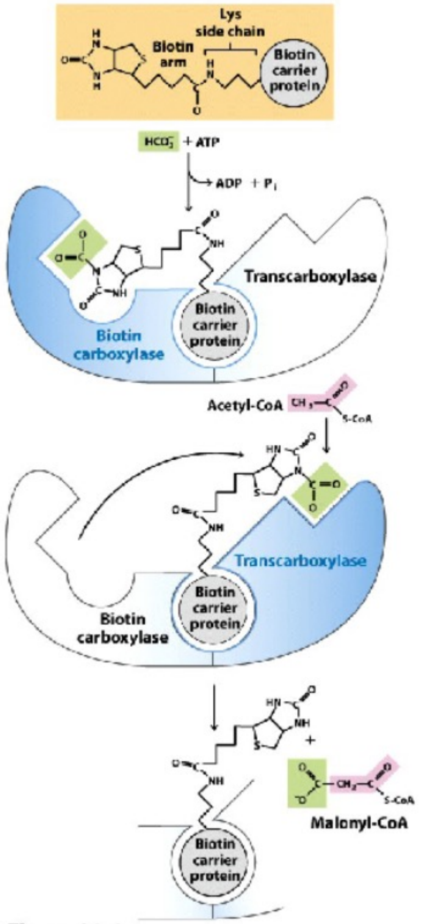
BCH II – Exercises 8

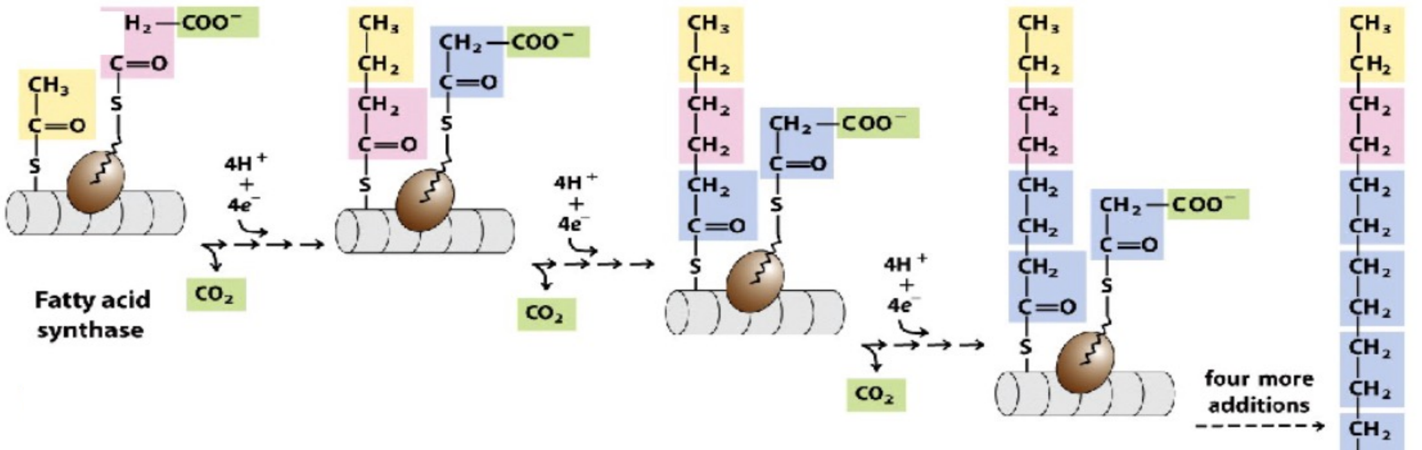
14/04/2025

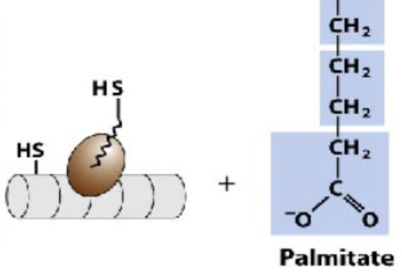
Question 1

Using your knowledge on the biosynthesis of fatty acids predict how palmitate will be labelled under the conditions described below and explain the reason for your answers:

(a) [14-C] acetyl-CoA (uniformly labelled) is added to a soluble (cytosolic) fraction from rat liver.



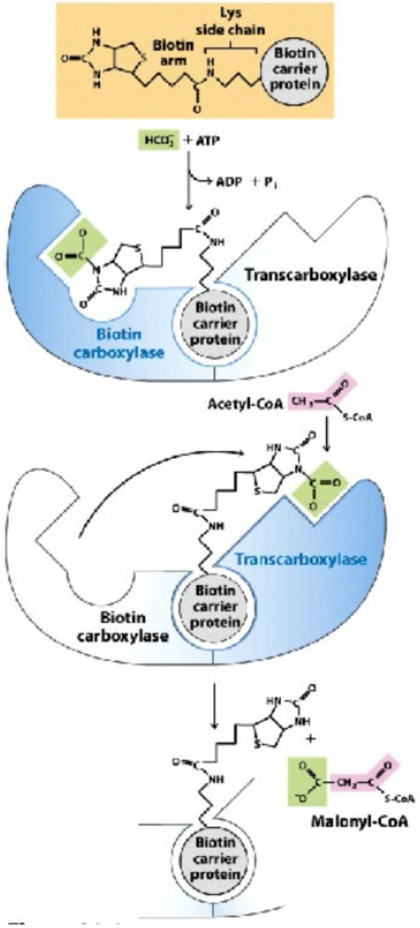


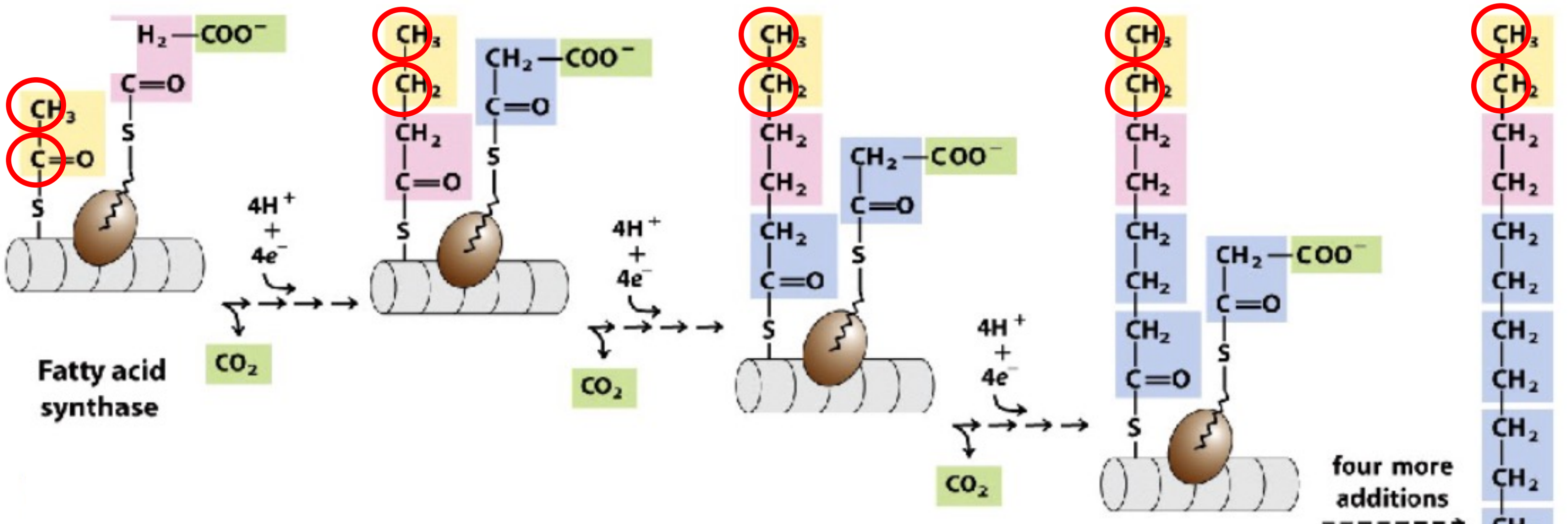


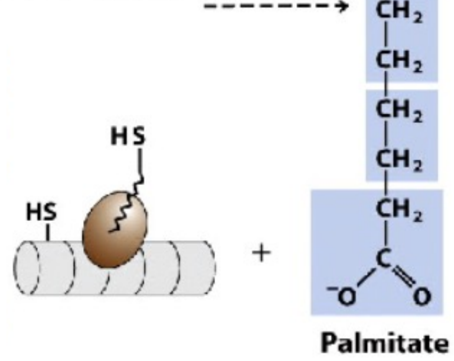
In a *soluble cytosolic fraction*, acetyl-CoA can be converted to malonyl-CoA by **acetyl-CoA carboxylase**. So, if all acetyl-CoA is uniformly labeled, then **both the acetyl-CoA (starter)** and the **malonyl-CoA (extenders)** derived from it will be labeled.

All Carbon atoms of the palmitate will be labelled because labelled acetyl-CoA can be converted into labelled malonyl-CoA

(b) A small quantity of [14-C] acetyl-CoA (uniformly labelled) is added to a soluble (cytosolic) fraction from rat liver in presence of an excess of unlabelled malonyl-CoA.





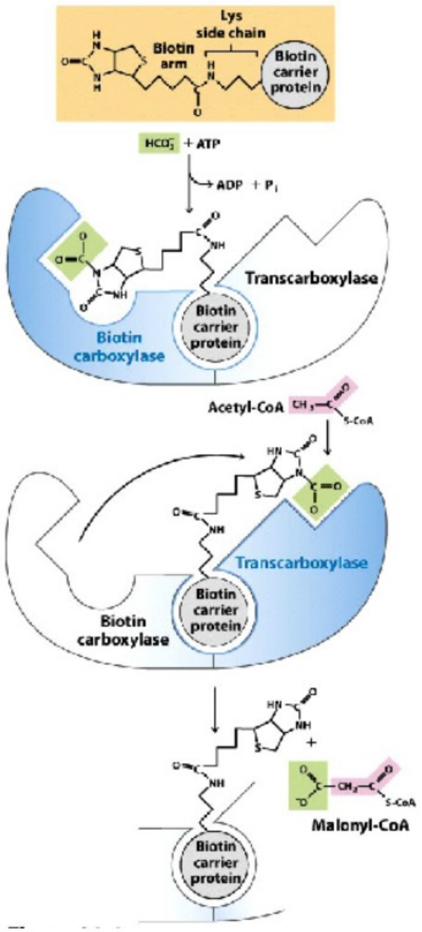


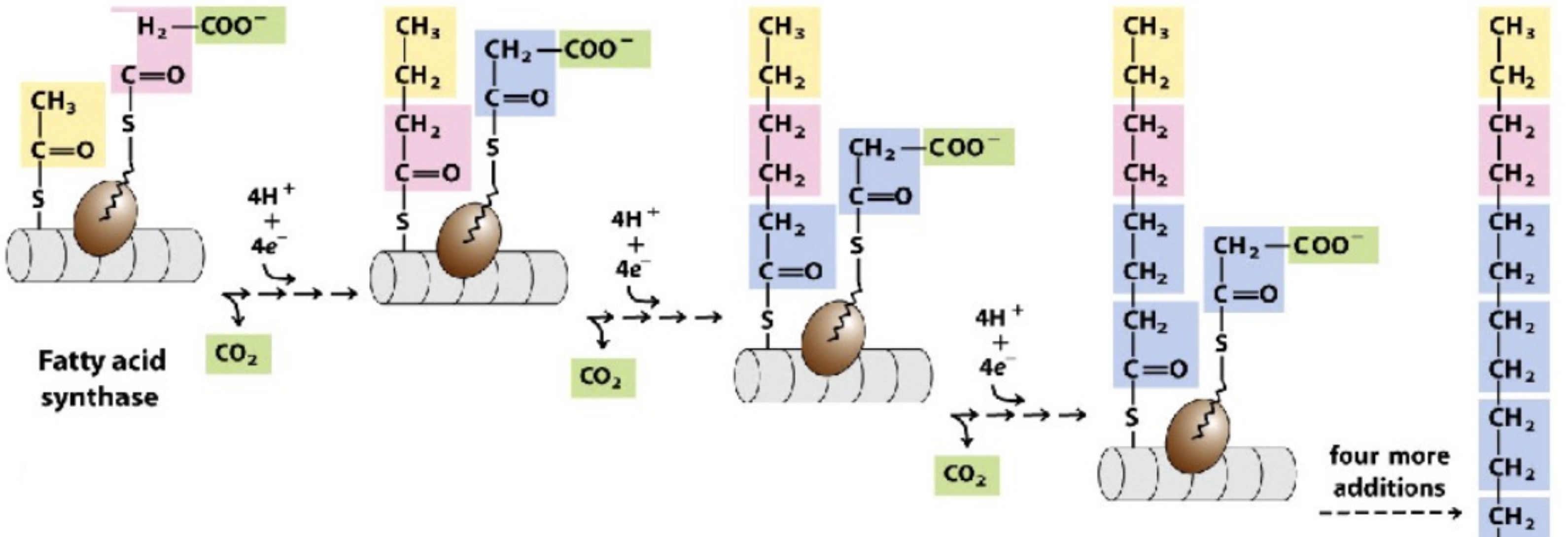
With a small amount of labeled acetyl-CoA and a large excess of unlabeled malonyl-CoA, labeled acetyl-CoA is unlikely to contribute to malonyl-CoA synthesis. Instead, it will only act as the **initial primer** (C15–C16).

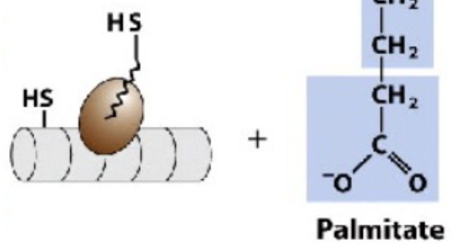
So, in **molecules that do incorporate labeled acetyl-CoA**, only the **two terminal carbons (methyl end)** will be labeled.

→ **C15 and C16**

(c) [14-C] malonyl-CoA (uniformly labelled) is added to a soluble (cytosolic) fraction from rat liver in presence of an excess of acetyl-CoA







In this condition:

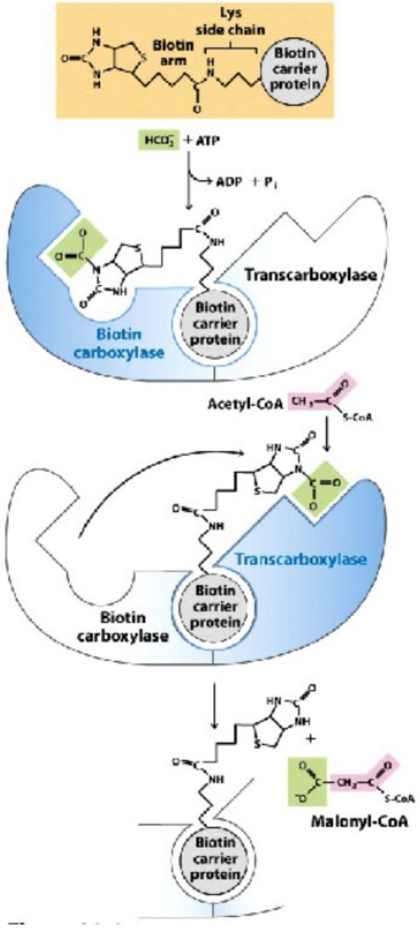
•Two pools of malonyl-CoA exist:

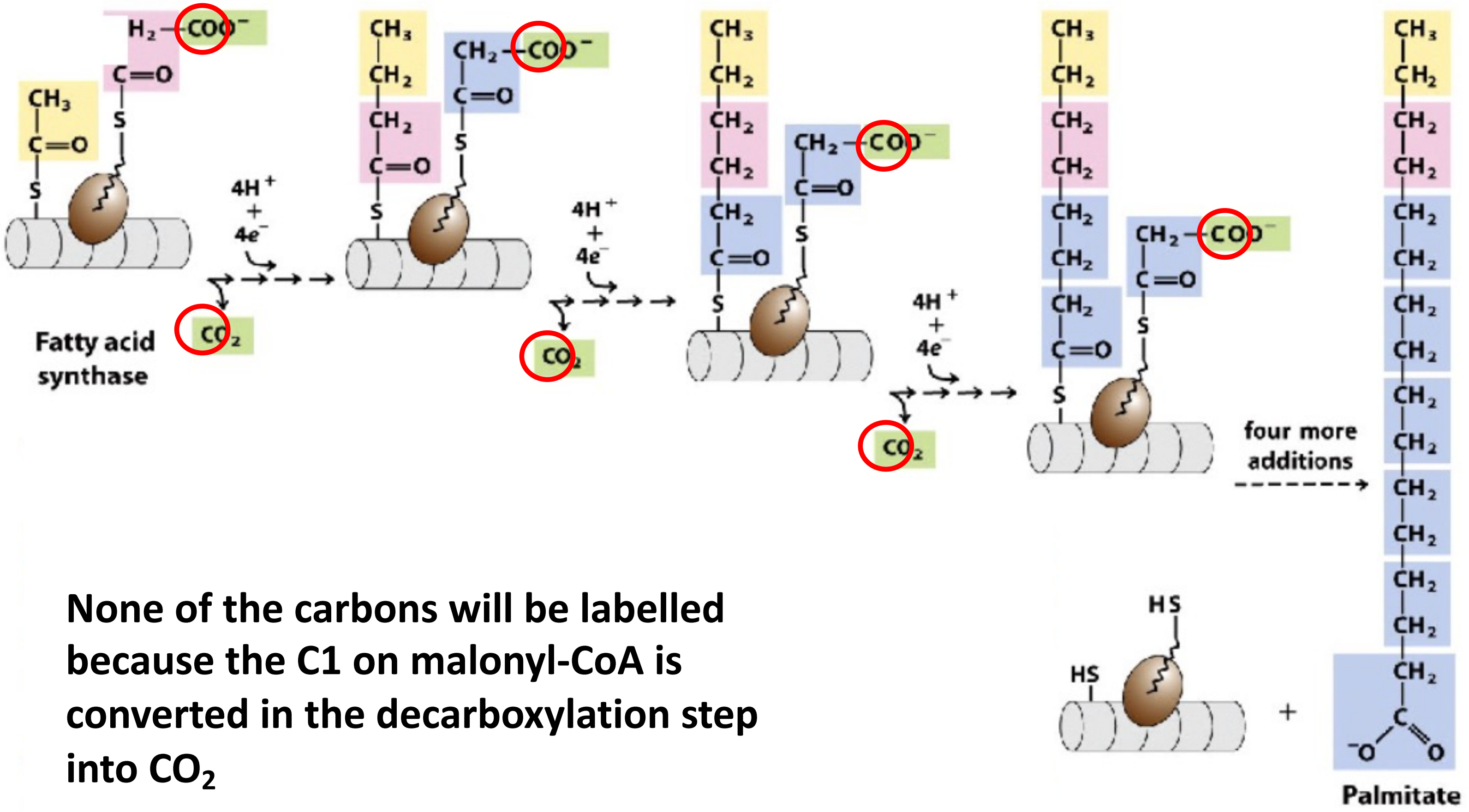
- Exogenous labeled malonyl-CoA (limited quantity)
- Endogenous unlabeled malonyl-CoA (produced from excess acetyl-CoA)

Therefore, the FAS machinery will draw from both pools, but most of the malonyl-CoA used will be unlabeled, simply because it's in excess.

→ Most of the palmitate molecules formed will be completely unlabeled. A small fraction may incorporate one or more 14C-labeled acetyl units (from malonyl-CoA), but we can't predict exactly which carbons would be labeled, or in how many molecules.

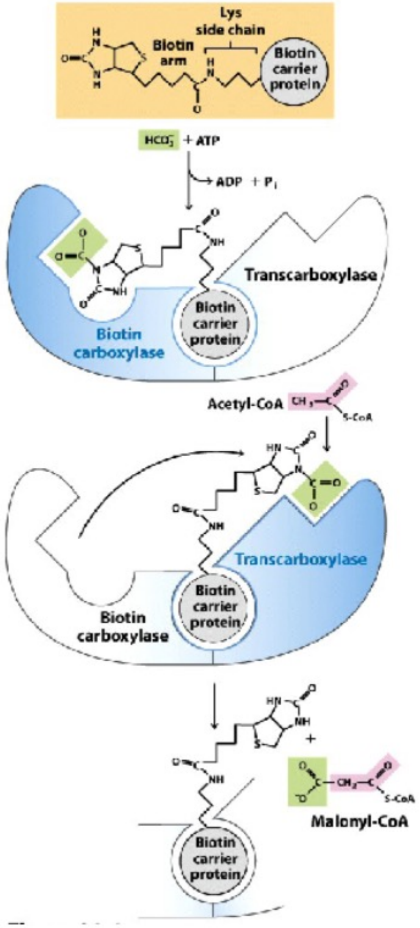
(d) [14-C] malonyl-CoA labelled on C-1 is added to a soluble (cytosolic) fraction from rat liver.

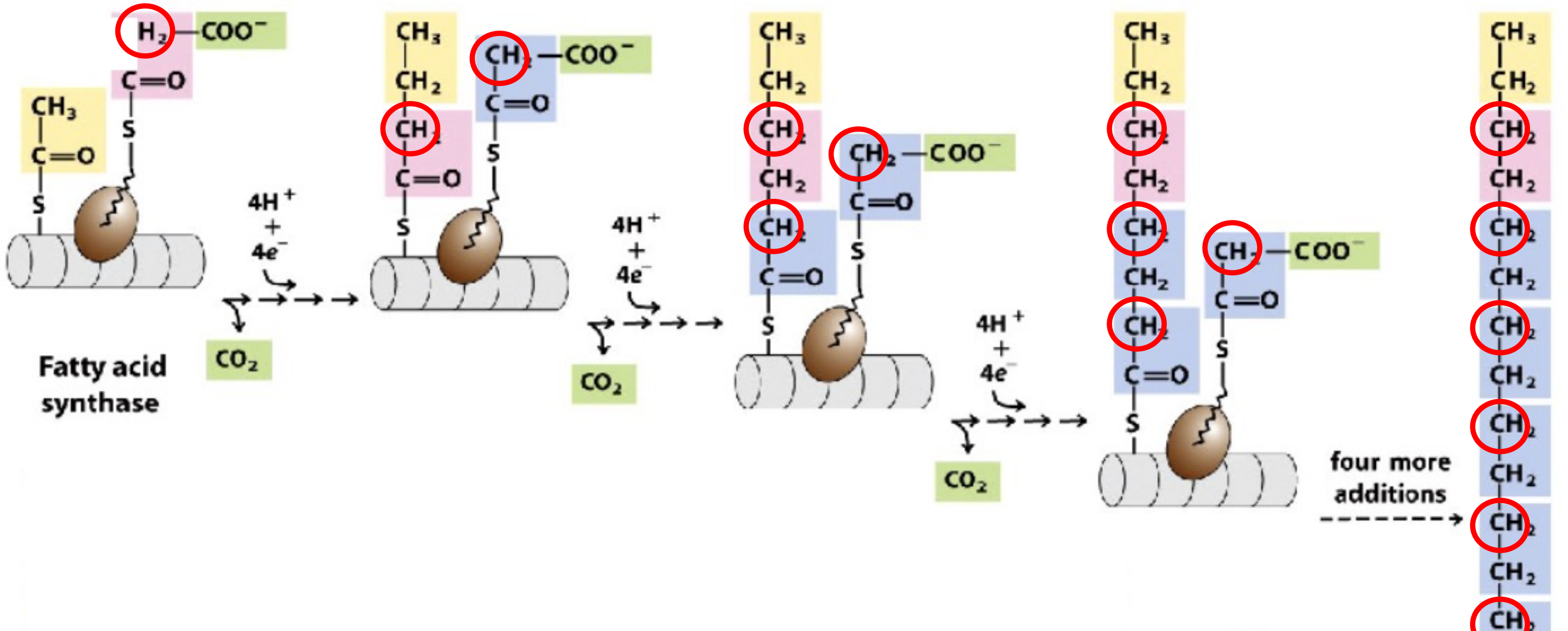


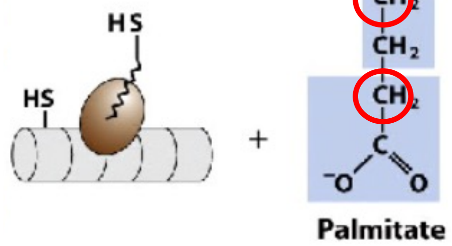


None of the carbons will be labelled because the C1 on malonyl-CoA is converted in the decarboxylation step into CO_2

(e) [14-C] malonyl-CoA labelled on C-2 is added to a soluble (cytosolic) fraction from rat liver.



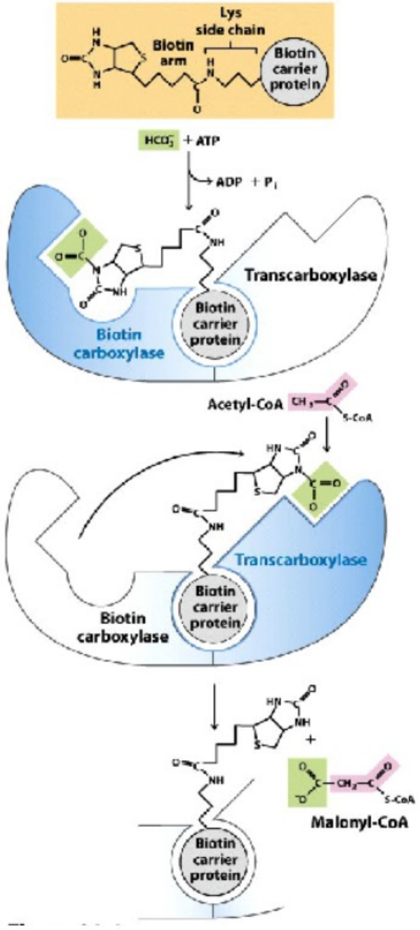


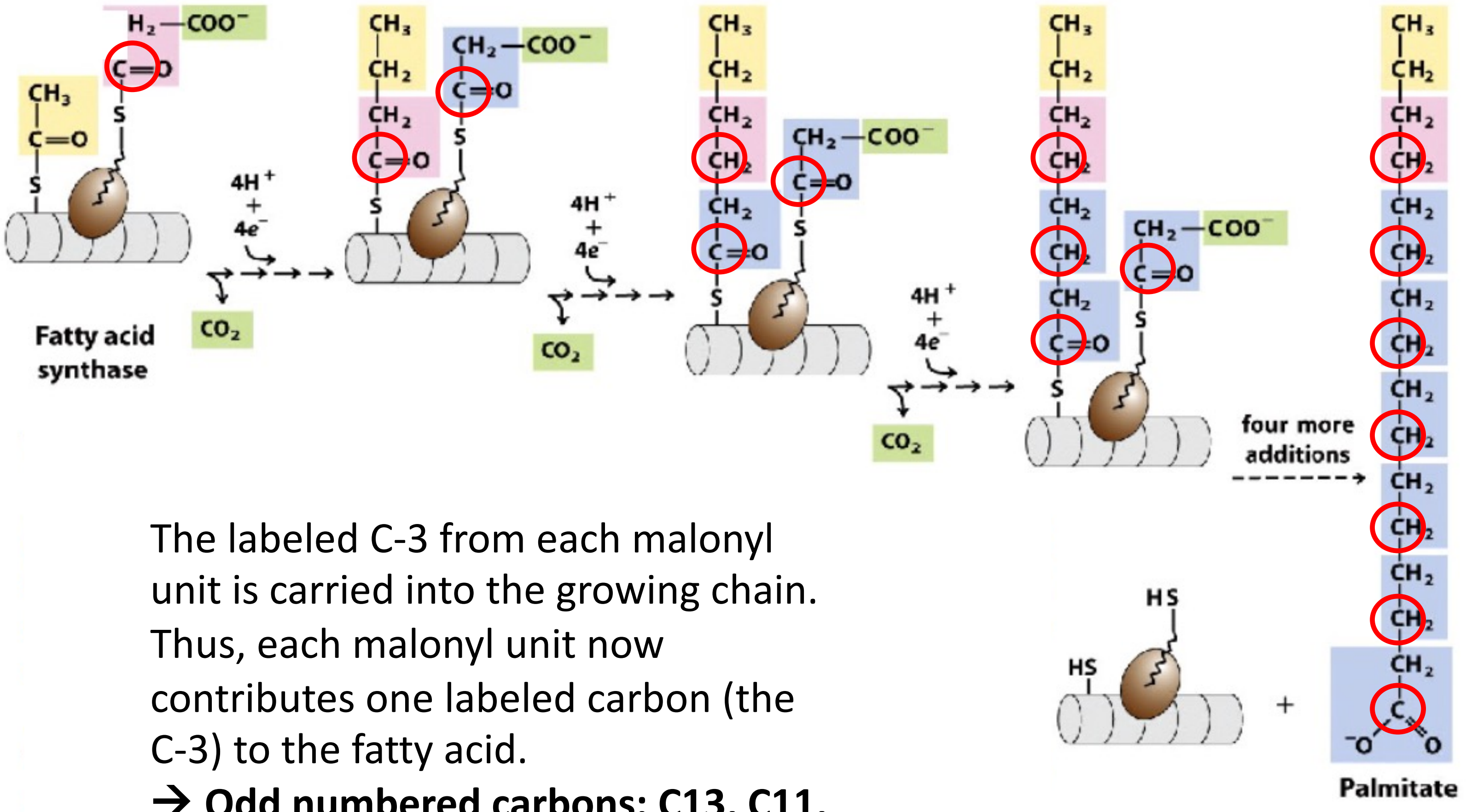


Each malonyl-CoA contributes two carbons per cycle; the C2-labeled carbon becomes part of the growing chain.

→ **Even numbered carbons: C14, C12, C10, C8, C6, C4, C2**

(f) [14-C] malonyl-CoA labelled on C-3 is added to a soluble (cytosolic) fraction from rat liver.



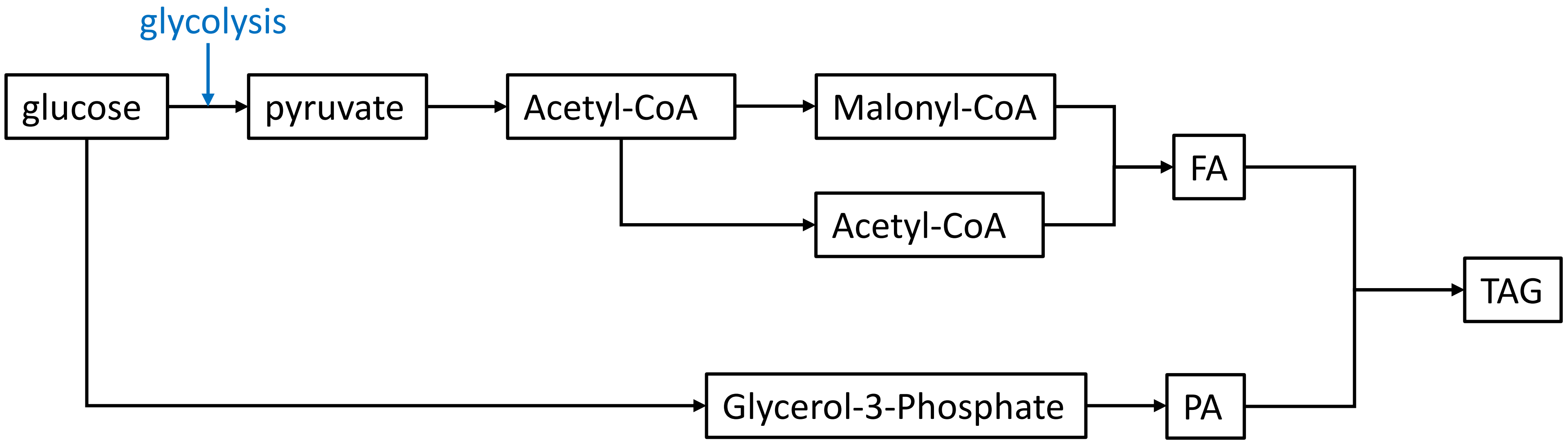


The labeled C-3 from each malonyl unit is carried into the growing chain. Thus, each malonyl unit now contributes one labeled carbon (the C-3) to the fatty acid.

→ **Odd numbered carbons: C13, C11, C9, C7, C5, C3, C1**

Question 2

After a glucose rich meal the excessive glucose that is not used to satisfy energetic needs, is transformed into fatty acids that are then stored into triglycerides. Can you describe the different paths that lead from glucose to triglycerides?



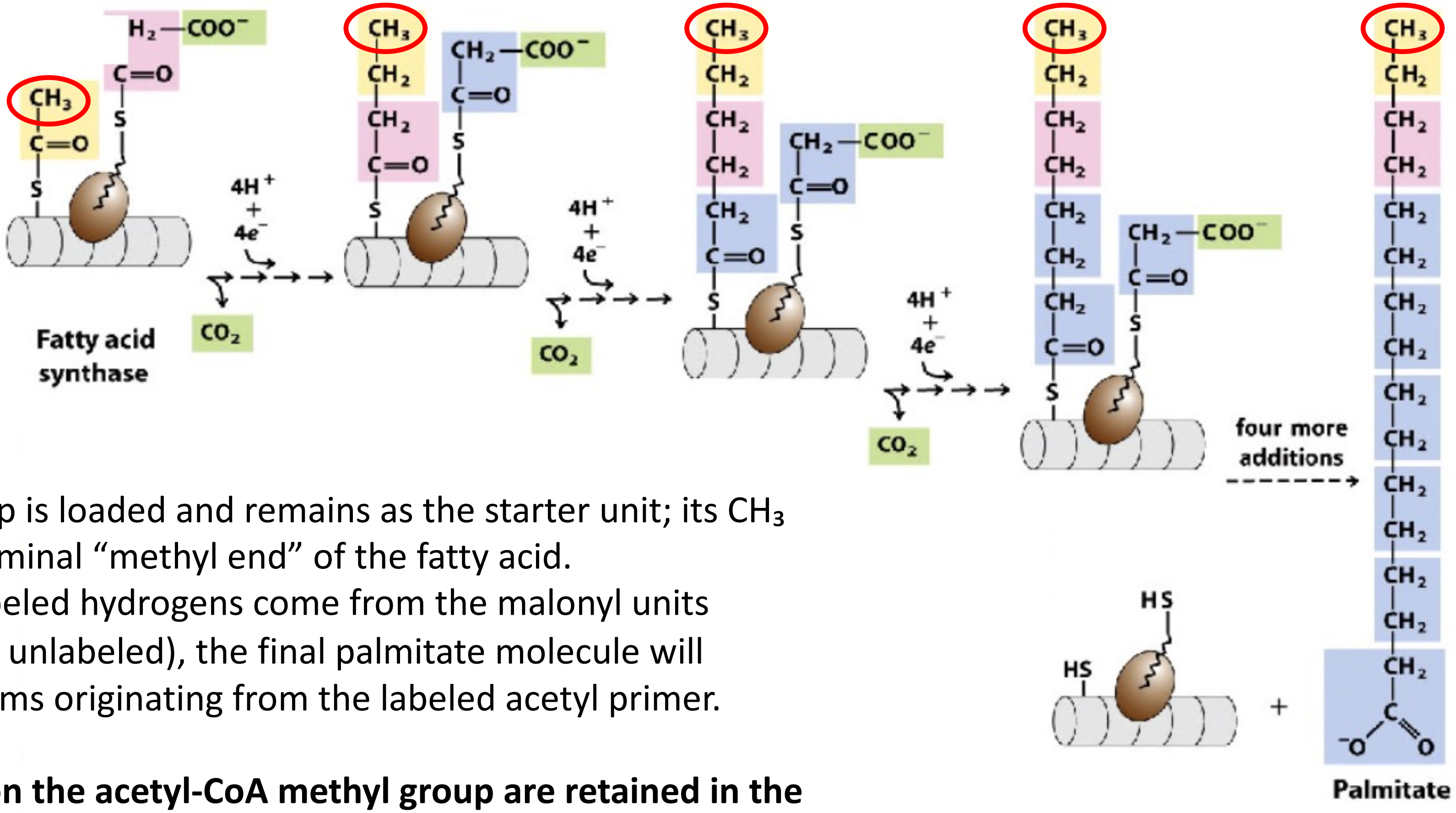
→ Glucose is first degraded through glycolysis to generate pyruvate. Pyruvate is converted into acetyl-CoA in the mitochondria, which is exported (as citrate) into the cytosol. There it is regenerated as acetyl-CoA and converted into malonyl-CoA. The FAS complex then synthesizes fatty acids (e.g. palmitate) using acetyl-CoA (as primer) plus malonyl-CoA (extensions) and NADPH.

→ Simultaneously, glycolytic intermediates form glycerol-3-phosphate. Acyl transferases esterify acyl chains in positions C-1 and C-2 of the Glycerol-3-Phosphate to produce phosphatidic acid which can be converted to triglycerides.

Question 3

In a mixture containing all the enzymes and cofactors required for fatty acid synthesis from acetyl-CoA and malonyl-CoA

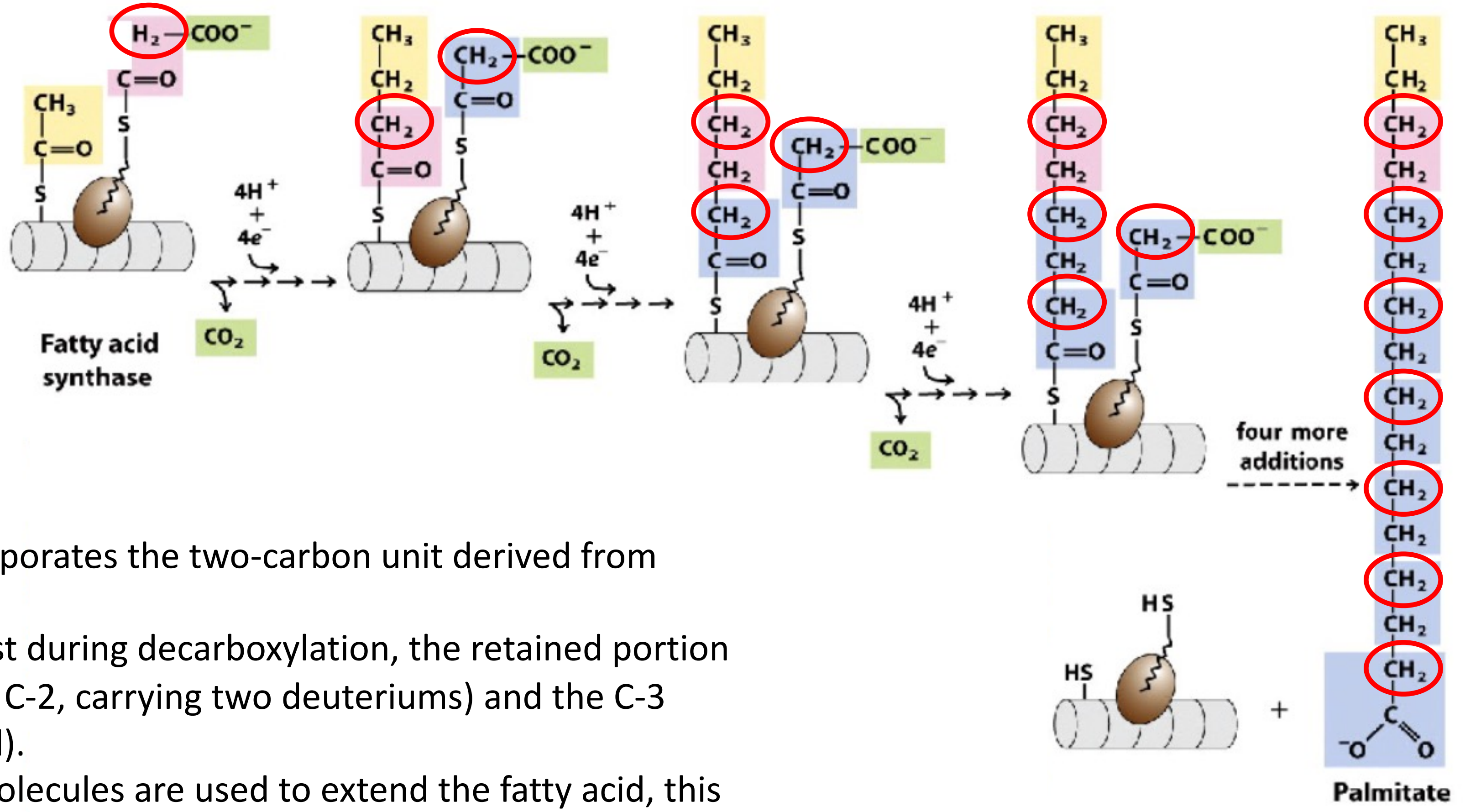
(a) we add acetyl-CoA labelled with deuterium (2-H) in the three positions on its methyl group and an excess of unlabelled malonyl-CoA. How many H atoms will be labelled in palmitate at the end of its synthesis? Explain why.



- In FAS, the acetyl group is loaded and remains as the starter unit; its CH_3 moiety becomes the terminal "methyl end" of the fatty acid.
- Since no additional labeled hydrogens come from the malonyl units (which are in excess and unlabeled), the final palmitate molecule will contain 3 deuterium atoms originating from the labeled acetyl primer.

→ 3 deuterium atoms on the acetyl-CoA methyl group are retained in the final palmitate.

(b) we add malonyl-CoA labelled with deuterium (2-H) in the two positions of C-2. How many Deuterium atoms will be incorporated into palmitate and in which positions? Explain why.

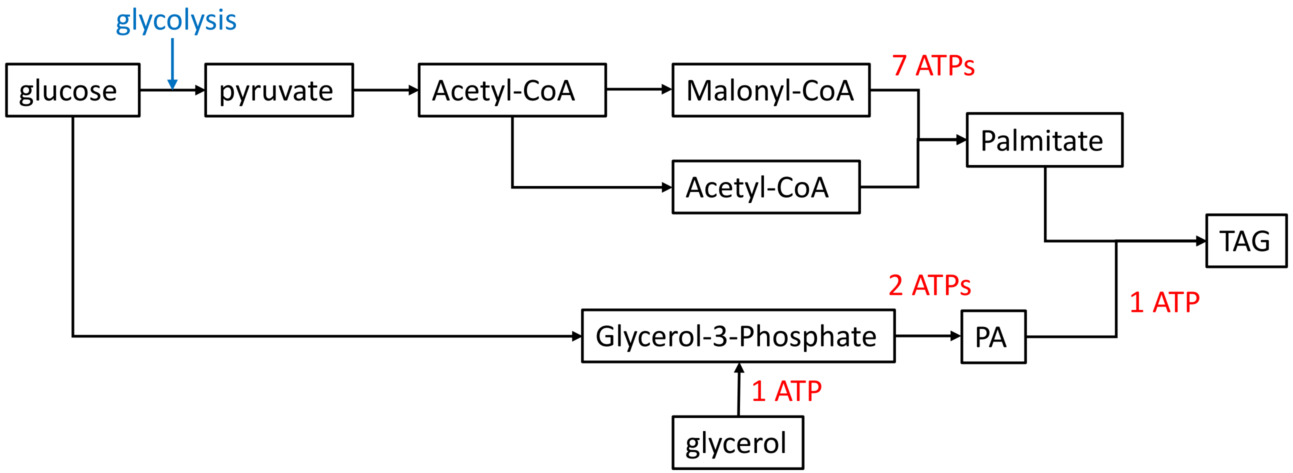


- Each extension cycle incorporates the two-carbon unit derived from malonyl-CoA.
- With C-1 (the carboxyl) lost during decarboxylation, the retained portion is the CH_2 carbon (originally C-2, carrying two deuteriums) and the C-3 carbon (which is not labeled).
- Because 7 malonyl-CoA molecules are used to extend the fatty acid, this labeling will appear in each two-carbon extension unit.

→ Total of 14 deuterium atoms: 2 at each of the following positions: C14, C12, C10, C8, C6, C4, C2

Question 4

How many ATP molecules are required to synthesise the triglyceride tripalmyticglycerol starting from glycerol and acetyl-CoA? Explain why.



To calculate the ATP cost we must consider:

1.Fatty Acid Synthesis:

- The overall reaction for palmitate synthesis (as given in the lecture) is:
  8 Acetyl-CoA + 7 ATP + 14 NADPH + 14 H^+ → Palmitate + 8 CoA-SH + 6 H_2O + 7 ADP + 7 Pi
- Thus, one palmitate molecule requires 7 ATP molecules.
- For a triglyceride with three palmitate chains, the FAS pathway alone consumes 3 × 7 = 21 ATP.

2.Glycerol Activation:

- To use glycerol in triglyceride assembly, it must be phosphorylated to glycerol-3-phosphate (by glycerol kinase), which requires 1 ATP.
- Acyl transferases esterify acyl chains in positions C-1 and C-2 of the Glycerol-3-Phosphate to produce phosphatidic acid using 2 ATP.
- To be converted into TAGs, PA needs to be dephosphorylated to produce diacylglycerol which is then the substrate of acyl transferases that catalyse the esterification of a third fatty acid to glycerol to for TAGs using 1 ATP.

Total: 7 x 3 + 1 + 2 + 1 = 25 ATPs

1. Fatty acid synthesis:

8 Acetyl-CoA + 7 ATP + 14 NADPH +14 H+ => Palmitate + 8 CoA-SH + 6 H2O + 7 ADP + 7 Pi + 14 NADP+

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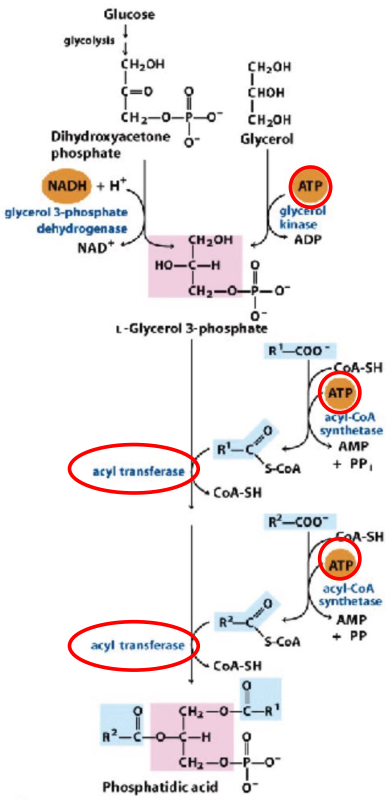
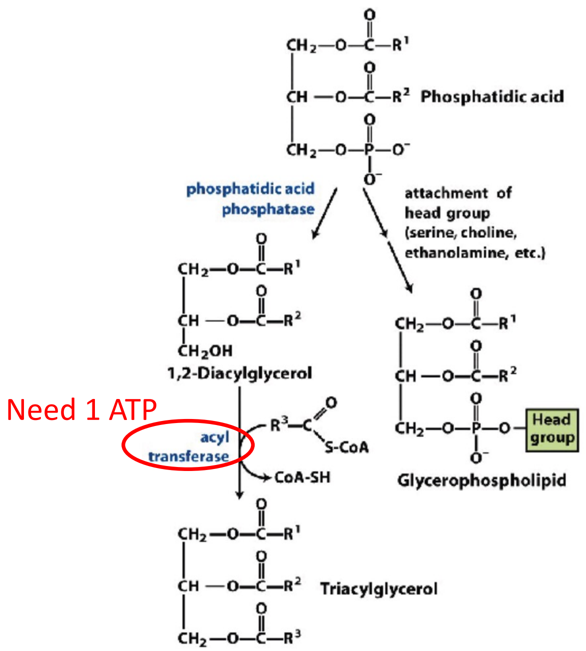
2. Glycerol Activation:





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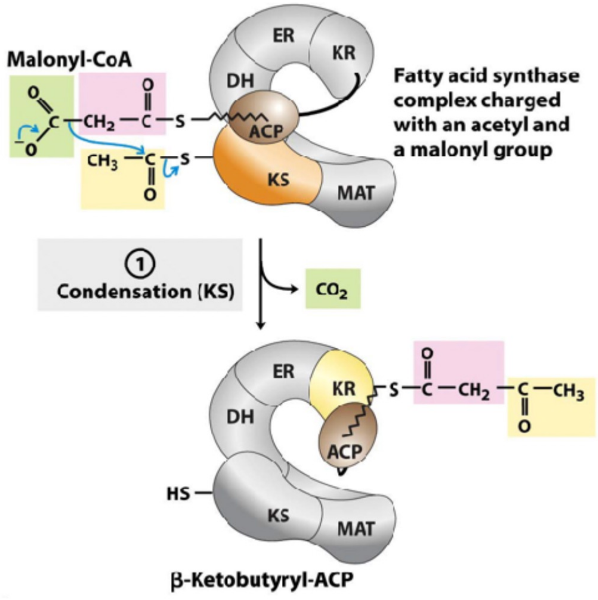
Question 5

In the reaction catalysed by the Beta-ketoacyl-ACP synthase a 4-carbon-atoms unit is generated by the condensation of a 3-carbon-atoms molecule and a 2-carbon-atoms molecule with release of a CO2 molecule. Explain what is the advantage of this strategy as compared with one where two 2-carbon-atoms molecules are condensed.



Facts to know:

1) This is the energetically driving step in FA synthesis
2) The released CO2 is the same that was added by the acetyl-CoA carboxylase to produce malonyl-CoA
3) the decarboxylation step makes this reaction exergonic
4) the mechanism of this reaction is similar to that of PEP-carboxykinase in gluconeogenesis

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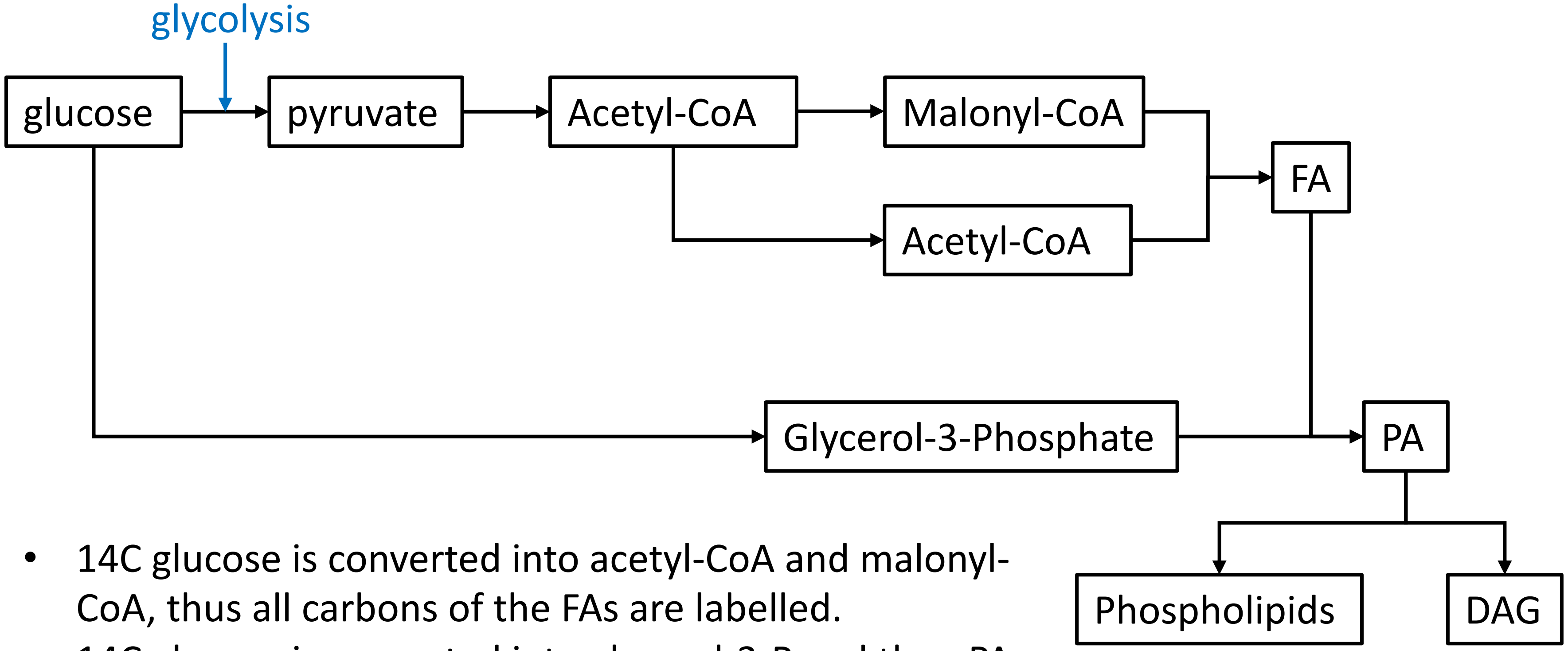
- **The decarboxylation reaction is exergonic => provide energy to the coupled reactions**
  ⇒ Energetically driving step.
- **The two 2-carbon atoms condensation strategy is much less exergonic => much less energy for the couples reactions,** could lead to less controlled product formation.

Question 6

A researcher has fed a rat with D-Glucose homogeneously labelled with 14-C on all its carbon atoms. After sacrificing the mice and extracting lipids from liver, she finds some radiolabelled diacylglycerol (C16:0/C20:5) has been produced. Assuming that 14-C incorporation has been complete can you predict which of the phospholipid carbon atoms are labelled? Explain why.



- 14C glucose is converted into acetyl-CoA and malonyl-CoA, thus all carbons of the FAs are labelled.
- 14C glucose is converted into glycerol-3-P and then PA, thus all carbons of phospholipids are labelled.