بسم الله الرحمٰن الرحيم

(وَفَوْقَ كُلِّ ذِي عِلْمٍ عَلِيمٌ)

Metabolism | Final 10

Metabolism GPL pt.2 + Cholesterol metabolism pt.1

Written by : DST , NST

Reviewed by : Tuqa Al-Soud

وَلِلَّهِ الْأَسْمَاءُ الْحُسْنَى فَادْعُوهُ بِهَا

المعنى: الشديد القوي الذي لا تنقطع قوته ولا تلحقه في أفعاله مشقة، ولا يمسه تعب.

الورود: ورد مرة واحدة في القرآن.

الشاهد: ﴿ذُو ٱلْقُوَّةِ ٱلْمَتِينُ﴾ [الذاريات:٥٨].

٥٩

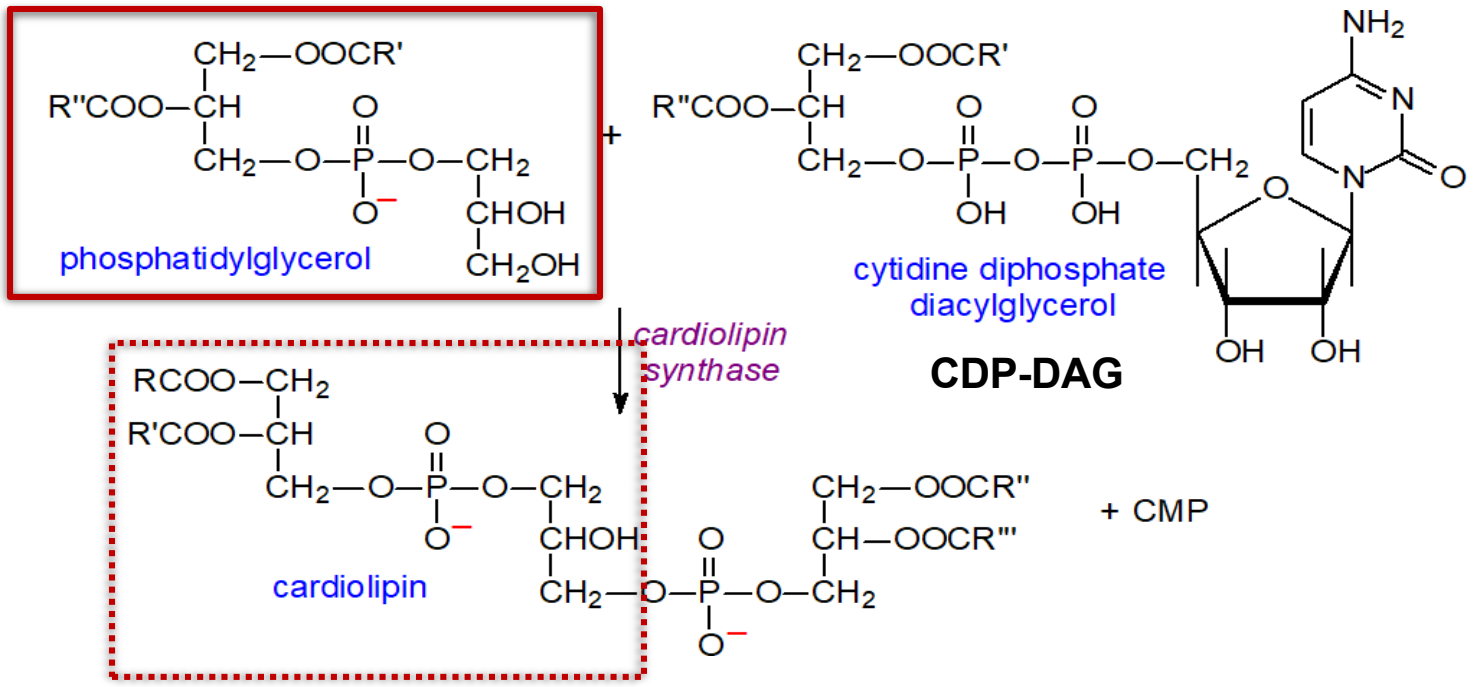
اضغط هنا لشرحٍ أكثر تفصيلًا

تذكير: رجب شهر الزرع؛ تكثر فيه الطاعات وتُمحى الزلات ، فما نزرعه اليوم نحصد أجره لاحقًا.أحسن فيه العمل، وابتعد عن المعصية،وجدّد التوبة قبل أن تُطوى الصحائف

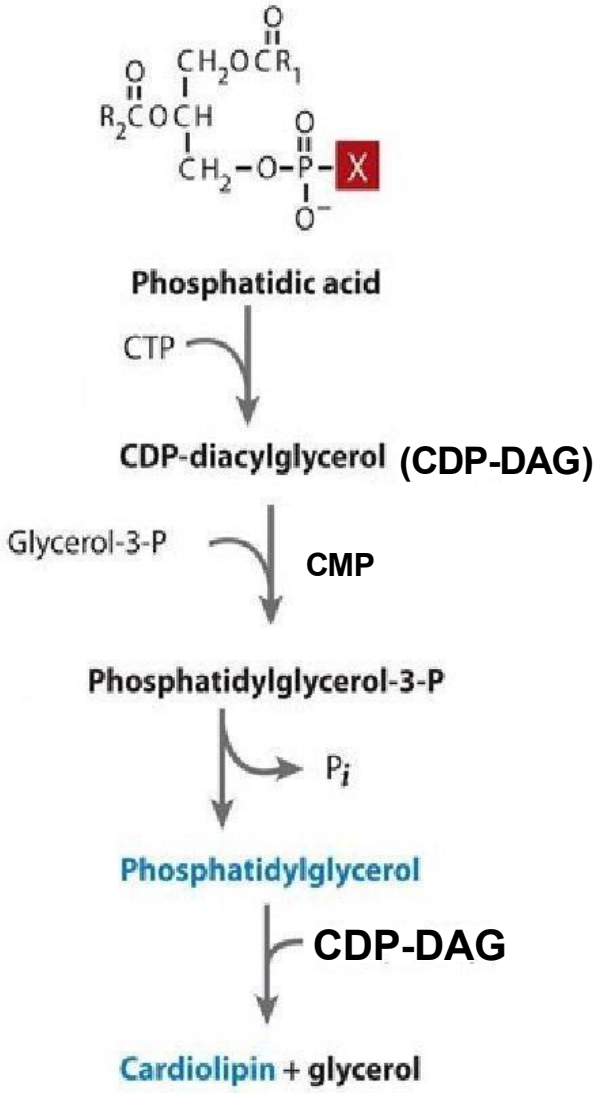
Phosphatidylglycerol and cardiolipin

- Same as PI in mechanism (as both are alcohols), but the only difference is that here glycerol is phosphorylated and then dephosphorylated.

- Phosphatidylglycerol is synthesized from CDP-DAG and glycerol 3-phosphate.
- Cardiolipin is synthesized by the transfer of DAG from CDP-DAG to a pre-existing molecule of phosphatidylglycerol.

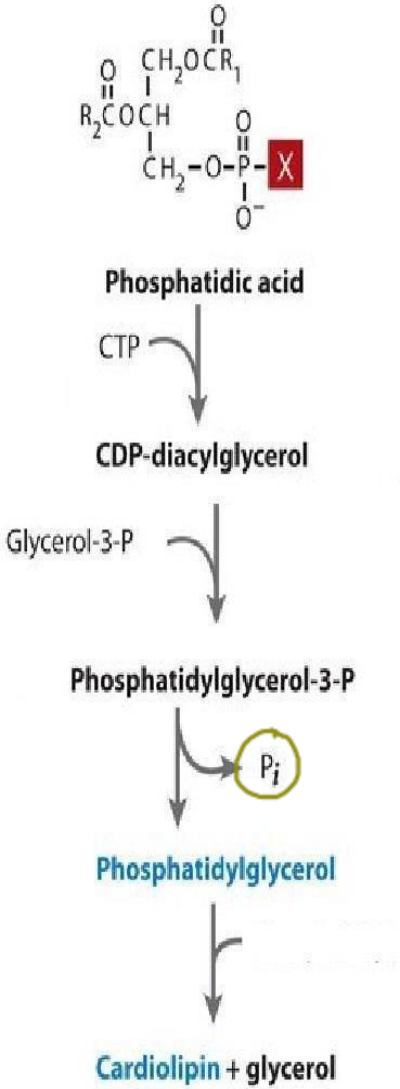


A phosphate group is required to allow the attachment, which then detaches



✓ Cardiolipin synthesis

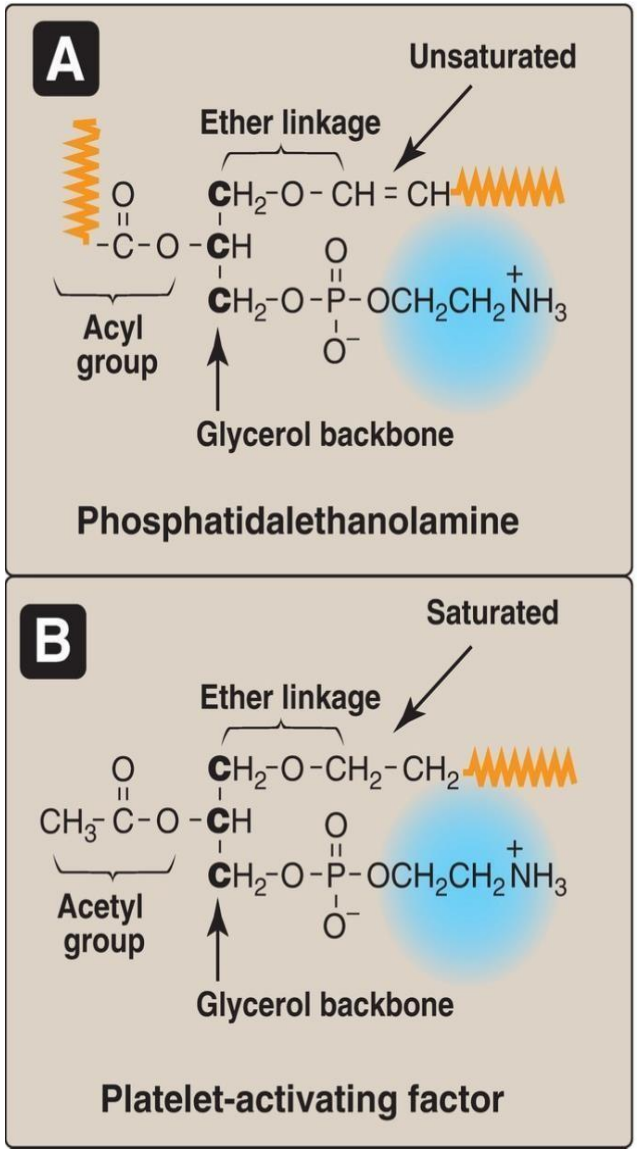
1. Cardiolipin synthesis begins with phosphatidic acid and phosphatidylglycerol as key precursors.
2. Phosphatidic acid is activated and converted to CDP-diacylglycerol, following a mechanism similar to other CDP-diacylglycerol processes.
3. A G3P molecule (glycerol always enters reactions in the form of G3P) is added to CDP-DAG .
4. CDP group is removed and released as CMP, then phosphatidylglecerol 3-phosphate is formed.
5. Phosphatidic acid (we obtain it from CDP-DAG) is then added to phosphatidylglycerol 3-phosphate (remove ,through the action of cardiolipin synthase, linking the molecules together to form cardiolipin (commonly found in the inner mitochondrial membrane).



Recall:
Cardiolipin =2 phosphatidic acids +1glycerol. Phosphatidylglycerol =phosphatidic acid + glycerol. So, what is left is adding a phosphatidic acid to the phosphatidylglycerol

Ether glycerophospholipids Ex. plasmalogens

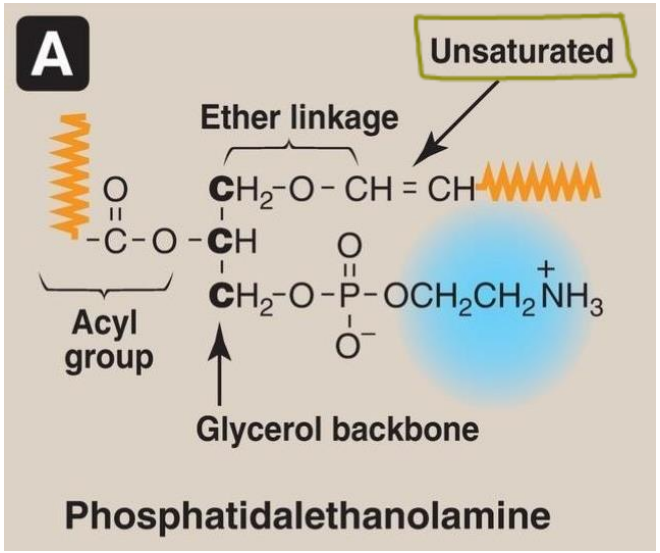
- The FA at carbon 1 is replaced by an unsaturated alkyl group attached by an ether linkage.
- Plasmalogens: PhosphatidAl**ethanolamine** (abundant in **nerve** tissue, is similar in structure to phosphatidylethanolamine.
- PhosphatidAl**choline** (abundant in **heart** muscle) is another significant ether lipid in mammals.
- Platelet-activating factor (**PAF**) has a **saturated** alkyl group in an ether link to carbon 1 and an **acetyl** residue at carbon 2 of the glycerol backbone.
  - Prothrombotic and inflammatory factor



- Differences in PAF:
1. Ethanolamine head group
2. FA on C2 is an Acetyl group (very short)
3. Ether linkage is present but the hydrocarbon chain is saturated.

Ether glycerophospholipids

- **Plasmalogens** are a type of phospholipid with a structure similar to regular phospholipids but with a key difference:
- They have an **ether linkage** at the ***first*** position of the glycerol backbone instead of an ester linkage , so it's not a fatty acid .
- The second carbon of glycerol backbone is connected by an ester bond to a fatty acid forming a tail .
- The third carbon is connected to phosphate and a head group (like ethanolamine , choline ,etc)

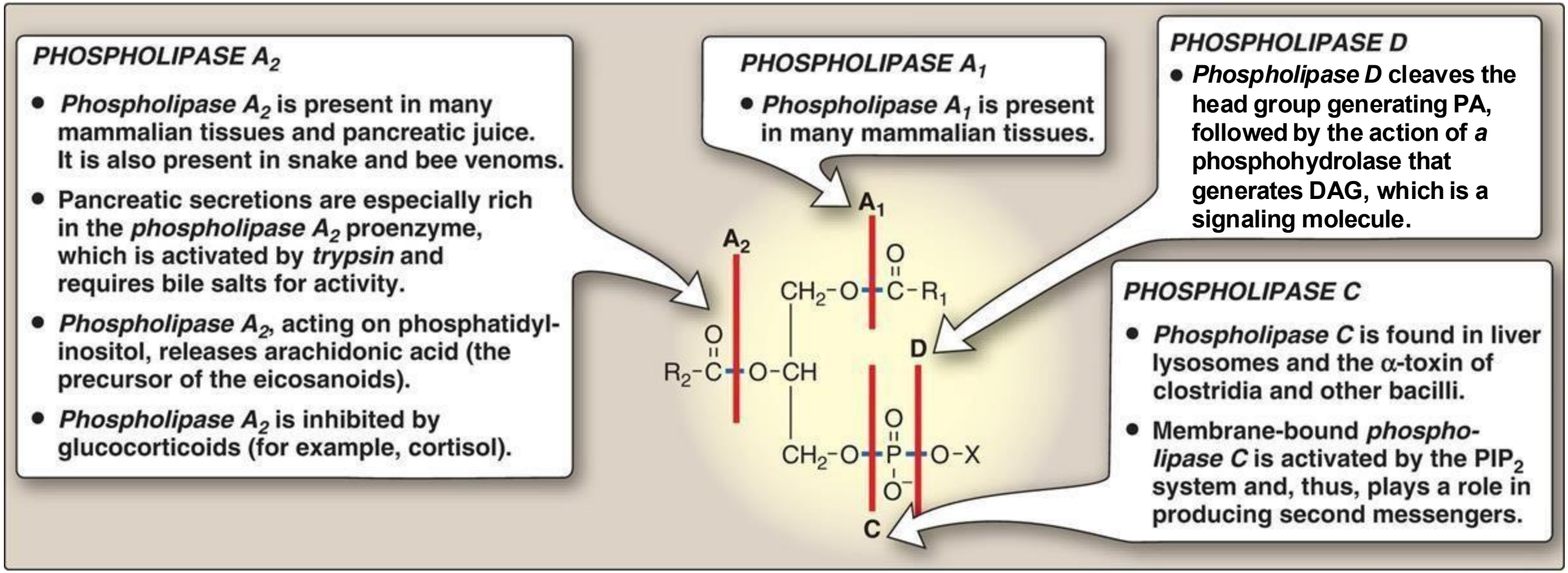


- Once an ether linkage is formed, its name changes from phosphatidylethanolamine to phosphatidalethanolamine (if the head group is ethanolamine) , that's what distinguishes plasmalogens .
- Plasmalogens serve as specialized forms of phospholipids with distinct structural features.

﴿ أَلَا بِذِكْرِ اللَّهِ تَطْمَئِنُّ الْقُلُوبُ ﴾

Degradation of Phospholipids

- The degradation process varies depending on the site of cleavage, not the head group.



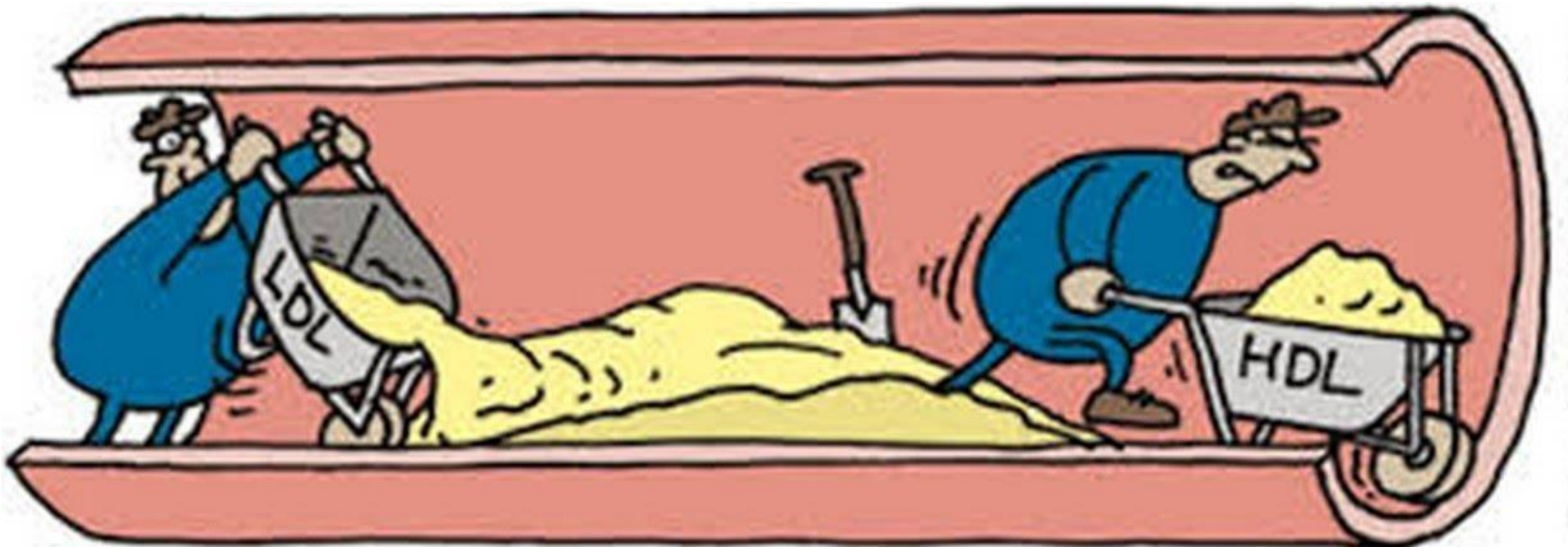
Degradation of Phospholipids

- Phospholipid degradation involves various enzymes, primarily **phospholipases**, which break down phospholipids in different ways depending on the purpose.
- The main types of phospholipases and their functions are:

1. ***Phospholipase A***:
   - Phospholipase **A1**: Removes the fatty acid attached to carbon **1** of the glycerol backbone.
   - Phospholipase **A2**: Removes the fatty acid attached to carbon **2**.
   - ✓ This involves the **hydrolysis of the ester** bond between fatty acids and glycerol.
   - ✓ It is important in the process of extracting arachidonic acid from its sources (we will learn this later)
   - ✓ It can be inhibited by cortisone (anti-inflammatory agent), whether it is naturally present in the body or administered externally, thereby preventing the synthesis of arachidonic acid.
2. ***Phospholipase C***: Removes the head group along with the phosphate group, leaving diacylglycerol (DAG).
   - ✓ This enzyme plays a key role in GPCR signaling pathways, as DAG is an important signaling molecule (activates protein kinase C).
3. ***Phospholipase D***: Removes only the head group, leaving behind phosphatidic acid, which can serve as a precursor for diacylglycerol.

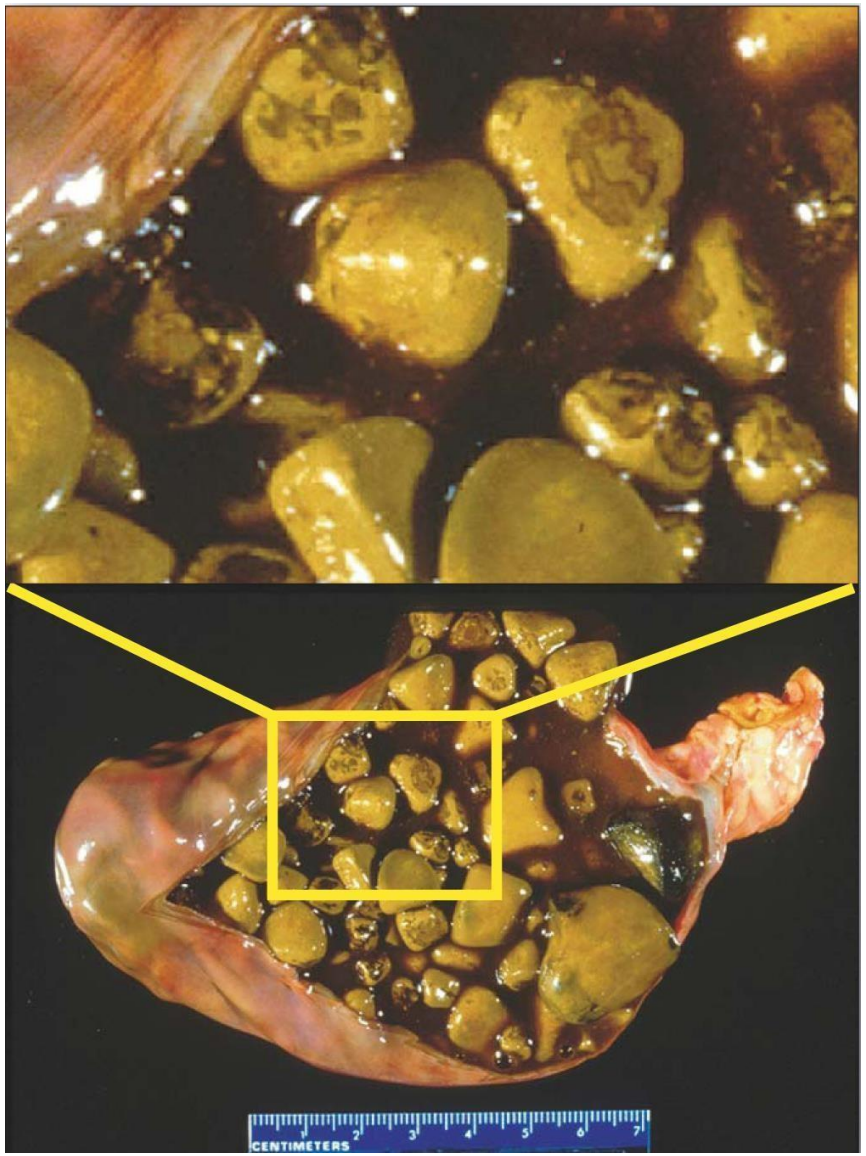
Summary

- ✓ Glycerophospholipids = glycerol + 2 FAs + phosphate group + head group.
- ✓ Different head groups result in distinct functions of different molecules.
- ✓ GPI anchoring of membrane proteins provides them with flexibility of movement through the membrane.
- ✓ PIP2 is cleaved by phospholipase C downstream in the GPCR pathway, producing DAG and IP3, which eventually activate protein kinase C.
- ✓ Surfactants reduce surface tension in the walls of the alveoli, preventing their collapse and easing our breathing.
- ✓ Phosphatidic acid, the parent GP, is synthesized from G3P by adding two FAs (-phosphate = G3P).
- ✓ PC and PE are produced by the addition of their head groups (carried by CDP) to DAG.
- ✓ PC, PE, and PS can be produced from each other through various reactions (methylation, decarboxylation, and transfer of head groups).
- ✓ PG and PI are produced by the addition of their head groups to DAG that is bound to CDP.
- ✓ Cardiolipin is produced by the addition of phosphatidic acid (carried by CDP) to PG.
- ✓ Ether glycerophospholipids (plasmalogens and platelet-activating factor) are similar to GP but have an ether bond at C1 of glycerol instead of an ester bond.
- ✓ Phospholipids are degraded by phospholipases, which have different types and produce different products.
- ✓ Phospholipase A removes FAs, phospholipase C removes the head group alongside the phosphate leaving DAG, and phospholipase D removes only the head group leaving phosphatidic acid.



Cholesterol Metabolism

Dr. Diala Abu-Hassan



Cholesterol was isolated from gall bladder stones in 1774

Why is it called cholesterol?

Chole ster ol

Chole: Bile

Stereos: solid

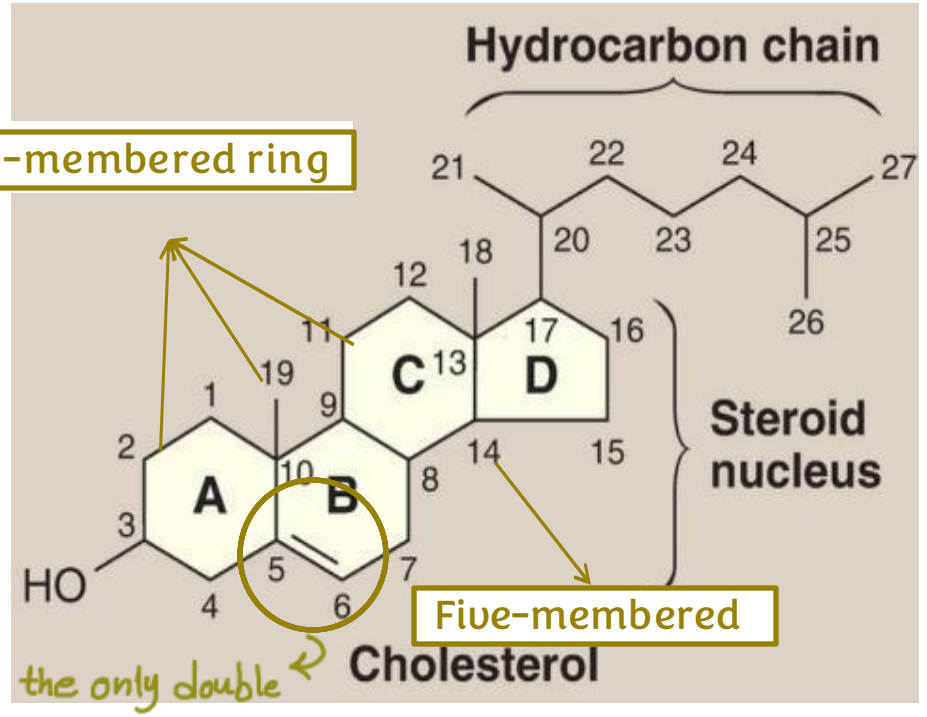
Cholesterols have a rigid solid structure due to the presence of steroid nucleus

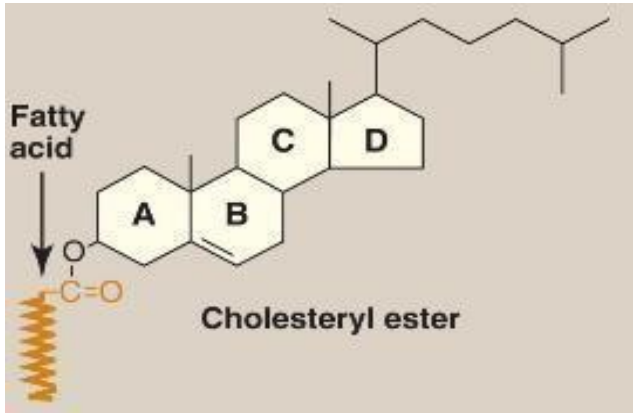
ol: alcohol

Cholesterol has an –OH group on carbon 3 & is the only functional group in a cholesterol molecule

Structure of cholesterol

- Cholesterol (steroid molecule) is a very hydrophobic compound.
  - ➢ It has an effect on membrane fluidity.
- It is a <u>27-carbon</u> molecule that consists of:
  - Four fused hydrocarbon rings (A–D) of 17 carbons called the steroid nucleus
  - Two methyl groups (C18 and 19)
  - Eight-carbon, branched hydrocarbon chain attached to carbon 17 of the D ring.
    - Ring A has a hydroxyl group (the only functional group) at carbon 3.(Add some hydrophilicity to the molecule)
    - Ring B has a double bond between C5 and c6.
- Most plasma cholesterol is esterified with a fatty acid attached at carbon 3.
  - ➢ Sometimes cholesterol will be esterified through its –OH group to a fatty acid forming cholesteryl ester , this reaction makes the structure more hydrophobic.





Sources and Elimination of Cholesterol

→ Synthesized
→ Diet

✓ Synthesis: ≈ 1000 mg → Mainly in hepatocytes , it is used in the synthesis of vitamin D , LDL , VLDL .

- **Liver**, Small Intestine, Adrenal Cortex …

✓ Dietary: ≈ 300 mg → Variable between individuals based on diet consumed.

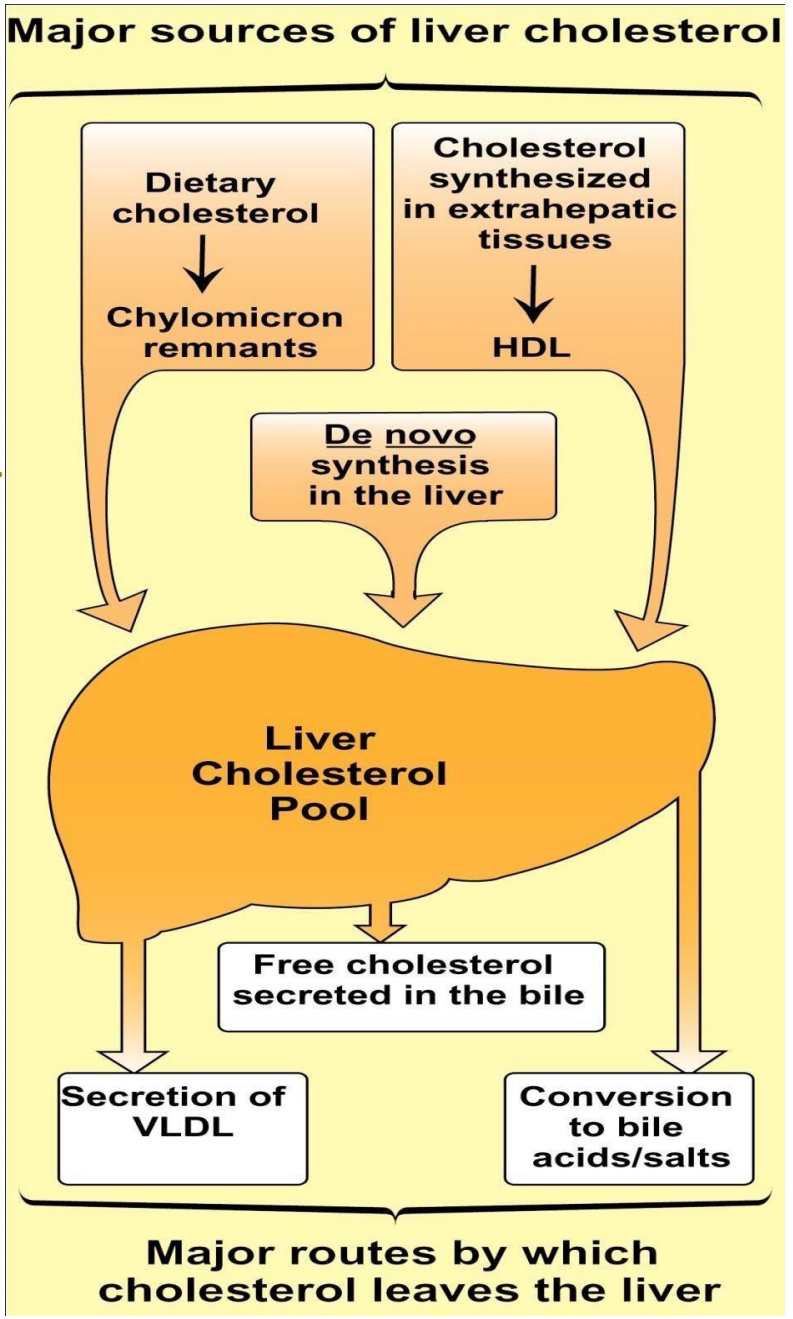
- The 1gram produced per day is enough for our needs (800-1000 mg) to produce different products.
- Cholesterol synthesis happens in other regions (extra hepatic tissue) like ***small intestine ,***
  adrenal cortex and ***glands that produce sex hormones***
- In the adrenal cortex, cholesterol is utilized to synthesize aldosterone, cortisol hormone, etc.

✓ Elimination: Via the Bile

- Cholesterol, Bile Salts

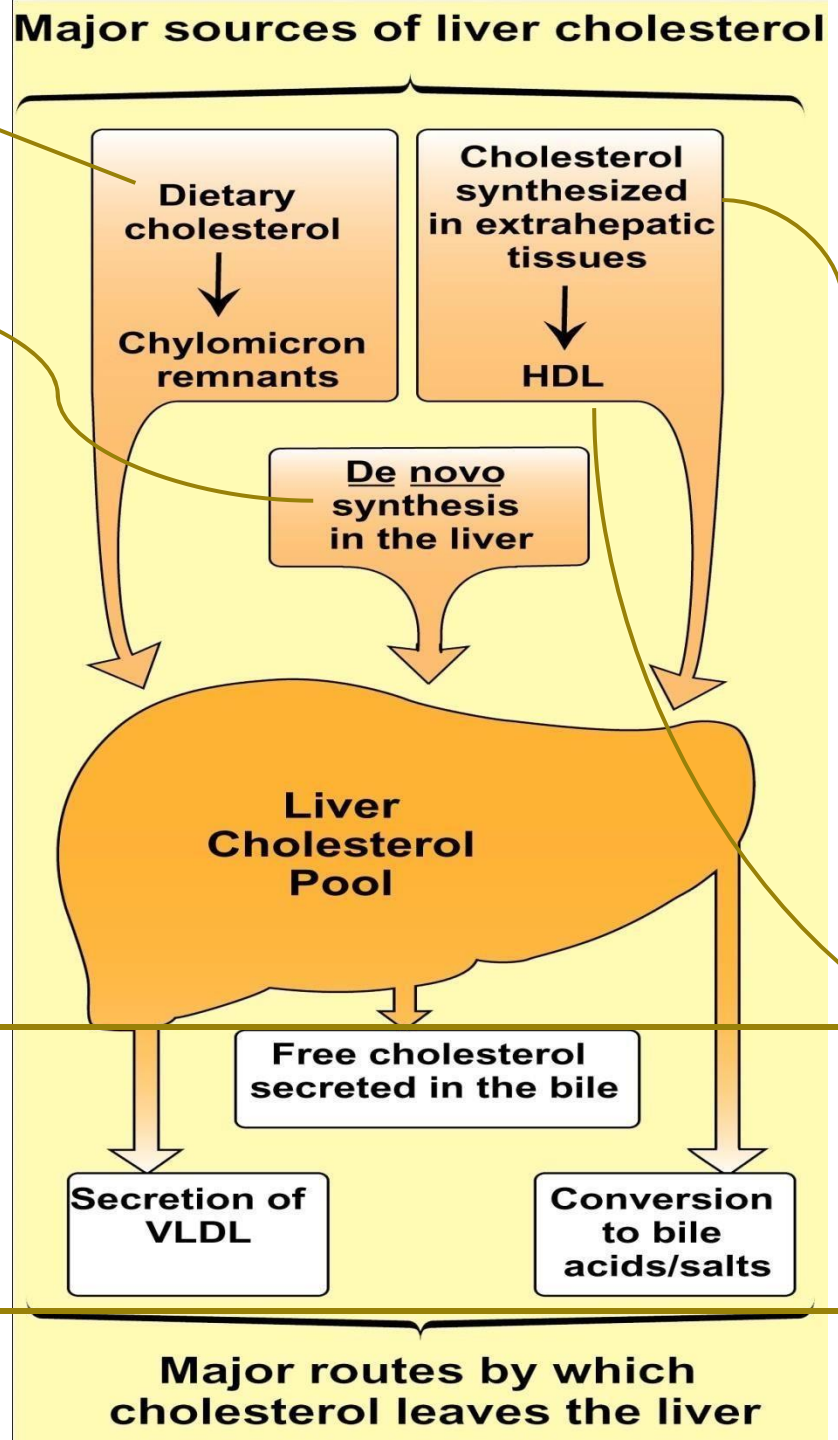
- **Complete elimination via bile secretions**: Cholesterol is secreted into bile, which is an intestinal secretion. A significant portion of the bile undergoes enterohepatic circulation (reabsorption), while a smaller portion is excreted in the feces (5%). This is the primary route of cholesterol elimination. This strategy was used by scientists searching for drug target ; by increasing the amount of cholesterol excreted in the feces, more cholesterol is consumed to compensate for the loss, thereby lowering cholesterol levels in patients with high cholesterol.

Explanation in the next slide …



As previously discussed

Synthesis of cholesterol in hepatocytes is from ***scratch*** (De novo synthesis).

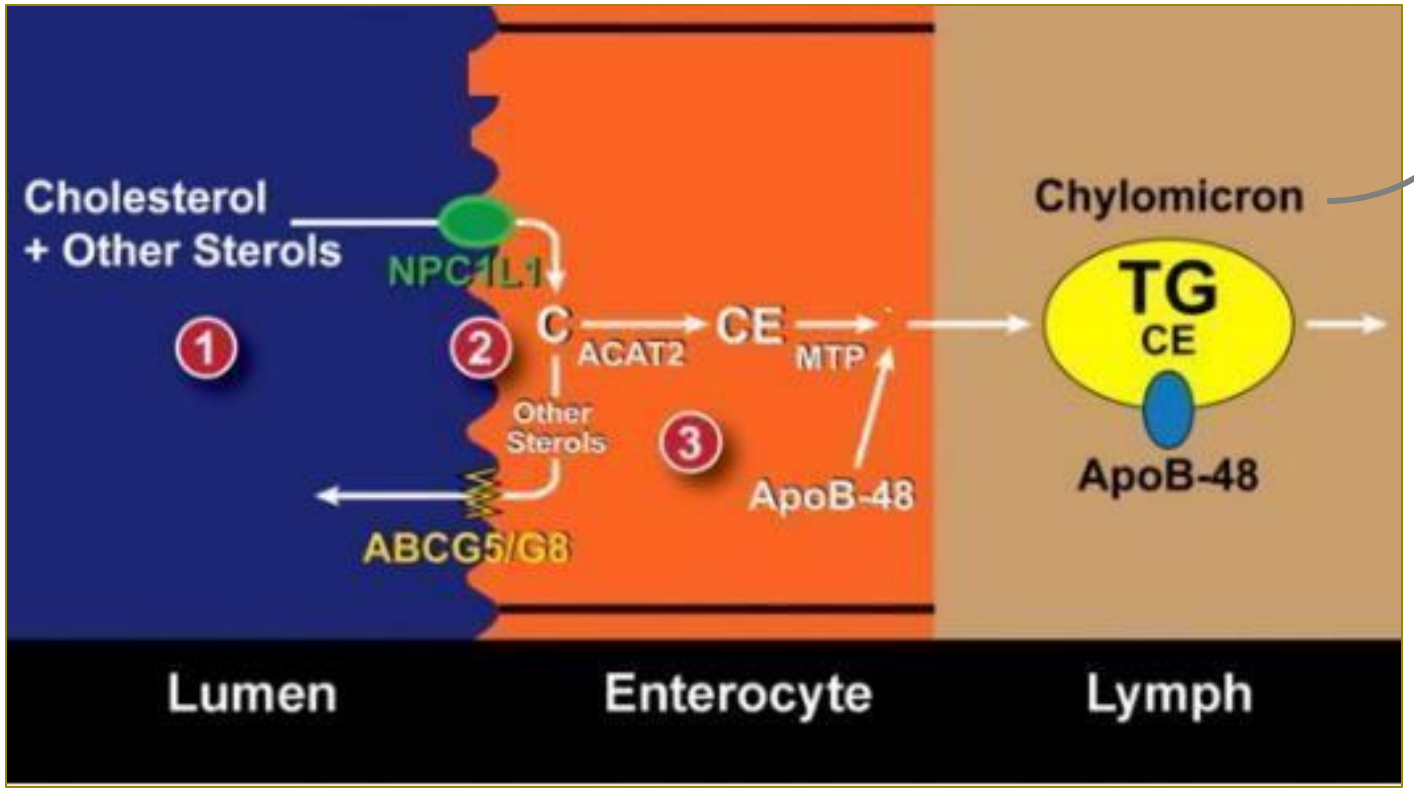


In extra hepatic tissue, it is most likely cycling, then using cholesterol to produce hormones or packaging cholesterol molecules in HDL.

HDL returns cholesterol molecules to sites of consumption most importantly the liver for synthesis of bile acids & salts as well as vitamin D.

Intestinal absorption of cholesterol

Cholesterol with phytosterols enter brush border through NPC1L1 (one way) to intestinal cells where cholesterol can be esterified to fatty acids forming cholesteryl ester (CE) then packaged to chylomicrons into the lymphatic system.



Majority of lipids in chylomicrons are TAG rather than cholesterol & cholesteryl ester – present in small amounts–

Cholesterol absorption depends on many factors:

- **Fiber** presence (lowers cholesterol absorption)
- **Plant sterols** –cholesterol like molecules present in plants– are absorbed via NPC1L1 then re-excreted into the intestinal lumen by ABCG5/8 transporter.

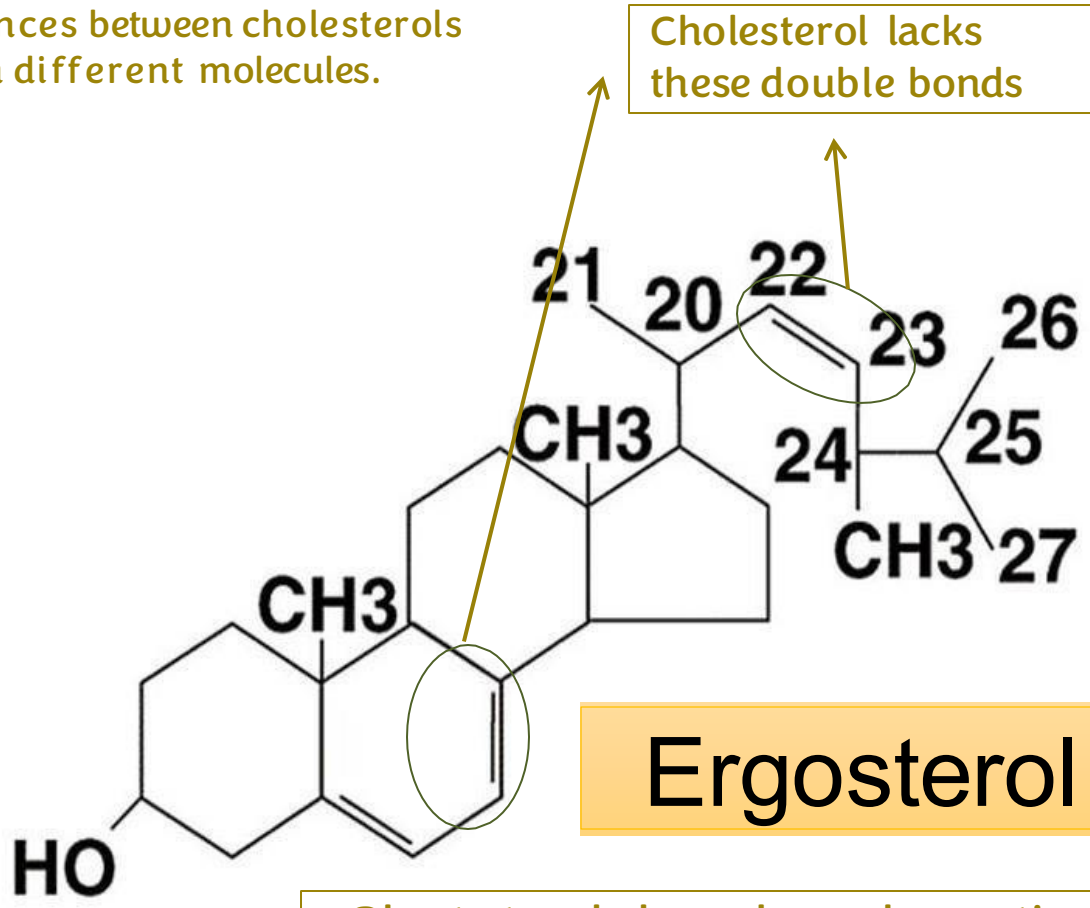
- Intestinal uptake of cholesterol is mediated by the Niemann-Pick C1-like 1 protein, the target of ezetimibe, and pumped out by ABCG5/8 (it excretes phytosterols without cholesterol)

- Defects in the efflux transporter (ABCG5/8) (accumulation of phytosterols and cholesterol) result in the rare condition of sitosterolemia increasing the risk of MI (myocardial infarction)

Plant sterols

There are some minor differences between cholesterols &phytosterols that make them different molecules.

- Plants manufacture phytosterols (substances chemically similar to cholesterol produced within plants
- They compete with cholesterol for absorption in the intestinal tract, thus potentially reducing cholesterol absorption
- Plant sterols (phytosterols) are poorly absorbed by humans (5% vs. 40% for cholesterol) and are actively transported back into the intestinal lumen.
  - Plant sterols reduce the absorption of dietary cholesterol.
    - A dietary strategies to reduce plasma cholesterol levels (an important protective mechanism).



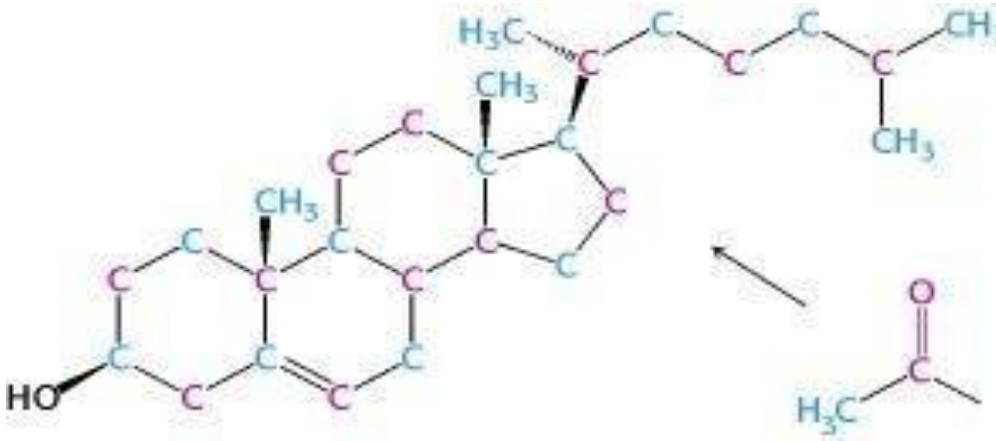
Plant sterols have low absorption ,enter then re-exit. Protect individuals from increases in cholesterol levels-hinders cholesterol absorption. Therefore , it's presence is considered beneficial

Cholesterol Synthesis Requires

- Anabolic -biosynthetic- pathway that happens in well-fed state that requires energy

- **Carbon source**: All the carbon atoms in cholesterol are provided by acetyl coenzyme A (CoA).
- **Reducing power**: NADPH is the reducing agent.
- **Energy**: The pathway is endergonic, and energy is provided by the hydrolysis of
  - The thioester bond of acetyl CoA (Cleavage of thioester bond gives off energy)
  - ATP
- O_2 ->especially in the last steps of synthesis.

- Synthesis requires enzymes in the cytosol, the membrane of the smooth endoplasmic reticulum (SER), and the peroxisomes.
- The pathway is regulated to balance the rate of cholesterol synthesis/excretion.



Generally, biosynthetic pathways happen in **cytosol**; Oxidation and degradation happens in mitochondria.

Synthesis of cholesterol starts in cytosol > ER membrane (SER) > peroxisome > smooth endoplasmic reticulum (SER) where final product is formed.

Stages in Cholesterol Synthesis

Acetyl CoA (C2)

↓

Mevalonate (C6)

↓

Isoprene Units (C5)

↓

Squalene (C30)

↓

Lanosterol (C30)

↓

Cholesterol (C27)

We will use 3 acetyl CoA molecules to form HMG-CoA then mevalonate.

Remove one carbon from mevalonate by decarboxylation to form isoprene unit.

We form a lot of isoprene units by repeating the process 6 times to get 6 isoprene units (6*5=30 carbons).

Binding the isoprene units in different reactions to form squalene -a linear structure with no cycles.

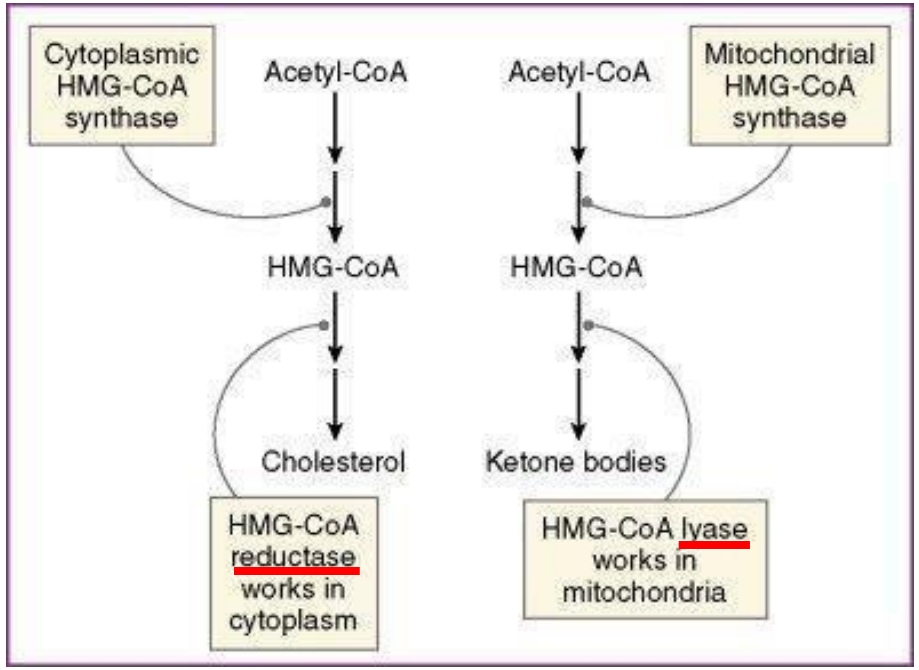
Cyclization and addition of -OH functional group occurs. Lanosterol is the first cyclic compound.

Modifying lanosterol gives the final product cholesterol.

Isoprene unit is a hydrocarbon chain , It is found in lipid-soluble vitamins, specifically vitamin K. Depending on the number of repeats, it forms different types of the vitamin.

Cholesterol synthesis, the first reactions in the cytosol…

- Similar to the synthesis of ketone bodies.
- Liver parenchymal cells contain two isoenzymes of the HMG CoA synthase.
  - A **cytosolic** enzyme for **cholesterol synthesis**.
  - A mitochondrial enzyme for ketone body synthesis.

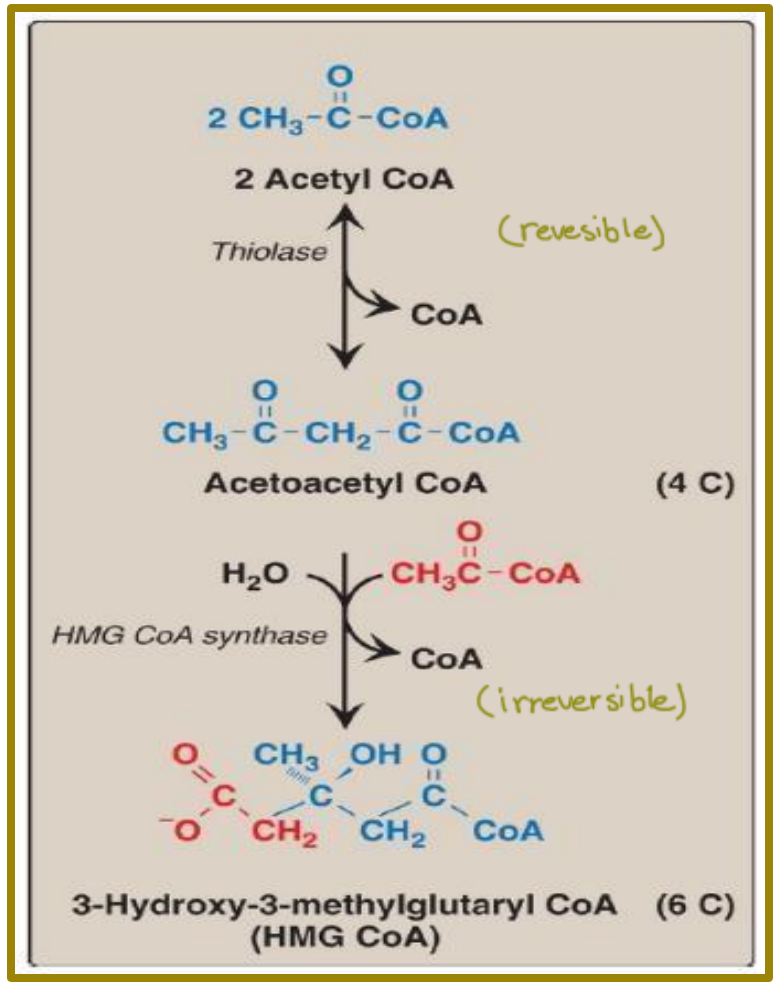


HMG-CoA in the cytosol > cholesterol synthesis
HMG-CoA in the mitochondria > ketogenesis

Same steps as ketogenesis, but with cytosolic enzymes of hepatocytes rather than mitochondrial enzymes.

Remember, in ketogenesis you continue the process by HMG-CoA lyase to produce ketone bodies.

Don't forget to study this figure :)

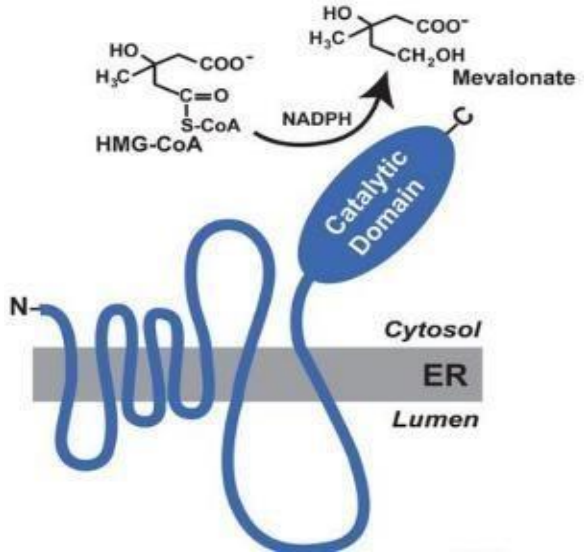


Cholesterol synthesis , synthesis of mevalonate

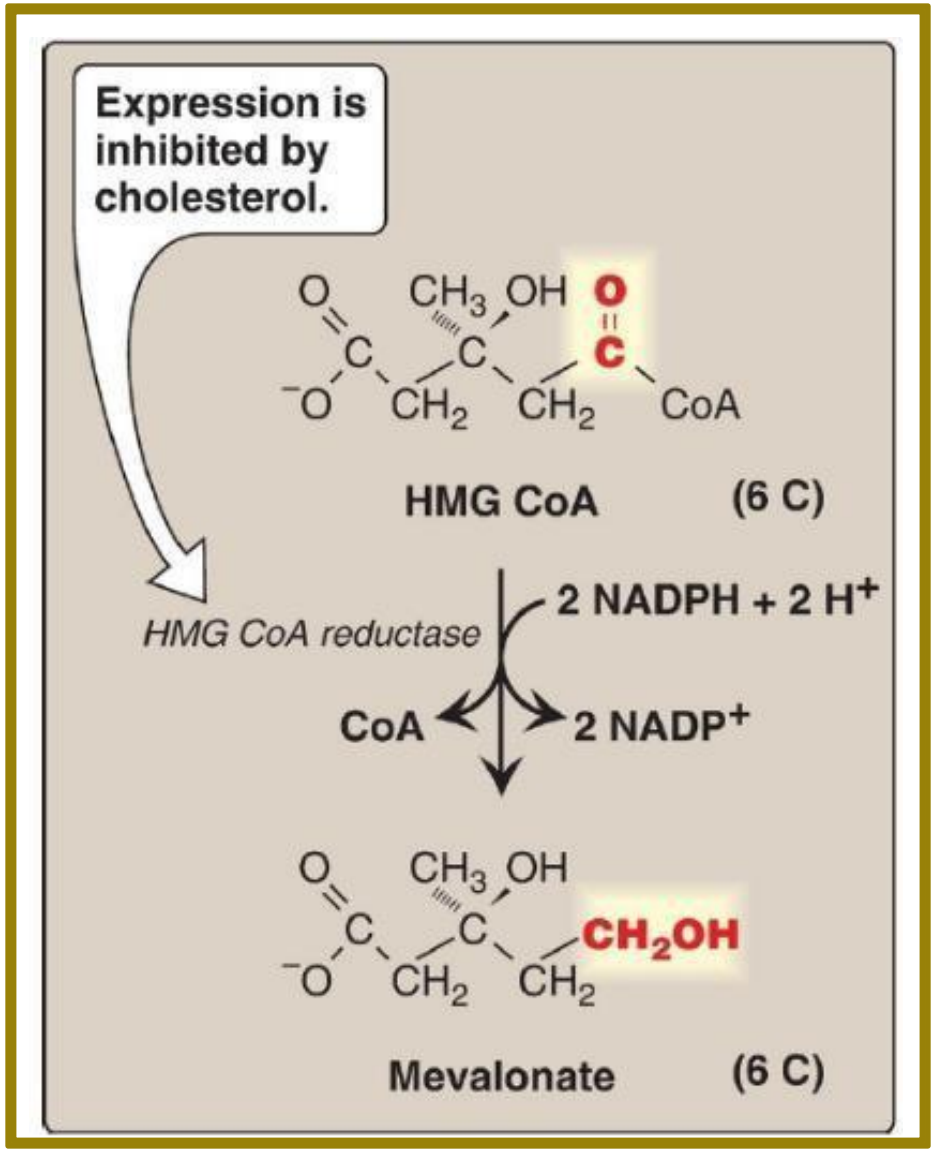
- HMG CoA is reduced to mevalonate by HMG CoA reductase.
  - A rate-limiting reaction and a committed step.
  - Two molecules of NADPH are oxidized.
  - CoA is released making the reaction irreversible.

➢ HMG CoA reductase removes CoA & reduces carbonyl to alcohol which is associated with oxidation of NADPH to NADP+.

- HMG CoA reductase is an integral membrane protein of the **SER**, with its catalytic domain projecting into the cytosol.

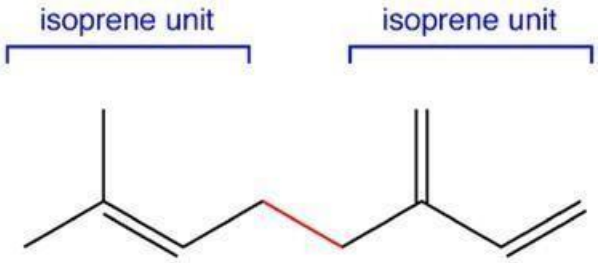


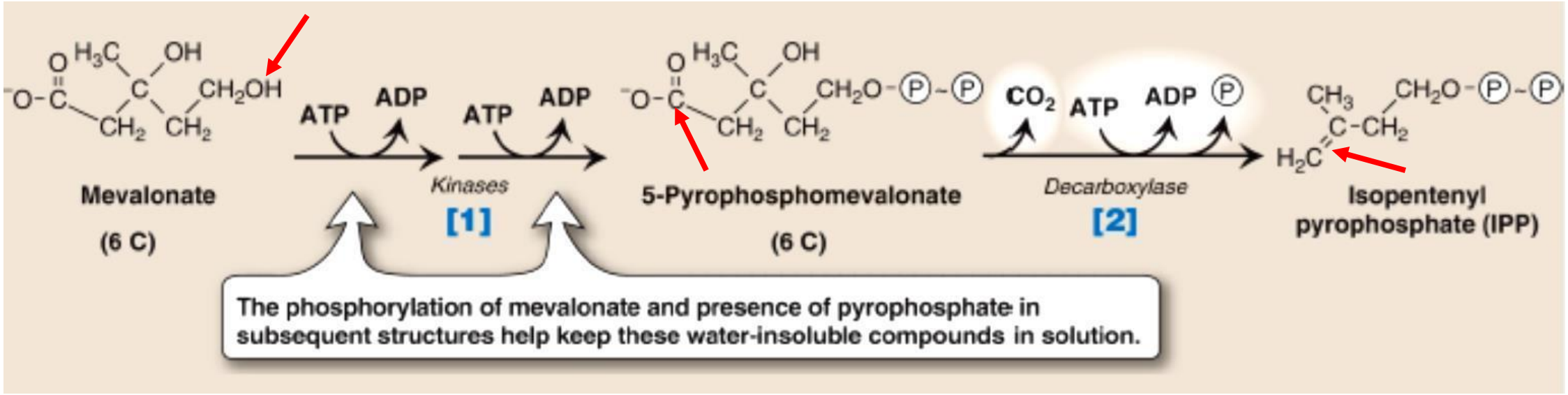
➢ HMG CoA doesn't enter SER but stays on the surface.



Synthesis of cholesterol-Isoprene unit (5Cs) in Peroxisomes

- Mevalonate is transported to the peroxisome.
- [1] Mevalonate is activated by transferring 2 phosphate groups from ATP. (Activation is done by adding **two** phosphates).
- [2] A five-carbon isoprene unit, isopentenyl pyrophosphate (IPP), is formed by the decarboxylation of 5 pyrophosphomevalonate.
  - The reactions require ATP.
  - IPP is the precursor of the isoprenoid family with diverse functions.
  - Nonsterol isoprenoids include ubiquinone (or coenzyme Q).



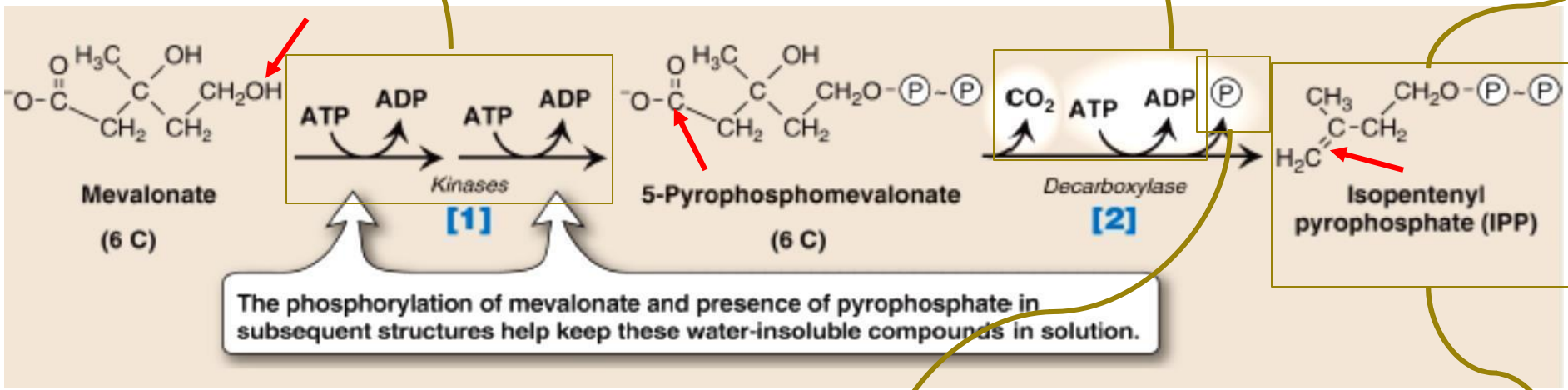


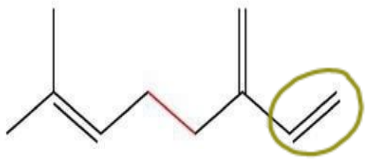
See the next slide...

Two phosphates from two ATP molecules are added to the -OH group by kinases.

Remove carboxyl group by decarboxylation which in this process requires ATP -usually decarboxylation reaction gives energy-.

We need to reform many IPPs to form the final product, cholesterol. IPP has a branch and a double bond, but the double bond is on carbon 1&2, rather than 2&3 as in isoprene units. It requires isomerization to move the double in IPP to the correct position.





We do not need the phosphate from the ATP for phosphorylation.

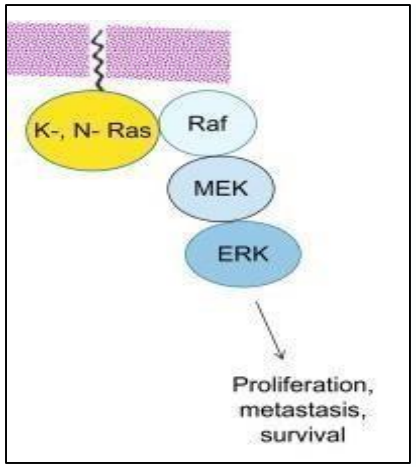
Reforming different bonds & forming a double bond to produce IPP molecule, which is a 5-carbon molecule.

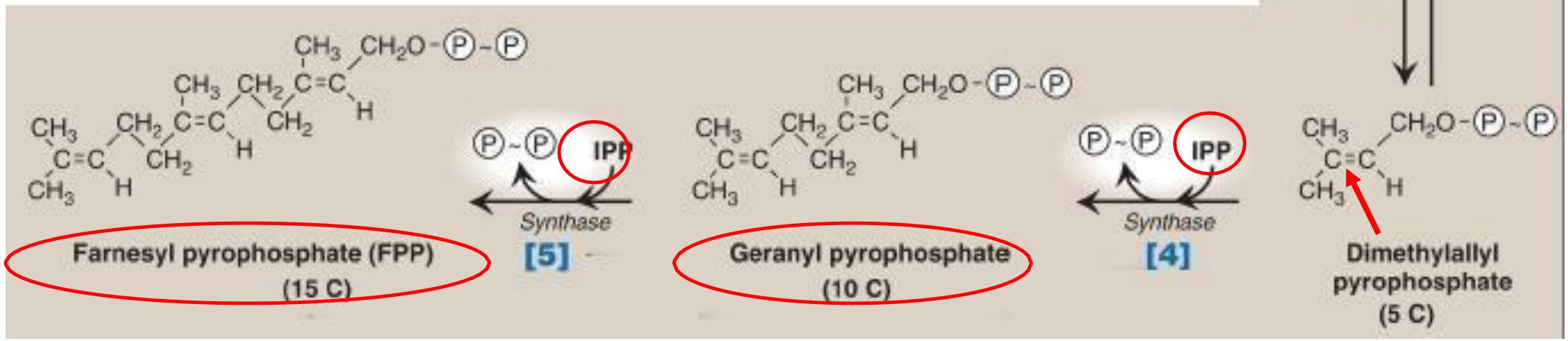
Synthesis of cholesterol- From 5 to 15 Cs in the Peroxisome

- [3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP)→ (Transfer double bond to carbon 2&3)

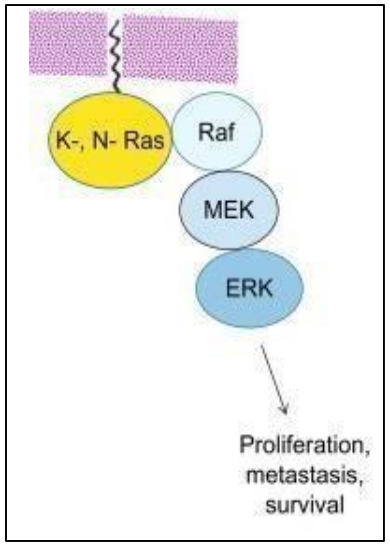
➢ Repeat process again to form another IPP molecule, using another three acetyl CoA molecules

- [4] IPP and DPP (both of them have 5C & 2 phosphate groups) condense to form 10-carbon geranyl pyrophosphate (GPP).
- [5] A second molecule of IPP then condenses with GPP to form 15 carbon farnesyl pyrophosphate (FPP).
  - Covalent attachment of farnesyl to proteins, a process known as prenylation, is one mechanism for anchoring proteins (for example, Ras) to the inner face of plasma membranes.





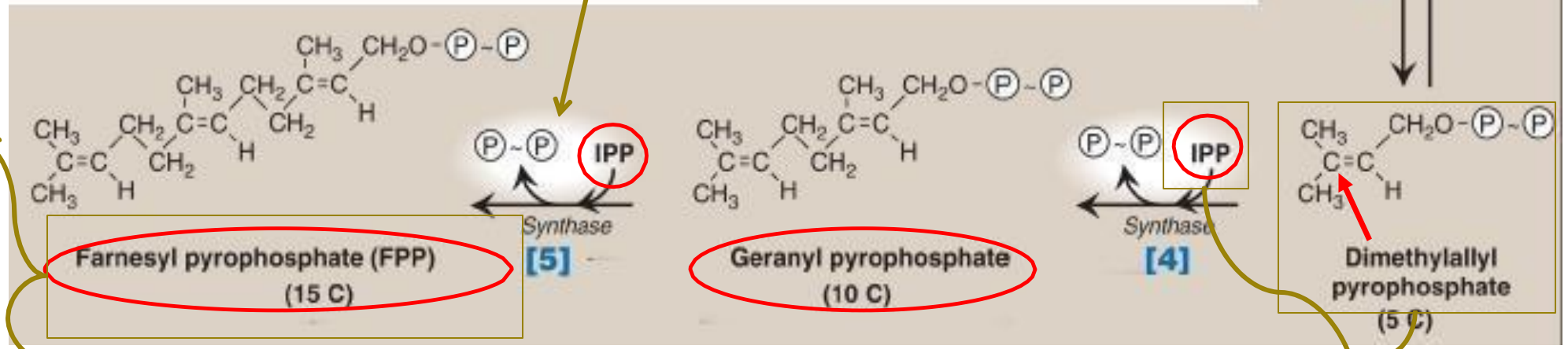
Anchoring by FPP happens on cytosolic side -inner leaflet- not from ECM side. Anchoring by phosphotidyl inositol happens on both outer &inner leaflet.



repeat process again.

1. Continue synthesis and give cholesterol

OR

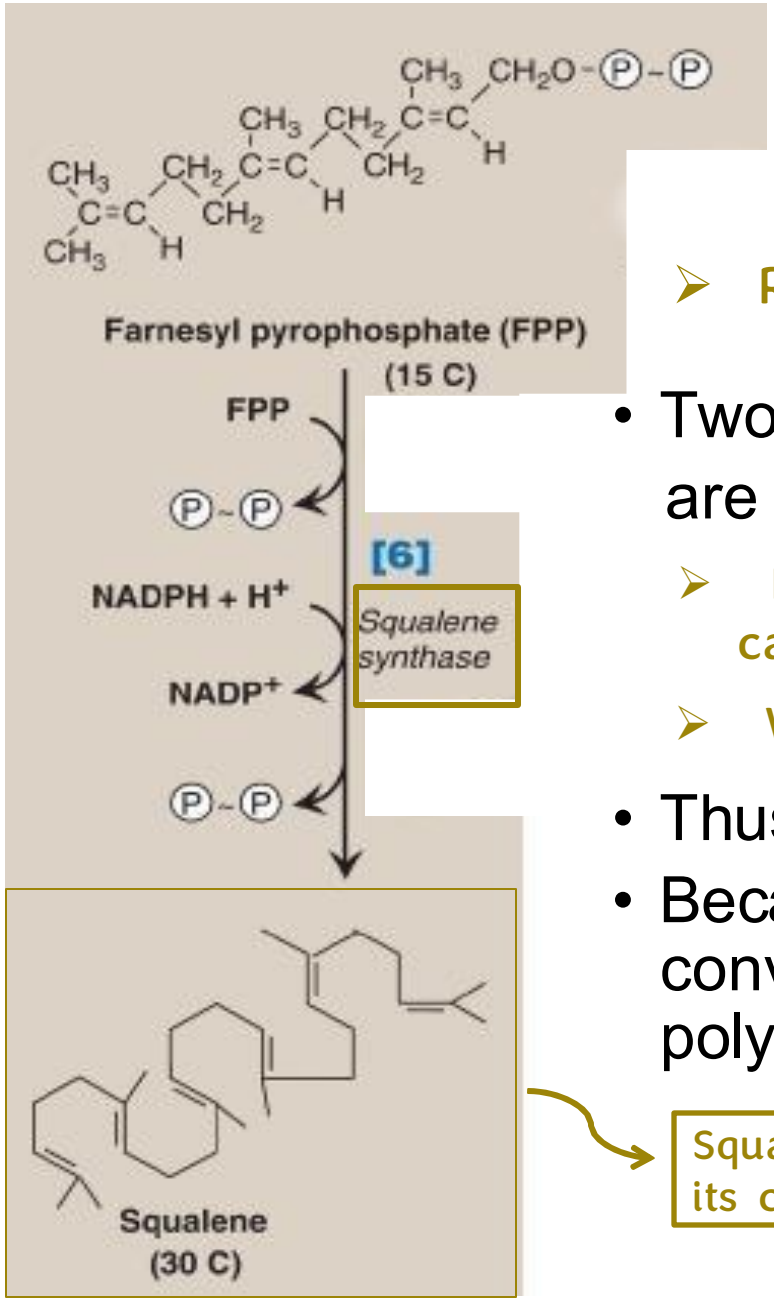


2. Used for attachment of proteins to membranes -has a hydrocarbon region- that gives it easy access to membrane structure easily (enabling it to be embedded in the membrane easily) .Attachment happens by covalent bond -strong bond- that provides some flexibility and lateral movement .

Are conjugated together by synthase enzyme, remove new phosphates from IPP during process.

All the figures that contain pathways must be studied :)

Synthesis of cholesterol- the synthesis of squalene in the SER



- Repeat all steps to produce another FPP.

- Two molecules of FPP combine, releasing pyrophosphate, and are reduced, forming the 30-carbon compound squalene
  - Reduction happens when both FPP molecules are combined which causes oxidation of NADPH molecule to NADP+.
  - We are still in the peroxisome.
- Thus, squalene is formed from six isoprenoid units.
- Because 3 ATP are hydrolyzed per mevalonate residue converted to IPP, a total of 18 ATP are required to make the polyisoprenoid squalene.

Squalene is a linear molecule, and its cyclization occurs in the SER.

بِكَرَمِكَ لا بجُهدي...

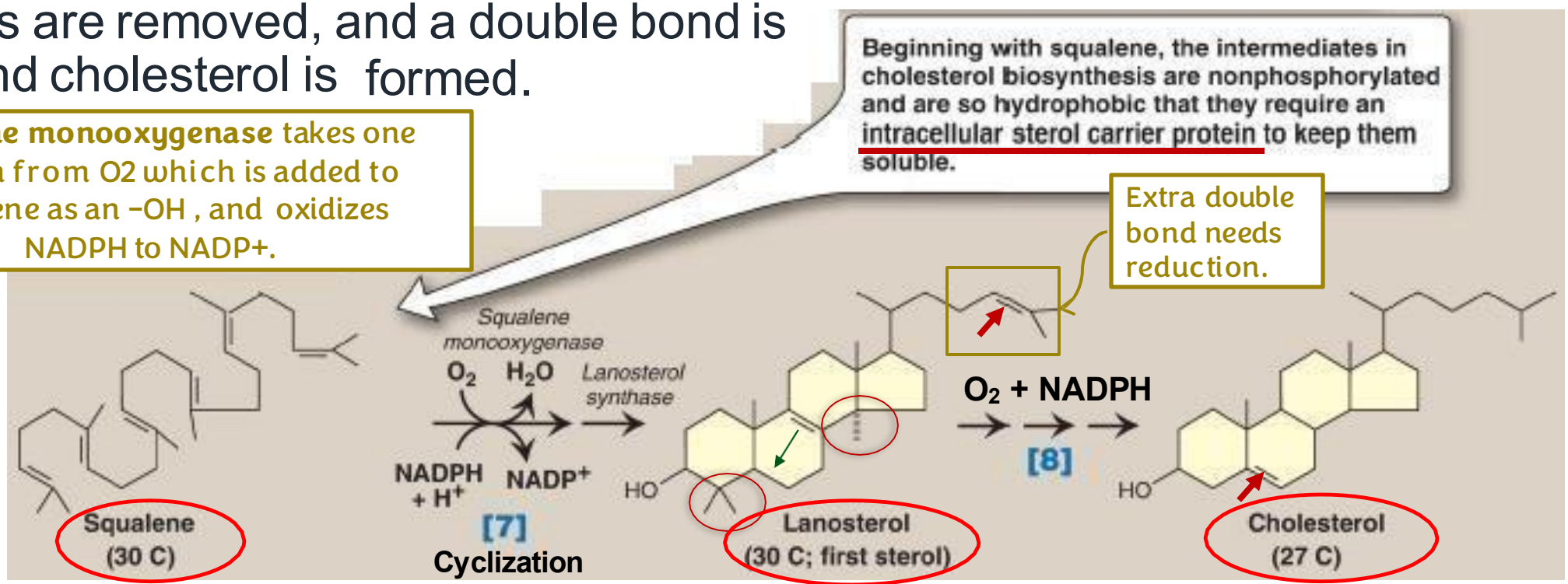
Synthesis of cholesterol- final steps in the SER

[7] Squalene is converted to the sterol lanosterol by **SER**-associated enzymes that use molecular oxygen (O2) and NADPH.

- The hydroxylation of linear squalene triggers the cyclization of the structure to lanosterol.

[8] The side chain of lanosterol is shortened, the methyl groups are removed, and a double bond is re-located, and cholesterol is formed.

Squalene monooxygenase takes one oxygen from O2 which is added to squalene as an –OH , and oxidizes NADPH to NADP+.



Monooxygenase reacts oxygen and the substrate, taking one oxygen atom from O_2 to oxidize NADPH and the substrate. The other oxygen atom is reduced. Monooxygenases are a source of ROS.

Linear

By **lanosterol synthase.**

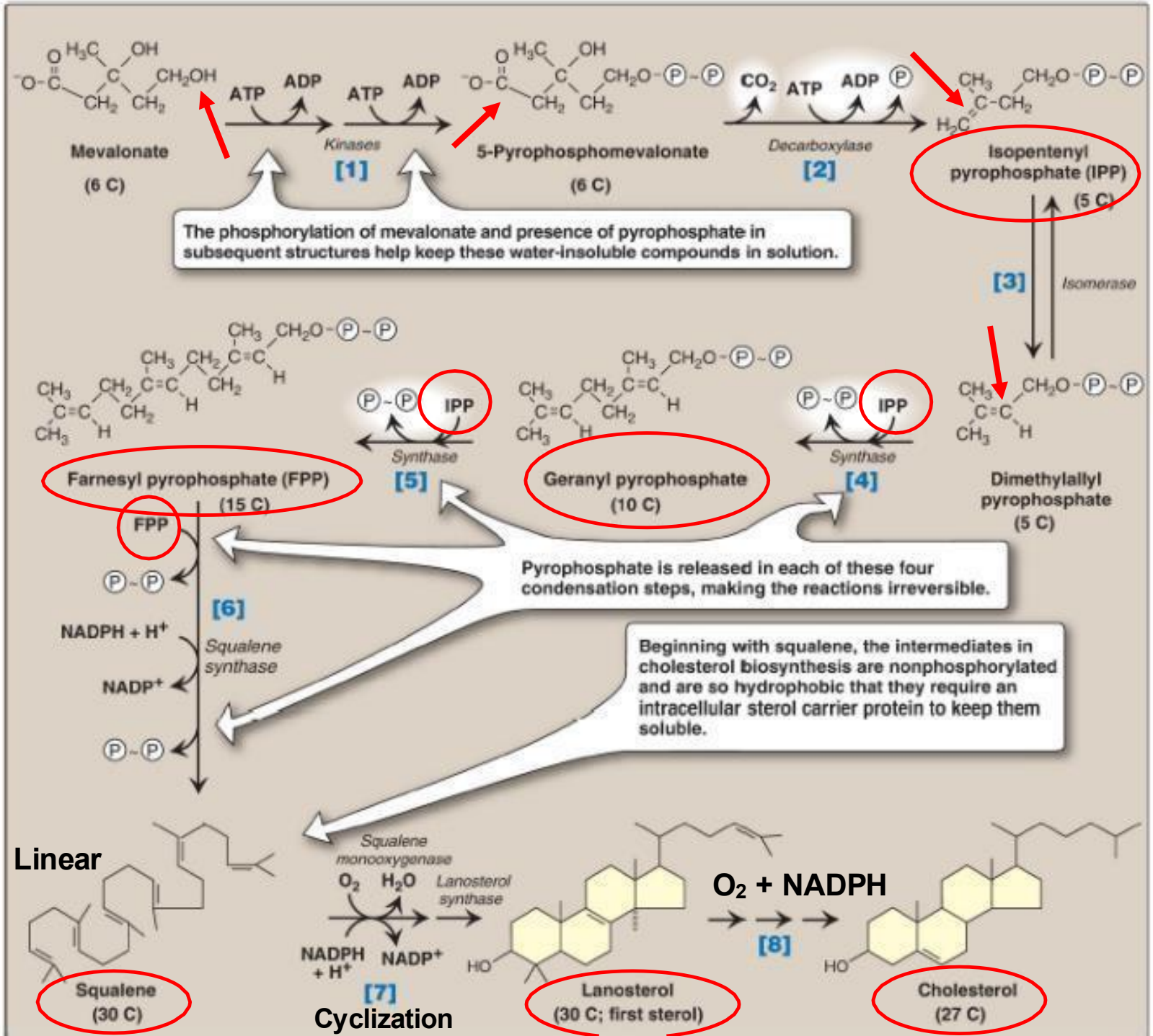
Lanosterol has 3 extra carbons and a double bond that needs repositioning.

Finally , it took us ages to get here :)

Synthesis of cholesterol

Cholesterol Synthesis from an Energy Perspective:

- No ATP is used before the formation of mevalonate.
- [1]: Two ATP molecules are required to phosphorylate mevalonate into 5-pyrophosphomevalonate.
- [2]: One ATP molecule is consumed during the decarboxylation reaction to produce the five-carbon units (IPP). Therefore, to produce one IPP, 3 ATP molecules are needed. Since we need 6 five-carbon units, this requires a total of 18 ATP.

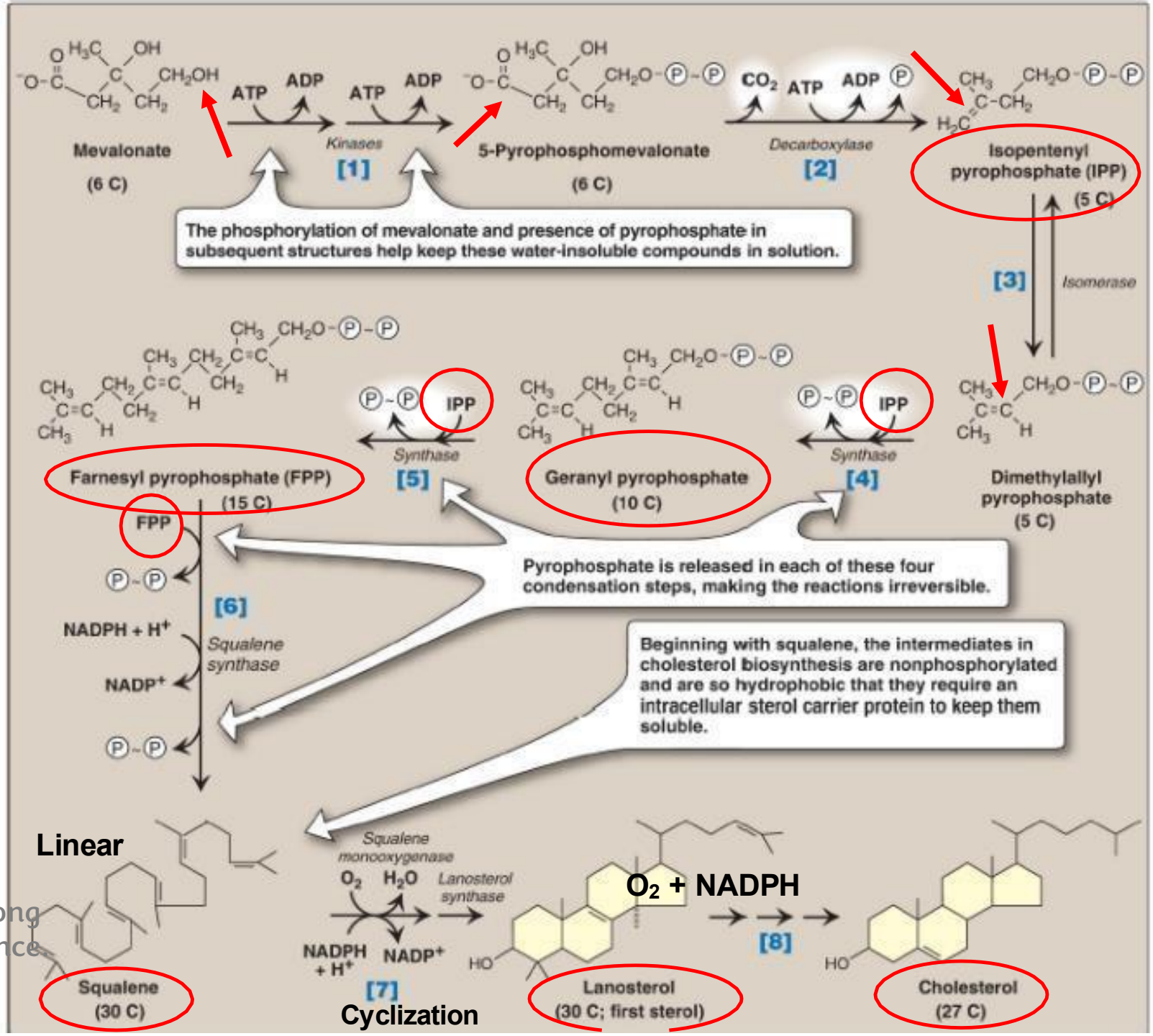


Synthesis of cholesterol

Cholesterol Synthesis from an Energy Perspective:

- To produce one mevalonate molecule, 2 NADPH molecules are used by HMG-CoA reductase, as discussed in slide 13. Since we need 6 IPP molecules, a total of 12 NADPH are utilized.
- In the last three steps, one NADPH is used in each step: in step [6] by squalene synthase, in step [7] by squalene monooxygenase, and in step [8].
- A total of 15 NADPH are needed.

❗Focus on the essential compounds ; as for the long compound names, they are not of great importance



رسالة من الفريق العلمي:

"كادَ الإنسَان يَموت منَ القَلَقِ ناسياً

أنَّ لهُ ربّا يَشُقّ الفَلَق"

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V0 → V1			
V1 → V2			