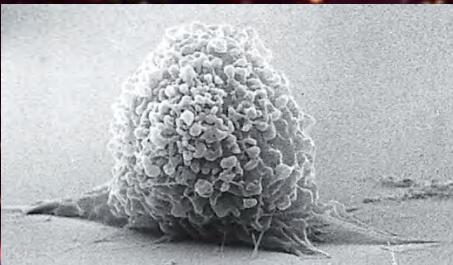
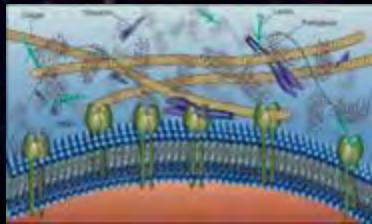


Lecture 11: cell walls, ECM, and cell-environment interactions

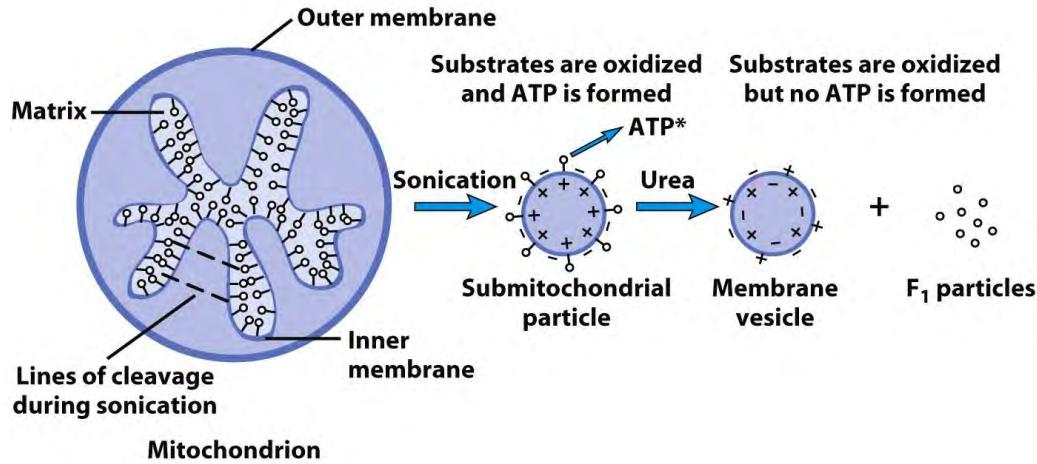
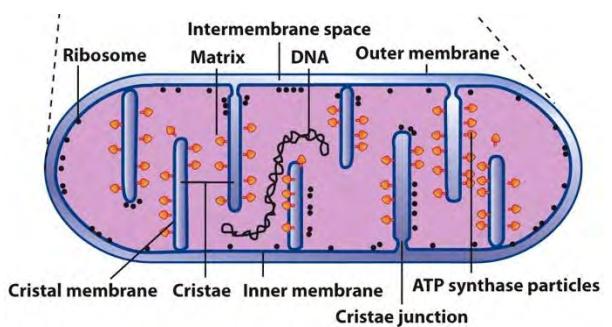
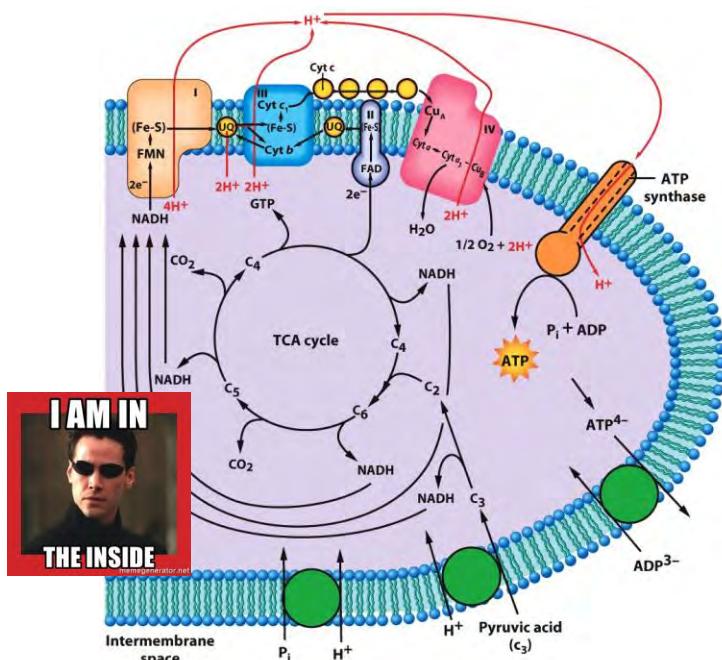
Now playing:
I've got you under my skin
Frank Sinatra



Lodish Chapter 20



Mitochondria minute!

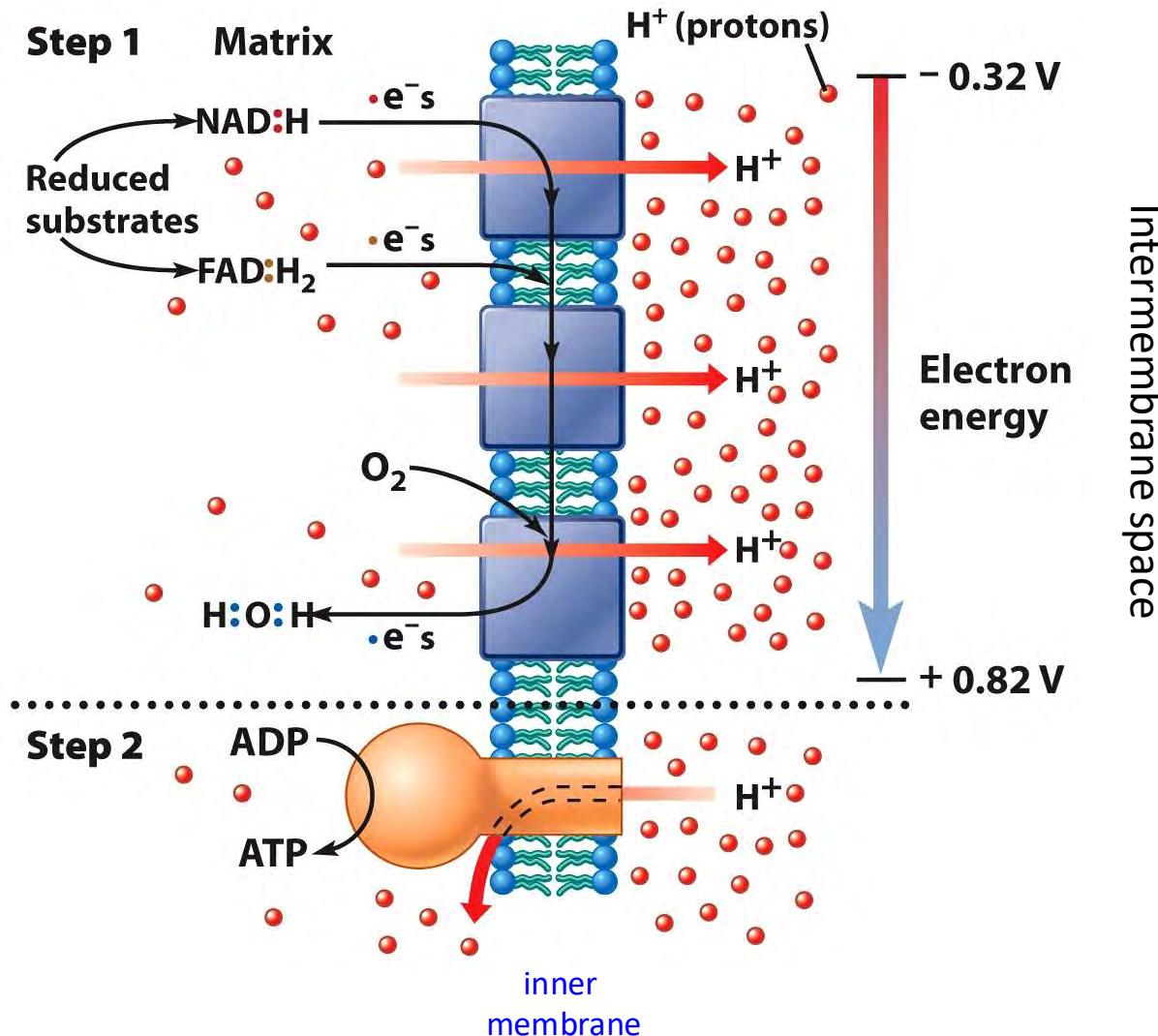


Submitochondrial particles can do some metabolic reactions, but not all.

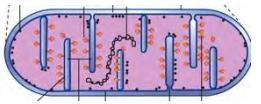
oxidative phosphorylation (ox phos)

ox phos - ATP formation driven by energy captured during **substrate oxidation**, and released by movement of electrons through the electron transport chain

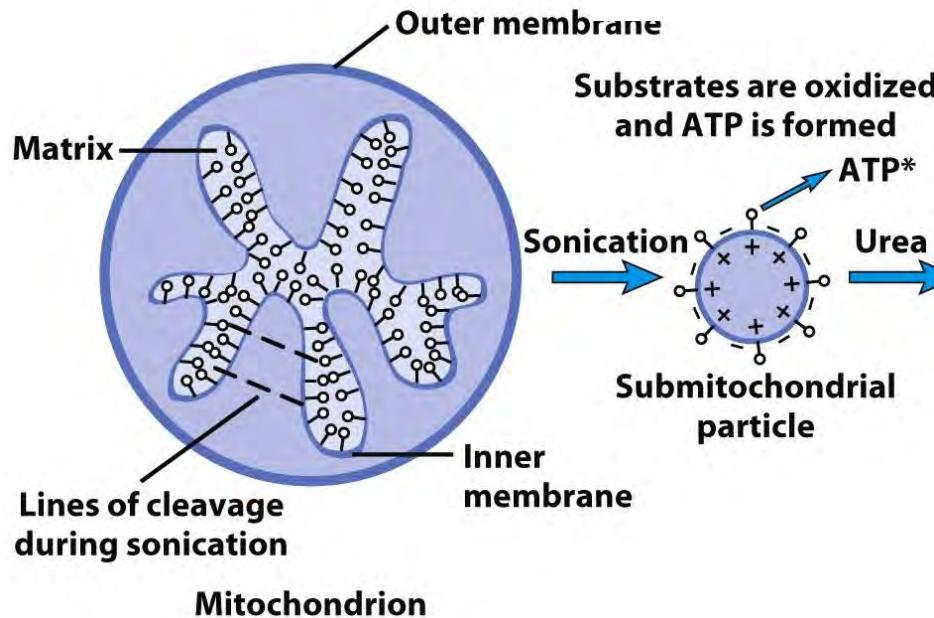
10^{26} ($>60\text{kg ATP}$) generated/person/day
(most phosphorylation events in the cell are *substrate phosphorylations*)



Prior clicker - experiments with submitochondrial particles



Which of the choices can your pure submitochondrial particle NOT do?

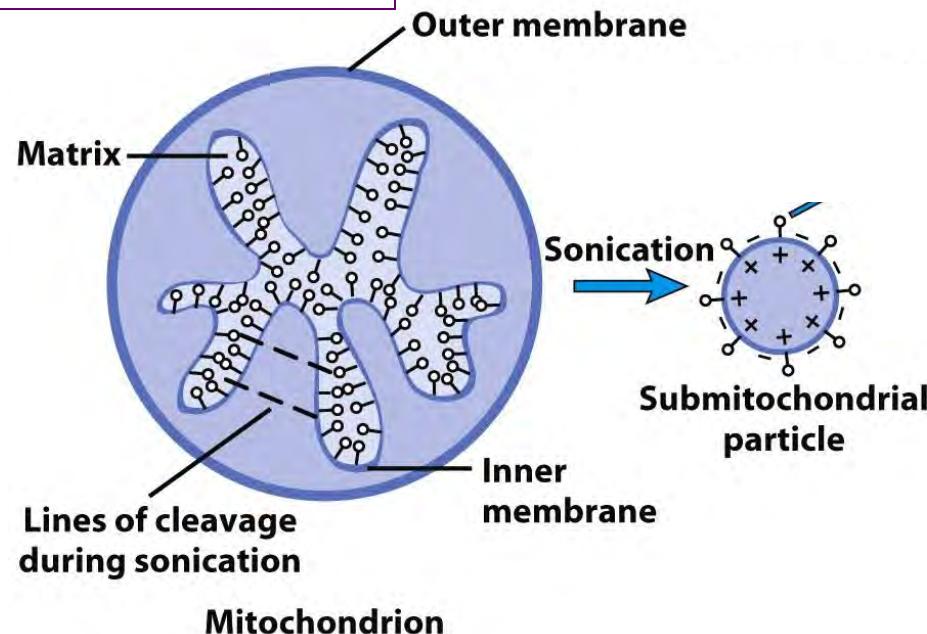


- a) catalyze all reactions of the TCA cycle
- b) oxidize NADH;
- c) produce H_2O from O_2 ;
- d) generate a proton gradient;
- e) synthesize ATP

Poll (warm up) –more experiments with submitochondrial particles

you treat one sample with a **protophore**, which makes the membrane leaky to protons

Which of the following can your pure submitochondrial particle NOT do now?

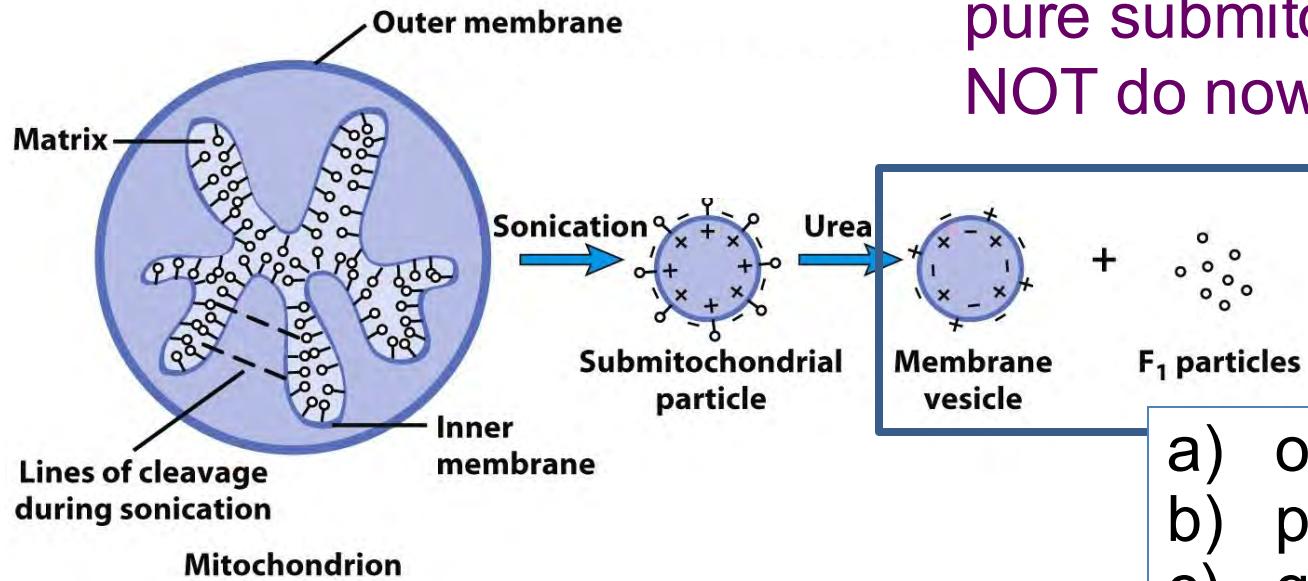


- a) oxidize NADH
- b) move electrons in the ETC
- c) synthesize ATP

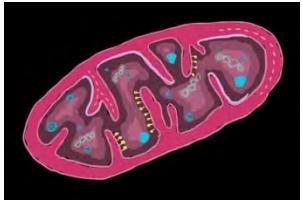
poll 2- still more experiments with submitochondrial particles

In a different experiment, you strip the particles of the F₁ subunits by urea treatment...
(this expt does not have the protophore)

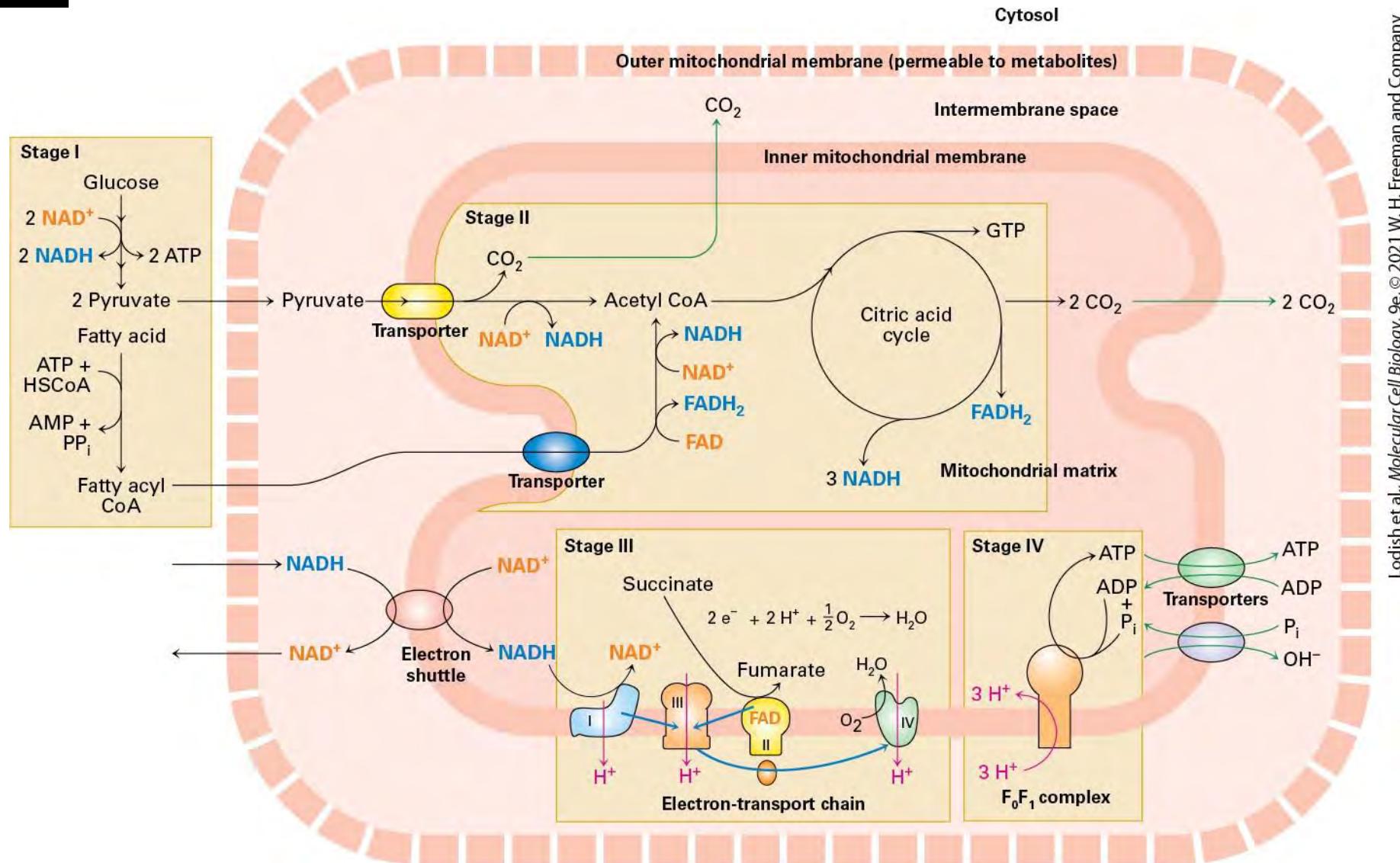
Which of the following can your pure submitochondrial particles NOT do now?



- a) oxidize NADH
- b) produce H₂O from O₂
- c) generate a proton gradient
- d) synthesize ATP
- e) two of the above



Oxidative metabolism wrap up



administrative notes

- Exam 1 average – 60%
- Keys to exam 1 are in your Akindi results email
- Poll everywhere results up to exam 1 are posted (please check)
- Final project benchmarks and dates are posted in Blackboard
- This week in discussion – extravasation of white blood cells
 - Fun fact: **Nobel prize in Physiology or Medicine** announced this week: awarded to **Mary E. Brunkow, Fred Ramsdell and Shimon Sakaguchi** for discoveries concerning peripheral immune tolerance that prevents the immune system from harming the body. (Regulatory T cells)

ECM and cell interactions– learning goals

by the end of this topic, you should

- compare and contrast cell wall and ECM
- know what kinds of molecules:
 - make up the ECM and what they contribute structurally
 - enable the different types of interactions between cells and the matrix or between different cells
 - be able to identify these molecules on a schematic
- be able to explain the underlying causes of blistering diseases
- be able to compare and contrast types of cell junctions/adhesions
- be able to describe and interpret the experiments used to characterize different adhesion molecules
- be able to describe the molecules contributing to cell interactions, including cytoskeletal components

cell walls

- with the exception of animal cells, most cells are surrounded by an outer protective layer
- bacteria, fungi, plants have cell walls – what is it made of?

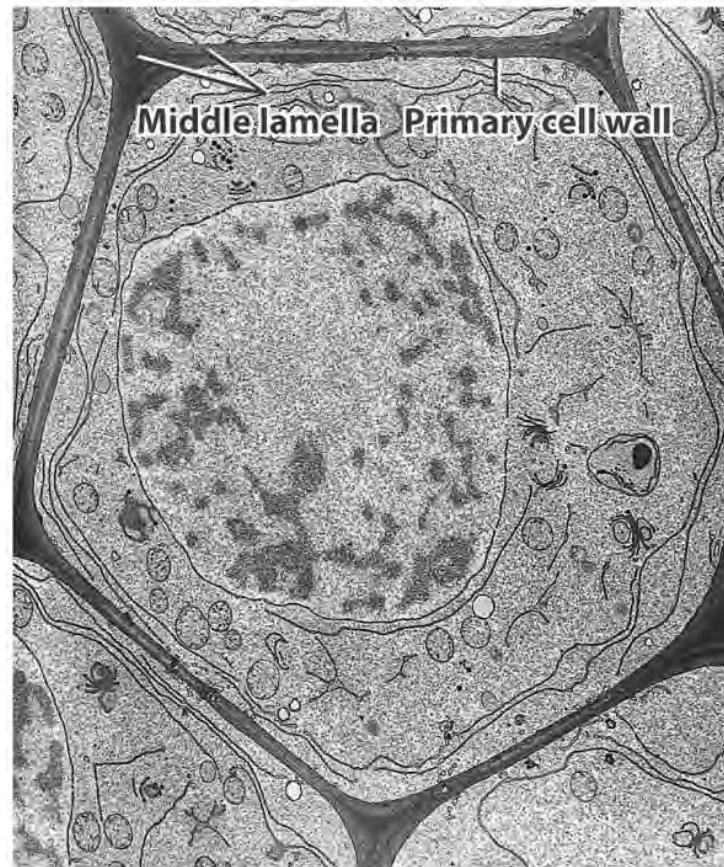
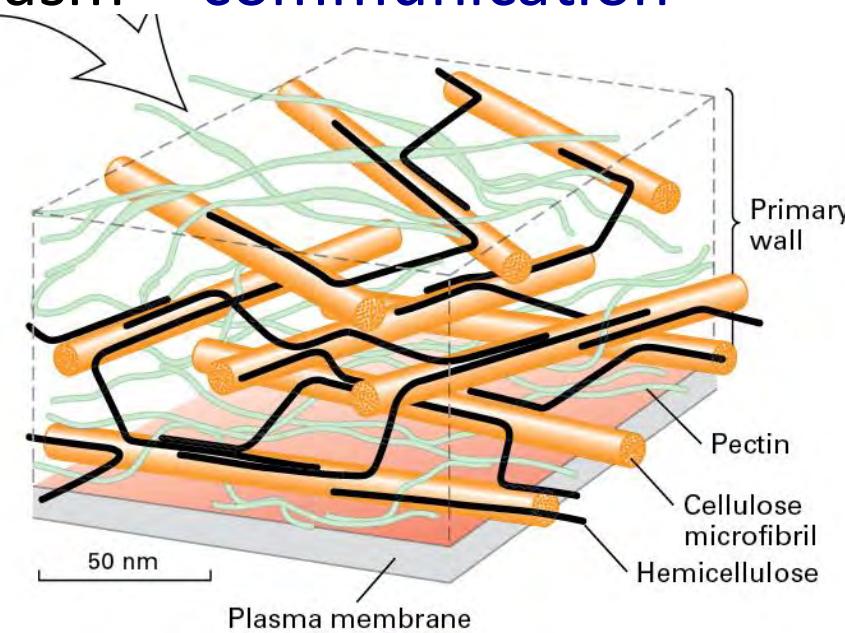
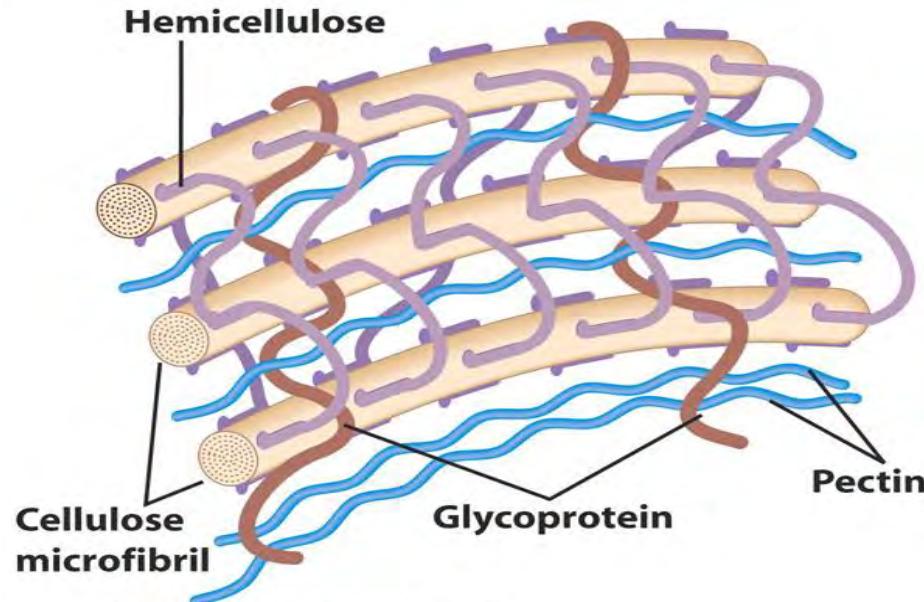
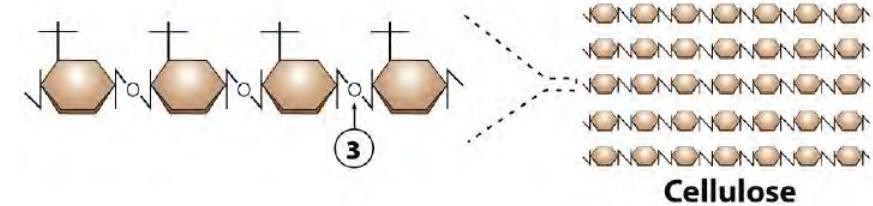


Figure 7-35a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

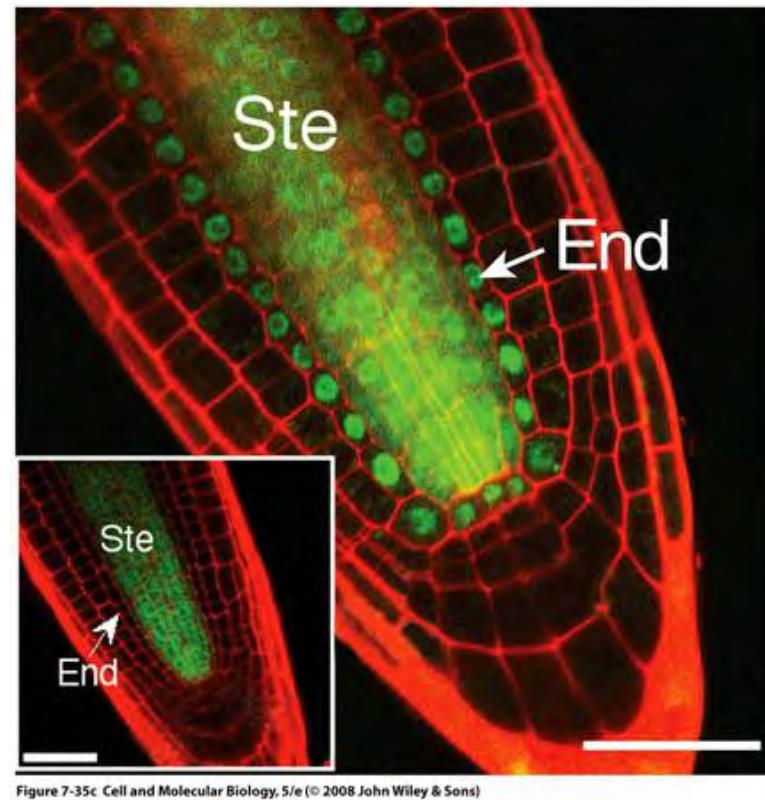
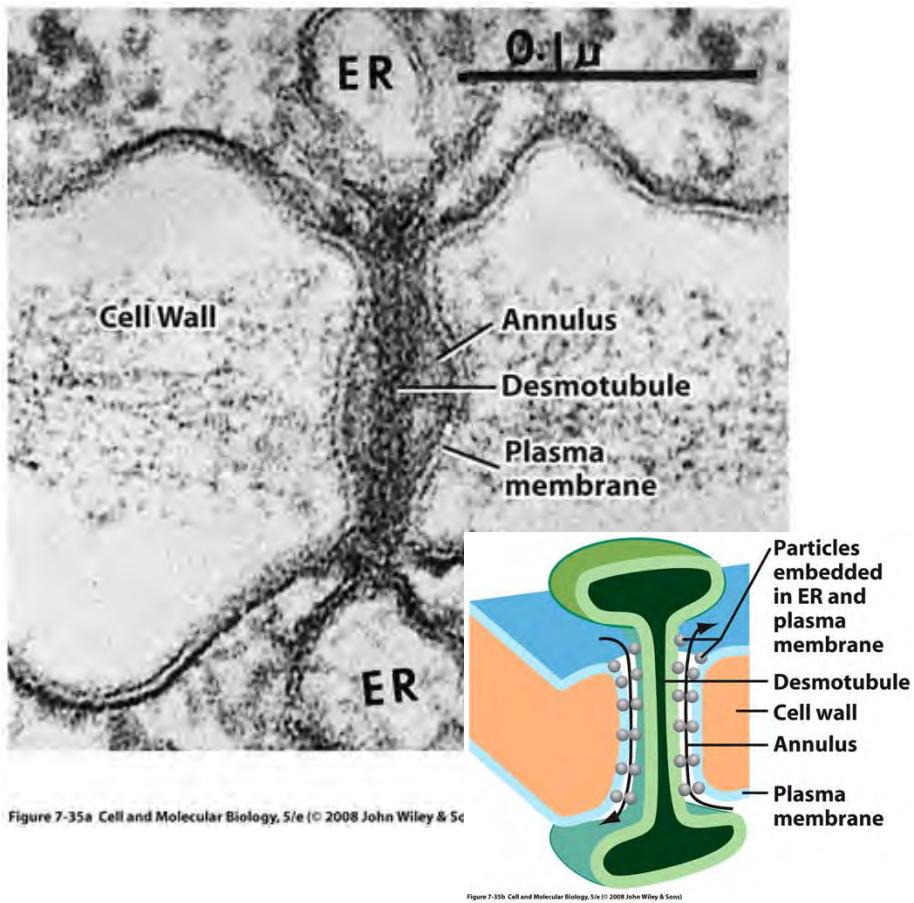
cell wall components and character

- pectins: polysaccharides, hold H₂O = **gel**
- microfibrils of cellulose: polysaccharides
 - gives the cell wall its structure = **strength**
- hemicelluloses: polysaccharides that cross-link cellulose = **mesh**
- proteins: Some span the cell wall and transmit signals from the outside to the cytoplasm = **communication**



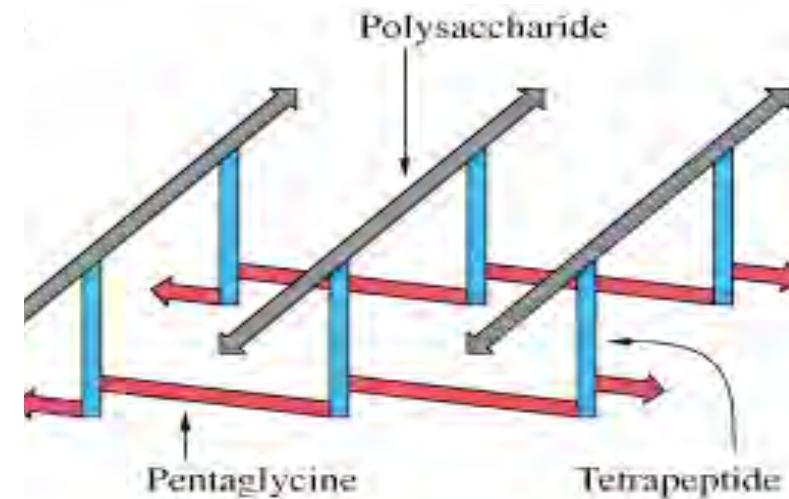
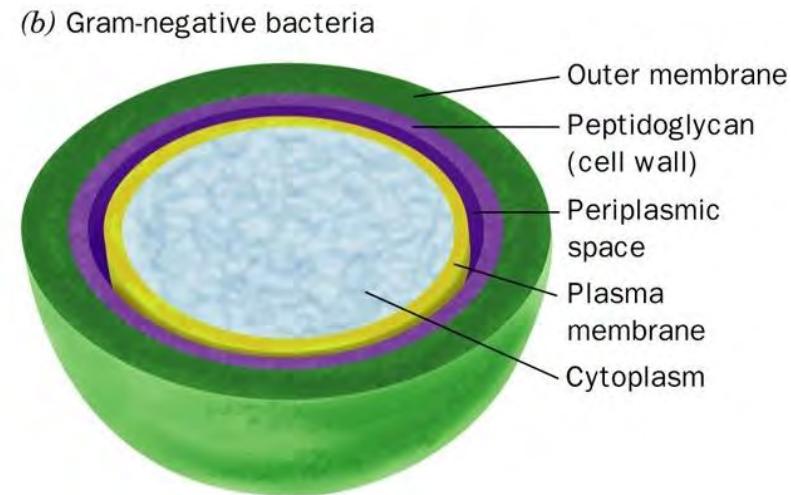
how do you communicate through walls?

- ***plasmodesmata***: cytoplasmic channels that pass through the cell wall to connect adjacent cells



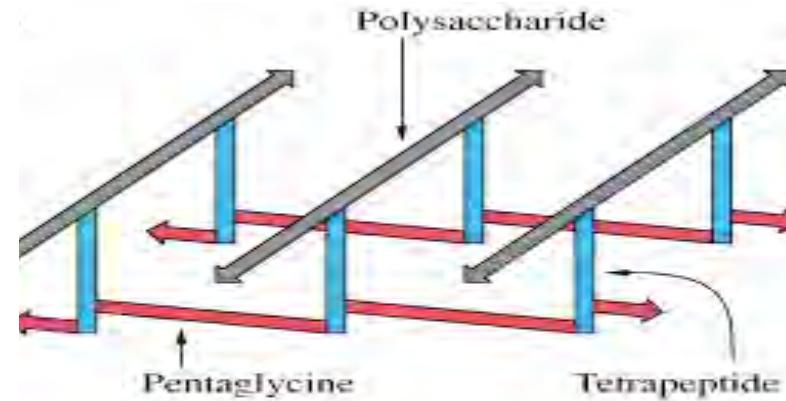
bacterial cell walls

- bacteria live in hypotonic environment (**osmotic pressure**)
- without a rigid cell wall they would burst
- **peptidoglycan cell wall** (polysaccharide chains connected by peptides)

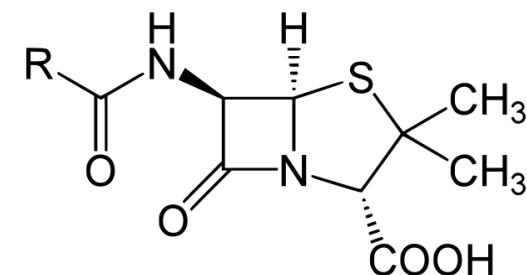


many antibiotics block cell wall production

- ex: Penicillin has a similar structure to the peptides that cross-link the polysaccharide chains of some bacterial cell walls



- Peptides are added to the polysaccharide chains by enzymes
- cell wall synthesis is continuous



Show of hands: have you ever taken an antibiotic like penicillin?

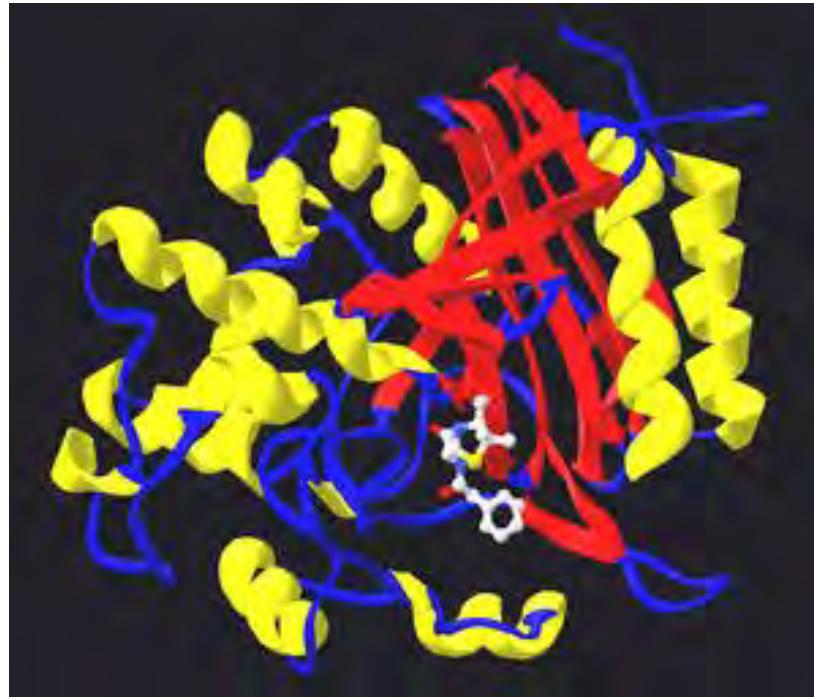
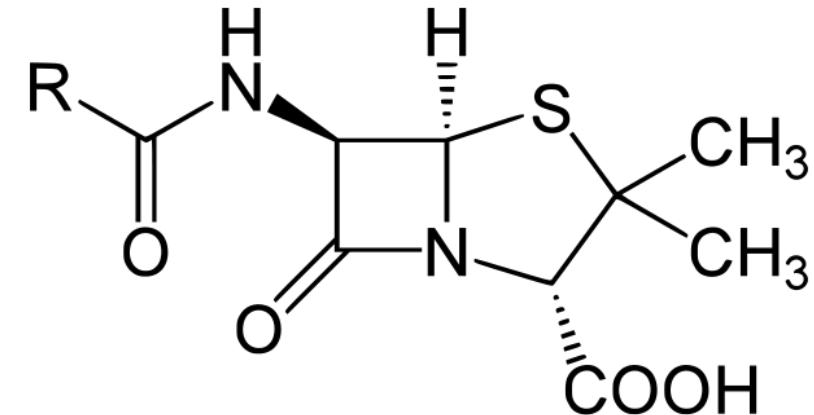


image: wikipedia commons-
enzyme inhibitor

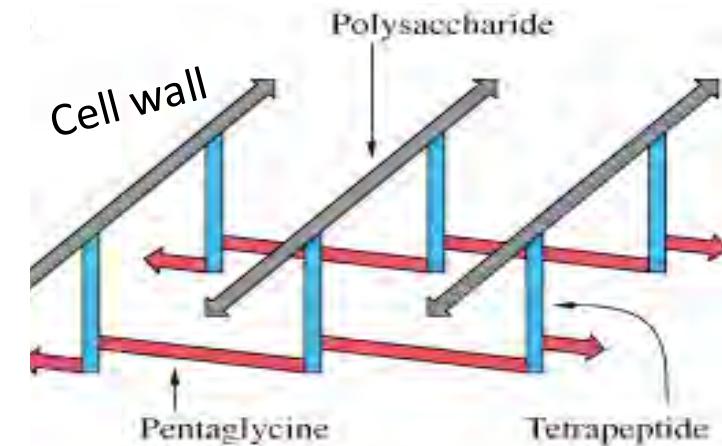


Review poll 3 - the bacterial transpeptidase enzyme is required for cross-linking peptides and polysaccharides of the cell wall. penicillin (white) covalently binds to the same site as the substrate for the enzyme transpeptidase, and is NOT RELEASED

what kind of enzyme inhibitor is this?



- A. allosteric
- B. competitive
- C. irreversible



bacteria exposed to penicillin often lyse (break open) relatively quickly.

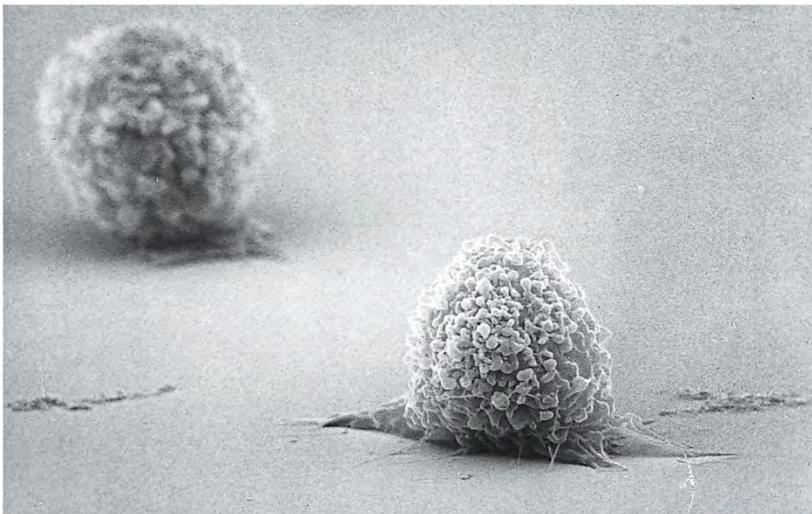
why?

what forces contribute?

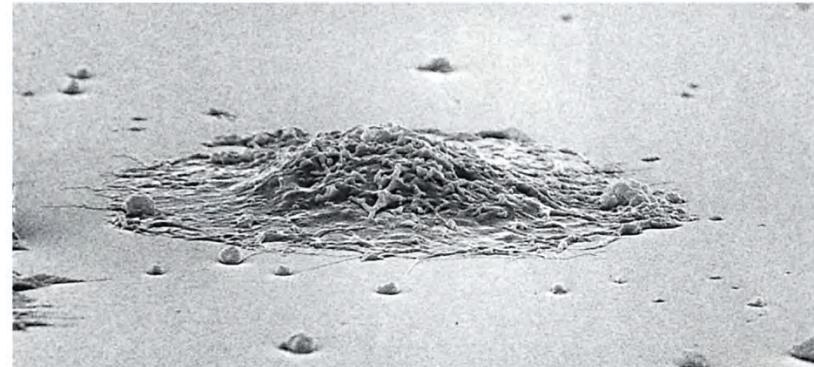
what does it mean about the *permanence* of the cell wall?

on the molecular level, how do you imagine bacteria can become resistant to this drug? (remember to keep selective pressure on bacteria by taking full course of antibiotics)

what does an animal cell do without walls?



From J. J. Rosen and L. A. Culp, *Exp. Cell Res.* 107:141, 1977.



From J. J. Rosen and L. A. Culp, *Exp. Cell Res.* 107:141, 1977.

how do multicellular organisms maintain
order and distinct tissues?

cellular interactions

multicellular organisms are more than just a mass of cells

- cells must adhere together → **tissues**
- tissues must be supported physically

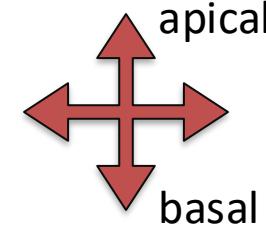
(**extracellular matrix or ECM**)

ECM is also a source of regulatory factors and facilitates cell migration

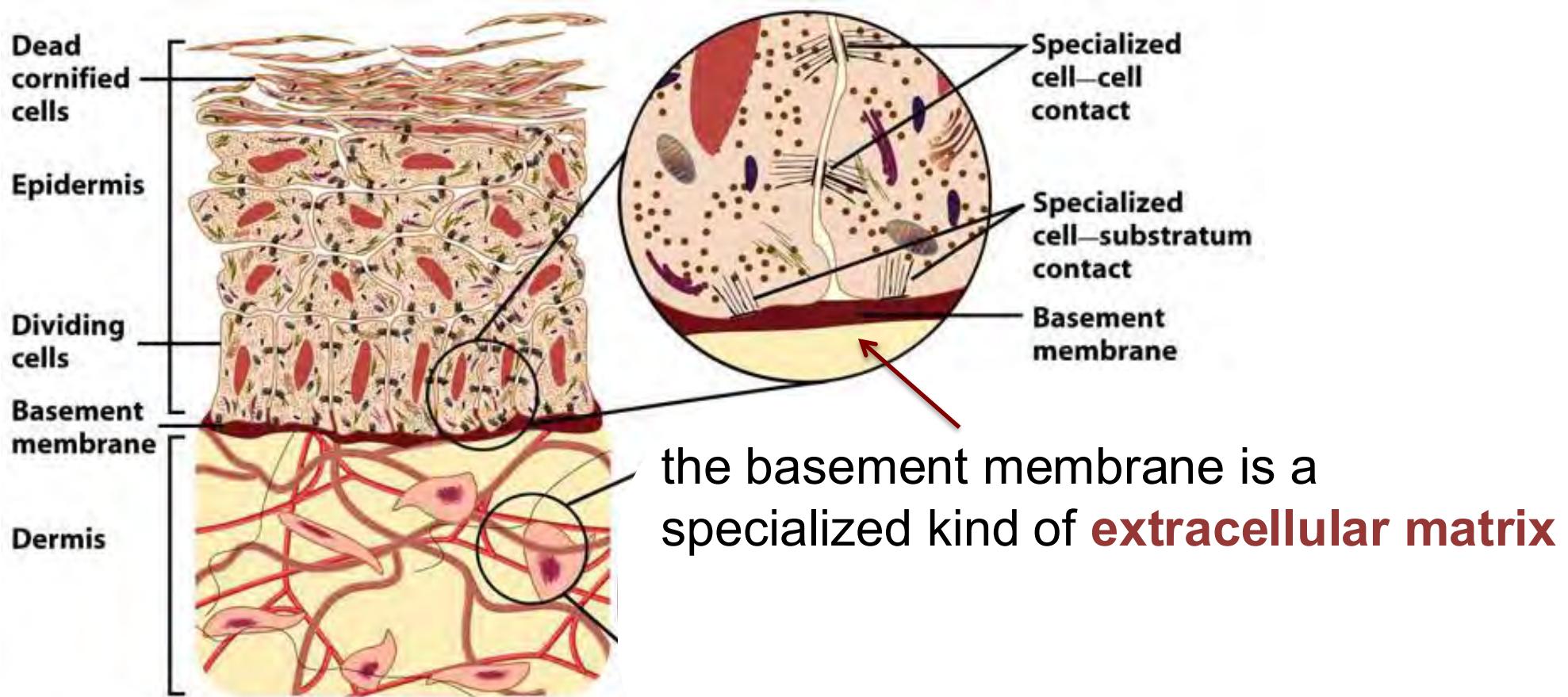
- cells within a tissue must be strongly adherent and be able to communicate (**cell junctions**)

Show me an epithelium!

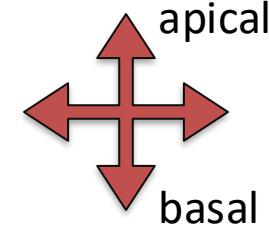
epithelial orientation



human skin tissue



epithelial orientation



human skin tissue

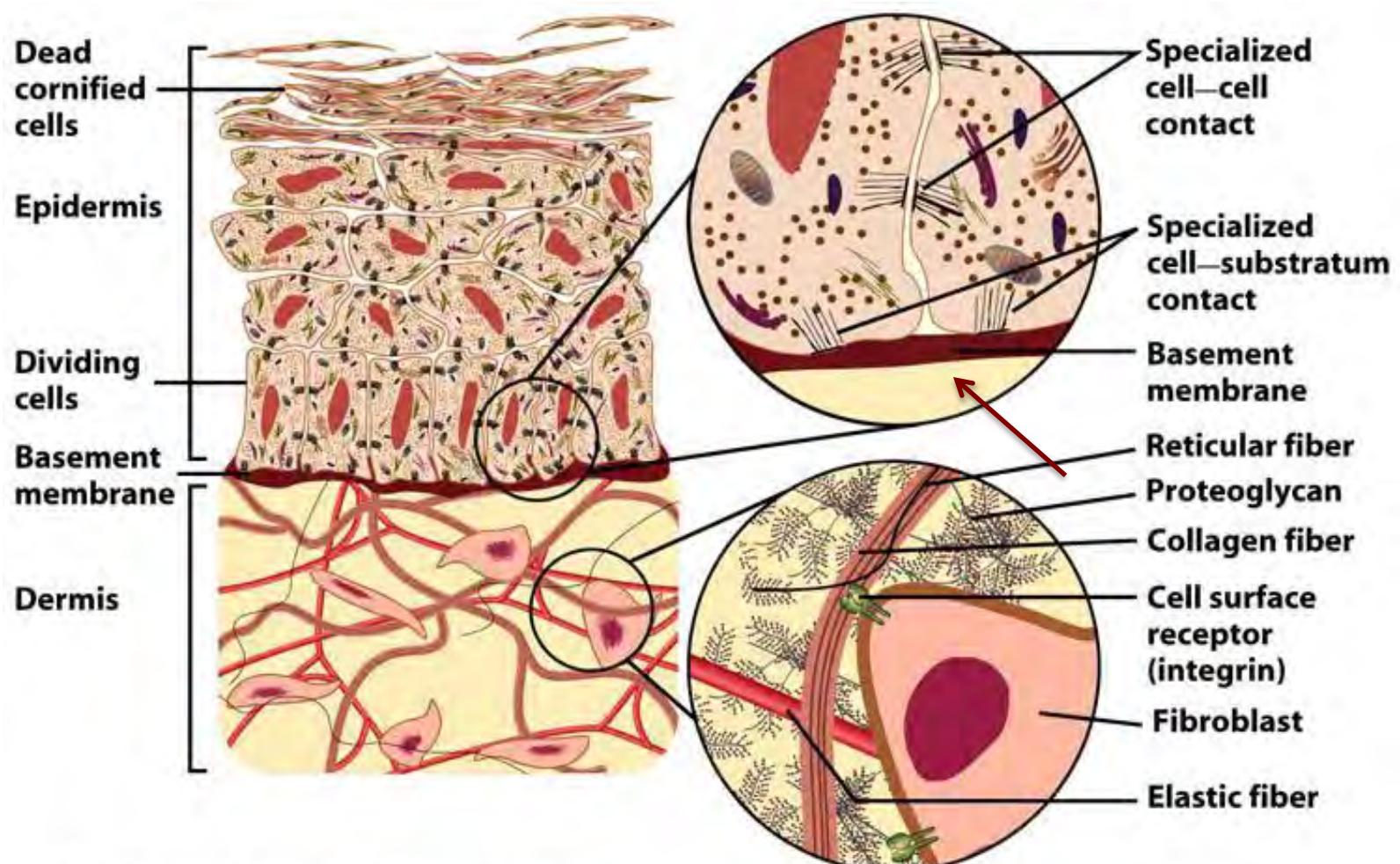
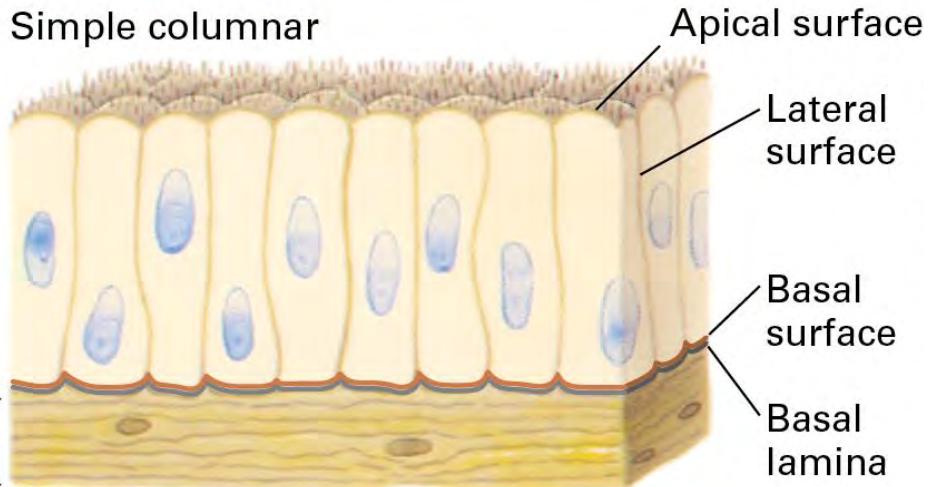


Figure 7-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons) the dermis is also largely **extracellular matrix**

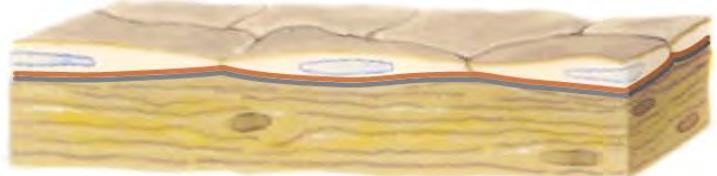
epithelia

(a) Simple columnar



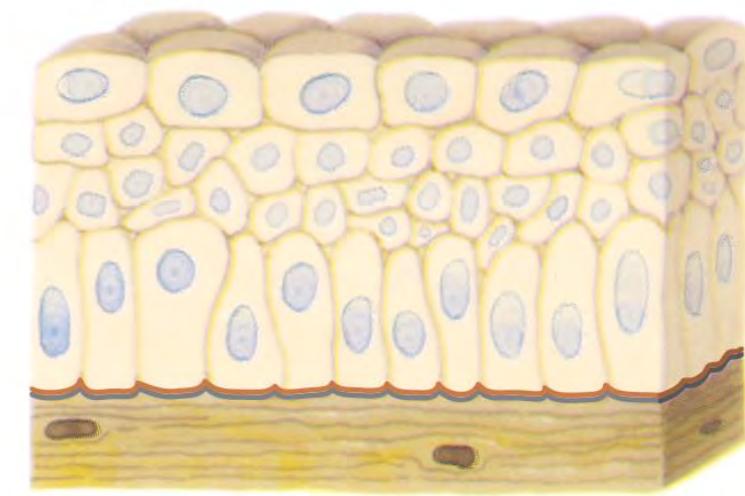
Stomach, gut

(b) Simple squamous



Blood vessels

(c) Transitional

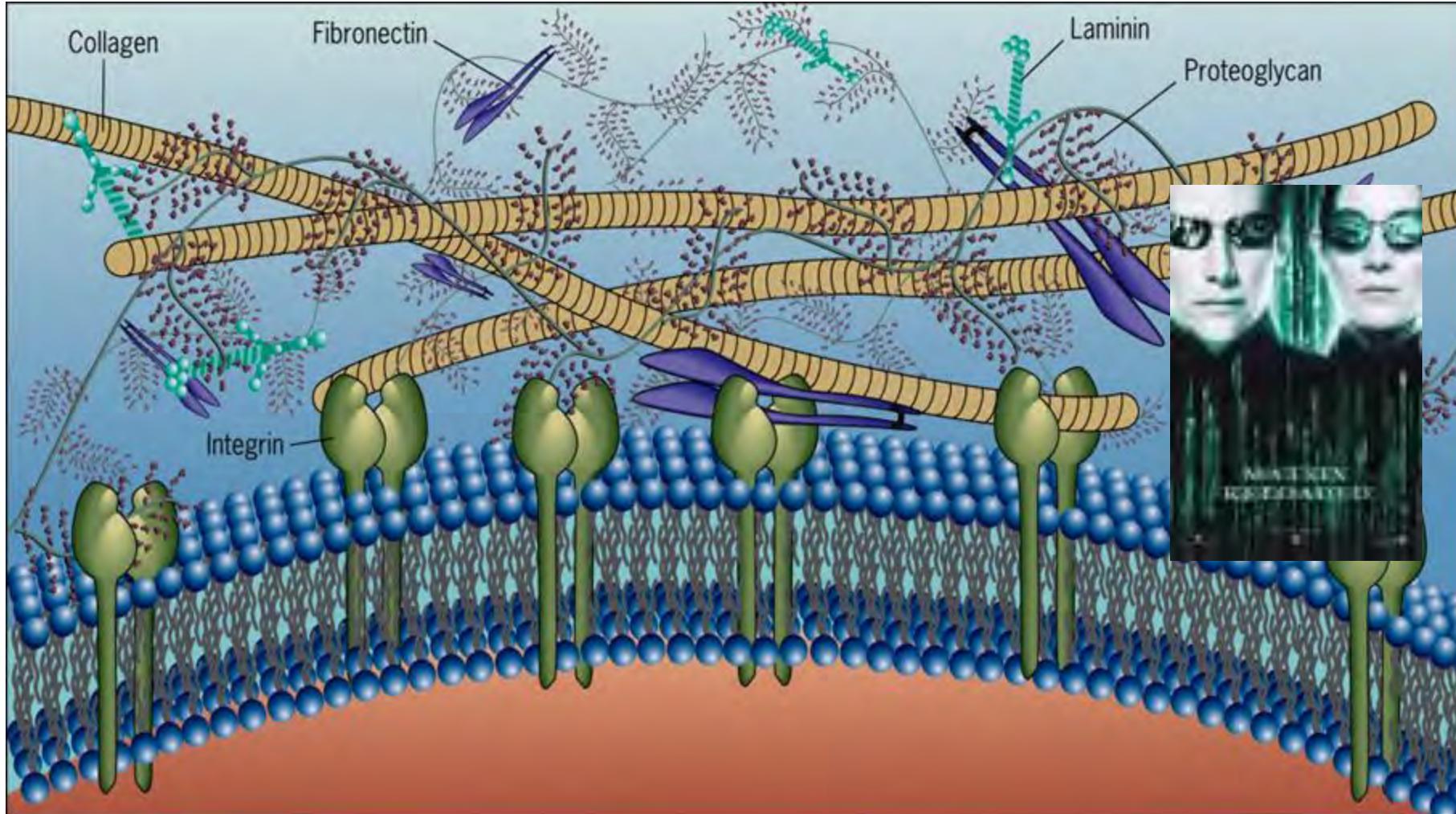


(d) Stratified squamous (nonkeratinized)



Skin (keratinized)

the extracellular “space” is not spacious



the **Extracellular Matrix (ECM)** is made up of many types of proteins and is required for many functions: tissue strength, morphology, cell adhesion/migration, cushioning, barrier, signaling

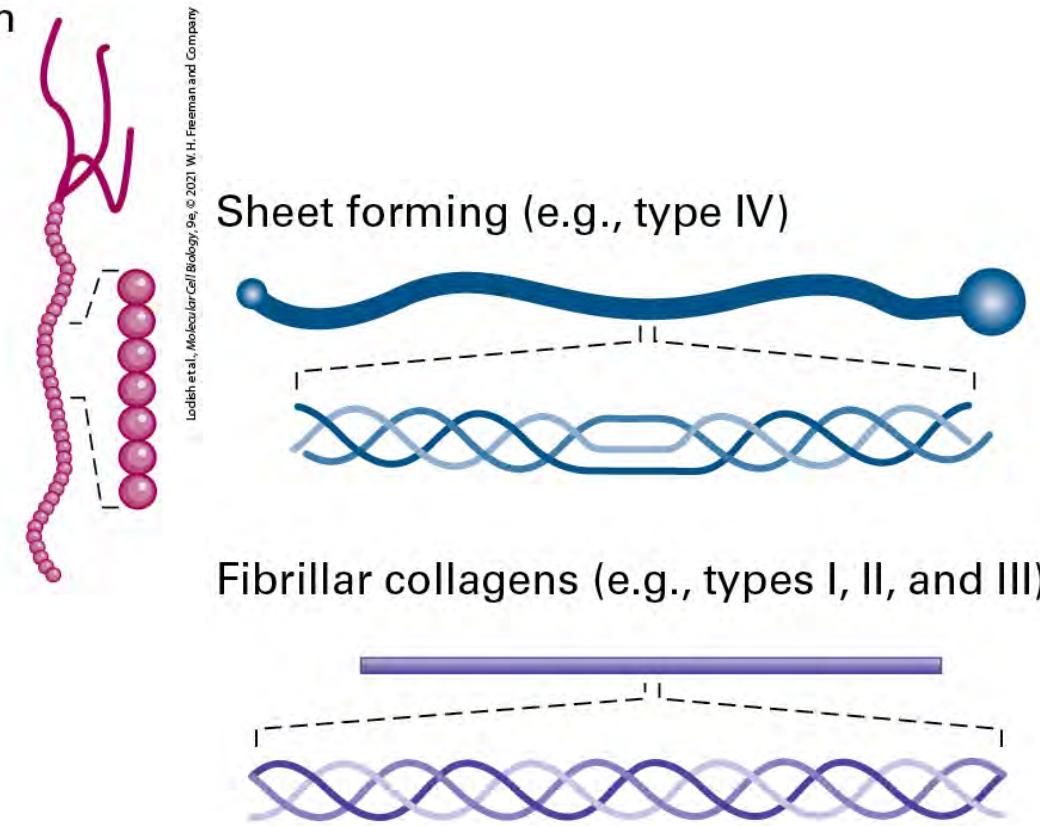
components of the Extracellular Matrix

- gel like components of ECM include the **glycosaminoglycans** and **proteoglycans** (and associated water). (polysaccharides)
- meshwork of the ECM is composed of **fibrous glycoproteins** (Collagen, Laminin and Fibronectin). These can be bundled for **strength**.
- **Integrins** (integral membrane proteins) mediate cell *adhesion* to the ECM – and **signaling**

these are made by local cells and secreted!

some components of the Extracellular Matrix

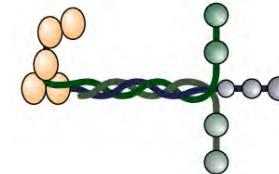
Perlecan



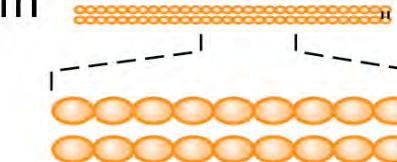
Sheet forming (e.g., type IV)

Fibrillar collagens (e.g., types I, II, and III)

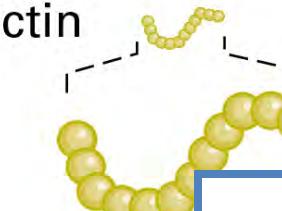
Laminin



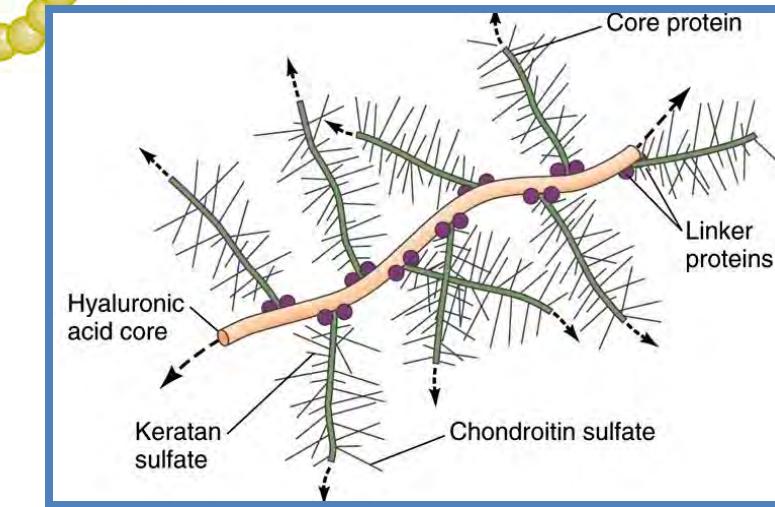
Fibronectin



Nidogen/entactin



glycosaminoglycans



proteoglycans – cells are sugar-coated

glycosaminoglycans: Long unbranched polysaccharide chains composed of repeating disaccharide units.

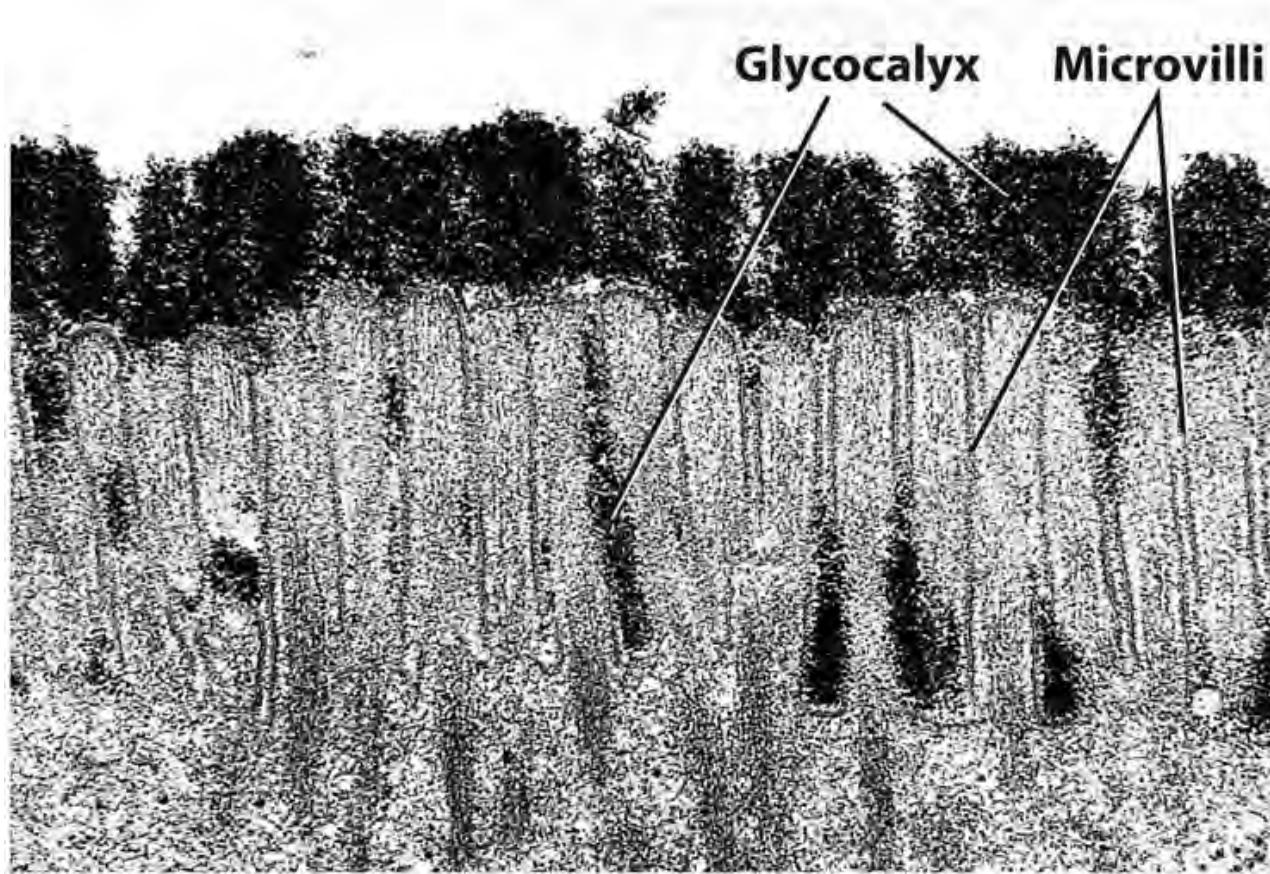


Figure 7-2b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Glycosaminoglycans attract water, occupy space and resist compression

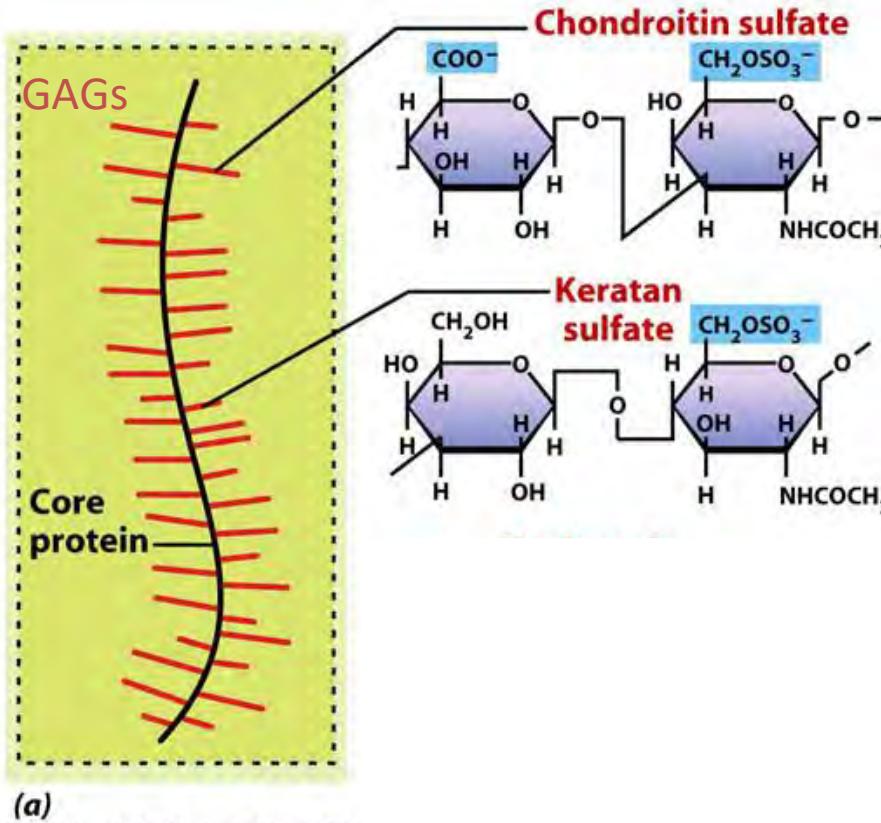
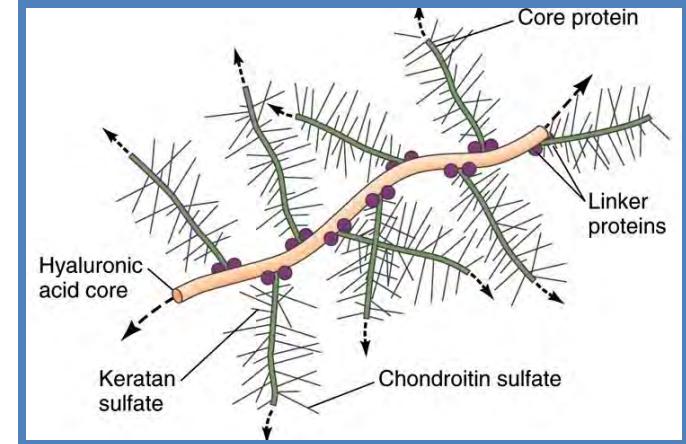


Figure 7-9a-c Cell and Molecular Biology, 5/e (1)

Proteoglycan complexes are very LARGE. a proteoglycan from cartilage may contain 30 keratin sulfate and 100 chondroitin sulfate chain



proteoglycans – cells are sugar-coated

glycosaminoglycans: Long unbranched polysaccharide chains composed of repeating disaccharide units.

Most glycosaminoglycans are covalently linked to **core proteins** = **proteoglycans**, which can link to **hyaluronic acid** and form giant proteoglycan complexes

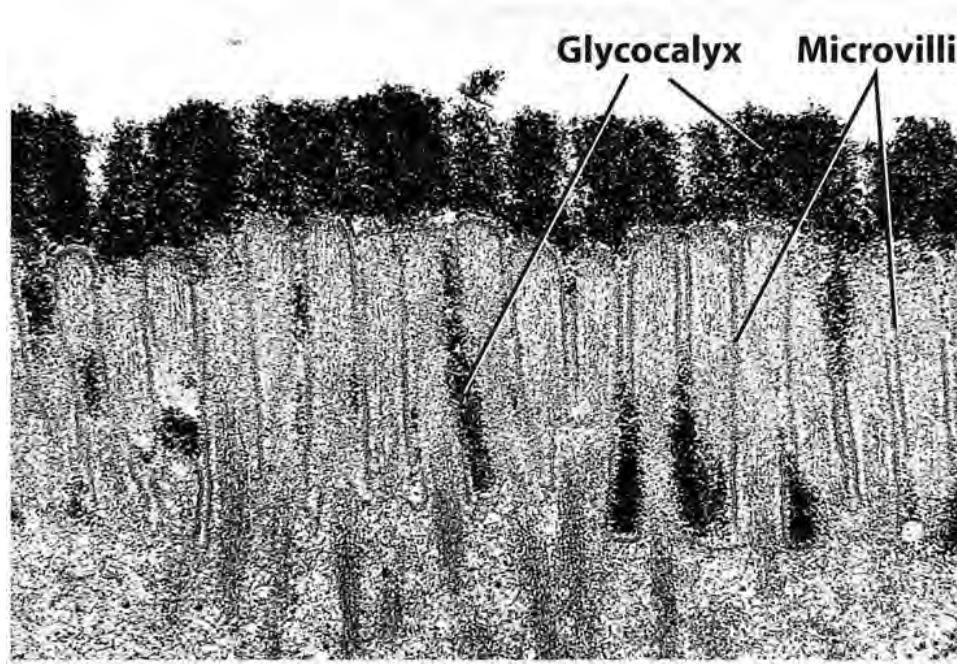
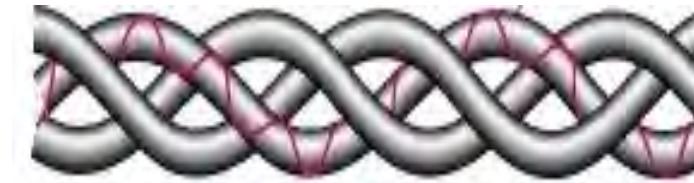


Figure 7-2b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

proteoglycans can be integral membrane proteins that link to the cytoskeleton or have a protease cleavage site close to the plasma membrane that can release the extracellular domain for signaling

Collagen protein structure

basic quaternary structure: **triple helix**



- Helix is 1 molecule
- Multiple molecules overlap. Overlaps stabilized by covalent bonds → **fibrils**
- Fibrils fit together in a repeating pattern to form fibers and sheets



- Fibers are very strong

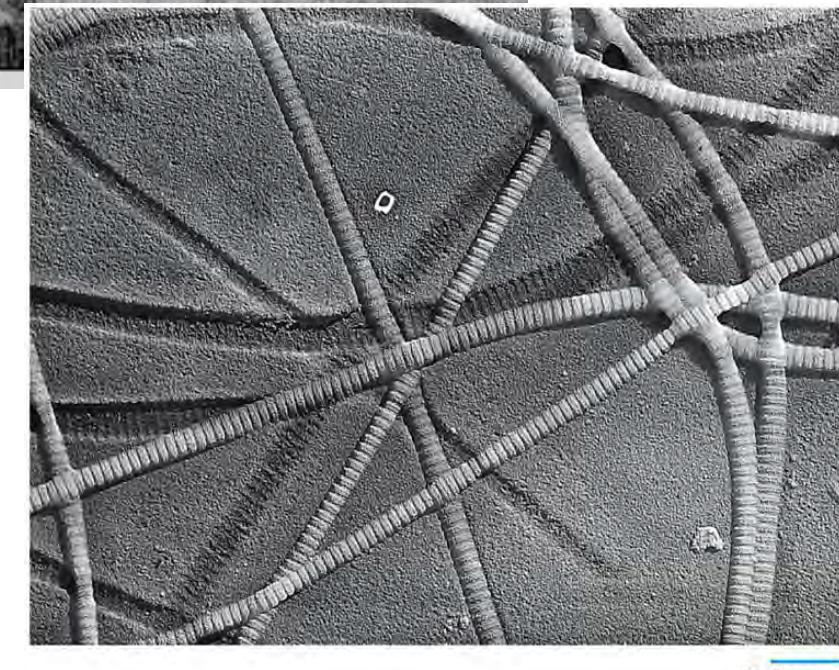
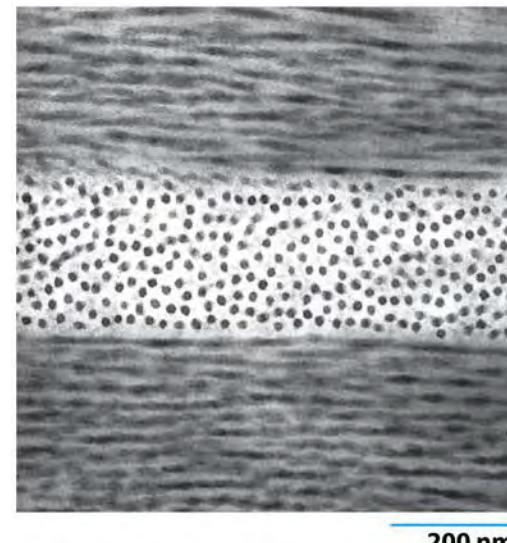
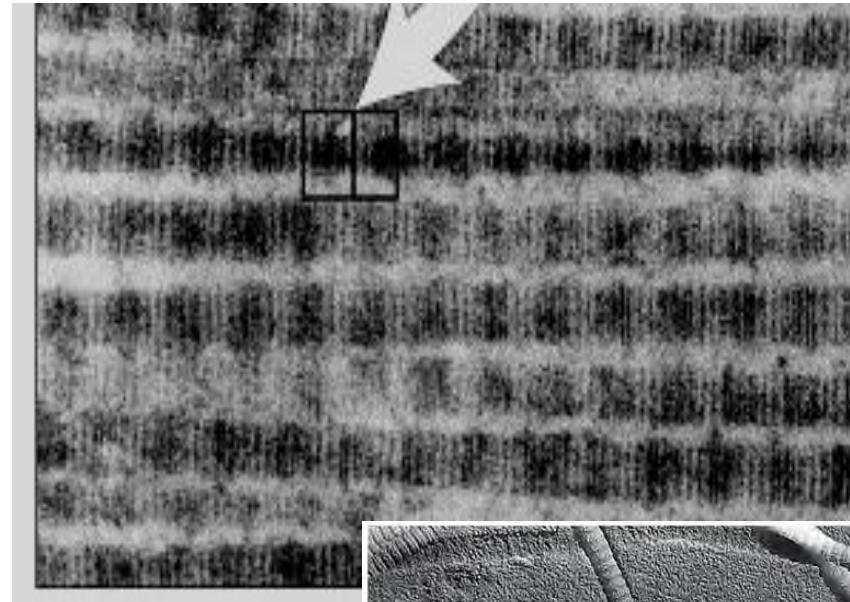
• STRENGTH and STABILITY requires post-translational modification- hydroxylation of proline

Collagen can be organized different ways

In bones and teeth the ecm is hardened by calcification.

In tendons the matrix is more fibrous and is maximized for tensile strength.

In the cornea the matrix forms transparent layers



types of Collagen

- Type I: connects muscle to bone (large fibers)
- Type II: major constituent of cartilage (small fibers)
- Type IV: forms the basement membrane (basal lamina)-
not organized in fibrils

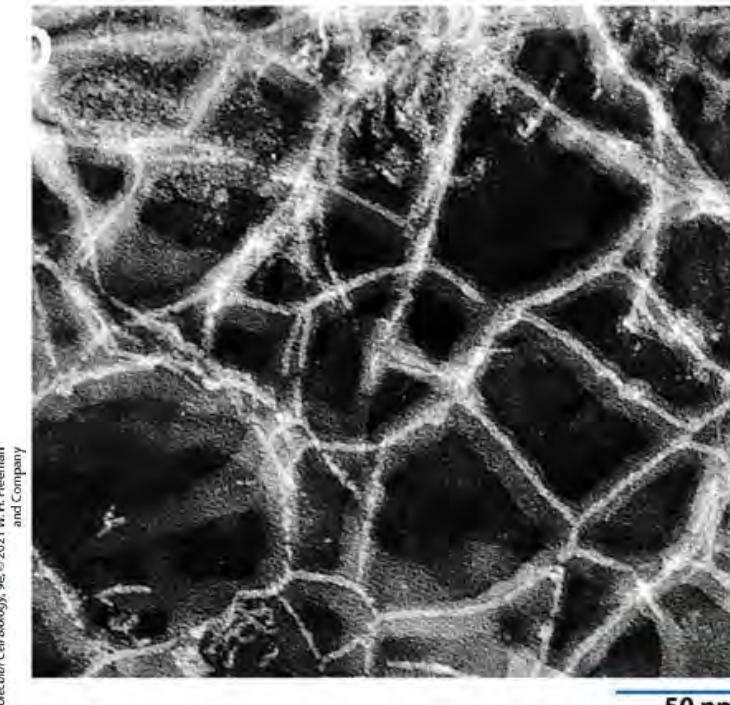
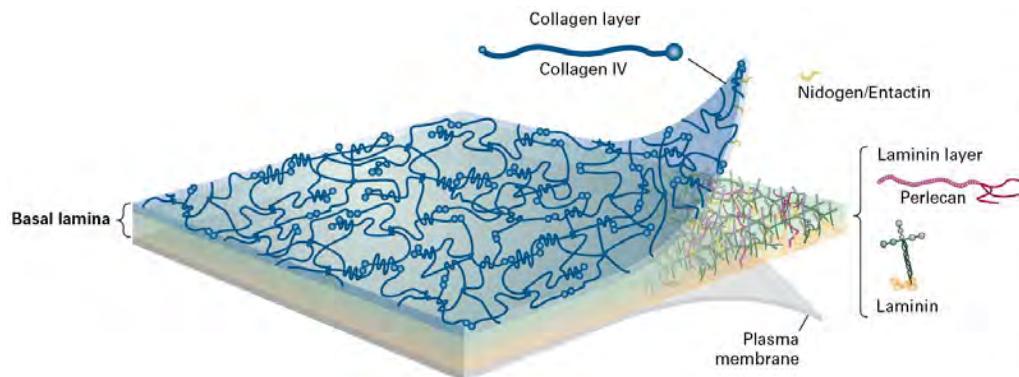
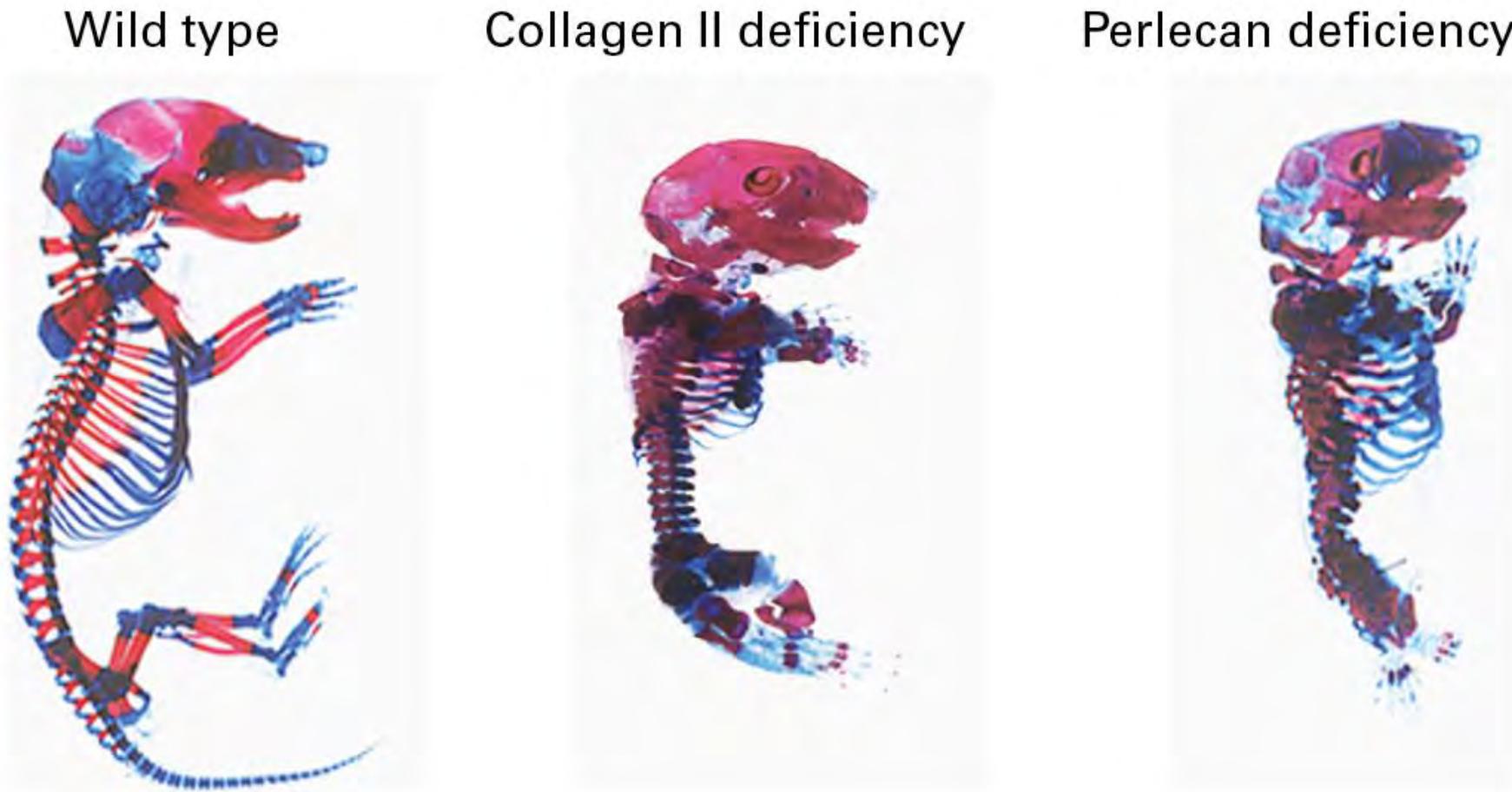


Figure 7-8 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

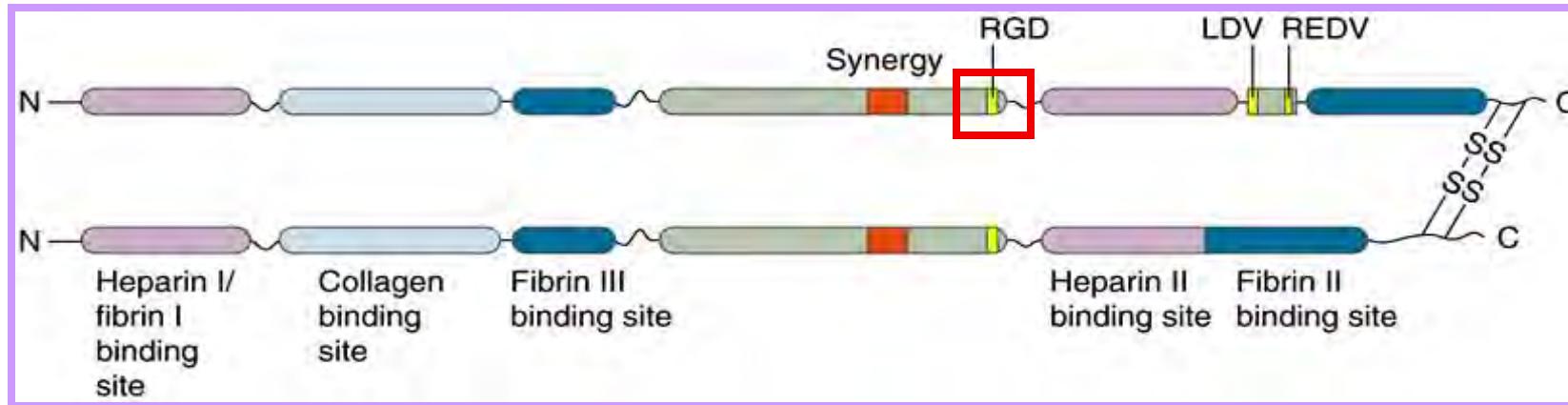
Lodish et al., Molecular Cell Biology, 9e, © 2021 W.H. Freeman and Company

ECM components are required for skeletal formation



Genetic test- knock out gene function

Fibronectin organizes the ECM



A dimer linked by disulfide bonds at the COOH terminus

Contains binding sites for cross-linking other components of the ECM and for binding cells.

For cell adhesion, integrin receptors bind to **RGD** tripeptide 

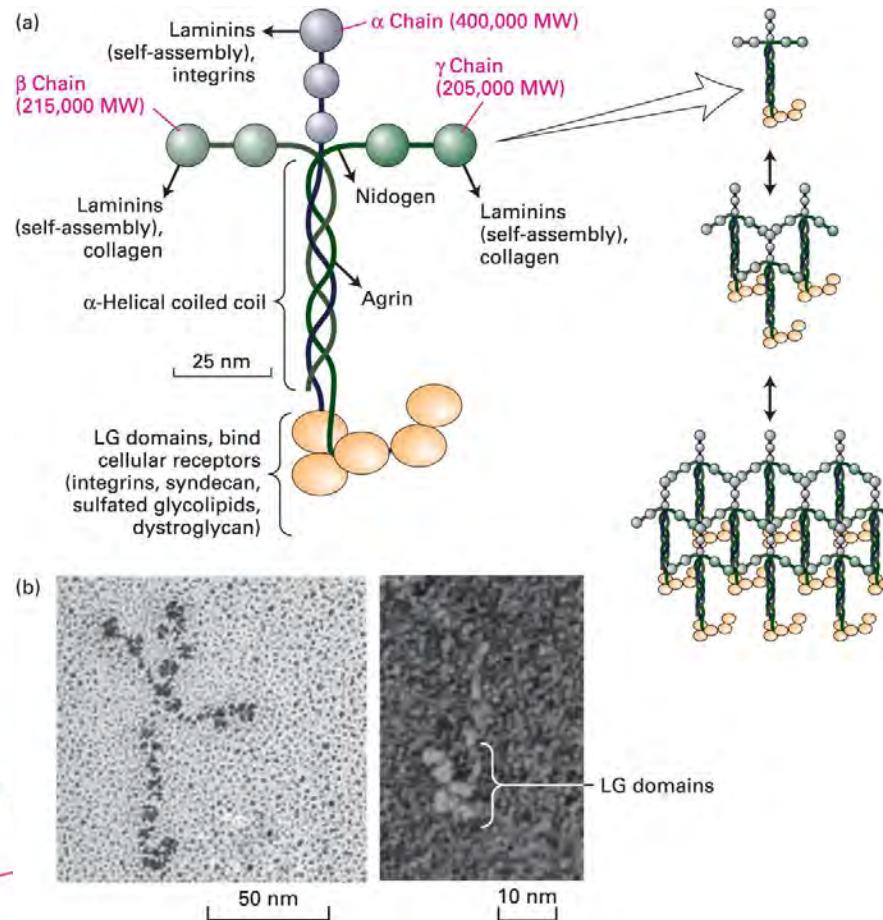
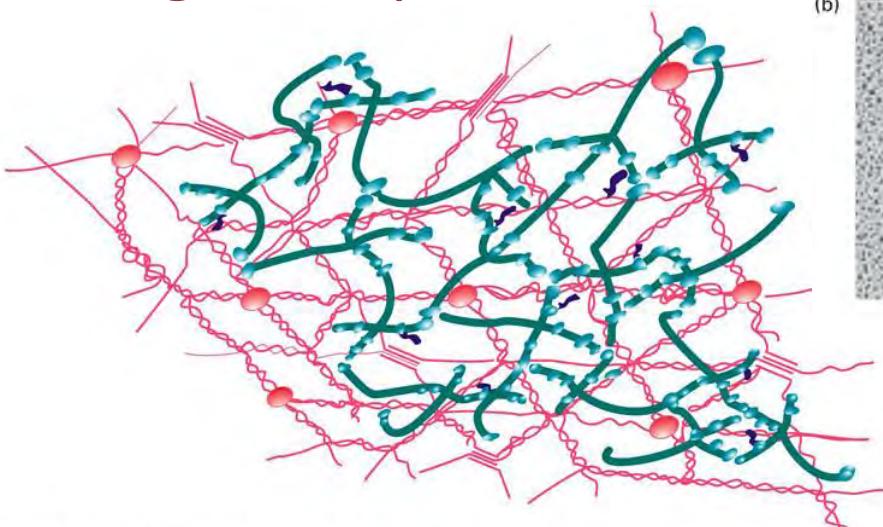
(other domains contribute).

Cells can migrate on fibronectin in a culture dish

ECM scaffold also depends on Laminins

Laminins are another family of glycoproteins that play an organizational role in the ECM. ALSO have RGD repeats

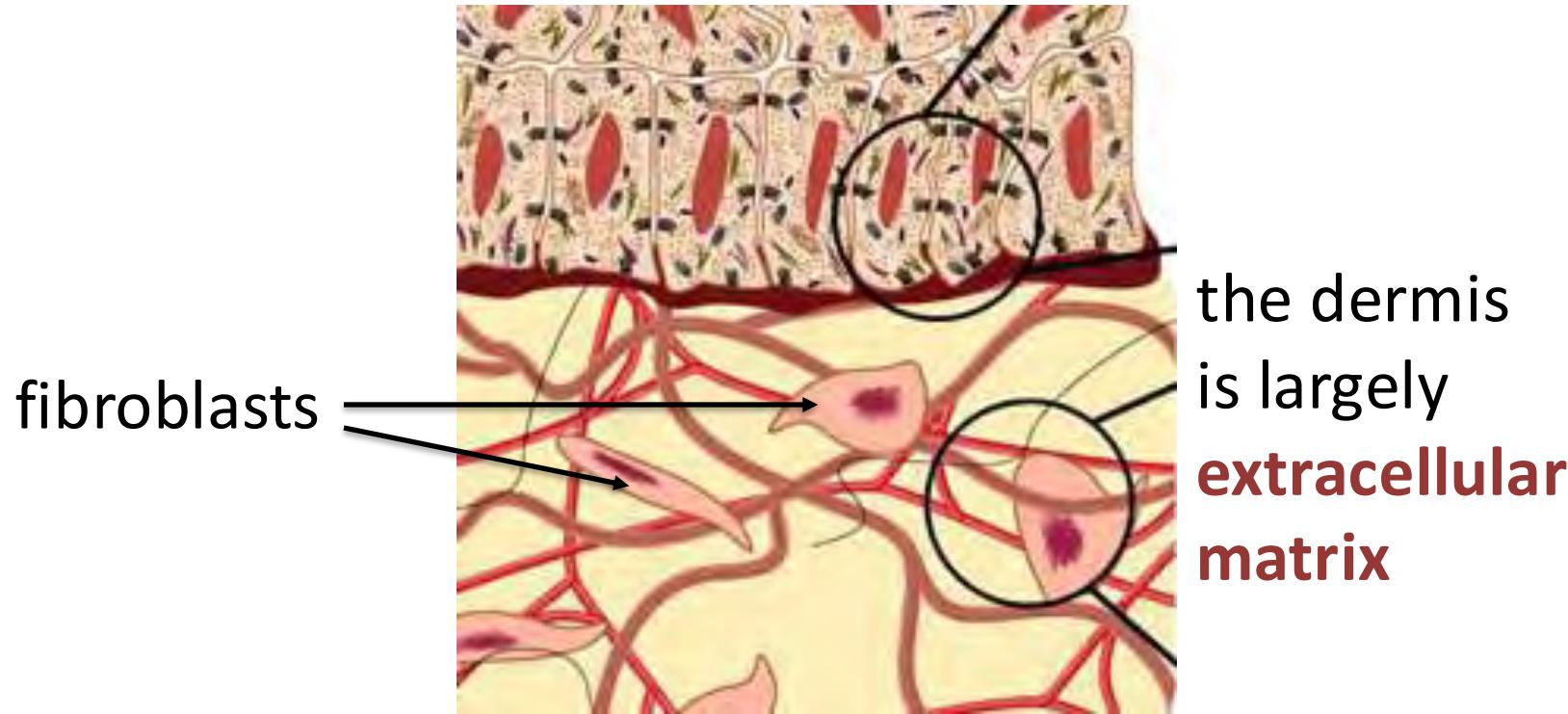
Collagen IV- pink



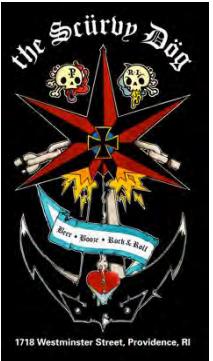
Part (b) courtesy of Jürgen Engel.

Lodish et al., Molecular Cell Biology, 9e, © 2021 W.H. Freeman and Company

how can cells move through the dermis?



Matrix metalloproteases (MMPs) digest ECM components
(and they are continuously replenished)



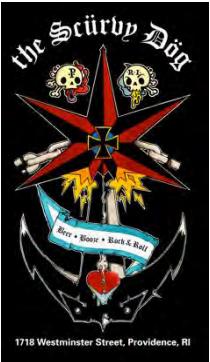
- Scurvy symptoms: internal bleeding, brittle bones, loose teeth, poor wound healing = weak ECM

Shout out- what
is a pirate's favorite
amino acid?

- 1) G
- 2) D
- 3) R



R- argggginine
G- glycine
D- aspartic acid



- Scurvy symptoms: internal bleeding, brittle bones, loose teeth, poor wound healing = weak ECM

poll 4- for what enzyme is vitamin C a co-factor?

- 1) hydroxylation of proline and lysine on collagen
- 2) transcription factor required to down regulate collagen production
- 3) cleavage of glycosaminoglycan



wrinkles in time

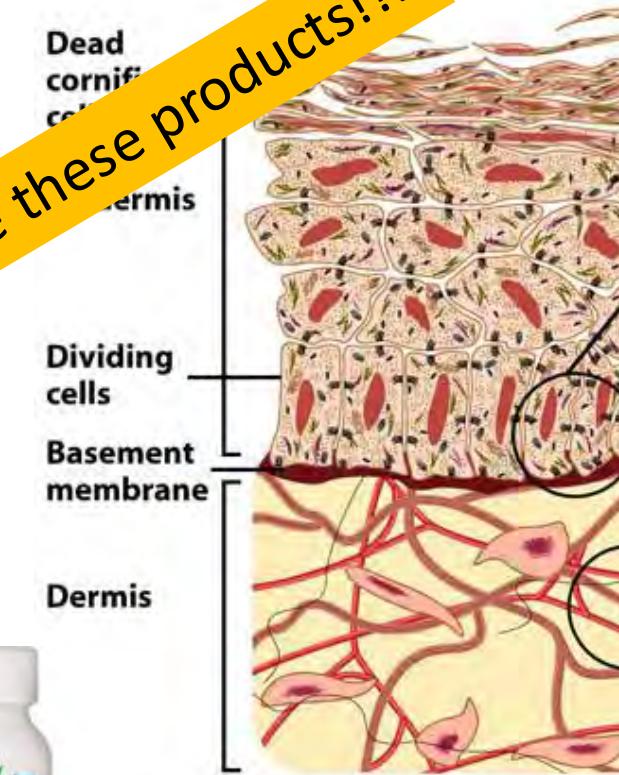


with age, remodeling/production of ECM slows...

food for thought:

- how does Collagen injection improve skin?
 - how does injection of hyaluronic acid help?
 - what layer of skin tissue must these substances be injected into to work?
 - would topical application of collagen be helpful?
 - oral?
- <http://www.collagen.org/dermalfiller.html>

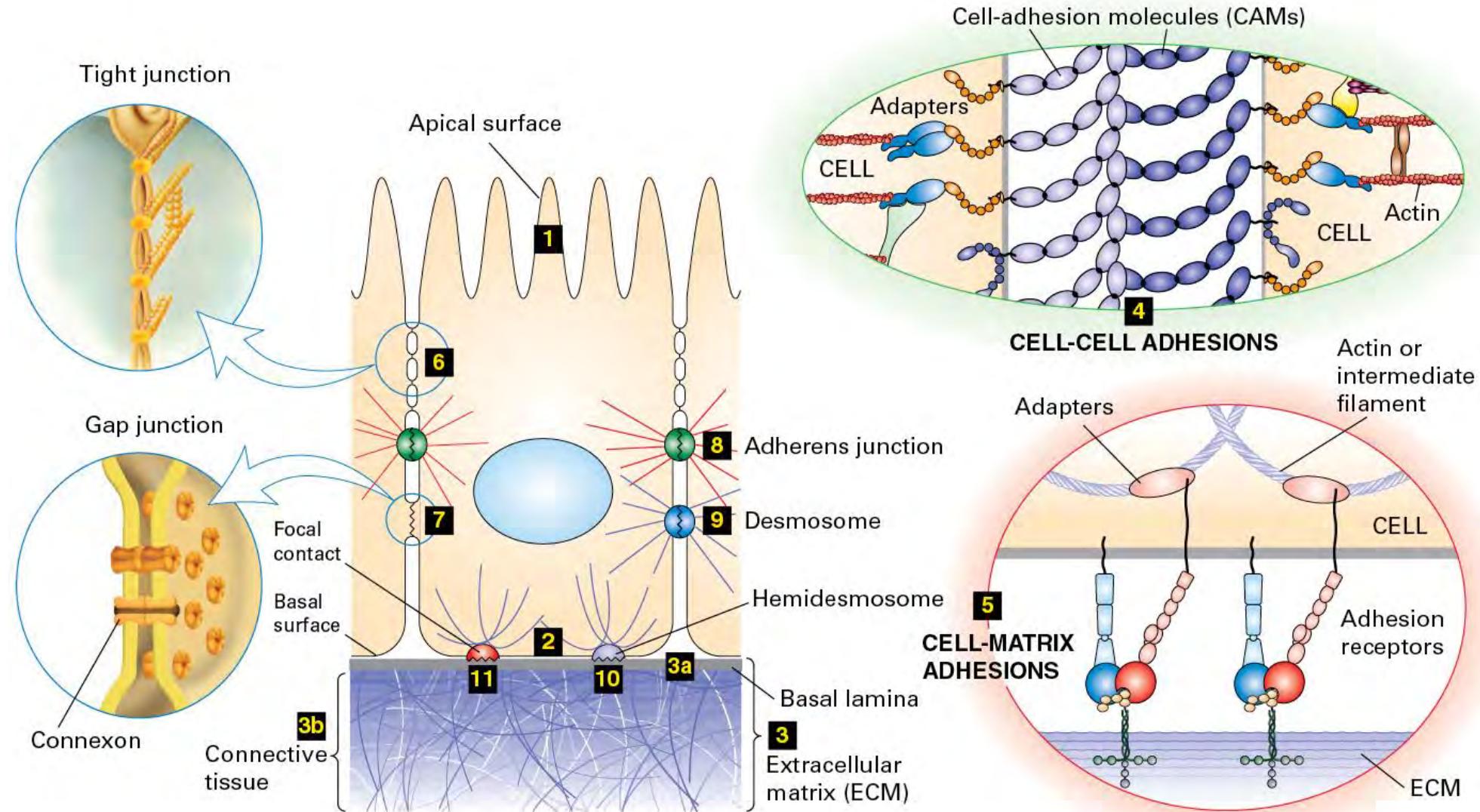
Dr. Starz-Gaiano does not endorse these products!!!



Cell Biology and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

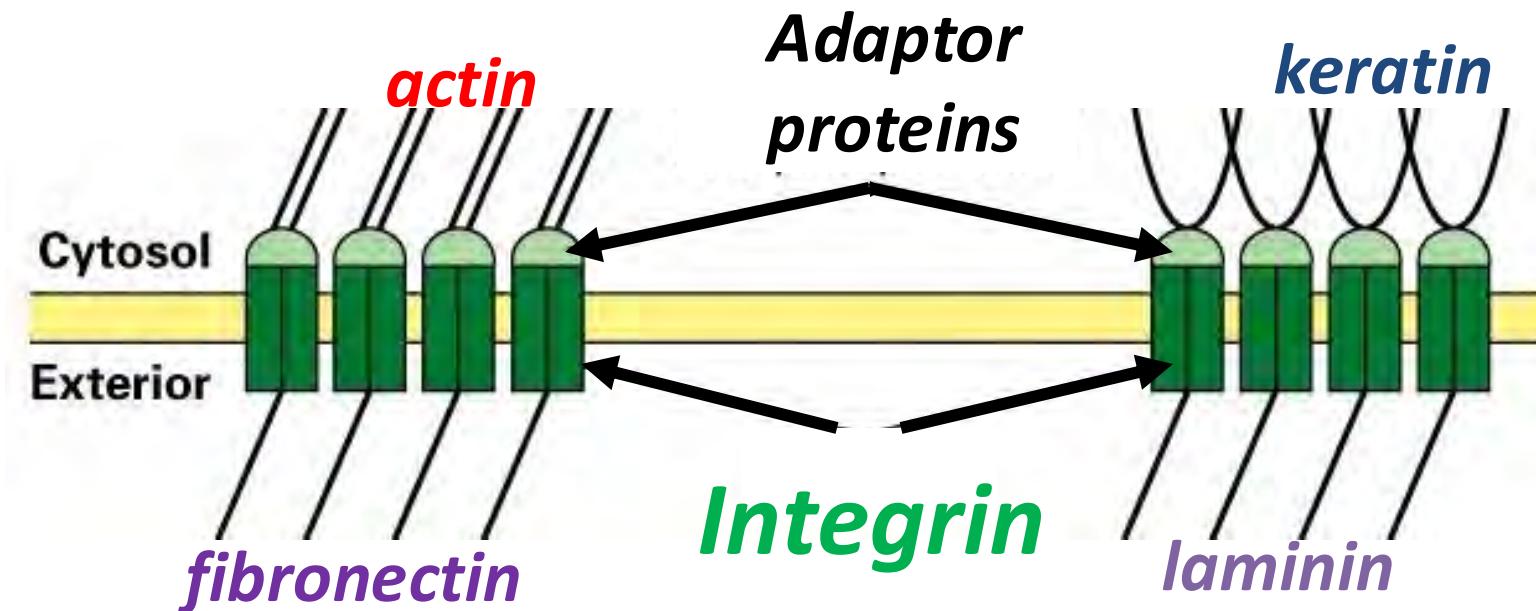
We have the foundation...

epithelial cells adhere to the ECM and to neighbors



how are cells attached to their ECM?

- ECM proteins (**fibronectin** or **laminin**) are connected to **integrins** on the cell surface, which are connected to adaptor proteins, which are connected to intracellular **cytoskeleton**



Preview of coming attractions :

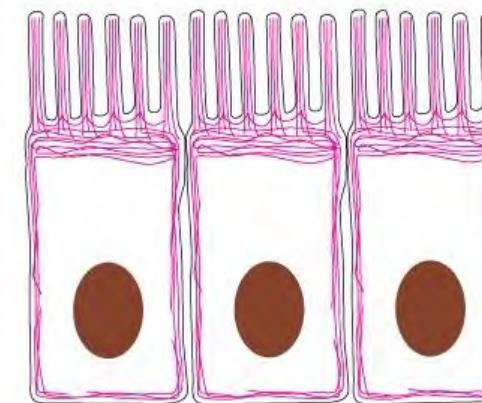
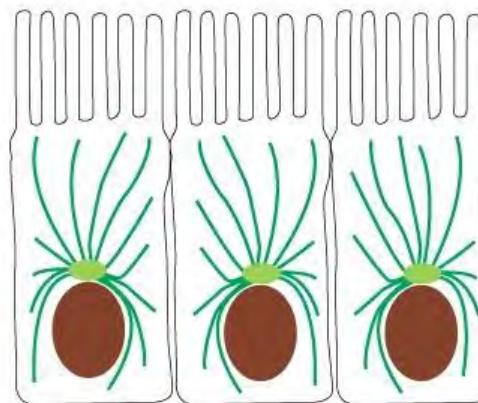
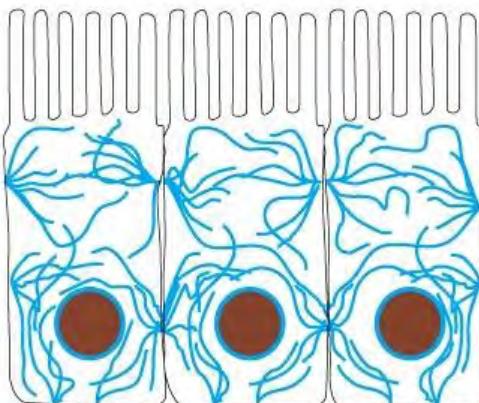
3 major cytoskeletal filaments:

intermediate filaments (like keratin)

microtubules

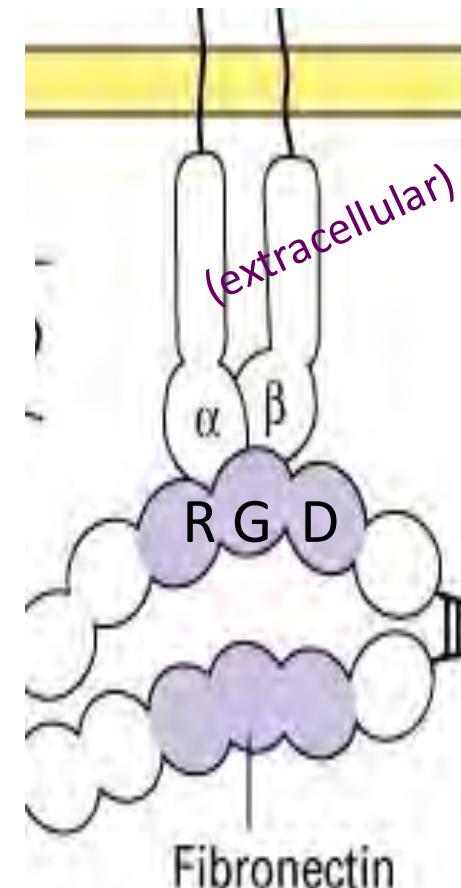
and

actin



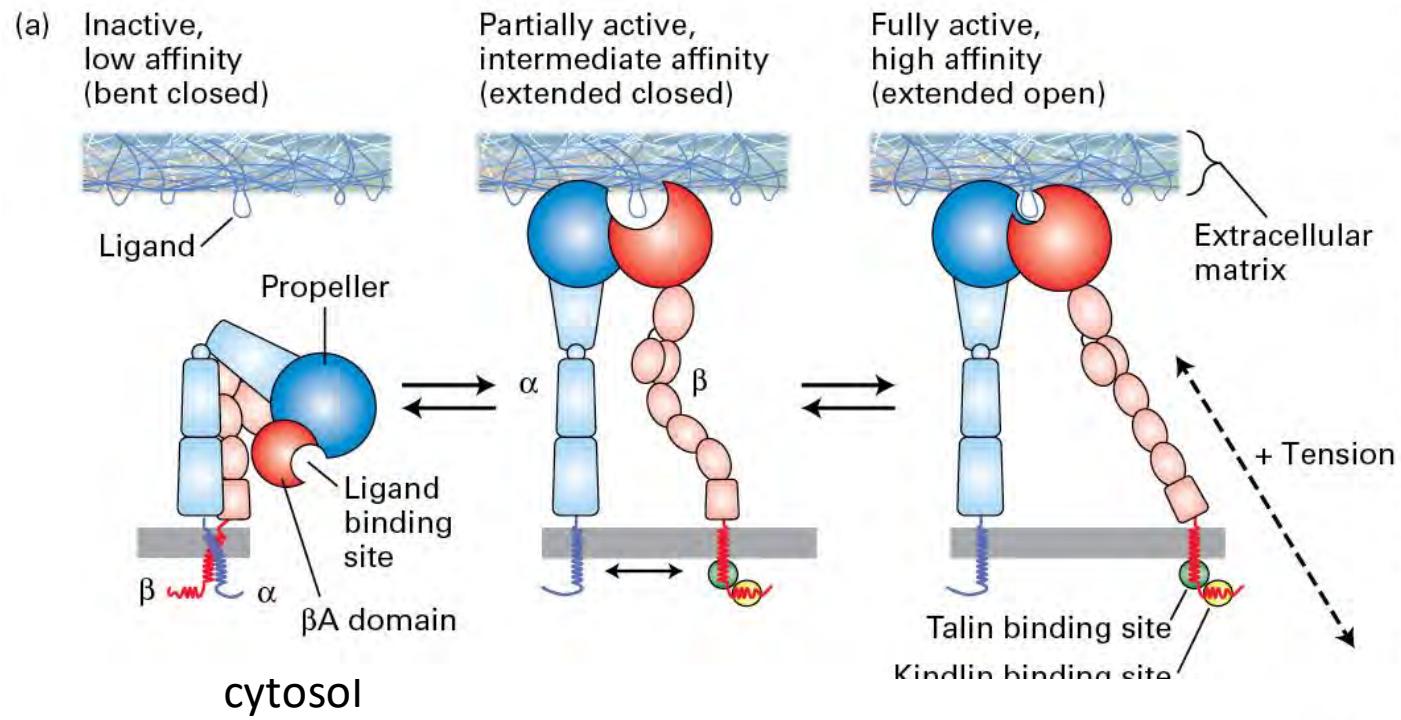
Integrins enable cells to bind to substrates

- Integrins contain two subunits ($\alpha+\beta$) and bind to ligands with the sequence **arg-gly-asp (RGD)** (e.g. fibronectin, ICAMS, etc.)
- Integrins mediated adhesion transmits signals from outside the cell to the cells' interior (**outside-in signaling**)
- Integrins mediate 2 types of cell-matrix adhesions, and some cell-cell adhesions



Integrins change conformation to bind a substrate

- Binding of **talin** between the cytoplasmic regions of the α and β chains causes a conformational change that activates integrins
- *inside-out-signaling* – cell is ready to attach



al., Molecular Cell Biology, 9e, © 2021 W. H. Freeman and Company

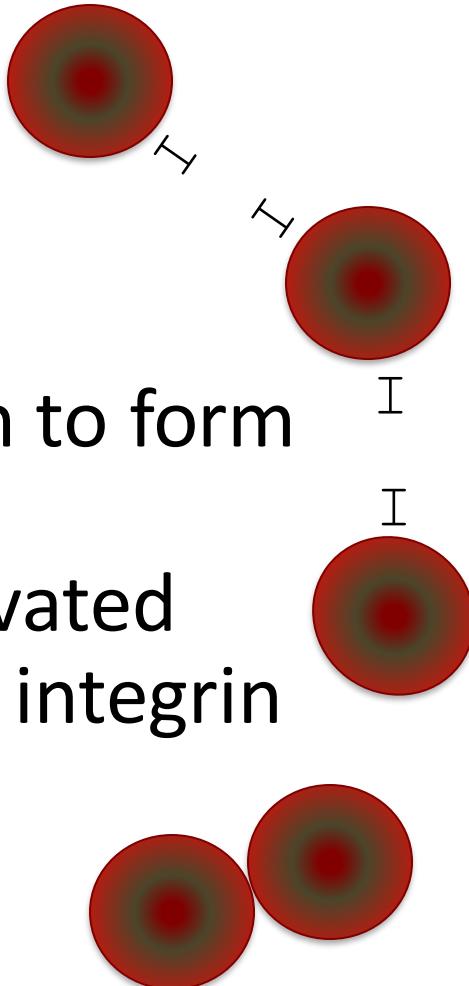


Integrins connect cells to the ECM

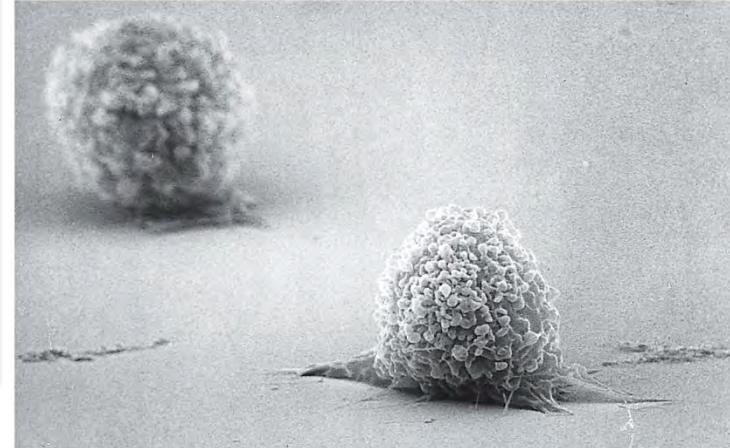
- transmembrane receptors
- Integrin/ligand binding is low affinity
- but (cluster) for strength
- must be activated to adhere
- many different integrins- each Integrin is an alpha/beta heterodimer
 - 18 α chains
 - 8 β chains

Integrins are important in cellular function

- Development
- Leukocyte extravasation
- Blood clotting
 - Platelets adhere to fibrinogen to form a clot
 - Platelet integrin must be activated
 - Genetic mutations in platelet integrin β chain \rightarrow clotting deficiency



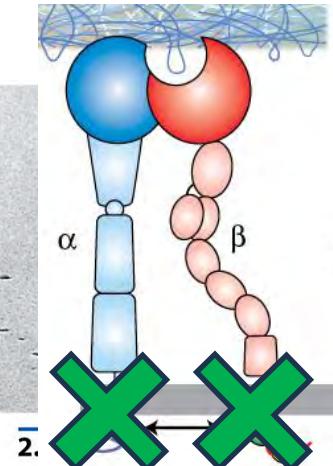
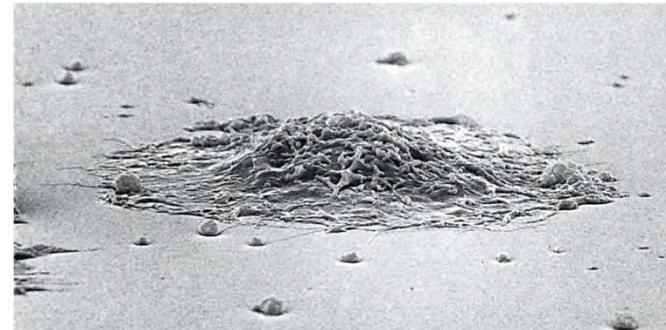
Cells placed on a slide coated in fibronectin will spread out and stick to the surface of the slide. This adhesion is mediated by Integrins.



2.5 μ m

From J. J. Rosen and L. A. Culp, *Exp. Cell Res.* 107:141, 1977.

Show of hands:
you have identified a mutant integrin, which is truncated and thus does not have the transmembrane domain. Will cells bearing this mutant integrin (and no others) stick down?



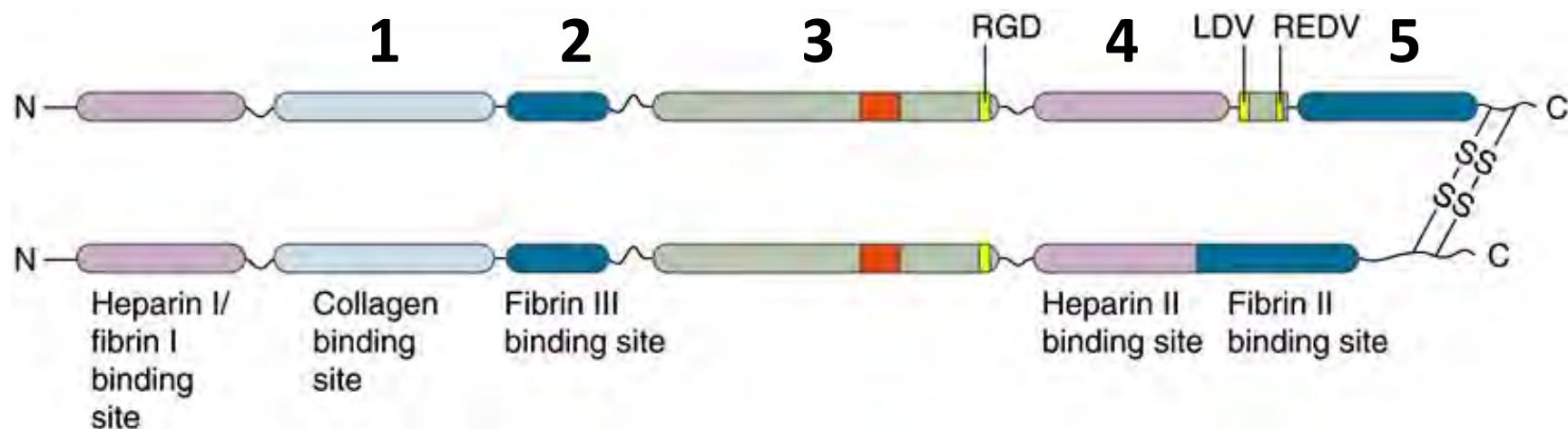
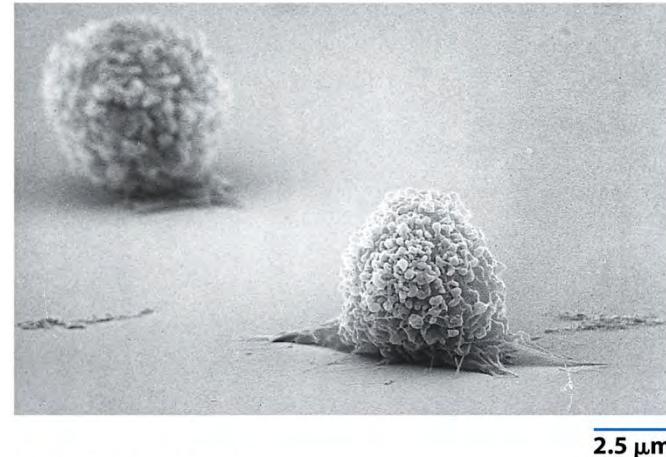
From J. J. Rosen and L. A. Culp, *Exp. Cell Res.* 107:141, 1977.

A. Yes

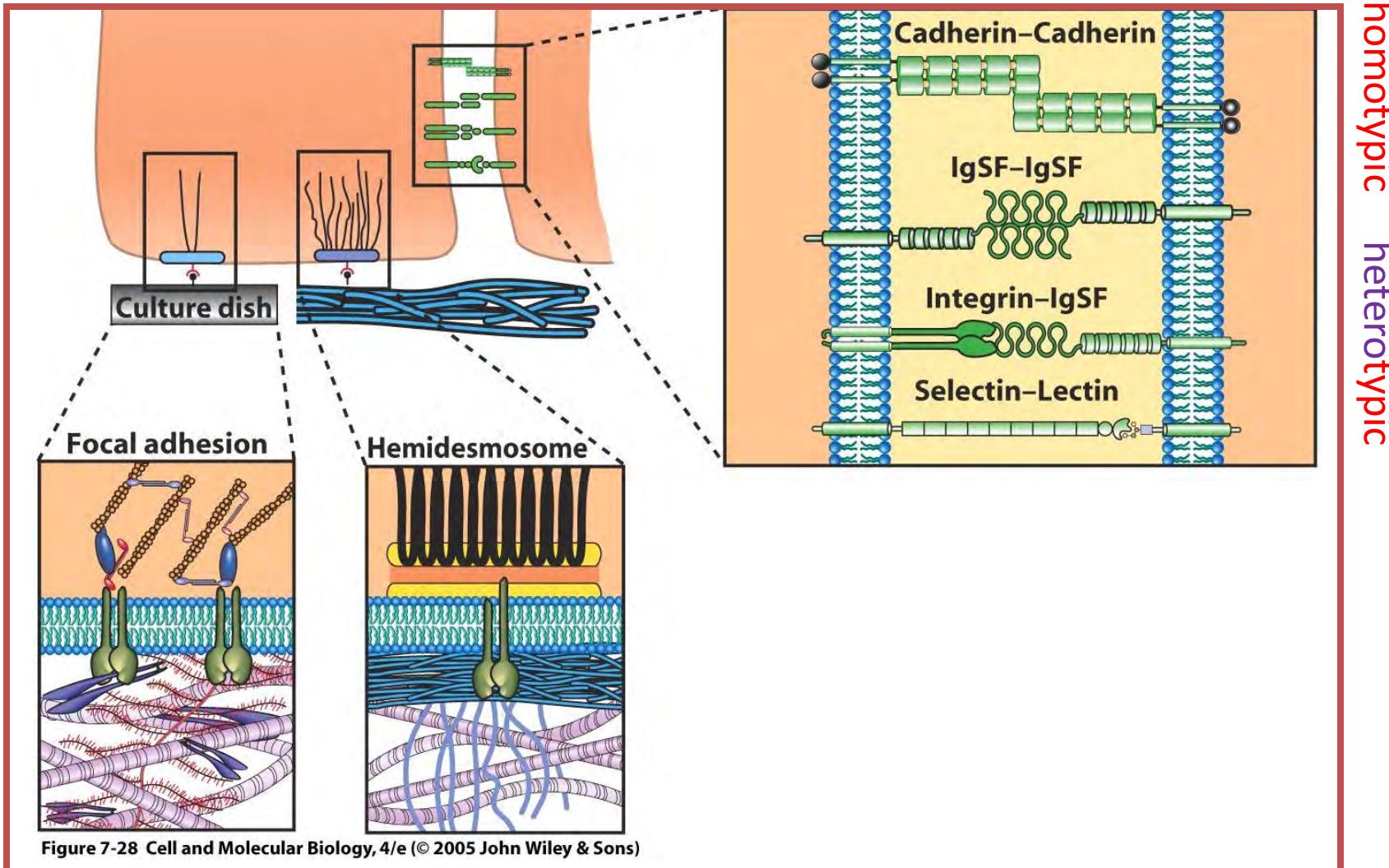
B. No

Cells placed on a slide coated in fibronectin will spread out and stick to the surface of the slide. This adhesion is mediated by Integrins.

Poll question 5:
prior to placing the cells on the slide, you mix into the media different purified domains from the fibronectin protein. which will prevent adhesion?



cell-matrix or cell-cell adhesions



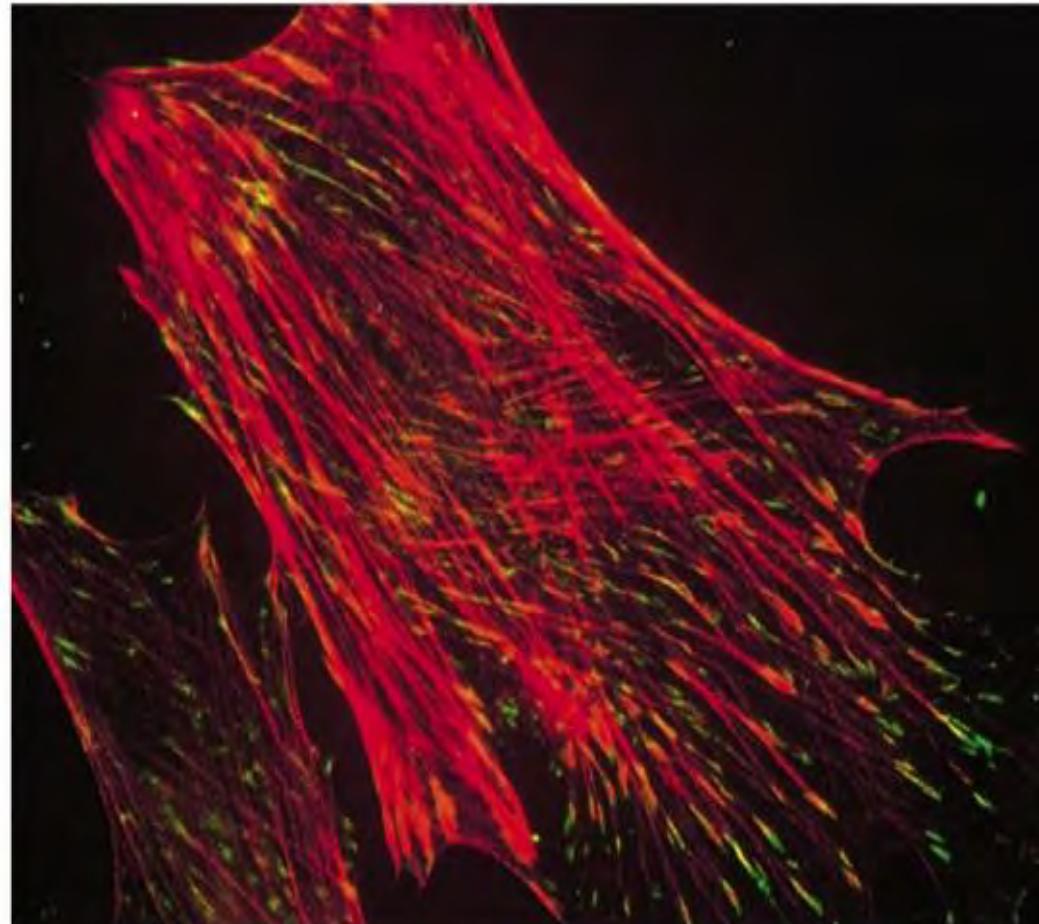
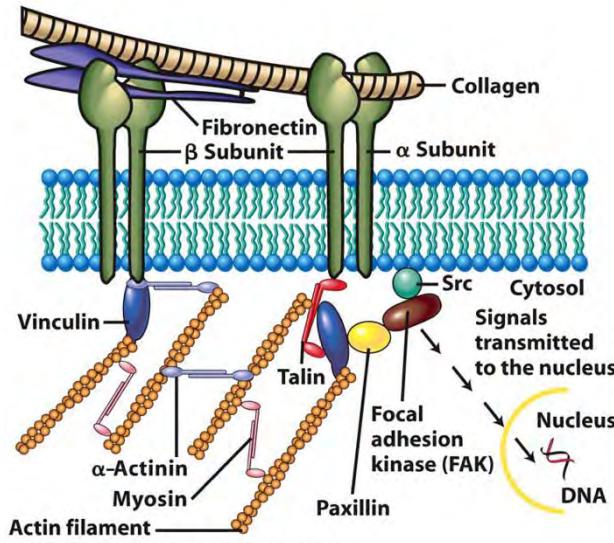
Cell-Substrate (or matrix)

Integrins associate with cytoskeleton in stress fibers when cells adhere to a substrate

Red = actin stress fibers

Green = integrin

Focal adhesion = point
of contact with substrate
(ECM) (actin filaments)



Integrins associate with cytoskeleton in hemidesmosomes = cell-matrix adhesions: basal side of cells (filaments) attach to basement membrane

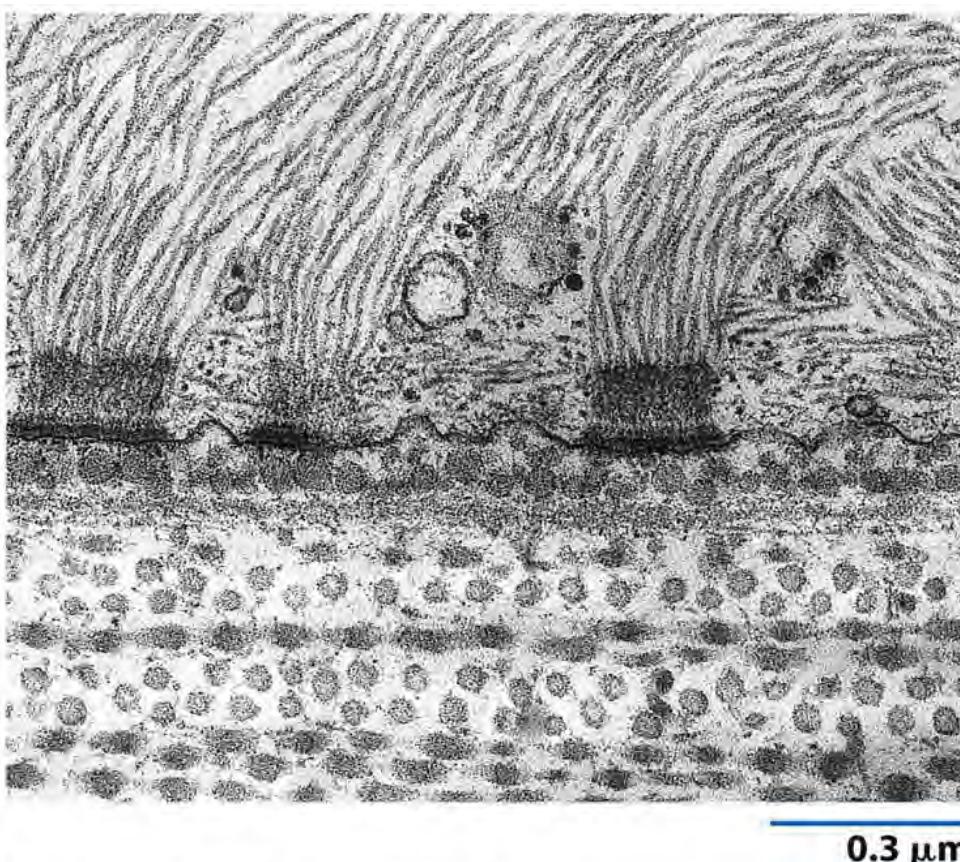


Figure 7-19a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

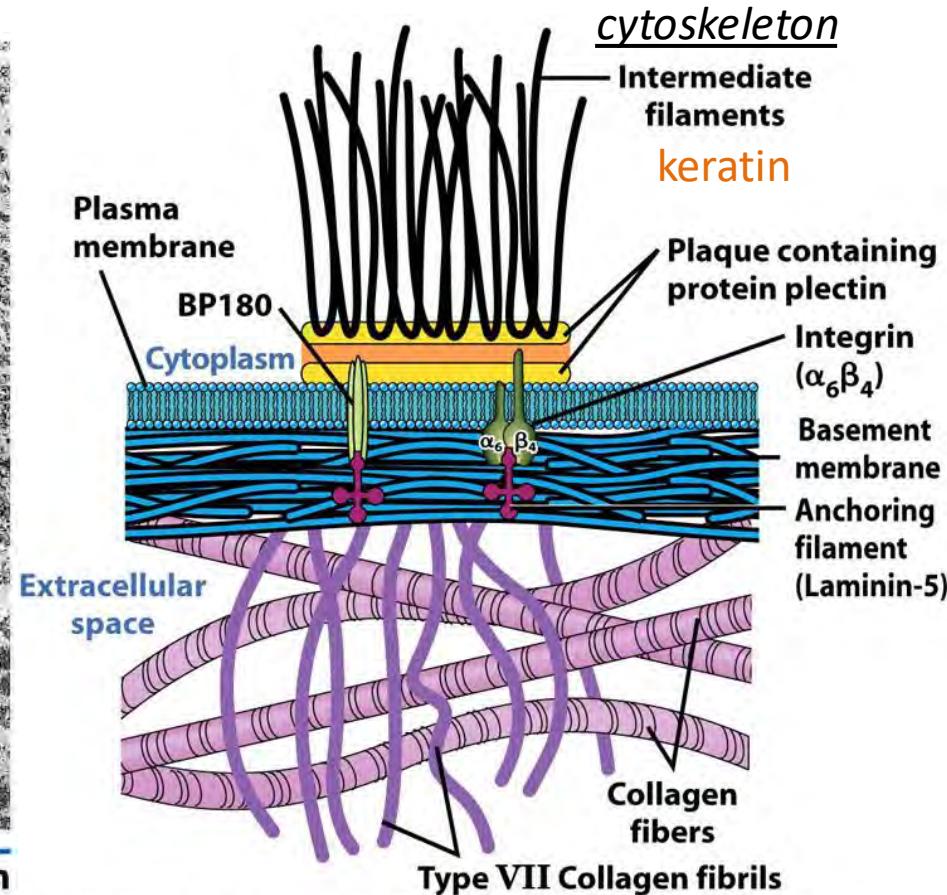
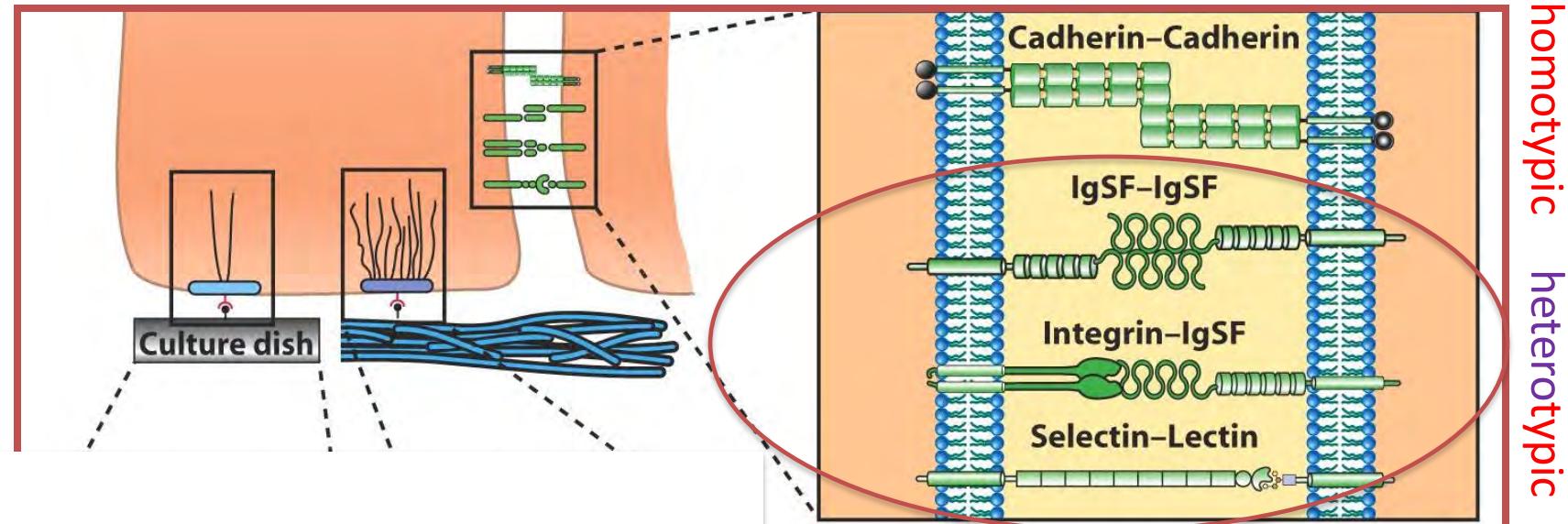


Figure 7-19b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

cells in a tissue do not just adhere to matrix

cell-matrix or cell-cell adhesions



Cell-cell adhesions require adhesion molecules

homotypic heterotypic

Cadherins mediate cell-cell adhesion (homotypic adhesion)

- Multiple types of cadherins; each type mediates adhesion of different types of cells (e.g. P, E, etc.)
- Extracellular domain has Ca^{+2} binding sites: Adhesion is Ca^{+2} -dependent
- Blocking antibodies can cause dissociation
- Cadherin are also in epithelial cell junctions

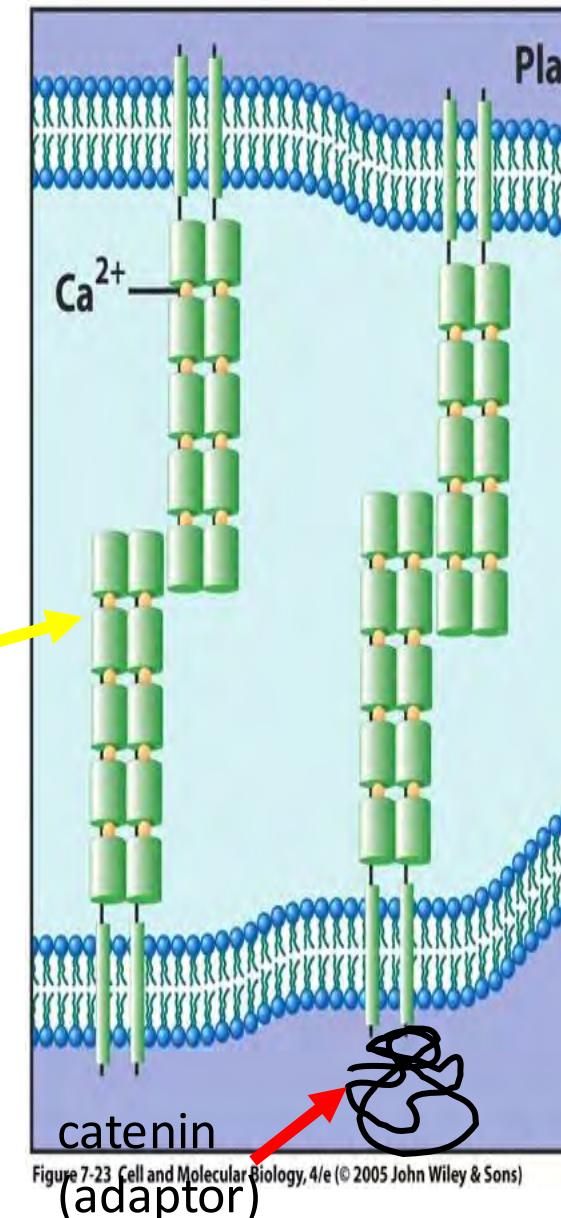
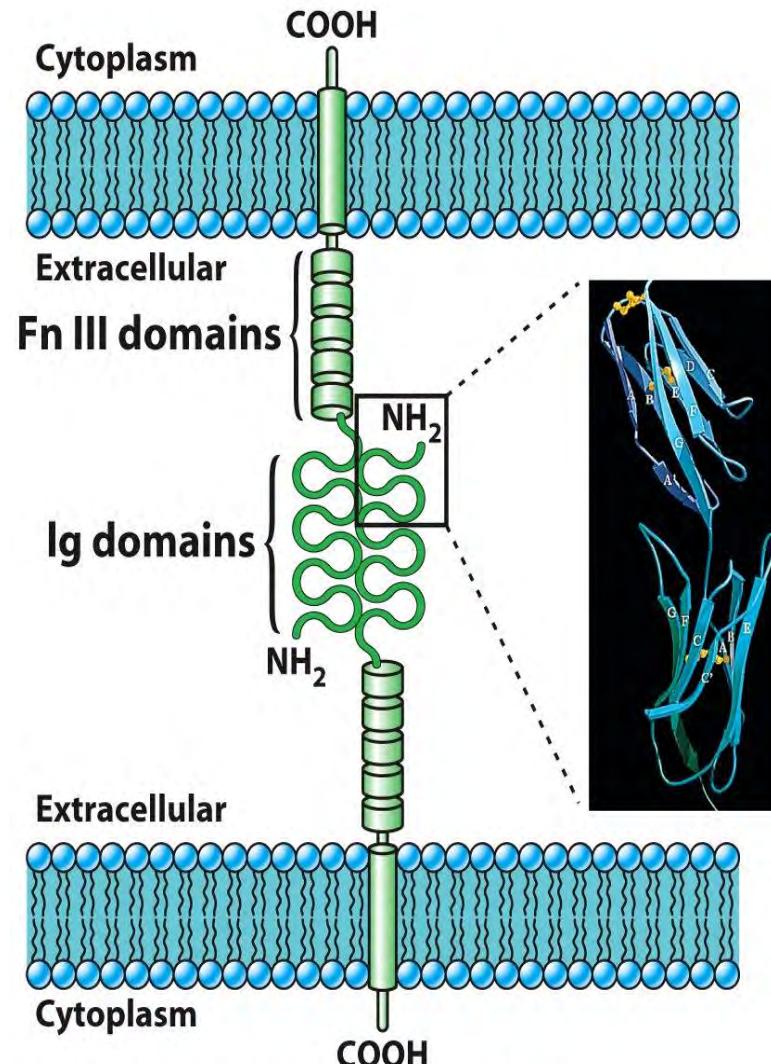


Figure 7-23 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)
catenin
(adaptor)

Ig-superfamily members (IgSF) can have homotypic or heterotypic adhesion



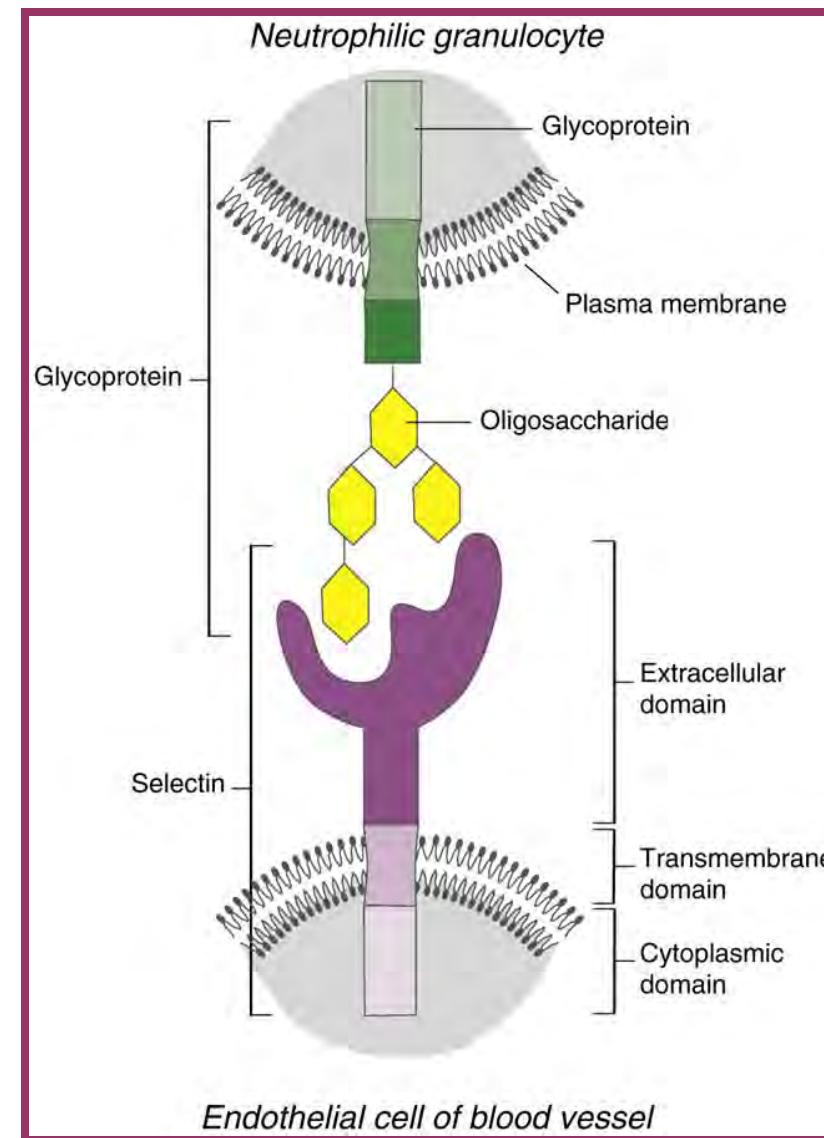
- Integral membrane proteins
- Multiple Cell Adhesion Molecules (CAMs) (L1)
- Ig domain of one N-CAM binds to opposing N-CAM
 - Or bind to integrins
- Ca⁺²-independent
- If antibodies to N-CAM are added to cultured neurons, the neurons dissociate

Selectins mediate cell associations/movement

- Lectin (protein) domain binds to carbohydrates on glycoprotein

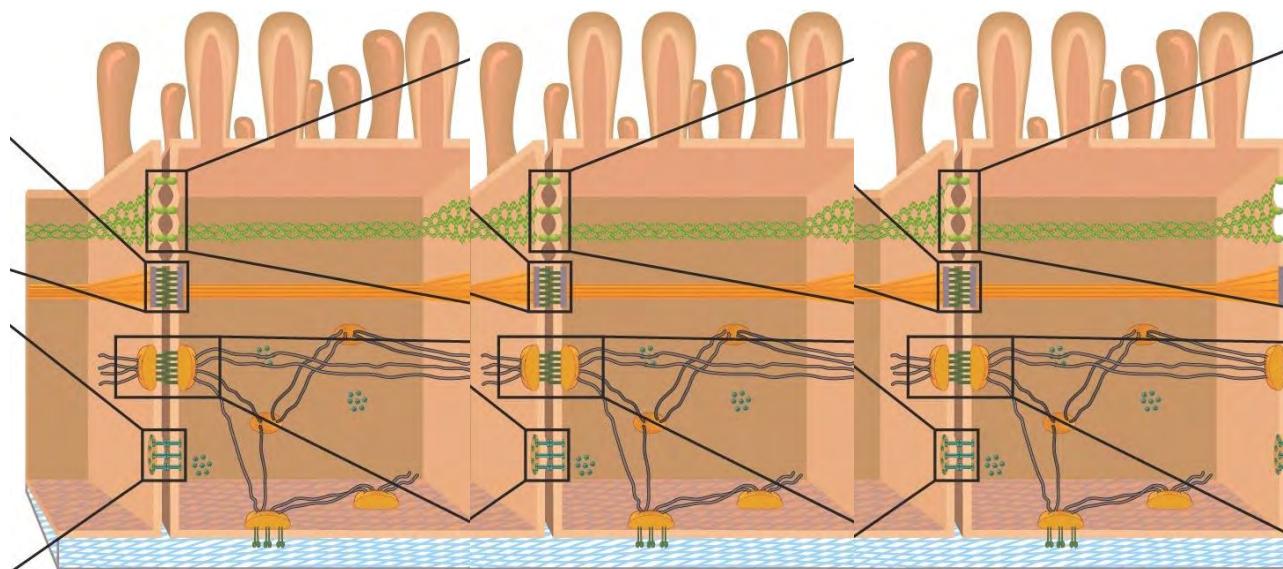
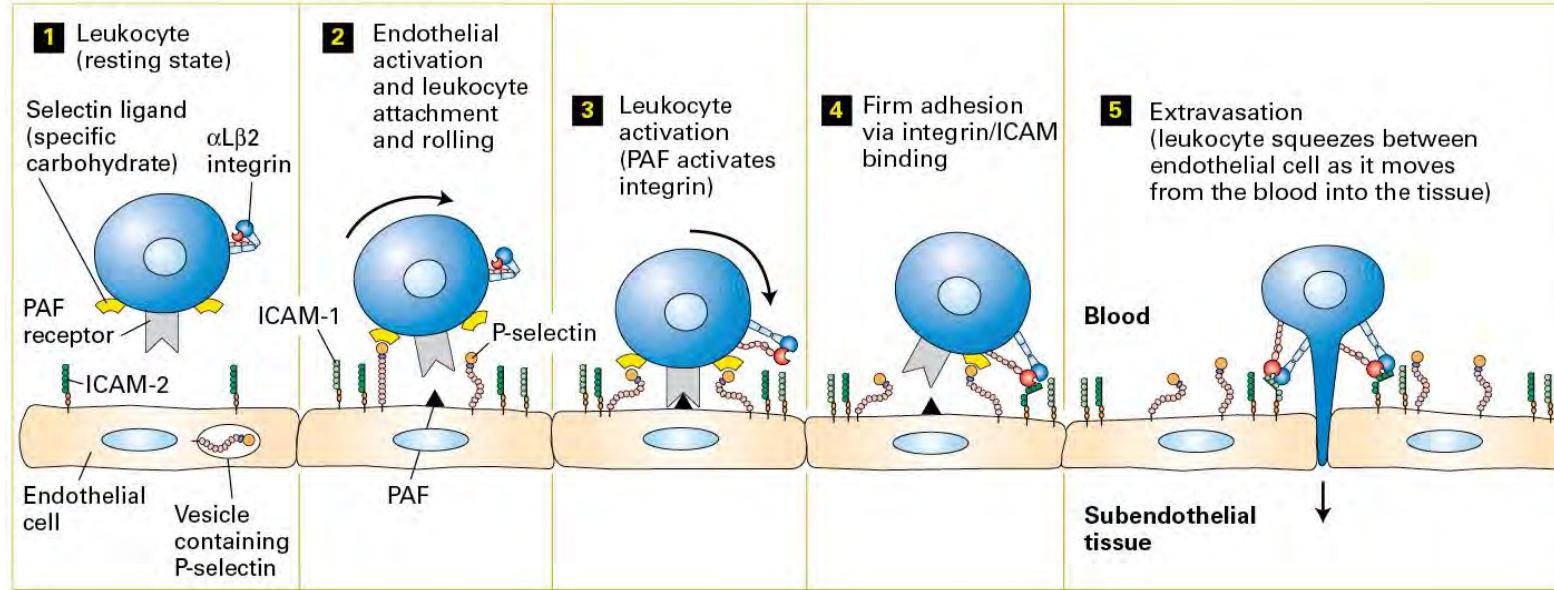
e.g: Leukocytes in extravasation

You will see more about this in discussion

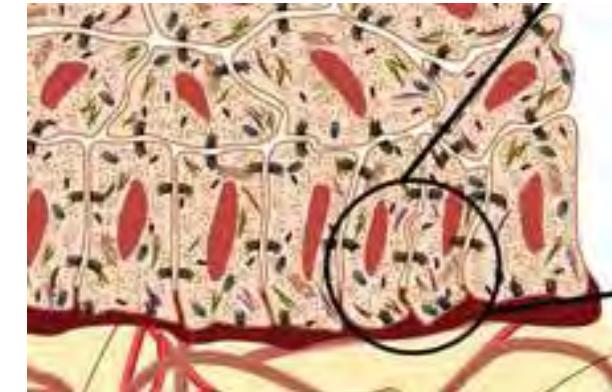


Extravasation in discussion

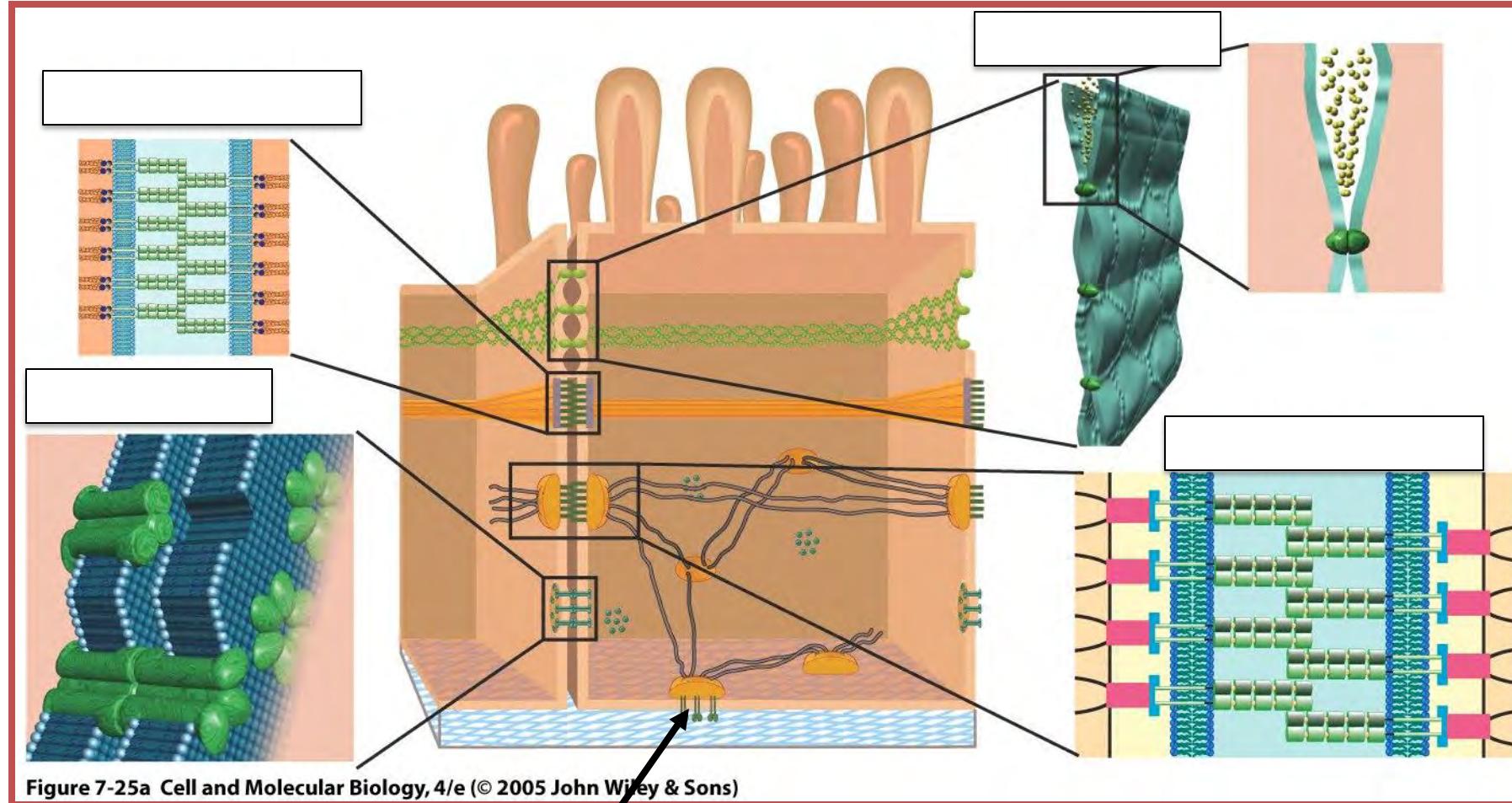
Cell-cell interactions depend on cell types and adhesion molecule profile



Skin is a type of epithelium



in epithelia, four types of cell junctions connect cells to each other (and link cytoskeletons)

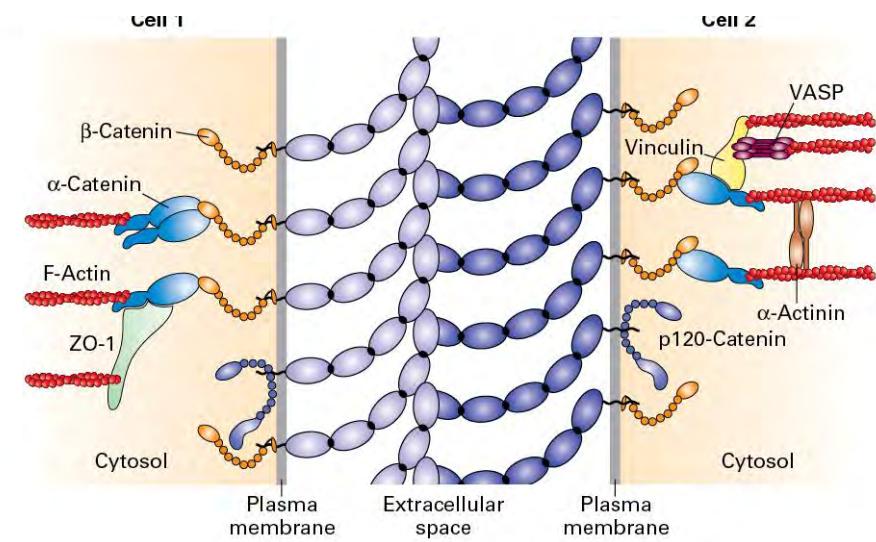
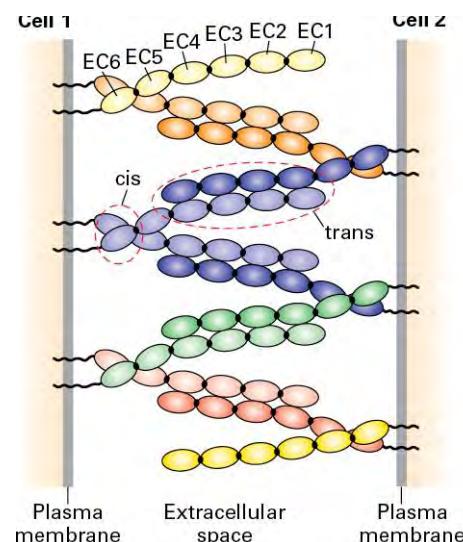
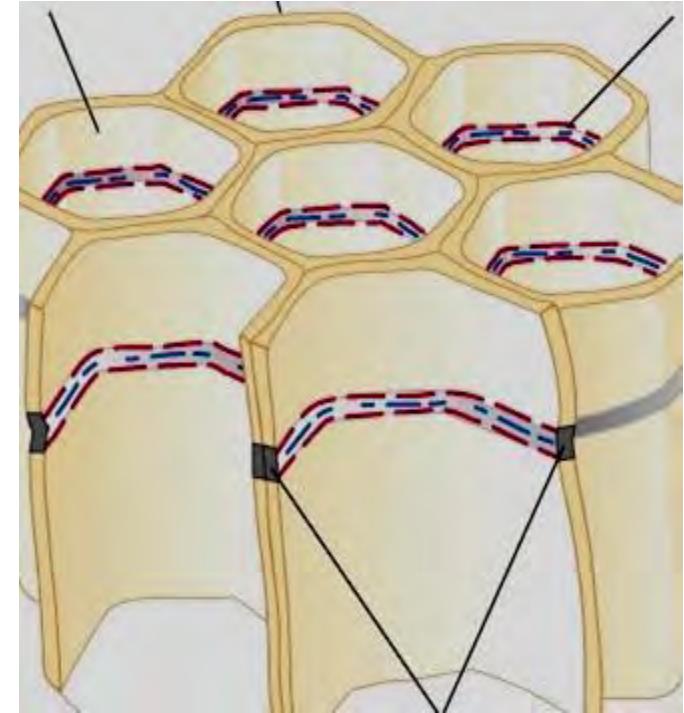


Hemidesmosomes – junctions with ECM and intermediate filaments
and focal adhesions (not shown)

Here's where we stopped for the day

adherens junctions

- adherens junctions coordinate cell-cell activity and help stabilize the epithelium
- **Actin-myosin filaments** form a “belt” around each cell that allows a group of cells to coordinate shape.
- they are frequently associated with tight junctions



adherens junctions couple the **actin** cytoskeleton of adjoining cells via...

the integral membrane protein

Cadherin

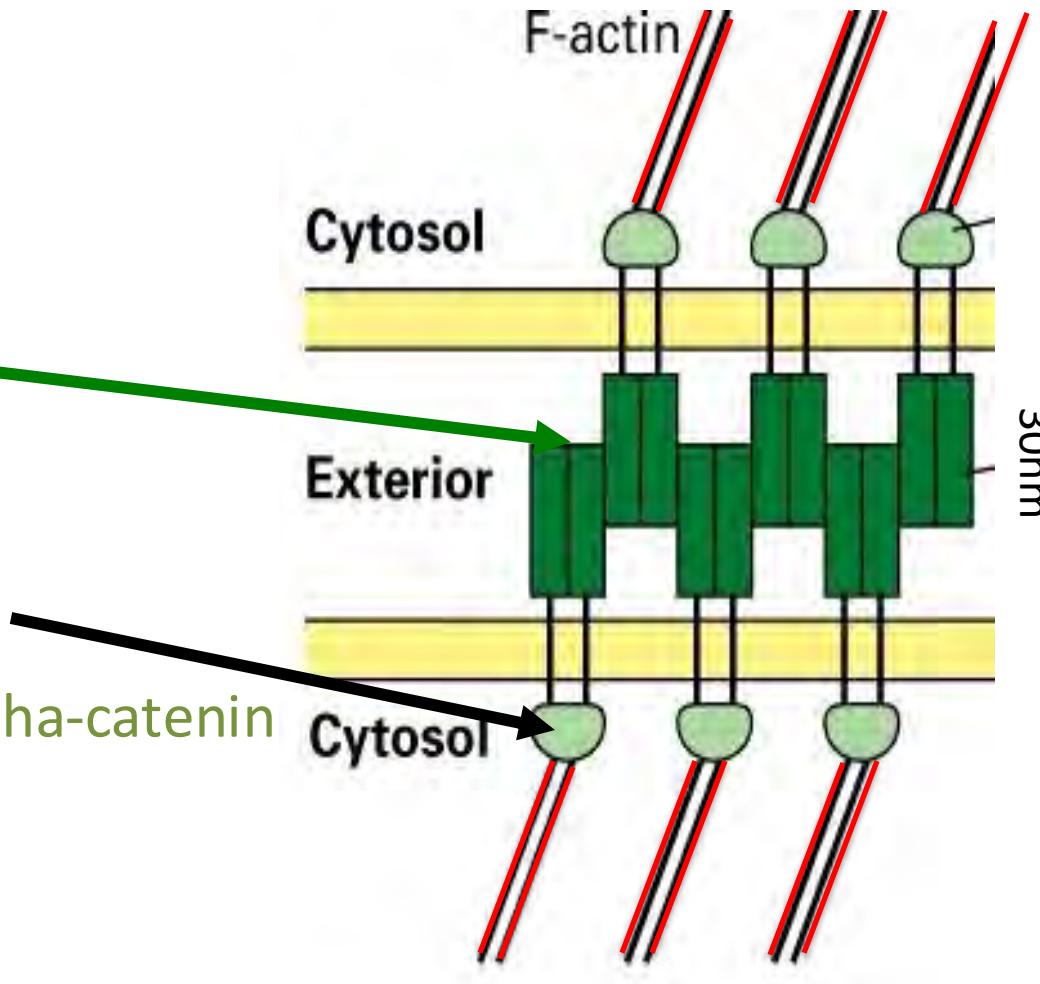
and adaptor proteins

such as **beta-catenin, alpha-catenin**

connect cadherin to

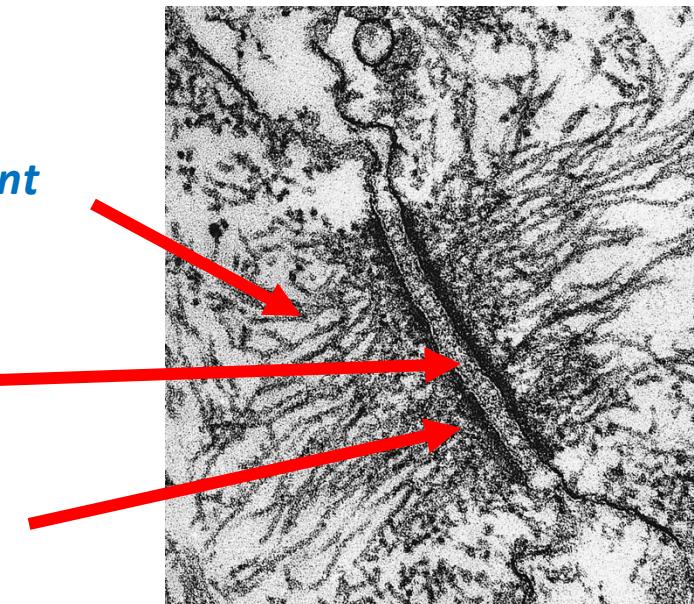
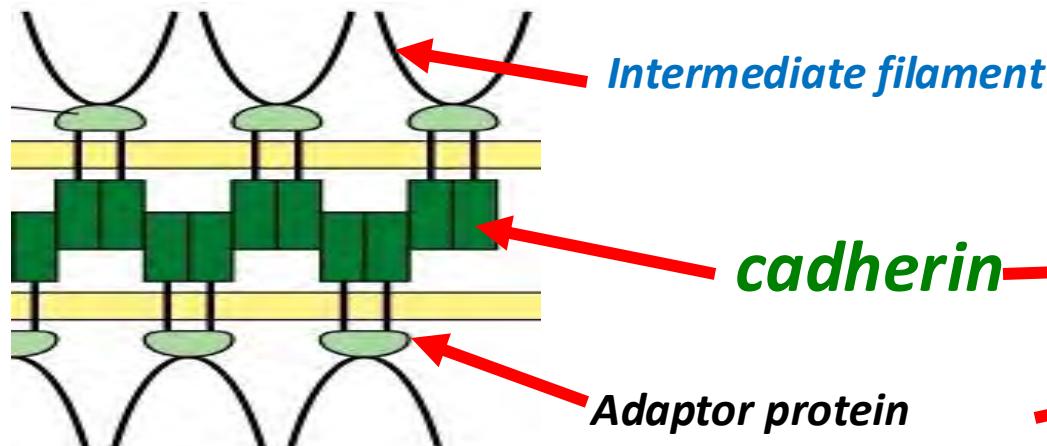
actin and myosin

filaments



desmosomes

- important cell-cell adhesions for **structural support/mechanical strength** of tissue (e.g. skin epithelia, heart muscle)
- 1 μ m discs (not belts)
- Cadherin proteins (called desmogleins&desmocollins) connect to adaptor proteins that connect to **cytoskeletal component intermediate filaments**
- (remember hemi-desmosomes? they also link to **intermediate filaments**)

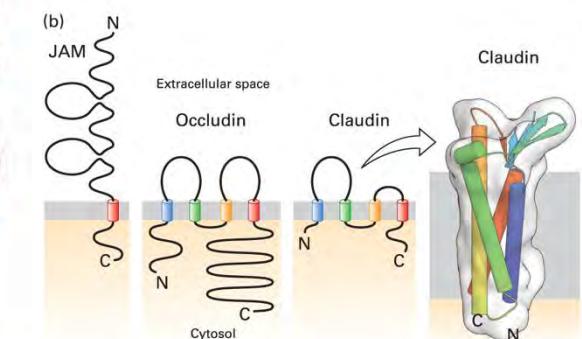
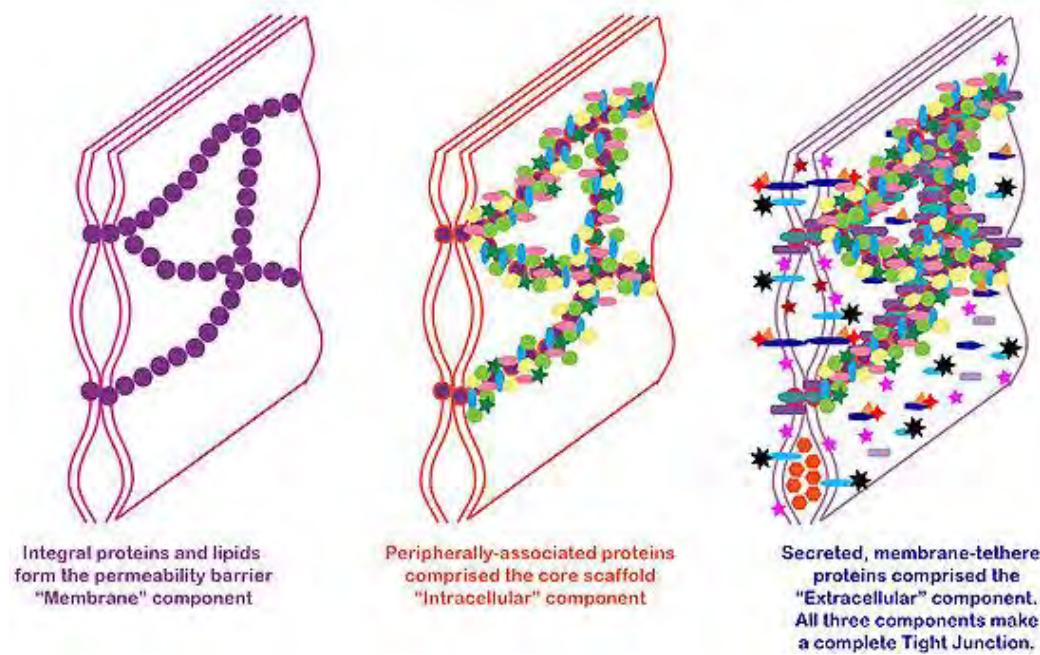
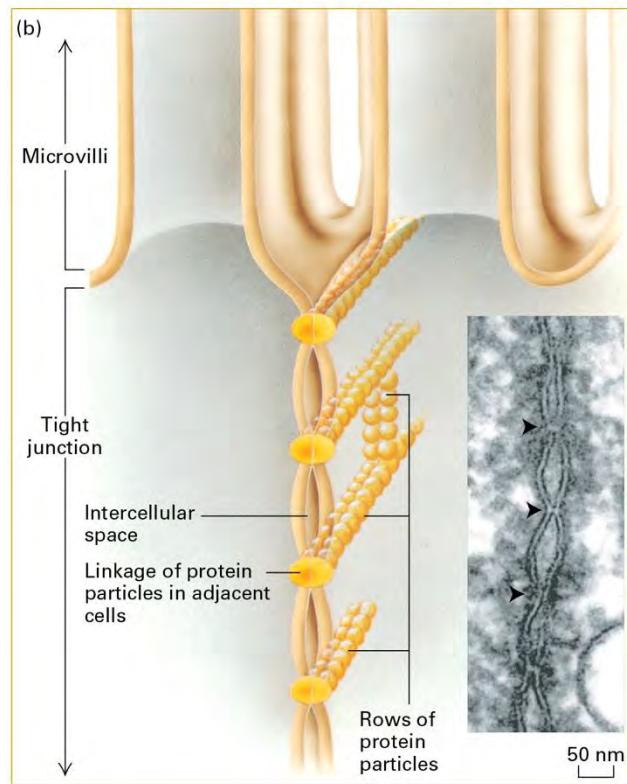
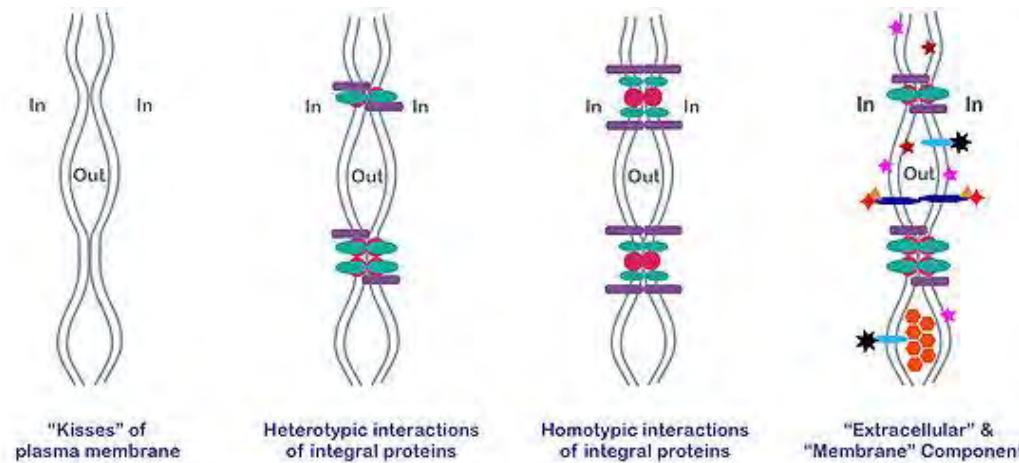




tight junctions

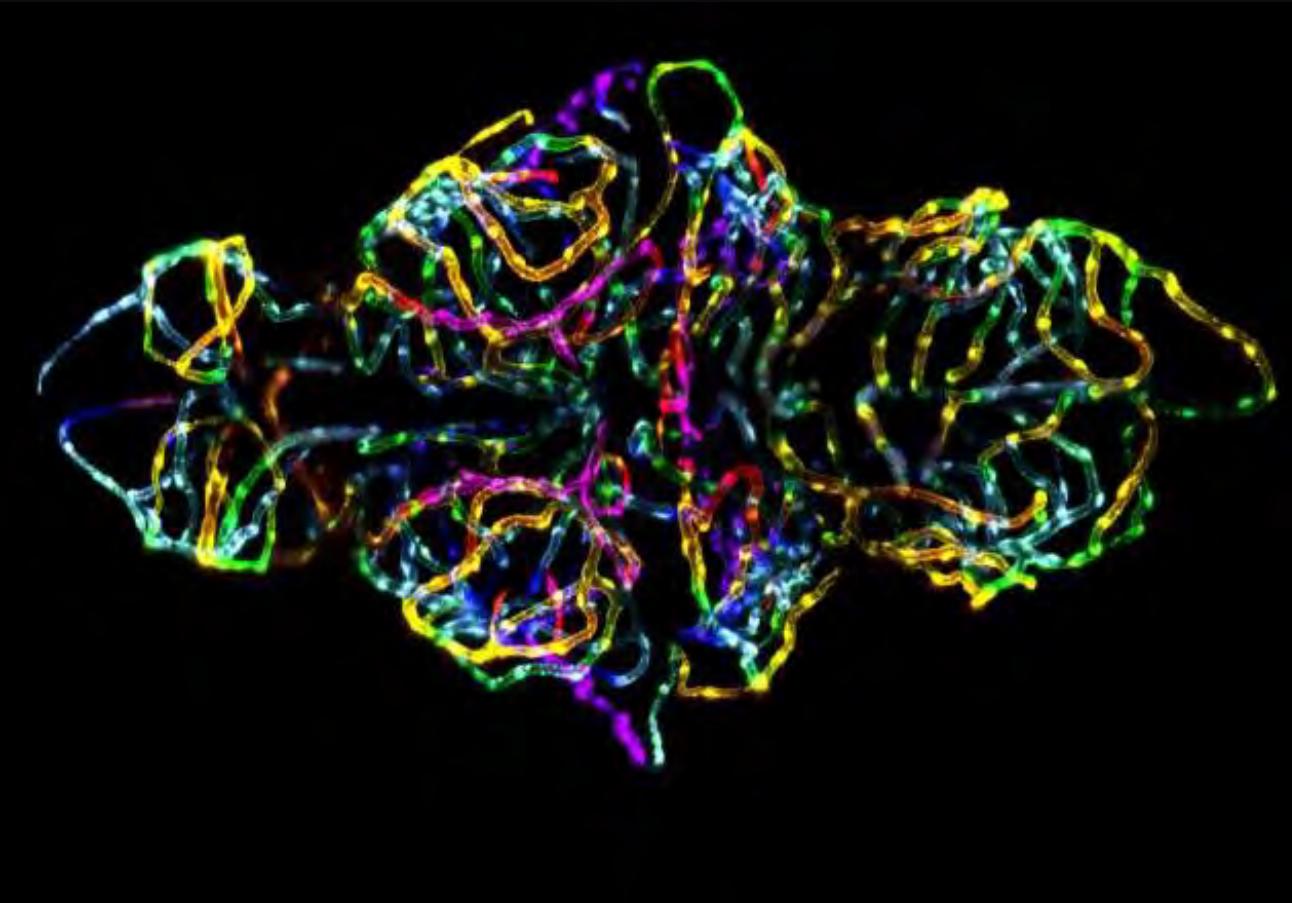
- hold cells together and make epithelial layers impermeable
- bands around cells that attach to a similar band on adjacent cells to prevent substances from slipping between
- localized **apically** ↑
- block lateral movement of membrane components = maintain cell polarity
- examples:
 - skin retains water
 - epithelial cells line the stomach and intestine. tight junctions are very important to make sure that the acid/food doesn't leak
 - tight junctions in capillaries in the brain make up the blood brain barrier, which blocks bacteria, drugs, etc

tight junctions form strands



integral membrane protein families
Occludin and
Claudin strongly bind to their counterparts on the adjacent cell

blood brain barrier



1ST PLACE 2012

NIKON

PHOTOMICROGRAPHY
COMPETITION

Dr. J. L. Peters & Dr. M. R. Taylor

St. Jude Children's Research
Hospital Memphis, Tennessee, USA

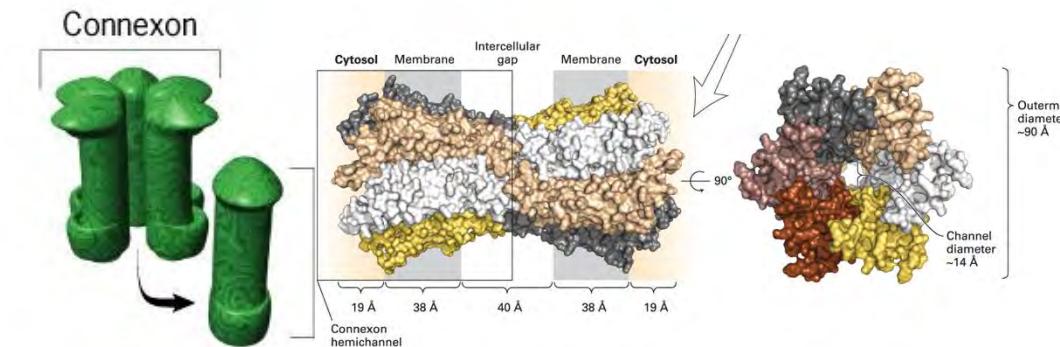
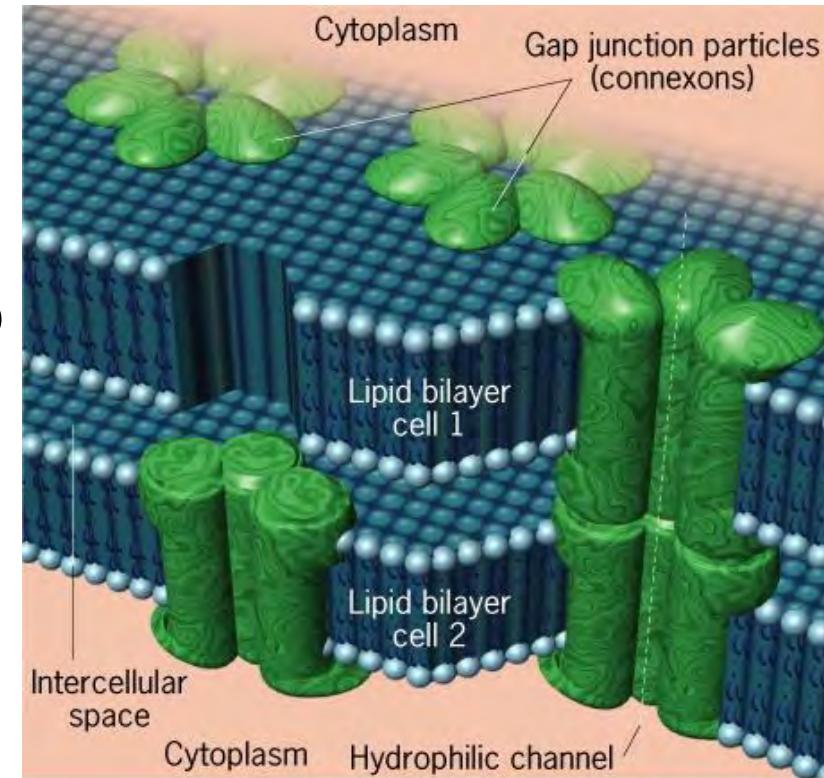
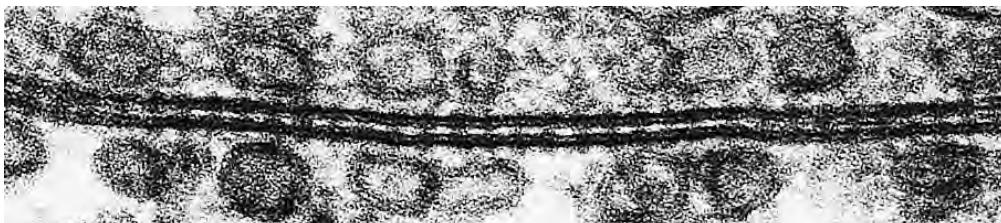
Subject Matter:

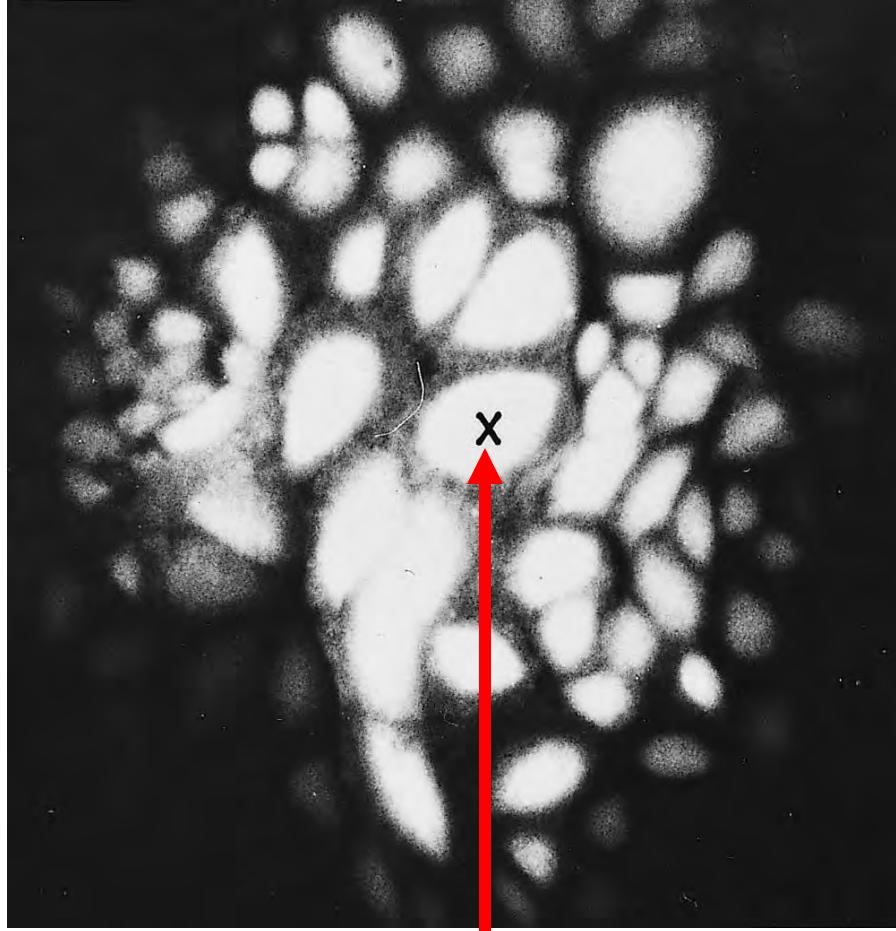
The blood-brain barrier in
a live zebrafish embryo

(20x) Technique: Confocal

gap junctions

- permit intercellular communication
- channels between cells that allow passage of small molecules (ions, amino acids, nucleosides, etc.) (continuous)
- major protein is connexin which forms channels through the plasma membrane
- regulated 1.5nm inner diameter



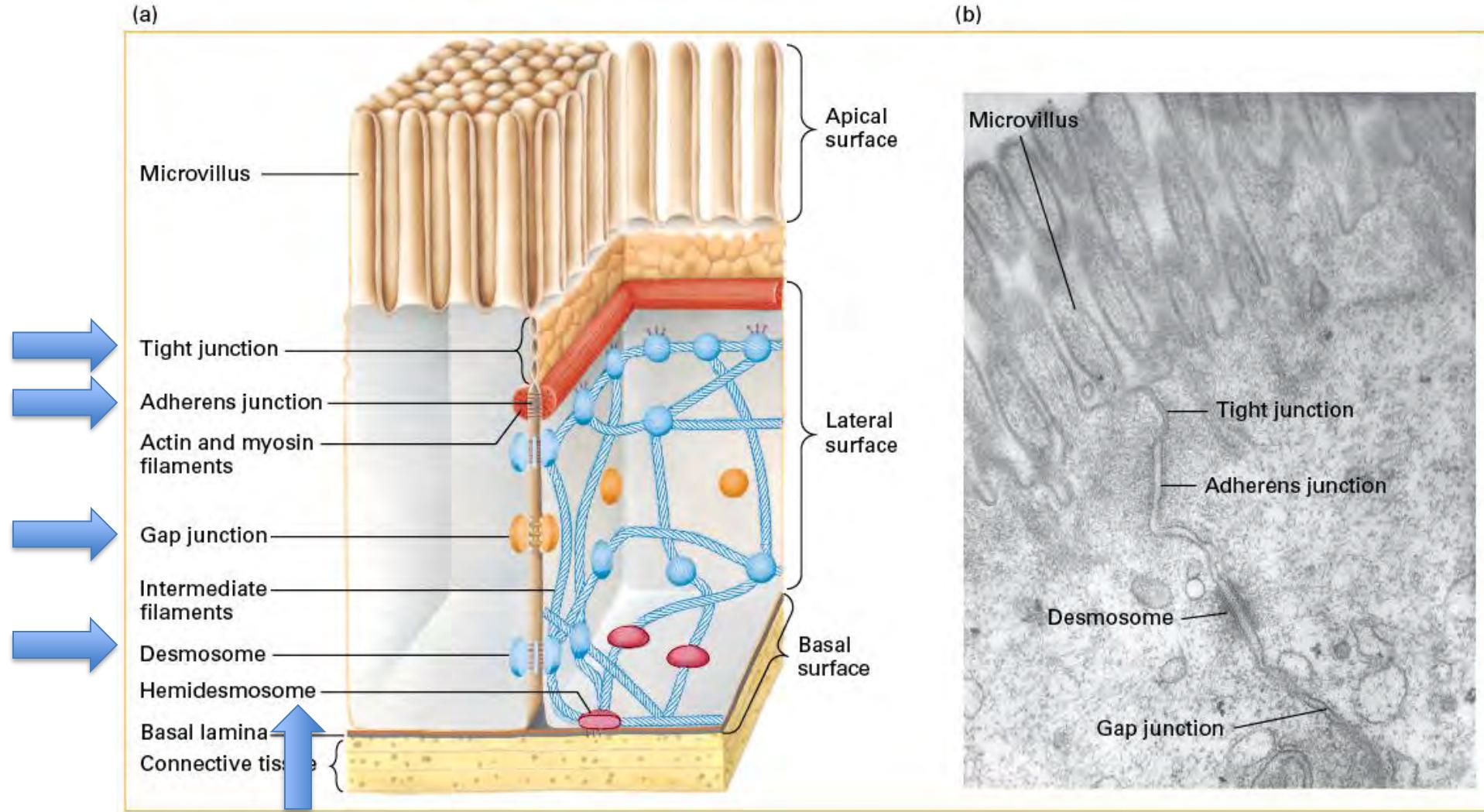


injected cell

**how do we know that
gap junctions are
passages between
cells?**

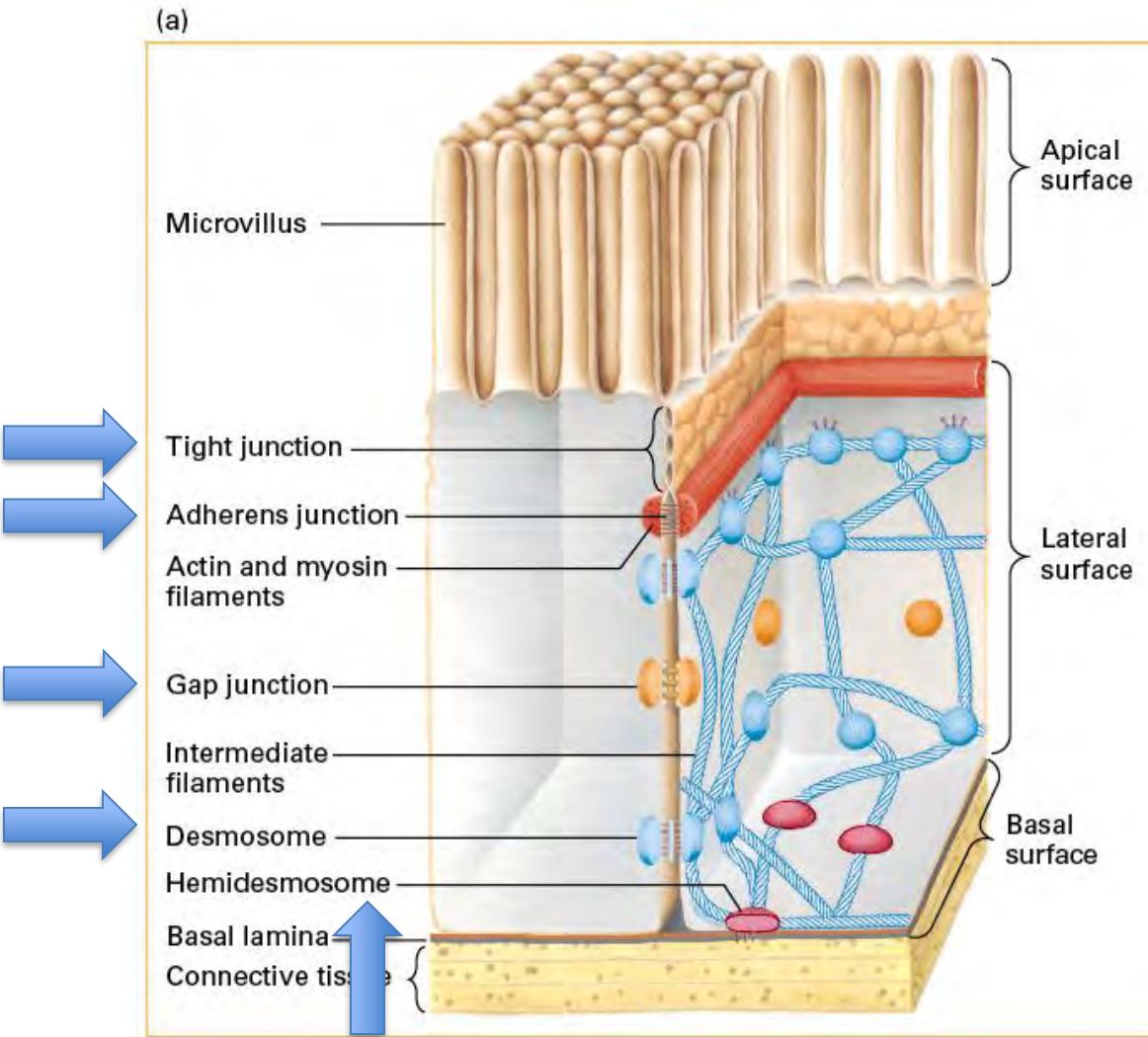
- inject fluorescently labeled small molecules into one cell
- observe that label traverses through gap junctions and into neighboring cells

major types of cell junctions



Two types do
NOT link to
cytoskeleton
(directly)

Last poll: which junction prevents water from leaking into your skin?



- a) Tight junctions
- b) Adherens junctions
- c) Desmosomes
- d) Gap junctions
- e) Hemidesmosomes

Coming up...

We go deeper into **cytoskeleton** in the next 3 lectures (exact order may differ from syllabus topics)