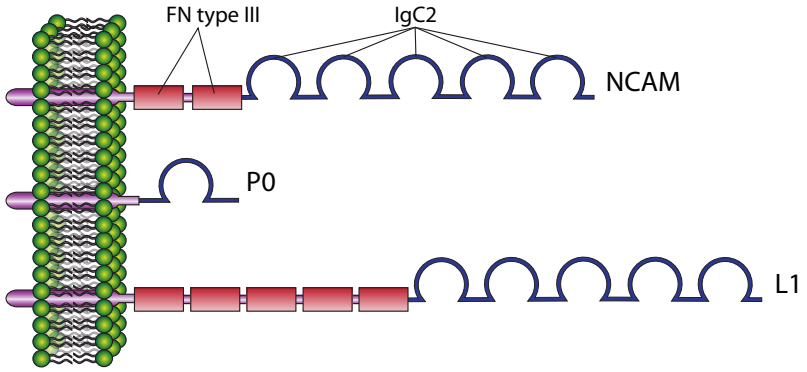


Figure 15. Ig superfamily cell adhesion molecules NCAM, P0, and L1 (top to bottom) are built with fibronectin Type II domains and Ig C2-like domains..

IgSF molecules are involved in a number of cellular processes requiring adhesion. The most obvious, of course, is the immune response in which an immunoglobulin, either secreted or on a cell, binds to a molecule foreign to the body. However, there are many other interactions outside of the immune system that involve IgSF molecules. One well-studied area is in neural development, where IgSF members L1 (L1CAM), NCAM and numerous others are expressed in specific patterns to control the routing of axons as

they make their way from the neuronal cell body to their eventual connections. This path can often be very long, crossing many different cell types, and taking several turns, so a robust guidance system is crucial to make a normal functioning nervous system. Specific combinations of cell adhesion molecules (also called axon guidance molecules in this case) direct these processes even through the extraordinary density of potential (but incorrect) nerve cell targets in the brain. IgSF molecules have been found to bind both homophilically and heterophilically, and for that matter, not just to other IgSF molecules, but to adhesion molecules of other structural families such as integrins.

Selectins

The last major cell adhesion molecule family to discuss is the selectins. Selectins bind heterophilically to oligosaccharide moieties on glycoproteins. In fact the name of the family is based on lectin, a generic term for proteins that bind sugars. The selectins, like cadherins and IgSF molecules are modular glycoproteins that pass through the membrane once. From C-terminus to N-terminus, the selectins are composed of a relatively short cytoplasmic domain, a single transmembrane domain, a series of CR (consensus repeat) or structural domains (from 2 in L-selectin to 9 in P-selectin), an EGF (epidermal growth factor) -like domain, and a lectin-like domain. The lectin-like domain binds a fairly specific oligosaccharide composed of sialic acid, galactose, GlcNAc, and fucose, of which the sialic acid and fucose are most important for recognition. Selectin-mediated adhesion is a Ca^{++} dependent process.

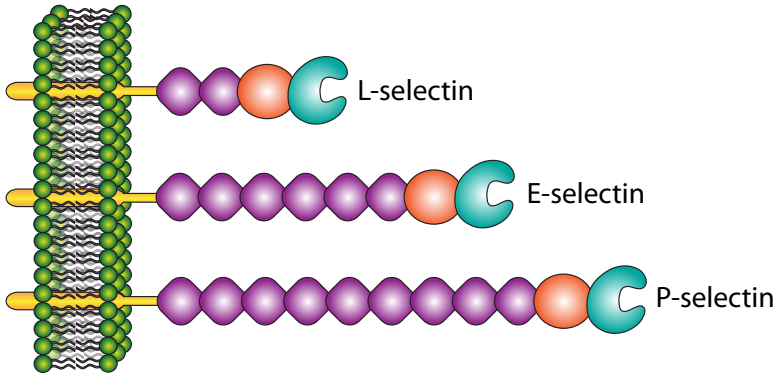


Figure 16. Selectins (L-, E-, and P-) are composed of between 2 and 9 short consensus repeat domains (purple), an EGF-like domain (orange), and the lectin-like binding domain (teal).

L-selectin, which is found on leukocytes, was the first discovered, by virtue of an interesting phenomenon called lymphocyte homing, in which lymphocytes were removed from various peripheral lymph nodes, labeled, and then injected back into the animal.

These lymphocytes would migrate back to the tissues from which they were derived without regard for the site of re-injection. The other two known selectins are E-selectin, which is expressed primarily on endothelial cells, and P-selectin, which is expressed on platelets and endothelial cells. The best-characterized ligand for selectins is PSGL-1, creatively named P-selectin glycoprotein ligand -1. Both E- and P-selectin are an important part of the inflammatory response, mediating the invasion of neutrophils from the bloodstream into the area of injury (fig. 17).

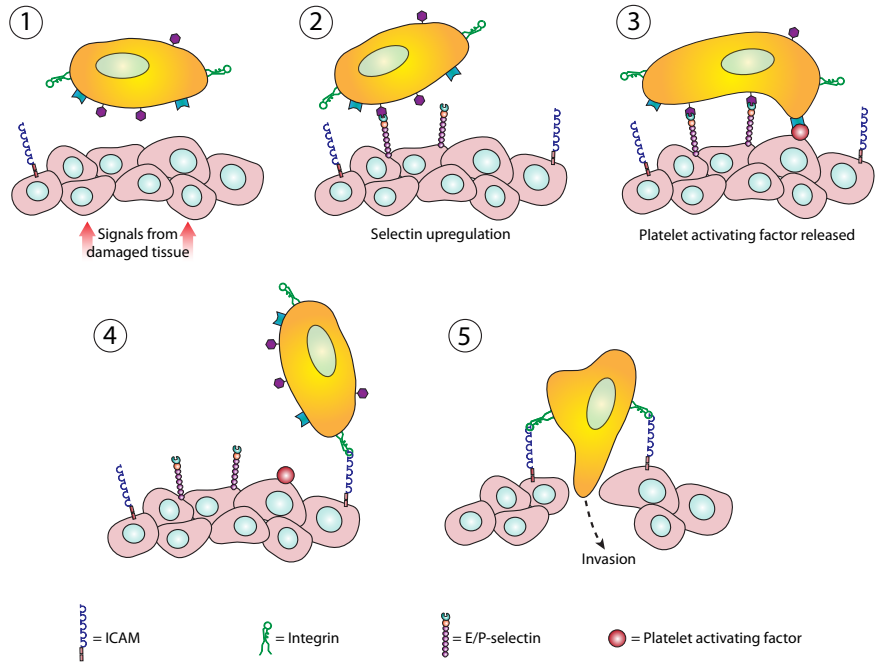


Figure 17. Neutrophil activation and invasion in the inflammatory response. First, endothelial cells in the blood vessel walls are activated by nearby damaged cells. These endothelial cells then begin to express E- and P- selectins, which bind onto neutrophils that are tumbling by in the bloodstream. This interaction slows the neutrophils down so that they are just rolling more slowly in partial contact with the endothelial cells. The endothelials release platelet activating factor, which activates $\alpha 1\beta 2$ and $\alpha M b2$ integrins on neutrophils. Those integrins can bind onto the ICAM (and IgSF molecule) on the endothelial cell surface, stopping their movement. Finally the neutrophil binds to ICAMs on two adjacent cells, then remodels its cytoskeleton as it squeezes between the two to exit the bloodstream and move to the lesion site.

Gap Junctions

Unlike the other types of cell-cell adhesion, the gap junction (sometimes called a nexus) connects not only the outside of two cells, it connects their cytoplasm as well. Each cell has a connexon (aka hemichannel) made of six connexin proteins. The connexins may be all of the same type, or combinations of different ones, of which there are 20 known in humans and mice. The connexon interacts with a connexon on an adjacent cell to connect the cytoplasm of both cells in a gap junction.

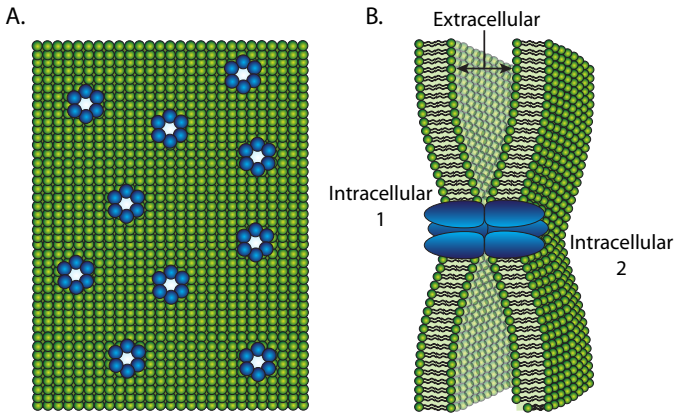


Figure 18. Gap Junctions.

The gap junction pore size varies depending on the type of connexins, but generally, the molecules under 1 kDa are able to pass through while larger ones can not. Therefore, cells connected by gap junctions are electrically connected (ions can freely pass), they can share cellular energy (ATP), and second messenger signaling molecules like Ca^{++} or IP_3, but not most proteins or nucleic acids. The pores are not always open, but are controlled by phosphorylation of several serines in the intracellular domains of each connexin.

Although they have now been found in most metazoan tissue types, they are particularly important in heart muscle. Here, the gap junctions insure efficient propagation of contractile signals so that the cardiac muscle can contract in synchrony. It is also important in cardiac development: gene knockout of connexin43, the primary heart connexin, leads to delayed looping of the ascending limb of the embryonic heart tube, which means malformations especially in the right ventricle, tricuspid valve, and subpulmonary outflow tract.

When most people, including most biologists, think about neuronal connections and synapses, they think of chemical synapses in which one cell signals to another by release of neurotransmitters. However, it is now well established that in the CNS, electrical synapses through gap junctions are a significant part of the repertoire of neural communication. The retina is an excellent example with numerous gap junctions between neurons. In fact, light-activated neurotransmitters can activate protein kinase pathways that phosphorylate connexins, thus altering conductance through the gap junctions. A striking example is the gap junction-based electrical coupling of cone photoreceptor neurons. They are coupled near the base of the cells, so that excitation of one drives excitation of several others. This is important in generating a clear visual signal because the primary reaction, phototransduction, is a dirty process. Due to the simple presence of random photons bouncing about, the signal to noise ratio of light-induced excitation is very low. However, because electrical coupling sums up the signal of near neighbors but not the background noise, the signal output from these neurons has an improvement in signal to noise ratio of ~77%! This topic is reviewed in Bloomfield and Volgyi, *Nature Reviews (Neuroscience)*, **10**:495-506, 2009.

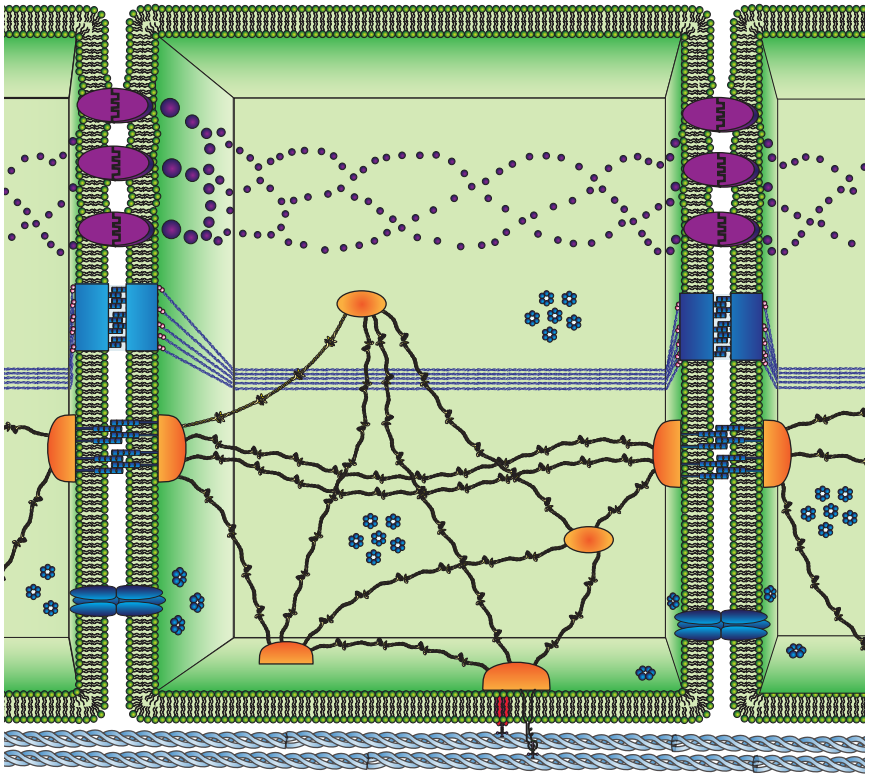


Figure 19. An overview of cellular adhesions. From top to bottom, there are tight junctions (purple), adherens junctions with f-actin (blue), desmosomes (orange) connected to intermediate filaments, and gap junctions (blue). There are also hemidesmosomes (orange) on the basal surface attached to the basement membrane.