Concordia

BIOL 266
FINAL EXAM
STUDY GUIDE

Exam Notes

BIOL 266: Cell Biology MIDTERM 1 REVIEW

UNIT 1 review

- Cell biology is the study of cells and their structure, function and behavior
- Reductionist approach: studying parts individually helps understand the whole thing.
- The cell is the fundamental unit of life and comes from preexisting cells
    1. Able to reroduce by themselves (organelles can't do this outside cell and viruses need a host)

Cells (5-20 micrometers) – tissue – organs – organ system – organism

BASIC PROPERTIES OF CELLS:

- Life; grow and reproduce — Highly complex and organized
- Possess genetic information — Capable of producing more of themselves
- Acquire and utilize energy — Chemical reactions called metabolism
- Move — Respond to stimuli
- Self regulate — Evolve

TYPES OF CELLS

- Eukaryotic (protists, animals and plants)
- Prokaryotic (bacteria)
    1. Nucleus not membrane bound

Prokaryotic cell features	Common features	Eukaryotic cell features
simple	Plasma membrane	Nuclear envelope
Divide by fission	Genetic information in DNA	Chromosome
Nucleoid region	Both store chemical energy as ATP	Membrane bound organelles
Less genetic material	Shared metabolic pathways (glycolysis, TCA)	Cytoskeleton with motor proteins
Single circular DNA with no histone proteins	Proteasomes of similar construction	Divide by mitosis + histones in DNA

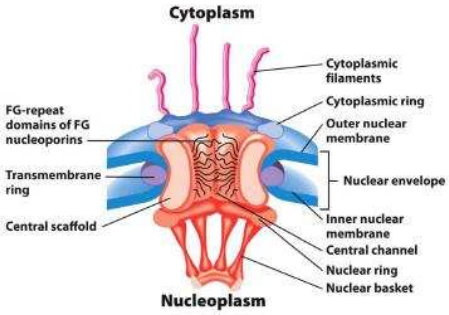
NUCLEUS: where genetic information gets stored surrounded by nucear envelope that contains pores. This is the diagram of a nuclear envelope.

1. Nuclear envelope:
    a. Inner and outer nuclear membranes
    b. Nuclear lamina:
        i. Supports envelope. Composed of lamins
        ii. Controlled by phosphorylation/dephosphorylation
        iii. Lamin a/c mutation causes progeria
        iv. Lamin b causes leukodystrophy
        v. Lamin binding protein emerin mutaitons causes muscular dystrophy
    c. Nuclear pore complexes: channels for molecule traveling between nucleus and cytoplasm

MOVEMENT THROUGH NUCLEAR PORE COMPLEX:

1. Passive diffusion
2. Energy dependent transport
    a. Proteins
        i. DNA-binding proteins
        ii. mRNA binding proteins
        iii. component of nucleus
        iv. ribosomal proteins
        v. importins (for other proteins)
    b. RNA
        i. mRNAs
        ii. tRNAS
        iii. 40s and 60s Subunits

NUCLEAR PORE COMPLEX:



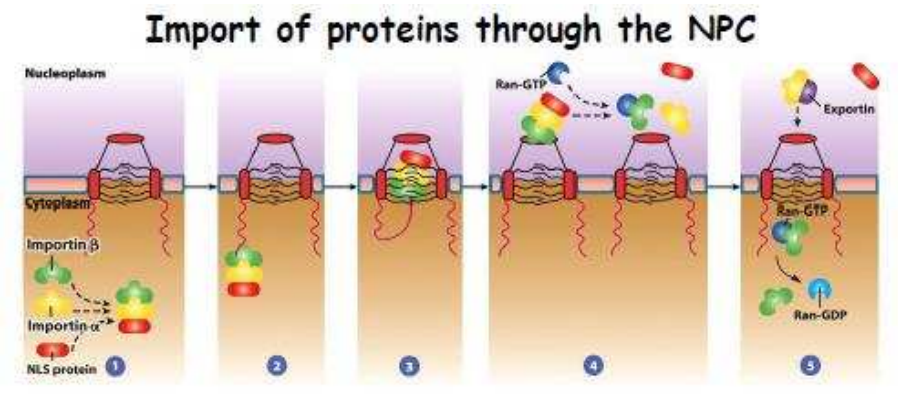
FG nucleoporins stops big molecules from passing through.

Central scafform holds complex to nuclear envelope.

8 cytoplasmic filaments

Importing proteins through NPC step by step:

1. Protein with NLS (nuclear localization signal) bind to NLS receptor (importin alpha/beta heterodimer)
2. Protein/importin complex associates with cytoplasmic filaments
3. Go through complex
4. Ran GTPase associates with complex
5. Rangtp importin beta complex goes back to cytoplasm where ran becomes ran GDP and goes back to nucleus
6. Importin alpha goes back to nucleus by protein called exportin



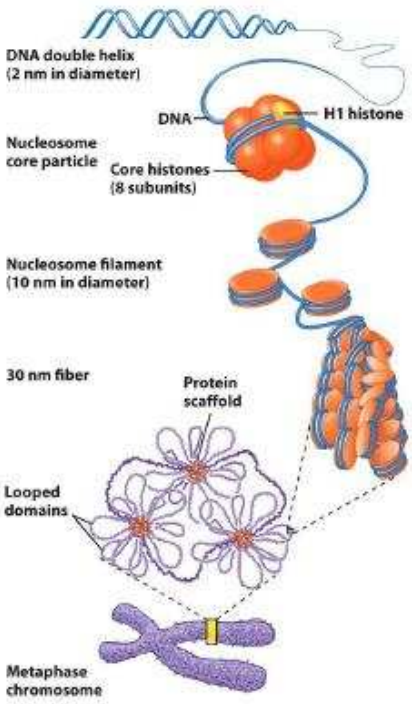
ORIGINS OF NUCLEUS: from ancient prokaryotic cell a nuclear membrane was formed.

INSIDE NUCLEUS:

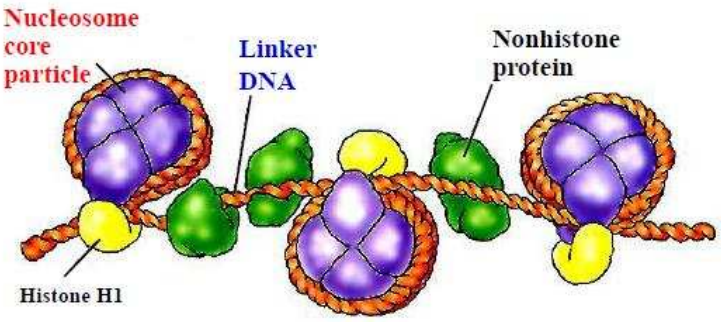
- nucleolus → where ribosomes are made
    1. ribosomal protein imported to nucleus
    2. proteins delivered to nucleolus and bind to preRNA
    3. pre RNA cleaved into rRNA
    4. proteins and rRNA assembled to 40s and 60s subunits
    5. subunits taken outside cytoplasm

TWO TYPES OF CELLS and their DNA packaging

- mitotic
    - chromatin: twice as much protein (histones + non histone of equal amounts) as DNA



▪ histones: small proteins that contain a lot of negatively charged amino acids (bind to DNA) types include (H1, H2A, H2B, H3, H4)
▪ basic unit of chromatin is nucleosome (DNA is wrapped around histones sealed by H1 histone + non-histone proteins serve as DNA linker



▪ When cells prepare to divide chromatin becomes chromosome!!!

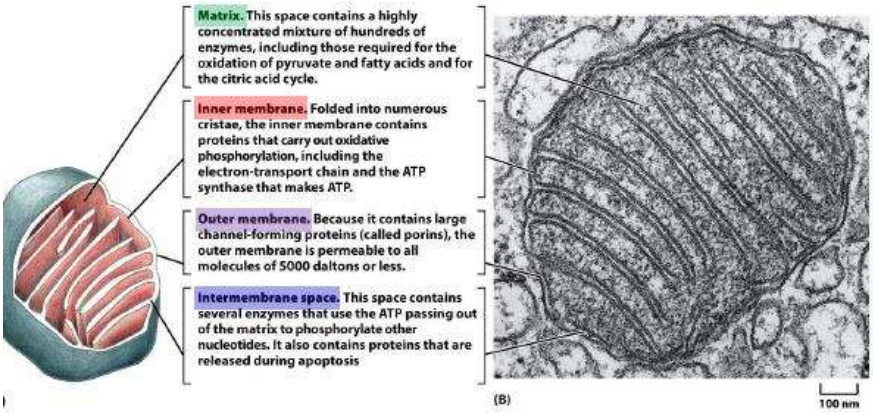
- non-mitotic
  - heterochromatin
    - constitutive heterochromatin: remains condensed all the time (around centromeres and telomeres)
    - facultative heterochromatin inactivated during certain phases of the organism's life (x-chromosome in females)
  - euchromatin: returns to dispersed state after mitosus

MITOCHONDRIA

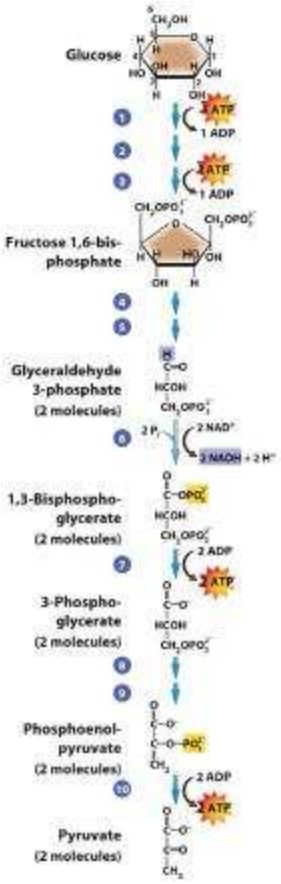
Power house of cell. Twi mitochondrion can fuse or can split by fission.

Function:

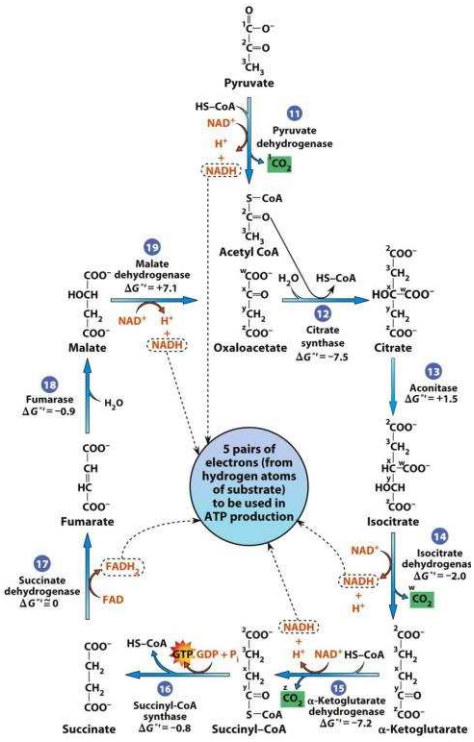
- produce ATP by oxidative phosphorylation



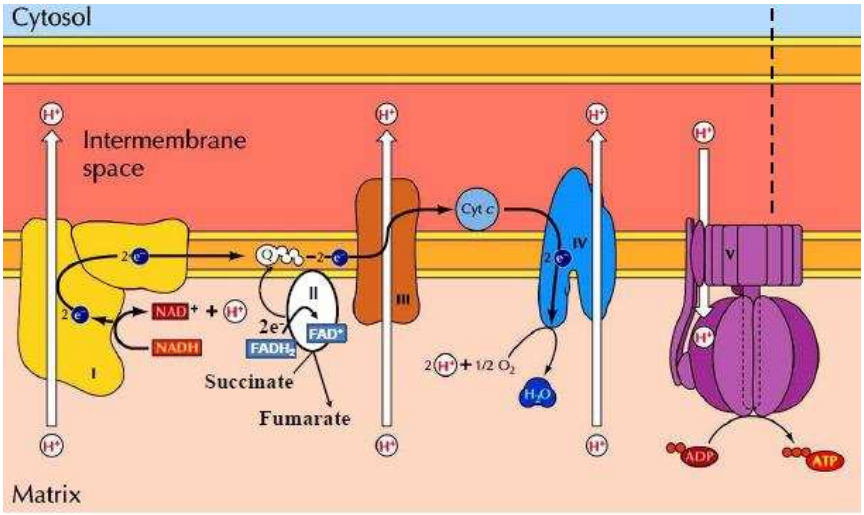
Cellular respiration:



1. Glycolysis
   a. Oxygen: oxidative phosphorylation
   b. No oxygen: fermentation
2. Oxydative phosphorylation
   a. Citric acid cycle (10 NADH, 2 FADH2, 2 ATP, 2 GTP)
   b. Electron transport chain (ends with ~30 ATP)



Electron transport



ATP production blocked by poison such as cyanide which bind to complex 4. In brown adipose tissue electron transport is uncoupled from ATP production and evergy is released directly as heat. Mitochondrial disorders affect muscle and nerve tissues greatly.

Buildup of reactive oxygen species makes

Heroine contaminated with MPTP which binds to complex 1 show PD symptoms

Yeast petite colonies (don't have mitochondria can't respire and therefore need fermentable carbon sources).

ORIGINS OF MITOCHONDRIA: endosymbiosis theory

- Outer membrane of both mitochondria and bacteria contain porins
- Inner membrane of bacteria and mitochondria contain lipid cardiolipin
- Mitochondria arise from other mitochondria by fission
- Contain single circular DNA
- Ribosomes similar

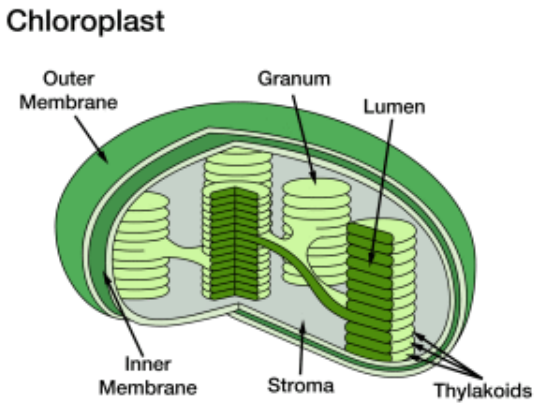
CHLOROPLAST

In plants. Both mitochondria and chloroplast contain permeable outermembrane and relatively permeable inner membrane. Largest organelle in plants where photosynthesis happens and sugars are transorted all over plant for cell respiration.

Stroma → mitochondrial matrix

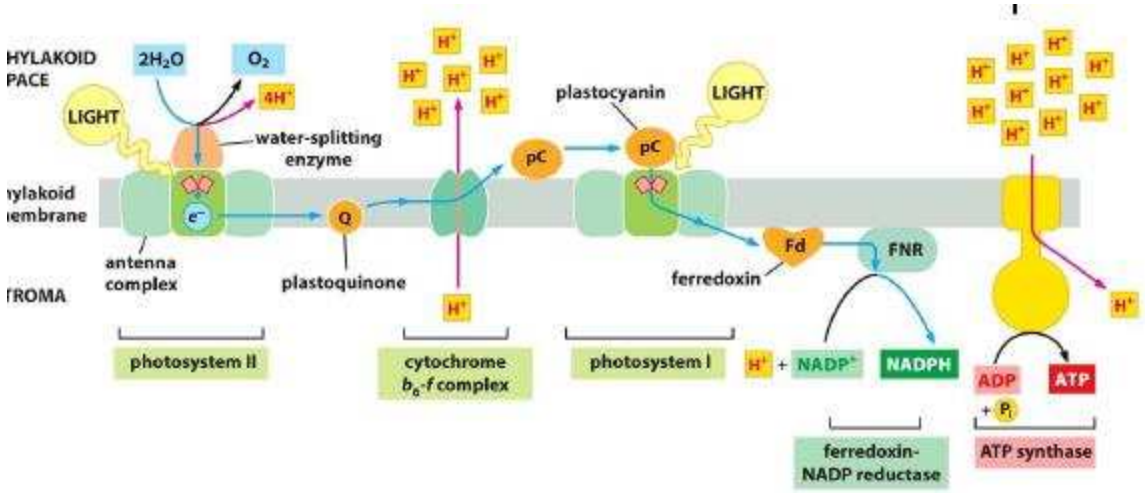
Thylakoid membrane grana → cristae

Not all plant cells have chloroplast, but all have mitochondria.



Photosynthesis:

1. Light reactions
    a. Absorption of light by green pigment called chlorophyll (transfer of photons to reaction center)
    b. Electron transport (note that PSI and PSII don't pump protons)



    c. Synthesis of ATP
2. Dark reactions
    a. Carbon fixation
        i. Rubisco is most abundant protein on earth because it is very inneficient at converting CO2
        ii. Consumes 18 ATP

Herbicides

- PSII (block electron transport)
- PSI (compete with ferrodoxin)

Mitochondria vs chloroplast

- Both have electron transport
- Both have DNA and ribosomes
- Both surrounded by double membrane
- Chloroplast → proton gradient
    - Terminal electron acception is NADPH
- Mitochondria → charge and proton gradient
    - Terminal electron acceptor is O2

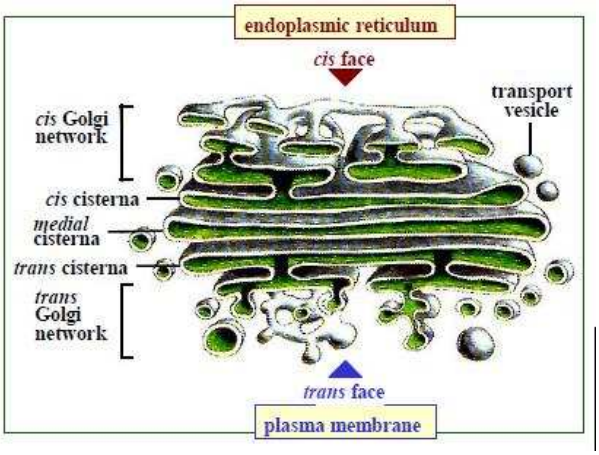
EUKARYOTIC CELLS ARE MADE OF ENDOMEMBRANE SYSTEM:

1. Endoplasmic reticulum: takes up most space
    a. Rough ER: ribosomes makes proteins for ER, golgi, lysosomes, secretory vesicles and plasma membrane. Carried by vesicles. (FORMS CISTERNAE)
    b. Smooth ER: synthesis of lipids for membranes carried by vesicles (TUBULES)
        i. Endocrine cells: sterioid hormone
        ii. Liver cells: detoxification of organic compounds
        iii. Muscle cell: sequestration oc Ca2+ ions from cytoplasm
    c. Transitional ER: region where vesicles exit to go to golgi (first through ER golgi intermediate space)

ER does quality control of folded proteins (checks for proper folding many times)

- Protein associate with calnexin removes terminal glucose
- If folded properly → exits
- Else it will gain glucose back and folding is re attempted
    - If can't fold many times it will be degraded
    - If lots of unfolded protein happen unfolded protein response activated
        - Stop translation
        - Protein degraded
        - Prolonged UPR → apoptosis

2. Golgi complex also forms cisternae. Contains many compartments.
    a. Where proteins are further glycosylated and sorted for exportation.
    b. Some lipids (glycolipids and sphyngomyelin) are made here.
    c. In plants where cell wall is made
        i. Cis golgi (faces ER)
        ii. Trans golgi (faces plasma membrane)
    d. Proteins enter through cis golgi and travel down. Types of transport:
        i. Vesicular: cis to trans golgi in vesicles
        ii. Cisternal maturation: cisternae mature as they move from cis to trans face while vesicles travel from trans to cis face.

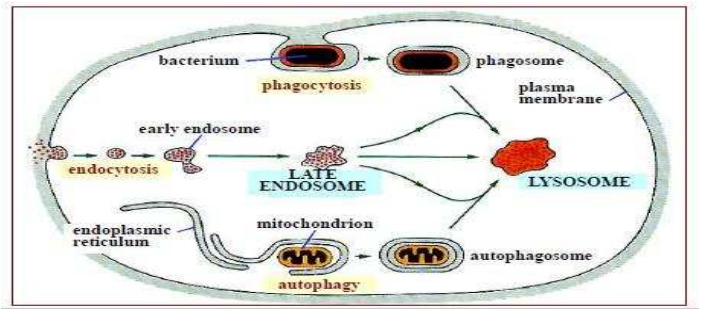


3. Endosomes
4. Lysosomes: membrane encolosed organelles where breakdown of many things occur. Acid inside. Membrane protein are highly glycosylated, protecting it from its own degradative enzymes
    a. Nucleic acid
    b. Proteins
    c. Lipids
    d. Carbohydrates

    Contain primary and secondary lysosomes (primary + membrane engulfed defective organelle -- autophagy)

    Pathways to delivering things to lysosomes

    1. Endocytosis
    2. Phagocytosis
    3. autophagy



    genetic diseases: gaucher disease (accumulation of undegraded defective material). Protein that degrades glucocerebroside doesn't work. Successfully treated with cerezyme
5. Vacuoles
6. Peroxisomes: site of synthesis and degradation of hydrogen peroxide. Where oxidation of very long chain fatty acids occurs and decomposition of hydrogen peroxide occurs.
    a. Oxidases
    b. Catalase
    c. Synthesos of plasmalogens (myelin lipid)
    d. Conversion of fatty acids into carbs in germinating seeds of plants (called glyoxosome instead of peroxisome)

    Mitochondria VS peroxisome similarities:

    Formed from preexisting organelles, import preformed proteins, oxidise fatty acids

    Mitochondria VS peroxisome differences:

    Single phospholipid bilayer in peroxisomes and don't have DNA or ribosomes

Peroxisomal disorders:

- Zwelleger syndrome: can't import peroxisomal enzymes so peroxisomes don't do anything.
- X-linked adrenoleukodystrophy (X-ALD): can't import ver long chain fatty acids so they accumulate in brain. Treated by gene therapy.

7. Cytoskeleton: protein filaments that:
    a. Provide structure
    b. Motility

    Microtubules: largest. Made of tubulin. Revealed by fluorescently labeled subunits.

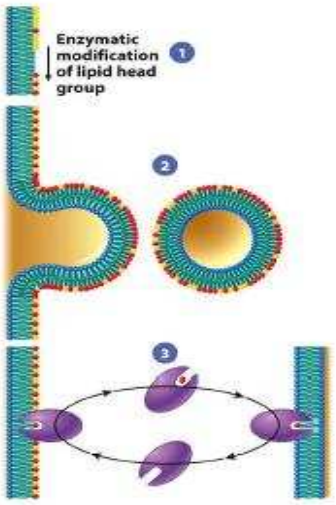
    Actin filaments: made of protein actin. Revealed by phalloidin.

    Intermediate filaments: most subunits

Orientation always remains the same.

How lipid composition changes in membranes:

1. Enzymatic modification of lipid head
2. Vesicle formation
3. Phospholipid transfer protein



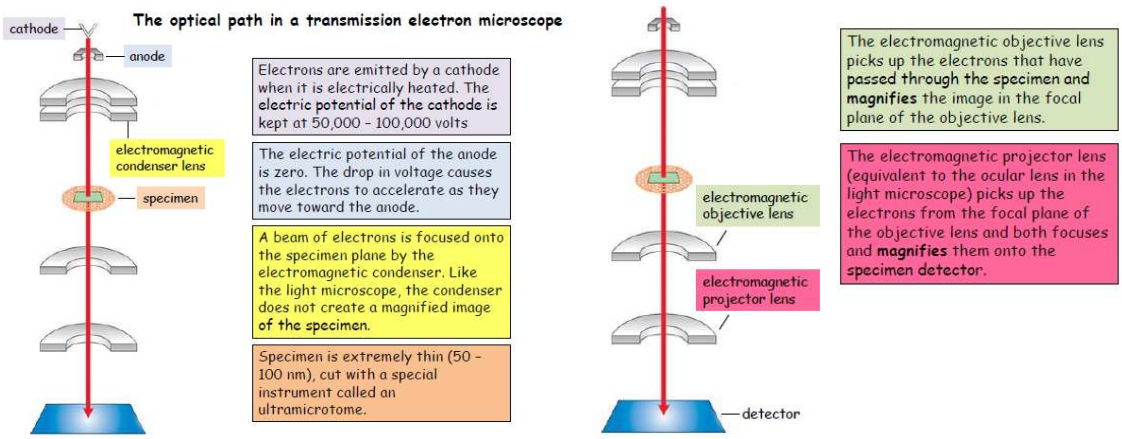
UNIT 2 review

Microscopes main types:

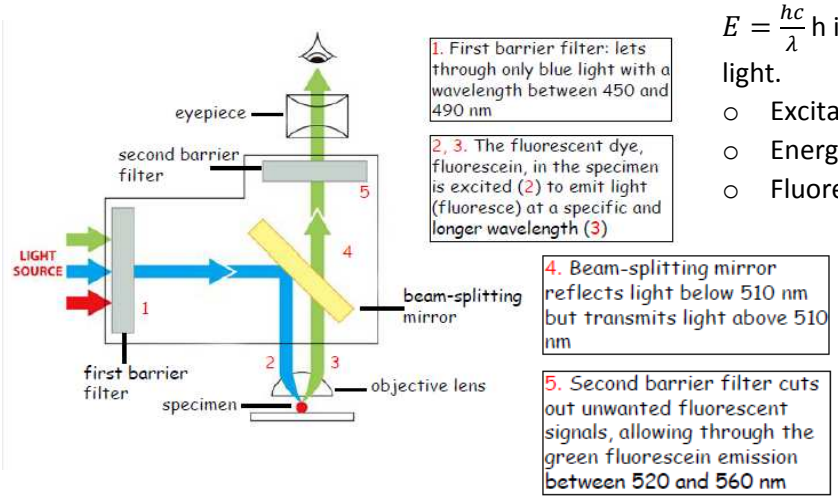
- **Compound:**
  - Total magnification = objective lens x ocular lens
  - Resolution is the most important properdy
    - $D = \frac{0.61\lambda}{nsin\alpha}$ where n is refractive index
    - $limitations\ like\ wavelength\ and\ angle\ of\ incidence$
    - N for oil is bigger. The smaller the D the better.

  Three main types:

  1. Brightfield: normal
  2. Phase contrast: higher n. refracted and inrefracted light form image. Requires phase plate.
  3. Differential interference contrast: for thicker samples
- **Electron: use electron instead of light.**
  - Larger, beam of electron shot downwards. Uses electromagnetic coils to magnify image. Vacuum.



- **Fluorescence: most utilized in cell biology. Absorbed light vs emited wavelength (higher)**



$E = \frac{hc}{\lambda}$ h is planck's constant. C is speed of light.

- Excitation
- Energy dissipation (relaxed)
- Fluorescent emission to bring back to groun state
  - Fluorescein gives green color
  - Tetramethylrhodamine emits red light

- **Confocal microscopy: improved fluorescent**
  - Focuses light on different levels and can make a 3D image. Laser passes through pinhole.
- **Immunifluorescent microscopy: revealing specific proteins in fixed cells.**
  - Primary antibody binds and secondary antibody and then fluorescein to amplify the signal.

1. Fix cells by:
   a. Cross linking: may interfere with fluorescence if glutaraldehyde is used because it autofluoresces.
   b. Precipitation: using cold organic solution. Dehydrates sample and therefore changes cell architecture.
2. Permeabilize cell: organic solution (precipitation does this already) or by treatment with detergent

In this type of microscopy must take into account:

a. Use different fluorescent dyes that emit different wavelengths
b. Use different antibodies

- **Immunogold electron microscopy: revealing specific proteins in fixed cells.**
  - Since gold has a high atomic number it deflects electrons highly.
- **revealing specific proteins in living cells: green fluorescent protein**
  - gene for protein goes inside cell and inside organelle and use fluorescence microscopy

1. TISSUE CULTURES: GETTING CELLS

a. Plate on dish and let grow. If theres multiple cell populations separate using FACS

2. SEPARATING CELLS: FACS

a. Antibody regognizes protein outside cell and binds fluorescent dye
b. Drop of single cell goes down. If fluorescent is detected; drop is negatively charged. Otherwise not charged
c. Passes through electric field

Cell lines are immortalized cells; by adding DNA that expresses telomerase.

Ability to grow indefenitely: transformed cell lines

Senescent cells: cells that stopped dividing. They do not express telomerase.

Growth media for cells:

Essential amino acids

Glutamine

Vitamins

Fatty acids

Glucse

Serum (hormones, transferrin, growth factors)

3. SEPARATING ORGANELLES

Differential centrifugation:

Nuclei is the heaviest densest material so it sediments at a speed of 1000g for 10 minutes. Remove post nuclei supernatant and recentrifuge the rest. Centrifuge of the rest will sediment together (golgi, mitochondria, peroxisomes and lysosomes- equilibrium density gradient centrifugation to separate)

Last centrifugation will give the microsomes and plasma membrane which must also go through equilibrium density gradient centrifugation to separate.

equilibrium density gradient centrifugation:

Gradient of sucrose used from top (lowest density) to bottom (highest density)

Lysosomes – plasma membrane vesicles – golgi – mitochondria – microsomes of ER – peroxisomes

1. Suspend in a buffer
2. Put inside density gradient
3. Centrifuge

Migrates to density position equal to that of organelle. Put hole at bottom to get everything.

4. STUDYING PROTEINS IN THOSE ORGANELLES

a. Enzymatic assays

Organelle	enzyme
Peroxisomes	Catalase, uricase
Mitochondria	Cytochrome c oxidase, succinate dehydrogenase
Lysosome	Acid phosphatase
Endoplasmic reticulum	Glucose – 6 – phosphatase
Golgi	UDP- galactosyl transferase
Plasma membrane	5' nucleotidase

b. Western blot analysis

Detecting a specific protein in a spample that contains many proteins.

Steps:

i. Separate proteins on a polyacrylamide gel (polyacramide gel electrophoresis)
   1. Without denaturing detergent – native gel electrophoresis; keeps protein complexes intact. (size shape and charge – amino acid composition)
   2. With dentaturing detergent – sodium dodecyl sulfate (SDS – negatively charged). No longer part of a protein complex. All have a similar charge since SDS binds so migration is only dependent on size → larger proteins migrate slower than small proteins.

Reducing agent to break bonds between SDS molecules included (beta – mercapteothanol or DTT (dithiothreitol))

ii. Transfer the proteins into a thin membrane from gel (nitrocellulose, PVDF)
iii. Probe the membrane with a specific antibody that will detect enzyme (primary and secondary antibody – fluorescent microscopy)
iv. Detect antibody

5. SEPARATING PROTEINS FROM EACH OTHER (ALL USE WESTERN BLOT)

a. Ion exchange chromatography

Separates based on charge (overall charge = sum of all charges at a certain pH)
Isoelectric point is the pH at which charge = 0
pH > isoelectric point -> negative charge
pH < isoelectric point -> positive charge

b. Size exclusion gel

Separates based on size. Depends if uses denaturing agent or not (monomers vs polymers) – smaller proteins pass through pores and larger no so they filter out faster

c. Affinity chromatography

Separates based on secific sequence. Specific protein gets attracted and gets stuck. The rest passes down. To get it break the bonds (near total purification in a single step)

6. STUDYING PROTEIN INTERACTIONS

a. Immuniprecipitation

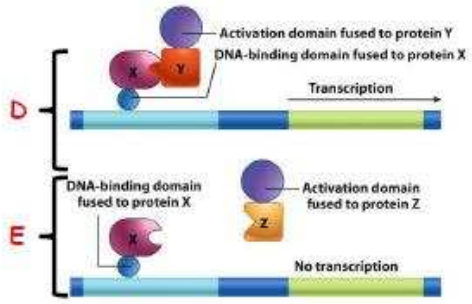
Similar to affinity chromatography; uses antibodies to bind to protein and then collected in beads. Non denaturing conditions. If other proteins are in the beads, they must interact with the certain protein.

b. Yeast two-hybrid

Two domains in trascription factor of yeast:

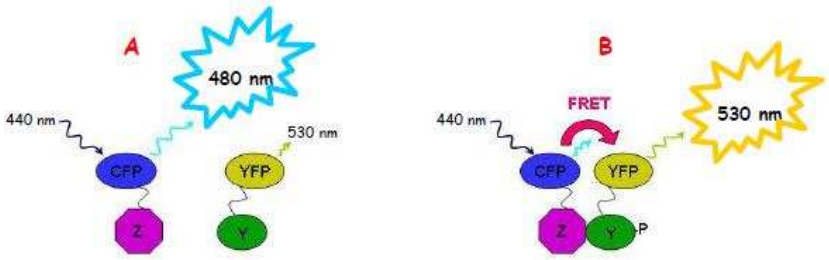
1. DNA binding domain
2. Transcription activation domain

DNA encoding each domain is bound to two different proteins. If proteins interact, transcription factor will be reconstituted and transcription of DNA will take place



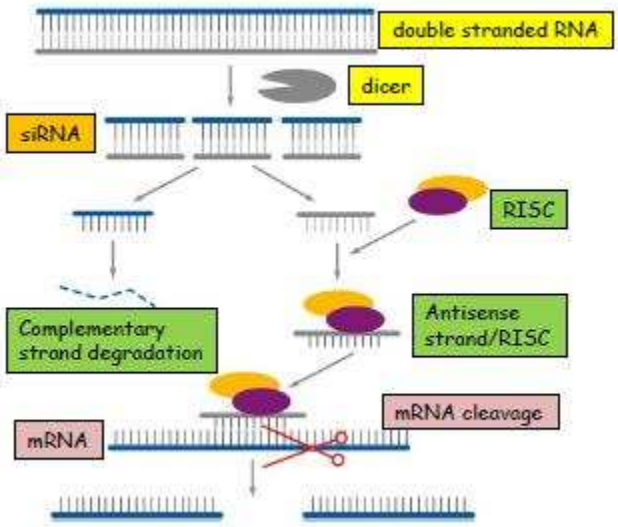
c. FRET (fluorescence resonance energy transfer)

Proteins bind to CFP and YFP. If proteins interact, CFP transfers energy to YFP and emmited wavelength will be different. Thwo proteins must be within 5nm for FRET to work



7. STUDYING PROTEIN FUNCTION

a. Gene silencing – RNA interference
   i. RNA related to mRNA encoding protein is cleaved by a dicer (results in small interfering RNA (siRNA) cannot be propagated)
   ii. Antisense strand associates with RISC (protein complex
   iii. Cleave mRNA associated with antisense strand/RISC complex

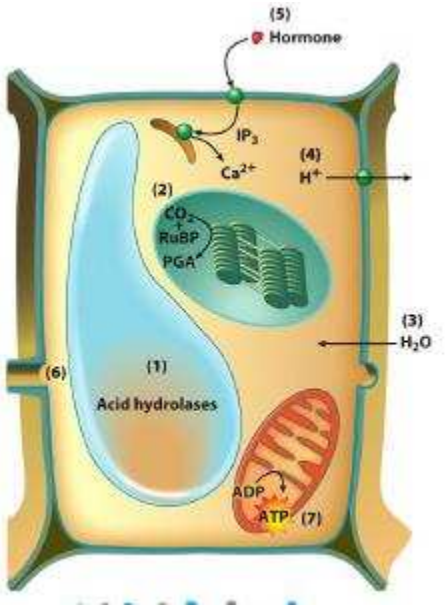


Without the mRNA there will be no protein – according to the central dogma of biology

UNIT 3 review

History of biological membranes

1. Cells must be surrounded by lipids since nonpolar molecules can't pass through
2. Arrange themselves in a layer
3. Twice the space so a bilayer
4. Proteins everywhere in bilayer
5. Proteins only in some places



Functions of biological membranes:

1. Compartmentalization
2. Biochemical activities
3. Selective permeability
4. Transport solutes
5. Respond to external signal
6. Intercellular interaction
7. Energy transduction (storing, producing…)

Plasma membrane specific functions:

1. Import expert molecules; waste goes out, nutrient go in
2. Receive information; proteins in membrane act as sensors
3. Capacity for movement and expansion when cells grow

Specific organelle membranes:

1. Spearate one aqueous phase (cytosol) to another (interior)
2. Selective barriers
3. Some small differences eps membrane proteins give them each a distinct function

ALL membranes contain proteins and lipids!!!

Lipid (amphipathic):

- Hydrophillic head
- Hydrophobic tail

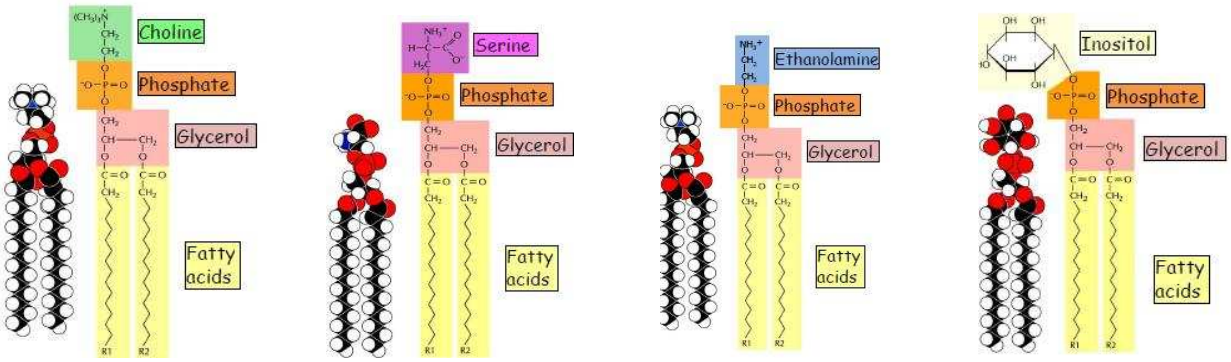
Types of lipids

- Fatty acids (simplest lipids): palmitate, stearate, oleate
    - Carboxyl group – hydrocarbon chain of different length
        - Saturated (no double bonds) vs unsaturated (one or more double bonds)

Types of membrane lipids:

1. Phosphoglycerides
    a. Phosphatidylcholine (most common type)
    b. Phosphatidylethanolamine

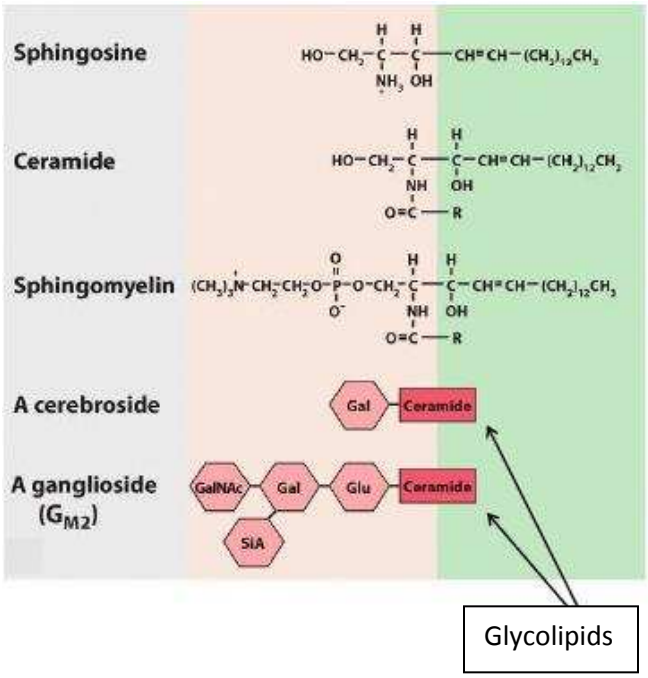
c. Phosphatidylserine
d. Phosphatidylinositol

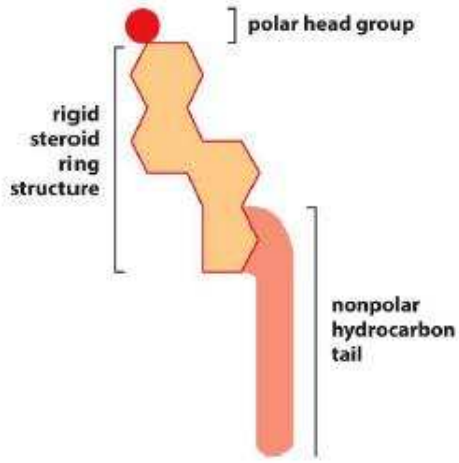


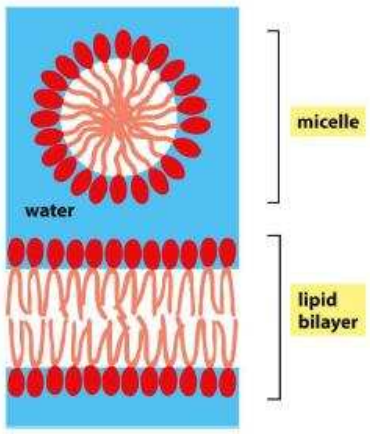
Fatty acid – glycerol → phosphoglyceride

Fatty acid – glycerol – phosphate → phosphatidic acid

2. Sphingolipids
    a. Sphingosine
    b. Ceramide (base)
    c. Sphingomyelin
    d. Cerebroside
    e. ganglioside
3. cholesterol







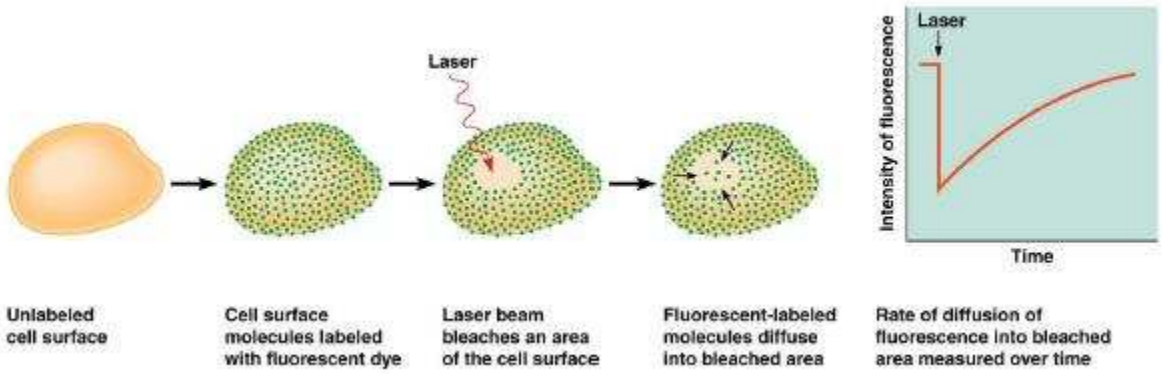
Hydrophillic molecules dissolve, hydrophobic ones don't. hydrophobic molecules create an energitically unfavorable environment. This is why bilayers form. One fatty acid tail makes a micelle; two makes a bilayer.

Lipids form bilayer called liposome.

Types of lipid mobility:

1. lateral diffusion: exchange spot with neighbor
2. flip flop
3. rotation: rare. Need flipases (except cholesterol)

Fluidity shown by FRAP (fluorescence recovery after photobleaching)



Bilayers don't fuse because it would take more energy to break down and refuse than stay as is. Membrane fluidity is also determined by types of lipids.

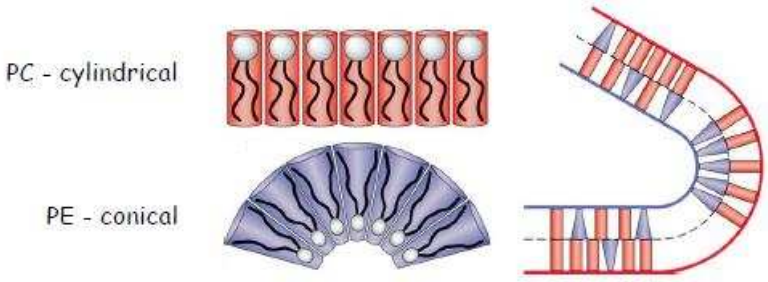
Length and degree of saturation of lipids.

More unsaturated = more fluid

Shorter = more fluid (because longer have more interactions between them)

More cholesterol = more fluid at low temp (less fluid at high temp)

Also; small polar head groups give a conical shape (phosphatidylethanolanine) which helps curve the bilayer. These are concnetrated on the inside of curve, while straight ones are on the outside. A part from that they are nicely spaced out throughout the layer.



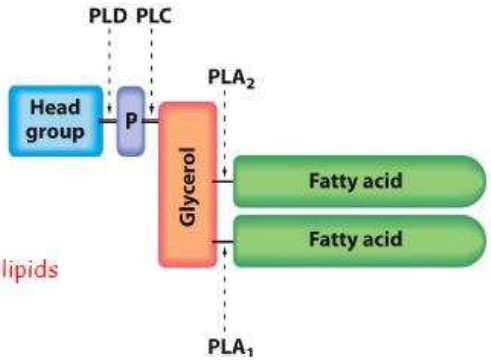
POSITIONS OF DIFFERENT LIPIDS:

1. Sphingomyelin and glycolipids are only outside. Produced by enzymes exposed to golgi lumen makin them face extracellularly.
2. Phosphatidylcholine is outside
3. Phosphatidylserine is inside as well as phosphatidylethanolamine (flipase)
4. Phosphatidylinositol are inside → cell signaling (flipase)

5. Cholesterol evenly distributed
6. Phosphoglycerides are produced on outer leaflet of ER. Scramblase transfers them to inner leaflet. Golgi flipases transfer lipids to specific pattern.

MEMBRANE ORIENTATION DOESN'T CHANGE (for lipids and proteins)!!!

Phospholipases break down lipids at different place. Different phospholipases break different bonds.



PLD – generates phosphatidic acid
PLA1 and A2 – generate lysophospholipids

Unit 4: Membrane proteins

Some facts about membrane proteins:

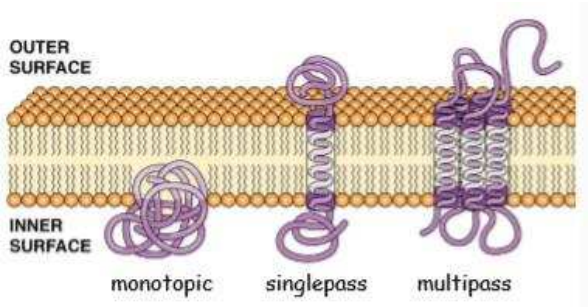
30% of all proteins in a cell are associated with a membrane

60% of targets of drugs are membrane proteins

50% of membrane weight is protein (much larger than lipids so there are 50-100 lipids per protein)

***Membranes are made of: Lipids (structural elements) and proteins (carry out specific functions)

Three main types of membrane proteins

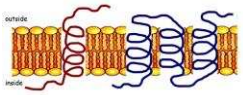


Integral proteins

pass through lipid. Amphipathic (hydrophobic inside bilayer and hydrophillic form domains outside bilayer). They form van der Waals interactions with the lipids.

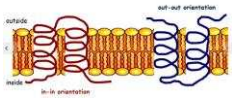
a. Monotopic
b. Single pass
c. Multipass

i. Odd number: in-out (one end inside one end outside)



- Usually the hydrophobic portion is made of **non-polar, non charged amino acids** and hydrophillic portion of **charged, polar amino acids**

ii. Even number: in-in or out-out (both ends on the same side)

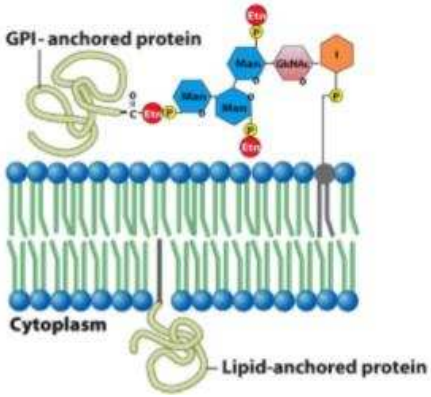


- Same as odd number

Lipid-anchored proteins

Protein is linked to a lipid which is embedded in the membrane. Two types:

1. GPI-Anchored: protein has covalent bond with glycosylated phosphatidylinositol (glycosyl phosphatidylinositol) → mainly external

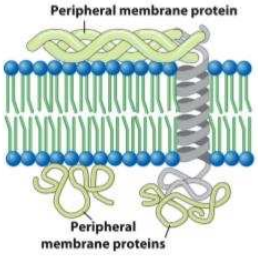


2. Fatty acid Anchored: protein is linked to fatty acid, usually myristic acid or palmytic acid.

   Can also be linked to multiple isoprene (5 carbon) groups (isoprenylated).
   Three groups → farnesyl
   Four groups → geranylgeranyl

   Common on small GTPases

Peripheral proteins

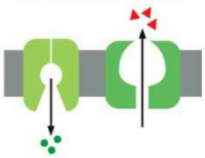


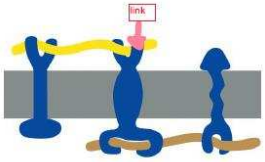
Weak electrostatic bonds that are easily soluble. These proteins are completely outside of the bilayer. Can either be attached to other proteins or attached to charged/polar head of bilayer.

Can be removed by agents that disrupt electrostatic bonds (high pH, high salt concentration)

Functions of membrane proteins

1. **Transport:** transport of charged molecules (example → H+ gradient in mitochondria) and large polar molecules (sugar, nucleortides and aa)

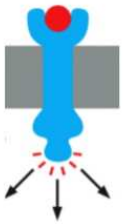




2. **Link:** linking two proteins that are cytosolic and noncytosolic

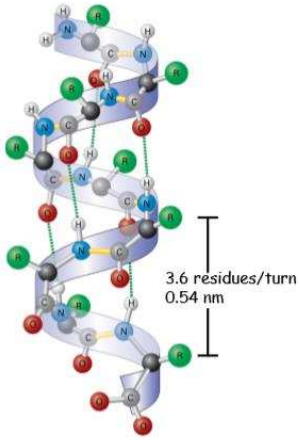
3. **Anchor:** anchoring two other proteins on either side of membrane

4. **Receptor:** detect chemical signals on face of membrane and relay them on other face. (for example, EFG binding to epidermal growth factor receptor)



5. **Enzyme**: catalyze reactions on either side of the membrane (example, adenylyl cyclase produces cAMP)

Proteins cross the membrane as an **alpha-helix**

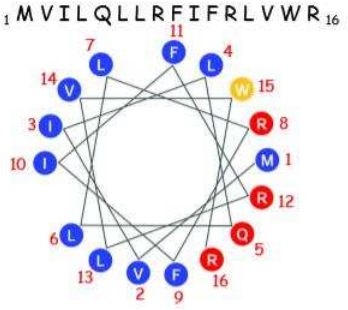


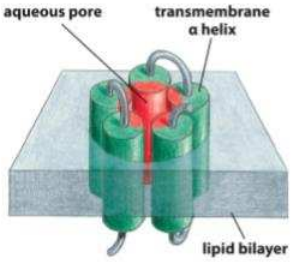
Alpha helices result from the slight negative charge of oxygen atoms and slight positive charge of hydrogen atoms within a chain. These two atoms form a hydrogen bonds together four residues away.

Proline residues arent found in alpha helices since the side chain doesn't allow spiral structure found in the alpha helix

Alpha helix that spans the bilayer once → all amino acid side chains are hydrophobic.

Often proteins that act as receptors for extracellular signals (signal molecule, an extracellular ligand, binds to extracellular part of intermembrane protein, while cytosolic part sends signal inside cell)



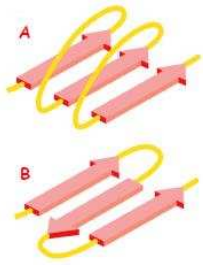


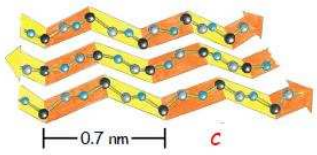
Some alpha helices can fold so that most of the hydrophilic side chains of amino acids are inside, making an amphipathic helix (making an aqueous pore)

Multipass proteins: form hydrophilic pore, and having its hydrophobic animo acids interacting with tails of lipids.

Pores: selective transport of large polar molecules or small charged molecules (ions) across membrane

Proteins can also cross the membrane as **beta-sheets/barrels**

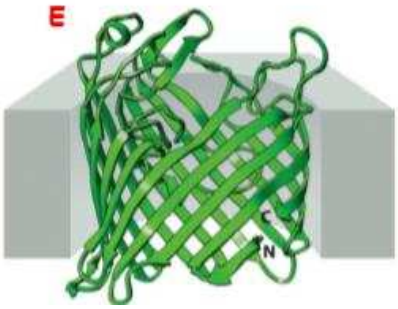




Beta sheets form hydrogen bonds with residues far away (neighboring strands)

- Parallel (A)
- Antiparallel (B)

Each strand can be kinked also (C)



Beta barrel: beta sheet curved around forming →

Outside the barrel → hydrophobic

Inside → hydrophillic

Again acting like a pore, transporting large polar molecules and small ions.

Solubilizing membrane proteins

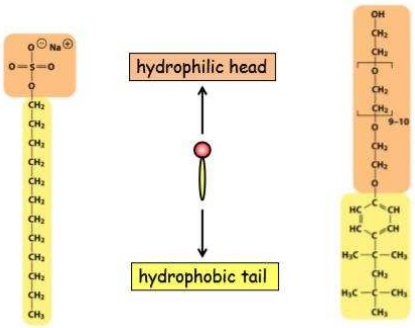
Transmembrane proteins can be solubilized by treatment that disrupt the bilayer. Must disrupt the hydrophobic interactions between lipid tails and hydrophobic portion of the protein.

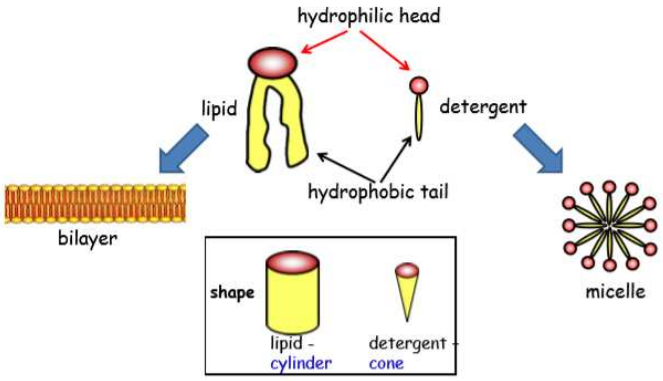
Most commonly used reagent are detergents: small amphipatic, lipid like molecules.

most commonly used detergents:

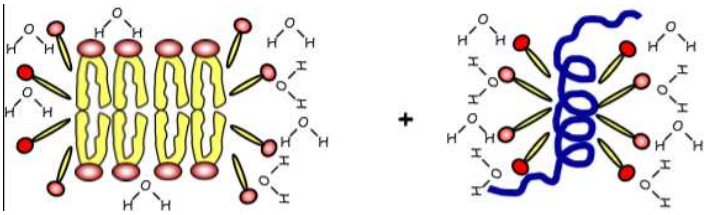
SDS (sodium dodecyl sulfate): strong ionic detergent (ionized polar head group) → left

Triton X-100: mild non-ionic detergent (non ionized polar head group) → right





Detergents only have on hydrophobic tail while membrane proteins have two tails. Detergents form micell where membrane lipids form bilayers. Detergents are coned where lipids are cylindrical.

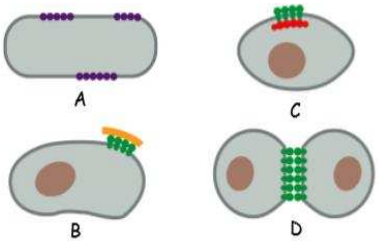


Mixing **excess detergent** with membranes hydrophobic ends interact with hydrophobic part of protein AND hydrophobic tail of lipids. Water molecules go around the hydrophilic head of the detergent making a protein-detergent protein.

Movement of the proteins in the bilayer

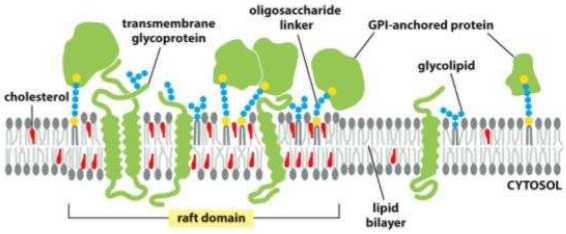
Movement of proteins can be restricted due to them forming a membrane domain by protein-protein interactions between different proteins:

a. Between similar proteins to cause aggregation
   - cluster of bacteriorhodopsin molecules on a halobacterium halobium
b. Between membrane protein and an extracellular protein
   - Can also use CAMs
c. Between membran protein and intracellular cytoskeleton
   - Membran proteins of erythrocyte held in place by anchoring to cytoskeleton
d. Between membrane proteins on the surface of two cells
   - Cellular adhesion molecules (CAMs) binding between cells.
     i. Homophilic: binding to same protein on both cells
     ii. Heterophilic: binding to different proteins on both cells



CAMs include: integrins, selectins and cadherins.

Immobilizing the membrane proteins accounts for the difference in diffusion constants (D) in the membrane. Fluidity of membrane also influences the mobility of the protein.



Lipid rafts: place on membrane that has high concentration of a cpecific lipid. At plasma membrane they are enriched in cholesterol and glycosphingolipids with long saturated hydrocarbon tails; making the raft rigid and thicker than surrounding proteins. It is also enriiched in GPI anchored proteins.

Unit 5: Membrane transport

Artificial membranes

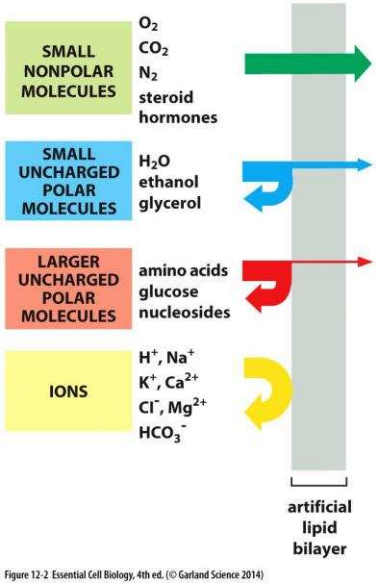
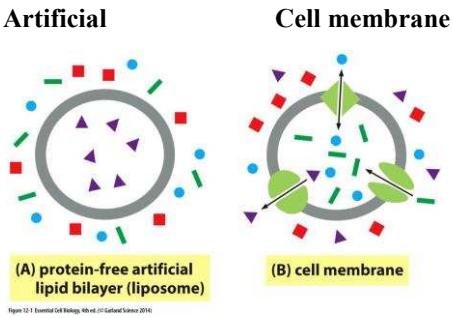


Figure 12-2 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Membranes are more permeable to smaller substances. Small non-polar molcules pass more easily, while it is harder for ions.

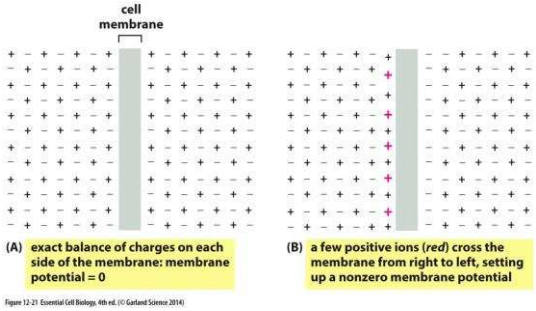
Synthetic bilayers are impermeable to large uncharged polar molecules and charged ions.

Cell membranes are peprmeable to these!! This is because they have membrane transport proteins.



Each type of membrane has its own set of transport proteins. These proteins determine which water soluble molecules can pass in and out of cell/organelle.

Ion concentration inside vs outside the cell:



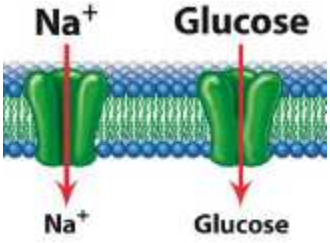
If it is imbalanced, cell can fall apart due to electric forces. Positive charges gather around the outside of the cell while negative charges are inside of the cell. This gives rise to negative membrane potential inside of the cell, but outside positive. This is a result of transporters that move specific ions from inside to outside (positive ones)

Na+ is balanced by Cl-

K+ is balanced by nucleic acids/proteins

Two types of transport

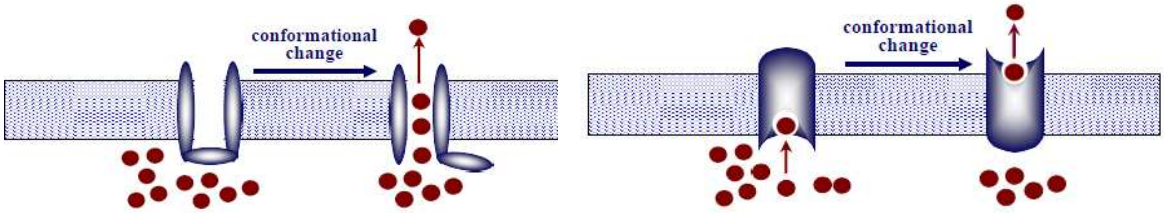
1. **Passive:** doesn't required energy, only concentration gradient (down its concentration gradient till equilibrium is reached)
    a. **Gases (O_2, CO_2), hydrophobic molecules (*benzene*) and small polar uncharged molecules (H_2O, *ethanol*);** dissolve in bilayer → diffuse across it → dissolve in the solution on the other side of membrane
    b. **For non-electrolytes**; rate of diffusion depends on partition coefficient and size (smaller diffuse faster)
        i. Partition coefficient: measure of ability to go from aqueous to non-aqueous solution
    c. **Large polar, uncharged molecules** (amino acids/nucleotides/sugars) and **charged molecules** can't dissolve in bilayer. They use facilitated diffusion through a protein. This prevents the molecules from interacting with the hydrophobic interior of the membrane. Doesn't require energy ONLY MEMBRANE PROTEINS.
        i. Mediated: interact with membrane protein
        ii. Non-mediated: doesn't interact with membrane protein



2. **Active**: transport against concentration gradient; requires energy. Requires membrane protein as well as energy (ATP → ADP)
    a. Example: proton pump of lysosome to make inside acidic
    b. Example: Na/K pump in plasma membranes to generate different concentrations

Classes of transport proteins

1. **ATP-powered pumps**: it couples hydrolysis of ATP to transport of molecule against its concentration gradient (1-1000 molecules/sec)
2. **Channel proteins (ions)**: transport ions down their concentration gradient through hydrophillic pore ($10^7 - 10^8$ *molecules/sec*). Two types of conformations LEFT:
    a. **Open:** allows many ions to pass through
    b. **Closed**



3. **Carrier proteins (transporters)**: bind water soluble molecules on one side and transport them to the other side. Involves conformational change (only 1 or few molecules at a time - $10^2 - 10^4$*molecules/sec*) RIGHT

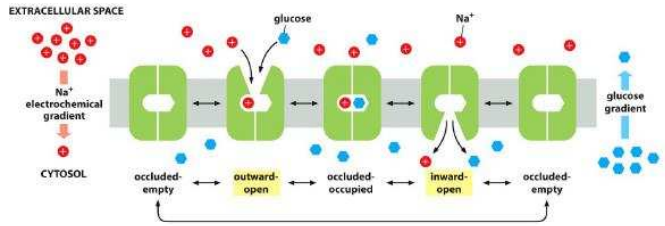
CHANNELS AND CARRIERS

- **Uniports:** work in passive diffusion, selective for one type of molecule

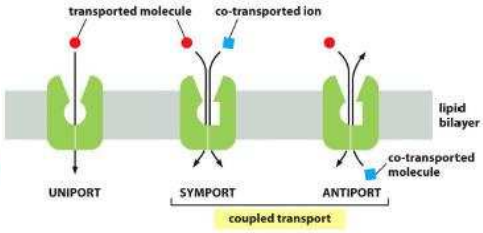
- Different from simple diffusion in that the rate of diffusion is higher for uniports, partition coefficient isnt important, limited by the number of uniporters in membrane, it is molecule specific.
  - Example: GLUT1; main uniporter for glucose in erythrocytes – blood cells. Glucose is converted to glucose – 1 – phosphate so that intracellular concentration of glucose is low uniport can keep functioning and importing it.
  - Km is a measure of enzyme affinity to substrates – GLUT1 has a 1.5mM for glucose and 30mM for galactose (lower Km, stronger binding)

- **Symports (same direction) + Antiports (opposite direction):** use existing electrochemical gradient instead of ATP. One molecule down its concentration gradient the other against. Conformation change occurs only when the two molecules are bound. This is called **secondary active transport**.
  - Example: reuptake of a neurotransmitter by an Na+ symporter and Na+/glucose symporter.

Na+/glucose symporter



symport/uniport/antiport



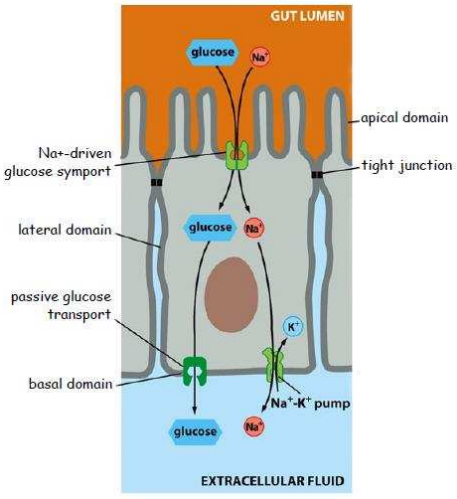
More about the Na+/glucose symporter

Using the free energy formula: $\Delta G = RT\ log\frac{[Na_{in}]}{[Na_{out}]}$ we find that for 1 mole of Na+, $\Delta G = -1.45kcal$ for the concentration gradient; add in the free energy of the membrane potential (-1.61 kcal) giving ~-3kcal

The symporter transports 2 sodium for each glucose, so overall free energy is

$$\Delta G = RT\ log\frac{[Glucose_{in}]}{[Glucose_{out}]} + 2RT\ log\frac{[Na_{in}]}{[Na_{out}]}$$

Setting the free energy to 0 (equilibrium) and substituting -6 for twice the sodium, we get the ration of glucose in:out = ~30 000.
This means that the symporter can generate an internal concentration 30 000x greater inside the cell than outside, just using sodium.



A negative ΔG means the equation is favorable.

If the symporter transported only 1 sodium, it would still give an in:out = 170 for glucose.

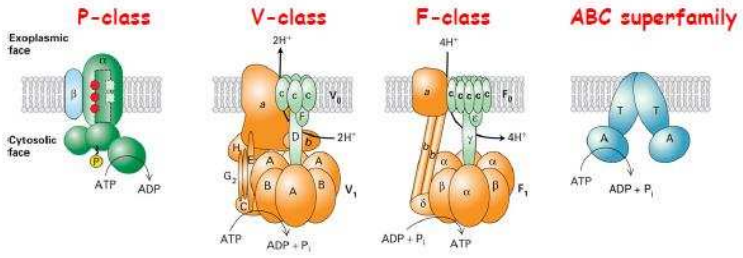
Na/K pump inside the gut epitheleal cells uses glucose symport
Higher concentration of glucose and lower concentration of sodium inside the cell (due to Na/K pump)
Glucose symport at apical domain, bringing glucose inside the cell.
Passive transport of glucose in basal domain (diffusion) to the blood to be sent to other tissues.
Tight junction proteins restrict membrane protein movement.

Many symporters and antiporters use Na+ gradient. This Na+ gradient is generated by an Na+/K+ pump with the help of ATPase. ATP → ADP + Pi

Resulting in 3Na+ in and 2K+ out against their concentration gradient.

This is one class of ATP-powered pump → P-class

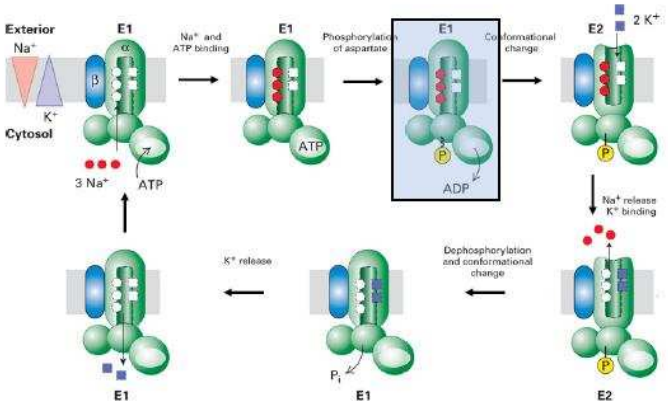
Classes of ATP-powered pumps



1. P-class pump: heterotetramer (2 subunits; alpha and beta) the beta subunit is phosphorylated during transport
    a. Na+/K+ ATPase
    b. CA2+ ATPase
    c. H+/K+ ATPase (acidifies stomach)
2. V-class proton pump: many subunits; transport only protons against gradient
    a. Lysosome
    b. Vacuole
3. F-class proton pump: like V-class, except generates ATP (mitochondria/thylakoid). Can operate in opposite direction under certain conditions.
4. ABC superfamily: biggest class. Transport sugar, amino acids, phospholipids, proteins. Responsible for multidrug resistance (chemotherapy; pumps drug out of cell)

P-class pump

More about the Na+/K+ pump



E1 conformation (intracellular):

- 3 Na+ binding sites (high affinity)
- 2 K+ binding sites (low affinity)

First, Na+ binds to binding sites, even if intracellular concentrations are low.
K+ does not bind even if intracellular concentrations are high.
Then ATP binds on the cytoplasmic side → hydrolized → phosphate binds to aspartate residue → ADP unbinds → E2 conformation change

E2 conformation (extracellular):

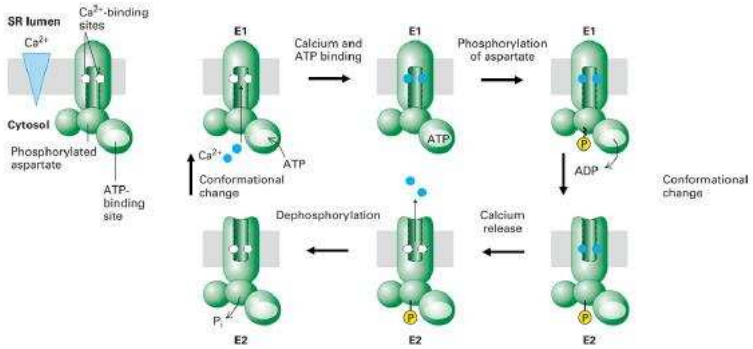
- Na+ binding site becomes low affinity → dissociate
- K+ binding site becomes high affinity → associate

Aspartyl-phosphate is hydrolized → E2 goes back to E1, cycle restarts

Ca2+ ATPase

E1 conformation:

- 2Ca2+ binding sites (high affinity)



Again same steps as Na+/K+ ATPase. (contraction of muscle cells, nerve cell neurotransmitter, fertilized oocute to start development, glycogen breakdown in liver cells)

Memrane transporters in health and disease

K+/H+ - ATPase: in perietal cells of stomach; produces acidic environment by pumping protons out of the cell. Target of drugs such as prilosec, nexium, prevacid. However, acidicity can also be neutralized by basic anions present in over the counter medication like Tums.

Na+/K+ - ATPase: inhibited by ouabain, found in plants and used in warfare arrows. Altering Na+ gradient and indirectly affects the Na+/Ca2+ exchanger that pumps Ca2+ out of the cell. This causes an accumulation of Ca2+ inside the cell and calcium is important to regulate muscle contractions. It eventually leads to heart problems, but in small amounts can be used to treat arythmia.

CFTR (cystic fibrosis transmembrane conductance regulator): member of the ABC superfamily. But it is not a pump since a pump will hydrolize ATP to movgainst their gradient. CFTR hydrolizes ATP and pens up to allow chloride to move DOWN its concentration gradient.

ABC transporters and more about CFTR

more than 100 members, 2 domains;

- Transmembrane domain
- Nucleotide binding domain

First CFTR that was discovered was called MDR (multidrug resistance) transport protein. It was identified in tumors that resisted chemotherapy. CFTR IS NOT A PUMP IT IS A Cl- TRANSPORTER!

Non functional CFTR causes mucus layer to thicken due to outward movement of ions, which draw water with them. This causes bacteria to accumulate and infections eventually.

Mutations of CFTR:

- ΔF508: → phenylalanine at position 508 is missing
  - 70% of mutations in US. Protein doesn't fold at body temperature, but it folds at lower temperature. VX-809 can bind to it and prevent its destruction.
  - Advantage to being heterozygous because it protects against cholera and typhoid fever. This is why most peple are heterozygous
- G551D: → G is glycine, D is aspartic acid
  - 4% of mutations. Channel reaches cell surface but doesn't open properly. Kalydeco treats it by keeping channel open.

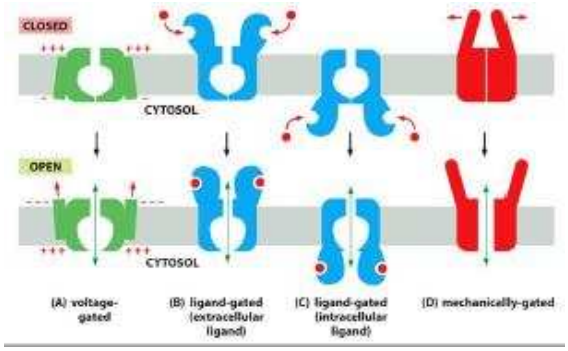
Ion channels

Properties that distinguish ion channels from a simple hole in membrane

1. **Selectivity** of ions. Depends on size and shape of ion and the charge of inner channel.
2. **Gating**: they arent open all the time. They only open in response to a stimulus for a short period of time. Doesn't need a conformational change for each ion, can allow 10^6 ions to pass through per second.

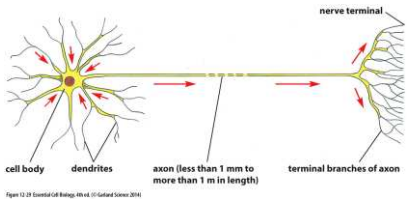
Types of gating (ways gates can open)

1. Voltage-gated:
   a. Respond to changed in electric potential across the membrane. Found in nerve, muscle, egg, plant cells.
   b. When the channel opens, membrane potential changes, which can activate or inactivate other voltage-gated channels.
2. Ligand-gated:
   a. Respond to the binding of a ligand
   b. Example: neurotransmitter, which is an extracellular ligand
   c. Example: inward rectifying potassium channel (K_{ir}) that respond to PIP_2 ligand



3. Mechanically-gated: respond to mechanical force → hair cells in ear.
    a. Sound waves cause channels to open, causing an inflow of ions that affect voltage gated channels that transmit signal to brain.

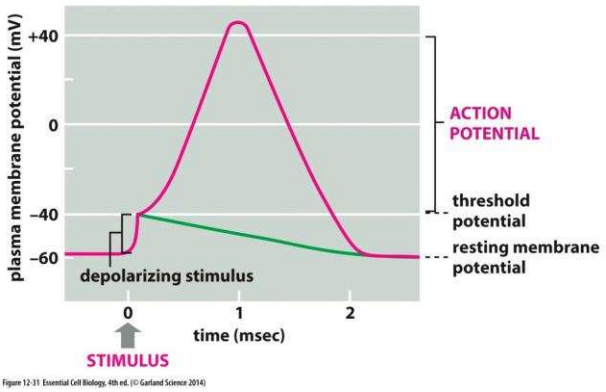
More about nerve impulses



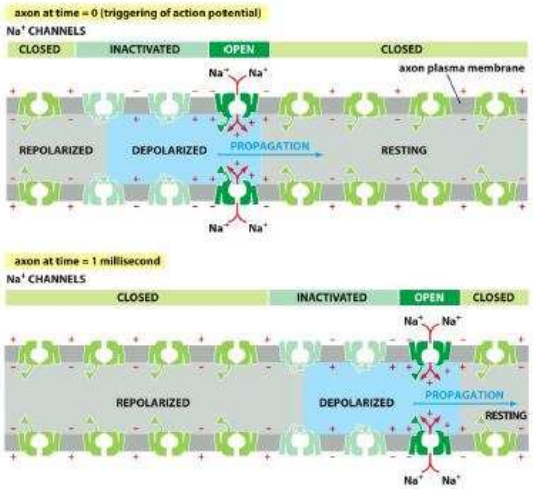
Neurons are a type of cell that have a membrane potential due to Na+ concentration in the membrane. The potential is ~-60mV.

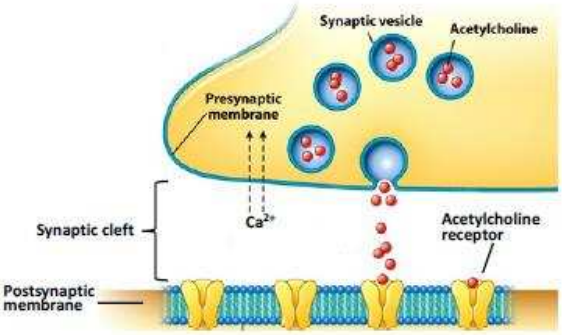
Steps:

1. Signal comes in from another neuron
2. Voltage gated channels open, depolarizing the membrane, opening other channels if enough, causing more depolarization.
3. At +40mV, electrochemical force of Na is 0, channels are inactive, causing the signal to move away.
4. If the threshold is surpassed, Na channels will open
5. Creating a monodirectional cascade
6. The nerve endings have voltage gated K+channels that open
7. Ions flow out
8. At nerve endings, Ca2+ channels open, Ca2+ gets in
9. Neurotransmitters are released through vesicles
10. Neurotransmitter binds to postsynaptic memebrane, those that don't are degraded or taken up again
11. Whole process starts again



Inside the neuron:





At the neuron ending:

Post synaptic receptors can be either:

- Ionotropic: receptor is the ion channel
- Metabotropic: not ion channel but signals ion channel

Unit 6 (I): Protein sorting to organelles

Introduction

PROTEIN	LOCATION
Na^+/K^+ ATPase	plasma membrane
RNA polymerase	nucleus
Proteases	lysosome
Catalase	peroxisome
ATP synthase	mitochondria
Hormones	extracellular space

Mammal cells contain up to 10 000 proteins, each must be localized to the correct organelle. Not every gene is expressed in all cells. These proteins need to find their right cell and place inside the cell.

On the left are some examples of proteins and the location where they must get to.

Proteins synthesized in eukaryotic cells:

- **Few** are encoded by mitochondrial/chloroplast DNA. They are synthesized on ribosomes inside of those organelles and incorporated directly in the compartments in them.
- **Most** are encoded by nuclear DNA. These are synthesized on ribosomes in the cytosol and are delivered to the right organelle from there.

Protein sorting is the process by which newly made proteins are directed to the correct location. Each protein has a different **sorting signal** (signal sequence from 3-60 aa – often removed when arrives at destination)

Main signal sequences to remember:

Function of Signal	Example of Signal Sequence
Import into ER	^+H_3N-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Retention in lumen of ER	-Lys-Asp-Glu-Leu-COO^-
Import into mitochondria	^+H_3N-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into peroxisomes	-Ser-Lys-Leu-COO^-

KDEL sequence for ER lumen

SKL sequence for peroxisomes

These signaling sequences are important for protein sorting.

Necessary: have to have the stretch of amino acid to get in

Sufficient: all you need to get in. take the amino acid stretch and replace in another molecule to see if it gets in.

Signal sequences are both necessary and sufficient.

Three steps of protein sorting

Main problem of import: how to transport hydrophillic molecules in impermeable membranes

1. Recognizing the signal sequence by a shuttling cytosolic receptor
2. Targeting the outer surface organelle membrane
3. Import of target protein t membrane/transport of protein across membrane

Three main mechanism of import to membrane-enclosed organelle

1. Transport through nuclear pores
    a. Specific proteins, which REMAIN folded during transport (ready to do what they need to do)
2. Transport across membranes
    a. ER, mitochondria, chloroplasts, peroxisomes
    b. Require protein translocators
    c. Proteins are unfolded to be able to cross membranes
    d.

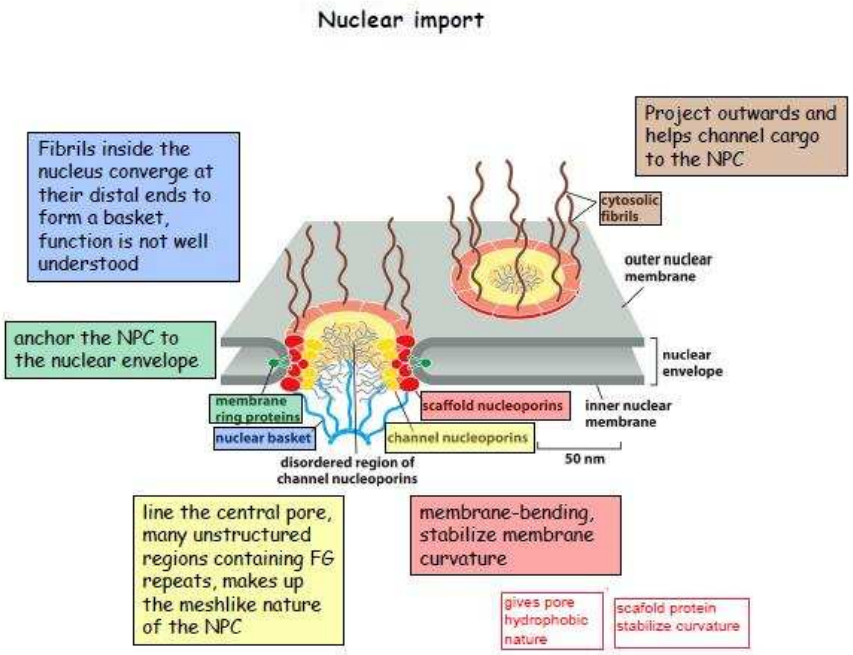
3. Transport by vesicles
    a. From ER → endomembrane system
    b. Vesicles transport proteins (folded) and pinch off membrane
    c. Fuse with target compartment

Nuclear import

Proteins enter through the nuclear pore complex.

- Made of copies of ~30 proteins → 500-1000 proteins
- Transports 1000 molecules/sec
- Bidirectional
- Small, water soluble molecules (40kDa) can move through it by passive diffusion

scafold protein stabilize the curvature



Nucleus targets by a nuclear localization signal made of basic residues

(P-K-K-K-R-K-V)

Those that have a NLS bind to NLS receptor (importin alpha beta heterodimer)

Protein complex associate with cytoplasmic filaments

Passes through NPC and associates with GTPase called Ran

Ran.GTP-importin beta complex goes back to cytoplasm (covered in midterm 1 review)

Ran gradient ensures directionalityof nuclear transport.

GTP-bound form → nucleus

GDP bound form → cytosol

Mitochondrial import

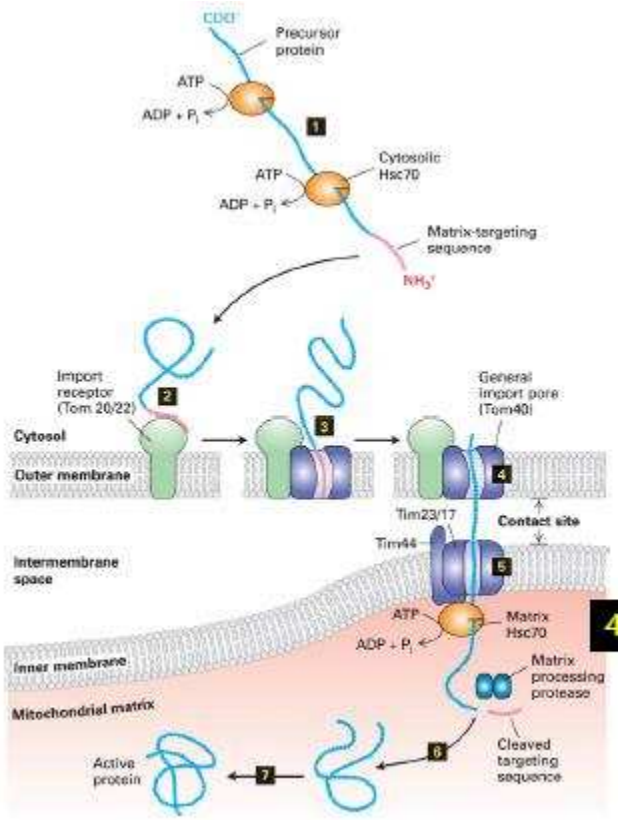
Import to matrix: usually N-terminal sorting sequence required. Rich in hydrophobic, positively charged, hydroxylated (Ser, Thr), lack acidic residues. Tend to form **amphipathic helix**.

Import to intermembrane space: second sequence required.

This type of transport only occurs when inner and outer membranes are in close contact.

STEPS:

1. Proteins kept unfolded by chaperone Hsc70, requiring energy in the form of ATP hydrolysis
2. Matrix targeting sequence interacts with receptor (TOM20/TOM22) in outer membrane
    a. OM stands for outer membrane
3. Receptor transfers protein to import pore in outer membrane (TOM40)
4. Protein passes
5. Protein connects to TIM23 and TIM27. Matrix Hsc70 binds to TIM44 and ATP hydrolysis occurs
6. Protease cleaves the target sequence
7. Protein folds into final conformation



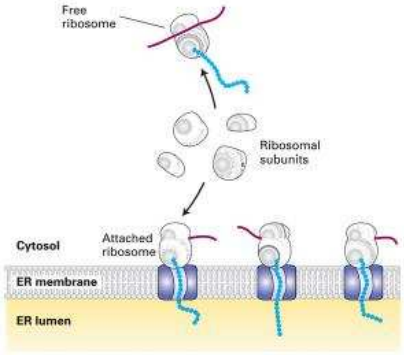
Note that H+ proton gradient is required also. This means that only mitochondria that respire can import proteins

For proteins that stop at the inter membrane space:

- Require hydrophobic targeting sequence that doesn't allow the protein to fully pass through TIM23/17
- Matrix targeting sequence gets cleaved and protein is released into the inner membrane

ER import

Transported across ER membrane BEFORE the protein is completely synthesized. Ribosome that synthesizes the protein attaches to the ER membrane, giving the ER a rough appearance (rough ER)

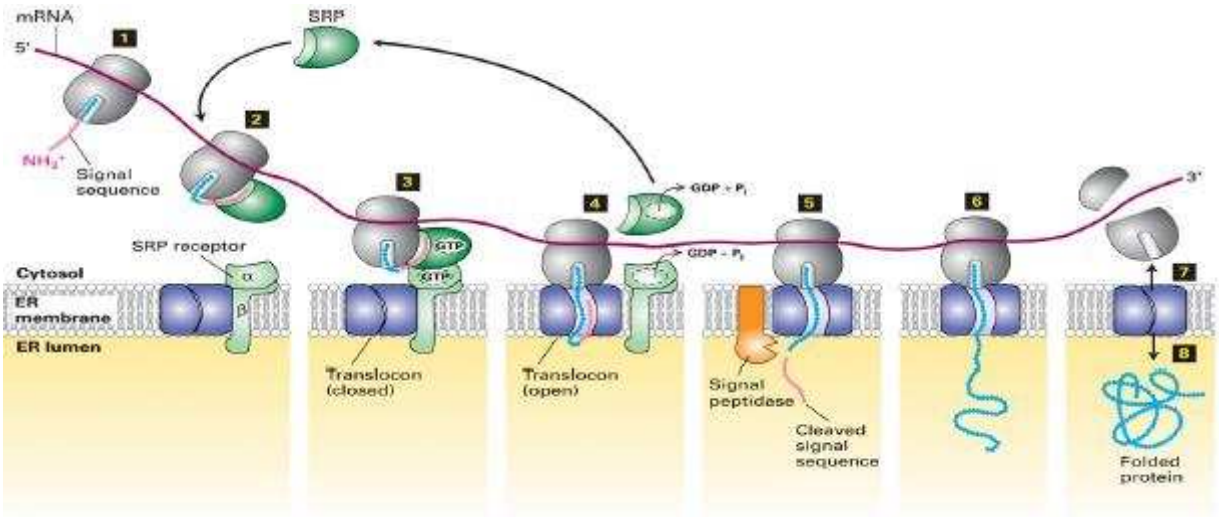


Types of ribosomes in cytosol:

- Membrane bound: attached to cytosolic surface of ER and synthesizes proteins into the ER
- Free: unattached and are synthesizing all other proteins

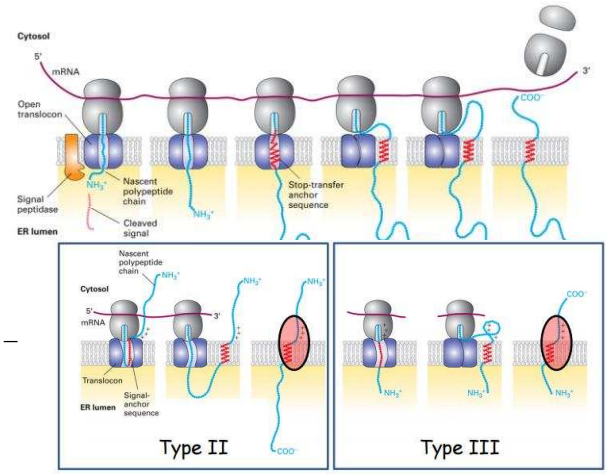
Steps of importing a SOLUBLE protein to ER lumen

1. Ribosome translates the protein
2. SRP (signal recognition particle) binds to ribosome/signal sequence and stops translation
3. SRP binds to SRP receptor (and GTP als binds to them both, enhancing the interaction)
4. GTP is hydrolyzed to GDP and protein/ribosome complex is transferred to translocon
5. Signal peptidase cuts the signal sequence
6. Translation resumes inside the lumen of ER
7. Ribosome is released and translocon closes
8. Protein folds



Types of membrane anchored protein (sorted in ER)

WITH RESPECT TO CYTOSOL

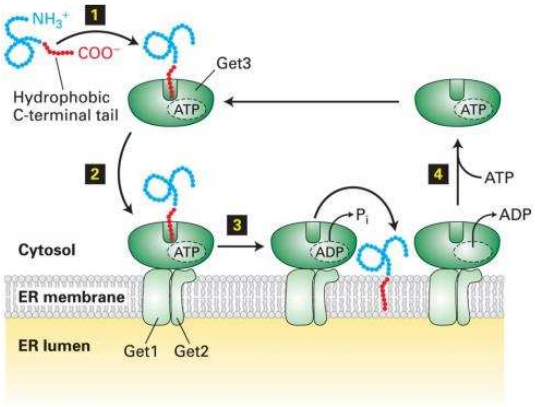


1. Type I:
    a. single pass.
    b. Signal sequence at N-terminus. Stop transfer anchor (STA)
    c. Uses SRP- SRP receptor to get to ER membrane. N-out – C-in
2. Type II:
    a. Single pass
    b. no cleavable signal sequence:
    c. uses SRP-SRP receptor to get to ER. N-in – C-out
3. Type III:
    a. Same as type II but N-out – C-in

hydrophobic sequence instead called signal anchor (SA) sequence. Position of SA sequence determined by position of positively charged residues relative to SA; since positive charges will always be in cytosol.

Between SA and N terminues → type II

Between SA and C-terminus → type III



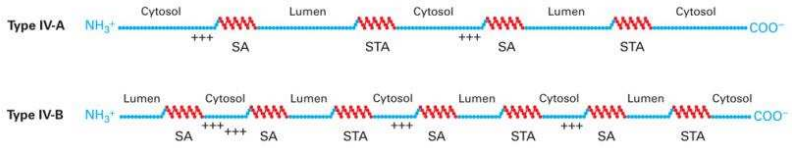
4. Tail anchored:
    a. single pass
    b. no cleavable signal sequence
    c. hydrophobic membrane at C terminus
    d. doesn't use SRP-SRP receptor but GET1/2/3 system to get to ER
    e. post translational insertion. N-in – C-out

STEPS:

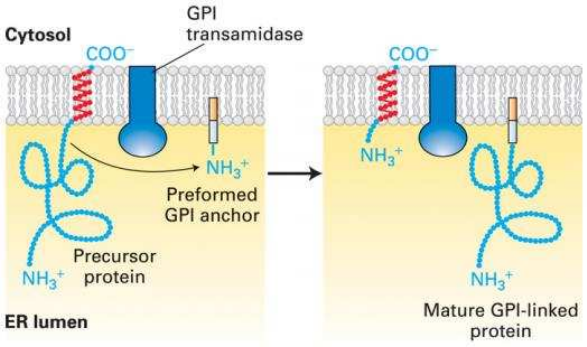
Hydrophobic tail binds to GET 4/5 and Sgt2 → tail then transfers to Get3 → Get 3 polypeptide binds to Get1 Get2 complex → Get3 hydrolyzes ATP and releases polypeptide →Get3 binds ATP and is released to membrane to get recycled.

5. Type IV:
    a. Multi pass
    b. No cleavable signal sequence
    c. Uses SRP SRP receptor for first domain but not the others
        i. Type IV-A → N-in – C-in
        ii. Type IV-B → N-out – C-in

Combination of STA and SA sequences. If first SA is type II → then protein will be Nin, If it is Type III → protein will be Nout



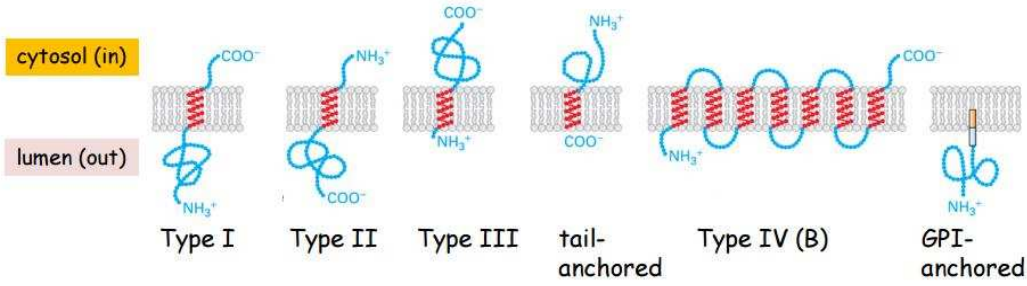
6. GPI anchored (glycosylphosphatidylinositol – is a lipid):
    a. Entire protein is lumenal (enter like type I)
    b. Cleaved signal at N terminus
    c. Uses SRP SRP receptor
    d. Anchored at C temrinus and then transferred to GPI anchor



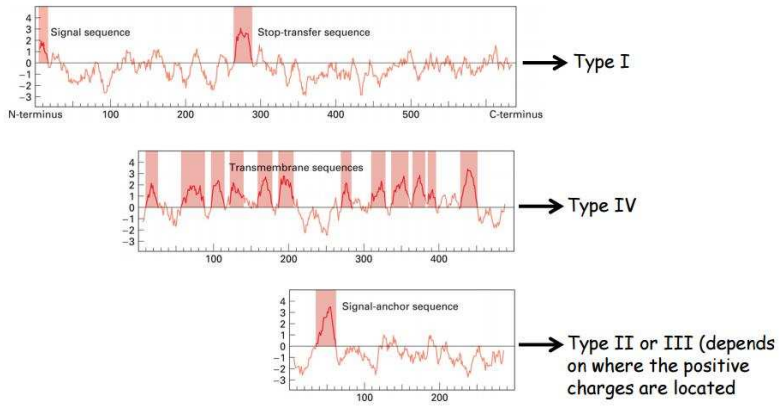
Enzyme called transamidase then

(i) Cleaves the protein within ER lumen
(ii) Transfers it to GPI anchor
    a. GPI anchor more readily diffuses to membrane
    b. GPI can act as a target signal

signal sequence	yes	no	no	no	no	yes
SRP/SRP receptor	yes	yes	yes	no	yes	yes



Hydropathic plot



Hydrophobic amino acids → positive index

Hydrophillic amino acids → negative index

Plot **for GPI anchored** will look like type 1 except stop transfer will be at the end of the plot.

Tail anchored plot will be like GPI except no signal sequence

Four modifications that occur in the ER

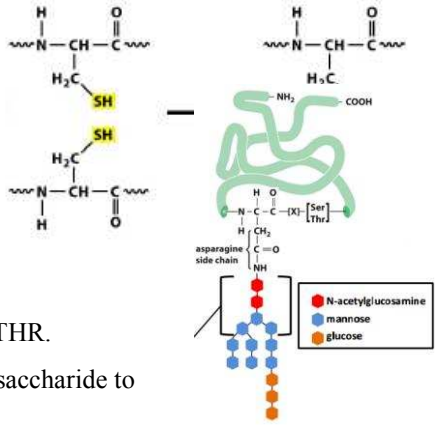
ER is starting point for

- Soluble proteins secreted from cell (hormones for example)
- Soluble proteins for golgi, lysosomes, endosomes (acid hydrolases)
- Membrane proteins that embed in golgi, lysosomes, endosomes or plasma membrane (Na/K-atpase)

To export, ER ensures the proteins are properly modified, folded and assembled. This is called quality control and these are the steps.

1. Disulfide bond formation:

Covalent bond between thiol groups of cysteine residues. Occur on same protein (**intramolecular**) or different proteins (i**ntermolecular**).



Depends on ER enzyme protein disulfide isomerase (PDI), so only secreted proteins orlumenal/extracellula domains of membrane undergo this.

These bonds stabilize protein structure (good for extreme pH or high level of protease)

2. Glycosylation: addition/processing of carbohydrates

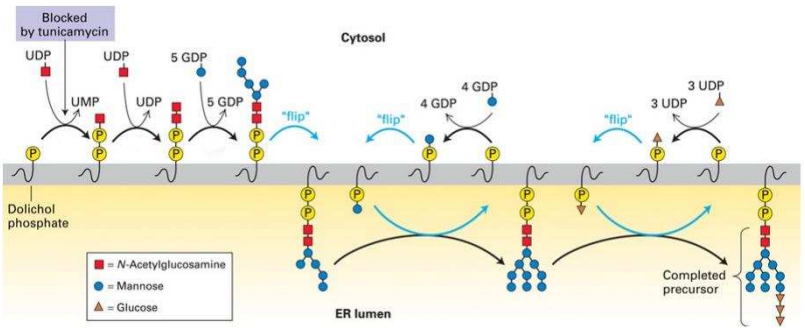
Begin with addition of oligosaccharide to ASN residue in ASN – X – SER/THR.

An ER membrane proein called **oligosaccharyl transferase** binds the oligosaccharide to the protein asparagine residue.

Then oligosaccharide assembled to lipid called **dolichol**

5 residues are called the core

Dolicol: 75-95 carbons. 2 GlcNAc and 5 mannose assembly occurs in cytosol surface of ER. The rest is added inside the lumen. Attachment of sugars is mediated by nucleotides (UDP-GlcNAc, UDP-glucose, GDP-mannose) Dolicol phosphate keeps flipping over.



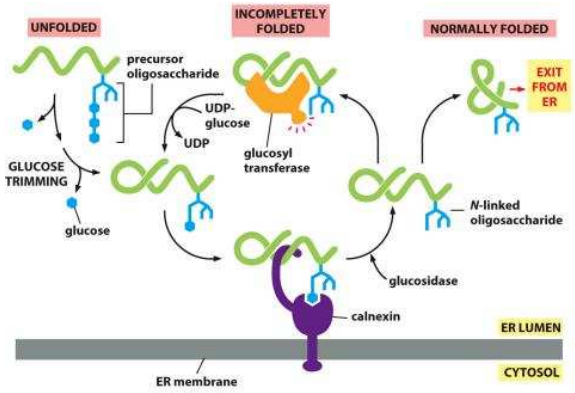
Tunicamysin blods the first GlcNac from attaching to dolichol, so protein never gets glycosylated and protein doesn't fold. Increases level of unfloded proteins in ER and causes unfolded protein response (UPR) to activate.

Glycosylation: promotes folding of protein, provides stability to proteins, promote cell-cell adhesion on plasma membrane proteins, act as transport signal (mannose-6-phosphate directs protein to lysosomes)

3. Folding + assembly of multisubunit complexes

Chaperones assist protein folding by preventing aggregation of hydrophobic stretches of aa. Two types of ER chaperones

- Classical chaperones: → Hsp70 (BiP), Hsp90, GRP94
- Carbohydrate-binding chaperones: → calnexin, calreticulin
    - Bind to monoglucosylated polypeptide → terminal glucose removes → folded proteins exit the ER, unfolded get glucose added back by glucosyltransferase



If mannose residues are removed, protein transports out of ER (dislocation) and is degraded in cytosol.

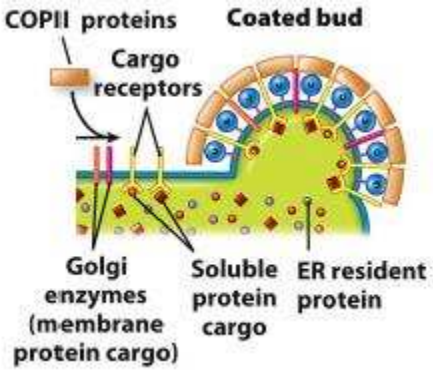
4. Proteolytic cleavage

Unit 6 (II): Protein sorting to organelles

Introduction

Mechanisms that control exit of proteins from ER:

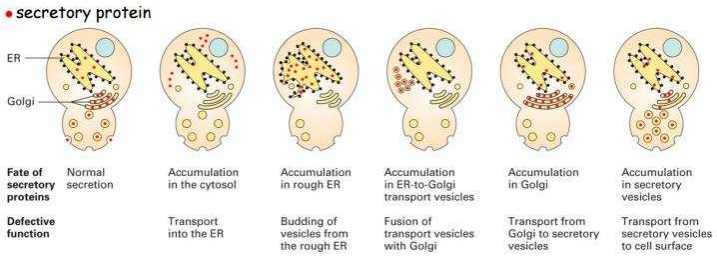
- Quality control: checks if protein is folded and assembled. If it is not ER chaperones retain it.
- Active cargo selection: specific cargo collected to region of ER and pinch off vesicle. Soluble cargo is recognised by bilayer membrane protein.
  Membrane cargo is recognized by cytosolic proteins and aid vesicle formation



For ER protein: Soluble protein contains signal called KDEL at C-terminus and interacts with KDEL receptor. This receptor moves from golgi to ER and binds KDEL containing proteins at golgi and released them in ER

For ER membrane proteins: signal is KKXX at the c-terminus of cytosol. This KKXX is recognized by COP I coat (protein needed for formation of vesicle)

Vesicle mediated transport



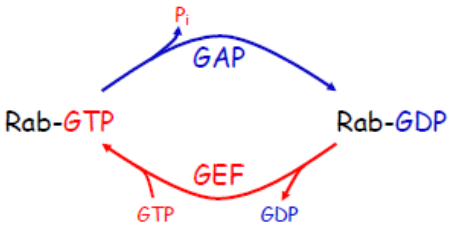
Conserved in all eukaryotes and yeast. Yeast temperature-sensitive mutants were used to identify the proteins needed for this process.

Main classes of vesicle coat:

- Clathrin:
  ○ at trans golgi for transport to lysosomes via endosomes
  ○ at plasma membrane for transport to endosomes
- COP I:
    - From cis golgi to ER
    - Between golgi cisternae
- COP II:
    - ER to cis golgi
    - At high temp, if COPII doesn't work, secretory proteins will accumulate in ER

Functions of protein coat on cytosolic surface

- Shapes donor membrane to a bid
- Capture cargo inside vesicles



Coat formation require GTP binding proteins called Rab.

Rab goes from active (GTP) to inactive (GDP).

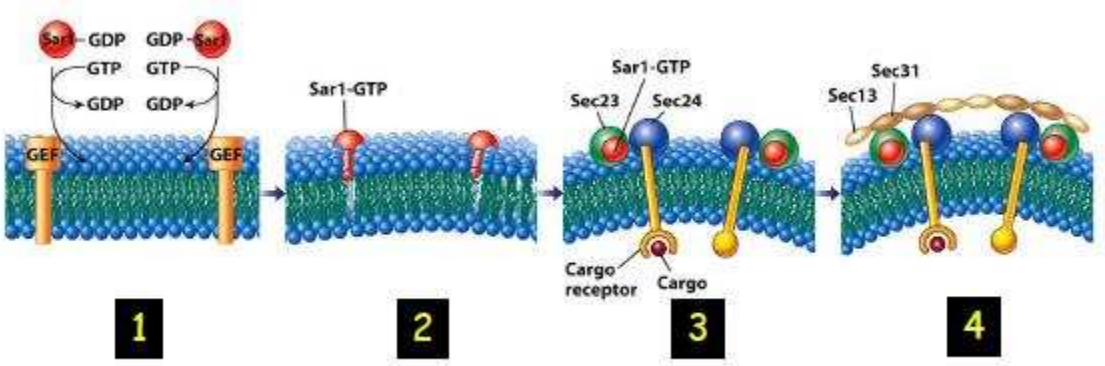
GAP (GTPase activating protein) hydrolizes the GTP

GEF (guanine nucleotide exchange factor) phosphorylates the GDP

STEPS OF COP II COAT FORMATION

Sec is for secretion

1. Rab protein SAR1 is activated by GEF
2. Activated Sar1 inserts into membrane and begins curving it
3. Activated Sar1 recruits Sec23 and Sec24 (inner portion of COPII coat), bending membrane more
    a. Sec24 binds membrane proteins
4. Sec23 and Sec24 recruit Sec13 and Sec31 (outer portion of COPII coat)



Formation of COP I coat at the golgi requires Arf1 activation (Rab protein) and COP I complex (7 subunits recruited as one unit)

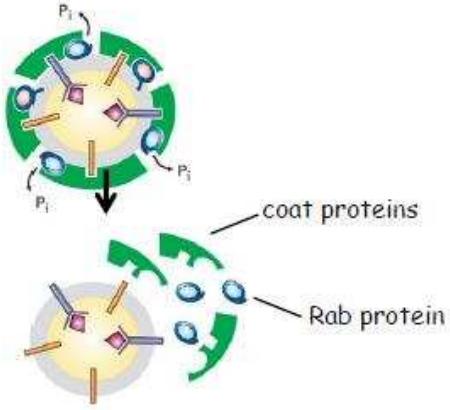
Vesicle fusion with target

Three types of transport vesicles have common features when fusing with target:

1. Vesicle coat must be completely or mostly removed from vesicle

For both COPI and COPII vesicles, removal of coat requires inactivation of the Rab protein.

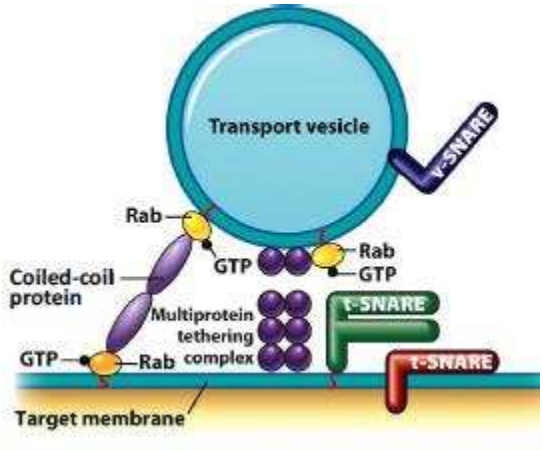
For clathrin coat, it is dependent on lipid composition (removal of phosphate from inositol phospholipids leads to uncoating)



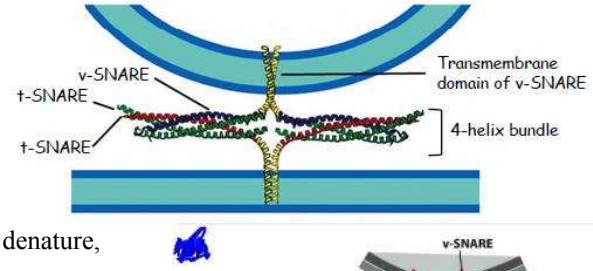
2. Vesicle must be specifically recognized by destination

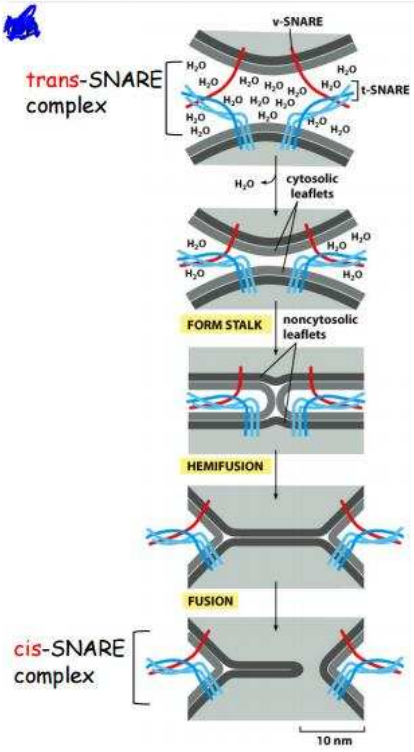
Rab proteins on vesicle and on target bind **effector proteins** that contribute to recognition of vesicle and target membrane. 3 steps:

1. Tethering: mediated by Rabs and their effectors, tethering factors and SNAREs (Reversible). Types of tethers (can be Rab effector):
    a. Multiprotein tethering complexes: made of 10 proteins, localize to disting organelles
    b. Coiled coil proteins: long alpha helical proteins that project long distances from target membrane



2. Docking: mediated by SNAREs pairing (stronger than tethering and Irreversible, will always proceed forward to fusion)
    a. Occurs at short distance (<<diameter of vesicle)
    b. V-SNARE for vesicle
    c. T-Snare for target membrane
    d. More than 35 SNARE proteins exist and all have a SNARE motif
    e. Snare bundles (image) do not denature, they form very strong bonds
    f. 4-helix bundle → 3 t-SNARE helices and 1 v-SNARE (trans SNARE complex)

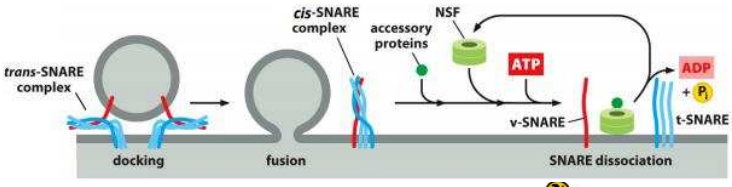




3. Fusion: driven by SNARE "zippering"
    a. When SNARES pair, it releases energy, which drives membrane fusion
    b. Steps:
        i. Outer leaf mix between vesicle and target
        ii. Expansion of hemifusion
        iii. Fusion of inner leaflet

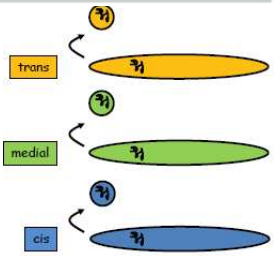
3. Vesicle and target must fuse and mix to deliver content

NSF (a hexametric ATPase) uses ATP to bind to cis SNARE complexes using accessory proteins called SNAP proteins. ATP hydrolysis breaks apart the cis SNARE complexes and allows them to be recycled.



Once the protein arrives in Cis-Golgi, two models of transport through golgi exist:

1. Vesicle transport model: golgi cisternae don't move and receive cargo in anterograde directed vesicles (ER-Golgi-PM)

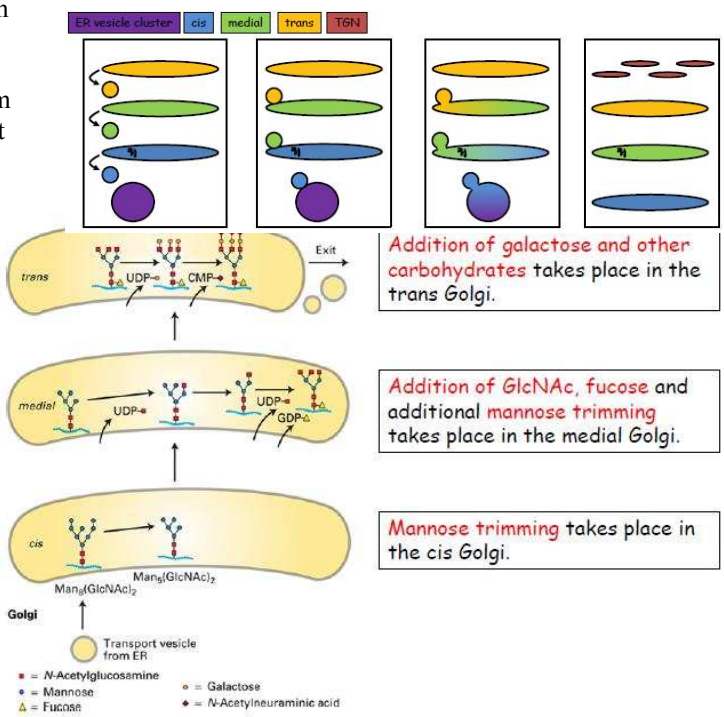


Cargo is packaged into vesicle and move from cis to medial to trans golgi everytime forming new vesicles.

2. Cisternal maturation: cargo doesn't move and passively matures as golgi enzyme travel in retrograde (trans → cis) vesicles

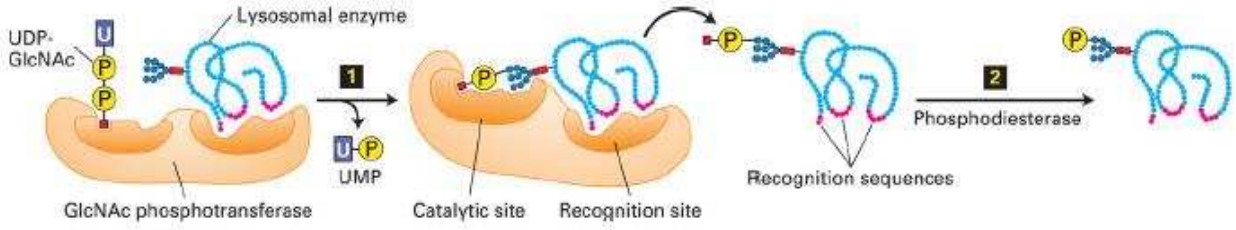
Vesicles bud from each golgi cisterna and contain golgi enzymes specific to the cisterna. Vesicles move backwards and deliver content there so the receiving cisterna becomes the compartment from which it received the enzymes. The cargo doesn't move, but its surrounding changed.

The golgi functions as a glycosylation factory. It modifies proteins, but not all proteins. Some yes some no. different things can happen to proteins.



One unique modification on soluble lysosomal enzymes result in the production of mannose-6-phosphate:

1. Addition of phospho GlcNac to one or more mannose residues
2. Removal of GlcNAc, leaving mannose 6 phosphate

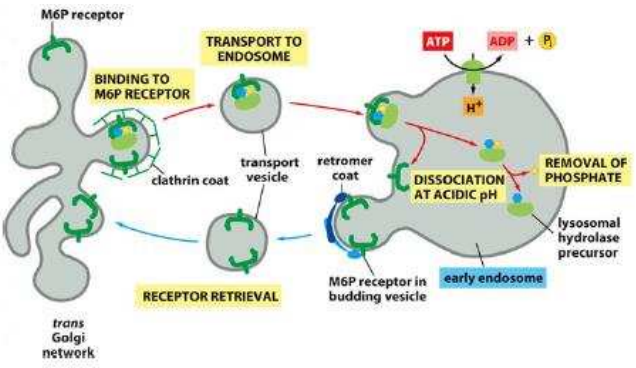


Delivery from trans golgi network to lysosomes

Soluble lysosomal enzymes contaning mannose-6-phosphate (M6P) are recognied by membrane bound M6P receptor:

- Bind to M6P residue at pH 6.5-6.7 (TGN pH)
- Releases residue at pH 6 (endosome pH)

M6P receptor recycles → enzyme go from endosome to lysozome



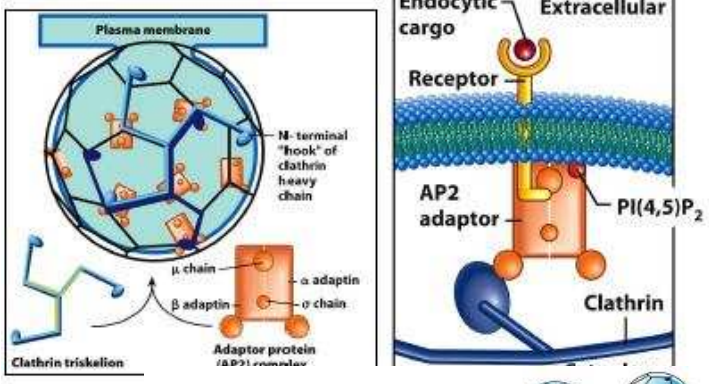
Endocytic pathway moves material inside the cell (extracellular to intracellular)

Clathrin: forms outer layer of coated vesicles and has triskelion appearance.

Adaptor protein: for inner layer of vesicle and engage cytoplasmic tail of receptor. PI(4,5)P (phosphatidylinositol 4-5 bisphosphate) facilitates binding to membrane.

Types of endocytosis:

- Bulk-phase endocytosis (pinocytosis): non selective, clathrin dependent or independent.
- Receptor-mediated endocytosis: selective, clathrin dependent. Initiated by binding of ligand to its receptor.



As vesicle forms, a GTP binding protein (dynamin) binds as a ring around vesicle. If GTP hydrolysis occurs, vesicle breaks free. If a non hydrolyzable form of GTP is used → vesicle continues to grow with a dynamin ring.

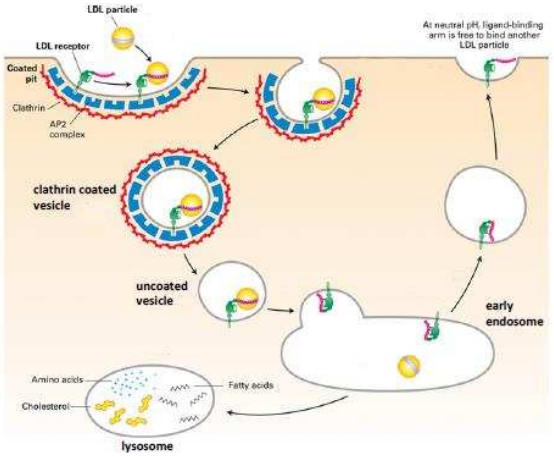
To uncoat, clathrin requires: modification of lipid that bind the adaptor proteins and energy (ATP hydrolysis by Hcs70 – heat shock protein)

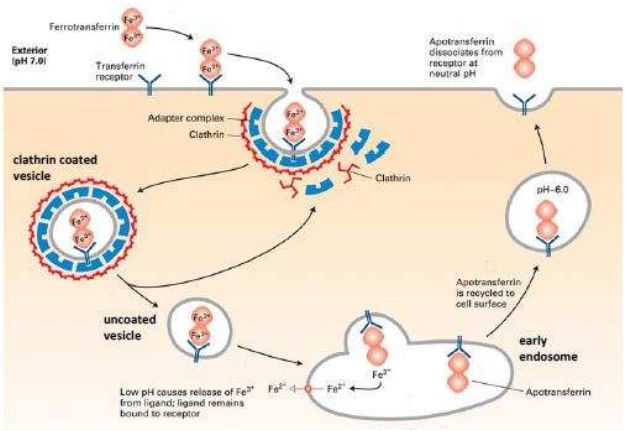
Uncoated vesicle becomes early endosome which undergo maturation to late endosome such that:

- Late endosome have a lower pH than early endosomes
- Late endosome associate with Rab protein (Rab7) early endosome with Rab 5
- Late endosomes are found near golgi in cell interior, early endosomes are found near plasma membrane
- Late endosomes are round/oval. Early endosomes are complex (tubulo-vacuolar)
- Late endosome become lysosome

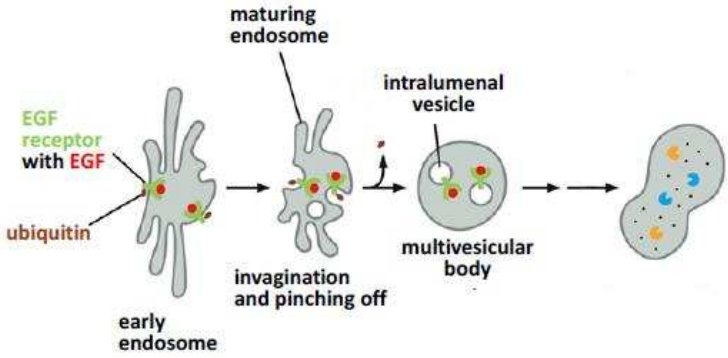
Fates of receptor/ligand complex

1. Low pH of early endosome causes dissociation of ligand from receptor. Receptor is taken back to cell surface and ligand goes to lysosome
    a. LDL/LDL receptor
2. Ligand and receptor do not dissociate and receptor bings the ligand back to cell surface
    a. Transferrin/transferrin receptor





3. Ligand and receptor are both sent to lysosome: receptor tagged with protein called ubiquitin. In matring endosome, invagination occurs and ubiquiting receptor ligand complex enter the invagination. Multivesicular body fuses with a lysosome.
    a. EGF/EGF receptor



Unit 7: Principles of cell signaling

Cells use extracellular signaling molecules that bind to receptor to communicate

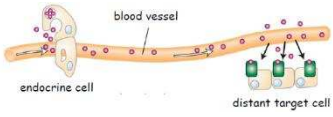
Signal transduction: converting extracellular signal to intracellular signal in target.

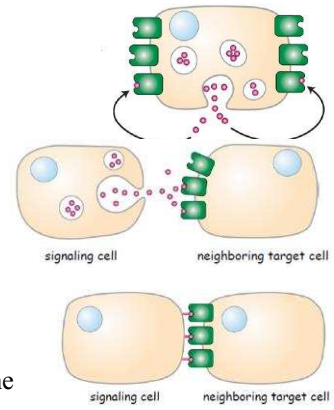
Six steps in signaling

1. Synthesis of signal molecule by cell
2. Release of molecule
3. Transport of signal to target
4. Detection of signal by receptor protein
5. Change in cellular metabolism/function/development
6. Removal of signaling molecule; terminates response

Signal types

1. Autocrine: respond to signal that they themselves release
    a. Common in cancer cells
2. Paracrine: affects cell close by
    a. Growth factor
3. Endocrine: affects cell far away
    a. hormones
4. Contact-dependent: membrane anchored signal in membrane of cell binds to receptor on another cell





Signaling molecule is NOT the signal. Signal depends on how target cell interprets the molecule.

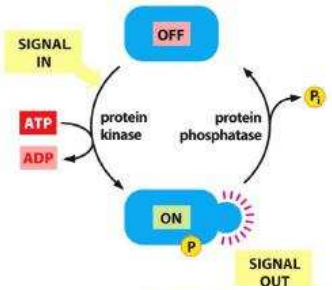
Intracellular signaling pathways

Signaling pathways are a cascade of events. Each protein alters the activity of the next.

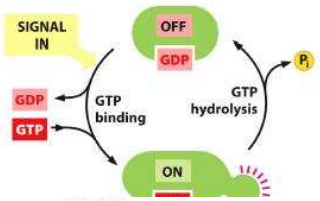
Small molecules → second messengers

Protein conformation is usually altered by phosphorylation. Kinases add a phosphate; phosphatases remove a phosphate.

Types of molecular switches after activation of cascade

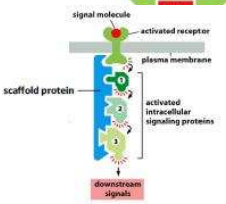


1. Phosphorylation/dephosphorylation
    a. Activates/inactivates enzyme
    b. Promote/interfere with protein-protein interactions
    c. Change subcellular location of protein
    d. Trigger protein degradation



2. GTP binding (g-protein coupled receptors)
    a. GTP binding protein is active when bound to GTP and inactive when bound to GDP
    b. When signal is absent → inactive
    c. Signal activates release of GDP and binding of GTP
    d. GTPase activity hydrolyzes the bound GTP to GDP and Pi
3. Assembly/disassembly of protein complexes

Many cascades contain multiprotein signaling complexes:



- Scaffold: bring other signaling molecues closer for sequential activation
- Receptor binds the other effector proteins and transmits signal (receptor is scaffold)

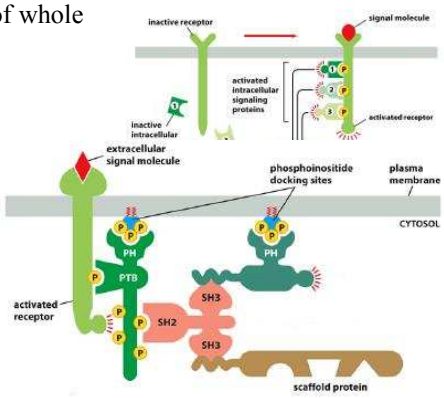
Interactions during signaling are mediated by protein domains; aa sequences that share similarity and function independently of whole protein.

Example: SH2 → binds to phosphate in a sequence

SH3 → binds to proline rich sequence

PH → bind to certain phosphoinositides

PTB → binds to phosphoryated tyrosine residues in sequence



4. Proteolysis (irreversible)

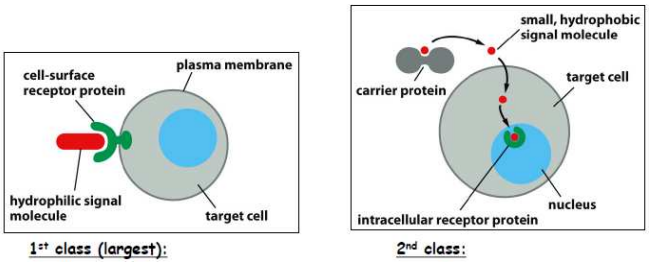
Intracellular protion of receptor is cleaved and acs as signal.

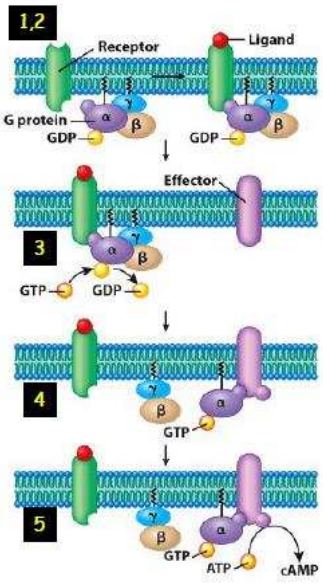
Steps of cascade

1. Relay signal → help spread it in cell
2. Amplify signal → to produce larger response
    a. 1 molecule of epinephrine leads to production of 100 x 10^6 molecules of glucose-1-phosphate
3. Distribute signal → to many effector proteins to have a complex response
4. Integrate signal → from other pathways before relaying them onward

Classes of EXTRACELLULAR signaling molecules

1. First class: extracellular signaling molecule too large and hydrophilic to cross plasma membrane
    a. Receptors are on outer surface
2. Second class: small and hydrophobic and can diffuse across membrane
    a. Receptors are inside





Types of extracellular surface receptors (class 1)

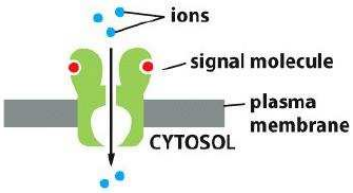
3 classes of ligand-triggered cell surface receptors:

1. G-protein coupled: in all cells
    a. G-protein linked receptor binds ligand. Ligand binding makes receptor bind with inactive heterotrimeric GTP-binding (G) protien
    b. G protein is activated
    c. Alpha subunit of G protein leaves receptor and binds effector enzyme
    d. Binding to G protein activates effector enzyme
    e. 2nd messenger generated (usually cAMP) → molecule that binds inside the cell

Some G proteins stimulate production of cAMP (Gs) others inhibit it (Gi)

Some G proteins stimulate production of diacylglycerol and inositol 1, 4, 5-triphosphate by activating phospholipase C (Gq)

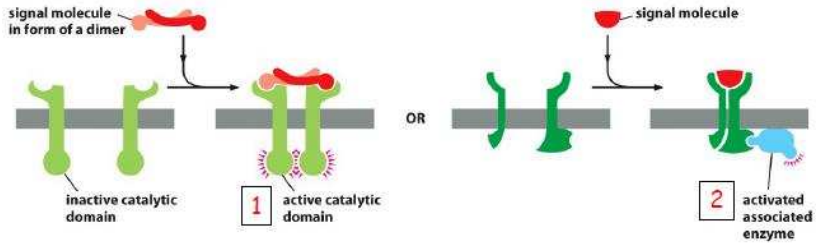
2. Ion-channel-coupled receptors: important in neurons



Ligand binding changes conformation of ion channel linked receptor and opens channel for ion flow (ions coming in could act as second messengers)

3. Enzyme coupled receptors:

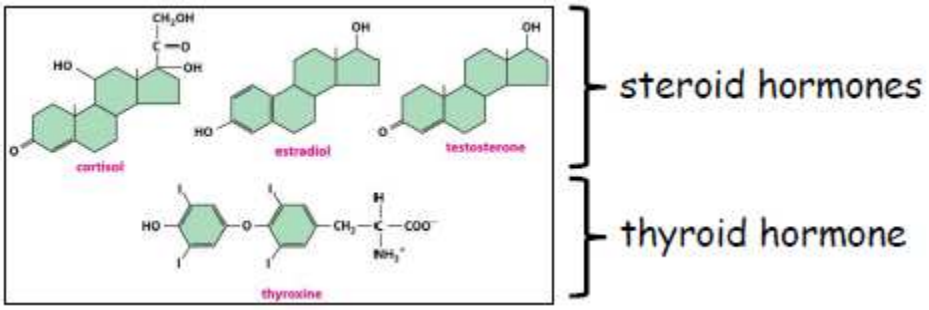
Binds extracellular ligand switching on an enzyme inside the cell. Enzyme is either (1) part of cytoplasmic domain of receptor or (2) one that associates with the cytoplasmic domain.



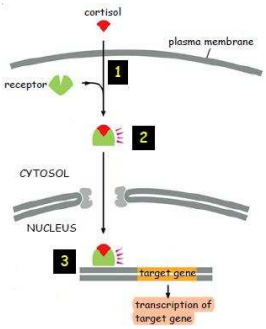
Extracellular receptors (class 2)

Receptors are either in cytosol or nucleus. Two groups of diffusable signaling molecule:

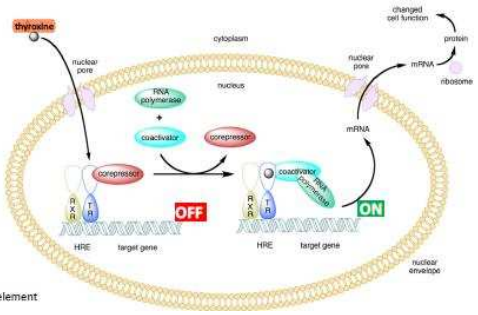
- Hormones: receptors are transcription factors → regulate expression of specific genes



  - Cortisol: produced by adrenal gland under stress and low blood glucose. Causes the body to produce glucose and suppresses the immune system.
    - Cortisol diffuses across plasma membrane and bind to receptor
    - Binding induces change in receptor; complex gets transported through nuclear pore
    - Receptor binds to DNA regulatory sequences, inducing expression

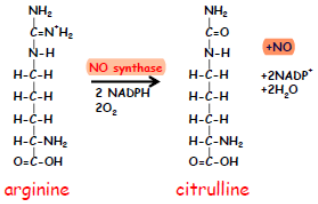


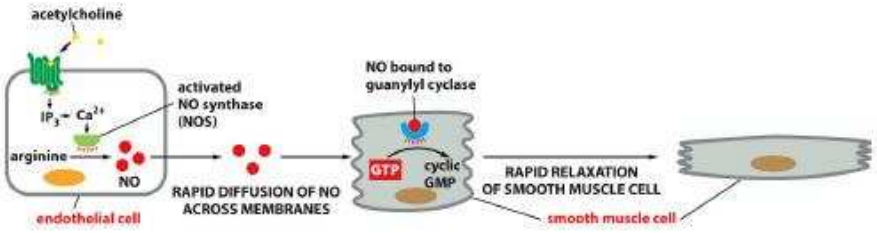
  - Thyroxine: produced in thyroid gland and increases MR and protein synthesis. Also transported through nuclear pores but not with receptor. receptor is inside the nucleus and is bound to DNA.
    - Absence of thyroxine → receptor binds to repressor to prevent gene transcription
    - Presence → thyroxine binds to a transcriptional activator which in turn binds RNA polymerase and the gene is then transcribed



HRE = hormone response element

- Nitric oxide (NO): produced by breakdown of ARG
  - Only local acting (paracrine signaling
  - Important in cardiovascular system (vasodilator)
  - NO is a vasodilator; it activates guanylyl cyclase which produces cGMP and muscles relax





Therapeutic perspective of NO:

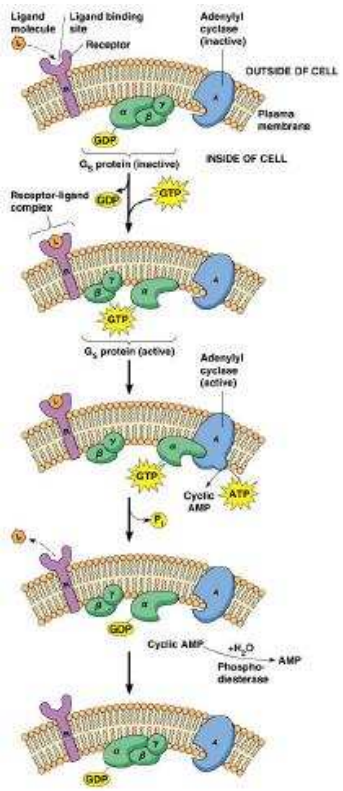
1. Nitroglycerin is used to treat angina → converted to NO and leads to vasodilation

2. Increase lifetime of cGMP increases blood flow. Inhibitors of phosphodiesterase 5 (sildenafil a.k.a viagra) achieve this.

Unit 8: Signal transduction pathways I

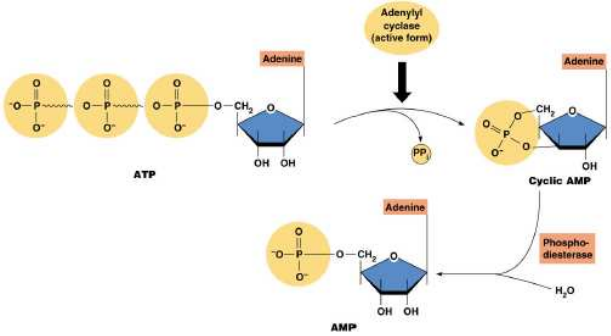
Gs-protein-coupled receptors signaling through second messenger cAMP

1. Ligand binds to receptor
2. Gs protein binds to receptor and is activated when GDP is replaced to GTP
3. Alpha subunit separates from the other subunits and binds to adenylyl cyclase
4. Active adenylyl cyclase makes cAMP from ATP
5. Signal is turned off by
    a. Hydrolysis of GTP → GDP + Pi
    b. Conversion of cAMP → AMP by phosphodiesterase



cAMP formation (cyclization reaction):

- Two phosphate removed from ATP
- Free end binds to its own sugar
- cAMP → AMP by phosphodiesterase



cAMP affects cAMP dependent protein kinase (Protein kinase A – PKA)

- inactive form of PKA is a tetramer made of two regulatory (R) and two catalytic (C) subunits
    - R subunit: two binding sites for cAMP. When cAMP binds C subunits activate and are released to phosphorylate target proteins

Rise in cAMP caused by Adrenaline is important in response to stress. Adrenaline binds to adrenergic receptors → beta adrenergic and Gc receptors; alpha adrenergic → Gi receptors

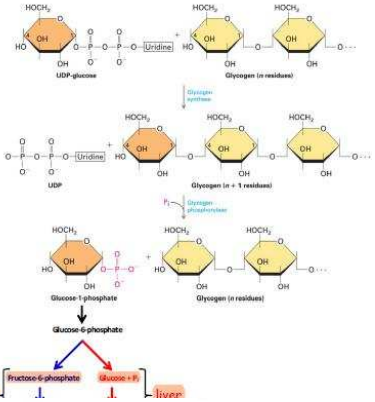
Extracellular Signal Molecule	Target Tissue	Major Response
Adrenaline	heart	increase in heart rate and force of contraction → Increased blood supply (nutrients) to tissues
Adrenaline	skeletal muscle	glycogen breakdown → energy
Adrenaline	fat	fat breakdown → energy

Glycogen metabolism

Glycogen is a polymer of glucose. This is how sugar is stored.

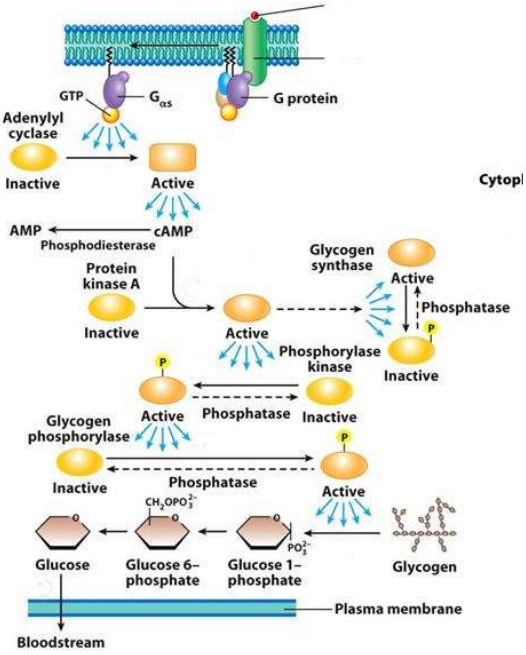
Glucose-UDP → glycogen with help of glycogen synthase.

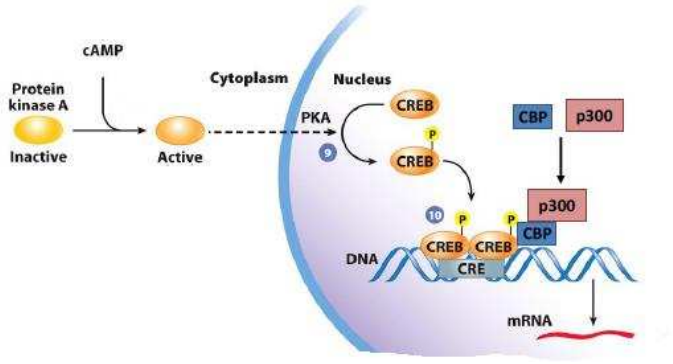
Glycogen → glucose-1-phosphate with help of glycogen phosphorylaze



Steps of adrenaline signaling:

1. Adrenaline/glucagon binds to receptor
2. Receptor is activated
3. Alpha subunit is activated
4. Adenylyl cyclase is activated
5. cAMP levels rise
6. cAMP binds to PKA
7. PKA targets glycogen synthase
8. PKA targets phosphorylase kinase (phosphorylated)
9. Phosphorylase kinase phosphorylates glycogen phosphorylase and glycogen breakdown starts





PKA pathway has both:

- Fast response: signal transduction involving cascade
- Slow response: cAMP induced gene transcription

PKA → active → nucleus → phosphorylates transcription factor (CREB) → binds to CRE → binds to CBP and p300 to activate transcription

ALL cAMP responsive genes have a DNA sequence upstream of gene called CRE (cAMP response element)

Regulation of adrenaline/glucagon signal

Turning off adrenaline/glucagon signal

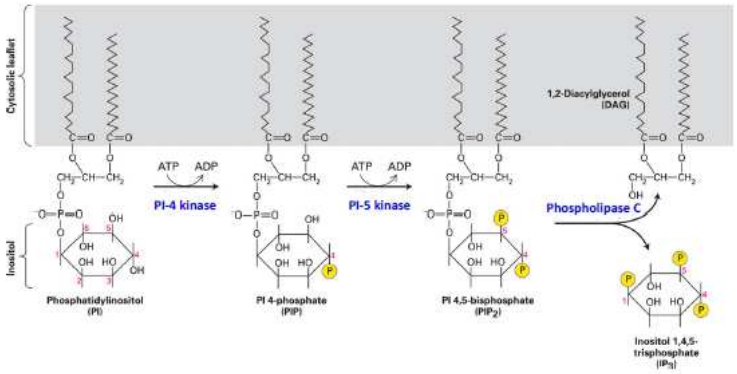
- Receptor and ligand affinity decreases when Gs alpha is activated
- Hydolysis of GTP on Gs alpha is increased by adenylyl cyclase (like GAP)
- Phosphodiesterase converting cAMP to AMP
- Beta-adrenergic receptor becomes substrate for both PKA and BARK (beta adrenergic receptor kinase)
- Lots of other points…

G-protein coupled receptors signaling through phospholipids

Some second messengers are derived from phosphotydilinositol. Inositol can be phosphorylated on 4 or 5th position on ring.

Phospholipase C hydrolyses inositol ring releasing two second messengers:

- Diacylglycerol (DAG): remain membrane bound
- Inositol-1,4,5-triphosphate (IP3): hydrophilic; can diffuse in cytosol



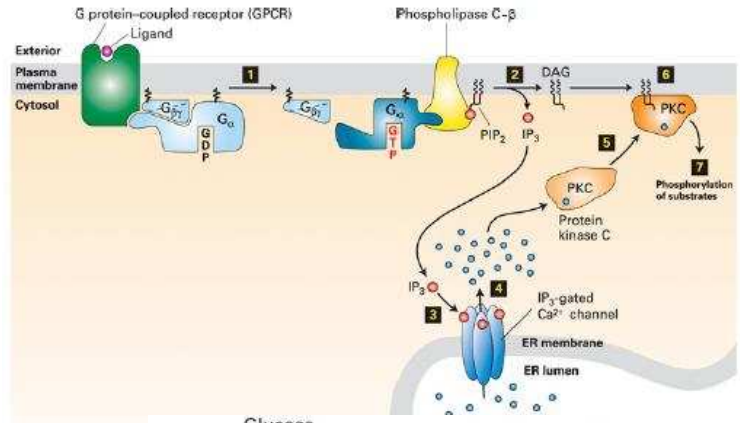
These trigger separate events.

Phospholipase C isoforms (cleave same bond anyway):

- PLC beta: activated through Gq associated receptor (Gq alpha subunit)
  - Example: vasopressin, acetylcholine, thrombin signal throught these
- PLC gamma: activated though tyrosine kinases (RTKs)

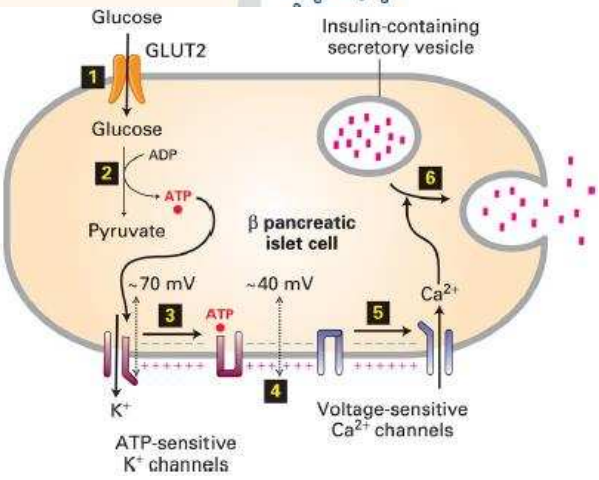
STEPS:

1. Ligand binds to receptor; activating Gq alpha and separating it from Beta gamma subunits
2. G q alpha activates PLC beta → DAG and IP3 are released
3. IP3 diffuses through cytosol to Ca2+ channel on ER
4. Ca2+ channel open → Calcium is released in cytosol
5. Calcium binds to PKC
6. PKC relocates to plasma membrane
7. PKC at the plasma membrane binds to DAG (which activates it)



Glucose as a signaling molecule:

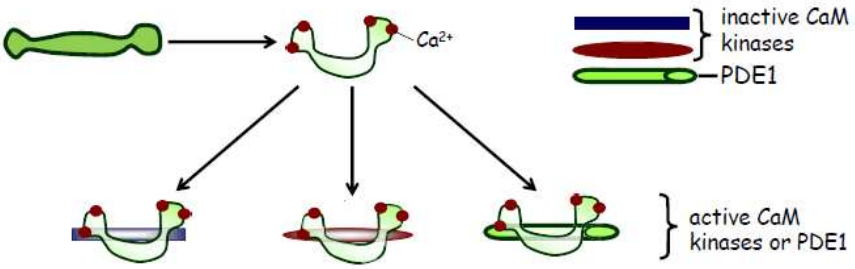
1. GLUT2 imports glucose
2. Glucose → pyruvate + ATP
3. ATP binds to ATP sensitive potassium channel, closing it
4. Because the channel is closed, depolarization occurs in plasma membrane
5. Depolarization opens the voltage sensitive Calcium channels
6. Increase of calcium triggers release of insulin through vessicles



Calmodulin (CaM) binds to Calcium producing different responses

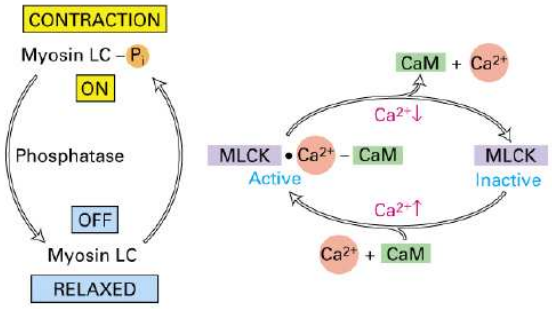
After bidning to calcium it either:

- Phosphorylates myosin light chain → activates myosin
- Phosphorylates and activates CREN → transcription of genes
- Binds to PDE1 to increae its activity → reduces level of cAMP in cytosol.



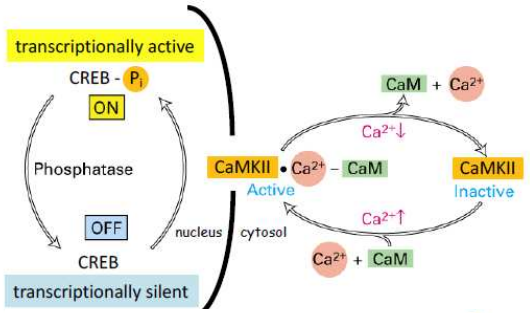
Regulation of muscle contraction by calcium and CaM

1. Rise in calcium causes it to bind to CaM
2. CaM.calcium activates myosin light chain kinase (MLCK)
3. MLCK phosphorylates light chain of the light chain of myosin
4. Chain binds toactin and initiates muscle contraction



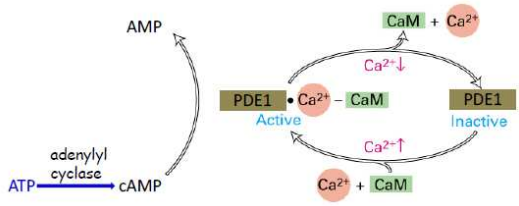
Regulation of CREB transcription activity by calcium and CaM

1. Rise in calcium causes it to bind to CaM
2. CaM.calcium activates CaMKII
3. CaMKII enters the nucleus
4. Phosphorylates CREB
5. CREB binds to DNA at CRE sequence and recruits p300 and CBP, activating gene transcription



Regulation of cAMP levels by calcium and CaM

1. Same as others
2. CaM.calcium activates PDE1 (in brain, heart, lung)
3. PDE1 converts cAMP to AMP



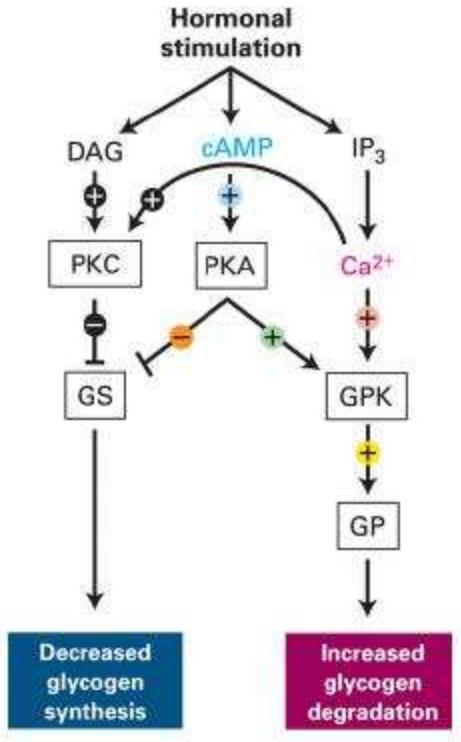
Calcium inhibits glycogen synthesis and activates degradation

Protein kinase C is activated through Gq preceptor. Converts glycogen synthase to its inactive form, stopping glycogen synthesis

Phosphorylaze kinase has 5 subunits: alpha, beta, gamma, delta. Delta is actually CaM. So increase in calcium leads to activation os phosphorylase kinase → active glycogen phosphorylase, breaks down glycogen.

So a rise in calcium inhibits glycogen synthesis and activates glycogen degradation.

Summary of how different second messengers (cAMP, IP3, DAG) affect glycogen metabolism

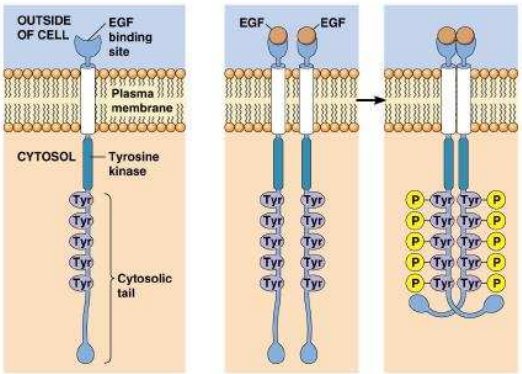


Unit 8: signal transduction pathways II

Some reseptors only need very low contentrations of ligands to be activate. These ligands are called growth factors, but also hormones.

Response is slow since they alter gene expression. Response involves phosphorylation of a downstream target or of receptor. Many of these receptors are tyrosine kinases (RTKs)

Ligand/growth factor	Receptor kinase activity
EGF (epidermal growth factor)	tyrosine
PDGF (platelet-derived growth factor)	tyrosine
TGFβ (transforming growth factor β)	serine/threonine
FGF (fibroblast growth factor)	tyrosine



RTKs are usually single membrane spanning, where G protein coupled receptors have 7. When ligand binds, aggregation of receptors occurs dollowed by autophosphorylation (one receptor phosphorylates TYR of other)

Ras-Raf-MAP kinase pathway

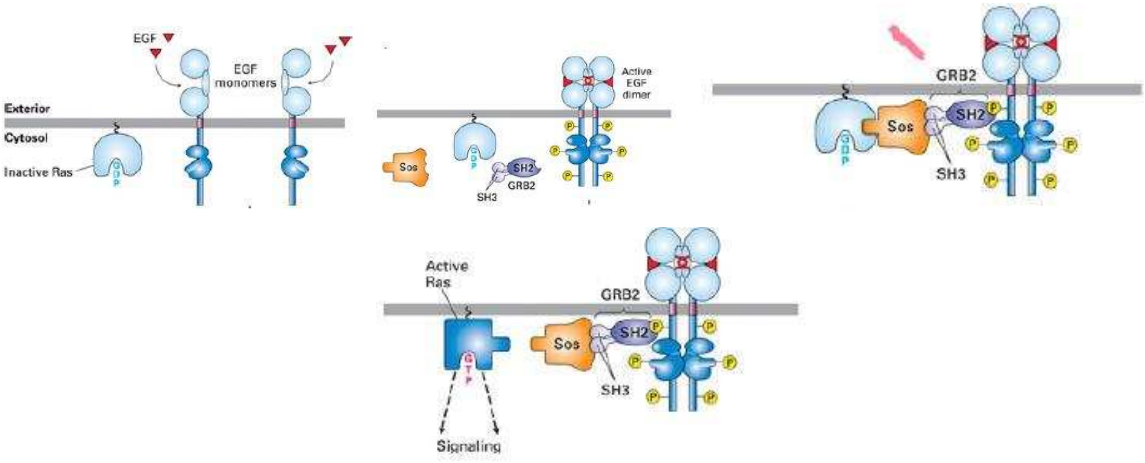
Ras- small GTP binding protein.

GRB2 – SH2 domain containing protein that binds to phosphotyrosine residues.

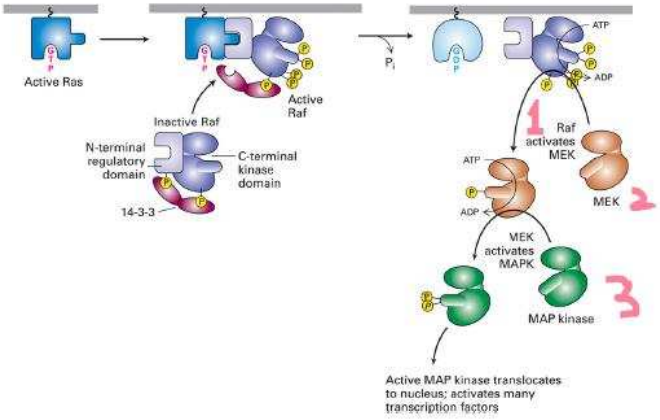
Sos (son of…)- nucleotide exchange factor for Ras, activates the GTPases

1. EGF binds to receptor → cascade → GTP binding protein Ras activates
2. EGF receptors aggregate
3. Autophosphorylation occurs
4. GRB2-Sos complex binds to activated recepton through SH2 domain of GRB2.

5. Ras associated with SoS
6. Sos activates Ras by releasing its GDP and helping uptake of GTP



7. Activated Ras binds to kinase Raf and protein 14-3-3 (inhibits Raf kinase)
8. Binding relieves inhibition activity of 14-3-3 and allows Raf phosphorylation
9. Ras hydrolysis GTP to GDP and released acivaed Raf
10. Activated Raf → phosphorylation/activation of MEK → phosphorylation/activation of MAP kinases (ERK1, ERK2)



MAP kinases mostly target transcription factors. This pathway can activate 160 genes called **early response genes** (needed for cell to enter and continue cell cycle). This pathway ensures cell proliferation, differentiation and survival.

All genes contain cis acting DNA sequences called Serum response element (SRE – activated by many growth factors in serum)

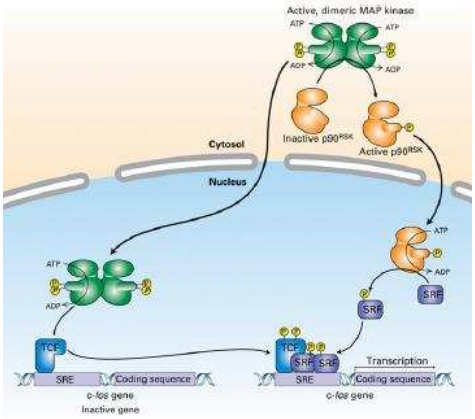
SRE binds to unphosphorylated forms of two transcription factors:

- TCF
- SRF

They are phosphorylated and in turn activate transcription

Steps:

1. MAP kinase phosphorylates $p90^{RSK}$
2. The activated MAP kinase and $p90^{RSK}$ move to the nucleus
3. MAP kinase phosphorylates TCF
4. $p90^{RSK}$ phosphorylates SRF
5. both bind to the SRE sequence of the gene



Summary of pathway:

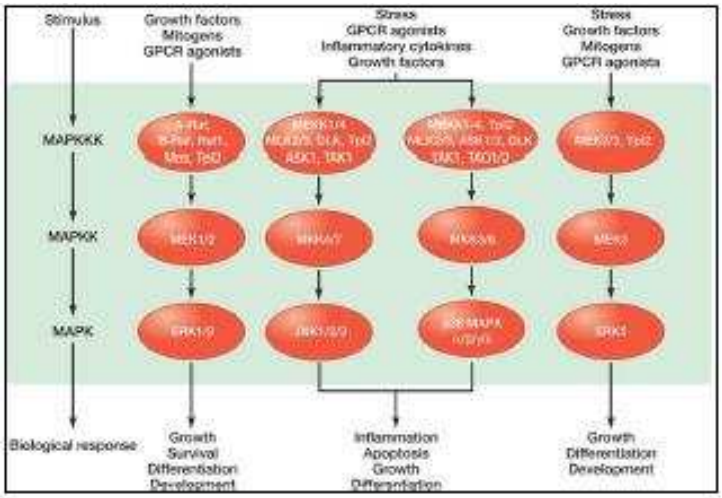
ERK gets phosphorylated by MEK so it is called MAPK

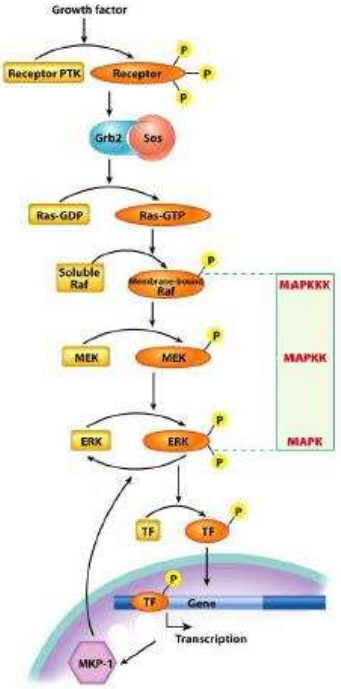
MEK gets phosphorylated by Raf so it is called MAPKK

Raf gets phosphorylated by Ras so it is called MAPKKK

(K is for kinase)

ALL SIMILAR PATHWAYS HAVE THIS KINASE STRUCTURE





How to silence cascade:

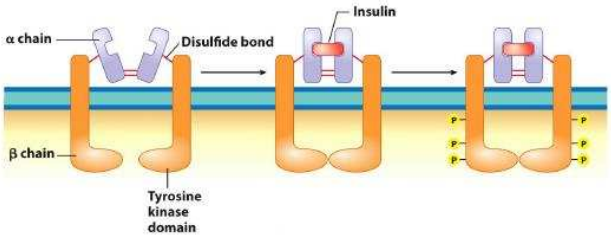
1. GTP hydrolysis by Ras (a mutated form of Ras that can't hydrolyze GTP → common in cancer cells)
2. Dephosphorylation of MAPK (MKP-1, made from the gene, acts on ERK1)
3. Inactivation of receptor by:
    a. Dephosphorylation
    b. Endocytosis and delivery to lysosome
    c. Antibody inhibition (therapeutic) → herceptin acts on the EGF receptor and is used to tread breast cancer

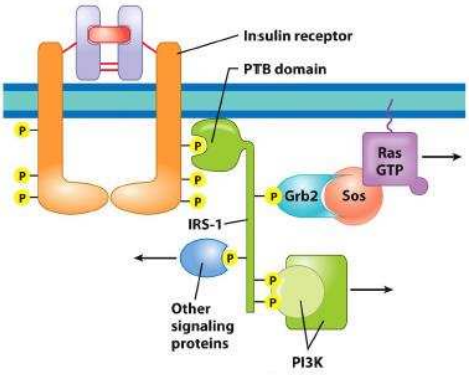
Insulin receptor pathway

Insulin receptor: heterotetramer: 2 alpha and 2 beta subunits.

Alpha subunits ae extracellular and form a pocket for insulin.

When insulin binds, shape changes in both alpha and beta subunits and the beta subunits gets autophosphorylates.





STEPS:

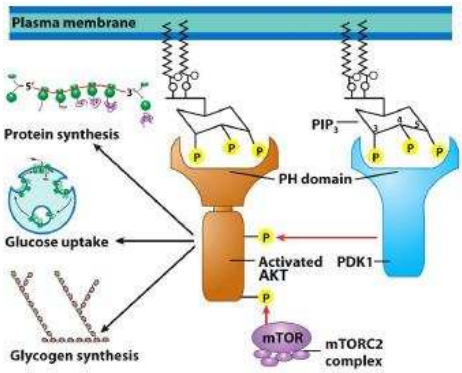
1. Beta subunits autophosphorylates
2. Beta subunit binds insulin receptor susbtrates (IRS1 and 2)
3. IRS1 activates several pathways once it is phosphorylated
    a. Ras: it binds to GRB 2, which binds to Sos, which binds to Ras GTP
    b. Phosphoinositide pathway: binds to PI3Kinase and phosphorylates phosphatidylinositol 4 bisphosphate to produce PIP 2 and PIP3, which are second messengers

PIP3: recruits two enzymes to form AKT and PDK1. These enzymes bind through their pH domains.

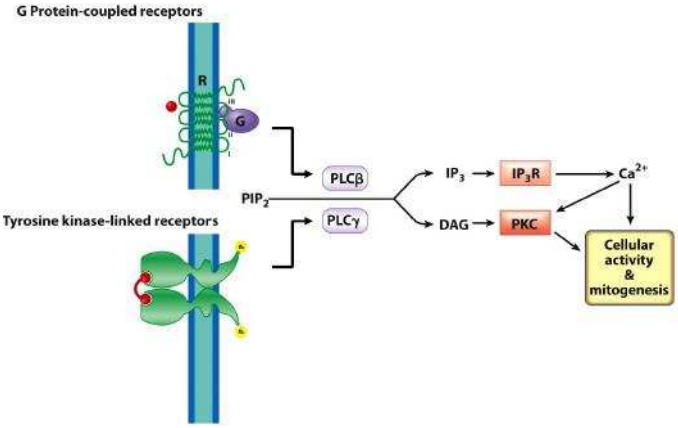
PDK1 and mTOR phosphorylates and activates AKT.

Activated AKT then dissociates from membrane and can do protein synthesis, glucose uptake (induce GLUT4) or glycogen synthesis

PTEN lipid phosphate can terminate PI3K pathway by converting PIP3 to PIP2



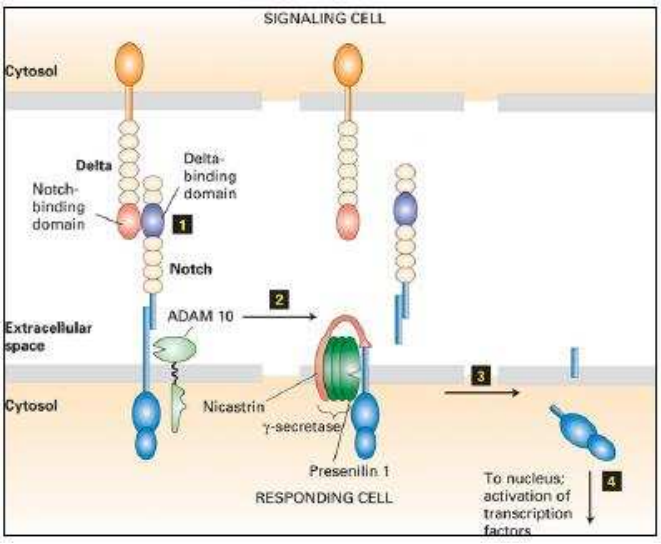
RTK can also activate phospholipase C (the gamma isoform), increasing the Calcium even more.



Signaling through proteolysis

Notch-Delta Pathway: important for cell fate determination

1. Delta ligand bind to notch receptor
2. Notch is cleaved by two proteases
    a. ADAM10: extracellular cleavage
    b. Presenilin 1: inside the membrane (alzeimer's)
3. Whatever pre----------senilin cut translocates to nucleus

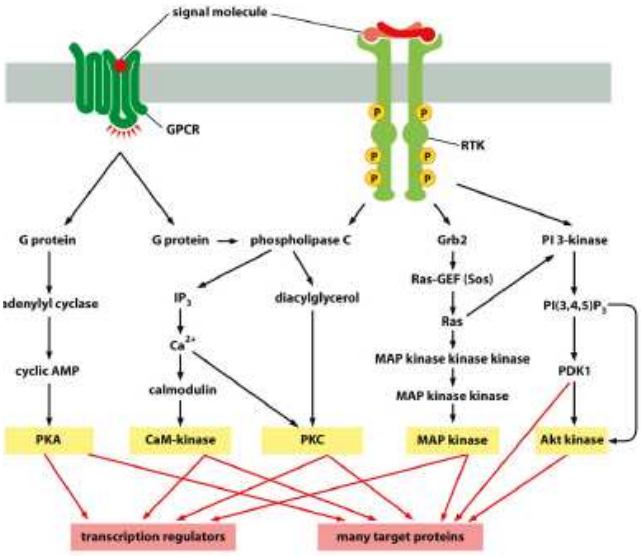


Convergence. Divergence and cross talk in signaling pathways

Convergence: when receptor can activate similar pathways. Different signals lead to expression of similar genes.

Divergence: when one receptor can activate many pathways

Cross talk: when part of one pathwayinfluences another



Studying a signaling pathway

1. Protein-protein interactions: co immunoprecipitation
2. Mutagenesis: determine key amino acids required for interaction and signaling
3. Bypass experiment: with mutant → determine order by finding if acts upstream or downstream