DEPARTMENT OF BIOTECHNOLOGY

Biosynthesis of Fatty Acid

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- De novo synthesis of fatty acids occurs in liver, kidney, adipose tissue & lactating mammary gland.
- Enzymes are located in cytosomal fraction of the cell.
- It is called as extramitochondrial or cytoplasmic fatty acid synthase system.

- Major fatty acid synthesized de novo is palmitic acid ($_{16}$C saturated fatty acid).
- It occurs in liver, adipose tissue, kidney, brain & lactating mammary glands.
- Acetyl CoA is the source of carbon atoms.

- **NADPH provides reducing equivalents – NADPH is produced from HMP shunt & malic enzyme reaction.**
- **Every molecule of acetyl CoA delivered to cytoplasm, one molecule of NADPH is formed.**
- **ATP supplies energy.**

Stages

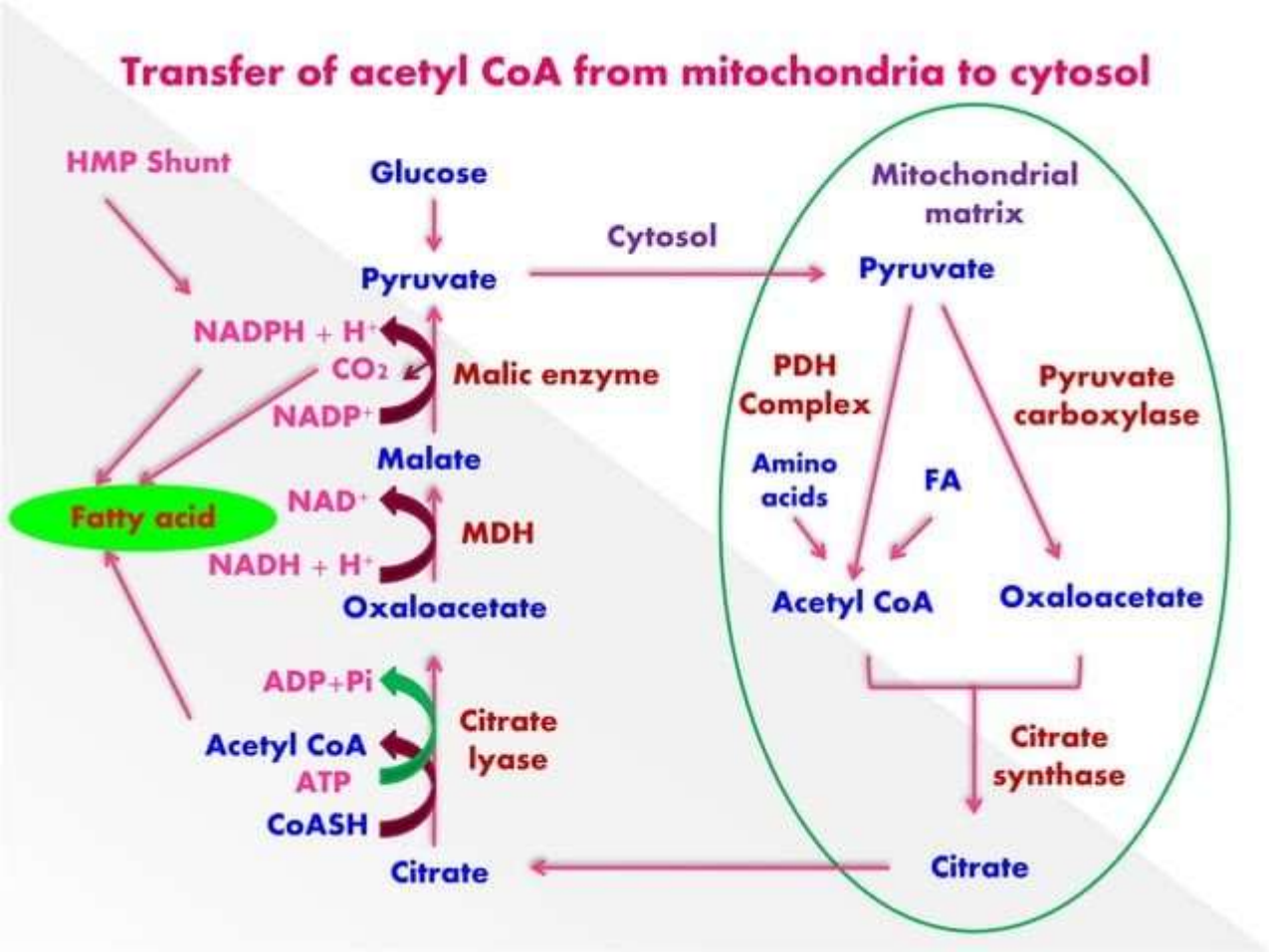
- Production of acetyl CoA & NADPH
- Conversion of acetyl CoA to malonyl CoA
- Reactions of fatty acid synthase complex.

Production of acetyl CoA & NADPH

- Acetyl CoA is the starting material for de novo synthesis of fatty acids.
- Acetyl CoA is produced in the mitochondria by the oxidation of pyruvate, fatty acids, degradation of carbon skeleton of certain amino acids & from ketone bodies.
- Mitochondria are not permeable to acetyl CoA.

- An alternate or a bypass arrangement is made for the transfer of acetyl CoA to cytosol.
- Acetyl CoA condenses with oxaloacetate in mitochondria to form citrate.
- Citrate is freely transported to cytosol by tricarboxylic acid transporter.

- In cytosol it is cleaved by ATP citrate lyase to liberate acetyl CoA & oxaloacetate.
- Oxaloacetate in the cytosol is converted to malate.
- Malic enzyme converts malate to pyruvate.
- NADPH & CO_2 are generated in this reaction.
- Both of them are utilized for fatty acid synthesis

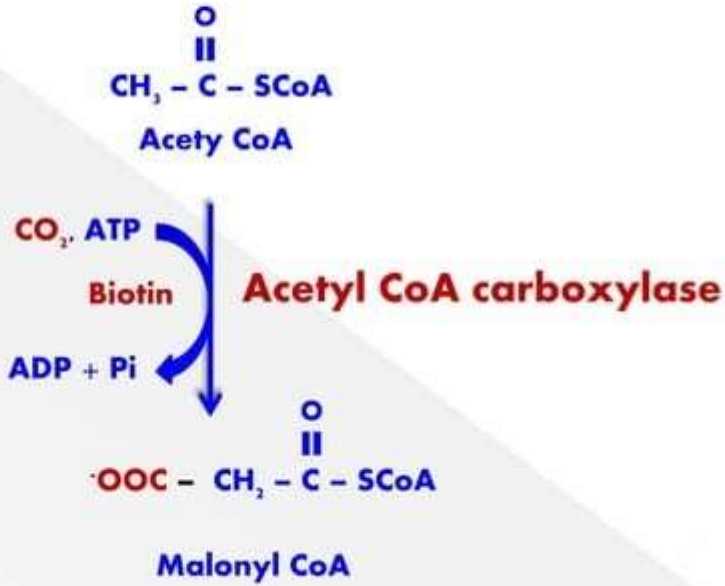

Transfer of acetyl CoA from mitochondria to cytosol
HMP Shunt
Glucose
Cytosol
Mitochondrial matrix
Pyruvate
Pyruvate
NADPH + H+
CO2
NADP+
Malic enzyme
PDH Complex
Pyruvate carboxylase
Amino acids
FA
Malate
Fatty acid
NAD+
NADH + H+
MDH
Acetyl CoA
Oxaloacetate
Oxaloacetate
ADP+Pi
Acetyl CoA
ATP
CoASH
Citrate lyase
Citrate synthase
Citrate
Citrate

- **Advantages of coupled transport of acetyl CoA & NADPH**
- **The transport of acetyl CoA from mitochondria to cytosol is coupled with the cytosomal production of NADPH & CO_2 which is highly advantageous to the cell for optimum synthesis of fatty acids**

Formation of malonyl CoA

- Acetyl CoA is carboxylated to malonyl CoA by the enzyme acetyl CoA carboxylase.
- This is an ATP-dependent reaction & requires biotin for CO_2 fixation.
- The mechanism of action of acetyl CoA carboxylase is similar to that of pyruvate carboxylase.
- Acetyl CoA carboxylase is a regulatory enzyme

Conversion of acetyl CoA to Malonyl CoA



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.

Advantages of Multi-enzyme complex

- Intermediates of the reaction can easily interact with the active sites of the enzymes.
- One gene codes all the enzymes; all enzymes are in equimolecular concentrations.
- The efficiency of the process is enhanced.

FAS Complex

- **First domain or Condensing unit:**
- **It is initial substrate binding site.**
- **The enzymes involved are β-keto acyl synthase or condensing enzyme (CE), acetyl transferase (AT) & malonyl transacylase (MT).**

Second domain or Reduction unit

- It contains the dehydratase (DH), enoyl reductase (ER), β-keto acyl reductase (KR) & acyl carrier protein (ACP)
- The acyl carrier protein is a polypeptide chain having a phospho-pantotheine group, to which acyl groups are attached in thioester linkage.
- ACP acts like CoA carrying fatty acyl groups.

Third domain or releasing unit

- It is involved in the release of synthesized fatty acid in the cytosol.
- Major fatty acid synthesized is palmitic acid.
- It contains thio-esterase(TE) or de-acylase.

Reactions

- The two carbon fragment of acetyl CoA is transferred to ACP of fatty acid synthase, catalyzed by the enzyme - acetyl CoA-ACP transacylase.
- The acetyl unit is then transferred from ACP to cysteine residue of the enzyme.
- The ACP site falls vacant.

- The enzyme malonyl CoA-ACP transacylase transfers malonate from malonyl CoA to bind to ACP.
- The acetyl unit attached to cysteine is transferred to malonyl group (bound to ACP).
- The malonyl moiety loses CO_2 which was added by acetyl CoA carboxylase.
- CO_2 is never incorporated into fatty acid carbon chain.

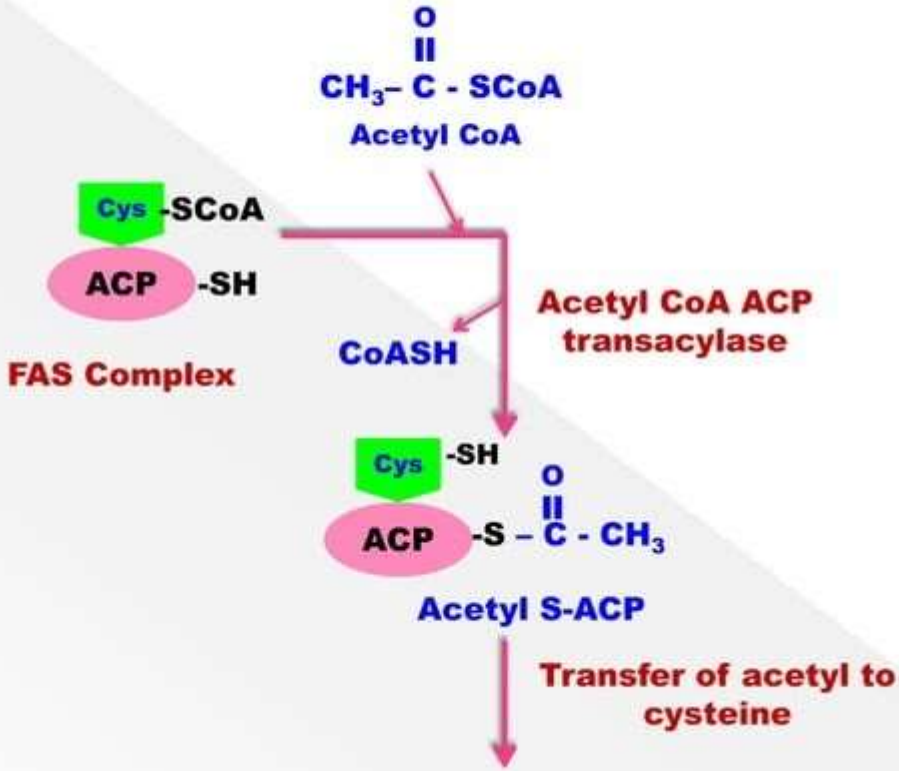
- The decarboxylation is accompanied by loss of free energy which allows the reaction to proceed forward.
- It is catalyzed by β-ketoacyl ACP synthase.
- β -Ketoacyl ACP reductase reduces ketoacyl group to hydroxyacyl group.
- The reducing equivalents are supplied by NADPH.
- β -Hydroxyacyl ACP undergoes dehydration.

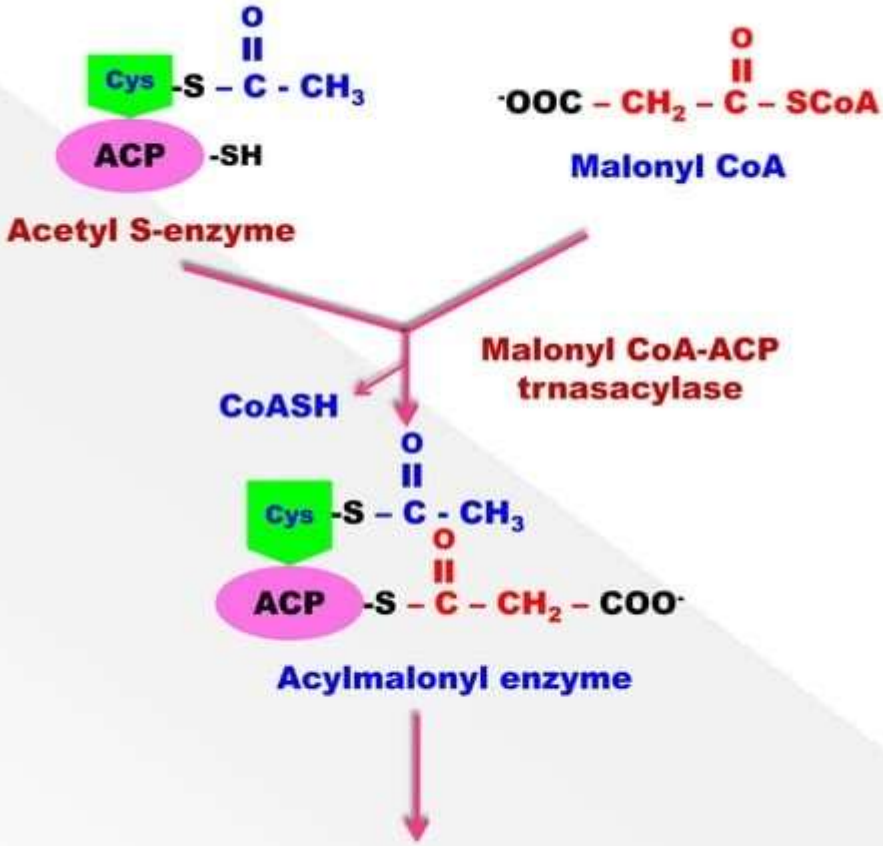
- A molecule of water is eliminated & a double bond is introduced between α & β carbons.
- A second NADPH-dependent reduction, catalysed by enoyl-ACP reductase occurs to produce acyl-ACP.
- The four-carbon unit attached to ACP is butyryl group.

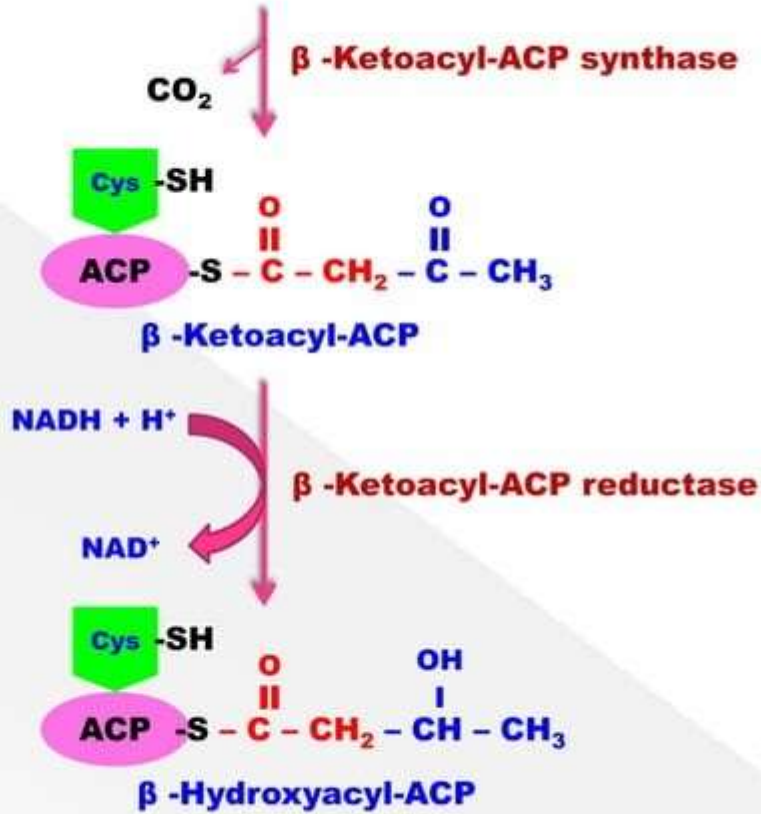
- The carbon chain attached to ACP is transferred to cysteine residue & the reactions of malonyl CoA-ACP transacylase & enoyl-ACP reductase are repeated 6 more times.
- Each time, the fatty acid chain is lengthened by a two-carbon unit (obtained from malonyl CoA).

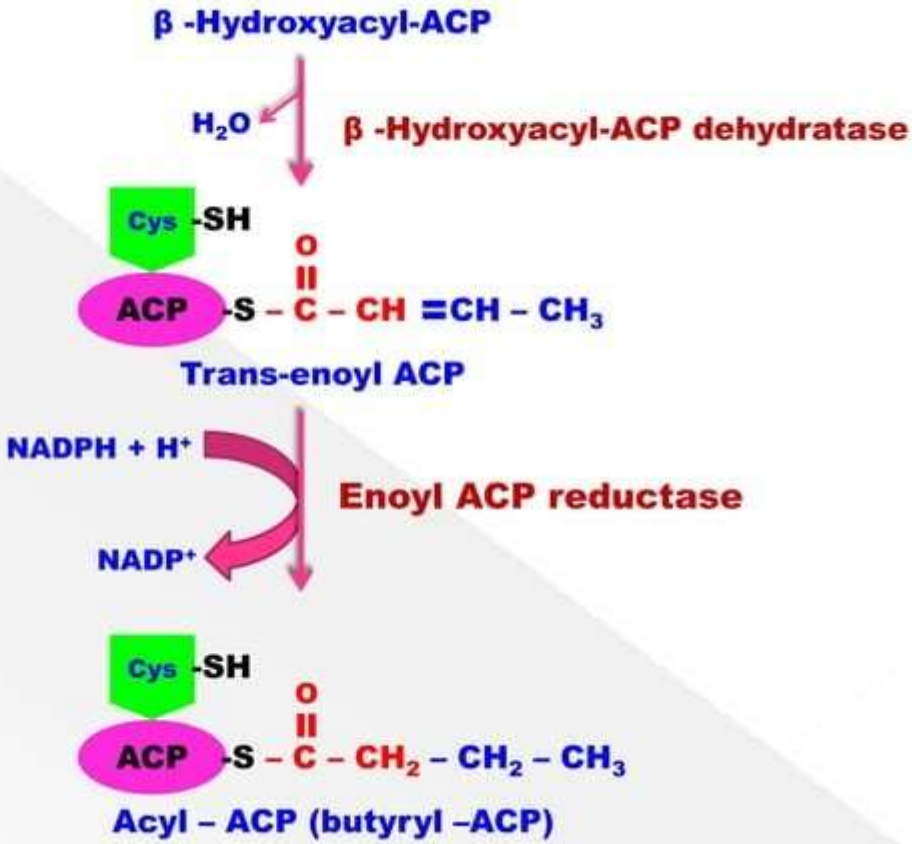
- At the end of 7 cycles, the fatty acid synthesis is complete & a 16-carbon fully saturated fatty acid-namely palmitate-bound to ACP is produced.
- The enzyme palmitoyl thioesterase separates palmitate from fatty acid synthase.
- This completes the synthesis of palmitate

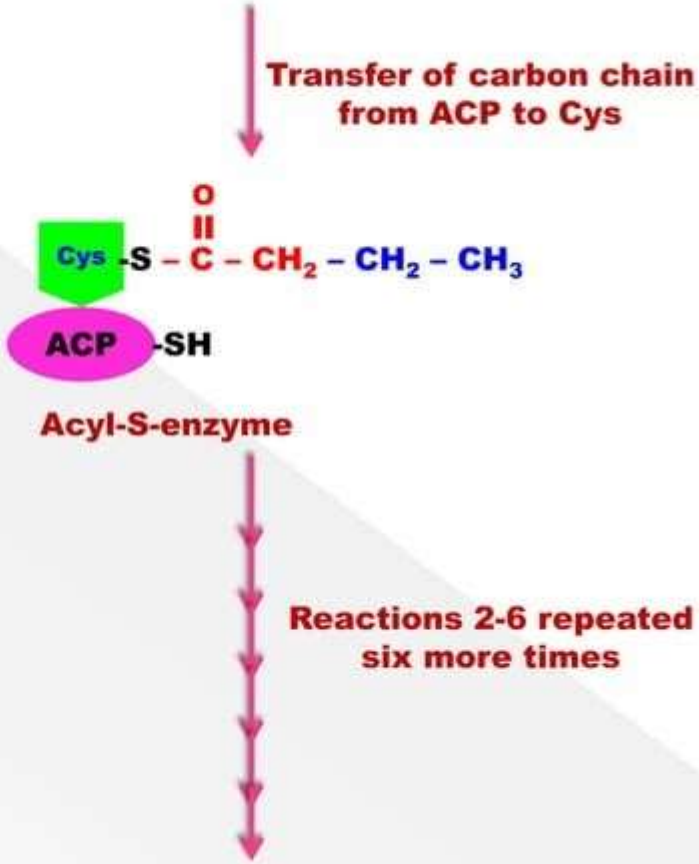
De novo synthesis of fatty acids

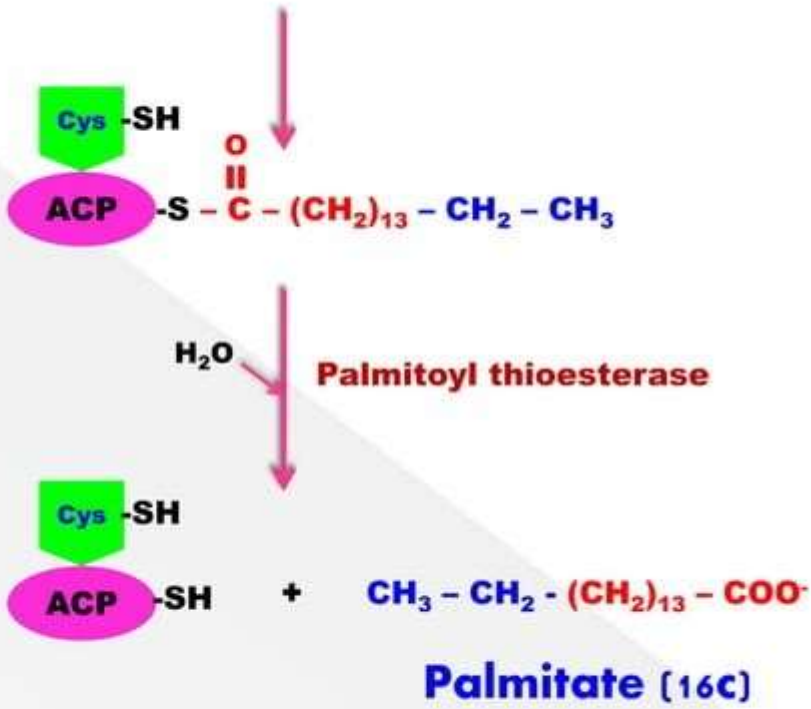



Cys
-S – C - CH3
O
ACP
-SH
Acetyl S-enzyme
-OOC – CH2 – C – SCoA
O
Malonyl CoA
Malonyl CoA-ACP
trnasacylase
CoASH
Cys
-S – C - CH3
O
ACP
-S – C – CH2 – COO-
O
Acylmalonyl enzyme


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
OH
ACP -S – C – CH_2 – CH – CH_3
β -Hydroxyacyl-ACP


β -Hydroxyacyl-ACP
H_2O
β -Hydroxyacyl-ACP dehydratase
Cys -SH
ACP -S – C – CH =CH – CH_3
O
Trans-enoyl ACP
NADPH + H^+
Enoyl ACP reductase
$NADP^+$
Cys -SH
ACP -S – C – CH_2 – CH_2 – CH_3
O
Acyl – ACP (butyryl –ACP)

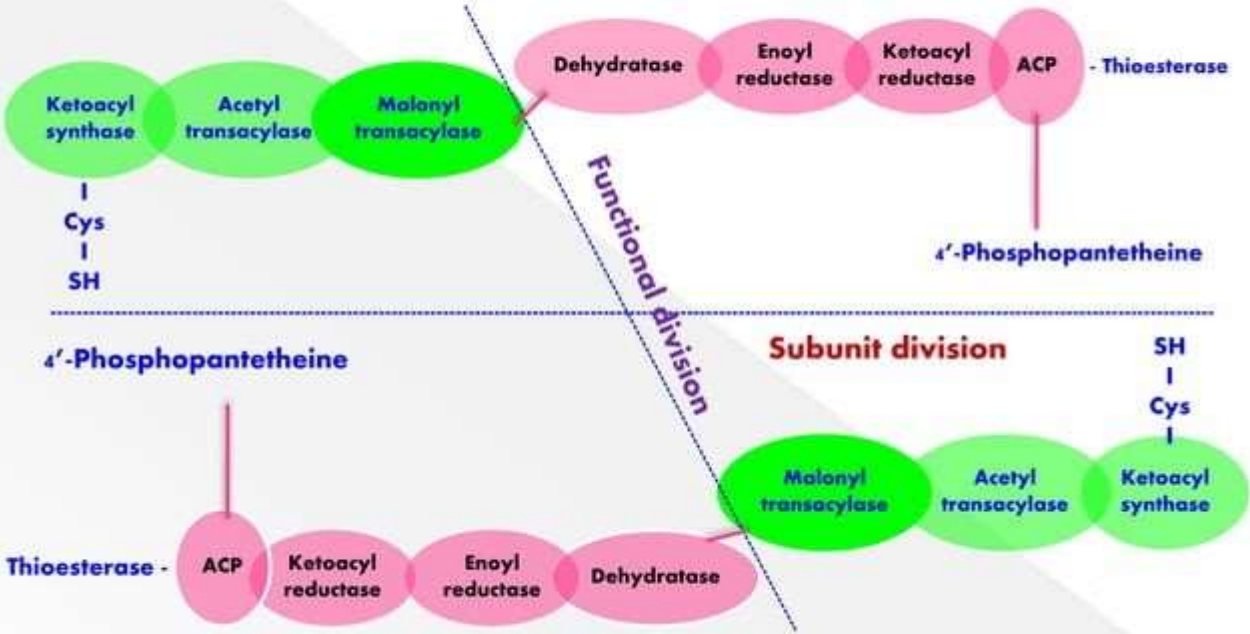

Transfer of carbon chain
from ACP to Cys
O
Cys -S – C – CH2 – CH2 – CH3
ACP -SH
Acyl-S-enzyme
Reactions 2-6 repeated
six more times


Cys -SH
ACP -S – C – (CH2)13 – CH2 – CH3
O
H2O
Palmitoyl thioesterase
Cys -SH
ACP -SH
+
CH3 – CH2 - (CH2)13 – COO-
Palmitate (16C)

Fatty acid synthase complex

- It is a multienzyme complex
- Fatty acid synthase is a dimer composed of two identical subunits (monomers),
- Each with a molecular weight of 240,000.
- Each subunit contains the activities of 7 enzymes of FAS & an ACP with 4'-phosphopantetheine SH group.
- The two subunits lie in antiparallel (head to tail) orientation

Fatty acid synthase - multienzyme complex



- The -SH group of phosphopantetheine of one subunit is in close proximity to the -SH of cysteine residue (of the enzyme ketoacyl synthase) of the other subunit.
- Each monomer of FAS contains all the enzyme activities of fatty acid synthesis.
- Only the dimer is functionally active.

- The functional unit consists of half of each subunit interacting with the complementary half of the other.
- FAS structure has both functional division & subunit division
- The two functional subunits of FAS independently operate & synthesize two fatty acids simultaneously

Significance of FAS complex

- The FAS complex offers great efficiency that is free from interference of other cellular reactions for the synthesis of fatty acids.
- There is a good coordination in the synthesis of all enzymes of the FAS complex.

Regulation of fatty acid synthesis

- Fatty acid production is controlled by enzymes, metabolites, end products, hormones and dietary manipulations.
- Acetyl CoA carboxylase:
- This enzyme controls a committed step in fatty acid synthesis.

- **Acetyl CoA carboxylase exists as an inactive protomer (monomer) or an active polymer.**
- **Citrate promotes polymer formation & increases fatty acid synthesis.**
- **Palmitoyl CoA & malonyl CoA cause depolymerization of the enzyme, inhibits the fatty acid synthesis.**

Dietary regulation

- Consumption of high carbohydrate or fat-free diet increases the synthesis of acetyl CoA carboxylase & fatty acid synthase, which promote fatty acid formation.
- Fasting or high fat diet decreases fatty acid production by reducing the synthesis of acetyl CoA carboxylase & FAS.

References

- Textbook of Biochemistry-U Satyanarayana
- Textbook of Biochemistry-DM Vasudevan

Thank You