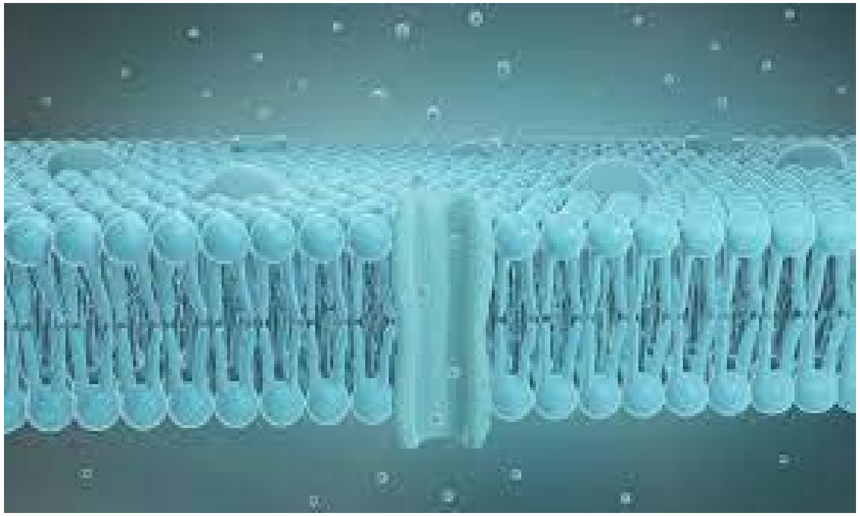
BMS2004 Biochemistry

Lecture 7: - Pentose phosphate pathway
- Lipid & biological membranes



Kwok-On LAI
Department of Neuroscience

Learning outcomes

- Describe **pentose pathway**, its importance and relation to disease

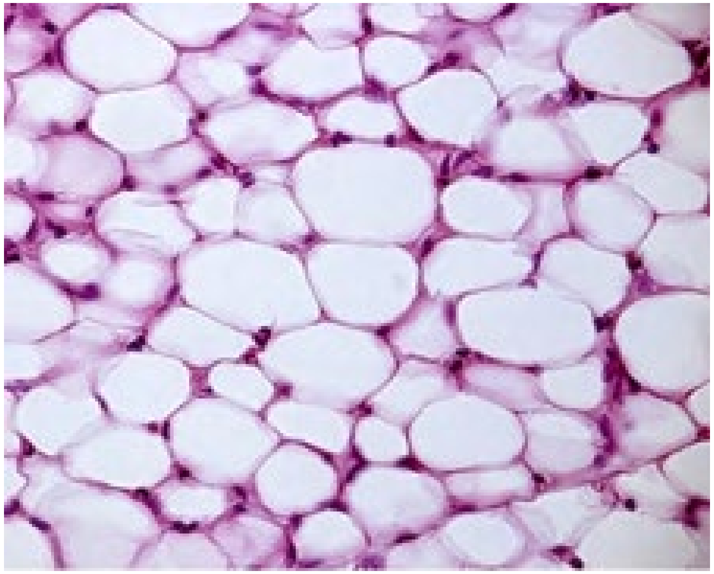
- Define the different types of lipids and their diverse functions in the body

- Describe the architecture of biological membranes (lipids and proteins)

- Recognize the curvature and dynamic of biological membranes and the proteins that underlie these properties

Lipid

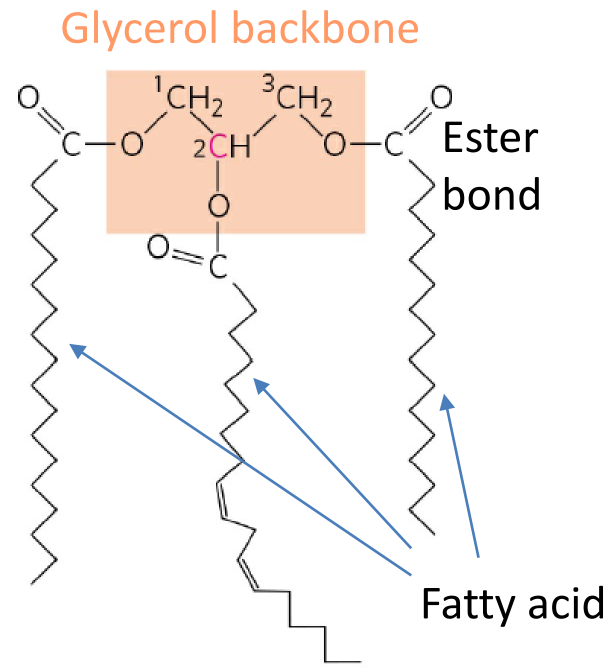

CENTRAL OBESITY
WAIST SIZE 80 CM
FOR WOMEN
WAIST SIZE 90 CM
FOR MEN
Balanced Diet + Regular Exercise
= Healthy Lifestyle
www.cheu.gov.hk



Lipids in diet

- Most of the fat found in food is in the form of triglycerides, cholesterol, and phospholipids

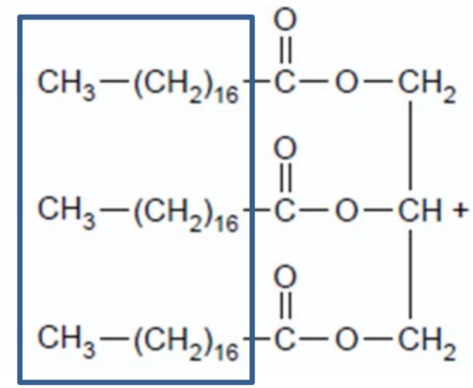
- Certain essential fatty acids must be obtained from diet; e.g. omega-3-fatty acid (Foods high in Omega-3 include fish, vegetable oils, nuts, flax seeds, flaxseed oil, and leafy vegetable)



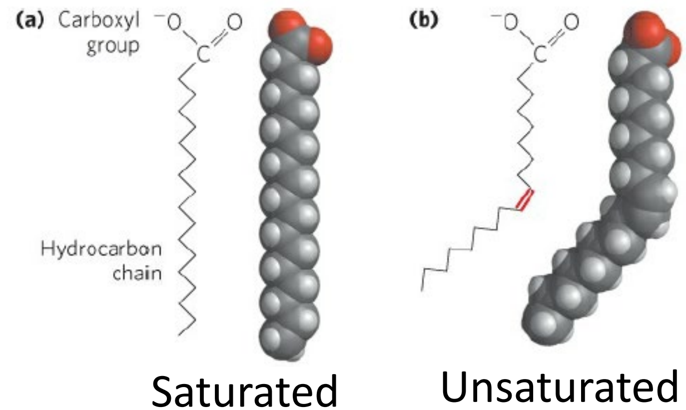
Triglyceride

Flax seeds 亞麻子

Types of fat in diet

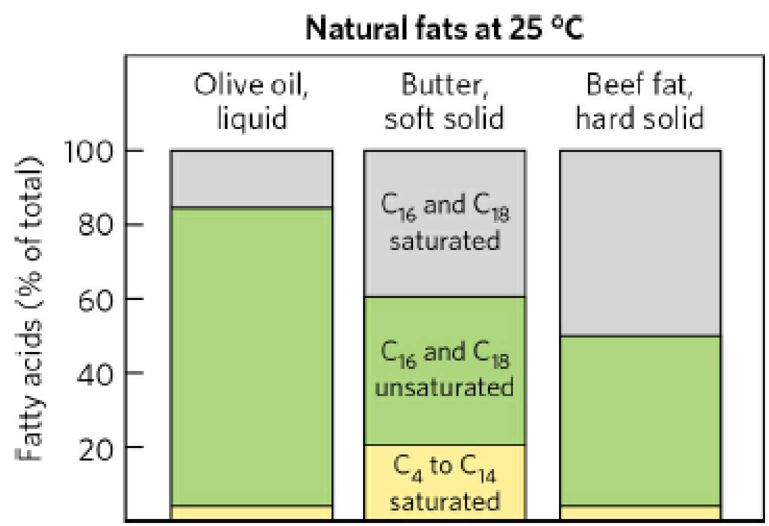


- Classified based on whether the fatty acids contain double-bonds; affect their melting temperatures (solid or liquid at room temp.)



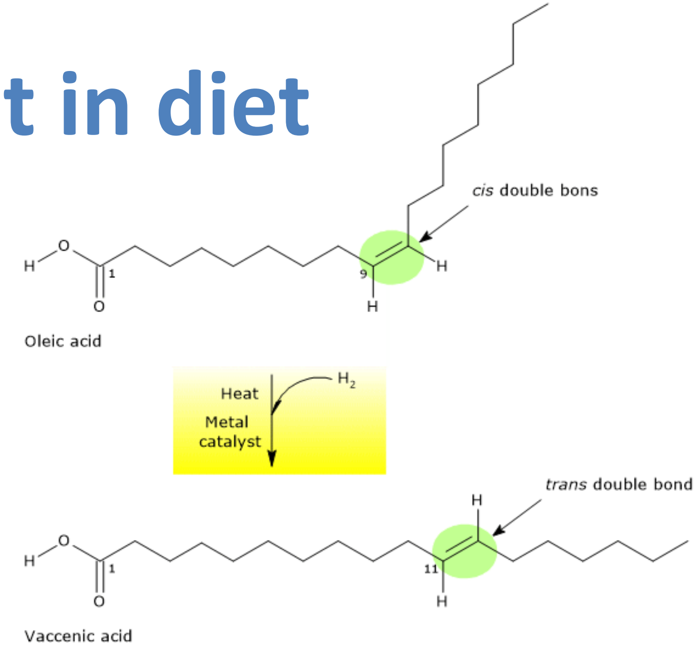
  - **Saturated fats**
    - Diets rich in saturated fats increase risk of coronary artery disease and atherosclerosis

  - **Unsaturated fats**
    - The healthiest fat sources in the diet
    - Polyunsaturated fats will lower both your LDL & HDL

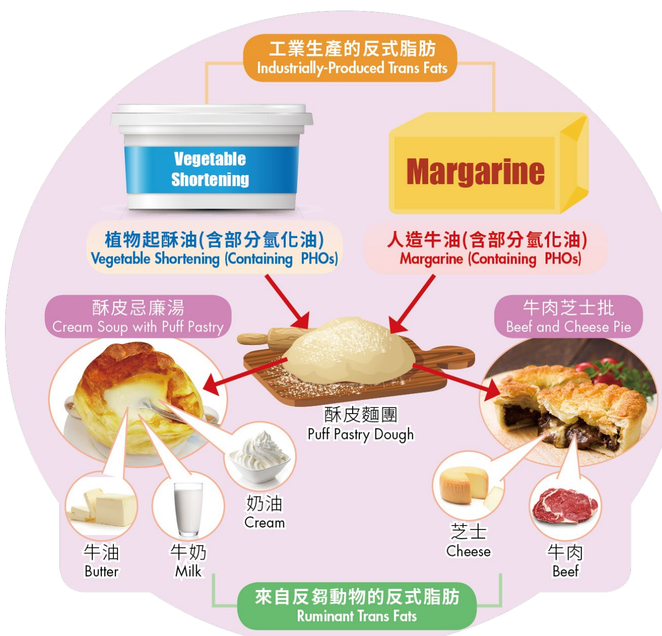


Types of fat in diet

- **Trans fat (unsaturated fat with trans isomer fatty acids)**
  - *Created in an industrial process to make oil more stable by hydrogenation*
  - *increases level of LDL and decreases level of HDL; diets rich in trans fat increase risk of coronary heart disease*
  - *WHO 2003: trans fat <1% of overall energy intake*



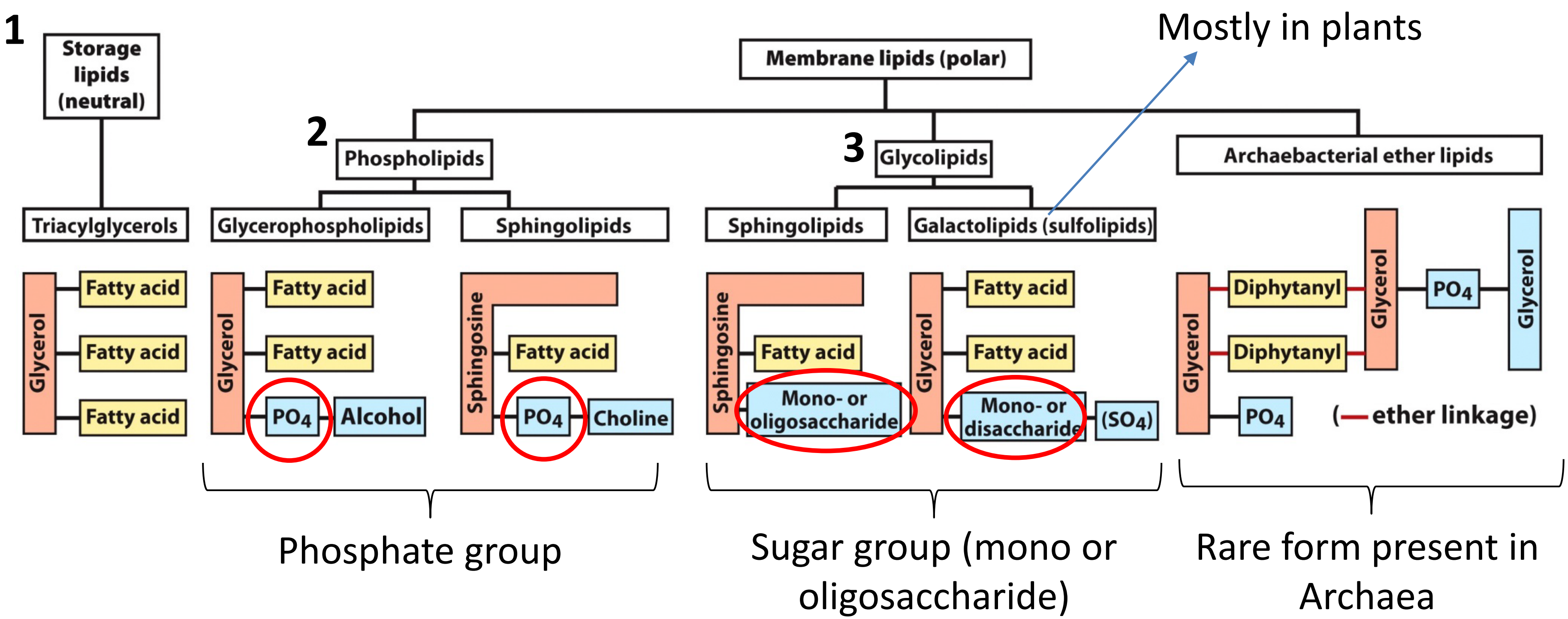
https://www.tuscany-diet.net/2014/06/27/trans-fatty-acids/



Function of lipids

- Lipids serve as structural components of cell membranes and in signaling pathway
- Rich energy source & storage (1g fat yields 9 Calorie, as compared to 4 Calorie of carbohydrate and protein)
- Cushion and protect the major organs
- Insulator, preserving body heat, and protecting against excessive cold
- Fat carries fat-soluble vitamins (Vitamins A, D, E, K)

Classification of lipids

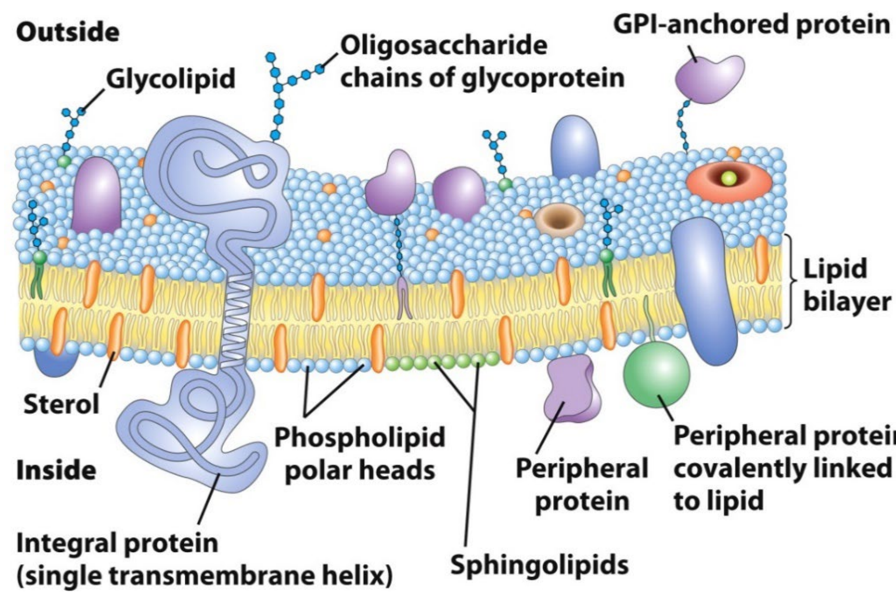


What are biological membranes?

- Forms: plasma membrane (cell membrane), organelle membranes

- Key components: lipids (sterols, glycerophospholipids, sphingolipids, glycolipids) and proteins (glycoproteins, proteins linked to lipids)

- Functions: cell integrity, formation of cellular compartments, signal transduction, barrier and uptake for specific substances, storage …



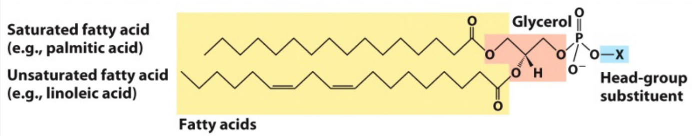
- Properties: flexible, dynamics, selectively permeable

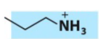
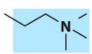
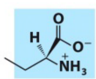
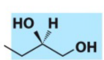
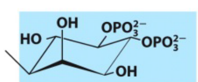
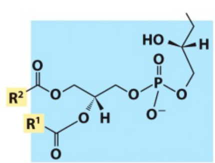
Membrane architecture

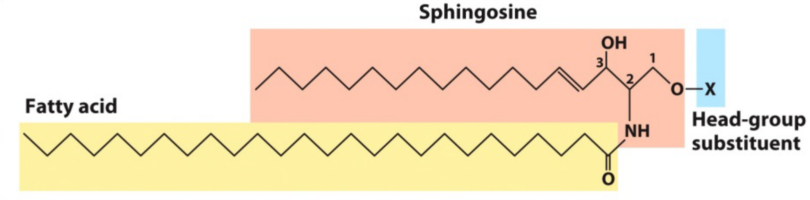
Key membrane lipids

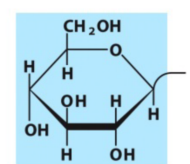
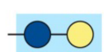
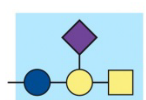
Glycerophospholipids: Glycerol as backbone; derivate named by X head group: "Phosphatidyl-" + X group; two fatty acid chains: one saturated one unsaturated.

Sphingolipids: sphingosine as backbone; one fatty acid chain connected through an amid linkage; also classified by X group.



Name of glycerophospholipid	Name of X—O	Formula of X	Net charge (at pH 7)
Phosphatidic acid	—	—H	−2
Phosphatidylethanolamine	Ethanolamine		0
Phosphatidylcholine	Choline		0
Phosphatidylserine	Serine		−1
Phosphatidylglycerol	Glycerol		−1
Phosphatidylinositol 4,5-bisphosphate	*myo*-Inositol 4,5-bisphosphate		−4*
Cardiolipin	Phosphatidyl-glycerol		−2



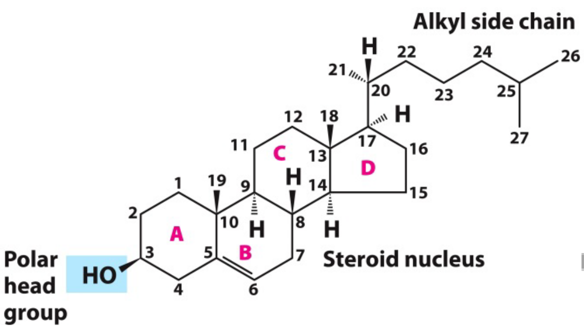
Name of sphingolipid	Name of X—O	Formula of X
Ceramide	—	—H
Sphingomyelin	Phosphocholine	$-P(=O)(O^-)-O-CH_2-CH_2-\overset{+}{N}(CH_3)_3$
Neutral glycolipids Glucosylcerebroside	Glucose	
Lactosylceramide (a globoside)	Di-, tri-, or tetrasaccharide	
Ganglioside GM2	Complex oligosaccharide	

Sterols

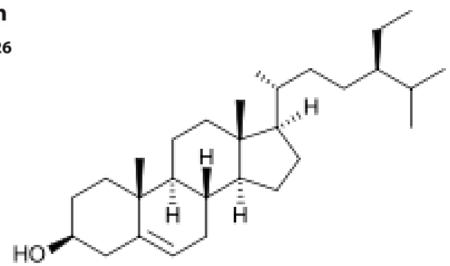
- Has a steroid nucleus , 4 fused rings rigid structure, and a hydroxyl group

- The sterol in animal membrane is cholesterol; Sterols in other species have similar nucleus structure but different alkyl side chains

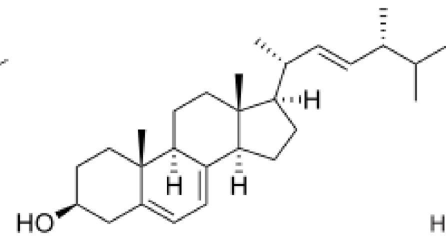
Cholesterol



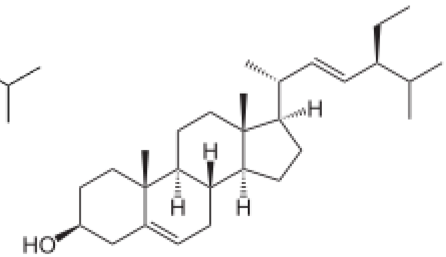
Sitosterol



Ergosterol

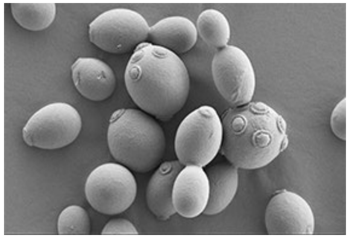


Stigmasterol



Animal

Plant



Fungi

Protist

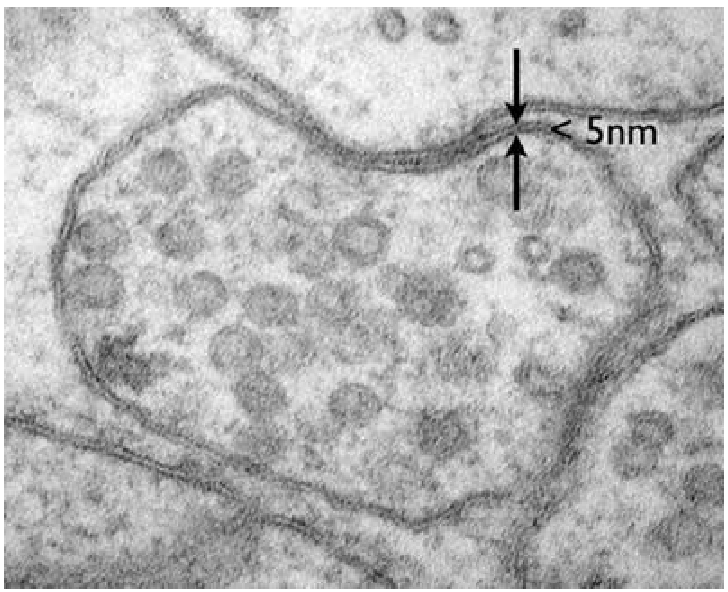
http://a-chien.blogspot.hk/2008/08/blog-post_4677.html

Key features of membrane architecture

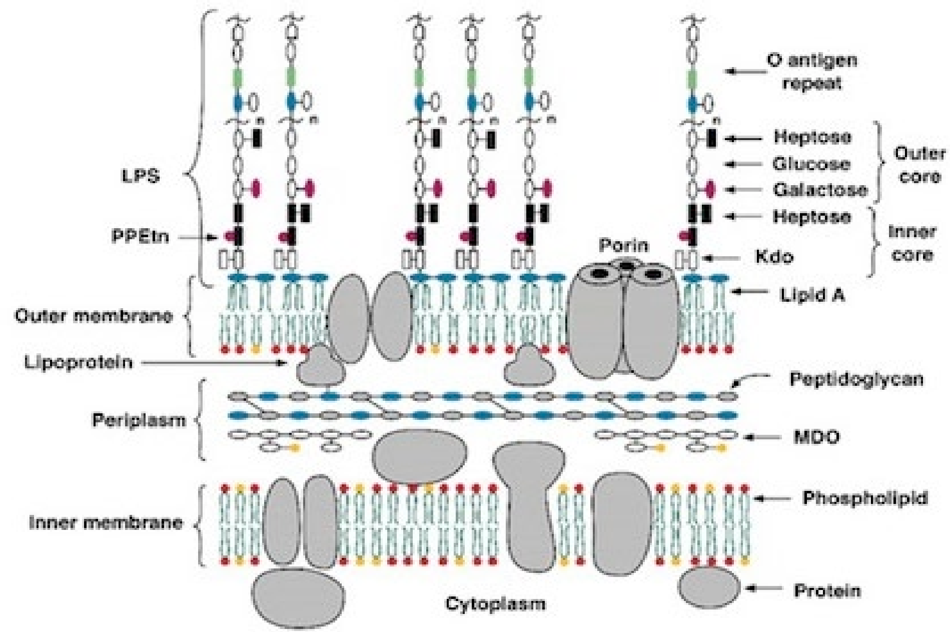
1. Bilayer structure

Trilaminar appearance (three layered structure) under electron microscope.
Typical thickness: 5 to 8 nm.

A neuromuscular junction in *C. elegans*.
Electron microscope



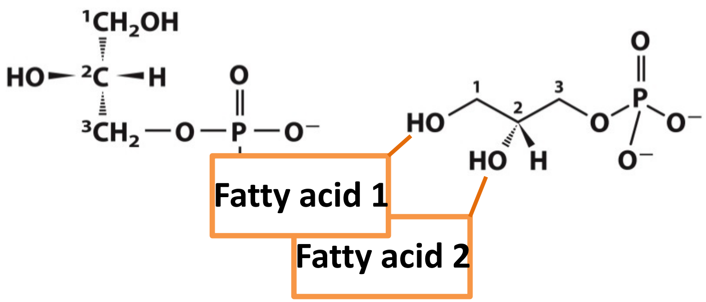
Schematic for *E. coli* membrane



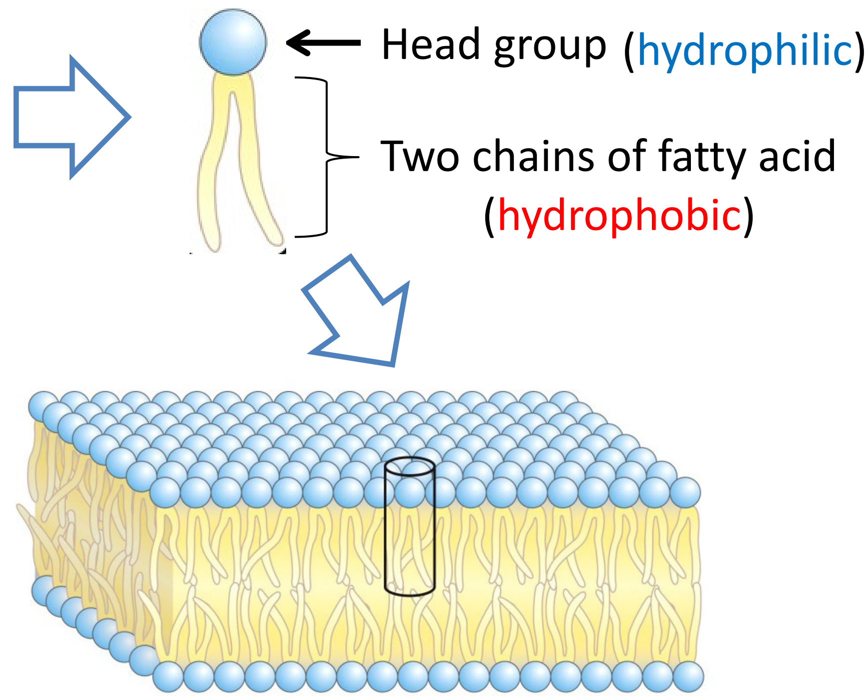
http://www.biochem.duke.edu/modules/biochem_raetz_lab/index.php?id=6
http://advanced-microscopy.utah.edu/education/electron-micro/

The basic structure of membrane lipid

- Membrane lipids are <u>amphipathic</u>: one end of the molecule is hydrophobic, the other hydrophilic
- Their hydrophobic interactions with each other and their hydrophilic interactions with water direct their packing into sheets (membrane <u>bilayer</u>)



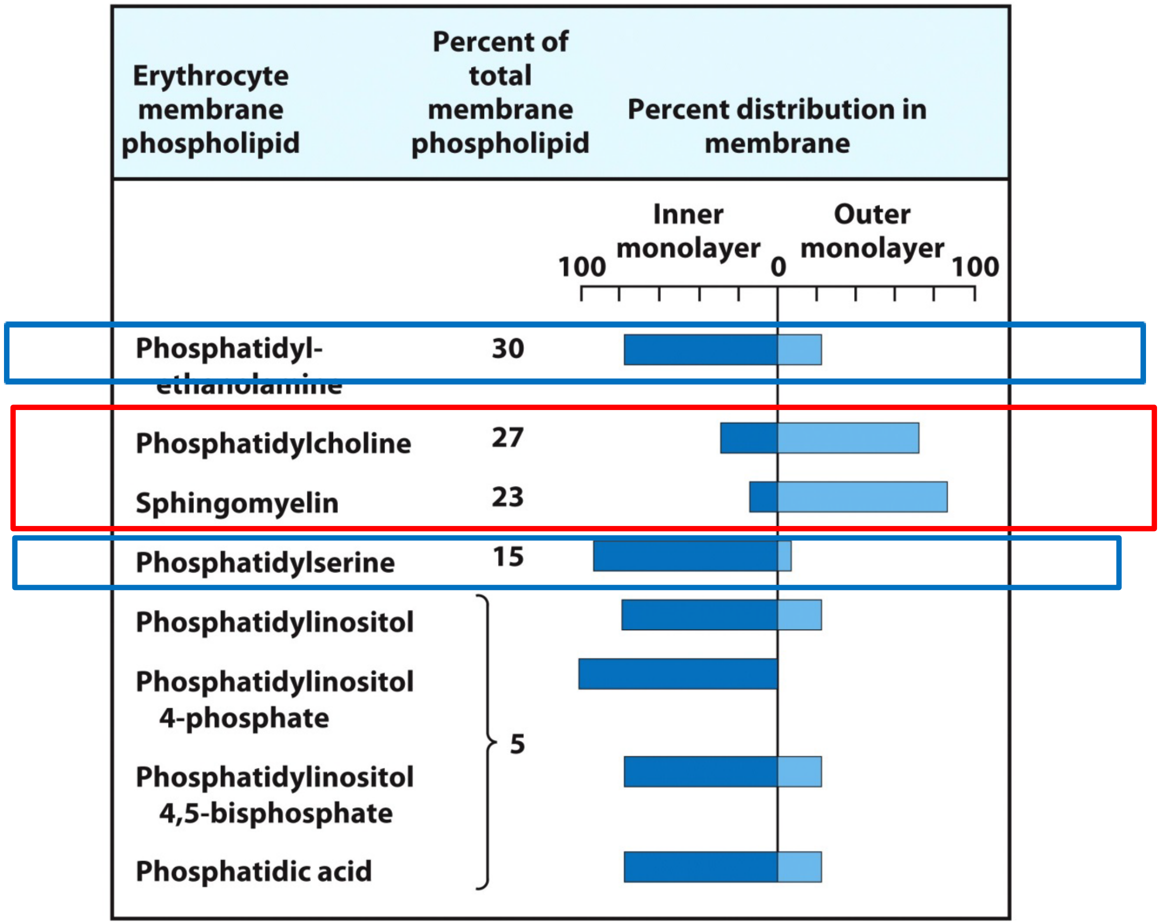
Glycerophospholipids are the most abundant lipids in biological membranes



Forming lipid bilayer

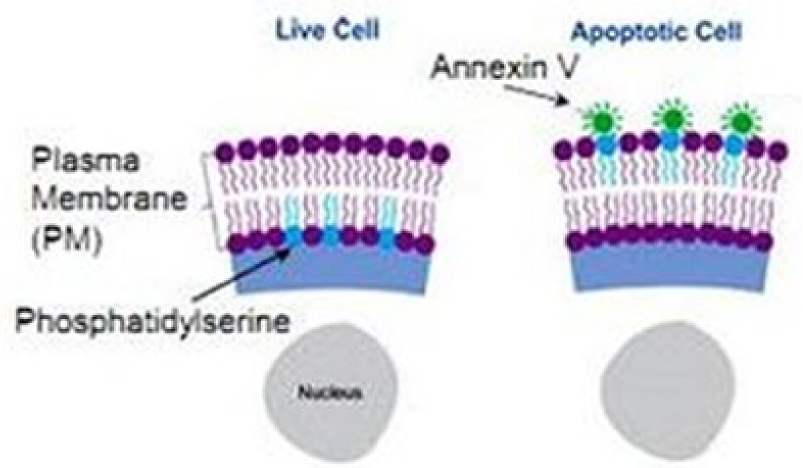
Key features of membrane architecture

2. Asymmetrical distribution of lipids between the inner and out membranes



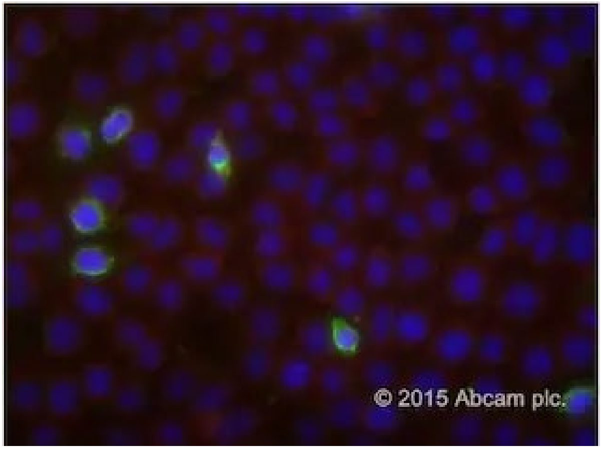
Inner membrane lipid as a marker for programed cell death (apoptosis)

- In the intermediate stage of apoptosis, phosphatidylserine is translocated from the inner to the outer leaflet of the membrane and becomes external to cellular environment



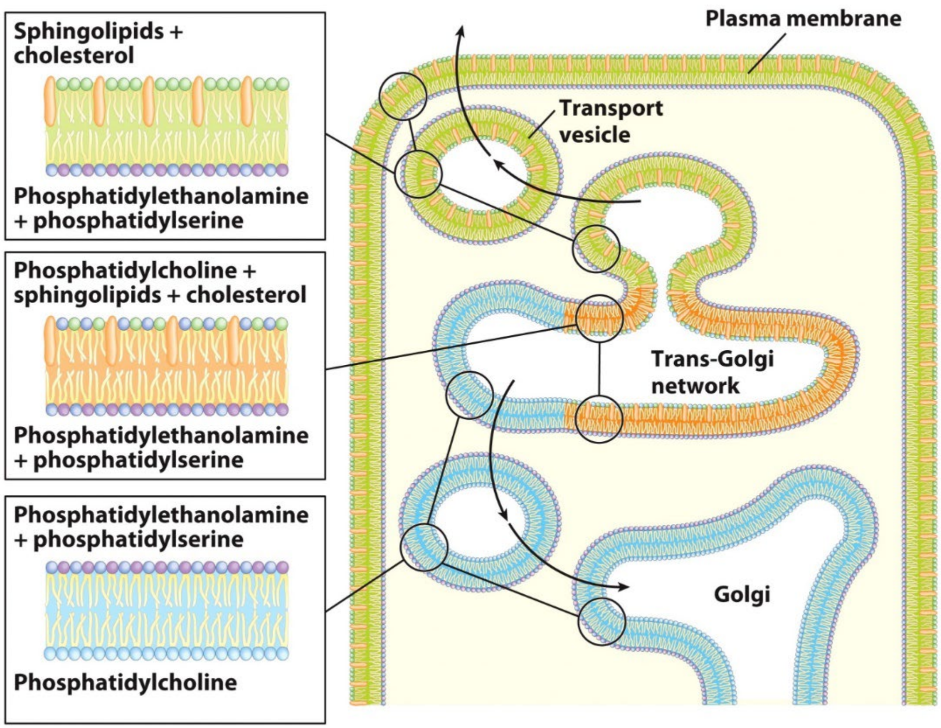
- Annexin V can bind to phosphatidylserine in the presence of Ca^{2+}

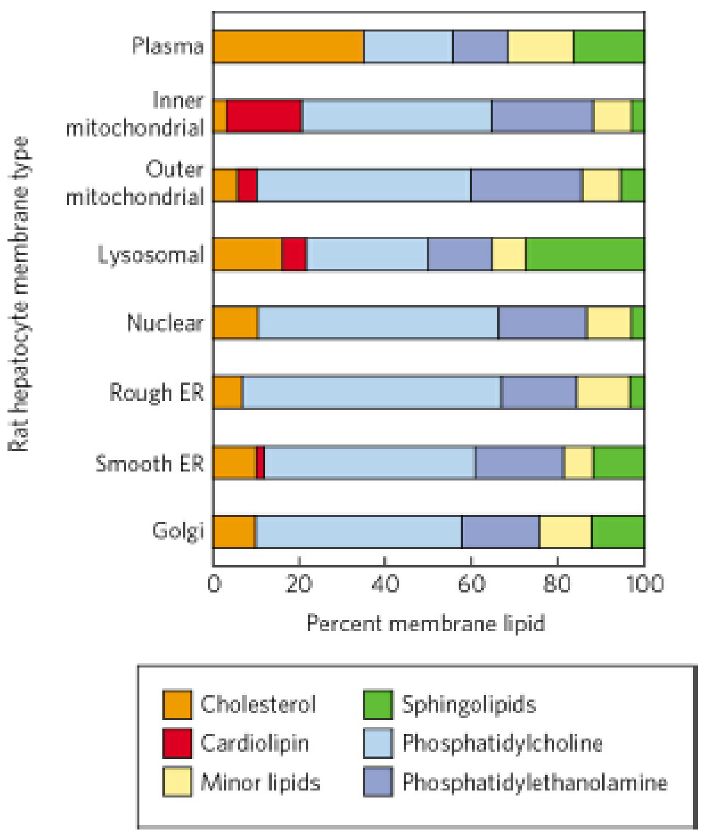
- Fluorescent labeled Annexin V can be used to quantity the number of cells with exposed phosphatidylserine, detected by fluorescent microscope



Key features of biological membranes

3. Each membrane has its own characteristic composition





Change of membrane composition at different temperatures

- Presumably to maintain a constant <u>fluidity</u> under various growth conditions (temperature affects the physical property of lipid)

    - <u>Saturated lipids</u>: more organized and less fluid
    - <u>Unsaturated lipids</u>: less organized and more fluid

TABLE 11–2 Fatty Acid Composition of *E. coli* Cells Cultured at Different Temperatures

	Percentage of total fatty acids*			
	10 °C	**20 °C**	**30 °C**	**40 °C**
Myristic acid (14:0)	4	4	4	8
Palmitic acid (16:0)	18	25	29	48
Palmitoleic acid (16:1)	26	24	23	9
Oleic acid (18:1)	38	34	30	12
Hydroxymyristic acid	13	10	10	8
Ratio of unsaturated to saturated[†]	2.9	2.0	1.6	0.38

Source: Data from Marr, A.G. & Ingraham, J.L. (1962) Effect of temperature on the composition of fatty acids in *Escherichia coli*. *J. Bacteriol.* 84, 1260.

*The exact fatty acid composition depends not only on growth temperature but on growth stage and growth medium composition.

[†]Ratios calculated as the total percentage of 16:1 plus 18:1 divided by the total percentage of 14:0 plus 16:0. Hydroxymyristic acid was omitted from this calculation.

Table 11-2
Lehninger Principles of Biochemistry, Sixth Edition

Saturated lipids →

← Unsaturated lipids

Key features of biological membranes

4. Presence of variety of proteins

- **Integral**

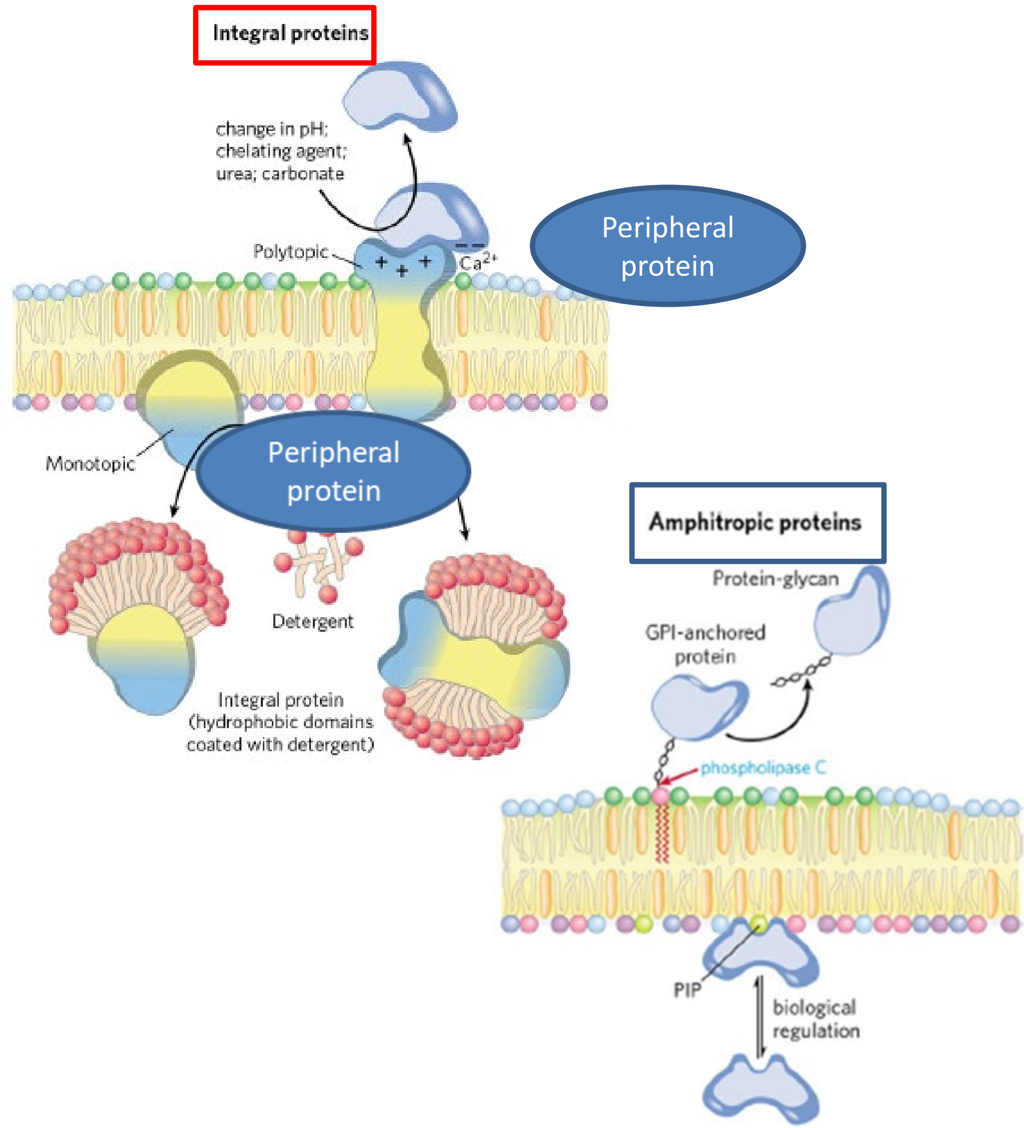
  - Embedded within the lipid bilayer

- **Peripheral**

  - Associate with membrane through electrostatic interactions and hydrogen bonding

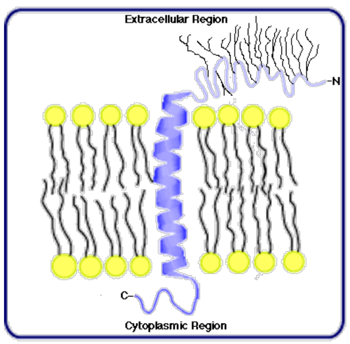
- **Amphitropic**

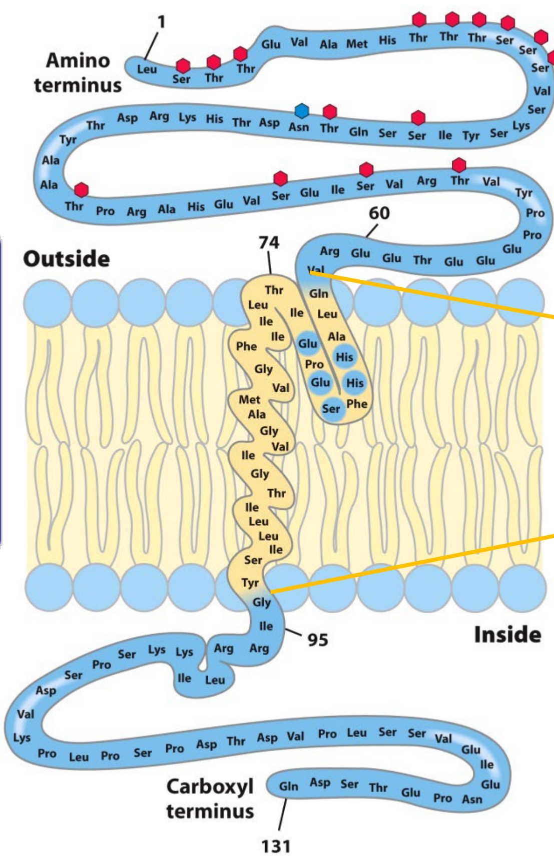
  - Associate reversibly with membranes and are found both in the membrane and in the cytosol



Transmembrane-spanning region of integral membrane proteins

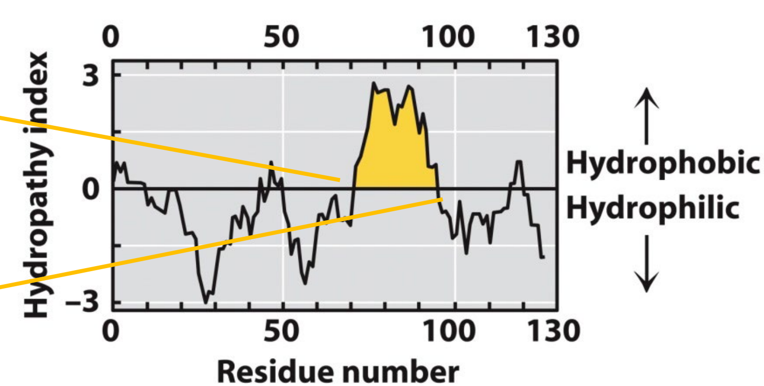
- e.g. Glycophorin, a segment of 19 hydrophobic residues form a transmembrane α helix.





- Hydrophobic region can be predicted from its sequence by "Hydropathy plot".
- Tools to calculate hydropathy profile: http://web.expasy.org/protscale/

Hydropathy plot of Glycophorin



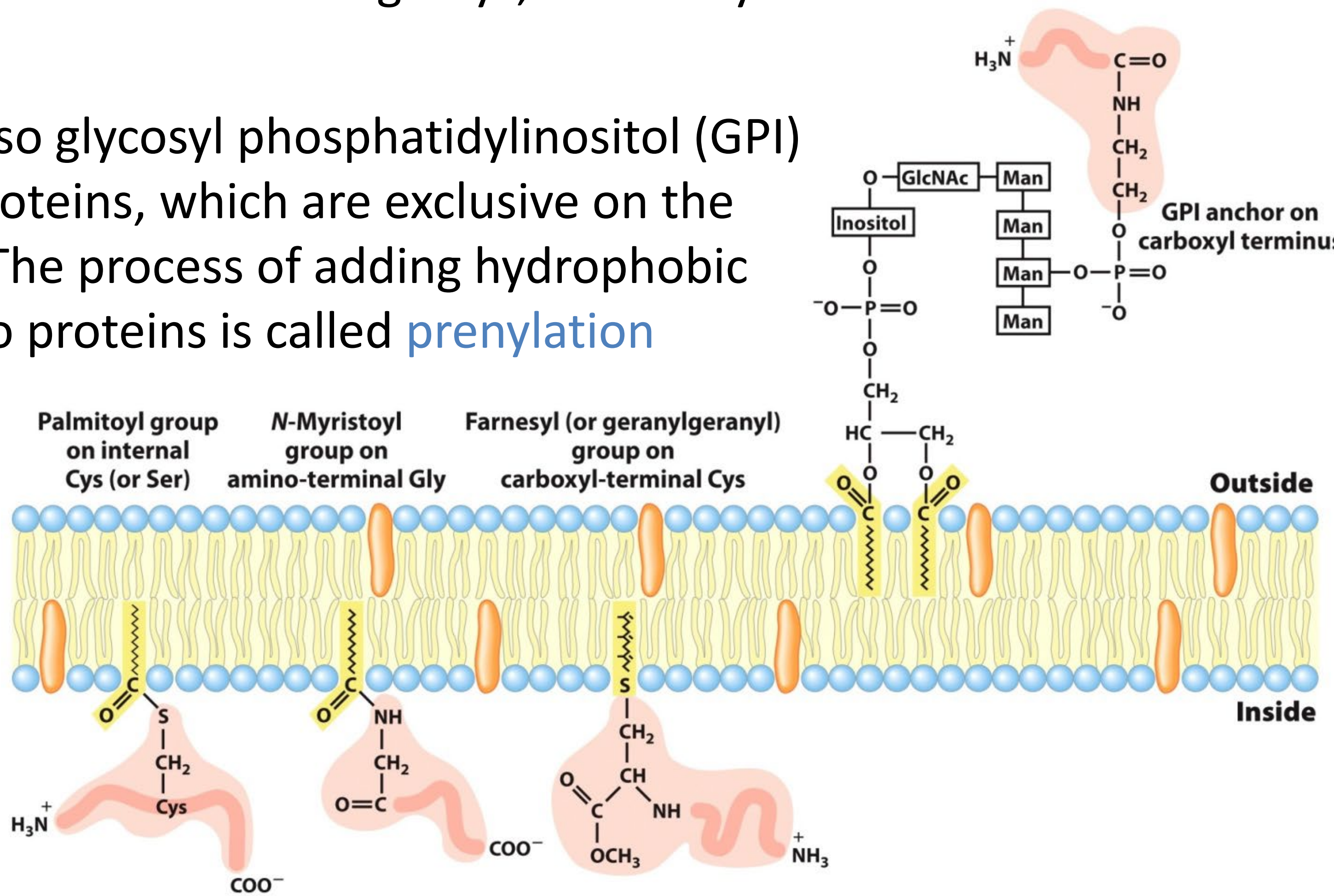
Putative transmembrane domain (~ 20 amino acids)

http://cr.middlebury.edu/biology/devlabbook/membranes/frap/membranes/chap6a.htm

Covalent binding of membrane proteins

- Besides non-covalent interactions (electrostatic, hydrogen bons, hydrophobic interactions), certain proteins can also be <u>covalently</u> linked to lipids of the membrane through Cys, Ser or Gly

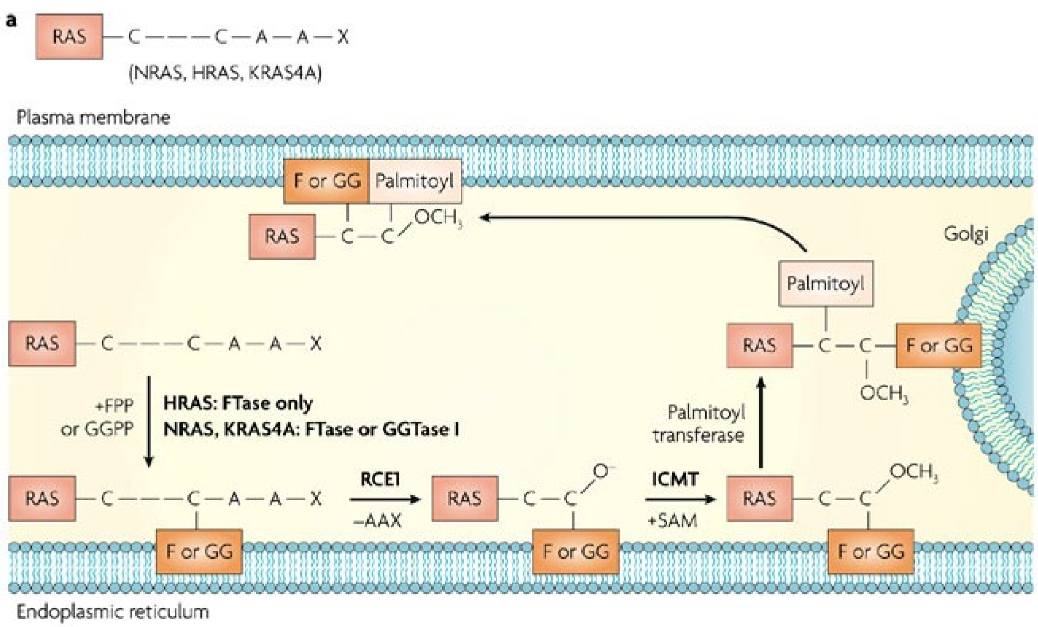
- There are also glycosyl phosphatidylinositol (GPI) anchored proteins, which are exclusive on the <u>outer</u> face; The process of adding hydrophobic molecules to proteins is called prenylation



Prenylation of the Ras proto-oncogene

- Ras, a small GTPase, is a proto-oncogene: mutations in *Ras* genes lead to the activation of Ras signaling pathway resulting in overproduction of genes involved in cell growth, differentiation and survival

- Ras goes through several steps of modification including prenylation of adding farnesyl (F) or geranylgeranyl (GG) groups which are required for its anchoring to the membrane



- Farnesyltransferase inhibitors have been investigated for cancer therapy but did not work out due to side effects and the redundant functions of F and GG.

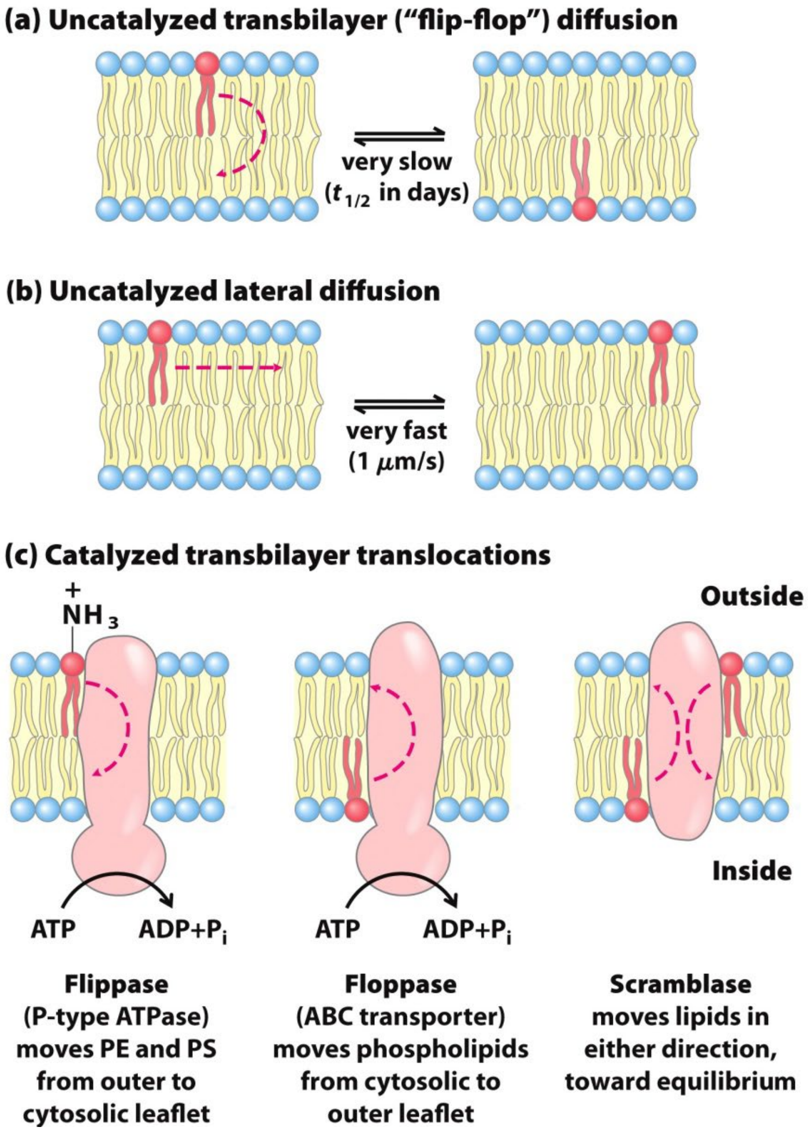
FPP, farnesyl pyrophosphate
FTase, farnesyltransferase
GGPP, geranylgeranyl pyrophosphate;
GGTase I, geranylgeranyltransferase I
SAM, S-adenyosyl methionine.

http://www.nature.com/nrd/journal/v6/n7/full/nrd2221.html

Membrane dynamics and flexibility

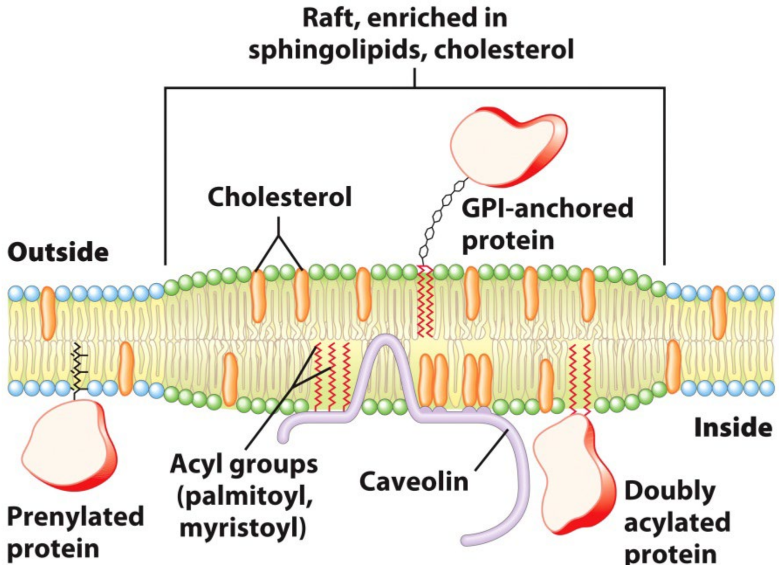
Membrane forms a fluid mosaic

Movement within biological membranes



- Lipids and proteins can rapidly diffuse laterally in the bilayer
- Transbilayer movement of lipids requires catalysis by flippase, floppase and scramblase. These enzymes contribute to the asymmetrical distribution of lipids in the two layers

Sphingolipids and cholesterols cluster together in membrane rafts

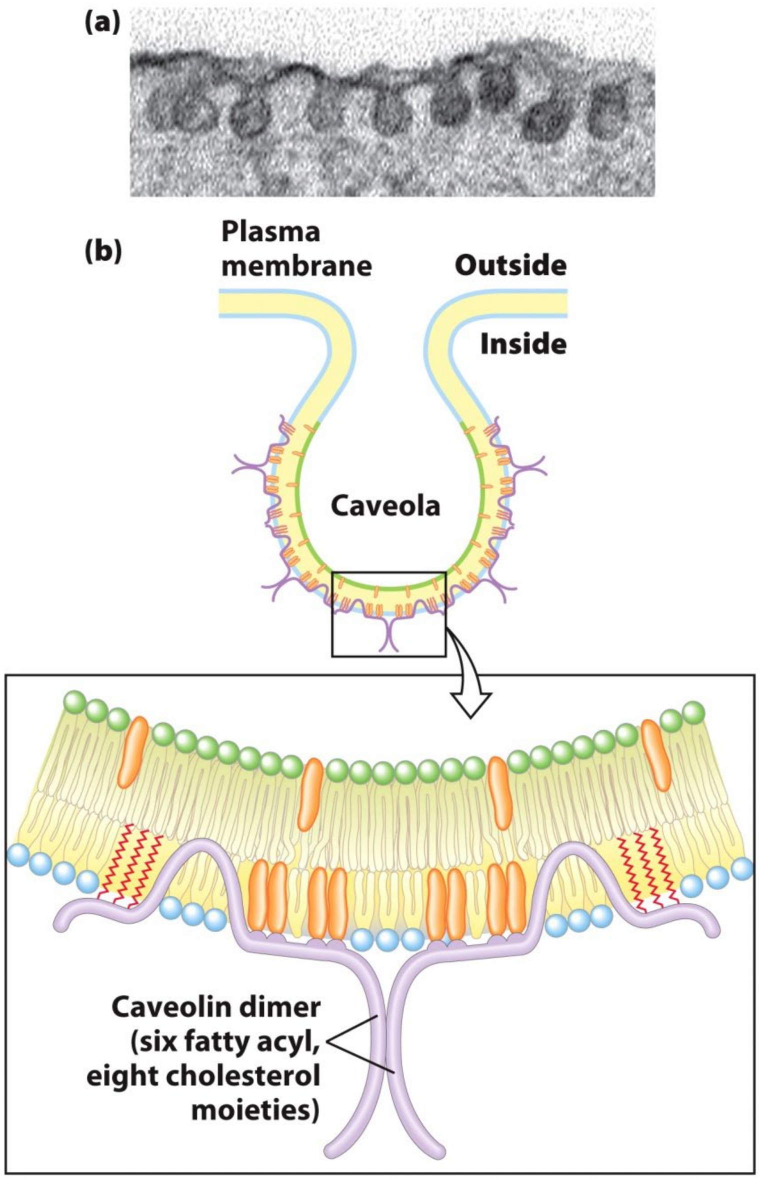


- Sphingolipids (contain long chains of saturated acyl groups) form stable association (<u>thicker and more ordered conformation</u>) with cholesterols. Like "rafts" moving in a "sea" of membrane

- Those "rafts" are enriched in
  1. proteins covalently linked to two chains of lipid through Cys residue
  2. GPI-anchored proteins

- "Rafts", as functional domains, facilitate biochemical processes, e.g. protein interactions and signal transduction

Caveolin and BAR domain proteins in shaping the membranes



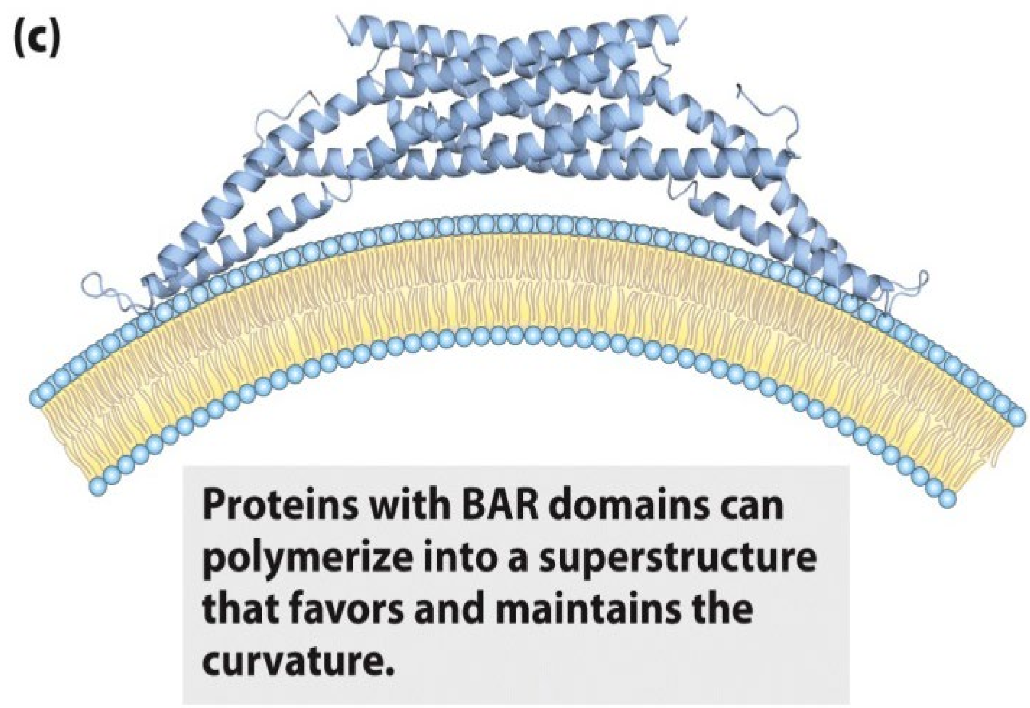
- Curvature is essential for maintaining membrane continuity and important for the processes of vesicle budding and membrane fusion

- Caveolin, an integral membrane protein located in the inner layer of membranes, forms dimers to force the formation of inward curvature of a membrane

Caveolin and BAR-domain proteins in shaping the membranes

- Proteins containing BAR domains can assemble into a crescent-shaped scaffold that binds to the membrane surface and forces curvature

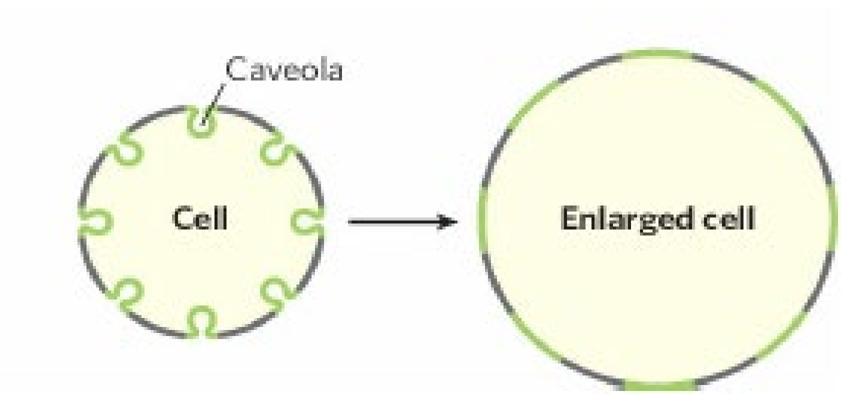
BAR domain polymerization

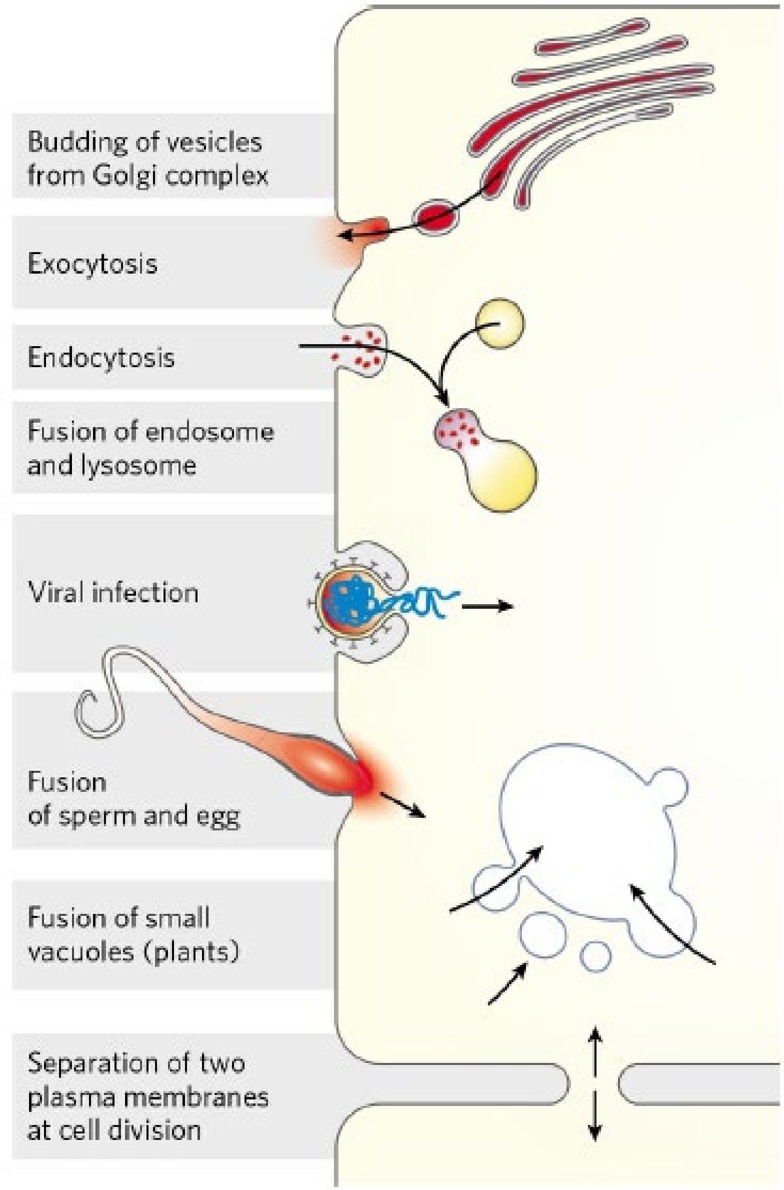


Inducing membrane curvature is important

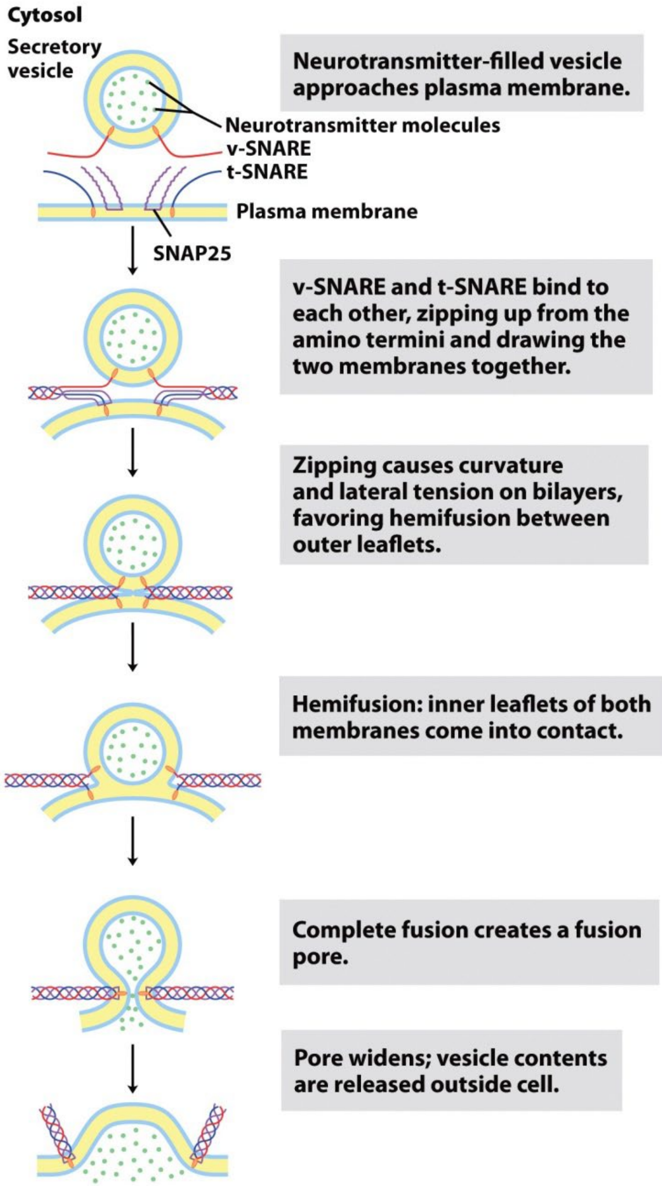
- <u>Membrane trafficking</u> underlies many important events

- Addition of membranes to expand the cell





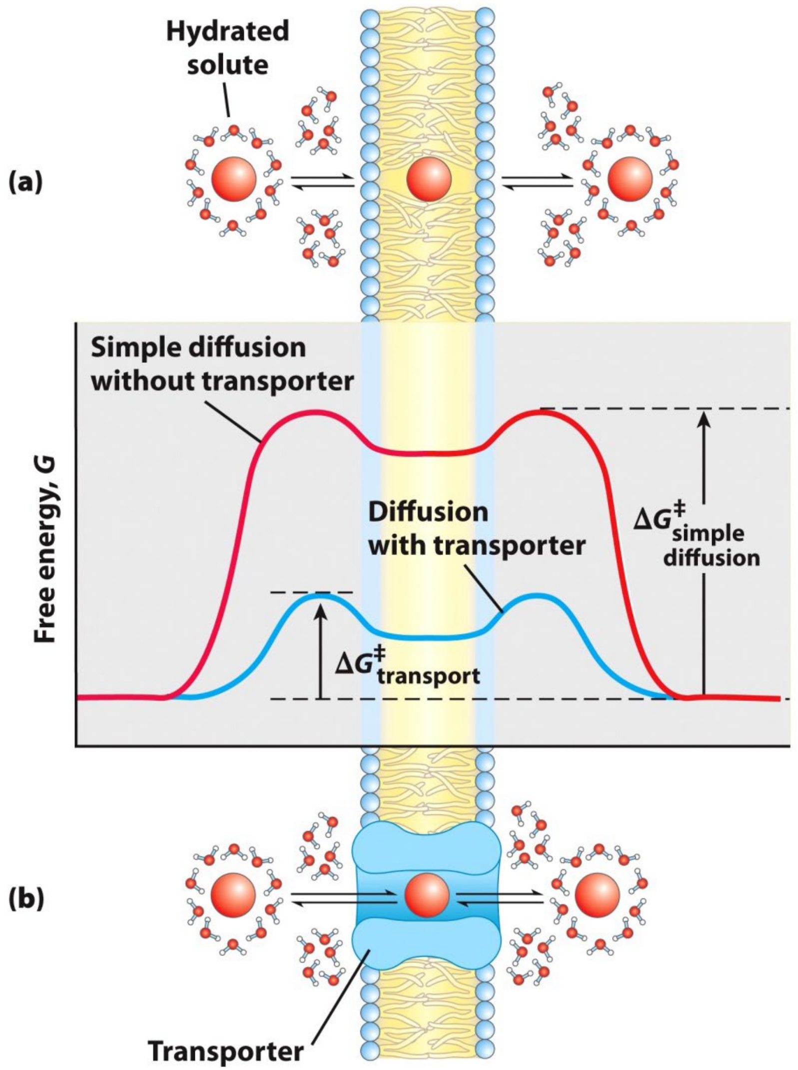
Fusion of membranes are mediated by cell surface proteins



- At neuronal synapses, neurotransmitters are released through **exocytosis**: fusion of -containing vesicles with plasma membranes involves SNARE proteins

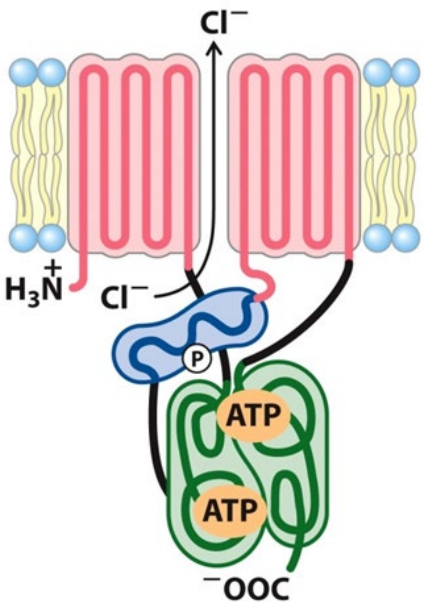
- v-SNAREs (on vesicles), t-SNAREs (on plasma membrane) and two SNARE25 form a bundle of long and thin rods made up of helices in a zipping-like action (facilitates the membrane fusion)

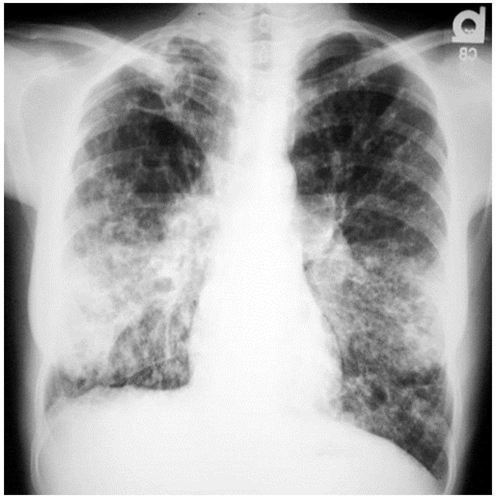
Membrane transport of solutes



- The energy (ΔG) required for a solute to go through the bilayer is very high
- Transporters and channels (all formed by integral membrane proteins) dramatically reduce the energy (ΔG) for transmembrane diffusion
- Could follow (passive transporter) or against (active transporter) the concentration gradient of the solute
- Active transporter: requires hydrolysis of ATP

ABC Transporters





- ATP-binding cassette transporters (ABC transporters) constitute a large family of ATP-dependent transporters that pump amino acids, peptides, proteins, metal ions, various lipids, bile salts and many hydrophobic compounds including drugs

- CFTR gene is an ABC transporter and an ion channel pumping out Cl^-

- Mutation affecting CTFR causes cystic fibrosis; severe obstruction of the gastrointestinal and respiratory tracts; mucus of internal surfaces of lung is abnormally thick

Summary (II)

Different types of lipids

- saturated vs unsaturated vs Trans fats; triglycerides, phospholipids, glycolipids, sphingolipids

Membrane functions and properties

- Asymmetrical distribution of lipids between the inner and out membranes
- Each membrane has its own characteristic composition; can be influenced by various environmental and growth conditions

Membrane proteins

- integral membrane proteins are held in the membrane by hydrophobic interactions
- proteins can be covalently linked to lipids

Summary (II)

Membrane dynamics

- Transbilayer movement; lipid raft; curvature formation; membrane fusion (exocytosis)

Membrane transport

- Transporters on the lipid bilayer for the transport of polar molecules and ions
- Active transport vs passive diffusion
- ABC transport; CFTR as example and its deficient function in cystic fibrosis

Study questions

1. Explain why cystic fibrosis may cause obstruction of respiratory and intestinal tracts

2. If the pancreatic duct is blocked by the CFTR mutation, how may it damage the pancreas?

Glucose utilization pathways

- Energy storage: Glycogen, starch, sucrose.
- Functional polymer: Polysaccharides (eg. glycoproteins, lipid polysaccharides).
- Energy generation: Glycolysis or pentose phosphate pathway.

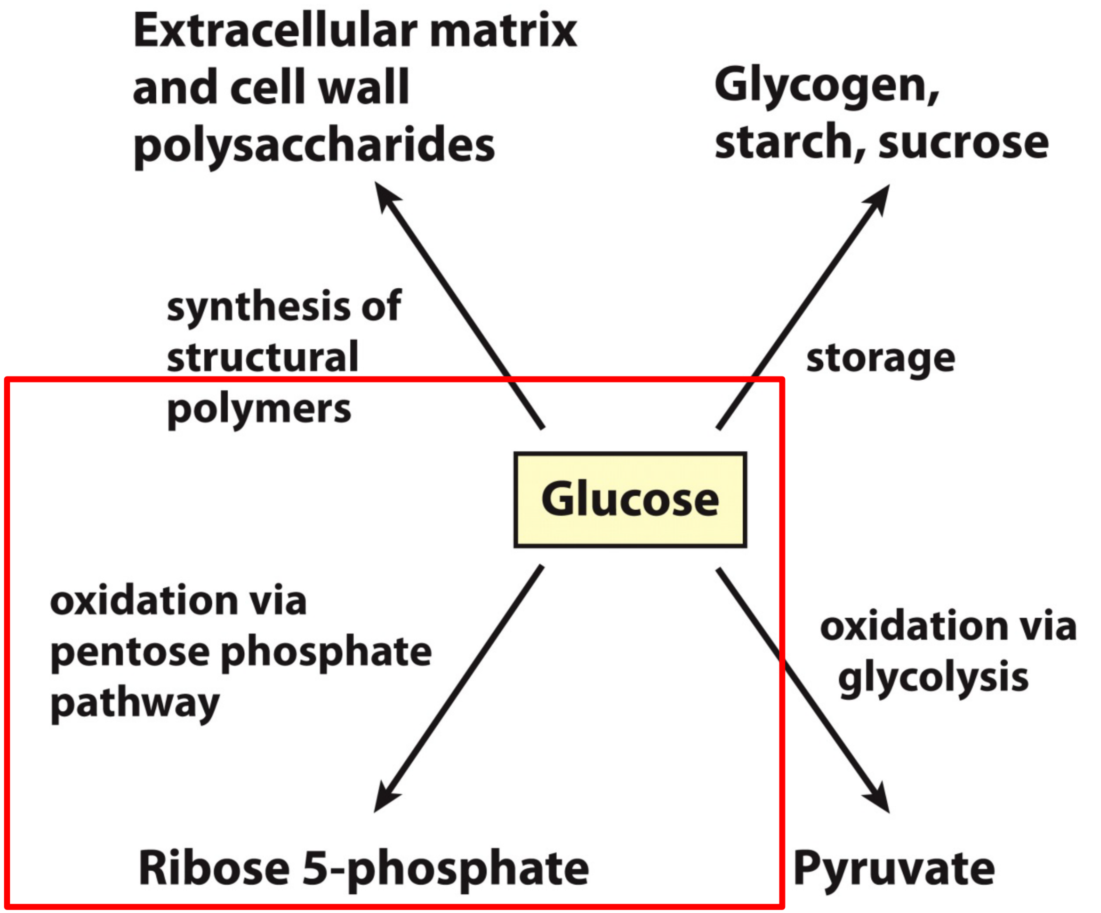


Figure 14-1
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Pentose phosphate pathway

- ***What?***

- The process serves to generate NADPH and 5-carbon sugar (pentose ribose 5-phosphate) from glucose 6-phosphate
- Also called phosphogluconate pathway or the hexose monophosphate pathway

- ***Why?***

- Produce precursors (ribose 5-phosphate) for DNA, RNA and coenzymes such as ATP, NADH, $FADH_2$ and coenzyme A
- Provide electron donor NADPH for reductive biosynthesis or countering damaging effects of oxygen radicals.

- ***Where?***

1. Rapid dividing cells: cells in bone marrow (stem cells and progenitor cells of blood cell lineage), skin, intestinal mucosa and tumor cells
2. Tissues that carry out extensive synthesis of fatty acid (liver, adipose, lactating mammary gland) cholesterol and steroid hormones synthesis (liver, adrenal glands, gonads)
3. Cells that are exposed to oxygen (such as?)

Overview of the pentose phosphate pathway

- The oxidative phase:
  - Oxidation of glucose-6-phosphate to ribose 5-phosphate
  - NADPH is generated

- The nonoxidative phase:
  - Interconversion of three-, four-, five-, six-, and seven-carbon sugars
  - Recycle excess five-carbon sugars back to glucose 6-phosphate

- All these reactions take place in the cytosol

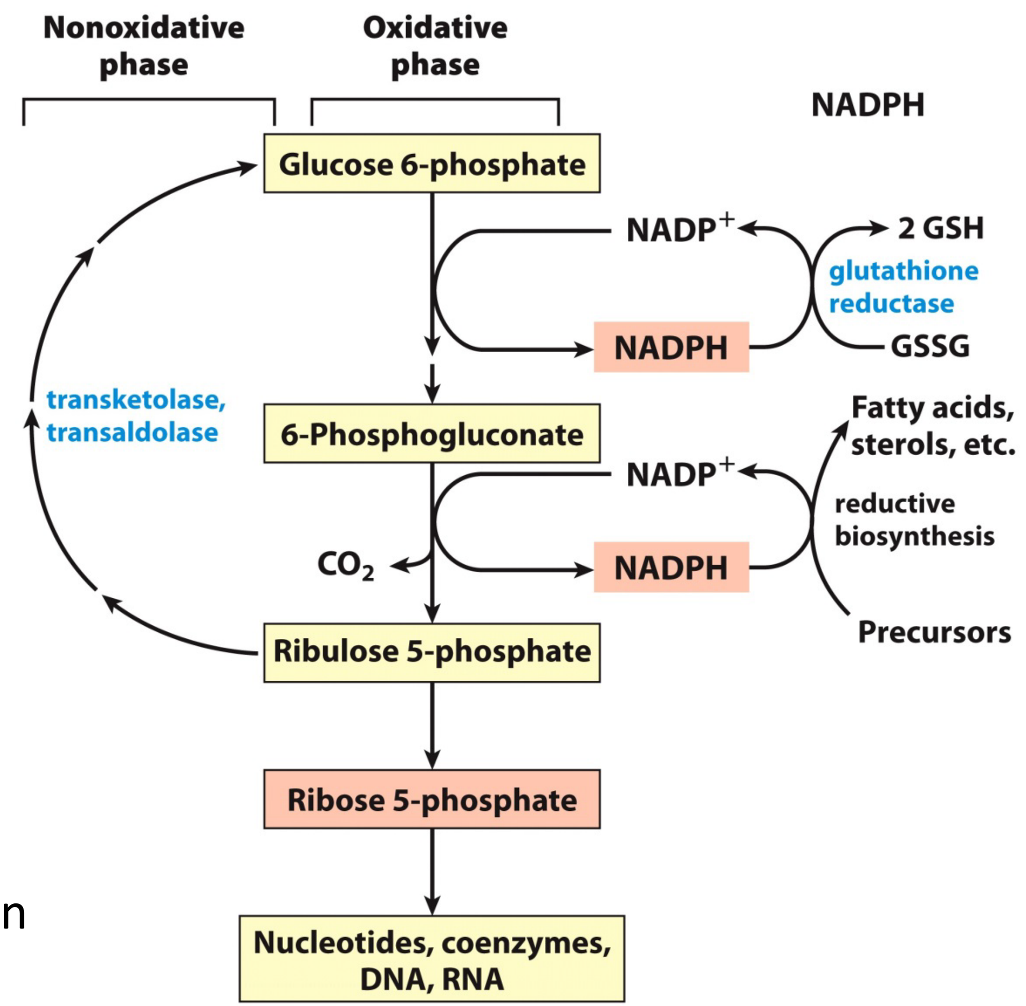

Figure 14-21
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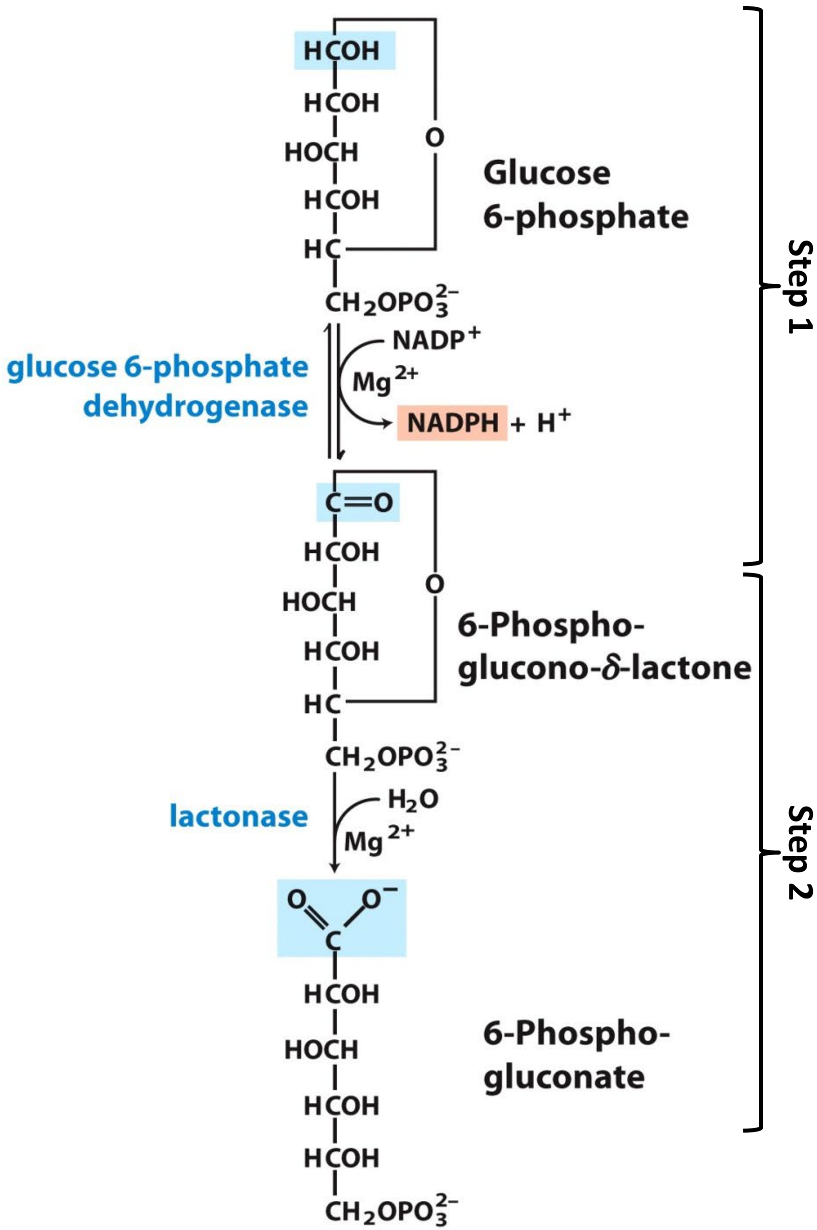
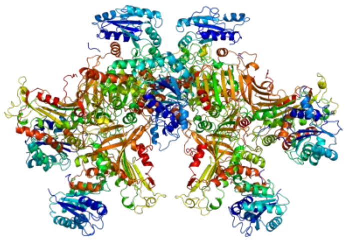
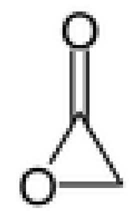
The oxidative phase



Step 1: Glucose-6-phosphate dehydrogenase



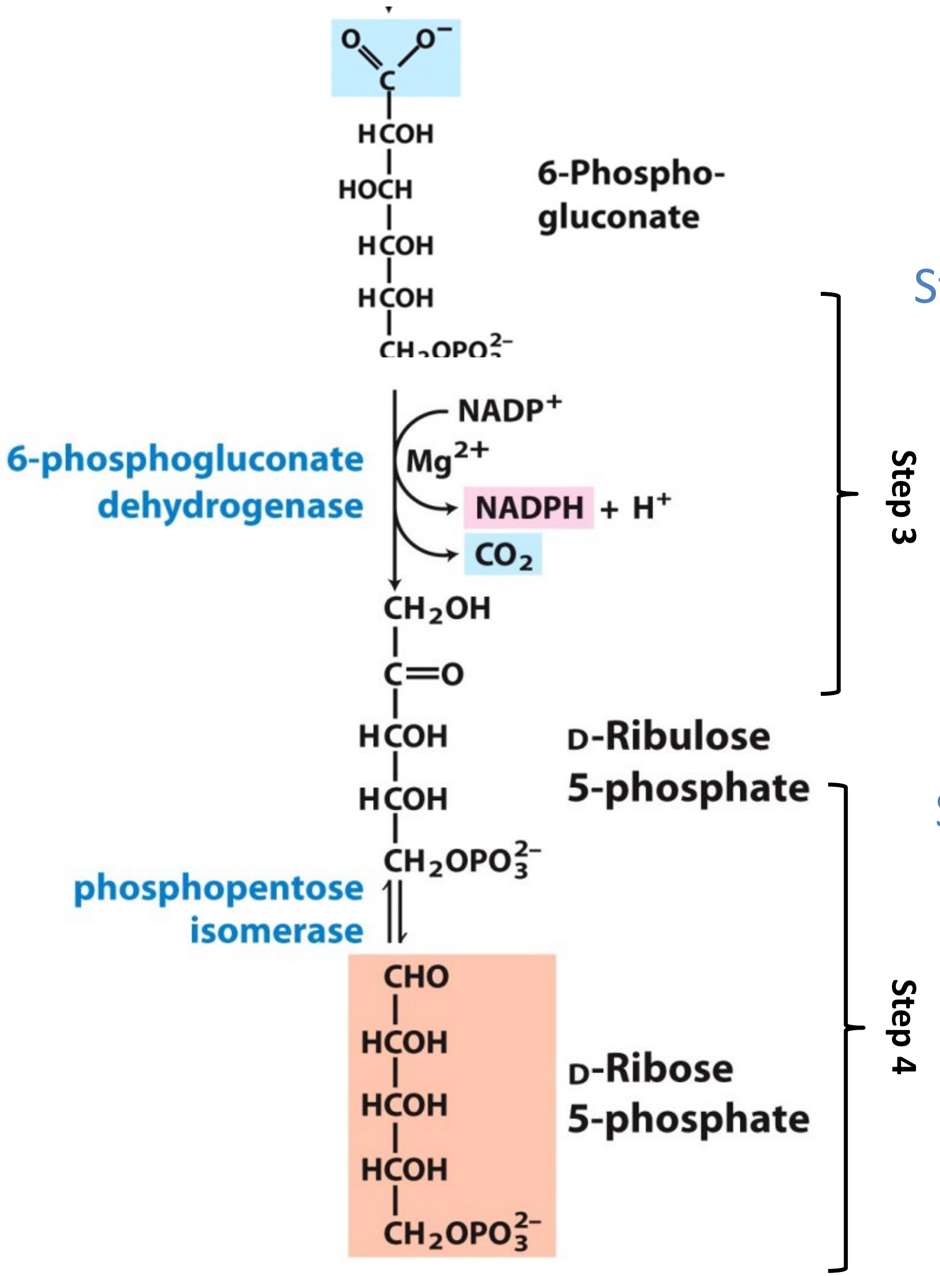
- Generates the first NADPH
- The product is 6-Phospho-glucono-δ-lactone. Lactone is a cyclic ester, which is formed from the condensation between a carboxylic acid (-COOH) and an alcohol (-OH).



α-acetolactone

Step 2: Lactonase

- Lactone is hydrolyzed by lactonase to produce a linear molecule: 6-Phosphogluconate.



Step 3: 6-Phosphogluconate dehydrogenase

- Generates the second NADPH and one CO_2
- Produces ketopentose ribulose 5-phosphate

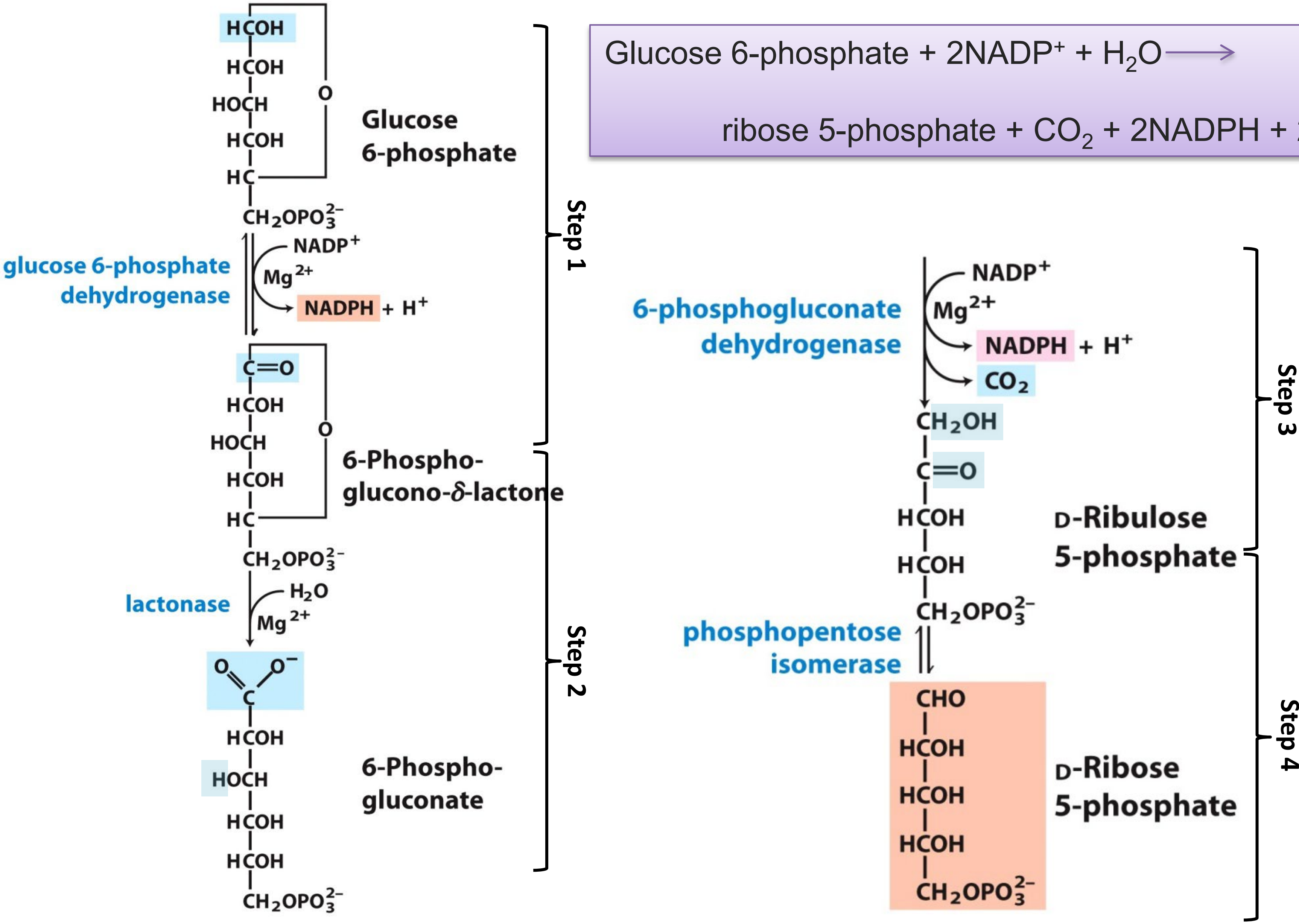
Step 4: Phosphopentose isomerase

- Produces pentose ribose 5-phosphate

The oxidative phase complete reaction

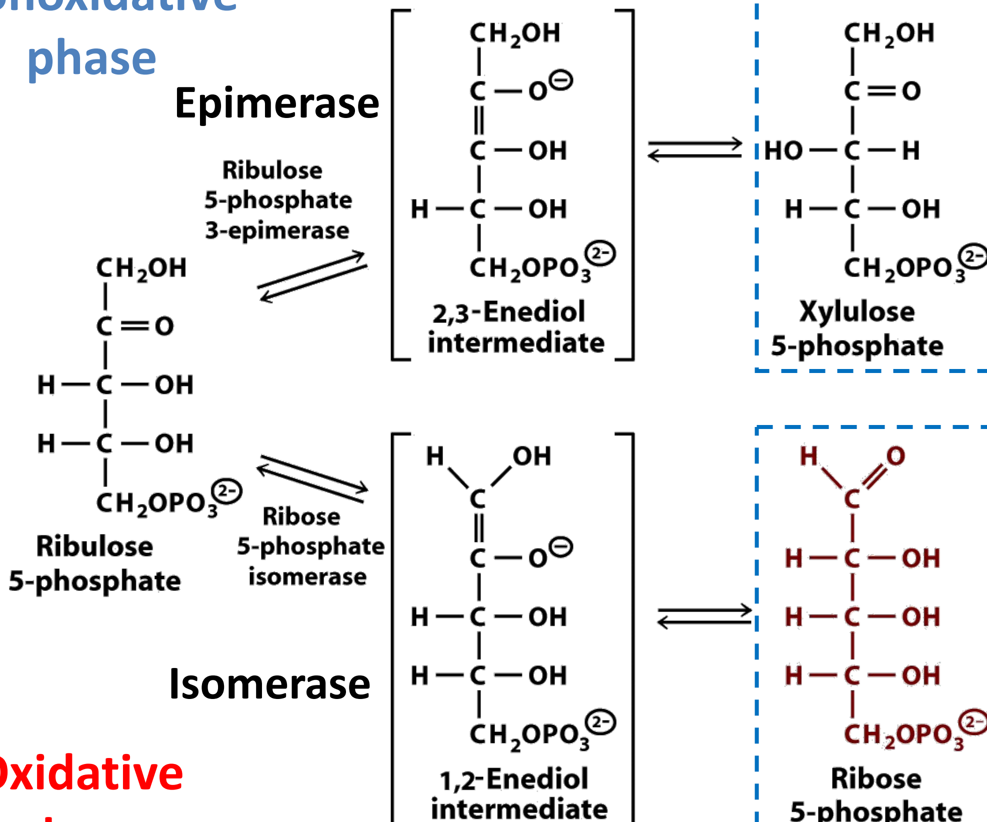
Glucose 6-phosphate + $2NADP^+$ + H_2O $\longrightarrow$

ribose 5-phosphate + CO_2 + 2NADPH + $2H^+$



The different fates of ribulose 5-phosphate

Nonoxidative phase



Oxidative phase

In some cases, more NADPH than ribose 5-phosphate is needed:

- most of pentose phosphates are converted back to glucose 6-phosphate

If both NADPH and nucleotides are required (e.g. in rapidly dividing cells):

- all ribulose 5-phosphate is isomerized to ribose 5-phosphate and the pathway is completed at this stage

The nonoxidative reactions pathway

Schematic for generating 5 hexoses (glucose 6-phosphate) from 6 pentoses

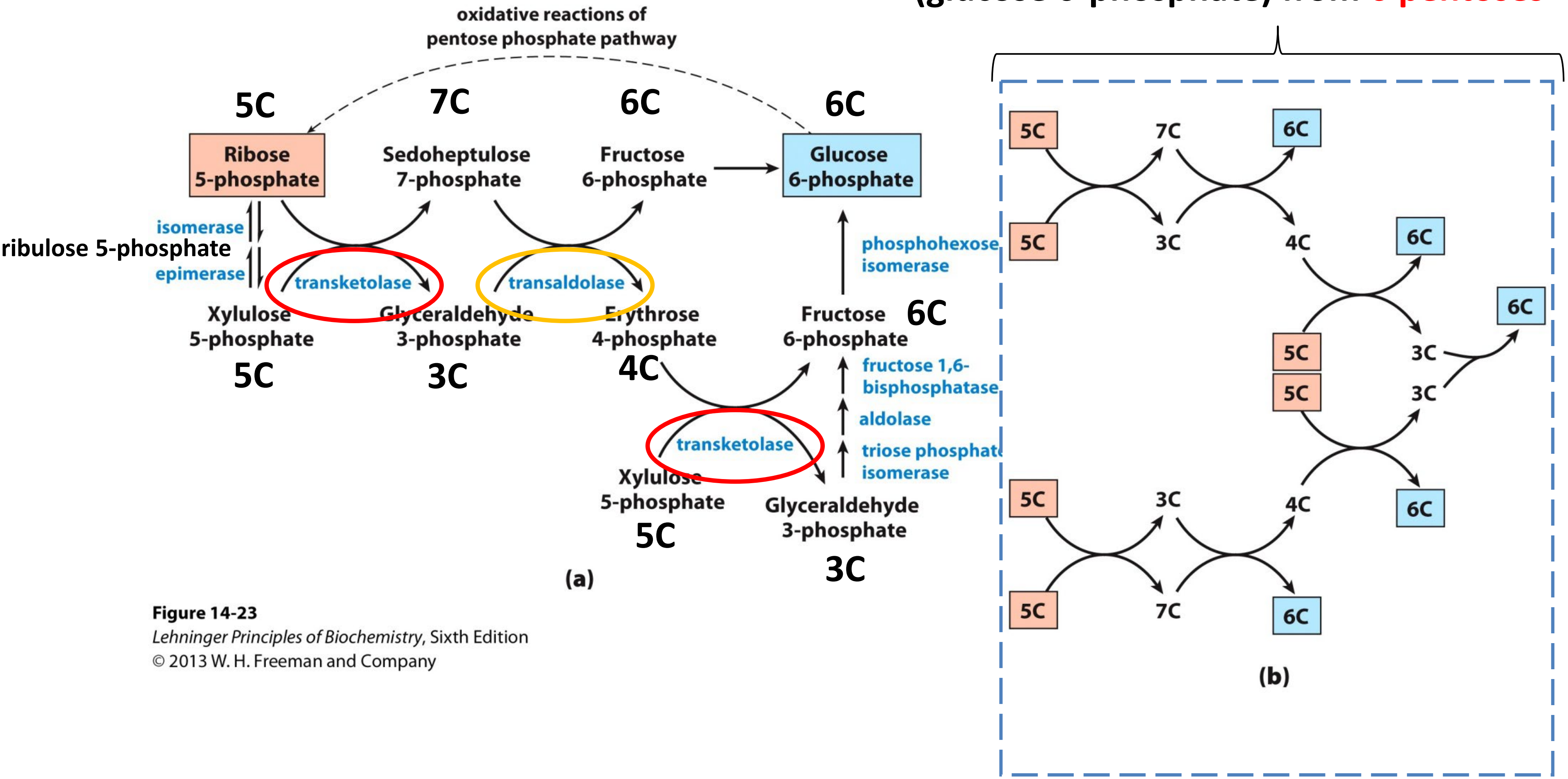


Figure 14-23
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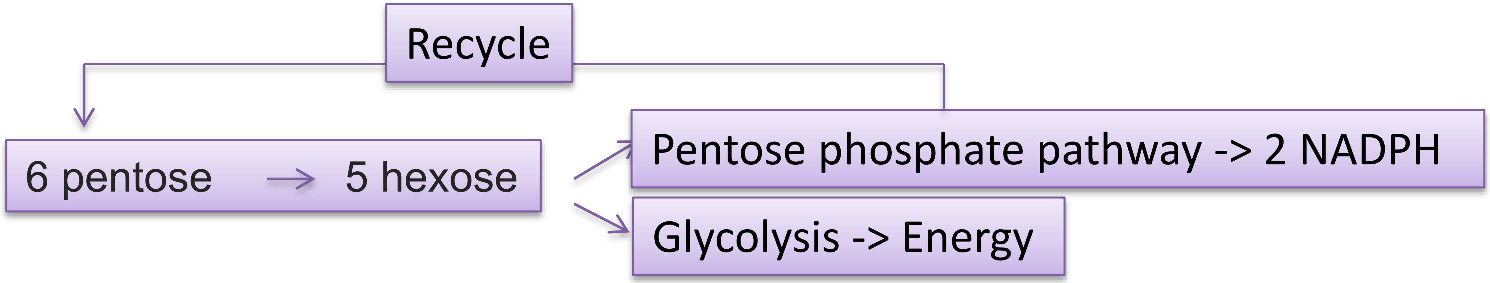
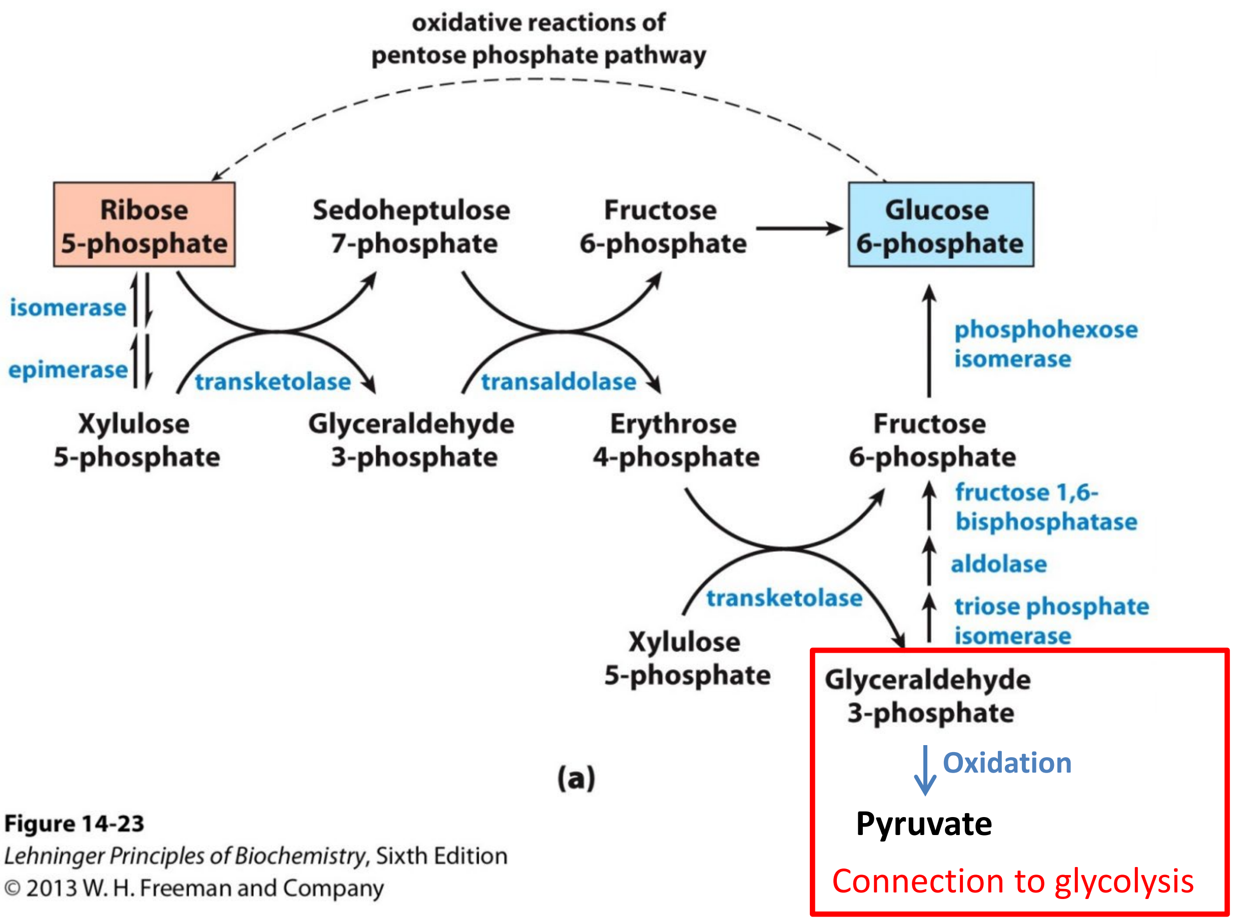
The nonoxidative reactions involve the enzymes **Transketolase** and **Transaldolase**

The nonoxidative phase complete reaction





(a)

Figure 14-23
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Regulation of pentose phosphate pathway

- The entering of pentose phosphate pathway is controlled by the level of $NADP^+$.

$NADP^+ \uparrow$

↓ Stimulate

Glucose-6-phosphate dehydrogenase

↓ Promote

Pentose phosphate pathway

↓

$NADP^+ \downarrow$ $NADPH \uparrow$

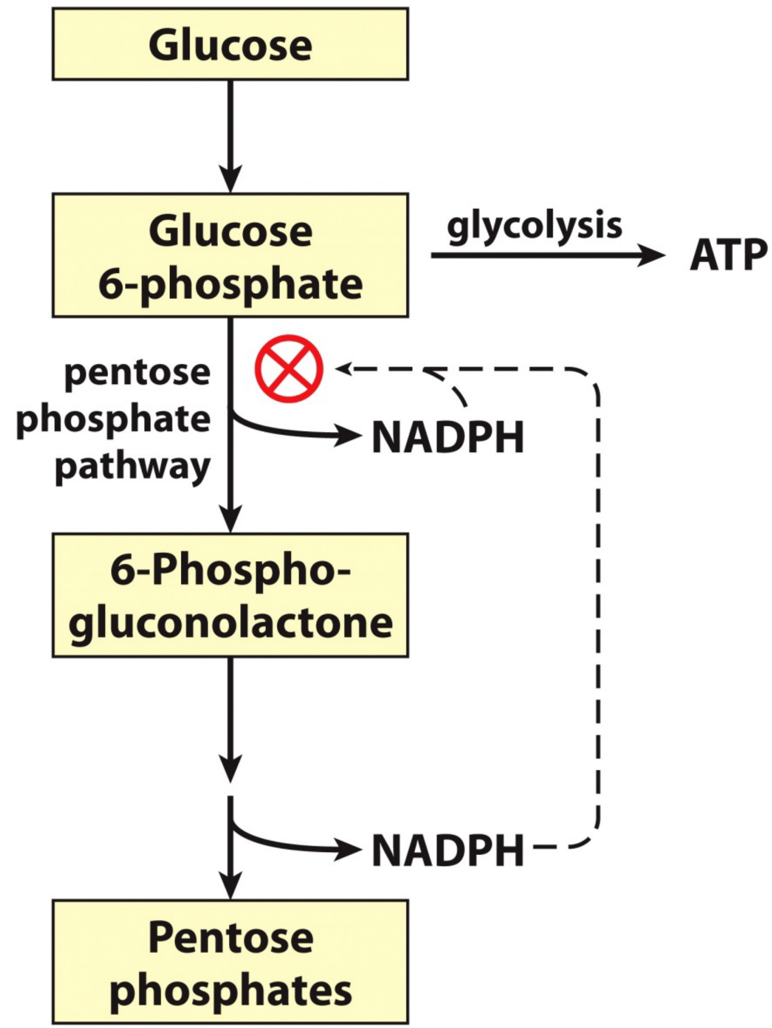

Figure 14-28
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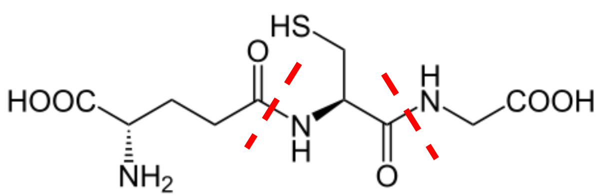
NADPH and glutathione in oxidative stress

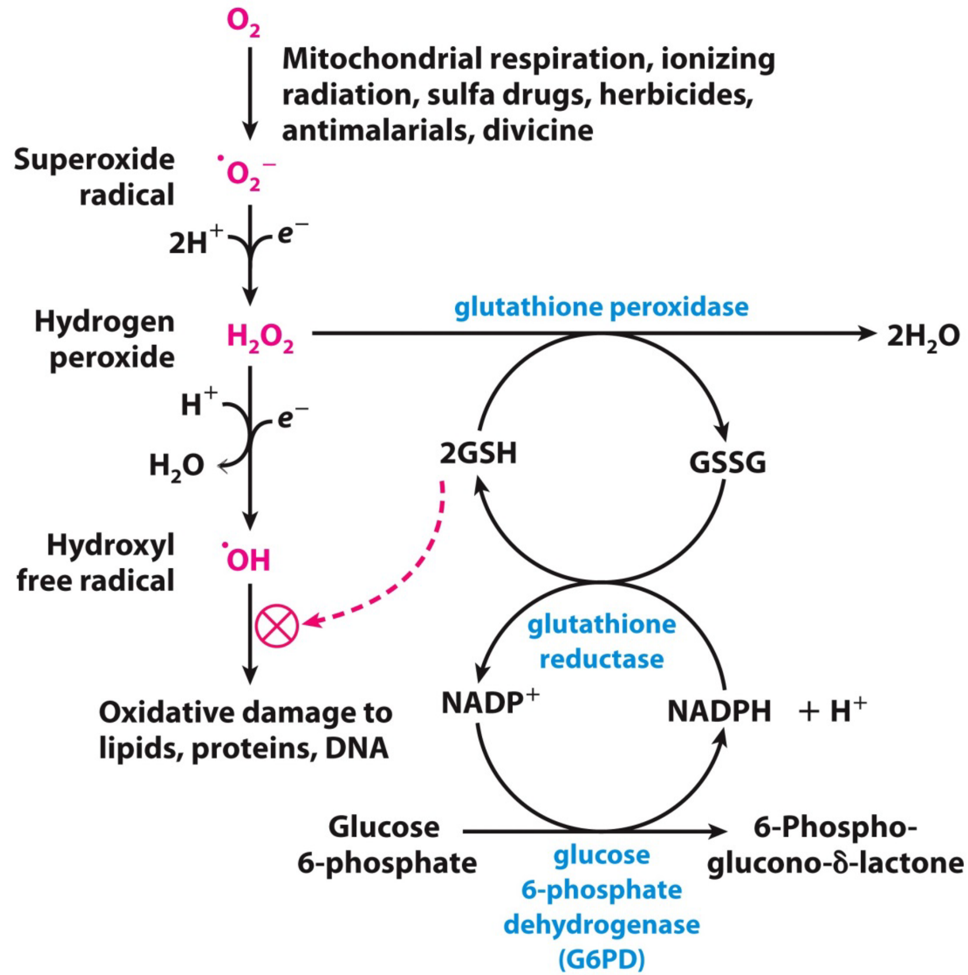
- Glutathione (GSH) is a short peptide composing of cysteine, glutamate and glycine

5 mM
In the cells



Glutamic acid Cysteine Glycine

- Detoxification of hydrogen peroxide (H_2O_2), which produces hydroxyl free radical (·OH), needs glutathione, glutathione peroxidase, glutathione reductase and NADPH



Box 14-4 figure 1
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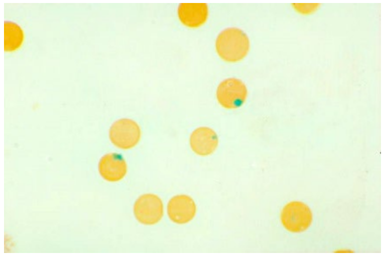
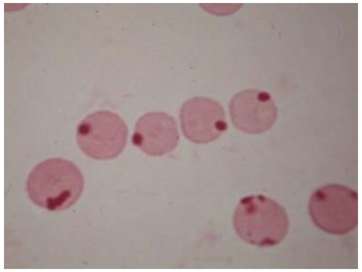
Hereditary anemia and Heinz body





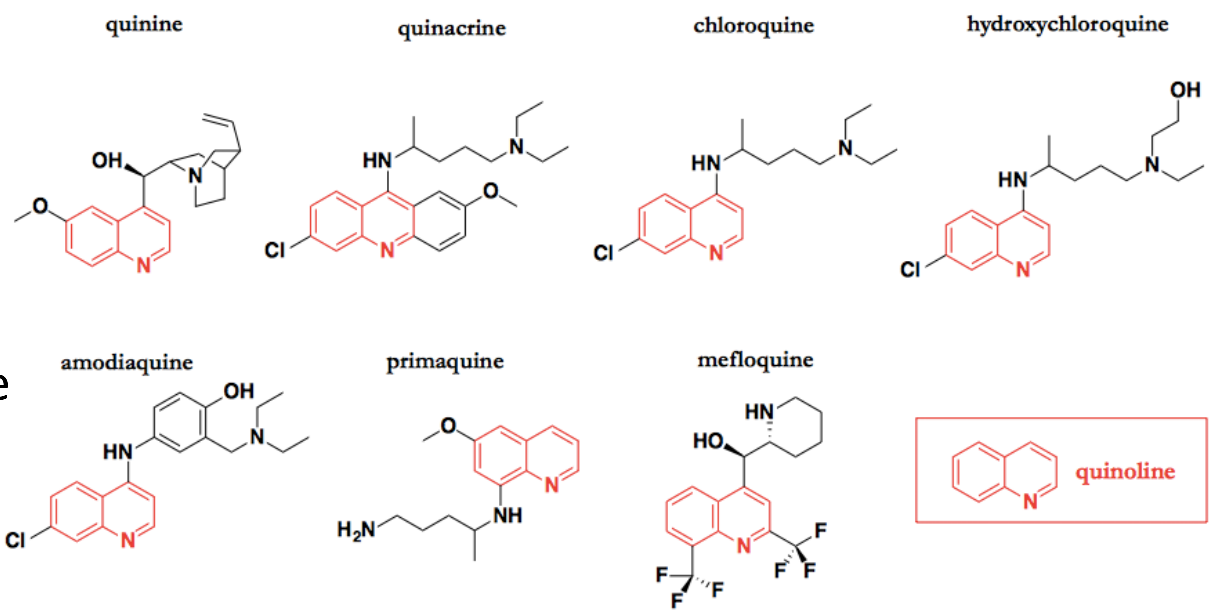
- G6PD deficiency person mostly asymptomatic but is more prone to oxidative damage induced by stresses and eating fava beans
- Red blood cells use lots of O_2 for the generation of ATPs; need NADPH to protect them from oxidative stresses
- Without G6PD and NADPH, free radicals attracts the globins which become denatured and forms a little ball (the "Heinz body")
- The gene for G6PD is on the X chromosome (thus males are much more likely to have full expression of the disease); the disease is more common in African Americans

http://www.pathologystudent.com/?p=1184
https://www.pinterest.com/pin/32081173428451507/
http://www.wikidoc.org/index.php/Heinz_body

Oxidative stress and malaria

- G6PD deficiency population have an advantage of resistance to parasite malaria infection because of higher level of oxidative stress in erythrocytes

- Quinoline derivatives stem from quinine (top left), a natural product isolated from the bark of the Andean cinchona tree



- Pharmaceutical **chloroquine** phosphate and hydroxychloroquine sulfate are approved by the FDA to treat malaria
- Treatment for COVID-19 not proven

Summary (I)

- The pentose phosphate pathway
  - generates two NADPH and a pentose ribose 5-phosphate from glucose 6-phosphate
  - contains an oxidative phase and a nonoxidative phase

- Importance:
  - for rapid dividing cells which use ribose 5-phosphate for the synthesis of DNA, RNA and coenzymes
  - for tissues that need NADPH for reductive biosynthesis or countering damaging effects of oxygen radicals

- Nonoxidative phase recycles ribose 5-phosphate (produces 5 hexoses from 6 pentoses)

- NADPH is required for the antioxidant function of glutathione
  - G6PD deficiency population is less resistant to oxidative damage but more resistant to malaria