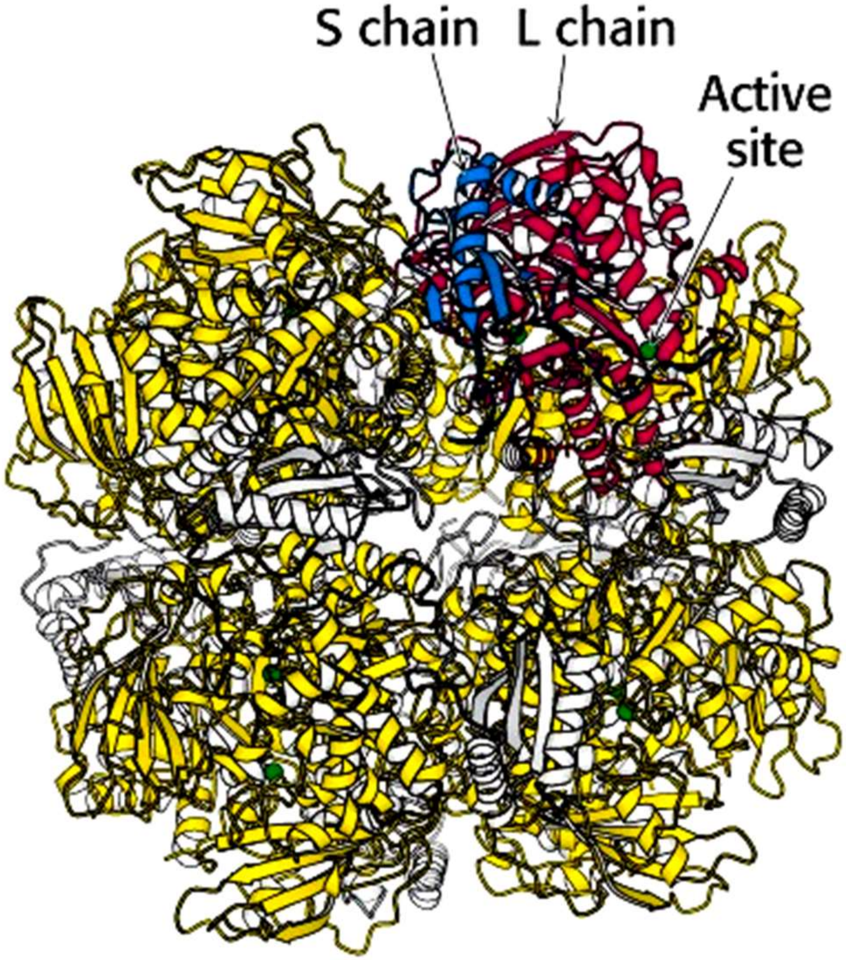
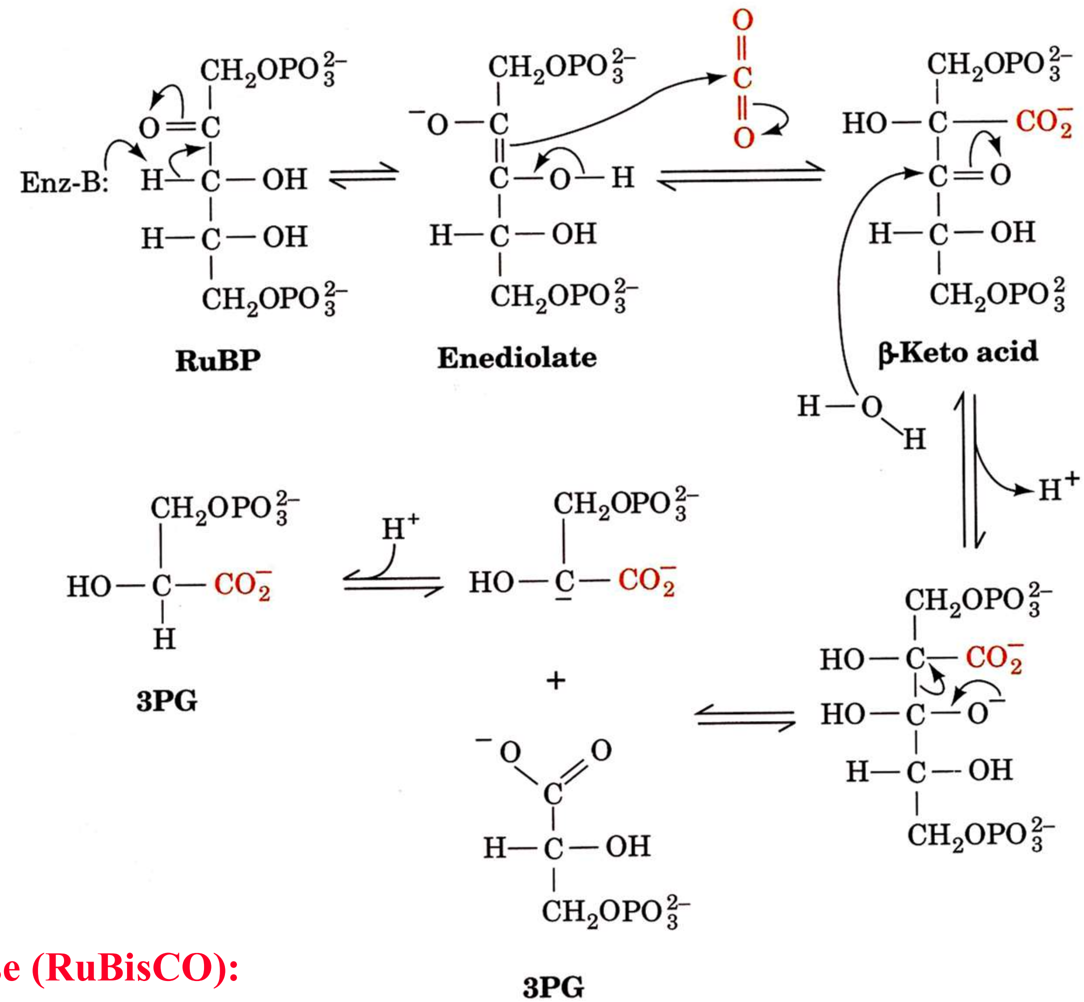
Meet the Most Abundant Protein on Earth





- ➢ **Ribulose bis-phosphate carboxylase/oxygenase (RuBisCO):**
- ♣ **8 large (L), 477-amino acid catalytic subunits + 8 small (S), 123-amino acid subunit (regulatory???) in square prism symmetry**
- ♣ **$k_{cat} \approx 3\ s^{-1}$ (slow!);**
- ♣ **comprises up to 50% of leaf proteins ⇒ most abundant in biosphere!;**
- ♣ **fixes ~10^{11} tons of CO_2 per year;**
- ♣ **has peculiar side reaction in which O_2 gets fixed instead of CO_2 (oxygenase activity!)**
- ♣ **reason possibly protection from O_2 at low [CO_2]?**

Reminiscent of pyruvate carboxylase, but no biotin!

But wait – how does this all work together? Regulation needed!

➢ Chloroplast stroma contain the enzymes of the Calvin cycles as well as those of glycolysis and the pentose phosphate pathway, which are used to generate ATP and NADPH
⇒ At night, the Calvin cycle has to be downregulated (through absence of activation) so that ATP and NADPH from the catabolic pathways do not get consumed in a futile cycle

Table 24-1 Standard and Physiological Free Energy Changes for the Reactions of the Calvin Cycle

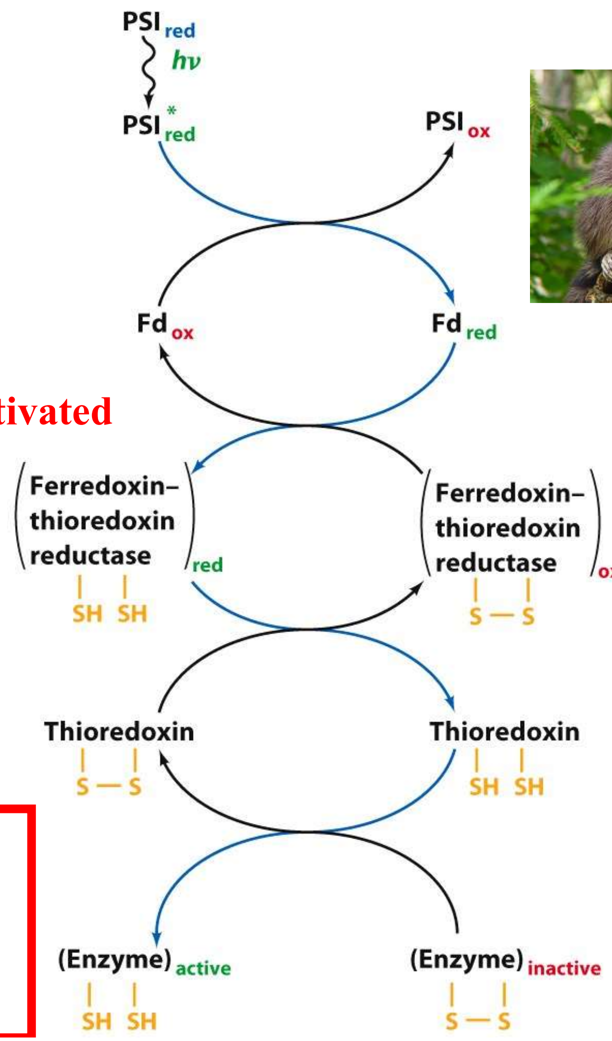
Step[a]	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	Phosphoribulokinase	−21.8	−15.9
2	Ribulose bisphosphate carboxylase	−35.1	−41.0
3 + 4	Phosphoglycerate kinase + glyceraldehyde-3-phosphate dehydrogenase	+18.0	−6.7
5	Triose phosphate isomerase	−7.5	−0.8
6	Aldolase	−21.8	−1.7
7	Fructose bisphosphatase	−14.2	−27.2
8	Transketolase	+6.3	−3.8
9	Aldolase	−23.4	−0.8
10	Sedoheptulose bisphosphatase	−14.2	−29.7
11	Transketolase	+0.4	−5.9
12	Phosphopentose epimerase	+0.8	−0.4
13	Ribose phosphate isomerase	+2.1	−0.4

most likely to be regulated

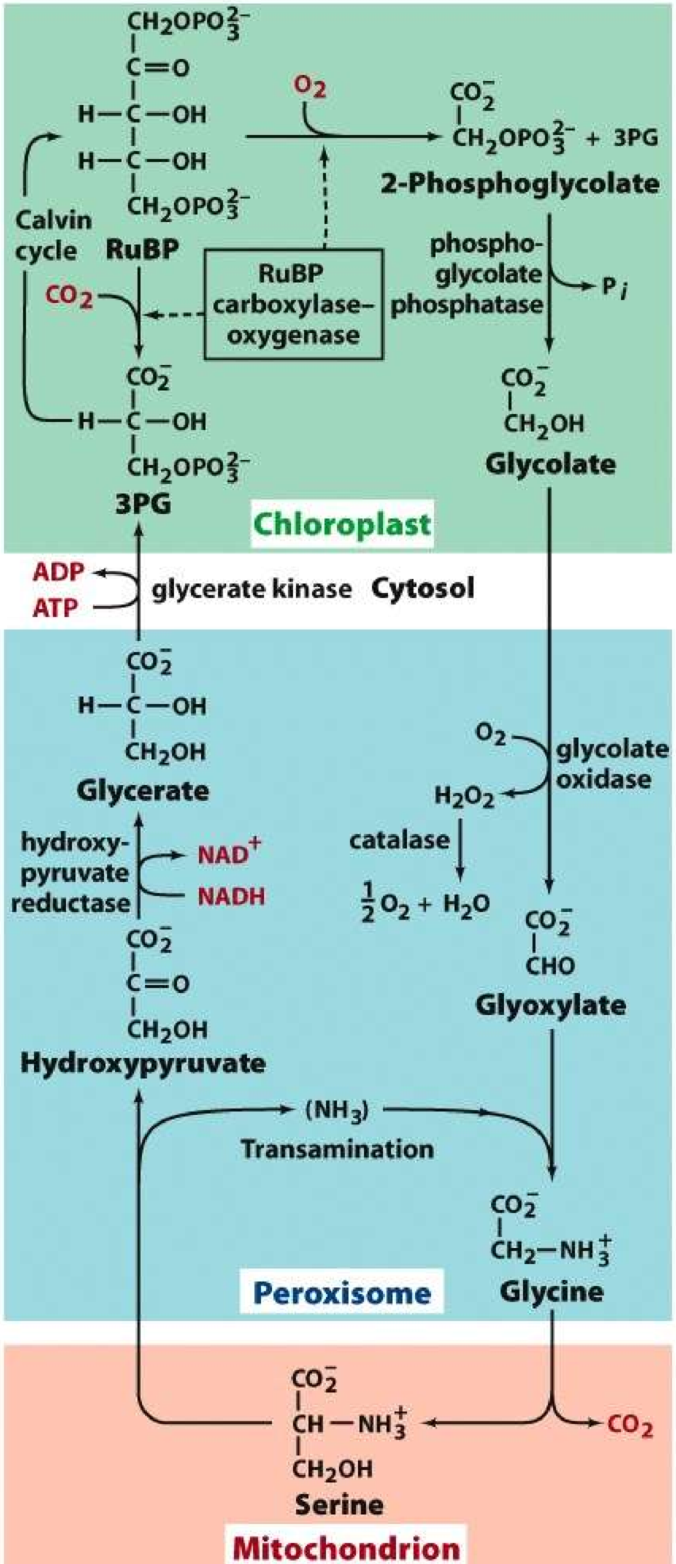
[a]Refer to Fig. 24-31.

Source: Bassham, J.A. and Buchanan, B.B., *in* Govindjee (Ed.), *Photosynthesis,* Vol. II, p. 155, Academic Press (1982).

Light activated through redox sensing:

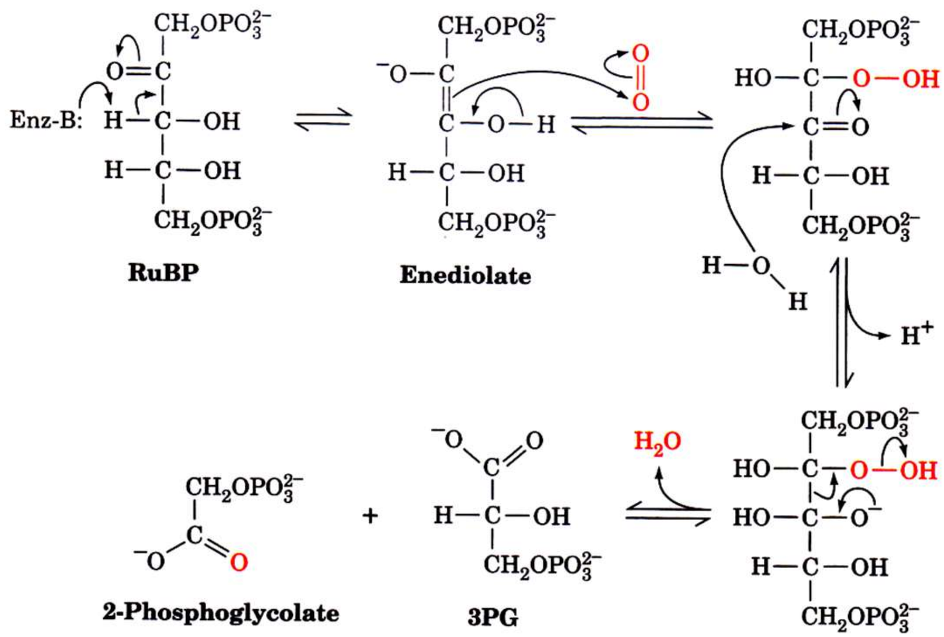


RuBisCO controlled by:
➢ pH (sharply optimal is 8.0, which is reached as protons are pumped from stroma to thylakoid lumen upon illumination)
➢ Mg^{2+} stimulation (as cofactor; proton influx into the lumen leads to Mg^{2+} efflux)
➢ 2-carboxyarabinitol-1-phosphate inhibition (produced only in the dark)



But another problem is less well controlled: Photorespiration

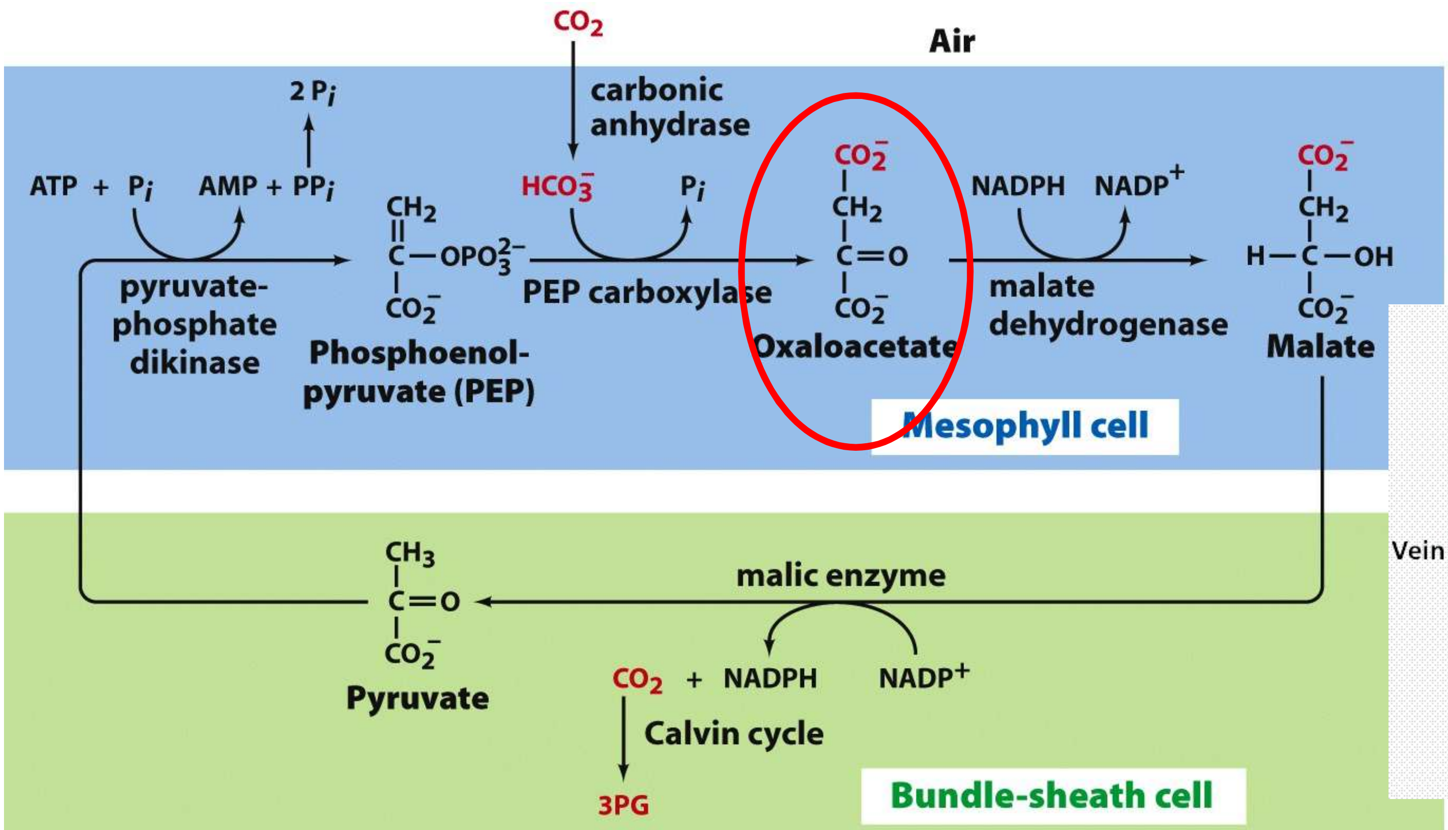
➢ A nasty side reaction of RuBisCO:

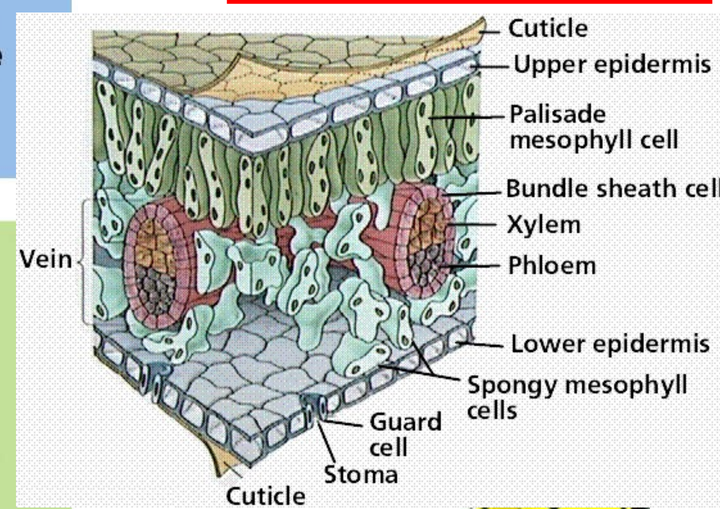


➢ Leads to consumption of O_2 (and ATP) and evolution of CO_2, independent of oxidative phosphorylation

How so-called C4 plants deal with it

- The CO_2 compensation point of ~40-70 ppm CO_2 (the normal atmospheric concentration is 330 ppm) saves many plants the trouble
- But the CO_2 compensation point increases with temperature (as O2 becomes a better substrate) so that tropical plants under hot and sunny conditions (i.e., ~5% of all plants, including corn) utilize the C_4 pathway below to increase their local concentration of CO_2 for the Calvin cycle





Chapter 24: What have we learned?

- Anatomy of chloroplasts, analogies to mitochondria
- Chlorophylls
- Absorption processes
- The bacterial photosynthetic reaction center and how it works
- Photosystems II and I and how they work
- The Z-scheme
- Making NADPH and ATP in photosynthesis
- Q cycles in Electron Transport Systems
- Light harvesting, segregation, regulation
- The Calvin cycle
- The RuBisCO mechanism, regulation, and what can go wrong
- The C4 pathway

Lipid Metabolism

Voet & Voet, Chapter 25

Major roles of lipids in cell structure and metabolism:

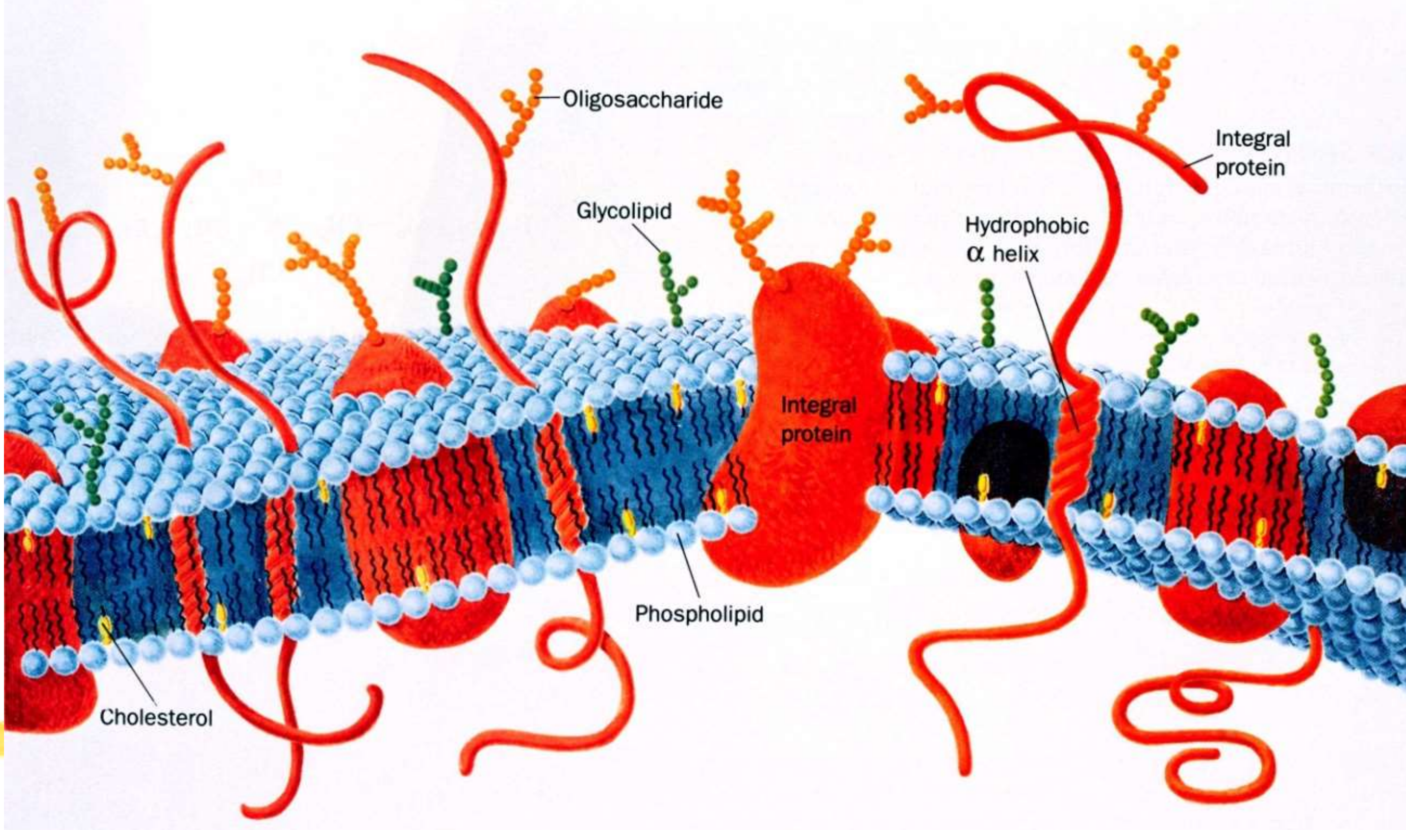
triacylglycerols: major form of stored energy in mammals

phospholipids, glycolipids, cholesterol: components of cell membranes

cholesterol: precursor of steroid hormones and bile salts

prostaglandins, prostacyclins, thromboxanes, leucotrienes, lipoxins: hormones and intracellular messengers

Fatty acid side chains: protein targeting to membranes



Fatty acids have 4 major physiological roles

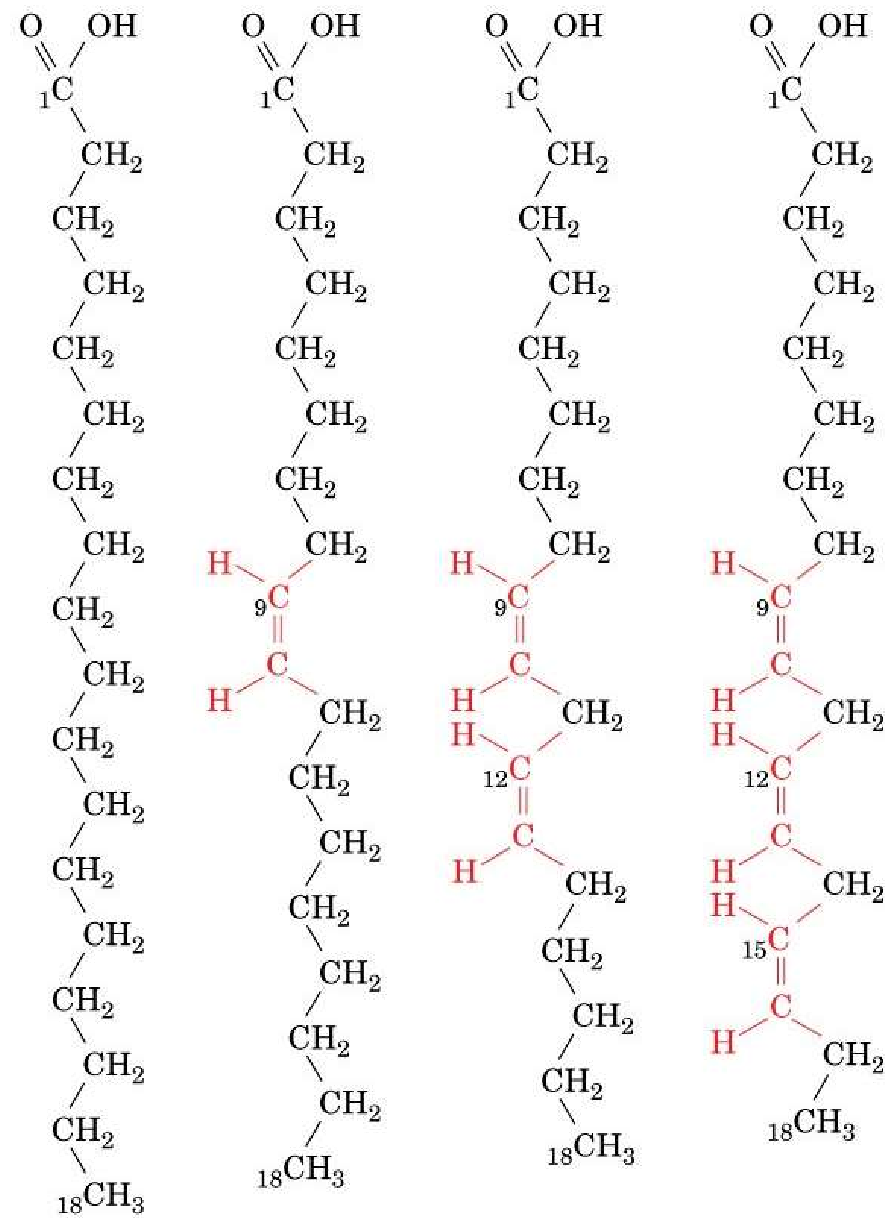
➢ components of phospholipids and glycolipids

➢ covalent attachment to proteins, protein targeting

➢ fuel and storage (triglycerides)

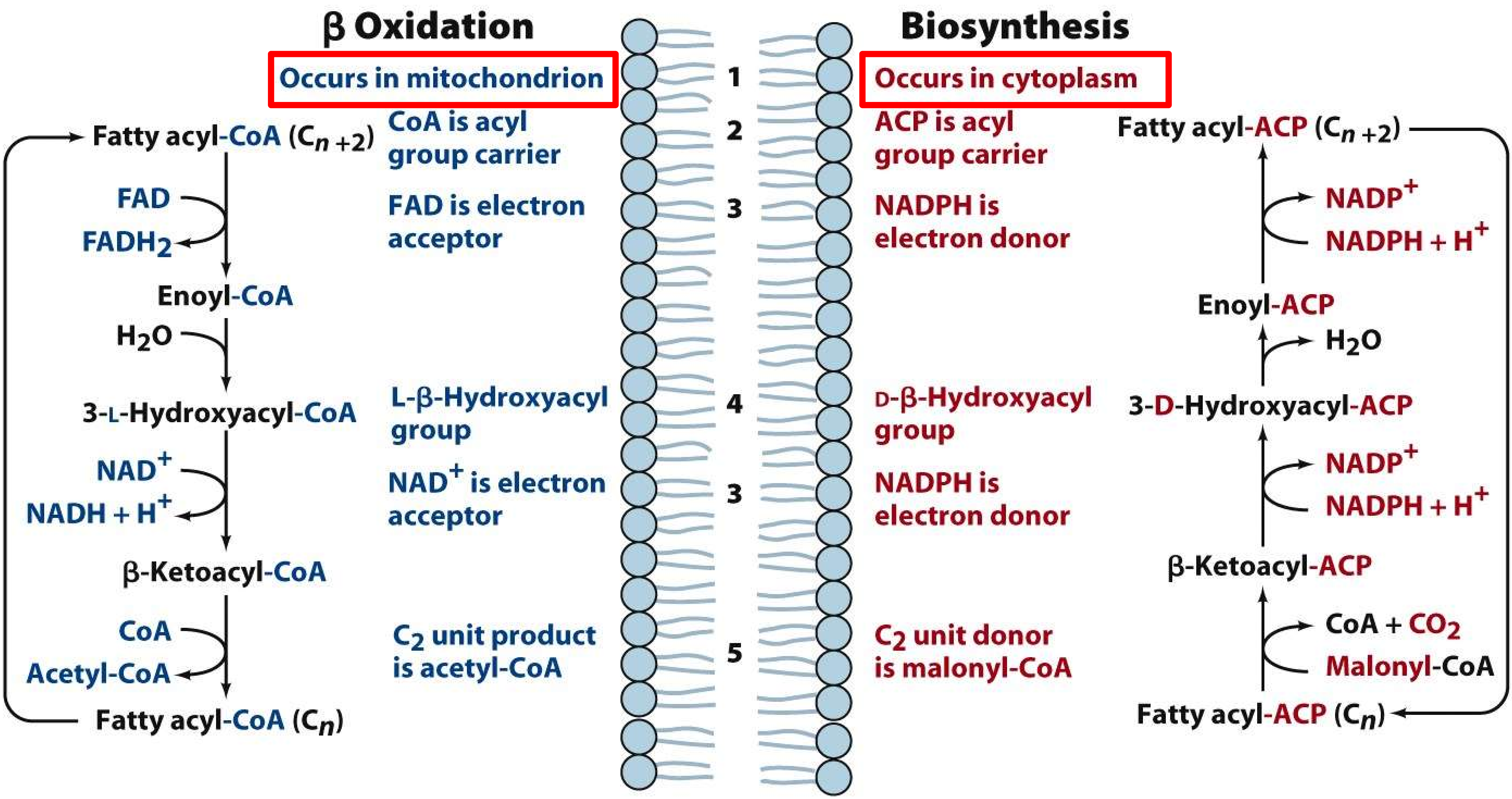
➢ hormones and intracellular messengers

Please note: double bonds (when present) are *cis* and unconjugated

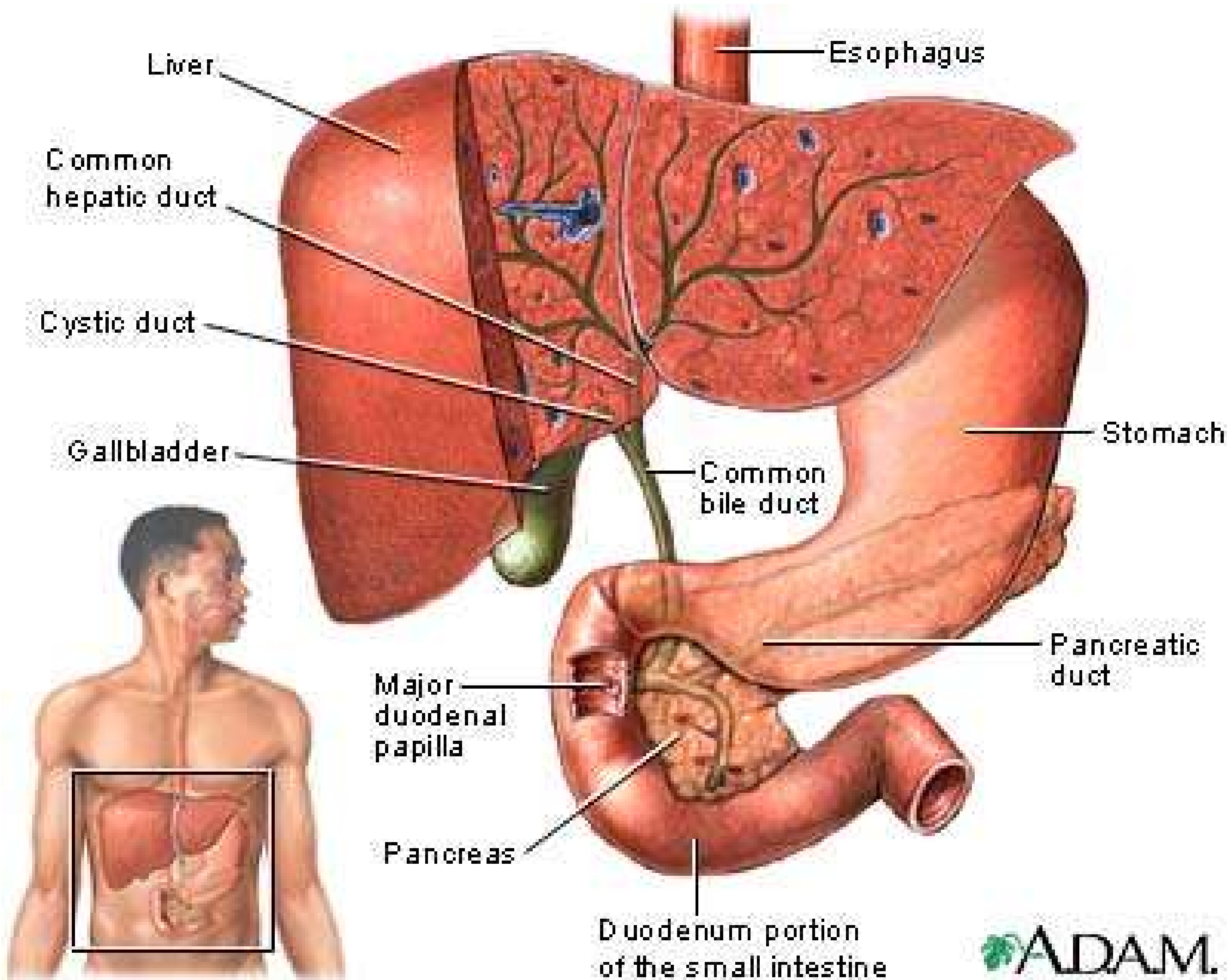


Stearic acid   Oleic acid   Linoleic acid   α-Linolenic acid

Sneak preview: Symmetry between fatty acid degradation and biosynthesis

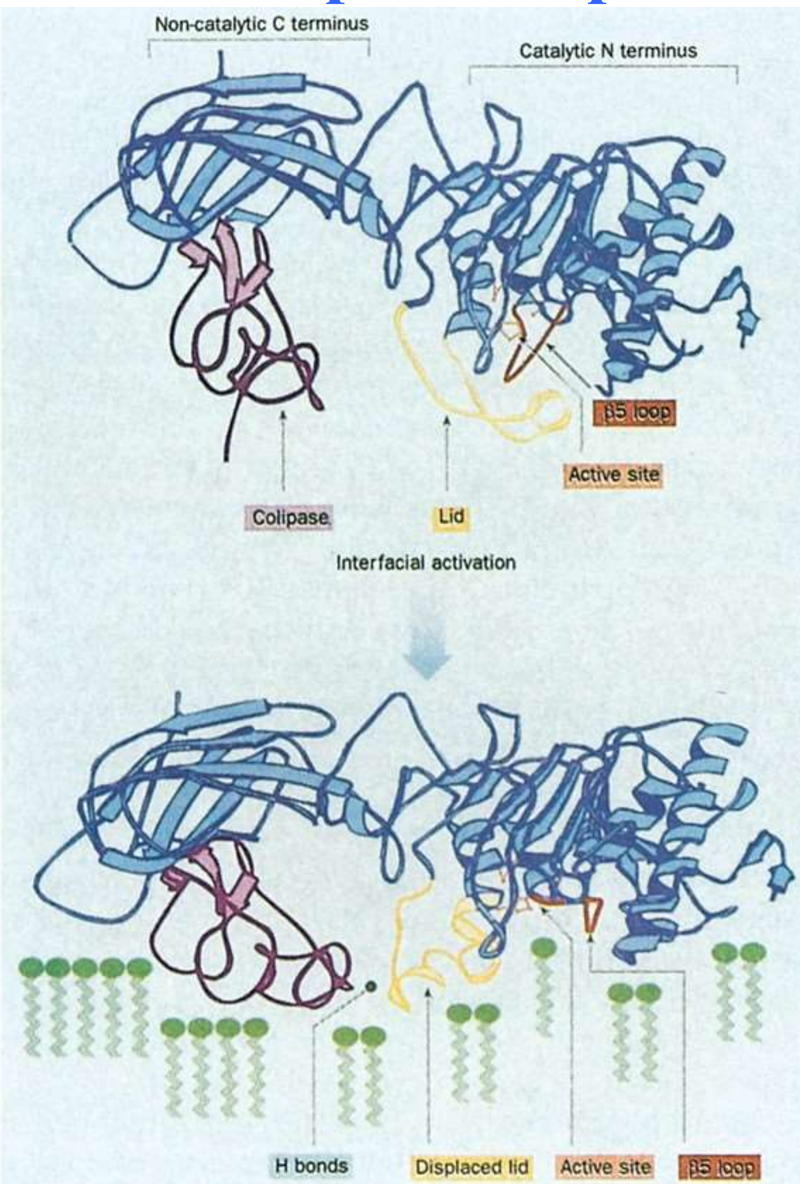


Digestion : Where It All Happens



Dietary Lipids are Digested by Pancreatic Lipases at the Lipid-Water Interface

pancreatic lipase-colipase complex

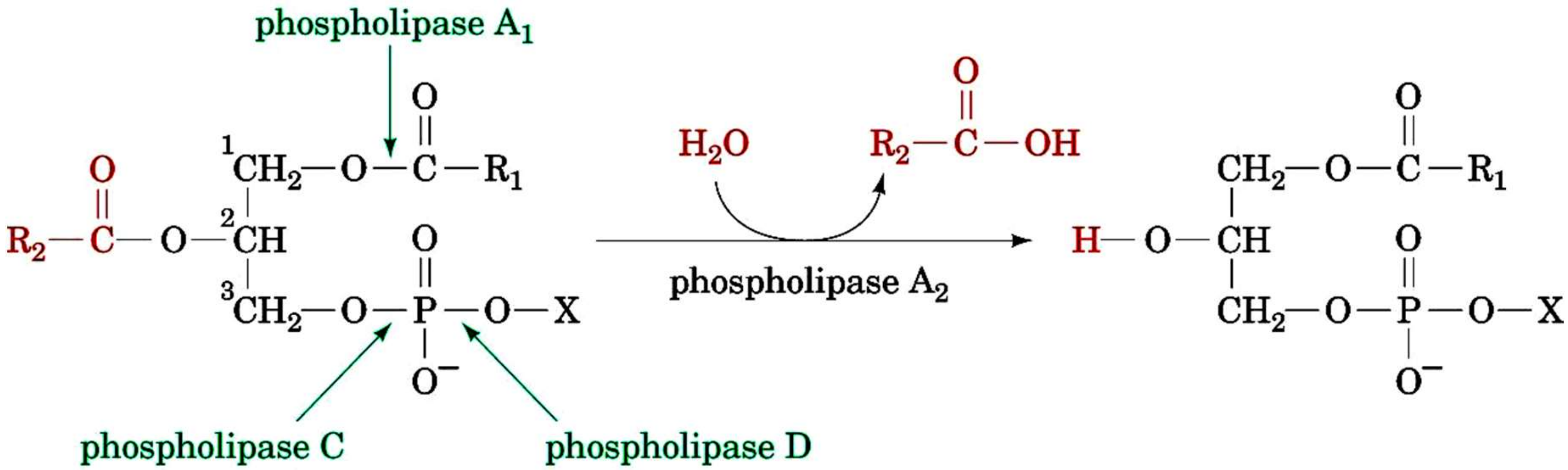


From Nature 362, 793 (1993). Used with permission

- **Catalyzes stepwise hydrolysis to form additional "soap": triacylglycerol → 1,2-diacylglycerol → 2-acylglycerol**

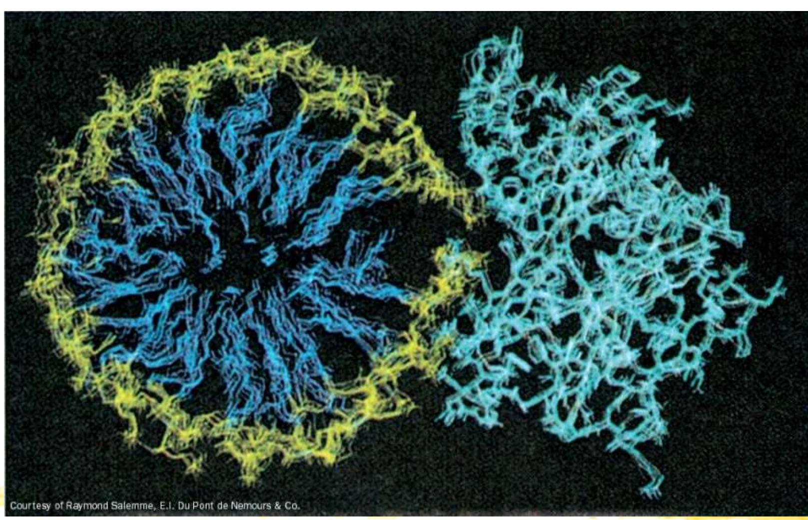
- **"Interfacial activation": The enzyme is only active in complex with micelles that open its lid (with the help of hydrogen bonding to the colipase)**

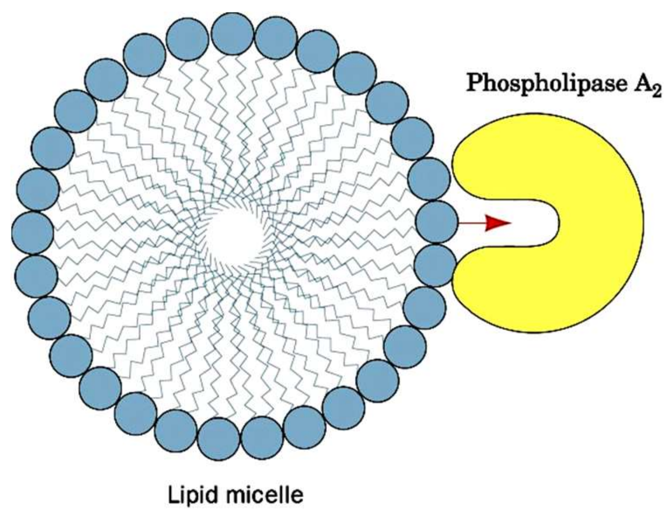
Phospholipids are Degraded by Pancreatic Phospholipases



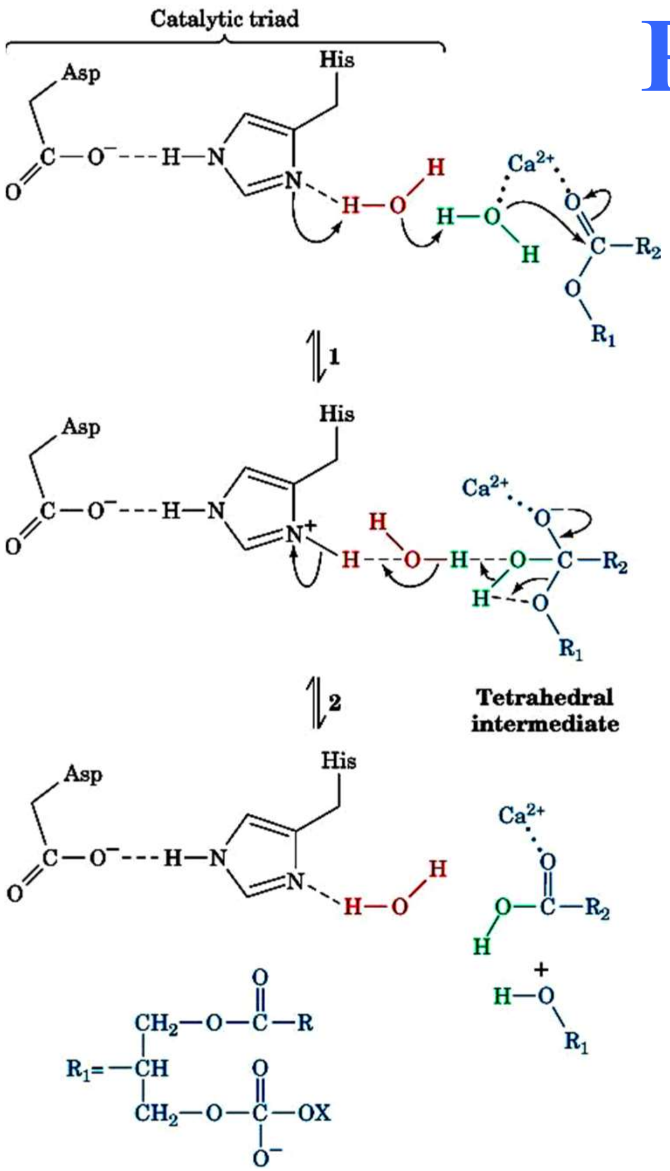
Phospholipid **Lysophospholipid**

hypothetical model

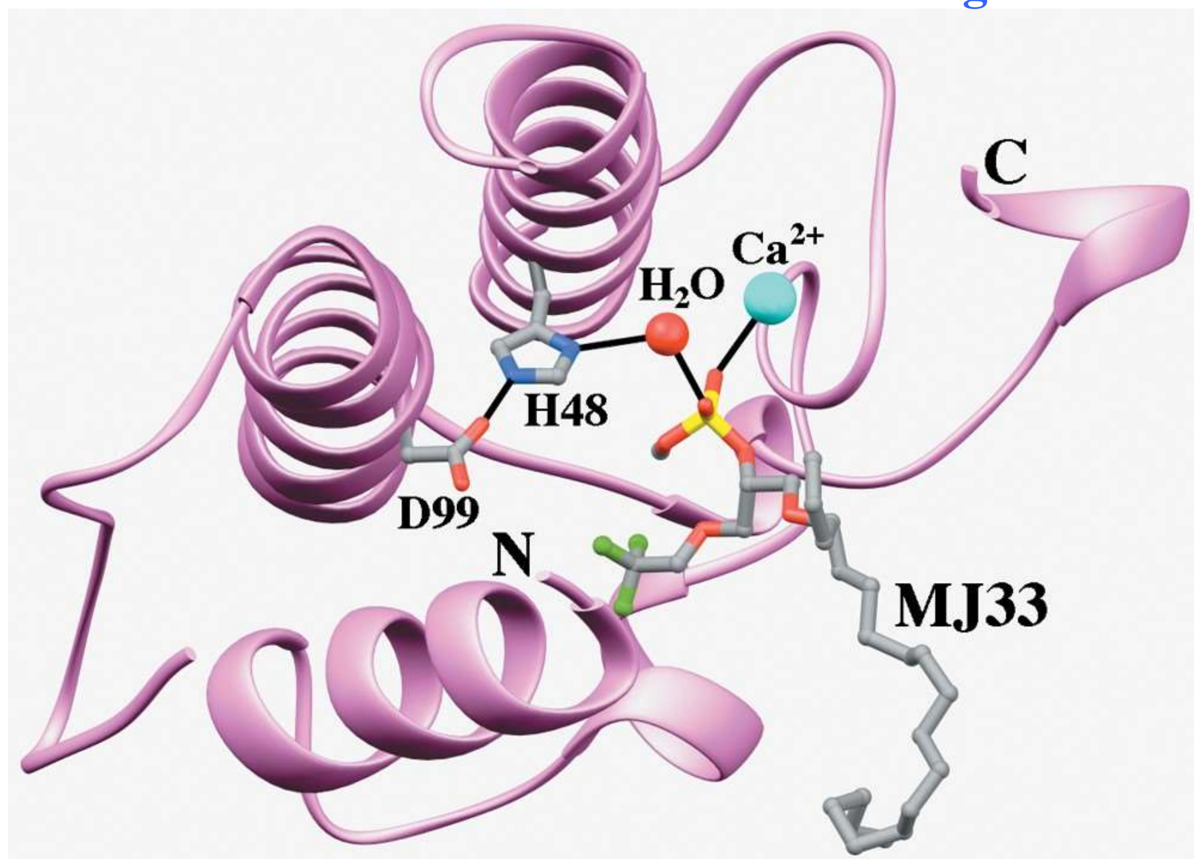




Active Site and Catalytic Mechanism of Phospholipase A_2

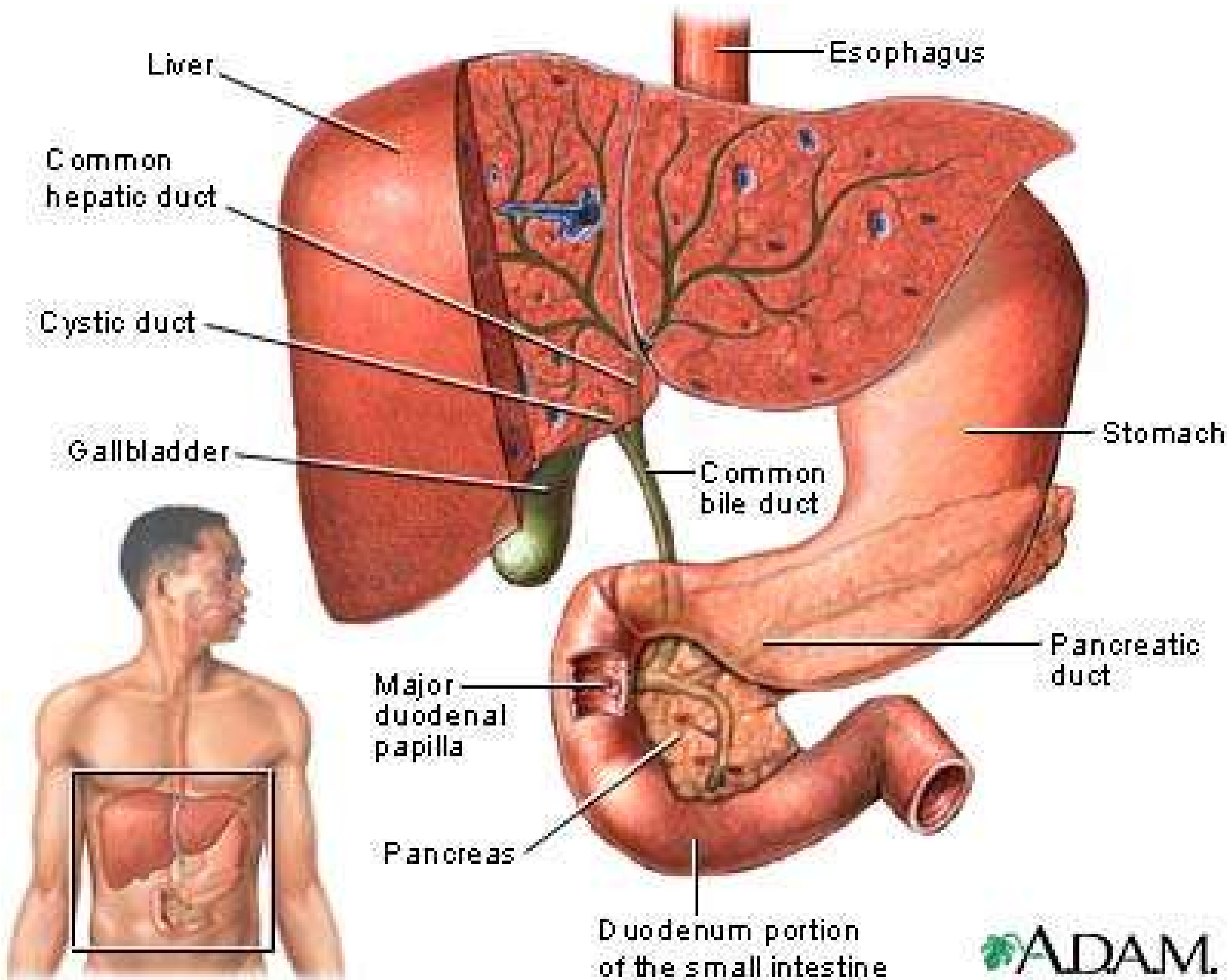


Cut-away view of active site with tetrahedral transition state analogue MJ33

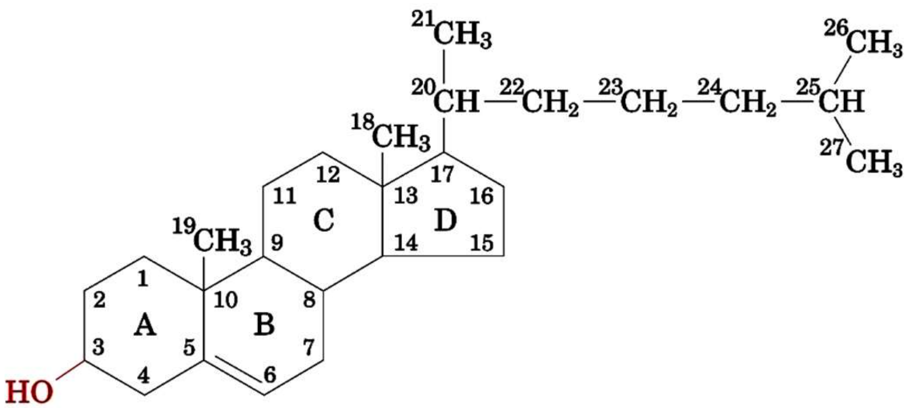


➢ **Bound Ca^{2+} participates in activation of reactive H_2O and stabilizes oxyanion of transition state**

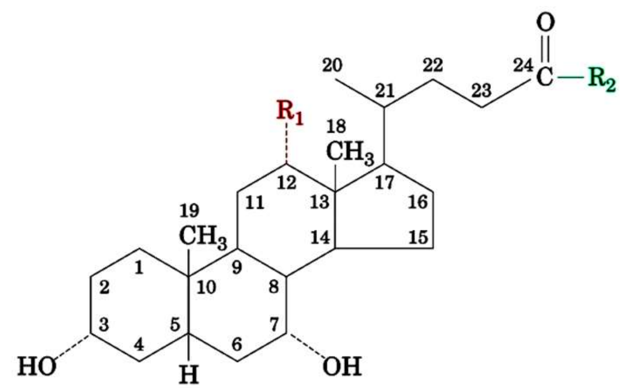
Digestion : Where It All Happens



Bile Salts Help Take Up Digestion Products and Lipid-Soluble Vitamins by Mucosa



Cholesterol

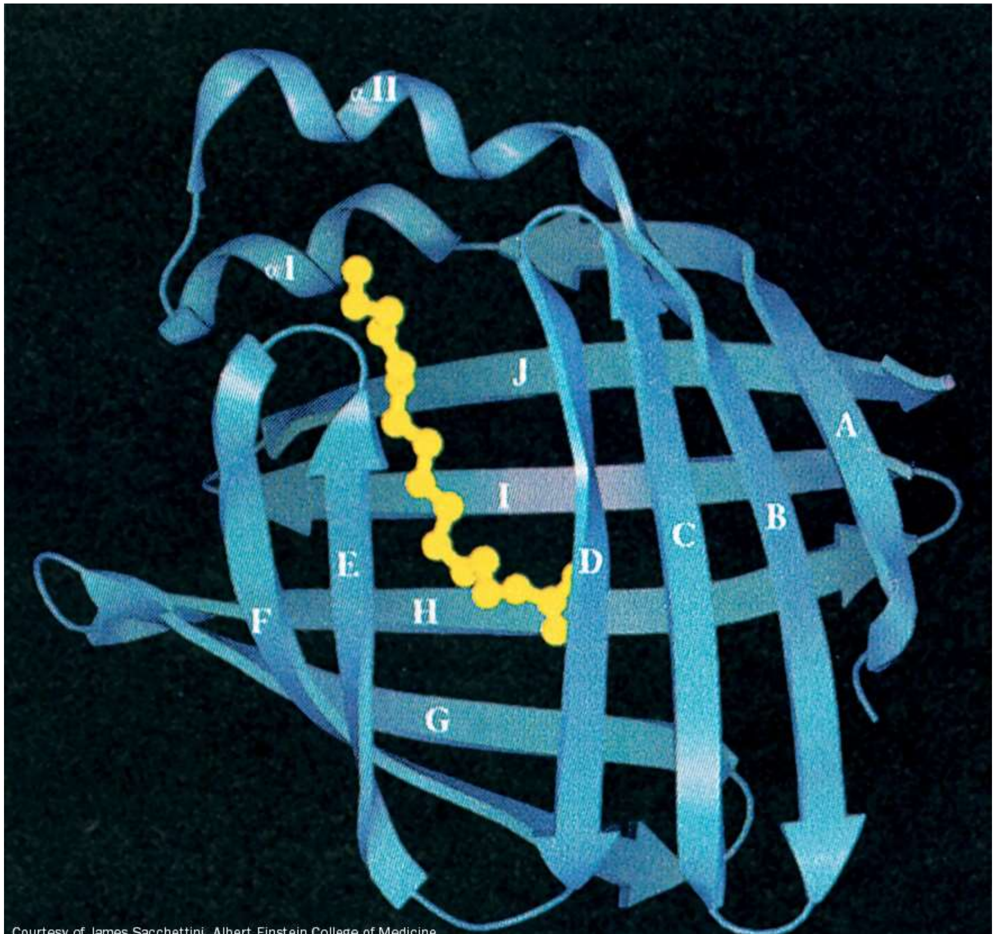


	R_1 = OH	R_1 = H
R_2 = OH	**Cholic acid**	**Chenodeoxycholic acid**
R_2 = NH—CH$_2$—COOH	**Glycocholic acid**	**Glycochenodeoxycholic acid**
R_2 = NH—CH$_2$—CH$_2$—SO$_3$H	**Taurocholic acid**	**Taurochenodeoxycholic acid**

Major bile acids and their glycine and taurine conjugates

- **Bile acids: synthesized in liver, passed to gall bladder, secreted into small intestine, re-adsorbed, taken up by liver**
- **The fraction that escapes re-adsorption is the only route for cholesterol excretion**
- **If bile acid production is defective due to liver disease, large amounts of fats are excreted into the feces (steatorrhea)**

Cytoplasmic Fatty Acid Binding Protein Ferries Fatty Acids Through Mucosa Cells



Fatty acid binding protein – bound here to palmitate in a "beta clam" – complexes fatty acids and protects cells from their detergent-like effects

- Inside the cells of the intestinal mucosa, fatty acids are converted back to triacylglycerols and packaged into chylomicrons along with cholesterol and vitamins

- Chylomicrons are released into lymphatic system and from there into the blood stream

Lipid Transport To and From Tissue

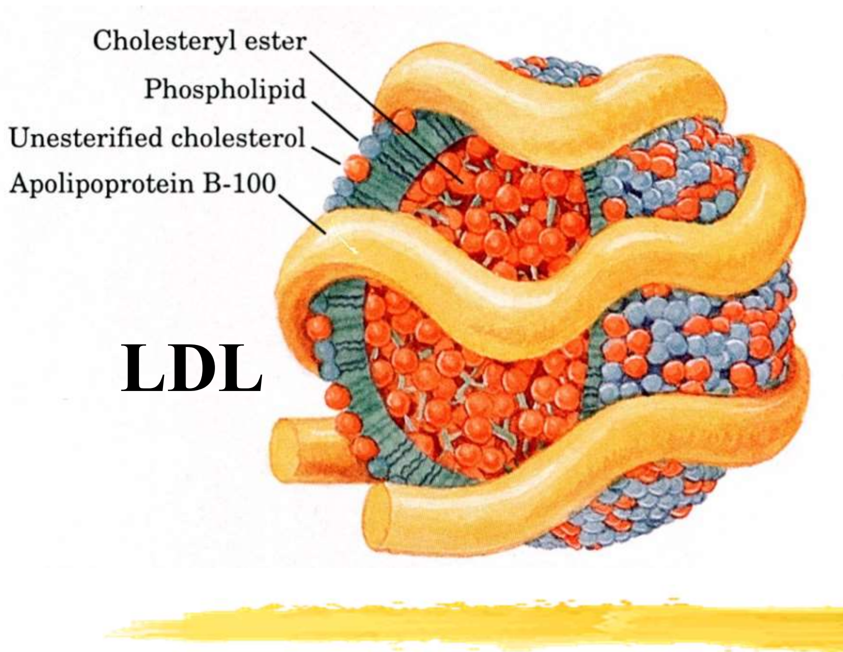
➢ Lipids are sparingly soluble in water ⇒ need to be transported as globular micelle-like particles = lipoproteins

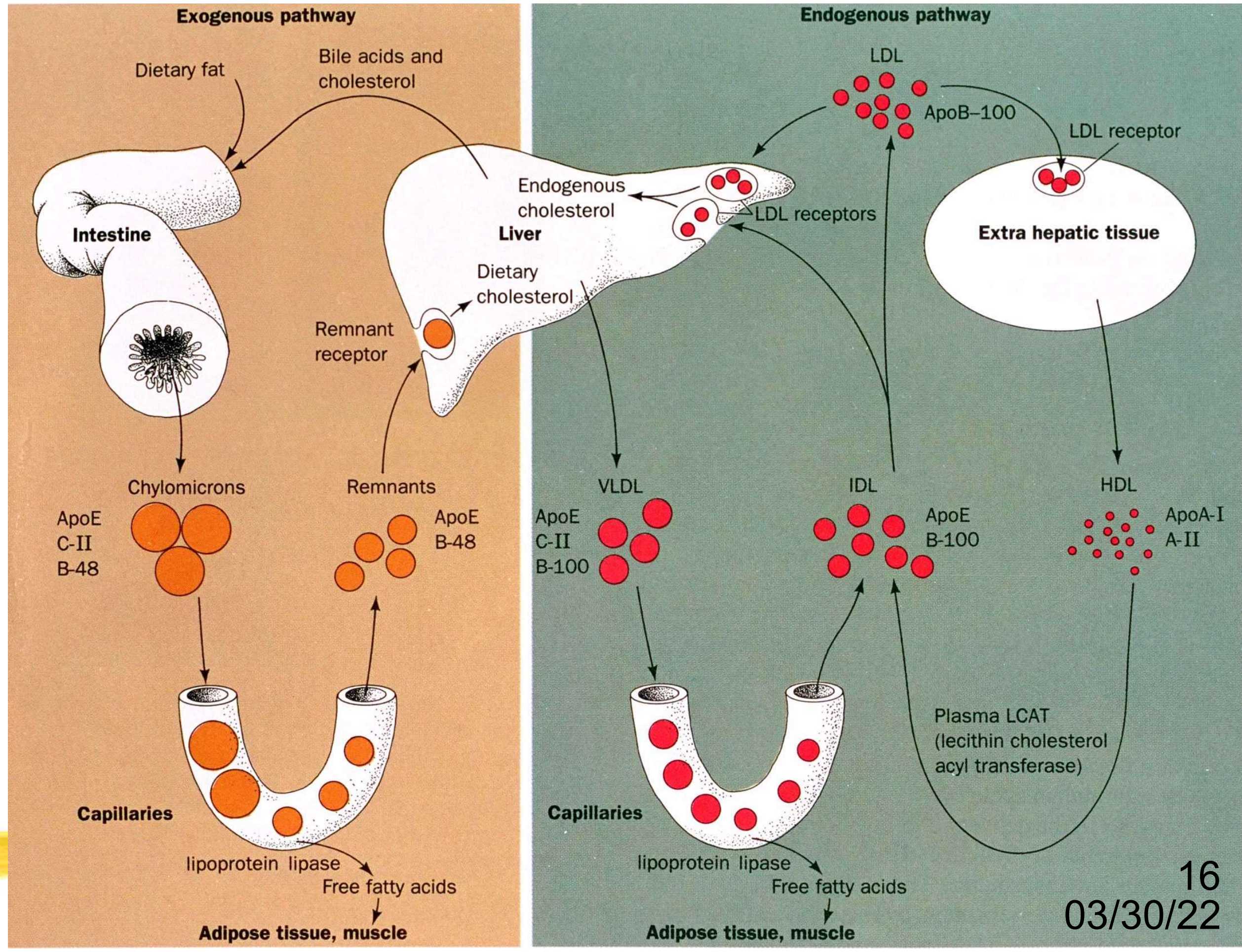
➢ **Chylomicrons:** transport exogenous triacylglycerols and cholesterol from intestines to tissues

➢ **Very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low density lipoproteins (LDL):** transport endogenous triacylglycerols and cholesterol from liver to tissues

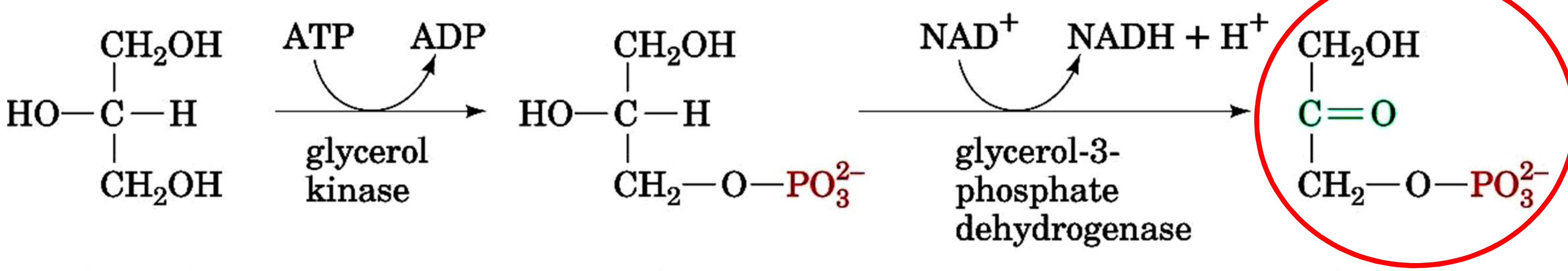
➢ **High density lipoproteins (HDL):** transport endogenous cholesterol from tissues to liver

Wrapped in α-helical, amphiphilic apolipoproteins





Glycerol from the Breakdown of Dietary and Endogenous Triacylglycerols is Transported to the Liver and Used in Glycolysis and Gluconeogenesis

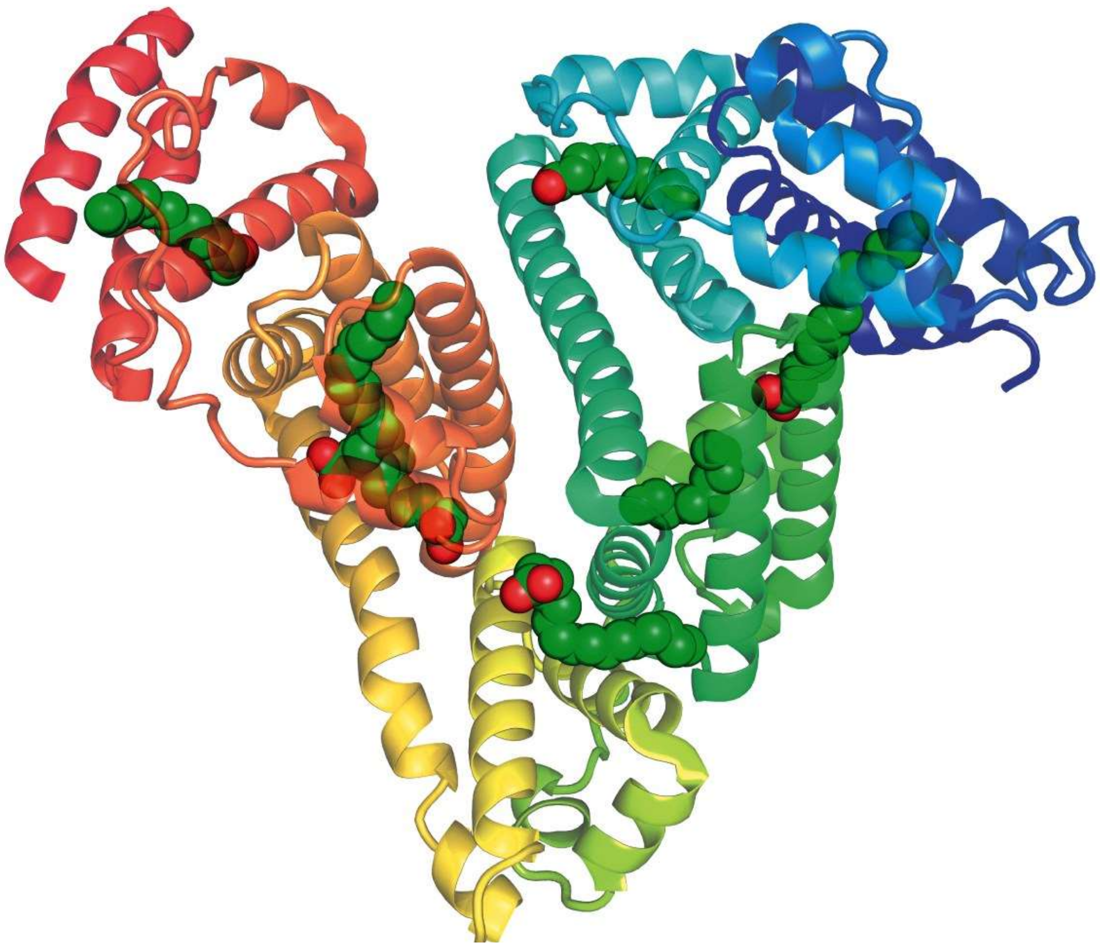


L-Glycerol

L-Glycerol-3-phosphate

Dihydroxyacetone phosphate

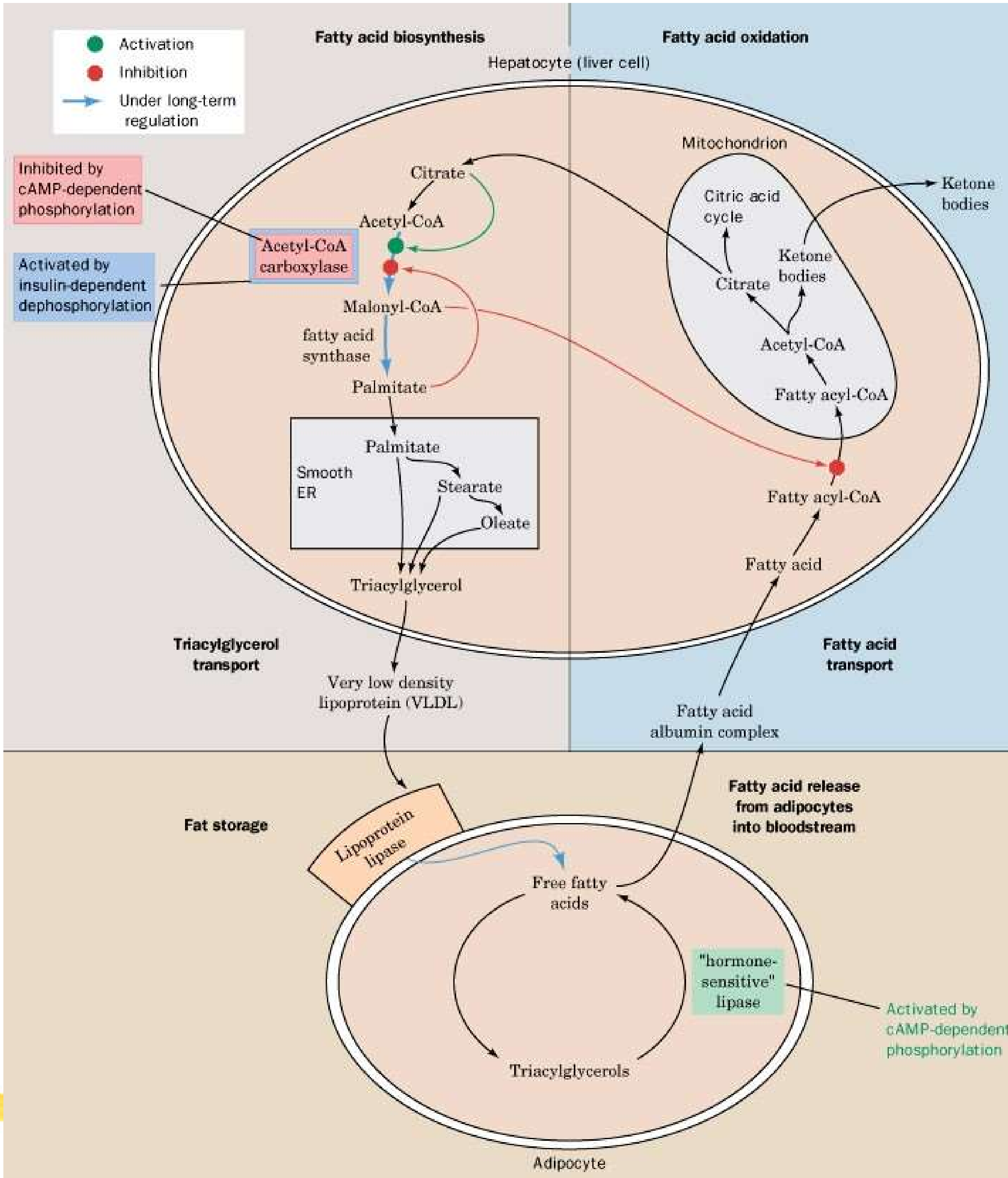
Fatty Acids Released From Adipose Tissue Are Ferried in the Bloodstream by Serum Albumin



Human serum albumin in complex with 7 palmitates

- The synthesis and degradation of triacylglycerols by adipose tissue is hormonally regulated
- Fatty acids are released into the bloodstream in complex with serum albumin
- Serum albumin carries a variety of insoluble molecules, including fatty acids, hormones, drugs

Sites of Regulation of Fatty Acid Metabolism



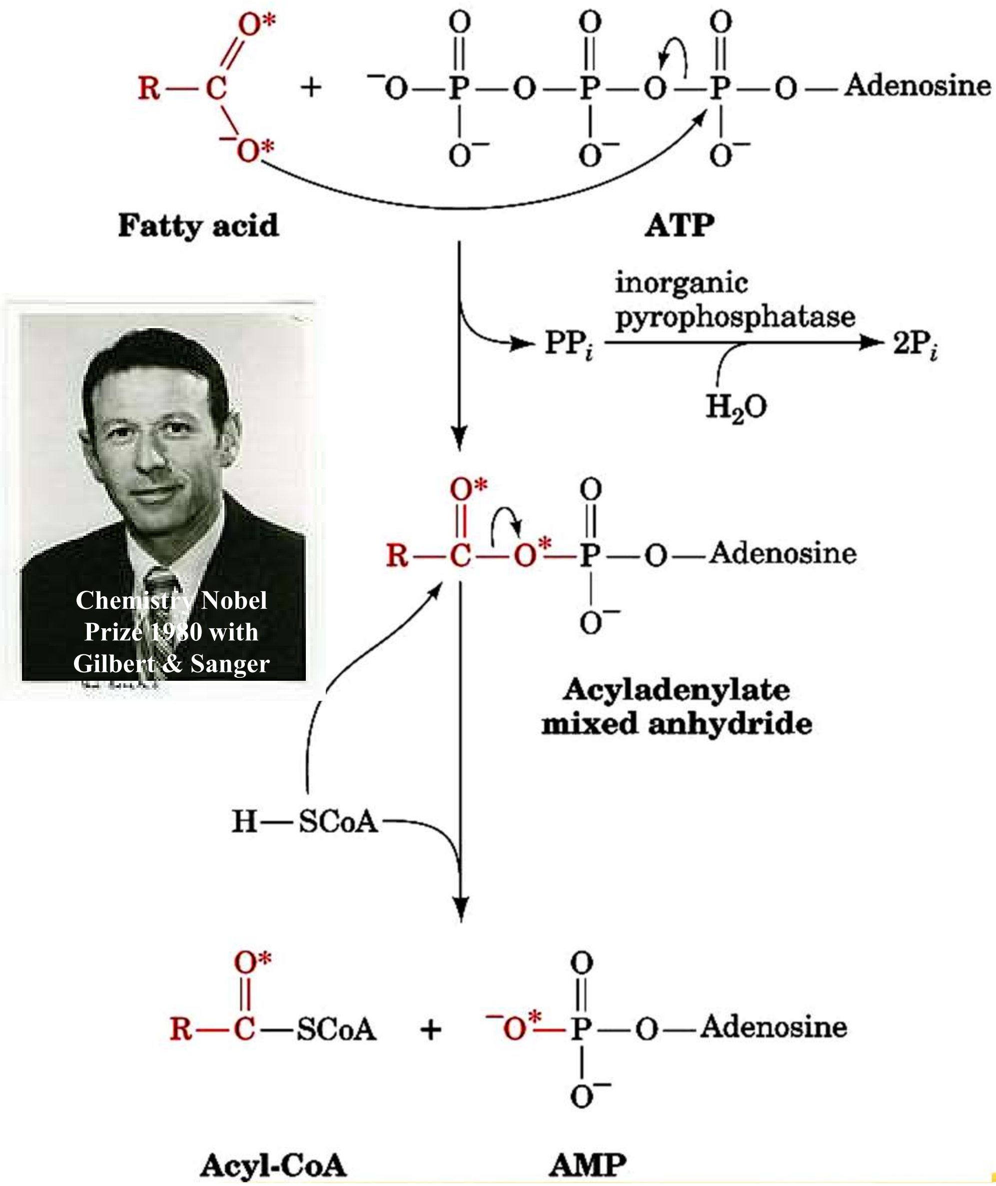
Overview: The utilization of stored triacylglycerols requires 3 processing stages

1) Hormone-sensitive lipase of adipose tissue liberates fatty acids, which are carried in the blood by serum albumin

2) At the consuming tissues, fatty acids are activated and transported into the mitochondrion for degradation

3) In the mitochondrion, fatty acids are broken down in a stepwise fashion to form acetyl~CoA, which is used in the TCA cycle

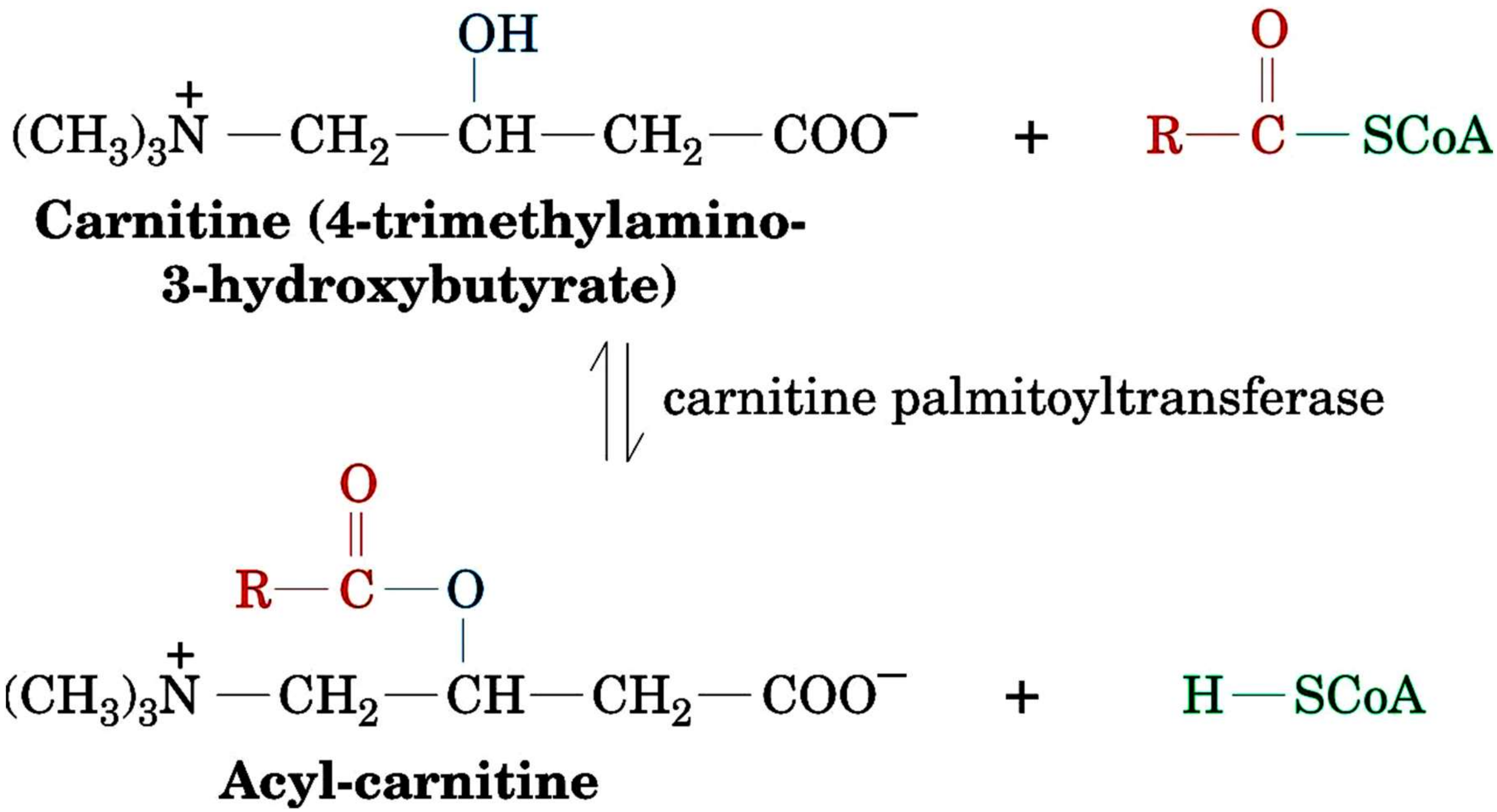
Step 2: Cytosolic Fatty Acid Activation On the ER or Outer Mitochondrial Membrane



- There are at least three different acyl-CoA synthetases in humans that act on fatty acids of different chain lengths
- Reaction is driven forward by hydrolysis of PP_i
- The acyl adenylylate is held tightly by the enzyme
- Mechanism was demonstrated by Paul Berg
- Acyl adenylates are frequently formed when carboxyl groups are activated in biochemical reactions

Fatty acid activation by acyl-CoA synthetase

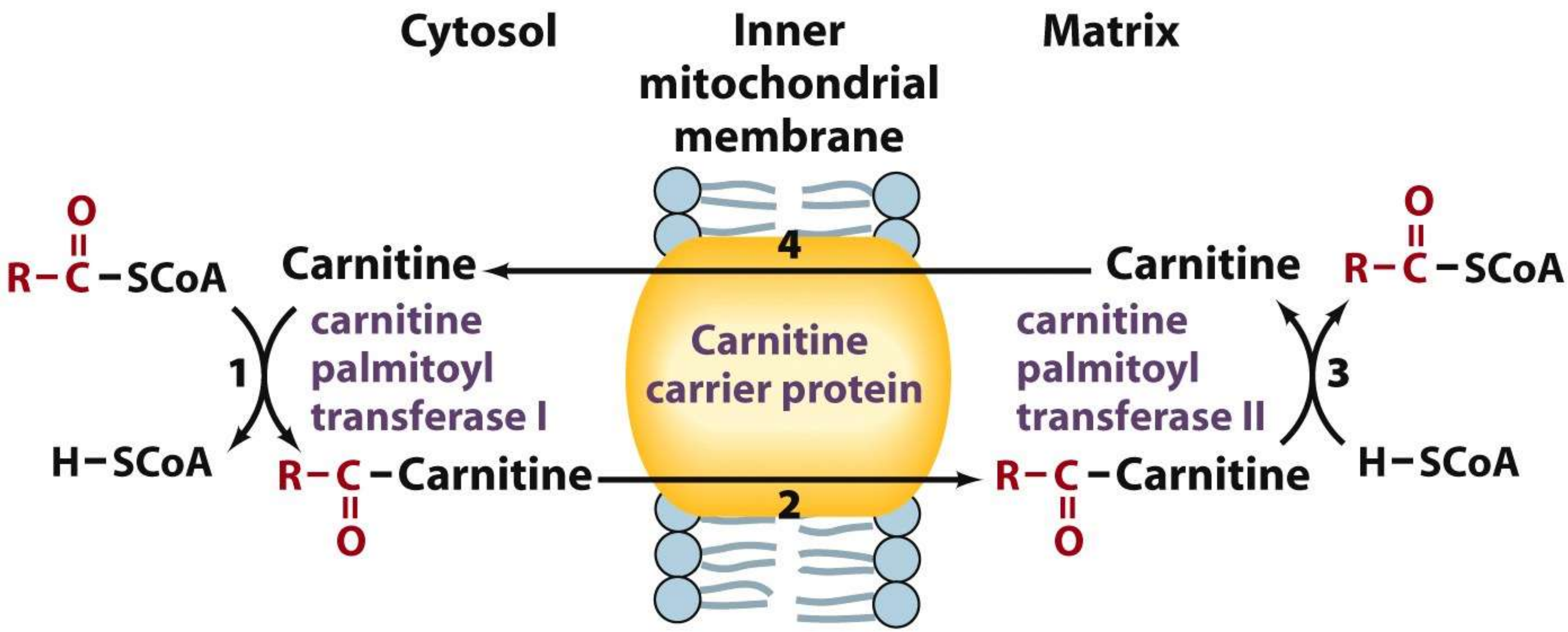
Carnitine Shuttles Long-Chain Activated Fatty Acids Into the Mitochondrial Matrix



Acylation of carnitine catalyzed by carnitine palmitoyltransferase

- **The equilibrium constant for this reaction is about 1. Normally, transfer of acyl group from an alcohol to a sulfhydryl group is thermodynamically unfavorable. Why does the O-acyl group in carnitine have such a high group transfer potential?**
Carnitine and its esters are solvated differently from most other alcohols and their esters because of the zwitterionic nature of carnitine.

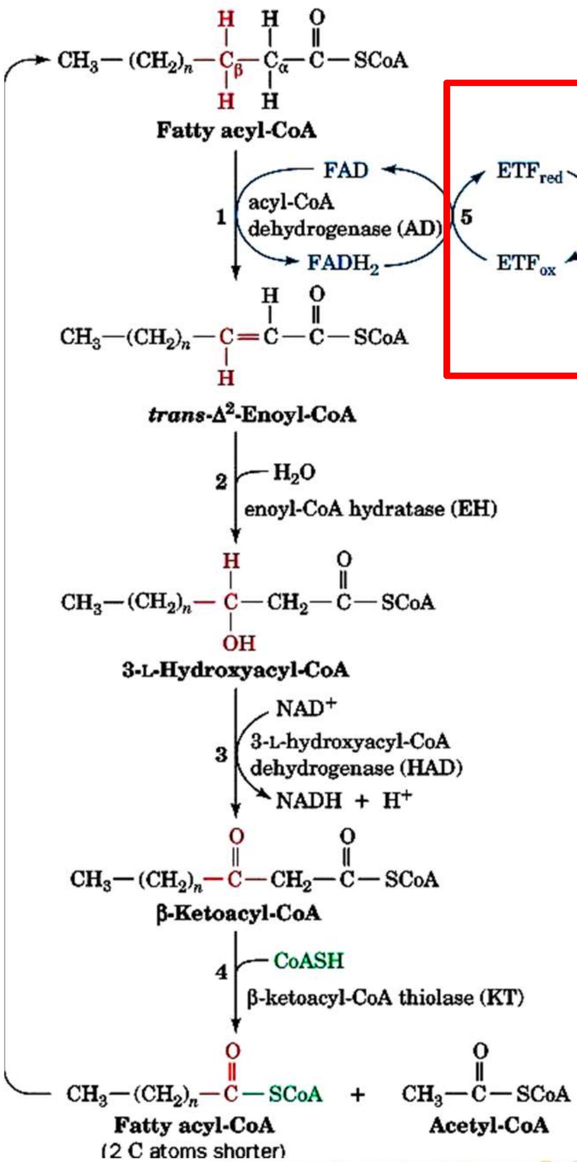
The Cell Maintains Separate Cytosolic and Mitochondrial Pools of CoA

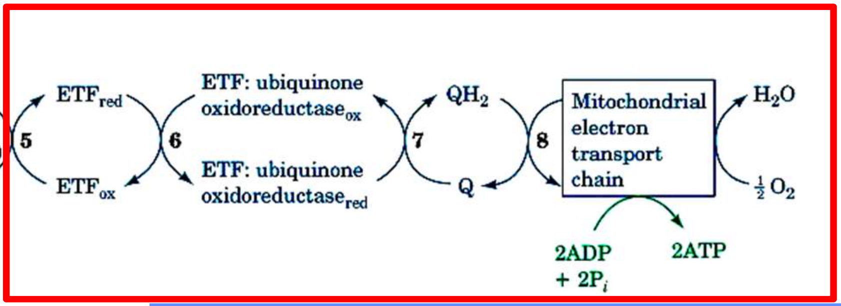


Transport (shuttle) of fatty acids into the mitochondrion

- ➢ Medium chain fatty acids (C_8-C_{10}) do not require carnitine to enter the mitochondrion
- ➢ Diseases of carnitine synthesis, transferase, or translocase (carrier protein) lead to symptoms ranging from muscle cramping to severe weakness and death; muscle, kidney, and heart primarily affected; muscle weakness during prolonged exertion is a key symptom, because body relies on long chain fatty acids for long-term energy

Step 3: One $FADH_2$, NADH, and Acetyl~CoA Are Generated Per Round of β-Oxidation





1. Acyl~CoA dehydrogenase (AD): 1st oxidation
   - 4 different enzymes for C_4-C_6 (short-chain), C_6-C_{10} (medium), ~C_{10}-C_{12} (long), and C_{12}-C_{18} (very long)
   - Deficiency of medium-chain AD may lead to SIDS due to imbalance between glucose and fatty acid oxidation
2. Enoyl hydratase (EH): hydration
3. 3-L-Hydroxyacyl-CoA dehydrogenase (HAD): 2nd oxidation
4. Ketoacyl thiolase (KT): Cleavage of the α,β bond to release acetyl-CoA and shortened acyl-CoA

Electron transfer flavoprotein (ETF) connects AD to mitochondrial electron transport chain (actual yield ~ 1.5 ATP)