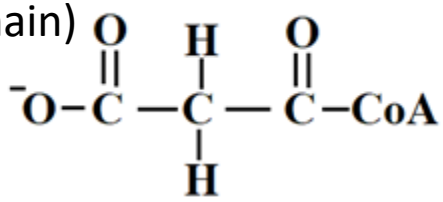
Fatty acid synthesis

- Mainly in **Liver Cells**, lactating Mammary gland, small amount in Adipose cell and Kidneys
- Occurs **in the Cytosol**, NOT mitochondrial Matrix
- Precursor (المادة الاولية): Acetyl-CoA (Carbon source)

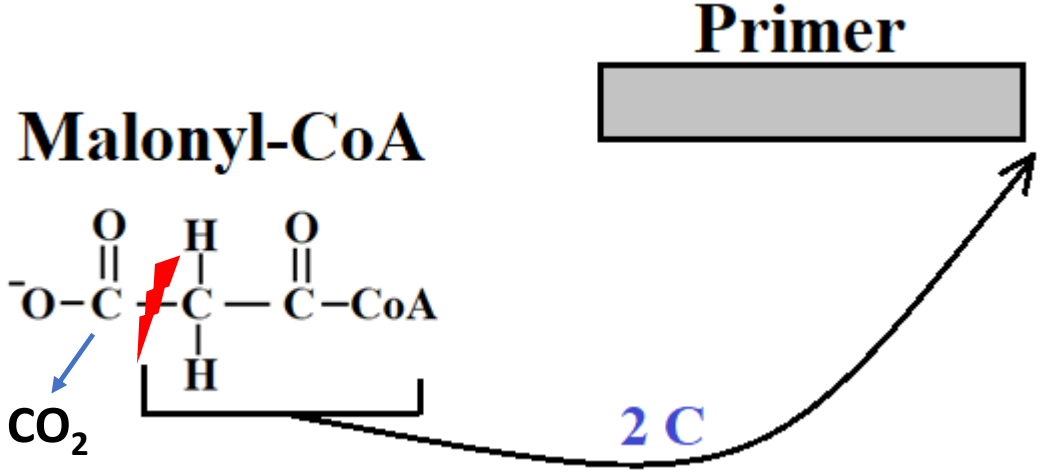
To synthesize Fatty acid we need

From Acetyl-CoA
- **Primer** (growing chain) (سلسلة اولية)
- **Malonyl-CoA**



- **NADPH (Reducing agent)**
- **Energy (ATP)**

During the Synthesis of fatty acid we successively (بشكل مستمر) add 2C to the Primer (these 2 Carbons from Malonyl-CoA)



In Eukaryotes the Enzyme that catalyze fatty acid synthesis ***Fatty acid Synthase*** (found in the cytosol)

- single polypeptide chain, synthesized from a single gene
- Contain 8 domains: 7 catalytic domains (7 activities) + ACP “Acyl-Carrier protein” domain

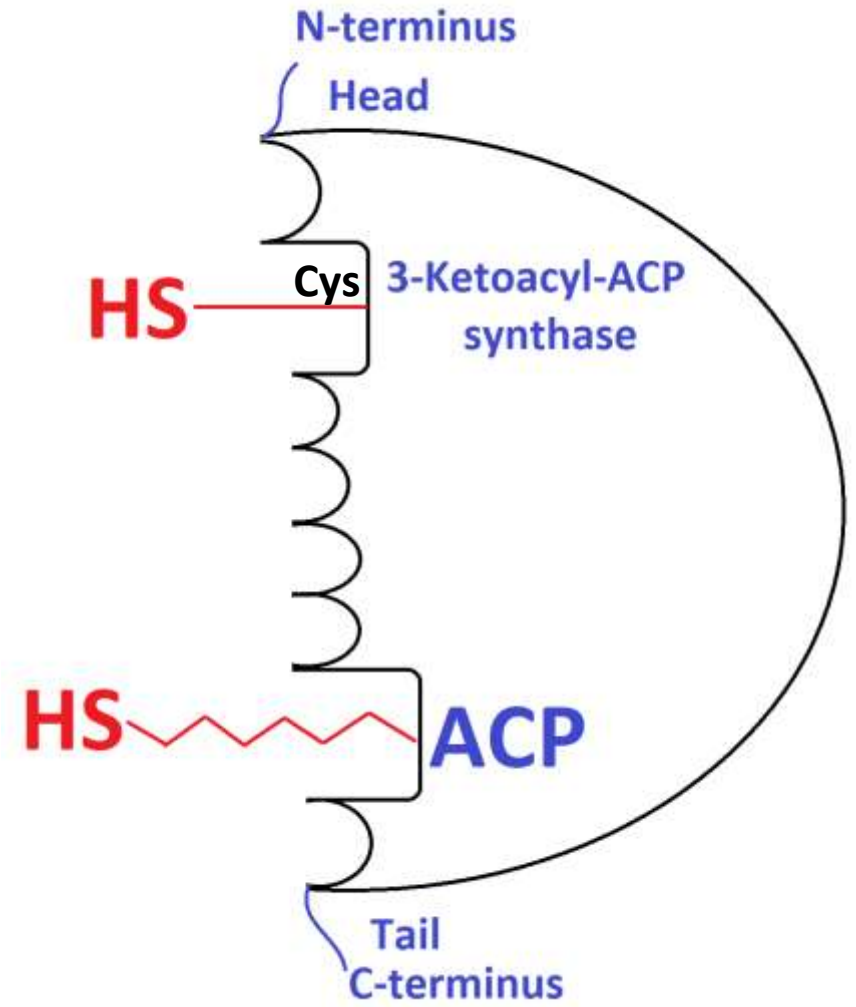
7 مواقع تحفيز

2 Domain has an SH group

3-KetoAcyl Synthase Domain (KS)
Cysteine SH
Also called condensing Enzyme (CE)

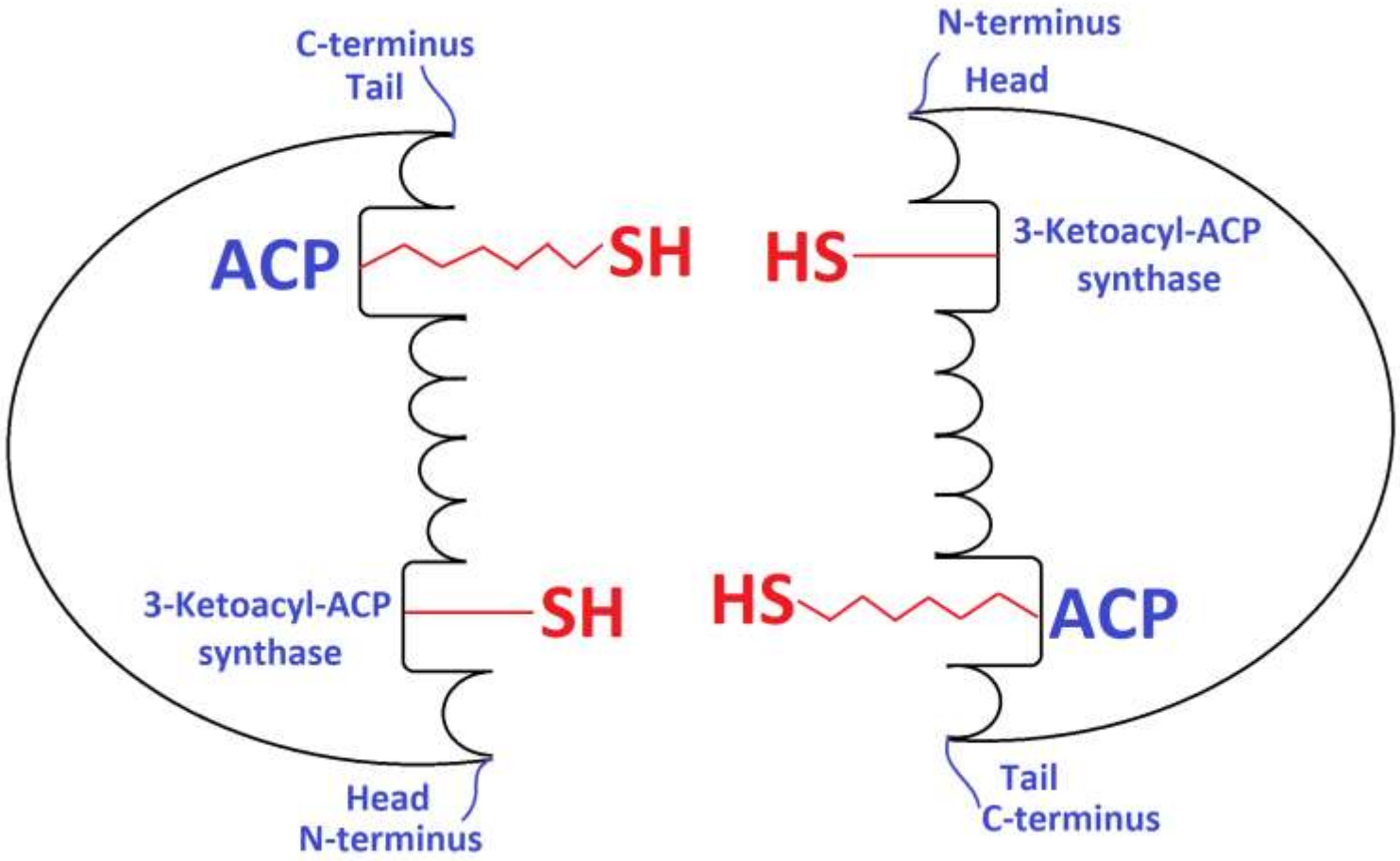
ACP
SH bound to pantothenate (Vitamin B5)
Not Cysteine

Note: Both CoASH and ACP contain Pantothenic acid (Vitamin B5) in their structure



Fatty acid synthase is **active only** in its ***Dimeric form*** (2 subunit not attached covalently to each other)
The 2 subunits attached ***Head-to-Tail***

2 fatty acids can be synthesized simultaneously



Remember

β-Oxidation Reactions

You will see the same structure in reverse direction

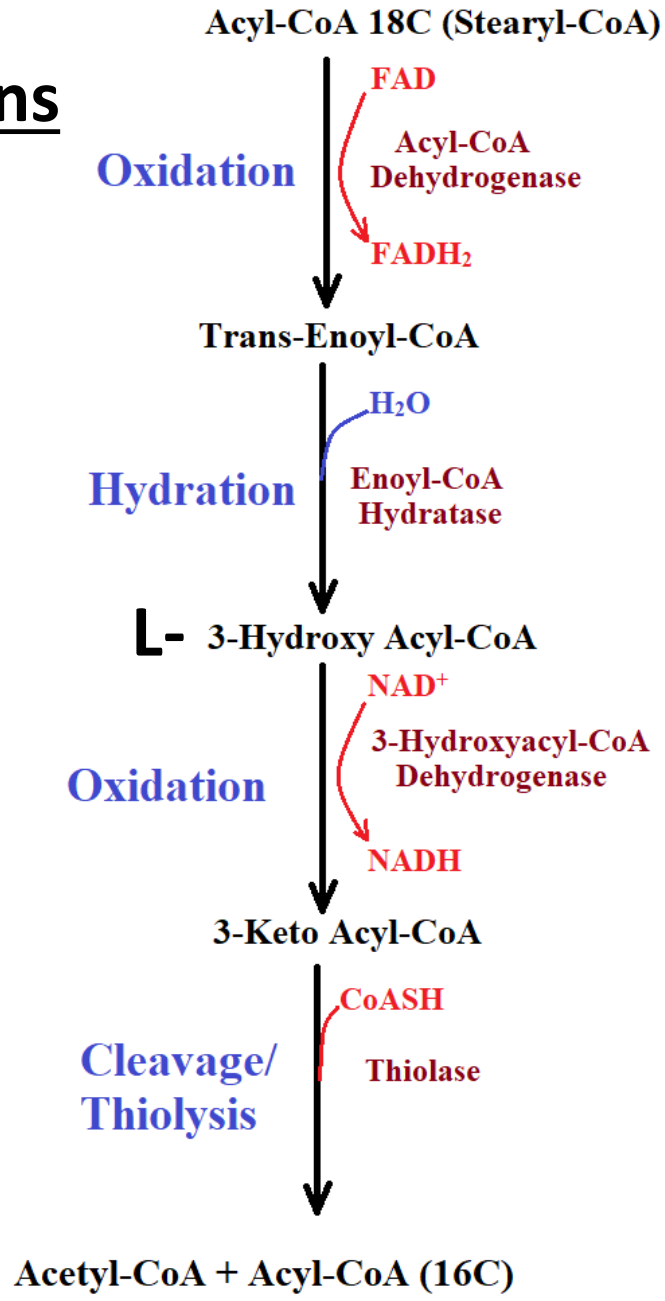
In fatty acid synthesis you will see

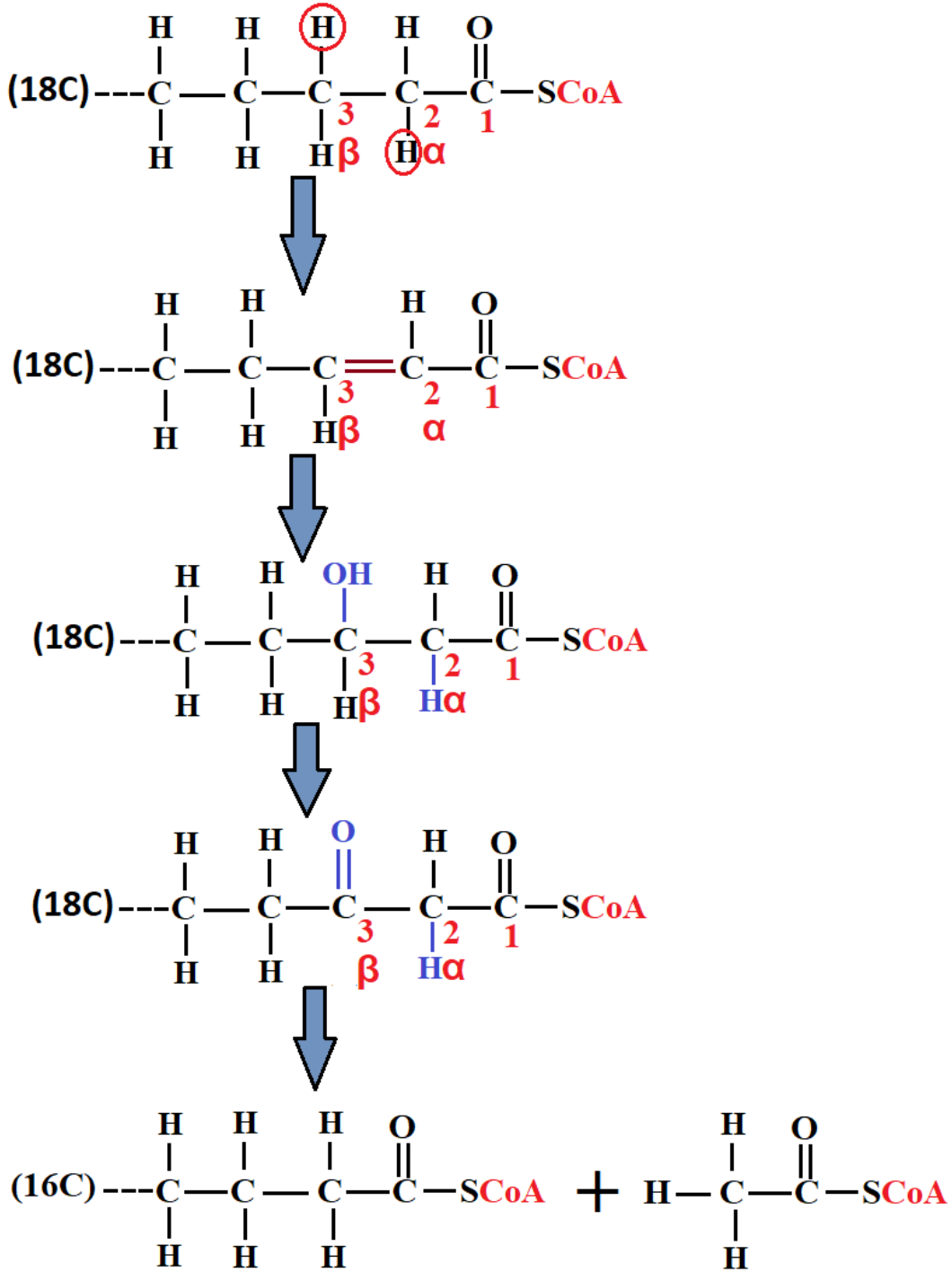
Joining

Reduction

Dehydration

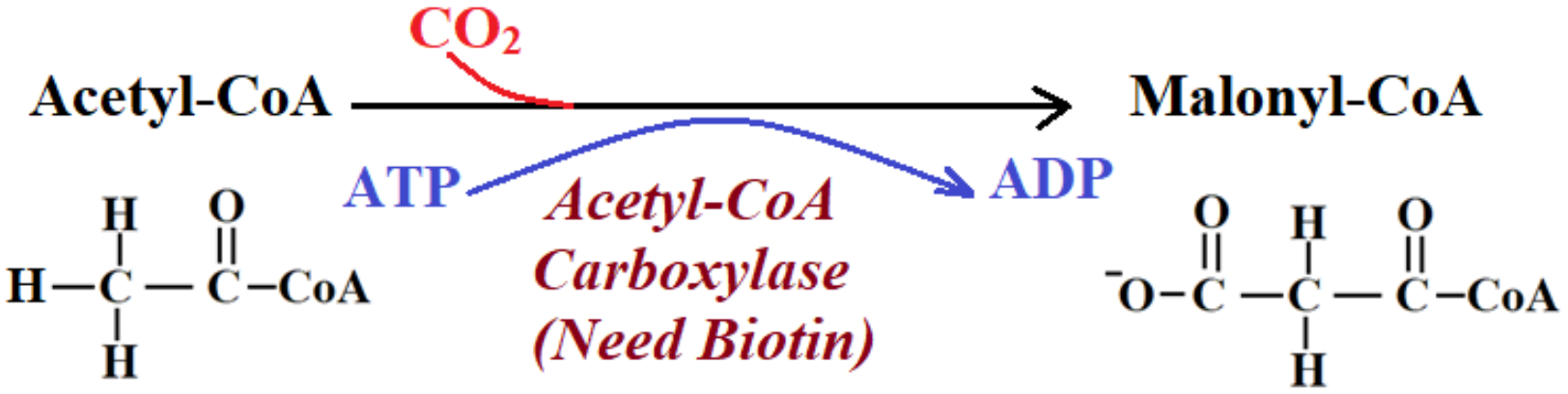
Reduction





Synthesis of Malonyl-CoA

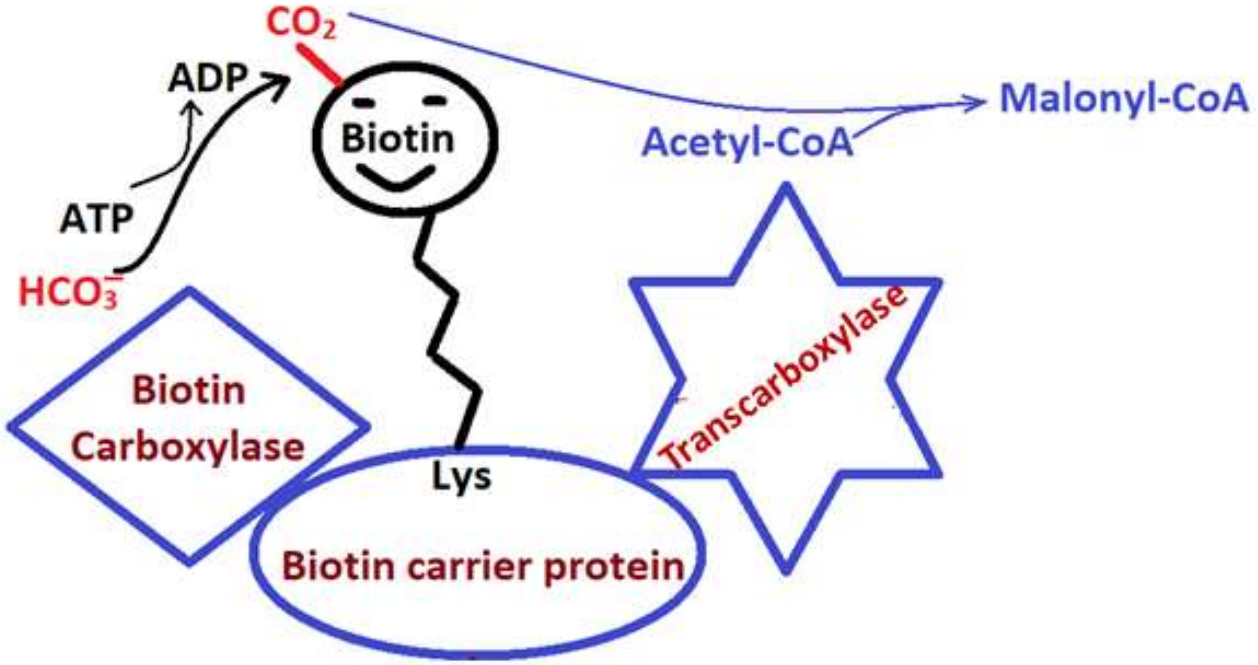
"The rate limiting and Control step of FA synthesis"



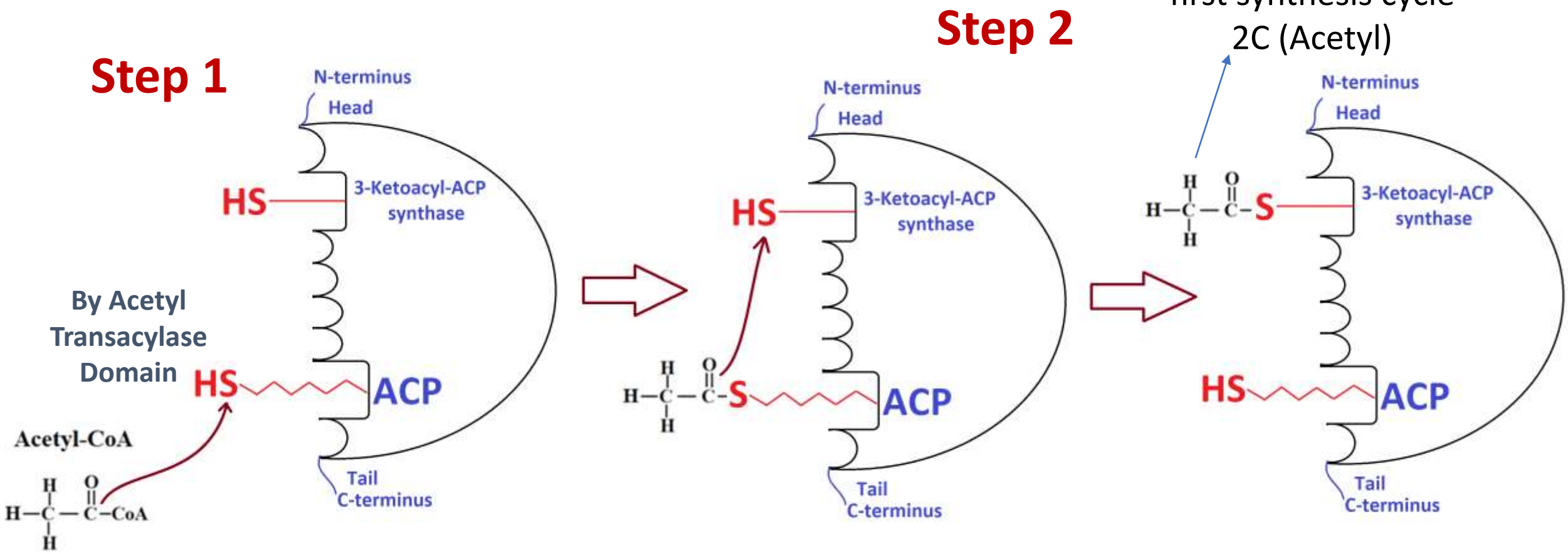
Acetyl-CoA Carboxylase in a multifunctional enzyme composed of 3 Domains with 3 activities:

1. **Biotin Carrier protein:** bind covalently to biotin by amide bond with Lysine
2. **Biotin Carboxylase:** attach CO_2 to Biotin, this require ATP, HCO_3^- is the source of CO_2
3. **Transcarboxylase:** Transfer CO_2 from Biotin to Acetyl-CoA forming Malonyl-CoA

Regulatory sites: Allosteric + Covalent



First synthesis cycle

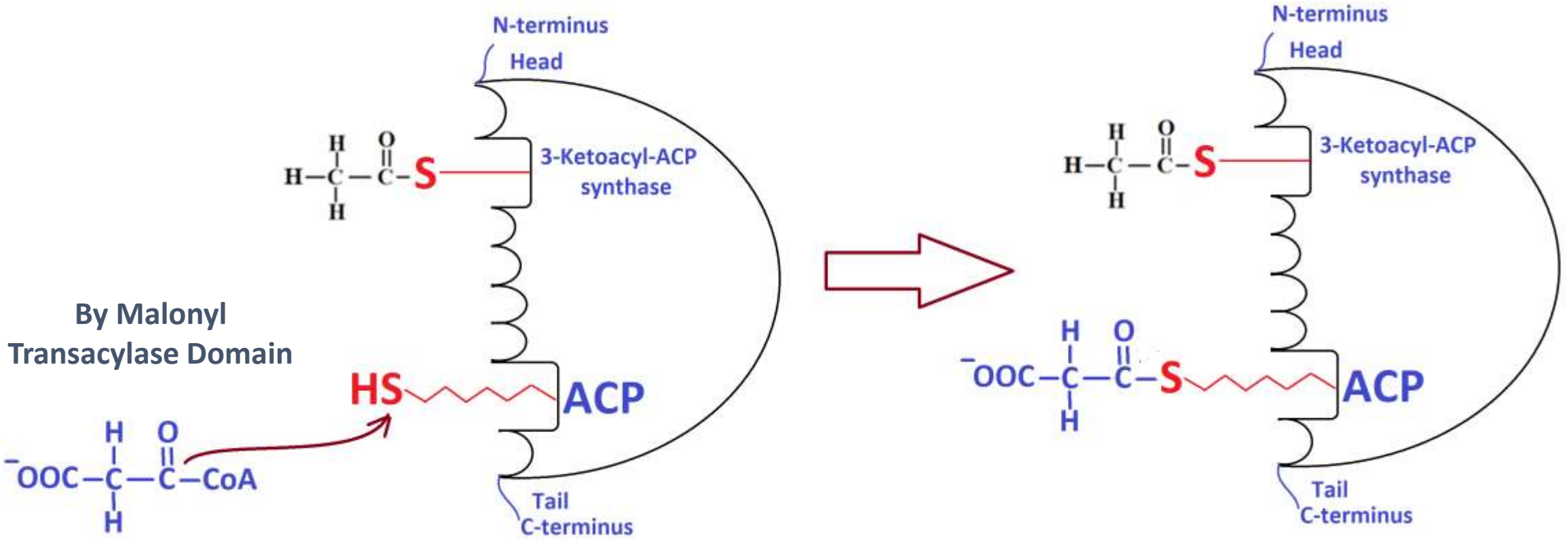


Step 1: Acetyl group (2C) is transferred fro acetyl-CoA to ACP by ***Acetyl Transacylase (AT) Domain***

فارغ

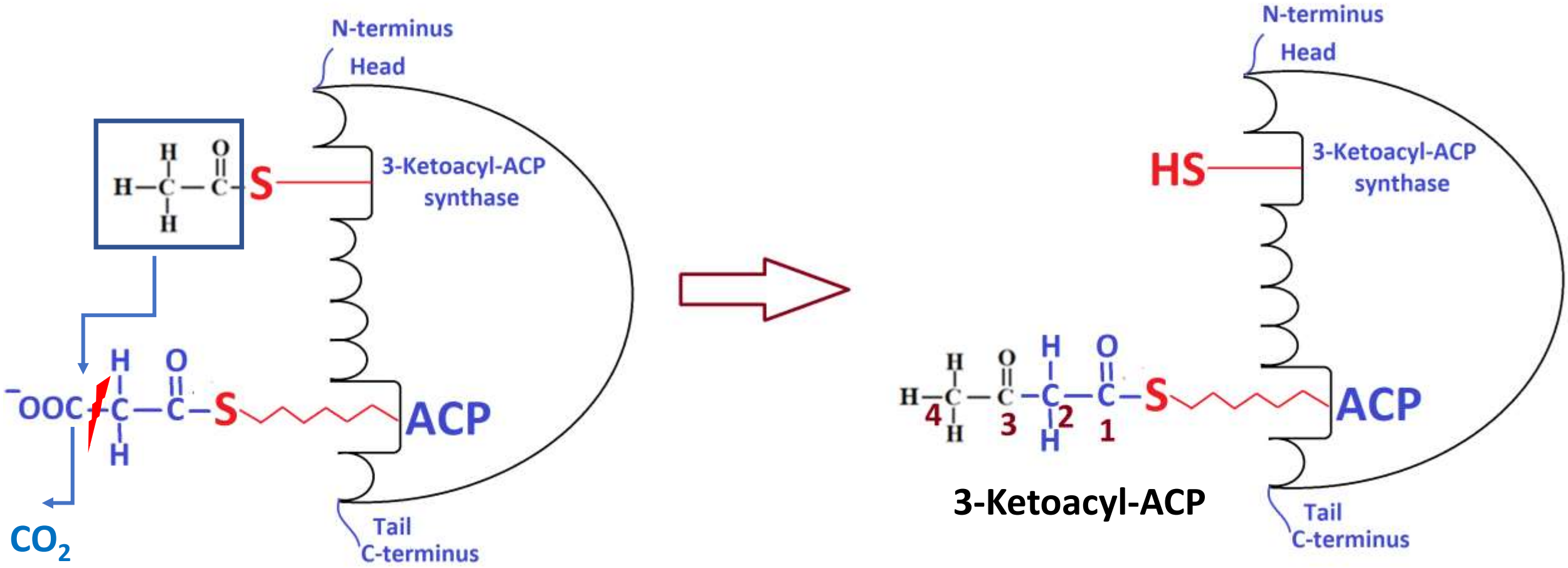
Step 2: the Acetyl group is transferred from ACP to **3-KetoAcyl Synthase Domain** leaving ACP vacant

Step 3



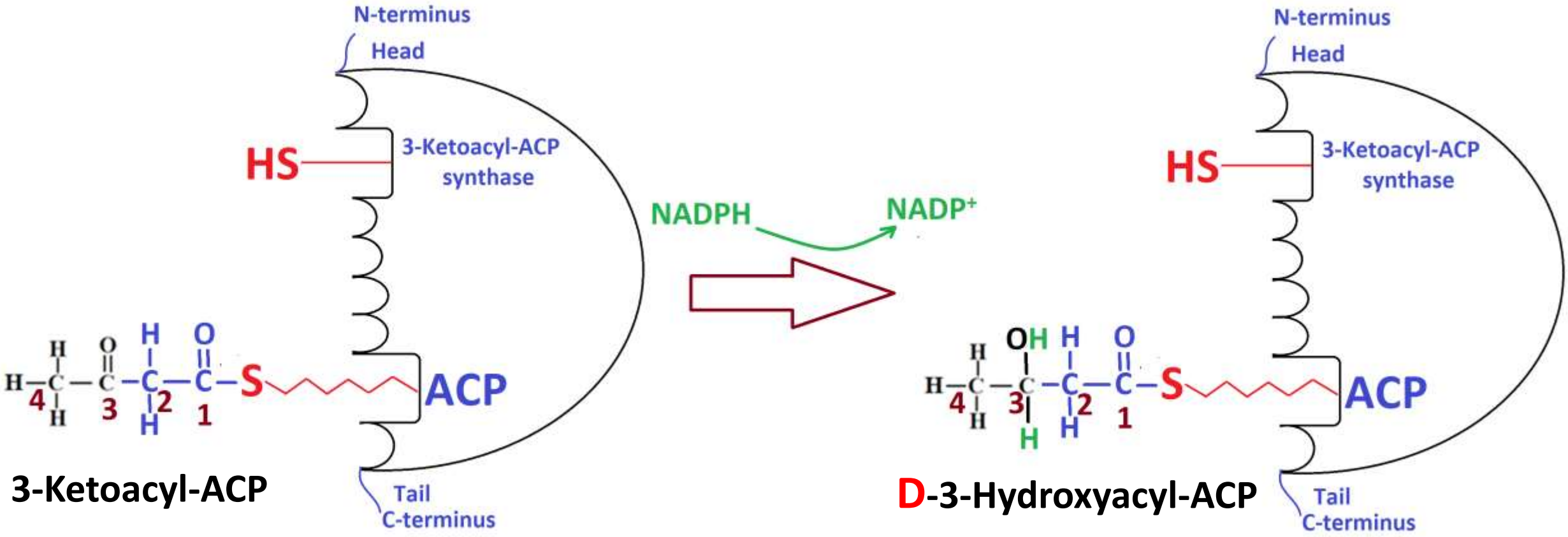
Step 3: Malonyl group (3C) is transferred from Malonyl-CoA to ACP by ***Malonyl Transacylase (MT) Domain***

Step 4 “Joining or Condensation”



Step 4: CO_2 is removed from Malonyl and the acetyl-group bound to **3-KetoAcyl Synthase** is transferred to the 2C remained from malonyl in the ACP forming 4C 3-Ketoacyl bound to ACP (3-Ketoacyl-ACP) this step by ***3-Ketoacyl-ACP synthase (KS) Domain (Condensation Step)***

Step 5 “Reduction”

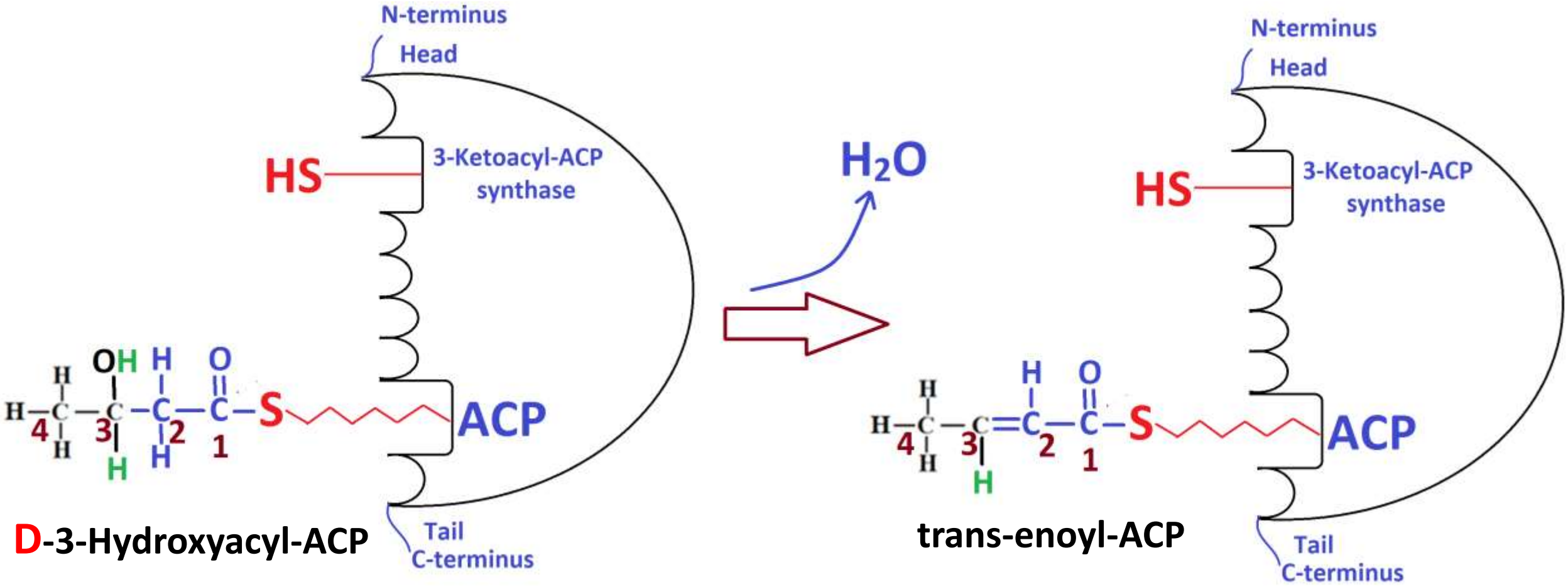


3-Ketoacyl-ACP

D-3-Hydroxyacyl-ACP

Step5: reduction of the Ketone group forming **D**-3-Hydroxyacyl-ACP by **3 -*Ketoacyl-ACP reductase (KR) Domain*** using NADPH as reducing agent

Step 6 “Dehydration”

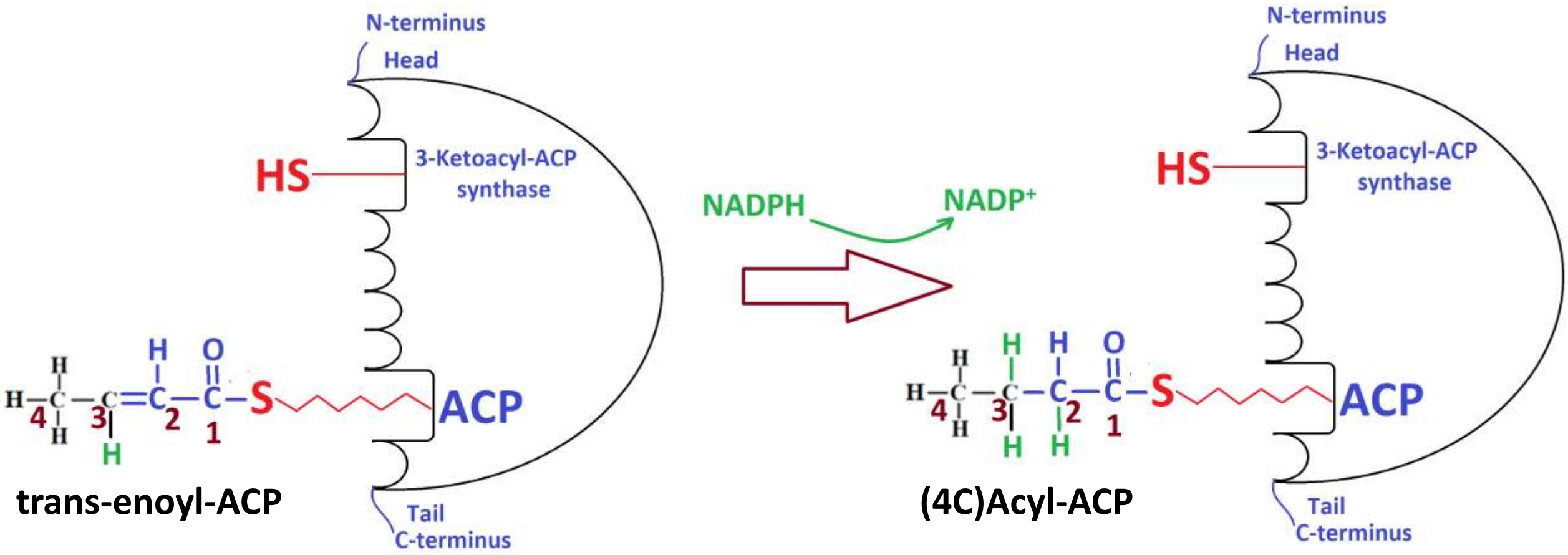


D-3-Hydroxyacyl-ACP

trans-enoyl-ACP

Step 6: dehydration forming trans-enoyl-ACP by ***3-Hydroxyacyl-ACP dehydrates (DH) domain***

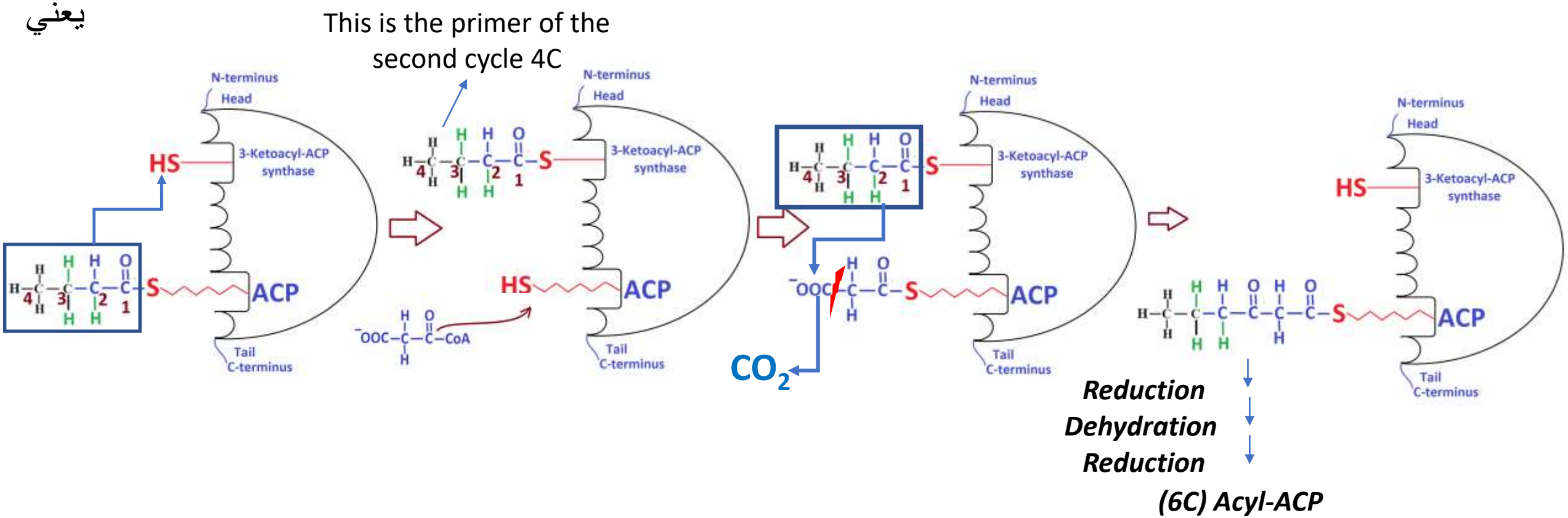
Step 7 “Reduction”



Step 7: reduction of the double bond forming 4C Acyl-ACP by ***enoly-ACP reductase (ER) Domain*** using NADPH as reducing agent مبروك صار عندك حمض دهني طوله 4 كربونات

Now we do a second cycle of synthesis repeating steps 2 – 7

يعني



The 4C acyl is transferred from ACP to 3-KetoAcyl Synthase Domain leaving the ACP vacant

Malonyl bound to ACP, remove CO_2 from malonyl and transfer the 4C acyl from **β-KetoAcyl Synthase Domain** to the 2C remained from Malonyl forming 3Ketoacyl-ACP then Reduction → Dehydration → Reduction

صار طوله 6 كربونات

# of Synthesis Cycles = $\frac{\#\ of\ Carbons}{2} - 1$

Each Cycle need 2 NADPH

Each Cycle Produce 1 H_2O

Each Cycle use Malonyl-CoA

Each Molonyl-CoA need 1 ATP

Total Acetyl-CoA used = $\frac{\#\ of\ Carbons}{2}$

One as Primer, the rest as Malonyl

In the cytosol we can do maximum **7cycles** producing **Saturated 16C acyl (Palmitoyl)** bound to ACP

After that the last Domain ***Thioesterase (TE) Domain*** hydrolyze the thioester bond between Pamitoyl and ACP releasing Palmitic acid (16:0)

- **The ultimate source of All Palmitate C is Acetyl-CoA but:**

- 2C is directly passed from Acetyl-CoA in the first step
- 14 passed through malonyl-CoA during the 7 synthesis cycles

Q: For synthesis of Pamitate (16:0) from cytosolic Acetyl-CoA?

-How many Cycles of synthesis (Condensation)? **7 cycles**

-How many Malonyl-CoA? 7 **Malonyl-CoA**

-How many Acetyl-CoA (as Acetyl-CoA)? **1 Acetyl-CoA**

-How many total Acetyl-CoA? **8 Acetyl-CoA**

-How many NADPH? **14 NADPH**

-How many H_2O? **7 H_2O – 1H_2O to release it from ACP = 6H_2O**

-How many ATP required? **7 ATP**

The overall reaction for palmitate synthesis:

8 Acetyl CoA + 7HCO_3^-+ 7ATP +14NADPH+14H^+ →→ Palmitic acid+ 8CoA+ 7CO_2 +14$NADP^+$+7ADP+7Pi+6H_2O

7 acetyl-CoA → 7 Malonyl-CoA

For more Elongation of the fatty acid? لزيادة الطول

The process occurs in the **smooth ER or Mitochonderia**, by enzymes called ***Elongases*** they successively add 2C at the carboxyl (head) side till we reach the desired length 18, 20, 22….

To make the fatty acid unsaturated → occurs in the Smooth ER

In the **smooth ER** we have Enzymes called ***Fatty acyl CoA Desaturases*** that add ***cis*** double bonds to long chain fatty acids

We have

Desaturase 9 → add double bond at C9 (C9 = C10)

Desaturase 6 → add double bond at C6 (C6 = C7)

Desaturase 5 → add double bond at C5 (C5 = C6)

Desaturase 4 → add double bond at C4 (C4 = C5)

Notes:

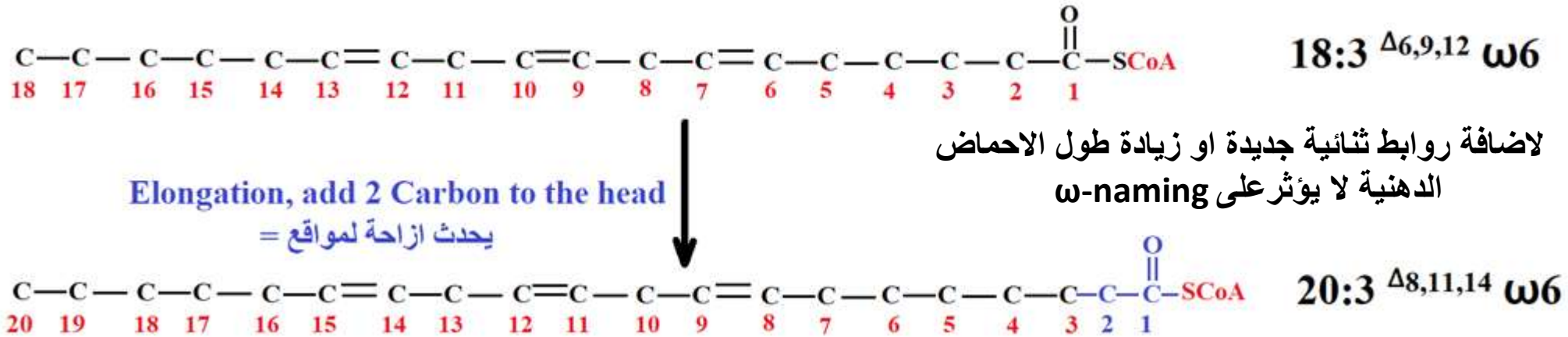
- The first double bond added is usually at C9 by desaturase 9
- all desaturases require O_2, NADH, Cytb5 and it's FAD-linked reductase
- We (Human) can NOT add double bonds beyond (past) بعد C9, that's why we cannot synthesize fatty acids containing double bonds beyond C9 and must obtained from diet we call them ***Essential fatty acids*** Linoleic Acid ($18:2^{\Delta 9,12}$) and Linolenic acid ($18:3^{\Delta 9,12,15}$)
- We can synthesize Arachidonic acid $20:4^{\Delta 5,8,11,14}$ from Linoleic acid so we call it ***Semi-essential***
- If low intake of linoleic acid → Arachidonic acid become essential

Examples

Stearic acid (18:0) by desaturase 9 become Oleic acid (18:1 $^{\Delta 9}$)

Palmitic acid (16:0) by desaturase 9 become Palmitoleic acid (16:1 $^{\Delta 9}$)

Note:



التعديلات

Q: write the product of the following Fatty acid Modifications?

1. **Elongation of 18:2$^{\Delta 9,12}$**
2. **The following series of modification on 18:2$^{\Delta 9,12}$**

Desaturase 6

Then elongation

Then desaturase 5

احفظ هاي خطوات تصنيع الناتج اللي رح يطلع معك

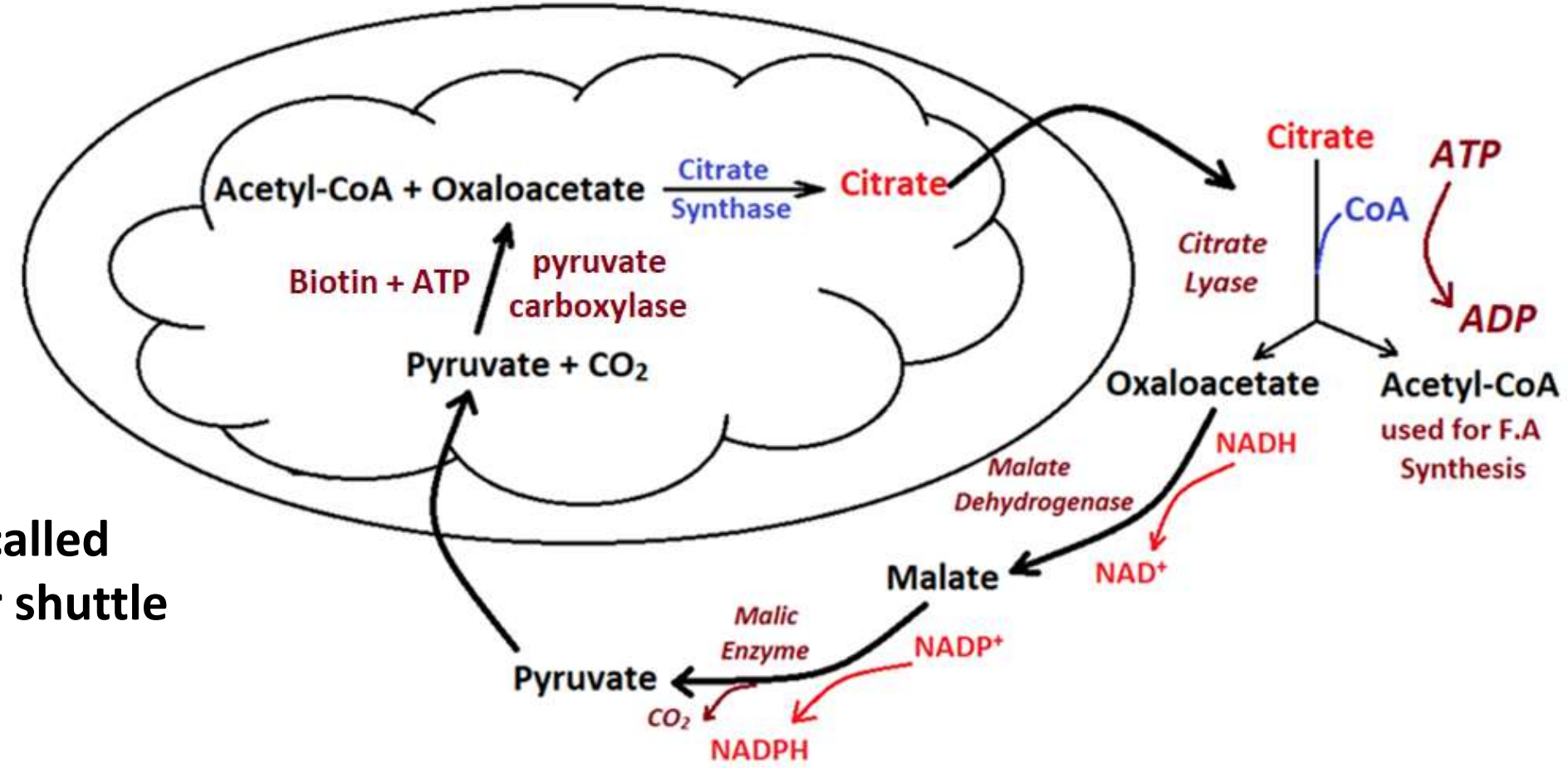
Important note

As we said; Fatty acids synthesis occurs in the **Cytosol** from **Acetyl-CoA**

But Acetyl-CoA used to synthesize fatty acids formed in the Mitochondrial Matrix from Pyruvate (carbohydrate)

So we should transfer Acetyl-CoA from Mitochondria to the Cytosol, **HOW?**

Acetyl-CoA can **NOT** cross the inner mitochondrial membrane (No Carrier)



This Process called
Citrate Shunt or shuttle

In Words:

1. Mitochondrial Acetyl-CoA produced mainly from pyruvate of Glycolysis is bounded to oxaloacetate forming Citrate by citrate synthase (1st step of TCA)
2. Citrate get out of Mitochondria to the Cytosol (So acetyl is transported as part of Citrate)
3. In the Cytosol Citrate is cleaved (يكسر) by ***Citrate Lyase*** which consume (يستهلك) ATP producing:

- Acetyl-CoA used for FA synthesis
- Oxaloacetate (OAA), this OAA is reduced (تختزل) to malate in the cytosol by ***Cytosolic Malate dehydrogenase*** (NADH is oxidized to NAD^+), then Malate is Oxidatively decarboxylated to Pyruvate by ***Malic Enzyme*** "$NADP^+$ is reduced to NADPH"

4. Pyruvate is return back to Mitochondria where it can be converted to Oxaloacetate by ***Pyruvate Carboxylase***

Note: for each acetyl-CoA transported to cytosol:

- Consume ATP
- NADH to NAD^+
- Produce NADPH

NADPH required for FA synthesis come from **PPP and from Cytosolic Malic Enzyme**

Excess Carbohydrate → Synthesis of Fatty acids

زيادة

So, in this case you want Citrate to get out of Mitochondria NOT to TCA cycle, How this happens?

Excess Carbohydrate→ Increase ATP (high energy charge) → inhibit TCA cycle → Increase Isocitrate and Citrate→ So Citrate accumulate and get out of Mitochondria

يتراكم

* High Cytosolic Citrate indicate high energy signal حضرتك ماكل كثير كربوهيدرات

So high Citrate and ATP enhance FA synthesis

تحفز

Regulation of F.A Synthesis

Control activity of **Acetyl-CoA Carboxylase**

(Malonyl-CoA الانزيم المسؤول عن صناعة)

a. *Allosteric Control*

This enzyme has 2 forms

Octamer: **inactive**

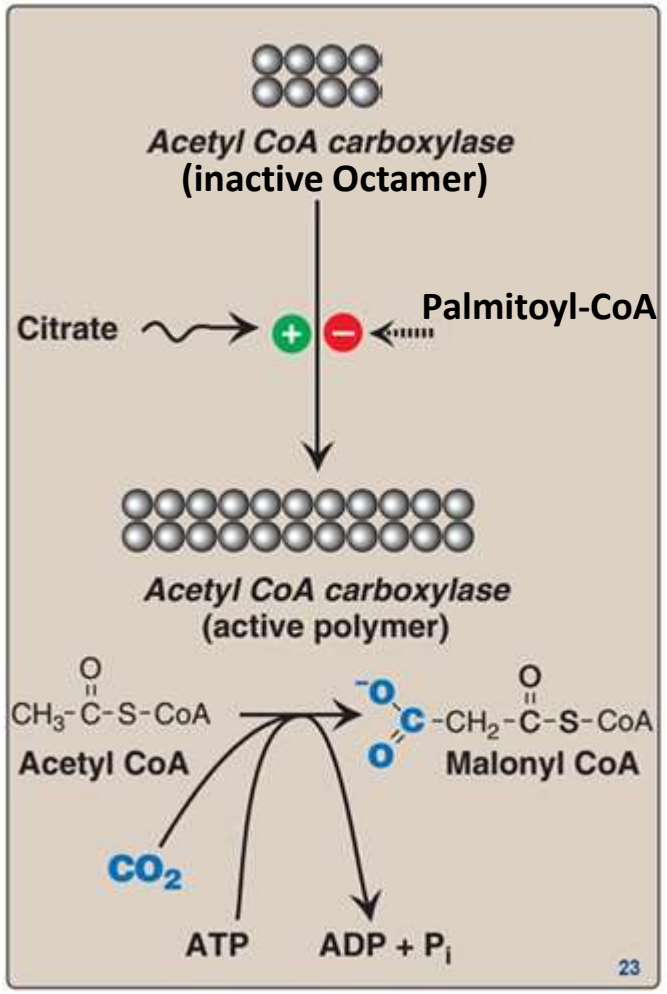
Filamentous polymer: **Active**

↑ **Citrate** → Induce polymerization of the enzyme → Activation

تحفز

↑ **Palmitoyl-CoA** "End-product" → inhibit polymerization → Inhibition

الناتج النهائي



b. Covalent control (phosphorylation)

Acetyl-CoA Carboxylase has 2 forms

- Phosphorylated → Inactive
- Dephosphorylated → Active

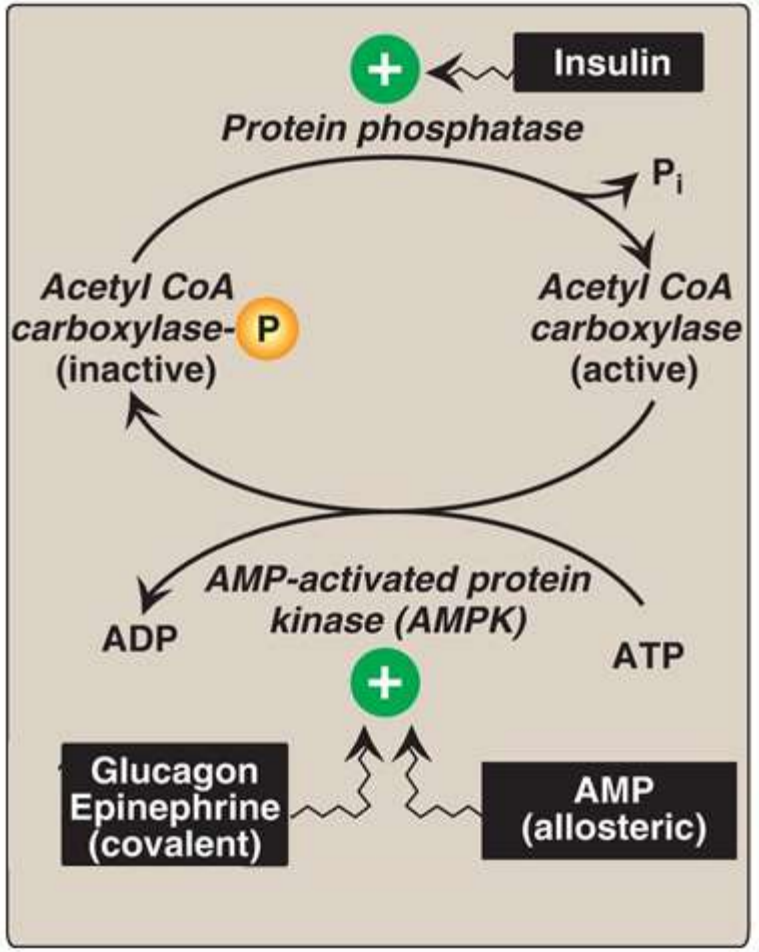
AMP-activated protein kinase (AMPK) phosphorylate Acetyl-CoA carboxylase → Inhibition

Protein phosphatase dephosphorylate Acetyl-CoA carboxylase → Activation

<u>AMPK is activated by:</u>

- Allosterically by AMP "high AMP indicate low energy so we cannot synthesize fatty acids"
- Covalently by phosphorylation through Glucagon and Epinephrine

AMPK انزيم اخر ينشط عندما تضيف عليه فوسفات



High Glucose → Insulin → activate protein phosphatase → dephophorylate and activate Acetyl-CoA Carboxylase → Activate FA synthesis

Low Glucose → Glucagon and Epinephrine → phosphorylate and activate AMPK → phosphorylate and Inhibit Acetyl-CoA Carboxylase → Inhibit FA synthesis

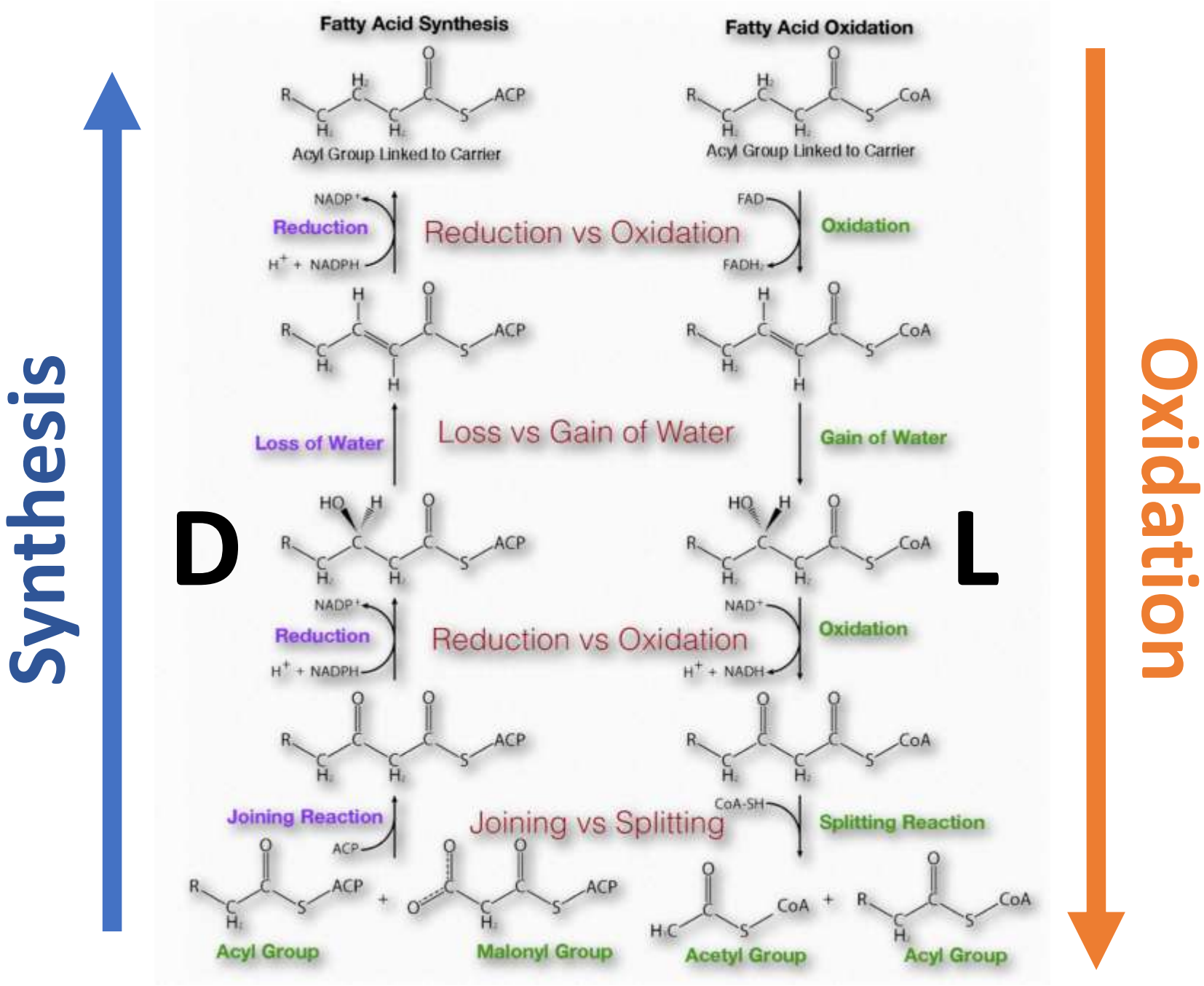
Summary:

- Citrate, Insulin, high carbohydrate diet "Glucose" → Activate acetyl-CoA Carboxylase →Activate FA synthesis
- Palmitoyl-CoA, AMP, low carbohydrate, Glucagon and Epinephrine → Inhibit acetyl-CoA Carboxylase →Inhibit FA synthesis

Regulation of Fatty acid catabolism (β-Oxidation)

↑ Malonyl-CoA→ Inhibition of FA catabolism by inhibiting ***CAT-I "Carnitine Shuttle"*** → Inhibit catabolism of Long chain FA

When we synthesize malonyl-CoA this mean that the cell need to synthesize Fatty acid, so fatty acid degradation should be stopped


Synthesis
Oxidation
Fatty Acid Synthesis
Fatty Acid Oxidation
Acyl Group Linked to Carrier
Acyl Group Linked to Carrier
NADP+
Reduction
H+ + NADPH
Reduction vs Oxidation
FAD
Oxidation
FADH2
Loss of Water
Loss vs Gain of Water
Gain of Water
D
L
NADP+
Reduction
H+ + NADPH
Reduction vs Oxidation
NAD+
Oxidation
H+ + NADH
Joining Reaction
ACP
Joining vs Splitting
CoA-SH
Splitting Reaction
Acyl Group
Malonyl Group
Acetyl Group
Acyl Group

VARIABLE	SYNTHESIS	DEGRADATION
Greatest flux through pathway متى اكثر اشي بتصير	After carbohydrate-rich meal	In starvation
Hormonal state favoring pathway	High insulin/glucagon ratio	Low insulin/glucagon ratio
Major tissue site	Primarily liver	Muscle, liver
Subcellular location موقعها بالخلية	Cytosol	Primarily mitochondria
Oxidation/reduction coenzymes	NADPH (reduction)	NAD^+, FAD (oxidation)
Product of pathway	Palmitate	Acetyl CoA
Repetitive four-step process	Condensation, reduction dehydration, reduction	Dehydrogenation, hydration dehydrogenation, thiolysis

Questions:

1. How many ATP required for the synthesis of Palmitate (16:0) from 8 Cytosolic Acetyl-CoA?

7 ATP for synthesis of 7 malonyl-CoA

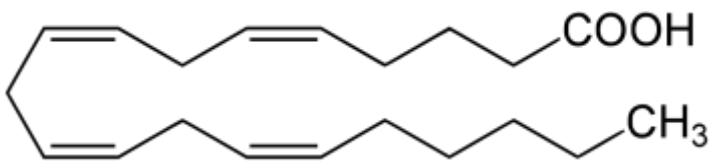
2. How many ATP required for the synthesis of Palmitate (16:0) from 8 Mitochondrial Acetyl-CoA?

15 ATP

(8 ATP to Transport 8 Acetyl-CoA from mitochondria to Cytosol + 7 ATP to convert 7 Acetyl-CoA to 7 malonyl-CoA

Eicosanoids: fatty acid derivatives

- variable group of compounds produced by almost all body tissues with a very wide range of functions
- Contain 20C atoms **"Eicosa = 20"**
- The **precursor** of all Eicosanoids is mainly **Arachidonic Acid 20:4$^{\Delta 5,8,11,14}$** (an ω6 fatty acid, semi- essential and synthesized from linoleic acid 18:2$^{\Delta 9,12}$)



- Eicosanoids can be classified into 4 main families:

a. **Prostaglandins (PG)**
b. **Thromboxans (Tx)**
c. **Leukotrienes (LT)**
d. **Prostacyclines (PC)**

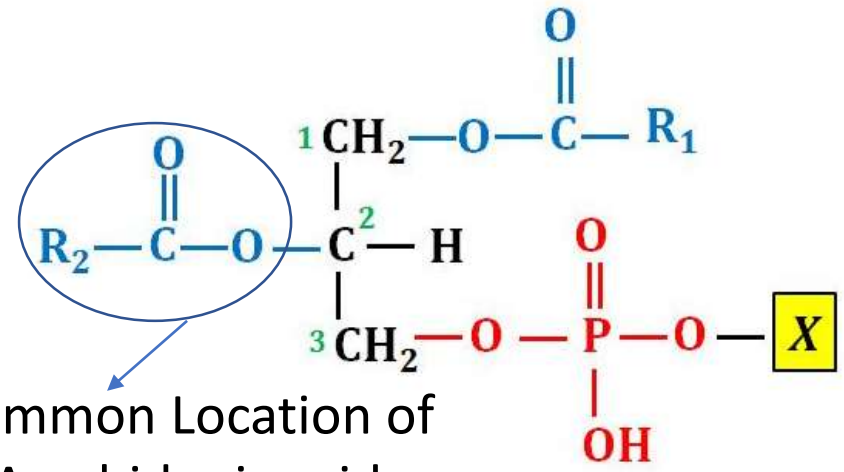
Functions of eicosanoids in general:

- Control smooth muscle contraction in the blood, bronchial tree, Uterus….
- Many LT and PG Induce inflammation "swelling, redness, fever, pain….."
- Thromboxanes control platelets aggregation

Dietary Linoleic acid → **Arachidonic acid**

added to C2 of Glycerol in glycerophospholipid

When the cell need to synthesize Eicosanoid we first release arachidonic acid from the phospholipids



Common Location of Arachidonic acid

Glycerophospholipids

" source of Arachidonic acid for Eicosanoid synthesis"

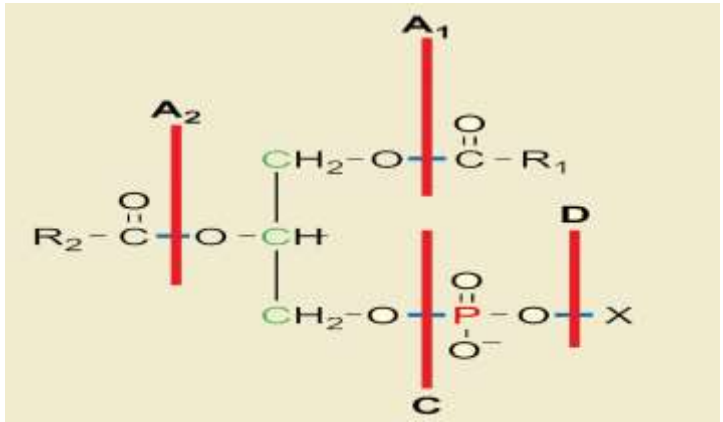
Hydrolysis of Glycerophospholipids is catalyzed by enzymes called Phospholipases; 4 types according to site of action

Phospholipase A1

Phospholipase A2

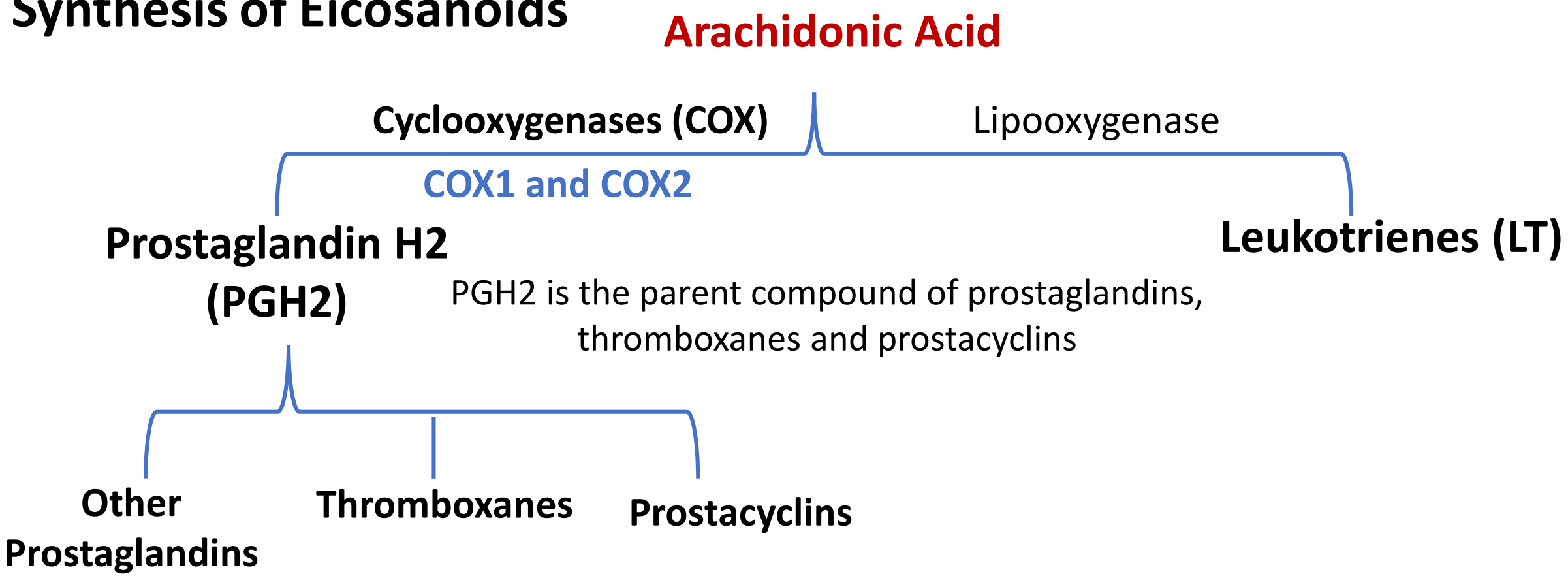
Phospholipase C

Phospholipase D



Phospholipase A2 is required to release Arachidonic acid for Eicosanoid synthesis

Synthesis of Eicosanoids



There are 2 types of COX enzymes:

i. **COX1:** constitutive enzyme "has constant concentration throughout the life of the cell" found primarily in the stomach, kidney, and platelets and synthesize the PG and Thx that are required for the normal functions of these tissues

ii. **COX2:** found in monocytes, macrophage, and smooth muscles, its an inducible enzyme synthesized in these tissue during inflammation it synthesizes PGs that induce inflammatory symptoms

If we inhibit COX1:

- Affect gastric lining → Gastric Ulcer
- Prevent Tx synthesis in platelets → prevent clotting

If we inhibit COX2:

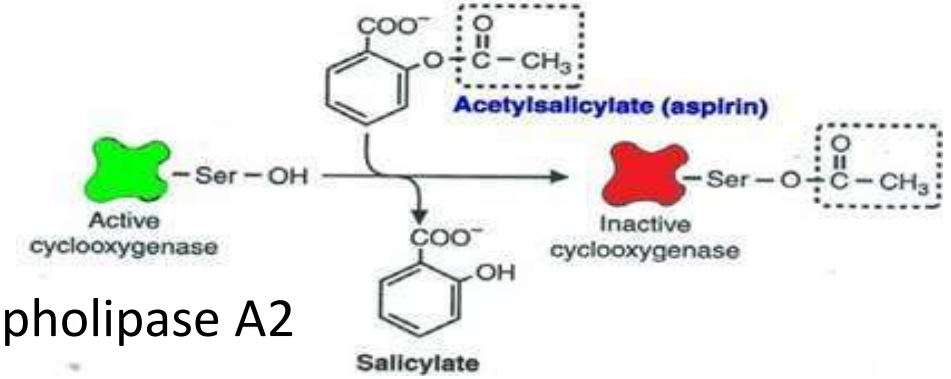
- Anti-inflammatory effect

-**Non-steroidal anti-inflammatory drugs (NSAIDs):** such as Aspirin, Ibuprofen….. These drugs **inhibit COX enzyme** so prevent PGs and Txs but not Leukotrienes

Aspirin "Acetyl-Salicylic acid": is nonselective COX inhibitor it irreversible inhibit both COX1 and COX2 by adding an acetyl group covalently to Serine residue in the active site of COX (suicide inhibitor)

Effect of Aspirin

- Prevent clotting due to inhibition of COX1 → baby aspirin 100mg after age of 50
- Analgesic and Antipyretic due to inhibition of COX2
- Side effect: Gastric Ulcer due to inhibition of COX1



Note: Steroidal anti-inflammatory drugs such as cortisol inhibit phospholipase A2 → no free Arachidonic acid → inhibit the synthesis of all Eicosanoids

Obesity and regulation of appetite (Hunger feeling)

- ***Obesity*** is increased fat and lipid storage in the body, many factors contribute to obesity such as the dietary habits, genetics…
- Obesity increase the risk of cardiovascular system, Diabetes, breast and colon cancer

➢ There are many hormones that regulate appetite:

a. Adipokines: peptide hormones released from adipocytes; include:

- **Leptin: Hunger suppressor**
- Adiponectin: regulate glucose level and fatty acid oxidation
- Resistin: induce inflammation, insulin resistance

b. **Ghrelin**: secreted by digestive tract (stomach) when its empty and its **Hunger stimulator,** which stimulate the hypothalamus to make you feel hungry

Leptin deficiency or Leptin receptor deficiency will increase the feeling of hunger and cause obesity