Lec. 2 /2 nd term Biochemistry II
3 rd stage
Biosynthesis of fatty acids

By

Ph. Dr. Safa W. Aziz

Introduction

Fatty acids have 4 major physiological roles:

- fatty acids are building blocks of phospholipids and glycolipids
- many proteins are modified by the covalent attachment of fatty acids, which targets them to membrane locations.
- fatty acids are fuel molecules

- fatty acid derivatives serve as hormones and intracellular messengers.

Fatty Acid Synthesis v/s Degradation

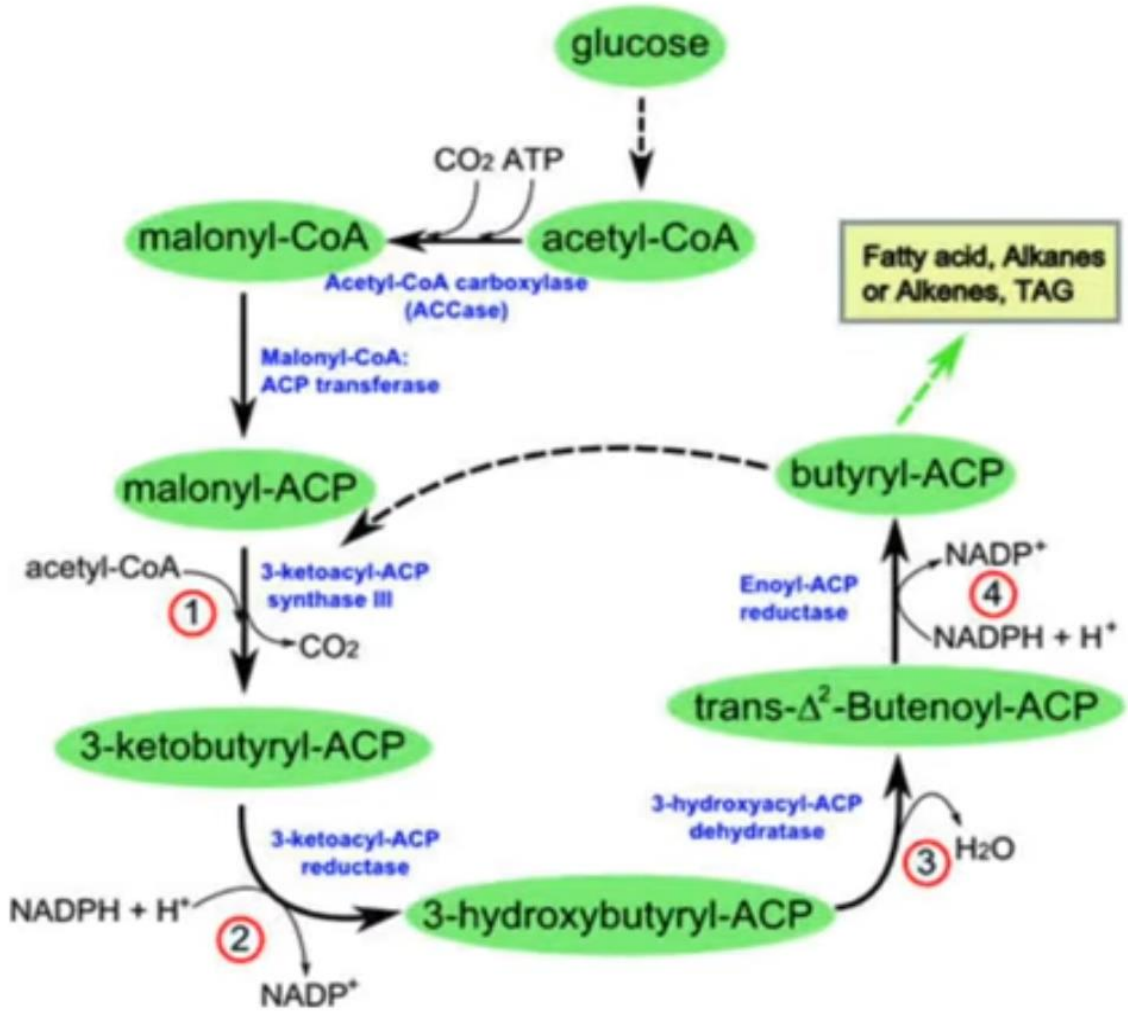
SYNTHESIS

- Cytosol
- Requires NADPH
- Acyl carrier protein
- D-isomer
- CO_2 activation
- Citrate ion
- Multi-enzyme complex
- 2 carbon units added, as 3 carbon malonyl CoA

DEGRADATION

- Mitochondria
- NADH, $FADH_2$
- CoA
- L-isomer
- No CO_2
- No citrate
- Enzymes as independent proteins
- 2 carbon units split off as acetyl CoA

Fatty Acids Synthesis



Fatty Acids Synthesis

Glucose ⬆️

Glycolysis
in Cytoplasm

2 Pyruvate

in Mitochondria

Acetyl COA (2C) + Oxaloacetate (4C)

(in Mitochondria)

Citrate Shuttle | Citrate synthase

Citrate (6C)

(in Cytoplasm)

ATP → ADP

Citrate Lyase

Acetyl COA (2C) + Oxaloacetate (4C)

(in cytoplasm)

Malonyl COA (3C)

ADP, ATP, CO_2

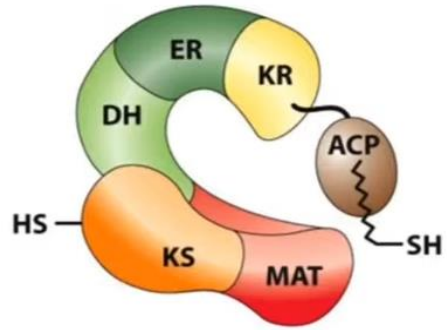
Acetyl COA Carboxylase | Biotin (B7)

stimulate by insulin

in Cytoplasm

inhibition by epinephrine, norepinephrine, glucagon, Cortisol

Fatty acids synthase



4 Starting Material

1_ Acetyl COA (in Cytoplasm)

2_ Malonyl COA

3_ NADPH

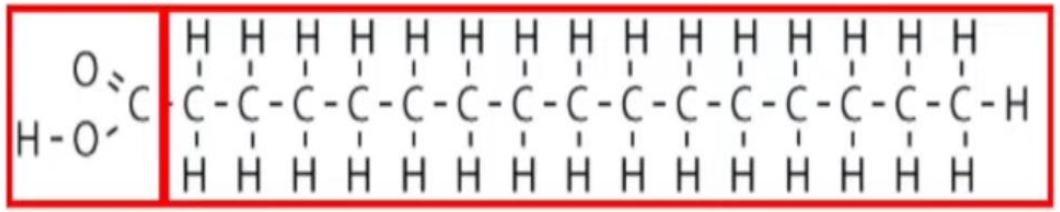
4_ Fatty acids synthase

Pyruvate

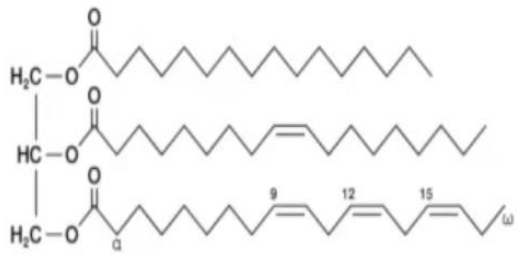
HMP Shunt

NADPH

- Fatty Acid
  - Carboxylic Acid
  - Chain of Hydrocarbons



- *Amphiphilic*
- Synthesis Requires:
  - 1) Acetyl CoA - cytoplasm
  - 2) NADPH (Pentose Phosphate Pathway)
  - *Occurs in the cytosol*
- Stored as Triacylglycerols (Triglycerides)
  - Adipose Tissue
  - Anhydrous
    - ↑ Energy/Mass



Biosynthesis

Fatty Acid biosynthesis is a stepwise assembly of acetyl-CoA units (in the form of malonyl-CoA units) ending with Palmitate (C-16).

It includes 3 steps :

The sites of Fatty acid synthesis are...

Liver

Kidney

Adipose tissue

Lactating mammary glands

Fatty acid synthesis occurs in three phases:

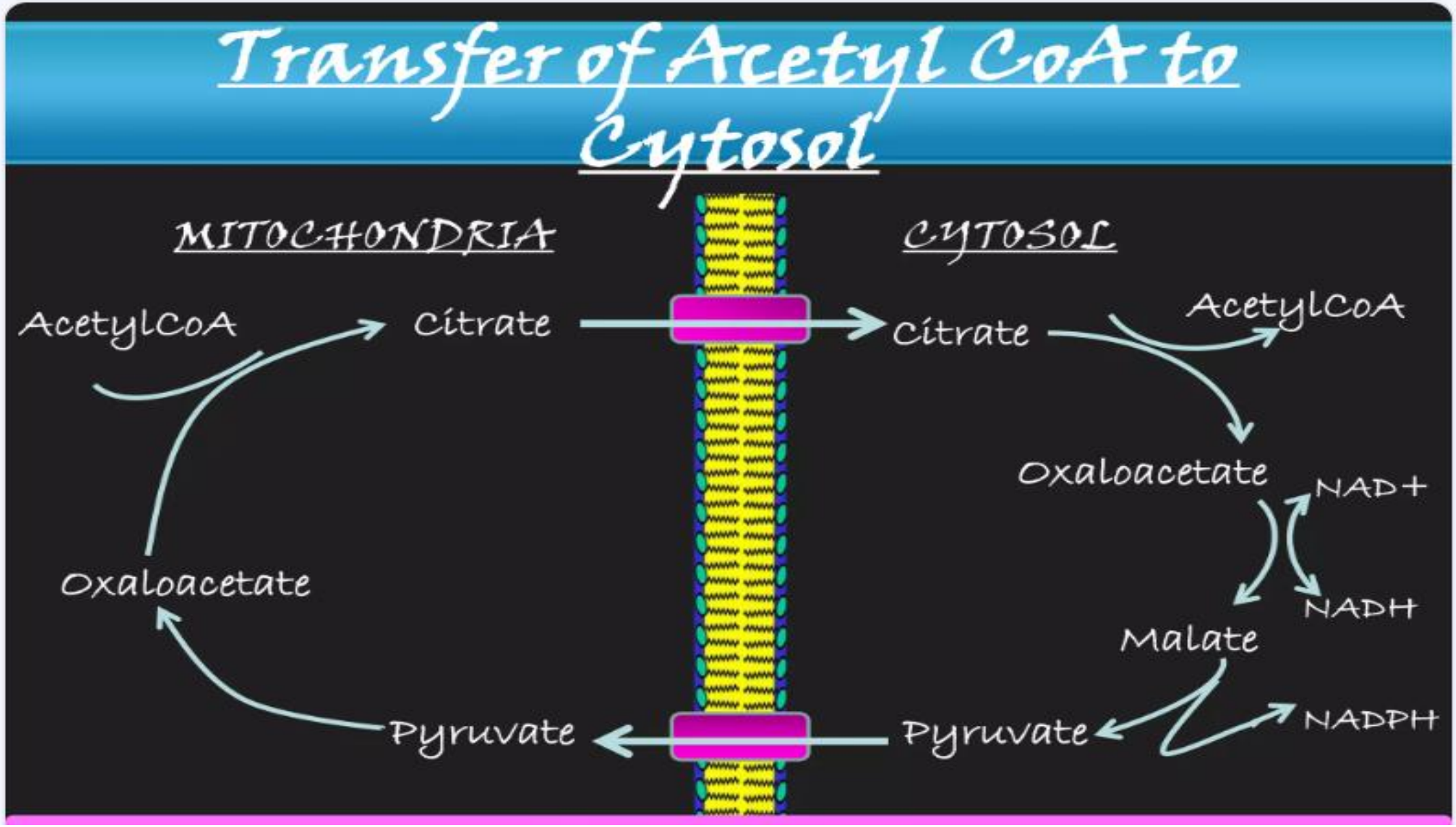
1. Transport of acetyl-CoA from mitochondria to cytosol.

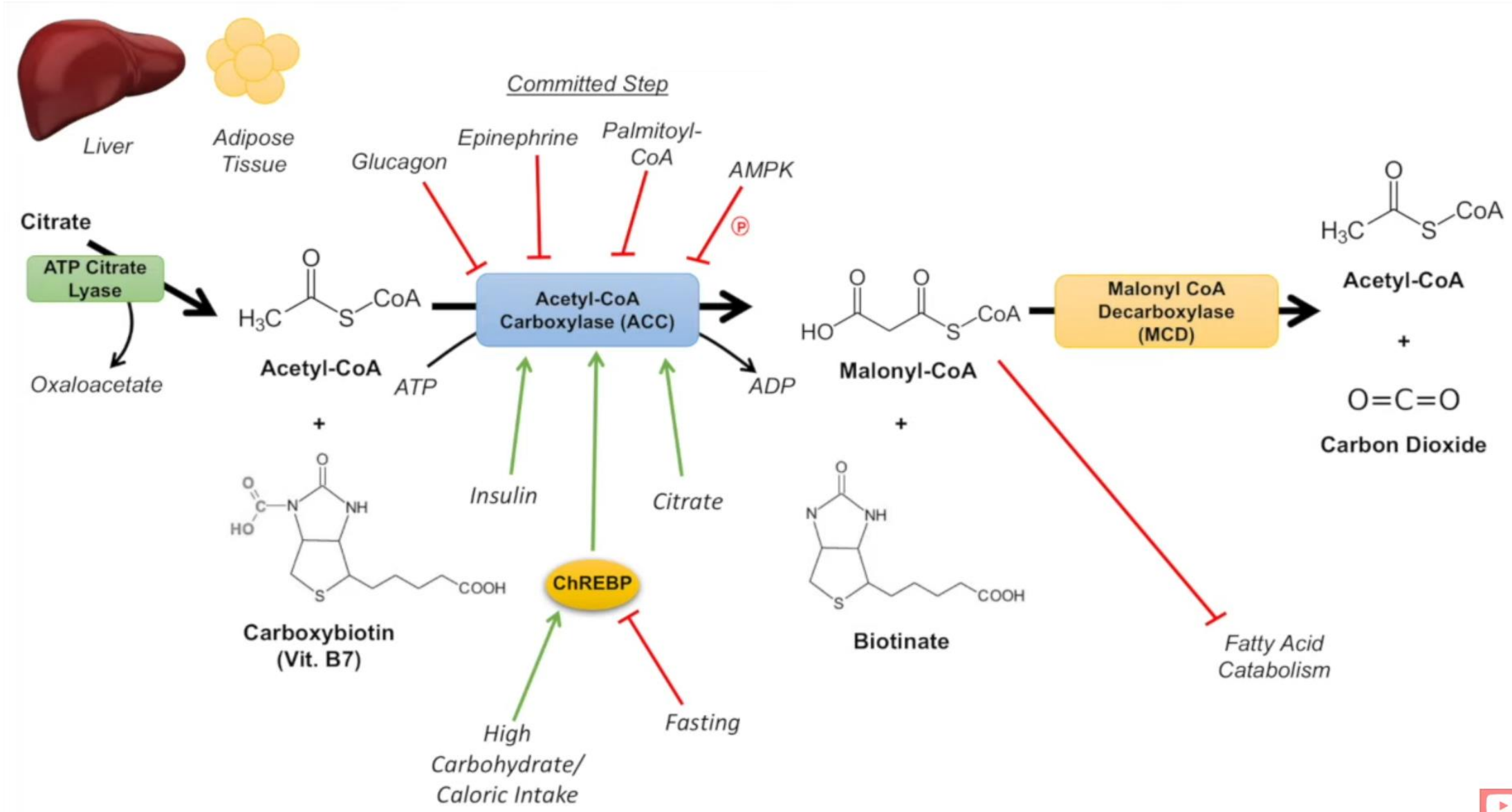
2. Carboxylation of acetyl-CoA to malonyl-CoA.

3. Reactions of fatty acid synthase complex.

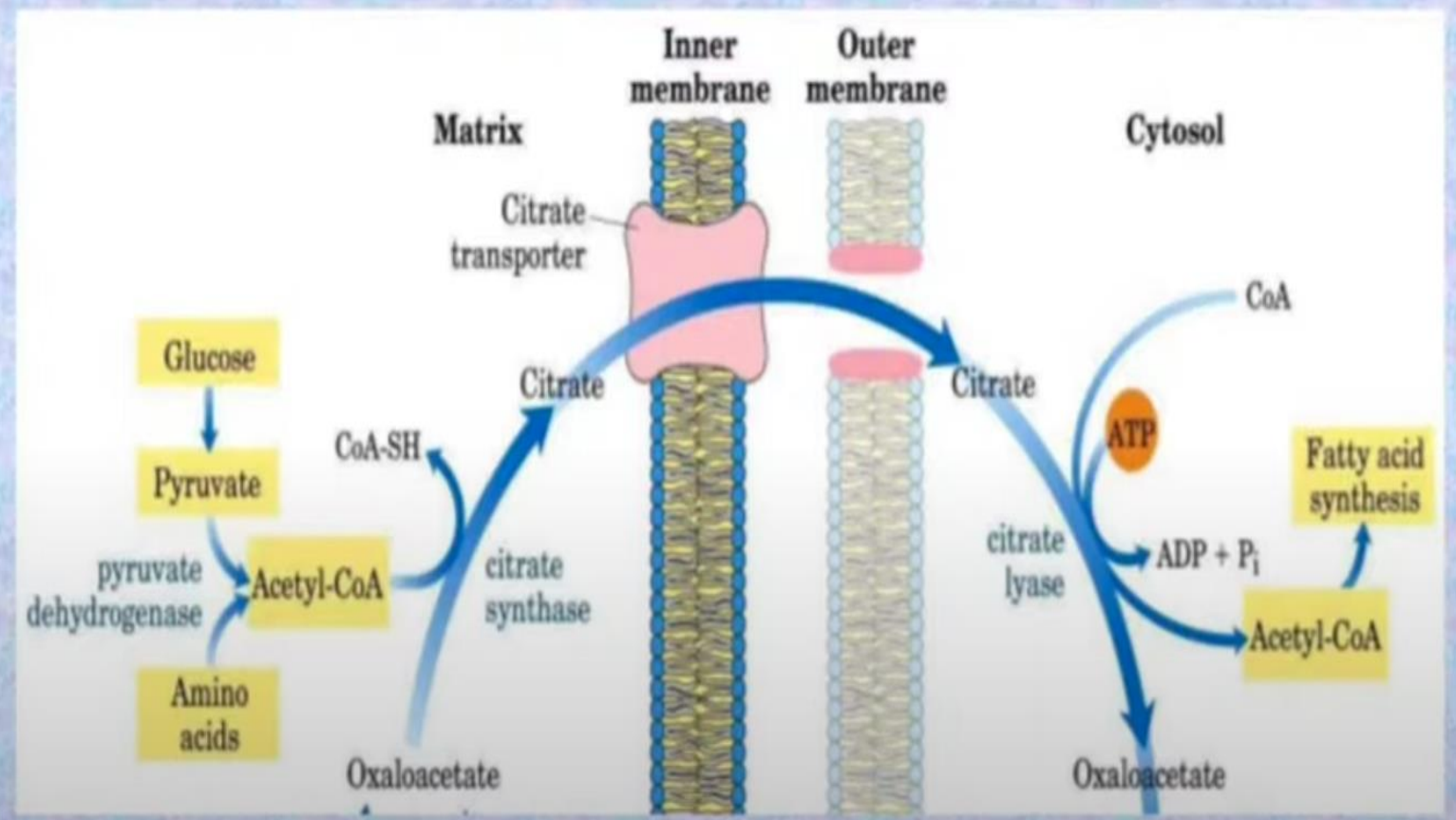
There are three systems for the synthesis of fatty acids

1. De novo synthesis of FAs **in cytoplasm**
2. Chain elongation **in mitochondria**
3. Chain elongation **in microsomes**


Transfer of Acetyl CoA to Cytosol
MITOCHONDRIA
CYTOSOL
AcetylCoA
Citrate
Citrate
AcetylCoA
Oxaloacetate
NAD+
NADH
Malate
NADPH
Pyruvate
Pyruvate
Oxaloacetate


Liver
Adipose Tissue
Committed Step
Glucagon
Epinephrine
Palmitoyl-CoA
AMPK
Citrate
ATP Citrate Lyase
Oxaloacetate
Acetyl-CoA
Acetyl-CoA Carboxylase (ACC)
ATP
ADP
Malonyl-CoA
Malonyl CoA Decarboxylase (MCD)
Acetyl-CoA
Carbon Dioxide
Carboxybiotin (Vit. B7)
Insulin
Citrate
ChREBP
Biotinate
Fatty Acid Catabolism
High Carbohydrate/ Caloric Intake
Fasting
SUBSCRIBE

TRANSPORTATION OF ACETYL CoA



Enzymes and Cofactors involved in fatty acids synthesis

Two main enzymes-

Acetyl co A carboxylase

Fatty acid Synthase

Both the enzymes are multi enzyme complexes

Coenzymes and cofactors are-

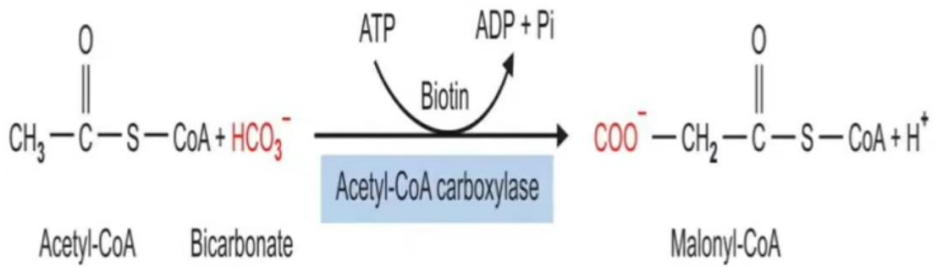
Biotin

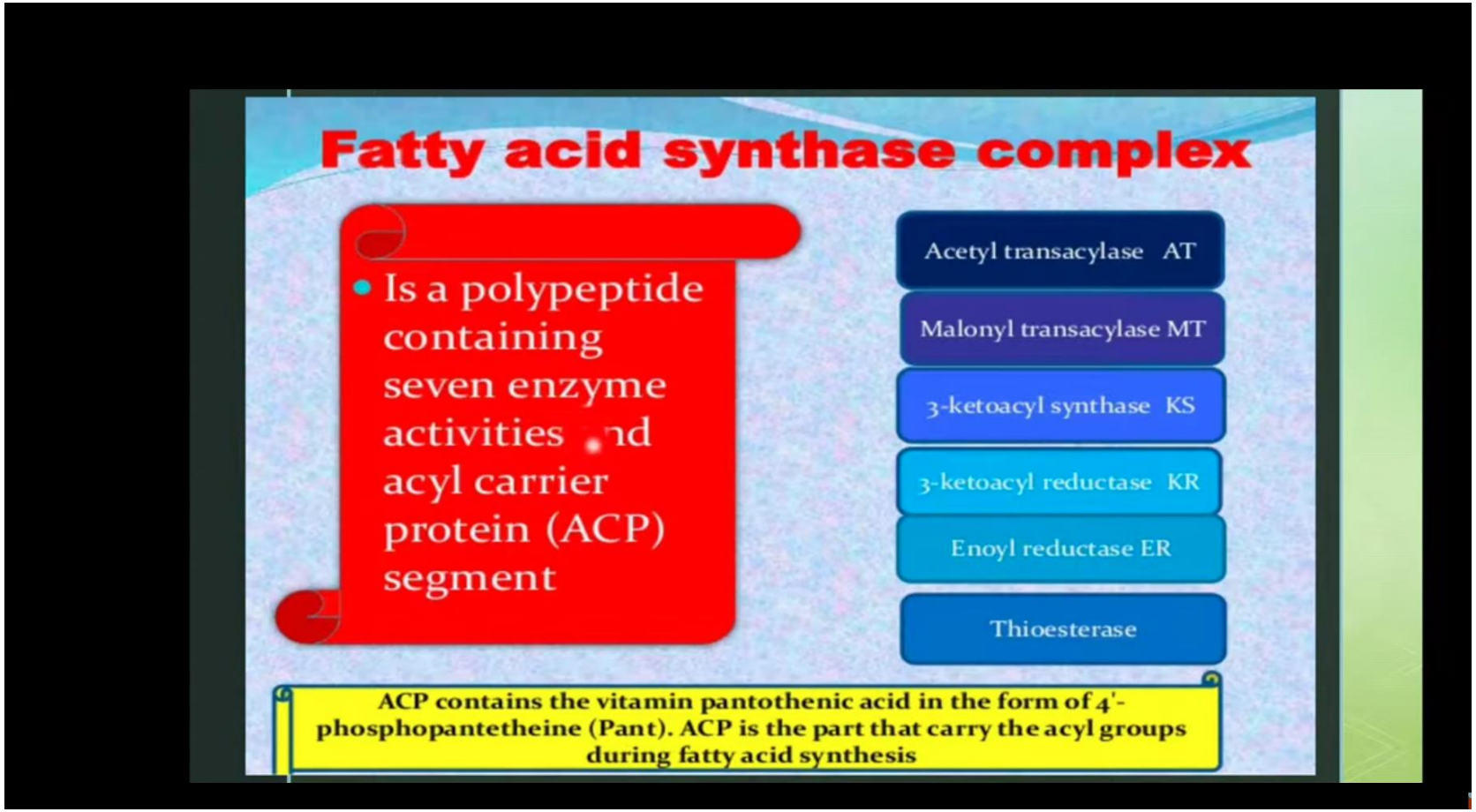
NADPH

Mn++

Mg++

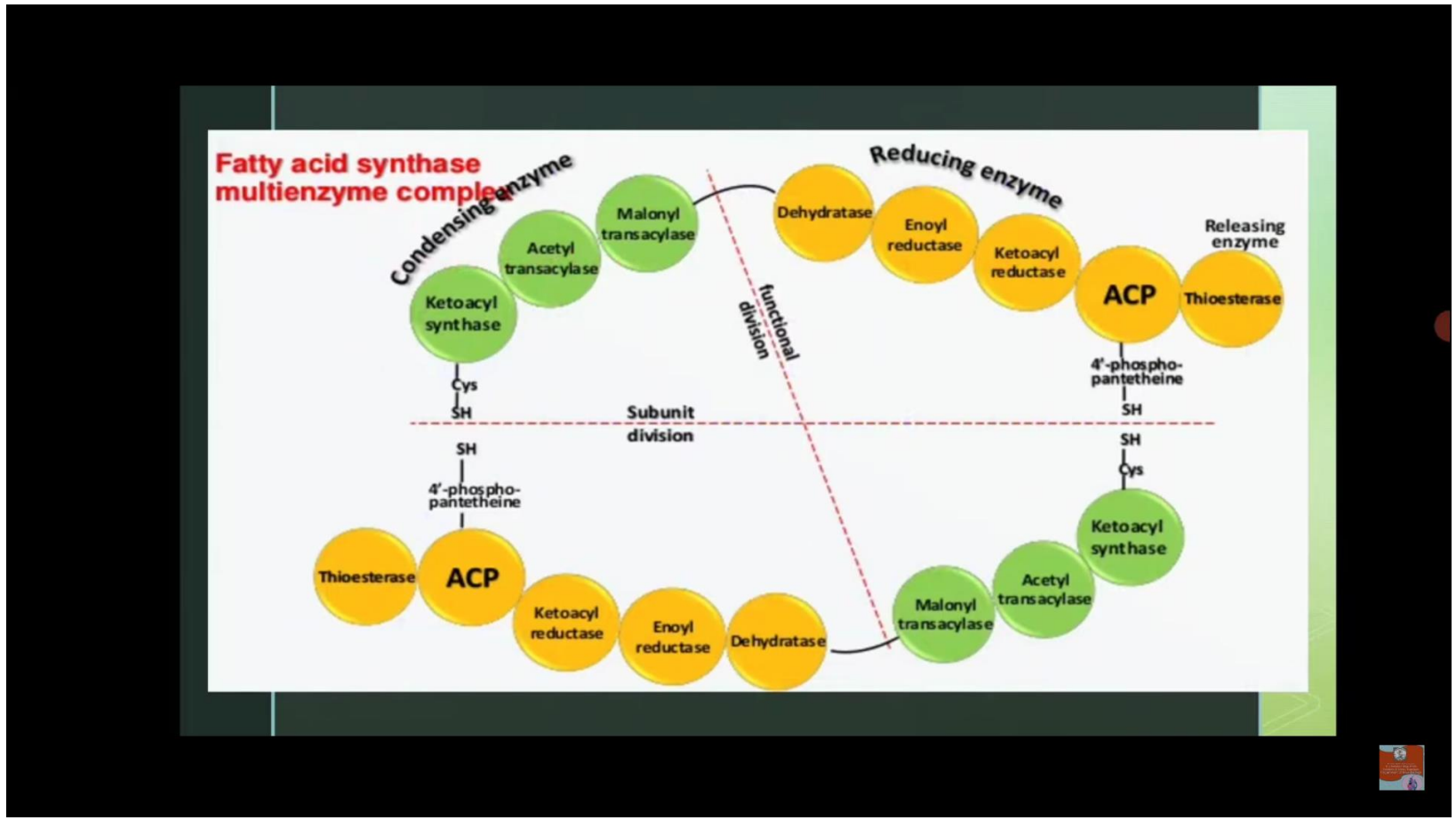
2. Carboxylation of Acetyl CoA to Malonyl CoA




Fatty acid synthase complex
Is a polypeptide containing seven enzyme activities and acyl carrier protein (ACP) segment
Acetyl transacylase AT
Malonyl transacylase MT
3-ketoacyl synthase KS
3-ketoacyl reductase KR
Enoyl reductase ER
Thioesterase
ACP contains the vitamin pantothenic acid in the form of 4'-phosphopantetheine (Pant). ACP is the part that carry the acyl groups during fatty acid synthesis

Reactions of fatty acid synthase complex

- Fatty acid synthase (FAS) - multifunctional enzyme.
- In eukaryotic cells, fatty acid synthase exists as a dimer with two identical units.
- Each monomer possesses the activities of seven different enzymes & an acyl carrier protein (ACP) bound to 4'-phosphopantetheine.
- Fatty acid synthase functions as a single unit catalyzing all the seven reactions.


Fatty acid synthase multienzyme complex
Condensing enzyme
Malonyl transacylase
Acetyl transacylase
Ketoacyl synthase
Cys
SH
Reducing enzyme
Dehydratase
Enoyl reductase
Ketoacyl reductase
ACP
Releasing enzyme
Thioesterase
4'-phospho-pantetheine
SH
functional division
Subunit division
SH
4'-phospho-pantetheine
Thioesterase
ACP
Ketoacyl reductase
Enoyl reductase
Dehydratase
SH
Cys
Ketoacyl synthase
Acetyl transacylase
Malonyl transacylase

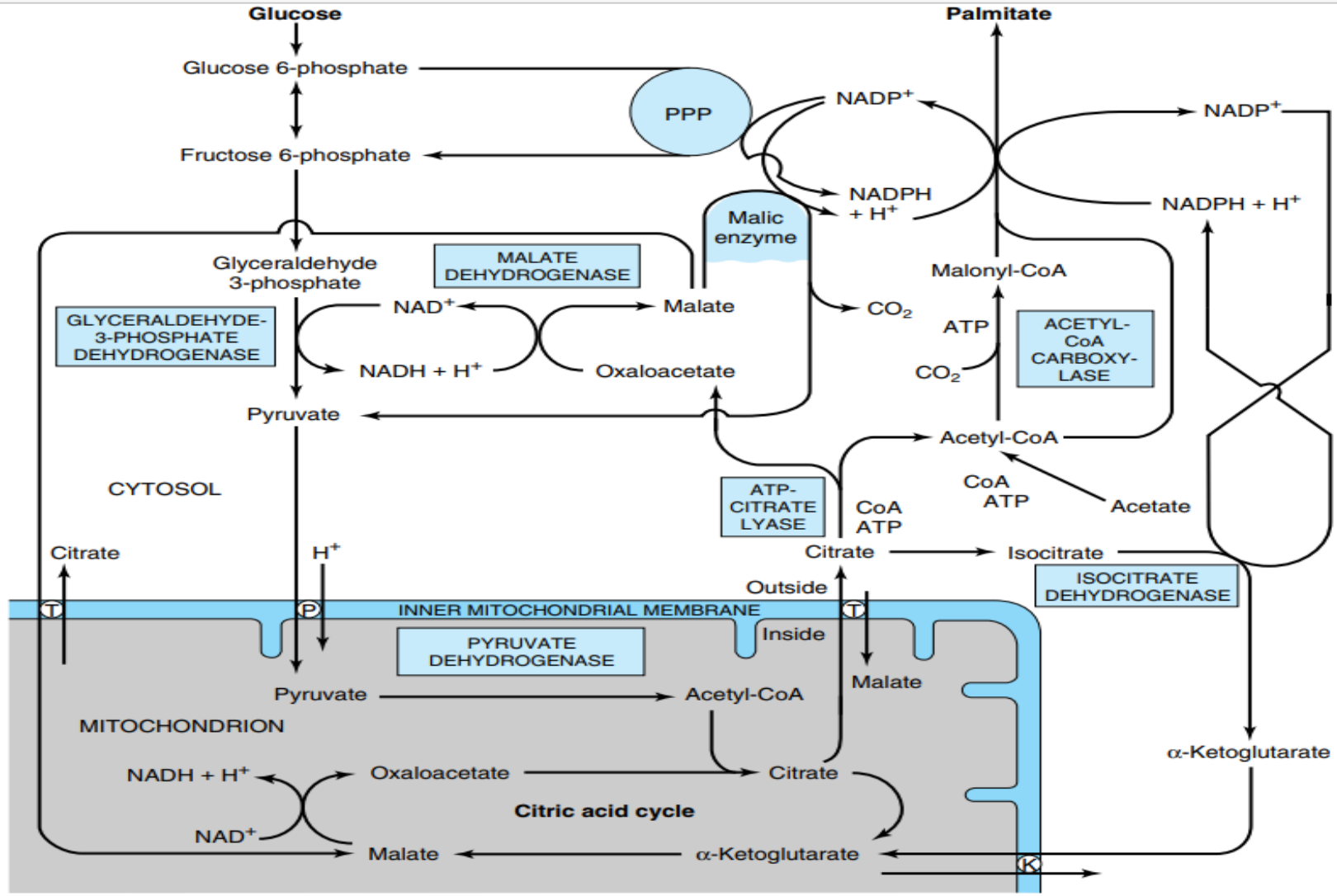
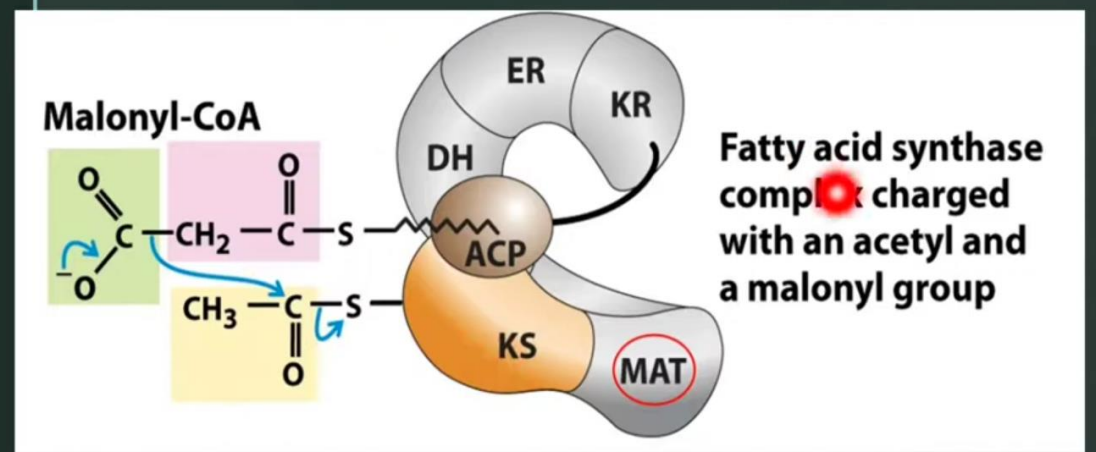


Figure 21–4. The provision of acetyl-CoA and NADPH for lipogenesis. (PPP, pentose phosphate pathway; T, tricarboxylate transporter; K, α-ketoglutarate transporter; P, pyruvate transporter.)


Malonyl-CoA
ER
KR
DH
ACP
KS
MAT
Fatty acid synthase complex charged with an acetyl and a malonyl group

3. Reactions of fatty acid synthase complex

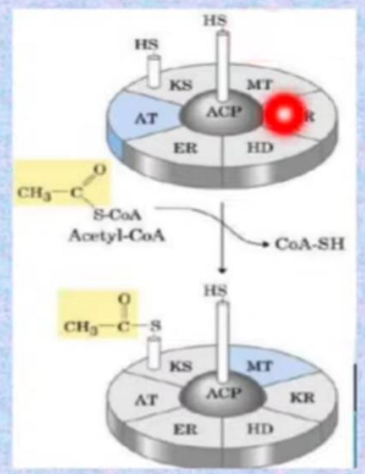
1. Condensation
2. Reduction
3. Dehydration
4. Reduction

The first a round in fatty acids synthesis

- To initiate FA biosynthesis, malonyl and acetyl groups are activated on to the enzyme **fatty acid synthase**.
- Initially, a priming molecule of acetyl-CoA combines with a cysteine —SH group catalysed by **acetyl transacylase**.
- Malonyl-CoA combines with the adjacent —SH on the 4'-phosphopantetheine of ACP of the other monomer, catalyzed by **malonyl transacylase** (to form acetyl (acyl)-malonylenzyme.

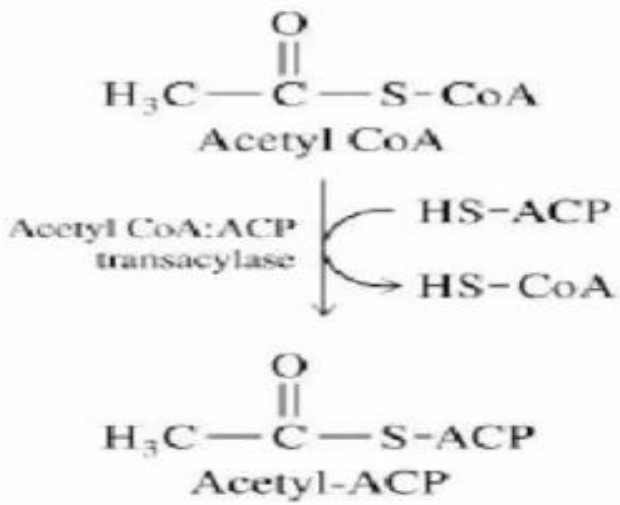
The activation of molnyl group

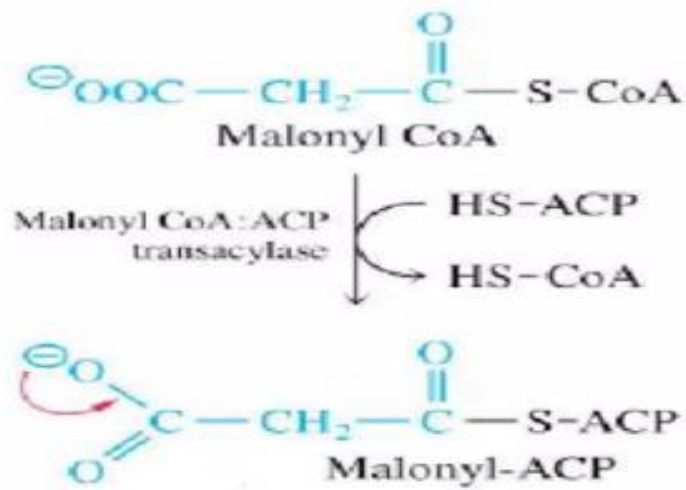
- The acetyl group from acetyl-CoA is transferred to the Cys-SH group of the-ketoacyl ACP synthase
- This reaction is catalyzed by acetyl- CoA transacetylase.



Activation

- ✓ Fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP.
- ✓ ***Acetyl transacylase*** and ***malonyl transacylase*** catalyze these reactions.
- ✓ **Acetyl CoA + ACP ⇔ acetyl ACP + CoA**
  Malonyl CoA + ACP ⇔ malonyl ACP + CoA

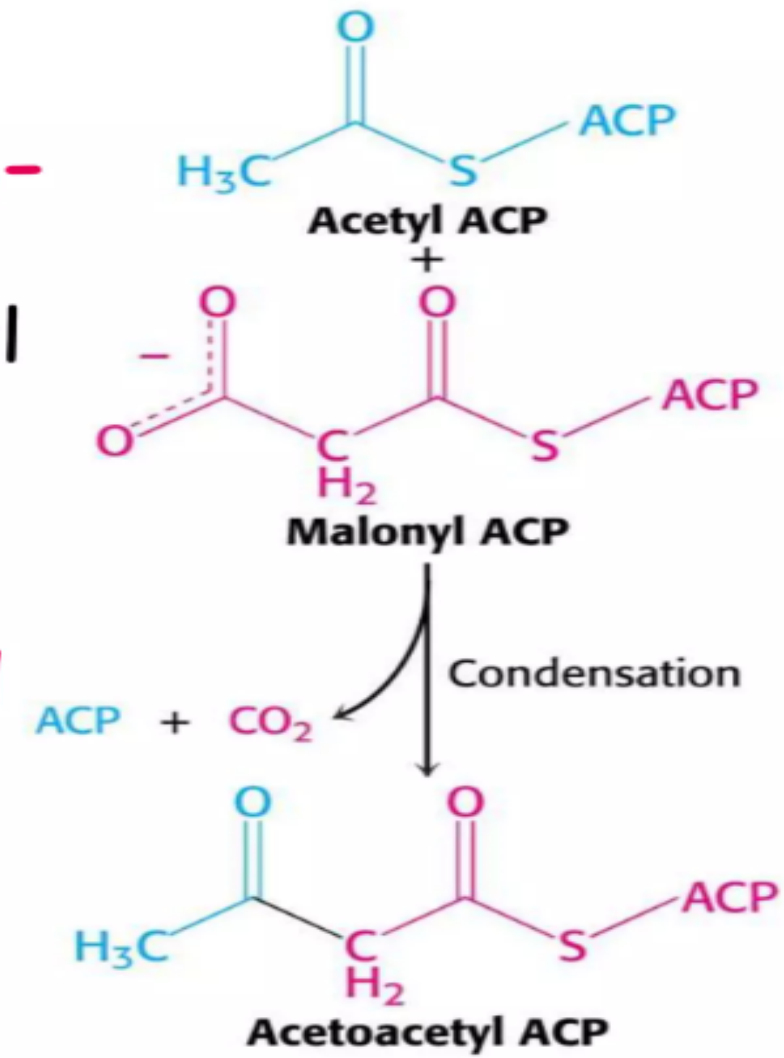




Condensation reaction-

Acetyl ACP and malonyl ACP react to form acetoacetyl ACP.

Enzyme - ***acyl-malonyl ACP condensing enzyme.***

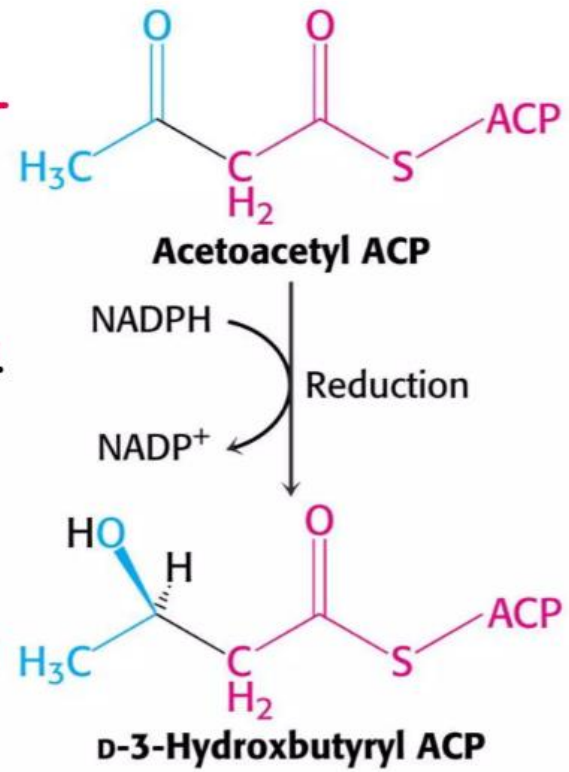


Reduction Reaction-

Acetoacetyl ACP is reduced to D-3-hydroxybutyryl ACP.

NADPH is the reducing agent

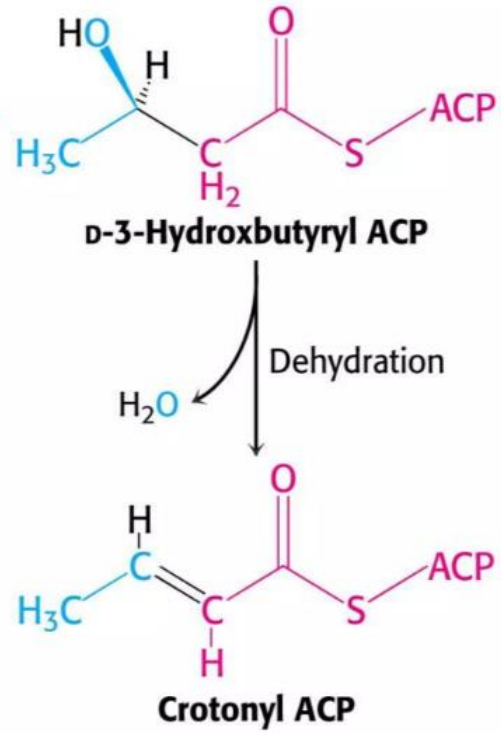
Enzyme: *β-ketoacyl ACP reductase*



Dehydration Reaction-

D-3-hydroxybutyryl ACP is *dehydrated* to form crotonyl ACP (trans-Δ^2-enoyl ACP).

Enzyme:
3-hydroxyacyl ACP dehydratase



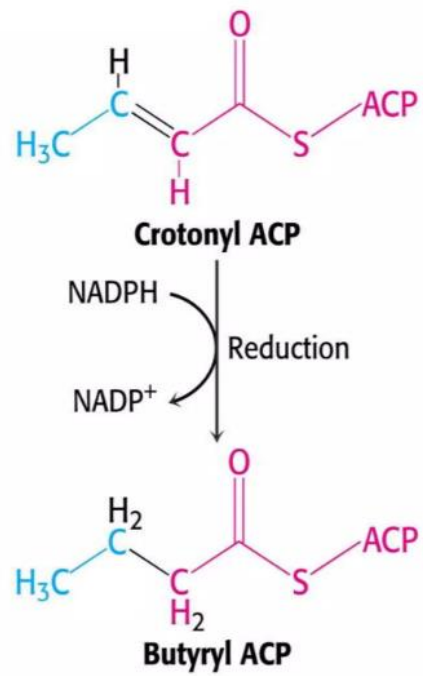
Reduction Reaction-

The final step in the cycle reduces crotonyl ACP to **butyryl ACP.**

NADPH is reductant.

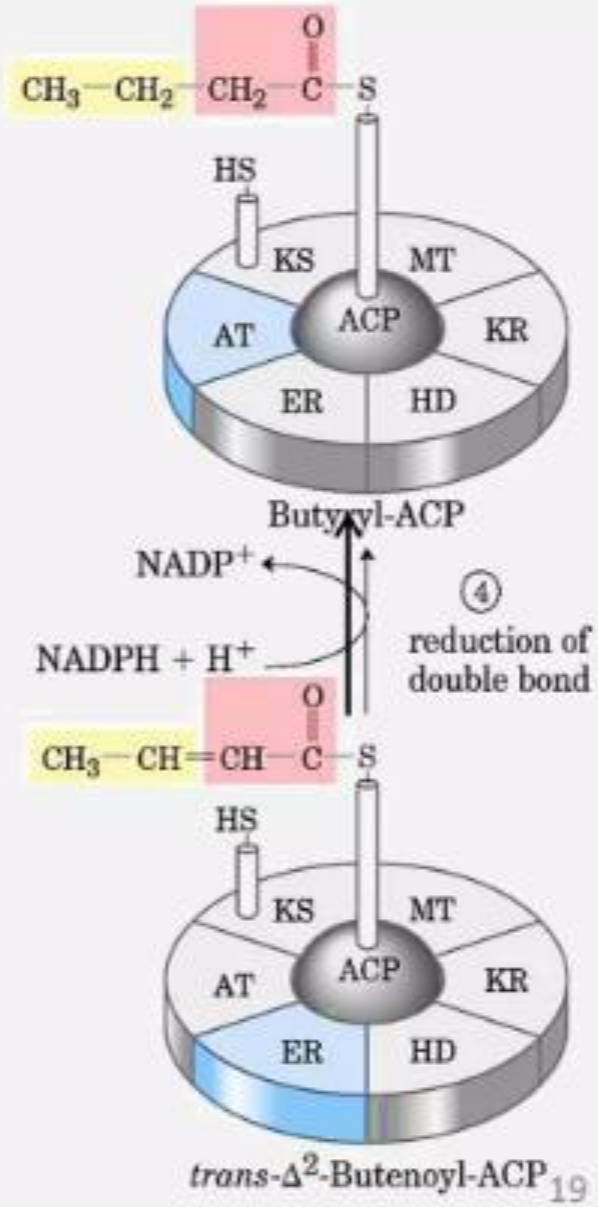
Enzyme - ***enoyl ACP reductase.***

This is the end of first elongation cycle (first round).



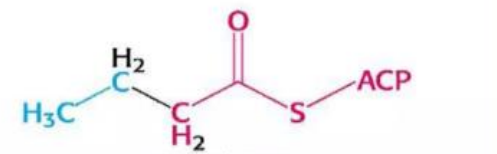
Step 4: Reduction

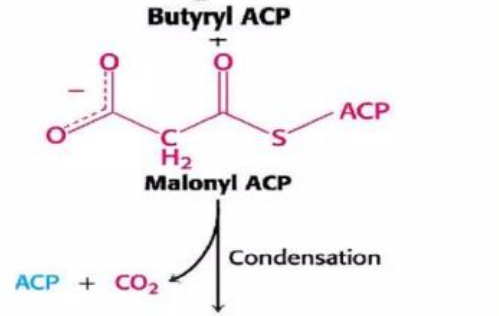
- Reduction of the double bond takes place to form butyryl-ACP.
- Reaction is catalyzed by enoyl-reductase.
- NADPH dependent reaction.



In the second round butyryl ACP condenses with malonyl ACP to form a C_6-β-ketoacyl ACP.

Reduction, dehydration, and a second reduction convert the C_6-β-ketoacyl ACP into a C_6-acyl ACP, which is ready for a third round of elongation.

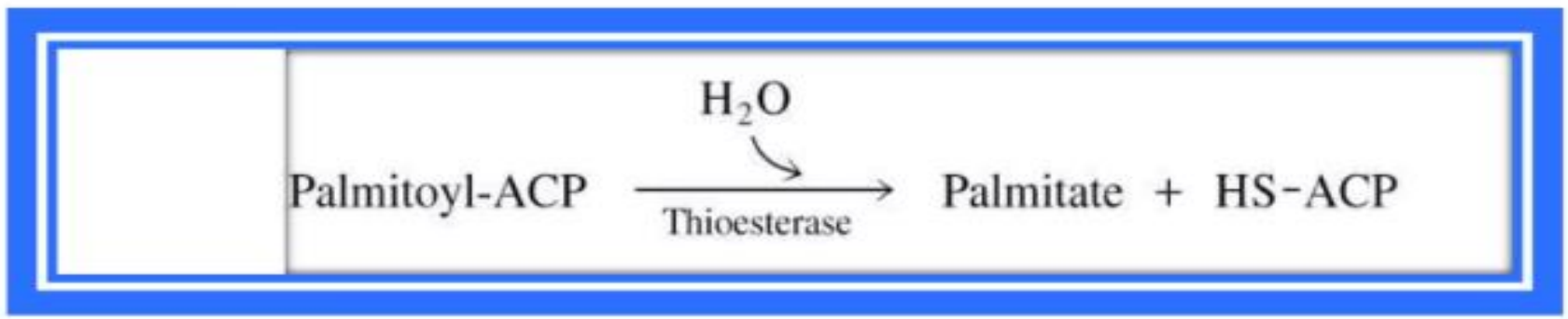




Termination

- Rounds of synthesis continue until a C_{16} palmitoyl group is formed

- Palmitoyl-ACP is hydrolyzed by a ***thioesterase***



Net Production

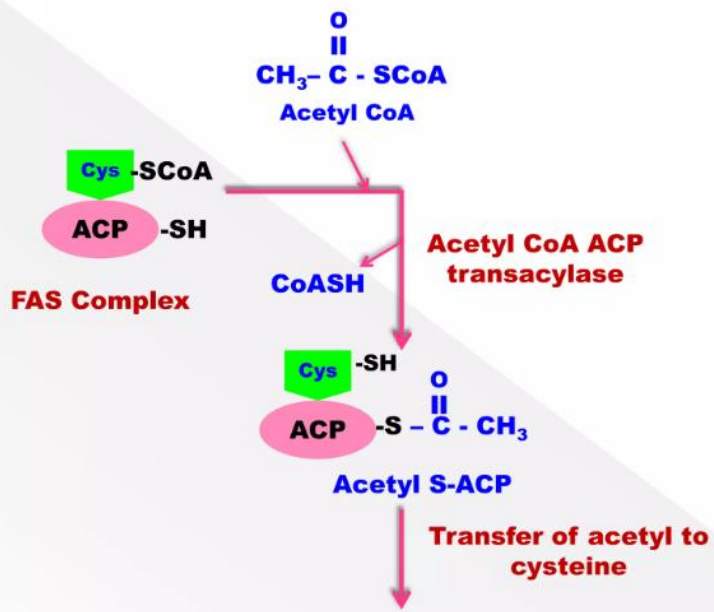
- **Net reaction-**

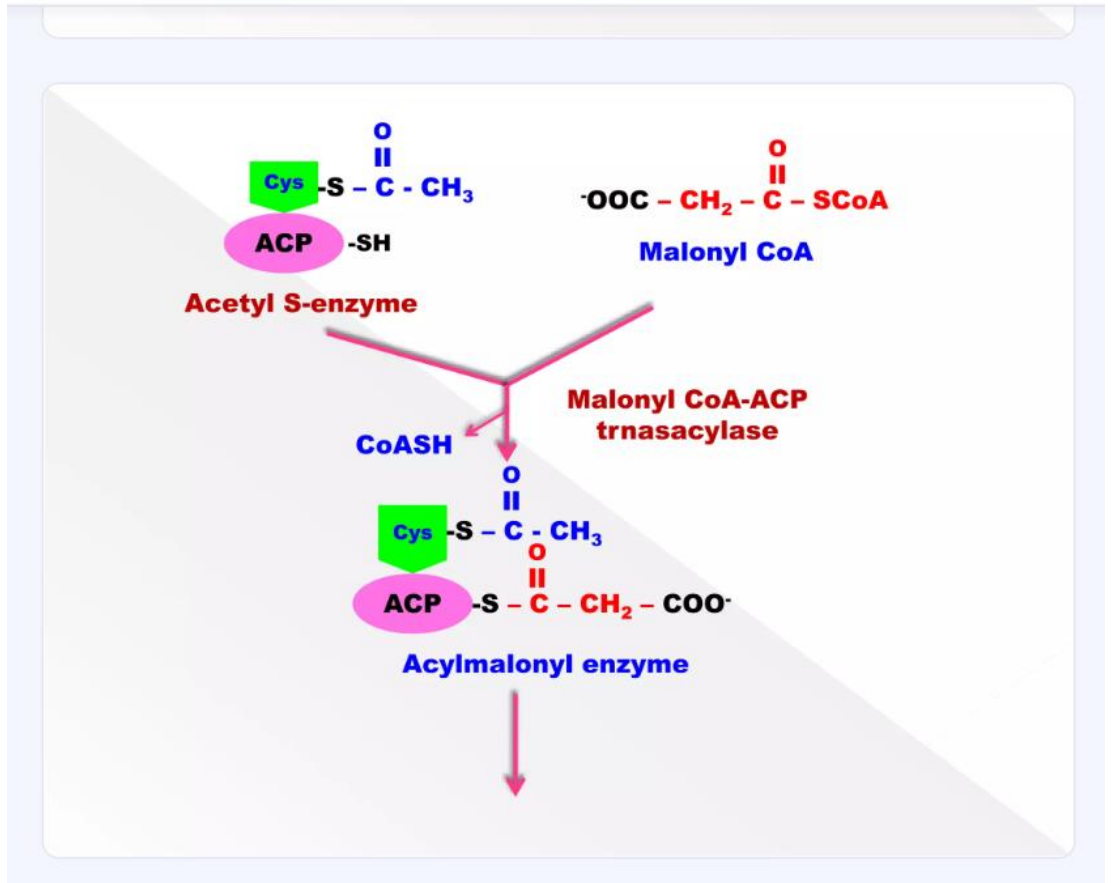
$$8\,\text{acetyl-CoA} + 14\,\text{NADPH} + 7\,\text{ATP} \rightarrow \text{palmitate} + 14\,\text{NADP}^{+} + 8\,\text{CoA} + 7\,\text{ADP} + 7\,\text{P}_i$$

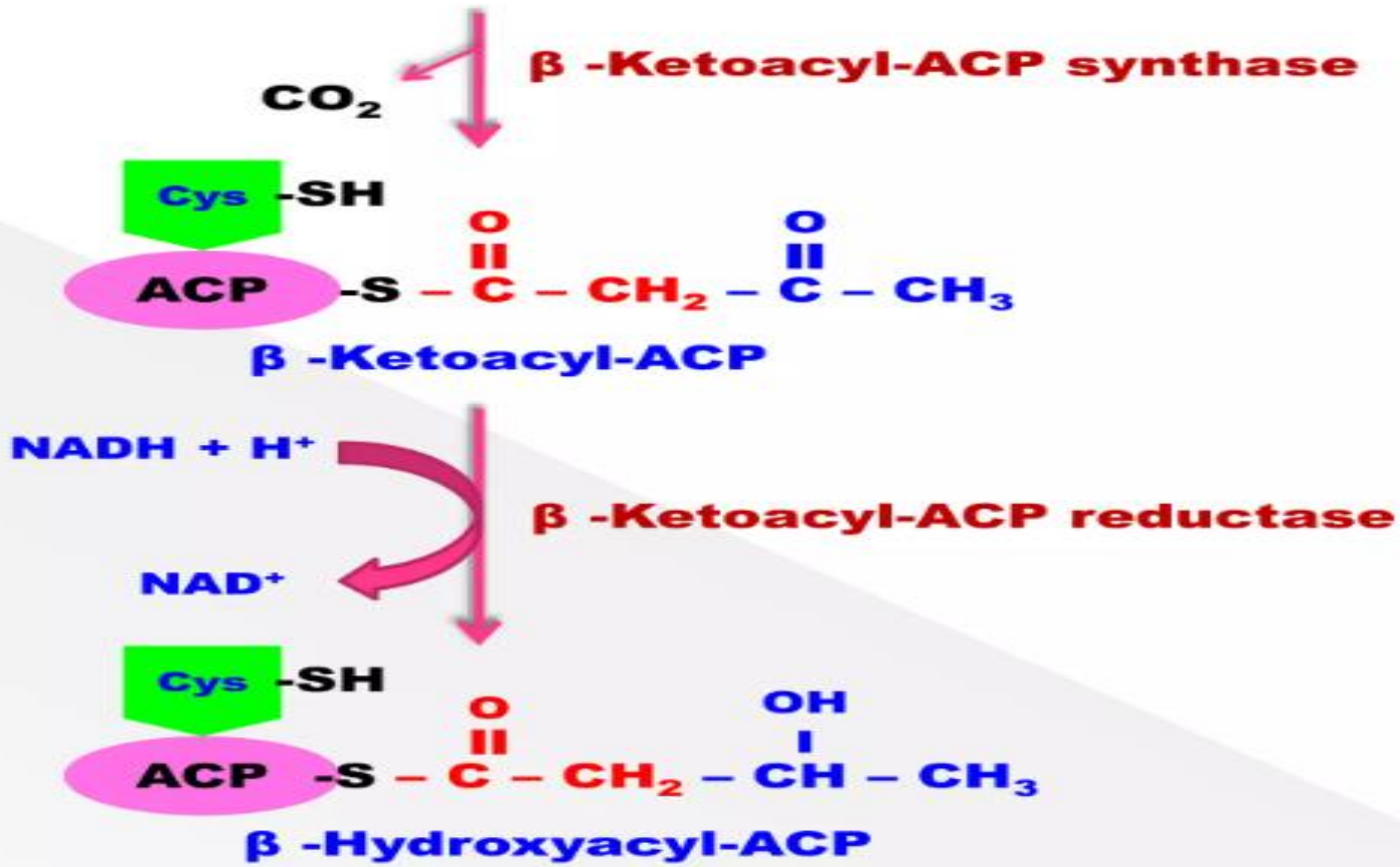
- **Over all Net Reaction-**

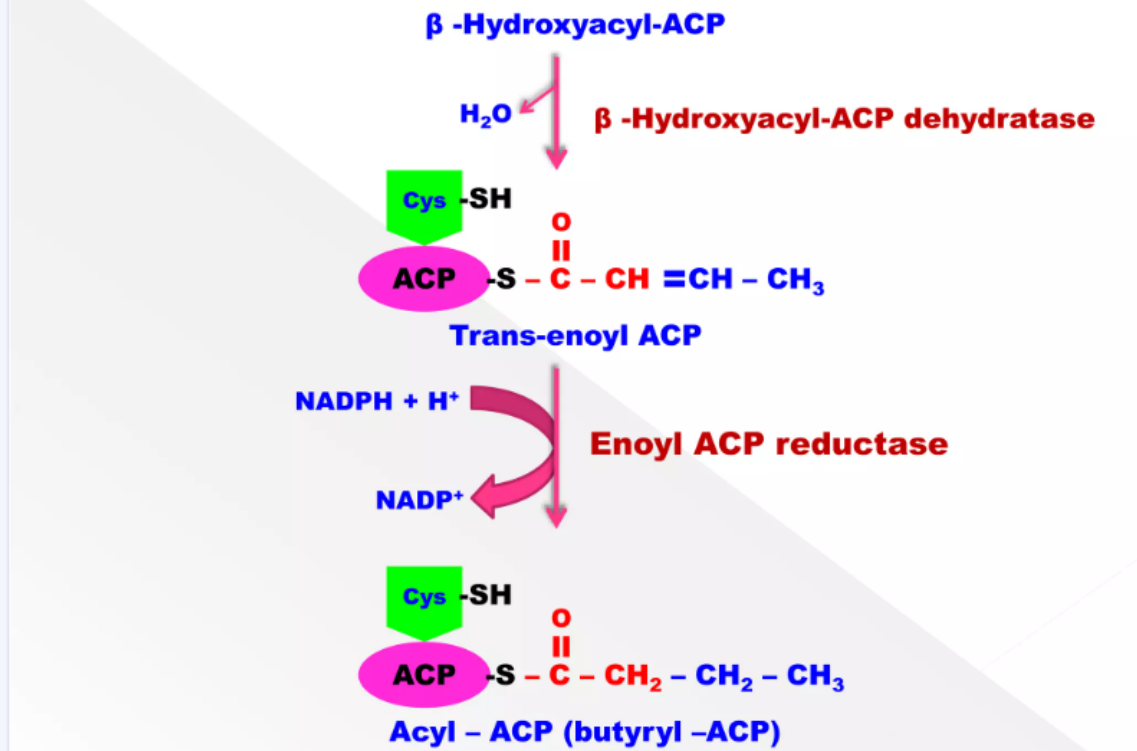
$$\text{acetyl-CoA} + 7\,\text{malonyl-CoA} + 14\,\text{NADPH} \rightarrow \text{palmitate} + 7\,\text{CO}_2 + 14\,\text{NADP}^{+} + 8\,\text{CoA}$$

De novo synthesis of fatty acids




Cys
-S – C - CH3
ACP
-SH
Acetyl S-enzyme
-OOC – CH2 – C – SCoA
Malonyl CoA
Malonyl CoA-ACP
trnasacylase
CoASH
Cys
-S – C - CH3
ACP
-S – C – CH2 – COO-
Acylmalonyl enzyme

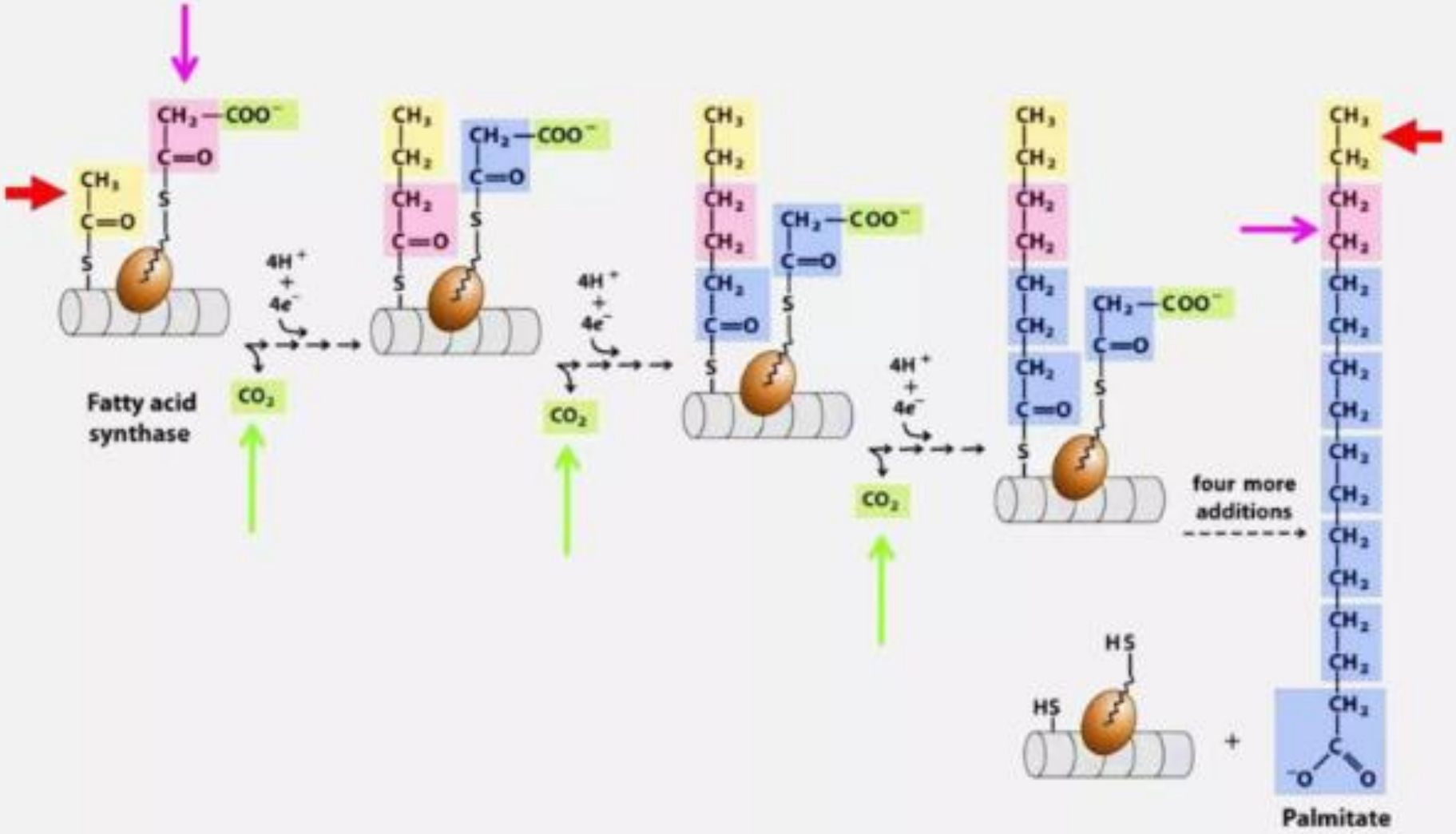

β -Ketoacyl-ACP synthase
CO_2
Cys -SH
ACP -S – C – CH_2 – C – CH_3
β -Ketoacyl-ACP
$NADH + H^+$
β -Ketoacyl-ACP reductase
NAD^+
Cys -SH
ACP -S – C – CH_2 – CH – CH_3
OH
β -Hydroxyacyl-ACP


β -Hydroxyacyl-ACP
H2O
β -Hydroxyacyl-ACP dehydratase
Cys -SH
ACP -S – C – CH =CH – CH3
Trans-enoyl ACP
NADPH + H+
NADP+
Enoyl ACP reductase
Cys -SH
ACP -S – C – CH2 – CH2 – CH3
Acyl – ACP (butyryl –ACP)

▪ Chain Elongation

- A new malonyl-CoA molecule combines with the —SH of 4'-phosphopantetheine, displacing the saturated acyl residue onto the free cysteine —SH group.
- The sequence of reactions are repeated until a saturated 16-carbon acyl radical (Palmityl) has been assembled.
- Catalyzed by enzyme Thioesterase (deacylase).

Chain Elongation

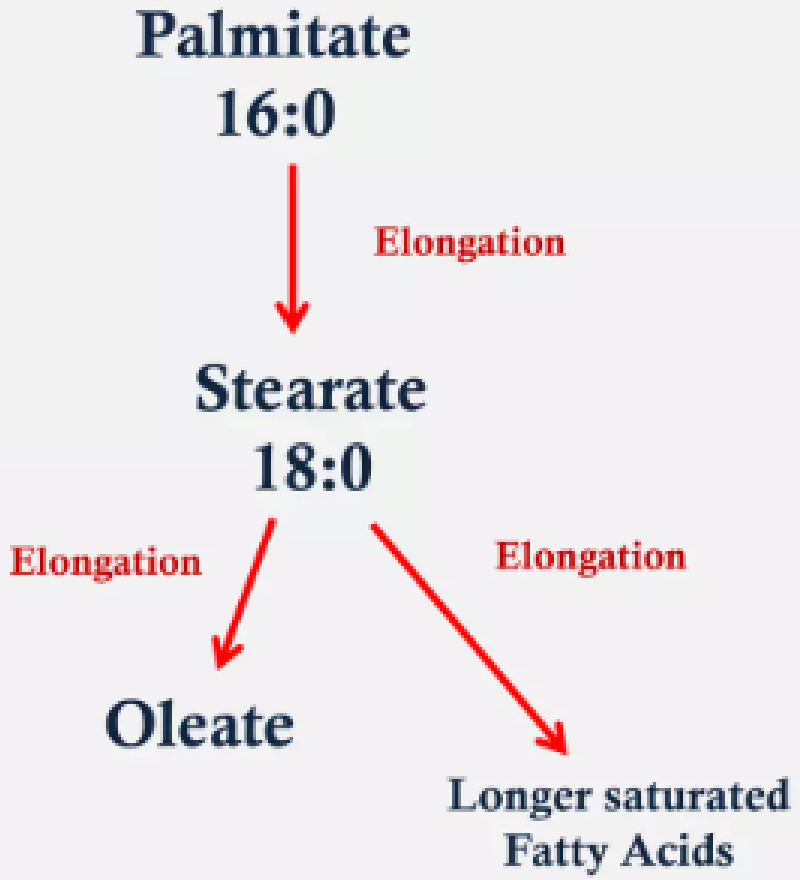


Result of fatty acid synthesis activity

- Seven cycles of condensation and reduction produce the 16-carbon saturated palmitoyl group, still bound to ACP.
- Chain elongation usually stops at this point, and free palmitate is released from the ACP molecule.
- Smaller amounts of longer fatty acids such as stearate (18:0) are also formed.

Fatty Acid elongation

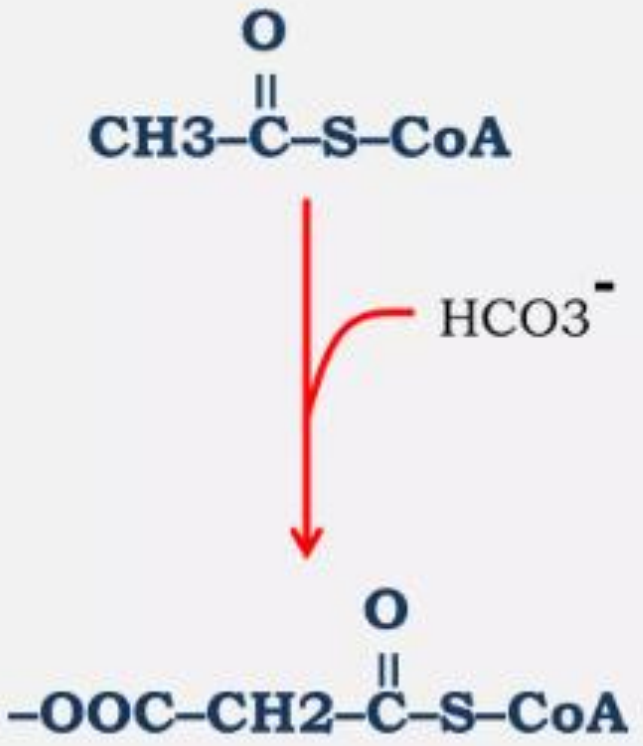
- Palmitate in animal cells is the precursor of other long-chained FAs.
- By further fatty acid chain length is elongated through the action of **FA elongation systems** present in the smooth endoplasmic reticulum and the mitochondria.



Regulation of Fatty acid biosynthesis

- The reaction catalyzed by acetyl- CoA carboxylase is the rate limiting step in the biosynthesis of fatty acids.

- Long-chain fatty acid synthesis is controlled in the short term by allosteric and covalent modification of enzymes and in the long term by changes in gene expression.



Conclusion

- Fatty acid biosynthesis takes place in cytosol and acetyl CoA is immediate substrate.
- Free palmitate is the end product.
- It is an ATP and NADPH dependent reaction.
- Rate of reaction is regulated by acetyl CoA carboxylase.
- **Net Reaction:**

$$8\text{Acetyl CoA} + 7\text{ATP} + 14\text{NADPH} \rightarrow \text{Palmitate} + 14\text{NADP}^{+} + 8\text{CoA} + 6\text{H}_2\text{O} + 7\text{ADP} + 7\text{Pi}$$

- • The synthesis of long-chain fatty acids (lipogenesis) is carried out by two enzyme systems: acetyl-CoA carboxylase and fatty acid synthase.
- • The pathway converts acetyl-CoA to palmitate and requires NADPH, ATP, Mn^{2+}, biotin, pantothenic acid, and HCO_3^- – as cofactors.
- • Acetyl-CoA carboxylase is required to convert acetylCoA to malonyl-CoA. In turn, fatty acid synthase, a multienzyme complex of one polypeptide chain with seven separate enzymatic activities, catalyzes the assembly of palmitate from one acetyl-CoA and seven malonyl-CoA molecules.
- • Lipogenesis is regulated at the acetyl-CoA carboxylase step by allosteric modifiers, phosphorylation/dephosphorylation, and induction and repression of enzyme synthesis. Citrate activates the enzyme, and long-chain acyl-CoA inhibits its activity. Insulin activates acetyl-CoA carboxylase whereas glucagon and epinephrine have opposite actions.