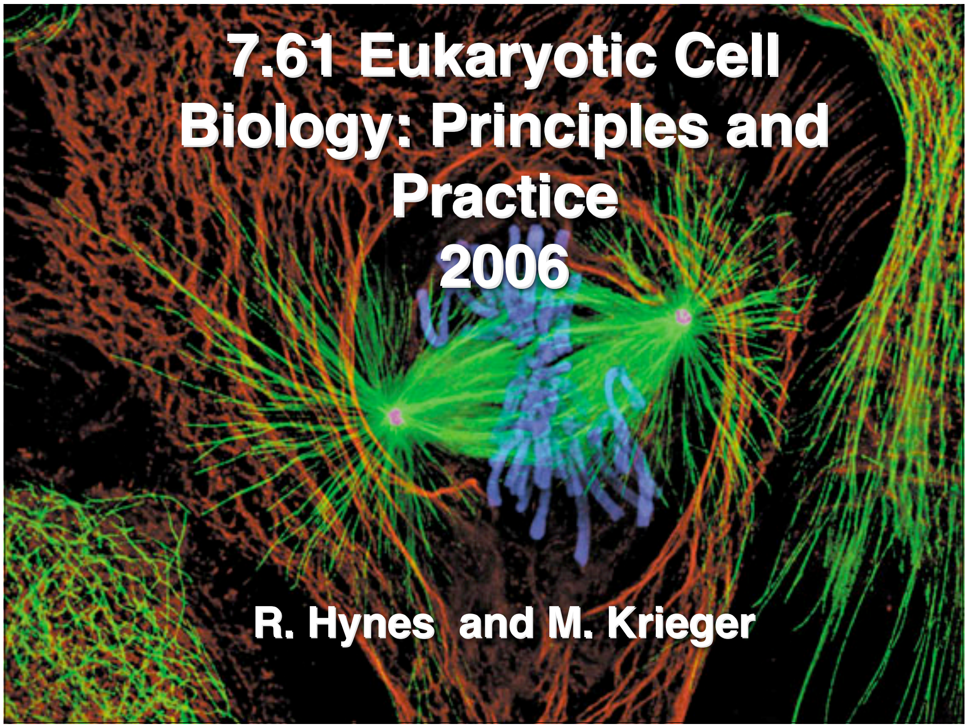


Aims/Focus of the Course

- Principles and Approaches of Modern Cell Biology
- Molecules ⟷ Cells ⟷ Tissues ⟷ Organisms
- How do we know what we think we know?
- If we don't know, how do we find out?
- What are the appropriate techniques/approaches?
- What are their strengths/limitations?

Experimental focus

Cell Biology in Context

- genomics ➔ sequence ➔ proteome
  - what do the proteins do?
  - how do they know where to go?
- development
- immunology
- neurobiology
- pathophysiology (cancer, cardiovascular disease etc.)
  a two-way street

All these have cells as their focus

How do cells work?
How do they communicate?
How does one study them?
Cellular organization & polarity are key
So are cell adhesion/migration

21stC Confluence of Techniques in Cell Biology

Biochemistry
Molecular Biology
Genetics
} Applied to cell structure & function

Classical cell biology – microscopy / EM – both ↑↑.
– cell fractionation
– real time imaging
– FRET, GFP, optical tweezers

Genomics / Evolution – in order to interpret the proteome need insight into how proteins function in cells

Structural biology – gives deeper insight into protein anatomy

Proteomics – mass spectroscopy, 2D gels etc.

Reverse genetics mutagenesis – overexpression /
– antisense, RNAi
– knockouts/knockins etc

Course Organization

- Lectures
- Readings/Discussions
- Minicourse structure

- Relevant background

  Biochemistry

  Genetics

  Molecular Biology

Questions of scale and methods of detection

Molecules

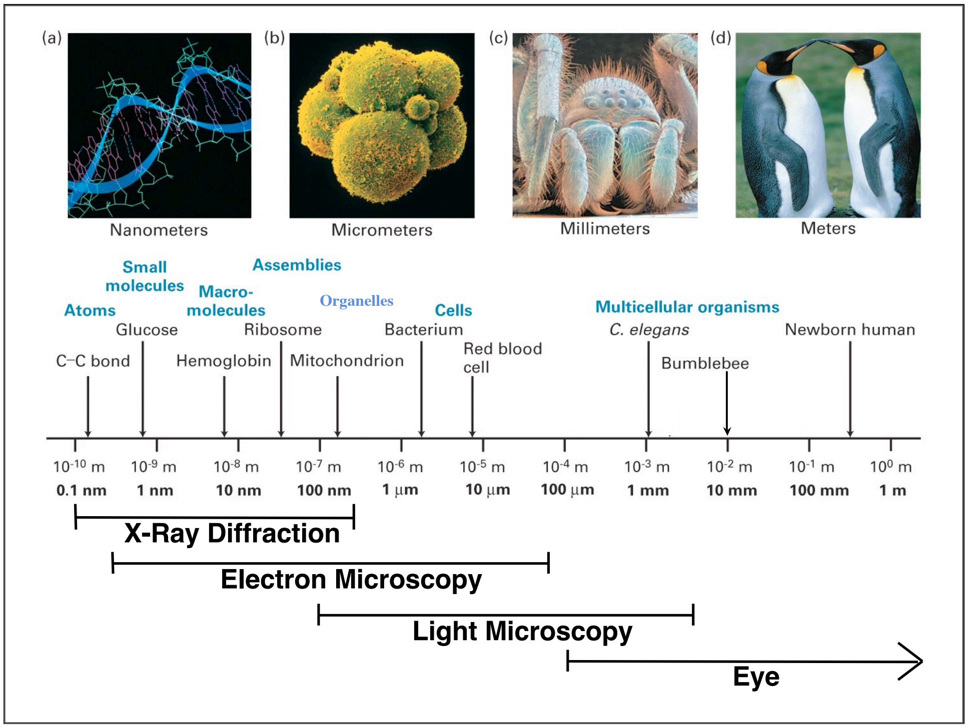
Molecular assemblies

Organelles

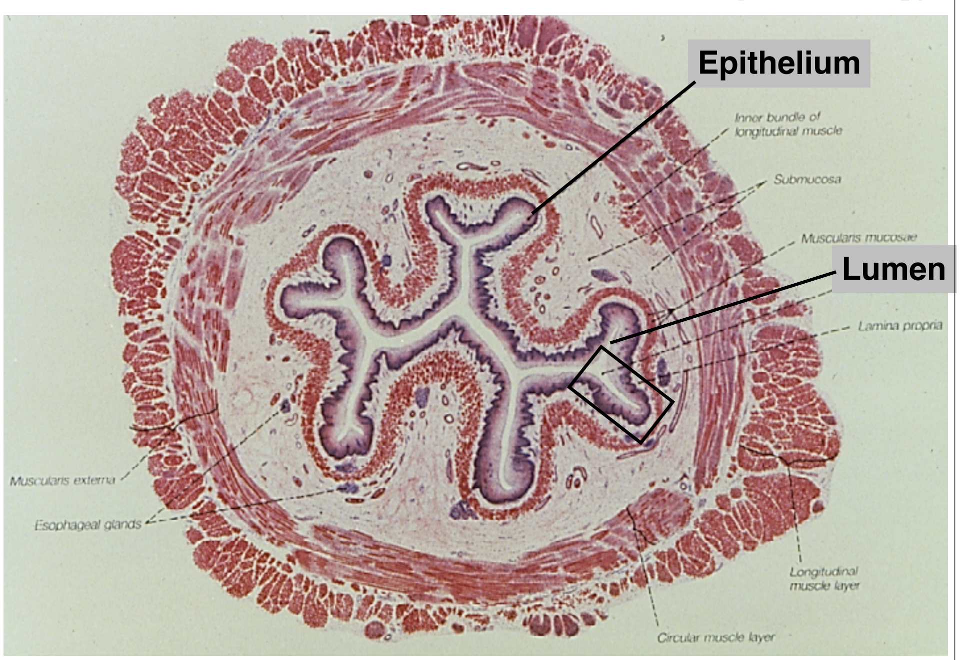
Cells

Tissues

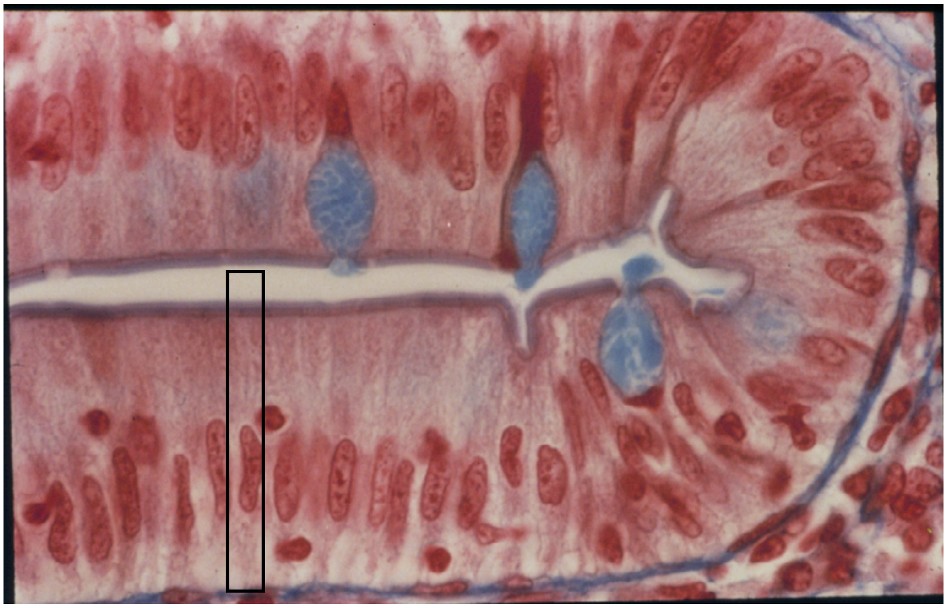
Organisms



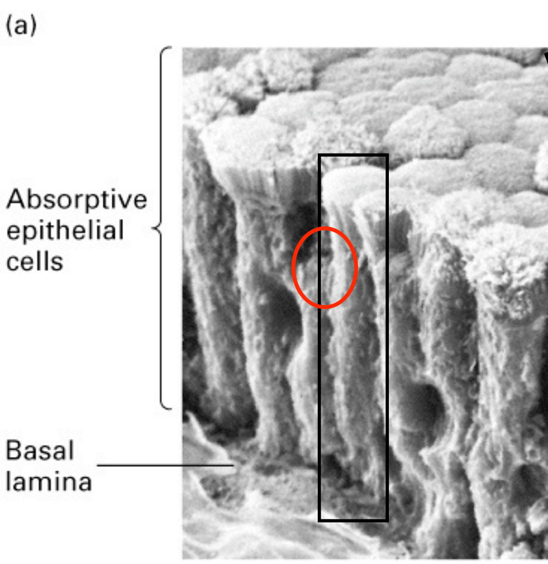
Histochemical stain of small intestinal cross section - light microscopy



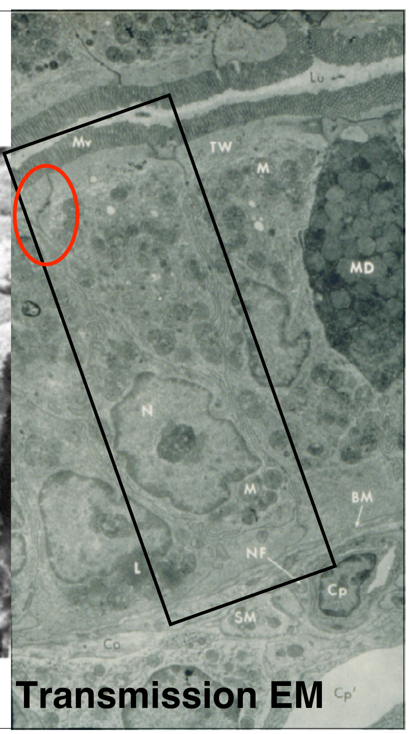
Higher power light microscopic view of intestinal epithelium



Low power EM of Intestinal Epithelium



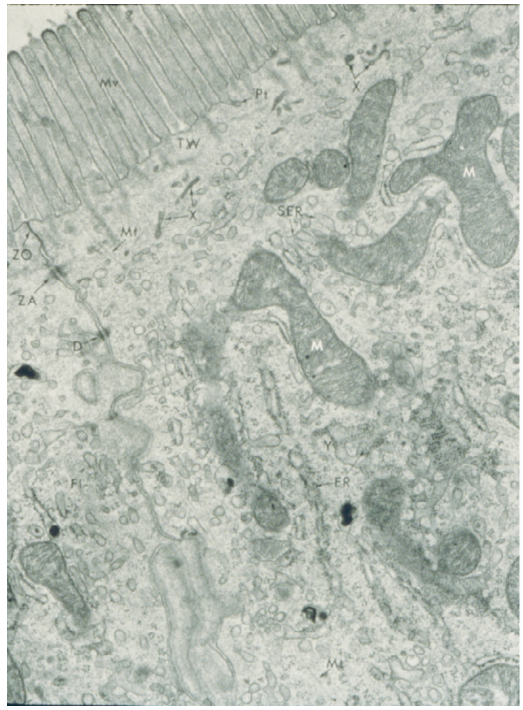
Scanning EM



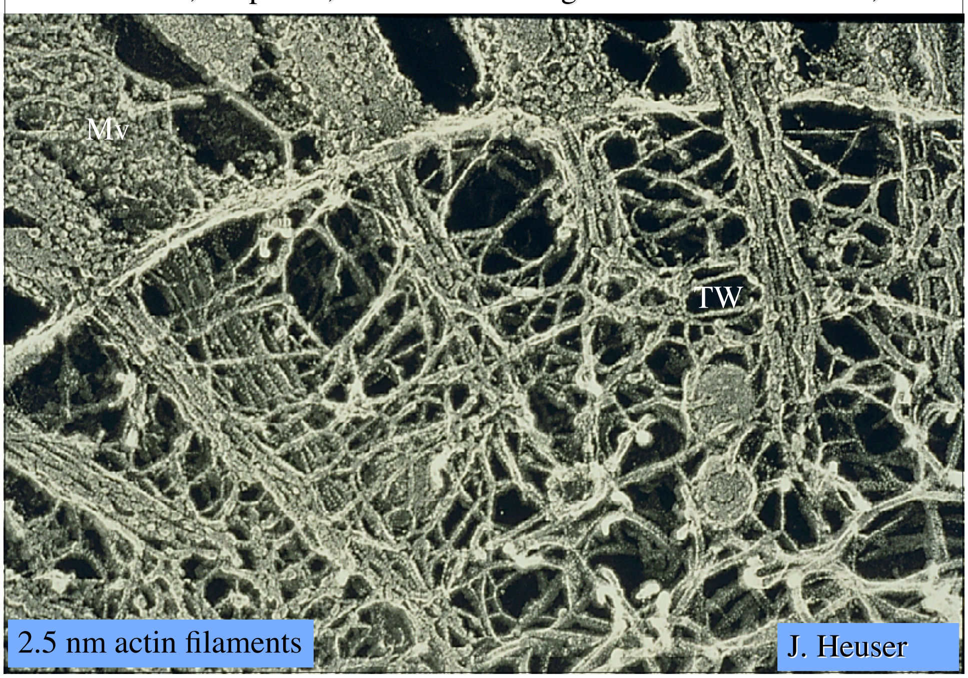
Transmission EM

Higher power EM of Intestinal Epithelium

Mv=microvilli, expand surface area, transporters
TW=terminal web
D=desmosome
ZA=zona adherens
ZO=zona occludens



Fast freeze, deep etch, metal coated image of microvilli and TM,



Higher power EM of Intestinal Epithelium

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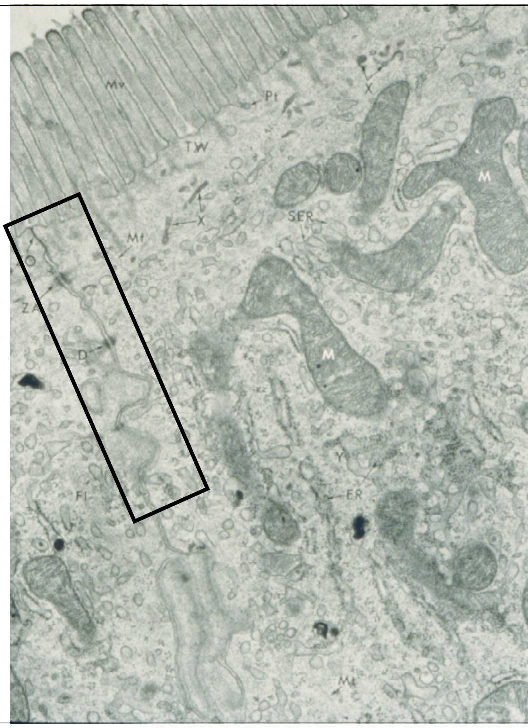
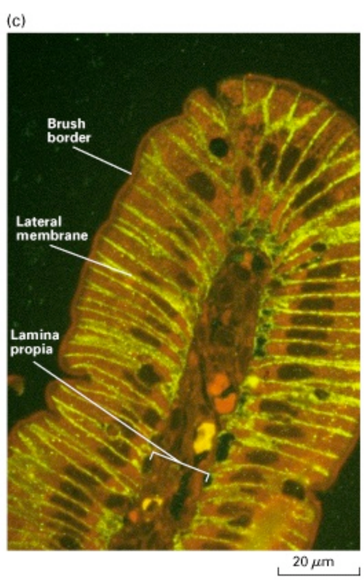
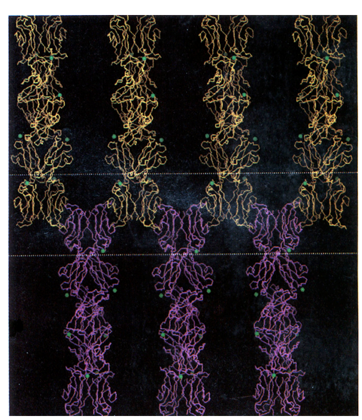
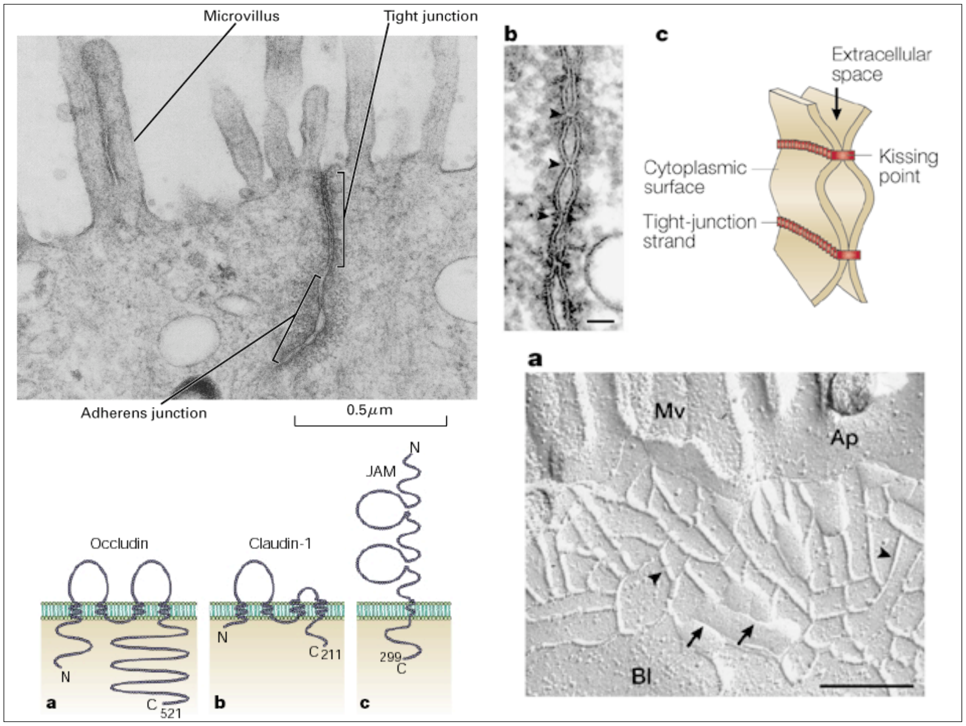


Intercellular adhesion



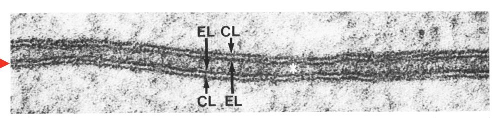
Staining for cadherins that mediate cell-cell adhesion





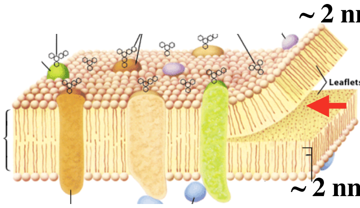
TEM - osmium tetroxide stain

Freeze Fracture



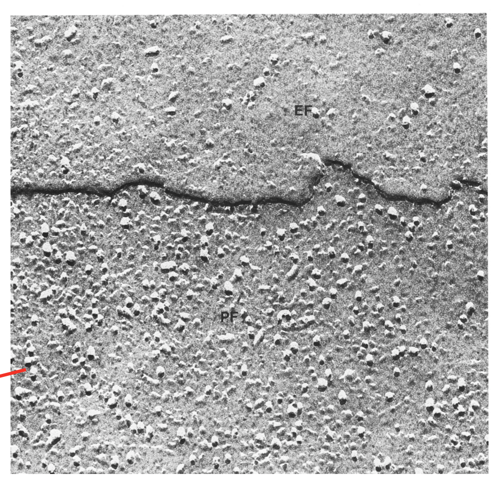
This micrograph (× 400,000) illustrates portions of the plasma membranes of two closely opposed cells. Membranes have a trilaminar structure in transmission electron micrographs. Each membrane consists of two parallel electron-dense lines separated by a lighter central zone. The electron-dense lines represent the phospholipid leaflets and are termed the exoplasmic (EL) and cytoplasmic (CL) leaflets of the plasma membrane. The exoplasmic (extracellular) leaflets contact the extracellular space (*) and the cytoplasmic leaflets contact the cytosol. The thickness of the plasma membrane is about 10 nm.

~ 2 nm



~ 2 nm

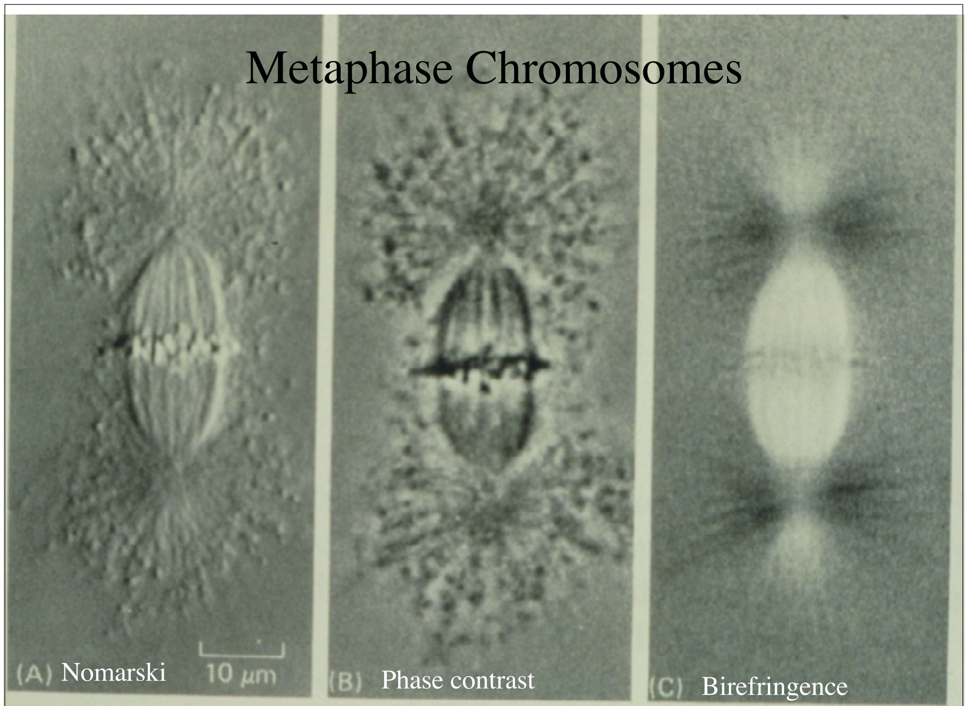
TEM - metal shadowing

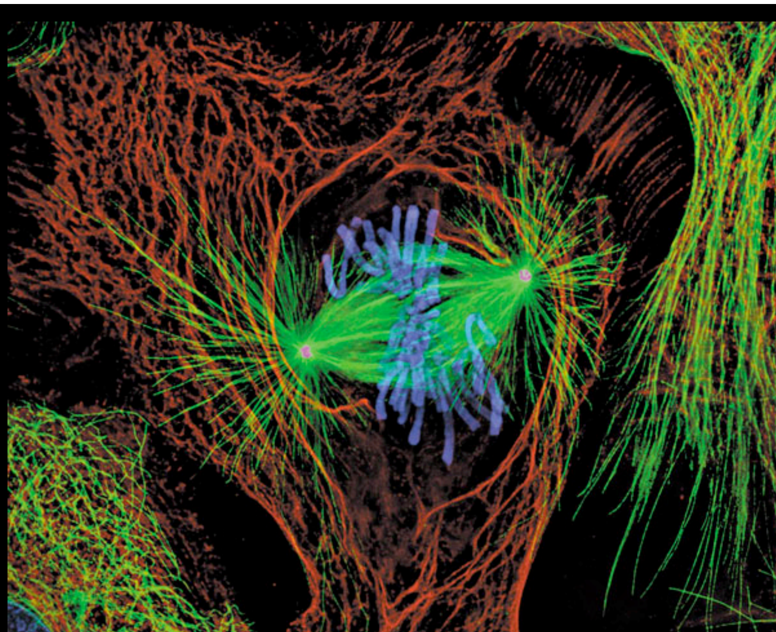


IMPs - intramembranous particles - integral membrane proteins

Different Sorts of Light Microscopy

- Phase contrast
- Nomarski
- Birefringence
- Immunofluorescence
  - fluorescently tagged antibodies
  - other tags allow EM visualization





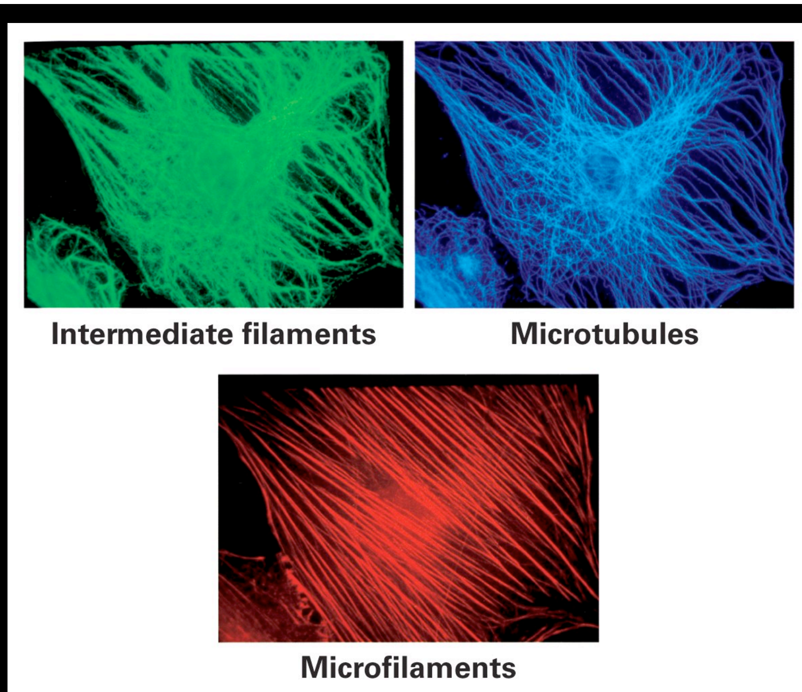
This image of a newt lung cell shows the metaphase cell stained for centrosomes (magenta), microtubules (green), chromosomes (blue) and intermediate filaments (red).

<u>Resolving power</u>: smallest detail resolved in imaging an ideal specimen, ~0.1 nm for EM, ~200 nm (0.2 μm) for LM

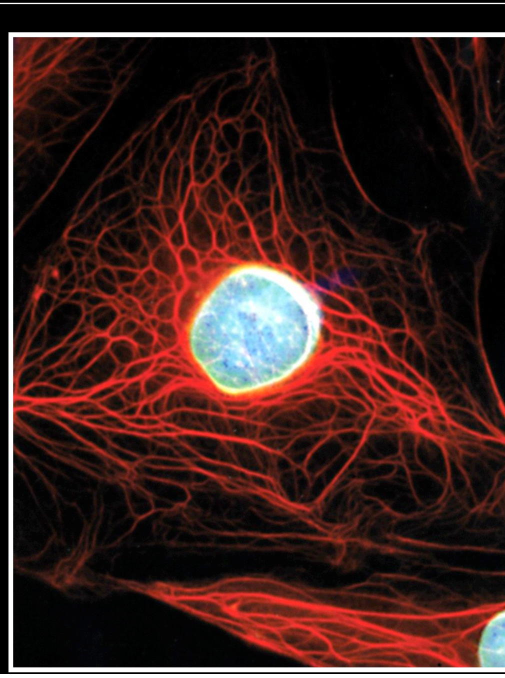
<u>Minimal resolvable separation</u> of incoherently illuminated points: $d_{min} = 0.61\lambda / n\sin(\alpha)$: λ = wavelength, n = refractive index (reason you use oil), α = aperture angle of lens

<u>Actual Resolution</u>: detail actually revealed in the image of a given specimen, requires contrast, not just resolution -> stains to highlight or contrast portions of specimen of interest

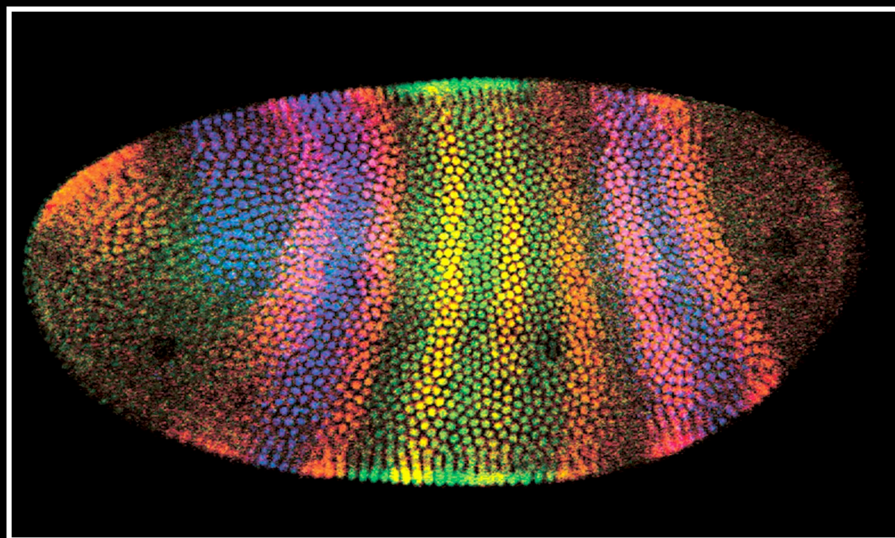
<u>Stains</u>: metals, fluorophores, reaction products (peroxidase, alkaline phosphatase), particle labels (ferritin, gold), shadowing, negative stain, autoradiography, antibodies (immunofluorescence, immunoEM).



Intermediate filaments: Keratin (red) and nuclear lamin (blue)



Fly Embryo: 3 nuclear antigens stained red, green, or blue



How many proteins are there in a cell?

How many proteins are there in a cell?

of genes in genome? 25-30,000

Let's assume 10,000 expressed in a given cell

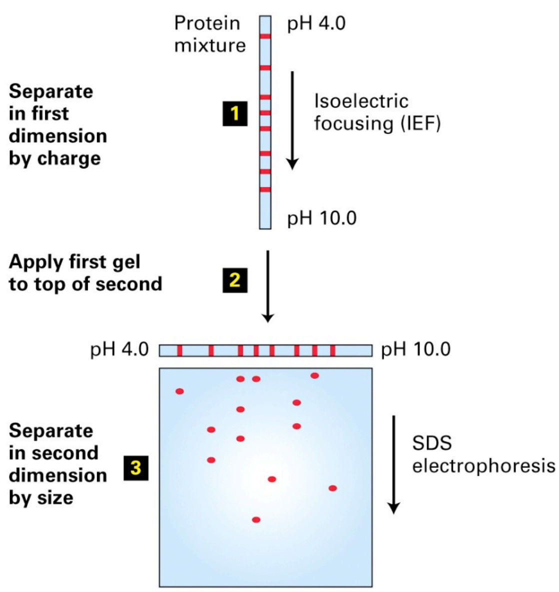
Amount of protein/cell? ~0.8 ng

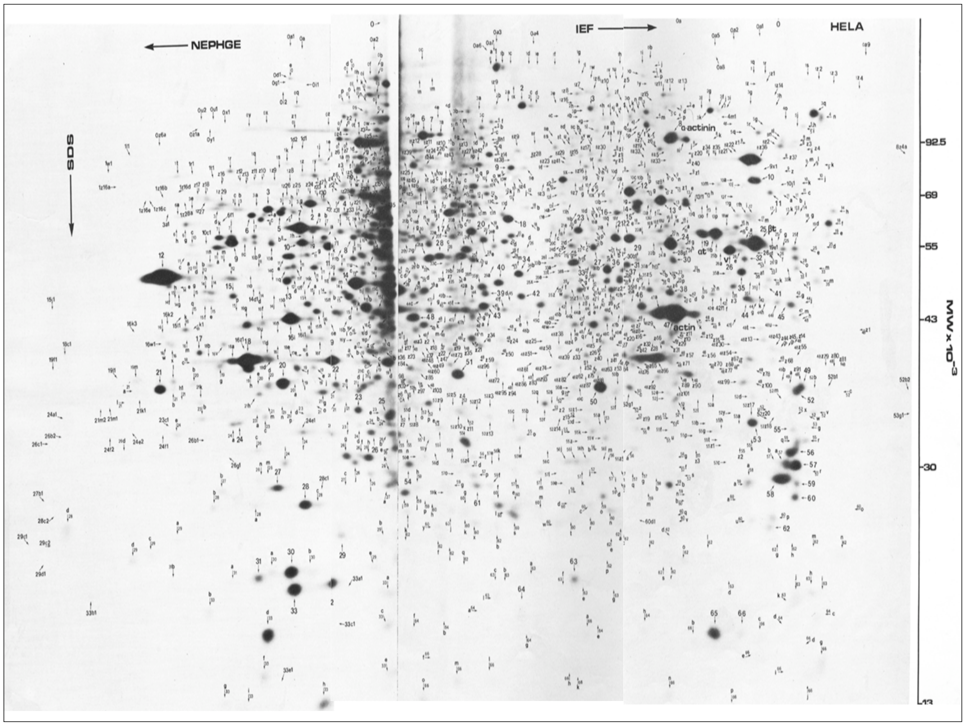
That converts to 10^{10} molecules of 50kDa per cell

If all were equally abundant - 10^6 ($10^{10}/10^4$)of each per cell

Of course, they vary widely in size and abundance

2D Gel Analysis





Abundant proteins are easy to purify -

Actin is 10% of many cells - 10x purification -> purity
BUT - most proteins are not as abundant

A <u>major</u> cell surface receptor - ~10^6 molecules per cell
or 10^{-4} of total cellular protein
So need 10^4 x purification

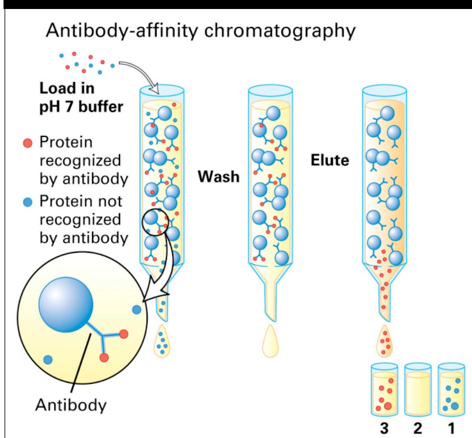
Assume 10% yield

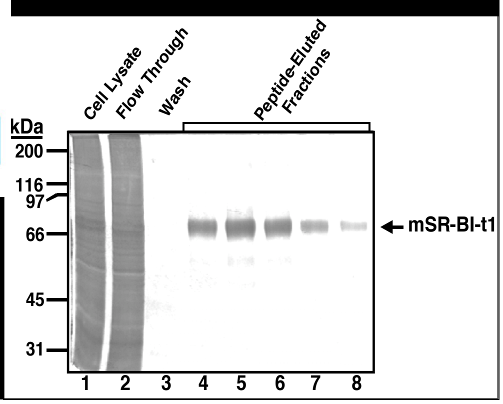
So 1g of cell protein (10^9 cells = 100 x 100mm dishes)
will yield 1g x 10^{-4} x 0.1 = 10ug
1pmol = 50ng of a 50kDa protein
But what if it were 100-1000 x less abundant?

A minor cell surface receptor ~100-1000 copies per cell - need 10^7-10^8 fold purification.
Animal tissue often a better source than cells

Alternative approaches:-
- affinity purification
- mass spectrometry
- clone the gene first
  - expression or functional cloning
  - genomics
- then express the protein
  - bacteria, yeast
  - animal cells
  - animals (transgenesis)

Purification of Membrane Proteins: immunoaffinity chromatography





Analyses of protein function

Biochemistry - purification and assay

Expression - transformation, transgenesis

Inhibition - genetics (forward and reverse)
- drugs, antibodies,
- "dominant negatives"
- RNAi, antisense
- knockouts, mutants

Membranes

Play key roles in cell function
- compartmentalization
- transport
- intercellular signalling
- cell adhesion

Membrane proteins have special properties both because of the membrane environment and in line with their different functions

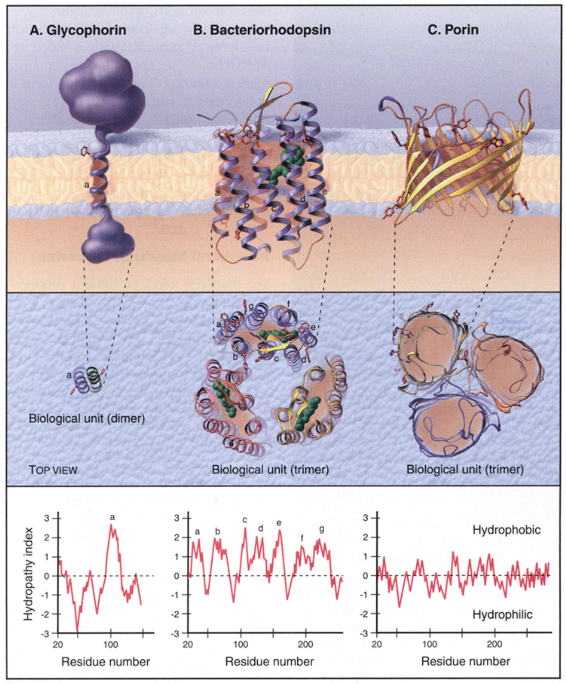
Representative Integral Membrane Proteins

side view

WEEK of Sep 18

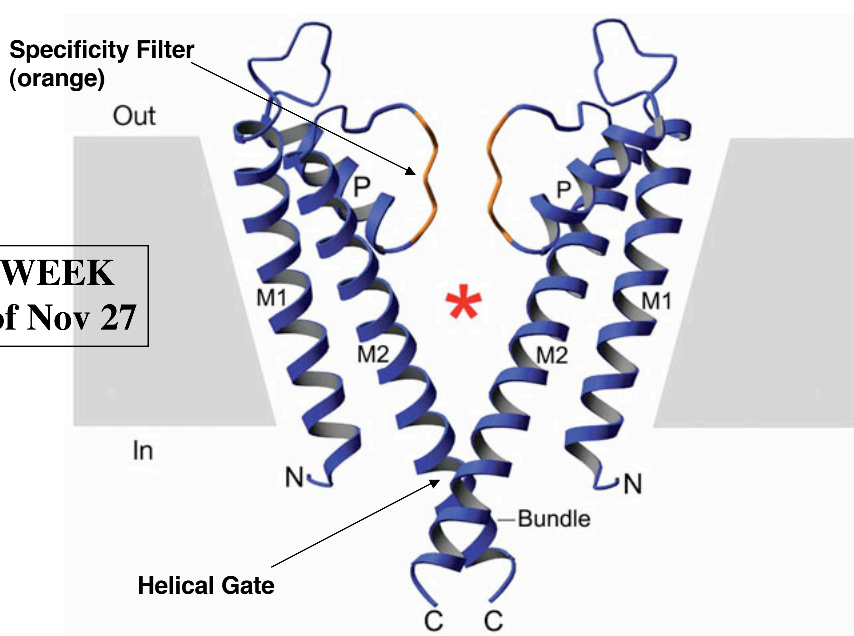
plan view

hydrophobicity plot/index

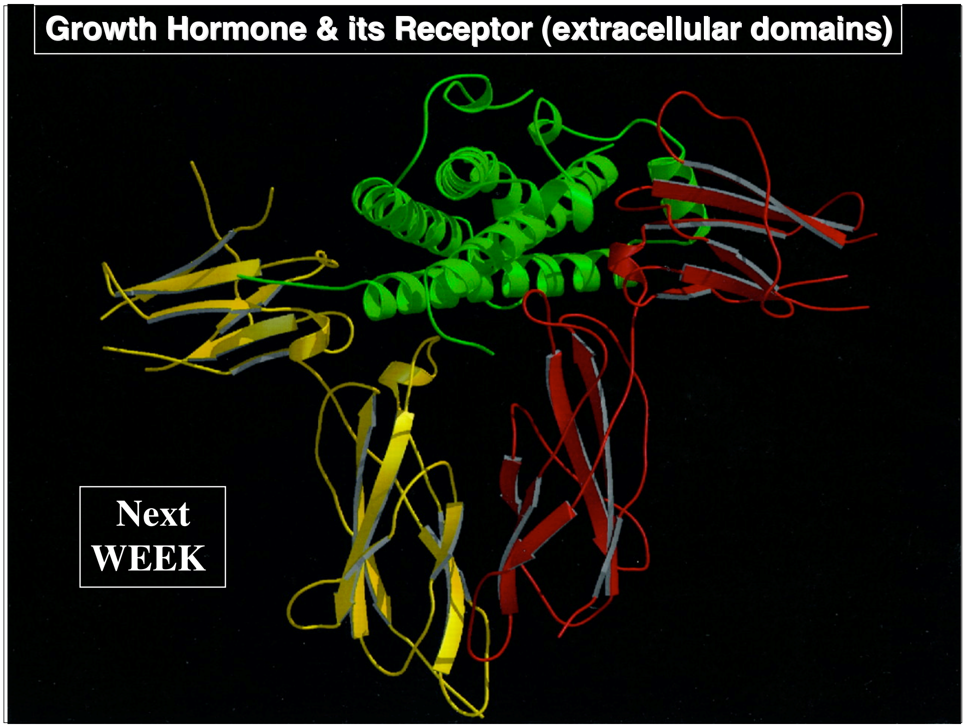


Two subunits of the KcsA K+ channel

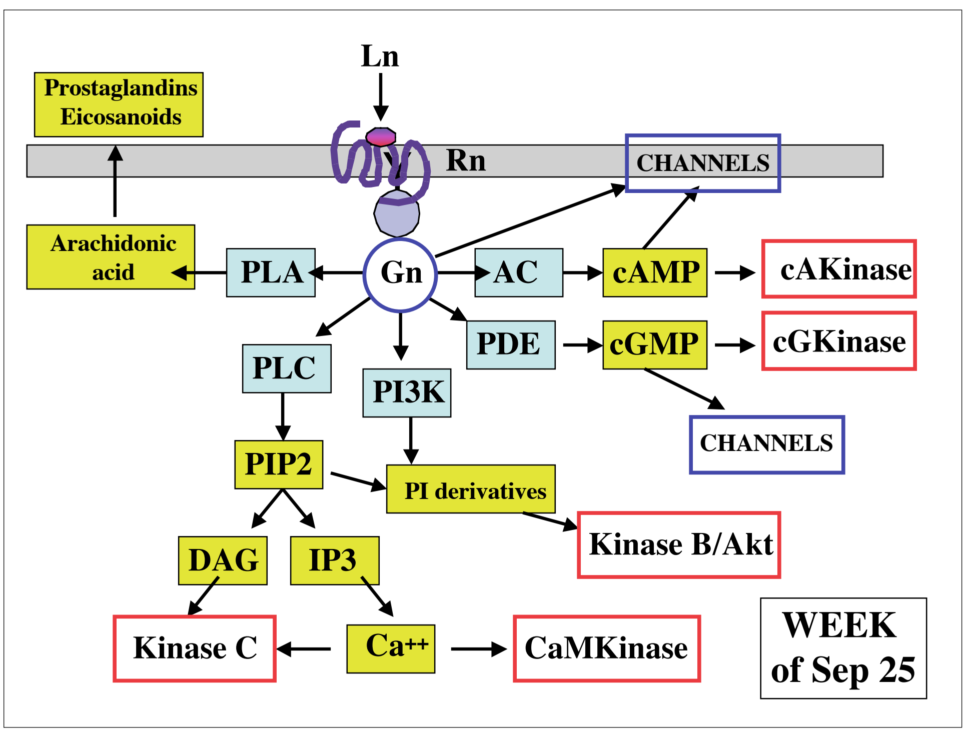
WEEK of Nov 27



http://www.nature.com/cgi-taf/DynaPage.taf?file=/nature/journal/v417/n6888/full/417523a_fs.html&filetype=&_UserReference=C0A804ED4654D463FE606837EB983D756FD8



Growth Hormone & its Receptor (extracellular domains)
Next
WEEK



Ln
Prostaglandins
Eicosanoids
Rn
CHANNELS
Arachidonic
acid
PLA
Gn
AC
cAMP
cAKinase
PDE
cGMP
cGKinase
PLC
PI3K
CHANNELS
PIP2
PI derivatives
Kinase B/Akt
DAG
IP3
Kinase C
Ca++
CaMKinase
WEEK
of Sep 25

Generic Signal Transduction Pathways

October

LIGANDS

RECEPTORS

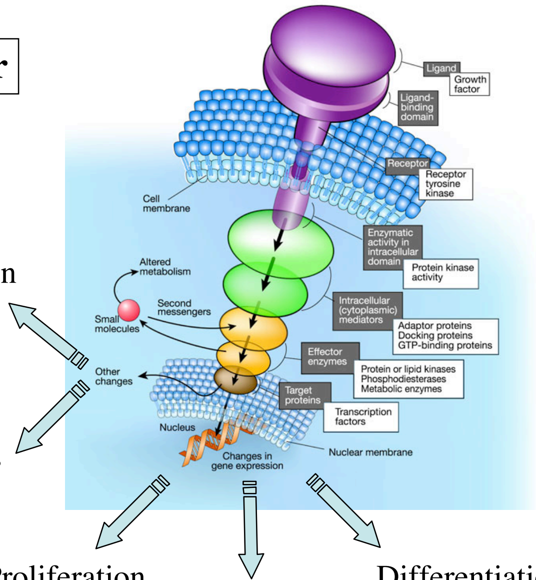
ADAPTORS

EFFECTORS

OUTPUTS

Cytoskeleton

Apoptosis



Proliferation

Survival

Differentiation

Membrane Trafficking and the Secretory Pathway:

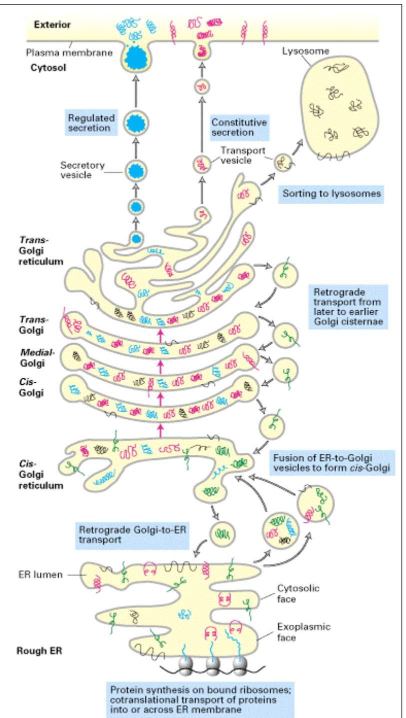
ER->Golgi->out

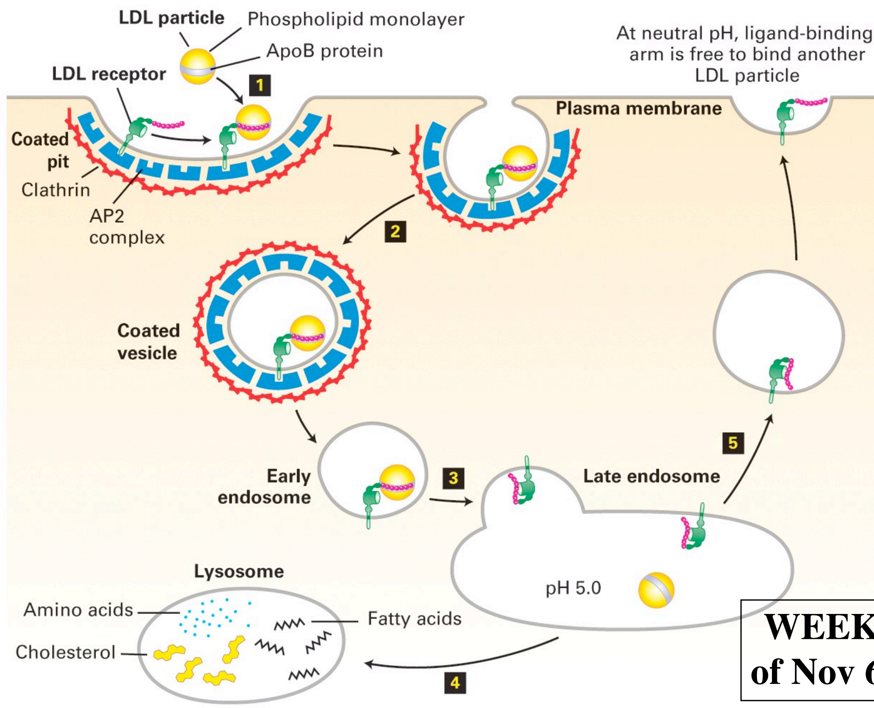
Sorting to different membrane-bounded organelles

How is this all orchestrated?

How do we know?

WEEK of Dec 4





WEEK of Nov 6

Image of outer surface of the cell by Heuser method:

Low Density Lipoprotein (LDL, 20 nm) bound to surface receptors clustered in 'Coated Pits'

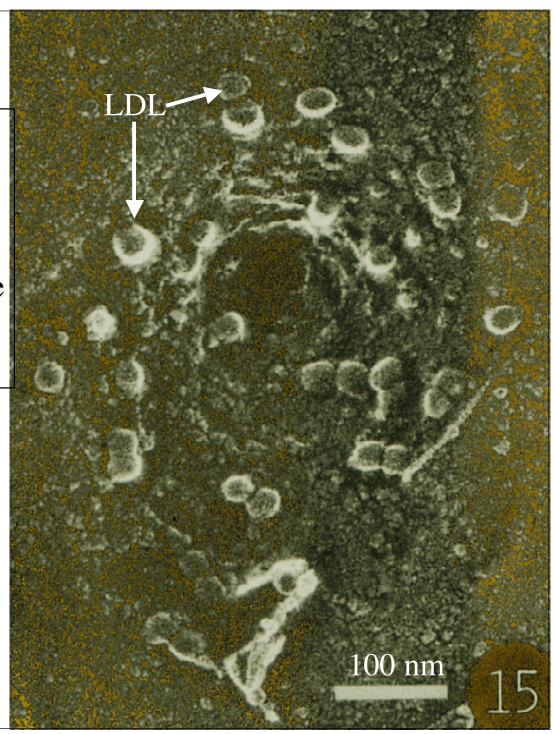
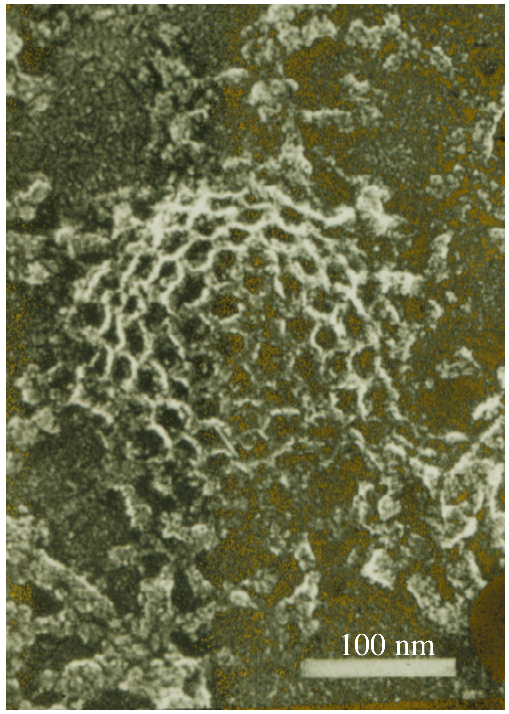
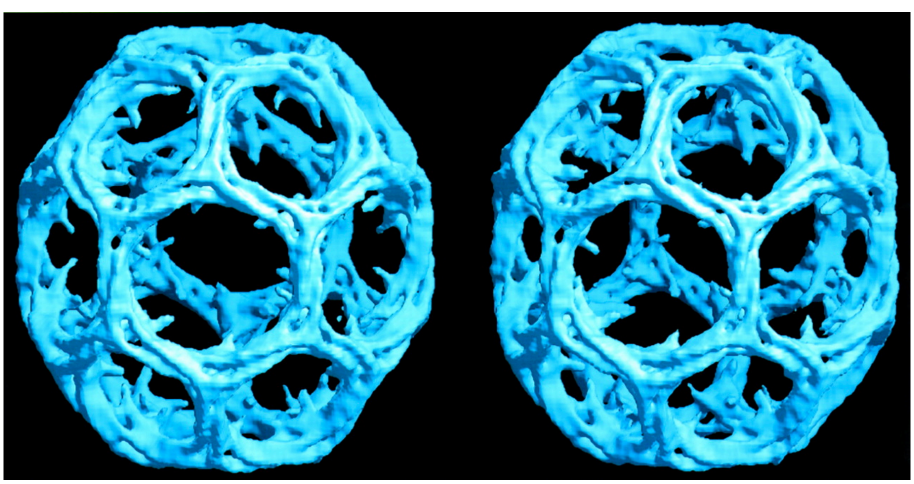


Image of inner surface of the cell by Heuser method:

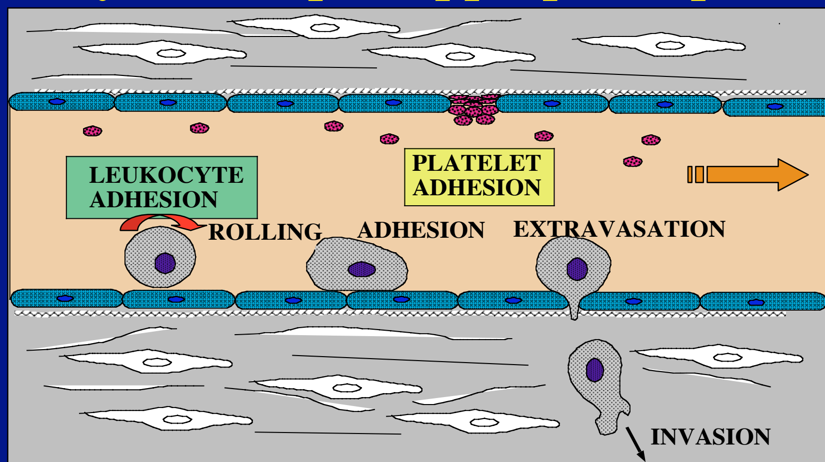
Clathrin Coated Pit



Reconstructed Image of clathrin shell from EM
21 A resolution

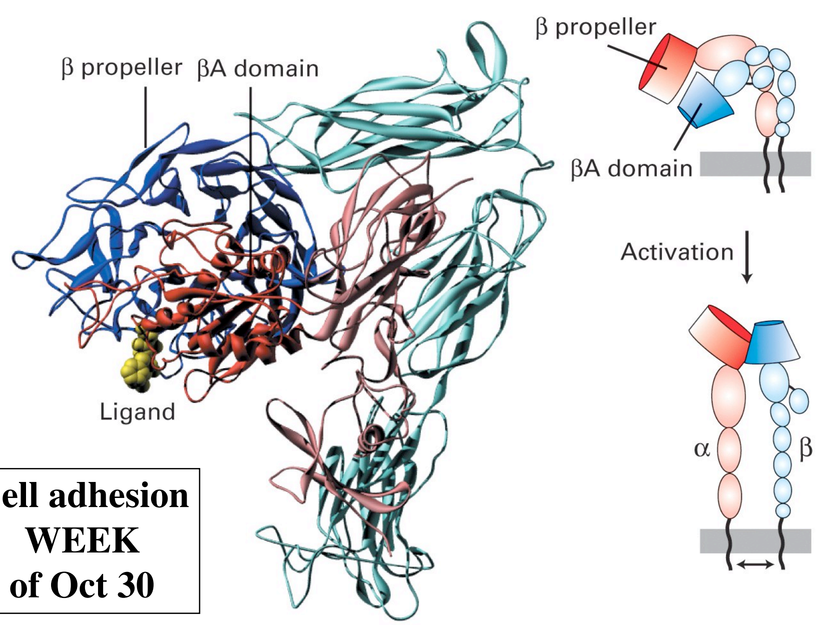


Blood cells circulate around the body and stop at appropriate places



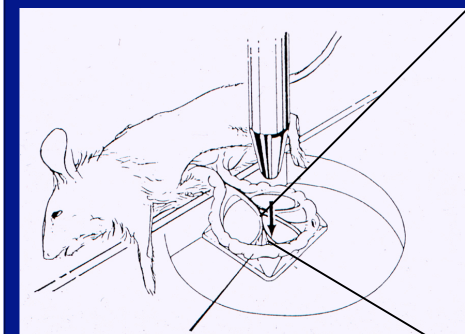
HOW DO THEY KNOW WHERE TO GO?

Integrin extracellular heterodimer



Cell adhesion WEEK of Oct 30

Intravital Microscopy of Leukocytes in Inflamed Venules



Atherton and Born, 1972

What molecules mediate this adhesion and

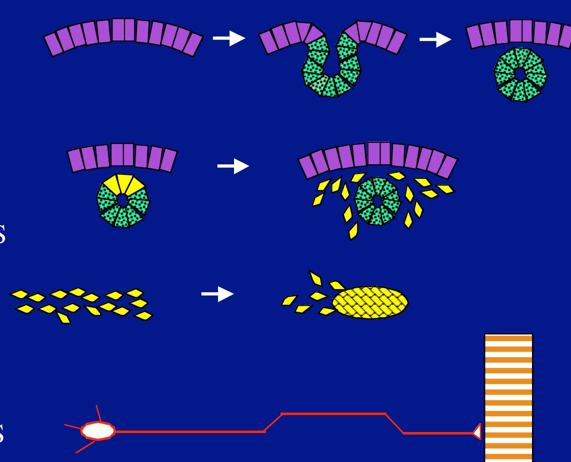
how is it controlled?

Some cells move about the body

SEPARATION of GROUPS of CELLS

MIGRATIONS of INDIVIDUAL CELLS

PROJECTION of NERVE PROCESSES

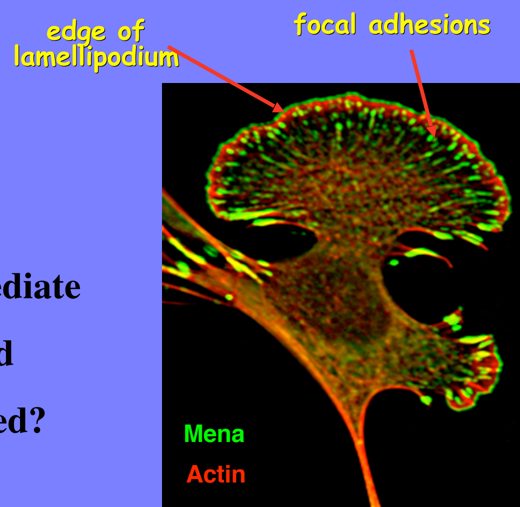


HOW DO THEY ATTACH and DETACH and HOW DO THEY MOVE?

Lamellipodial Protrusion

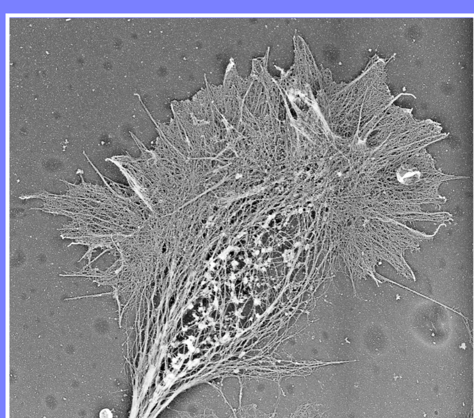
Cell motility WEEK of Nov 20

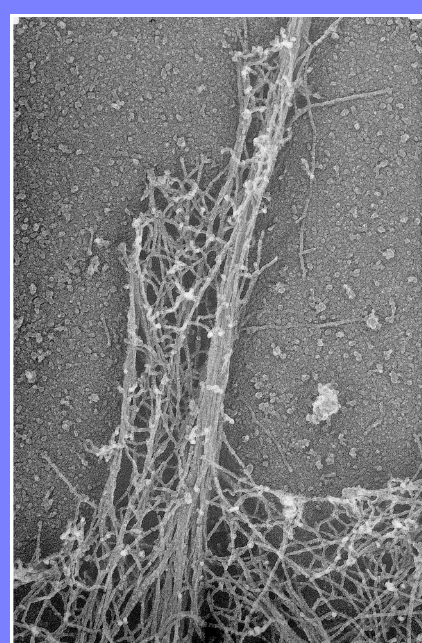
What molecules mediate cell motility and how is it controlled?



Frank Gertler

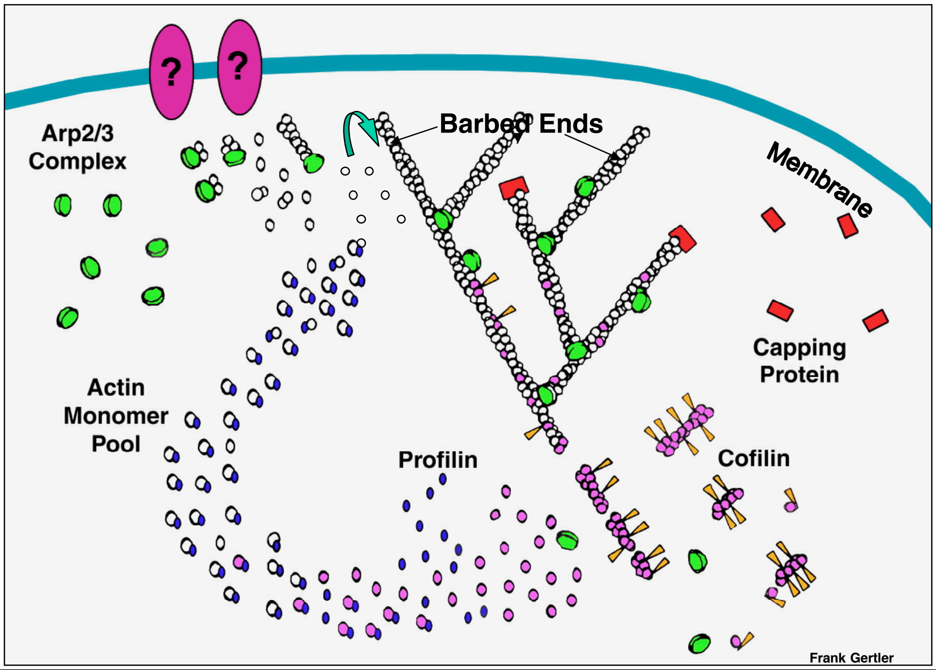
Hippocampal Growth Cone





Geraldine Strasser

Actin Assembly Drives Lamellipodial Protrusion



Neurons

Cell migration to reach appropriate places

Axonal projections to appropriate targets

Synaptic communication

Organization of pre- and postsynaptic membranes