lecture 12- cell adhesions (continued) , cytoskeleton overview, intermediate filaments,

now playing:
blister in the sun
violent femmes

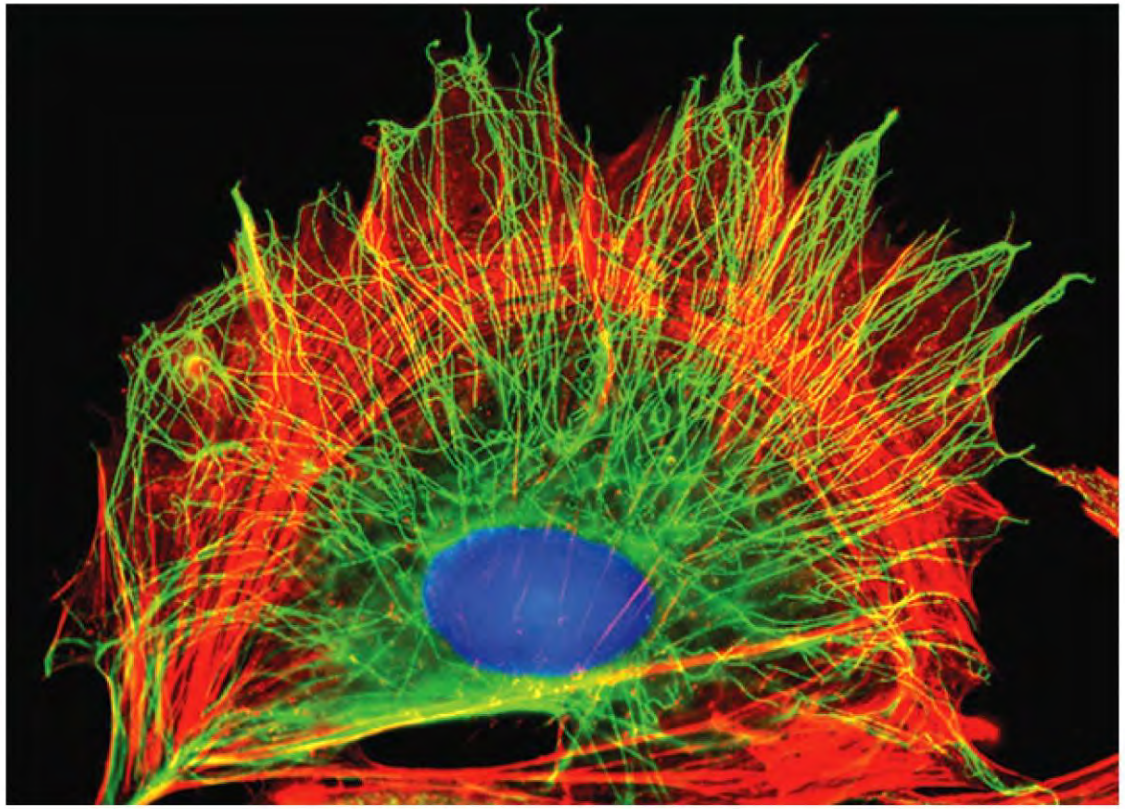
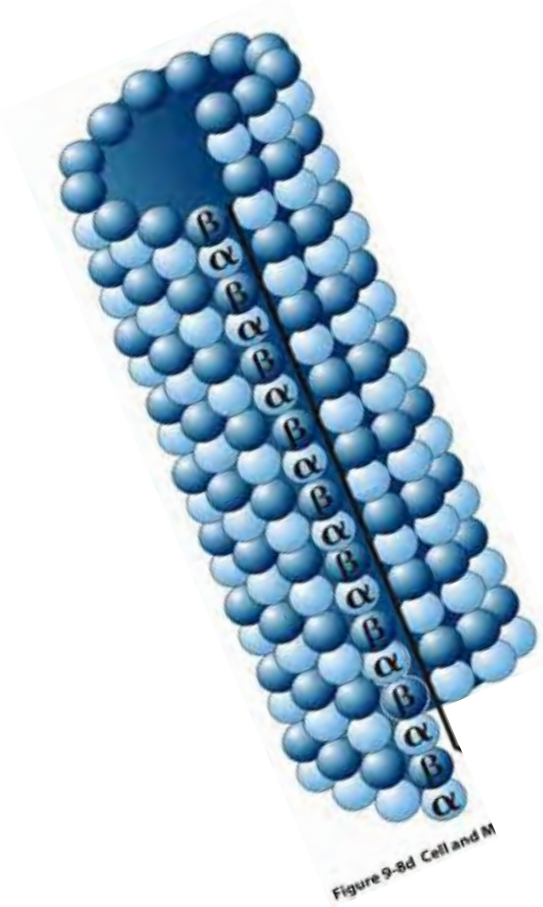
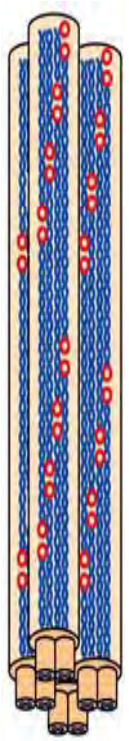


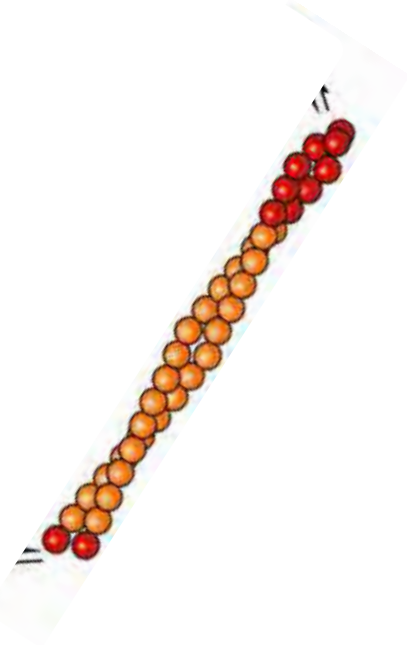
Figure 17-1 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Lodish chapters 20, (17) 18

cytoskeleton is made up of polymers – which allows cells to be dynamic!







But sometimes cells just need stability….

cell-matrix or cell-cell adhesions

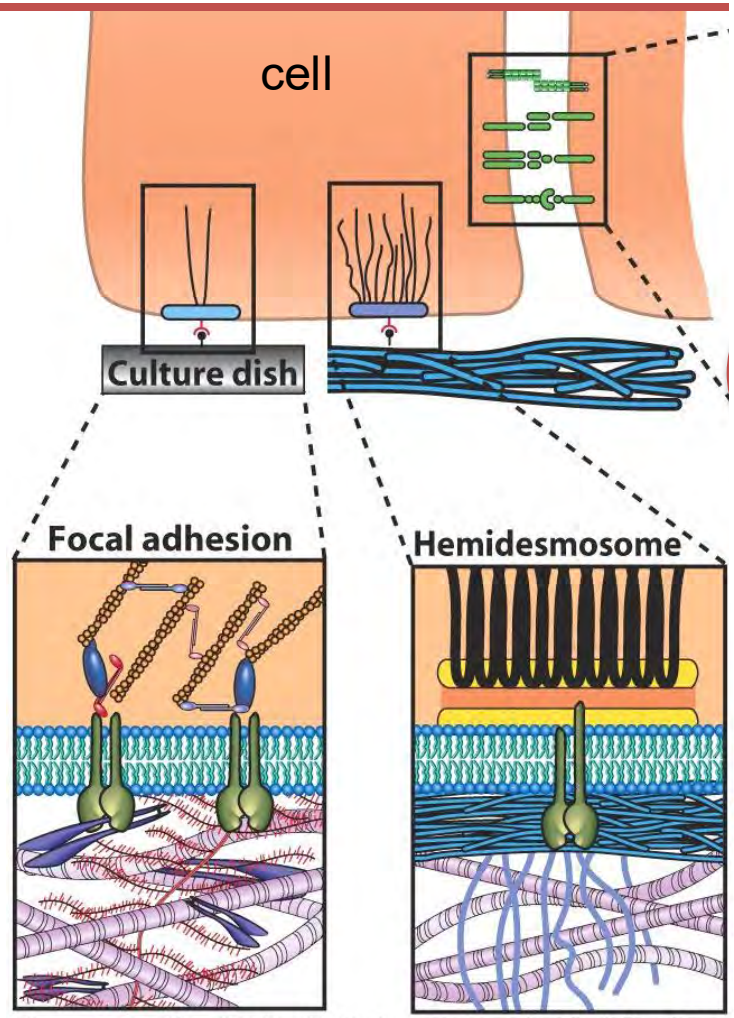


Figure 7-28 Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Cytoskeleton

Adhesion molecule

Cell-Substrate (or matrix)

administrative

Exam 2 is Oct 21 – why not start studying today?

PollEv.com/msg303
Send **msg303** to **22333**

warm up poll question

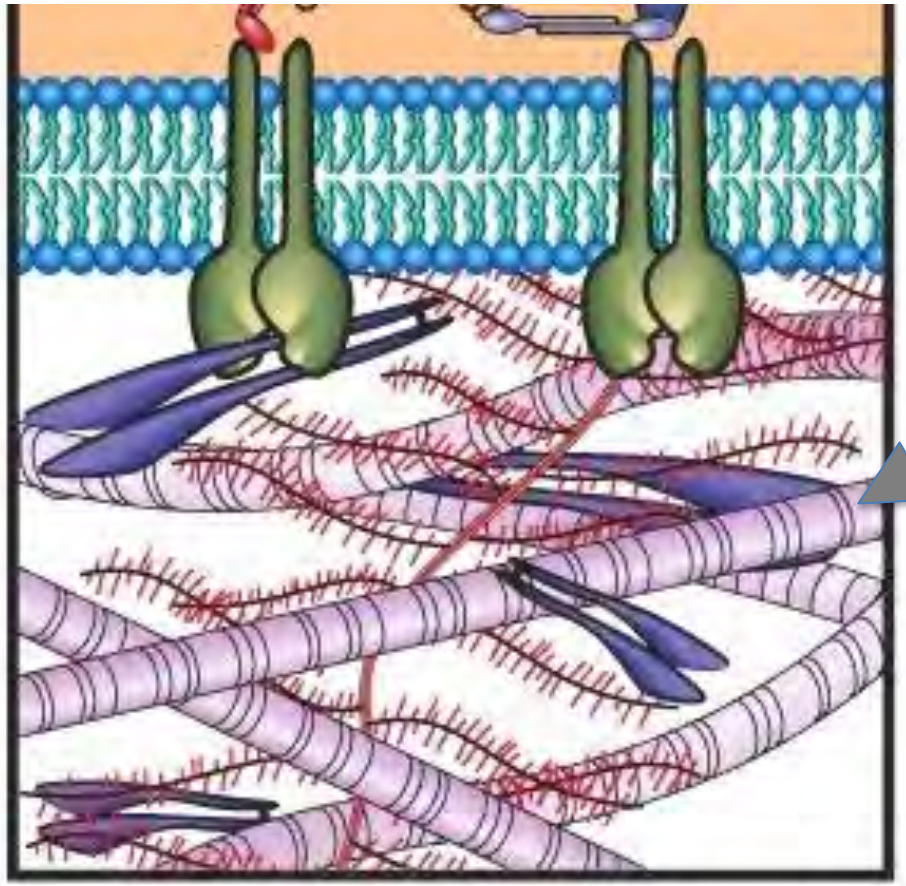


Figure 7-28 Cell and Molecular Bio

Name that ECM protein!

a) Integrin
b) Fibronectin
c) Collagen
d) Cellulose
e) Hyaluronic acid

PollEv.com/msg303
Send **msg303** to **22333**

warm up poll question

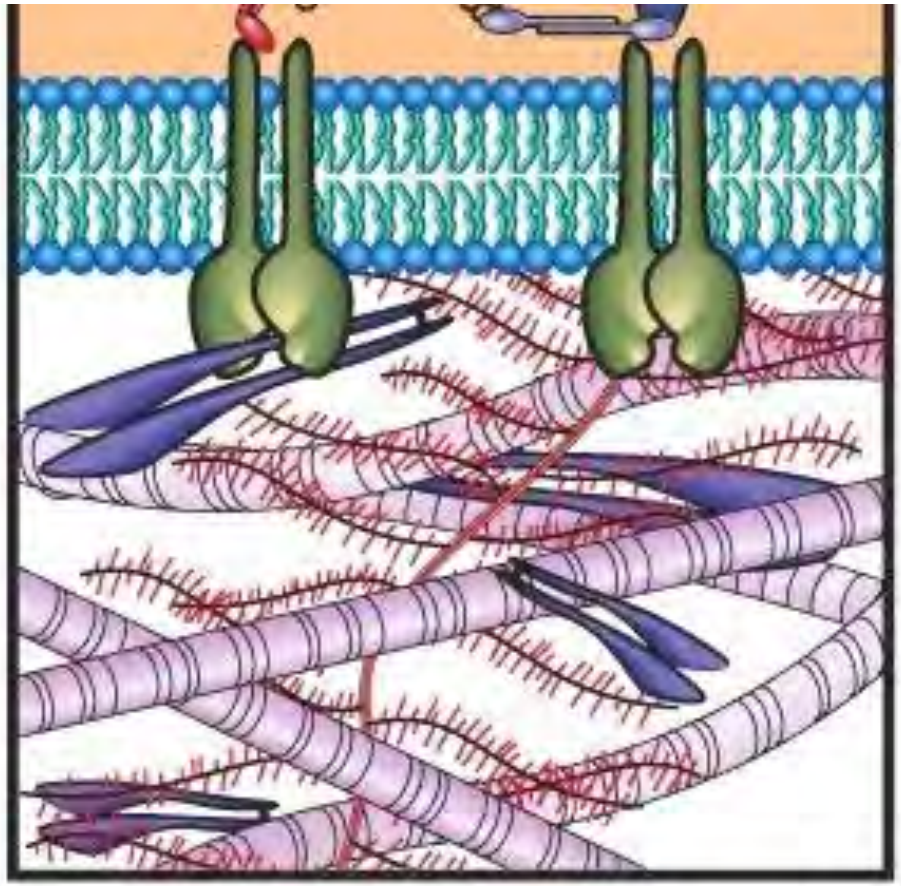


Figure 7-28 Cell and Molecular Bio

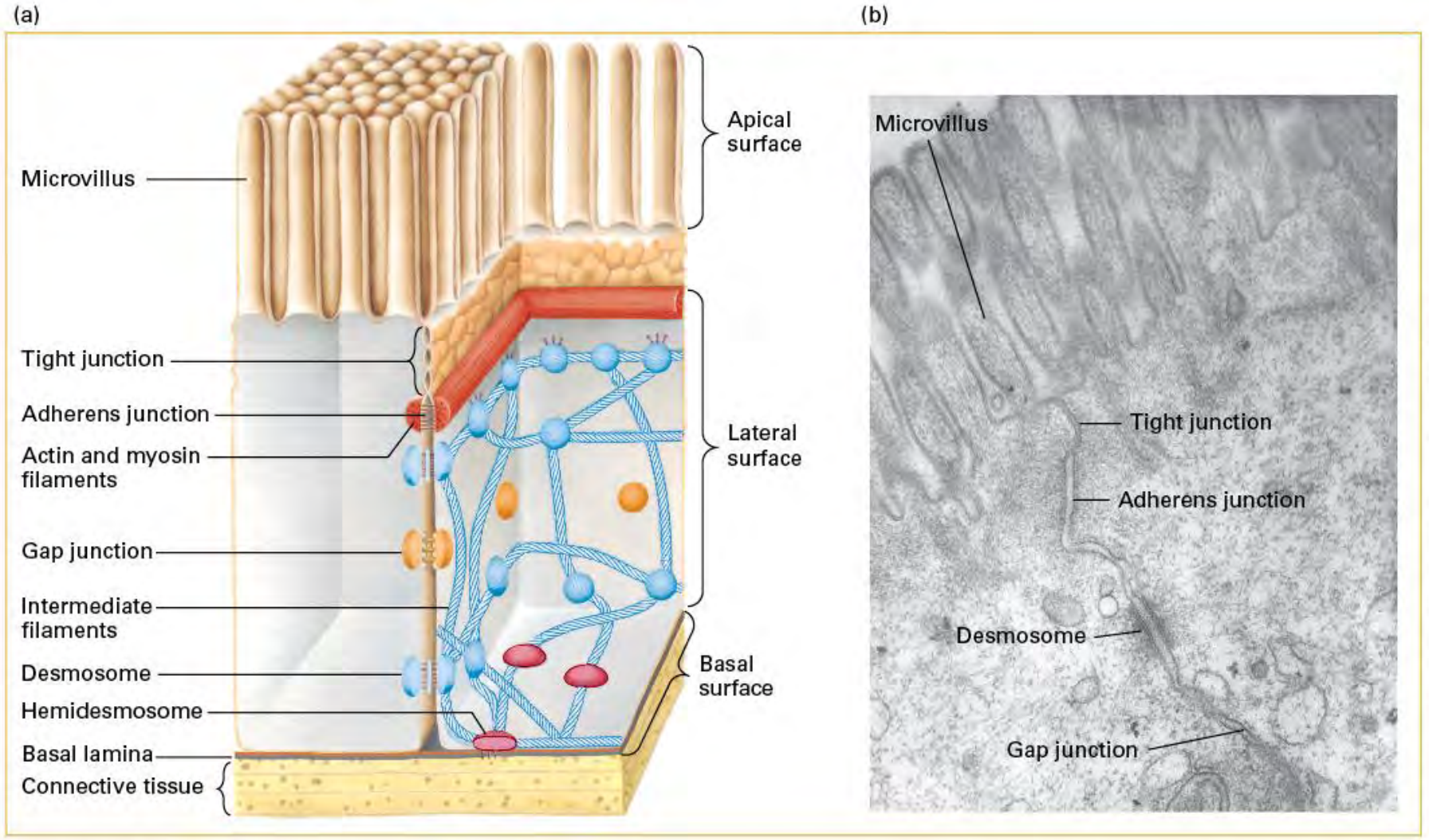
Which of these answers is not in the ECM (and not even a protein)?

a) Integrin
b) Fibronectin
c) Collagen
d) Cellulose
e) Hyaluronic acid

learning objectives

- be able to: compare and contrast types of cell junctions/adhesions
- describe and interpret the experiments used to characterize different adhesion molecules
- describe the molecules contributing to cell interactions, including cytoskeletal components
- by the end of this section, you should be able to:
  - describe 4 major functions of the cytoskeleton and know which proteins contribute to these functions
  - describe, compare, and contrast the 3 different types of filaments in the cytoskeleton (ie, know their roles, and to *what specialized structures they contribute*)
  - describe intermediate filaments, know to which cellular structures they contribute, and explain how their molecular structure relates to function

major types of cell junctions



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

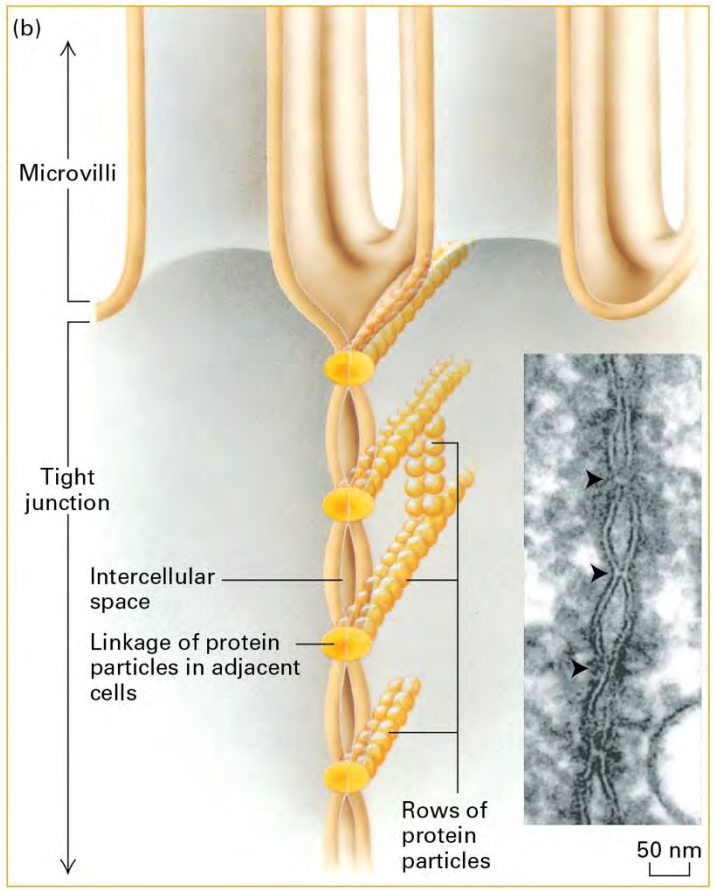
Two types do NOT link to cytoskeleton (directly)

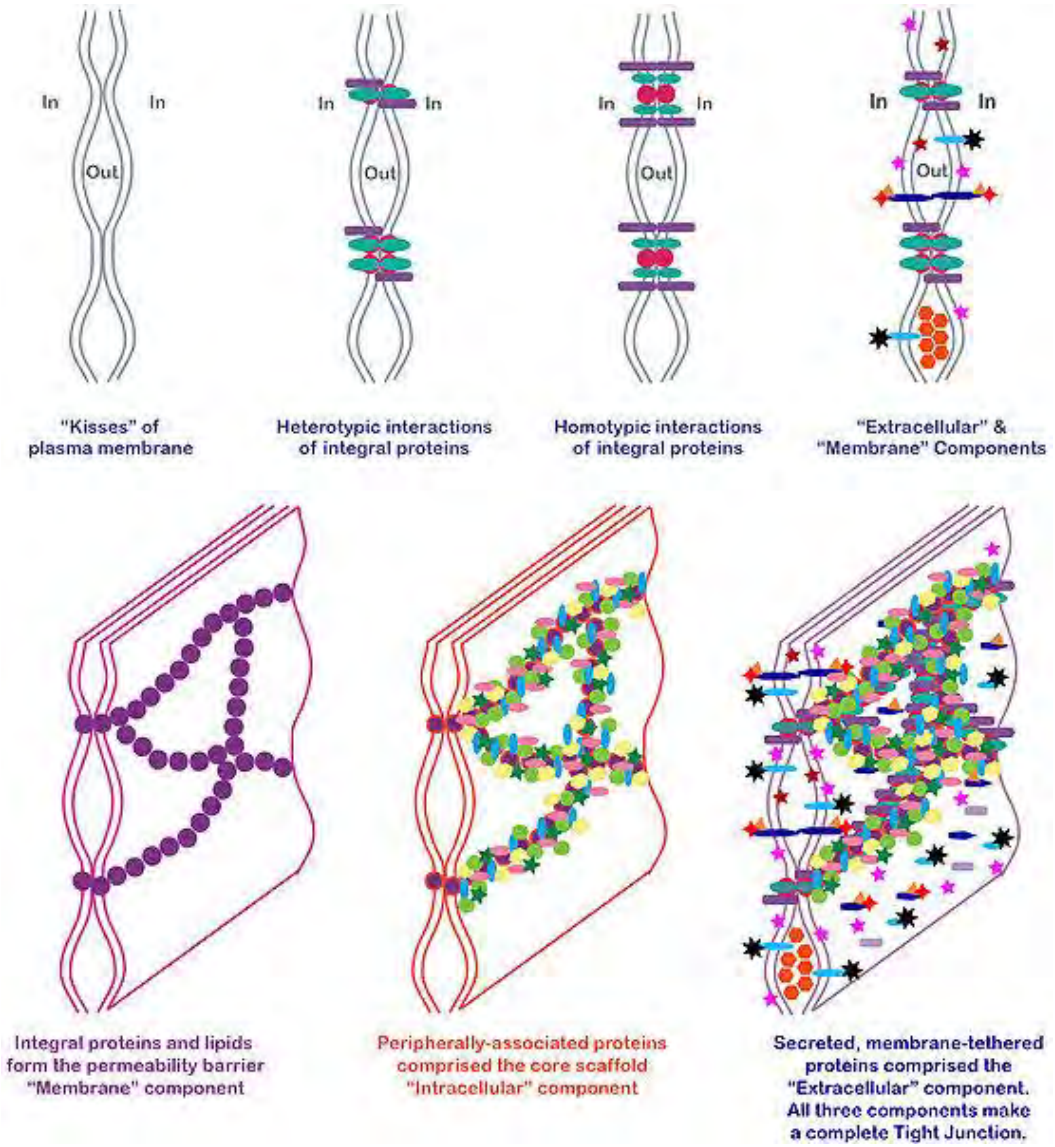
Which way is up?

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

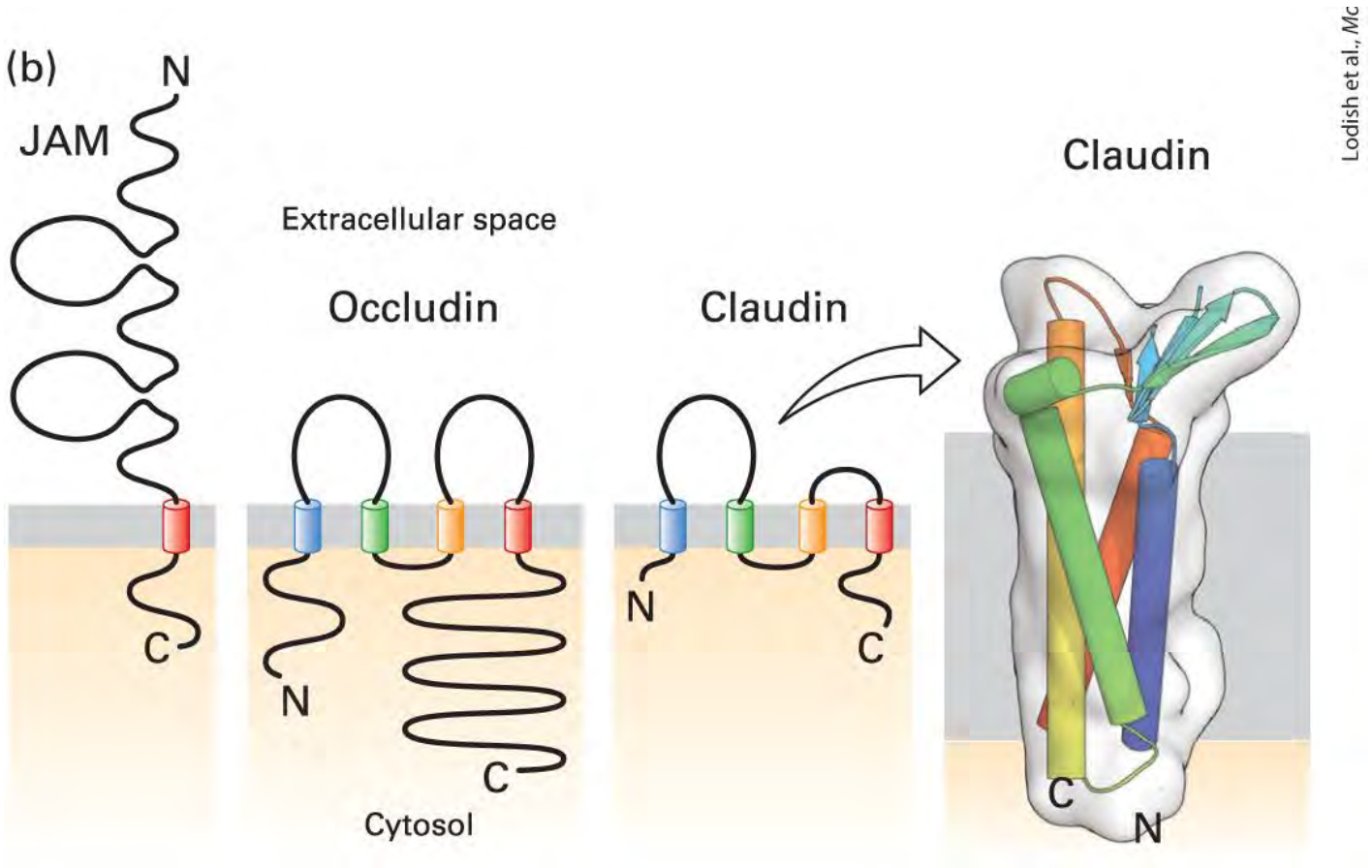
tight junctions form strands





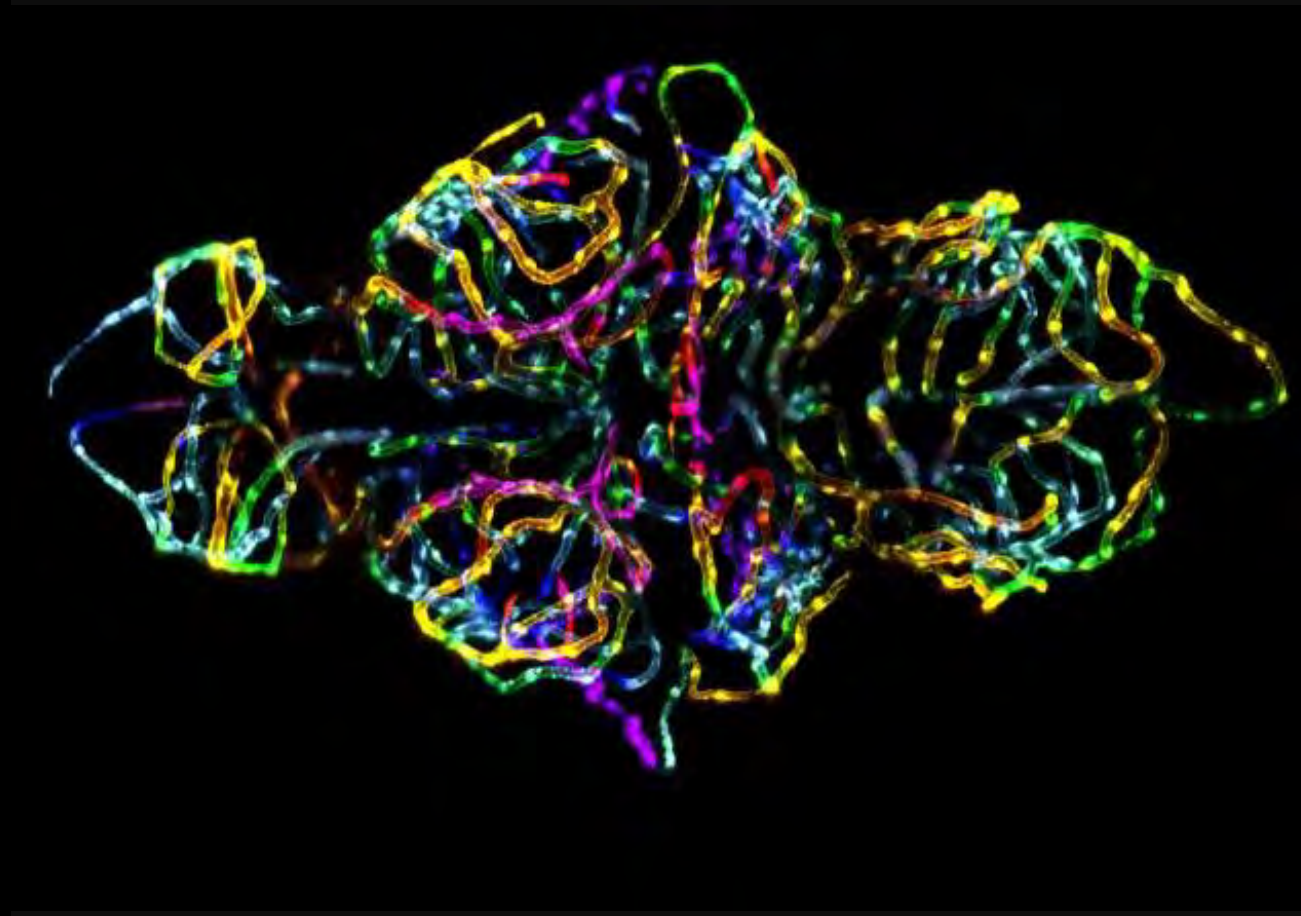
http://www.biology-direct.com/content/1/1/37/figure/F1

tight junction adhesion molecules



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

example: 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

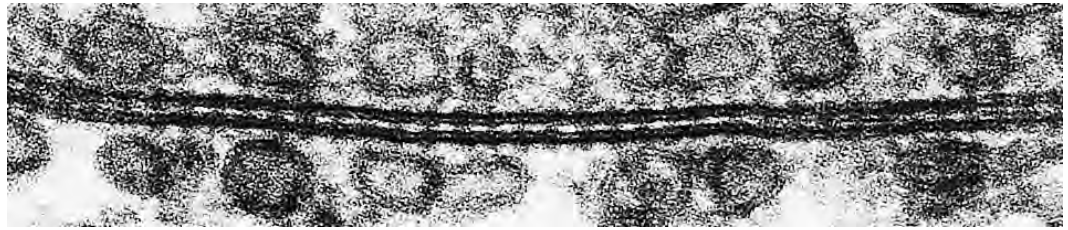
Subject Matter:

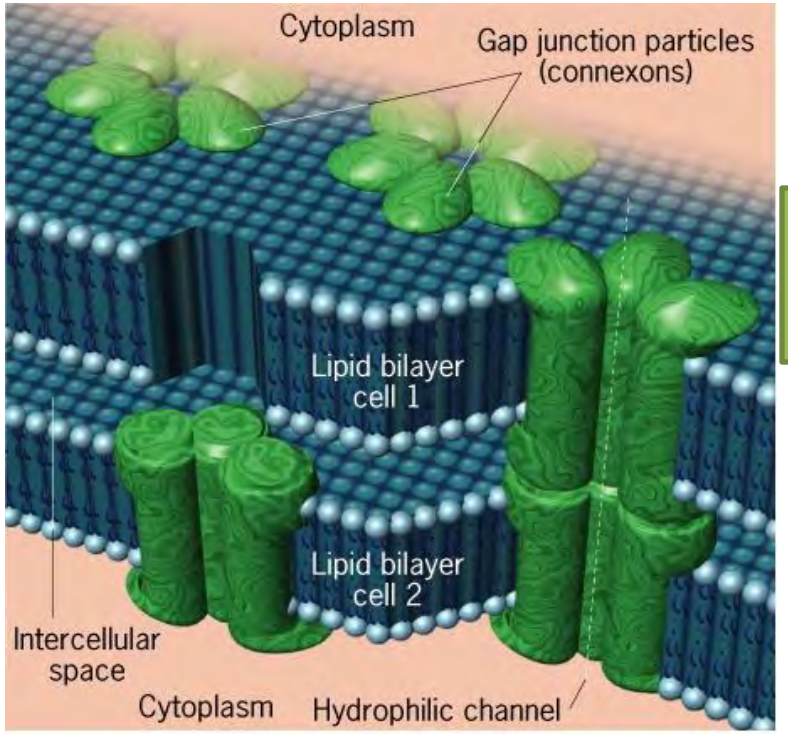
The blood-brain barrier in a live zebrafish embryo (20x)Technique:Confocal

http://www.nikonsmallworld.com/galleries/entry/2012-photomicrography-competition/1

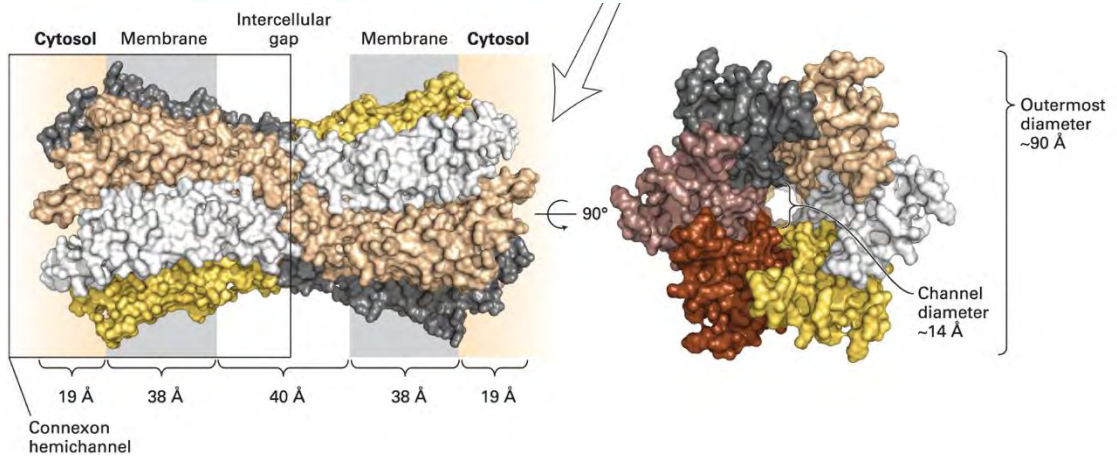
gap junctions

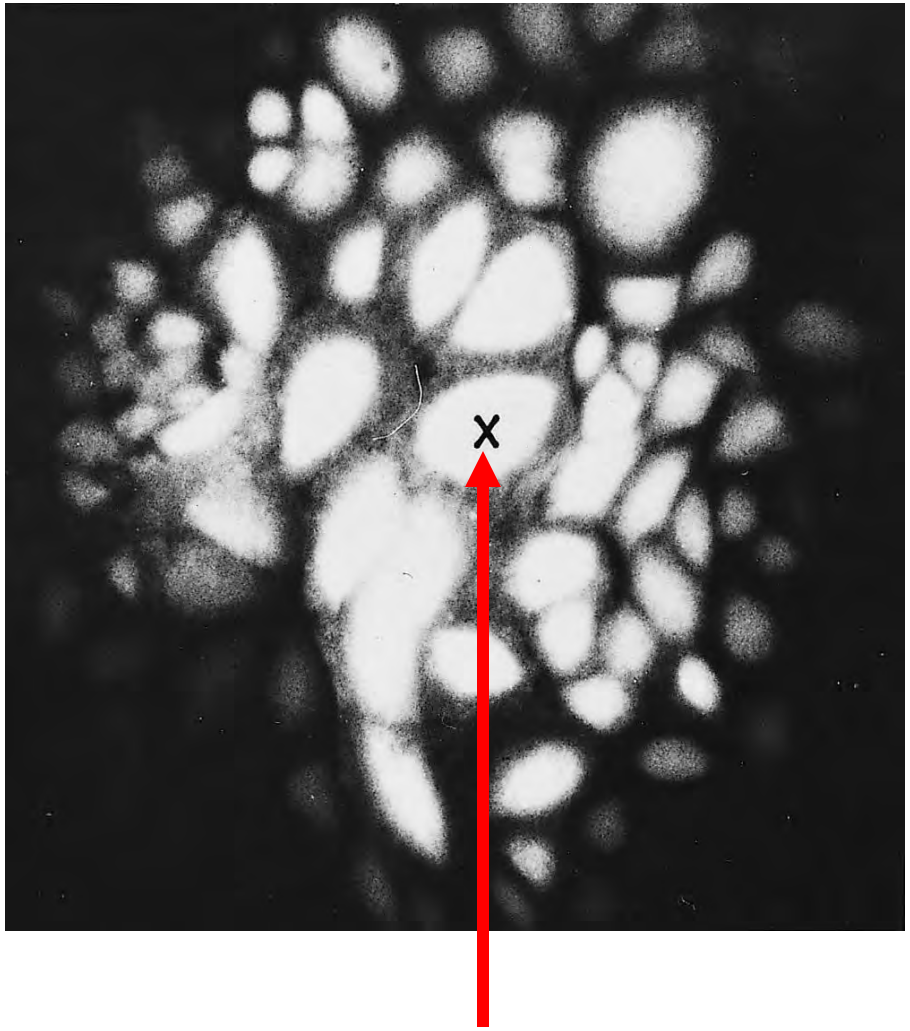
- permit intercellular communication
- channels between cells that allow passage of small molecules (ions, amino acids, nucleosides, etc.) (continuous)
- major protein is ***connexin*** which forms channels through the plasma membrane
- regulated 1.5nm inner diameter





what similarities and differences to plasmadesmata?



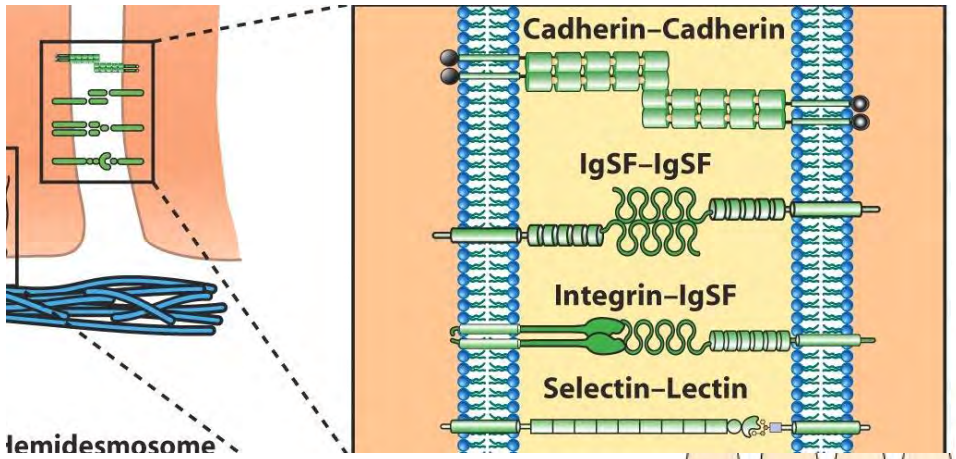


how do we know that gap junctions are passages between cells?

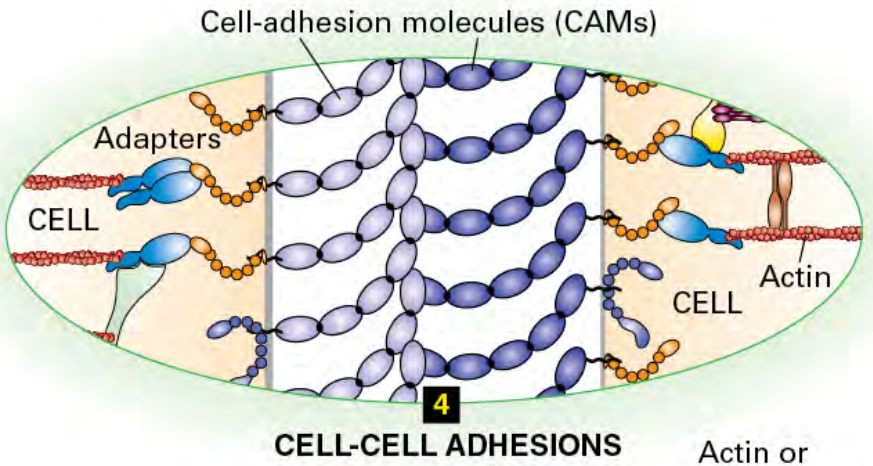
- inject fluorescently labeled small molecules into one cell
- observe that label traverses through gap junctions and into neighboring cells

cells adhere to the ECM and to neighbors

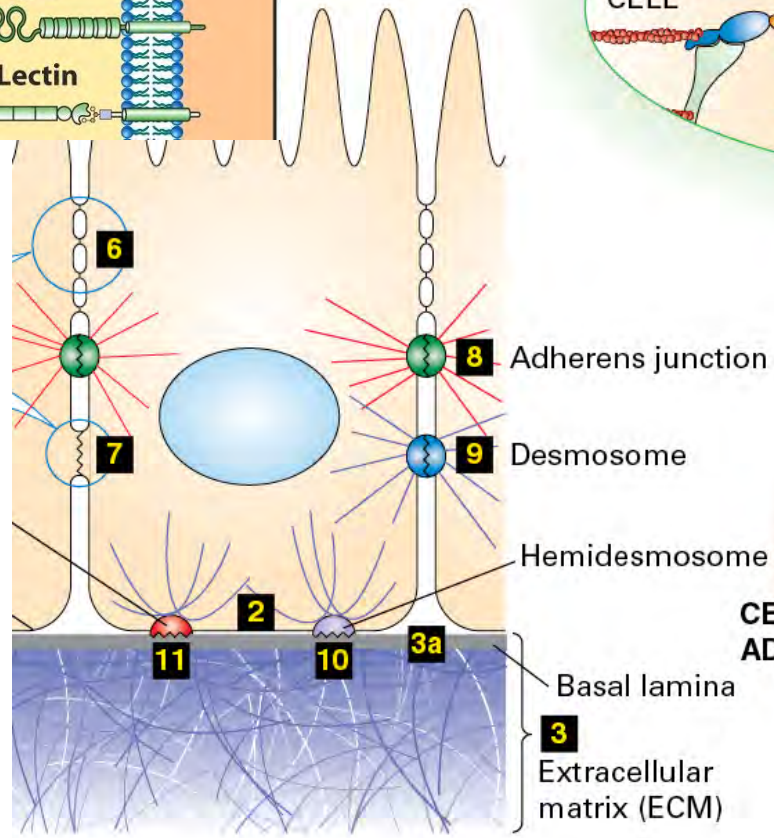
Adhesion molecule

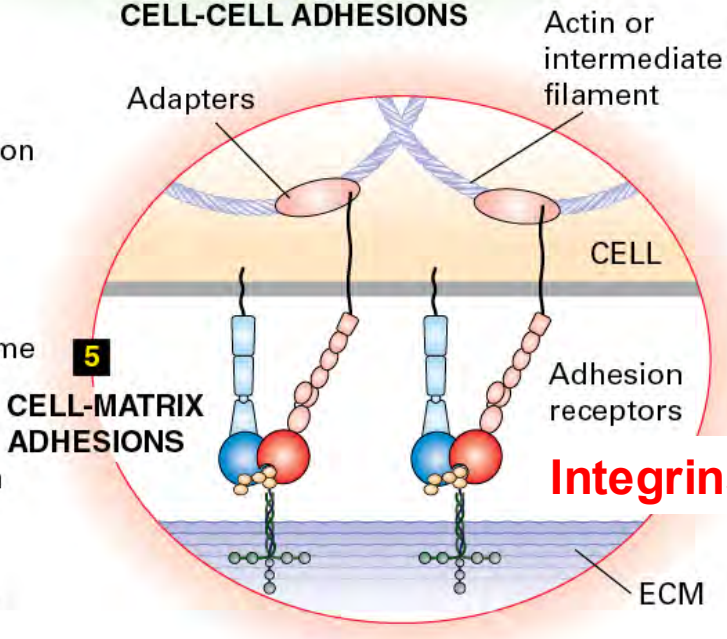


Adhesion molecule



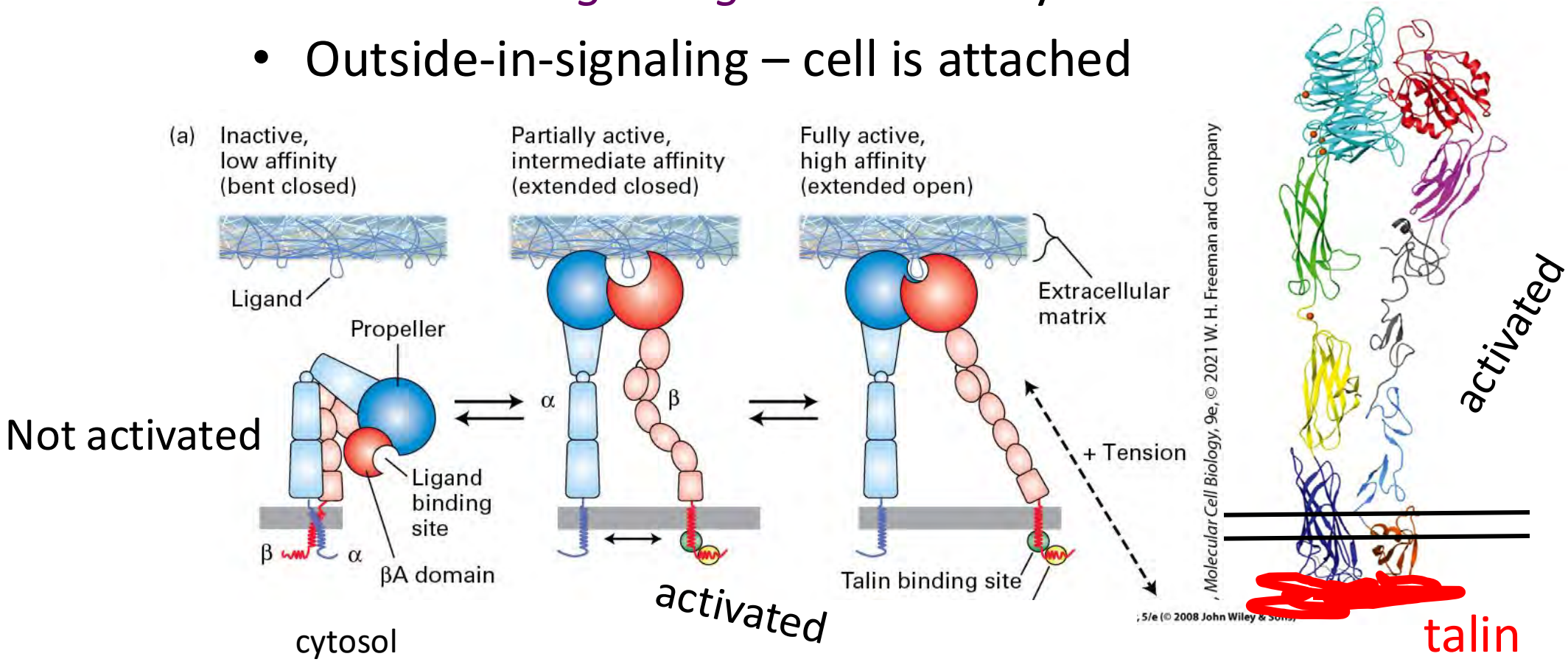
stable interactions (epithelial) mediated by **cell adhesion molecules** are connected to the cytoskeleton





Flashback: integrins can bind to RGD-containing ECM

- Integrins change conformation to bind a substrate
- Can also bind Ig-Superfamily members
- *inside-out-signaling* – cell is ready to attach
- Outside-in-signaling – cell is attached

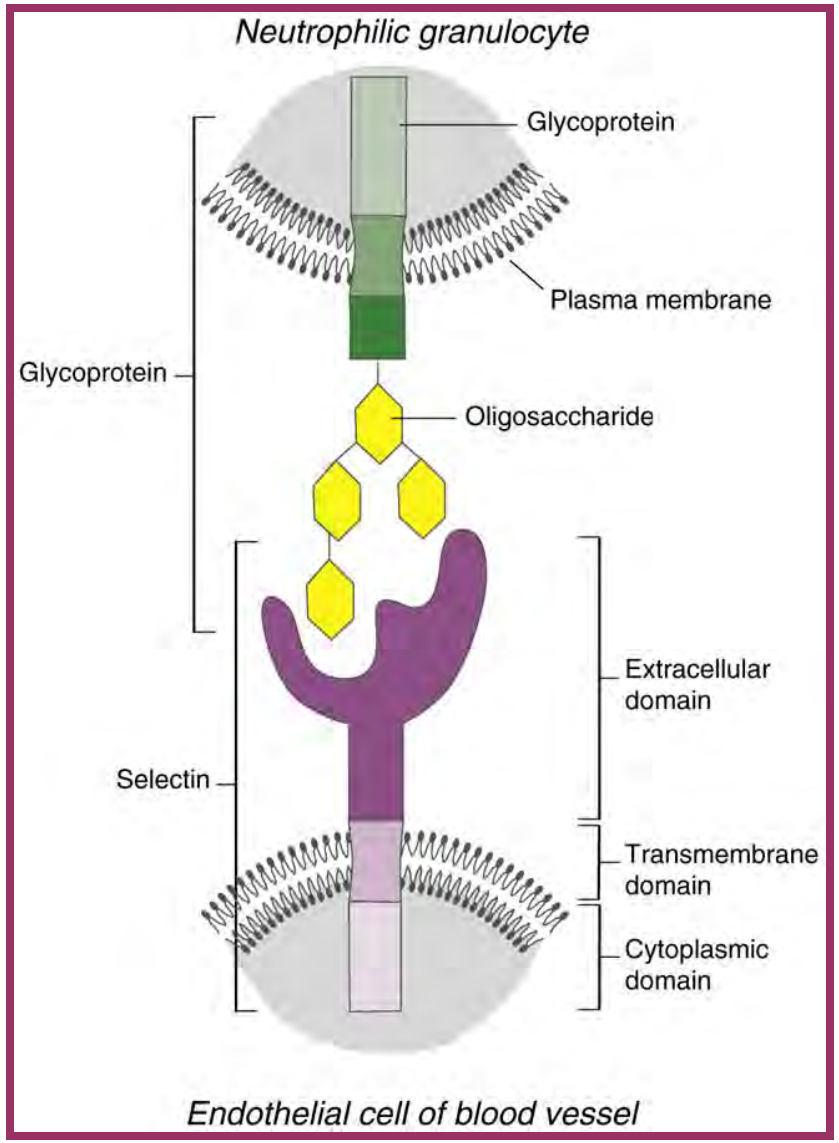


Selectins mediate cell associations/movement

- Lectin (protein) domain binds to carbohydrates on glycoprotein

e.g: white blood cells in extravasation

You will see more about this in discussion



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

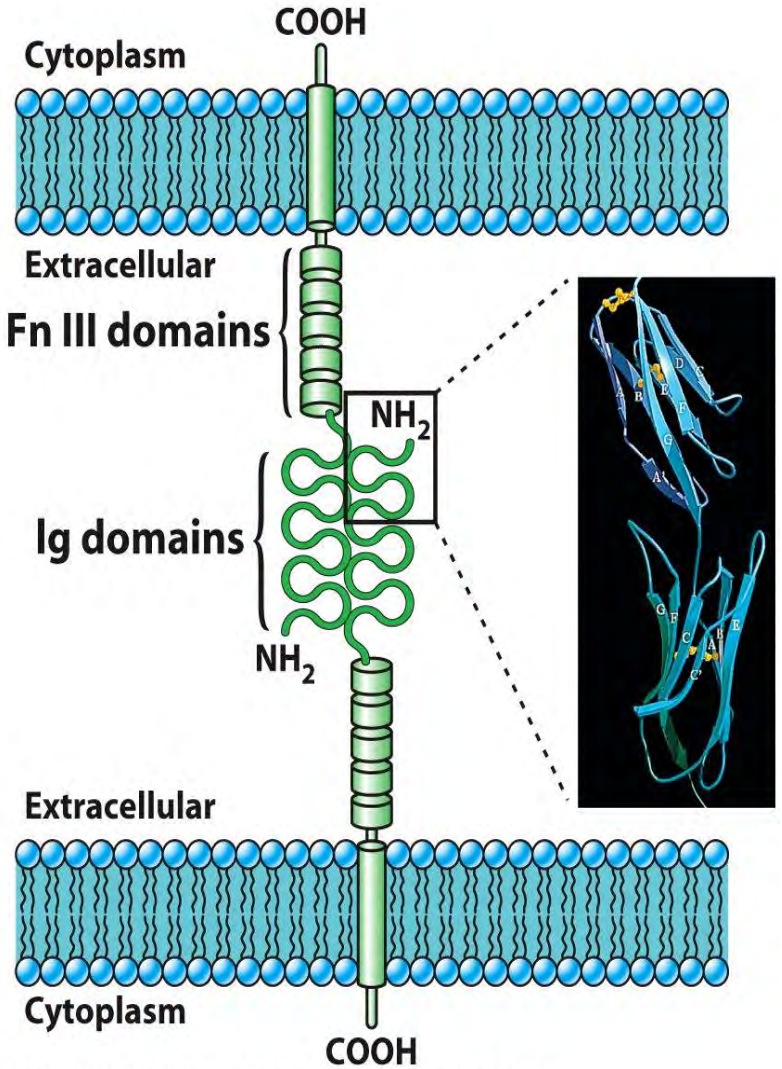
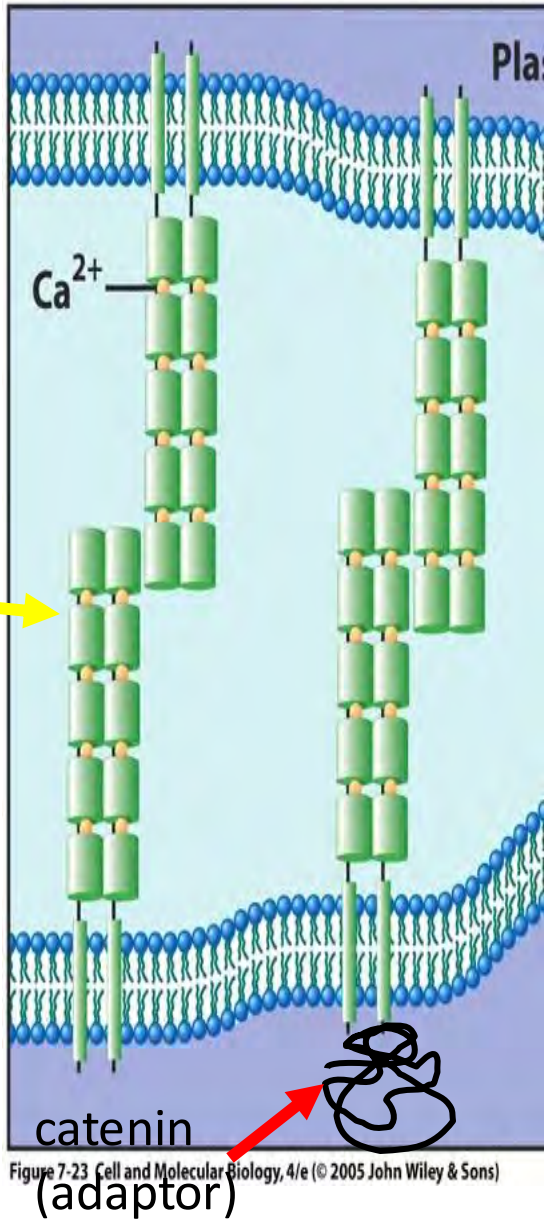


Figure 7-22 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

- Integral membrane proteins
- Multiple Cell Adhesion Molecules (CAMs) (L1)
- Immunoglobulin (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

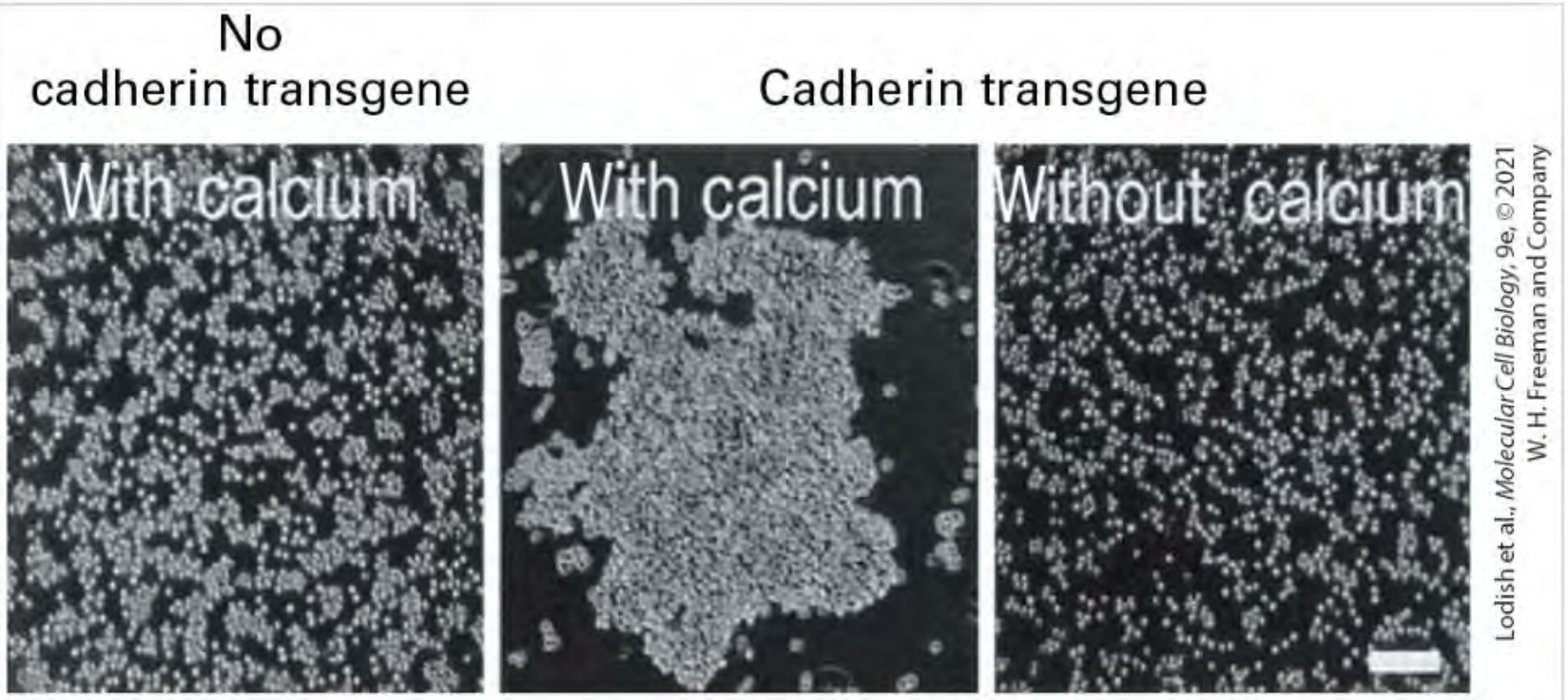
Cadherins mediate cell-cell adhesion (homotypic adhesion)

- 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.
- Blocking antibodies can cause dissociation
- Cadherin are also in epithelial cell junctions



a cadherin experiment

Adhesion is Ca^{+2}-dependent



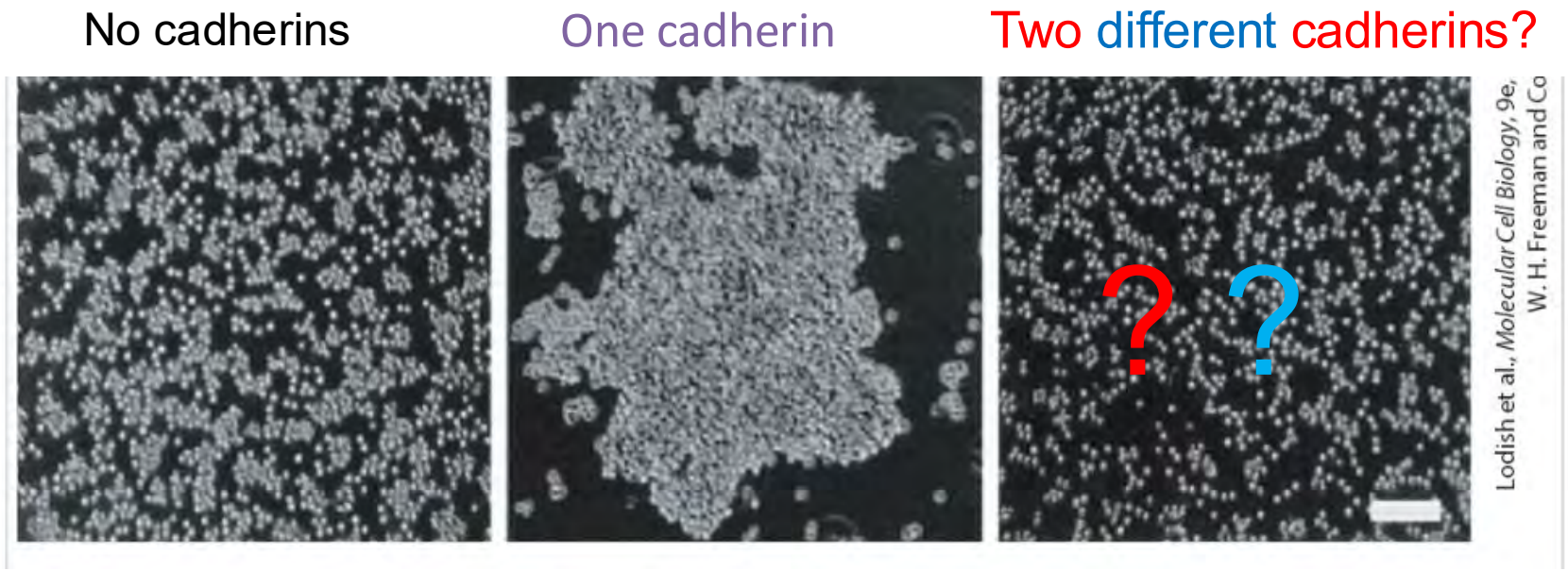
EXPERIMENTAL FIGURE 20-12 E-cadherin mediates Ca^{2+}-dependent adhesion of L cells. U

poll

another cadherin experiment

What happens if you add E-cadherin to some cells and N-cadherin to other cell then mix them?

a) they don't stick
b) they stick weakly
c) they clump into two groups



in epithelia, four types of cell junctions connect cells to each other and link to cytoskeleton

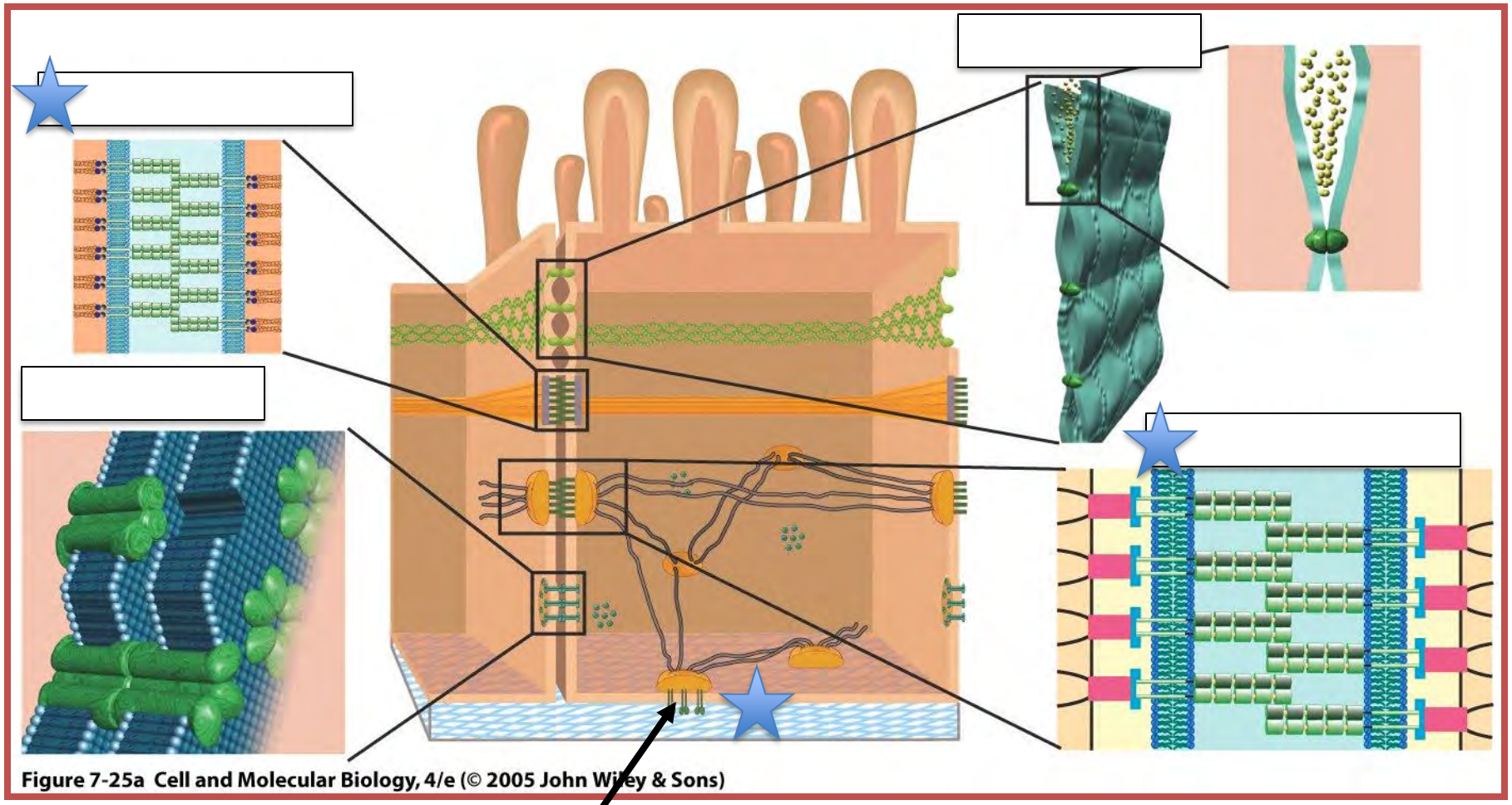
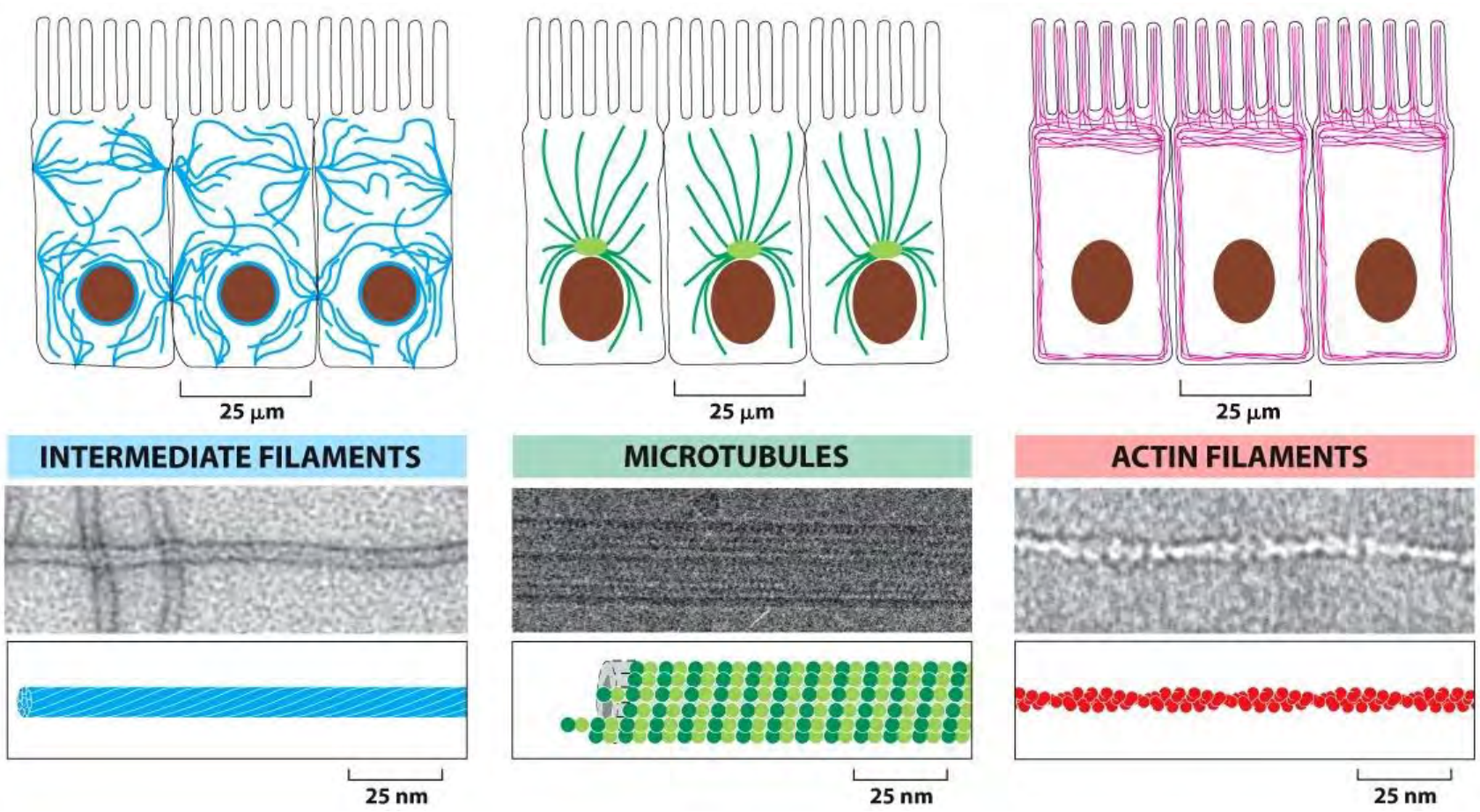


Figure 7-25a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Hemidesmosomes – junctions with ECM and intermediate filaments

and focal adhesions (not shown)

three types of cytoskeletal filaments



adherens junctions couple the actin cytoskeleton of neighboring cells

the integral membrane protein

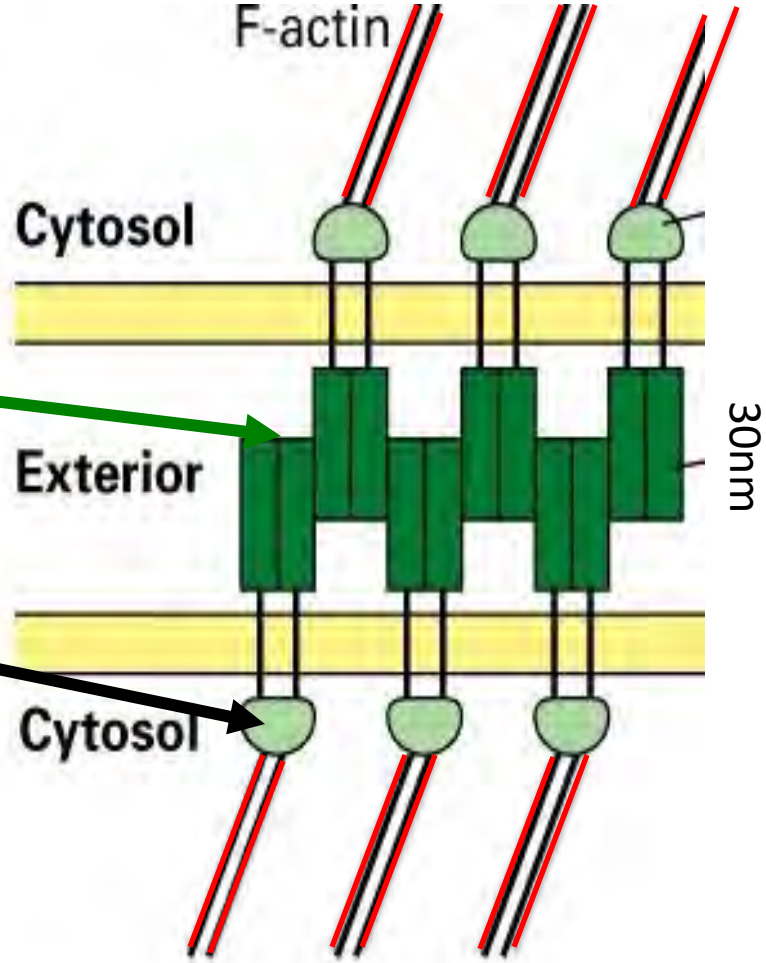
Cadherin

Adhesion molecule

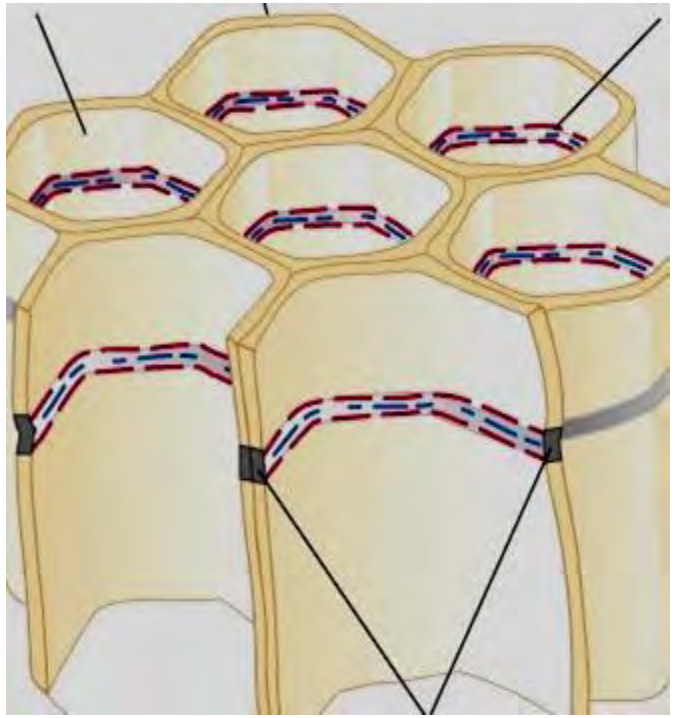
and adaptor proteins

such as beta-catenin, alpha-catenin connect cadherin to

actin filaments

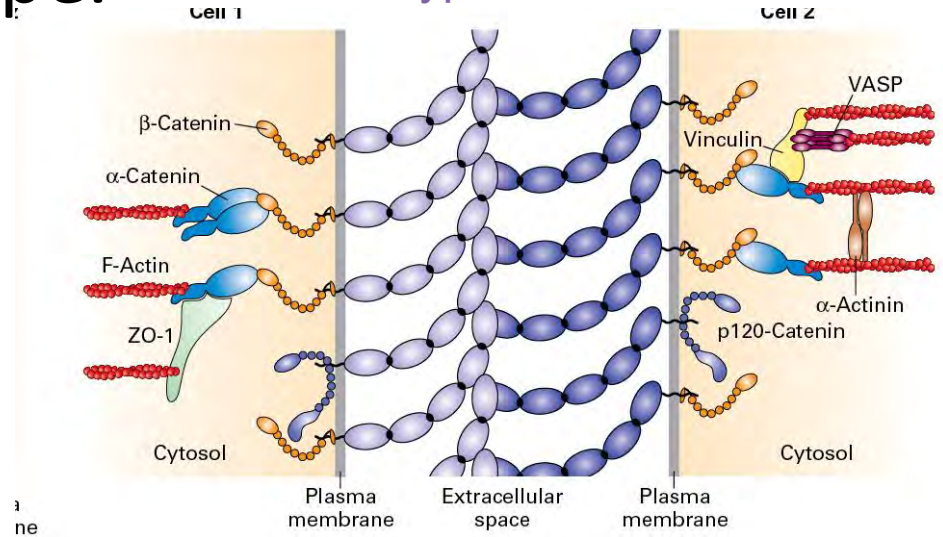


adherens junctions



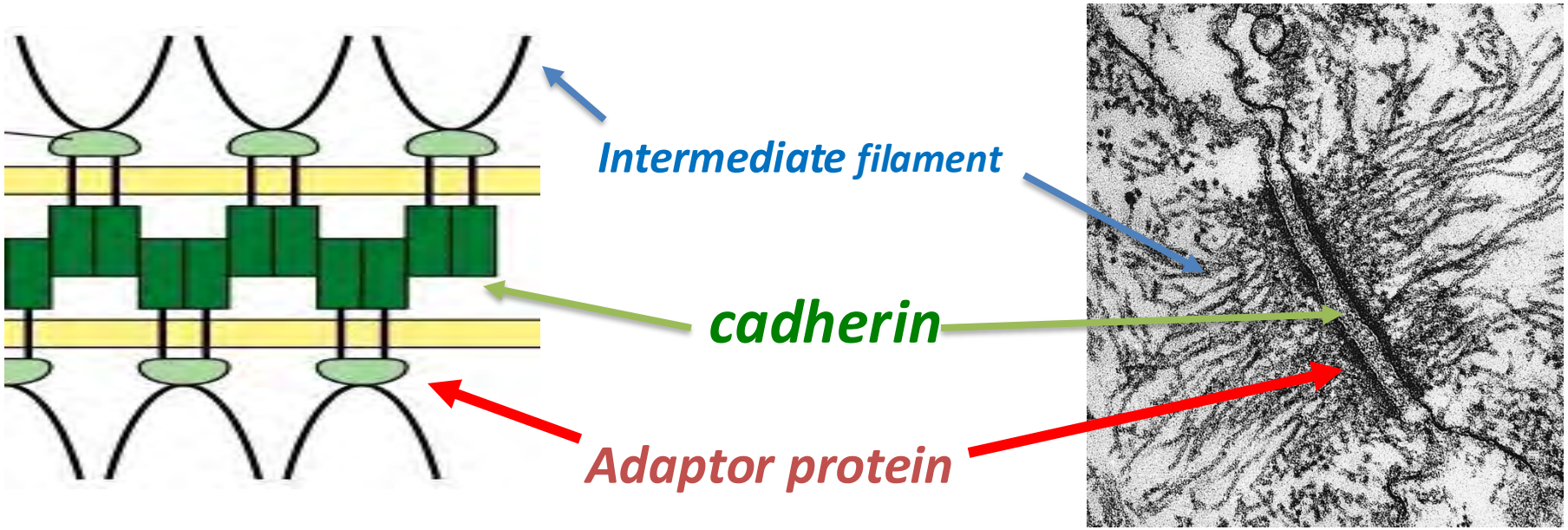
- adherens junctions coordinate cell-cell activity and help stabilize the epithelium
  - Actin-myosin filaments form a “belt” around each cell that allows a group of cells to coordinate shape.
  - they are frequently associated with tight junctions

Homotypic adhesions



desmosomes

- important cell-cell adhesions for structural support/mechanical **strength** of tissue (e.g. skin epithelia, heart muscle)
- 1μm 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)



recall

Integrins associate with cytoskeleton in **hemidesmosomes** = cell-matrix adhesions:

basal side of cells (filaments) attach to basement membrane

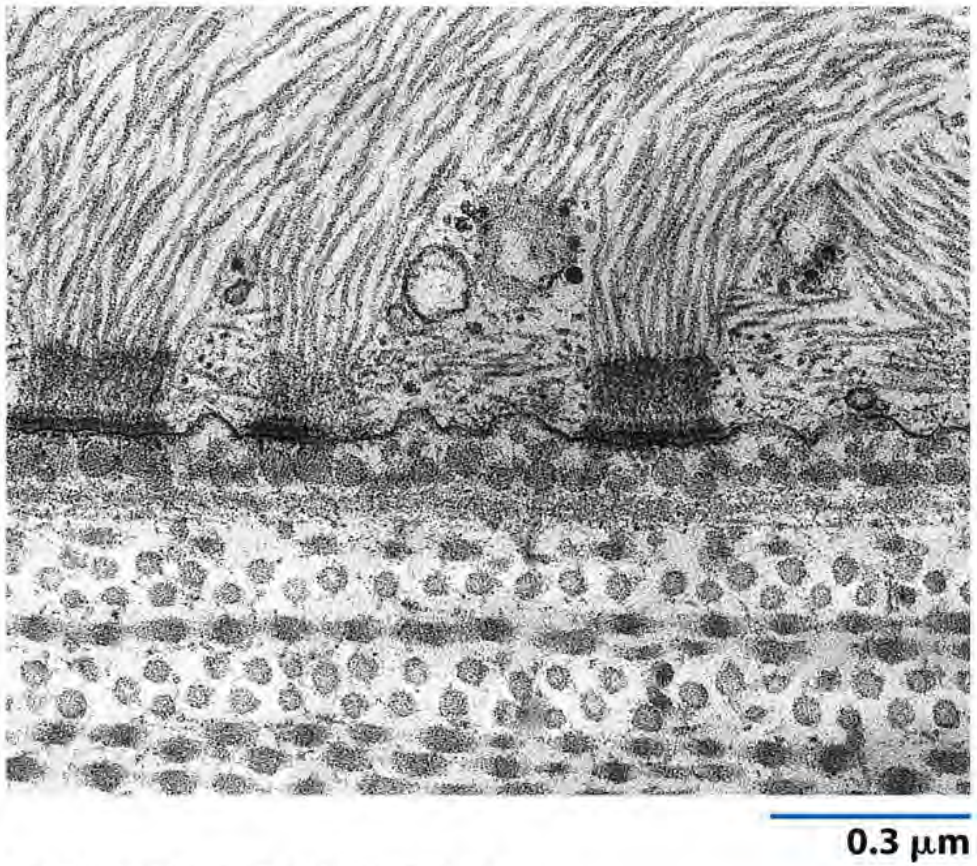


Figure 7-19a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

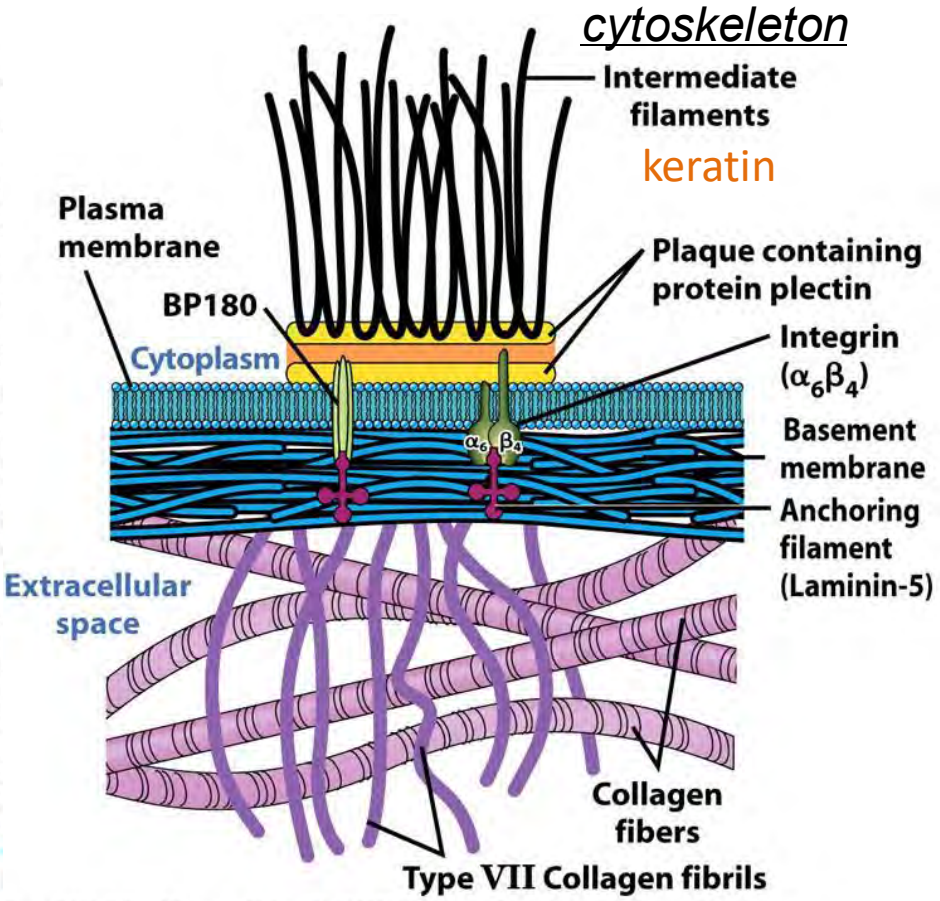


Figure 7-19b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

cytoskeleton overview

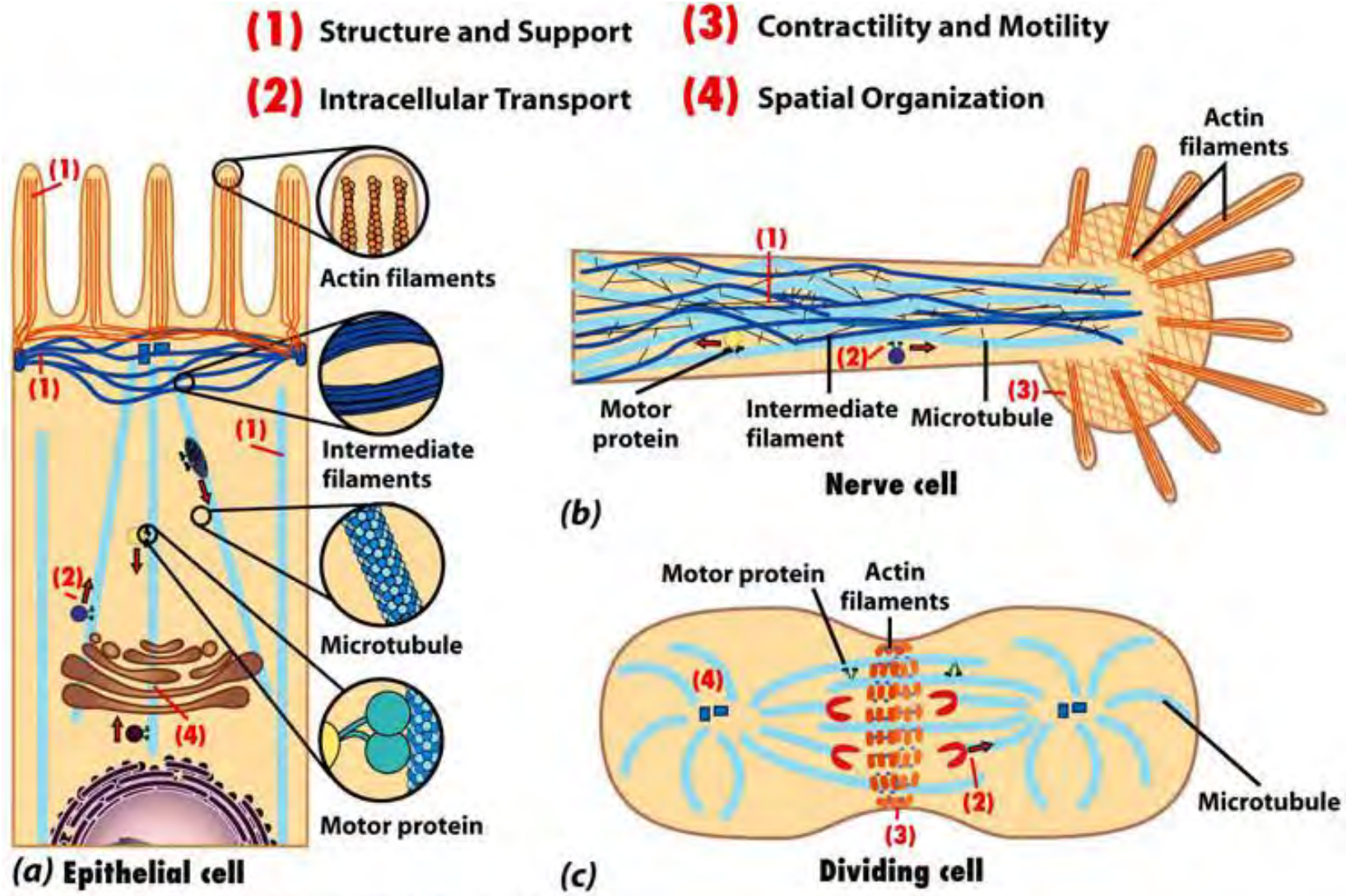


Figure 9-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

three type… oskeletal filame… nd…

Intermediate Filaments

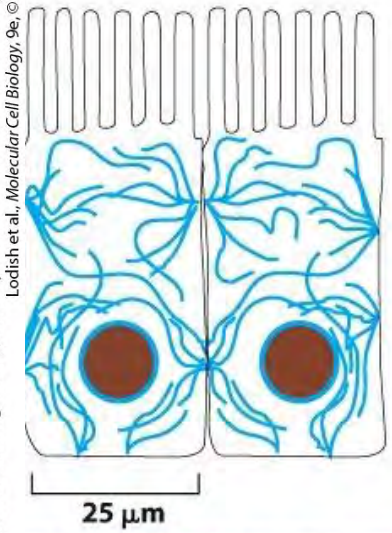
- IF subunits don't bind a nucleotide
- Great tensile strength
- Assembled onto preexisting filaments
- Less dynamic
- Unpolarized
- No motors
- Cell and tissue integrity

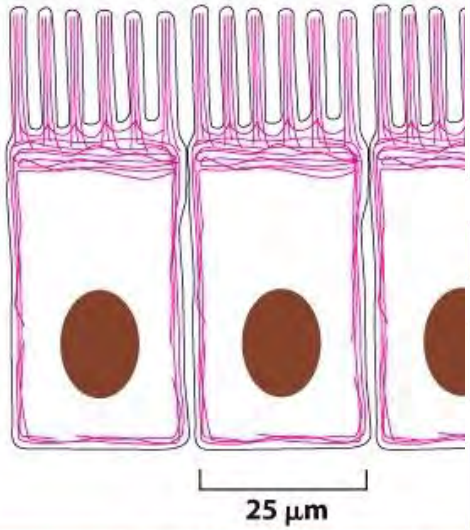
Microtubules

- αβ-Tubulin binds GTP
- Rigid and not easily bent
- Regulated assembly from a small number of locations
- Highly dynamic
- Polarized
- Tracks for kinesins and dyneins
- Organization and long-range transport of organelles

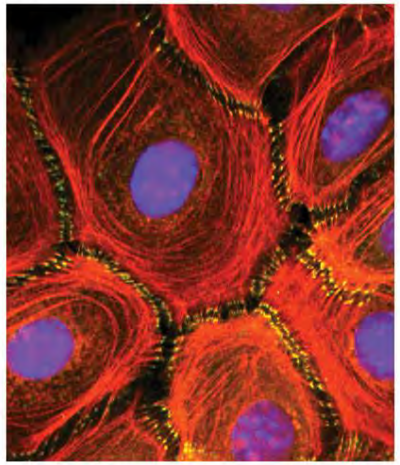
(a) Microfilaments

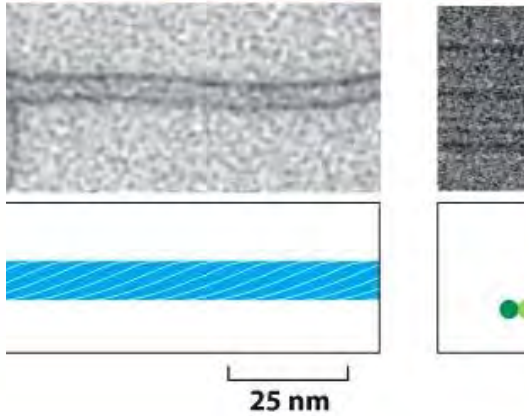
- Actin binds ATP
- Form rigid gels, networks, and linear bundles
- Regulated assembly from a large number of locations
- Highly dynamic
- Polarized
- Tracks for myosins
- Contractile machinery and network at the cell cortex



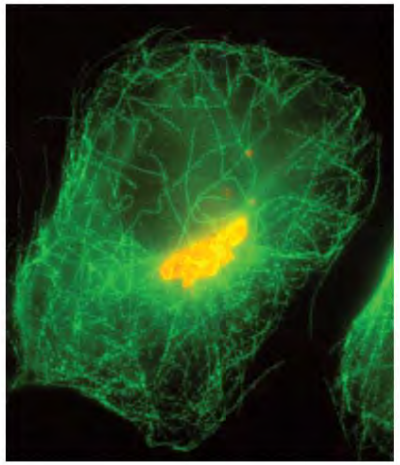


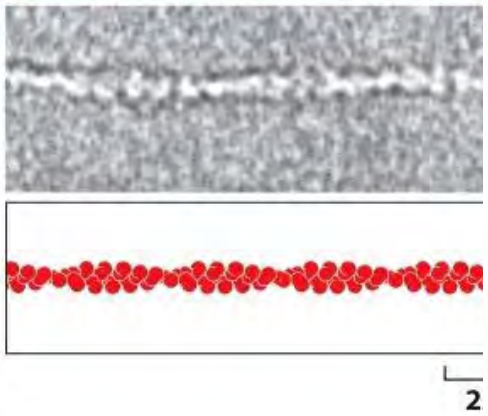
(d)



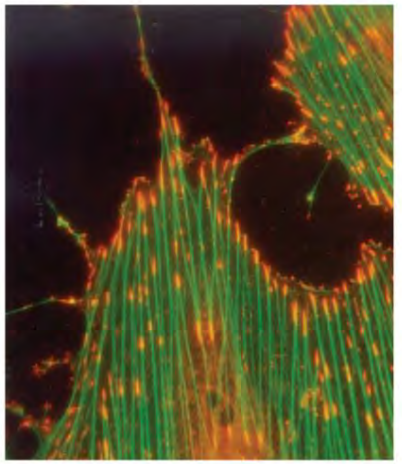


(c)



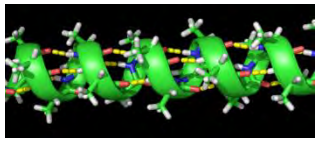


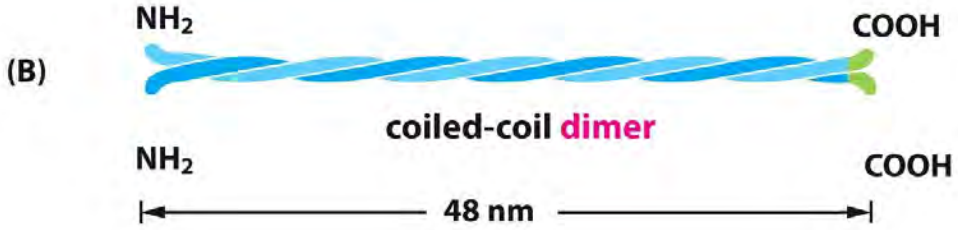
(b)

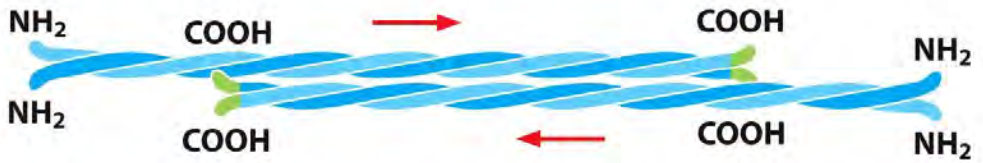


intermediate filaments

- **non-polarized** fibers of proteins = __ **nm in diameter**
- **mechanical support**, nuclear lamina, neurofilaments of axons
- heterogeneous- >65 genes but structurally similar
  - keratin, vimentin, desmin, lamin, nestin, NeuroFilaments

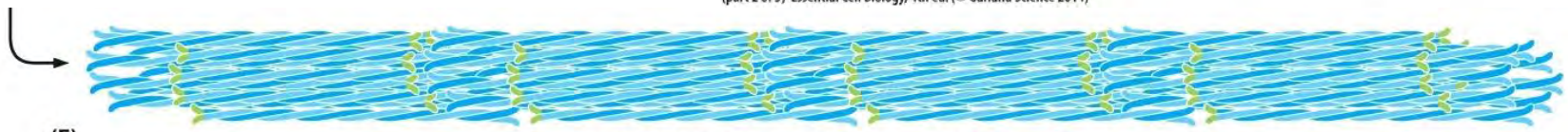






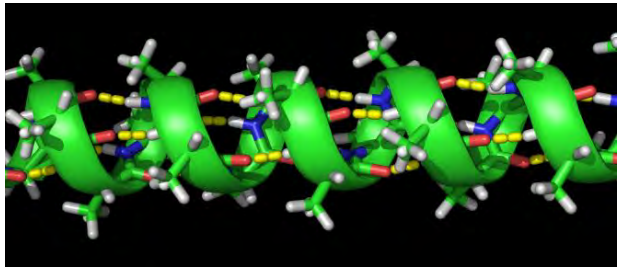
staggered tetramer of two coiled-coil dimers

(part 2 of 3) Essential Cell Biology, 4th ed. (© Garland Science 2014)



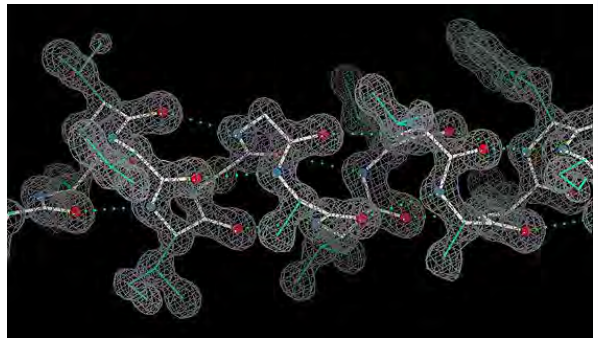
addition of 8 tetramers to growing filament

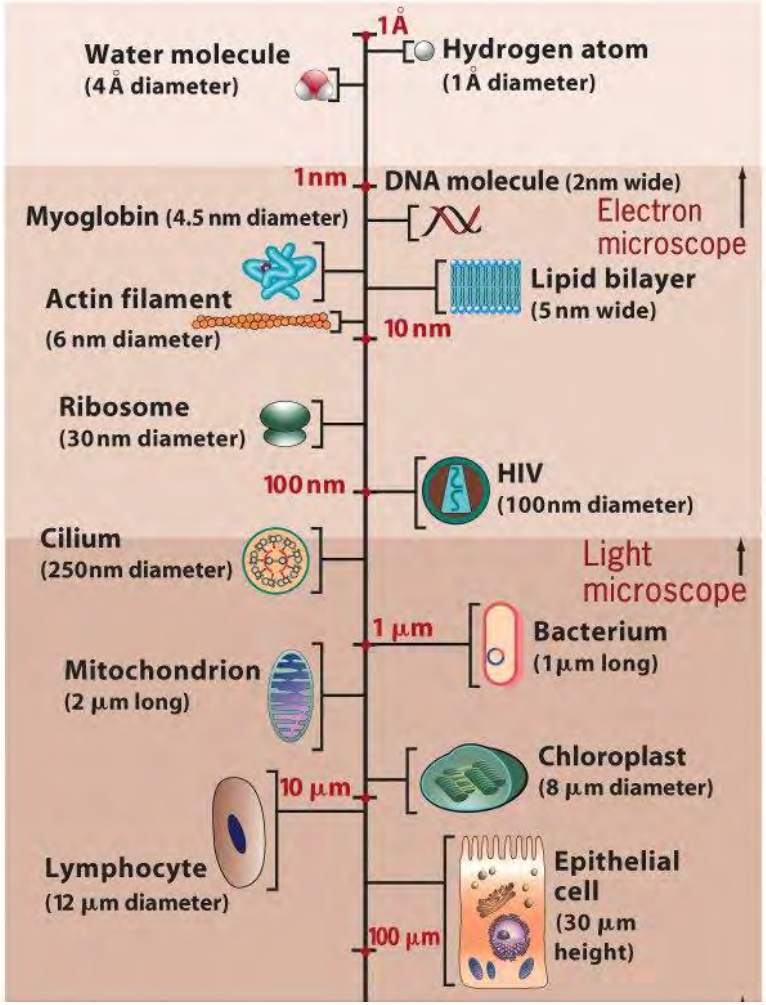
an aside on alpha helices



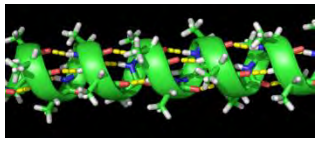
5 Angstroms

- side chains face outward, but not much space in the middle





intermediate filaments



- **non-polarized** fibers of proteins = __ **nm in diameter**
- **mechanical support**, nuclear lamina, neurofilaments of axons
- heterogeneous- >65 genes but structurally similar
  - keratin, vimentin, desmin, lamin, nestin, NeuroFilaments
- **most stable cytoskeletal structure**; subunits incorporated in middle;
- does NOT require GTP or ATP to form

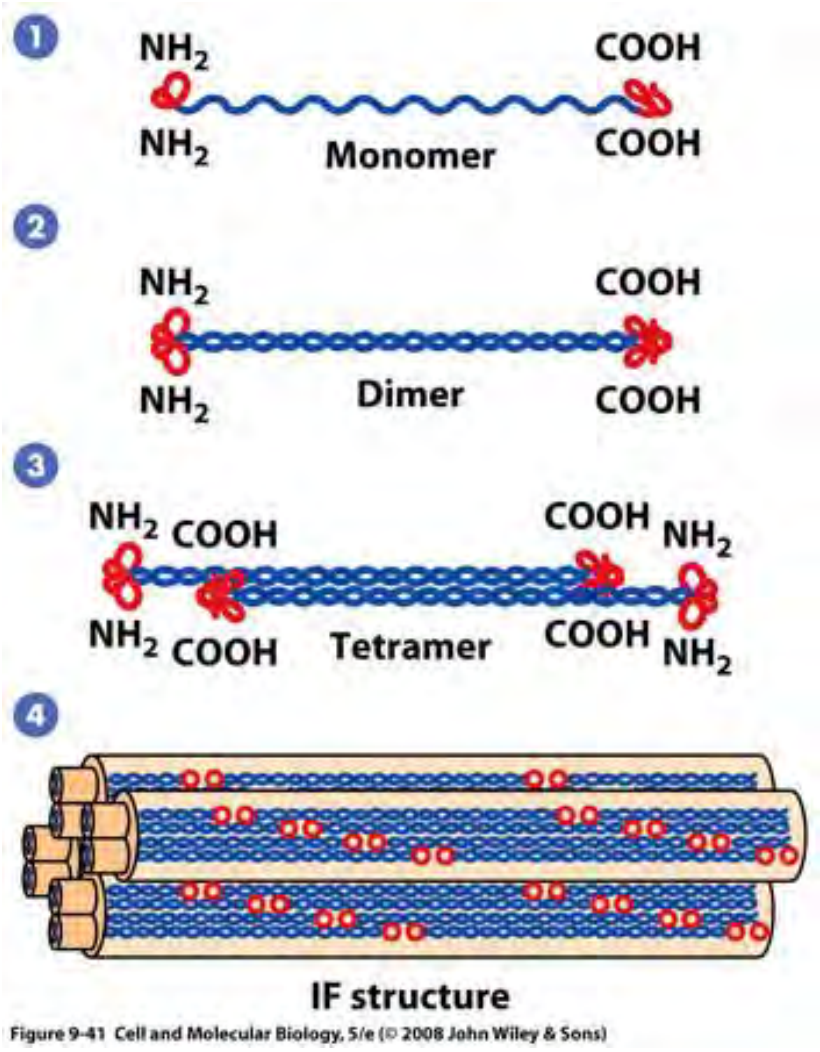
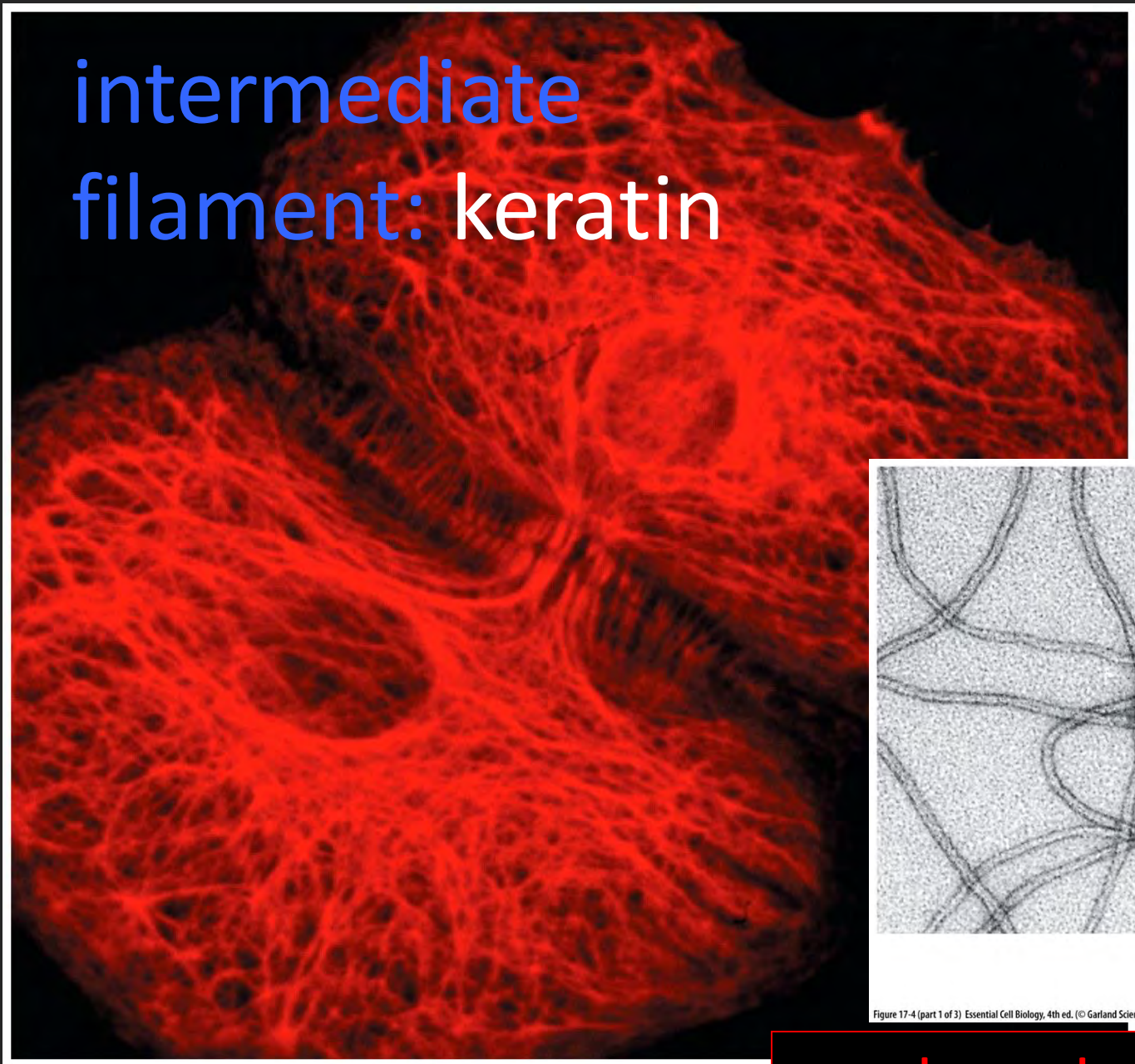


Figure 9-41 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

intermediate filament: keratin



From Pierre A. Coulombe and M. Bishr Omary, *Curr. Opin. Cell Biol.* 14:110, 2002

note flexibility in shape

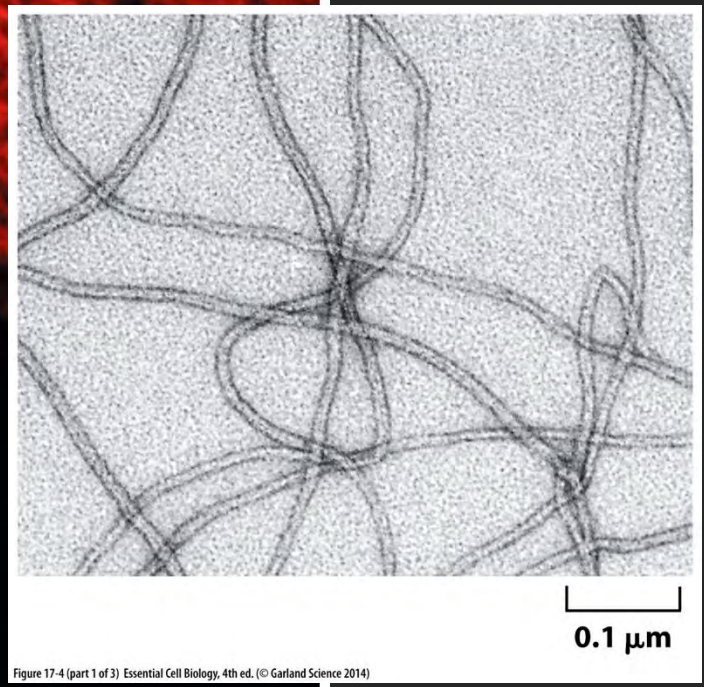
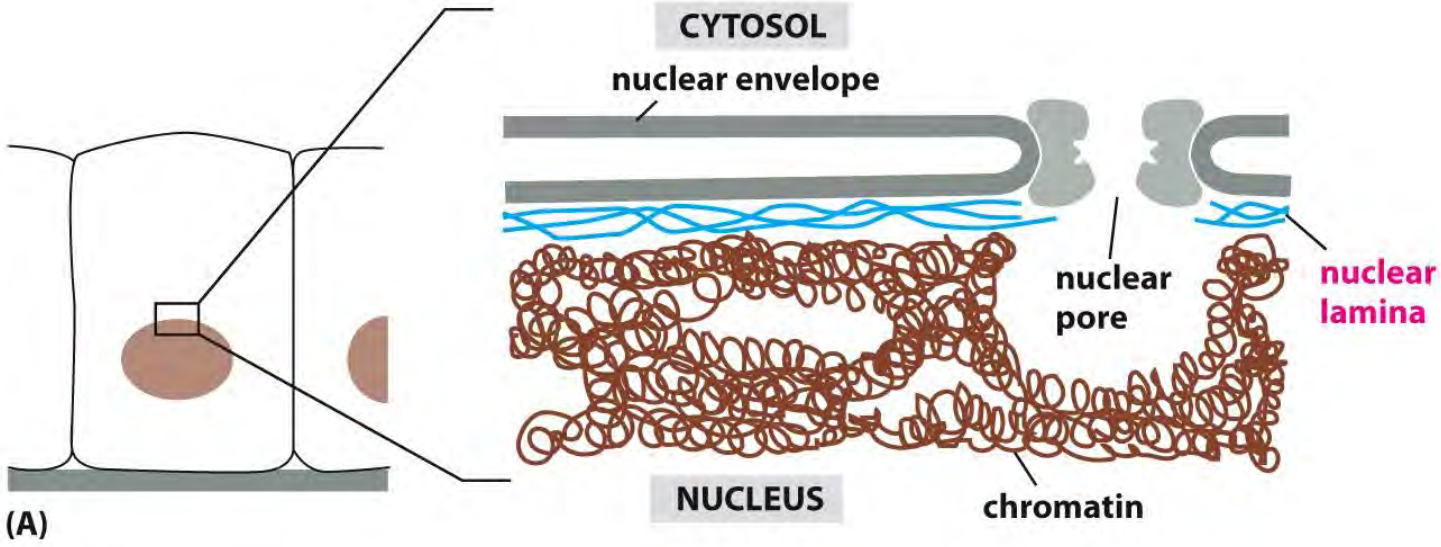
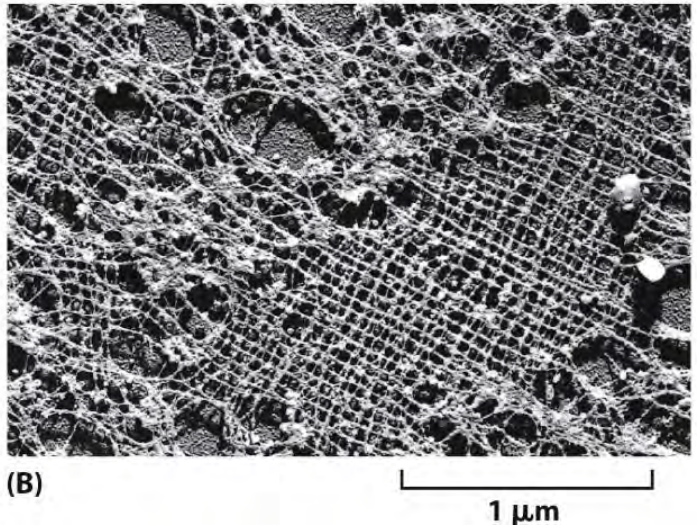


Figure 17-4 (part 1 of 3) Essential Cell Biology, 4th ed. (© Garland Science 2014)

- cultured skin cells

intermediate filaments
provide structure to nuclei





nuclear lamins

<- what kind of image is this?

intermediate filaments provide strength to tissues

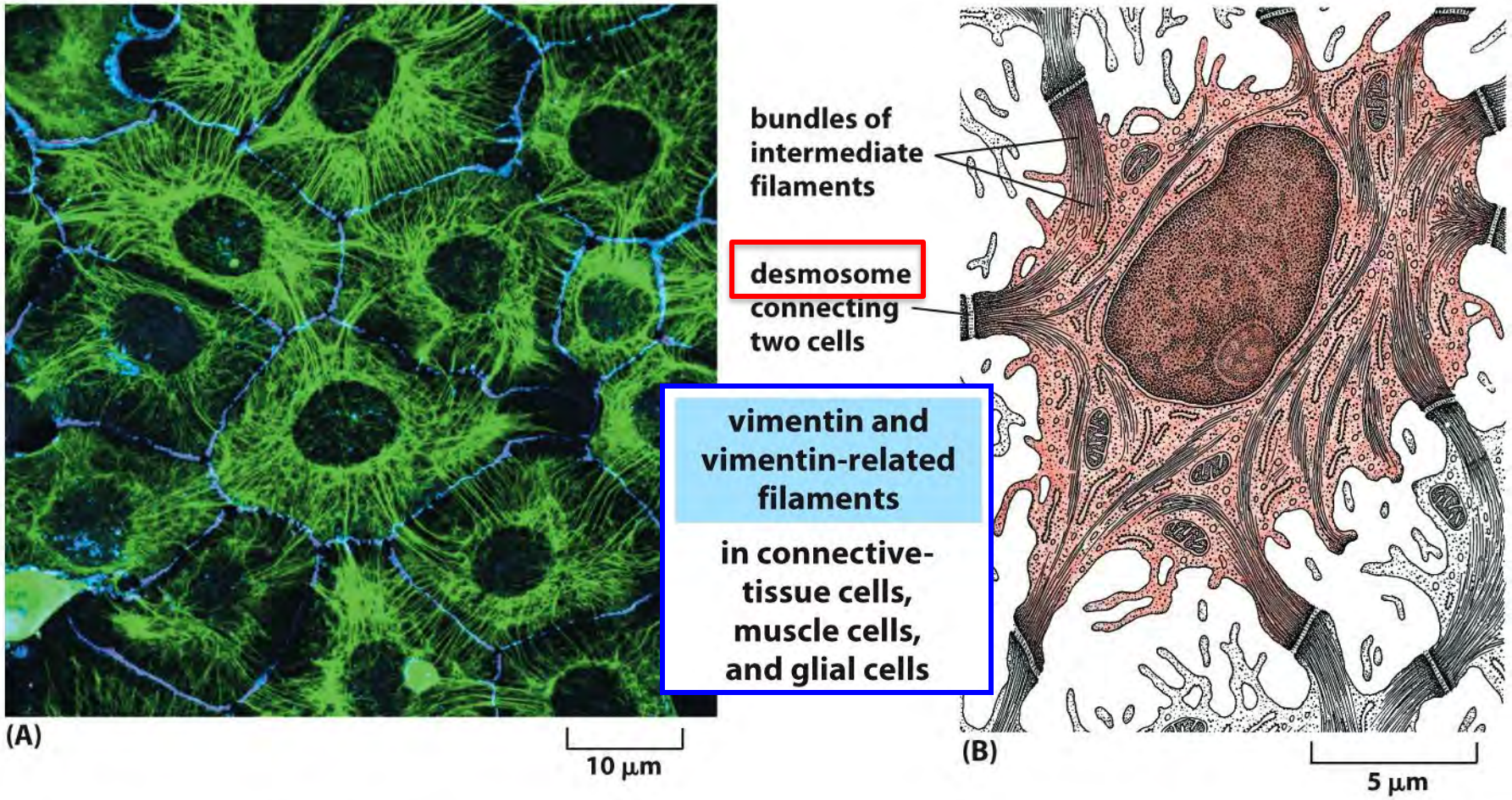
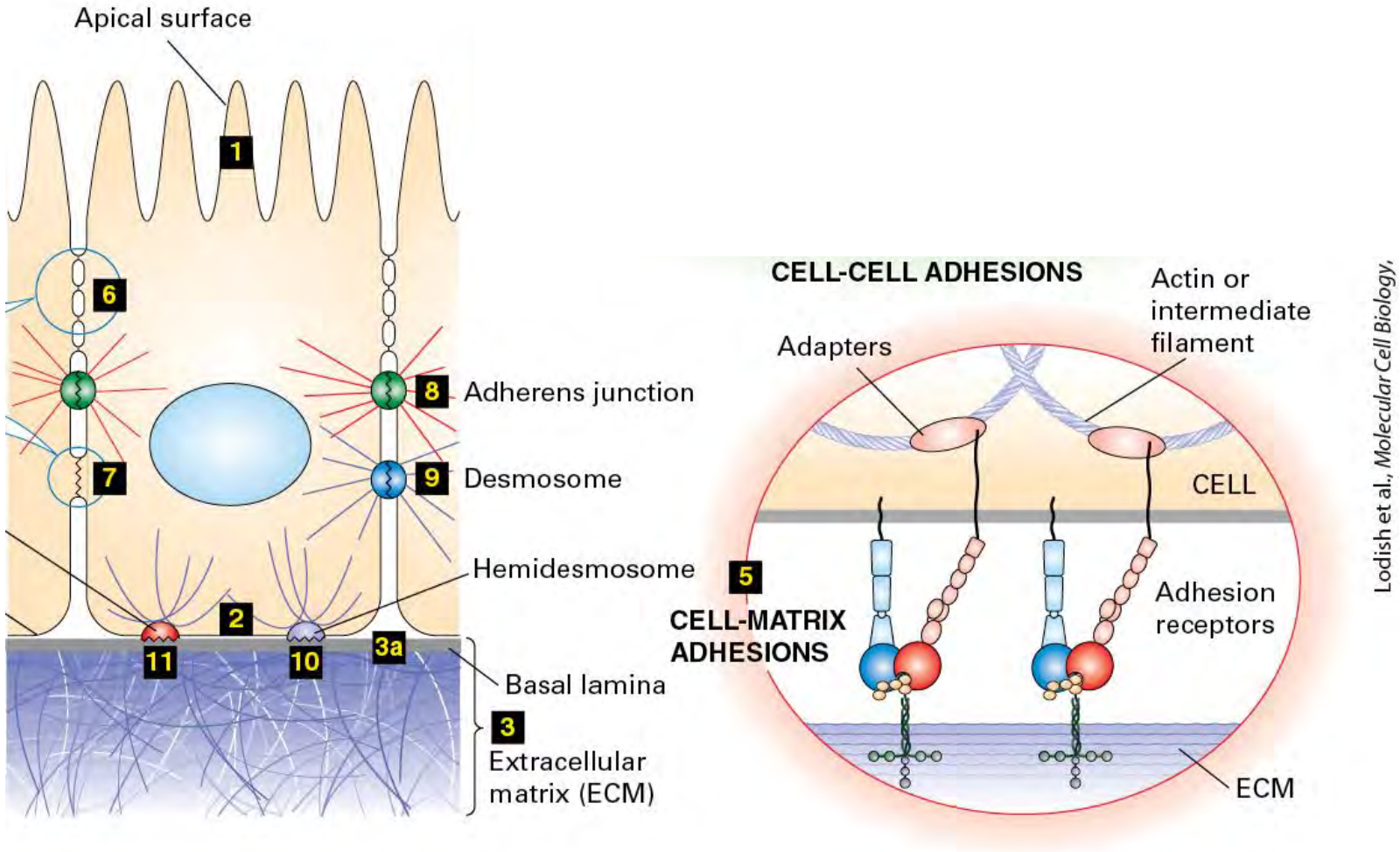


Figure 17-3 Essential Cell Biology, 4th ed. (© Garland Science 2014)

cells adhere to the ECM and to neighbors

two of these adhesions require intermediate filaments

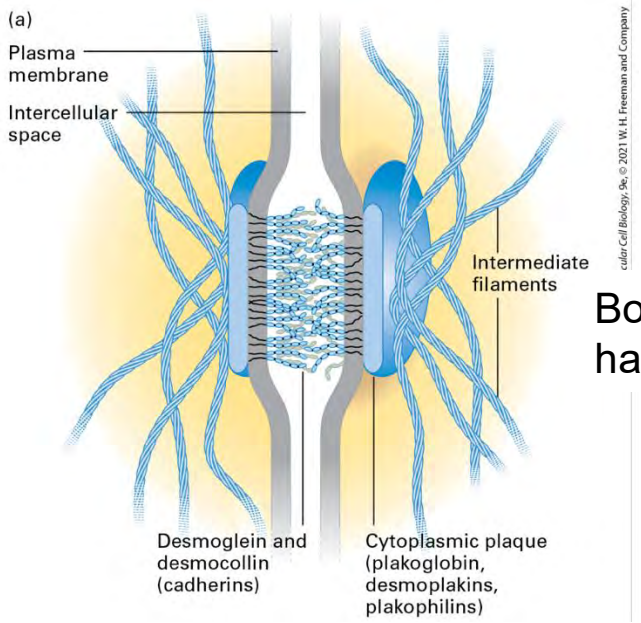


desmosomes & hemidesmosomes

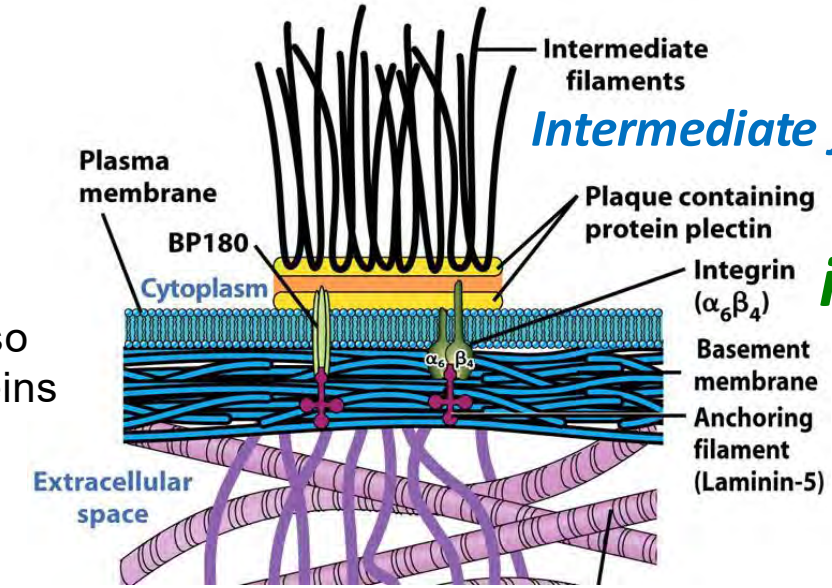
- important cell-cell adhesions for structural support/mechanical **strength**
- desmosomes use Cadherins proteins to bind other cells and connect to intermediate filaments
- hemi-desmosomes use Integrin proteins to bind ECM and link to intermediate filaments

Intermediate filament

cadherin



Both adhesions also have adaptor proteins



integrins

major types of cell junctions - three connect to the cytoskeleton

Adherens junctions use cadherins to link to ACTIN

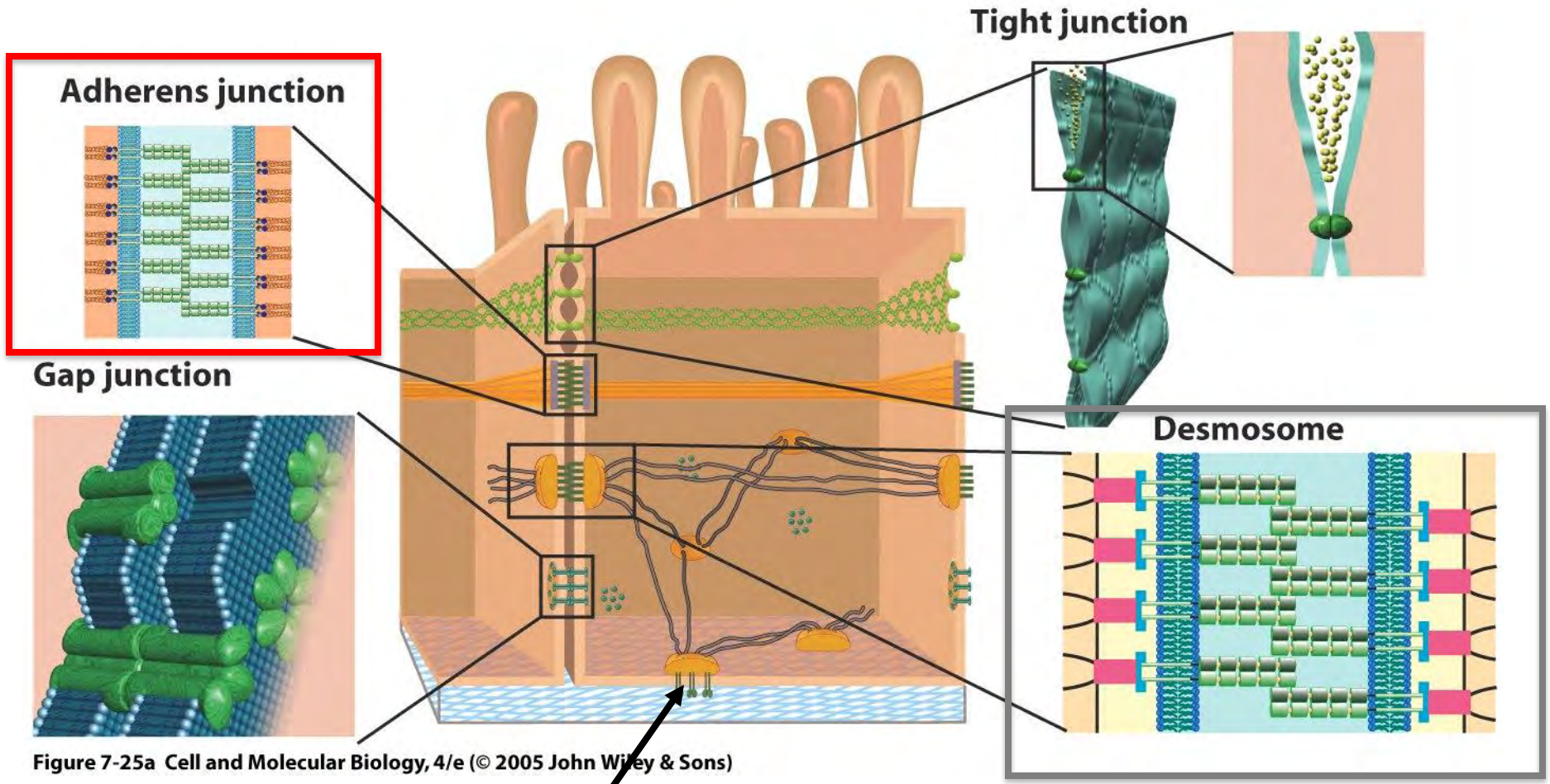
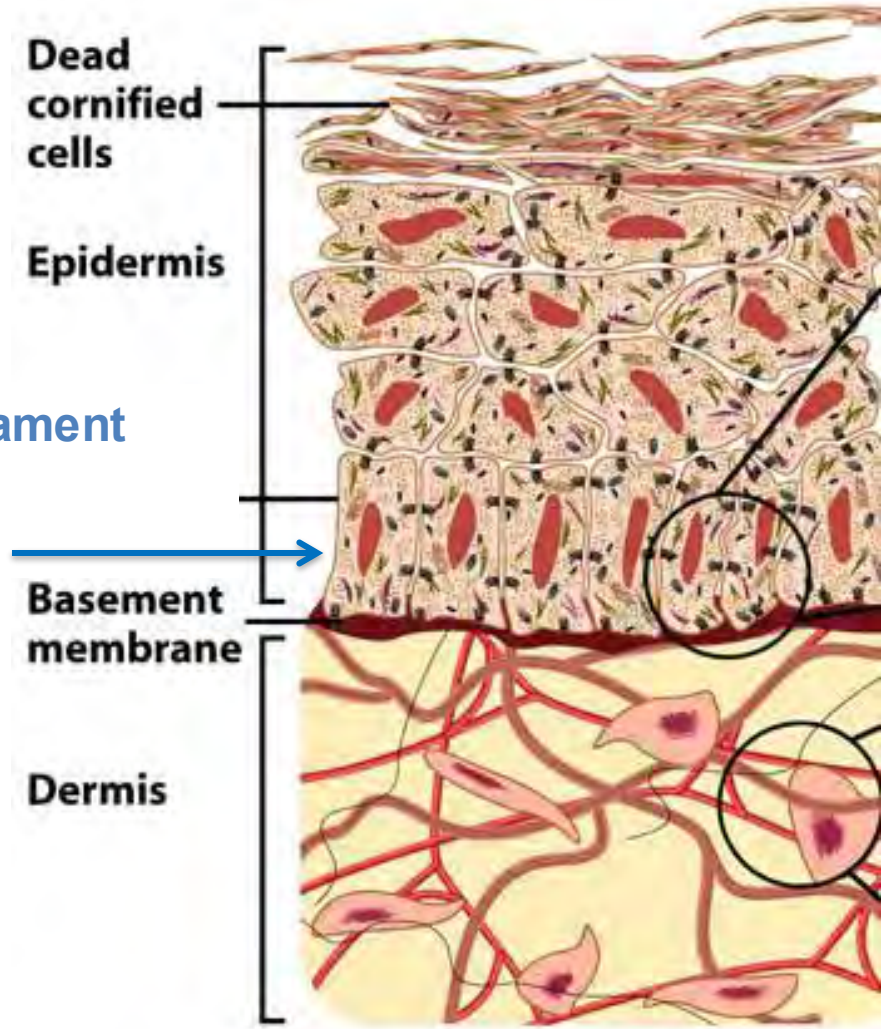


Figure 7-25a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

And **focal adhesions** to the ECM use integrins associated with actin (not shown)

Hemidesmosomes – junctions with ECM and intermediate filaments

human skin tissue



Intermediate filament Keratin5/K14 expressed here

Figure 7-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

blistering disease caused by mutations in intermediate filament proteins

epidermolysis bullosa (EBS)

Pfendner and Lucky 2006

50 in 1 million live births – genetic autosomal disease – mutations in Keratin K14 or K5 polypeptides

most occurrences of EBS are due to point mutations (usually missense) in either Keratin 5 or K14. how could missense mutations in K5 and K14 lead to this autosomal *dominantly* inherited disease?

poll 4: what is a missense mutation?

a) one that results in a new AA codon
b) one that results in a stop codon
c) one that alters the reading frame
d) one that prevents the transcript from being made

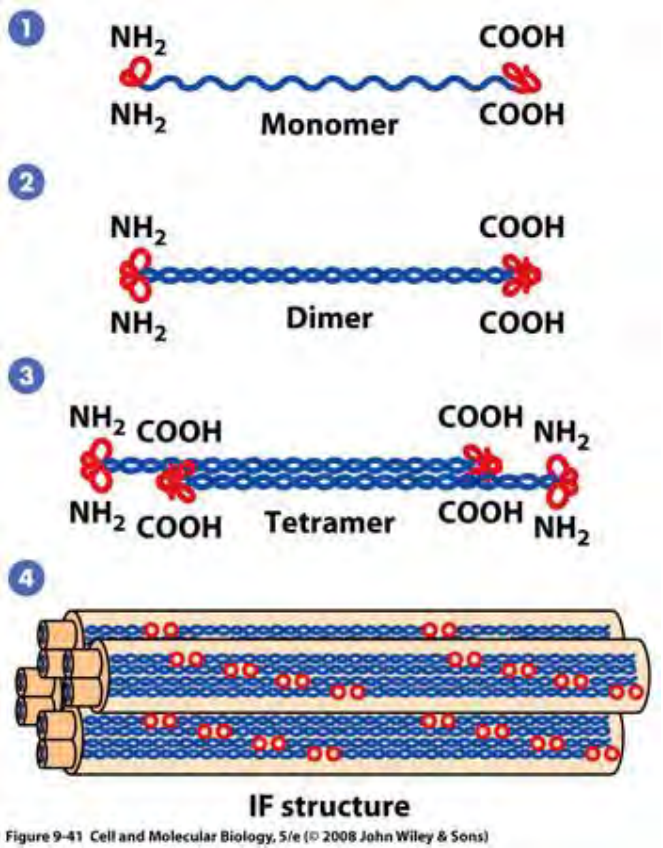


Figure 9-41 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

What's autosomal dominant?

most occurrences of EBS are due to point mutations (usually missense) in either Keratin 5 or K14.
how could missense mutations in K5 and K14 lead to this autosomal *dominantly* inherited disease?

- what about IF assembly helps you understand what might occur in these cells?

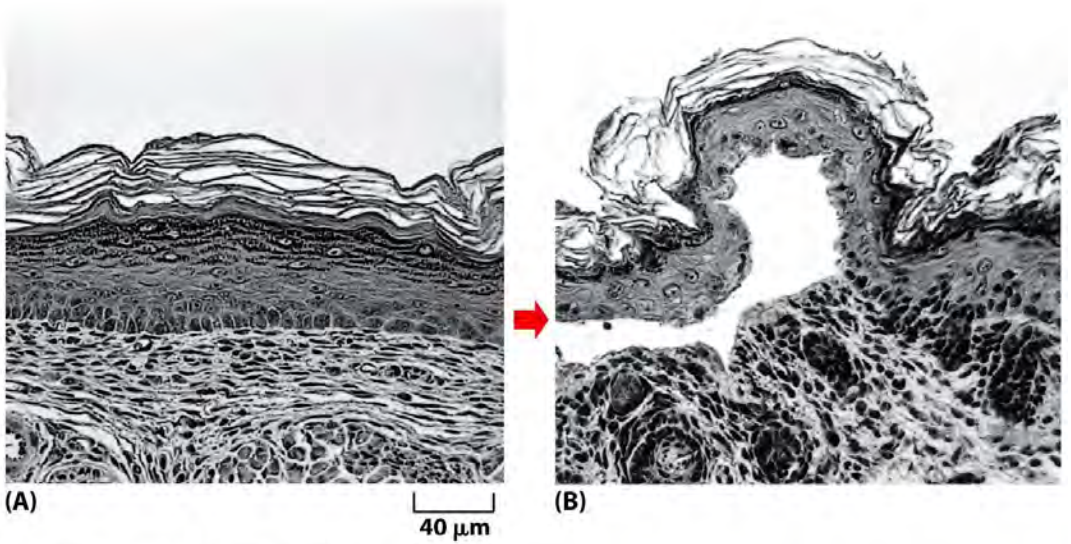


Figure 17-6 Essential Cell Biology, 4th ed. (© Garland Science 2014)

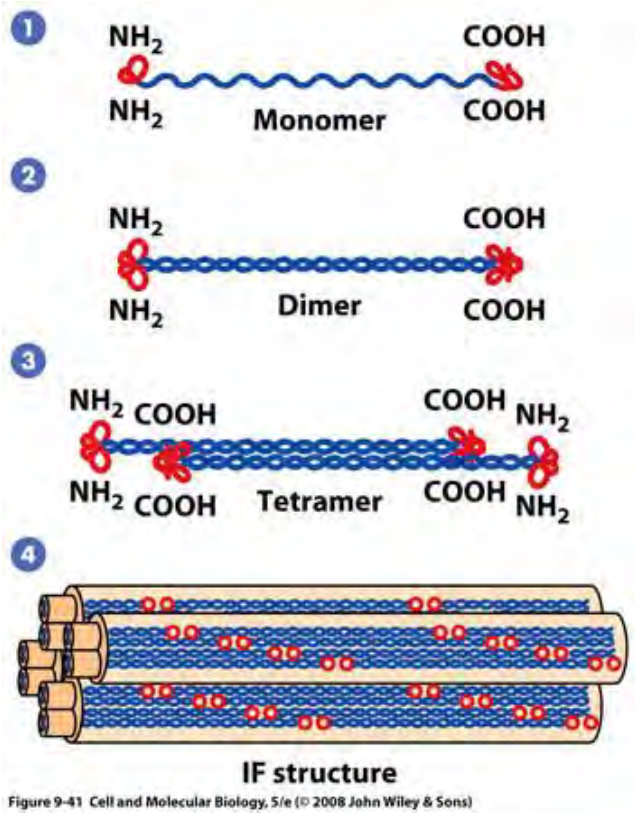


Figure 9-41 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

(are the cells breaking away from basement membrane or being broken?)

blistering disease can also be an auto-immune disease – antibodies block protein function (bullous pemphigoid)

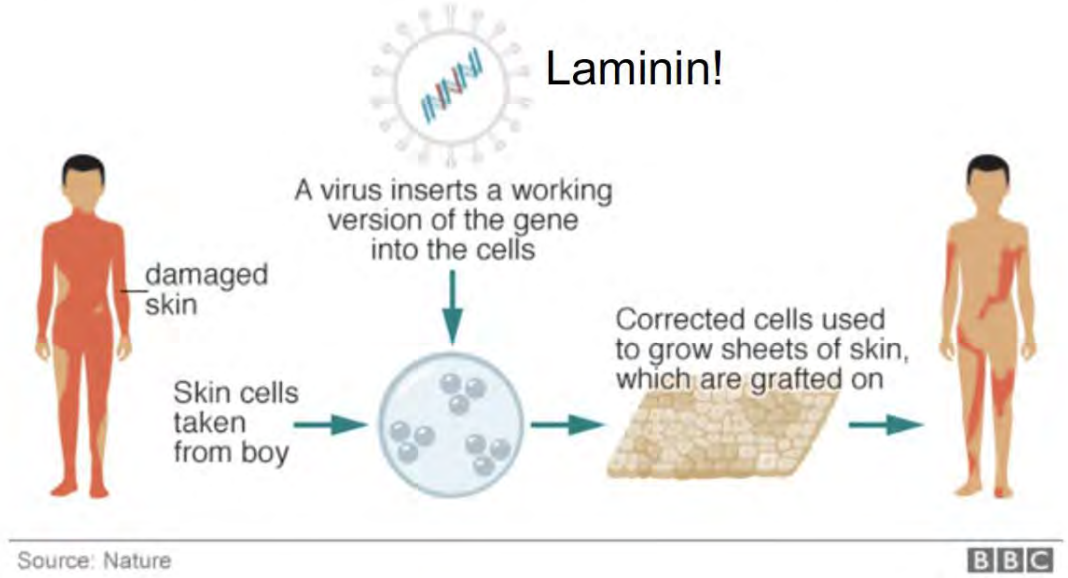
poll 5: What's the most likely antigen for this kind of disease?

A. keratin
B. vimentin
C. occludins
D. integrins
E. connexins

there are several classes of *auto-immune diseases* that develop later in life and cause similar severe blistering

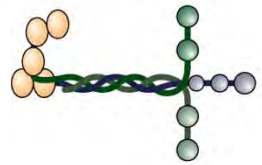
Interesting case in the news … "butterfly child"

- https://www.bbc.com/news/health-41914101



Where did we see Laminin?

Laminin

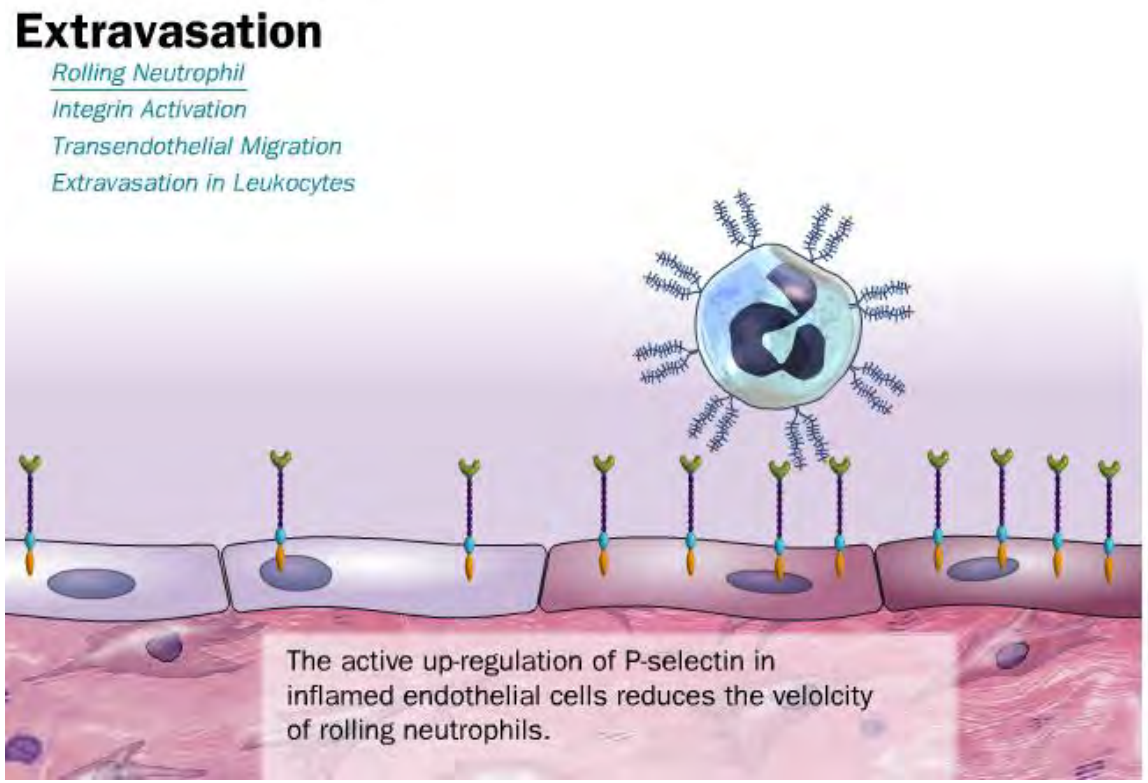


Article | Published: 08 November 2017
Regeneration of the entire human epidermis using transgenic stem cells
Tobias Hirsch, et al… & Michele De Luca
Nature **volume551**, pages327–332 (16 November 2017)

Primary paper: https://www.nature.com/articles/nature24487

Exploring cell junctions- case study: extravasation

Neutrophil- Type of white blood cell (leukocyte)



Endothelium- Type of epithelium - lining inside

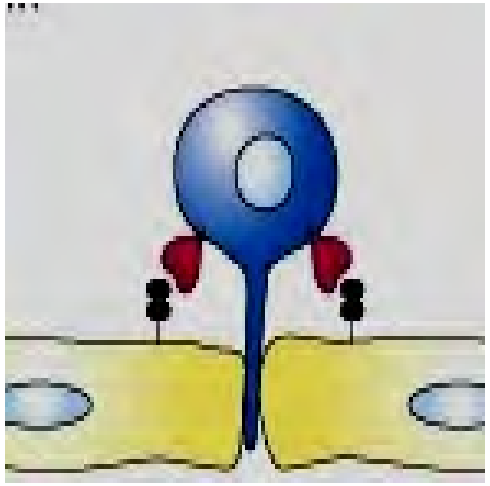
a topic for discussion

a leukocyte responding to an invader must escape from the bloodstream

the leukocyte moves between the **endothelial** cells of the blood vessel =extravasation (post capillary venules)

poll 6: what kinds of junctions would you expect those **epithelial** cells to have?

a) adherens junctions
b) gap junctions
c) tight junctions
d) hemidesmosomes
e) all of the above

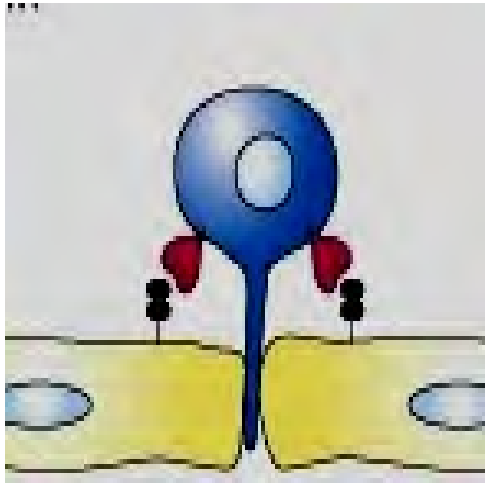


a leukocyte responding to an invader must escape from the bloodstream

poll question:

which of the following junctions is the major barrier to the leukocyte?

a) gap junctions
b) tight junctions
c) desmosomes
d) hemidesmosomes
e) all of the above

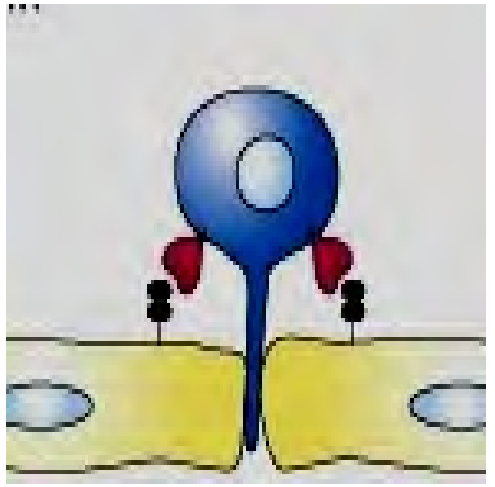


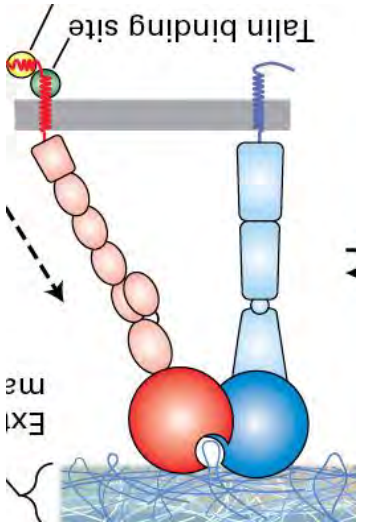
a leukocyte activates integrins to escape from the bloodstream

poll question :

which of the following junctions also contain integrins?

a) adherens junctions
b) gap junctions
c) desmosomes
d) hemidesmosomes
e) two of the above





three type… oskeletal filame… nd…

Intermediate Filaments

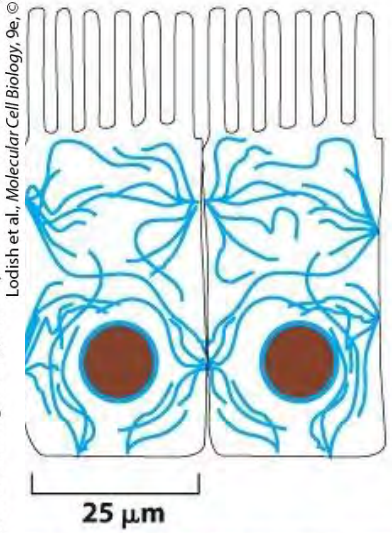
- IF subunits don't bind a nucleotide
- Great tensile strength
- Assembled onto preexisting filaments
- Less dynamic
- Unpolarized
- No motors
- Cell and tissue integrity

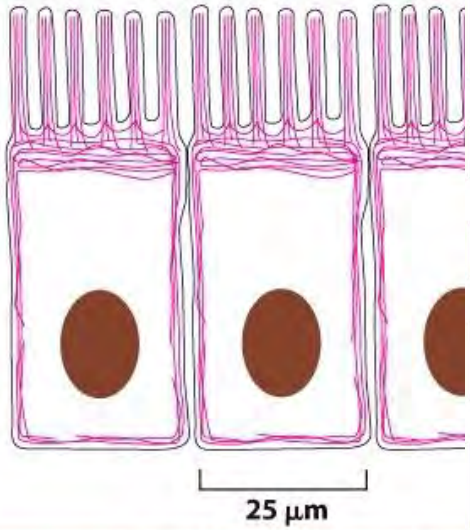
Microtubules

- αβ-Tubulin binds GTP
- Rigid and not easily bent
- Regulated assembly from a small number of locations
- Highly dynamic
- Polarized
- Tracks for kinesins and dyneins
- Organization and long-range transport of organelles

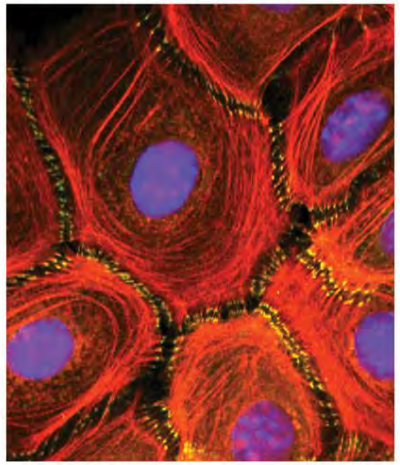
(a) Microfilaments

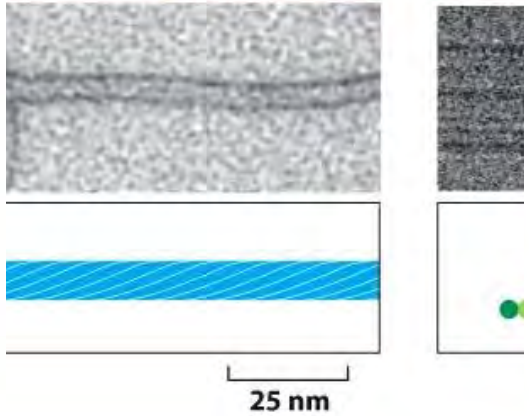
- Actin binds ATP
- Form rigid gels, networks, and linear bundles
- Regulated assembly from a large number of locations
- Highly dynamic
- Polarized
- Tracks for myosins
- Contractile machinery and network at the cell cortex



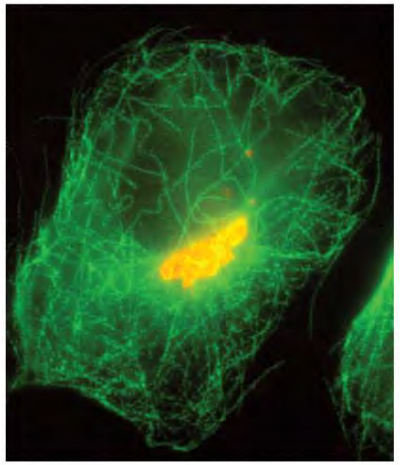


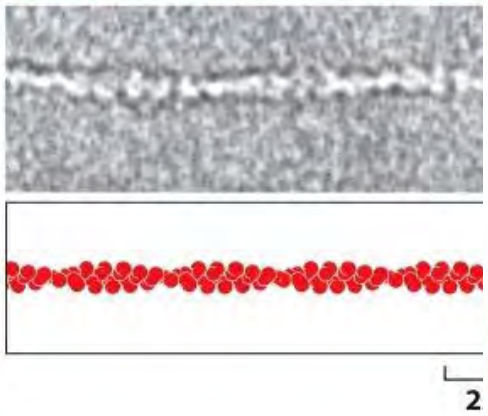
(d)



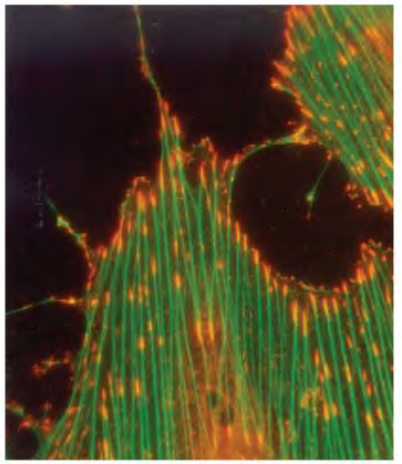


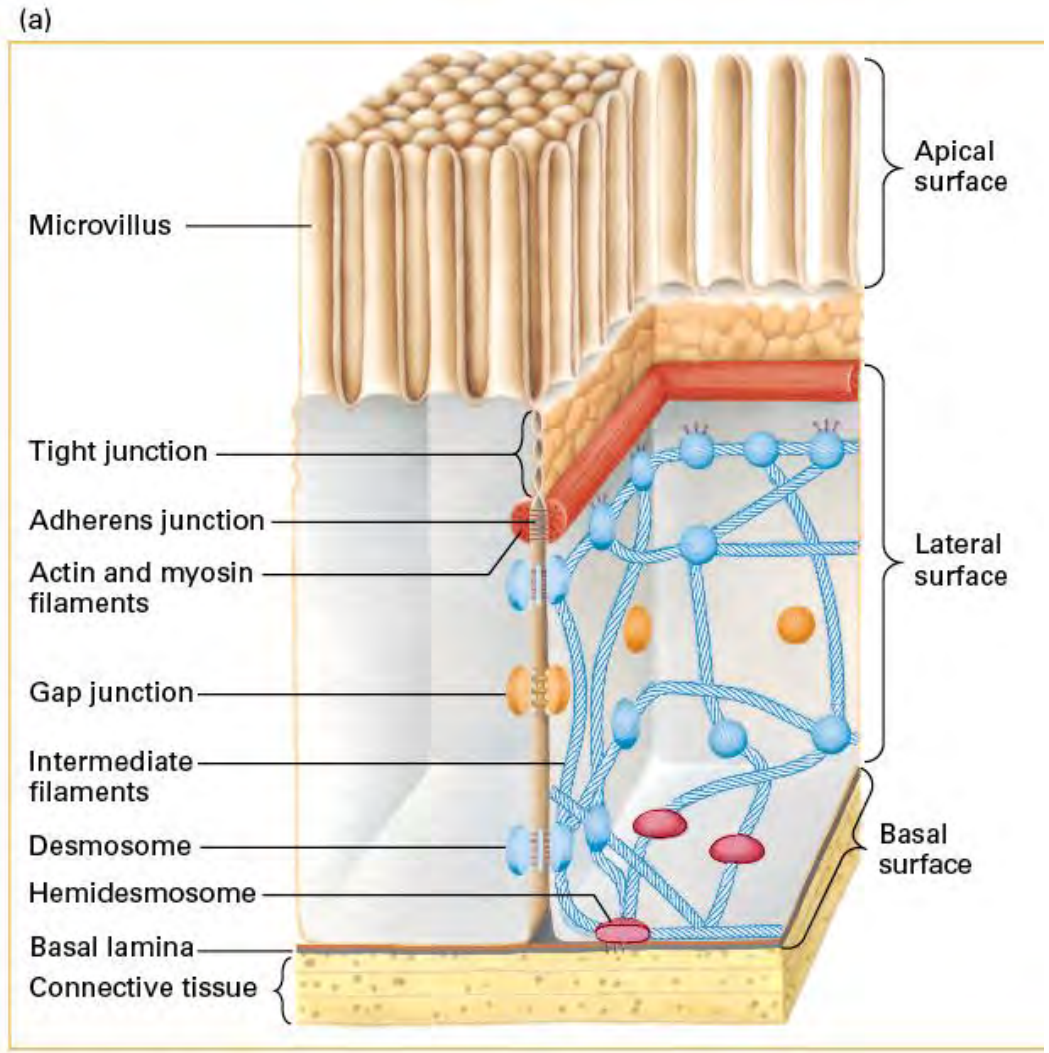
(c)

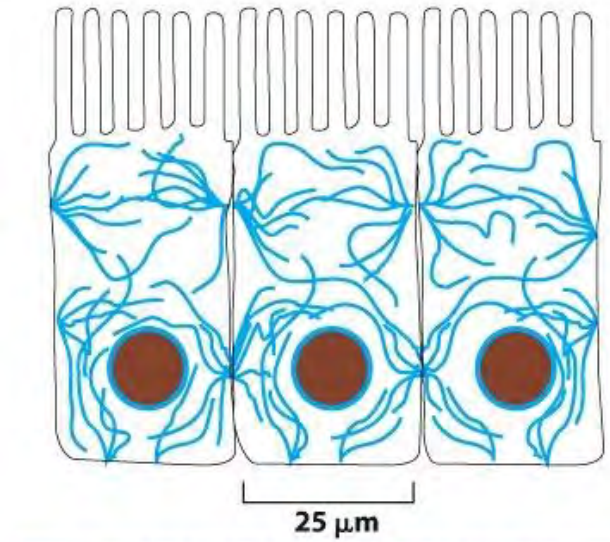




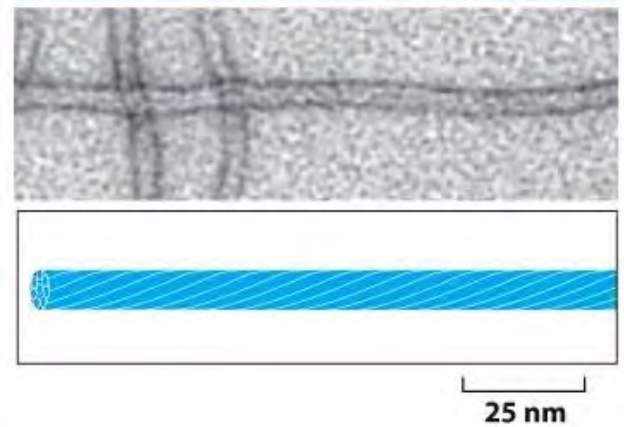
(b)





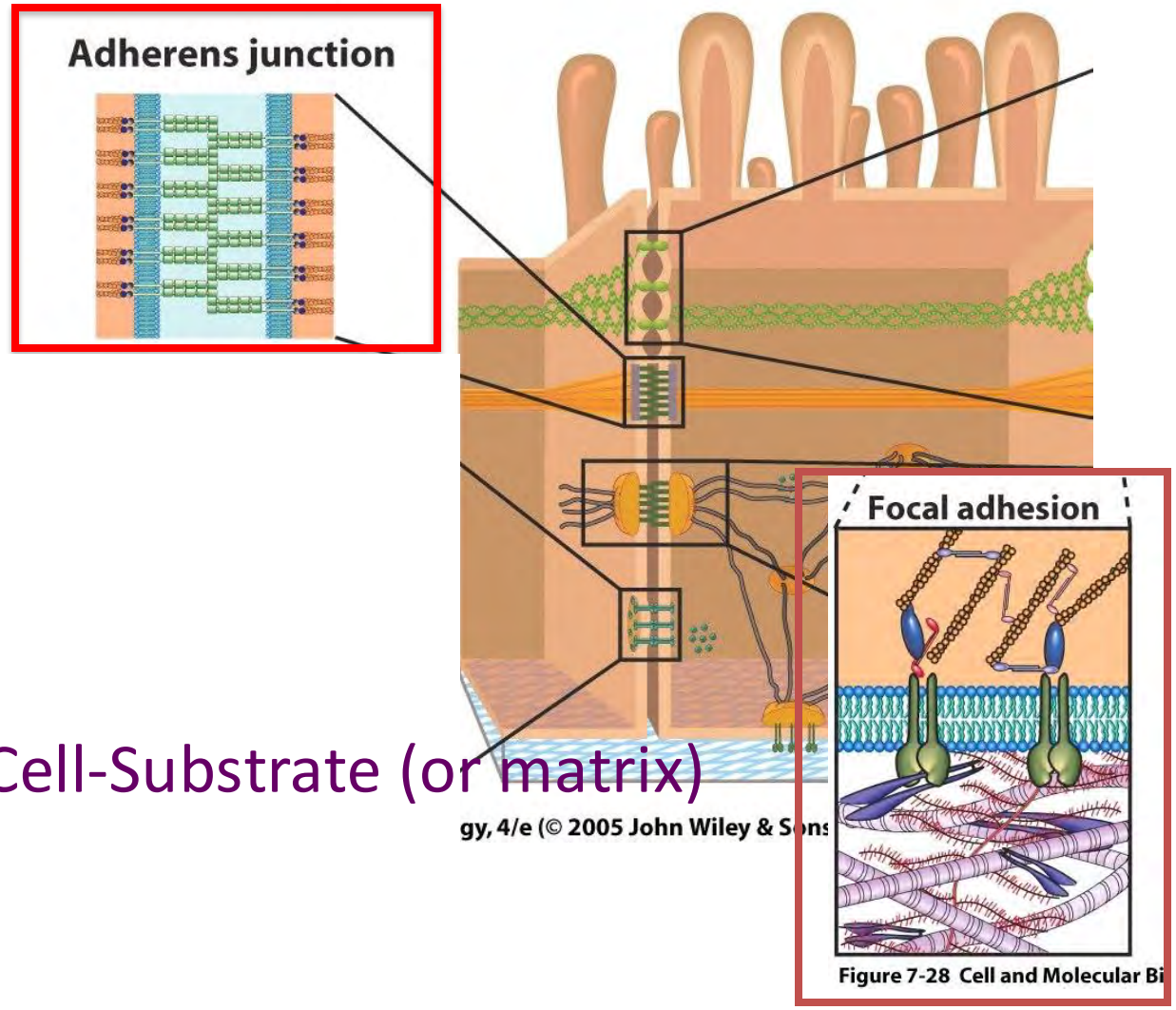


INTERMEDIATE FILAMENTS



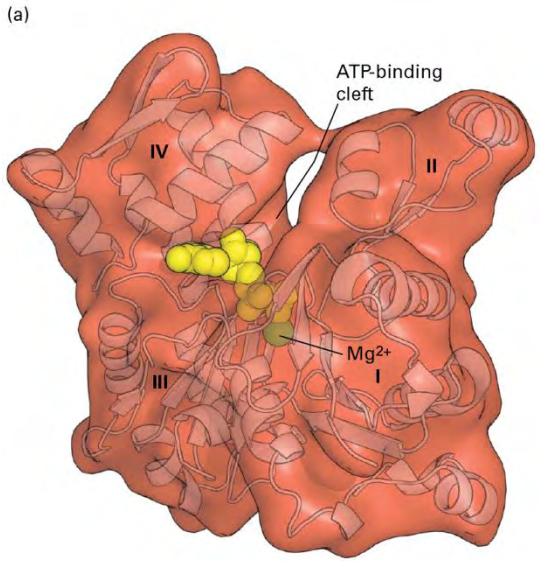
major types of cell junctions - two connect to the actin cytoskeleton

Adherens junctions use cadherins to link to ACTIN

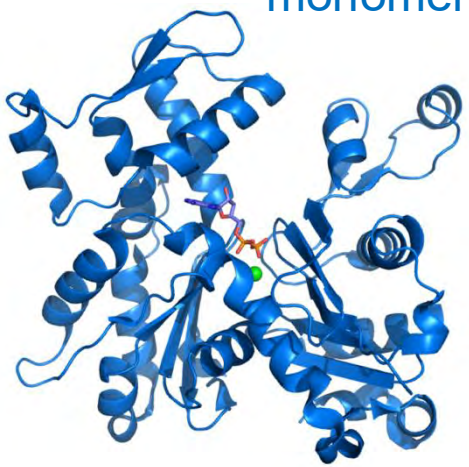


focal adhesions to the ECM (or on a petri dish) use integrins associated with actin

microfilaments = actin filaments
= F-actin



G-actin monomer



wikipedia

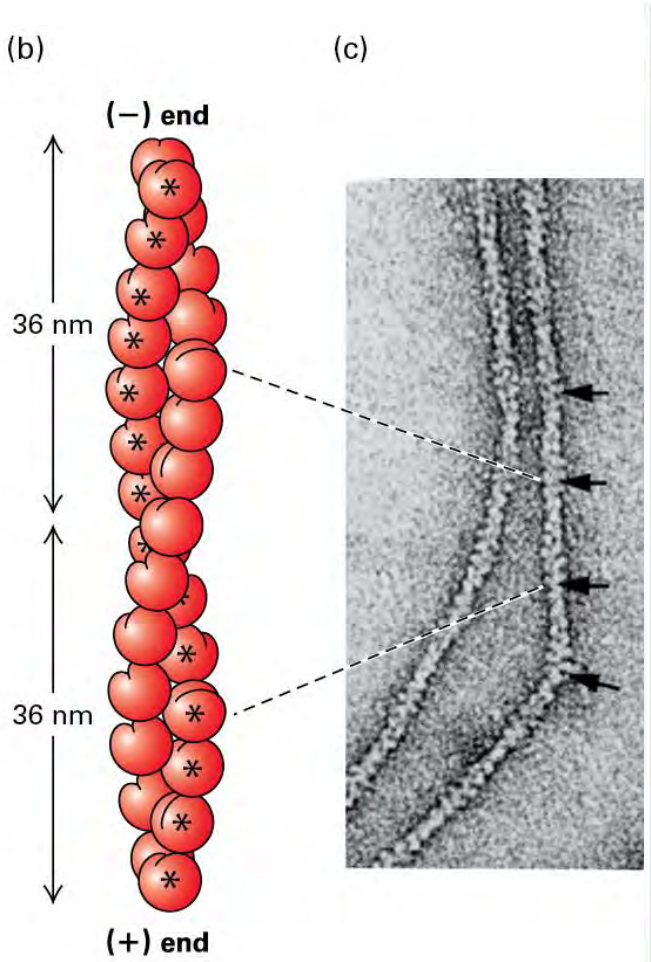


Figure 17-29abc Essential Cell Biology, 4th ed. (© Garlanc

Figure 9-44a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

polarized polymers of actin protein monomers (G-actin) = ___ **nm in diameter**

required for

- cell motility/dynamics (fill cell protrusions)
- AND transport
- AND structural (think: junctions)

assembly requires ATP–

hydrolyzed within polymer (ADP bound)

can form strands, bundles, branches dependent on context and associated proteins

microtubules

- *polarized* +/-, hollow tube ___**nm diameter**
- made up of α and β subunits (heterodimers) that can be added and released (13 protofilaments)
- stabilized by associated proteins

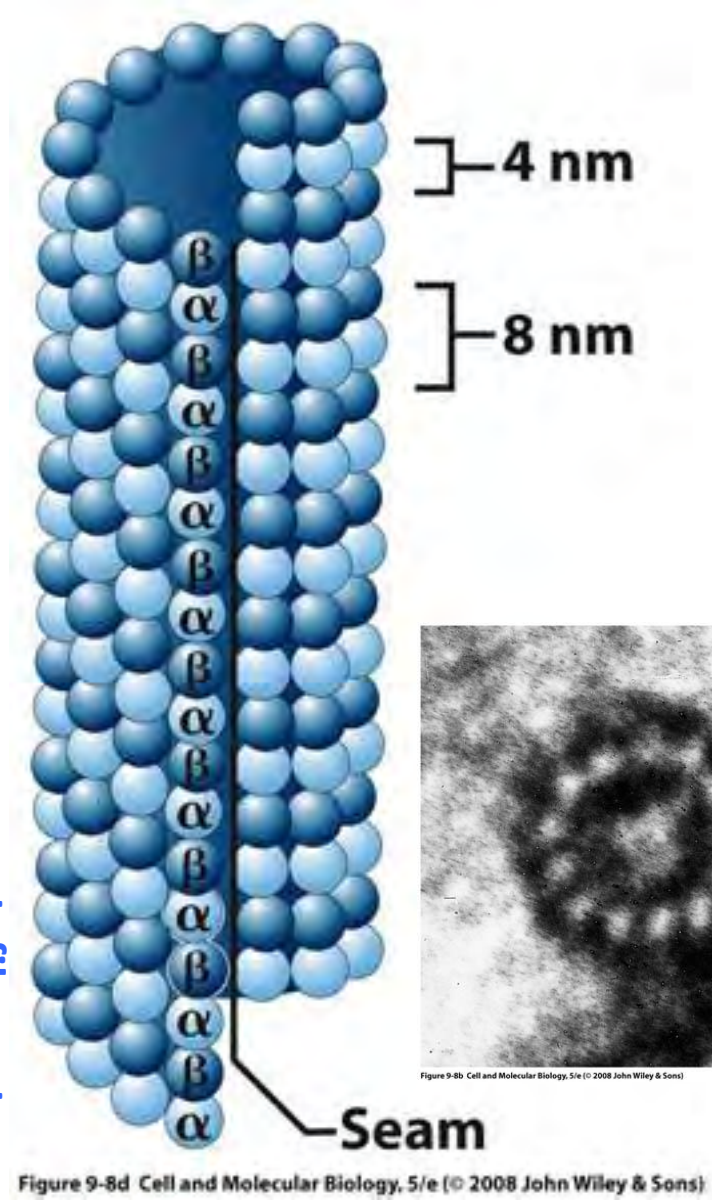
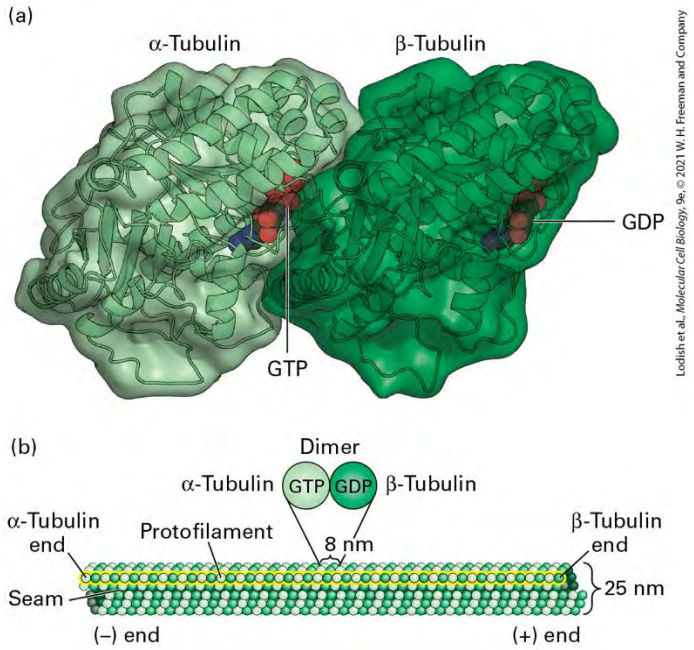


Figure 9-8d Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Figure 9-8b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



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

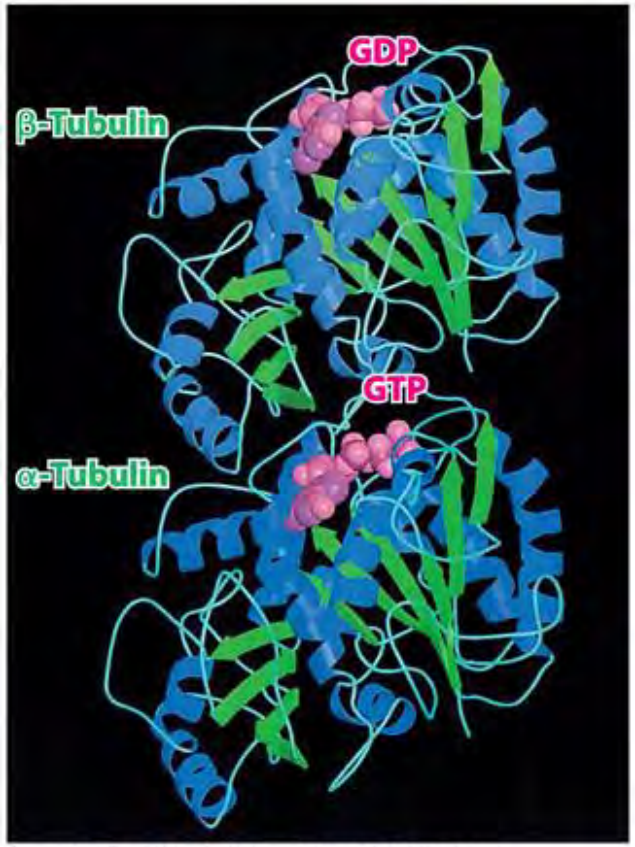
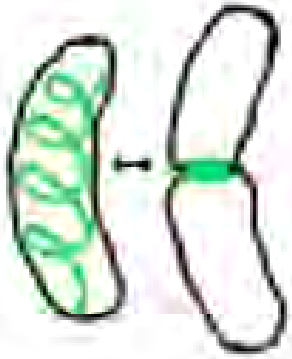
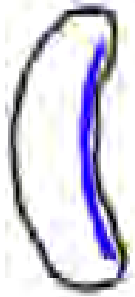


Figure 9-8c Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

prokaryotes have cytoskeletal filaments too!

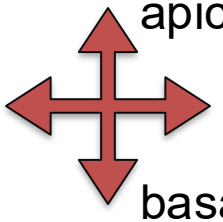
	Division	Polarity	Shape
Eukaryotes	Tubulin	Actin	Intermediate filaments
Prokaryotes	FtsZ	MreB	CreS
Caulobacter localization			
	polymerizes, uses GTP, strong 3D structural similarity	polymerizes, strong 3D structural similarity	strong primary AND 3D structural similarities (nuclear lamin and cytokeratin)

Based on - Gitai, Z. (2005). "The New Bacterial Cell Biology: Moving Parts and Subcellular Architecture". Cell 120 (5): 577-586.

Next time- making cytoskeleton move

human skin tissue
epithelia

epithelial orientation



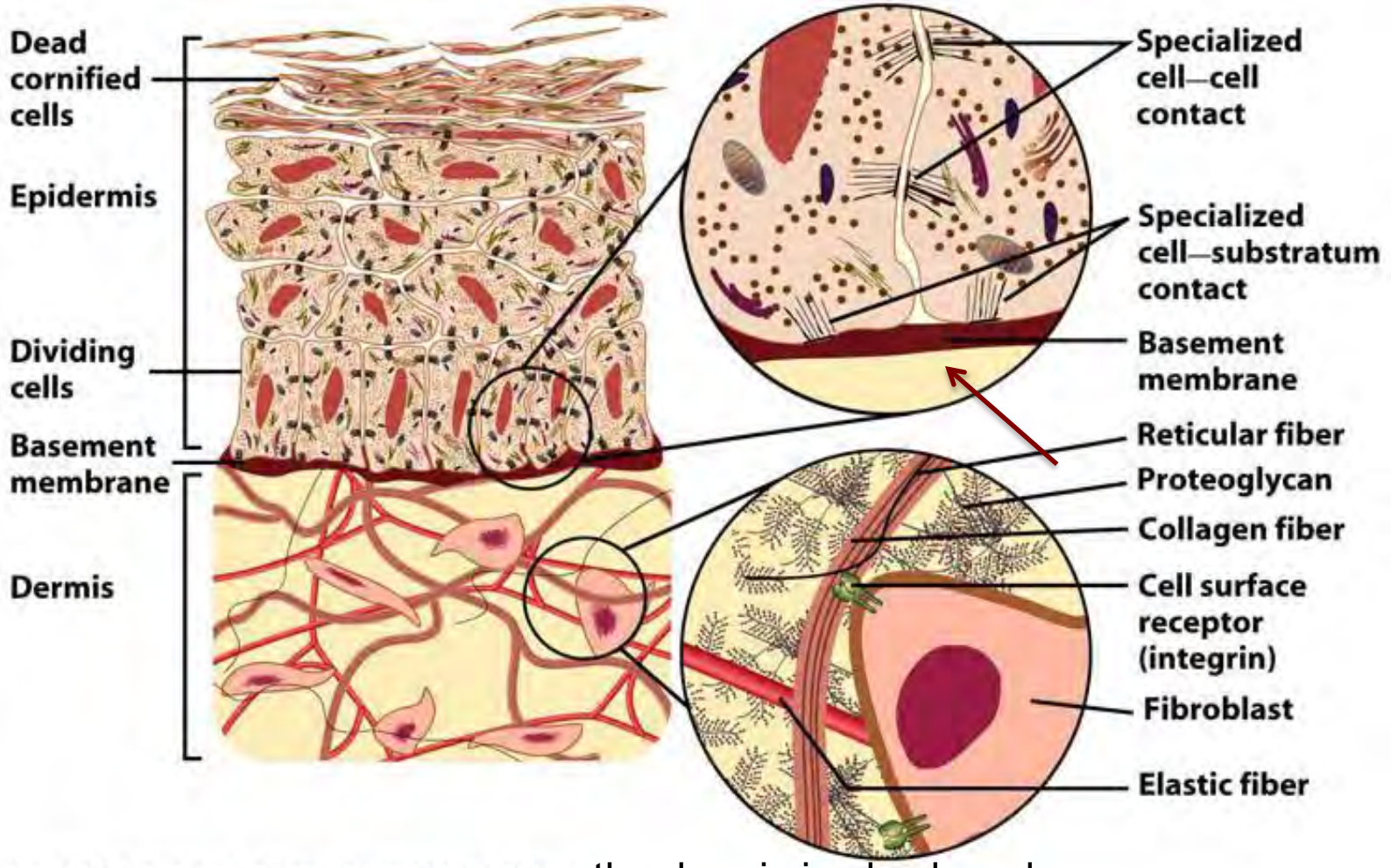


Figure 7-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

the dermis is also largely **extracellular matrix**