Lipid metabolism lecture 1 of 3

Fatty acid metabolism: Fatty acid synthesis

Ahmed Salem, MD, MSc, PhD, FRCR

Lipids metabolism	1. Fatty acids metabolism a. Fatty acid synthesis b. Fatty acid catabolism 2. Cholesterol synthesis 3. Eicosanoids synthesis from fatty acids

Introduction

- FAs are synthesised whenever there is caloric excess in diet
- Main pathway for synthesis is called **de novo fatty acid synthesis**
- Immediate substrate for synthesis: **acetyl coA**
- Final product: **palmitic acid (16C, saturated)**
- Synthesis needs: NADPH, ATP, biotin & bicarbonate (CO_2)

Biosynthesis of FAs

- Pathway is called **Lynen's pathway** (Feodor Lynen → Nobel prize)
- Glucose by glycolysis → pyruvate (cytosol)
- Pyruvate by PDH→ acetyl-CoA (mitochondria)

Acetyl – CoA derived from glucose & others is used for synthesis of FA by:

1) **The extramitochondrial (cytosolic) system (site of FA synthesis)**
2) The mitochondrial system
3) The microsomal system

FA elongation

Extra-mitochondrial biosynthesis
=De Novo synthesis of FA (main synthesis occurs via this route)

- It is the main synthesis pathway of palmitic acid (C16, saturated) from acetyl-CoA

- All other FA are made by modification of palmitate, so called **stem fatty acid**

- **Site:** The **cytoplasm** of many organs including:
  - **Liver (most imp), adipose tissue, brain, lactating mammary gland [major sites]**
  - Lung [minor site]

Transport of acetyl co A to cytoplasm

- Starting point of de novo synthesis is **acetyl coA** (formed in mitochondria)
- Inner membrane not freely permeable to acetyl co A:
  - → acetyl CoA units are delivered to the cytoplasm as citrate
  - → citrate transported from **mitochondria via tricarboxylic acid transporter**
- In cytoplasm, citrate is cleaved to oxaloacetate & acetyl coA
- Oxaloacetate can return to mitochondria as malate or pyruvate

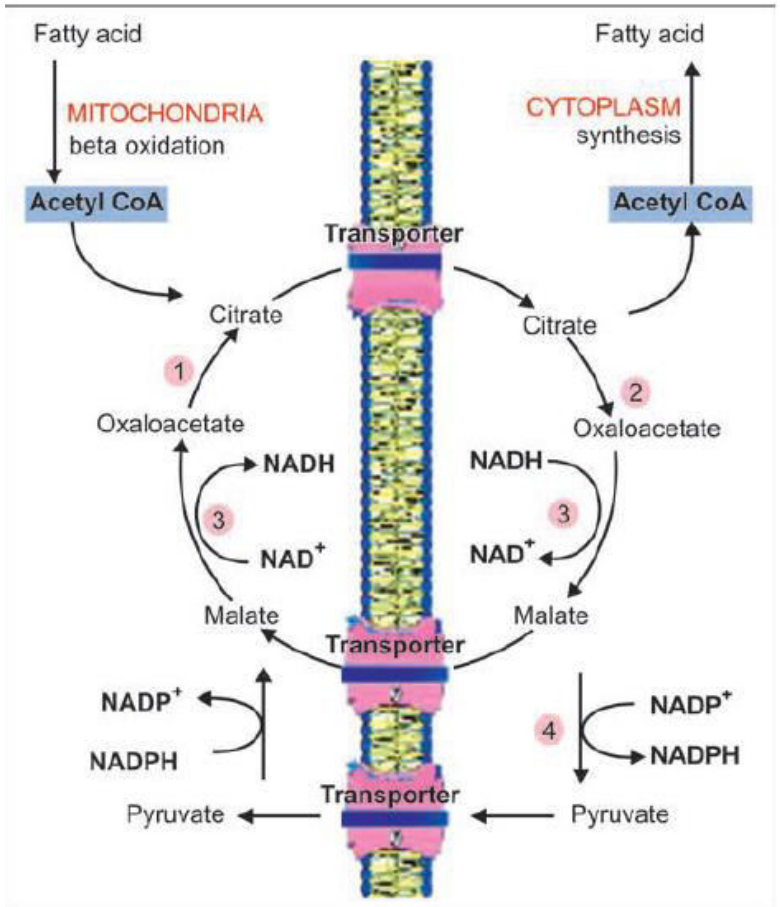
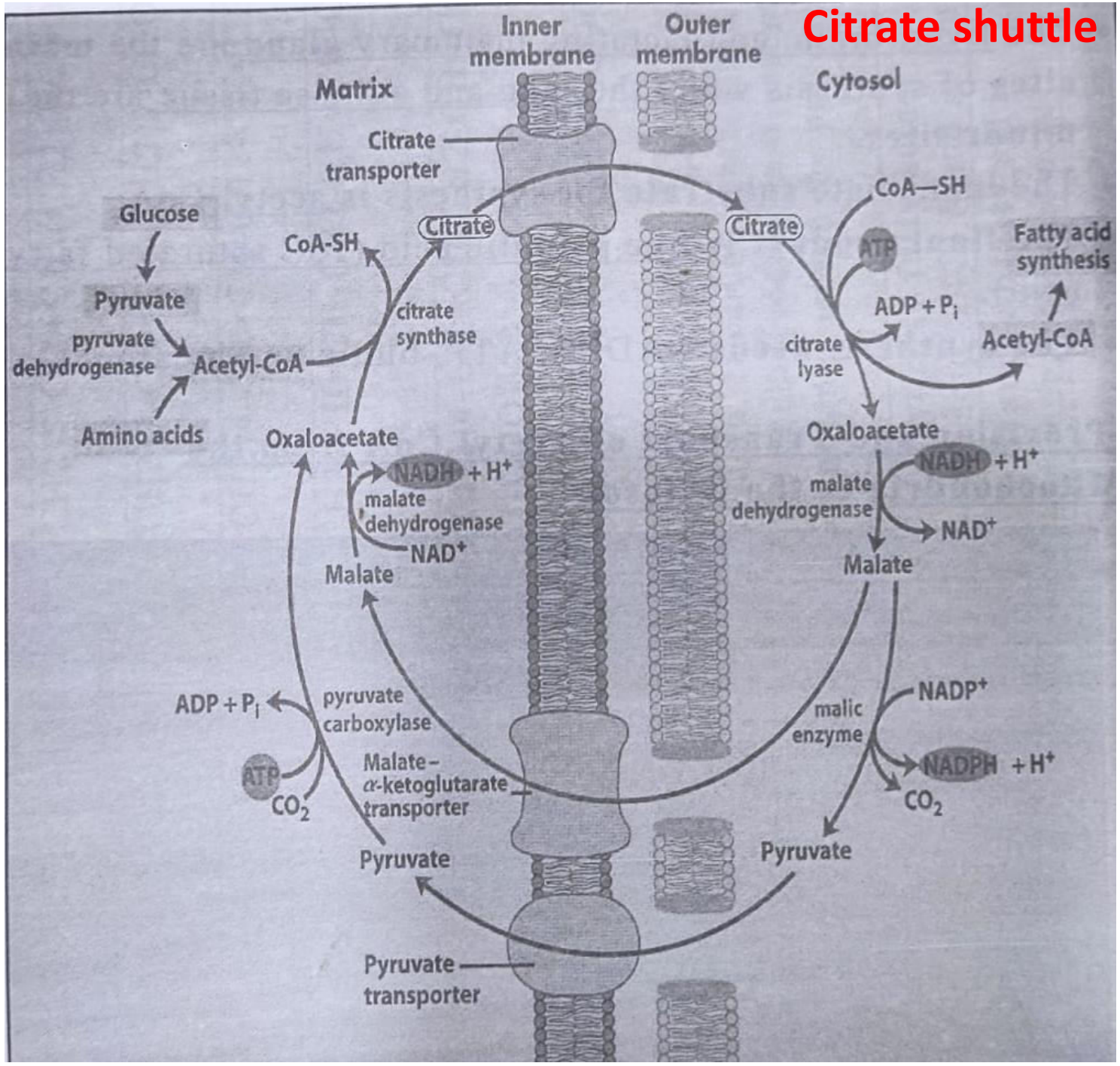


Fig. 11.13. Transfer of acetyl CoA from mitochondria to cytoplasm by malate–oxaloacetate shuttle. 1 = citrate synthetase; 2 = ATP–citrate lyase; 3 = malate dehydrogenase; 4 = malic enzyme

Citrate shuttle



Key facts about FA synthesis

- FA synthesis takes place in cytosol and uses NADP as co-enzyme for redox reactions
- **Citrate shuttle** is responsible for moving acetyl coA from mitochondria to the cytosol
- NADPH is an important co-enzyme for de novo FA synthesis; sources:
  - Main source of NADPH is PPP (both FA synthesis and PPP occur in cytosol; no permeability barrier)
  - Malic Enzyme: The reaction helps to transfer cytoplasmic oxaloacetate to the mitochondria

$$\text{Malate} + \text{NADP}^+ \rightarrow \text{Pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+$$

- The building block for FA synthesis is malonyl coA (3C)
- FA synthesis in each reaction cycle adds 2 carbons that are derived from malonyl coA following decarboxylation
  - Acetyl (2C) coA is used as a primer for C15 and 16 in palmitate → even number FA
  - If propionyl (3C) coA is used as a primer → odd n FA is formed
  - Short chain FA is formed if chain is released before reaching 16 carbons as in mammary glands

Steps of de novo FA synthesis:

1. The initial step of FA biosynthesis including carboxylation of acetyl CoA to produce malonyl CoA

This step needs **biotin**, Mn^{2+}, and an enzyme; **ACC** *(acetyl CoA carboxylase) and biocarbonate*

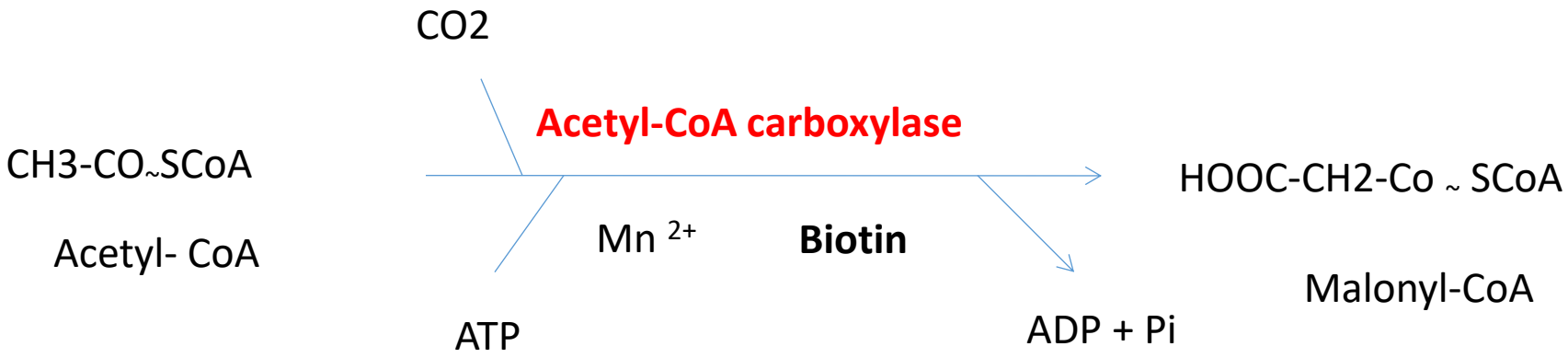
Rate limiting step of FA synthesis

The enzyme is allosterically regulated, the major effectors being:

→citrate (positive)

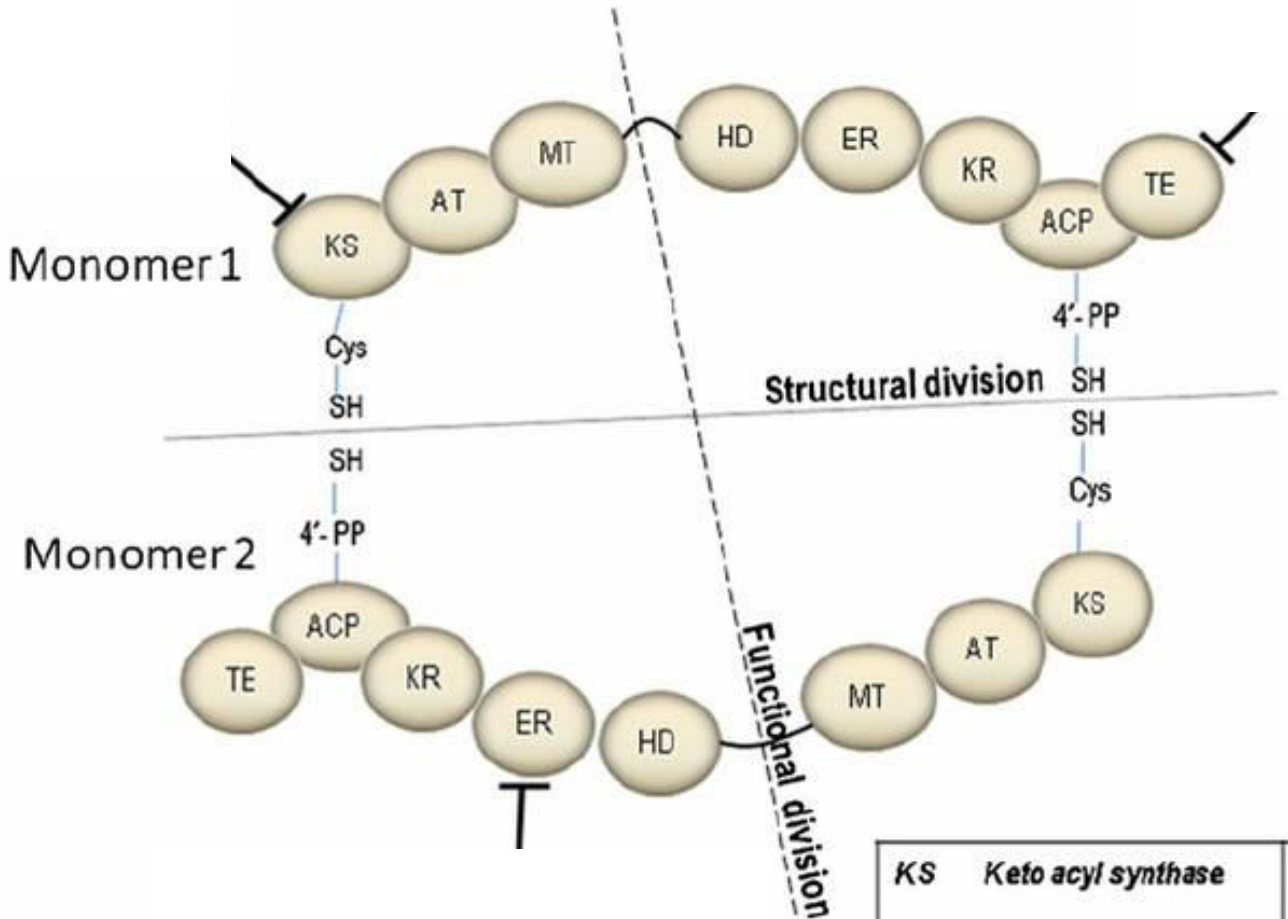
→ palmitoyl CoA (negative)

- 8 acetyl-CoA (C2) → 1 palmitic acid (C16)
- 7 of these 8 acetyl-CoA are converted to malonyl-CoA (2C)

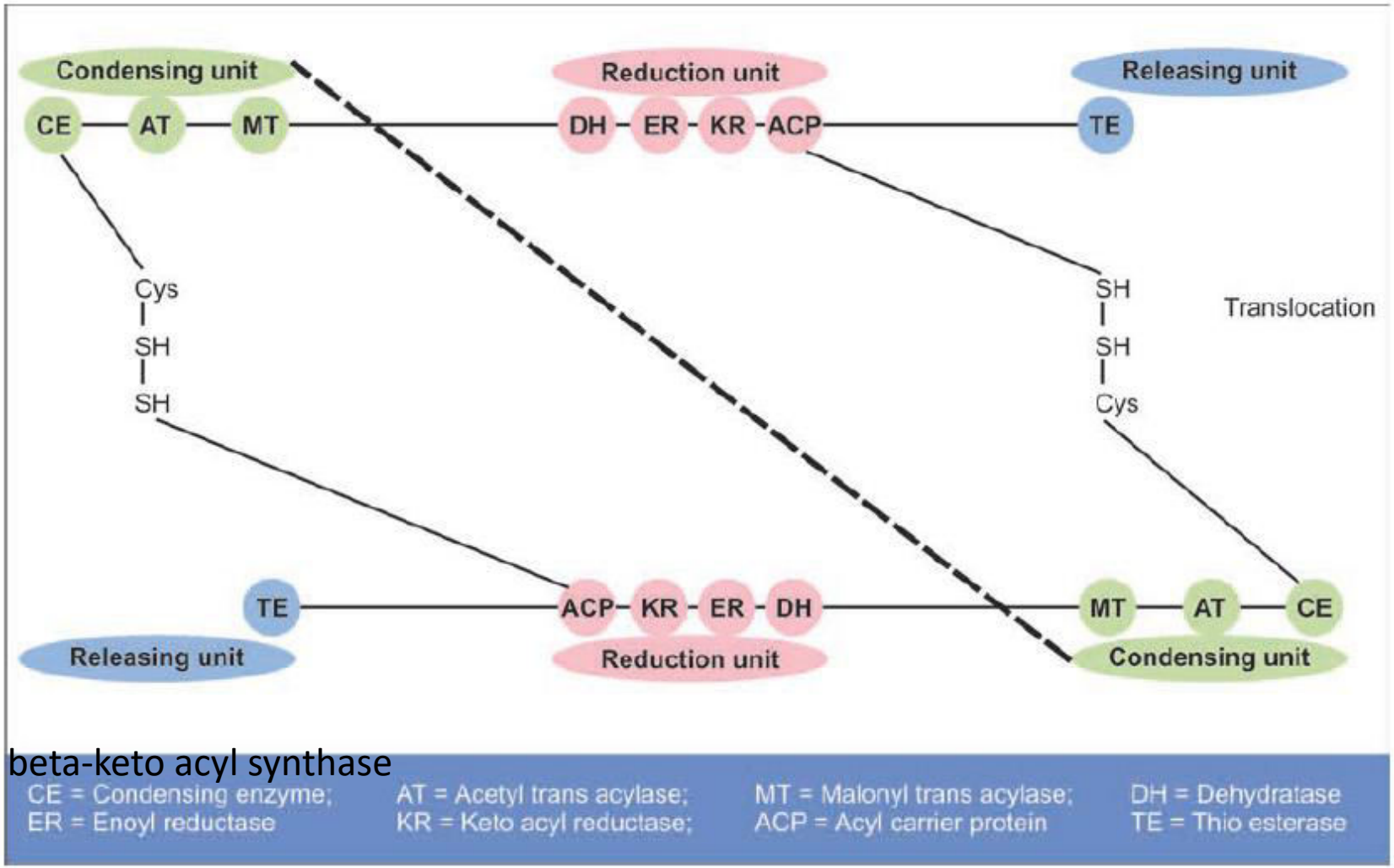


The fatty acid synthase multienzyme complex (responsible for all other steps of FA synthesis):

- This system exists as a multi-enzyme complex
  - The active form is a dimer composed of 2 identical monomers opposite to each other
  - → It is a polypeptide containing 3 domains with 7 enzymes
- Each monomer contains two SH groups, one attached to acyl carrier protein (ACP), the second is provided by cysteine and attached to the enzyme 3-ketoacyl synthase
- This dimer is arranged head to tail, so the SH group of ACP of one monomer is very close to the SH group provided by 3-ketoacyl synthase (condensing unit) of the second monomer.
- **1st Domain or Condensing Unit**
  - It is the initial substrate binding site
- **2nd Domain or Reduction Unit**
  - The acyl carrier protein (ACP) is a polypeptide chain having a phospho-pantotheine group, to which the acyl groups are attached in thioester linkage.
  - → ACP acts like the CoA carrying fatty acyl groups
- **3rd Domain or Releasing Unit**
  - It is involved in the release of the palmitate synthesised



KS	*Keto acyl synthase*	*ER*	*Enoyl reductase*
AT	*Acetyl transacylase*	*KR*	*Keto acyl reductase*
MT	*Malonyl transacylase*	*ACP*	*Acyl carrier protein*
HD	*Dehydratase*	*TE*	*Thioesterase*

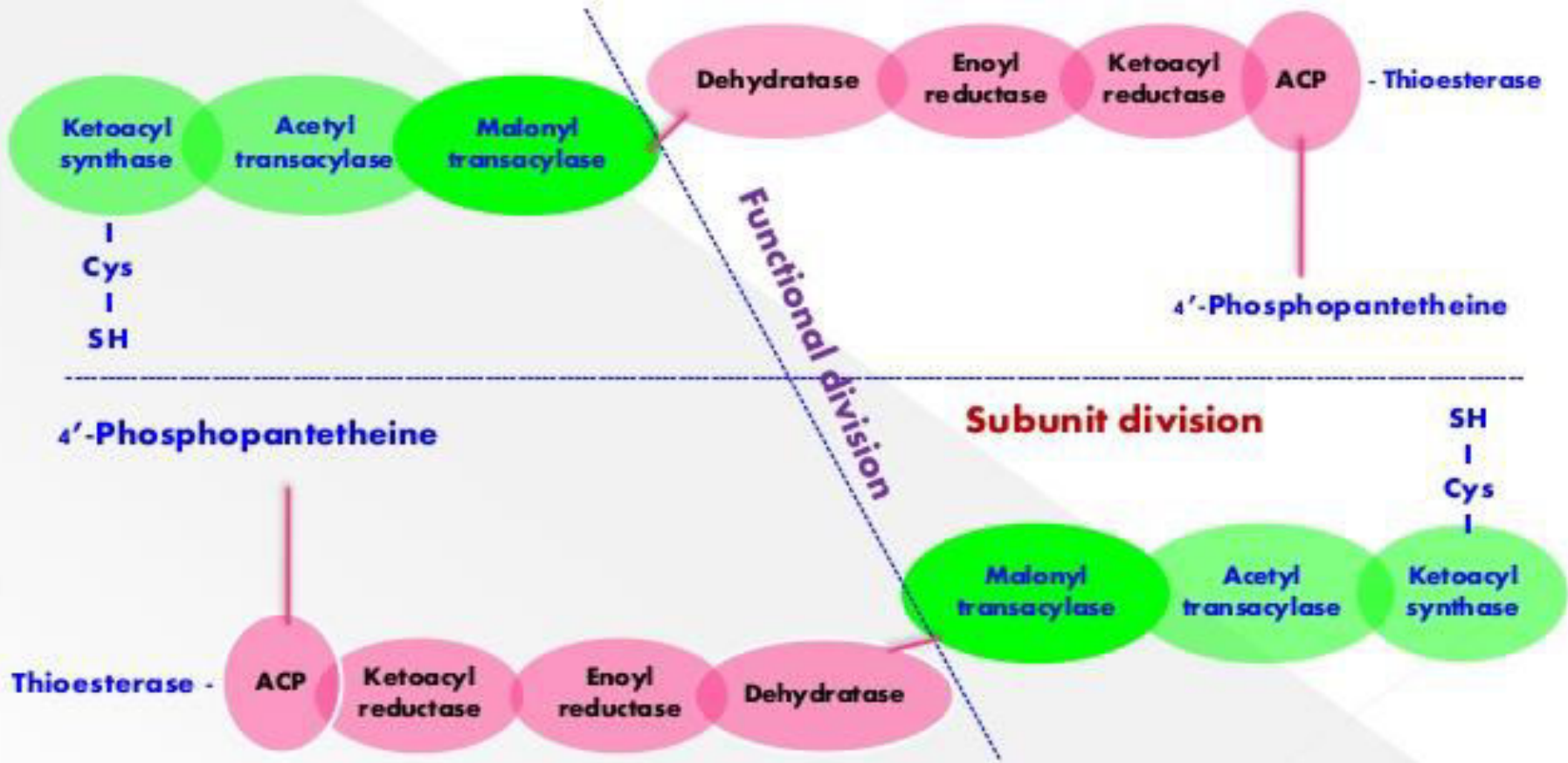


beta-keto acyl synthase

Advantages of Multi-enzyme Complex

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

Fatty acid synthase - multienzyme complex



Step 2: Three C and Two C Units are Added

- **Step 2A:**

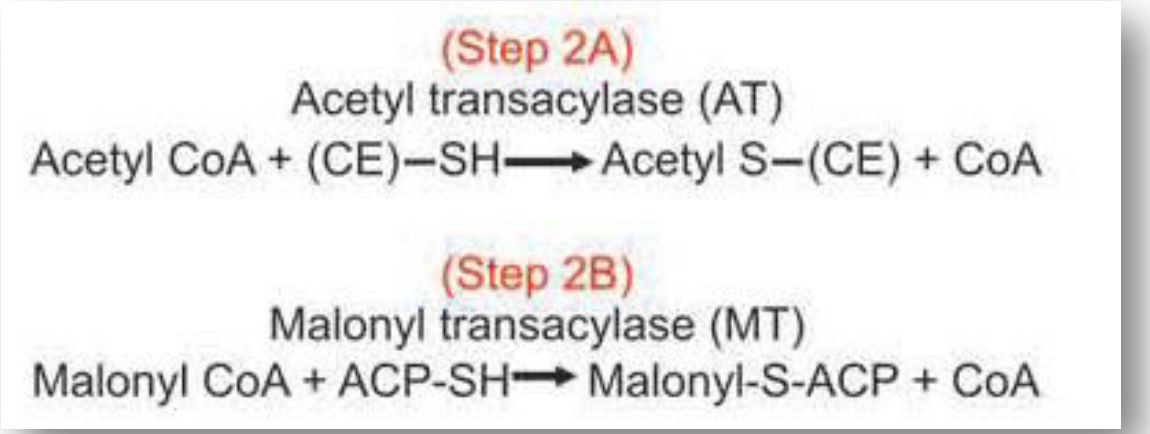
A priming molecule of acetyl coA combines (transfer of acetyl group) with –SH of cysteine of one monomer of the enzyme

  - This is catalysed by acetyl transacylase

- **Step 2B:**

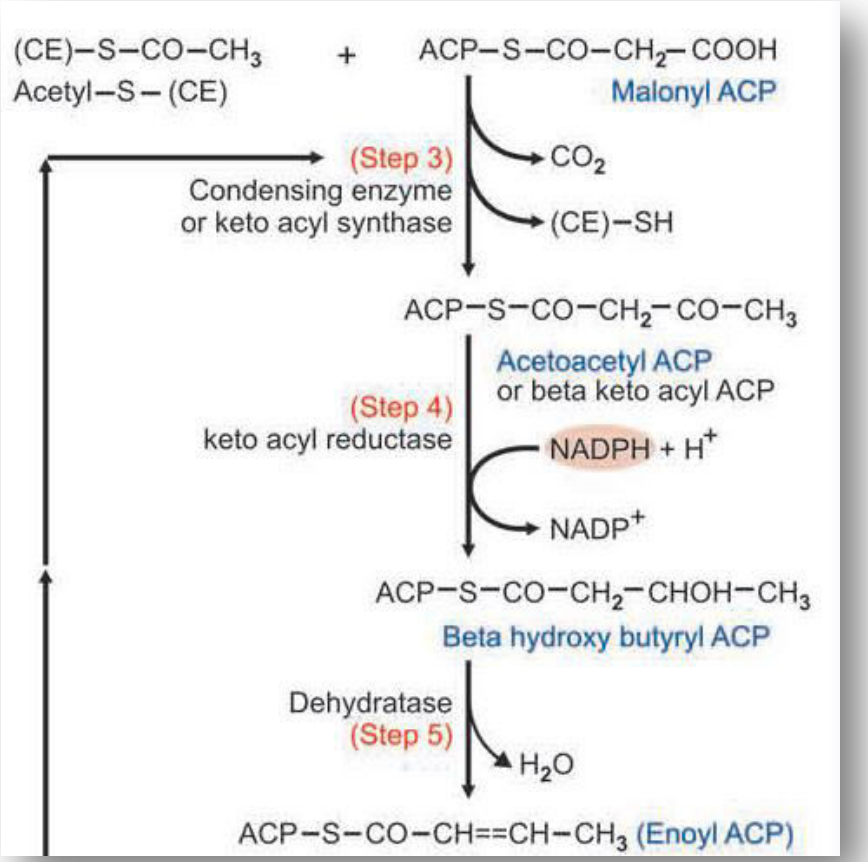
A malonyl coA molecule combines with the –SH of phospho-pantothenyl of the ACP in the other monomer of the synthase complex

  - This is catalysed by malonyl transacylase



Steps 3-5

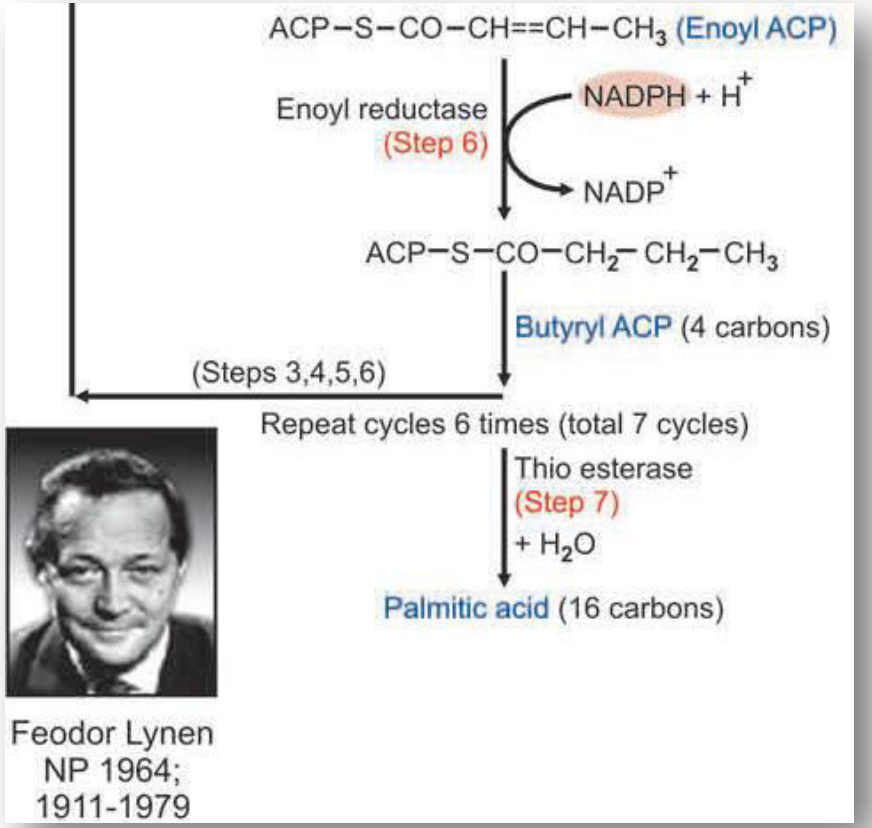
- **Step 3 (condensation):** the acetyl group attacks the malonyl residue
    - Catalysed by 3 ketoacyl synthase (condensing enzyme) → acetoacetyl enzyme
        →Leads to liberation of CO2
- **Step 4 (reduction):** The acetoacetyl ACP is reduced by NADPH dependent beta-keto acyl reductase
    → to form beta-hydroxy fatty acyl ACP
- **Step 5 (dehydration):** by a dehydratase to form:
    → enoyl ACP otherwise known as (alpha beta unsaturated acyl ACP)



Step 6 and cycling

- **Step 6 (2nd reduction):** The enoyl ACP is again reduced by enoyl reductase (ER) utilizing a 2nd molecule of NADPH to form butyryl ACP

- **Cycling of Reactions:**
  - The butyryl group (4C) is now transferred to the SH group of the condensing enzyme on the other monomer and;
  - A 2nd malonyl CoA molecule binds to the phospho-pantothenyl SH group
    - The sequence of reactions (steps 3,4,5,6) are repeated
    - The cycles are repeated a total of 7 times, till the 16-carbon palmitic acid is formed



Feodor Lynen
NP 1964;
1911-1979

Release of palmitic acid

- **Step 7 (palmitic acid is released)**
  - The thio-esterase or de-acylase activity (TE) releases palmitate from the multi-enzyme complex
  - The end point is Palmitic acid (16 C) in liver & adipose tissue
  - In lactating mammary gland, the end products are Capric (10 C) and Lauric (12 C) acids
    - Mother's milk contains these medium chain fatty acids

So to form palmitic how many acetyl CoA Malonyl CoA NADPH+H Used?

$$1\ \text{Acetyl CoA} + 7\text{Malonyl CoA} + 14\text{NADPH} + 14H^{+} \rightarrow$$
$$1\text{Palmitate} + 7CO_2 + 14NADP^{+} + 8\text{CoA} + 6H_2O$$

Elongation of FA chains

- Occurs by a major microsomal system at the surface of endoplasmic reticulum
    - Using malonyl coA as 2C donor & NADPH as a reductant
    - Reaction similar to de-novo FA synthesis (addition of 2C) but different as activities appear on individual enzymes (not part of multi-functional enzyme) → **coA esters** used
- Another minor system of elongation lies in mitochondria
    - Uses acetyl coA as acetyl donor
    - Reactions are reversal of FA oxidation (except that NADPH is used in saturation of double bond c.f. FADH2 in beta oxidation)
- Brain have additional ability for chain elongation
    - Producing very long FA chains C22-24 during myelination

Fasting & DM (due to low insulin activity) abolish chain elongation

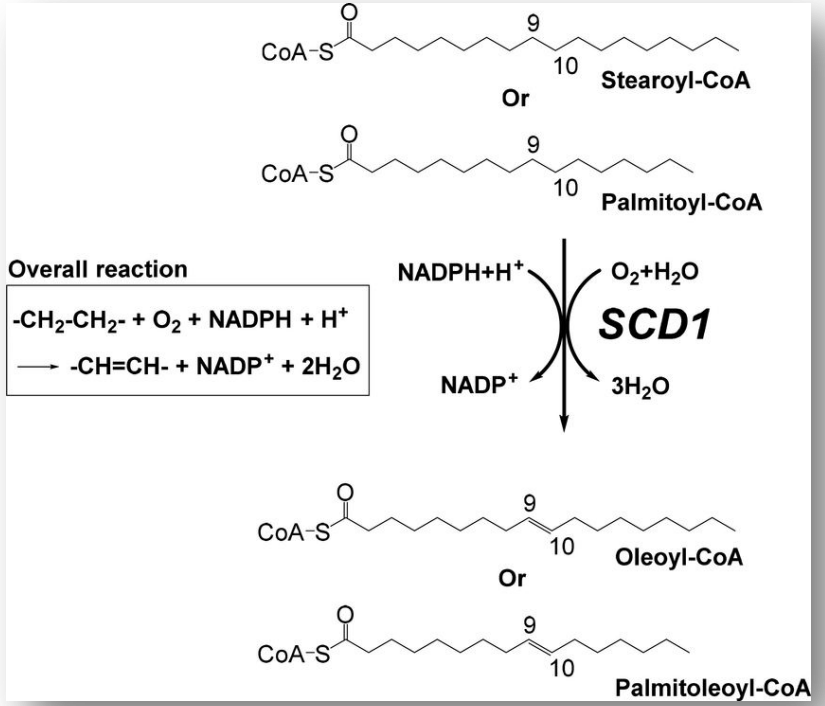
Desaturation of FA chains

- Saturated FA precursors of the 2 most common mono-unsaturated FAs:
  - Palmitate → palmitoleate C16:1 (delta 9)
  - Stearate → Oleate C18:1 (delta 9)

- Enzymes (desaturases) present in ER of liver & adipose tissue
  - Responsible for desaturating FAs (i.e. adding cis double bonds)
  - Introduce double bonds in newly synthesised FA by O2 dependent pathway
    - Require NADPH or NADH, cytochrome b5, FAD-linked reductase

- Human has C9, 6, 5 & 4 desaturases but LACK ability to introduce double bonds from C10 to the ω end of chain
  - This is basis of essentiality of linoleic and linolenic acids

- Desaturation & elongation is pathway to arachidonic acid (20: 4, δ5,8,11,14) from dietary linoleic acid (18:2, δ9,12)



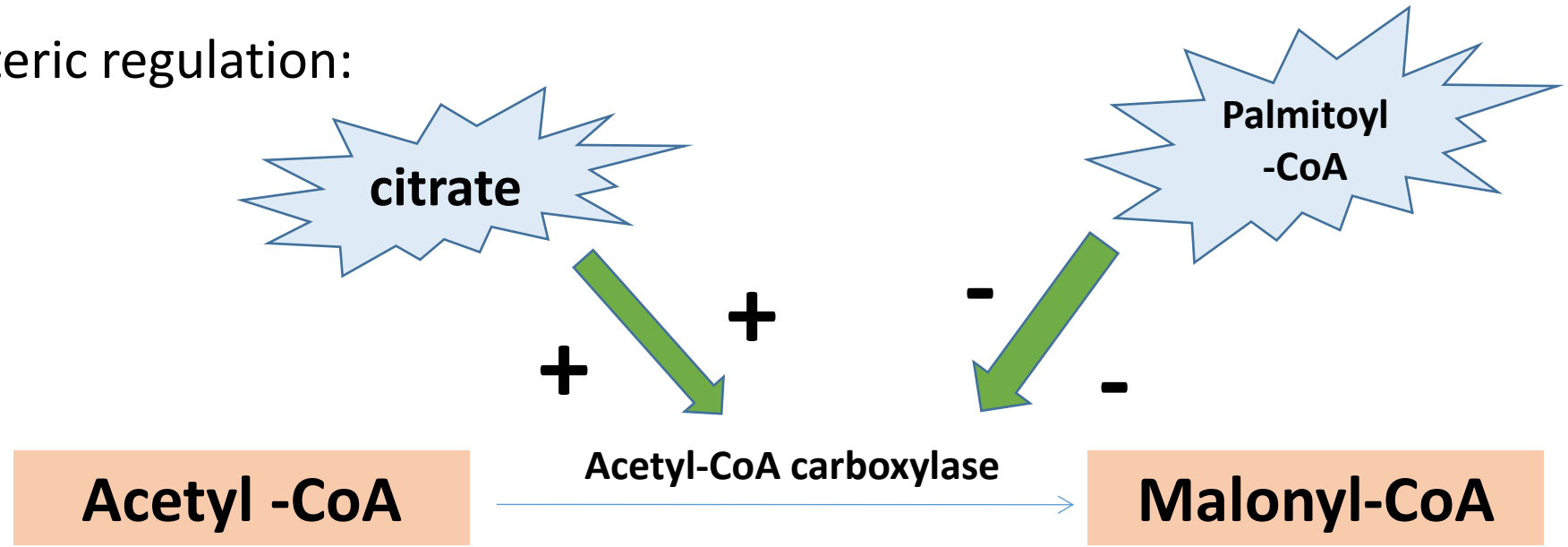
Regulation of De Novo synthesis of FA

- Acetyl –CoA carboxylase is the key enzyme:

Fatty acid synthesis occurs when carbohydrate is abundant and the level of fatty acids is low

The availability of citrate in the cytoplasm is the most important regulatory factor producing a short-term effect

Allosteric regulation:



3. Acetyl –CoA carboxylase (ACC):

The active form is the dephosphorylated:

- Insulin, suppresses cAMP, so it activates acetyl CoA carboxylase
- Adrenaline and glucagon have the reverse effect (phosphorylate or inactivate ACC)
- ACC is inactivated by AMP activated protein kinase (AMPK)
    - AMPK is allosterically activated by rise in AMP relative to ATP

- Feeding status:

CHO feeding stimulates insulin secretion which induces the synthesis of acetyl-coA.

Fasting → ↓↓ insulin and ↑↑ adrenalin and glucagon→ ↓↓ glucose uptake and utilization, so fasting inhibits FA synthesis.

3. Insulin Favors Lipogenesis

Insulin enhances the uptake of glucose by adipocytes and increases the activity of pyruvate dehydrogenase, acetyl CoA carboxylase and glycerol phosphate acyl transferase (see Table 24.4). Insulin also depresses the hormone sensitive lipase (Fig. 11.16).

Long term regulation of ACC (dietary manipulation)

- High caloric diets → ↑ ACC synthesis → ↑ FA synthesis

- Fasting/ high intake of polyunsaturated FAs, prolonged biotin deficiency → ↓ ACC synthesis → ↓ FA synthesis

- Long term regulation occurs at genetic level by changing rate of synthesis/ degradation of enzyme
  - In DM → FA synthesis is impaired (restored to normal with administration of **insulin**)
  - Stimulatory effect of FA synthesis in mammary glad through **prolactin**

Insulin, glucagon affect short and long term control of ACC

Well-fed state	During fasting
Lipogenesis increased Lipolysis inhibited Lipoprotein lipase active Insulin inhibits HS-lipase	Lipogenesis inhibited Lipolysis increased Glucagon activates HS-lipase FFA in blood increased

Synthesis is not the opposite of oxidation

	Beta-oxidation	Fatty acid synthesis
Site	Mitochondria	Cytoplasm
Intermediates	Present as CoA derivatives	Covalently linked to SH group of ACP
Enzymes	Present as inde-pendent proteins	Multienzyme complex
Sequential units	2 carbon units split off as acetyl CoA	2 carbon units added, as 3 carbon malonyl CoA
Co-enzymes	NAD^+ and FAD are reduced	NADPH used as reducing power

Triacylglycerol (TAG)

- **TAG:** FAs + glycerol

- Liver and adipose tissue are major sites of TAG synthesis
    - In adipose tissue → for storage of energy
    - In liver → secreted as VLDL & transported to peripheral tissues

- Synthesis of TAG needs activation of glycerol & FAs
    - Active form of glycerol is glycerol 3-P
        - **In liver & adipose tissue:** glycerol is produced from glucose via DHAP (from glycolysis)
            - This is active in presence of insulin
        - **In liver only:** glycerokinase phosphorylates glycerol directly
    - Active form of FA is fatty acyl coA (via thiokinase enzyme by reaction btwn acetyl coA & FA)

- Synthesis of TAG (reaction btwn activated FAs and activated glycerol)

- **Fate of TAG:**
    - In liver → exported as VLDL (bound to cholesterol, phospholipids and protein)
    - In adipose tissue → provision of energy when needed