Cells to Tissues

Scientific Foundations

Learning objectives

1. Students should be able to list the molecules that mediate attachments between cells and their role in maintaining tissue integrity.
2. Students should be able to list the components of the extracellular matrix and describe their role each plays in tissue integrity.
3. Students should be able to develop reasonable arguments for the relative importance of cadherins and integrins in tissue repair and the progression of cancer.

From single cells to multicellularity

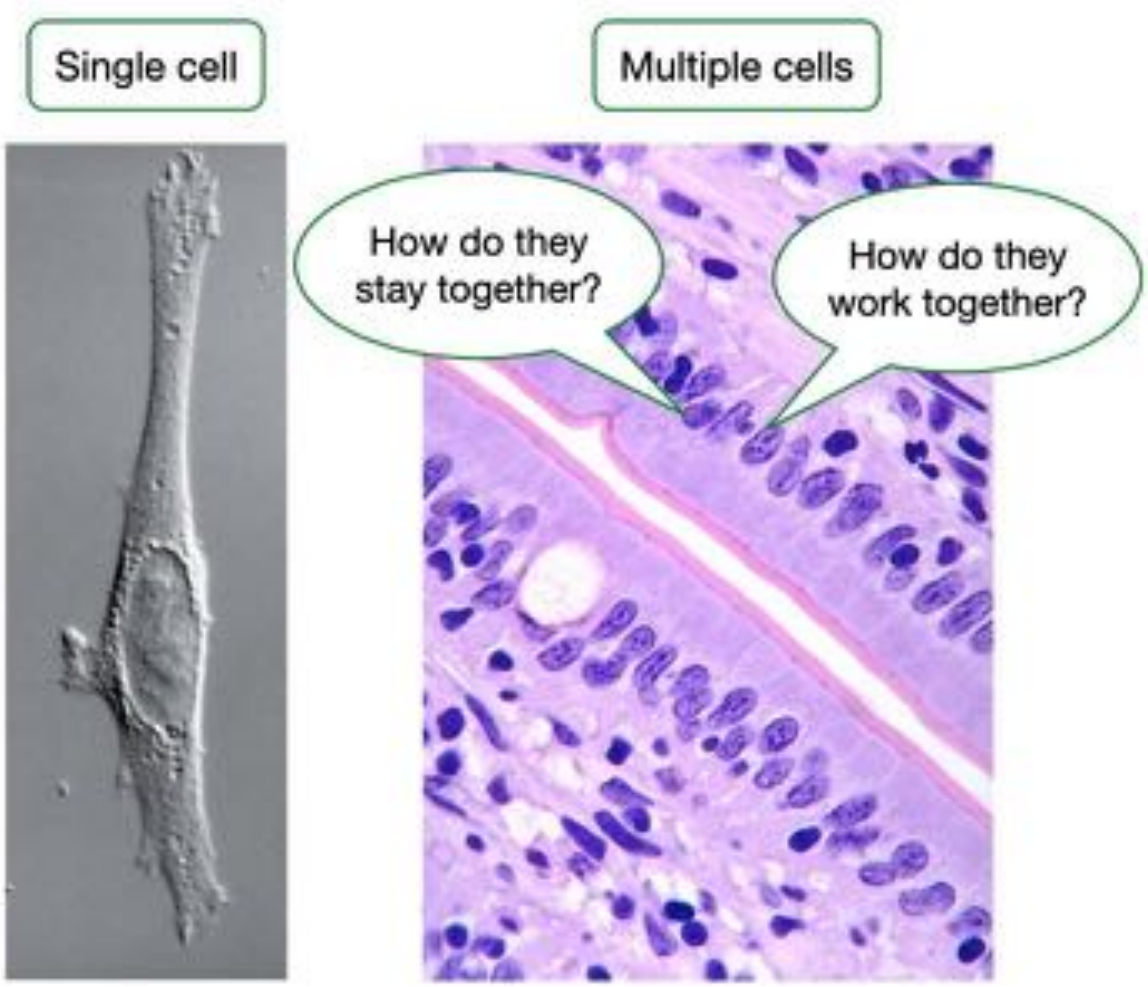
The vast majority of life forms in this world exist as single cells (e.g. bacteria and yeast). We, however, are multicellular organisms in which cells work together to perform specific tasks that contribute to our health and survival. The highest ordered structures in the body are the organs, each of which has a unique architecture and performs a defined set of functions. Organs are composed of distinct structures called tissues that are composed of cells and extracellular matrix. There are four recognized tissue types:

- Epithelia
- Muscle
- Nervous Tissue
- Connective Tissue

Each of these tissues has a unique structure and a distinct set of activities. Central to the structure and function of each tissue are the cells that compose it. Tissues are placed into one of these four categories based on a set of shared characteristics. We will describe in detail these characteristics when we discuss each tissue type. Although each tissue type has a set of unique features that define it, these features can vary slightly depending on where the tissue is located in the body. For

example, the intestine and kidney both have epithelia, but the architecture and biochemical activities of the epithelium differ in the two organs to meet the needs of the intestine or kidney.

Working in a group rather than as an individual allows cells to perform more advanced functions but places limits on the behavior of the cells. In particular, cells in tissues must relinquish some of their autonomy to maintain the integrity and overall function of the tissue. Cells in tissues must also generate four essential properties for the integrity of tissues: adhesion, renewal, identify and communication.



From one cell to ensembles of cells.

Cells in a tissue must adhere to each other while preventing cells that don't belong to the tissue from sticking. Cell adhesion maintains the mechanical integrity of tissues and allows cells to communicate with each other. Adhesion in tissues is mediated by interactions between individual cells and between cells and an external complex of proteins and sugars called the extracellular matrix (ECM).

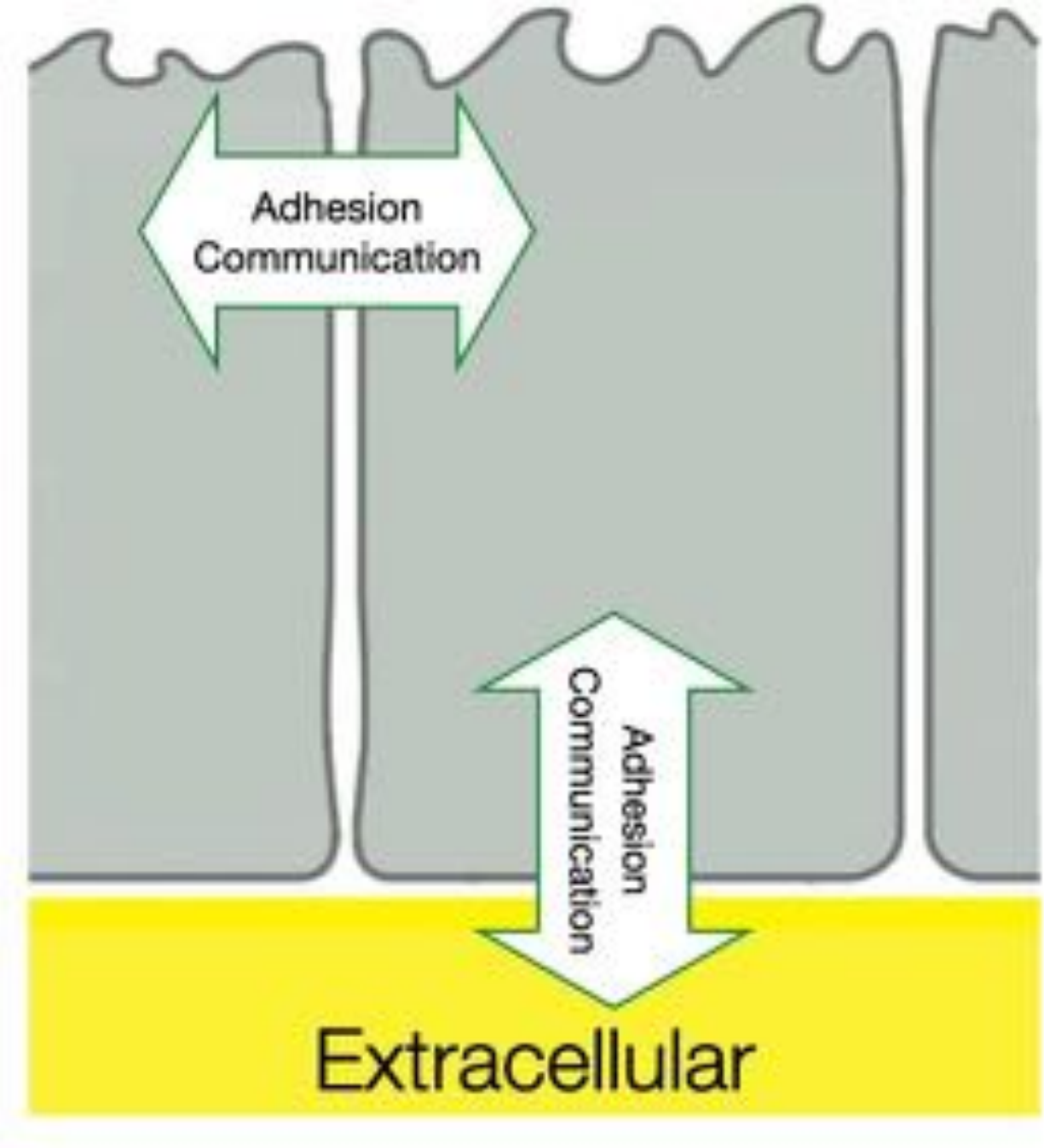
A second essential property of tissues is the regulation of cell proliferation. In many cells that live as individual cells (e.g. yeast), cell proliferation depends primarily upon the existence of nutrients. If enough essential nutrients exist in the environment, an individual cell will continue to divide until the supply of nutrients is exhausted. Tissues on the other hand need to create and maintain a precise architecture and size and therefore, must control when and where its cells divide. In addition, cells have a limited lifespan and depending on the type of tissue and its location, the cells within the tissue die (undergo apoptosis) at different rates. To maintain their integrity, tissues must be able to generate new cells at the same rate as existing cells are lost. Importantly, if the rate of cell division outpaces the rate of cell loss, a tumor could develop in the tissue.

When cells join a tissue, they must assume the identity of the other cells in that tissue. This means the activities and behavior of the cell must match and integrate with its neighboring cells. Most cells in a tissue have a common gene expression profile that produces the proteins and structures which perform the specific biochemical reactions to generate the functions of the cells.

Lastly, communication is critical for tissues. Communication provides cells in a tissue with information about when to divide, which genes to express and how tightly to associate with adjacent cells and the ECM. Information is passed between cells, from the ECM and from small molecules that are produce locally or in another part of the body.

Multicellularity requires several key interactions

In order to function in a tissue cells must remain attached to each other. Several proteins, described below, connect cells in a tissue. These cell-to-cell attachments, however, are not sufficient to maintain the mechanical integrity of a tissue. Cells also form connections to protein components of the extracellular matrix. The proteins and sugars in the extracellular matrix are adapted to resist tension and compression to provide mechanical integrity to tissues.

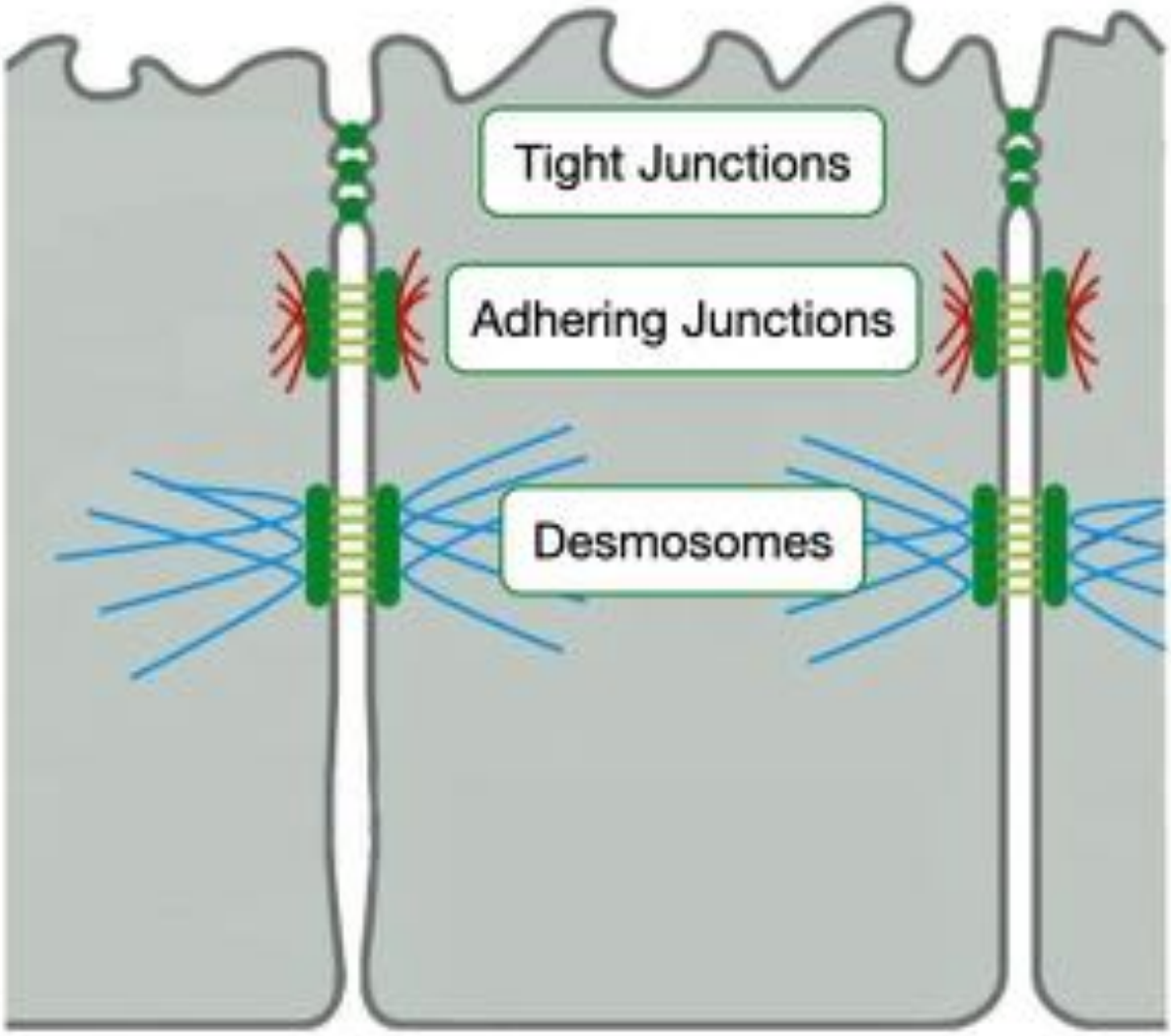


Adhesion and communication are critical for the integrity and function of tissues.

The attachments between cells and between cells and the ECM not only important for adhesion but also communication. The attachments regulate many activities in cells and are critical for maintaining cell identify and tissue integrity.

Cell to cell adhesion

Cell to cell interactions are largely mediated by three protein complexes in the cell membrane: tight junctions, adhering junctions and desmosomes. This session will discuss adhering junctions and desmosomes. Tight junctions will be presented in the lecture on epithelia.

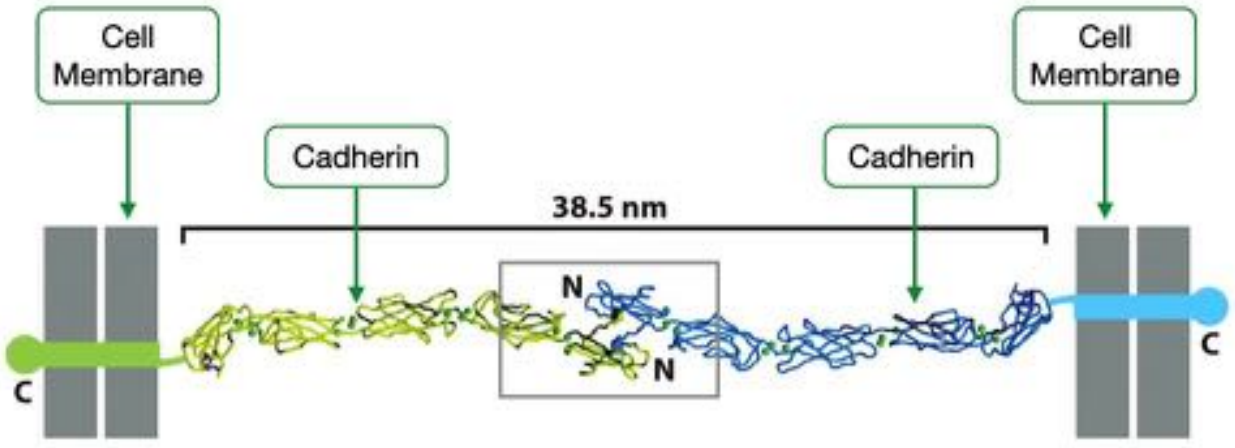


Three complexes generate intercellular adhesion

The architecture of adhering junctions and desmosomes is similar. Both use members of the same family of proteins, called cadherins, to form interactions between adjacent cells, and both associate with the cytoskeleton within cells. Adhering junctions interact with actin filaments; desmosomes interact with intermediate filaments.

Cadherins

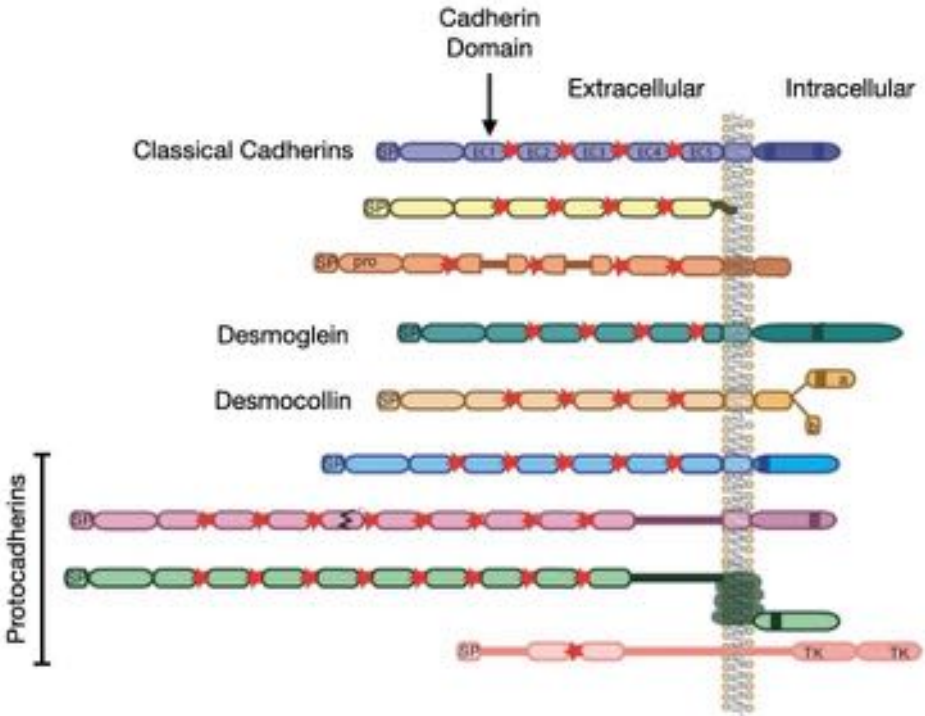
Cadherins are integral membrane proteins that mediate adhesion between cells in adhering junctions and desmosomes. Most cadherins are single transmembrane proteins that contain multiple copies of an extracellular domains called the cadherin domain. Interaction between cadherins in adjacent cells usually occurs through most N-terminal cadherin domain. The mechanisms by which cadherins interact is critical for maintaining cell adhesion in tissues and the integrity of tissues.



Cadherins in adjacent cells interact via their N-terminal domains.

Cadherin structure and interactions

Cadherins are a large family of proteins consisting of over 100 different types. Cadherins are divided into four classes: classical, desmosomal, protocadherins and unconventional. Most of the cadherins you will encounter will fall into the classical or desmosomal classes. The classical class contains different types of cadherins including, E-cadherin, N-cadherin and VE-cadherin. The desmosomal class contains, desmoglein and desmocollin. All members of the cadherin family have cadherin domains and most pass membrane once, but some span the membrane multiple times.



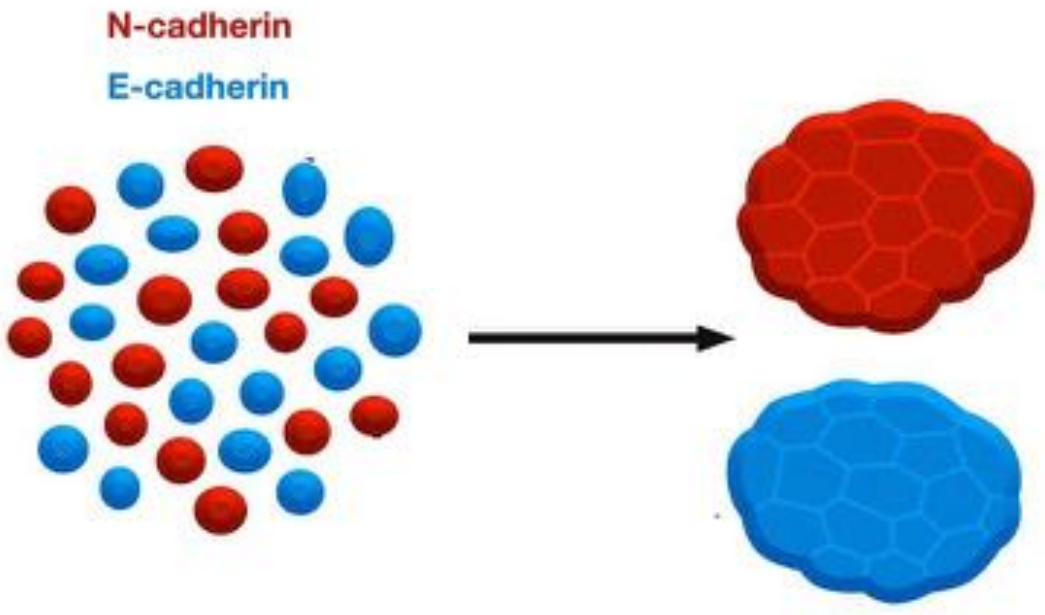
Cadherins comprise a large family of proteins.

One important feature of classical cadherins is that they most often interact homotypically, that is one type of cadherin preferentially associates with the same type of cadherin rather than a different type of cadherin. For example, E-cadherins interact with other E-cadherins but not N-cadherins.

The homotypic interaction between members of classical cadherins helps tissues maintain their cellular identity. Cells in epithelia tissue express E-cadherin which allows them to associate with each other but not cells with a different type of cadherin. Likewise, cells in nervous tissue express N-cadherin but not E-cadherin, allowing cells in nervous tissue to associate while not allowing cells expressing E-cadherin to integrate into the tissue. Thus, any cell expressing the wrong type of cadherin would not be able to interact with other cells in the tissue.

Homotypic cadherin interaction and segregation of cells

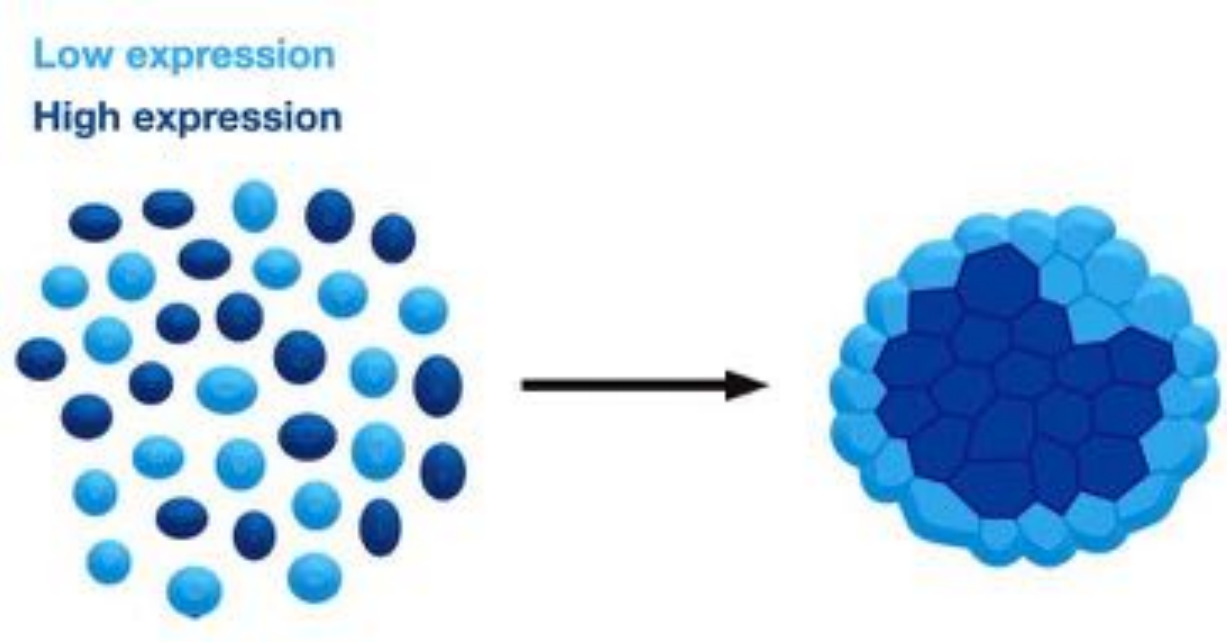
This principal of homotypic interaction of cadherins and segregation of cells can be demonstrated experimentally. Identical cells were engineered to express either E-cadherin or N-cadherin. The cells were mixed in culture and allowed to grow. Over time the cells expressing E-cadherin clustered away from the cells expressing N-cadherin which had also formed clusters. The results show that cadherins are sufficient to hold cells together to form a group and that distinct groups of cells can be formed by expressing different types of cadherins. Thus, tissues can maintain integrity by triggering cells to express the same type of cadherin.



Cells can be sorted by the types of cadherins.

Expression level of cadherin and segregation of cells

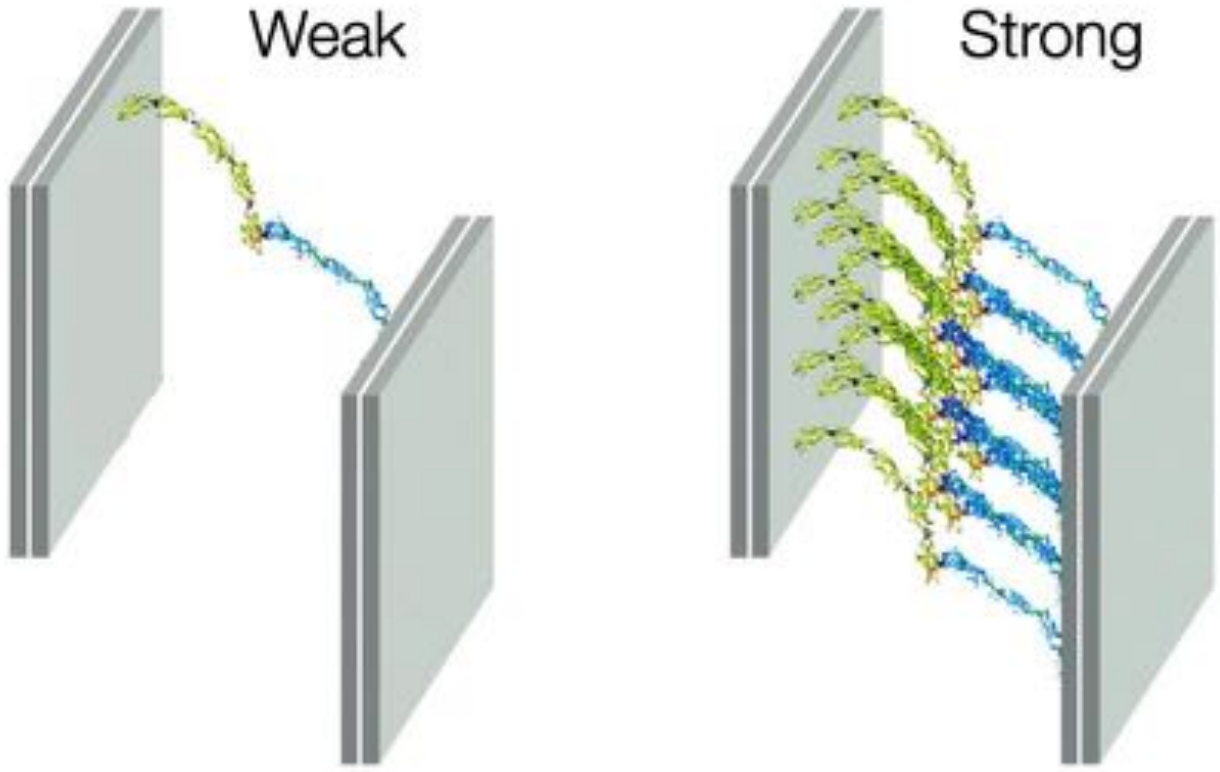
A related principle of cadherin interaction is that the strength of an association between cells can be modulated by changing the amount of cadherin in the cell membrane. Similar to the experiment above, identical cells were engineered to express cadherin, but in this experiment the cells made the same type of cadherin, and therefore, all the cells should interact with each other. Instead, the researchers changed the amount of cadherin in the cells by expressing the cadherin from a strong or weak promoter. The cells were mixed in culture and allowed to grow. As expected, the cells formed groups, but the cells that expressed a high level cadherin clustered in the center of the groups, whereas the cells expressing low amounts of cadherin localized to the periphery of the groups. Thus, even when cells express the same type of cadherin, the arrangement of those cells in a group can be altered by altering the amount of cadherin in the cell membrane.



Cells can be sorted by the expression level of cadherins.

Strengthening the interaction between cadherins via the cytoskeleton

The interaction between any two cadherins is weak and not nearly strong enough to maintain associations between adjacent cells. The strength of association between adhering junctions and desmosomes in adjacent cells comes from the clustering of many cadherins in one region of the cell membrane. The combined interaction of thousands of cadherins in one domain, either an adhering junction or desmosome, is sufficient to hold adjacent cells together.

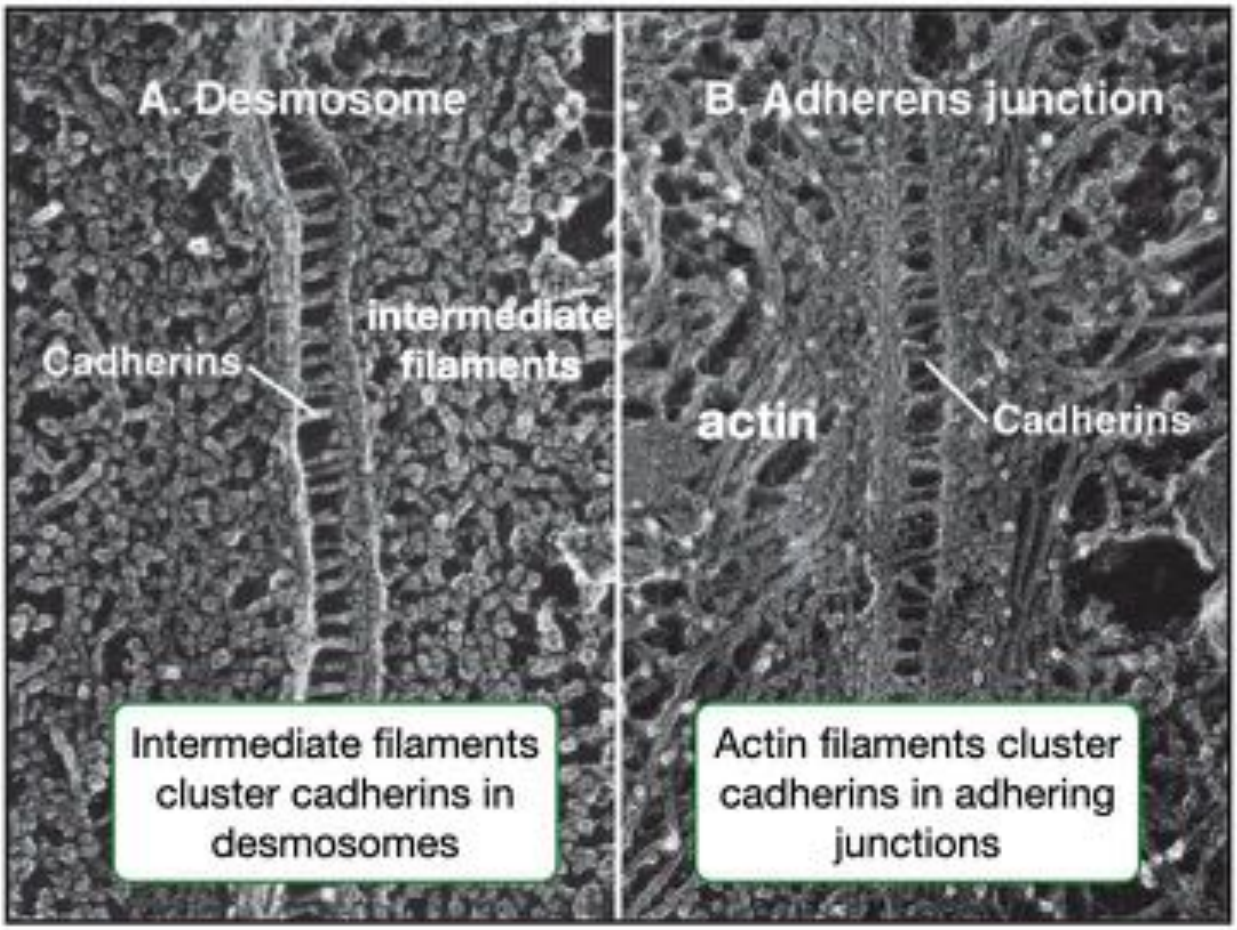


Clustering of cadherins increases strength of interactions between cells.

Recall that proteins and lipids in the cell membrane diffuse rapidly due to thermal energy, so something must hold cadherins in one regions of the cell membrane or they would end up distributed throughout the cell membrane and not able to form sufficiently strong interactions to hold cells together.

Interactions with the cytoskeleton clusters cadherins

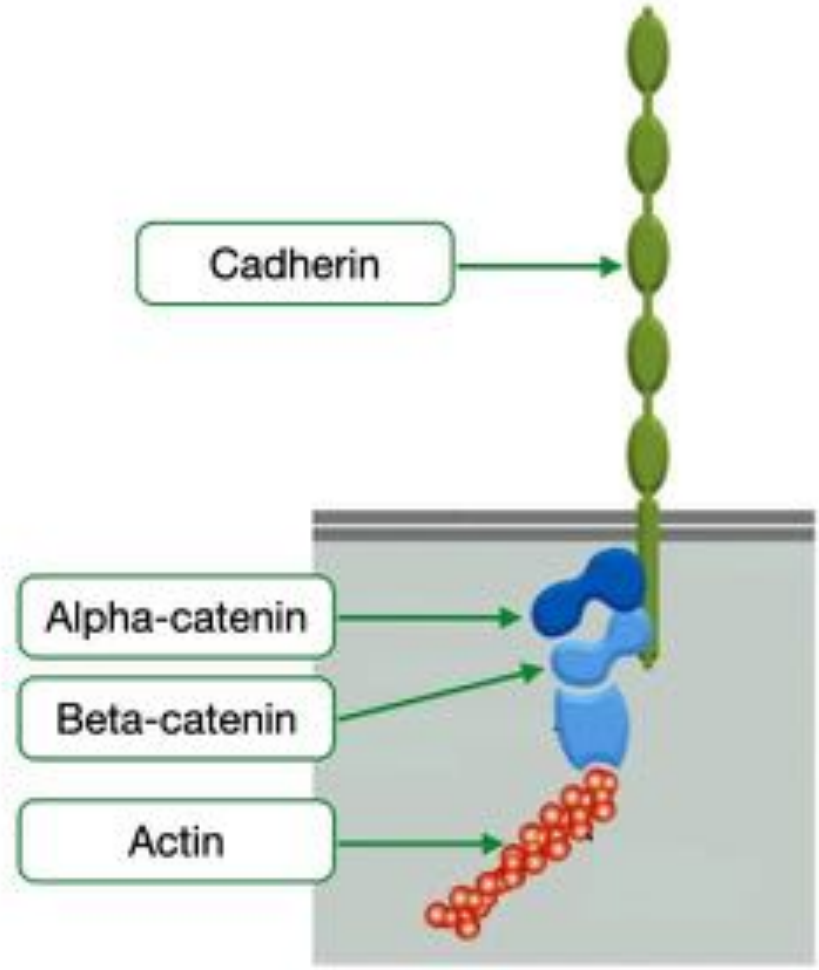
The key to keeping cadherins in one region of the cell membrane is their interaction with the cytoskeleton which inhibits the diffusion of cadherins with in the cell membrane. In adhering junctions cadherins associate with actin filaments, whereas in desmosome, cadherins associate with intermediate filaments.



Links to cytoskeleton cluster cadherins in desmosomes and adhering junctions.

Adhering junction

In adhering junctions, a set of proteins that include alpha-catenin and beta-catenin link cadherins to actin filaments. Although the interaction between cadherins, the catenins and actin filaments may appear to serve only structural purposes, recent research has shown that beta-catenin is a transcription factor and its activity increases during several cell signaling events. We will explore the role of beta-catenin in cell signaling pathways in the session on Cell Communication.

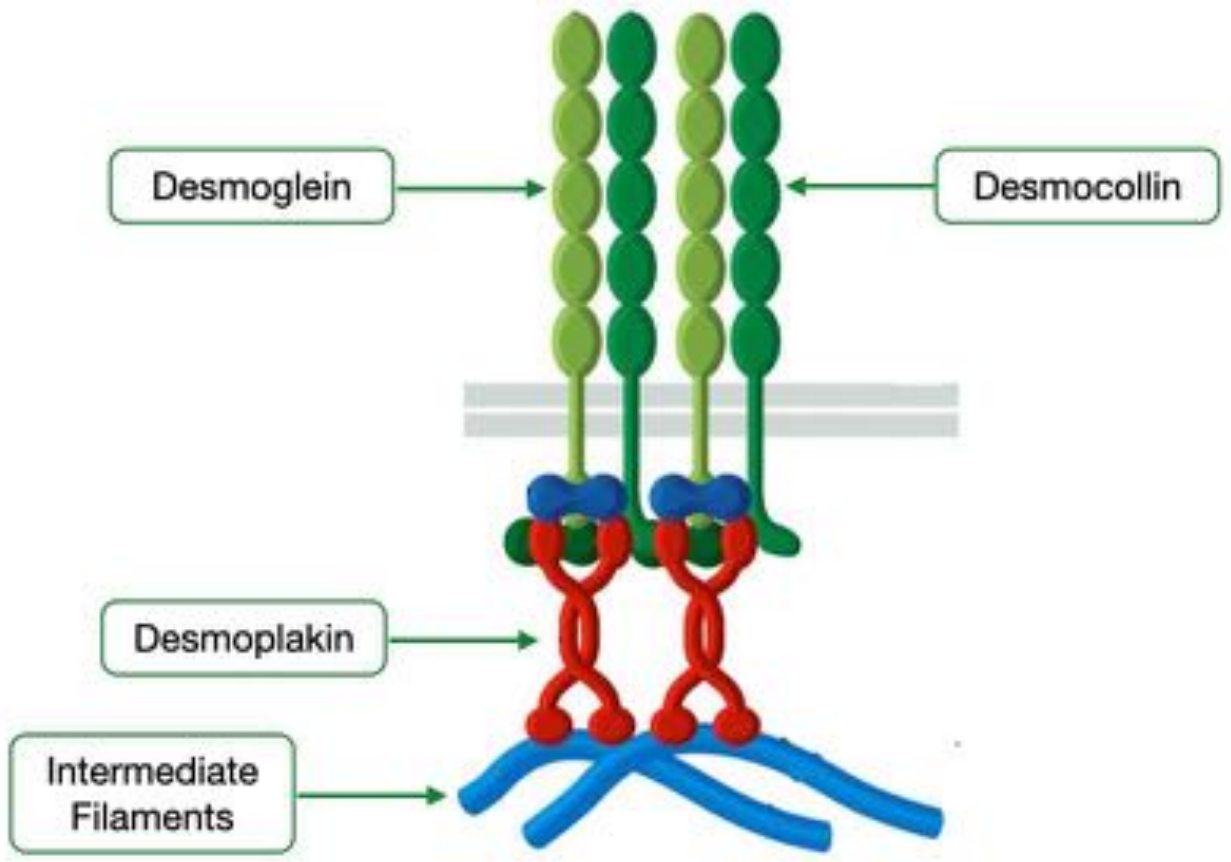


Catenins link cadherins to actin filaments in adhering junctions.

Desmosomes

Desmosomes also use cadherins to generate intercellular adhesion. Desmosomes contain desmoglein and desmocollin and in contrast to adhering junctions, the cadherins interact heterotypically. Thus, desmosomes must contain at least one desmoglein and one desmocollin.

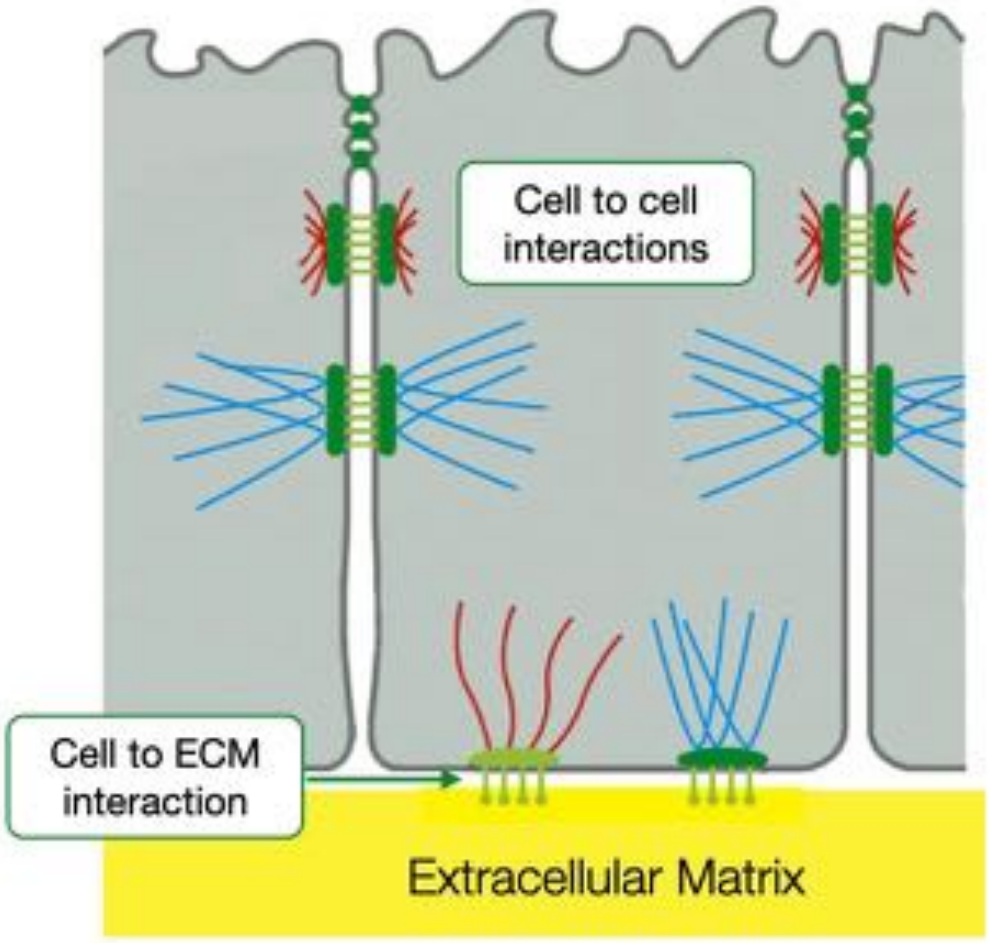
The cadherins in desmosomes are also linked to the cytoskeleton but instead of actin filaments, desmoglein and desmocollin are linked to intermediate filaments via a set of proteins. The significance of associating with intermediate filaments rather than actin filaments is to generate greater mechanical strength in a tissue. Recall that intermediate filaments are more robust than actin filaments and allow cells to stretch without breaking when exposed to low external forces but resist large external forces. Consequently, desmosomes are prominent in cells that are exposed to strong external forces. For example, the cells in your skin contain numerous desmosomes, but cells in other parts of the body where external forces are more mild contain few if any desmosomes.



In desmosomes, cadherins are linked to intermediate filaments.

Cell adhesion to an extracellular matrix

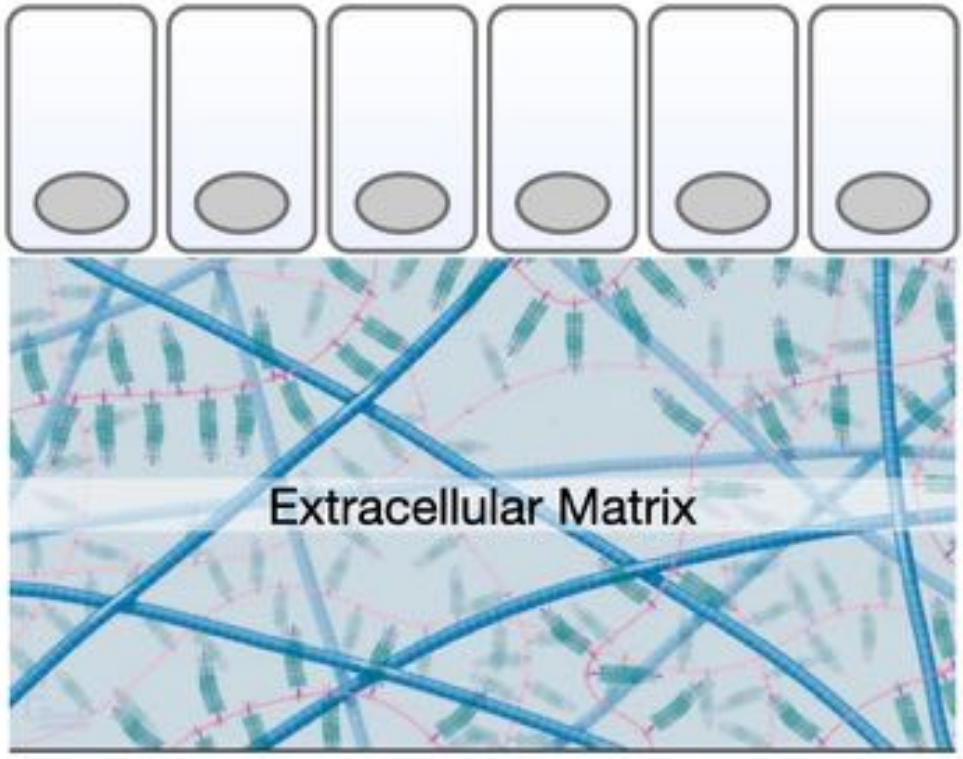
Although the cell-to-cell interactions generated by cadherins are sufficient to hold cells together in culture conditions, in tissues cells must also associate with an underlying matrix of proteins and carbohydrates called the extracellular matrix (ECM). The ECM provides mechanical integrity to tissues by forming a common substratum to which cells in the same tissue can attach. In addition, the ECM regulates many important cellular activities, including growth, division and differentiation.



Interactions between neighboring cells and between cells and ECM hold tissues together.

Components of the extracellular matrix

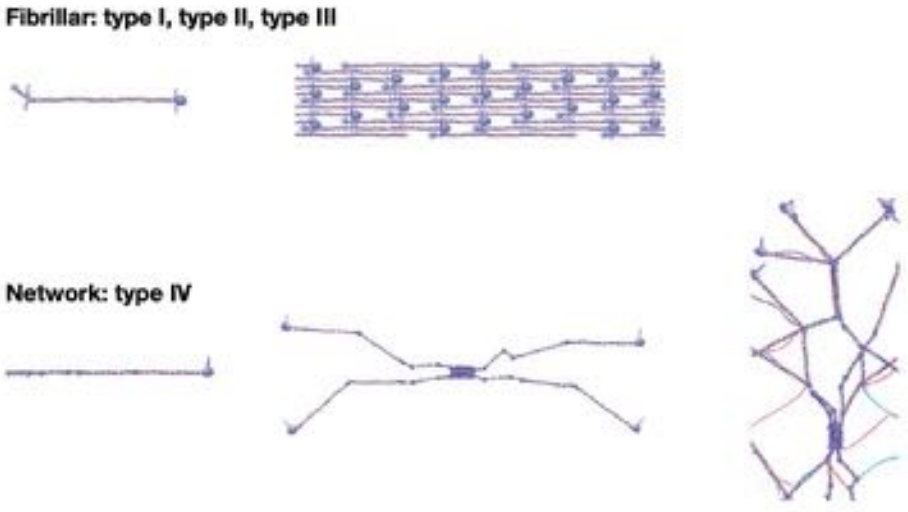
The extracellular matrix contains many different proteins and carbohydrates and the amount of those components in the ECM will vary depending on the tissue. However, most ECMs contain several classes of proteins which are described in more detail in the lecture on extracellular matrix. Below we provide a brief description as a refresher.



Extracellular matrix provides a common framework to support a group of cells.

Collagen

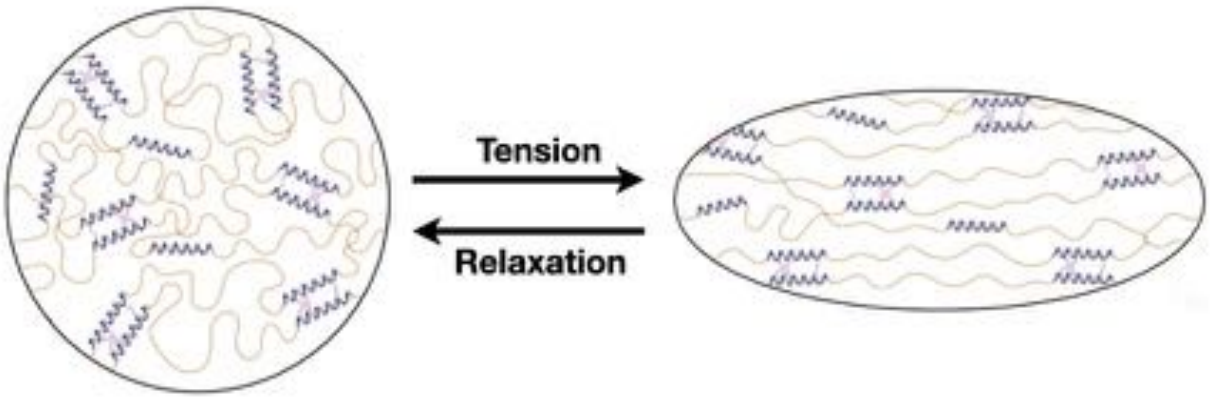
Collagen is the most abundant protein in the human body and is a major component of the ECM. Collagen represents a large class of proteins which polymerize to form fibers or networks. Collagen serves two primary functions. Collagens that form fibers resist tension that is applied to an extracellular matrix or tissue. Collagens that form networks organize other proteins in an extracellular matrix.



Collagens are a large family of proteins that form fibers or networks.

Elastic Fibers

Similar to collagen, elastic fibers resist tension but they also generate a recoil force that returns a tissue to its original shape after tension has been removed. The structure of elastic fibers will be presented in detail in the lecture on extracellular matrix.

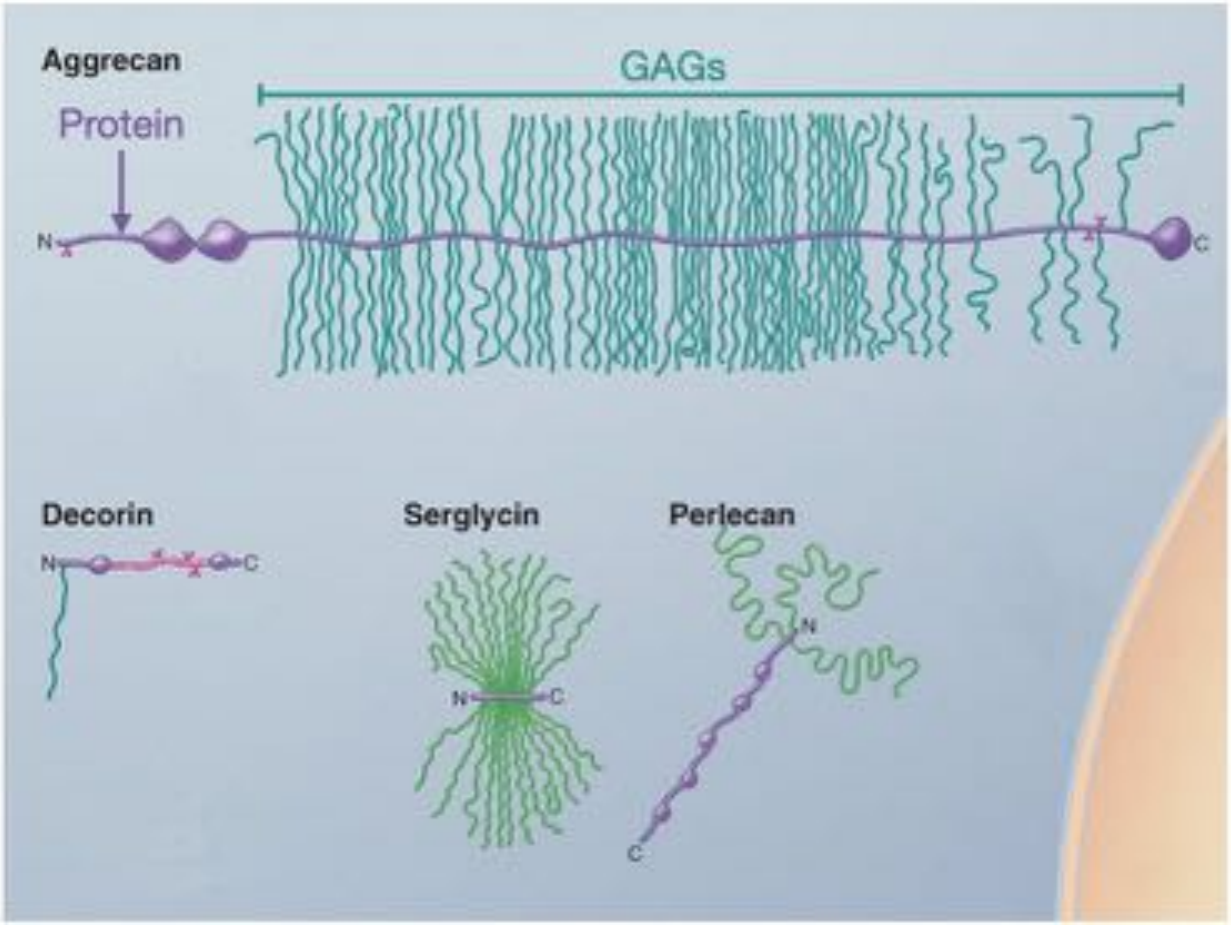


Tension generates order in elastin networks that provides energy for recoil.

Most extracellular matrices will have a mix of collagen and elastic fibers. Those they need to strongly resist tension and not become stretched under tension will contain predominantly collagen. Those matrices that need to stretch and recoil will have more elastic fibers.

Proteoglycans

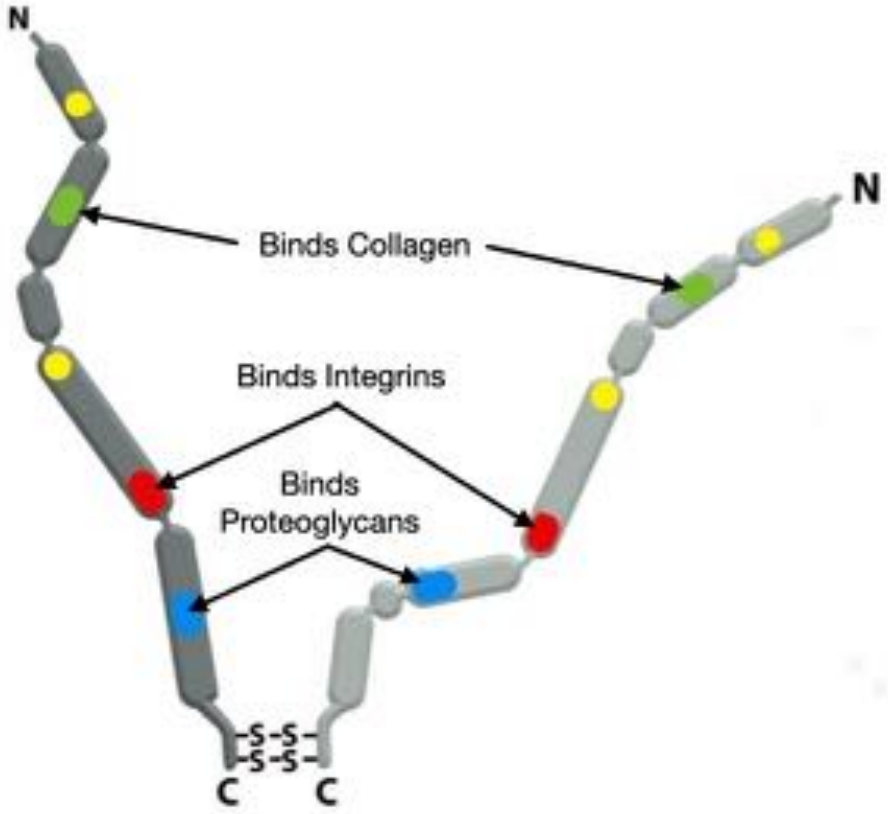
Proteoglycans are proteins that have large amounts of sugar side-chains. These side chains can grow to enormous lengths and many proteoglycans are more carbohydrate than protein. Proteoglycans serve several functions in an extracellular matrix. One is to resist compression. The sugar side chains are negatively charged and attract positively charged sodium. Water follows sodium into a matrix with proteoglycan and the retention of water in the matrix helps it resist compression from an external force.



Proteoglycans are single polypeptide with several attached glycosaminoglycans.

Glycoproteins

The ECM also contains several glycoproteins. These proteins differ from proteoglcyans by having relatively fewer carbohydrates attached. One of the most important glycoproteins is fibronectin. Fibronectins is a modular proteins and contain several different functional domains. Fibronectin is a homodimer and contains separate domains for binding collagen, proteoglycans and Integrins (see below). As such, fibronectin functions as a molecular glue that links different components within the ECM and links cells to the ECM.



Fibronectin is a glycoprotein in the ECM that functions as molecular glue.

Cell to ECM interactions

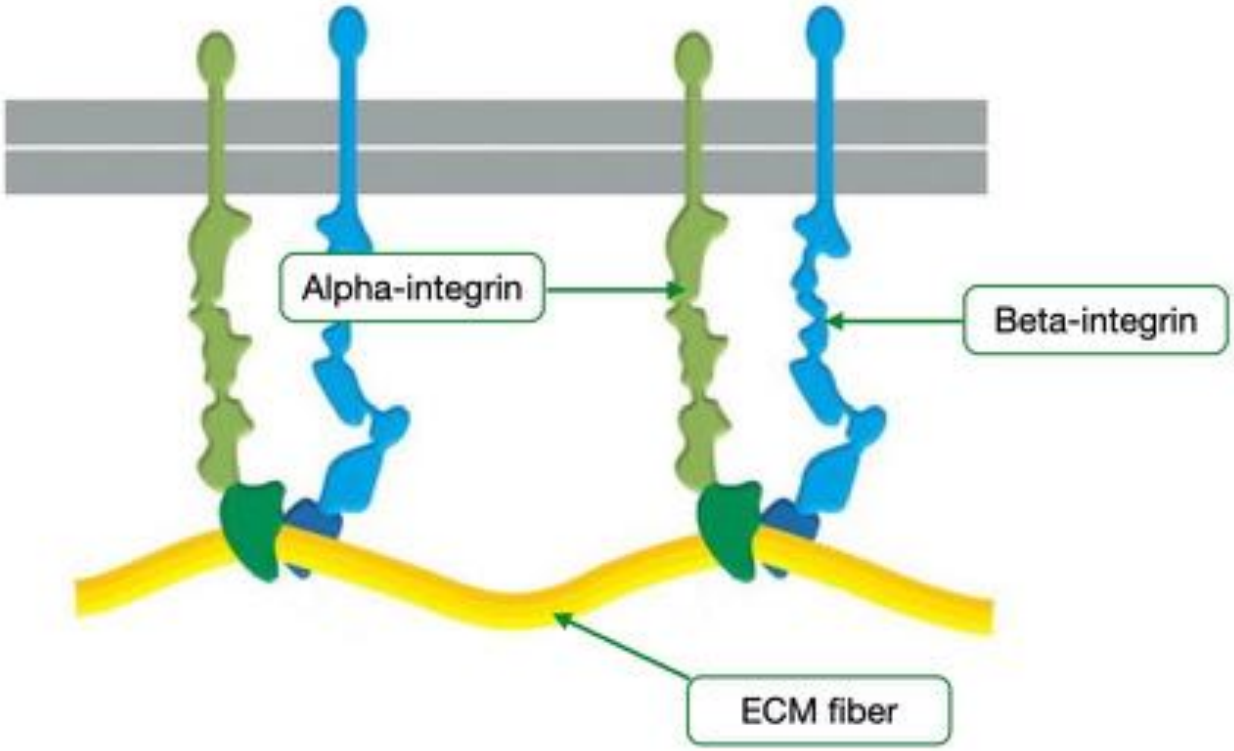
Cells need a mechanism to attach to the ECM. Similar to the cell-to-cell interactions described above, cells use integral membrane proteins in the cell membrane to attach to the ECM. These proteins are called integrins.

Integrins

Integrins are heterodimers comprising an alpha subunit and a beta subunit. Both subunits span the cell membrane once. Our genome encodes 18 different alpha integrins and 8 different beta integrins. These subunits combine to form 24 different pairs of integrins. Each integrin pair has unique properties but fall into different classes based on which protein the alpha-beta pair bind. For example, one class of integrin pairs binds collagen whereas a different class binds fibronectin. In addition, some integrins bind a protein called laminin which will be described in the lecture on epithelia.

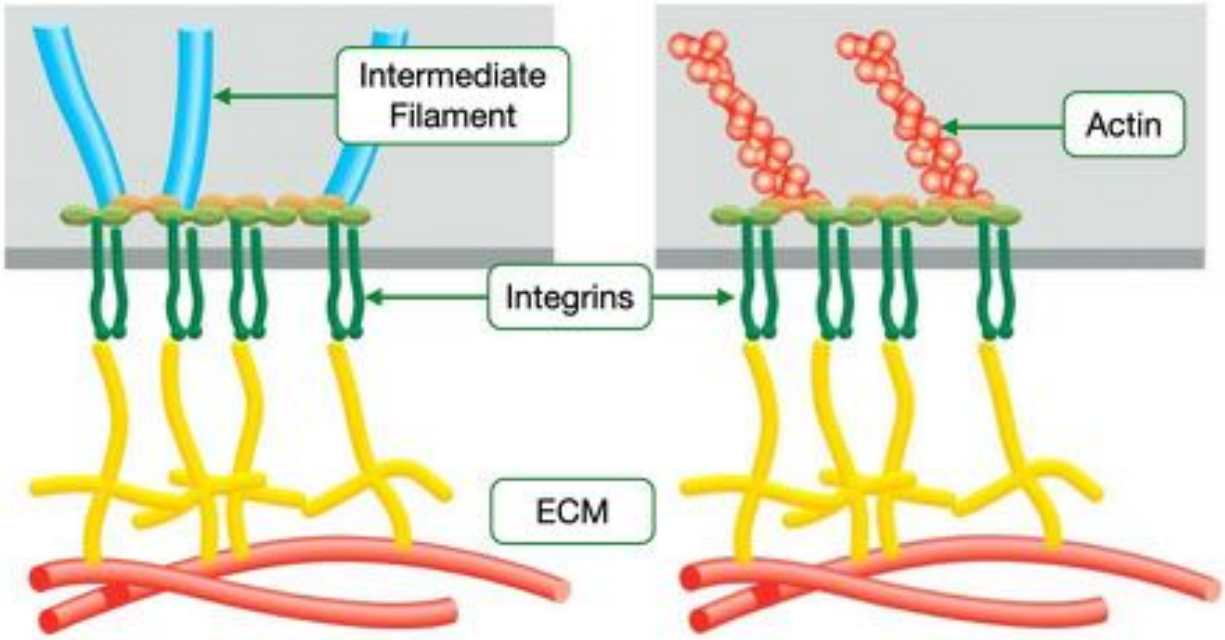
To promote tissue integrity, cells will express integrins pairs that match the components of the extracellular matrix and generate stronger adhesion between cells and the ECM. In some tissues,

the cells synthesize the components of the ECM to which they will attach. Thus, a specific gene expression pattern in cells can create an extracellular matrix and the integrin pairs that best bind to that matrix.



Integrins are cell surface receptors that link fibers of the extracellular matrix to the cytoskeleton.

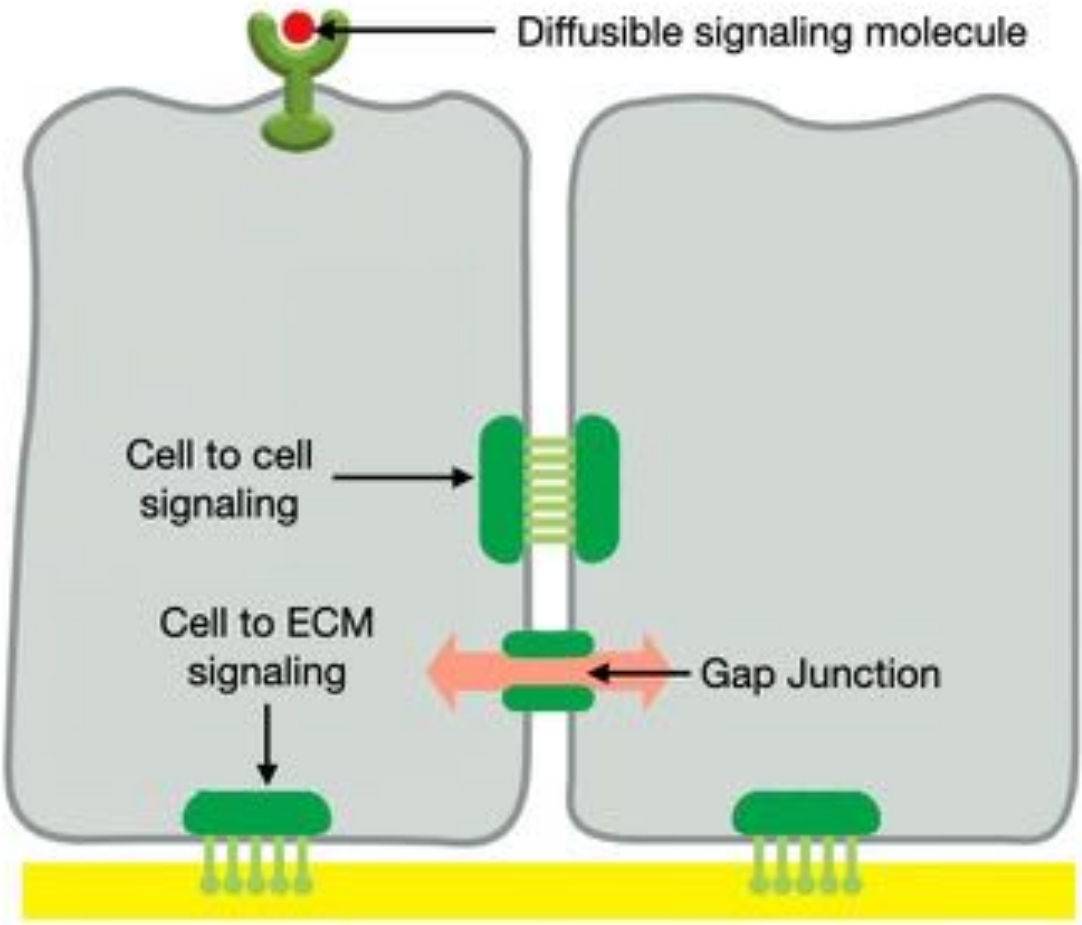
Similar to cadherins, integrins are linked to the cytoskelelton. In most cells integrins are linked to actin filaments via a set of intermediary proteins including talin, vinculin and alpha-actinin. In cells subject to strong external force, integrins associate with intermediate filaments. These integrins form a structure called a hemi-desmosome because it resembles half of a desmosome in electron micrographs.



Integrins in cells under mechanical stress link ECM to intermediate filaments.

Communication in tissues

Because most tissues perform specific functions under certain conditions, the activities of the cells in a tissue must be coordinated. In addition, the rate of growth and division of cells in a tissue must produce enough cells to replace those that are lost but also be tightly controlled so that too many cells are produced which could change the structure and integrity of the tissue. Overproduction of cells also increases the risk of development of a tumor.

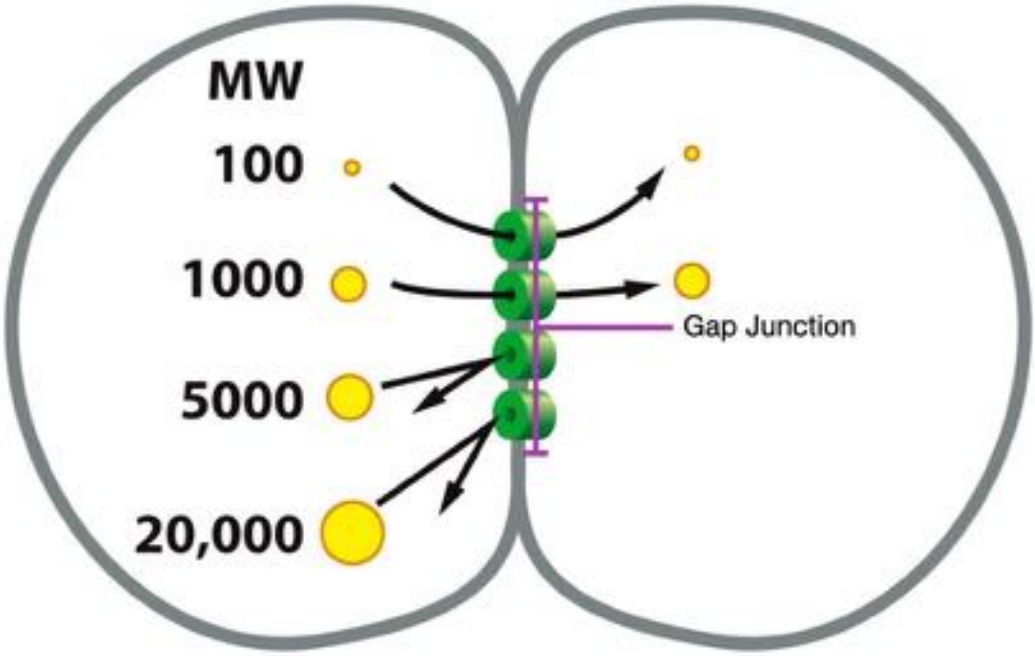


Cell communication regulates cell division and coordinate activities between cells.

There are three primary mechanisms through which cells communicate and tissues regulate the activity of cells: gap junctions, diffusible signaling molecules, and via cell-to-cell or cell-to-ECM interactions.

Gap junctions

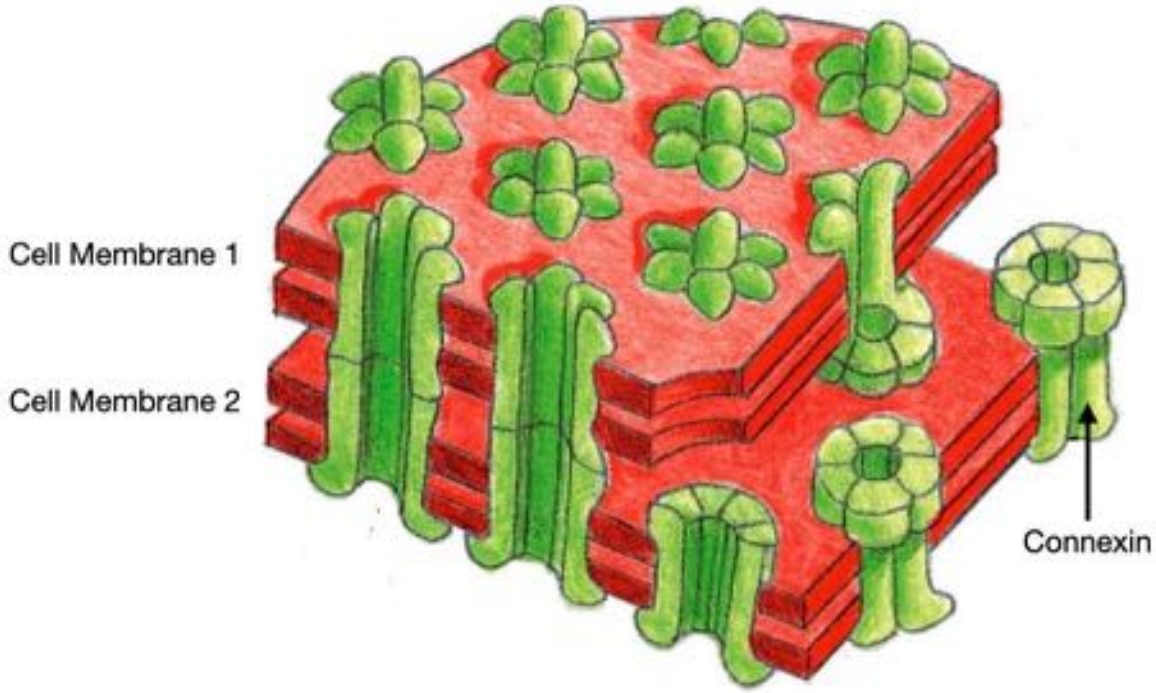
Gap junctions are a collection of protein pores in the cell membrane that interconnect adjacent cells. The individual pores of gap junctions are size restrictive: molecules smaller than 1000 Da can freely diffuse through pores while larger molecules are prevented from passing. Thus, gap junctions allow ions and other small molecules (e.g. ATP) to diffuse freely between the cytoplasm of adjacent cells. Because ions and small molecules are often involved in triggering signaling reactions within cells, gap junctions are effective means for a signaling event at one cell to pass throughout a collection of cells



Gap junctions allow diffusion of small molecules between neighboring cells.

Connexins

Connexins are proteins that make up the pores in gap junctions. 6 connexins assemble in the cell membrane of one cell to form a pore that interacts with a complex of 6 connexins in neighboring cells to form a continuous channel between the two cells. Connexins are a large family of proteins some of which have been associated with diseases (e.g. connexin 26 and deafness).

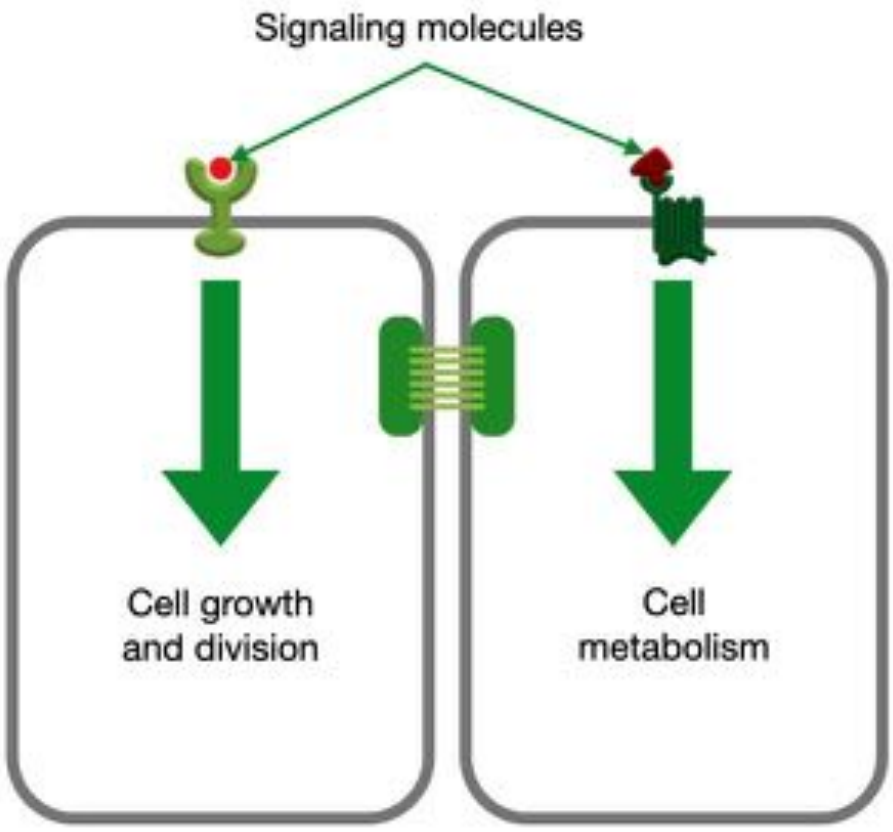


Connexins are transmembrane proteins that form ~1.5 nm pores between cells.

An important feature of some connexins is that they close when cytosolic calcium levels are high. Cytosolic calcium is kept at very low concentrations and is important trigger for many biochemical reactions and biological processes (e.g. muscle contraction). Cells keep cytosolic calcium at low levels to tightly regulate these reactions and processes. When a cell incurs damage to its cell membrane, calcium flows into the cell due to the strong electrochemical gradient favoring calcium movement into cells. By closing when cytosolic calcium levels remain high for prolonged periods, connexins prevent the spread of potential harmful molecules and ions from damaged cells into viable cells.

Diffusible signaling molecules

In any tissue, cells are bathed in a variety of molecules that regulate the activity of those cells. These molecules can be produced locally by cells within the tissue or by cells in another region of the body. Many factors impact how these signaling molecules affect the activity of cells within a tissue, and the details of how these molecules change the behavior of cells will be discussed in the session on Cell Communication.

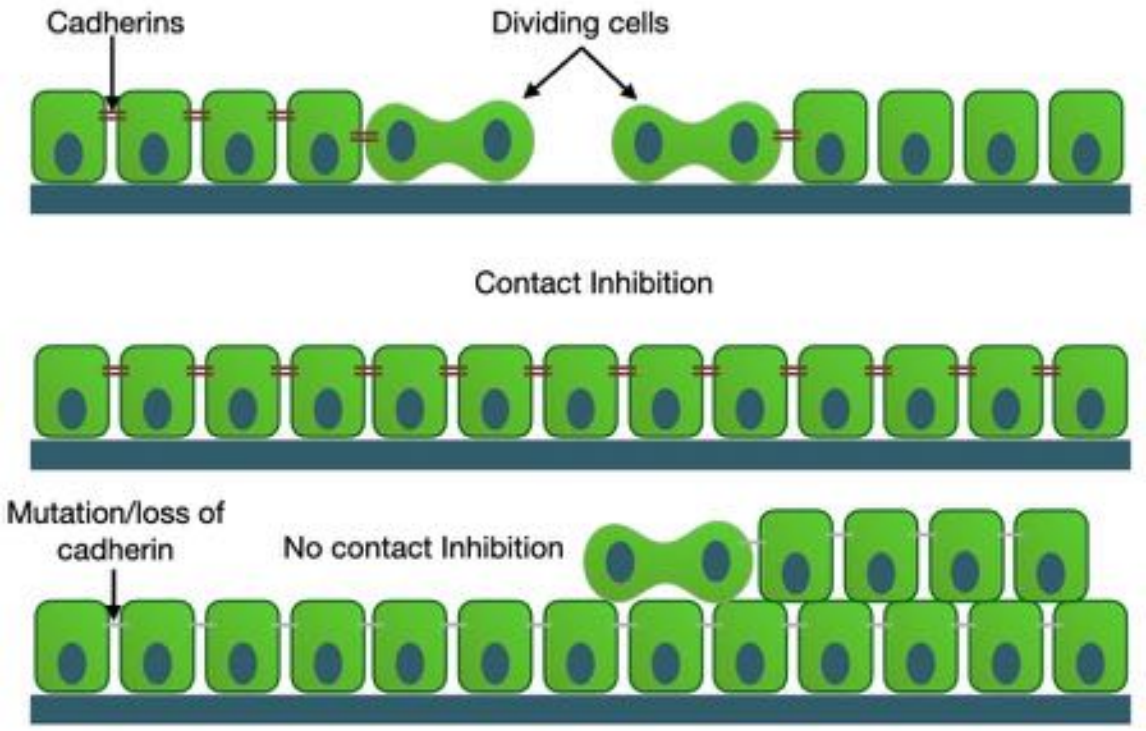


Signaling molecules in the ECM regulate cell behavior and activity.

One key factor in cell signaling is the concentration of the signaling molecule that surrounds the cells. Higher concentrations of a signaling molecule are more likely to trigger a change in the activity of cells. In many tissues, components of the ECM regulate the concentration of signaling molecules by binding signaling molecules and preventing them from interacting with the cells in the tissue.

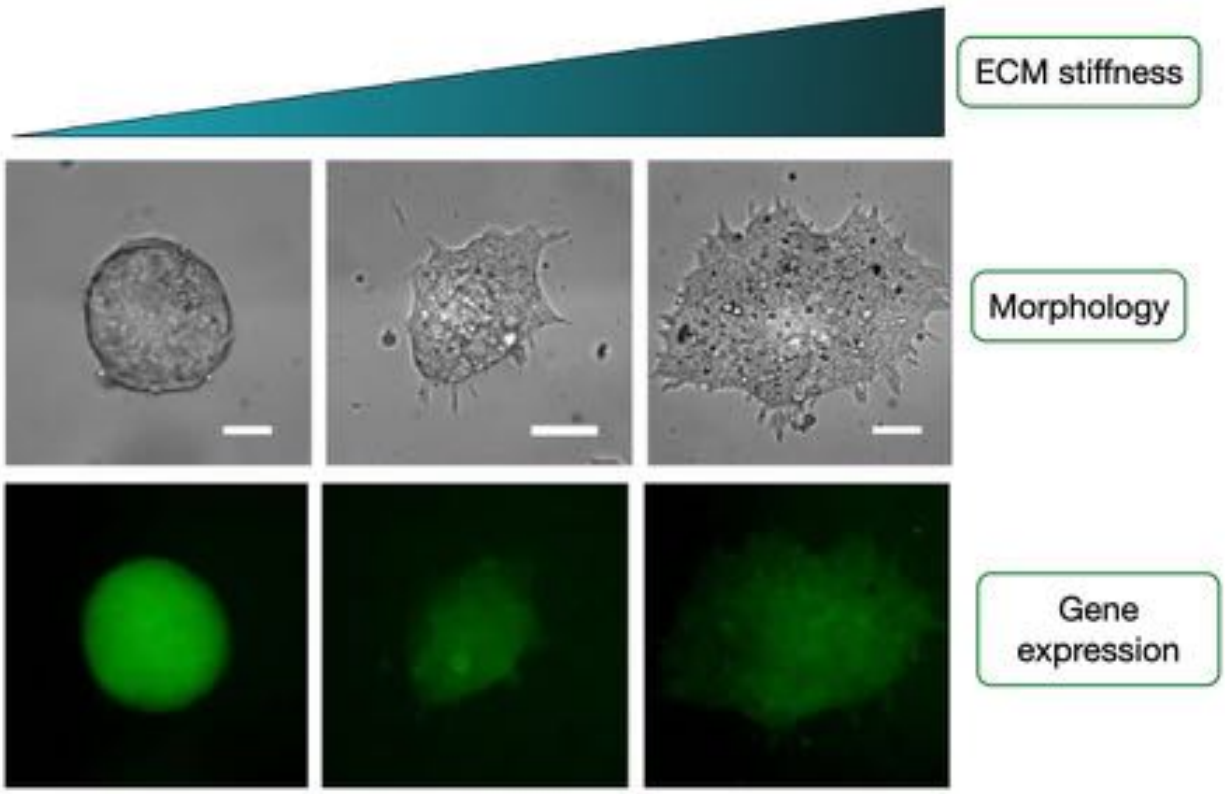
Communication via cell-to-cell and cell-to-ECM interactions

Besides maintaining the physical integrity of tissues, cell-to-cell and cell-to-ECM interactions also affect the behavior of cells. For example, interactions between cadherins in adhering junctions is known to inhibit the proliferation of cells. This behavior is often referred to as contact inhibition as was first observed in cell grown in vitro. Cells that express cadherin will grow and divide along the surface of tissue culture flask, but upon making complete contact with neighboring cells, the cells will stop dividing and form a single layer of cells. If cells lack cadherin or express a mutated cadherin, they will not stop dividing when they make complete contact but will continue to multiply and start growing on top of each other. Thus, signaling through cadherins in adhering junctions can regulate cell division and help tissues prevent over proliferation of cells.



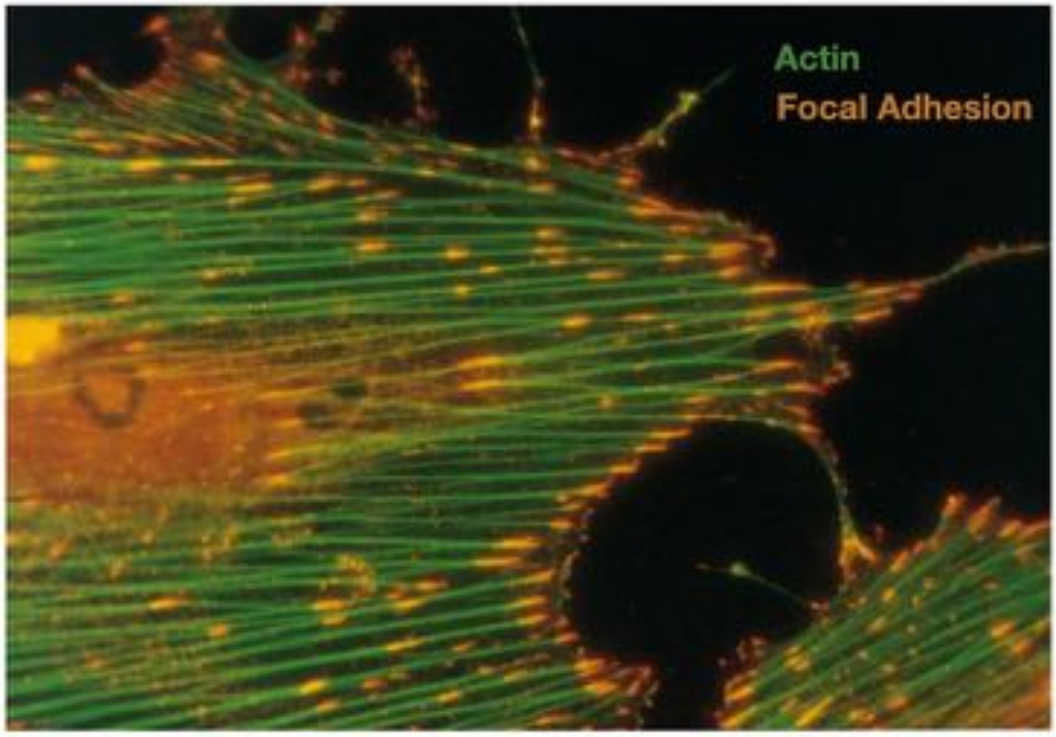
Cadherins regulate cell division.

The interaction between integrins and the extracellular matrix also affects the behavior of cells. As described in the lecture on extracellular matrix, the stiffness of the extracellular matrix has been found to influence cell behavior, including cell division, cell motility and cell differentiation. The example below shows how the stiffness of the ECM can affect both the shape of a cell and its gene expression profile.



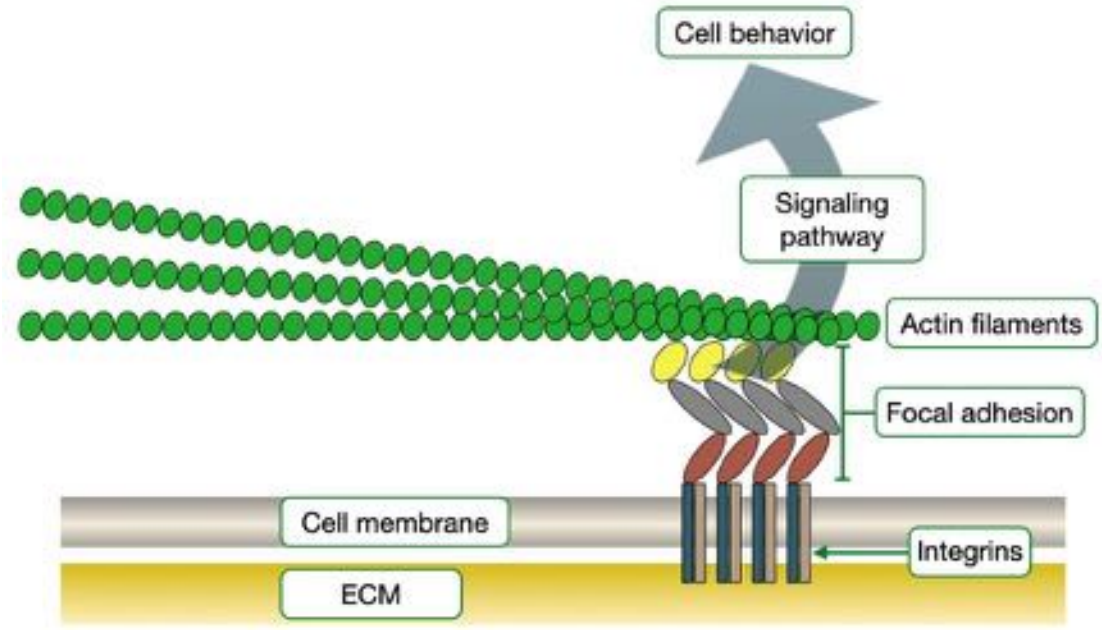
Extracellular matrix regulates cell behavior.

In many cells, integrins cluster into small domains within the cell membrane called focal adhesions. These clusters serve as platforms for several different signaling pathways. Exactly how the interactions affect the behavior of the cells depends upon the type of integrin and how tightly the integrins are bound to the extracellular matrix.



Cells connect to extracellular matrix at sites called focal adhesions.

Thus, in addition to providing structural support, the interaction between Integrins and the extracellular matrix also establishes and maintains cell identity. Cells that wish to join a tissue have to express the correct set of integrins that bind the components in the extracellular matrix of the tissue. Upon forming a strong interaction with the the extracellular matrix, signaling from focal adhesions can control gene expression and determine which biochemical pathways are active in a cell.



Integrin attachment to ECM helps determine and maintain cell identity.