Membranes and membrane transport

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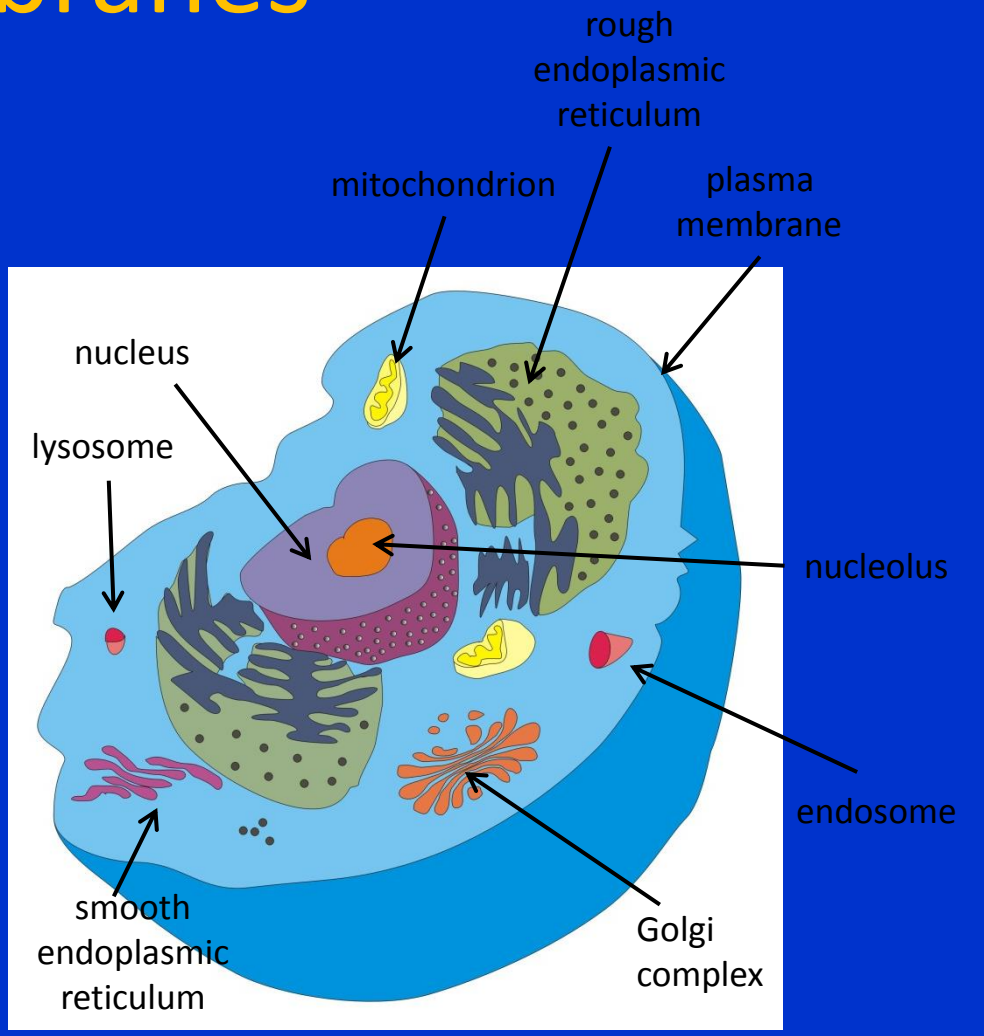
Membranes

Human body – more than 10 000 mld cells

Surface area of membranes
~ km^2

Functions:
- compartmentalization (mt, ER, nucleus…)
- barrier (protection)
- regulation of transport (in and out)
- medium for membrane proteins
  - information transfer
  - enzymatical processes
  - connections to other cells
- cell shape formation
- cytoskeleton anchoring



Compartmentation

- Intracellular fluid (ICF)

  2/3 of total body water

  1. production, storage, and consumption of E in the cell
  2. environment for cell repair
  3. cell replication medium
  4. special functions

- Extracellular fluid (ECF)

  plasma + interstitium

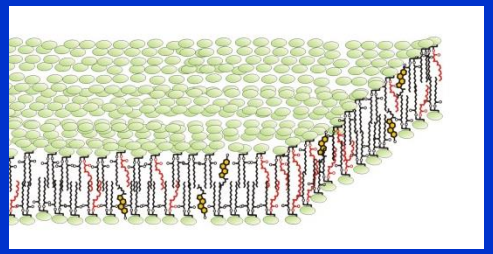
  1. mainly transport

compound	ECF	ICF
Na^+	140 mmol/l	10 mmol/l
K^+	4 mmol/l	140 mmol/l
free Ca^{2+}	2.5 mmol/l	0.1 mmol/l
Cl^-	100 mmol/l	4 mmol/l
HCO_3^-	27 mmol/l	10 mmol/l
PO_4^{3-}	2 mmol/l	60 mmol/l
glucose	5.5 mmol/l	up to 1 mmol/l

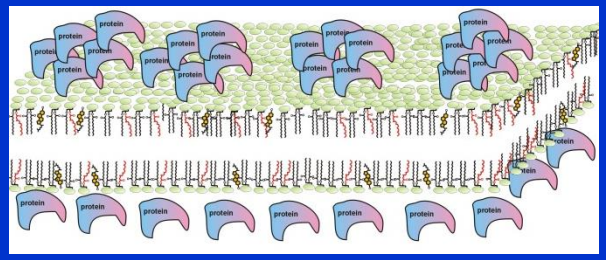
Structure of membranes I

1925: Gortel and Grendel

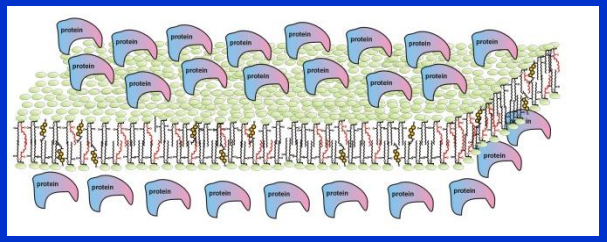
- membranes contain lipids:



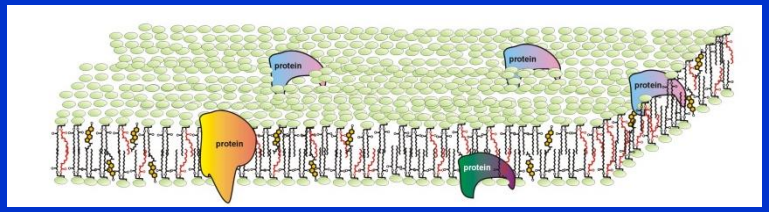
- and proteins: 1935: Danielli and Dawson



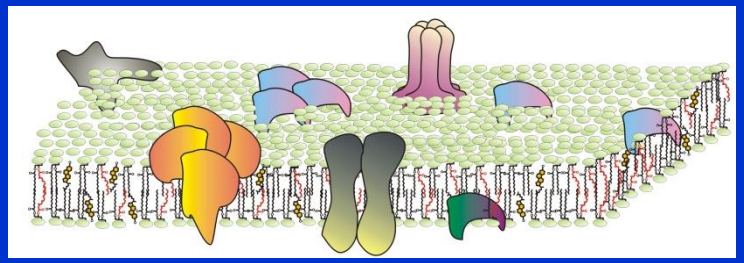
1966: Robertson



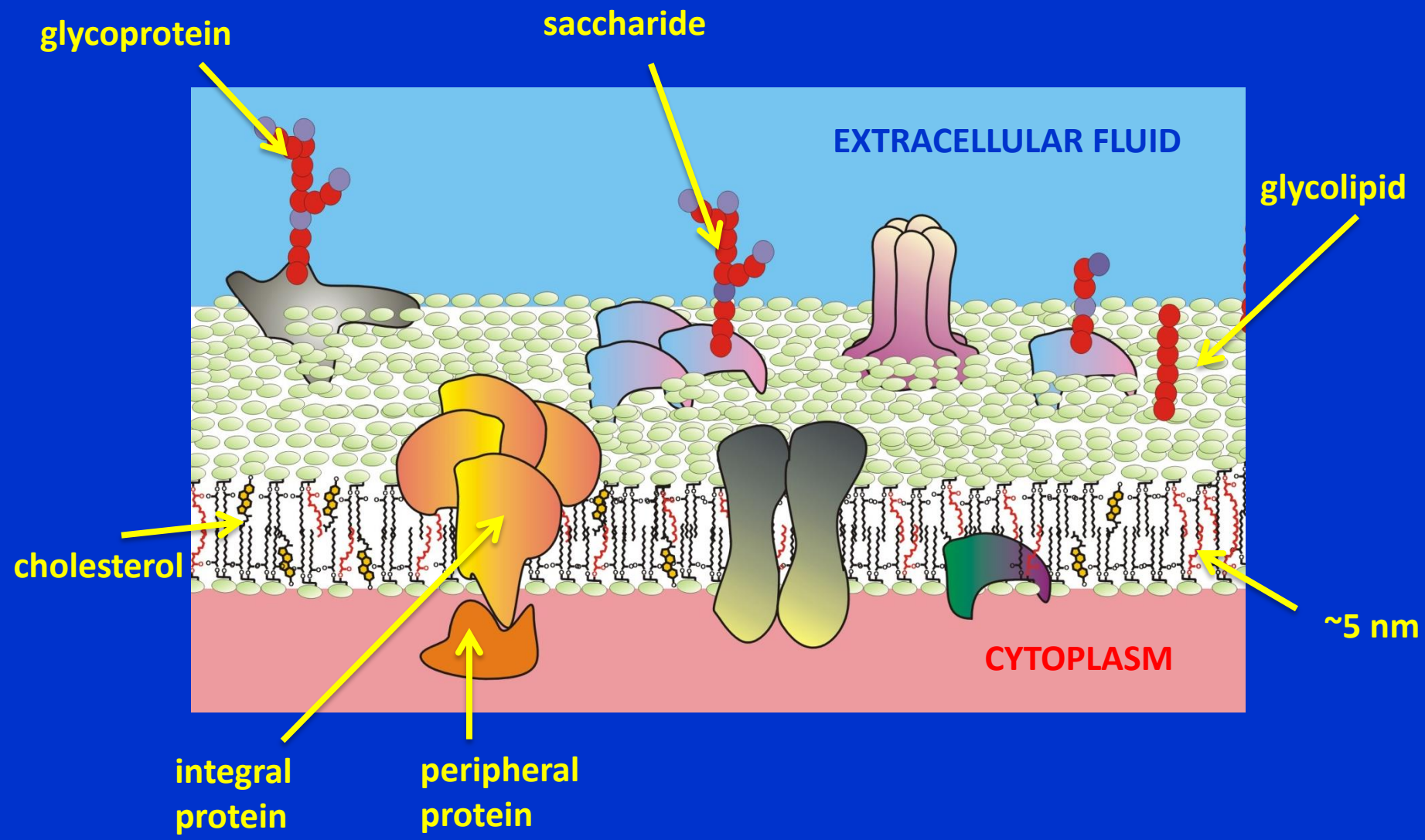
- are fluid: 1972: Singer and Nicolson



mosaical: 2005: Engelman



Structure of membranes II



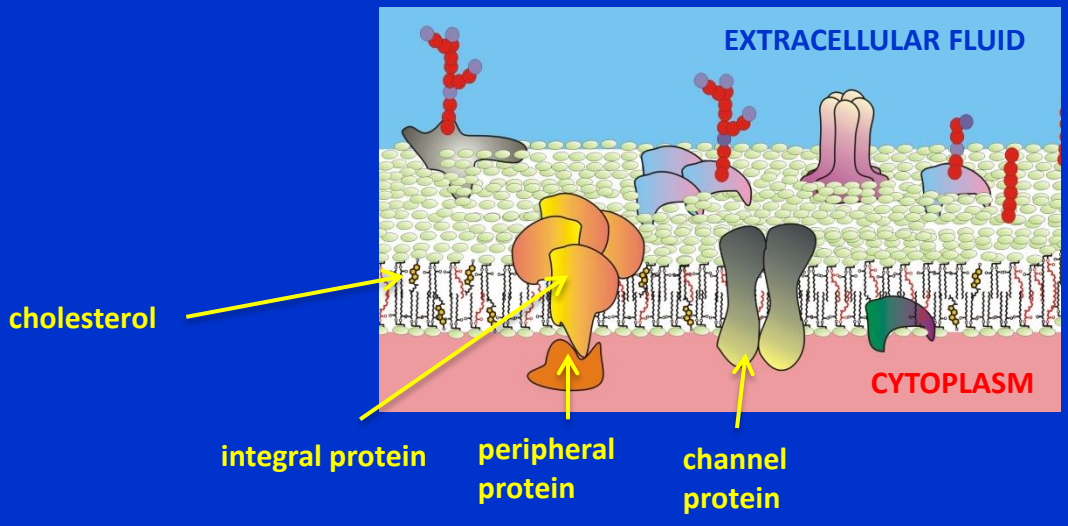
Membrane component function

Lipids

- amphipathic molecules – formation of membrane structure
- cholesterol – keeps membrane fluidity (T, composition)
- DPG (CL) – in mt important for H^+ gradient transfer

Proteins

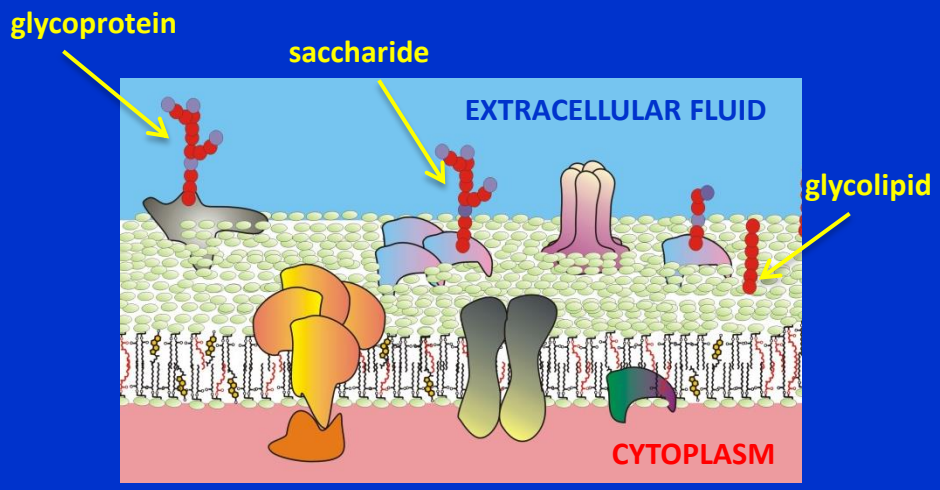
- integral proteins – enzymes, pumps (active transport)
- peripheral proteins - glycoproteins – chemical receptors
- channel proteins – transfer molecules across membranes



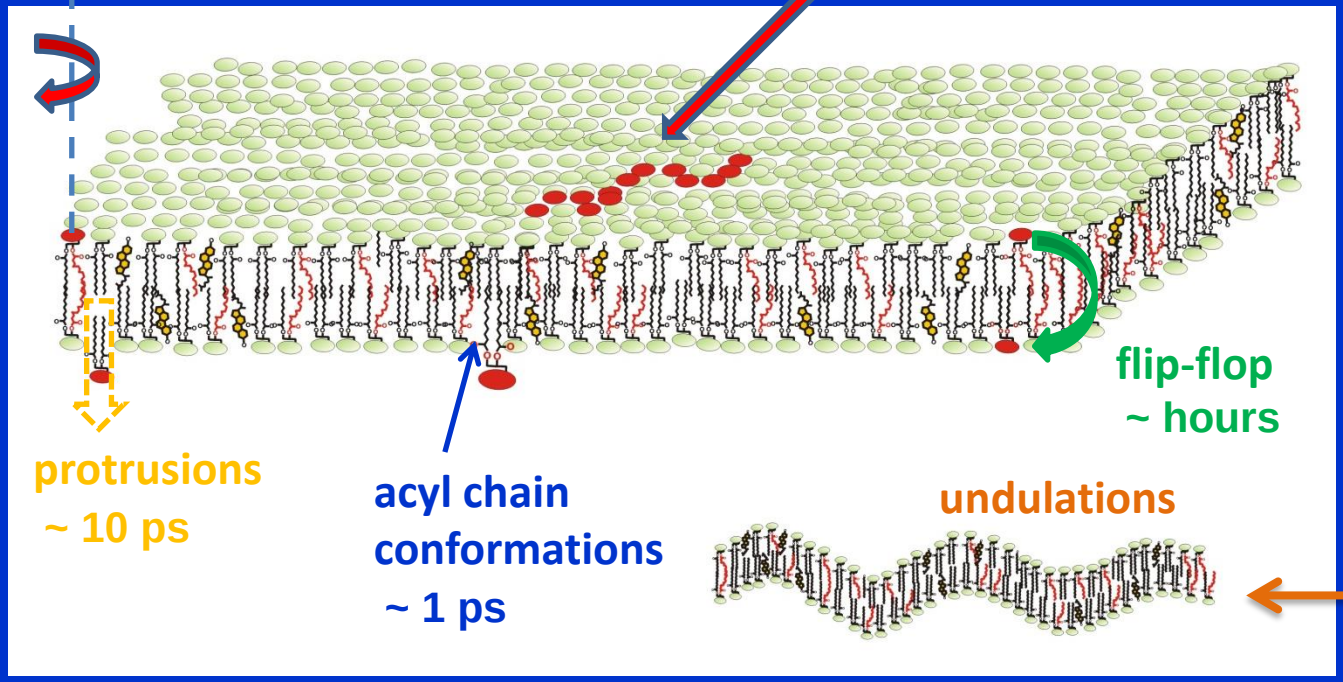
Membrane component function

Saccharides

- structural parts of glycolipids or glycoproteins
- saccharide moiety always oriented exofacially

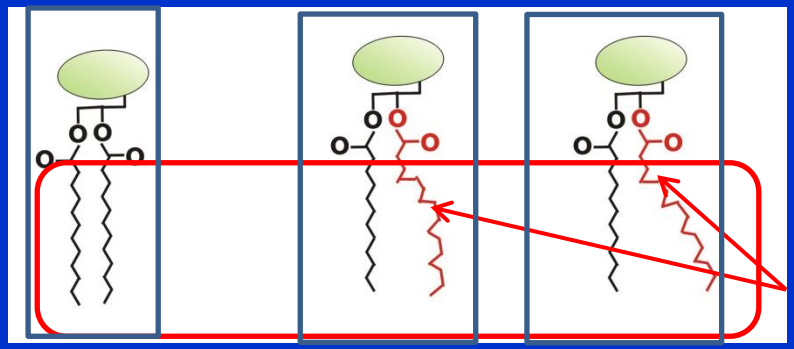


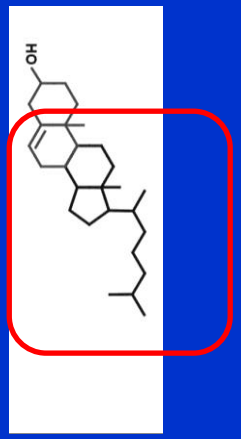
Membrane structure – lipids



Lipid structure of biological membranes

- nonpolar part: fatty acid/sterane skeletons





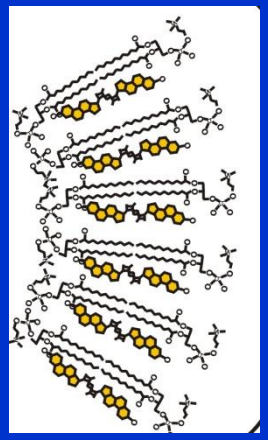
- polar part: phosphate/bases(saccharides) sphingosine/base (saccharide)

glycerolipids

glycerophospholipids

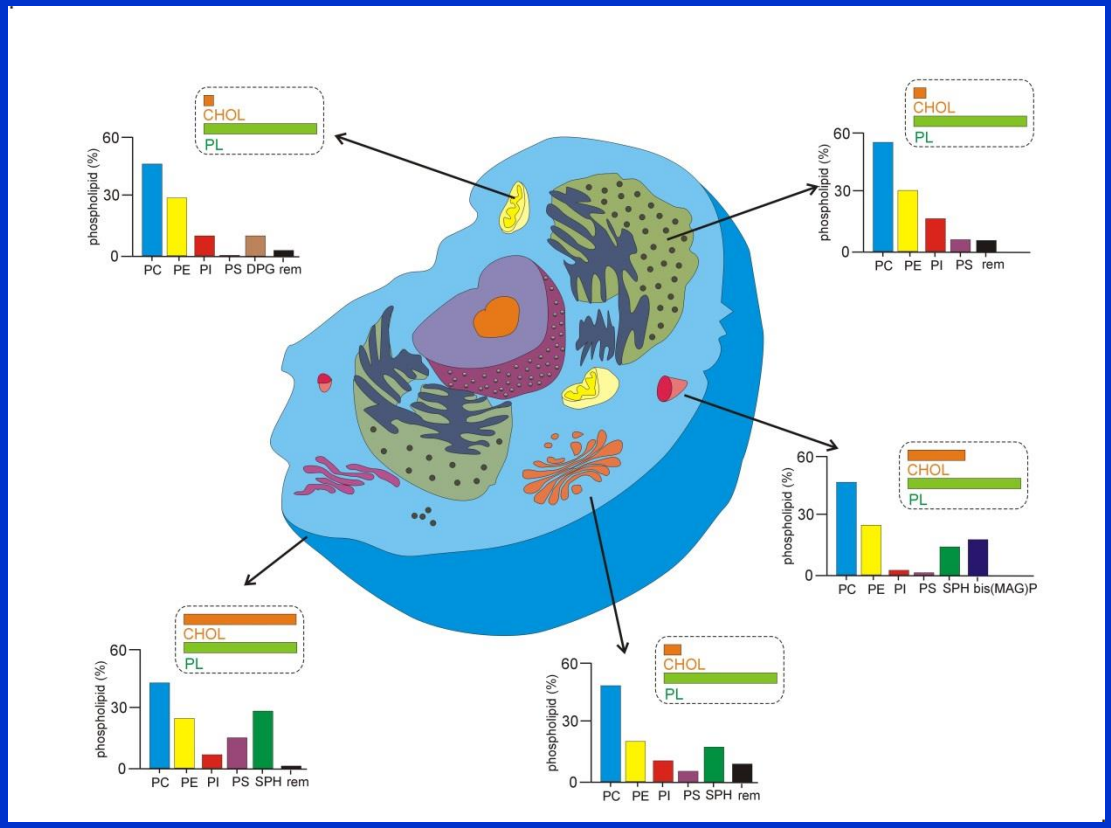
sphingolipids

sterol lipids



Membrane composition - lipids

- membrane lipid composition of individual membranes of subcelullar structures is different



Lipid – protein bonds

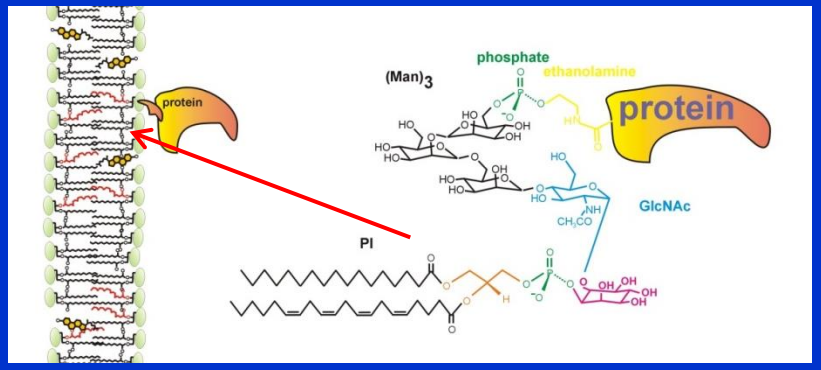
A. GPI-anchored proteins – on outer face

bonded via short oligosaccharide to glycophosphatidylinositol (GPI)

various receptors

enzymes

adhesive proteins



B. other anchoring: on cytoplasmatic face

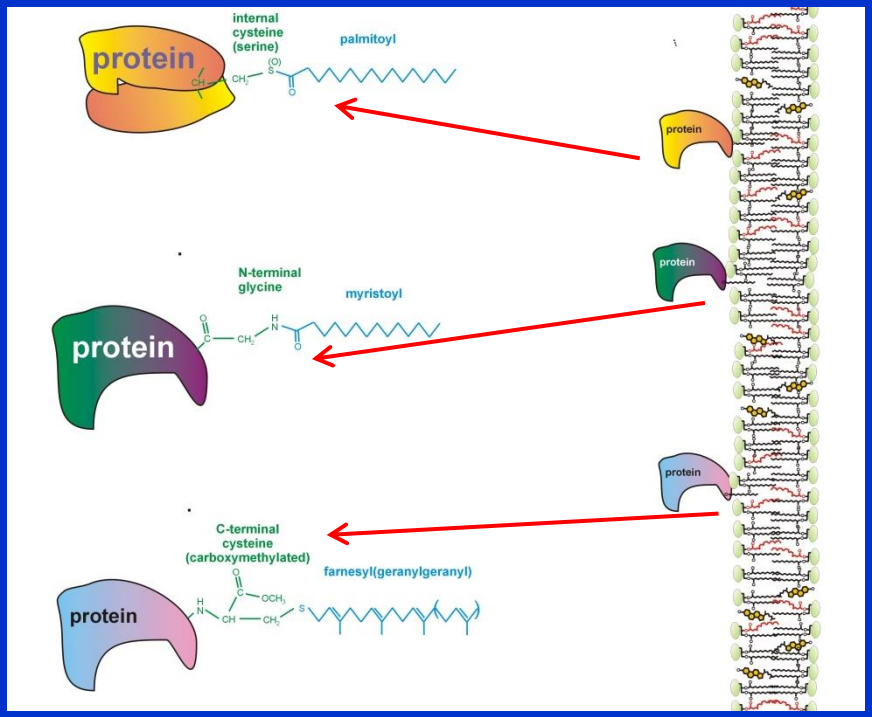
bonded via long hydrocarbon chain

proteins Src & Ras

- normal to malignant transformation

Gγ subunits, rhodopsin kinase

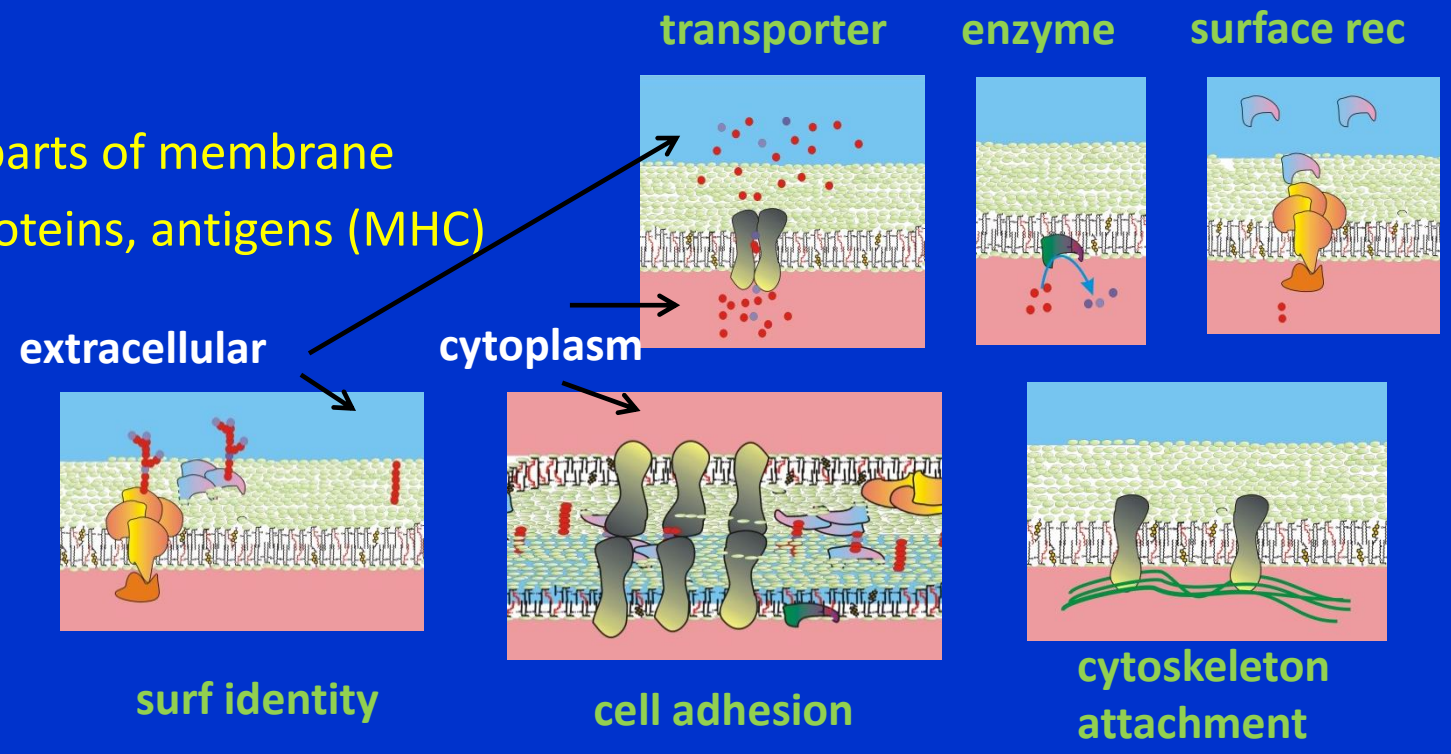
centromeric proteins, nuclear lamins



Membrane proteins

5539 human genes encode membrane proteins, which is approximately 26 % of human protein encoding genes) (Fagerberg 2010)

- receptor dependent channels/transporting molecules
- receptors
- enzymes
- structural parts of membrane
- bonding proteins, antigens (MHC)



Membrane composition - proteins

- protein composition of individual membranes of subcelullar structures is different
- protein/lipid ratios also variable: plasma membranes of hepatocyte (0.85), erythrocyte (1.1), myeline (0.23), mitochondrial membranes – inner (3.2) and outer (1.1)

- some typical proteins can be used as markers of isolated membranes

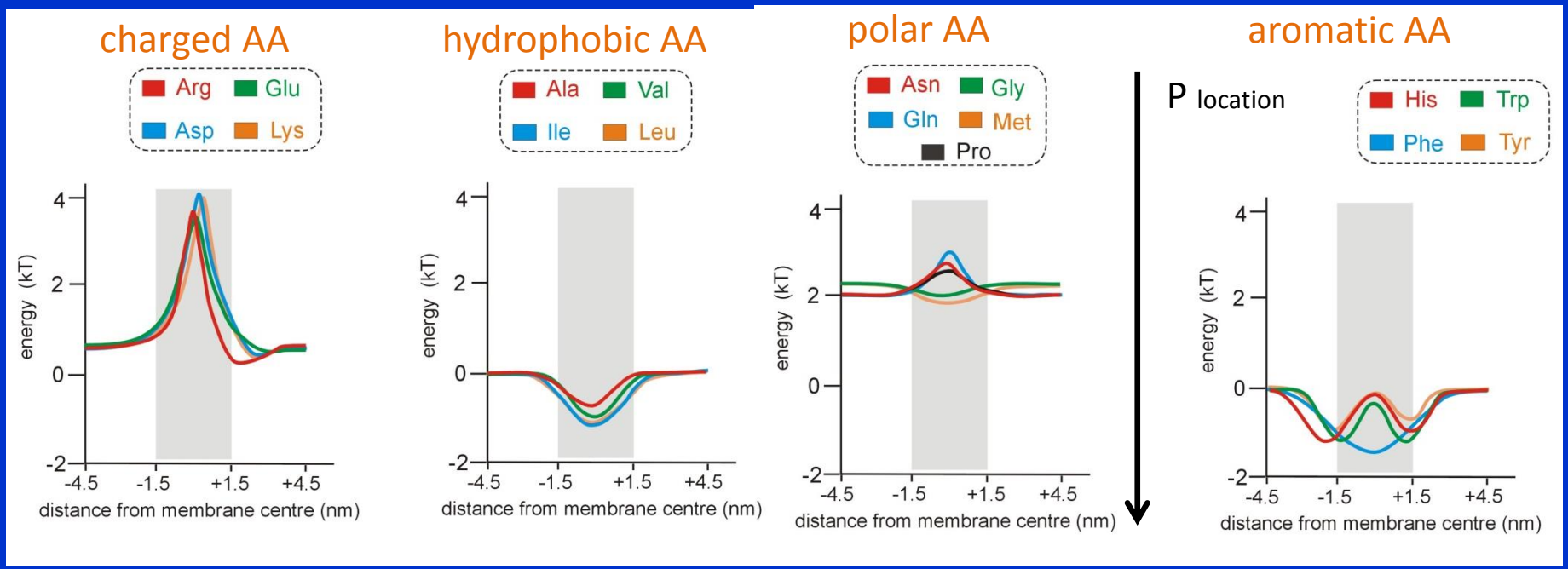
structure	protein (enzyme)
plasma membrane	adenylyl cyclase, Na^+/K^+ ATPase
endoplasmatic reticulum	glucose-6-phosphatase
Golgi apparatus	
cis	GlcNAc transferase I
medium	Golgi mannosidase II
trans	galactosyl transferase
mitochondrial membrane (inner)	ATPase

Protein structure of biological membranes

hydrophobic AA - prefer inner membrane space

hydrophilic AA – prefer outer membrane space

low probability of AA location



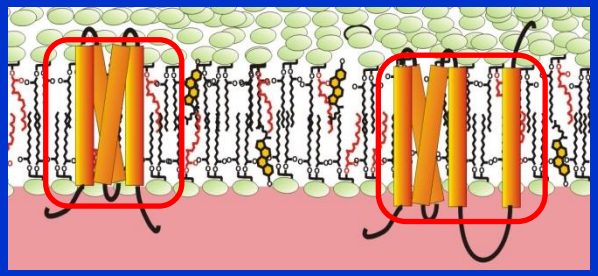
high probability of AA location

Protein structure of biological membranes

α-helix

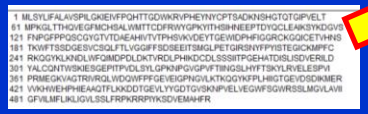
minimalization of hydrophilicity of peptide bonds

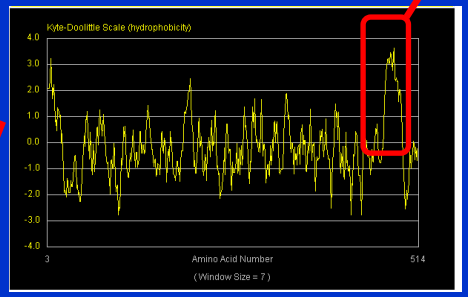
transmembrane part of protein (~20 AA)



hydropathic index

calculations for consecutive sequences of 20 AA in peptide





hydrophobic region = transmembrane domain

hydrophobicity

http://www.vivo.colostate.edu/molkit/hydropathy/

Membrane proteins

Integral proteins

majority of membrane proteins

strong interactions with membrane PL – detergent

globular and amphiphatic

two hydrophilic ends with hydrophobic part

inserted into or span through membrane

can span membrane once or more times

(= transmembrane proteins)

transporters, ion channels, receptors, G-proteins…

Peripheral proteins

no direct interactions with membrane PL

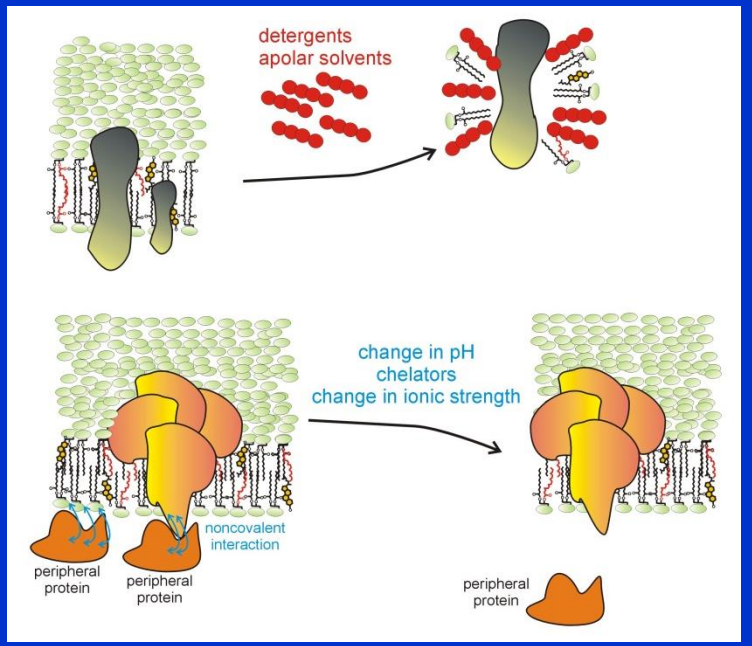
– salts with ionic strength

interactions with hydrophilic parts of integral proteins

spectrin, ankyrine….

Lipid anchored proteins

bound to lipid bilayer via lipid-modified amino acids



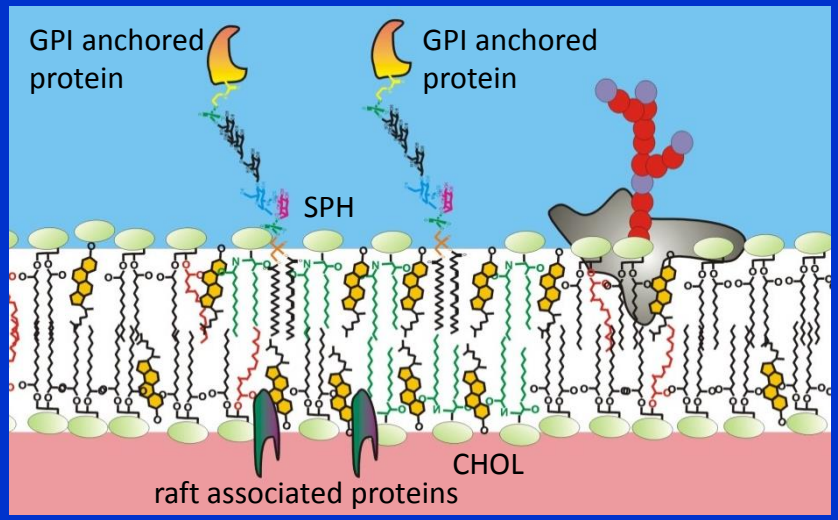
Membrane structures – lipid rafts

1973 membranes are not homogeneous – in ery there are regions that are resistant to detergents

1980 membrane lipids have assymetric distribution

1994 GPI-proteins in CH/SPH rich membrane regions

2006 Pike: Membrane rafts = small (10–200 nm), heterogeneous, dynamic domains rich on CH and SPH, which are able to compartmentalize cellular processes



Membrane structures – caveolae

Caveolae = main type of membrane rafts

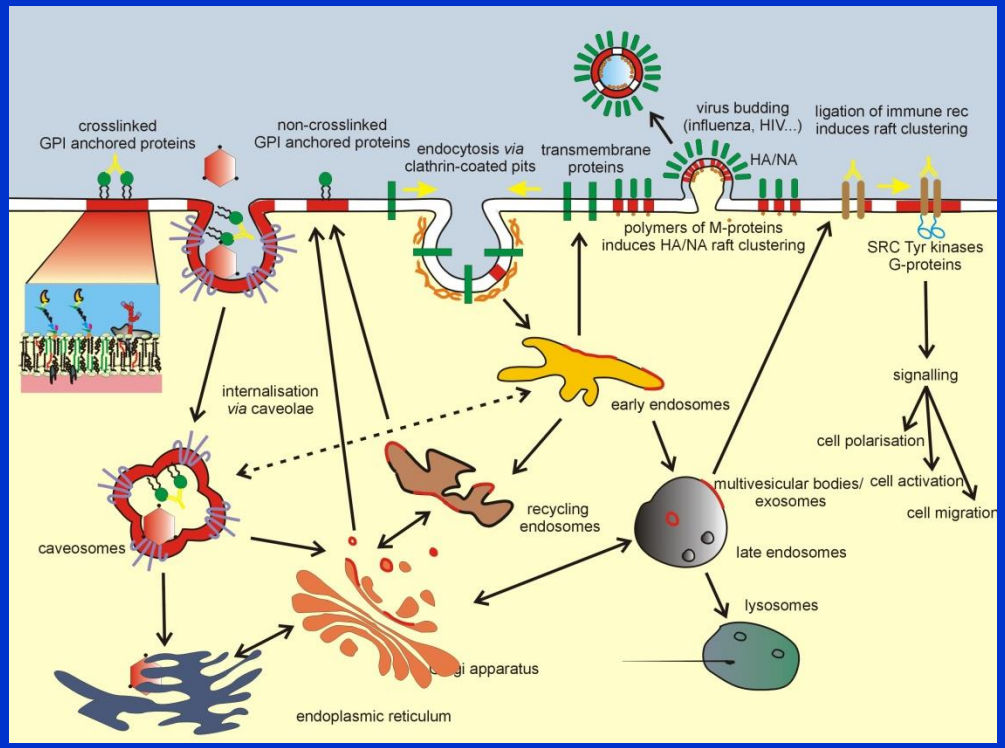
invaginations of cellular membranes containing caveolin

<u>Caveolins</u>

three isoforms of caveolin – 1,-2,-3

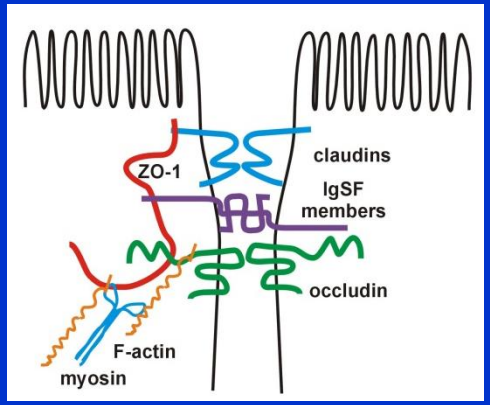
protein is palmitoylated

causes raft accumulation



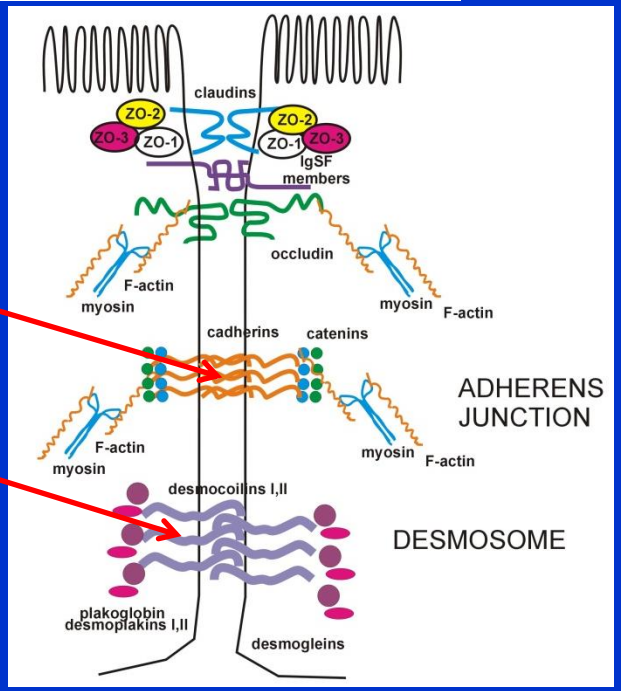
Membrane structures – tight junctions

on apical face of epithelial cells
belt-like, circumferential structure
prevent diffusion of macromolecules
between cells
= crucial for function of epithelial barrier



cell „sticking“ is further strenghtened by
adherent regions
„adherent junctions“
desmosomes

- initial adhesive attractions of cells
- do not close cell layer



Membrane structures – microvilli

microvilli = stable protrusions from plasma mebrane

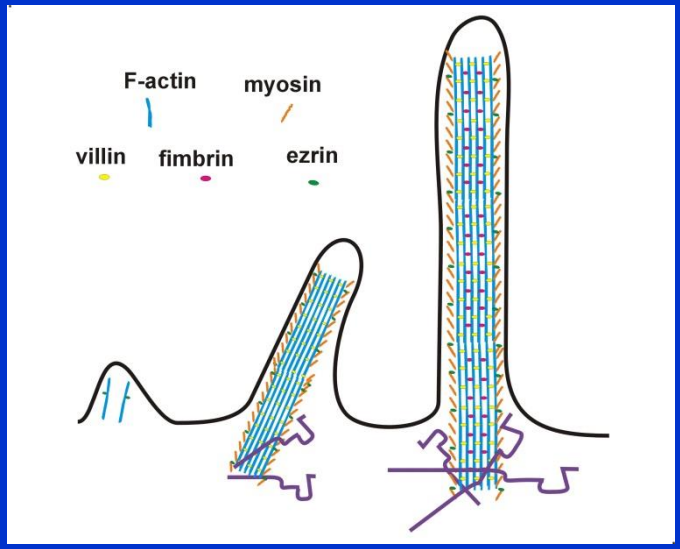
high membrane surface area – absorption

increased interaction with informative molecules – signal perception

coated with glycocalyx – glycoprotein layer

supported with actin filaments, which are crosslinked by fimbrin and villin

villin – important for microvillar morphogenesis



Membrane structures – gap junctions

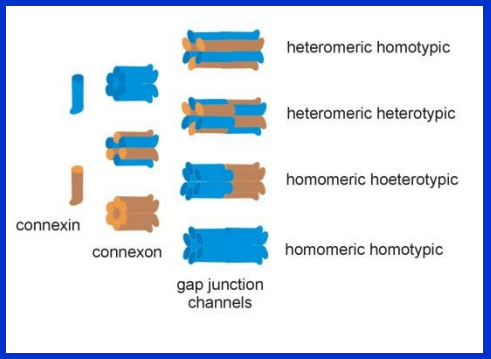
gap junctions = structures enabling direct transfer of small molecules (to 1200 Da) between adjacent cells

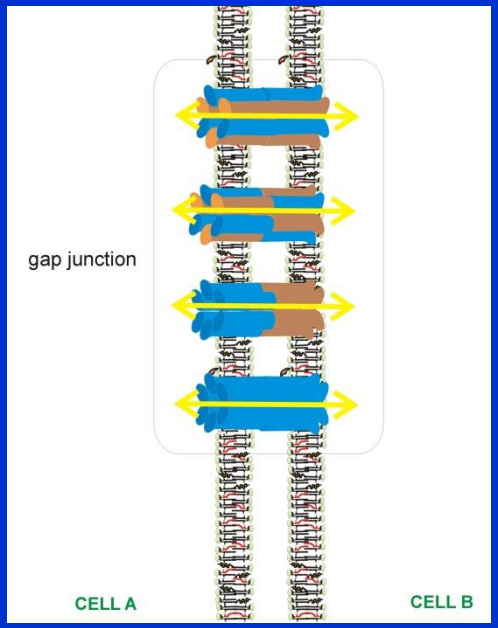
contain connexins

6 connexins$_{cell1}$ + 6 connexins $_{cell2}$ = connexon

one region of gap junction contains more connexons

connexon channel formation needs 2-4 nm proximity of membranes





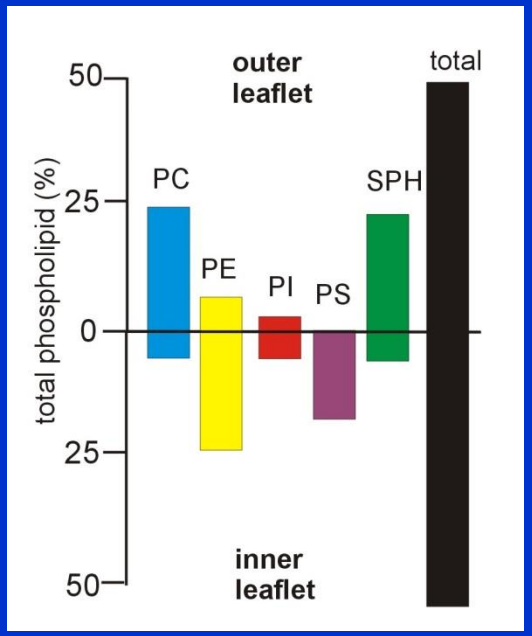
Membrane assymmetry

lipids

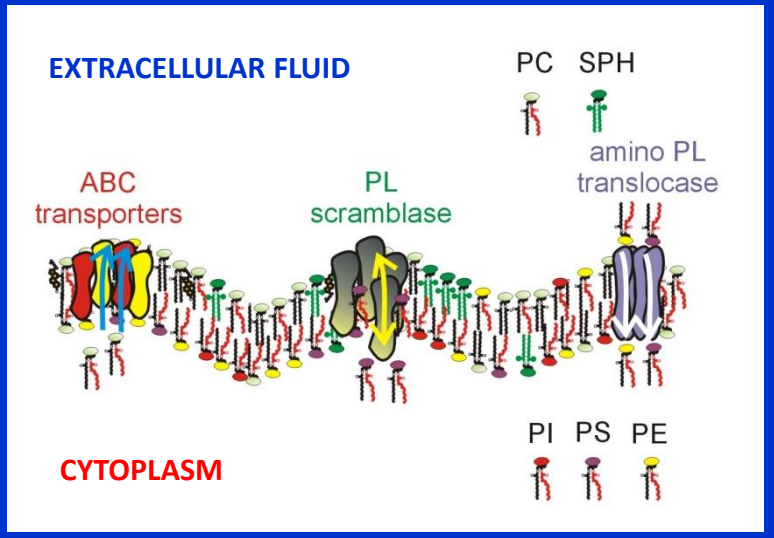
generally choline PL (PC, SPH) on outer side of membrane

aminoPL (PE, PS) on inner side of membrane (anionic PL)

mammalian PM



assymetry is kept



Membrane assymmetry

proteins

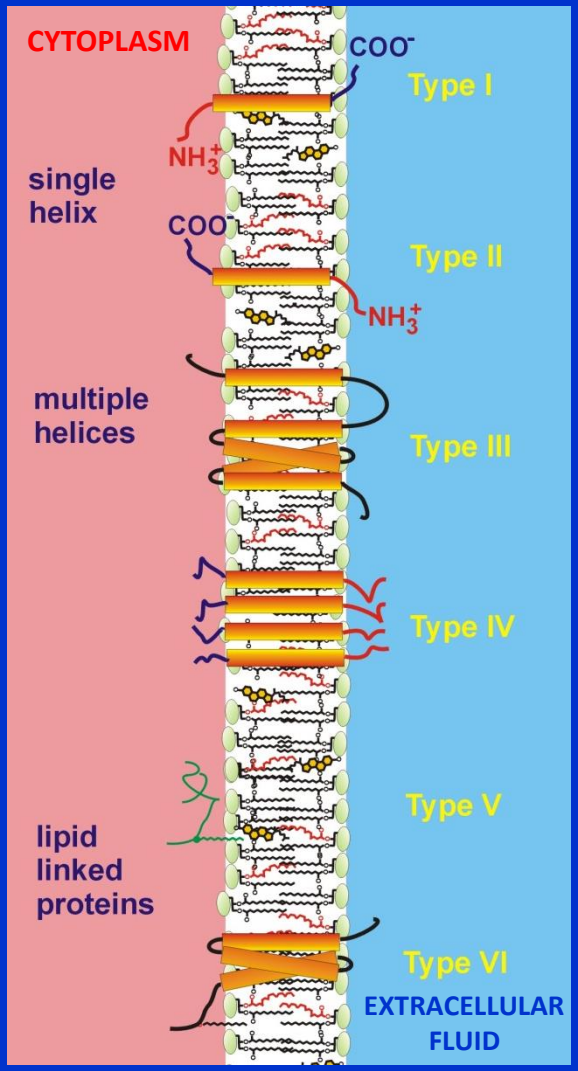
assymetry of peripheral proteins is based on their interaction with membrane structures

assymetry of integral proteins results from membrane orientation

(types I-VI of integral proteins)

saccharides

saccharide moiety always on exofacial side



G-proteins

G proteins

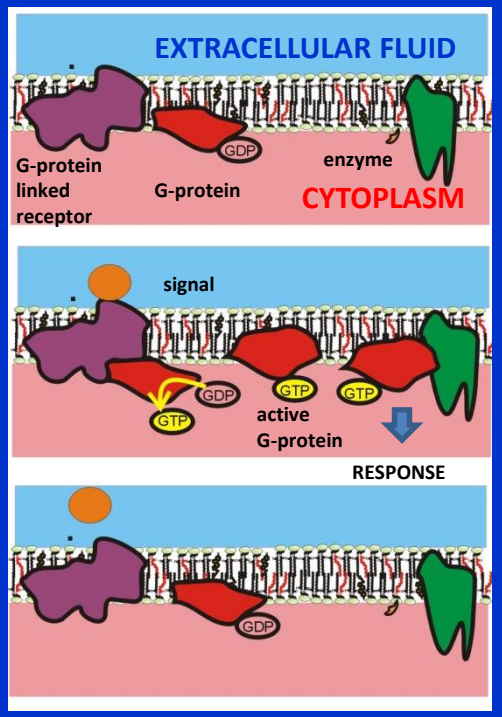
heterotrimeric complex with subunits α_s, β, γ

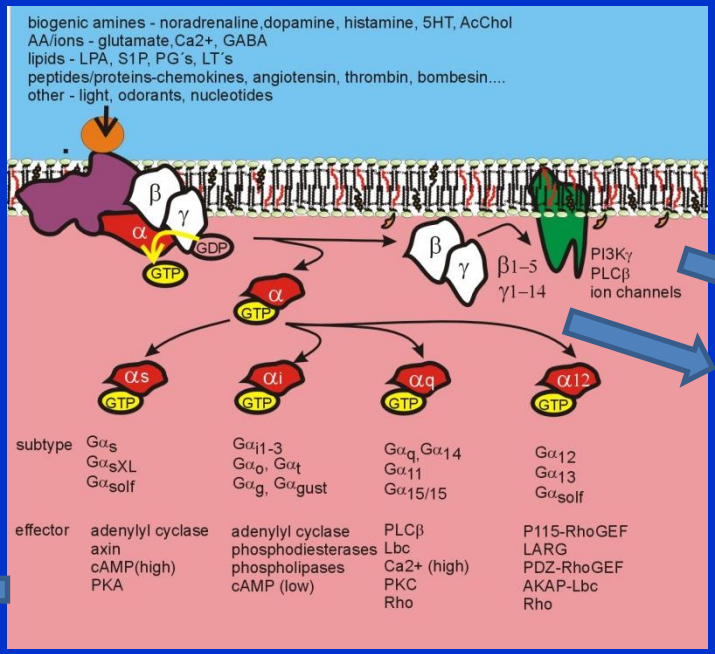
subunits $\beta\gamma$ anchored with prenylation

GPCR

receptors coupled with G-proteins

typically 7 membrane helices





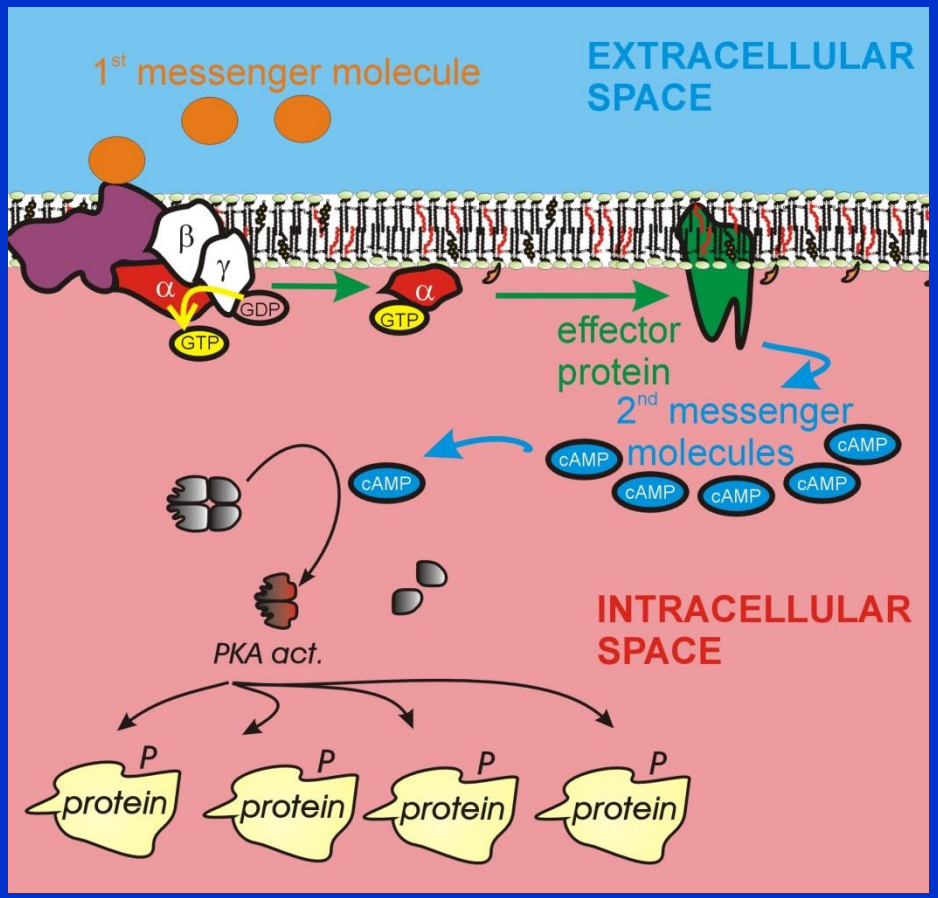
cellular responses

cellular responses

Second messengers

Second messengers = molecules that

1. **relay signals received** at receptors on the cell surface (hormones, growth factors, etc.) — to target molecules in the cytosol and/or nucleus
2. greatly **amplify the strength** of the signal synthesized/released from stores
3. cause (= **presence is signal**) changes in the biochemical activities (protein characteristics) **within the cell**



Second messengers

Types of second messengers:

I. Cyclic nucleotides

cAMP by adenylyl cyclase (*needs* G-protein) from ATP

adrenaline, glucagon, LH…

cGMP by guanylyl cyclase (*does not need* G-protein) from GTP

ANP, NO, hv in retina...

II. Lipids

IP_3 and DAG from PIP_2 by PLC

DAG recruits PKC

IP_3 releases Ca^{2+} from ER/"calciosomes"

TSH, vasopresin, GABA, angiotensin...

III. Ca^{2+}

Ca^{2+} released by IP_3 rec/ryanodine rec

high Ca^{2+} binding to troponin C, calmodulin

muscle contraction, T/B cell activation, exocytosis

IV. Other (eicosanoids, Mg^{2+}, Ser-P, Chol-P, NO, CO, H_2S…)

Saccharide structure of biological membranes

Membrane saccharides

- always on **exofacial side** of membrane
- monomer sequences are considerably variable
- **2 - 10% weight** of the membranes

I. Glycolipids

usually minor part (~7% of saccharides in glycolipids)

II. Glycoproteins

majority of saccharide moiety ~93% of saccharides in glycoproteins

Saccharide structure of biological membranes

x proteoglycans: saccharide >> protein

I. Glycoproteins

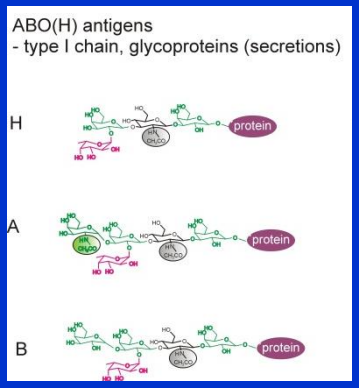
Protein + short saccharide part (can be branched , < 15 units)

1. Sialic acid usually on end – negative charge
2. Bonding with several AA: 2 types of linkage

important for half-life of serum glycoproteins

N-glycosidic bond to Asn

O-glycosidic bond to Ser/Thr



Functions of glycoproteins in membranes

determination of immune response

host-virus/bacteria interaction

HIV entry into immune cells: *via* chemokine rec

Helicobacter pylori adhesion to gastric mucosa cells: *via* Lewis blood antigen

play a role in cell adhesion

Saccharide structure of biological membranes

II. Glycolipids

short (branched) oligosaccharide chain + ceramid → sphingolipids

(+ prenol, + acylglycerol)

Functions of glycolipids in membranes

1. membrane anchors, cell distribution targeting
2. cell recognition

   Erythrocytes – ABO antigen system

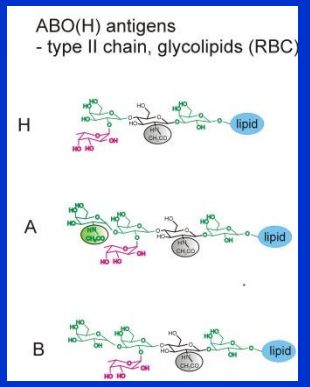
3. parts of lipid rafts (cell signalling functions)

   involved infectious diseases

   cholera toxin

   influenza virus binding to gangliosides)

if secreted into serum: glycoproteins with ABO oligosaccharides



Membrane transport

Small molecules

I. Passive transport

1. Simple diffusion
2. Facilitated diffusion
   - 2.1. transporting molecules/peptide structures
   - 2.2. via ion channels

II. Active transport

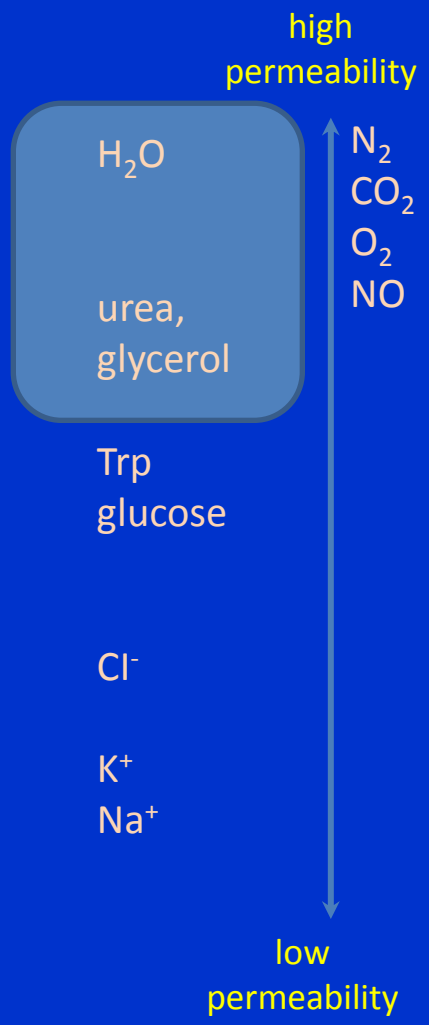
1. most often via ATP coupled transporters

Large molecules/supramolecular structures

not transferred across membrane

I. Endocytosis

II. Exocytosis

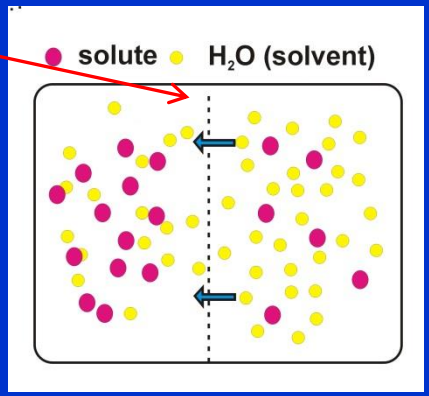


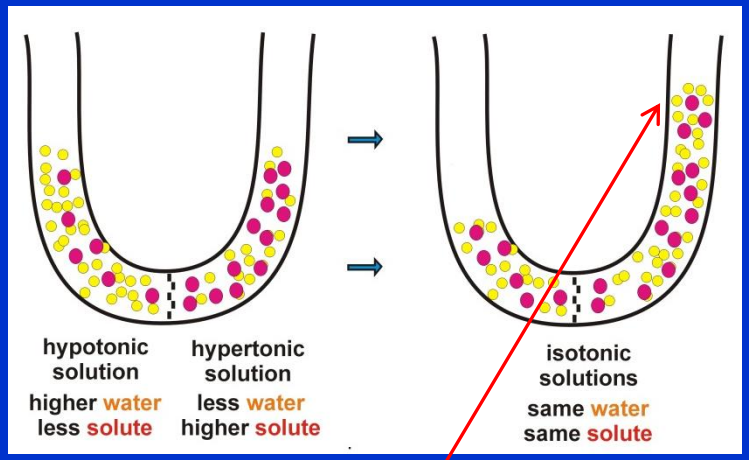
Osmosis

Osmosis = passive process, when molecules of water move from area with higher concentration of water into the area with lower concentration

semipermeable membrane does not allow solute transfer

= molecules of water dilute solute





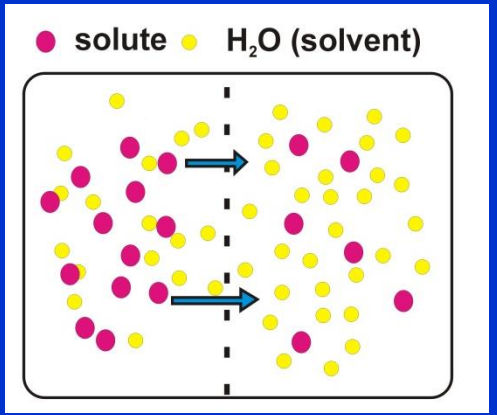
Reverse osmosis

E must be supplied

pressure on the semipermeable membrane

(sea water desalting; high pure water production)

Facilitated diffusion



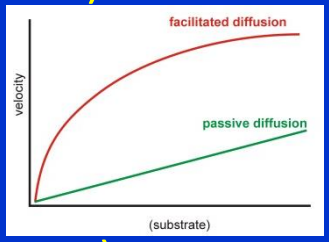
Diffusion = passive process, when the molecules from the area
with higher concentration move into the area
with lower concentration

Facilitated diffusion (ion channels/transporters)

movement across membrane is augmented with ion channels/transp. proteins

also passive = no need for E (voltage gated neuronal ion chanels)

saturable system



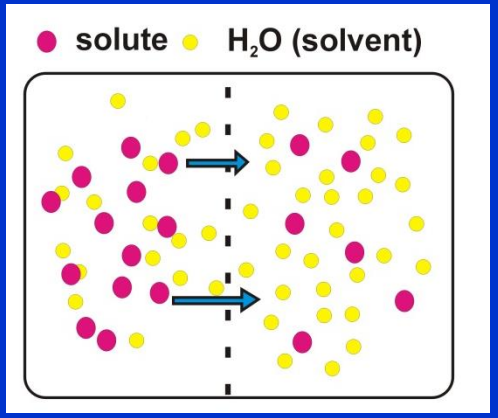
velocity of facilitated diffusion is proportional to:

1. concentration gradients across membrane
2. number (N) of active transporters/channels (e.g. hormones)
3. affinity of linkage between structure transporting and being transported
4. velocity of conformational changes of transporting structure

insulin – increases N of Glu transporters via mobilization from intracelular space

growth hormone, estrogens - increasing N of AA transporters

Simple diffusion



Diffusion = passive process, when the molecules from the area with higher concentration move into the area with lower concentration

Simple diffusion

v diffusion ~ concentration gradient

- persistent supply of molecules + removal away

(lungs: O_2 + CO_2; intestine: glucose lumen + port. circulation)

~ area – area increase

(lung alveolae; mt cristae; intestinal villi…)

~ temperature (not easily accomplished)

~ electric potential across membrane – abstraction of ionts

(formation of ATP)

~ hydrostatic pressure over membrane - (…?..)

1/~ distance – more folds

(thin membranes – max. 7-10 nm; foldings – more membranes)

Membrane transporters

Uniport

transport of one type of molecule

(GLUT1, Valinomycin)

Symport

coupling of transfer of 2 different molecules

gradient of 1st molecule ⇒ transfer of 2nd molecule

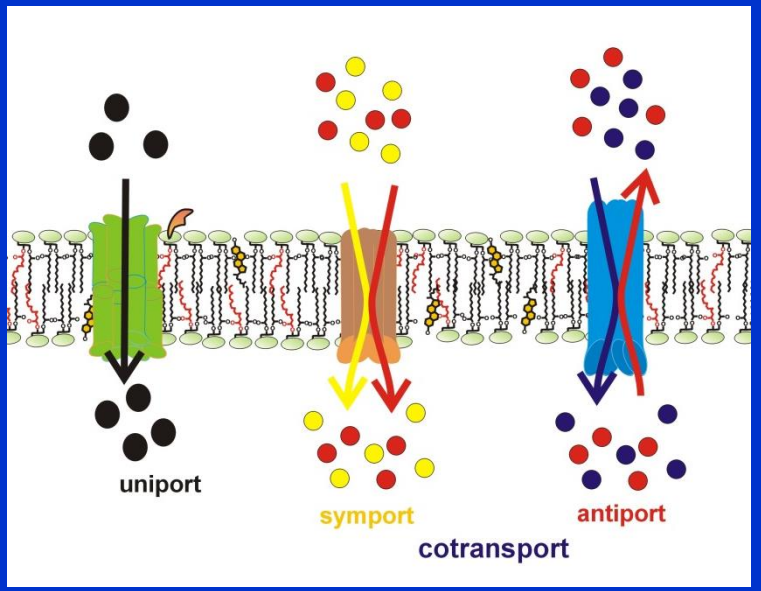
(glucose – Na^+ symport)

Antiport

confers only exchange (S_1 for S_2)

usually ping-pong kinetics

(ATP/ADP 1:1.....)



Membrane transporters

Transport of glucose into myocytes/adipocytes

GLUT4 regulated by insulin

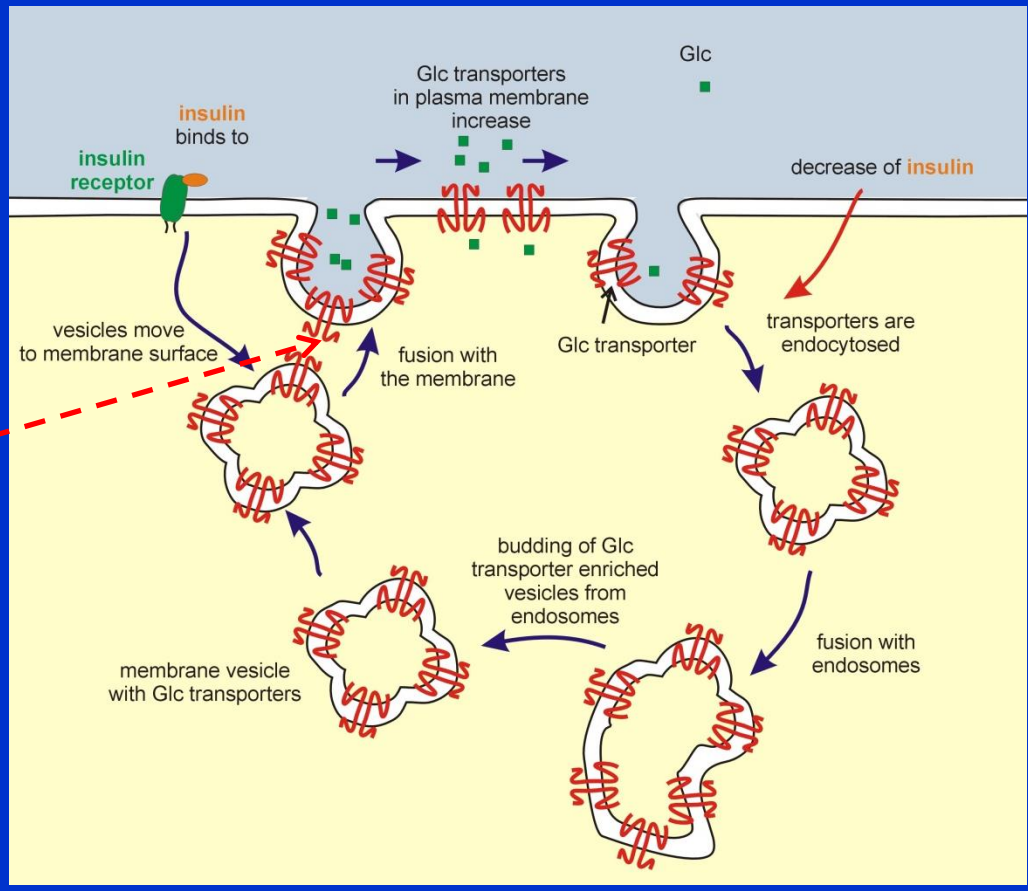
increase $[glucose]_{plasma}$ > 5 mmol/l

↓

increase $[insulin]_{plasma}$ DM type I

↓

mobilization of GLUT4



Ion channels

Transmembrane structures resembling pores (diameter cca 6 nm)

specific for given ion (Na^+, K^+, Ca^{2+}, Cl^-)

mechanism of selectivity varies

working transiently

– open/closed channel

Relative permeability to the three voltage gated channels ($P_{ion\ X}/P_{ion}$)

Ion X	Na^+ channel	K^+ channel	Ca^{++} channel
Li^+	0.93	0.018	0.0024
Na^+	1	0.010	0.0008
K^+	0.086	1	0.0003
Rb^+	0.012	0.91	
Cs^+	0.016	0.07	0.00002
Ca^{++}	too small	too small	1
Sr^{++}			0.67
Ba^{++}			0.40
Tl^{3+}	0.33	2.30	
NH_4^+	0.16	0.13	

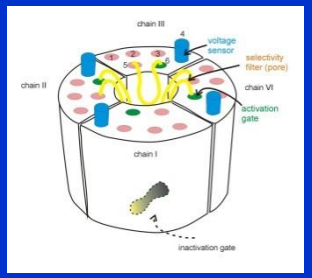
Ion channels

Transmembrane structures resembling pores (diameter cca 6 nm)

regulation of ion channel activity

1. ligand gated channels (neurotransmitters in synapses)
   inhibition: AcChol – tubokurarin, cobrotoxin
2. voltage gated channels (Na^+ in neurons)
   inhibition: Na^+ - tetrodotoxin, K^+ - dendrotoxin
3. mechanically gated channels – change in pressure, touching
   membrane curvature change (osmotic homeostasis)
4. light gated channels (types of rhodopsins - phototaxis)

voltage gated Na^+ channel in neurones



flow velocity of ionts ~ diffuse velocity (10^6 -10^7 ionts/s)

Ionophores

small cyclic molecules/complexes capable of destroying the ion gradients

synthetized in some microbes and animals

1. Shuttle system

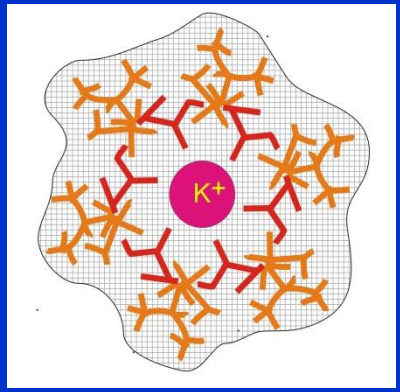
   packing the ion and carrying across membrane

   selectivity mechanisms also variable

   valinomycin

   specifically for K^+

valinomycin



2. Channel formation → cell destruction

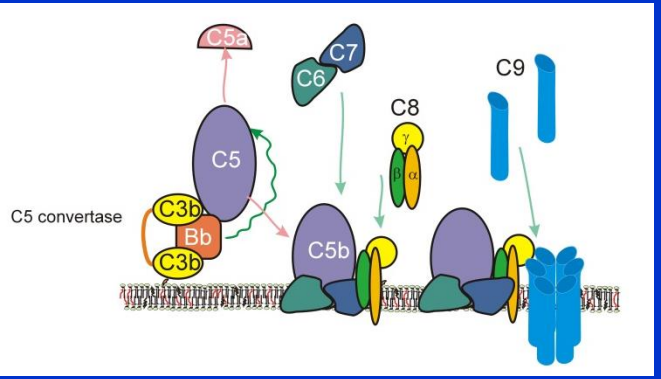
   antibiotics – *gramicidine*

3. Formation of huge pores

   diptheria toxin

   activated parts of complement

complement activation



Aquaporins

Augment transport of H_2O across membranes

Selective for H_2O (some also with glycerol) , ionts are not passed through (even H^+)

protons – H_2O interaction with Asn prevents H^+ further relay

Aquaporin	location	function
AQP-1	proximal renal tubule	fluid reabsorption
	lung	water homeostasis
	eye	secretion of aqueous humor
	CNS	secretion of cerebrospinal fluid
AQP-2	renal collecting duct	water permeability
AQP-3	renal collecting duct	water retention
AQP-4	CNS	reabsorption of cerebrospinal fluid
		regulation of brain oedema
AQP-5	salivary/lachrymal glands	fluid secretion
	alveolar epithelium	fluid secretion
AQP-6,7,8,9	kidney, fat cells testis, liver, leukocytes	? transport of glycerol ? not established

ADH regulates N of pores (reabsorption of H_2O)

mutation

lower reabsorption

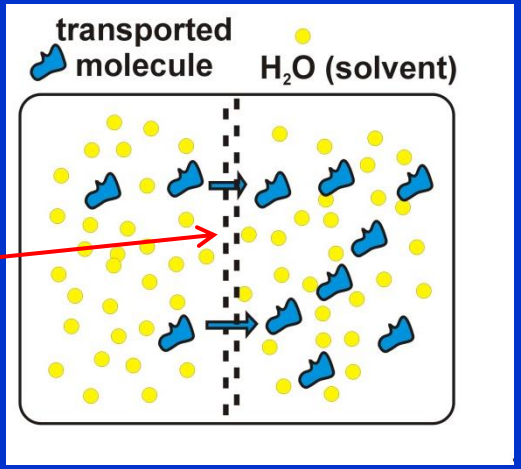
Active transport

Active transport = active process, when molecules from the area

with lower concentration move into the area

with higher concentration

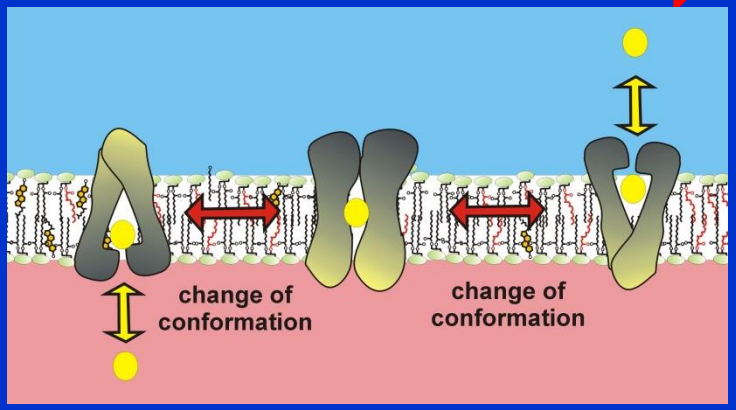
with the help of membrane pumps and E



Membrane not permeable for transported molecule

Membrane proteins - pumps

1. Specific for transported molecule
2. Binding of transported molecule ⇒ conformational change of protein



3. In the membrane, there is not open space left
4. Transport kinetics can be described with Michaelis-Menten approach
5. Number of transported molecules at a time reaches units of ionts

Energetics of active membrane transport

Hydrolysis of ATP – most often used process in mammals

Na^+-K^+ pump: consumes up to 33% of resting E of mammalian cells (67% in neurons) – keeping of electrochemical gradient is highly important

Types of ATP-coupled active transporters

1. Type P

   Ca^{2+} ATPase in SR

   Na^+/K^+ ATPase in PM

2. Type F

   mt ATPase - oxidative phosphorylation

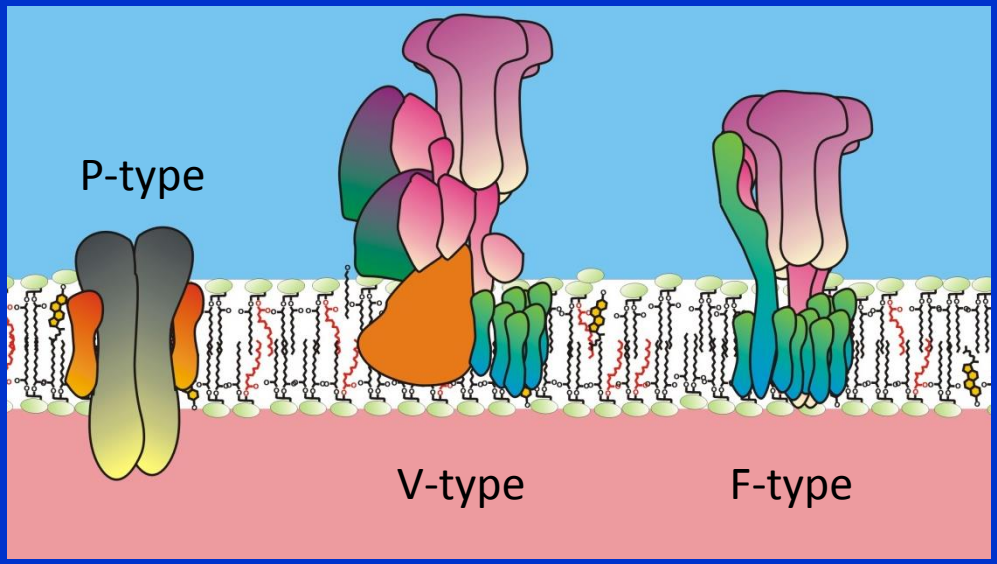
3. Type V

   ATPase H^+ into lysosomes

4. ABC transporters

   cystic fibrose transporter in PM

   multidrug resistance (MDR-1) in PM



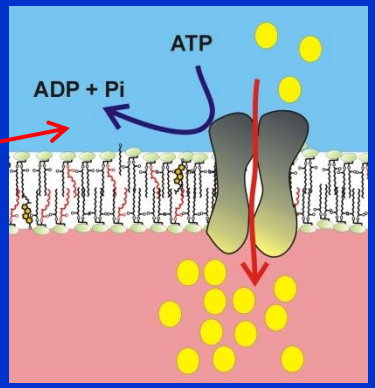
Energetics of active membrane transport

Active transport must be coupled with exergonic process

1. hydrolysis of ATP
2. absorption of light
3. oxidative reaction – electron transfer

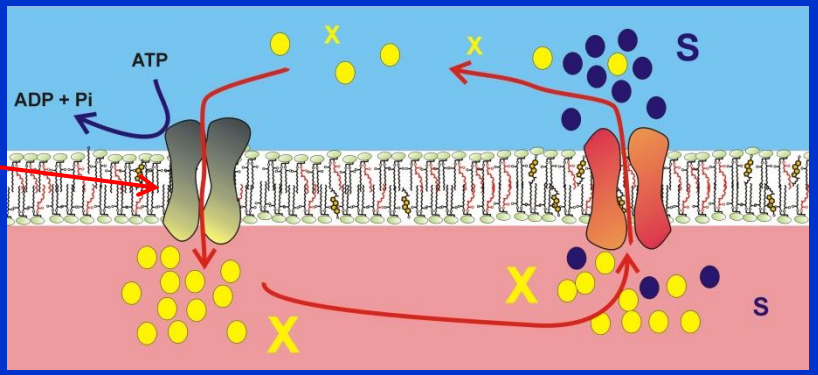
Primary active transport

accumulation of solute is directly coupled with exergonic reaction



Secondary active transport

endergonic transport of solute (S) is coupled with exergonic flow of another solute (X) initially transported via primary active transport

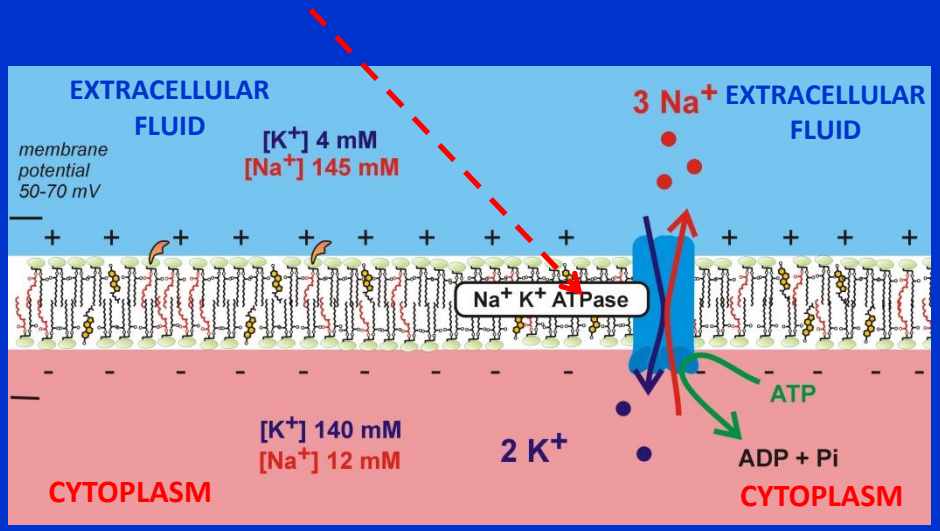


Energetics of active membrane transport

Na^+/K^+ ATP pump: consumes up to 33% of resting E of mammalian cells (67% in neurons) and maintains:

1. negative inner potential of cell (2 K^+ for 3 Na^+)
2. favourable concentrations of K^+ and Na^+

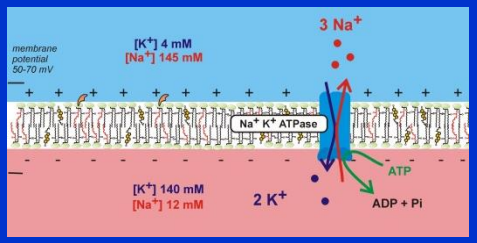
Inhibited by cardiac glycosides (ouabaine, digitalis)



Energetics of active membrane transport

Na^+/K^+ ATP pump: consumes up to 33% of resting E of mammalian cells

can be coupled with other transports:



Oral rehydration therapy

- used in acute diarrhea/cholera

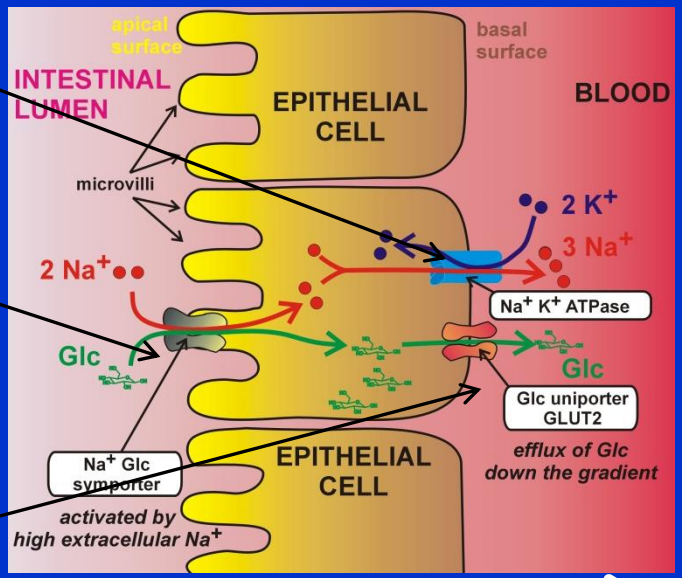
Na^+/K^+ ATP pump maintains low Na^+ within epithelial cells

The patient is provided with solutions with glucose **and** Na^+ = substrates for Na^+/Glc **co**trasport

Na^+ is driven to blood (ATP pump)

glucose is transported via GLUT2

Cl^-/H_2O are driven via osmotic gradient



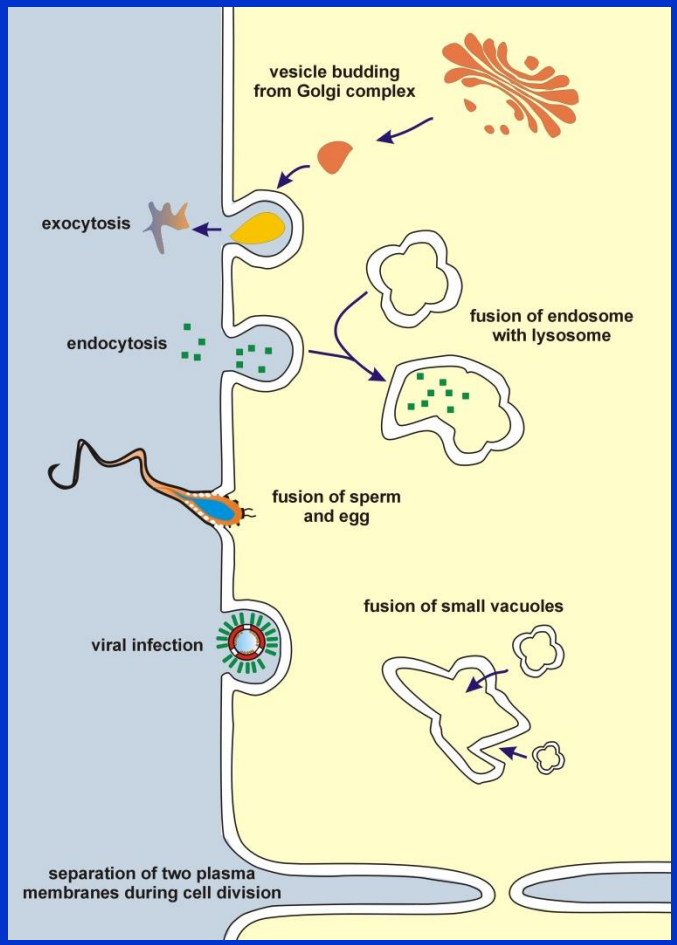
overall transport of:

1. Na^+
2. glucose
3. Cl^-
4. H_2O

Fusion of membranes

Membrane are able to fuse without disruption of cell membrane integrity

1. vesicle budding from Golgi complex
2. exocytosis
3. endocytosis
4. fusions of endosomes/lysosomes
5. fusion of sperm and egg
6. viral infections
7. vacuole fusions
8. cell division – separation of PM



Endocytosis

Internalizing process for large molecules/structures

nutrient intake (after processing)

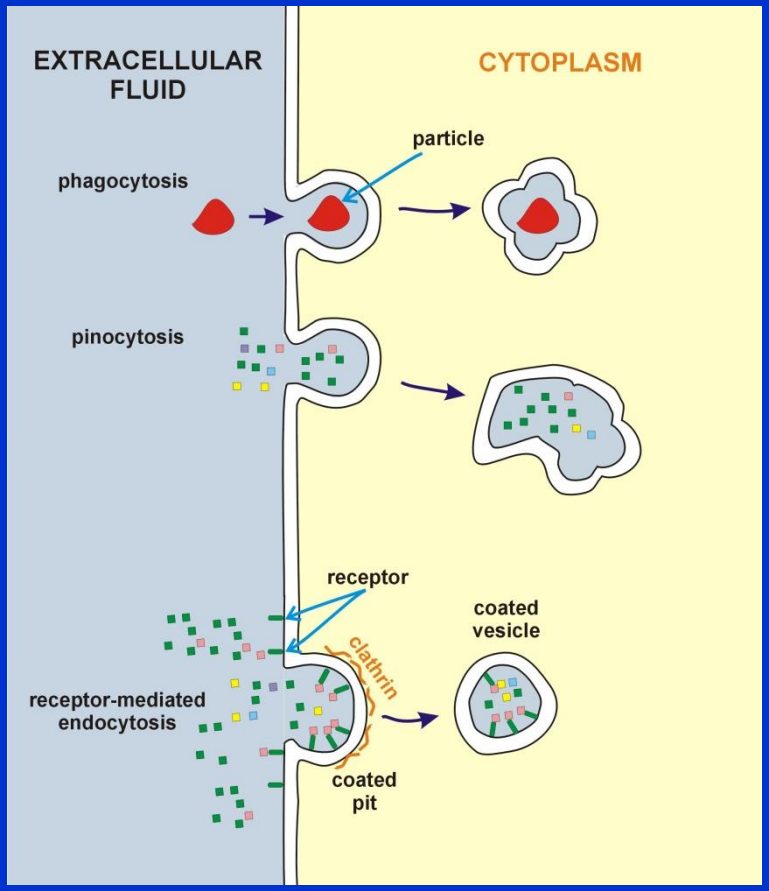
receptor storage

part of immune defense

1. Random endocytosis
2. Receptor mediated endocytosis

A. Pinocytosis

B. Phagocytosis



Pinocytosis

All cells have this ability, usually for fluid-phase substrates

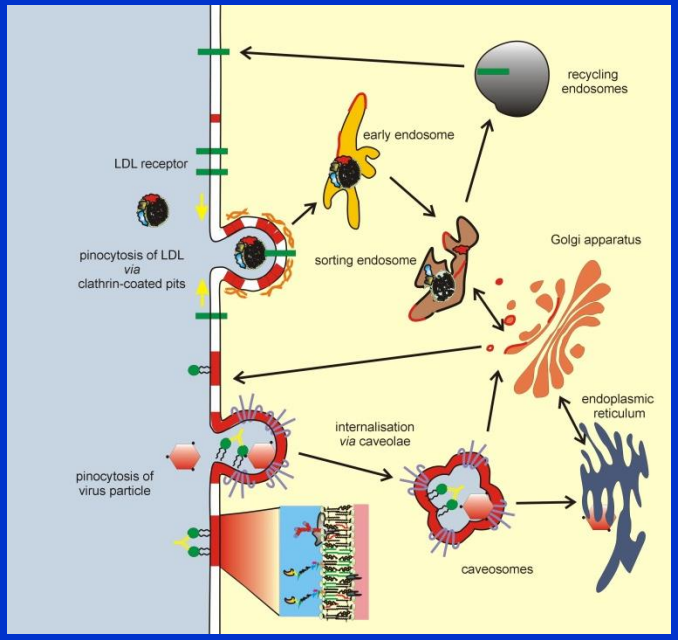
ingested fluids directed into endosomes

Fluid phase pinocytosis

- Non-selective process, very rapid
- Solute entry into cell is proportional to its concentration within extracellular fluid

Absorptive pinocytosis

- Receptor mediated process
- Invagination of membrane – coated pits (clathrin)
- (LDL uptake; absorption of extracellular GlyProt)
- (infection with HIV; hepDNA; poliomyelitis)



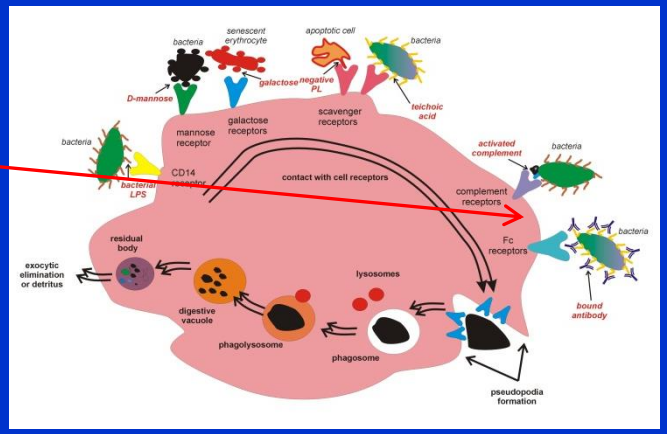
Phagocytosis

Only specialised cells (macrophages, granulocytes)

Large particles (viruses, cells, cell debris … solid particles)

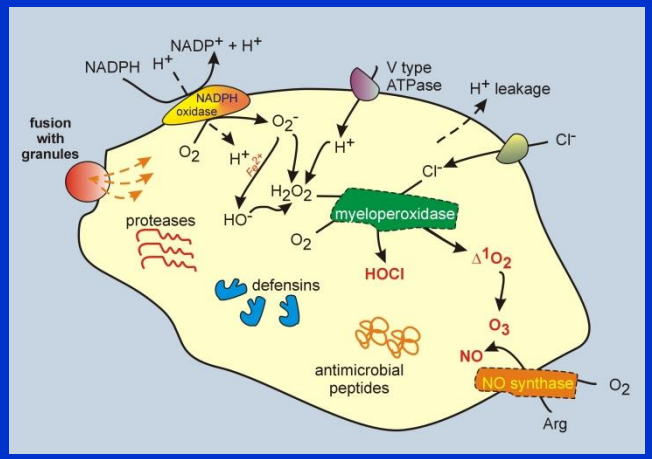
process can be accelerated

via opsonization (IgG…)



Destructive mechanisms in <u>phagolysosomes</u>

1. Oxygen radical formation
2. NO formation
3. Antimicrobial peptides (proteases…)
4. Fe binding (lactoferin)



Exocytosis

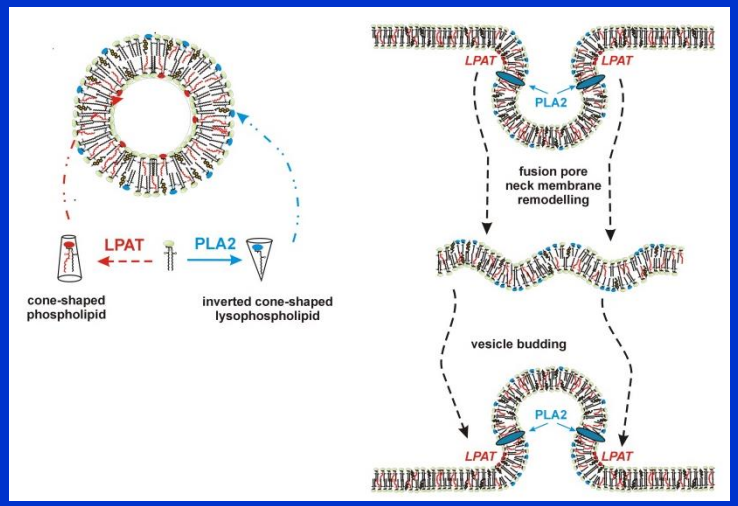
Most cells use for macromolecule expelling into extracellular space

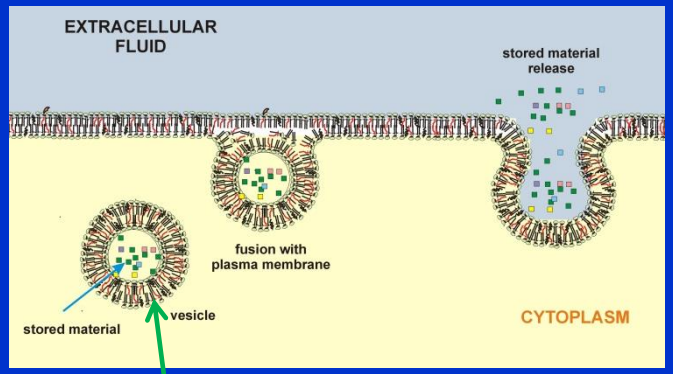
1. attaching to the cell surface – peripheral proteins (antigens)
2. part/joining extracellular matrix – collagen, glycosaminoglycans
3. information transfer through extracellular space – hormones etc.

Remodelation of membranes (Golgi – vesicles – PM)

often triggered by changes in Ca^{2+} concentrations

(Ca^{2+} dep channels; hormonally controlled)





sometimes special coating proteins needed

Further reading

Textbooks

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