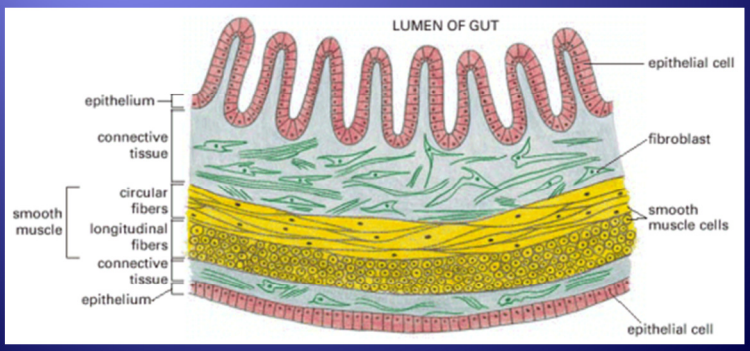
Session 6

CELL ADHESIONS (CELL-CELL & CELL-MATRIX)

Integrating Cells into Tissues

- Cells differentiate into distinct "types"
  - Phenotypes: morphology, biochemical, mechanical, physiological, development
  - Mechano-connections by cell-cell and matrix contacts



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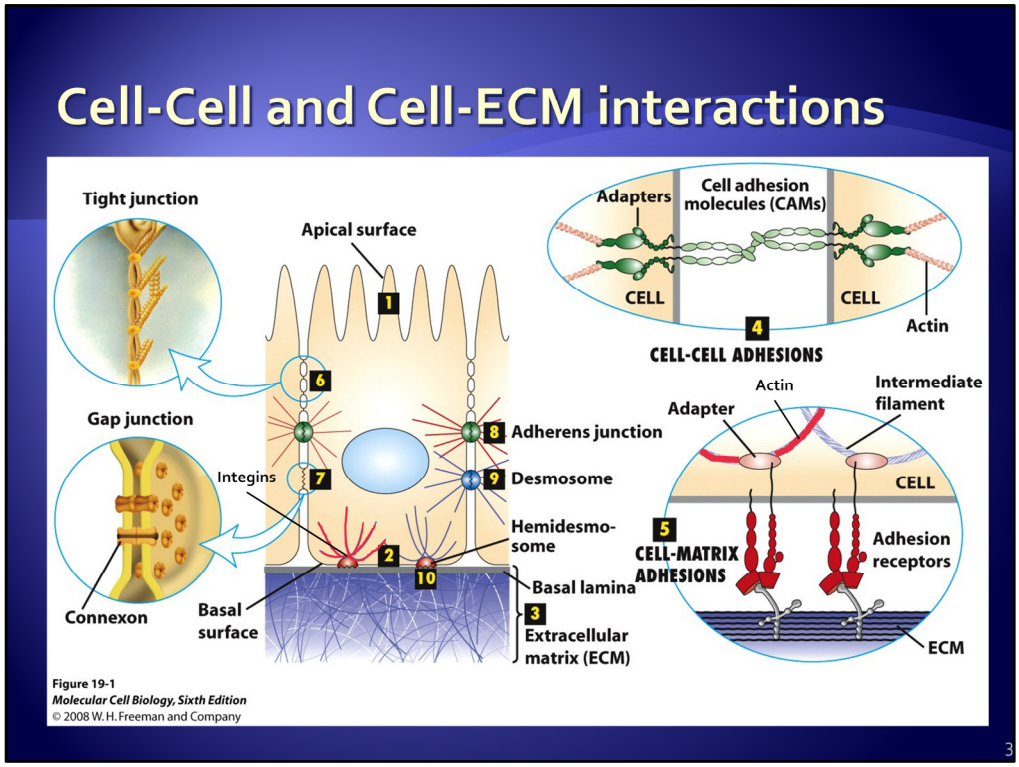
We are 10 trillion cells and each one is a little different. Many have common characteristics in morphology, biochemical, mechanical, physiological, or their spatial origin in the development from an embryo known as phenotypes. An example is the GI tract where you have epithelial cells that form a tight barrier of mechanically connected cells that absorbed nutrients and secretes chemicals for digestion. The fibroblast underneath the epithelial cells form the connective tissue that supports the epithelial cells. Although these cells are not directly connected together, they reside within an extracellular matrix that connects them together. Underneath the fibroblasts is a layer of smooth muscles that involuntary contract to move the food through the system and to prevent it from going backwards (not good!). Outside the muscle is the adventitia which is the outer covering of an organ and also contains fibroblasts and epithelial cells.

These cells are connected to each other through cell-adhesion molecules (CAMS) and to the surrounding extracellular matrix (noncellular material). There are distinct mechanical properties of rigid bones, flexible joints, and contracting muscles that allow us to move and stand tall against gravity. We have layers of protective barriers and sealants. We also form flexible compartments for vital fluids.

Because cell adhesions are intrinsically associated with the cytoskeleton and signaling pathways, a cell's surroundings influence its shape and functional properties ("outside-in") and likewise, cellular shape and function influence a cell's surroundings ("inside-out"). In a sense, the mechanics of the environment affects a cell's own mechanics and vice-versa.



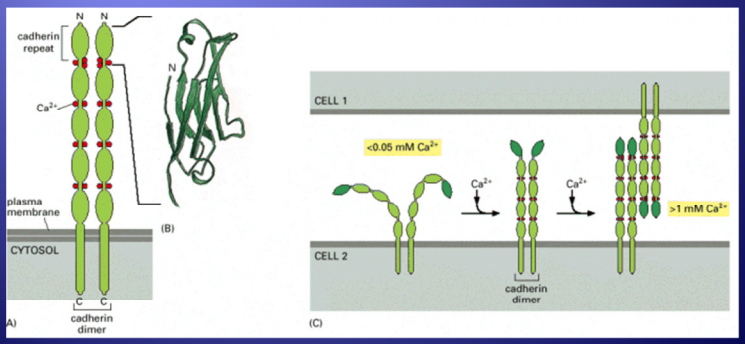
1) The apical surface of these epithelial cells contain cilia that project into the lumen of the gut
2) These cells rest on the extracellular matrix (ECM) through integrins and hemidesmosomes that connect to the cytoskeleton
3) Large protein and glycan molecules form the ECM

The cells in the tissue are connected by
4) cell-cell adhesions
5) cell-matrix adhesions
With special transmembrane proteins that enable a velcro-like connection between other cells and ligands in the matrix.

Cell-cell adhesions have different types
6) Tight junctions, as their name implies, form a tight seal between the cells that prevent diffusion from solutes in the lumen into the underlying tissue
7) Gap junctions provide a passage way for Ca2+ ions or other small molecules to transit from one cell to the next. These structures are important in muscle contraction where the signal to activate the sarcomeres in one cell can propagate through the gap junctions to activate contraction in the neighboring muscle
8) Adherens junctions are one of the first structures to form between neighboring cells through cadherin proteins that connect to the actin filaments in the cytoskeleton. The formation of adherens junctions subsequently allows tight junctions to form.
9) Desmosomes are similar to adherens junctions but use plakin proteins to link neighboring cells structurally by their intermediate filaments

Homotypic Cell-Cell Adhesions

- Cadherins : *c*alcium *adherin*g
- Transmembrane proteins
- Types: E-, N-, P-, VE-, and T-cadherin.



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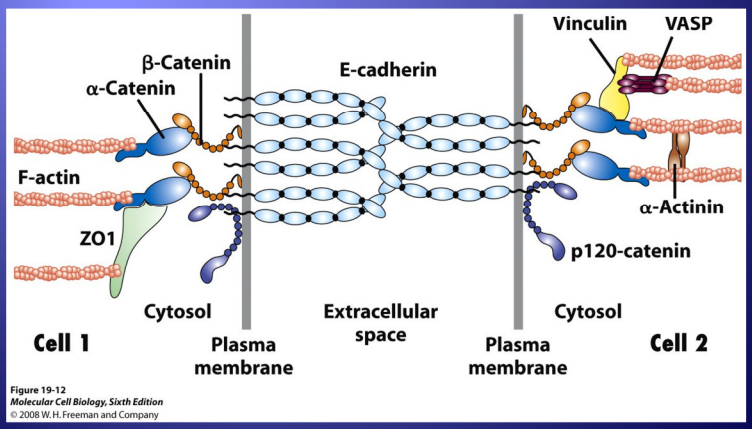
Cadherins are transmembrane proteins whose exoplasmic domains cluster to form dimers through calcium-binding site that form a stiff-rodlike structure. The dimers then can form homotypic connections with neighboring cells.

Classical cadherins
E-cadherin – epithelia
N-cadherin - neurons, heart, skeletal muscle, lens, and fibroblasts
P-cadherin - placenta, epidermis, breast epithelium
VE-cadherin –endothelial cells (vascular epithelium)
T-cadherin - neurons, muscle

Cadherin-CSK connection

- Adaptor proteins (e.g. β-catenin) connect adherens junctions to actin filaments



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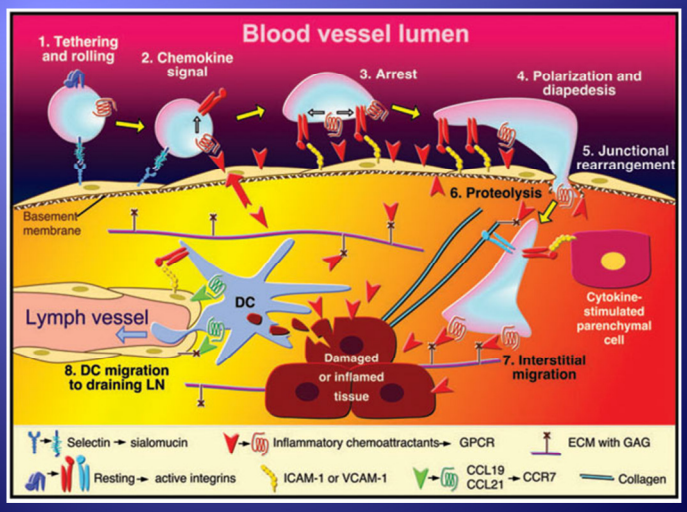
The cytosolic domain of cadherins link to the actin cytoskeleton by adapter proteins that are essential for strong adhesions. Lack of expression to the adaptor proteins (e.g., alpha-catenin or beta-catenin) can occur spontaneously in tumor cells.

Heterotypic Cell Adhesions

- Leukocyte rolling and transmigration



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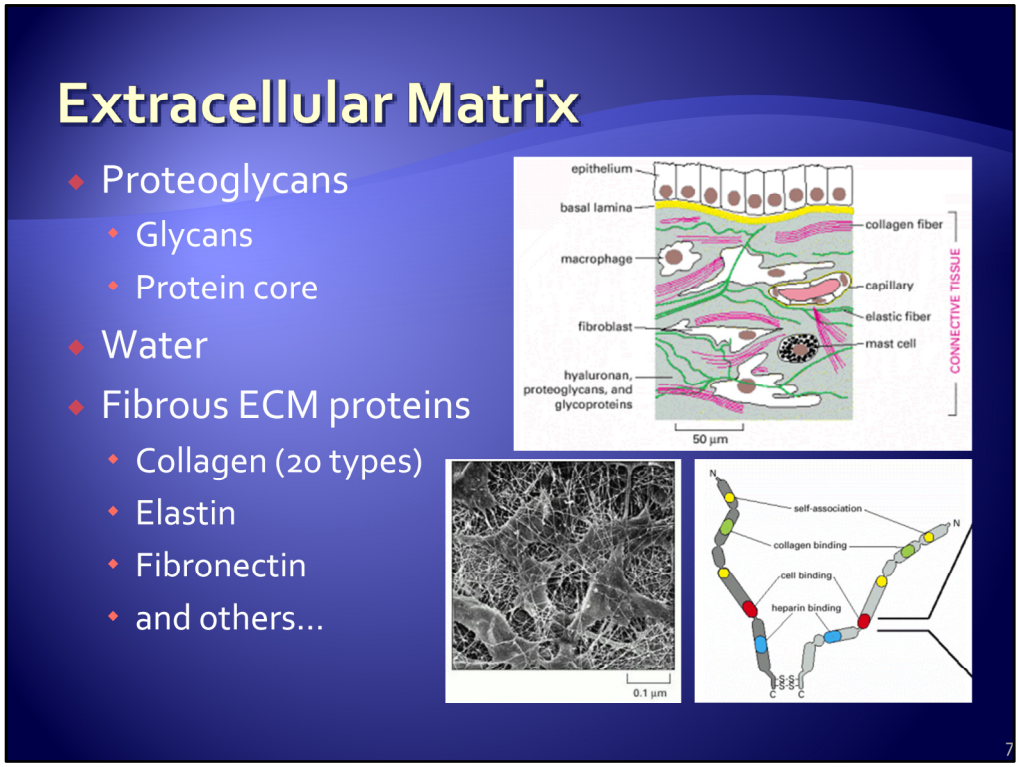
Leukocytes (WBC) patrol the vasculature and look for signs of inflammation or infection. They become tethered to heterotypic cell adhesions and rolling along the surface of endothelial cells by weak interaction. Once they receive the chemical signals (chemokines and chemoattractants), they will change their cytoskeletal shape to become polarize and migrate between the tightly packed endothelial cells (diapedesis/transmigration) to get to the damage or infection in the tissue and clean up the mess.

The whole process of leukocyte transmigration is a complex orchestration of heterotypic adhesion molecules (selectrin, integrin, ICAM, VCAM, etc.) that help the leukocyte halt the rolling process and become activated and arrested at the site above the damaged tissue before mounting the migration process through the tissue.



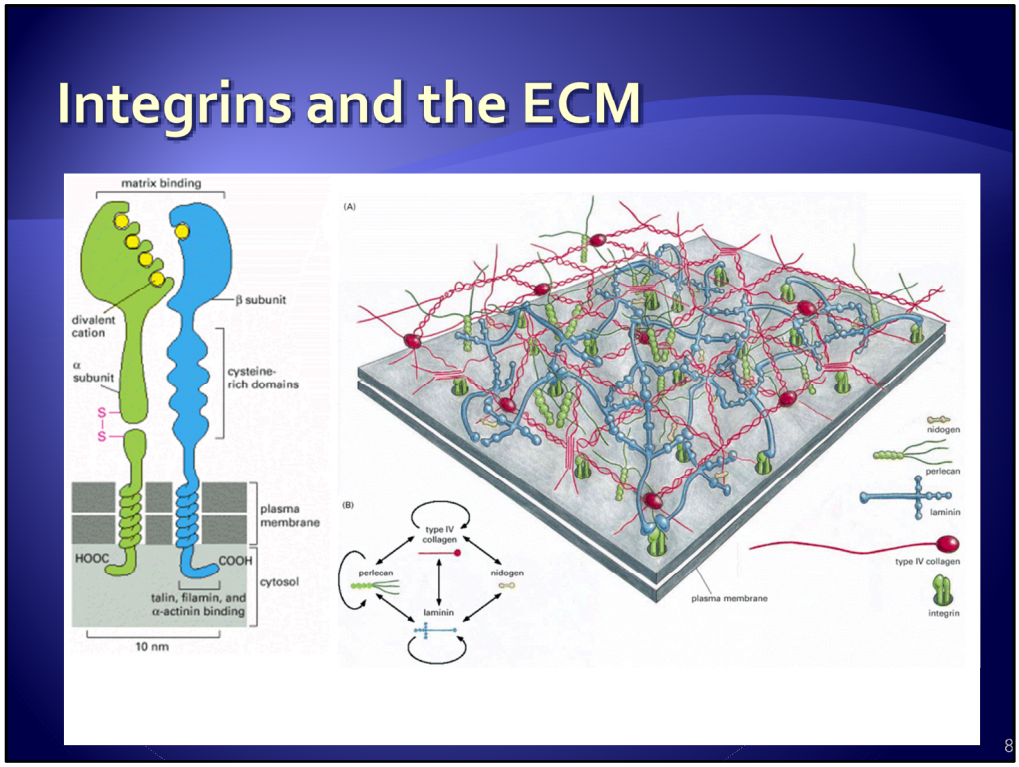
The macromolecules that constitute the extracellular matrix are produced locally by cells residing inside the matrix. In most connective tissues, the matrix molecules are secreted by cells called fibroblasts. In cartilage, it is chondrocytes and in bone, it is osteoblasts. These cells help to organize the matrix and the orientation of the cytoskeleton can influence the orientation of the matrix produced outside.

Proteoglycans are have lots of water molecules around them and are a gel-like substance in which the ECM protein fibers are embedded. The proteoglycan gel resists compressive forces; the elastomeric fiber EM proteins resists tension. The electron microscopy micrograph shows fibroblasts in the ECM. The collagen fibril are distinctly apparent but the proteoglycans that form the hydrated gel of the interstitial void was removed during the fixation.

Of the ECM proteins, collagen is the most abundant. Collagen I is the most abundant and is the primary ECM of b*ONE*. Collagen II is found in the cartilage (car*TWO*lage). Collagen IV does not form fibrils like the others and instead is meshlike sheet that forms the basal lamina of epithelial cells (floor = *FOUR*).

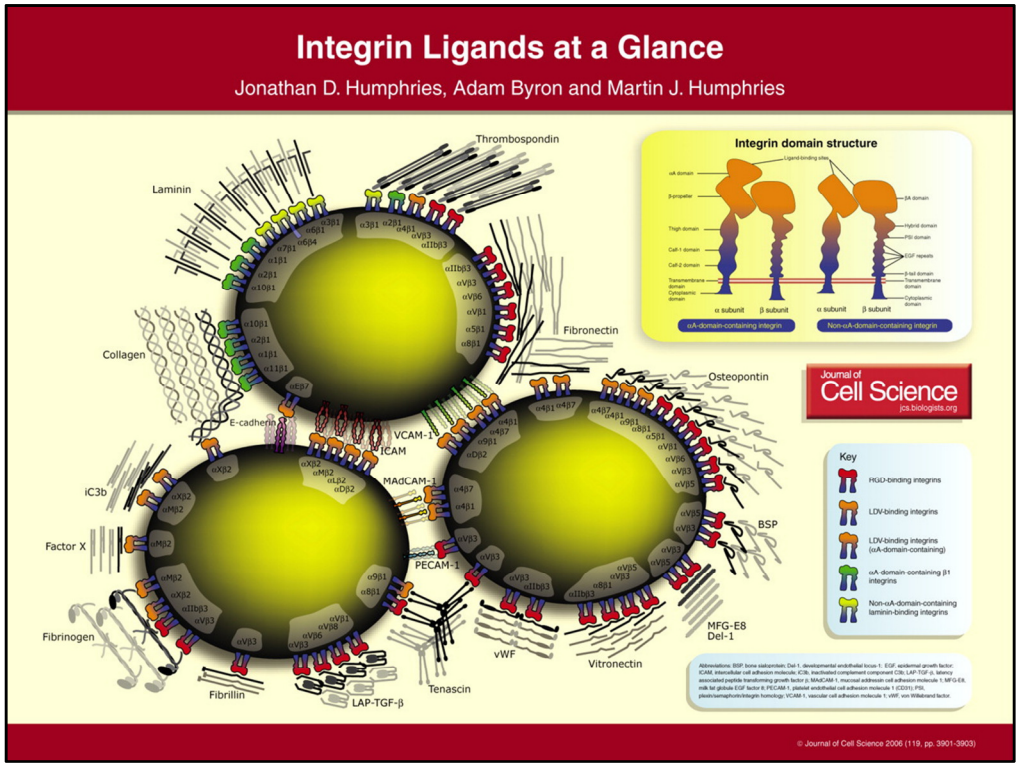
Elastin provides the elasticity of skin, lung, and blood vessel.

Fibronectin is a dimer with disulfide bonds at one end and many distinct domains for cell or ECM binding. It is a multifunctional matrix protein that was discovered by its presence on the surface of normal fibroblasts in culture and which helped them tightly adhere to the culture dish, but it is absent from the surfaces of tumor cells. Fibronectin is essential for migration and differentiation in embryo morphogenesis. It is also present in blood and helps with wound healing by promoting blood clotting and helps the migration of white blood cells to the affected area.



How a cell binds to the ECM is through integrins. Each integrin is a dimer of an alpha and beta subunit that is a transmembrane protein having an extracellular domain to bind to the ligands in the CM and a cytosol domain that connects to the cytoskeleton.

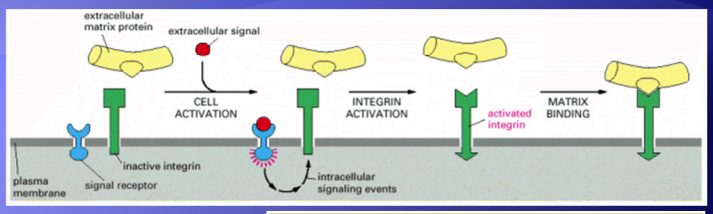
Integrins bind to ligands in a manner that depends on extracellular divalents cations (Ca2+ or Mg2+). Not all ECM proteins are integrin-binding and instead bind to other ECM proteins to make a cross-linked structure.



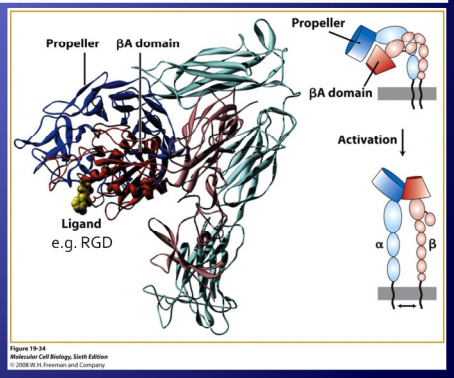
Integrins function as adhesion receptors that mediate cell-matrix interactions. There are at least 24 integrin heterodimers –two noncovalently associated transmembrane gycoproteins composed of one of 18 alpha subunits and one of 8 beta subunits that form in various combinations. The combination of one alpha with one beta allows the integrin receptor to bind to different ligands in the matrix. Many matrix proteins are recognized by multiple integrins. It is the combinatorial diversity that allows for a relatively small number of components to serve a large number of distinct functions.

Integrin Activation

- Cells not always sticky



- Extracellular signals induce conformational changes in integrins that increase binding affinity ("inside-out")
- Important for platelets and leukocytes



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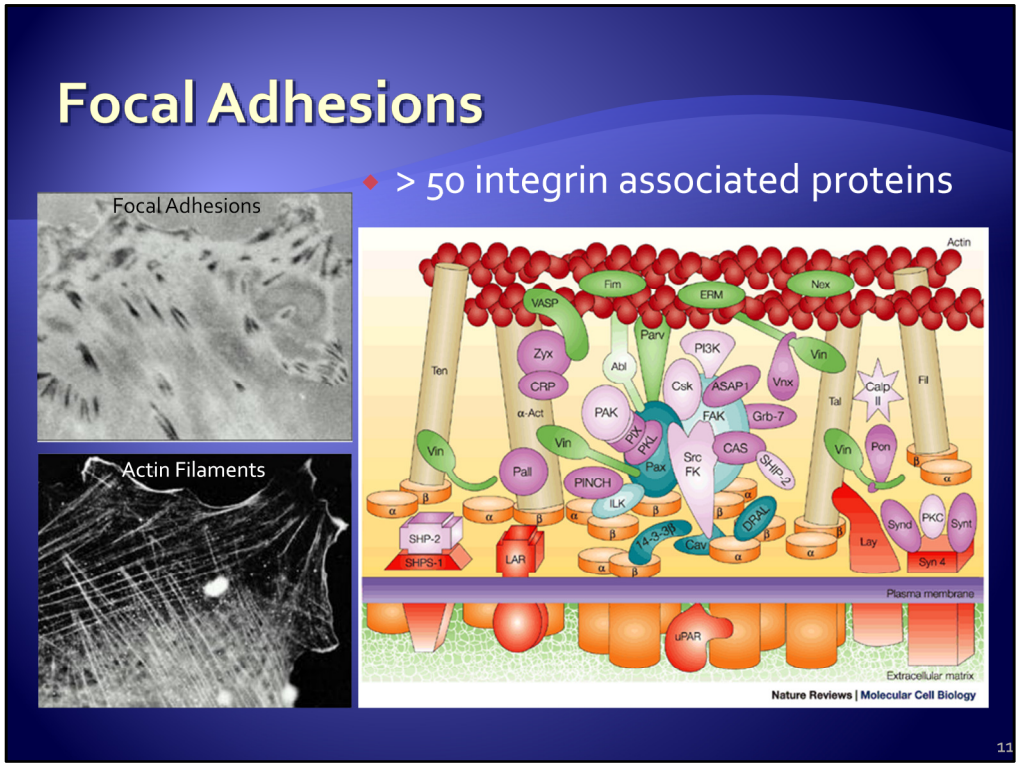
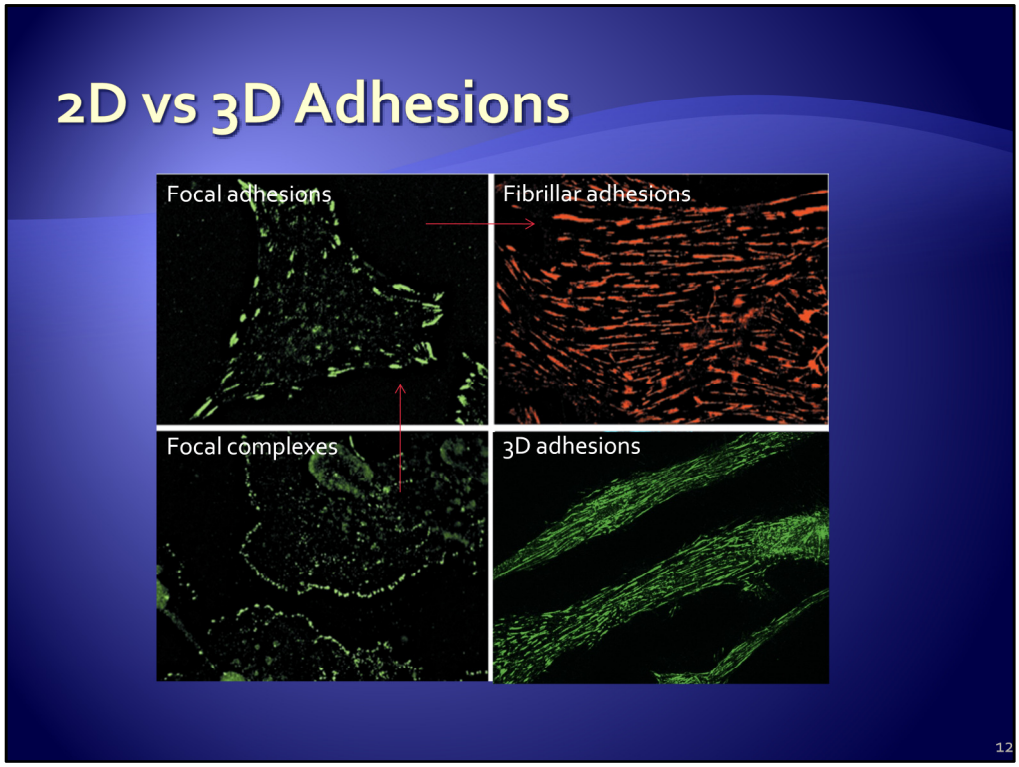
In many cases, the binding affinity of integrins is modulated by cells. When the appropriate extracellular signal is presented to the cell, the activation of intracellular signaling events will cause integrins to undergo a conformational change that increase their stickiness to ligands in the ECM. In most cases, the integrins will bind to RGD amino acid sequence domains (Arginine, Glycine, Aspartic Acid) in the extracellular matrix proteins. Platelets and white blood cells in particular rely on this feature to remain inactive or rolling until the right clotting or inflammation signal presents itself and the cells attach and do their jobs.

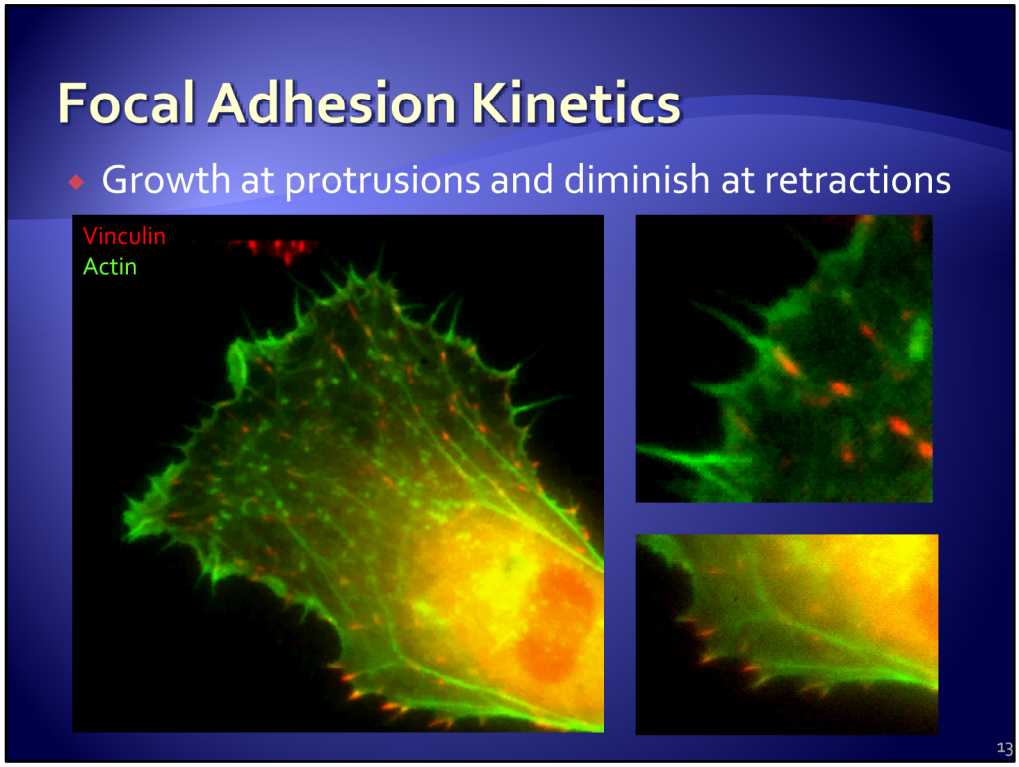


It is believed that single ligand-bound integrins need to cluster together to form a strong, stable adhesion to mechanically connect the cytoskeleton to the extracellular environment. The locations where clustered integrins come together is the focal adhesions. This are the spot-welds of closest contact with the underlying substrate and have direct association to the actin filaments through linking proteins like Talin (Tal), Vinculin (Vin), alpha-actinin (α-Act). Other proteins like Src, Focal Adhesion Kinase (FAK), and PAK have kinase activity and lead to "outside-in" signaling pathway activation that can lead to global changes in cell proliferation or cell shape changes. Paxillin (Pax) is an adaptor protein that serves as a scaffold that allows all the focal adhesion associated proteins to come together and increase their activation.

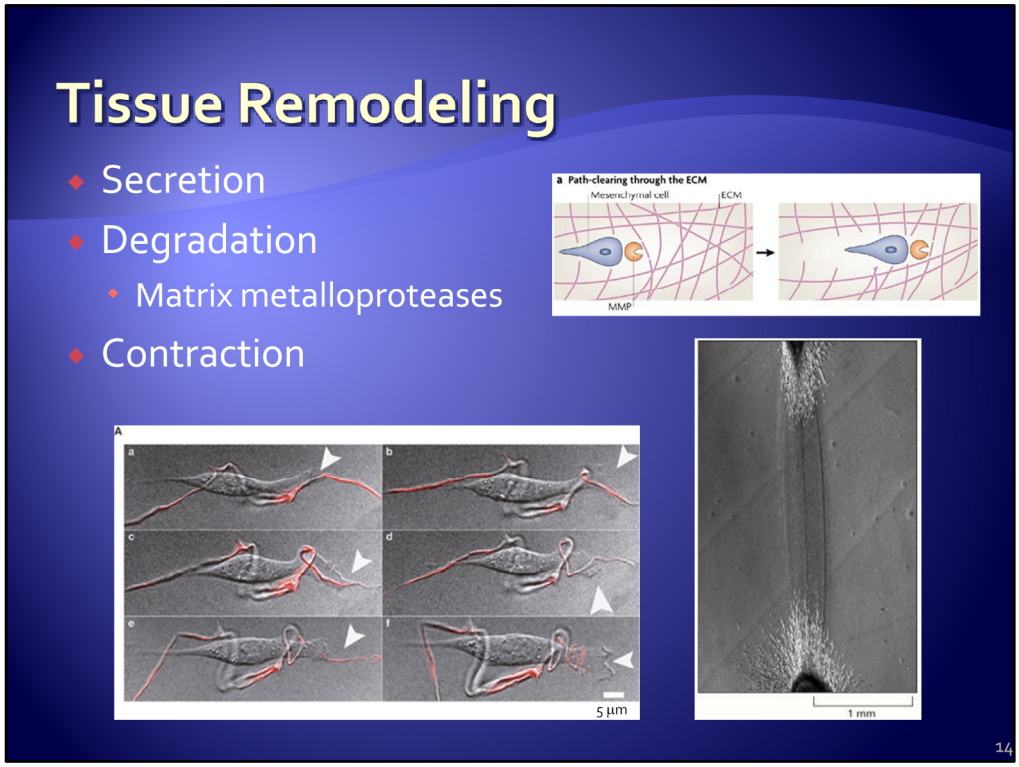


The same proteins that form focal adhesion structures seen in tissue cultures are observable in cells in 3D culture but they do not have the same structural look. When integrins first cluster together, they have the appearance of focal complexes which as transient structures typically at the lamellapodia that may disappear or grow larger and stronger into focal adhesions. The development of focal complexes into focal adhesions is stimulated by Rho and actin-myosin contraction. In vivo, not all cells form focal adhesions but some do, e.g. endothelial cells and smooth muscle. Toward the inside of cells is the fibrillar adhesions which closely associated with fibrillar proteins of the ECM (collagen fibrils or fibronectin fibrils).

Edna Cukierman and Ken Yamada from NIH published a paper in Science entitled “Taking Cell-Matrix Adhesions to the Third Dimension” where the cultured fibroblasts in 3D matrices. They found that the adhesion structures looked more similar to fibrillar adhesions in both size, alignment to ECM fibrils, and focal adhesion associated proteins.



These videos show focal complexes forming at the leading edge. The adhesions grow in size until they become focal adhesions that can connect to stress fibers and support he actin-myosin forces that propel a cell forward (traction forces). At the points of retraction, the focal adhesions appear to slide, which may be due to re-arrangement of the ECM or slippage between the integrin-ligand bonds. You can also see the diminishing in size of the focal adhesions which decreases their strength and promotes retraction.



All ECM proteins come from cells. The mechanical forces that a cell experiences can upregulate the production of MMPs which are proteases digest the matrix proteins. Some are highly specific like collegenases which cleave collagen and help to keep the structure integrity of the matrix intact but promote cell migration.

In the video, the mechanics of lamellipodia formation can pull collagen fibrils in culture. On the right is a picture of two pieces of embryonic chick heart (rich in fibroblasts as well as heart muscle cells) that were cultured on a collagen gel for 4 days. A dense tract of aligned collagen fibers has formed between the explants, as a result of the fibroblasts in the explants tugging on the collagen.

Major Themes from Introductory

- Bio-Chemo-Mechanical Themes
  - Fundamental building blocks
  - Chemical-mechanical relationships in biology
  - Structure-function relationships
- Architectural Themes
  - Whole is great than the sum of the parts
  - Versatile and multifunctional components
  - Emergent properties
  - Connectivity is intra- and intercellular
- System Dynamics Themes
  - Flux within stable structures
  - Multi-input, multi-output behaviors
  - Feedback loops lead to homeostasis or commitment to cell function

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