Discoveries and Traits of Eukaryotes, Prokaryotes, and Vira

History

Disease Origins

- Hippocrates (15th C)– est. medical school to teach disorder diagnosis & prognosis
- disorders caused by imbalance in 4 humors: phlegm, blood, yellow bile, black bile
- 17th C study of cadavers and environmental causes of disorders (eg painters, nuns)
- Percivall Pott – London surgeon (Bartholemew's hospital), identified first carcinogen in soot by studying case histories of chimney sweeps with scrotal sores (Pott's tumor, disease, & fracture)

Microscopes & Cell Theory

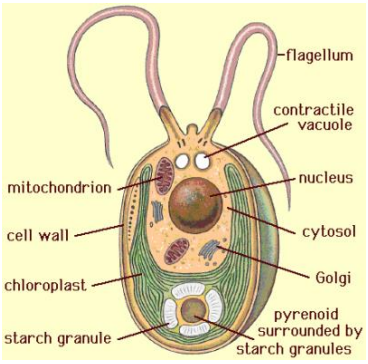
- cell theory originated at development of higher resolution instruments
- microscopes: compound light, transmission electron, scanning electron, & scanning tunneling
- Robert Hooke – 1665 micrographia of cork, contributions to optics, published book of plates of etchings (eg hairy mold); cellulae (remnants of dead cells in plants)
- van Leeuwenhoek - 17th C, first described RBCs, animacules (protozoa), and building blocks (cells)
- Schleiden (1838, plant cells) & Schwann (1839, animal cells) determined that observations were both of cellular units (solved problem of scale, 1st tenet of cell theory)
- by light microscopy: nuclei, mitochondria, chloroplasts, golgi bodies & cell walls (not membrane) visible
- trial & error development of dyes that are water soluble & are retained by specific substances (eg chromatin, membranes)

Hepatocyte Slide

- water coats proteins to allow concentrations to increase in specific regions
- mitochondria & chloroplasts appear more numerous due to cross sections observed
- composition observed can be explained by protein sorting

Eukaryotes

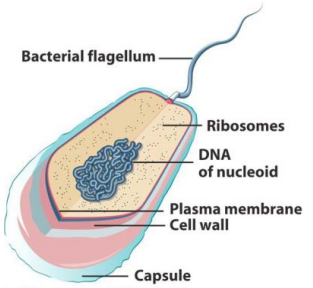
Basic Components



- eukaryotes: plasma membrane, cytoplasm, nucleus, chromatin (1.8 m), nuclear envelope, organelles (RBCs: nucleus is extruded from the cell once it leaves the bone marrow)
- plant cells: cell walls, vacuole, & chloroplasts
- sizes: 20um cell, 5um nucleus, axa up to 1m → molecular movement along cytoskeleton via motor proteins (hydrolysis) rather than by diffusion
- cilia move fluids during fetal development that determines which side of the body organs develop, cilial disorder leads to respiratory problems
- cilial movement change in paramecium results from calcium channels changing concentrations
- basic types: plant cells, animal cells, protists, unicellular algae, fungi

Prokaryotes

Basic Components



- components: flagellum, ribosome, nucleoid, plasma membrane, cell wall, capsule
- exist in wide range of natural conditions (eg atmosphere, thermophiles, gut flora, etc)
- plasma membrane (selective permeability, specialized surface functions) → liquid space → cell wall (rigidity & ion concentrations) → capsule
- if wall disrupted by cellulose digesting enzymes (eg from snails) & membrane broken down by detergent, nucleoid would relax
- bacterial flagellum – nonpliable; rotating propeller
- 1 – 5um, 1.5mm DNA in nucleoid; copied every 20 minutes; 3200 genes in E. coli

Intestinal Prokaryotes

- most gut flora are nonpathogenic & helpful (E. coli is most common)
- live mostly in proximal colon
- help in digestion: sterile animals in experiment needed 30% more food to maintain weight; break down non-digestible tissues into absorbable matter
- functions: metabolic (ferment dairy residue & mucus; salvage energy as short-chain fatty acids; production of vitamin K; absorption of ions), trophic (control of epithelial cell proliferation & differentiation via short-chain fatty acids); development & homeostasis of immune system, protective (biofilm barrier effect against pathogens)

Exceptions

- *Thiomargarita nambiensis*/sulfur pearls of Namibia (can swell up to 0.75mm)
- monocytes form phagocytic vesicles around pathogenic bacteria; vesicles deliver them to intracellular chamber with lysosomal enzymes for degredataion; *Ehrlichia chaffeensis* inhibit transport to chamber & proliferate within vesicle, conferring protection against immune response; carried by the Lone Star tick
- *Legionella* bacteria invade amoebas or macrophages of respiratory track (entry via phagocytosis); bacteria release enzymes that cause the phagocytes to form channels/whorls rather than vesicles around the pathogen

Vira

Peyton Rous (1911)

- determined that firbosarcoma resulted from a biological substance
- ran substance from chicken tumors through infectious agent-retaining Buchner funnel; could not determine nature of virus until invention of electron microscope
- first cancer-causing gene determined by studying how Rous Sarcoma (retrovirus) infects cells

Basic Components

- not living; complex structures; multiply in cells (require machinery of living organism)
- structures: protein envelope & nucleic acids, may have lipid bilayer from host cell
- bacteriophage – 0.2um; head & neck regions with legs that grip the plasma membrane and inserts the nucleic acid through sugar-uptake port (e.g. Lambda – DNA virus)

Life Cycles

- lysogenic: viral DNA becomes part of cell's own chromosome
- lytic: if DNA is damaged (eg by UV rays), cell produces new vira

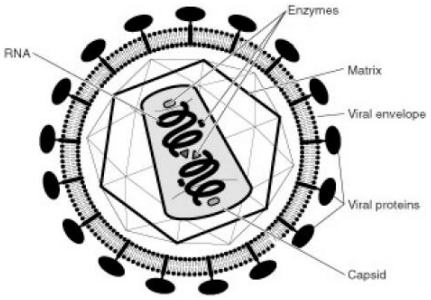
- ratio of CI and CRO proteins regulates if/when lytic cycle begins

Examples (DNA vs. RNA)

- DNA: adenovirus: childhood infection, icosahedron (20-sided) with spikes that bind to plasma membrane, must be internalized into the cell and its DNA incorporated into the nucleus
- DNA vira tend to have more complicated life cycles & contain more genetic material
- RNA: TMV: cylindrical capsid containing 1 spiral RNA; travels between cells via plasmadesmata

Retrovira

Basic Components



- lipid bilayer, protein capsid, 2 non-complementary RNA molecules (mostly identical, cannot form double helix)
- protein capsid, matrix, and nucleocapsid (+1 other in some cases) fold RNA
- enzymes (don't exist in host cells): viral polymerase (reverse transcriptase (2 subunits) + ribonuclease), protease, integrase; targeted by anti-viral drugs
- nucleic acids have only 3 genes → multiple proteins after cleavage (gag → capsid + matrix; pol → integrase, protease, polymerase; env → 2 gp envelope proteins (inserted into membrane))

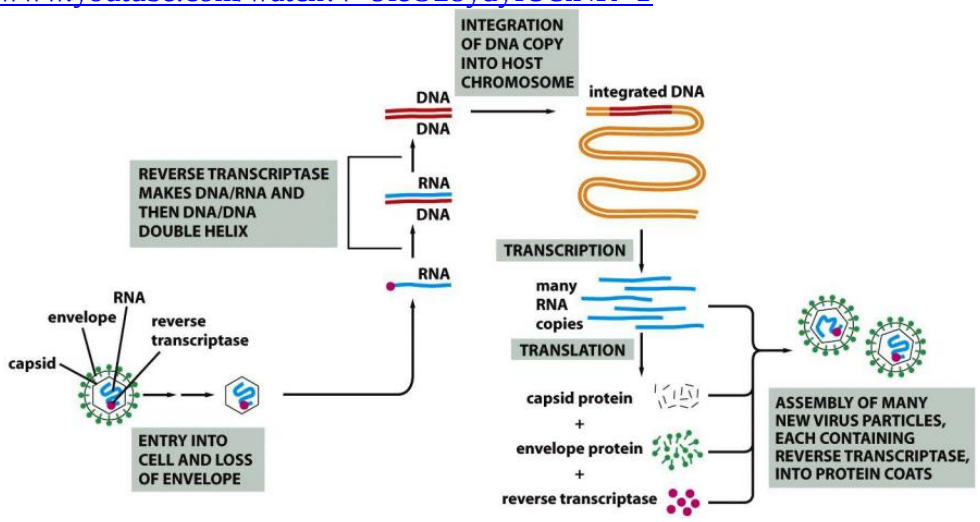
- go against Watson/Crick central dogma; discovery of recombinant DNA technology
- e.g. HIV & sarcomas

Life Cycle

- infection: host cells that a virus can infect depends on surface proteins of cell (ID tags to determine target)
  i) fusogenic proteins – surface proteins on host cell interact with viral proteins and cause the membranes to fuse
  ii) gp120 binds to cd4 membrane protein & coreceptor (β-chemokine (CCR5) on macrophages, α-chemokine (CXCR4) on helper T cells) → receptor for growth factor that causes inflammatory response; gp41 fuses cell membranes
  iii) capsid enters & disintegrates, releasing reverse transcriptase, integrase, & RNA
- reverse transcriptase: RNA → complementary DNA → opposite strand → RNA degraded
- integrase
  i) turns generated DNA into loop, covers ends with preintegration protein complex, coordinates Mg ions at active site to hydrolyze a nucleotide from each 3' end
  ii) nuclear localization signals (sequence of amino acids that allows a protein that has them to bind to a carrier protein to get through the nuclear pore complex)
  iii) can also insert when nuclear envelope disintegrates during prophase
  iv) positions hydroxyl groups over host DNA phosphorous groups 5bp apart & splices in
  v) ligates host DNA to insert at certain 4 nucleotide sequence (10^9 nucleotides in human genome = essentially random)

vi) host repair mechanisms fill gaps with complementary bp

- new vira are generated by host cell (translation, assembly at plasma membrane, budding)
  i) proteins move to staging zones marked by membrane areas with gp120
  ii) RNA & proteins bud off; immature virion reassembles capsid (15-30mins, protease must finish cleaving gag products) & infects next cell
- http://www.youtube.com/watch?v=9leO28ydyfU&NR=1

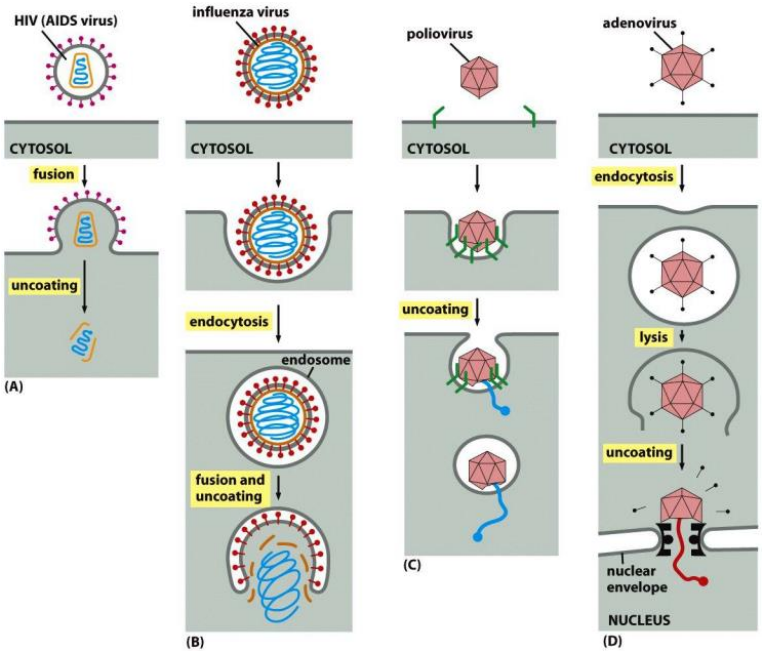


Anti-viral Drugs

- cell does not recognize foreign RNA, DNA, or antigens; recognizes viral glycoproteins, but these evolve rapidly in HIV
- targets:
  i) viral polymerase with nucleotide analogs that specifically target reverse transcription of polymerase gene
  ii) bind to active site of viral protease (specific folding & substrate requirements)
  iii) viral integrase
- HIV resistance
  i) case study of patient with HIV & multiple myeloma; radiation & chemotherapy to kill bone marrow stem cells; bone marrow transplant; lost all traces of HIV
  ii) non-functional CCR5 receptor protein (10% of Northern Europeans); there may exist a pool of helper T cells that have CCR5 receptor or only CCR5 receptor is targeted in early-stage infection
  iii) viral evolution: low viral polymerase fidelity leads to mutations that can be drug resistant

Methods of Host Cell Entry

- fusion (see HIV lifecycle)
- binds to receptor; threads nucleic acids in
- receptor-mediated endocytosis (internalized into coated vesicles)
  i) endocytic vesicle fuses with particle when pH changes due to lysosomal enzymes & uncoats
  ii) lysis & uncoating



Dynamics of Biomembranes

Overview

- Basic structures similar
- Compositional differences (primarily in proteins) account for functional differences
- Classes of lipids in the membrane
- Development of the fluid mosaic model (key experimental observations)
- Classes of membrane proteins
- Evidence for membrane fluidity
- Effect of lipid composition on membrane fluidity

Membrane Architecture

Components

- purpose: what enters and leaves the cell, how the cell responds to signals from other cells, sense the environment, transmit neural signals & action potentials
- lipid bilayer, membrane proteins, fluidity
- membrane proteins – determination of the fluid-mosaic model
- under electron microscope, all organelle & cell membranes look similar
- plasma membrane: 45% by mass lipids, 45-50% proteins, & 5-10% carbohydrates (attached to lipids & proteins)

Unit membrane (pancreatic secretory cell)

- used to study protein sorting (secretion of digestive enzymes)
- polarized structure; nucleus → rough ER & golgi body → intermediate & secretory vesicles

Electron Microscopy

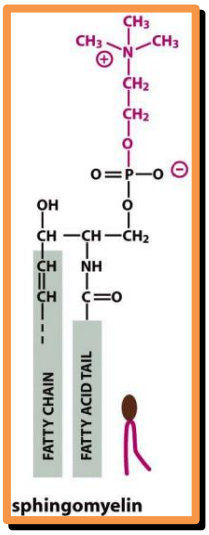
- fix the cell (treat with aldehyde & organic solvent to keep all molecules in place by creating bonds between macromolecules in the cell)
- stain (harsh & oxidative, e.g. osmium tetraoxide) [osmium – heavy atom that dissolves in lipids; dissolution at different levels in different tissues creates contrast]
- slice thin cross section (infuse with epoxy resin, slice with microtome diamond knife)

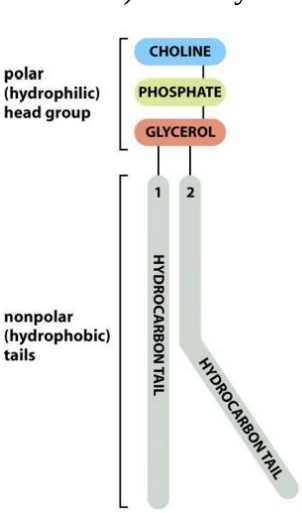
- pass accelerated electrons through cell and take picture from fluorescent screen
- assumption that structures observed were there naturally and not artifacts from preparatory process
- electron microscopes lead to discovery of structure of membranes & vira

Lipids

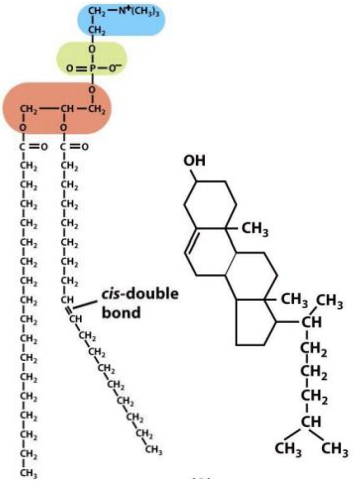
Types

- lipid – hydrophobic small molecules used for energy storage, cell membranes, and signaling; derived from ketoacyl and isoprene groups; amphiphilic
- phospholipids (main membrane lipid)
  - i) polar head group (choline + phosphate + glycerol), nonpolar tails (2 fatty acids (14-24C, usually 16-18C carbon chain & carboxylic acid))
  - ii) phosphatidyl = phosphate + choline
  - iii) e.g. phosphoglyceride (most common in animals) & sphingomyelin
- sterols (second main membrane lipid)
  - i) polar head group (-OH), rigid steroid ring structure, nonpolar hydrocarbon chain
  - ii) no cholesterol in bacteria & inner mitochondrial membrane (phagocytosis mitochondrial formation theory)
- glycolipids
  - i) extracellular signals & signaling; deposited on outer layer of membrane
  - ii) derivative of sphingomyelin
  - iii) none in bacteria or inner membranes, except where synthesized (Golgi apparatus)
  - iv) carbohydrate - $C_m(H_2O)_n$

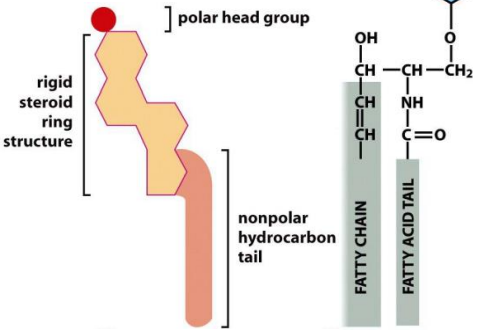




phosphoglyceride



cholesterol



glycolipid

Table 10–1 Approximate Lipid Compositions of Different Cell Membranes

LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	*E. COLI* BACTERIUM
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	28	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	44	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	23	27	30

Orientation of Lipids in the Membrane

- 1917 – Langmuir (1932 Nobel Prize in Chemistry), studied interaction of fluids (biofilms)
- Langmuir trough – drop arachidonic acid into water; forms monolayer with polar heads of oil molecules pointing down; decrease surface area by squeezing layer together; measure pressure needed to determine isotherm
- 1925 – Gortel & Grendel – extracted all lipids from RBC membranes with acetone and compared known total surface area to measured lipid surface area (using Langmuir trough)
- used RBC because these cell fragments only have 1 membrane
- challenge: 1cc of blood, 5x10^9 RBC (half the volume of blood) → assuming RBCs are spheres, what would be the surface area of the lipids on a Langmuir trough?
  V = 0.5cm^3/5x10^9 = 4/3*pi*r^3; r = .00000287941m; SA = 4*pi*r^2 = 1.04188 x 10^-10
  SA = 2 * 5x10^9 * SA = 1.0418m^2

Lipid Bilayer Features

- membrane width: ~30 Angstroms, ~60 Angstroms after interaction with osmium petroxide
- hydrophobic ends are never exposed (liposome bilayer sphere is lowest energy state)
- liposomes are being adapted for drug delivery
- fluidity: change composition of membrane in response to environmental factors
  i) chain length (decreases – more chain interactions) & unsaturation (increases – kinks in chain mean fewer interactions)

Lipid Behavior

- spontaneously form micelles/liposomes (25nm-1um) & black membranes (hole in partition between two aqueous compartments)
- lateral movement: gold-particle label → extremely rapid
- flip-flop: spin label detected by electron spin resonance spectroscopy → once per month
- phospholipid translocators catalyze flip-flopping since synthesis occurs only in cytosolic leaflet

Proteins

Basic Structure

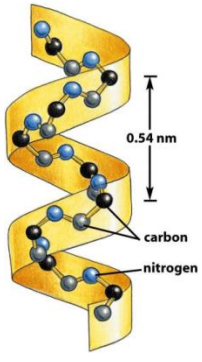
- polypeptide assembly: dehydration that converts carboxylate (-) & primary amine (+) to secondary amide
- polar & nonpolar side chains on alternating sides of polypeptide backbone
- polypeptide traits: two ends (amino & carboxylic), peptide bond, side groups
- H bonding: low energy state; since nonpolar oils break H bonds, water will bead when placed on oil layer

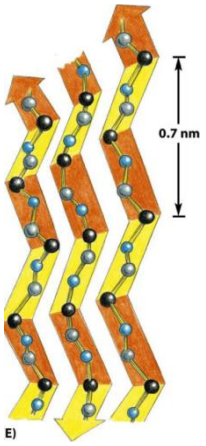
Linus Pauling (1901-94)

- alpha-helix secondary structure (Nobel Chemistry Prize 1954)
- Xray diffraction: dehydrate protein & bombard with Xrays; patterns reveal underlying structure
- anti-nuclear proliferation (Nobel Peace Prize 1963): warned of effects of Xrays (e.g. irradiation of arthritic tissues used as treatment through 1950s); demonized by military
- taking vitamin C & other antioxidants to help colds

Secondary Structures

- alpha-helix
  i) right-handed turn (beta-helix is left-handed)
  ii) H bond between amino H and carbonyl O every fourth amino acid
  iii) R groups sticking outwards (all must be nonpolar in lipid bilayer)
  iv) 1.5 Angstroms between each amino acids





v) how many amino acids would be needed to cross the plasma membrane?: 20
vi) e.g. bacteriarhodopsin

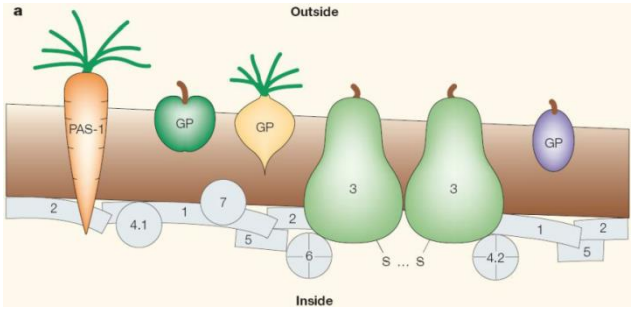
- beta-pleated sheets
  i) antiparallel & parallel defined by directions of backbones relative to each other
  ii) H bonds between chains (similar to alpha-helix)
  iii) R groups face upwards or downwards from plane
  iv) e.g. pore beta barrels

Fluid Mosaic Model

Davson & Danielli model (1935)

- by 1950's, it was known that membranes had proteins & general protein structure; but this model was assumed to be true until 1970s
- membrane proteins coated exterior & interior of membrane; suggested that osmium bonded with proteins to produce "railroad track" appearance

Singer & Nicholson model (1972)



- fluid mosaic model; proteins were globular structures that floated in sea of lipids
- pears are pores with disulfide bonds
- mosaic: how was it established that proteins were stuck in lipid bilayer rather than coating it?
  i) Freeze fracture method (revealed integral nature of membrane proteins)
  ii) measured conformations of proteins in membrane (tend to be globular, which indicated they wouldn't be flat and coat the membrane)
- fluid: how do we know that the membrane is not static?
  i) Heterokaryon membrane protein assay
  ii) Photobleaching assay

Freeze Fracture Method

- revealed integral nature of some membrane proteins
- liquid nitrogen quick freezing maintained structures (water doesn't crystallize)
- ragged cut with dull (glass) knife – reveals outer membrane, inner membrane, & cell interior
- exposed to vacuum to remove water, then cast in metal (platinum-carbon vapor), then studied by electron microscopy

Integral & Peripheral Proteins

- integral proteins – cannot be removed by ionic solution; only detergent can extract them from the membrane (hyrophobic domains)
- peripheral proteins – ionically bond to a molecule in the membrane (lipid, protein, or sugar); ionic solution can extract these from membrane
- membrane proteins are usually made in ribosomes on ER, which thread polypeptide into ER membrane, which packages it into a vesicle and sends it to the cytoplasm where it is folded
- some (integral & peripheral) proteins are covalently linked to a lipid anchor (e.g. signaling molecules, which need to move laterally) → are neither integral nor peripheral because they do not have ionic bonds (e.g. myristoyl, palmitoyl, farnesyl anchors)
- all are generally glycosylated on extracellular surface (cell-cell interaction, binding, signaling molecules bind to glycosulated

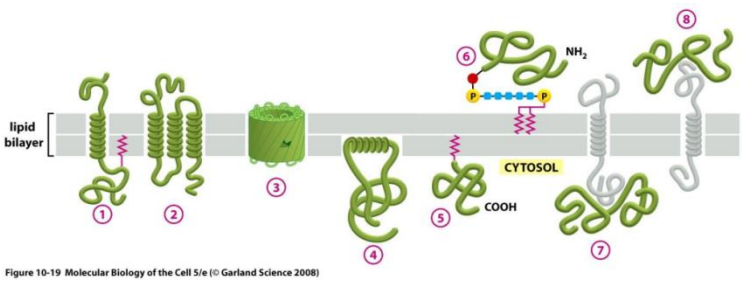


Figure 10-19 Molecular Biology of the Cell 5/e (© Garland Science 2008)

domains, connection to extracellular matrix) → sugars added at protein production center, such as Golgi body (lymphocyte slide – ruthenum red dye binds to sugar groups)

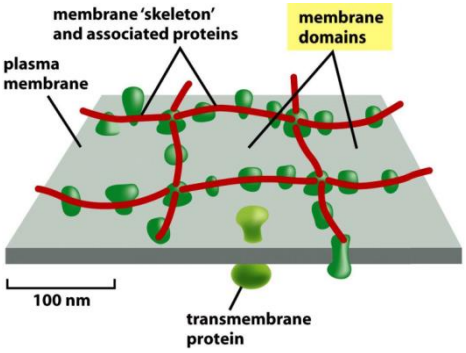
- hydropathy plot – determines transmembrane/hydrophobic segments

Membrane Fluidity

- protein movement and lipid fluidity
- phagocytosis requires vesicle to bud off from membrane
- secretory vesicles must leave Golgi body & ER and fuse with plasma membrane
- fluidity allows membranes to stay fluid (not form gel/solid) under all conditions
- two assays to measure lateral motility of proteins in membranes:
  i) Frye & Edidin (1970) : mouse-human heterokaryon via retroviral (sendai virus) fusogenic proteins (can also be achieved by exposure to polyethylene glycol); bind to species antibodies with fluorescent markers; incubate and observe colors
  ii) heterokaryon – chromosomes will mix and mitosis will divide them up → further divisions → chromosome loss → hybrids still viable for some time
  iii) Schlessinger et al (1976): FRAP method - fluorescent recovery after photobleaching; bind fluorescent markers (ConA linked to dye) to surface proteins (binds to sugars) on rat embryonic cells (myocytes), bleach with laser beam, and measure membrane with phototubes after recovery (55% recovery after 15 minutes)
  iv) FLIP method - beam continuously illuminates one spot and loss of light from other areas on membrane measured

Fluidity Exceptions

- epithelial cells: basal lamina, edges of cell fused with tight junction; glucose travels from gut to blood through from apical to lateral surface & diffuse across basal lamina into capillaries
- proteins restricted to apical surface will not travel to lateral of basal membranes
- RBC: fibrous proteins anchored to integral proteins; proteins can move within sectors (inner leaflet; connected to cytoskeleton)



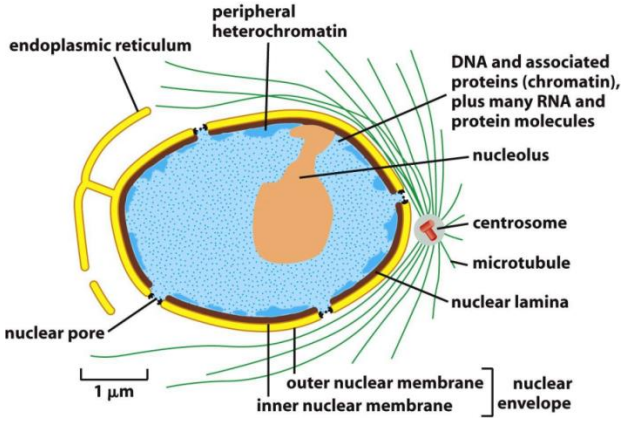
Temperature

- lipid bilayer: supportive with dynamic structures; crystal-like
- transition temp (Tm): changes from fluid to solid/gel state as mobility of membrane lipids drops
- how can you modify the Tm (esp. in organisms which cannot regulate their body temperature)?
- the more ordered the membrane structure, the higher the Tm
- enzyme synthesis induced by environmental temperature drops
  i) unsaturation: decreases Tm (cis-double bond kinks keep phospholipid cylinders apart)
  ii) e.g. bacteria in soil, cold-tolerant pansies, carps when placed in cold water, prior to hibernation to keep systems functioning
  iii) shorter fatty acids have fewer (lateral hydrophobic and van der Waals) interactions within and between layers
  iv) e.g. membranes can drop from 18C to 16C
- cholesterol: polar OH near polar head group; greater concentrations increases fluidity by decreasing organization; animal mechanism; nonpolar rings and chain
- **how do you design an experiment to test these concepts?**

The Nucleus

Overview

- Structure and Roles of Envelope
- Function of the Nucleolus
- The Nuclear Lamina
- Chromatin Composition and Packaging
- Karyotype Analysis
- Prokaryotic Chromosomes



Structures

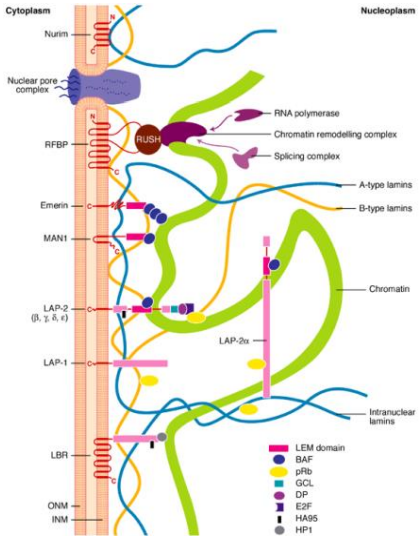
Basic Composition

- usually 5um in a 20um cell and round (except WBC nuclei are globular; absence of these structures indicates a malignancy)

Nucleolus

- stainable region (organelles are membrane-bound); area of different density
- nucleolar organizers – site of transcription of clusters of ribosomal genes; ribosomal proteins enter nucleus from cytoplasm to join rRNA for beginning of ribosomal assembly
- ribosomes: particles that provide surface for mRNA translation
  i) ribosome subunits in animal cells: 40S (18S RNA & 33 proteins); 60S (5S + 5.8S + 28S RNA & 49 proteins); different in other cell types
  ii) Svedberg – size & folding; rate of moving from surface to end of centrifuge tube
- humans may have up to 10 nucleoli which can coalesce to form 1 or 2
- tend to be small or nonexistant in terminally differentiated nongrowing cells

Nuclear Envelope



- microtubule production site
- two continuous lipid bilayer membranes; outer membrane can be continuous with other cellular membranes (e.g. ER, may hold ribosomes to produce nuclear proteins)
- dark areas are chromatin regions; anchored to lamina on nucleoplasm face; discontinuities indicate nuclear pores

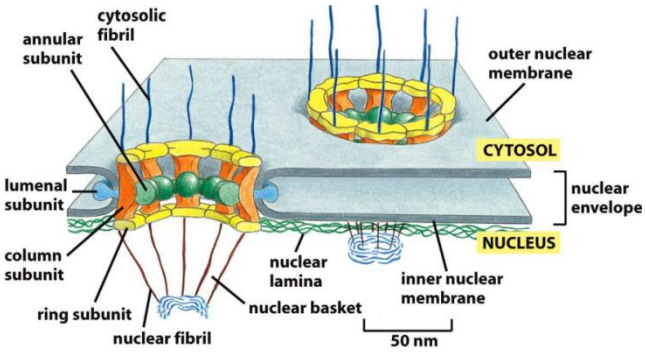
Nuclear Lamina

- fibrous proteins associated with integral proteins on inner membrane
- provides structural rigidity (against centrifugation & detergent)
- 450 molecules of 30 different proteins
- alpha lamin criss-crosses nuclear pores
- falls apart during mitosis because lamina proteins are phosphorylated (lamin + enzyme + ATP); association with each other changes; phosphorous groups removed after cytokinesis
- integral proteins may be transcription factors, thus are bound with chromatin by lamina

Nuclear Pore Complex

- densities vary (e.g. 3-4K in mammalian cells; several million in amphibious eggs) due to size & activity
- ATP, GTP, ions, water, and small proteins (<20,000mw) can diffuse easily through spokes of nuclear basket
- how would you design an experiment to determine how large molecules move in and out of the nucleus (must follow fate from cytoplasm to nucleoplasm without blocking pores)?

i) Inject non-nuclear protein (albumin – control for injection location & damage) & large nuclear protein linked with electron micrograph visible tag (e.g. gold particle or other heavy metal) into cytoplasm; take pictures over time to show that proteins align with pore complex



- motor (shuttle/RAN) proteins bind to specific amino acid sequence (nuclear localization signal) on large nuclear protein → motor protein becomes associated with fibrils → GTP hydrolysis
  i) same method for entry and exit
  ii) protein may bind to 2 RAN or to another protein which binds to a RAN
- pores are flexible to accommodate larger molecules & are bound to lamina
- active, energy-dependent movements: mRNA to cytoplasm & proteins to nucleus
- rate of ~1000 subunits per second; flow can be bidirectional

Eukaryotic Chromosomes

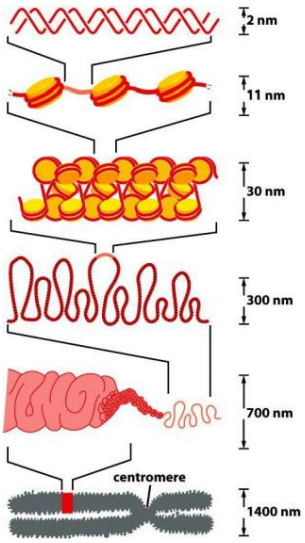
Gene Composition

Organism	Base Pairs (kb)	Length (mm)	Shape
SV40	5.1	0.0017	circular
Lambda	49	0.0166	linear
E. coli	4,000	1.36	circular
Yeast	13,500	4.6	17 chrom. (hap)
Drosophila	165,000	56	4 chrom. (hap)
Human	2,900,000	990	23 chrom. (hap)

- 3.2 x 10^9bp in human genome → 25000 genes → 27000bp per gene → 1300bp per coding sequence (1.5%)
- DNA types: transposable elements, regulatory sequences, high-copy repetitive elements, pseudogenes, replication origin
- scale: if a nucleus were a tennis ball, the chromatin would be 25 miles long → shortening about 10,000x to fit into chromosome
- chromosome evolution phylogenetic tree via conserved synteny

Protein Composition: Histones & Nucleosome Folding

- DNA forms: heterochromatin (may be purely structural, e.g. centromere & telomeres); euchromatin (extended, expressable)
- weight ratios relative to DNA: ~1 histone (purely structural); ~0.7 non-histone proteins
- octramer of H2A, H2B, H3, & H4 (nucleosome); H1 (binds to linker DNA to hold nucleosome together), H5 (RBC in birds only)
  i) histone fold structural motif: 3 alpha-helices connected by 2 loops
  i) H3-H4 dimers form tetramer, then bind to H2A-H2B dimers
  ii) named by peak orders from chromatography column
- DNA wrapped in left-handed coil 1.7x around histone core (110 A wide x 55 A high)
  i) rich in basic amino acids (e.g. lysine & arginine) → positive charge (binds to negatively-charged phosphate groups on double helix backbone) + 142 H bonds
  ii) N-terminal tails extend out of core; subject to covalent modification
  iii) T-A dinucleotide minor grooves preferred on inside → tighter binding
- fairly small for proteins; highly conserved



Dynamic Nucleosome Structure

- nucleosomes unwrap every 250ms, exposing binding sites
- ATP-dependent chromatin remodeling complexes loosen DNA-histone binding, revealing

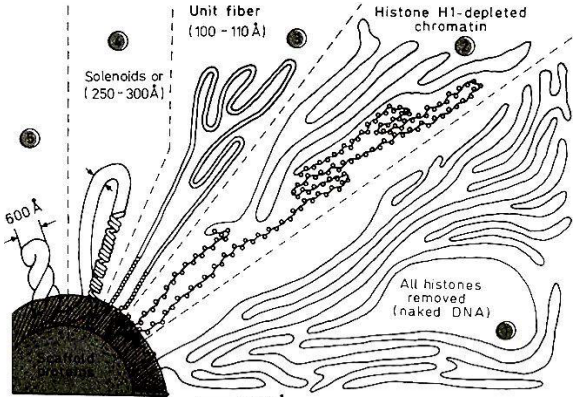
binding sites by sliding DNA forward or allowing histone exchange (H2A-H2B or all 4) via negatively-charged histone chaperones

Cell Cycle

- G0 (most cells) → G1 → checkpoint 1 (no turning back) → S → checkpoint 2 → G2 → M
- Prophase: chromatin condensation, nuclear envelope disintegrates, microtubules fall apart

Higher-order Folding

- issues: condensation & expression
- isolate nuclei in G1 & treat with different salt concentrations (since that protein-DNA interactions are ionic) → examine under electron microscopy
  - i) zigzag model: 100 A-thick nucleosome beads on DNA-thick strand → histone tails + H1 → 300 Angstrom-thick strand (solenoid)
  - ii) endonuclease clips linker DNA → separate electrophoretically → determined length of DNA associated with one nuclesome



  - iii) ~200 nucleotide nucleosome → nuclease digests linker DNA (allow digestion to progress, since histone will protect DNA from endonuclease) → dissociation in ionic solution → 165 nucleotide DNA strand & octameric histone core
  - iv) Loemmli – isolated metaphase chromosomes, extracted histones with salt solution → scaffolding (non-histone; holds DNA in chromosome shape & remaining DNA in looped domains
  - v) staining: bands due to folding & protease

Chromosomal Abnormalities

Karyotypes

- chromosome – sister chromatids linked at kinetochore
- karyotype analysis – detecting chromosomal aberrations
- goal: freeze in metaphase & disrupt spindle fibers
- growing cell culture → colchicine (freezes in prophase, 12-20 hours) → fix cells in methanol-acetic acid (kills cells) → splash onto slides (breaks apart cells) → trypsin solution (protease, 10-15 min, clear proteins that could mask stain-binding sites) → stain with Giemsa

  - i) colchicine – disintegrates microtubule polymers; plant enzyme defense mechanism against predators; allows cells to progress to mitosis, then freezes them there
  - ii) G-banding – Giemsa stains heterochromatic, late-replicating, AT-rich DNA (dark regions); does not stain euchromatic, early-replicating, GC-rich DNA (light regions)
- spectral karyotyping – label chromosome-specific nucleotide sequences with fluorophores covalently linked to cDNA (hybridization) instead of Giemsa stain; must first increase temperature and change solvent to denature protein; sequences found from human genome project

Philadelphia Chromosome

- Nowell & Hungerford (1960, Upenn) – shortened chromosome 22 in chronic myeloid leukemia due to t(9;22) reciprocal translocation
- chronic phase: elevation in leukocyte count → crisis phase: WBC count too high, RBC count too low (anemic), kidney failure (too many WBC proteins)
- now diagnosis by bone marrow sampling; check if ch9 is too long and ch22 is too short; number of abnormal cells indicate stage of disease progression
- Philadelphia chromosome: ch22 chromosome (BCR, high expression) + ch9 section (ABL

kinase (phosphorylation) which induces cell proliferation) → fused protein
- o new cancer drugs target ABL by binding to kinase active site
- o reciprocal translocations cause >350 disorders, esp. in hematopoetic cells

Prokaryotic Chromosomes

- o foreign DNA: if viral DNA enters cell, histones will associate with it & condense the chromosome
- o bacteria don't have histones (although archea have histone-like protein)
- o condensation to nucleoid
  - i) supercoiling via toposiomerases ligating, twisting, & rejoining DNA
  - ii) negative & positive

Vira Reading
vira usually use receptor molecules, commonly with a coreceptor, to enter the cell
goals: recognition & attachment, enter host cell, release genome
enveloped vira use membrane fusion; nonenveloped vira use pore formation & membrane disruption
similar to SNARE-mediated intracellular vesicle fusion
vira must regulate fusion to only fuse with appropriate host cell membranes & not with each other
HIV – binding to receptor causes conformational change which exposes fusion protein
influenza virus – receptor-mediated endocytosis; when endosome acidifies, membranes fuse, releasing viral DNA & capsid proteins
H+ pumped into endosome enters virion through ion channels → triggers uncoating of RNA & exposure of fusion protein
polio – binds to receptor, then forms pore (exposes hydrophobic projection) through which it extrudes its RNA
adenovirus – receptor-mediated endocytosis → reducing environment of endosome activates viral protease which uncoats DNA → protein lyses endosome membrane → releases some of the viral DNA & capsid proteins

bacteria phagocytosis
tuberculosis – alveolar macrophages contain infection within tubercule lesion, which is walled off by fibrous capsule & is calcified
bacteria can induce phagocytosis in a nonphagocytic cell by expressing an adhesion which binds to cell adhesins as if the bacteria were another host cell or the ECM
cells attempts to form cell junction by moving actin & cytoskeletal components to site of attachment → since bacteria are comparatively small, attempt leads to uptake of bacterium (zipper mechanism)
trigger mechanism: bacterium injects effector molecules into host cytoplasm → activates Rho-family GTPases, which stimulate actin polymerization OR severe actin filaments & rearrange cross-linking proteins → localized ruffling on cell surface which traps bacterium in actin-rich profusions which form the macropinosome

intracellular eukarytoic parasites
must expend own energy to infect host cell
Malaria & Toxoplasma: extends microtubule conoid into host cell → cell invaginates → compartment does not have transmembrane proteins, does not participate in vesicle traffic or fuse with lysosomes, takes up metabolic intermediates & nutrients from cytosol

Trypansoma: binds to cell receptors, causes Ca2+ elevation in cytosol → signal recruits lysosomes to fuse with cell membrane → parasitic enzyme removes saliac acid from lysosomal glycoproteins and coats own membrane with cell sugars → forms pore to lyse lysosome
microsporidia: polar tube extends into host cell & delivers spore contents via osmotic pressure

intracellular parasites alter vesicle traffic
can escape endosome, prevent fusion with lysosomes, or surive in phagolysosome
Listeria: releases lysteriolysin O, which is inactive at neutral pH & degraded by host proteasome
methods: Toxoplasma, prevent endosome maturation (TB), arrest before lysosomal (Salmonella), enclosure in layers of rough ER (Legionnaire's), use exocytotic compartment that fuses with Golgi body vesicles (Chlamydia)

viral maturation (budding)
usual route: membrane proteins travel from ER to Golgi to plasma membrane, post-translation modification en route
poliovirus replication: increases lipid production & prevents ER transport to increase budding
Herpes: coated in ER, uncoated, then recoated in Golgi
Vaccinia: coated in Golgi (2 membranes), then again (4), then released (3)

membrane reading
lipid rafts – domains of sphingomyelin; thicker to accommodate certain membrane proteins; stabilized by certain proteins; help organize membrane proteins
    lipid anchors control membrane localization of some signaling proteins
lipid droplets – neutral lipids are contained in phospholipid monolayer which breaks off from ER containing proteins (e.g. for lipid synthesis)
lipid asymmetry – leaflets are differently charged (e.g. phosphatidylcholine & sphinogmyelin on outside, phosphatidylethanolamine & phosphatidylserine [charged] on inside); helps to convert extracellular signals to intracellular ones
    e.g. PKC requires negative charge
lipid head groups may be modified to create protein-binding sites
    e.g. phosphate groups are added to phosphatidylinositol to recruit cytosolic proteins to membrane
phospholipases translate external signals to internal ones by cleaving phospholipids to generate short-lived intracellular mediators
apoptosis – phosphatidylserine is moved to outer leaflet by translocators or scramblase

Membrane Transport

Overview

- Diffusion: Simple and carrier-mediated
- Approaches in studying transport (how to differentiate between types; nature of proteins involved in each, glucose & ions)
- Active transport (e.g. ions & glucose in intenstinal cells, LDL uptake by receptor-mediated endocytosis, sports drinks, cholera)
- Trans-cellular transport
- Ion gradients and membrane potential
- Sensory response (how proteins are inserted into membranes, ionic currents on neurons)

Passive Transport

Diffusion

- towards equilibrium: chance of particles colliding with membrane on higher concentration side is higher than from lower concentration side, leading to a net movement of particles
- simple/passive – lipid soluble, permeable membrane (O2, CO2)
  i) Vin is proportional to [Sout], Vout is proportional to [Sin]
  ii) V = flux = movement/(area*time)
  iii) Net flux = Vin – Vout = K([Sout] - [Sin])
- carrier-mediated – channels or carriers (translocators: GLUT & glycerol channel)

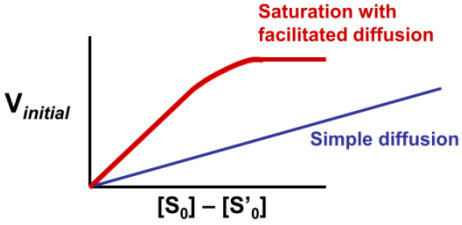
Methods for Testing These Kinetic Differences

- Whole cells
  i) put RBCs in water → uptake & expansions will cause holes in membranes to form and leak out hemoglobin & glucose metabolism molecules→ centrifuge → return to correct ionic concentration solution → holes close & membrane returns to original shape → RBC ghosts
  ii) empty vesicle allows you to study flow in and out of; glucose will not be modified
- subcellular vesicles: digest bacterium cell wall → fragment membrane via mechanical agitation (e.g. sonic oscillation) → vesicles form with diluted cell contents
- artificial membranes: put phospholipids, detergent, & membrane protein in dialysis bag → as detergent seeps out, liposomes form to create artificial vesicles
- assay setup: cell/vesicle/membrane fragment suspension → incubate with radioactively tagged peptides (must be at different concentrations in and outside of cell) → pellet via centrifugation → compare concentrations in pellet and in solution

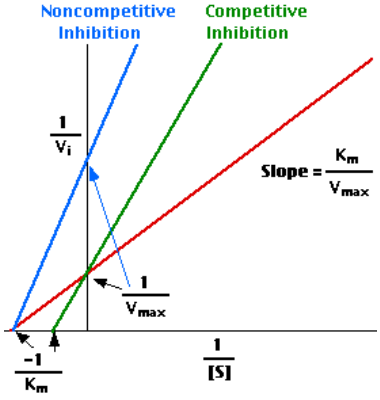
Facilitated Diffusion: Properties

- Channels, saturable, flux in direction of equilibrium
- Mutation blocks specific transport (glycerol channel/facilitator protein)
- Ligand-specific carrier (glucose translocator protein)
- Physiological regulation of facilitated diffusion (glucose carrier translocation in adipocytes)
- Water movement through kidney tubules

Facilitated Diffusion: Enzyme Saturation Kinetics



- rate reflects ability of the carrier to bind to individual molecules (graph: concentration vs. rate of uptake) (Vmax)
- faster rate of saturation = functions maximally at lower concentration; higher affinity for substrate (Km = conc difference at Vmax)
- competitive inhibition – binding of an

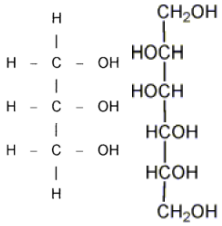


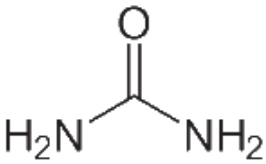
inhibitor to an active site prevents enzyme from binding to substrate

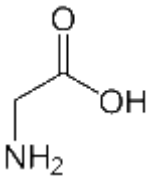
- Lineweaver-Burk plot: 1/V vs. 1/S, x-int at -1/Km, y-int at 1/Vmax

Glycerol Channel in E. coli

- aquaporin (aqueous channel, 10^9 water molecules can diffuse across)
- glycerol channel is a non-specific (allow molecules of a certain size to diffuse across)
- 6 alpha-helical segments that form a cylinder
- allows molecules to move between cytoplasm & periplasm (compartment between membrane and wall, usually concentrations outside cell are higher)
- highly selective: allows movement of sugars & water, but not ions → selectivity loops in center of channel (potential explanation: positively charged side chains)
- facilitator protein: glycerol, straight-chain sugars, urea, glycine







Methods for Testing Competitive Inhibition

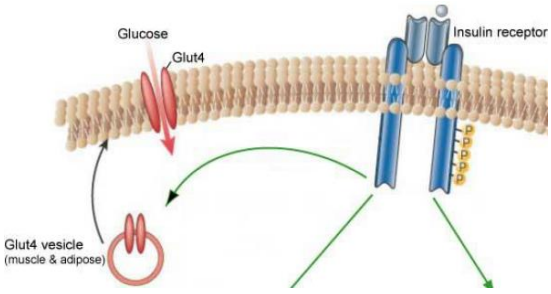
- trace radioactive substrates
- purify channel in liposome, observe that channel functions maximally at higher concentration when incubated in two substrates
- mutate gene for receptor and observe that all substrates move slower/fail to move

Glucose Translocator Protein

- fate of glucose: metabolized via glycolysis in mitochondria into ATP or turned into glycogen if in excess
- structure: binding site for ring formation of glucose/other 6-carbon sugars → equilibrium drives conformation change towards cytosol or towards blood
  i) 12 alpha-helical transmembrane segments form cylindrical wall (similar to most transporter proteins)
  ii) slightly modified versions in humans
- in most cells, flow is unidirectional: glucose is deposited in cell & phosphorylated into glucose-6-phosphate (first step of glycolysis, catalyzed by hexokinase)

GLUT varieties

- Km – concentration of glucose at which transporter is functioning at half the maximal rate
- GLUT 1 & 3: 1mM, most cells → highest affinity
- GLUT 2: 15-20mM, liver, intestines & pancreas → lowest affinity (excess glucose stored as glycogen in liver)
- GLUT 4: 4-5mM, adipocytes & muscle
- in blood tests: normal concentration (long after meal but long before fasting) is ~100mg/dL = 5mM → brain and heart, but not liver, is at saturation, allowing vital structures to have glucose even at low concentrations
  i) 85mg/dL = fasting; 250mg/dL = too high

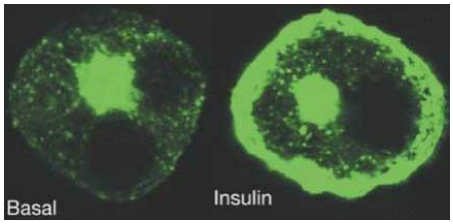


Hormonal Regulation of GLUT receptors

- pancreatic hormones affect metabolism of variety of tissues
- low blood glucose → glucagon released by alpha cells → liver releases glucose into blood;

reverse glycolysis, glucose formation from amino acids, breakdown of glycogen

- high blood glucose → insulin released by beta cells → fats cells take in glucose from blood
- green fluorescent proteins (GFP) – produced by jellyfish; used as protein marker in living organisms

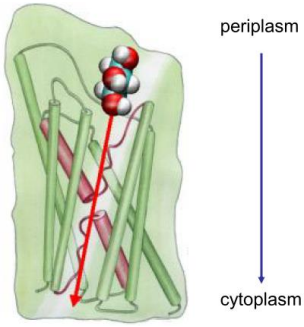


i) append GFP gene to coding sequence for GLUT carrier via viral vector

ii) when adipocytes were transfected with this construct, observed that GLUT 4 was redistributed from GLUT4 vesicles to cell surface (vesicles fused) when insulin is added to culture (e.g. after a meal)→ vesicles reform when insulin is removed

iii) insulin receptors → GLUT4 receptors move to membrane + cell growth & differentiation

Mammalian Aquaporins



- structure: similar to glycerol channel in E. coli; 28kD tetramer; 1 NPA selectivity loop from each side; glycoprotein
- 10 different types: 2 found in kidney; 2 others (6 & 7) involved in glycerol movement in adipocytes; some involved in water vapor concentrations in alveoli; some in eye lens (differences in distribution & rates)
  - i) aquaporin 7 knockout mice → obesity
  - ii) fatty acid breakdown in adipocytes: glycerol cannot be released
- regulation of water movement through kidney tubules:
  - i) renal capillaries → water, glucose, ions are filtered out → tubules deposit into bladder
  - ii) water retention: kidney cells will pick up water from distal tubules and redeposit into capillaries
  - iii) hypothalamus cells detect concentration of water around them → signal vasopressin (ADH; 9 amino acid chain) release from pituitary gland → binds to receptor → aquaporin 2 vesicles will merge with plasma membrane → increases rate of water through kidney cells
- cell culture: suspend in aqueous solution with amino acids, vitamins, fetal cow serum (FCS), incubated at 37degC
- selectivity loops: C=O on one side, hydrophobic on the other → too narrow for ion
- 2 asparagines occupy oxygen valencies → prevent H+ diffusion between H2O molecules

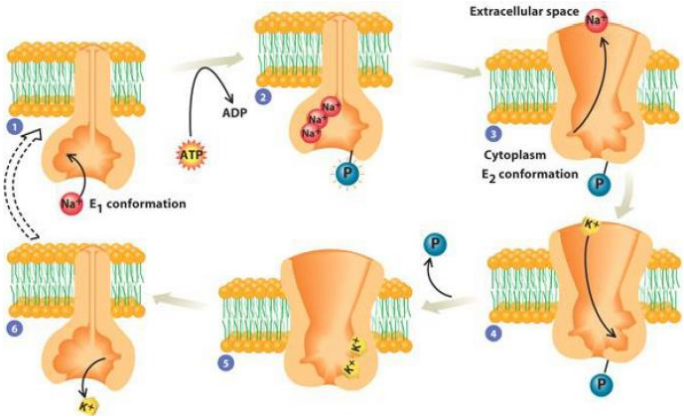
Active Transport

Types

- active transport – energy-requiring; movement against concentration gradient
- carriers show saturation kinetics
- concentration gradient kept constant: charge differences/membrane potentials are energy source to drive reactions (neurotransmission, movements of amino acids & glucose, muscle contraction) or deactivate digestive enzymes
- primary, secondary, & trans-cellular
- uniport, antiport, & symport

Primary Active Transport: Sodium & Potassium Gradient

- in a typical mammalian cell, extra (Na+, Cl-, HCO3-, Mg2+, Ca2+), intra (K+)
- Jen Skau (1957, 1997 Nobel Prize): discovered that activity of one ATPase could be manipulated by changing Na+ & K+ concentrations
- Na+-K+ ATPase in neurons – 3Na+ out of cell,



2K+ into cell

- 3Na+ into E1 conformation pump → conformation change by additional charge hydrolyzes ATP to ADP, Pi added to pump → change to E2 conformation, affinity for Na+ decreases → 2K+ into pump → change to E1, Pi released → affinity for K+ decreases
- 10 alpha-helical regions; alpha (ATP & ion binding sites) & beta subunits

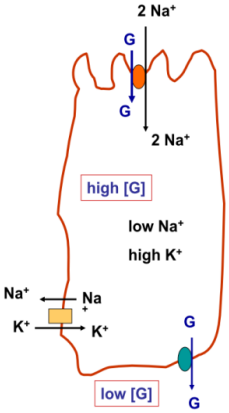
Cardiac Glycosides

- e.g. ouabain & digoxin → Digitalis
- plant seeds known for millenia to help with heart pain
- inhibit Na+-K+ ATPase by binding to first extracellular segment to freeze in E2 conformation
- helps patients with myocardial infarction (narrowing of vessels leading to heart → lack of oxygenated blood reaching heart → weakening of heart muscles) & atrial fibrillation (atria don't fully contract)
- mechanism: raised levels of intracellular Na → inhibition of Na-Ca antiport → raised levels of intracellular Ca → greater uptake into sarcoplasmic reticulum → greater Ca release on stimulation, leading to faster & more powerful myocyte contraction and longer refractory period (heart rate regulation)

Proton Pumps

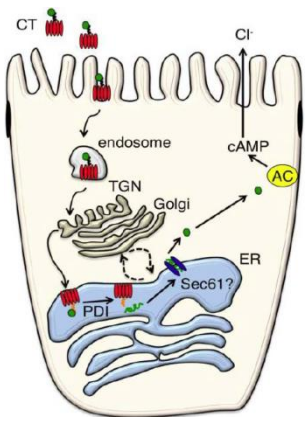
- enzymes that hydrolyze ATP & move protons into a compartment, decreasing the pH → intracellular digestion
- degredative/hydrolytic enzymes in lysosomes work optimally at pH4-5 to protect against accidental damage by enzymes outside of lysosome
- primary – movement of substance is directly related to hydrolysis of ATP

Secondary Active Transport: Glucose in Intestinal Cells



- secondary/coupled – energy generated by another process associated with ATP hydrolysis
- intestinal structures
  - i) transcellular transport: glucose must move against concentration gradient from lumen through epithelial cells into blood vessels
  - ii) lumen → epithelial layer → connective fibers → smooth muscle → more connective fibers → epithelial layer → peritoneal cavity
  - iii) columnar cells: elongated base, ruffled surface to increase absorption area, tight junctions
  - iv) tight junctions – e.g. claudins (crosses membrane 4 times), claudins of one cell interact with claudins of another cell ionically (Ca2+) at kissing points
- glucose carrier
  - i) Unlike with GLUT carriers, glucose is moving against concentration gradient
  - ii) Na+-K+ ATPase on basolateral face (side near capillaries) creates ion gradient → transport protein (apical face) brings glucose + 2Na+ into cell → moves out of cell into blood via GLUT2 (basal face)
  - iii) symport: two substances brought in together; one along it concentration gradient and another against its concentration gradient; also used for amino acid uptake in intestinal cells & glucose transport in kidney cells

Cholera & Gatorade



- water & Cl- moves along with Na+
- gatorade: hydration effective because allows more rapid uptake of ion, glucose, & water
- water moves with ions so osmotic pressure does not change

- vibrio cholera: severe dehydration; cholera toxin (A+B subunits) are internalized by intestinal cells leading to ion release into lumen
- toxin binds to surface receptors, receptor-mediated endocytosis → endosome delivers CT to rough ER → subunit A is released into cytoplasm → covalent modification of G-protein → AC generates cAMP → PKA activated → PKA phosphorylates CFTR (cystic fibrosis), which opens Cl- channel → ions & water leave cell
- treatment: 18g sugar & 3g salt in 1L water; salt packets sold with sodas

Methods for Testing Coupled Receptor

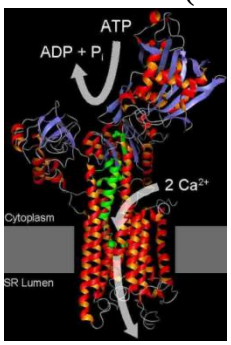
- cut longitudinal strip of intestine → dangle between two chambers → put different substances on lumen/apical facing chamber
  i) seal two wells together with tissue layer & drill holes in sides for solution insertion
  ii) KCl in both chambers
  iii) radioactively tag molecules to observe movement between chambers
  iv) required for movement: apical chamber = glucose + Na + no ouabain

Trans-cellular Transport

- infant immune system cannot recognize foreign substances
- antibody/immunoglobin subunits: 2 light-chain & 2 heavy-chain
- variable antigen binding region & FC segment (common region)
- IgA antibodies generated in mother's lymphocytes & secreted into milk → pup FC receptors on apical face of intestine transport antibodies via endocytic vesicles → internal immunity against intestinal pathogens
- IgG transport across placenta during human pregnancy

Calcium Pump

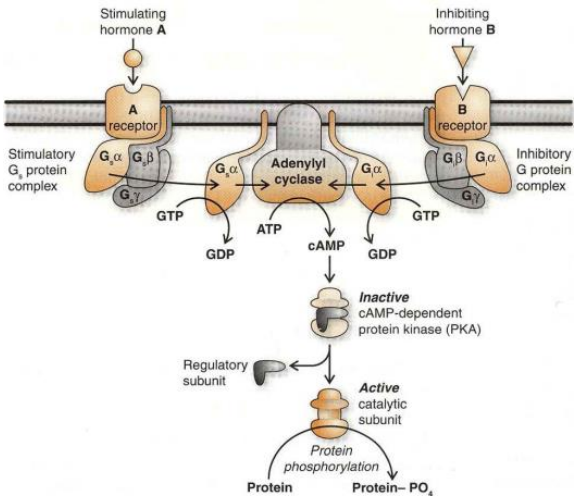
- moves 2Ca outside of cell or inside of smooth ER
- same mechanisms as Na-K ATPase
- ATP hydrolysis twists helices slightly such that Ca ions face other side of membrane
- (heart) muscle contraction:



i) depolarization of membrane/voltage change (low intra Na & Ca → Na increased by slow leakage into cell (heart) or neuron)
ii) voltage-gated channels open to allow Ca to move into cytoplasm from sarcoplasmic reticulum & extracellular space (ES)
iii) Ca binds to actin & myosin active sites to allow fibers to move past each other
iv) relaxation when Ca is pumped back to ER/ES (Ca ATPase moves Ca into ER & ES, Na-K ATPase moves Na out of cell, Na-Ca anti-port moves Ca out of cell & Na into cell along concentration gradient)
v) cardiac glycosides work primarily in cardiac (non-striated/smooth) muscle

Second Messenger Signaling: cAMP

- signaling molecules: hormones released from cell to signal other cells downstream (eg insulin & vasopressin)
- receptors allow all-or-nothing response (only small concentrations needed, more effective than carriers, link to receptor is very weak & will not convey transport)
- 2 signal bind to 2 receptor → cross-link receptors → conformational change → amplification of second messenger signal
- G-protein: fatty-acid linked membrane proteins
- 2 hormones bind to receptors → 2 G-alpha subunits drop

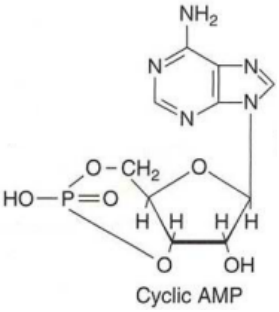


G-beta & G-gamma subunits → both bind to AC via GTP hydrolysis → 3'-5' cAMP production → cAMP binds to & removes regulatory subunit on PKA, activating PKA → protein phosphorylation → cellular response

- cAMP phosphodiesterase degrades cAMP → response ceases
- inhibition of signal via downregulation of AC

Vasopressin

- also uses G-protein pathway → increases aquaporin-2 vesicles delivery to membrane
- Nephrogenic Diabitis Insipidus: defective aquaporin-2 channel OR defective vasopressin receptor→ dehydration
- Neurogenic Diabitis Insipidus: not enough vasopression produced (defective pituitary cells or hypothalamus cannot detect osmolarity of blood)
- glucagon also uses PKA pathway → glycogenesis (reversal of glycolysis)



Cyclic AMP

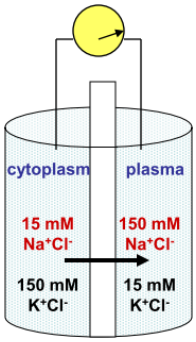
Membrane Potential & Sensory Systems

Overview

- Membrane potential: Nernst & Goldman equations
- Gated channels: voltage, ligand, & mechanically
- Neurons: action potentials & events at neuronal junctions
- Active transport in biological systems

Membrane Potential

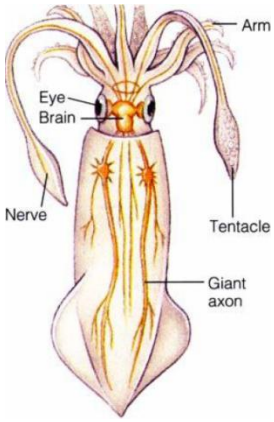
Electrical Potential vs. Chemical Gradient



- membrane semipermeable to cation, but not anion
- cation will move down concentration gradient until equilibrium is established
- equilibrium: energy given by moving down concentration gradient equals the energy taken by generating an electrical potential
- equilibrium potential of cation = when chemical potential = electrical potential
- experiment: put cytoplasm & plasma with usual Na+ & K+ concentrations in compartments separated by K+-selective membrane → K+ will move out towards plasma, registering -59mV (Vint - Vext) on volt meter @ 25degC and positive charge in plasma
- if Na+-selective, Vk = 59mV

Nernst Equation

- German physical chemist – 1920 Nobel prize in chemistry
- VK = - RT/zF * ln([K]in /[K]out) = RT/zF * ln([K]out /[K]in)
- R (gas constant) = 1.987 cal/mol*K, T = 278+degC, z = valence of ion , F (Faraday's constant) = 23,062 cal/V-mol
- in biological systems: VK = - 61.4mV*log10([K]in/[K]out) @ 37degC



Squid Axon

- siphon pushes animal away from predators
- 2 giant axa (1mm in diameter) that run along animal to operate siphon
- speed velocity of axon by increasing diameter or myelination
- what is the potential across the membrane?

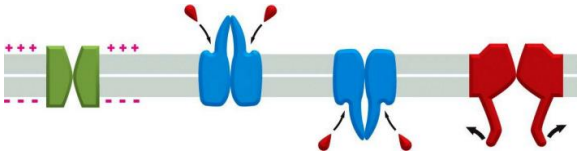
Goldman Equation

- Vm = RT/F*ln([(Pk)[K+out] + (Pna)[Na+out] + (Pcl)[Cl-in]]/[(Pk)[K+in] + (Pna)[Na+in] + (Pcl)[Cl-out]]

- z is factored out
- relative permeabilities: if Pk = 1.0, then Pna = 0.04 & Pcl = 0.45
- squid axon: Vm = -60.12mV @ 20degC

Example Problems

- table of permeabilities → predict using Goldman equation
- open an Na channel → permeability increases so much that can use Nernst equation



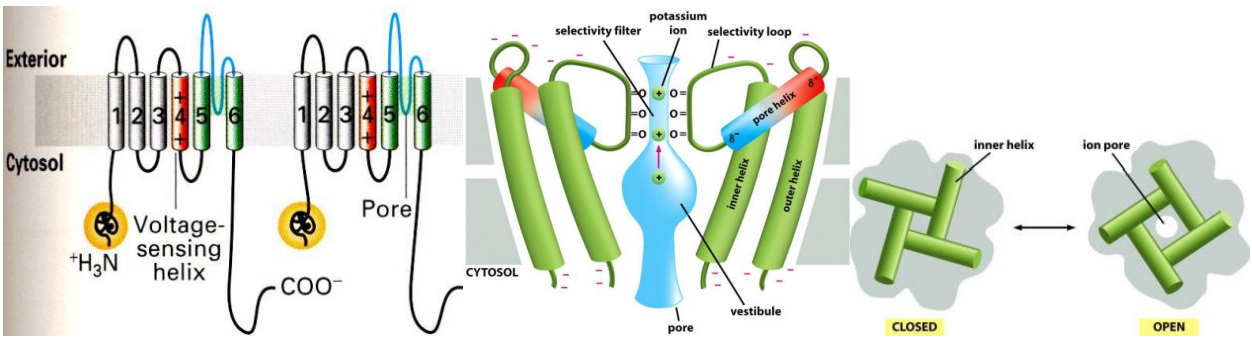
Gated Channels

Reflex Circuit

- circuit
  - i) reflex: neural signal which bypasses higher-level reasoning
  - ii) reflex circuit: afferent neuron → interneuron → efferent neuron
  - iii) conduction changes mechanical to electrical to mechanical potential
  - iv) ganglia – mass of nerve cell bodies
- mechanically-gated:
  - i) sensory receptors in skin mechanically detect distortion
  - ii) distortion opens channels & allows movement of Na+ across membrane
- voltage-gated:
  - i) changes conformation based on change in membrane potential
- ligand-gated
  - i) efferent neurons: neurotransmitters open channels across synapse
  - ii) e.g. acetylcholine receptor increases permeability of ion channels

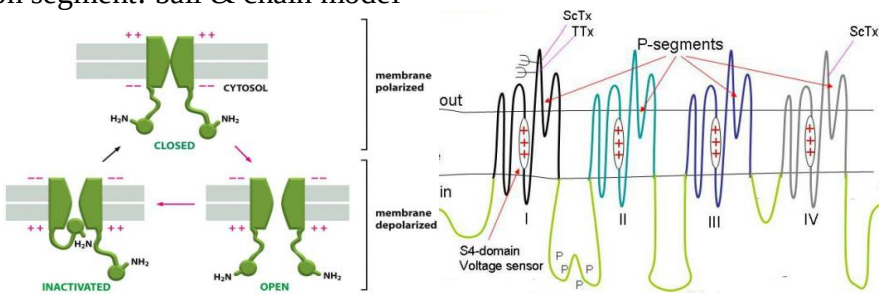
Voltage-gated K+ Channel

- structure
  - i) tetramer of 6 helical segments; helix 5 & 6 are dipolar hydrophilic pore
  - ii) S4 sensor domain/paddle that lines channel → voltage-sensing & positively charged → angle changes based on change in membrane potential
  - iii) compare to aquaporin (4 membrane-spanning regions) & GLUT4
- selectivity
  - i) S4 electronegative carbonyls oriented towards pore center to solvate hydration shell
  - ii) 5 residues with carbonyls on P loop
  - iii) selective for K+ because larger than Na+; energetically unfavorable
- discovery by Rod MacKinnon (2003 Nobel Prize in Chemistry)
  - i) Tarantula toxin (short peptide) causes partial paralysis by binding to S4 paddles, preventing angle chance and blocking nerve conduction
  - ii) 2-4 toxins per channel
  - iii) where it binds to determines orientation of changes in sensor paddles

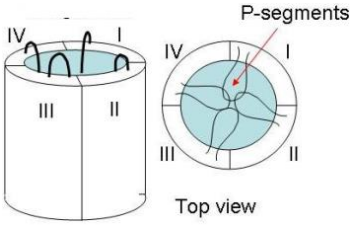


Sodium Channel

- novocaine: interferes with Na+ uptake by binding to cytoplasmic face of Na+ channel
- opens at -40mV → inactivation segment blocks channel, regardless of membrane potential
- inactivation segment: ball & chain model



Conductance Channels

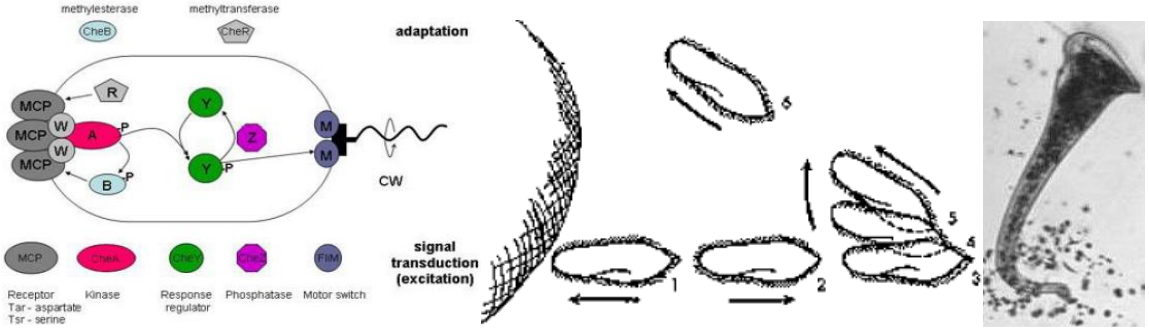


- ion-selective: 4 subunits; e.g. K+/Na+/Ca2+
  - i) e.g. K+ & Ca2+ have 6 alpha-helical segments with S4 voltage sensor & P-segment pore
  - ii) selectivity loops use charge & dimension
  - iii) K+: 4 separate subunits; Na+ & Ca2+: 1 subunit with 4 domains
- charge-selective: 5 subunits; differentiate between cations & anions
- non-selective: 6 subunits; gap junction; e.g. membrane contact conducts action potential in electrical synapse
- similar structures have different opening & closing mechanisms and selectivity

Physiology of Neurons

Sensory Systems in Unicellular Organisms

- Bacterial flagellum – rigid, rotating, motor at base can move in corkscrew (forward) or windmill (backwards) depending on attractant or repellant substrate binding to receptor
  - i) 5 classes of chemoattractants (e.g. amino acids)
  - ii) chemotaxis regulated by 3 switch proteins due to phosphorylation of chemoproteins
- Paramecium – hundreds of cilia (like in eukaryotes); swims forwards; cilia will reverse if bumps into object
  - i) experiment: fix paramecium on slide, count rate of cilial beating & take electrical readings
  - ii) cilial coordination due to mechanically-gated Ca2+ channel opening & closing
- Stentor
  - i) Large enough that can be seen under dissecting scope
  - ii) Cilia at base of oral funnel, eat by endocytosis, tentacle at base allows organism to adhere to substrate
  - iii) Poke with thin glass rod → organism will contract → habituation
  - iv) Mechanical channels → elevates intracellular calcium → longitudinal proteins contract
  - v) Habituation: G-protein becomes associated with channel such that channel no longer opens

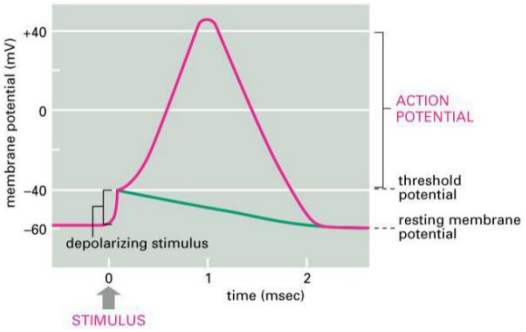


Structures

- dendrite (sensory segments) → cell body → combination/filtration at axon hillock → axon (can be 1mm-1m long) → terminal branches → synapse
- neurotransmitters: synthesized in cell body, shuttled via motor proteins along microtubules to axon terminus
- external stimulus → mechano-sensory receptors → interneuron/spinal ganglia → motor neuron → contraction of voluntary muscle
- Mechano-sensory: membrane pore tethered to collagen outside cell & structures in cell → distortion opens channel
- Sensory neuron: sensory dendrites at peripheral end, axon along nerve tracts, AP, nerve terminals at proximal end, cell body just outside spinal cord

Action Potentials

- pinch at sensory dendrite → mechanosensitive sodium channels open → change in potential measured by distal electrode
- resting potential: -65mV

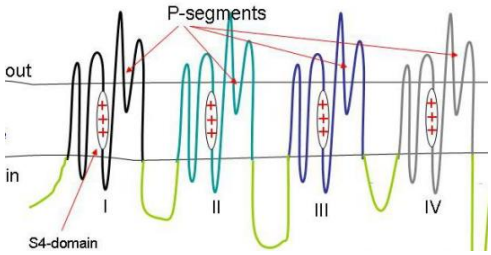


- threshold potential (-40mv): stimulus must be large enough that potential change is steep enough to open voltage-gated sodium channels
  - equilibrium potentials: -90mV for K+, +58mV for Na+
  - action potential: potassium leak channel open → -65mV → depolarization reaches electrode, sodium gates open → +40mV, sodium gates inactivate → potassium gates open → -80mV hyperpolarization (refractory period due to channel inactivation)→ potassium gates close → Na/K-ATPase reestablishes resting potential
- Na+ in cell diffuses forward along axon, activating sodium gates
- membrane potential moves toward equilibrium potentials of Na+ & K+
- hyperpolarization enforces unidirectionality of action potential propagation

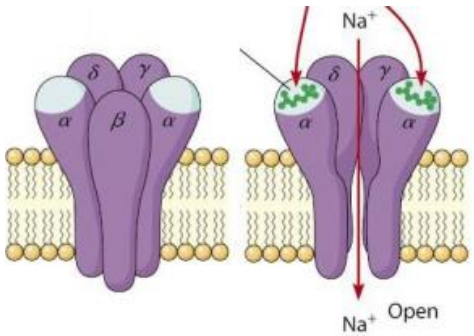
Patch Clamping

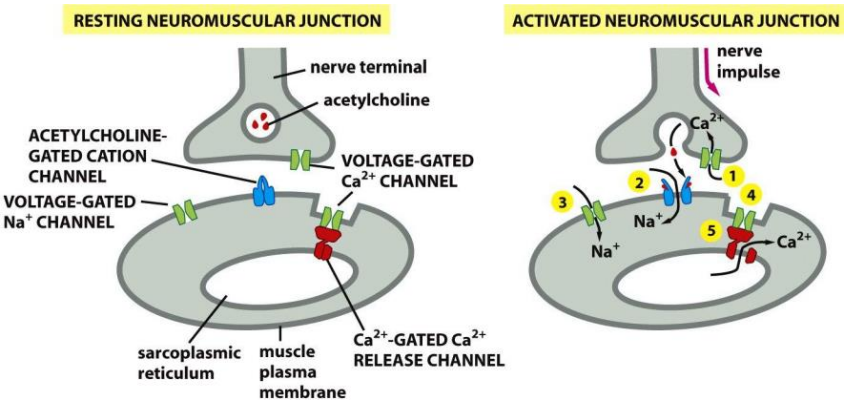
- form tight seal on membrane with glass micropipette (polished to prevent puncture) & apply gentle suction to detach 1um patch of membrane with ion channels
- attach patch to electrode with differential amplifier; set ionic concentrations in experimental intracellular & extracellular compartments, use drugs, or adjust voltage to measure current
- used to measure voltage at which channels open, determined all-or-none response (complete or gradual opening of channel)

Neurotransmission at Neuromuscular Junction



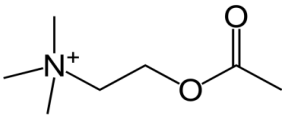
- action potential opens Ca2+ channel on presynaptic membrane
- vesicles fuse with presynaptic membrane (Ca2+ allows V-SNAREs & T-SNAREs to twist together)
- acetylcholine binds to ligand-gated cation channel
  i) 2 alpha + 1 beta, gamma, delta subunits; acetylcholine binds to 2 alpha subunits
  ii) 20mV force pushing K+ out < 128mV force pushing Na+ in → Na+ moves in
  iii) can be regulated by other factors via phosphorylation
- voltage-gated Na+ channel opens
- voltage-gated Ca2+ channel opens → Ca2+ gated Ca2+ release channel in sarcoplasmic reticulum opens

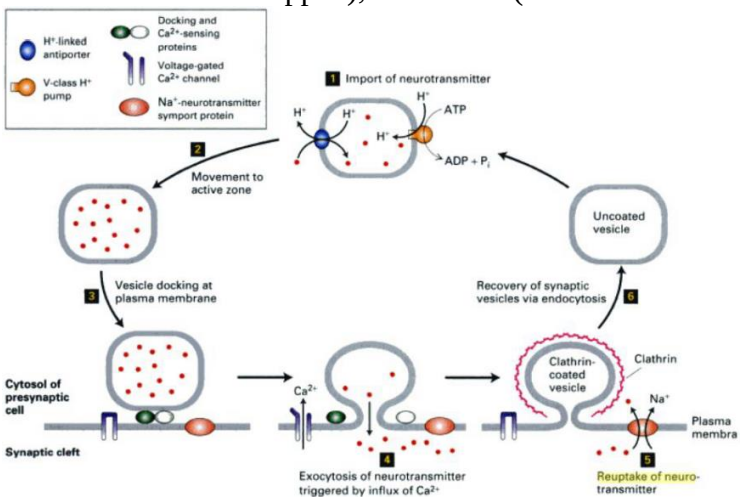




Degredation & Reuptake

- clathrin-coated vesicles buds off from presynaptic membrane on cytoplasmic side
- reuptake of neurotransmitter via Na+ symport
- v-class proton pump in uncoated vesicles creates proton gradient at expense of ATP hydrolysis
- neurotransmitter pumped into vesicle via H+ antiport
- entire release & reuptake cycle in 1 minute
- acetylcholine esterase
  i) degrades acetylcholine into acetate & choline before reuptake
  ii) inhibitors: SARIN (complexes with enzyme; experience muscular spasms because stimuli are not stopped), Malathion (insecticide version)

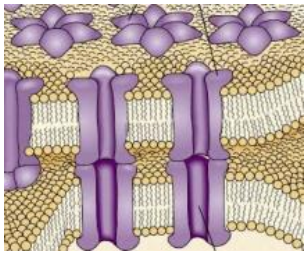




Myelination

- in 99% of vertebrates
- evolved as a method to increase speed (20x-100x) in smaller diameter neurons
- glial cells – supportive cells; Schwann (PNS) & oligodendrocytes (CNS)
- prevents (K+?) leakage to allow saltatory conduction of AP in nodes of Ranvier
- autoimmune/dystrophy diseases tend to destroy myelination, degenerating conduction speeds, muscle strength, & neural health (e.g. multiple sclerosis)

Electrical Synapse

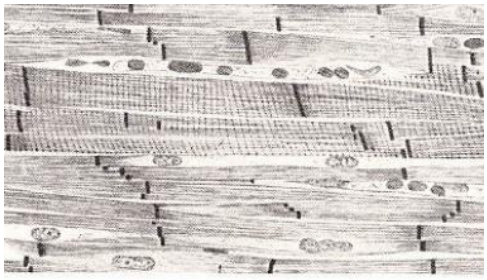


- transmission of depolarization by cell-cell contact
- 6 connexins for connexons which form 3.5nm wide gap junctions
- used in fast-reacting rapid transmission neurons (e.g. squid ink release)

Heart Muscle

- muscle types: cardiac, striated/skeletal, & smooth

- experiment with cardiac muscle cells in culture: contracted individually, then contracted in unison after forming cell bodies
- membrane channels leak Na+, which initiates AP (30bpm)
- heart structures: atria contract, then ventricles contract → 2 functional subsystems
  i) intercalated discs (low resistance bridges) & cell branching
  ii) Sinoatrial (SA) Node cells depolarize the fastest (60/7bpm) → depolarization spreads to other cells
  iii) if SA node damaged, AV node (slower) will sustain heartbeat, otherwise other cardiac cells will beat at 40bpm (too slow)



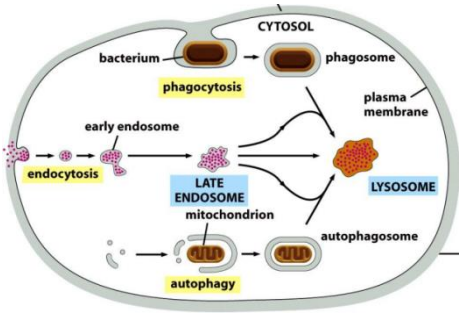
Endocytic Pathways

Overview

- General endocytic pathways
- Lysosomes: enzymes & acidification, lysosomal disorders
- Paracytic pathogens
- Receptor-mediated endocytosis: LDL uptake, familial hypercholesterolemias
- Vesicle targeting

General Endocytic Pathways

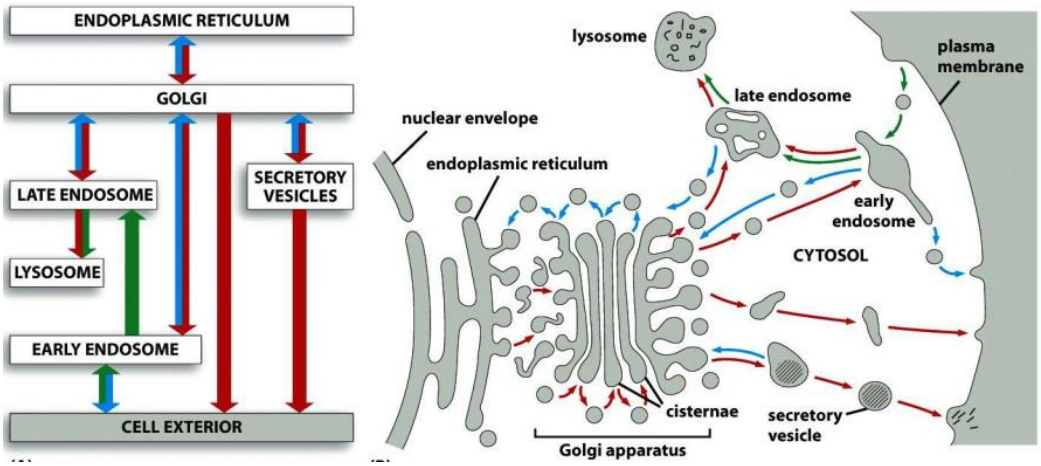
Types



- phagocytosis – (phagosome/large vesicle)
  i) membrane recognizes foreign tissue & brings pathogen into cell
  ii) e.g. amoeba – pseudopodium takes up food
- pinocytosis – bring extracellular fluid into the cell (small vesicle)
  i) constitutive process: budding in constant
  ii) e.g. endothelial cells (line capillaries)
  iii) artery/vessel – endothelial cells surrounded by connective tissue
  iv) BBB – epithelial cells on capillaries near brain are very selective
  v) liver – epithelial cells are less selective (selectivity based on tissue function)
- receptor-mediated endocytosis (RME) – coat protein forms coated pit to bring in substrate (midsize vesicle)
  i) highly selective (e.g. for hormones, LDL)
  ii) various fates for receptors (can be digested or returned to membrane)
  iii) recycling: e.g. transcytosis of GLUT receptors
- autophagy – digestion of organelles due to damage or starvation
  i) vesicles nucleate & extend to surround organelle & fuse (double-membrane)

Membrane Traffic & Recycling

- RME types: RNA vira, LDL uptake, iron uptake
- components:
  i) early endosome: acidification via proton pumps, sorting (pH = 6)
  ii) late endosome: more acidic, presence of lysosomal enzymes; lysosomal in nature; particles to be lysed take this route (pH = 5)
- pathways:
  i) general pathway: sorting → uncoating → motility → tethering → fusion
  ii) green arrow (endocytic): buds off → early endosome → late endosome → lysosome
  iii) red arrow (biosynthetic/secretory): rough ER → GB (adds or removes sugars, sorts by destination) → lysosome via late endosome, early endosome, PM, or ES via secretory vesicles
  iv) blue arrow (backflow): early endosome → PM or GB OR late endosome or secretory vesicles → GB
- traffic moves between ER and GB and within layers of GB
- traffic coordinated by coat proteins: clathrin, COP I, COP II
  i) most vesicles have protein coats, with the exception of secretory vesicles and some others



Lysosome Structure & Function

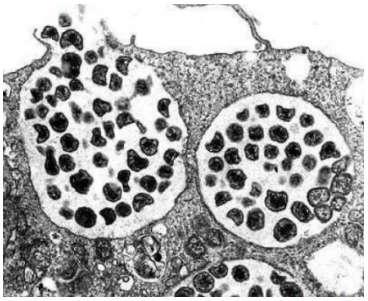
- membrane-bound compartments (0.2-0.5um)
- hydrolytic enzymes – inserts water between monomers
- if functional at nonoptimal pH, would cause inflammation in blood and joints
- nonspecific: will degrade any macromolecule which fuses with it
- transport vesicle is acidified before delivery to lysosome
- acid hydrolases: nuclease, protease, glycosidase, lipase, phosphatase, sulfase, phospholipase
- lysosomal transport proteins return digested products to cytosol
- heterogeneity: late endosome fuses with lysosome, which fuse with each other

Hurler's Syndrome

- alpha-L-iduronidase deficiency (should break down bond between iduronic acid and a galactose derivative)
- faulty degredation of complex sugars dermatan (flexibility in heart valves, blood vessel support) & heparan sulphate (connective tissue)
- rare recessive genetic disorder: 1 case per 75,000 in Ireland; 1 case per 250,000 in Germany
- accumulation of nonmodified sugars leads to mental development problems, death soon after childhood, abdomen distension, liver enlargement, heart valve inflexibility, gargoyle-like features, hunchback, and compromised CNS remodeling

Receptor-Mediated Endocytosis

Bacterial Blocking

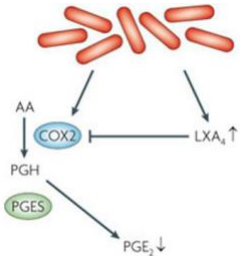


- pathogenic bacterial protective mechanisms: hide in phagosome by blocking membrane fusion → blocks pathway of delivery to late endosome & prevents membrane recycling → allowed to proliferate within vesicle & macrophage lyses
  - can also alter signaling by secreting proteins
  - blocks early endosome acidification but still sorted
  - e.g. Ehrlichia chaffeensis (bacteria proliferate within vesicle, macrophage lyses) & Legionella (redirected to & proliferate in ER)
    i) potential host cells: leukocytes & macrophages
- e.g. Mycobacterium tuberculosis – infection remains without antibiotics or vaccination
  i) internalized by macrophages in alveoli
- experimental methods: randomly knockout bacterial genes to identify which are responsible for pathogenic traits

- macrophage: “professional endocytic vesicle” - uses & recycles ~200% of membrane surface per hour

Preventing Merging

- cyclooxygenase 2 catalyzes conversion of arachadonic acid into protoglandin E2 precursor
- SYT-2 present on vesicles (perhaps also on target compartments) is regulated by PGE2 and elevated Ca2+ levels (like SNAREs)
- TB upregulates/produces Lipoxin A4, a COX2 inhibitor
- blocks apoptosis (mitochondria release cytochrome c, which degrades & packages the cell) → necrosis

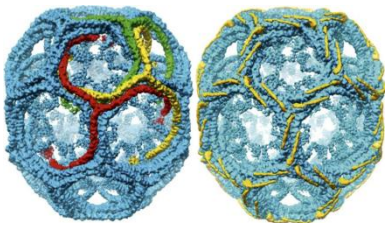


Housekeeping vs. Signaling Receptor Classes

- Housekeeping
  - i) allows uptake of necessary nutrients
  - ii) e.g. RBC precursors obtain Fe2+ and transferrin to make hemoglobin → acidification changes transferrin affinity for iron, not receptor affinity → receptor & carrier protein returned to PM
- Signaling
  - i) internalization & degredation turns off hormone response
  - ii) erythrocyte growth factor from kidney → proliferation → surface cleared of growth factor and receptor
  - iii) sequestration of endosome membrane into internal compartments

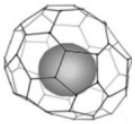
Clathrin-Coated Pits

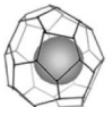
Overview



- discovery
  - i) Keith Porter (1960’s, Southwestern Medical School) – study of yolk (lipid-protein energy complex) pinocytosis by mosquito oocytes
  - ii) bind to vesicle surfaces which bud from membrane → discovery of coated pits
  - iii) not all pinocytic vesicles are clathin-coated
- pits drag in peripheral membrane proteins as well
- clathrin-coated vesicle route: early endosome → trans-Golgi network, PM, or multivesicular body → microtubule-mediated transport → late endosome → endolysosome → lysosome
- on RME & vesicles with lysosomal enzymes budding from Golgi destined for late endosome
- receptor-coat recognition by mutliubiquitylation

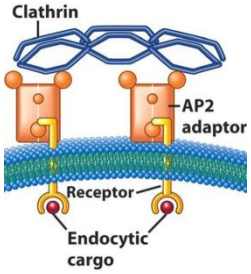
Composition





- 3 heavy chains (180kDa) + 3 light chains (25kDa) = triskelion
- heavy-chain legs interact with those of other triskelia on cytoplasmic face of plasma membrane
- experiment: extraction from rat neuronal tissues (axon terminals)
  - i) 36-60 triskelia per vesicle, pentagons – heptagons, 31 – 50nm diameter

AP2 adaptor protein complex



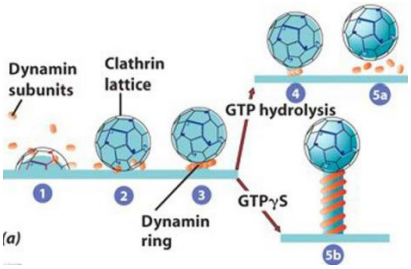
- affinity of receptor on cytosolic face for AP2 & of epsin for AP2 not affected by ligand binding
- AP2 on vesicles which bud from PM, AP1 on vesicles which bud from GB
- 4 subunits, alpha & beta adaptin and sigma & mu chain
- beta unit binds to clathrin, mu chain binds to receptors
- binds to phosphoinositides, which label membrane as endosome

- epsin – binds to alpha subunit of AP2 & membrane lipids; involved in vesicle curvature

Receptor Binding

- coat assembly & cargo selection → bud formation → vesicle formation → uncoating
- ATP hydrolysis → chlathrin conformational change → uncoating before fusion with endosome
- uncoating: HSP70 (heat shock protein, mw = 70)
- HSP name from Drosophila genes which are upregulated when temperature increased

Dynamin



- dynamin form spiral ring around base of bud → GTP hydrolysis induces conformational change → dynamin proteins released & vesicle formation
- study with GTP-gamma-S replacing GTP → neck elongation rather than budding
- GTP-gamma-S: gamma phosphate group replaced with a sulfate, which will allow dynamin to bind to it but not to hydrolyze it

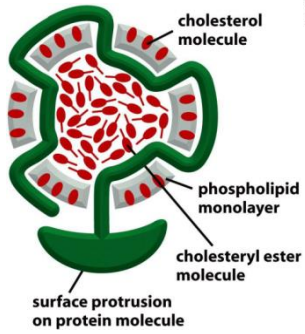
Familial Hypercholesterolemia

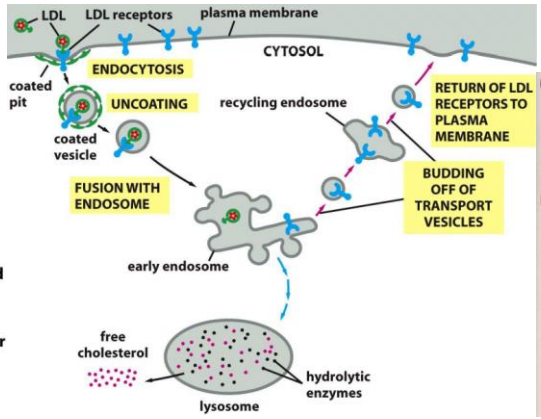
Disease Mechanism

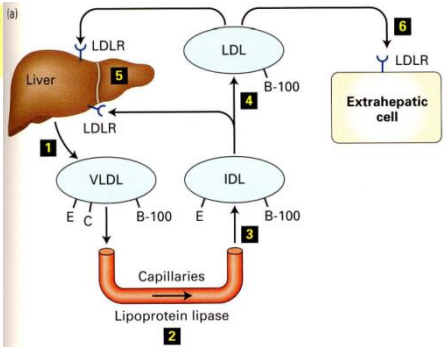
- Brown & Goldstein (Southwestern Medical School; Nobel 1985) studied cholesterol metabolism in fibroblasts from such patients
- good model of RME
- types: heterozygous: 2-3x normal levels (300-400mg/dL) → heart problems in 50s, or homozygous recessive: 6-7x normal levels (>500mg/dL) → heart problems in 20s/30s
- normal levels: LDL @ 100mg/dL (high @ 160mg/dL), HDL @ 40-60mg/dL
- associated with cardiovascular disorders & arterial sclerosis
- potential mutations: receptor LDL binding site or AP2 binding site; Apoprotein B; no receptor; receptor not transported to GB/PM

LDL Pathway

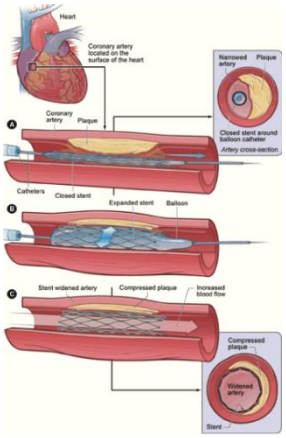
- cells can synthesize cholesterol or produce LDL receptor to uptake from blood
- difference between HDL & LDL: centrifugation (banding from when particle density equals the density of the fluid)
- fusion with early endosome → acidification lowers affinity & sorts → hydrolytic enzymes release free cholesterol → process just like for iron uptake
- receptor recycling up to 150 times, once per 20 minutes
- LDL: sphere of 1500 cholesteryl esters covered by phospholipid-cholesterol monolayer associated with apoprotein B-100
- carrier protein moves cholesterol in chylomycra from intestines to liver → release of VLDL (B-100 + triglycerides + cholesterol) into circulation → lipids removed & added, changing to IDL and then LDL → uptake by extrahepatic tissue (eg steroid hormone secreting tissues) for use or excess uptake by liver
- liver: modification of absorbable fat → gall bladder (detergents) → secretion back into intestines for reabsorption or removal





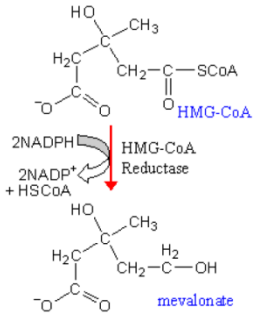


Atherosclerosis



- artery wall: smooth muscle cells; inner lining: endothelial cells on connective tissue fibers
- deposition of lipids between endothelial cells & muscle cells (lipid streaks) → damage to endothelial lining due to turbulence at vessel branch → LDL adheres to lipid streaks → macrophages will attempt to phagocyse lipids → necrosis of macrophages due to 'overeating' (foam cells) → repeat
- muscle cells can repair by growing over plaque by occluding lumen OR plaque can become unstable, break off, and block smaller vessel downstream
- common corroted artery: branches to internal (brain) & external
- intervention: coronary balloon angioplasty & stent placement; introduce catheter into femoral artery → reduces pain due to angina

Statins

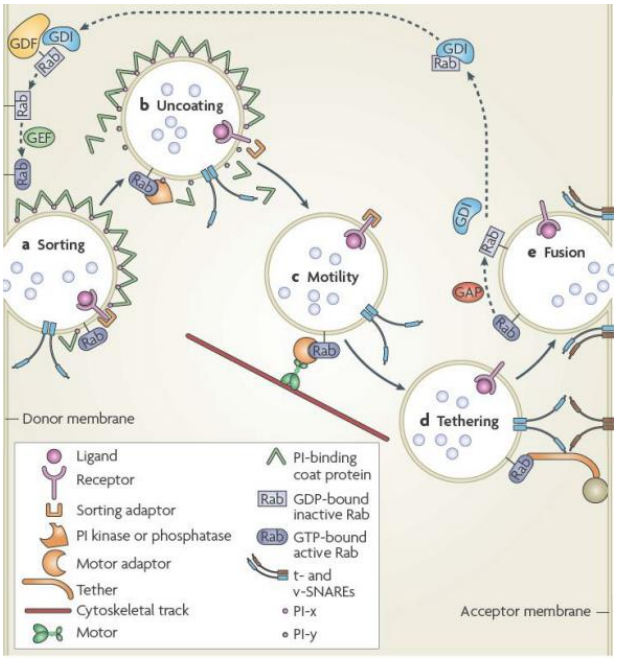


- mechanism of action: inhibits synthesis in all tissues & liver increases receptor production (already normally 70% of all LDL receptors)
- discovery in microorganisms
- rate limiting step: reduction of HMG-CoA → mevalonate as catalyzed by HMG-CoA reductase; competitively inhibited by statins
- Lipitor: atorvastatin
  - i) reduction by 25-60% (dosage-dependent) within 2 weeks
  - ii) also prevents neurodegenerative disorders
- Zitia – blocks transport of cholesterol from intestines to lymphatic system
  - i) given as secondary drug in addition to statin
- another method: liver transplant in children (works because children are vigorous & have not yet had time to have had cardiovascular damage)

Vesicle Motility & Fusion

Motility

- Steps:
  - i) sorting: sorting adapter is bound to vesicle
  - ii) uncoating: RAB (GTPase) is exposed, adapter falls off
  - iii) motility: RAB binds to motor adapter, which binds to motor, which moves along micotubule

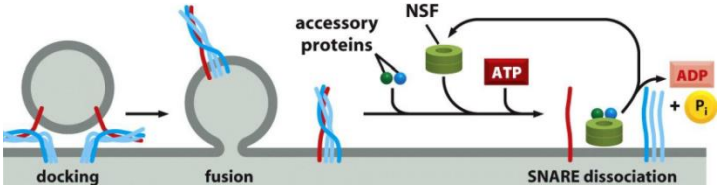


iv) tethering: RAB binds to tether, v- & t-SNAREs associate
v) fusion: GAP (RAB GTPase activating protein) causes hydrolysis of GTP on RAB, releasing it

- Components:
  i) motor proteins: ATPase for each step, classes to move in either direction
  ii) microtubules provide cell body & axon shape
  iii) GAP pathway: GDI (GAP dissociation inhibitor) factor binds to RAB → RAB falls off & returns to PM & binds to GDF (GDI dissociation factor) → GDI & GDF fall off, RAB is bound to PM → GEF (PM-bound) removes GDP & replaces with GTP
  iv) different varieties of RAB (~40) for different target membranes & cell types

Fusion

- Steps:
  i) outer leaflets merge (form stalk) → outer leaflets move apart (hemifusion) → inner leaflets merge (fusion)
  ii) NSF (ATPase) + accessory proteins dissociates SNAREs
- Components:



  i) v-SNARE: pair of synaptobrevin molecules
  ii) t-SNARE: pair of 1 syntaxin & 2 Snap25 molecules
  iii) 35 families of SNARES; in well studied strains, Ca2+ plays an important role
  iv) names derived from synapse
- botulism & tetanus toxins: cause muscle spasms by cleaving SNAREs, preventing Ach release into synapse
- HIV insertion requires conformational change of fusion proteins in neck

Sorting Newly Synthesized Proteins

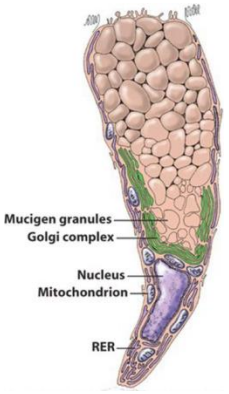
Overview

- Pancreatic model
- Assembly of secretory vesicles
- Signal sequences
- Integral membrane proteins
- Core glycosylation
- Sorting lysosomal enzymes
- Transfer into mitochondria
- Movements through the nuclear envelope

Pancreatic Model

Goblet Cells

- found in intestinal glands
- secrete mucins (glycoproteins) though apical face (polarized cell) into intestinal lumen
- mucins are lubricant & protectant against digestive enzymes



- most of cell energy dedicate to synthesis & sorting of mucins
- good model of protein sorting because most proteins destined for one central compartment (secretory vesicles)
- pancreatic acinar cell: cholecystokinin hormone released when food reaches duodenum → signal transduction → secretory vesicles merge with PM → digestive enzymes released into pancreatic duct → intestines

Protein Synthesis

Protein Translation

- 5' → 3', NH2 → COOH
- 5'end of an mRNA becomes associated with small subunit → subunit 'crawls' to first codon → tRNA brings first amino acid to complex → large subunit associates with complex → second tRNA arrives → peptide bond forms → ribosome moves forward one codon, and first tRNA falls off → repeat until termination signal → dissociation

Locations of Protein Synthesis

- in eukaryotes: cytosolic, rough ER, mitochondria & chloroplasts
  i) ER-bound ribosomes (synthesis-dependent): secreted proteins, integral PM, ER, GB, lysosomes
  ii) cytosolic ribosomes (synthesis-independent): cytosol, mitochondria, chloroplast, nucleoplasm, peroxisomes
- most proteins for mitochondria & chloroplasts are produced elsewhere (5-8% produced on them)
- advent of electron microscopy → discovery that volume of rER related to function of the cell (e.g. secretory cells had large amounts of cytoplasm dedicated to rER)
  i) orientation: large subunit on membrane surface
  ii) synthesized protein is sent through channel in large subunit
  iii) suggests that proteins destined for secretion are pushed though membrane into cisternae
- smooth ER:
  i) continuous with rER; transitional area where transport vesicles bud off
  ii) proteins associated with sER: lipid synthesis; detoxicification → concentration of these enzymes rise in response to classes of drugs (drug habituation & liver problems); calcium storage (e.g. sarcoplasmic reticulum)

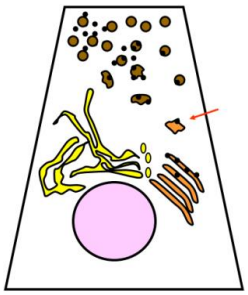
Identifying Transport Molecules

- GlcNAc transferase I mutant GB + viral glycoprotein → add wild type GB → 3H-GlcNAc added
- observe diffusion rates via GFP & FLIP/FRAP
- block certain pathways via mutants
- coat specialization: various organelles have various compositions of PIP enzymes
- coat recruitment by PIPs & GTPases; falls off due to PIP & GTP hydrolysis
  i) GTPases: Arf (COPI & clathrin) & Sar1 (COPII)
- vesicle targeting by Rab & SNAREs

Protein Pathways: ER

George Palade

- Rockfeller University; Nobel Prize in Medicine for defining secretion pathway
- pulse chase studies: trace radioactive amino acids through slices of pig pancreas
  i) setup: Erlenmeyer flasks with pancreatic cells in buffer (salts, oxygen, etc)

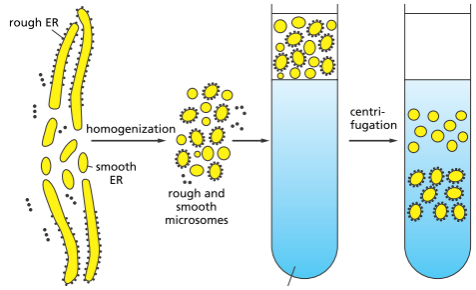


ii) pulse: addition of 3H-Leucine substrate → uptake by cell (wait 3 min)
iii) chase: add large concentrations of nonradioactive leucine to dilute any radioactivity in later synthesis

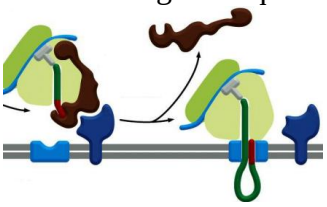
- autoradiography:
  i) wash off nonradioactive proteins → fix on slide (kills cell) → dip in silver-bromide photographic emulsion → incubate in dark, look under microscope for activated particles
  ii) protons in radioactive material can interact with silver particles in film (discovery of radioactivity: rock next to film)
  iii) look at slices that had been incubated for 3 min, 10 min, 20 min, 1 hour, 2 hours
- progression: (3 min) synthesis on rER → (10 min) condense into exit sites, which enter transport vesicles which fuse into transport intermediates → (20 min) vesicles between rER & GB → (1 hour) sugar addition & sorting on GB → (2 hours) condensing vesicles in cytoplasm → secretory granules → apical face (all travel of transport vesicles along microtubules)
- current method: insert GFP via viral vector; difficult to observe because there is no chase, so use inhibitor to restrict proteins to one compartment, then relieve inhibitor and continue observation

Gunther Globel

- student of George Palade; won Nobel Prize 20 years later for defining pathway for proteins destined for PM and organelles
- repeated experiment in cell-free environment
- lyse rabbit immature RBC → extract will translate any mRNA
- eg glycolipid enzyme (not secreted, 60kD) & albumin (secreted, 64kD → version produced was 68kD)
- added proteins to rER extraction → add protease → proteins were degraded, indicating that proteins did not enter rER
- secretory proteins have signal sequence: add rER before translation → signal proteins meant that mRNA was only translated by ribosomes on the rER → albumin proteins entered rER and size was reduced to 64kD when addressing signal peptide removed
- ER fragmentation: disrupt cells via homogenation → form microsomes → sedimentation in sucrose gradient



Signal Sequences



- signal recognition particle (SRP; multiple proteins + RNA) binds to signal sequences (amino end or internal) on nascent peptide when it emerges from large subunit
  - translation pauses by preventing binding by elongation factor
  - SRP attaches to SRP receptor on ER membrane
  - translation continues and is threaded through translocon/sec61 membrane channel
- enzyme allows SRP & SRP receptor to be replaced & recycled
- ER signal sequences: 6-15 amino acids

Table 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
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 plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
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-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

Translocon
- studied extensively in bacteria, conserved in eukaryotes
- channel cannot remain open or else Ca2+ & other molecules would leak into the cytoplasm
- alpha-helical plug displaced & protein threaded through
- signal peptidase cleaves signal peptide, allowing it to diffuse out of seam into ER membrane

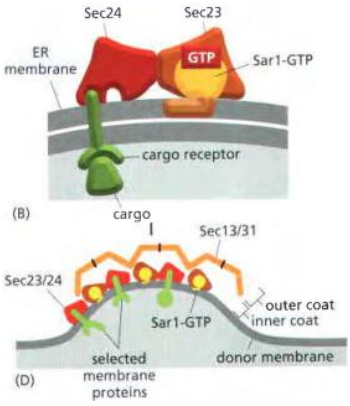
Protein Folding
- BiP chaperone: prevents folding, then sequentially fall off (ATPase) to allow folding in stages
- protein disulfide isomerase: breaks & reforms disulfide bonds
- misfolded proteins are degraded; remain bound to chaperones and are secreted for degredation by cytoplasmic proteases

Integral Proteins
- stop-transfer segment binds to hydrophobic stop-transfer-peptide-binding site & moves laterally through seam after translation
- internal start-transfer/signal sequence: final orientation such that positively-charged amino acids are on cytosolic face
- anchor sequence: alpha-helical; tend to have sequence of charged amino acids on the carboxylic end to prevent continual threading
- multipass model: multiple internal start & stop transfer sequences
- orientation of integral membrane proteins depend on insertion into ER membrane (standard procedure is NH2 end goes into ES)

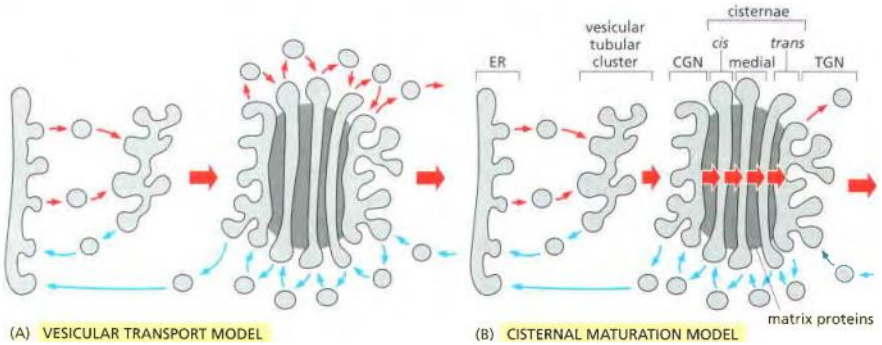
Protein Pathways: Golgi Body

Golgi Body
- 7-8 parallel membranes near nucleus
- cis-Golgi network (CGN, close to ER, microtubules) → cis cisternae → medial cisternae → trans cisternae → TGN (where vesicles bud from, microtubules)
- functions: sugars attached in ER or GB, enzymes in various lamellae modify sugars, addition of sorting tag

COPII Coats
- coats on vesicles moving from ER to Golgi
- Sar1-GEF activates GTP, which recruits Sec23 & 24; Sar1 GTP hydrolysis releases coat
- exit signals on cargo protein & on cargo receptor
- Sec13 & 31 bind to Sec23 & 24



Lamellan Maturation Model
- different lamellae have different enzymatic compositions
- retrieval transport: Golgi enzymes must be moved backwards from trans to medial to cis lamealla via medial vesicles with COPI
- vesicular tubular clusters form (homotypic membrane fusion) from COPII ER to GB vesicles, move along microtubule via motor protein towards cis network
- alternate hypothesis: vesicular transport molecule; would require cargo to be found in transcis-ternal vesicles rather than in cisternae alone



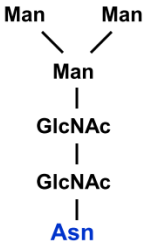
Retrieval Transport:

- COPI coats on vesicles moving throughout Golgi network & back to ER (returns membranes & proteins which should not have been transported to ER)
- soluble ER resident protein in GB binds to KDEL (Lys-Asp-Glu-Leu), which binds to KDEL receptor, which binds to COPI coat; loses affinity somehow in ER
- other retrieval signal: KKXX
- kin recognition aggregation mechanism

Sorting Newly Synthesized Proteins (cont'd)

Glycoproteins

Overview



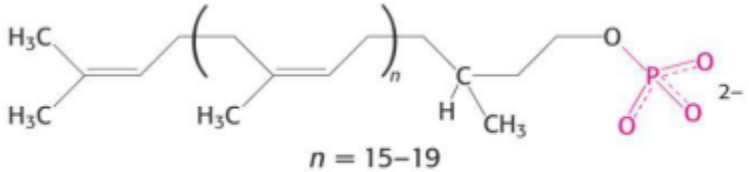
- Purpose: folding by preventing aggregation & mediating chaperone binding; interact with membranes & receptors
- sugars (all 6-carbon rings): glucose, mannose, galactose, fucose, N-acetylglucosamine (GlcNAc), N-acetyl-neuraminic acid (NANA)
- N-linked: Sugars attached to aspargine (Asn – X – Ser/Thr) (rER & Golgi)
  - i) core glycosylation standard sequence
- O-linked: Sugars attached to serine or threonine (Golgi)

Golgi Sequence

- cis network: phosphorylation of oligosaccharides on lysosomal enzymes
- cis cisternae: removal of mannose
- medial cisternae: removal of mannose & addition of GlcNAc
- trans cisternae: addition of galactose & NANA
- trans network: sulfation of tyrosines & carbohydrates
- sorting in cis and trans networks
- destinations: lysosomes, plasma membrane, secretory vesicles

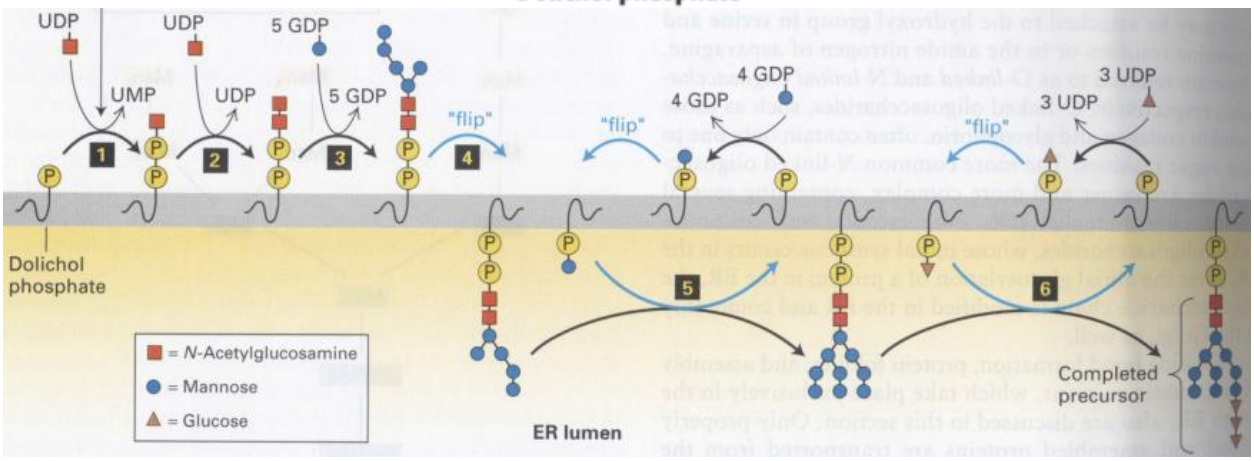
rER N-linkage Pathway

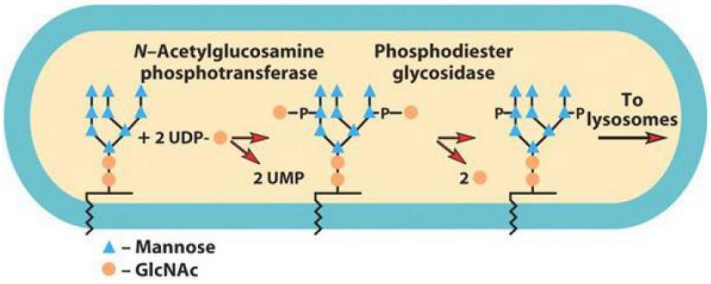
- transfer of a chain of sugars to the nascent protein → donor: Dolichol pyrophosphate → sugar chain trimmed while in ER → further modifications in Golgi
- dolichol phosphate: 15-19C depending on organism, changes orientation in ER membrane (via enzyme) during core glycosylation
- in lumen, enzyme will recognize correct signal & add 2 N-acetylglucosamine + 9 mannose + 4 glucose → glucose sequentially removed → 1 branched mannose removed → to cis-Golgi
- GB held together by fibrous proteins in Golgi matrix, including microtubules for transport



$n = 15–19$

Dolichol phosphate





Lysosomal Enzymes

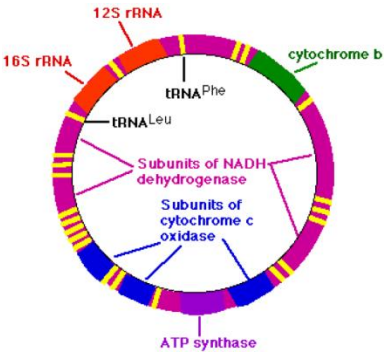
- addition of phospho-mannose: (cis network) GlcNAc phosphotransferase recognizes lysosomal enzyme
- specificity for amino acid sequence which identifies peptide as lysosomal
- mannose-6-phosphate receptor: clustered in regions in trans network (like coated pits) attached to clathin coats → delivery to endosomal compartment (may be late or early)→ dissociation at acidic pH & removal of phosphate → receptor returned to GB trans face in retromer-coated vesicle
- retromer coat: vesicles that return acid hydrolase receptors
  i) requirements: receptor, curved membrane, phosphoinositide endosome marker
  ii) binds as dimer to stabilize curvature

I-cell disease (Mucolipidosis II)

- autosomal recessive lysosomal storage disorder
- I – inclusion bodies in fibroblasts
- defective gene for N-acetyelglucosamine phosphotransferase → lysosomal enzymes are secreted rather than sent to lysosomes (should attach GlcNAc-P to terminal mannose at expense of UDP)
- symptoms: coarse facial features, skeletal abnormalities, cardiovascular problems, death within first 10 years due to cardiovascular disease or infectious diseases (pneumonia)
- some lysosomes are exocytosed due to stress, release of molecules (melanocytes), or product too large to be digested
- zymogens: lysosomal enzymes with inhibitory domain at N-terminus which is removed by proteolysis
- since some lysosomal enzymes are not secreted, suggests M6P-independent pathway

Mitochondria & Chloroplasts: Structure, Endosymbiosis, & DNA

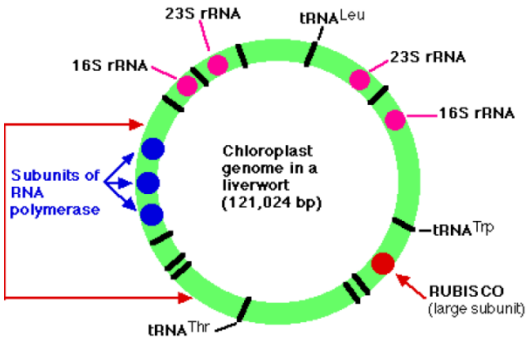
Mitochondria



- mitochondrial cristae for oxidative phosphorylation (cytochome oxidase), matrix (citrate synthase), intermembrane space (adenylate kinase), outer membrane (monoamine oxidase)
- mtDNA: ~5% of DNA in mitochondria; circular & ds in humans; 13 polypeptides, 22 genes for tRNA, 2 genes for mitochondrial rRNA
- features: detected in whole cell, different from nuclear DNA, recoverable from the organelle, 10^5 bp, usually identical between mitochondria
- homogenize liver tissue & centrifuge to extract mitochondria → incubate in 3H-leucine → ribosomes on mitochondria translated mtDNA
- proteins: ATP synthase, some subunits of cytochrome c (but not all), 4 subunits of NADH dehydrogenase (but not all?), cytochrome b; all hydrophobic
- DNA in matrix, protein synthesis on inner membrane
- 1.5 bya endosymbiosis of bacteria when oxygen became available in the atmosphere

Chloroplasts

- palisade cells near leaf epidermis & above stomata absorb light
- plastids: pigment-containing organelles
- structure: inner & outer membrane, stroma (like matrix), thylakoid membranes (like inner membrane)



- clDNA: 10^6 bp (10x size of mtDNA), 40 polypeptides, 4rRNA, & 37 tRNA
- 3.5bya bacteria appear; 3.2bya photosynthetic bacteria appear; 2.2bya oxygen-metabolizing bacteria appear

Endosymbiosis

- DNA closer to bacterial DNA (no introns), ribosomes inhibited by bacterial ribosomal inhibitors (streptomycin) but not by cytoplasmic ribosomal inhibitors (cycloheximide), sequence information, no histones
- *Elysia chlorotica*: ingests cytoplasm of filamentous algae & internalizes chloroplasts
  i) selective distribution of the chloroplasts by the gut cells
  ii) chloroplasts can be maintained for months due to lateral transfer of DNA from chloroplast to slug

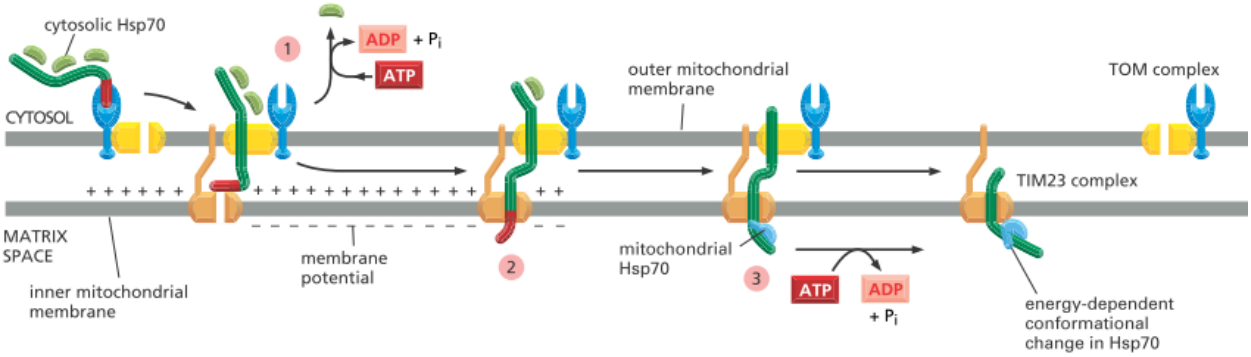
Post-translational (synthesis-independent) Transport

Experiments

- isolate mitochondria in radioactive proteins, observe translocation into organelle by applying protease
- what else besides organelle is needed for translocation?
- what types of proteins are automatically incorporated?
- how could a non-organelle protein be modified to be incorporated?

Mitochondria

- signal peptide binds to receptor → threaded through membrane via TOM complex as cytosolic HSP70 chaperones are released at expense of ATP→ translocation into matrix via TIM complex simultaneously at contact points if destined to become an inner protein → binds to HSP70 mitochondrial chaperone proteins → signal peptide cleavage (MPP – mitochondrial processing peptidase) & folding (mtHSP70)
- contact points: membranes become parallel; have different compositions
- transit peptide: N-terminal, 20-50aa, form helix with opposite faces hydrophobic & hydrophilic
- TIM22 for integral inner membrane proteins; TIM23 for matrix proteins; other unique translocons for other types of proteins (e.g. cytochrome c, SAM & OXA complexes)
- ATP & proton gradient across inner membrane established for oxidative phosphorylation is used as a translocation energy source



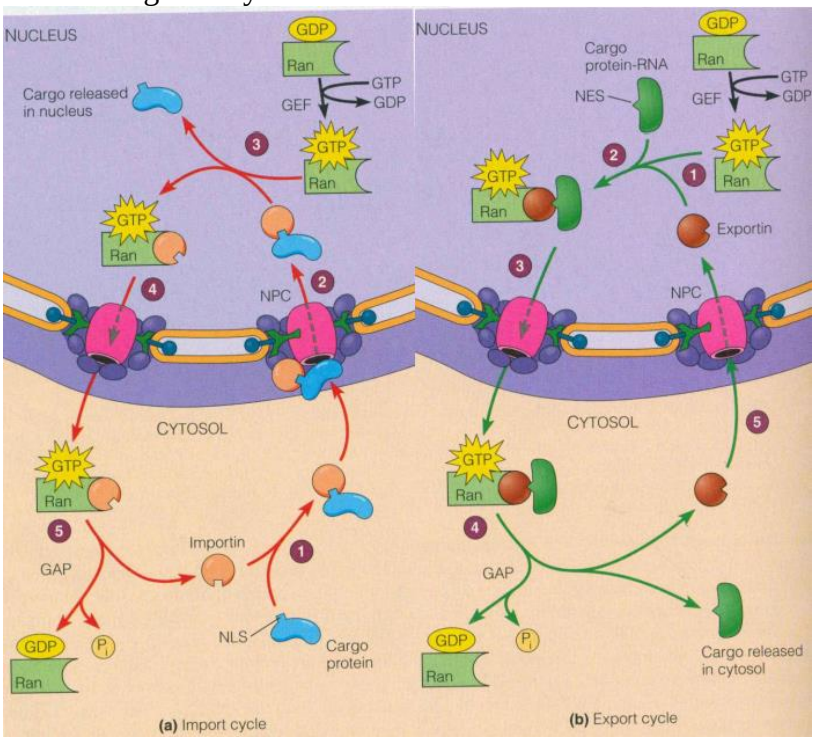
Chloroplasts

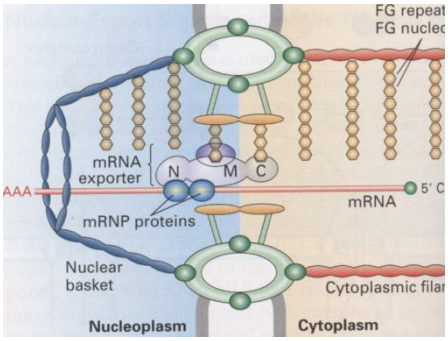
- TOM → TOC, TIM → TIC
- similar mechanism; translocation only requires electrochemical gradient across thylakoid membrane, otherwise only ATP & GTP hydrolysis

- additional signal sequence needed to target for thylakoid space/membrane, unmasked when first signal sequence in cleaved
- several thylakoid transport routes differentiated by energy source & chaperone proteins
- peroxisomes & vacuoles also have post-translational transport

Nuclear Envelope

- nuclear pore complexes: 30x size of ribosome
- nucleoli assemble 20,000 ribosomes/min; 5-6 subunits & 100 histones pass through each envelope/min
- nuclear localization signal (6-30aa hydrophilic) & nuclear export signal
- proteins 20kD – 60kD (e.g. histones) can diffuse through the nuclear pore
- major players: signal on cargo, nuclear pore complex, importin (alpha & beta subunits), exportin, Ran GTPase; GAP (GTP accelerating protein), GEF (GTP exchange factor)
- GTP proteins determine directionality & shuttle proteins determine rate of transport
- mechanism docking & transport of complex through pore unknown; somewhat mechanical
- different from other types of protein transport: nuclear localization signal can be anywhere on protein, protein can be completely folded before entry, localization signal is never removed, allowing reentry after mitosis



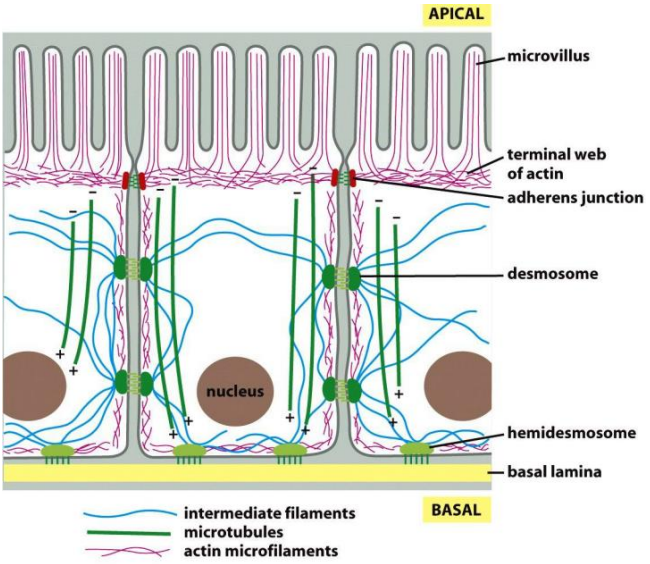


Cytoskeleton & Motility

Overview

- Microtubule-Associated Motors
  - i) Chromosomal Partitioning in Mitosis
  - ii) Axoplasmic Transport
  - iii) Ciliary Motion
- Actin-Based Motility
  - i) Muscle Contraction
  - ii) Cytoplasmic Traffic

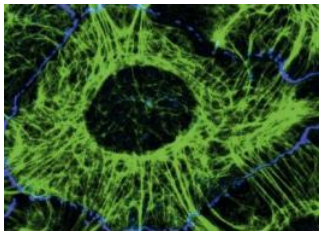
Structures



Classes of Filaments

- actin microfilaments (7nm): motor protein tracks, along plasma membrane, in microvilli, terminal web between adherens junctions, for cell surface shape & cell motion
- intermediate filaments (10nm): between desmosomes & hemidesmosomes, primarily for mechanical resistance to tearing, no polarity
  i) nuclear lamin, keratins, neurofilaments
  ii) strong lateral bonds allow twisting (other filaments would break)
- microtubules (25nm): motor protein tracks, + (beta) end at nucleus & - (alpha) end at apical face give the cell polarity, motility, cytoplasmic organization, spindle fibers, cell structure
- motor proteins: kinesins & dyenins (microtubules), myosin (actin)

Microtubule Structures

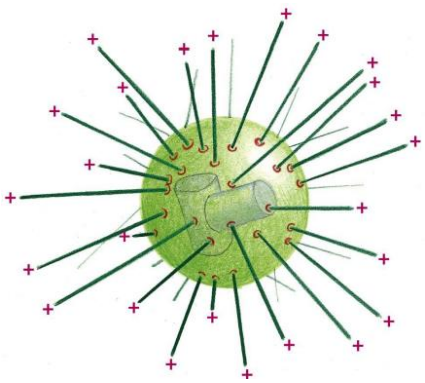


- staining: (keratin intermediate filaments are green on slide)
  i) fix cells: alcohol makes more pores & fixes lipids, aldehyde fixes proteins
  ii) stain that binds selectively to fiber (e.g. primary & secondary antibodies)
- alternating alpha & beta subunits (tubulin heterodimer) form a string (protofilament), 13 (or 11) of which form a column; addition is always to + end
- end charges act as vectors to give directionality for motor proteins
- filament formation due to GTP hydrolysis on beta subunit

Polymerization

- weak noncovalent bonds within protofilaments and lattic structure of filaments provides thermal stability with dynamic ends
- + and – ends have different kon and koff rates
- kon rate depends on the critical concentration, whereas koff is a constant (dynamic instability for microtubules/treadmilling for actin)
- lag phase → growth phase → equilibrium state
- ATP/GTP ‘cap’ when addition is faster than nucleotide hydrolysis (loss of cap = catastrophe)

MTOC (centrosome)



- if you dissociate all microtubules, reassembly will begin from MTOC and elongate outwards (also, ER & GB will fall apart)
- + end moves outwards towards PM from gamma-tubulin ring (seeds – end, nucleation is rate limiting step)
  i) experiment: linked gold particles to anti-gamma-tubulin antibodies to determine structure)
  ii) three tubulin types in cell-free system causes microtubule formation
- colchicine – ancient Egyptian drug; used as treatment for gout & cancer; disaggregates mictotubules which locks cells in mitosis, leading to apoptosis

Centrioles

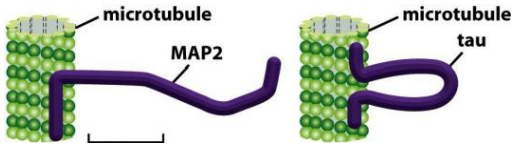
- in centrosome matrix orthogonal to each other
- not found in plants, fungi, & some oocyte cells; instead, found in most animal cells (during microtubule production & mitosis)

- not responsible for seeding microtubule formation (always seeds from center of cell)
- other times, role of centrioles as related to microtubules is more clear
  i) during mitosis, organelle doubles & pulls microtubules out from MTOC
  ii) found as base of cilia (basal body) associated with core microtubules
- structure: 9 triplets of microtubules, proteins associated with spoke & holding triplets together (in some cells (e.g. Drosophila oocytes) doublets instead of triplets)

Microtubule Intracellular Motility

Microtubule-Associated Proteins

- along axon: microtubules + (intermediate) neurofilaments → parallel architecture due to associated proteins
- functions: regulate growth & stability, change filament dynamics,
- types: XMAP215 stabilizes + end, Catastrophin severs MT, & Stathmin destabilizes MT
- drugs used for cancer treatment:
  i) actin: phalloidin, cytochalasin, swinholide, latrunculin
  ii) microtubule: taxol, colchicine, vinblastine, nocodazole
- mechanism not clear, but known to stabilize architecture in certain locations
- MAP2 → dendrites, tau → maintains proper distance between microtubules in axon

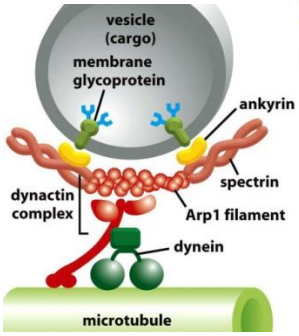


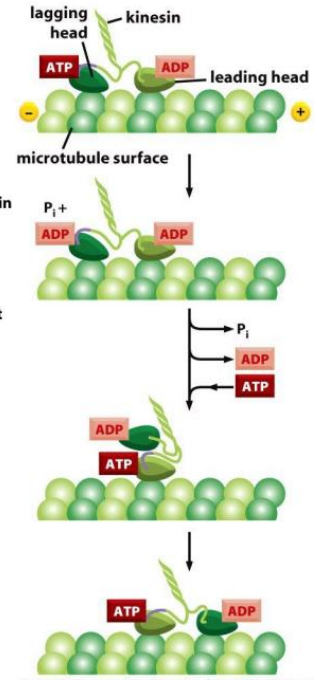
Movements along Axon

- diffusion could take months
- experiment: radioactive amino acids introduced near dendrites of dorsal root ganglia (base of spine) → traced to cell body, where neurotransmitters are synthesized → bolus (spike) moves towards distal axon (anterograde) → bolus may also move back towards cell body (retrograde)
- exudates of giant squid axon: observe that organelles & vesicles line up along microtubules & move when ATP is present

Microtubule Motor Proteins

- both have heavy chains (bound to tubulin) & light chains (bound to molecule)
- kinesin
  i) isolation by R. Vale: matrix of column chromatography is microtubules, nonmotor proteins will flow out, use ATP to release motor proteins
  ii) processive movement by binding to beta-tubulin
  iii) anterograde + end movement (N & M types)
- dynein
  i) multiple light chains bind to accessory proteins
  ii) retrograde – end movement
- reconstituted cell-free system: attach silica bead to kinesin & dyenin in solution with microtubules; observe movement in both directions along microtubules
- responsible for movement of vesicles, organelles, spindle fibers, & cilia
  i) eg kinesin move towards PM & ER, dynein moves towards Golgi complex





Axonal Transport & Neurodegenerative Disorders

- tauopathies: 1 in 10,000; can occur before 55; causes dementia & palsies; overphosphorylation of taus, leading to dispersement & tangling of microtubules (neurofibrillary tangles)
- due to either inherited mutation or neurotoxic substance exposure

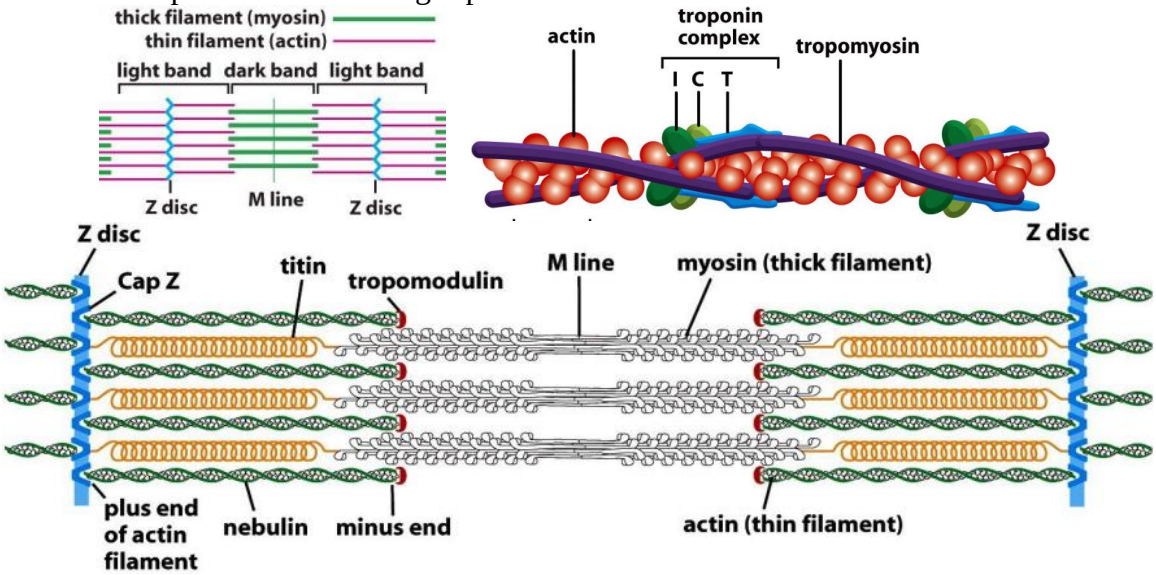
Actin & Myosin Intracellular Motility

Thin Filaments

- actin
  - i) 7nm, in all eukaryotes, most abundant cytoplasmic protein, highly conserved
  - ii) F(ilamentous)-actin: polymer of G(lobular)-actin (has + and – end, 43kD, hydrolyzes ATP when polymerizing)
  - iii) typically found in bundles along PM, nearing adhering matrix to anchor
  - iv) nucleation from ARP complex in cortex along PM
  - v) cross-linking by filamin & spectin
- type 2 myosin
  - i) moves towards + end, in all animal cells, best studied in striated muscle, 17 different types in various different cells
  - ii) actin-dependent ATPase
  - iii) 6 polypetides: 2 coiled alpha helices form neck when bound to 4 light chains at N-terminus
  - iv) can form thick filaments (15nm)
  - v) variety in motor proteins due to variety in cargo-bearing tail domains
- others:
  - i) F-actin, tropomyosin, & troponin (I, C, & T subunits)
  - ii) titin: largest known protein (38,000 amino acids)

Striated Muscle

- myofiber: multi-nucleated cells (<10cm) from merger of myoblasts during embryonic development, join in bundle to form myofibrils
- sarcomere: 2 light bands (actin thin filament, - end towards center) & 1 dark band (myosin thick filament) between Z disc
- myosin heads on either side of M line (bare region) point in opposite directions
- each myosin surrounded by 6 actin
- rigor mortis: Ca slowly leaks out of sarcoplasmic reticulum and causes myosin binding, but ATP depletion prevents release from power stroke
- depolarization runs down t-tubules (membrane invagination) along SR → voltage-gated Ca2+ channels open → troponin binds Ca2+ → tropomyosin displaced → myosin head binds to actin → Ca2+ pump returns Ca2+ to SR
- muscle cramps: lactic acid changes pH + lack of ATP



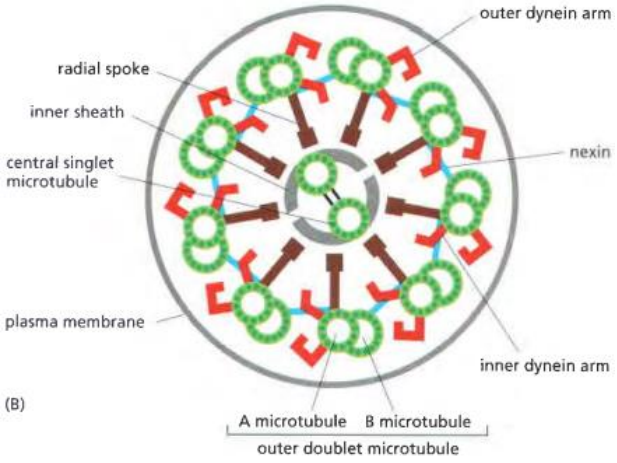
Microfilaments
- cell cortex (below plasma membrane) & stress fibers (adhesion)
- differences: myosin class & what it adheres to
- non-muscle myosin: I & V

Cytoplasmic Streaming
- cell cortex, where no movement occurs, is separated by actin filaments of same polarity
- cytoplasm moves in circles around vacuoles; mixing allows diffusion to occur
- myosin-coated latex beads stream & ATP-dependent
- mono-polar mini-myosin

Cell Motility

Extension
- cell crawls by extending pseudopodia (cortical), which reach out & adhere (stress fibers)
- contraction of cortical myosin II at back end of cell to push cytoplasm forwards
- chemoattractants bind to receptors, which activate Rac pathway (actin forwards) and Rho pathway (antagonistic, myosin contraction)

Cilial Movement
- ionic currents change when membrane encounters an obstacle, changing direction of cilial beating
- flagella & cilia move by bending core axoneme and shifting microtubule doublets bound by linker proteins
- classes: motile (respiratory tract, ova duct, CS fluid), primary (sensory: fluid motion in ear, kidney), & nodal ()
- structure: 9 + 2 continuous with triplet basal body, assembly outward from basal body
  i) A (complete) + B (incomplete) doublets; inner & outer dyenin arms (first ever isolated); radial spokes; nexin ring
  ii) motor proteins attached to A tubule move along adjacent B tubule → sliding leads to organelle bending

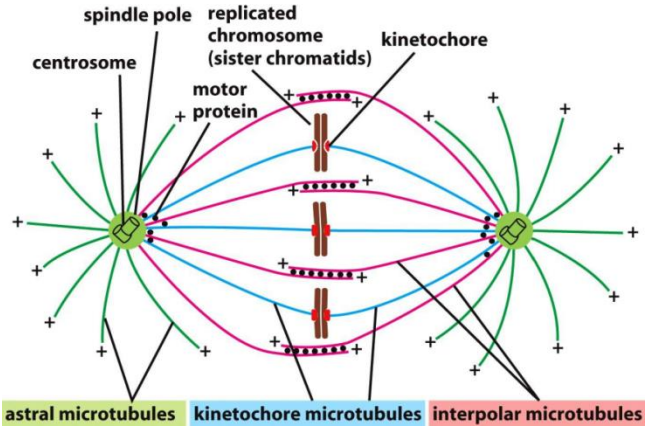


Potential for Genetic Defects
- dyenin, radial spokes
- Kartagener's Symdrome/Ciliary Dyskinesis: autosomal recessive, 1/20,000, situs inversus (body symmetry is reversed in 50% of individuals), chronic otitis, male infertility, repiratory tract disease
- body symmetry: notochord forms during gastrulation; nodal cells at bottom; nodal cilia creates gradient of morphogens which determine morphology

Mitotic Microtubules

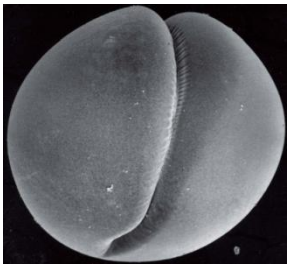
Phases of Mitosis
- before mitosis, centrioles separate and replicate (ghosts appear)
- + end of spindle fibers bind to chromosomes (polar fibers become kinetochore fibers)
- cohesin holds sister chromatids together & binds
- at kinetochore: protein layers, CENP-E (MT-binding protein), dynein, MCAK (mitotic kinesin)
- actin homolog pushes chromosomes apart during bacterial replication

Anaphase



- importin delivers kinesin-14, which moves along Ran gradient towards spindle poles
- multiple forces pull chromatids apart:
  i) depolymerization of tubulin at plus end of kinetochore microtubule
  ii) kinesin motors slide interpolar fibers over each other (while spindle fibers are elongated - teloscoping)
  iii) dyenin motors pull astral fibers towards PM cortical fibers (move towards centromere)
  iv) all exert tension on kinetochore fibers
- cohesin (4 subunits) cleaved by separase (mutation causes ploidy problems)
- progressive depolarization at kinetochore caused by depolymerization

Telophase & Cytokinesis



- actin & myosin filaments of the contractile ring
- nuclear lamin reforms
- midbody: remaining interpolar microtubules span cleavage furrows → snaps → membranes become continuous
- contractile ring (actin + class 2 myosin) forms around equator between poles to form cleavage furrow
- unknown: why the cleavage furrow forms at the equator