Cell-Cell and Cell-Matrix Interactions: Focus on Integrins

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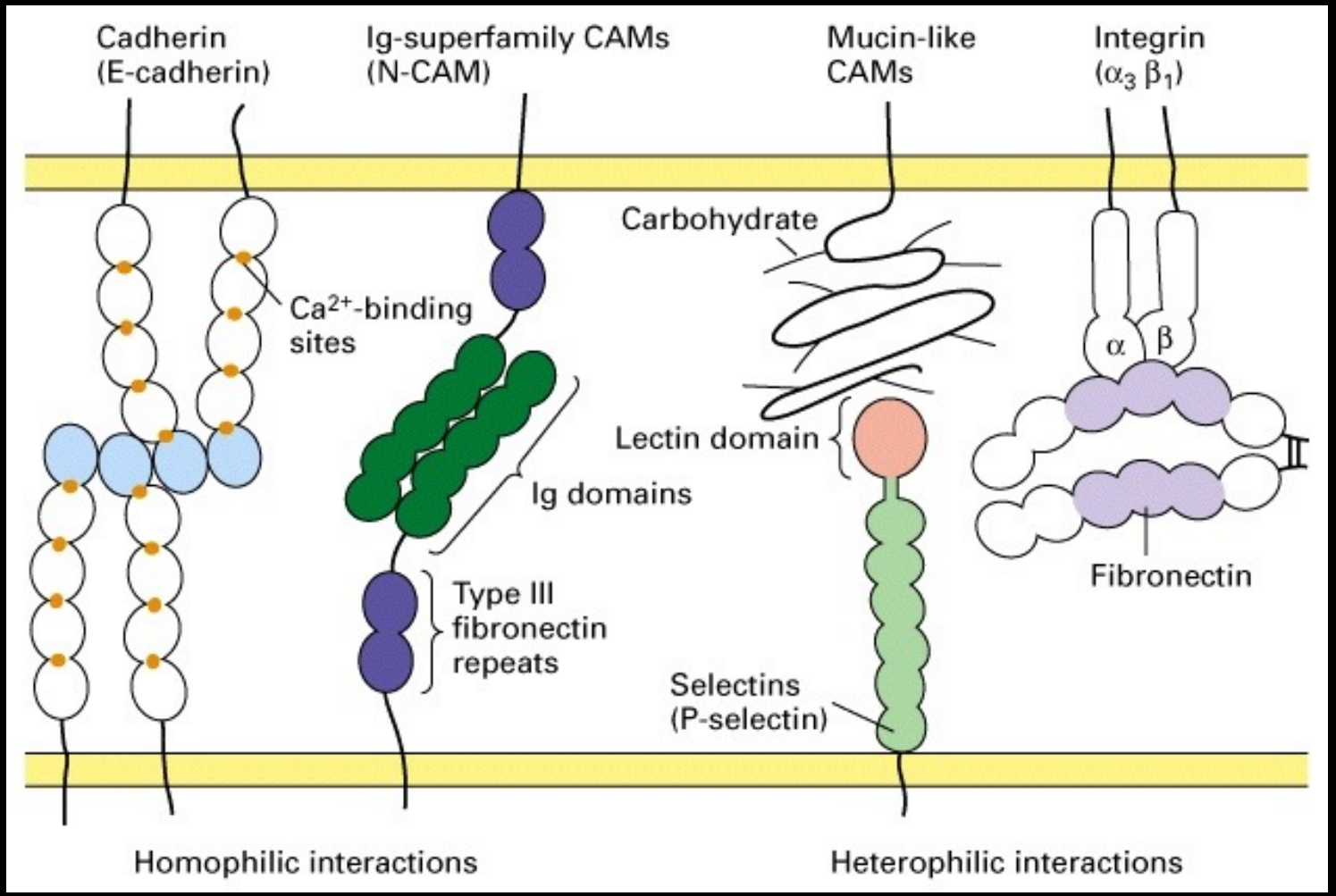
Goals of lecture

1. Context: Cell adhesion and ECM molecules
2. Integrins: molecular structure & function
3. Activation of integrins: mechanism and roles in physiology
4. Examples of human disease based on integrin dysfunction

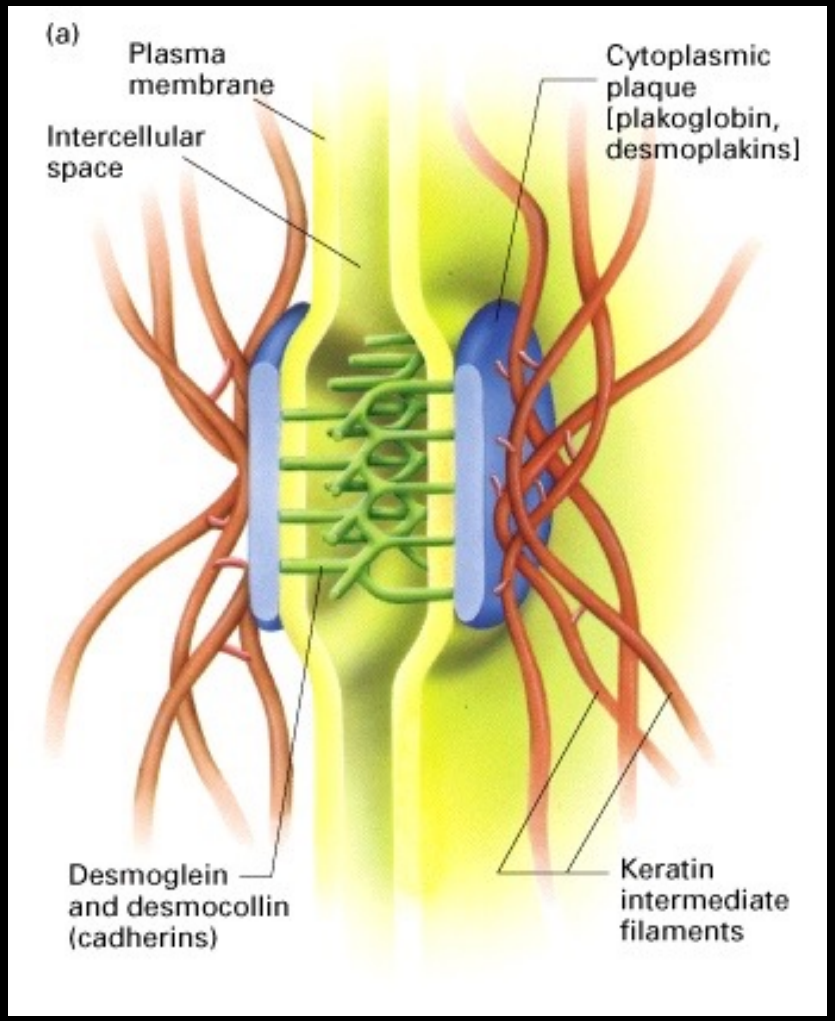
Reading in Textbooks

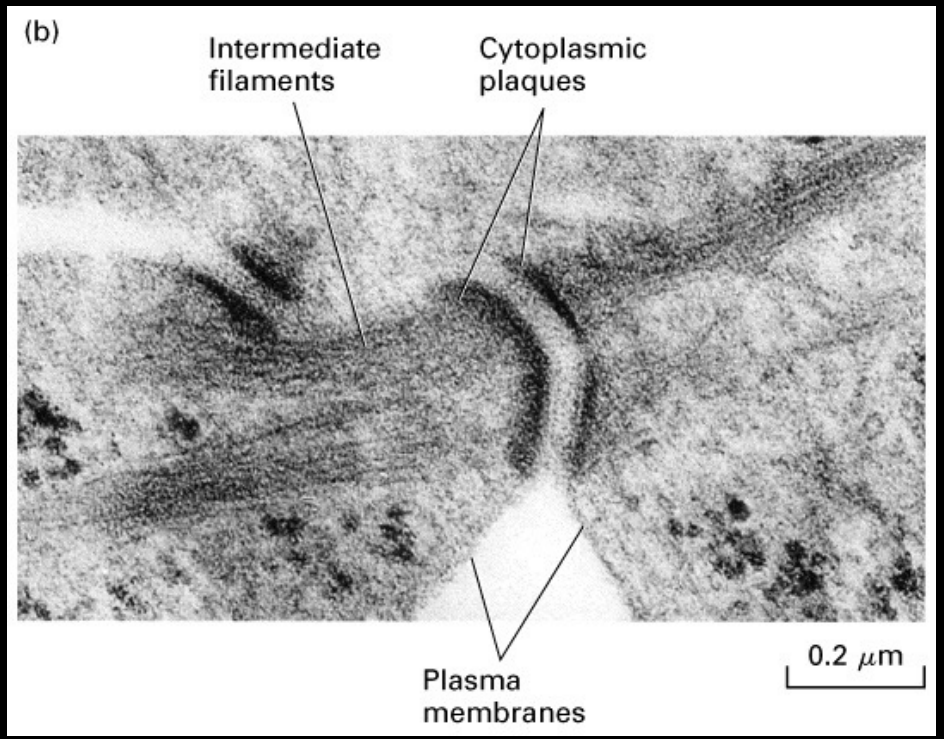
- Alberts et al., Molecular Biology of the Cell, 6th ed., 2014, Garland.
  - Chap. 19 - Cell Junctions and the ECM.
  - Available as eBook, https://bit.ly/2AR19DR.
- Lodish et al., Molecular Cell Biology, 8th ed., 2016, Macmillan.
  - Chap. 20 - Integrating Cells into Tissues.
  - Available as eBook and hard copy, https://bit.ly/2Qy9Hog.
- Pollard et al., Cell Biology, 3rd ed., 2016, Elsevier.
  - Section VIII: Cellular Adhesion and the ECM. Chaps. 28-32.
  - Available as eBook and hard copy, https://amzn.to/2OwoAWC.

Four principal classes of cell-adhesion molecules (CAMs)

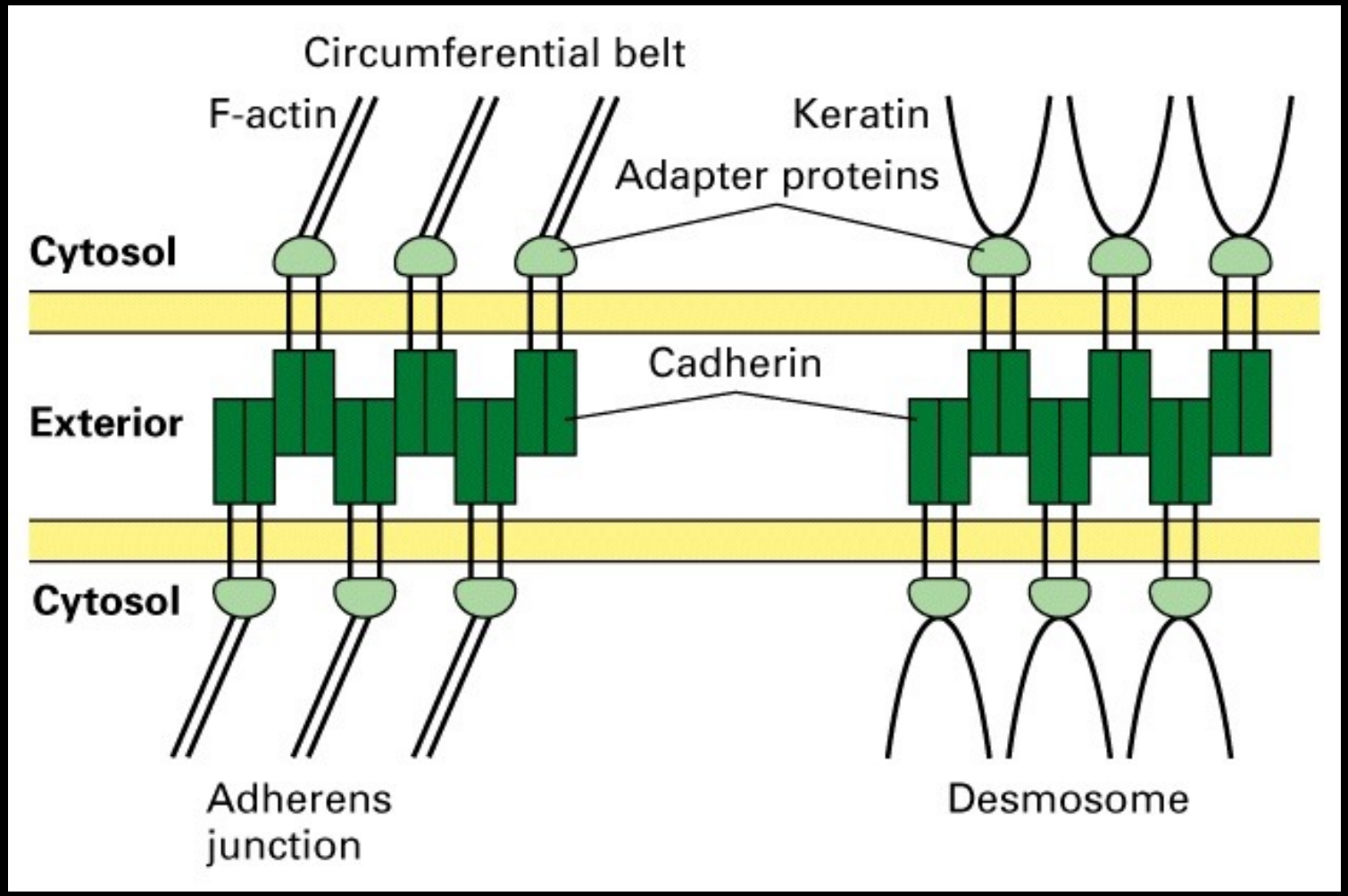


Desmosomes provide strength and rigidity to epithelial layer



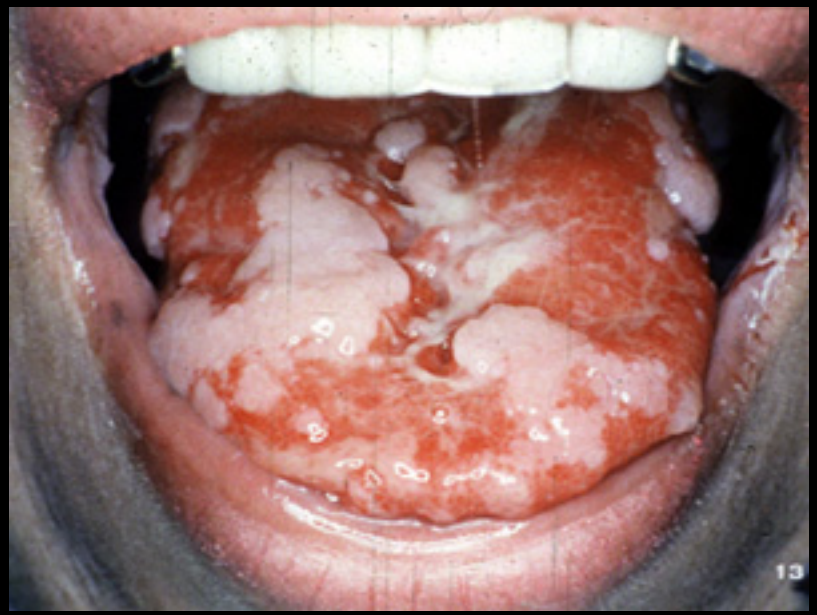


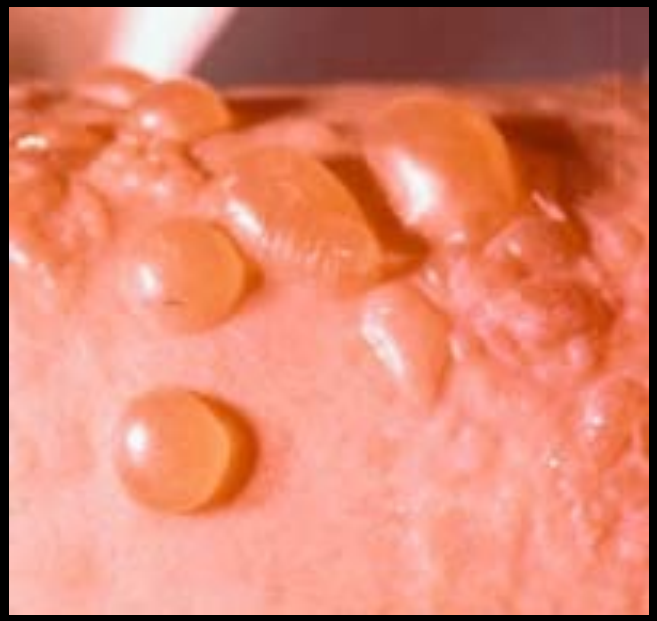
Cadherin junctions connect cells: Adherens Junctions and Desmosomes



Pemphigus Vulgaris: Disease of Desmosomes

Auto antibodies to desmoglein 1 and 3 (type of cadherin)
Disrupt adhesion between epithelial cells
Involvement of skin and mucous membranes



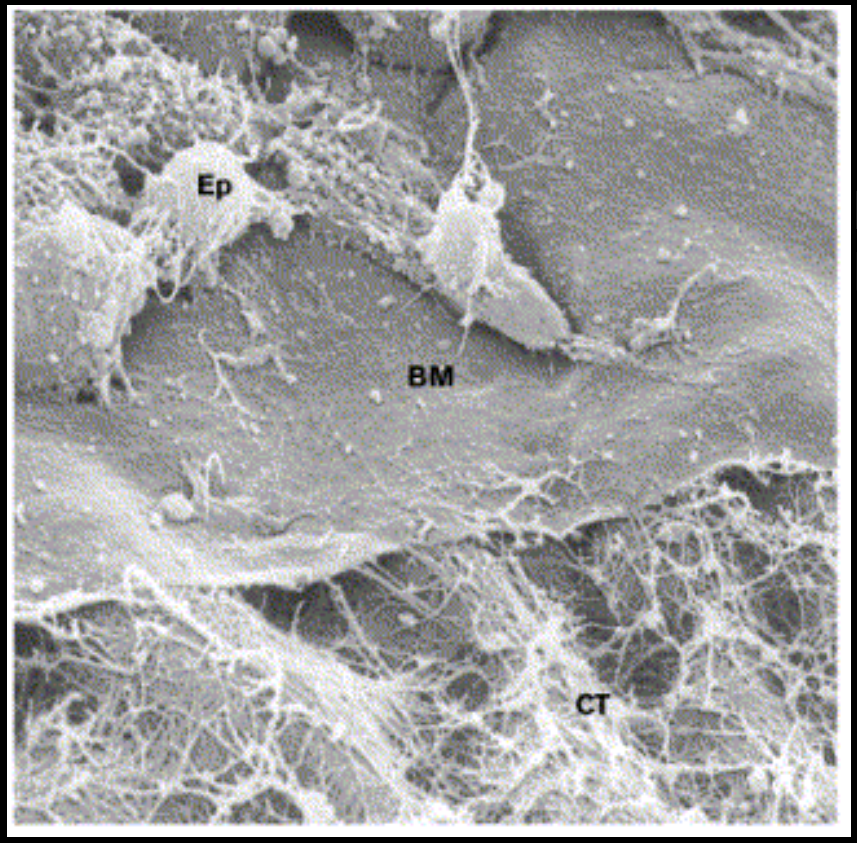


Basement Membranes

- Specialized layers of extracellular matrix surrounding or adjacent to all epithelia, endothelia, peripheral nerves, muscle cells, and fat cells
- Originally defined by electron microscopy as ribbon-like extracellular structures beneath epithelial cells

Basement Membrane

Epithelial Cells

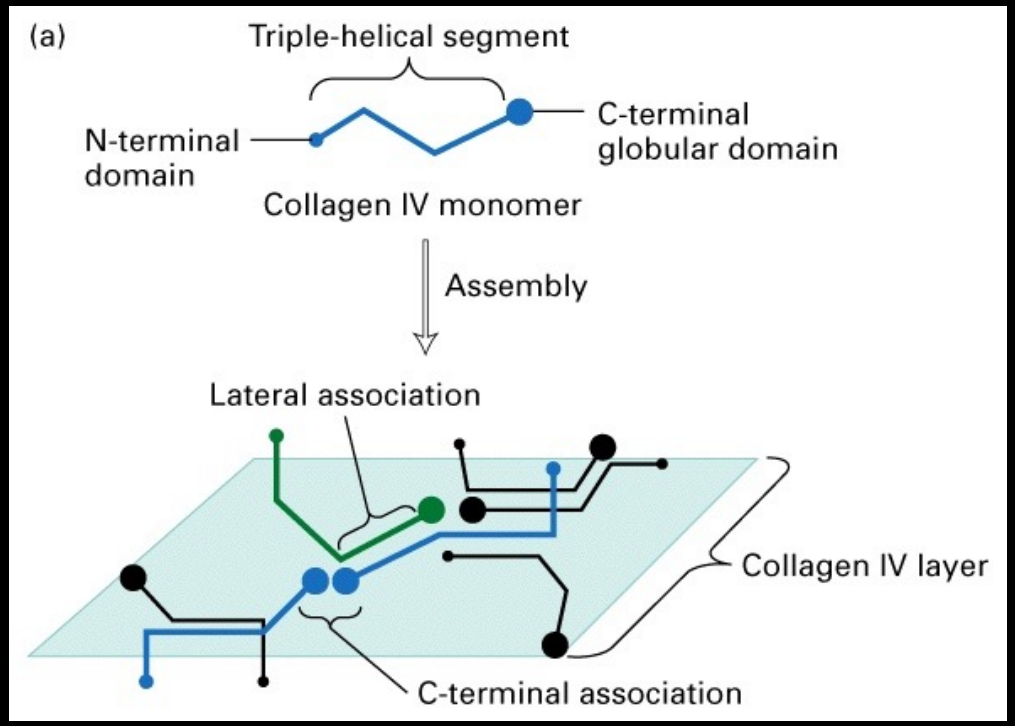


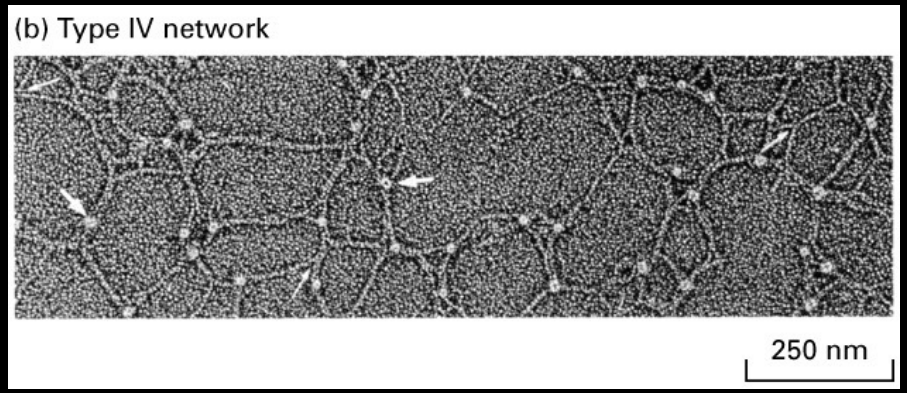
Basement Membrane

Connective Tissue

Schwarzbauer, J. 1999. Basement membranes: Putting up the barriers. *Curr Biol.* 9:R242-4.

Basement membrane
Sheet based on type IV collagen

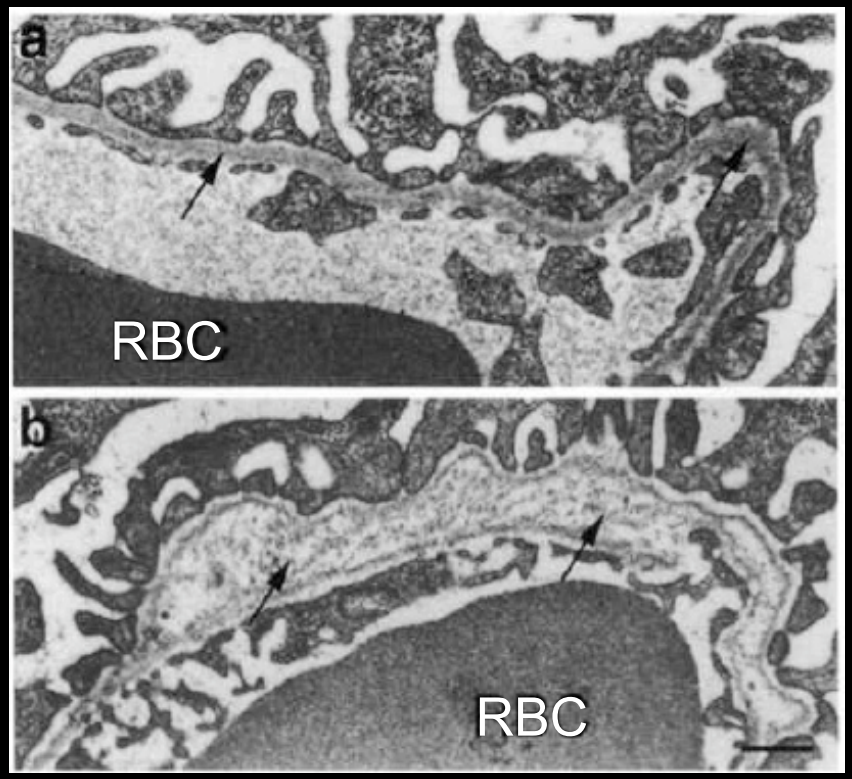




Type IV Collagen Mutations and Human Disease

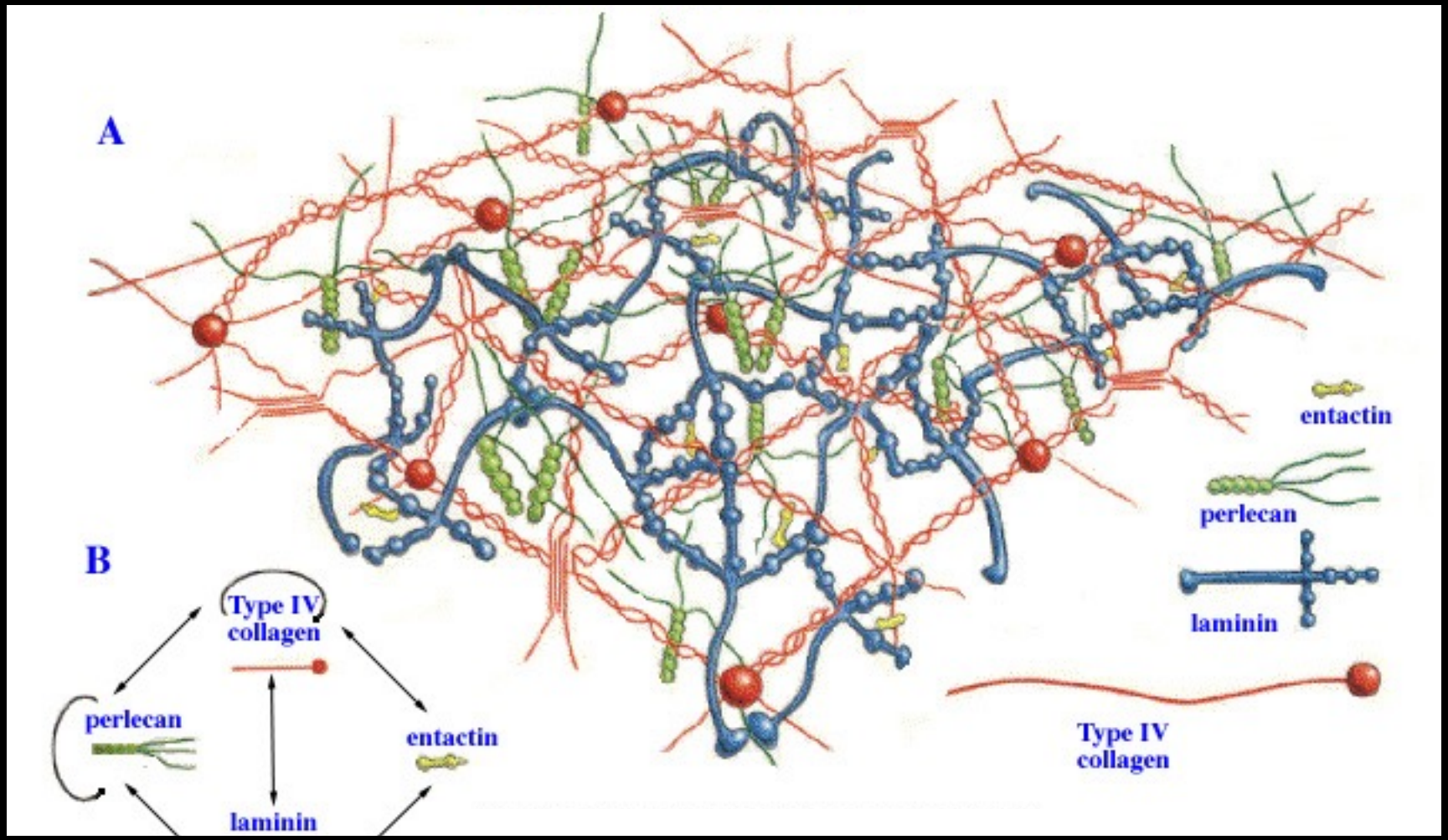
- *COL4A1* mutations
  - Small vessel disease / retinal vascular tortuosity
  - Hemorrhagic stroke
  - Porencephaly (brain cysts)
  - HANAC syndrome
- *COL4A3/A4/A5* mutations
  - Alport syndrome / hereditary glomerulonephritis

Kidney Glomerular BM



Miner JH, *J Cell Biol* 1996.135:1403-13

Components of Basement Membrane

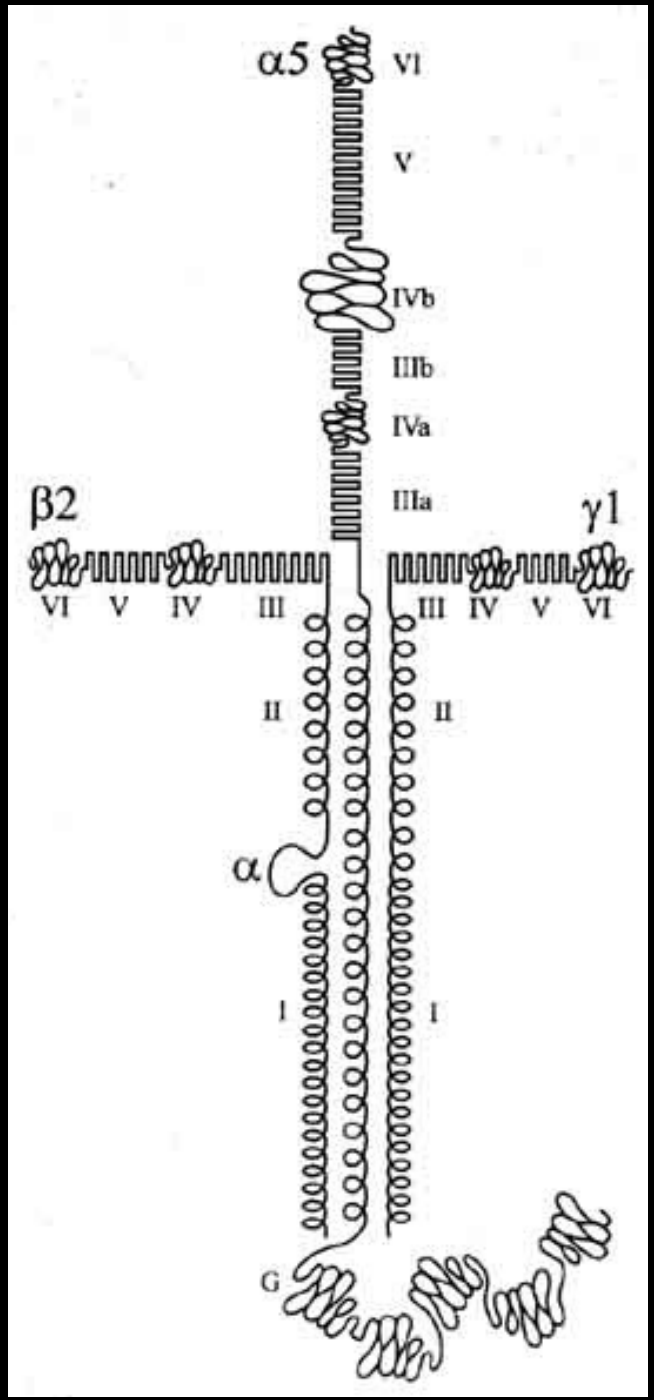


Fredrik Skarstedt and Carrie Phillips

Laminin

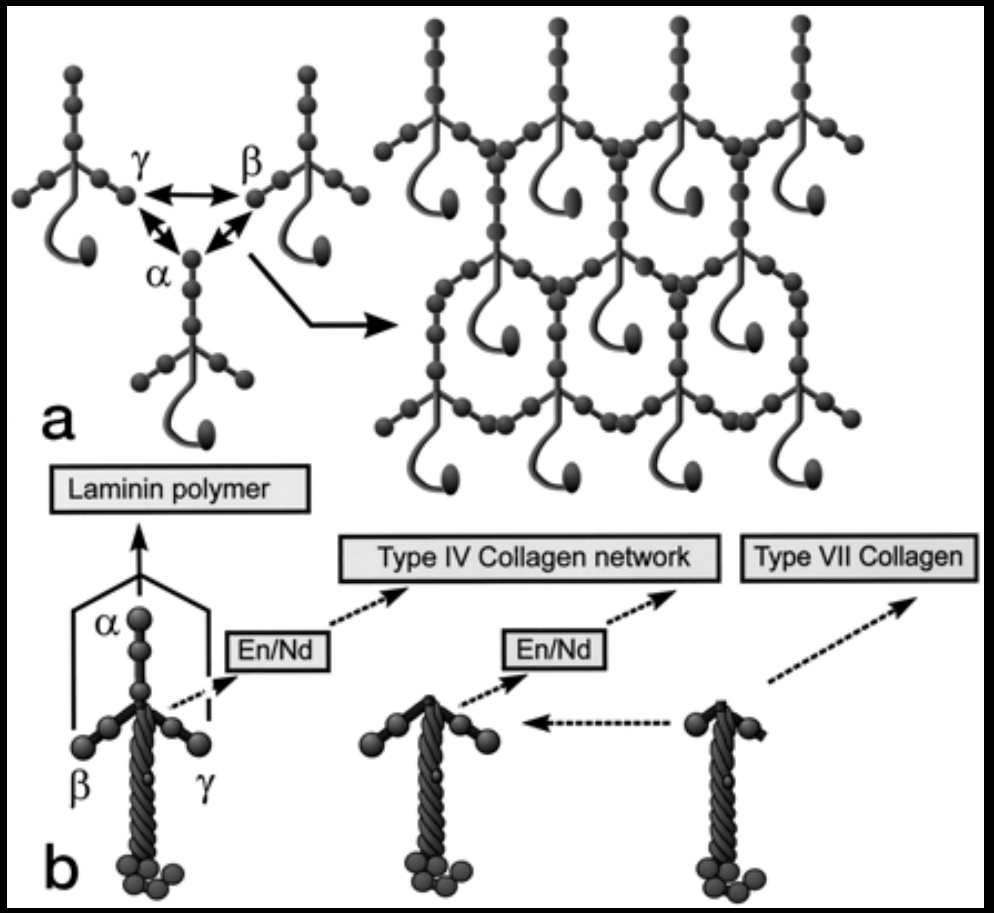
Heterotrimers: Alpha, Beta and Gamma Subunits

- 400 to 800 kDa cruciform, Y, or rod-shaped macromolecules.
- Major glycoprotein of basement membranes - Necessary.
- Chains are evolutionarily related.
- 5 alpha, 4 beta, and 3 gamma chains are known. They assemble with each other non-randomly.
- 15 heterotrimers described to date.



Laminin Trimers Polymerize

- Laminin chains assemble into trimers in the ER and are secreted as trimers into the extracellular space.
- Full-sized laminin trimers can self-polymerize into a macromolecular network through short arm-short arm interactions.
- The α subunit LG domain is left free for interactions with cellular receptors.



Laminin Mutations in Mice (M) and Humans (H) Have Serious Consequences

Lama1, Lamb1, Lamc1: Peri-implantation lethality (M)

Lama2: Congenital muscular dystrophy (M, H)

Lama3, Lamb3, Lamc2: Junctional epidermolysis bullosa (skin blistering) (M, H)

Lama4: Mild bleeding disorder, motor / nerve terminal defects (M); cardiac and endothelial defects (H)

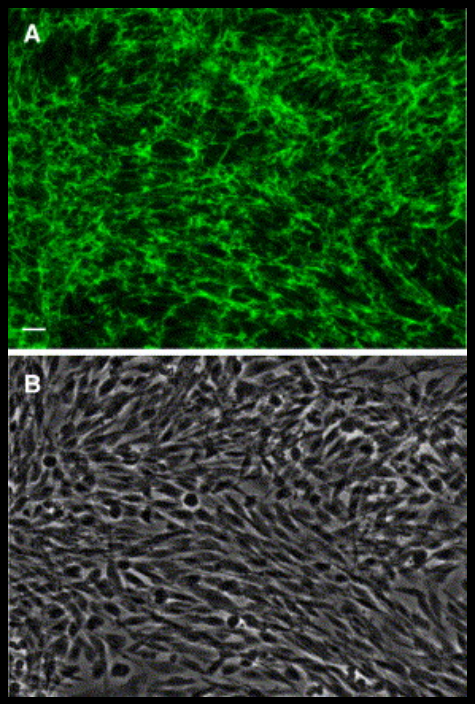
Lama5: Neural tube closure, placenta, digit septation, lung, kidney, tooth, salivary gland defects (M)

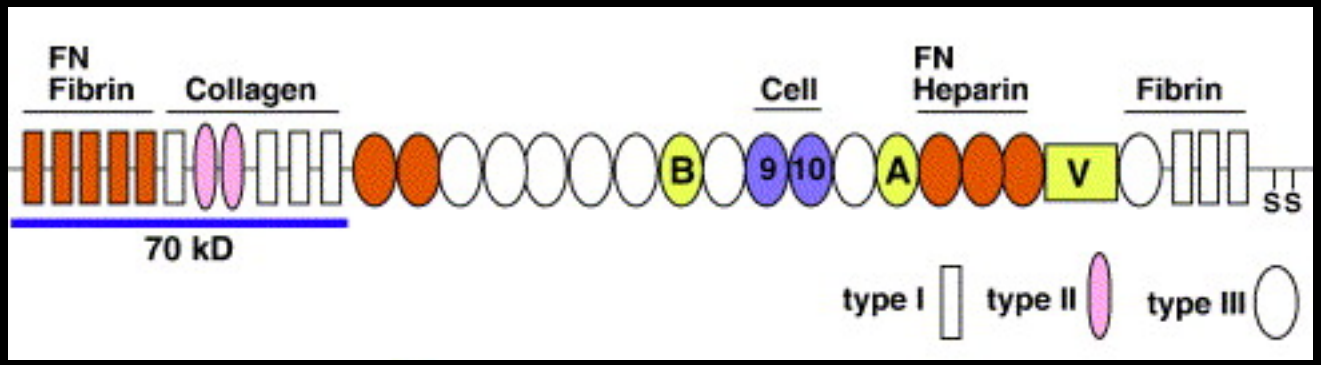
Lamb2: Neuromuscular junction and kidney filtration defects (M); Iris muscle, neuromuscular, kidney filtration defects (H; Pierson syndrome)

Lamc3: Brain malformations, autism spectrum disorder? (H)

Fibronectin (FN)

- Glycoprotein associated with many ECMs and present in blood (plasma)
- Composed of multiple domains of different types
- Alternative splicing generates many isoforms; they heterodimerize via S-S covalent bonds
- **Fibroblasts synthesize FN, secrete it, adhere to it, and respond to its presence**
- Harbors **"RGD"** motif - ligand for various integrins, especially $\alpha 5\beta 1$
- Knockout mice die as embryos with defects in vasculature and heart development

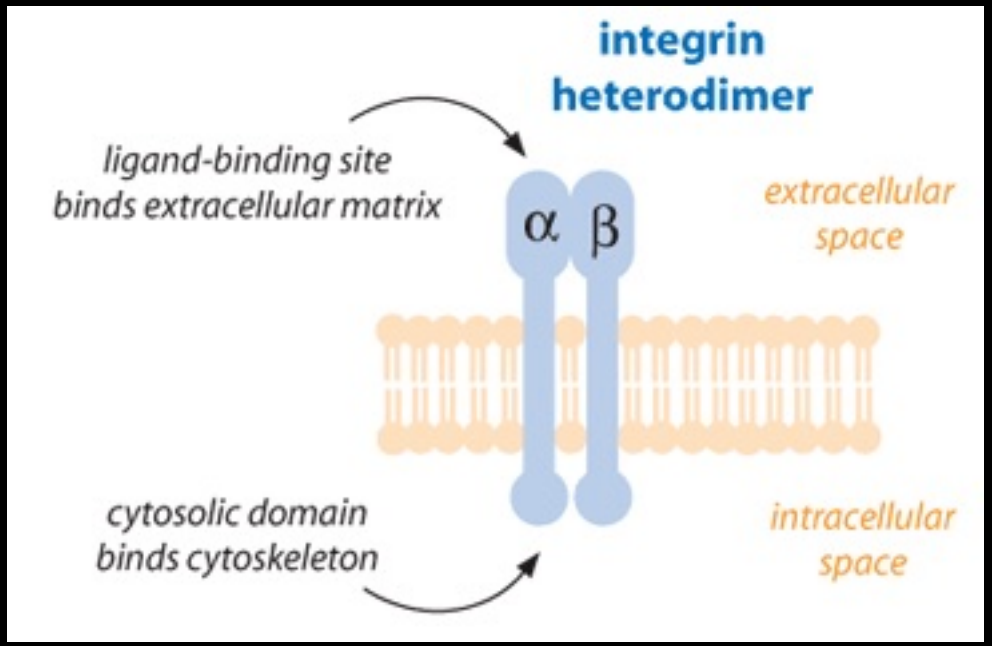




Mao, Y. & J. E. Schwarzbauer. 2005. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biol.* 24:389-399.

Integrins

- Large family of transmembrane receptors for ECM and cell-surface proteins.
- Heterodimers of alpha and beta subunits
- Both subunits contain single-pass transmembrane domains.
- 16 different α chains and 8 different β chains => 22 distinct heterodimers.
- Cytoplasmic tails of both subunits create cell signals, in response to ligand binding.

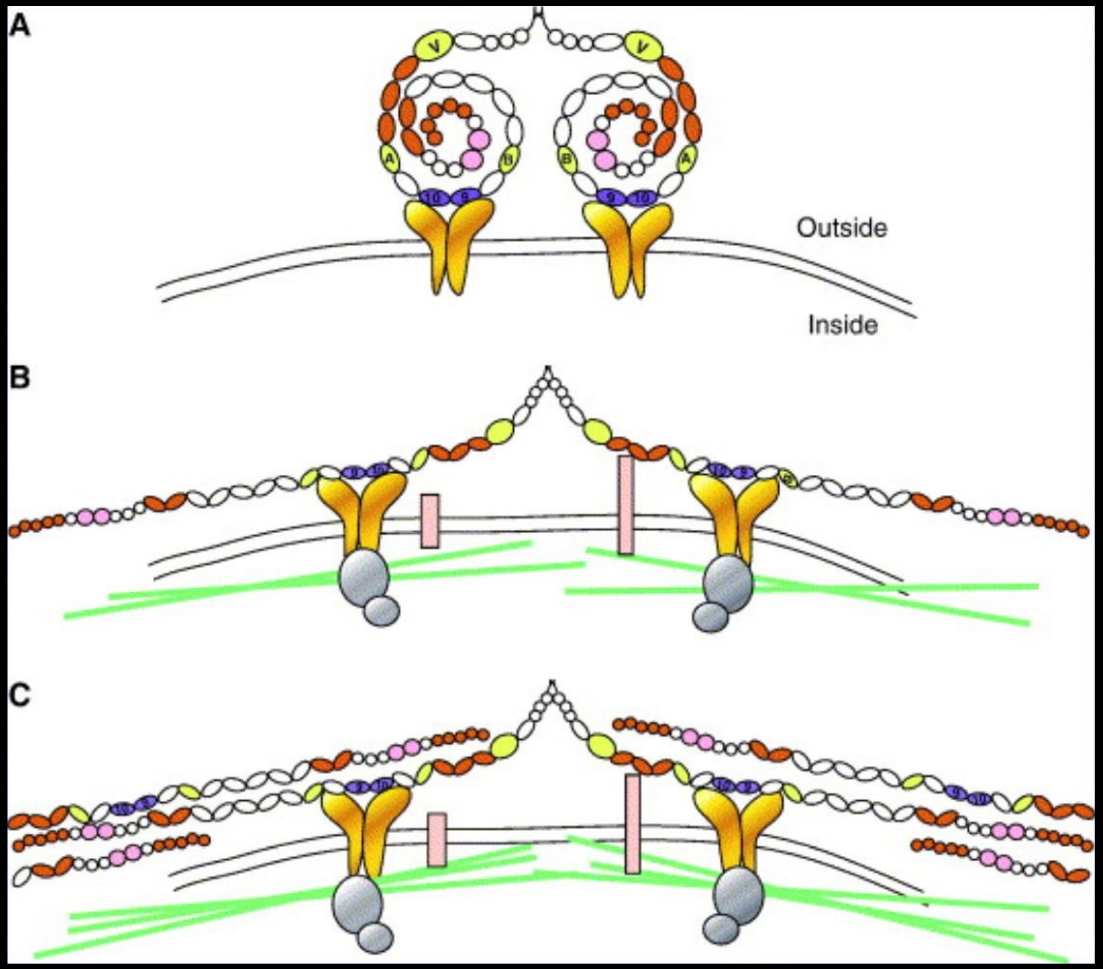


Integrins Direct FN Fibril Formation

Compact soluble FN binds integrin

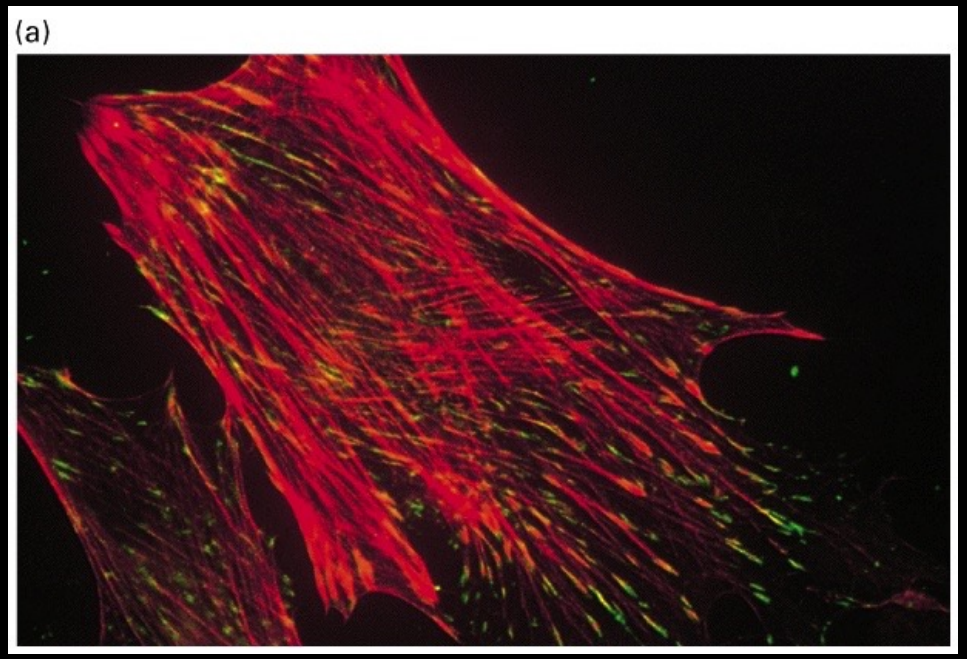
FN binding induces reorganization of actin and signaling

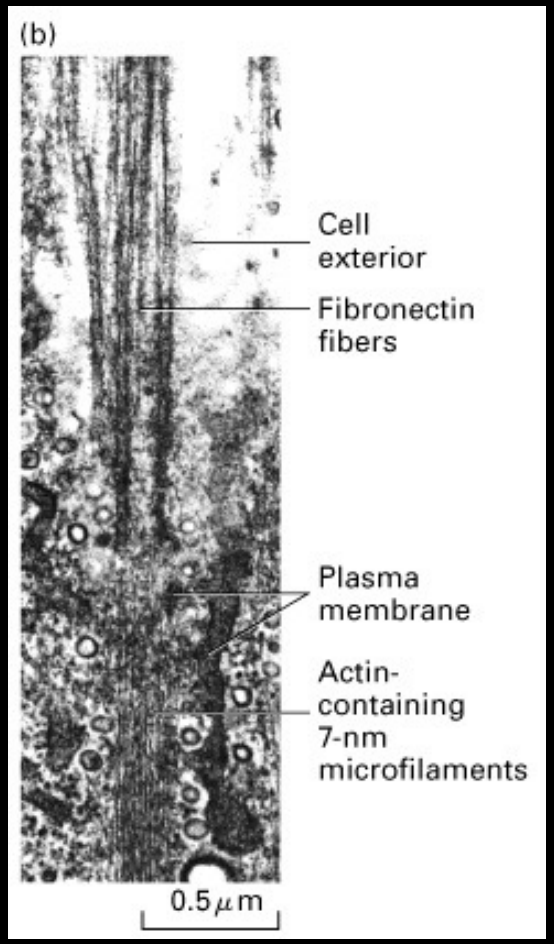
Cell contractility leads to changes in FN conformation, exposing FN interaction domains and allowing fibril formation



Mao, Y. & J. E. Schwarzbauer. 2005. Fibronectin fibrillogenesis, a cell-mediated matrix assembly process. *Matrix Biol.* 24:389-399.

Integrins were Discovered at “Focal Adhesions”





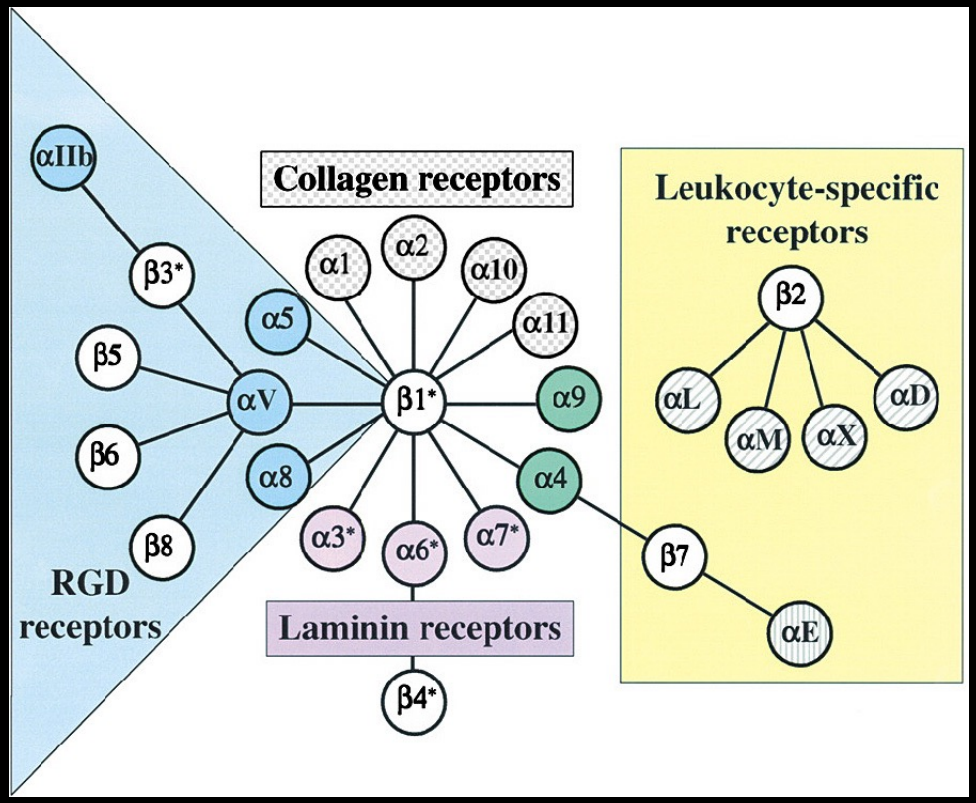
What is the connection across the cell’s membrane that links the sites of ECM contact to the cytoskeleton?

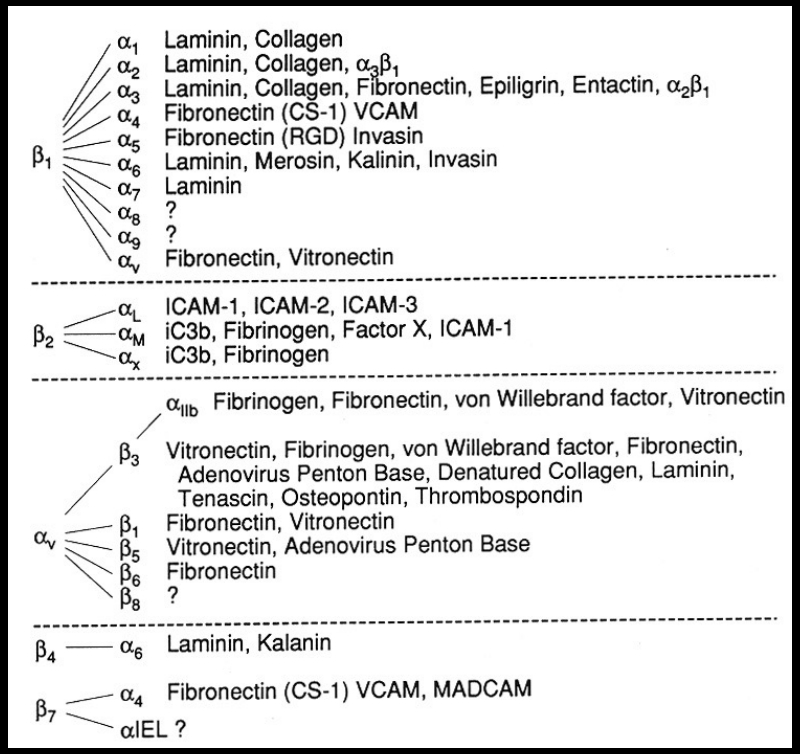
Researchers found monoclonal Abs that blocked attachment and cytoskeletal organization. These Abs found to bind to transmembrane receptor proteins - now called integrins.

Integrins bind to Ligands

- Some integrins bind to a specific site on ECM proteins, composed of amino-acid residues Arg-Gly-Asp (RGD).
- These ECM proteins include fibronectin, vitronectin, and tenascin.
- They function as ligands for the integrin receptor.
- Ligand binding requires divalent cation, e.g. Ca^{2+}.

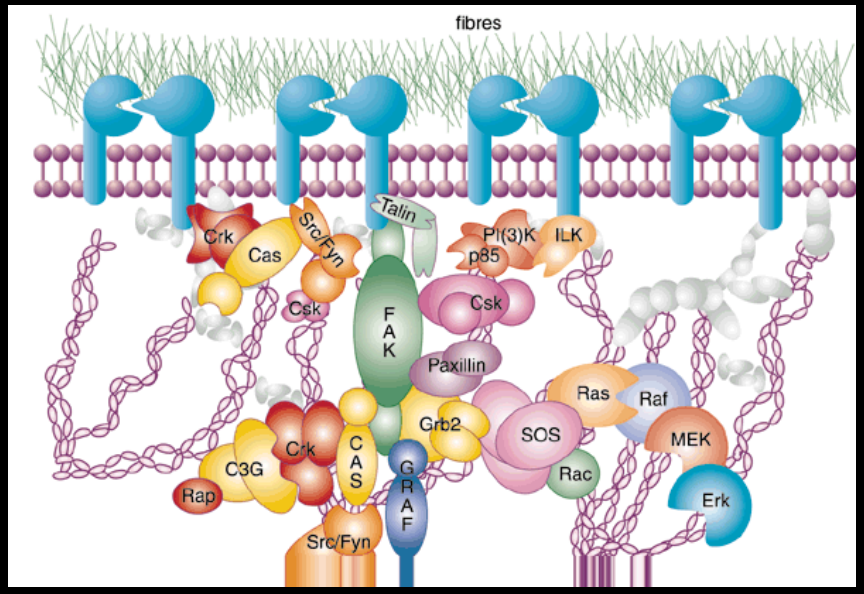
Integrin Family Members and Their Ligands



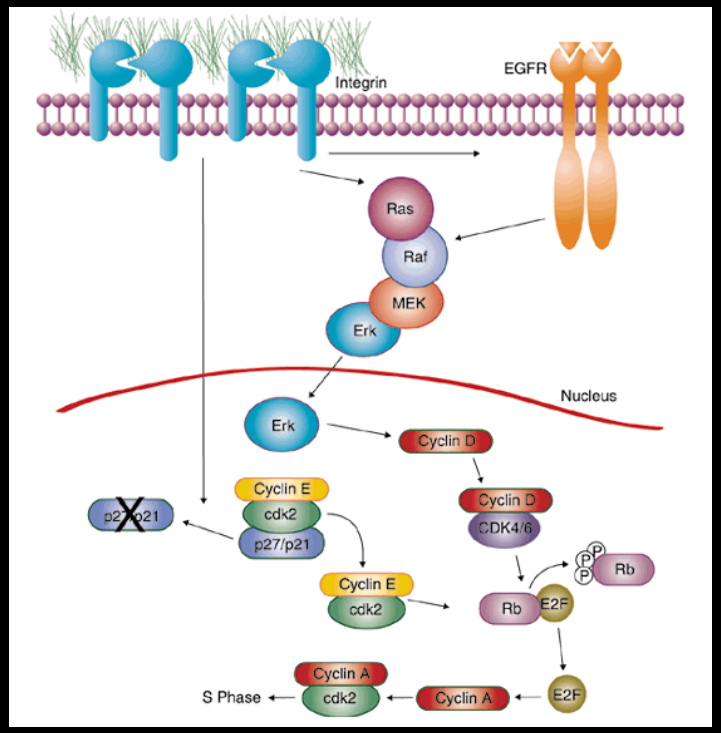


Hynes (2002) Cell 110:673

Integrin Signaling Pathways



Signal transduction proteins associated with, or activated by, integrins. Signaling molecules, such as FAK, bind to and recruit additional signaling molecules, creating a complex signaling network that is intimately connected to the cytoskeletal network.



Integrins and growth factor receptors cooperate in cell cycle regulation. Both growth factors and cell adhesion are required for transmitting signals to the Ras/Raf/Mek/Erk signaling pathway.

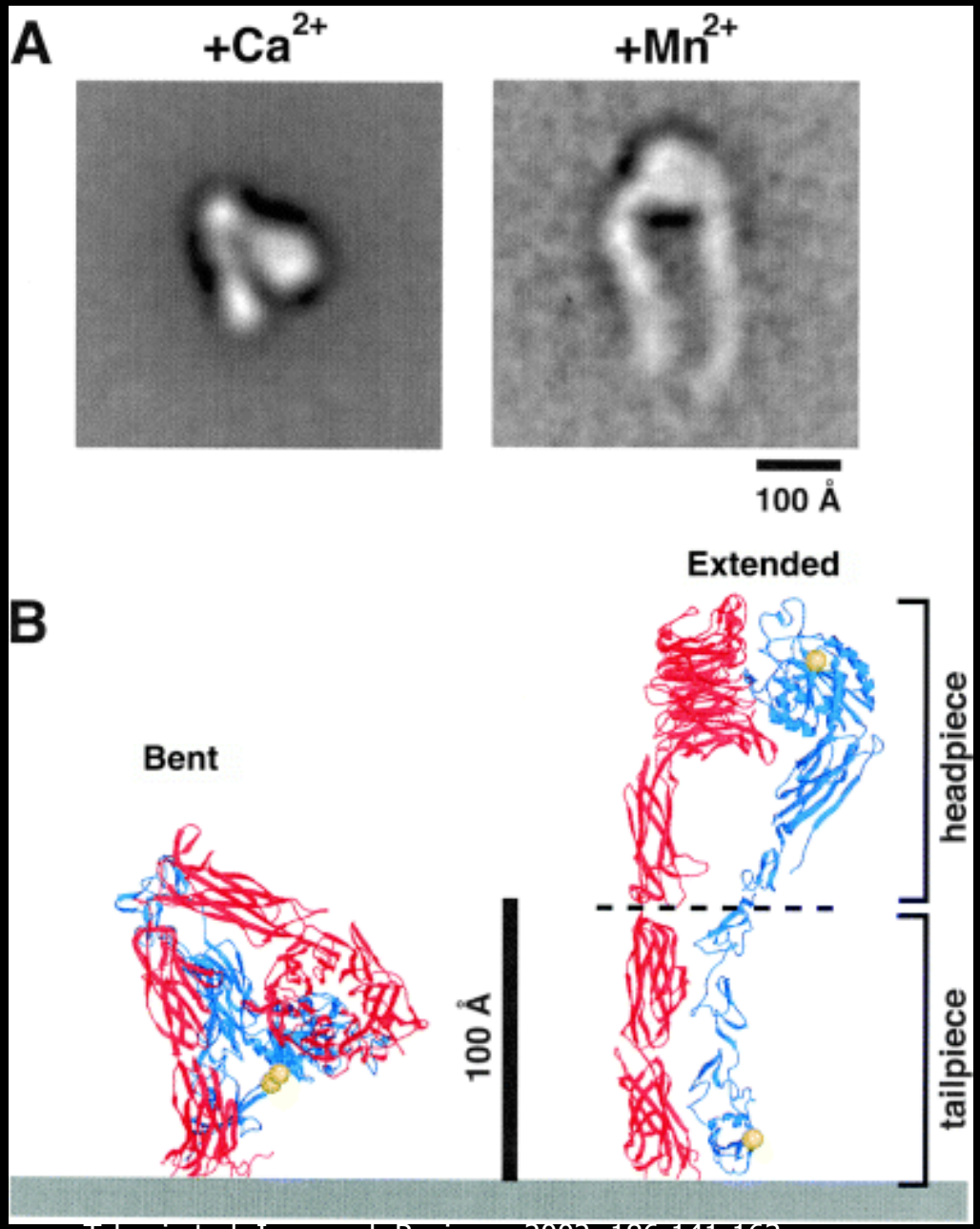
Miranti and Bruge (2002) Nature Cell Biol. 4:83

Activation of Integrin

Activation increases integrin affinity for ligand. Makes the binding site more accessible to ligands

A) EM images of the purified extracellular domain of an integrin. (Ca2+ inhibits ligand binding, Mn2+ stimulates artificially.)

B) Drawing of how images relates to integrin conformation.

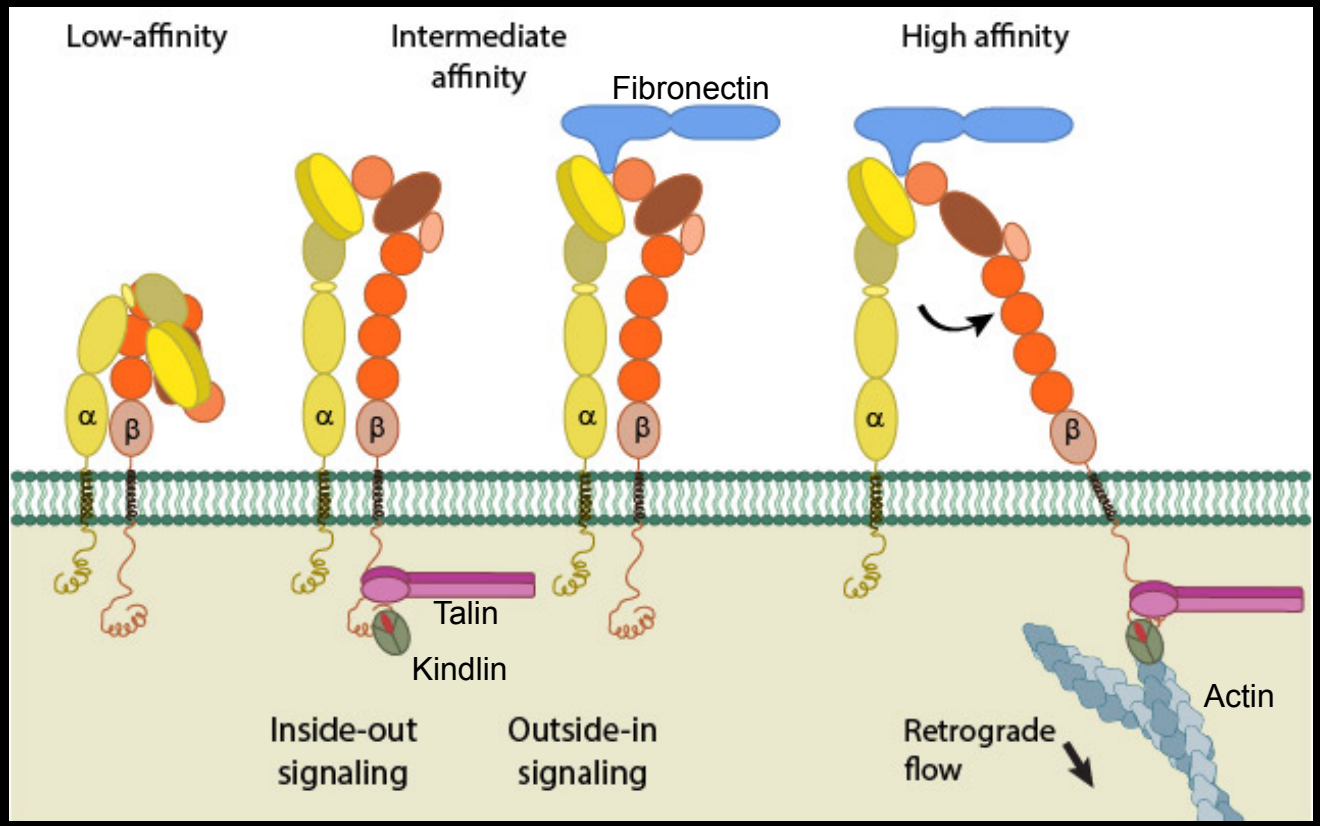


Takagi et al. Immunol. Reviews; 2002, 186:141-163.

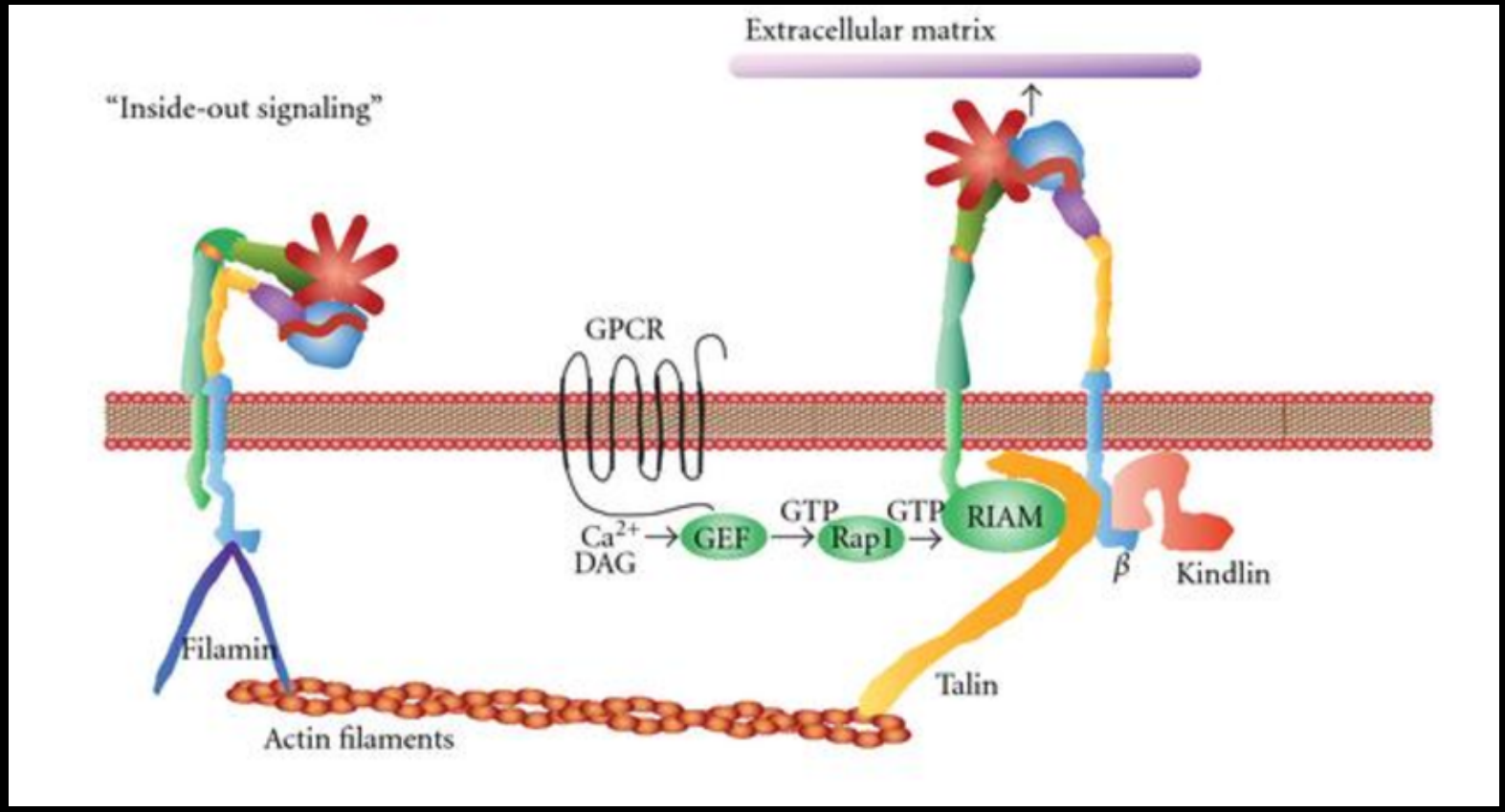
Integrins display “Inside-Out” and “Outside-In” Signaling

Inside-out signaling: Initiated by G protein-coupled receptors that activate kinases leading to RAP1 activation.

Outside-in signaling: Initiated by ligand binding and clustering of integrins leading to activation of Src kinase.



Signaling Pathways Leading to "Inside-Out" Activation



Integrin activation by extracellular agonists, ie, inside-out signaling involves G protein-coupled receptors (GPCRs), kinase cascades and activation of GDP > GTP exchange on Rap1, a small G protein.

Integrins in Human Physiology & Disease

Hemostasis
Osteolysis
Angiogenesis
Metastasis
Hematopoiesis
Immune Surveillance
Inflammation

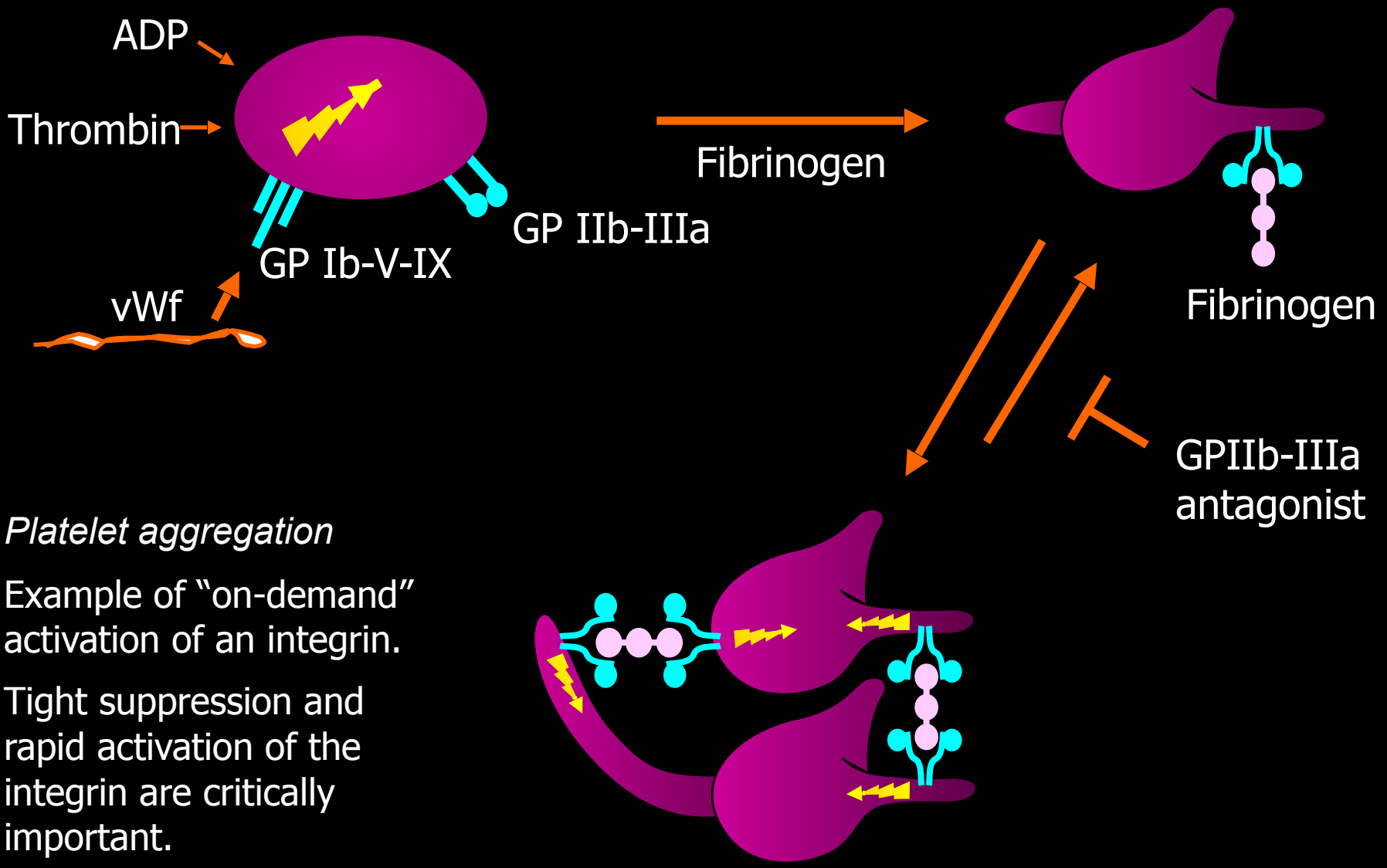
Integrins and Disease

Diseases treated w anti-integrin therapies:

- Crohn's Disease
- Inflammatory bowel disease
- Ulcerative colitis
- Rheumatoid arthritis
- Ischemia-reperfusion injury
- Thrombosis
- Autoimmune diabetes
- Cancer and metastasis
- Occlusive stroke
- Stem cell mobilization (leukemias)
- Psoriasis
- Multiple Sclerosis
- Asthma
- Osteoporosis
- Graft vs host disease
- Atherosclerosis
- Transplant rejection
- Tumor-dependent osteoporosis leading to fracture

Diseases with autoimmune or immunological component

Platelet Aggregation Depends on Integrins

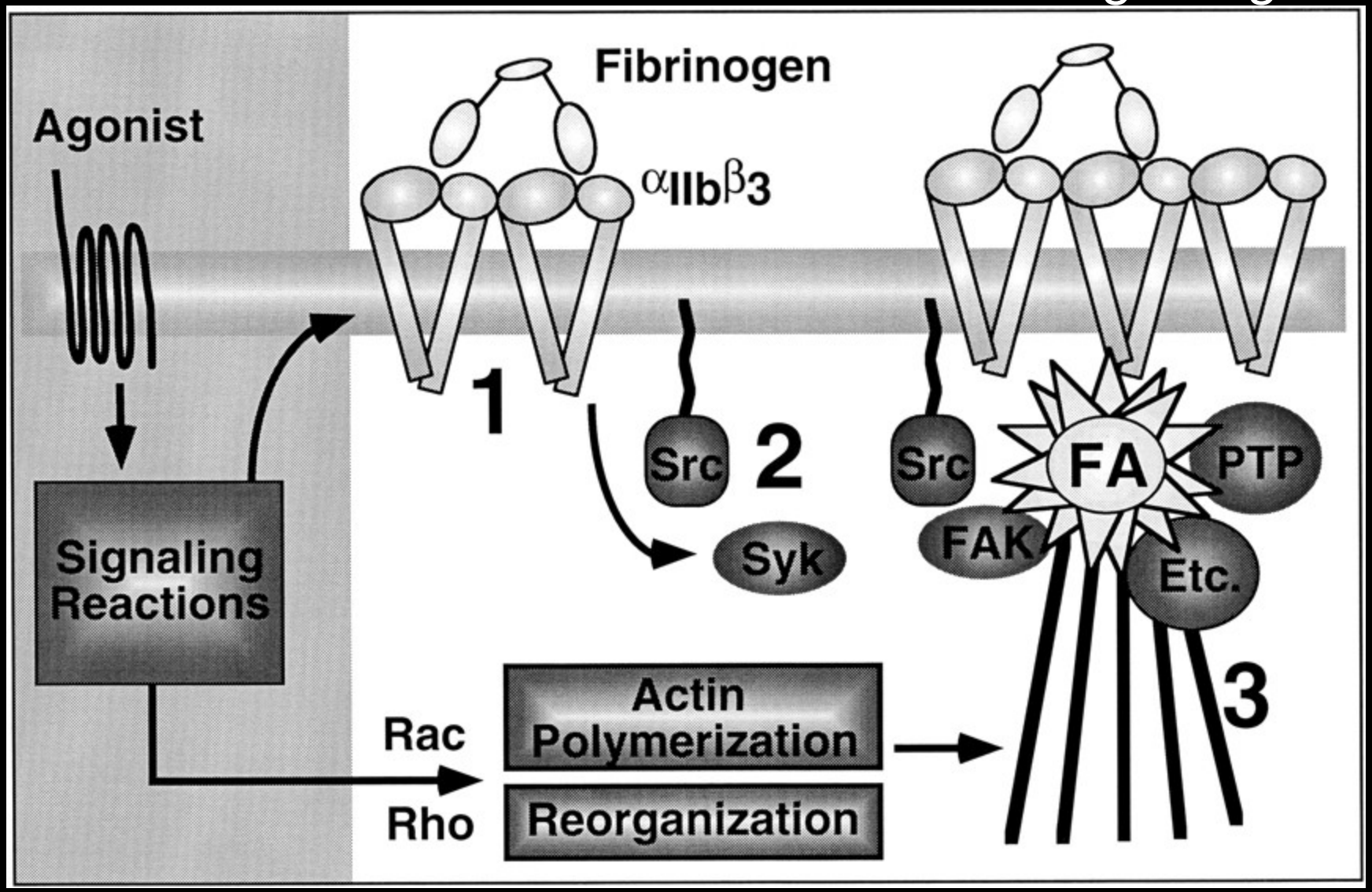


Platelet aggregation

Example of "on-demand" activation of an integrin.

Tight suppression and rapid activation of the integrin are critically important.

Platelet Activation involves “Outside-In” Signaling



Activation of tyrosine kinases (Src and Syk) promote platelet activation, spreading and aggregation.

Glanzmann's Thrombasthenia

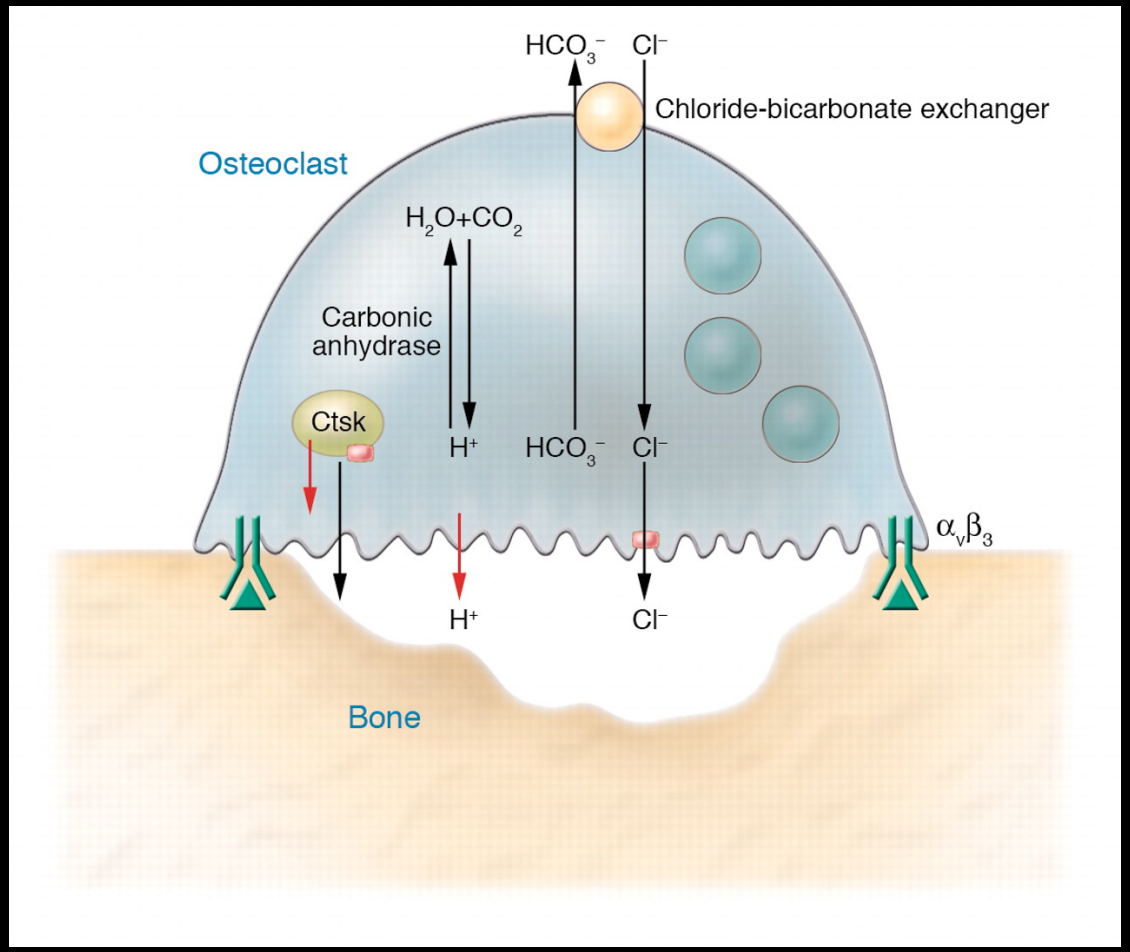
- Platelets - low number in blood and fail to aggregate
- Patients bleed excessively upon wounding, dental work, menstruation. Childbirth can be fatal.
- "Classical" Glanzmann's - mutations in either chain of the platelet fibrinogen receptor glycoprotein IIb/IIIa, or αIIbβ3.
- Treatment: Platelet transfusions or bone marrow transplantation.
  - Transfusion: Mismatched donor platelets can induce anti-platelet Abs, which exacerbate the problem.
  - Bone marrow transplant: Possible graft vs. host disease.

Function of αvβ3 - the “other” β3 integrin

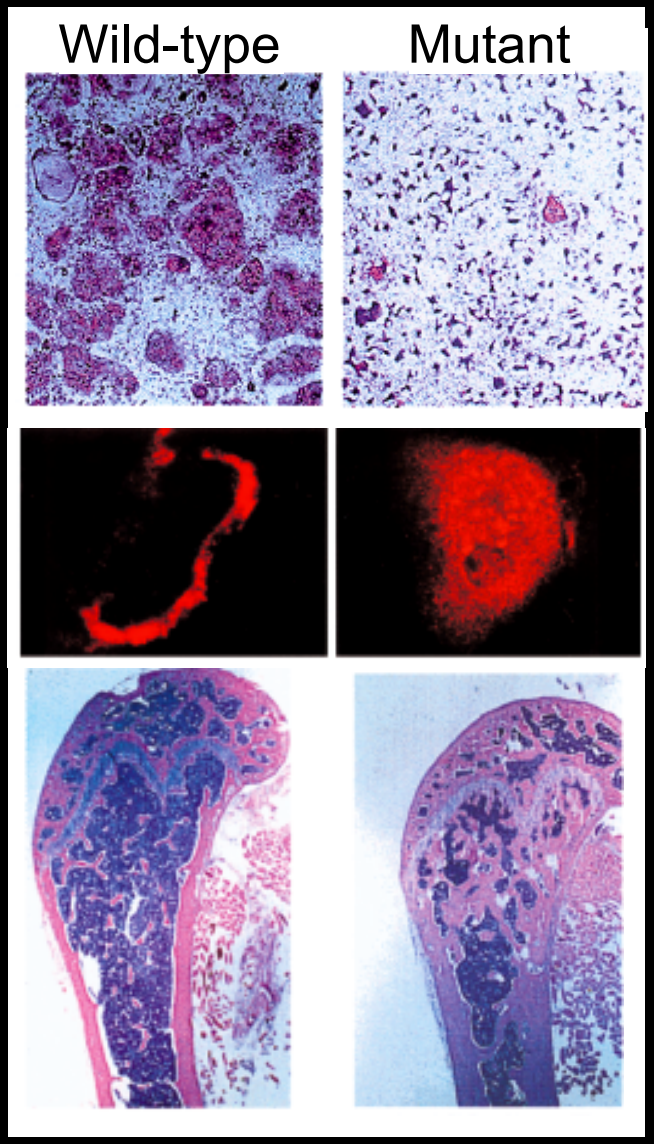
- Osteoclasts- critical for adhesion to bone and bone resorption
- Vascular endothelium- proliferation and survival of vascular endothelial cells during pathologic angiogenesis
- Tumor cells - adhesion, survival, invasion
- Macrophages - phagocytosis of apoptotic cells
- Neutrophils - extravasation and oxidative burst

Osteoclasts use Integrin αvβ3

Osteoclast integrin binds to RGD-containing osteonectin on bone matrix. Forms one huge circumferential “focal adhesion” that makes a seal. Cell secretes acid and degradative enzymes to digest the bone.



Defective Osteoclasts in Mice Lacking Integrin β3



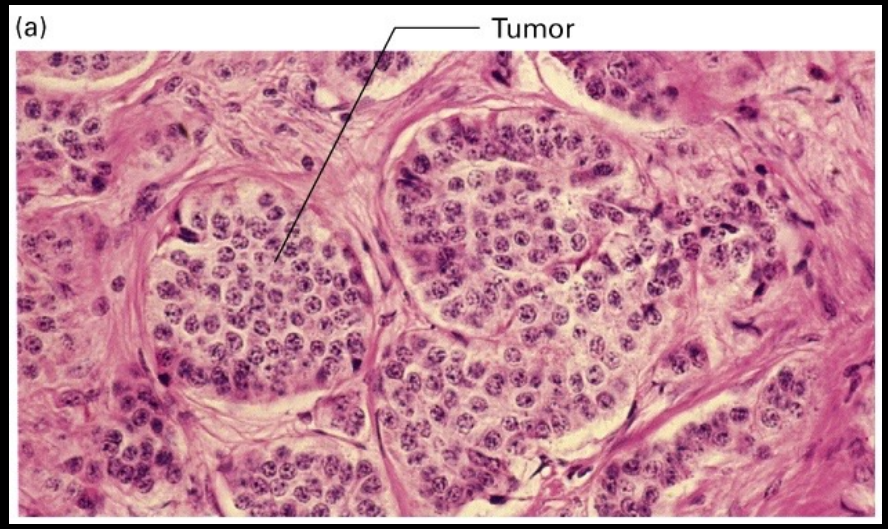
Osteoclasts (big cells): Not large, not spread

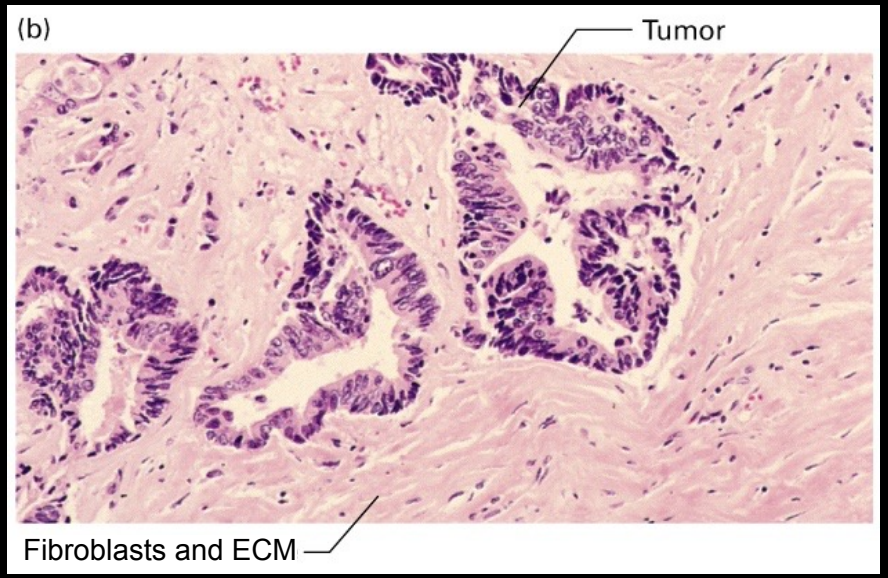
Defective Ring of Actin Filaments at the Seal

Marrow Space: Increased Trabecular Bone

McHugh *et al*. 2000. Mice lacking β3 integrins are osteosclerotic because of dysfunctional osteoclasts. *J Clin Invest.* 105:433-440.

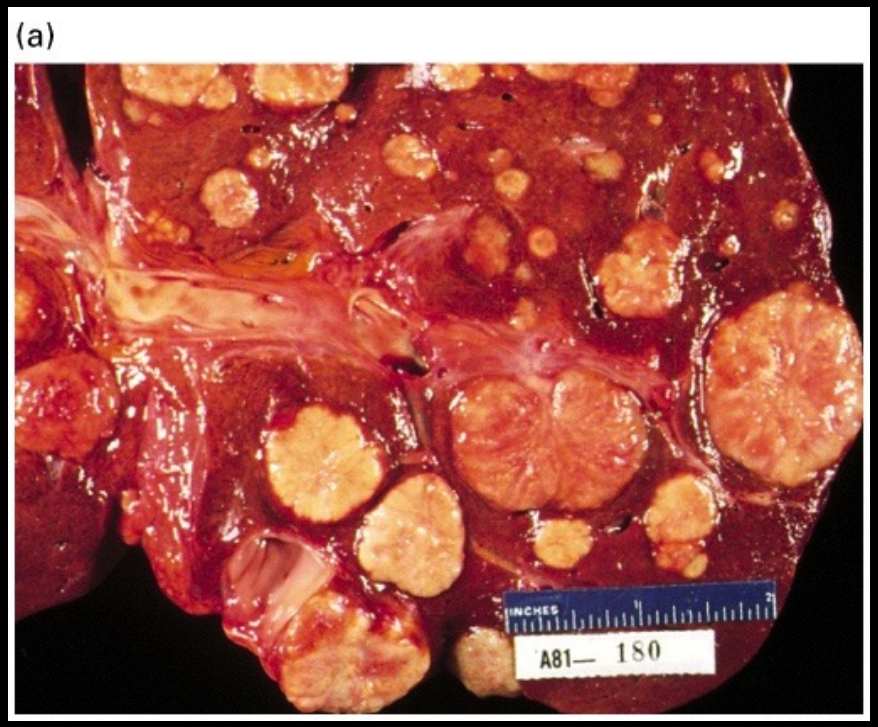
Integrins and Cancer

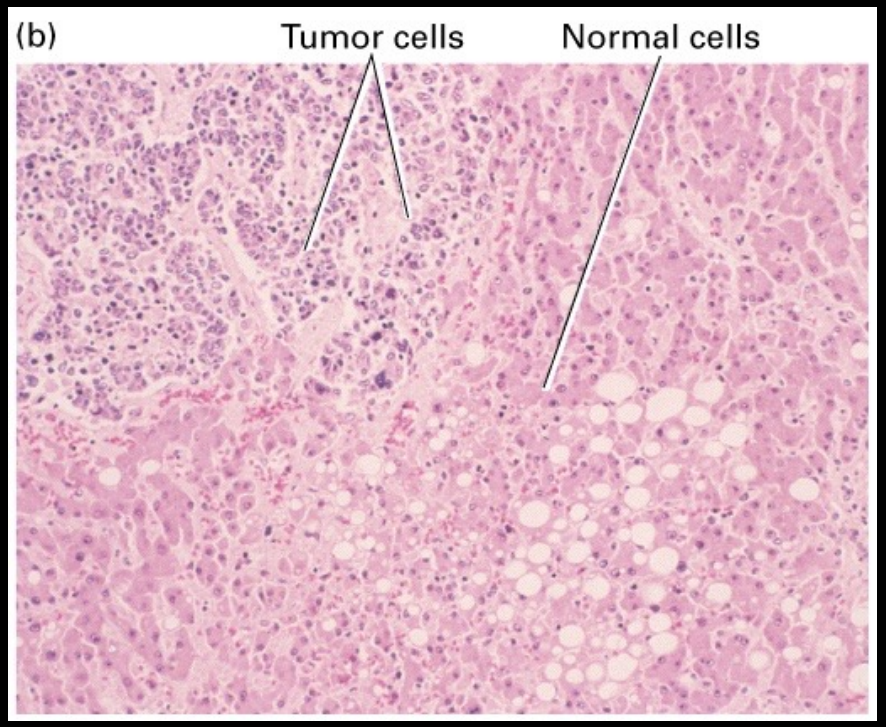




Left: Benign tumor, surrounded by basement membrane.
Right: Malignant tumor, invading surrounding interstitial tissue.

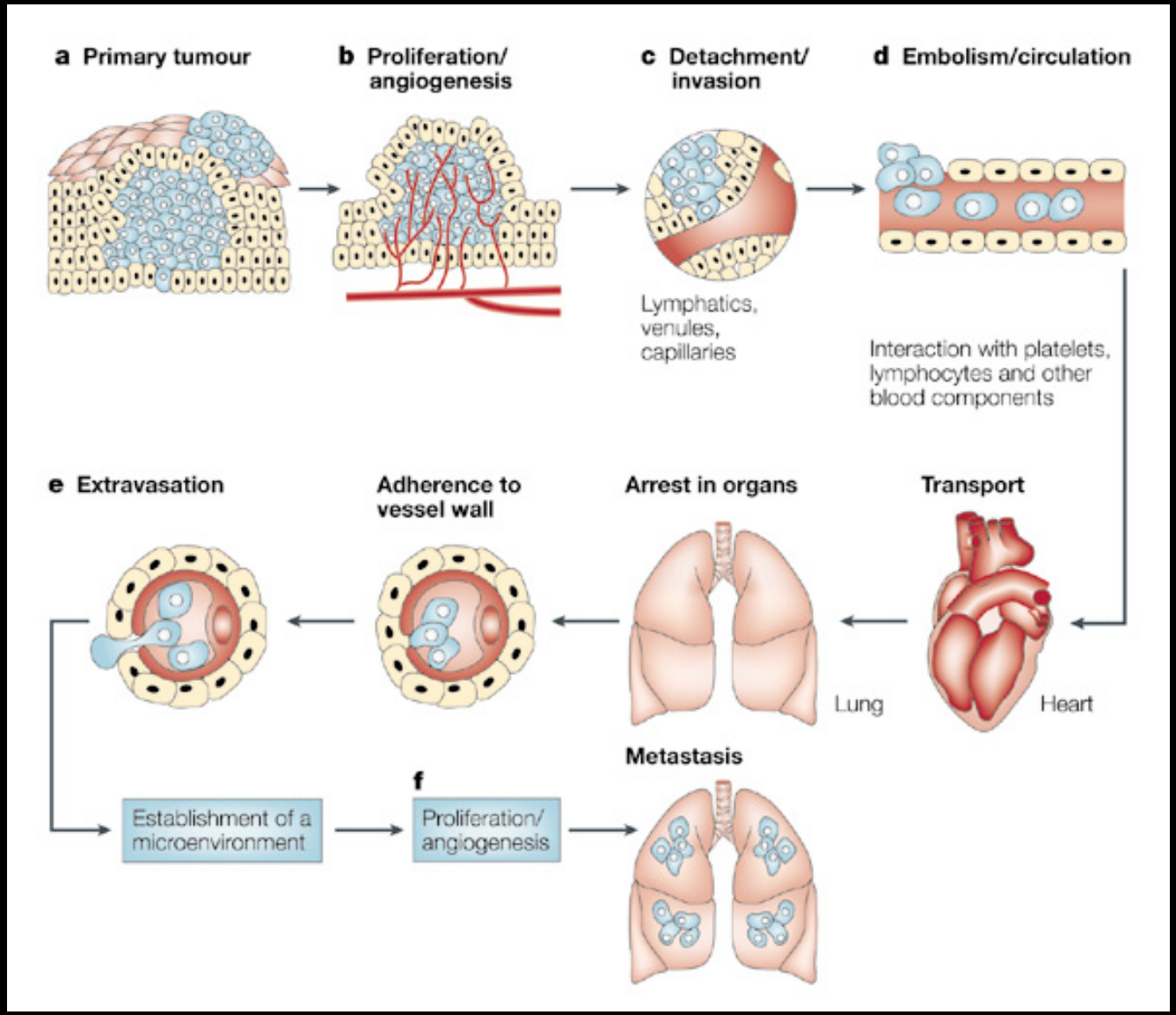
Malignant cancers: Invade surrounding tissue then spread to other organs





Decreased cell-cell interactions, increased protease production, and formation of new blood vessels. Note absence of basement membrane barriers in (b).

Integrins Have Roles in Many Steps of Metastasis

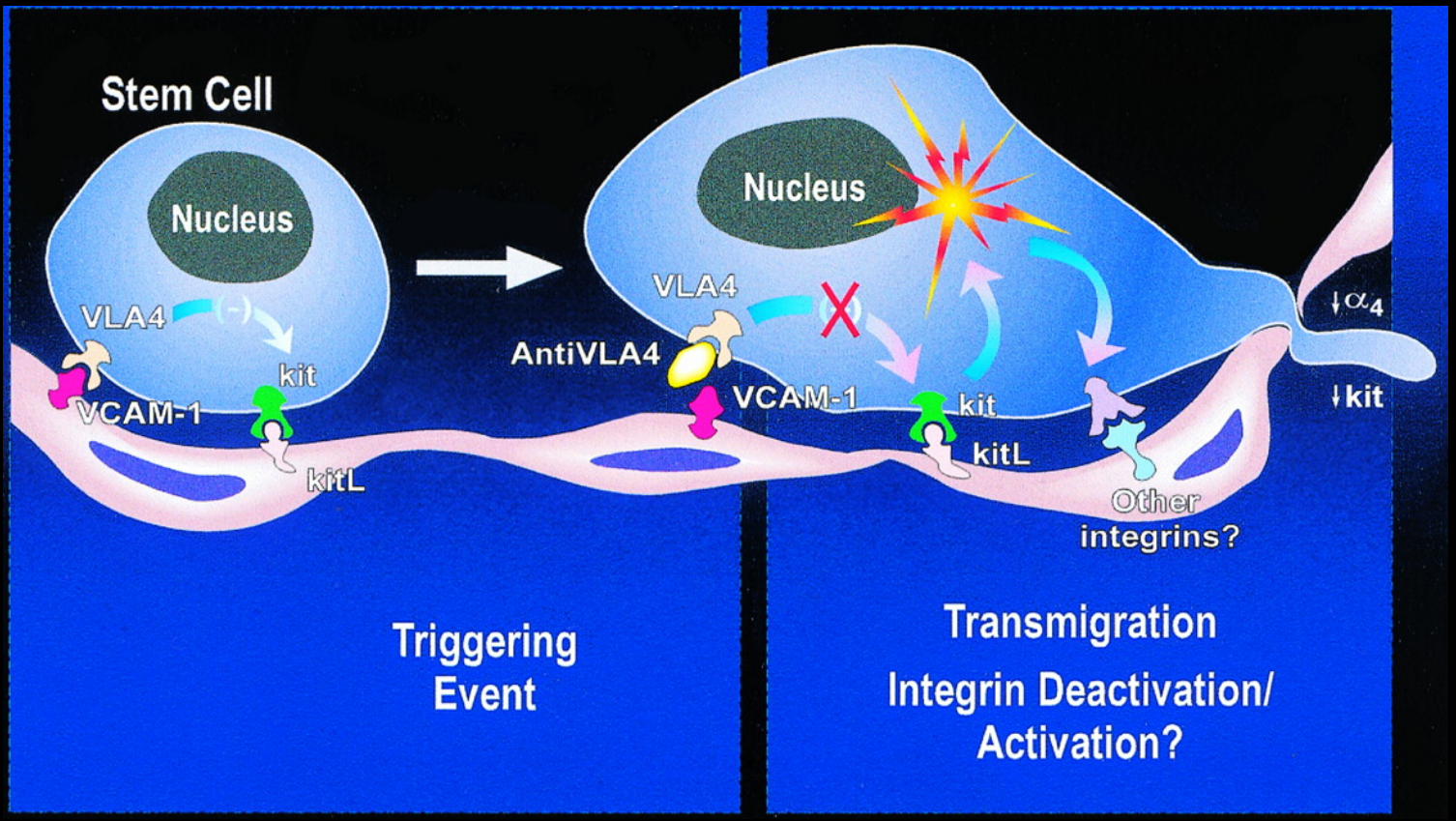


Fidler, I. J. 2003. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. *Nat Rev Cancer.* 3:453-458.

Proteins Involved in Metastasis

- Integrins: Receptors for cell - extracellular matrix (ECM) interactions
- Cadherins: Receptors in cell-cell interactions
- Matrix Metalloproteases (MMP): Degradation of ECM and adhesion proteins
- Growth factors and their receptors
- Angiogenic factors: VEGF, FGF.
- Transcription factors and their regulators

Releasing Stem Cells from the Bone Marrow



Integrin (VLA4) on hematopoietic stem cell (HSC) binds VCAM in bone marrow “niche.”

Receptor (CXCR4) on HSC binds SDF1 on osteoblast / stroma.

G-CSF, α4β1 inhibitors or CXCR4 antagonists disrupt these interactions, releasing stem cells into the circulation, for harvest.

Therapies with Proteins Targeting Proteins

Receptors, including integrins, are targets for therapies called “biologics”. Biologics are proteins produced by living cells (bacteria, animals, plants). They affect interaction of ligands with cell surface receptors.

These “drugs” pose many challenges in development, delivery, safety and ultimately cost. They challenge the traditional pharmaceutical definitions of “composition” and patentability.

For the companies and FDA, how does one define a biological as “generic”? How does one define and regulate a related molecule?

Types of Biologics

Ligands

-insulin purified from pig or cow pancreas - early example

-growth factors and hormones produced in animals or cultured cells

Monoclonal Antibodies (mAbs)

-first raised in mice vs human antigens

-now “humanized” to decrease recognition as immune targets

Decoy Receptors

-soluble, extracellular domains of receptors bind ligand in circulation

-prevents ligand from reaching receptor on cell surface

-examples: soluble VEGF receptor, TNF-α receptor

Nucleic Acid Biologics

-gene therapy (vectors a concern)

-antisense approaches: siRNA, antisense oligos, miRNAs

Lecture Summary

- Cell adhesion molecules have dynamic and essential functions in human physiology, derangements of which often cause disease.
- Cell-adhesion molecules (CAMs) involved with structural or surveillance roles are always “ON” - cadherins, mucins and selectins.
- Integrins are a class of highly regulatable CAMs. They bind soluble or matrix-bound ligands, including fibrinogen, fibronectin and thrombospondin, as well as cell-associated ligands of the IgG family like I-CAM and V-CAM.
- Integrins exists in active and inactive states. Can be activated by inside-out signaling.
- Mutations that affect integrin expression, activity and regulation cause a number of human diseases. Integrins are targets of mAbs and other therapeutics.
- Biologics are a growing class of drugs that take advantage of the high affinity and selectivity of protein-protein interactions to modify the function of integrins and other cell surface receptors accessible to large macro-molecules.