MEMBRANE STRUCTURE

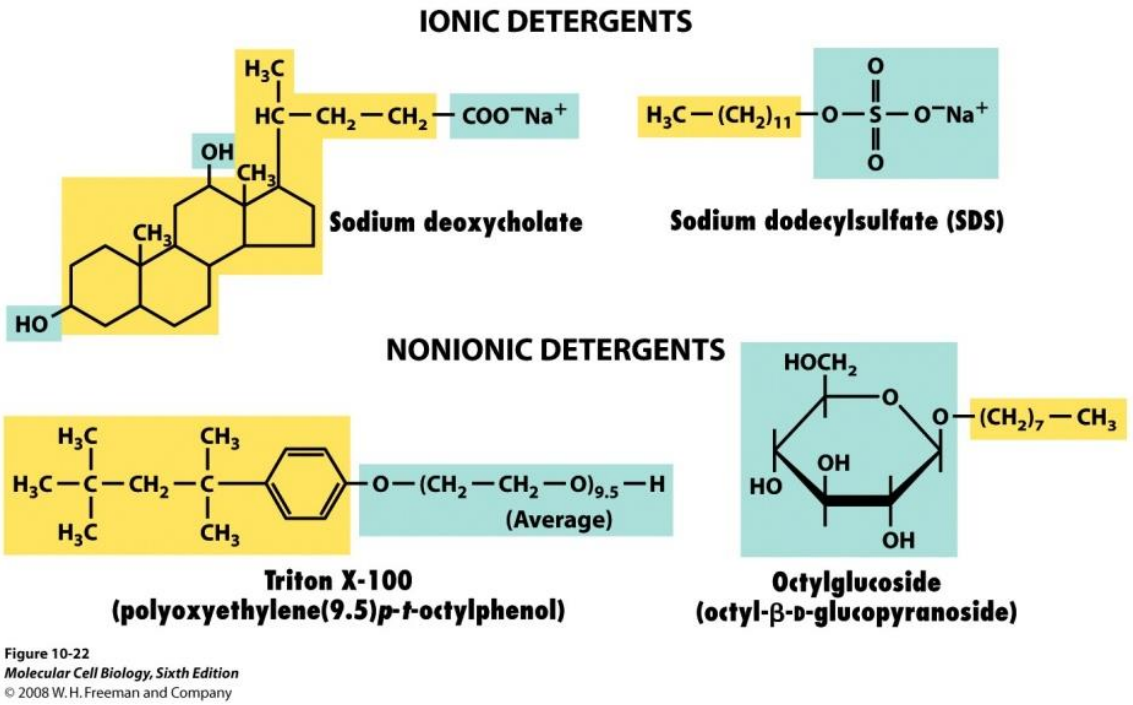
LECTURE 3

Chapter 10

SOLUBILIZATION OF MEMBRANE PROTEINS

Detergents are amphipathic molecules that intercalate into the lipid bilayers and solubilize the membrane proteins and lipids.
The hydrophobic part interacts with the hydrocarbons and the hydrophilic part interacts with water.
Non ionic detergents lack a charged group unlike ionic detergents.
Critical Micelle concentration (CMC) is characteristic for each detergent and depends on its hydrophobic and hydrophilic groups.
Ionic detergents denature proteins but non-ionic detergents do not. Ionic detergents can break ionic and hydrogen bonds because of their charge. Ionic detergents bind to hydrophobic regions of membrane and water-soluble proteins

Hydrophobic – yellow, Hydrophilic - blue



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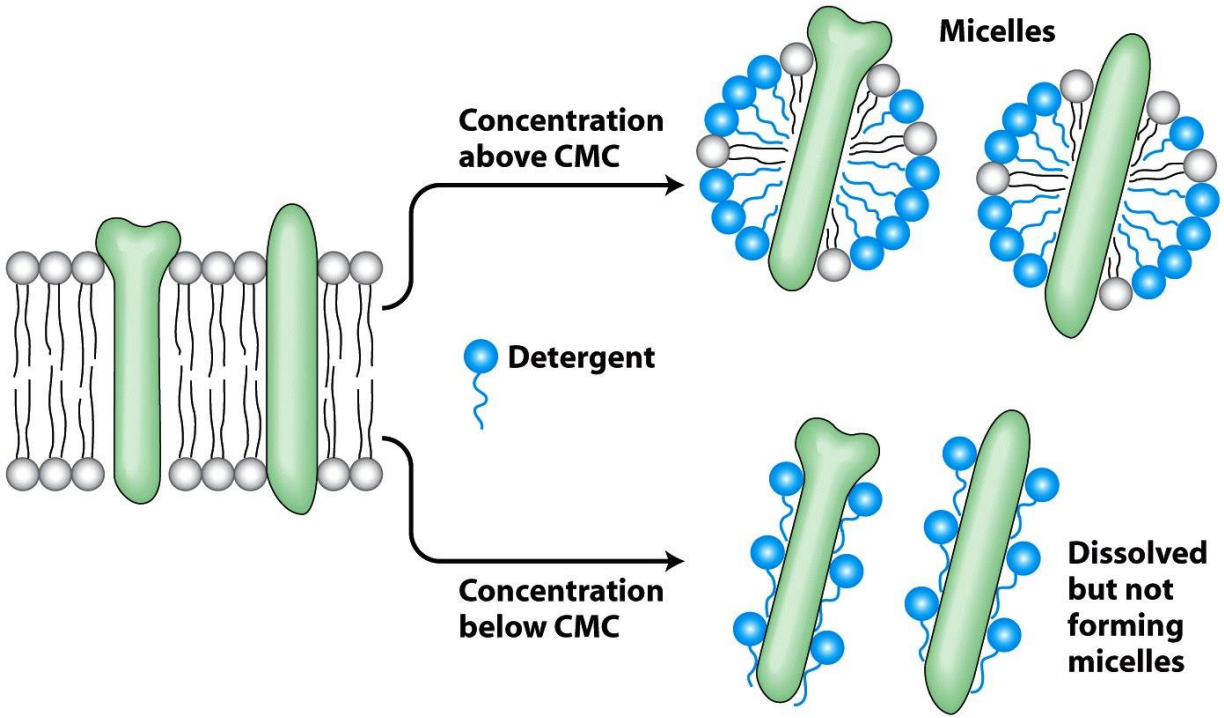
Non ionic detergents are useful in extracting membrane proteins before they are purified.

HOW NONIONIC DETERGENTS SOLUBILIZE MEMBRANE PROTEINS?



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When separated from membranes, the hydrophobic regions of integral membrane proteins are exposed and they tend to interact with one another causing the formation of aggregates and precipitate from solution. Nonionic detergent prevents this aggregation and aids in solubilization of proteins.

How do you remove **Peripheral membrane proteins ?**

Removed from membranes using high salt solutions – these break the ionic bonds

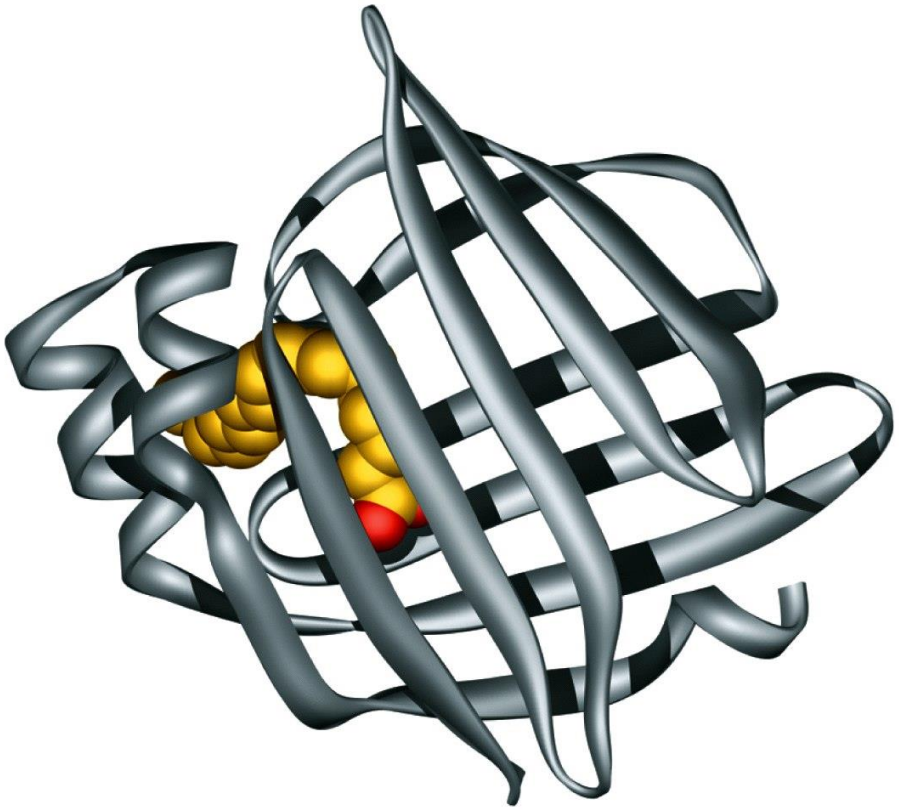
These proteins are soluble in aqueous solutions and need not be solubilized using nonionic detergents

FATTY ACID SYNTHESIS

- 14, 1,6, 18 and 20 carbon fatty acids
- Saturated fatty acids are made from acetyl CoA by two cytosolic enzymes - acetyl CoA carboxylase and Fatty acid synthase
- Palmitoyl CoA can be elongated to C-18 or C-24 in ER or mitochondria
- Desaturase enzymes introduce double bonds – present in ER
- Fatty acyl CoAs' are soluble in aqueous solutions
- Smooth ER synthesizes diacylglycerophospholipids
- Sphingosine and N-acyl sphingosine (ceramide) takes place in the ER
- Addition of a polar head group to ceramide is in the Golgi

- How do fatty acids move around?
  - Bound to Fatty acid binding proteins
  - Fatty acid binding proteins have a Beta – sheet pocket

BINDING OF FATTY ACID TO THE FATTY – ACID-BINDING PROTEIN (FABP)



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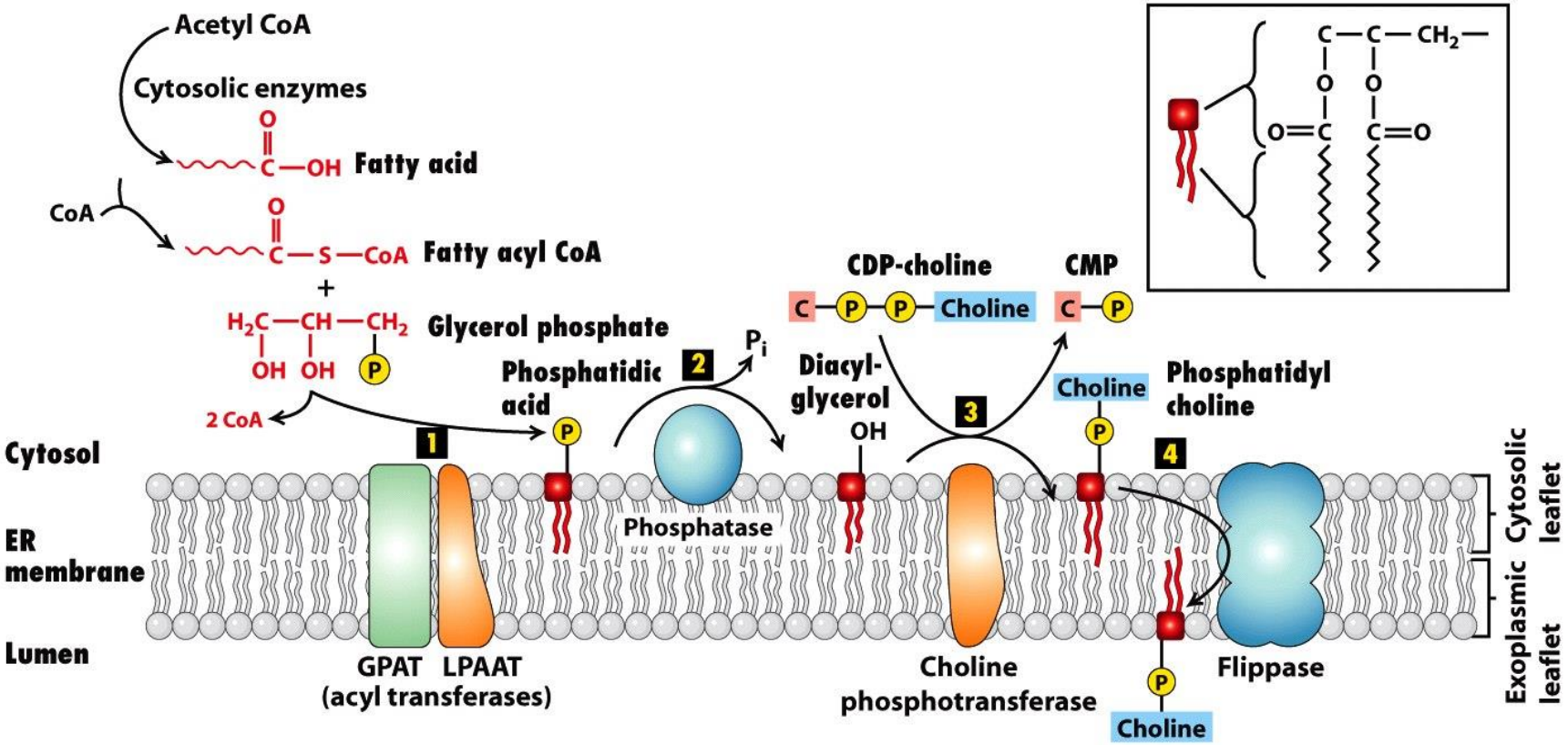
FABP expression is regulated by levels of fatty acid.
FABP levels are high in active muscles and in adipocytes.

PHOSPHOLIPID BIOSYNTHESIS AND FLIPPASES



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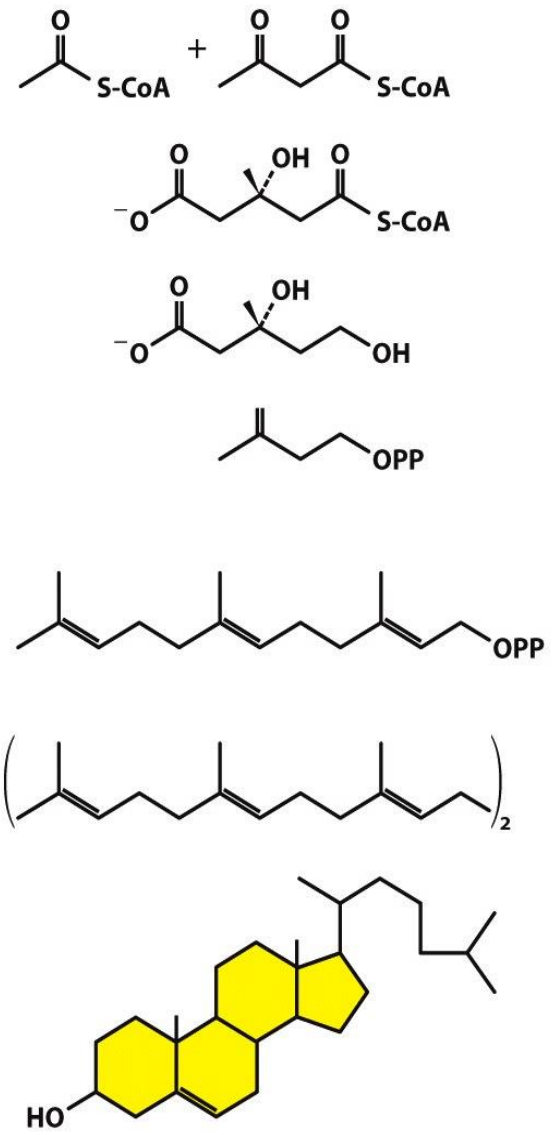
CHOLESTEROL BIOSYNTHESIS



Acetyl CoA + acetoacetyl CoA

↓

HMG-CoA

↓ **HMG-CoA reductase**

Mevalonate

↓

↓

Isopentenyl pyrophosphate (IPP) → Isopentenyl adenosine; Many other isoprenoids

↓

Farnesyl pyrophosphate → Dolichol; Heme; Ubiquinone; Vitamins (A, E, K); Chlorophyll; Lipid-anchored proteins (Ras)

↓

Squalene

↓

Cholesterol → Vitamin D; Bile acids; Steroid hormones; Cholesterol esters; Modified proteins (Hedgehog)

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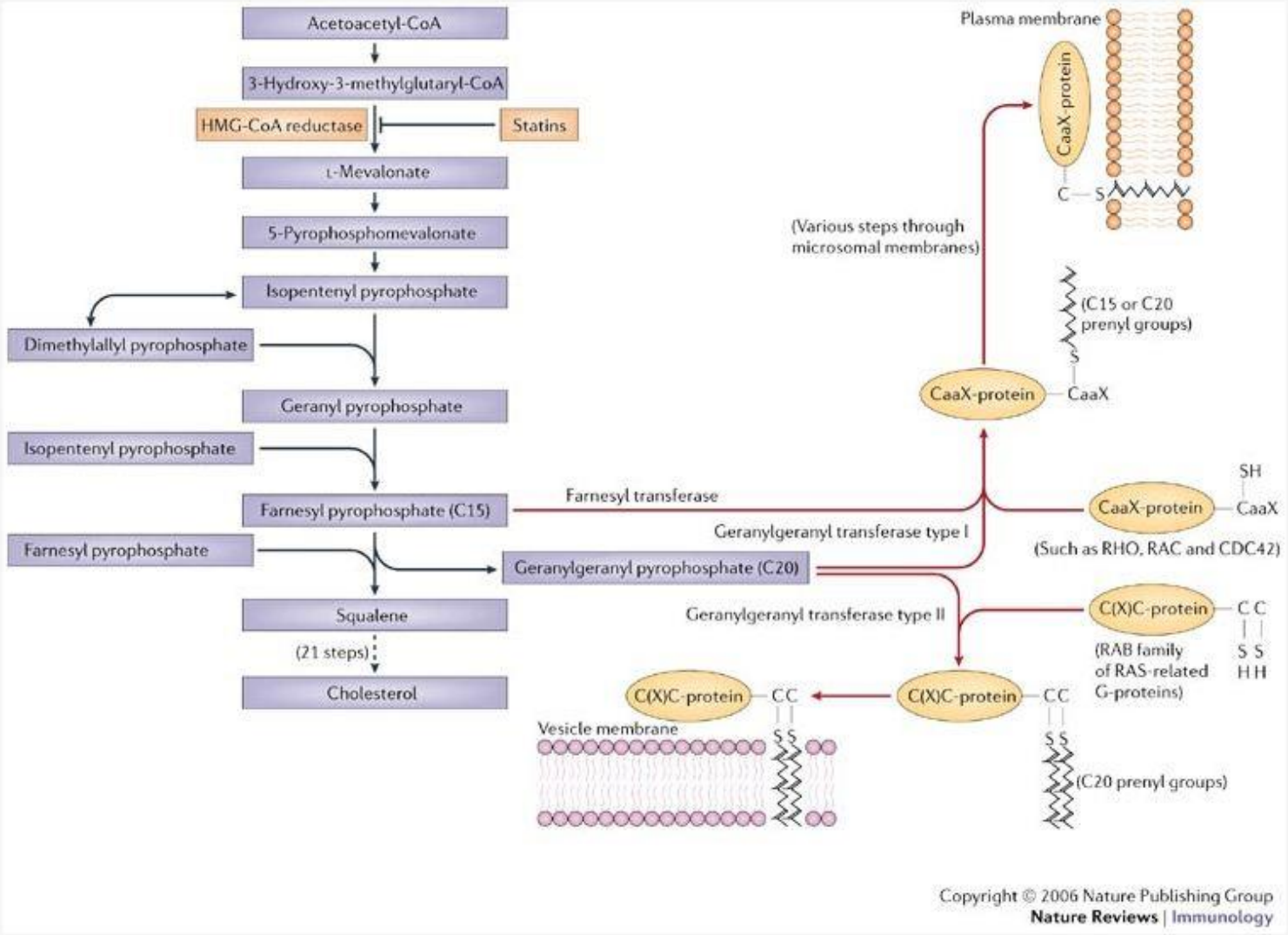
RATE LIMITING ENZYME – HMG CoA reductase

HMG-CoA is synthesized in the cytosol
Rate limiting step is catalyzed by ***HMG-CoA reductase.*** This enzyme is located in the ER membrane. This enzyme has a water-soluble catalytic domain.
It has 8 trans-membrane helices embedded in the membrane. 5 of these form the sterol-sensing domain.

What happens when cholesterol levels are high?

When cholesterol levels are high, cholesterol binds to the sterol sensing domain and this causes the protein to bind to two other membrane proteins – Insig-1 and Insig-2. These induce the enzyme to be ubiquitinated and degraded by the proteasome pathway.

How do STATINS work?

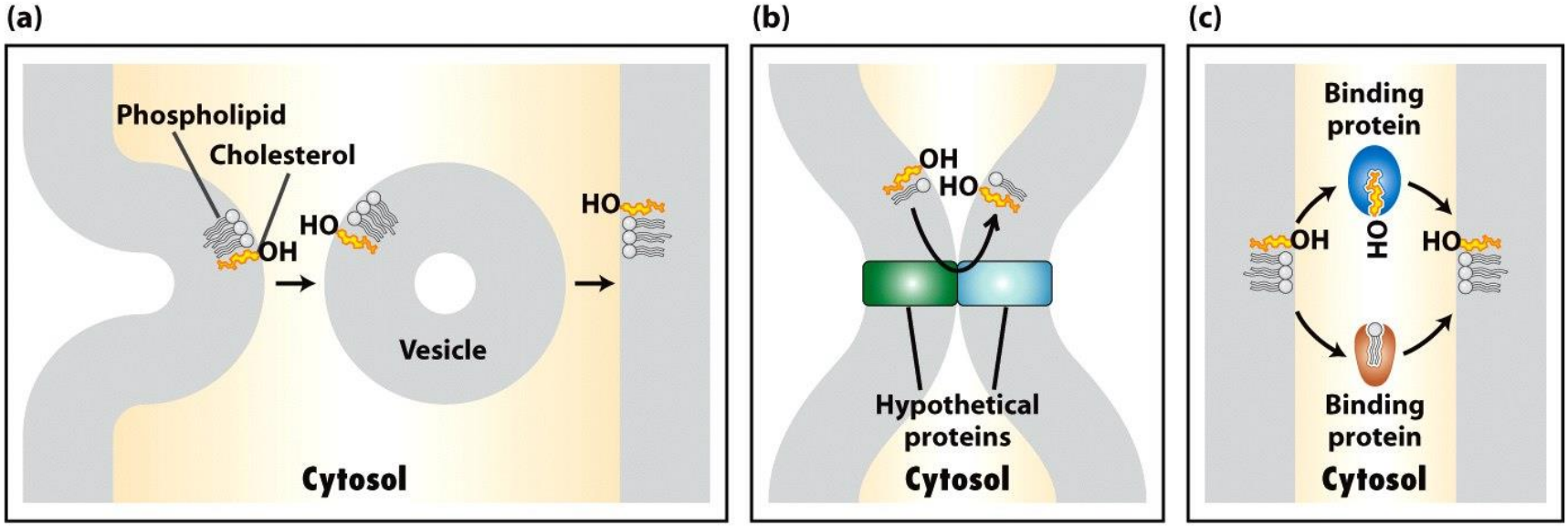


Statins inhibit the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to L-mevalonate through competitive inhibition of the rate-limiting enzyme HMG-CoA reductase. This inhibition results in a decrease in the downstream biosynthesis of cholesterol and other intermediate metabolites, including the isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These isoprenoid pyrophosphates serve as essential adjuncts in the post-translational modification of numerous key proteins that function as molecular switches, including the small GTPases RAS, RAC and RAS homologue (RHO). These proteins

Atherosclerosis

- Deposition of lipids, cholesterol and other extracellular material in the inner wall of arteries
- Distortion or changes in arterial wall structure
- These may lead to clots

MECHANISM OF CHOLESTEROL AND PHOSPHOLIPID TRANSPORT BETWEEN ORGANELLES



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