LIPID METABOLISM

Stages And Reaction Steps Of Beta Oxidation Of Fatty Acids

Three Stages Of Beta Oxidation For Oxidation Fatty acid Palmitate

Stage I
Activation of Long Chain Fatty acid (Acyl Chain) To Acyl-CoA In Cytosol

– Palmitate to Palmitoyl-CoA

In Cytosol

Stage II

Translocation of Activated Fatty acid From Cytosol into Mitochondrial Matrix

Through Role of Carnitine

(Carnitine Shuttle)

Stage III

Steps of Beta Oxidation Proper

In Mitochondrial Matrix

- Oxidation Reaction
- Hydration Reaction
- Oxidation Reaction
- Cleavage Reaction

Stage I
Activation Of Fatty acid In Cytosol
Is a Preparative Phase

Site Of Fatty acid Activation

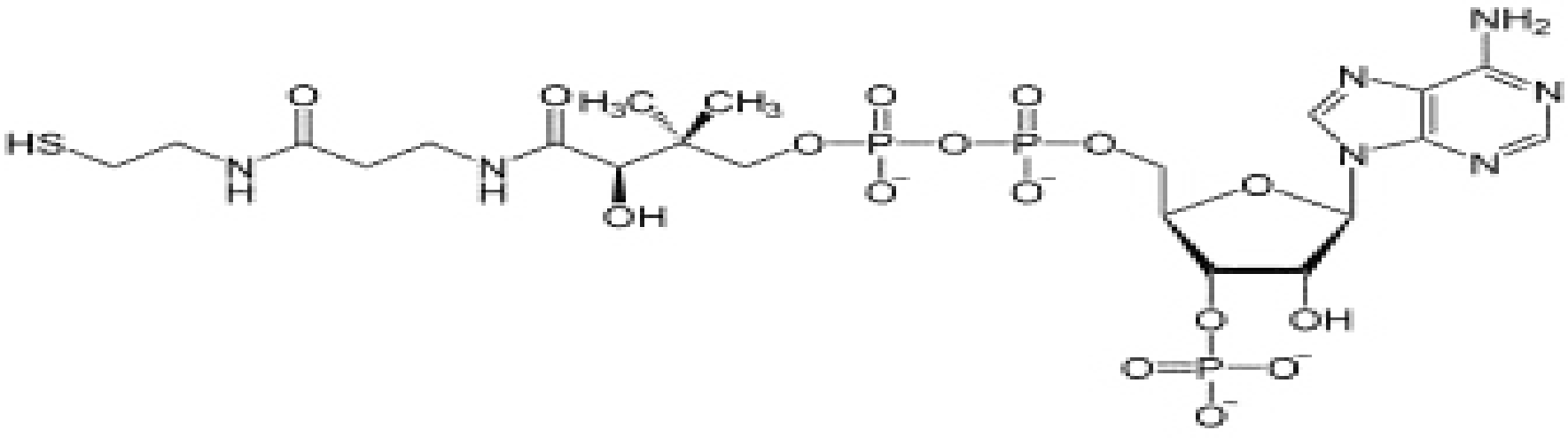
- Fatty acid(Acyl Chain) is activated in **Cytosol** to **Acyl-CoA** .

Requirements of FA Activation

- **Enzyme:**
  - **Thiokinase /Acyl CoA Synthetase**
- **Coenzymes/Cofactors:**
  - **CoA-SH derived from Pantothenic acid**
  - **ATP**
  - **Magnesium ions (Mg^{++})**

CoezymeA (CoA-SH) Activates Fatty Acids for Beta Oxidation

CoA Helps in Activation of Fatty Acid



- A **long chain** Fatty acid is termed as **Acyl chain.**

- **Every Fatty acid** which undergoes β Oxidation of Fatty acid is **first activated** to **Acyl-CoA.**

- **Activation of a Fatty acid means:**

- Linking of **Acyl Chain** to **Coenzyme A** to form **Acyl-CoA with a high energy bond.**

- **During Activation of Fatty acid (Acyl Chain)**

- '**H**' of **CoA-SH (Coenzyme A)** is substituted by **Acyl chain**

- To form **CoA-S Acyl**, i.e. **Acyl-CoA** an activated Fatty acid.

- Thus **CoenzymeA** is a **carrier of Acyl chain** in an activated fatty acid.

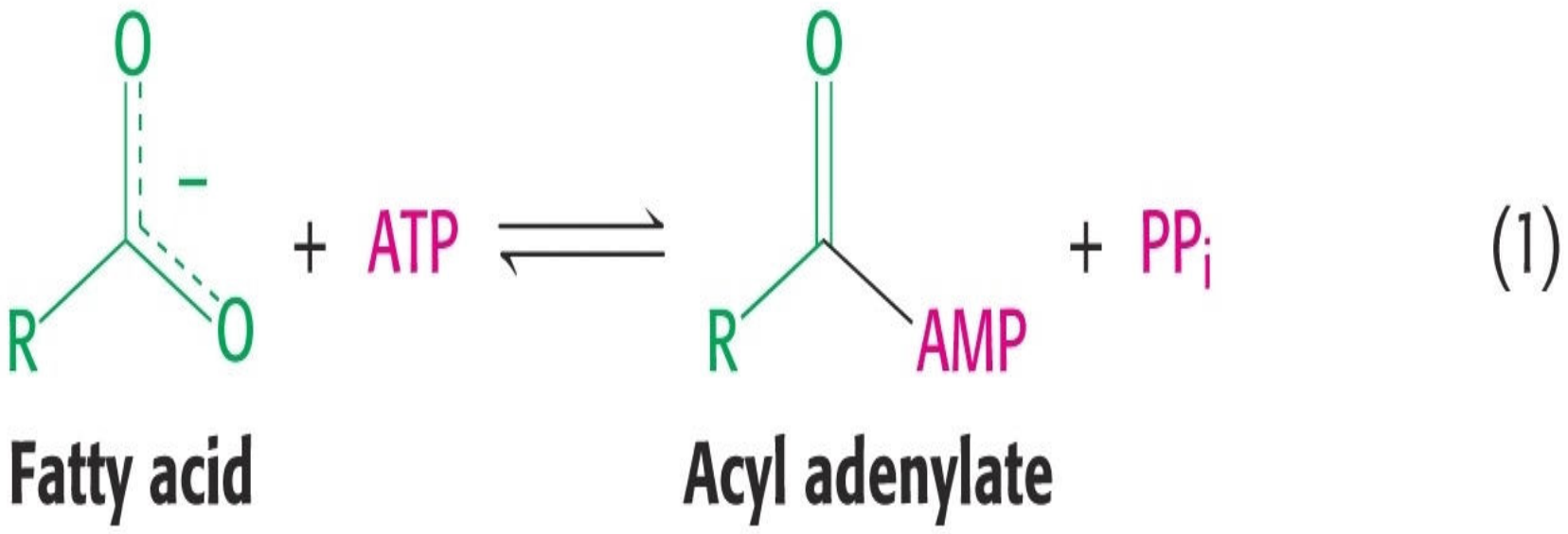
Steps Of Fatty Acid Activation

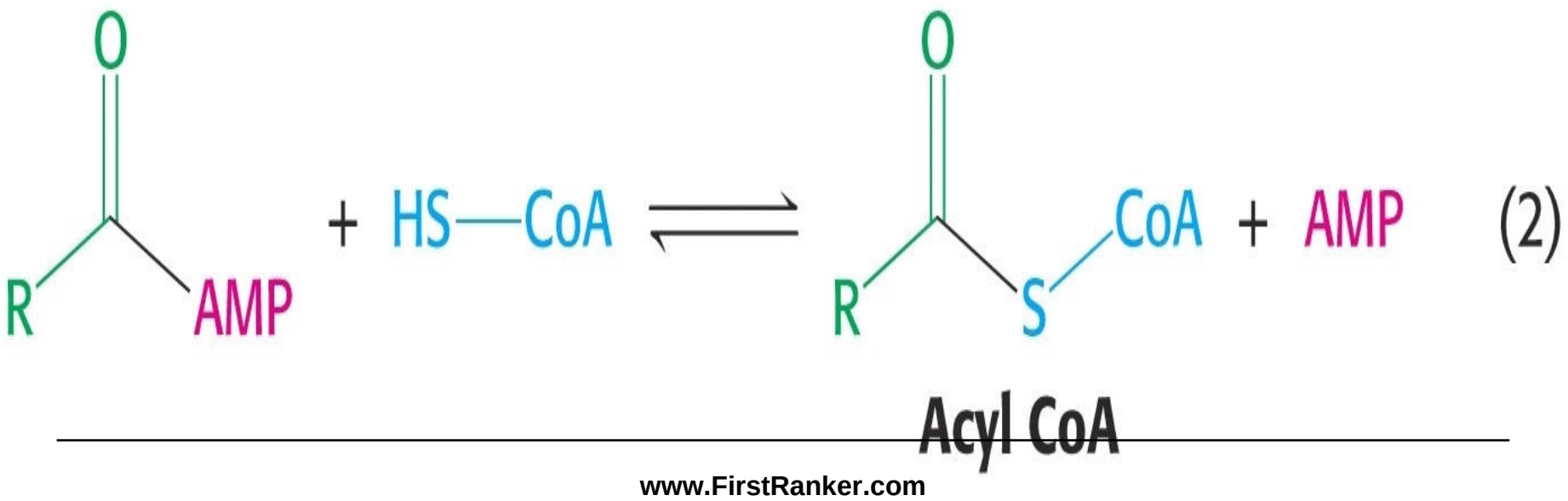
Activation Of a Fatty Acid

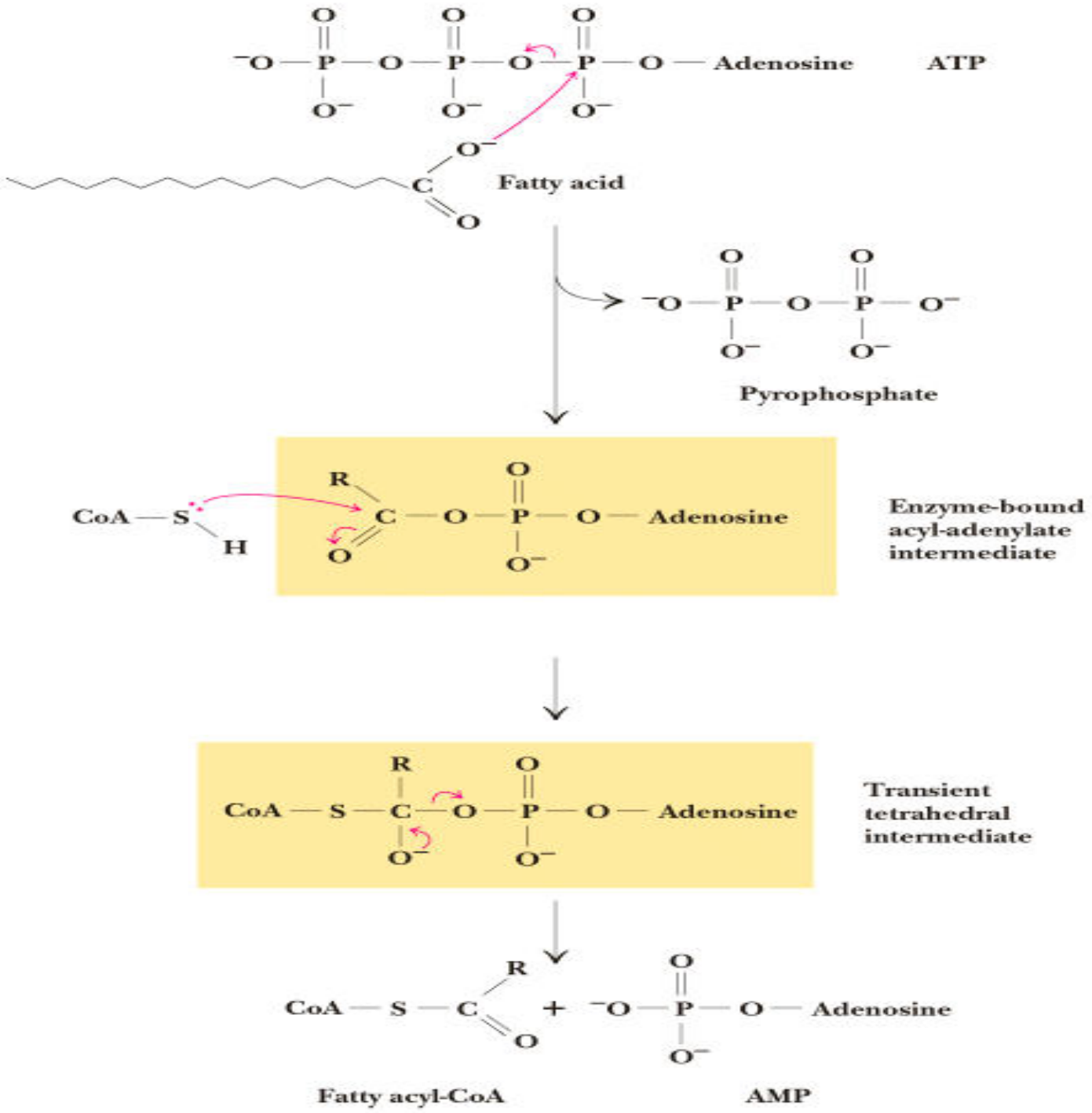
Is ATP Dependent

Converts ATP to AMP

Hence Requirement is equivalent to 2 ATPs







Acyl-CoA Synthetase/

Fatty Acid Thiokinase

condenses Fatty acids with CoA,

with simultaneous hydrolysis of ATP to AMP and PP_i

- An **Acyl-CoA is an activated energetic compound having high energy bond in it.**

- Thus formation of Acyl–CoA is an **expensive energetically**

Fatty acid Activation

- Activation of Fatty acids is **esterification of Fatty acid with Coenzyme A**
- In presence of **Acyl-CoA Synthetase** (**Thiokinase**) **forming an activated Fatty acid as Acyl-CoA.**
- This process is **ATP-dependent**, & occurs in **2 steps.**

- During the activation of Fatty acid **ATP is converted to AMP and ppi.**
- **Two high energy bonds** of **ATP** are **cleaved** and **utilized** in this activation which is **equivalent to 2 ATPs.**

- Subsequent hydrolysis of PP_i from ATP drives the **reaction strongly forward.**
- Note the **Acyl- Adenylate** is an **intermediate** in the mechanism.

- There are **different Acyl-CoA Synthetase** for fatty acids of **different chain lengths**.

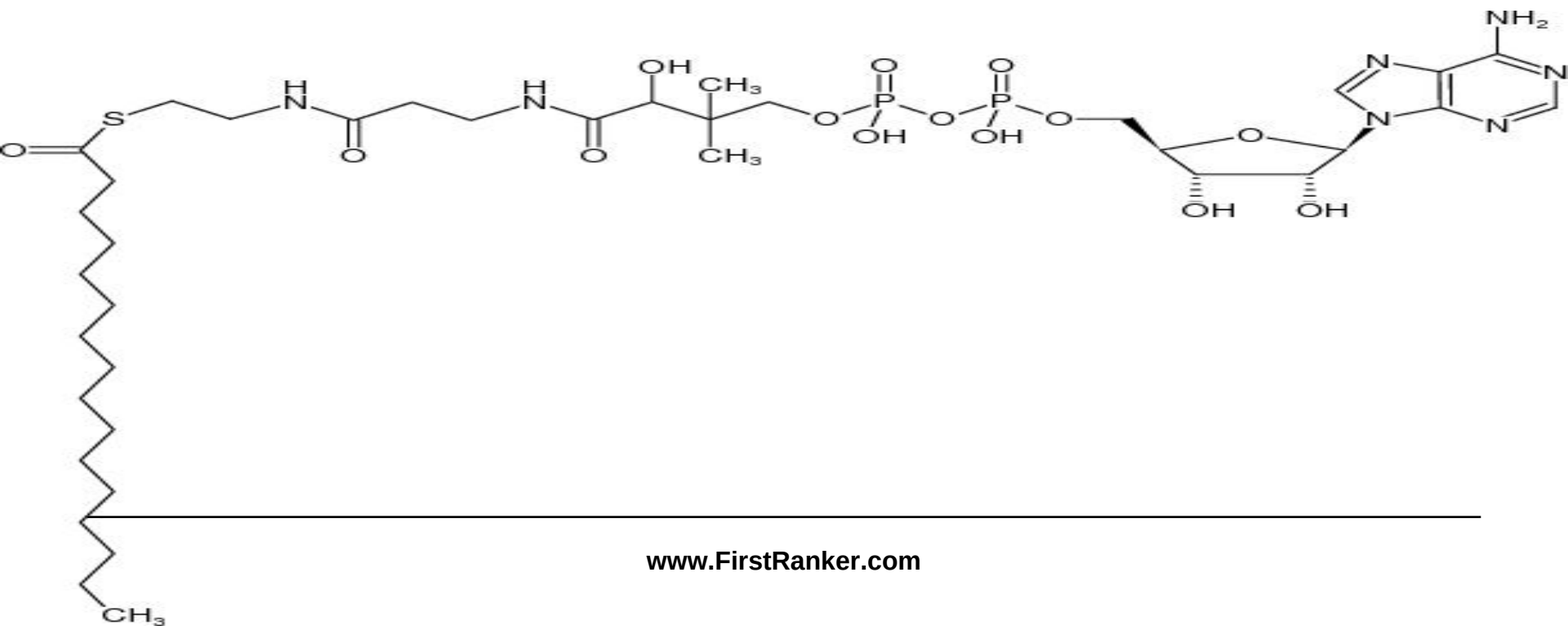
Activated Fatty Acid (Acyl-CoA) is a High Energy Compound

Which Facilitates Second Stage Of Beta Oxidation Of Fatty Acid

Stage II
Translocation Of Acyl-CoA From Cytosol Into Mitochondrial Matrix With The Help Of Carnitine

- β-oxidation proper occurs in Mitochondrial matrix.

- **CoA is a complex structure.**
- **CoA** part of Palmitoyl-CoA is **impermeable to inner membrane of Mitochondria**



- **Long-chain Fatty acids more than 12 Carbon atoms cannot be directly translocated** into the Mitochondrial matrix.

- However **short chain Fatty acids are directly translocated** into the Mitochondrial matrix

- **To translocate an activated long chain Fatty acid** (Acyl-CoA) from **cytosol to mitochondrial matrix**

- **Across mitochondrial membrane** operates a **specialized Carnitine Carrier System.**

What Is Carnitine?

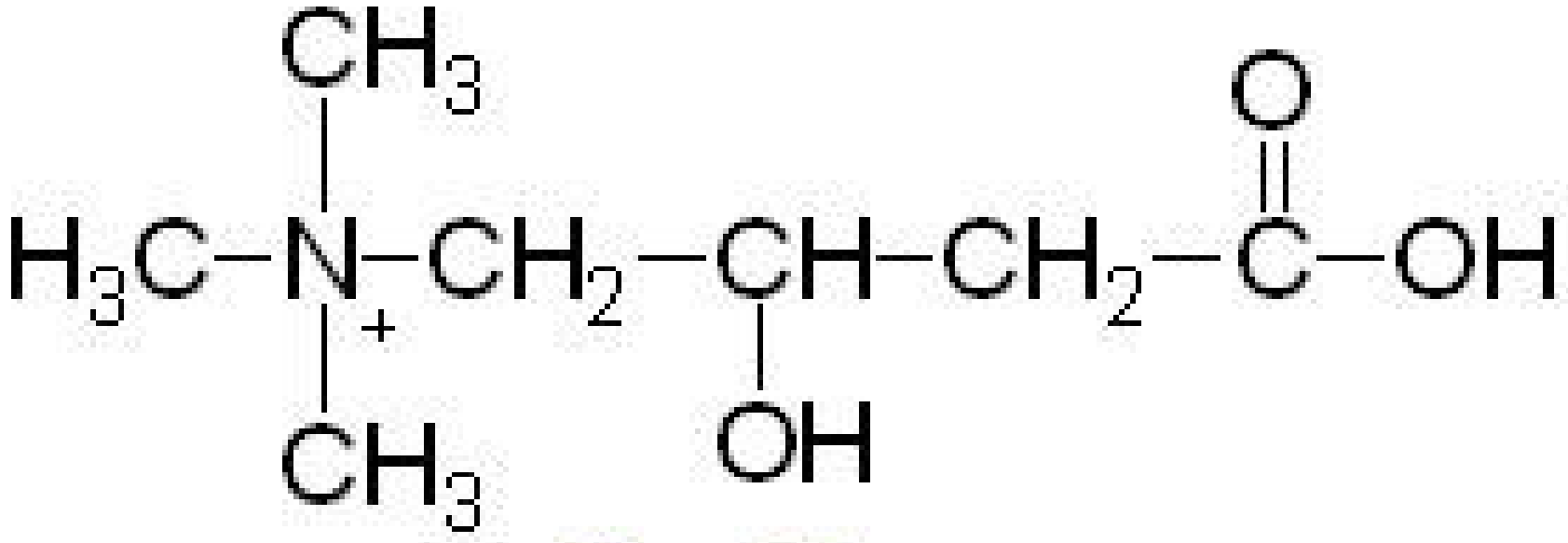
- **Carnitine** is a **functional, Non Protein Nitrogenous** (NPN) substance.
- Carnitine is **biosynthesized** in the body **by amino acids Lysine and Methionine.**

Carnitine chemically is

β Hydroxy- γ Tri Methyl Ammonium Butyrate

OR

3-Hydroxy 4- Tri Methyl Ammonium Butyrate



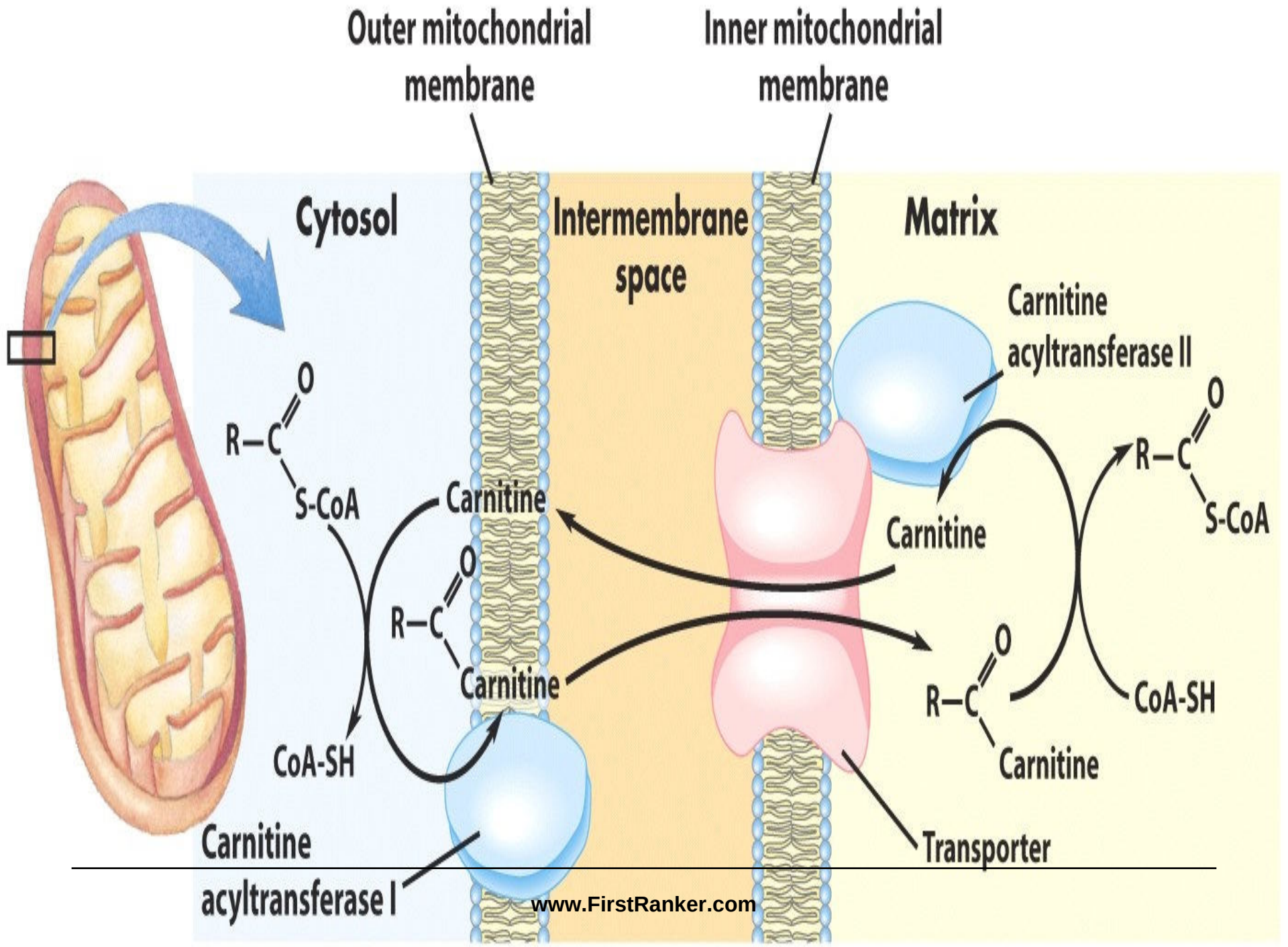
Carnitine

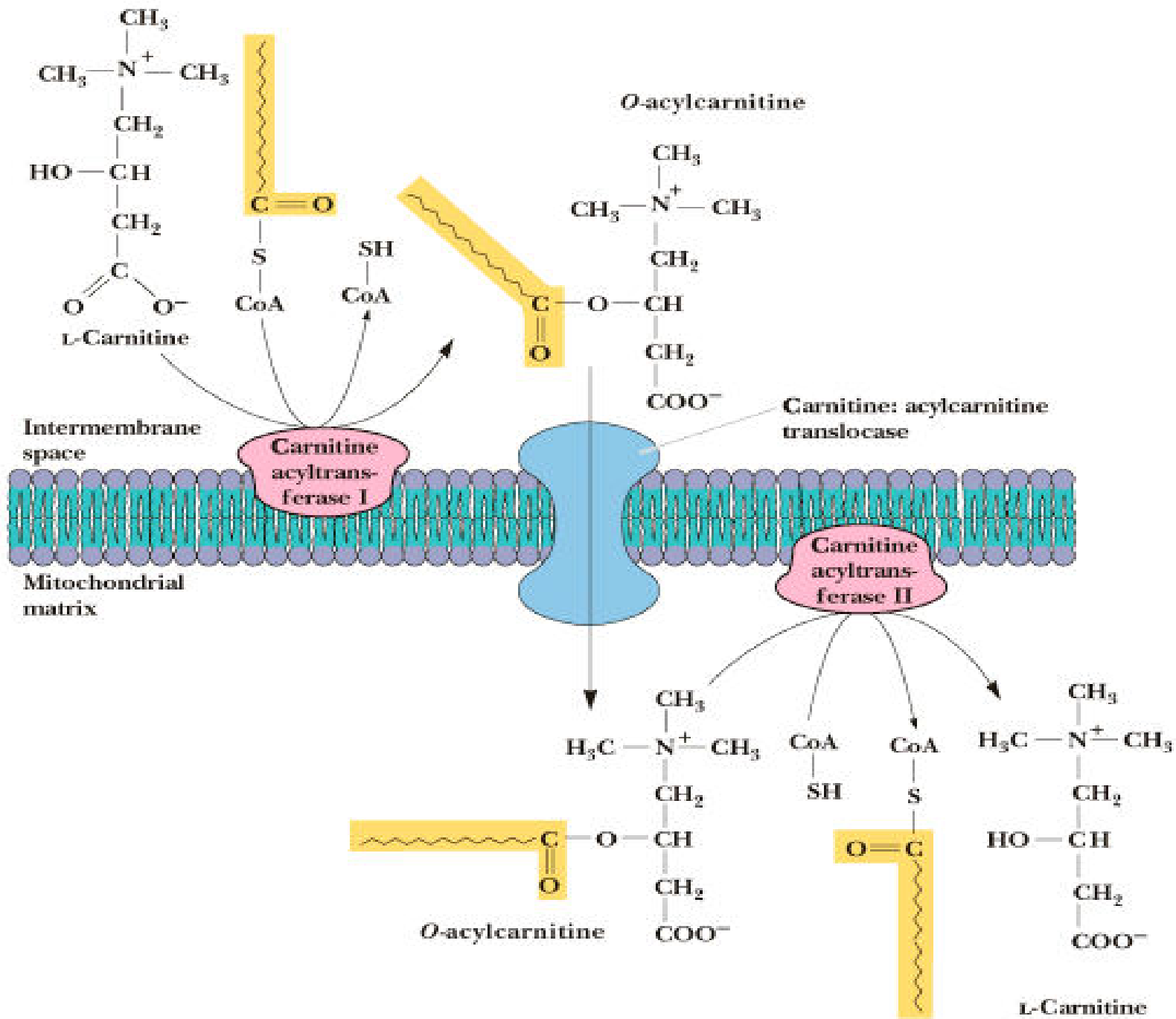
- **Long chain Acyl CoA** traverses an inner mitochondrial membrane with a special transport mechanism called **Carnitine Shuttle**.

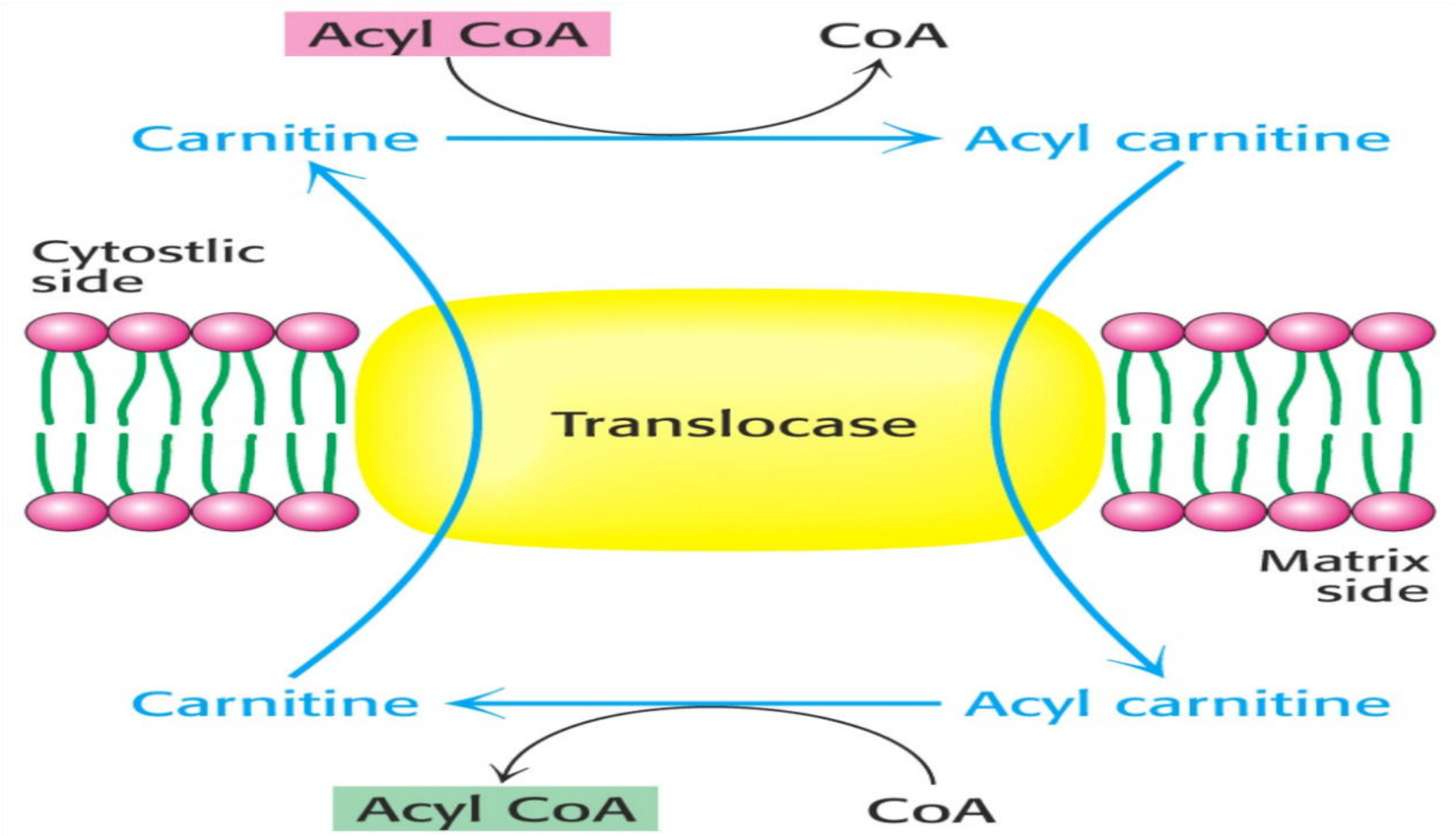
Significance Of Acyl-CoA Formation

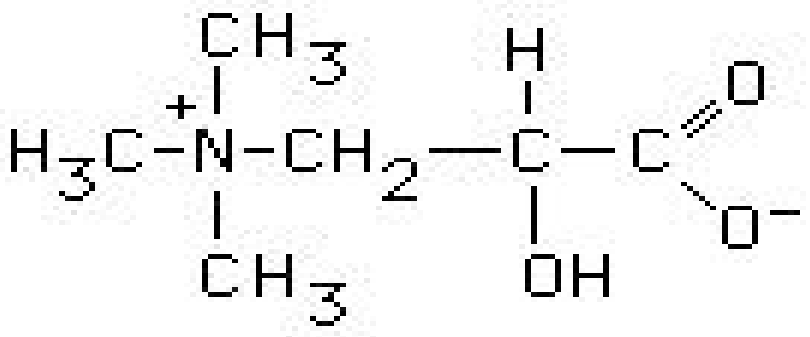
- High energy bond of Acyl-CoA releases high energy which helps in condensation of Acyl with Carnitine for further translocation.

Mechanism Of Carnitine In Transport Of Fatty Acyl CoA From Cytosol To Mitochondrial Matrix

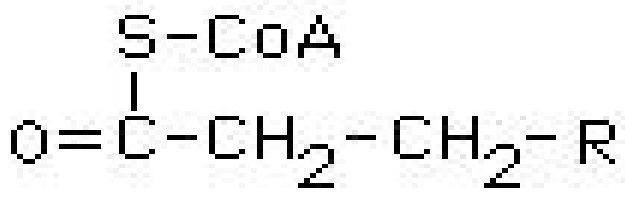




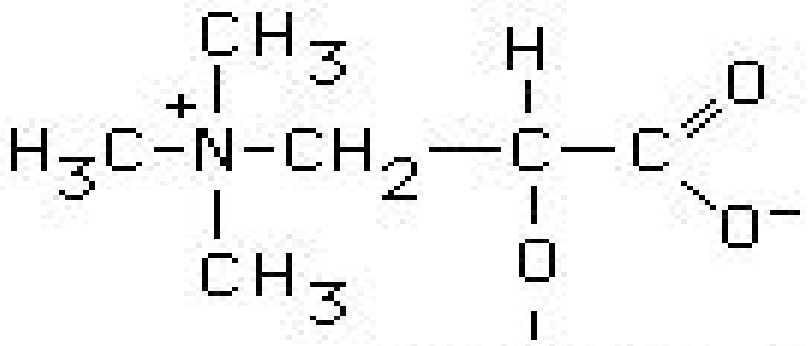




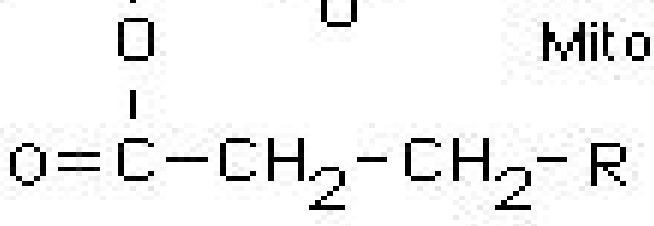
CARNITINE



Fatty Acyl CoA
Greater than 12 Carbons



ACYL CARNITINE
Can now traverse the Inner Mitochondrial Membrane



- Acyl-CoA a high energy compound **cleave its high energy bond in second stage.**

- Bond energy released is used up for **linking Carnitine to Acyl chain to form Acyl-Carnitine.**

- Long-chain FA are converted to Acyl Carnitine and are then transported

- Acyl-CoA are reformed inside an inner membrane of mitochondrial matrix.

- Acyl groups from Acyl-COA is transferred to Carnitine to form Acyl-Carnitine catalyzed by **Carnitine Acyl Transferase I (CAT I)**

- **CAT I is present associated to outer mitochondrial membrane**

- **Acylcarnitine is then shuttled across** an inner mitochondrial membrane by **a Translocase** enzyme.

- Acyl group is linked to CoA of Mitochondrial pool in mitochondrial matrix by **Carnitine Acyl Transferase II (CAT II) to regenerate Acyl-CoA in mitochondrial matrix.**

- Finally, Carnitine is returned to cytosolic side by **Protein Translocase**, in exchange for an incoming Acyl Carnitine.

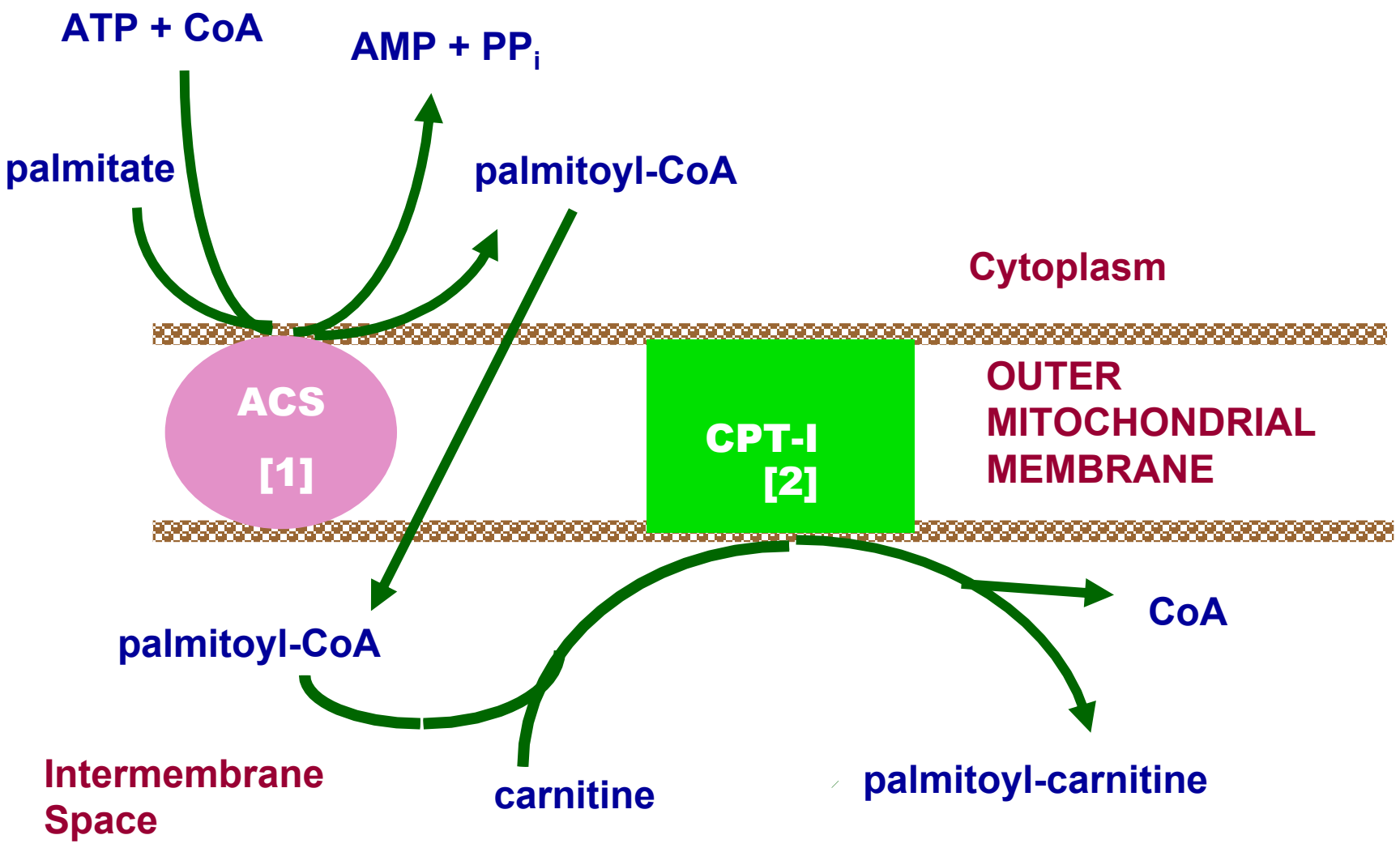
Points To Remember

- Cell maintains two separate pools of Coenzyme-A:
  - **Cytosolic pool of CoA**
  - **Mitochondrial pool of CoA**

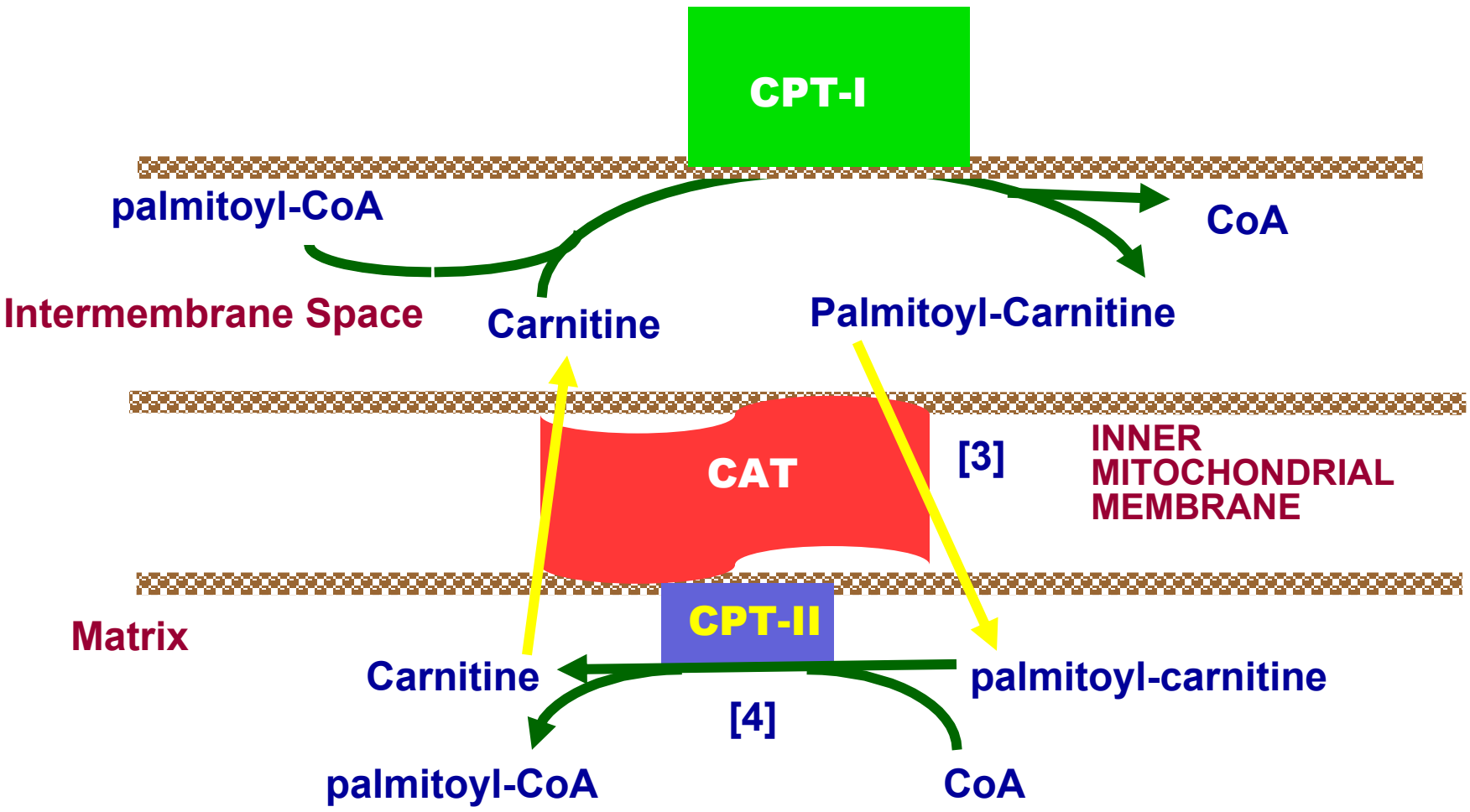
- **CoA is complex structure cannot transport across Mitochondrial membrane**

- **CoA linked to Fatty acid in Mitochondria is different from that CoA used for Fatty acid activation.**

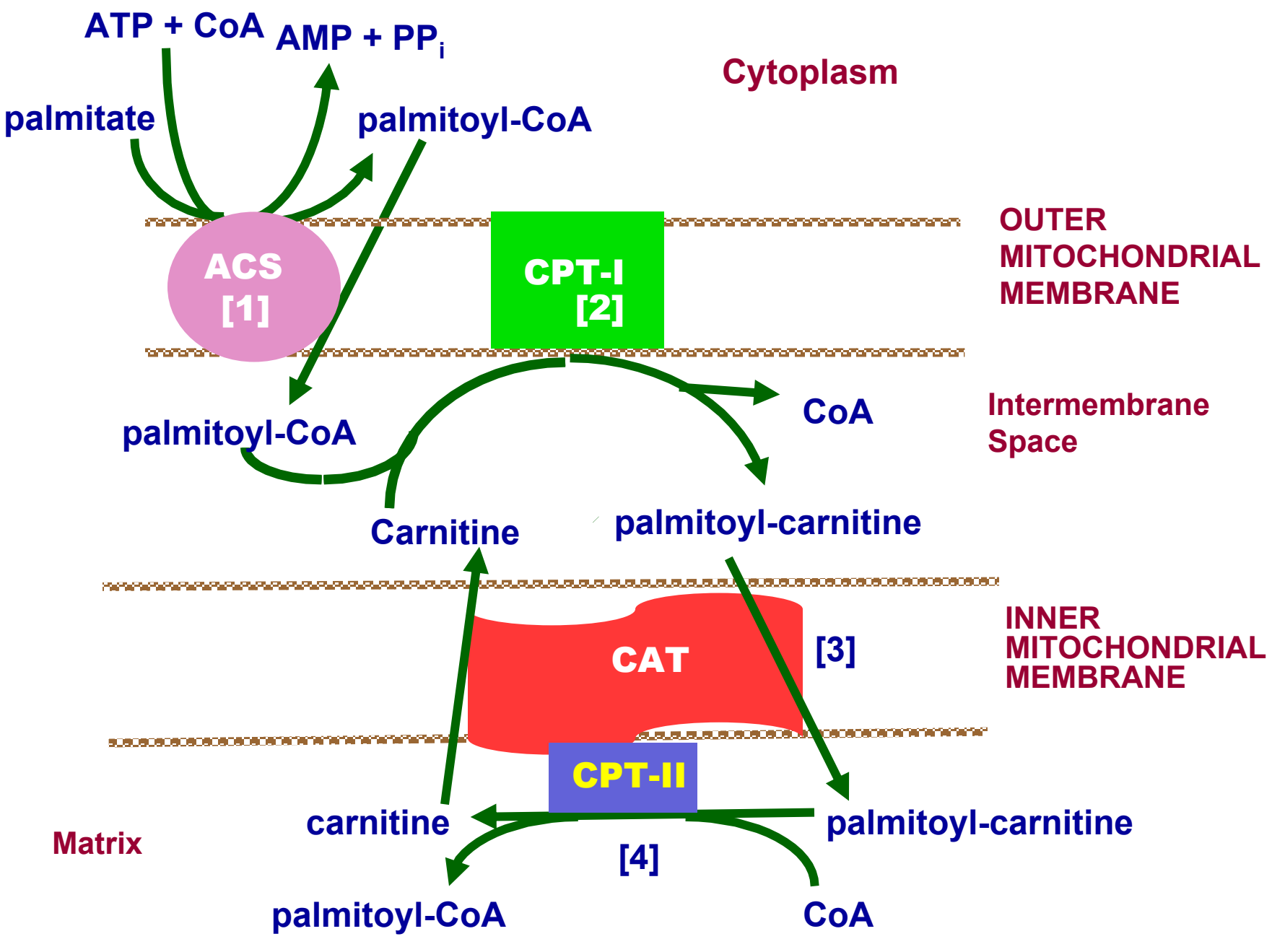
Translocation of Palmitoyl-CoA Across Mitochondrial Membrane

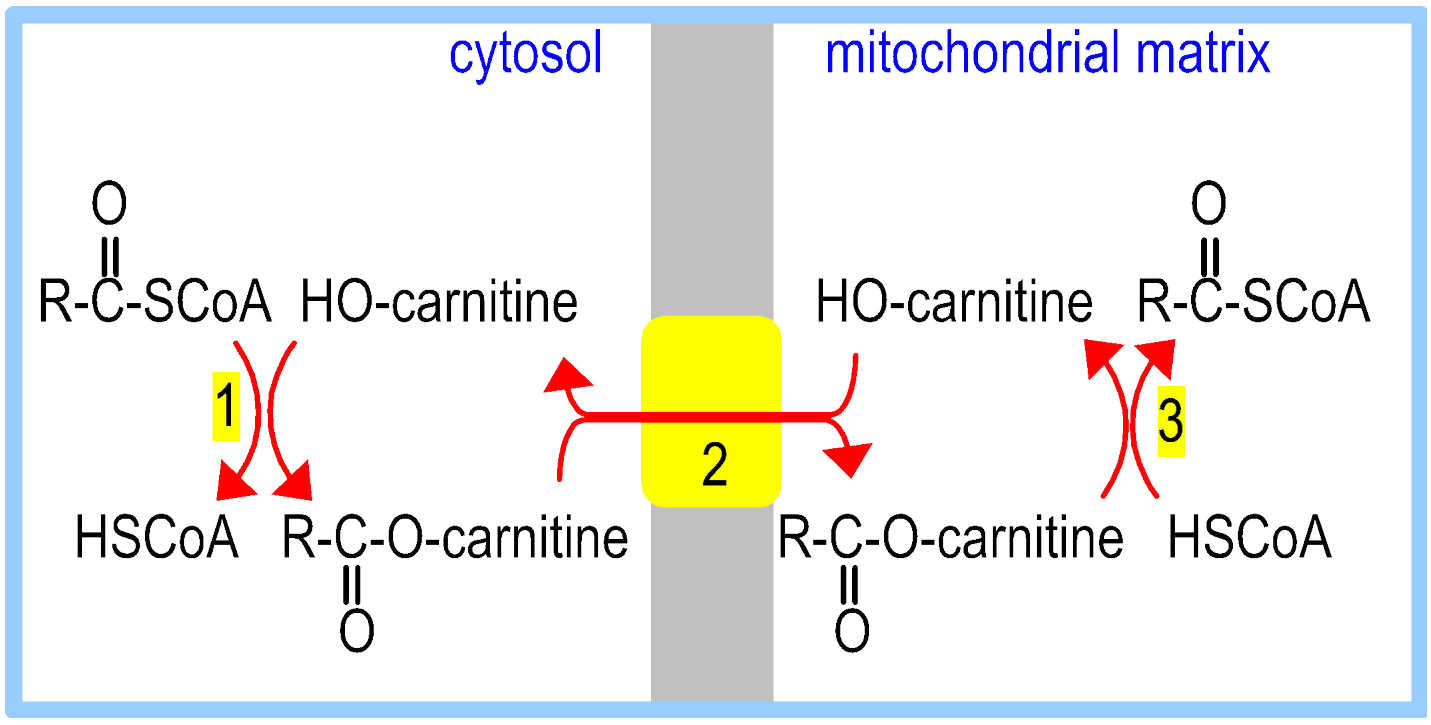


Activation of Palmitate to Palmitoyl CoA and conversion to Palmitoyl Carnitine



Mitochondrial uptake via of Palmitoyl-Carnitine via the Carnitine-Acylcarnitine Translocase (CAT)

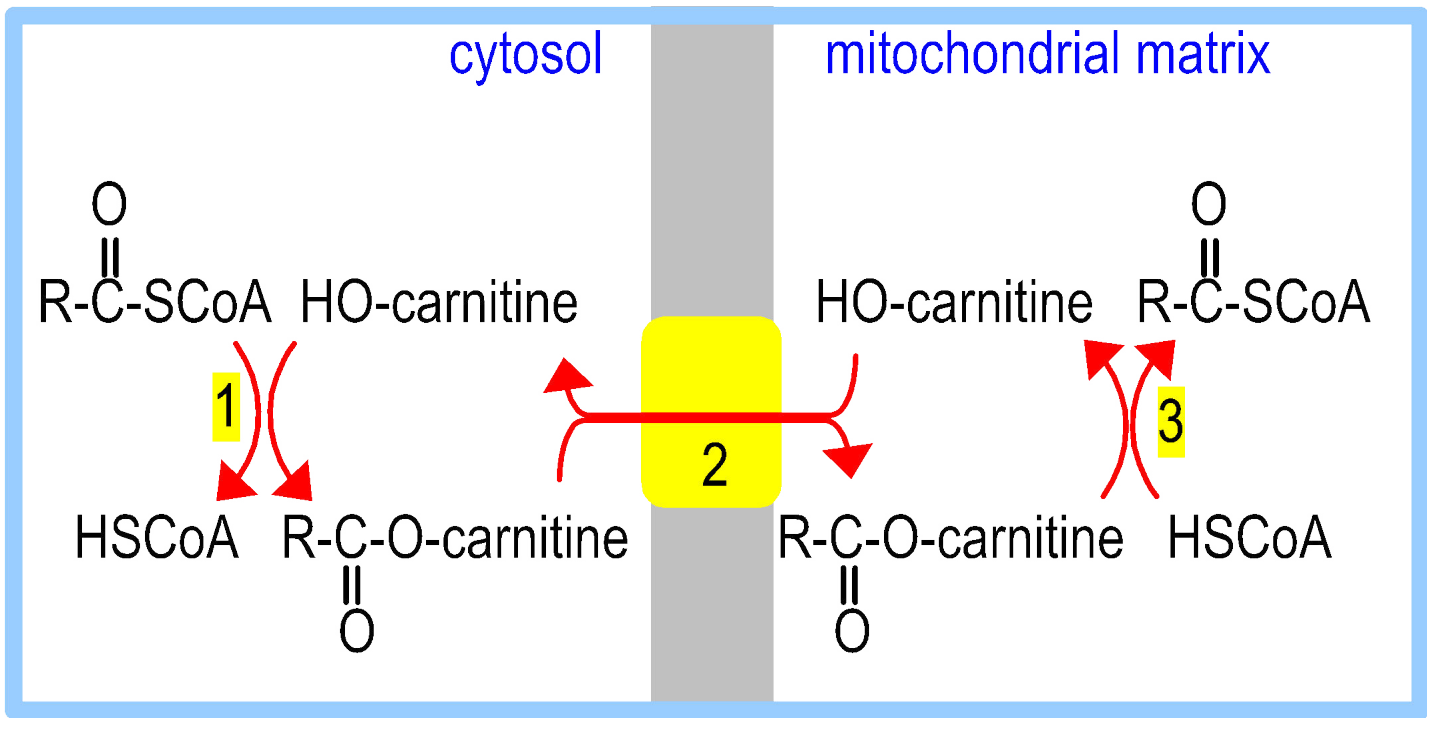




Carnitine-mediated transfer of the fattyAcyl moiety into the mitochondrial matrix is a 3-step process:

1. **Carnitine Palmitoyl Transferase I**, an enzyme on the cytosolic surface of the outer mitochondrial membrane, transfers a fatty acid from CoA to the OH on Carnitine.

2. An **Translocase**/**Antiporter** in the inner mitochondrial membrane mediates exchange of Carnitine for Acylcarnitine.



3. **Carnitine Palmitoyl Transferase II**, an enzyme within the matrix, transfers the fatty acid from Carnitine to CoA. (Carnitine exits the matrix in step 2.)

The fatty acid is now esterified to CoA in the mitochondrial matrix.

Stage III
Steps of Beta Oxidation Proper/Cycle
In Mitochondrial Matrix

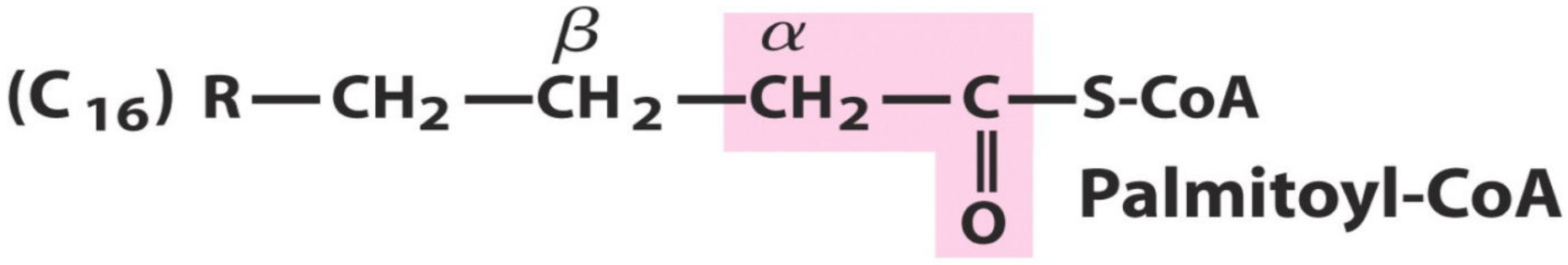
- **Oxidation Reaction**
- **Hydration Reaction**
- **Oxidation Reaction**
- **Cleavage Reaction**

Site/Occurrence Of β – Oxidation Proper

- In **Mitochondrial Matrix** of Cells.

- **After translocation of Acyl-CoA in Mitochondrial matrix.**

Mechanism Of Reactions Of Beta Oxidation Proper of Palmitoyl-CoA



Step I: Oxidation by

FAD linked Acyl CoA Dehydrogenase

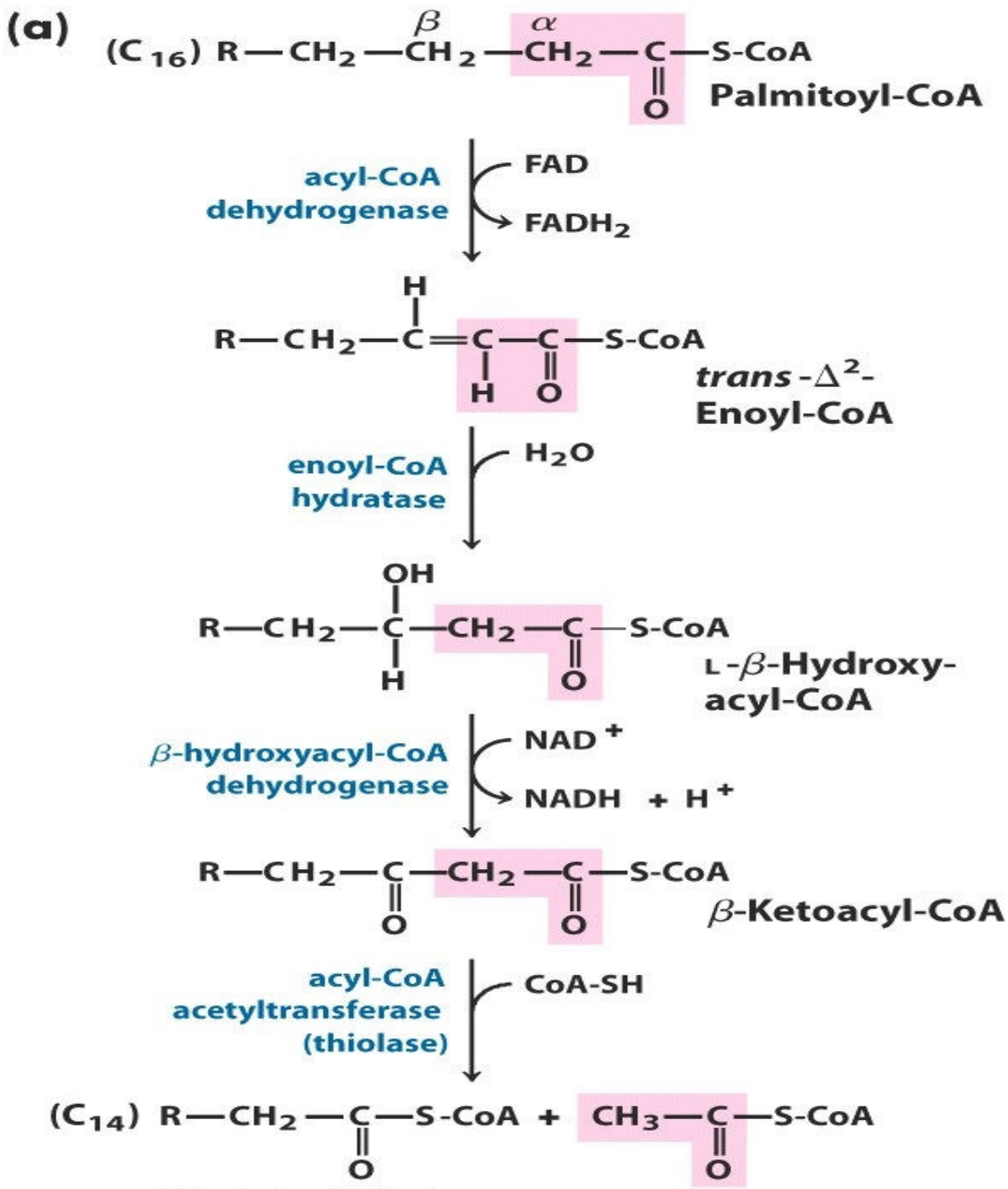
Step II: Hydration by **Enoyl CoA Hydratase**

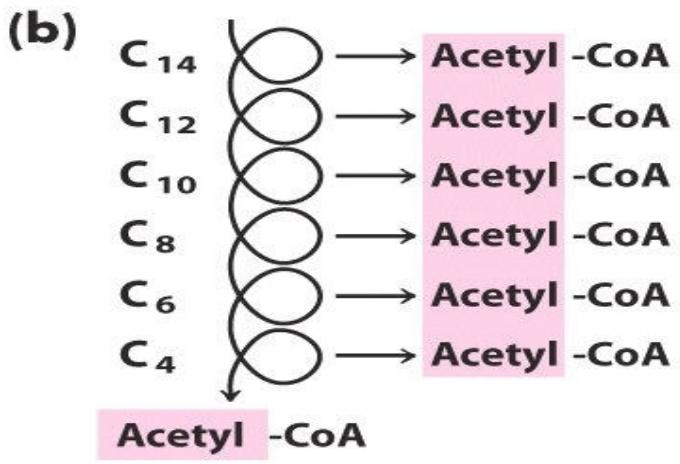
Step III: Oxidation by ***NAD linked***

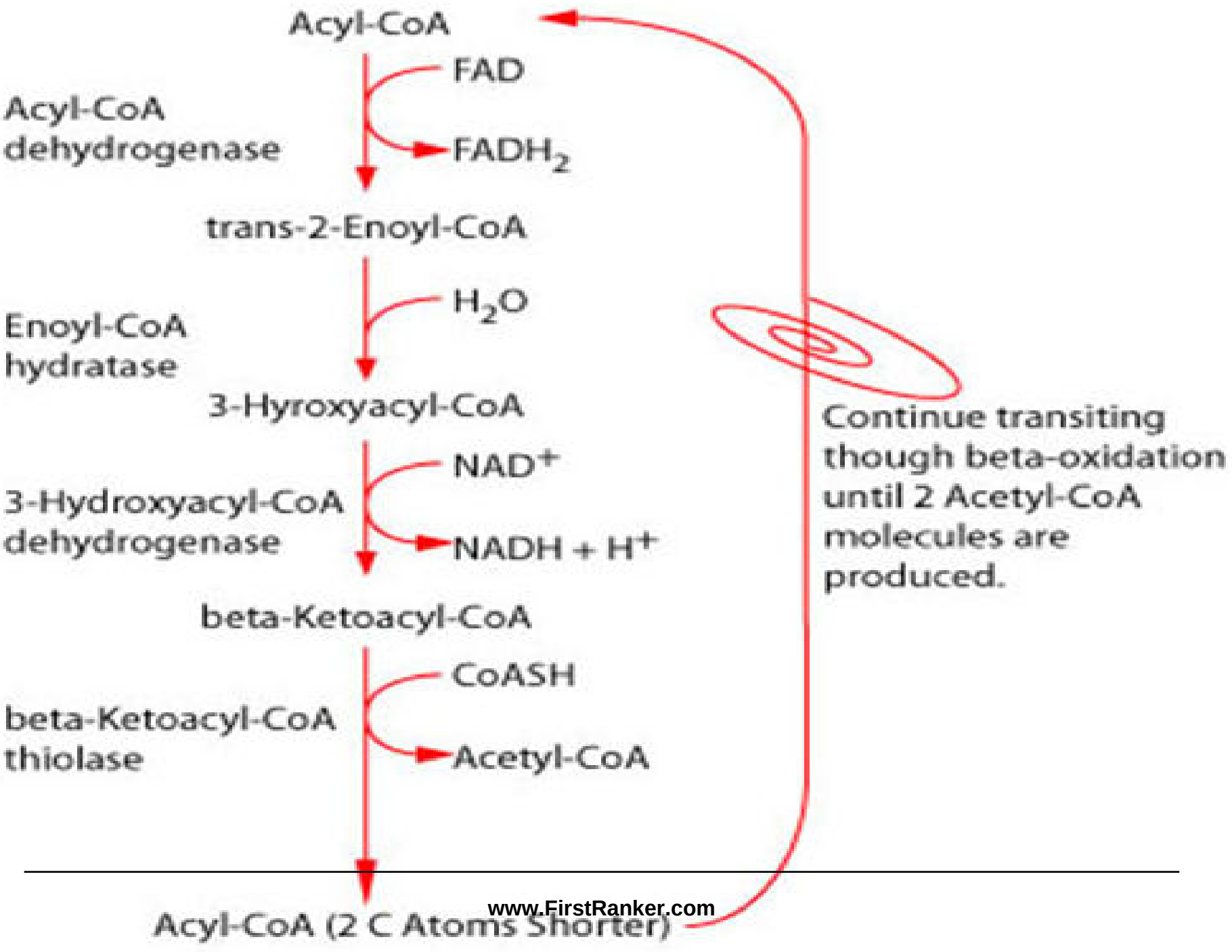
Beta Hydroxy Acyl CoA Dehydrogenase

Step IV: Thiolytic Cleavage by Keto

Thiolase







Palmitoylcarnitine

inner mitochondrial membrane

Carnitine translocase

respiratory chain

Palmitoylcarnitine

matrix side

1.5 ATP

2.5 ATP

Palmitoyl-CoA

oxidation — FAD → $FADH_2$

hydration — H_2O

oxidation — NAD^+ → NADH

cleavage — CoA

recycle 6 times

$CH_3-(CH)_{12}-C(=O)-S-CoA$ + Acetyl CoA

Citric acid cycle → 2 CO_2

β-Oxidation of Palmitoyl CoA

Beta-oxidation of fatty acids

CYTOPLASM | MITOCHONDRIAL MEMBRANE | MATRIX

ATP → AMP+PP

+HS-CoA

Fatty Acid → Acyl-CoA

Carnitine

HS-CoA

Acyl cartinine

HS-CoA

Acyl-CoA

FAD → $FADH_2$ + $\frac{1}{2}O_2$ → H_2O + 2 ATP

-2H Acyl-CoA-dehydrogenase

Enoyl-CoA

Enoyl CoA-hydratase + H_2O

β-oxyacyl-CoA

NAD → $NADH + H^+$ + $\frac{1}{2}O_2$ → H_2O + 3 ATP

-2H β-hydroxyacyl-CoA dehydrogenase

β-ketoacyl CoA

+HS-CoA Thiolase

Acetyl CoA

Kreb's cycle

Acyl-CoA, shortended by two carbon atoms

Activation

Membrane transport

β-oxidation

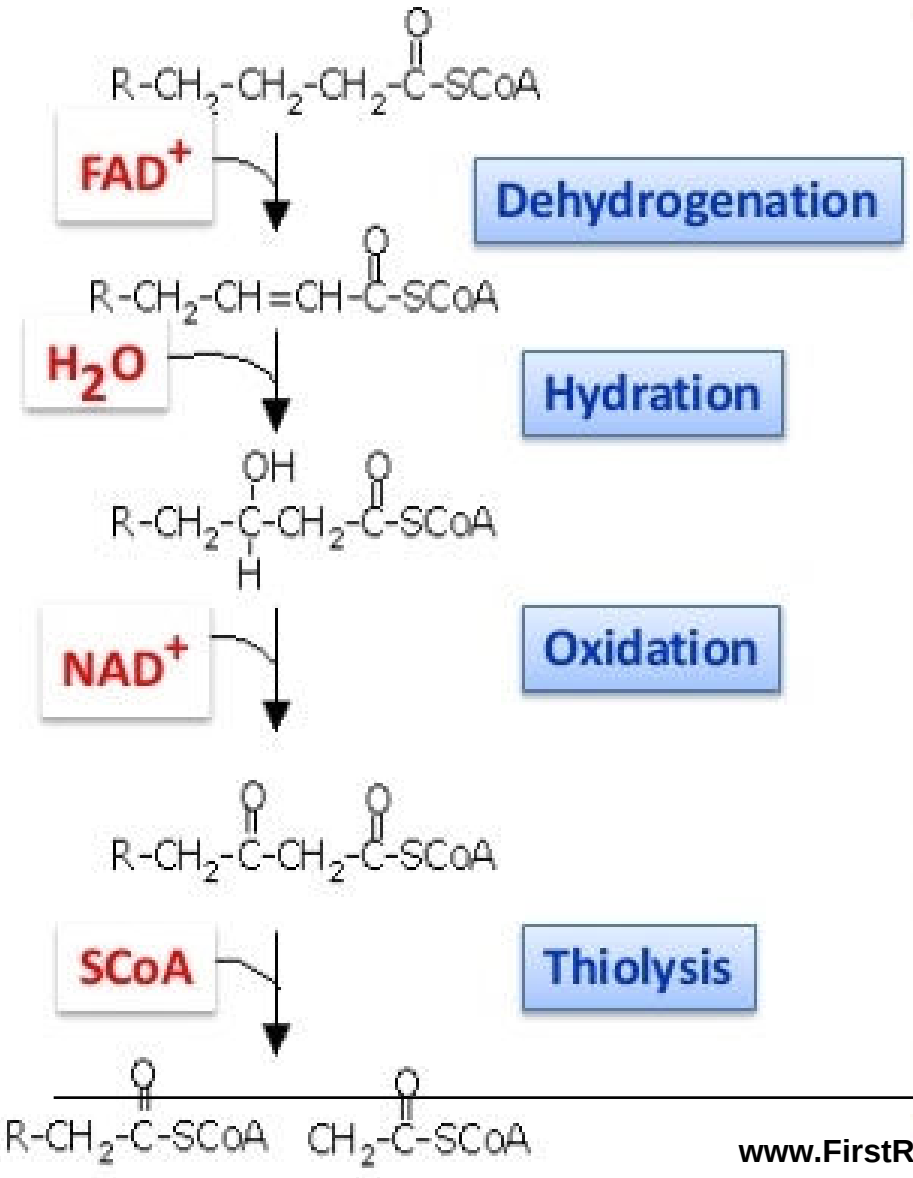
Every cycle of β-oxidation includes 4 reactions.
Result : formation of 5 ATP molecules and splitting of Acetyl-CoA molecule

- Strategy of **First 3 reactions** of Beta Oxidation proper is to
- **Create a Carbonyl group** (C=O) on **β-Carbon atom (CH2)** of a Fatty acid.
- This **weakens bond between α and β Carbon atoms** of Fatty acid.

- **Fourth reaction** cleaves **"β-Keto ester"** in a **reverse Claisen condensation reaction.**

- **Products** of Each turn/cycle of **beta oxidation proper are** :
  - **Acetyl-CoA**
  - **Acyl-CoA** with **two carbons shorter**

The Four-Step Beta-Oxidation Cycle of Fatty Acids



Each cycle through, releases a single acetyl-CoA molecule, reducing the initial fatty acid by two-carbon units.

Beta-oxidation can convert fatty acids having an even number of carbon atoms in their acyl chain, completely to acetyl-CoA.

For example, a fatty acid with 16 carbons in its acyl chain is converted to 8 acetyl-CoA molecules via beta-oxidation.

Step 1

Role Of
Acyl-CoA Dehydrogenase
To Bring
Oxidation of the C_α-C_β bond
of Fatty acid

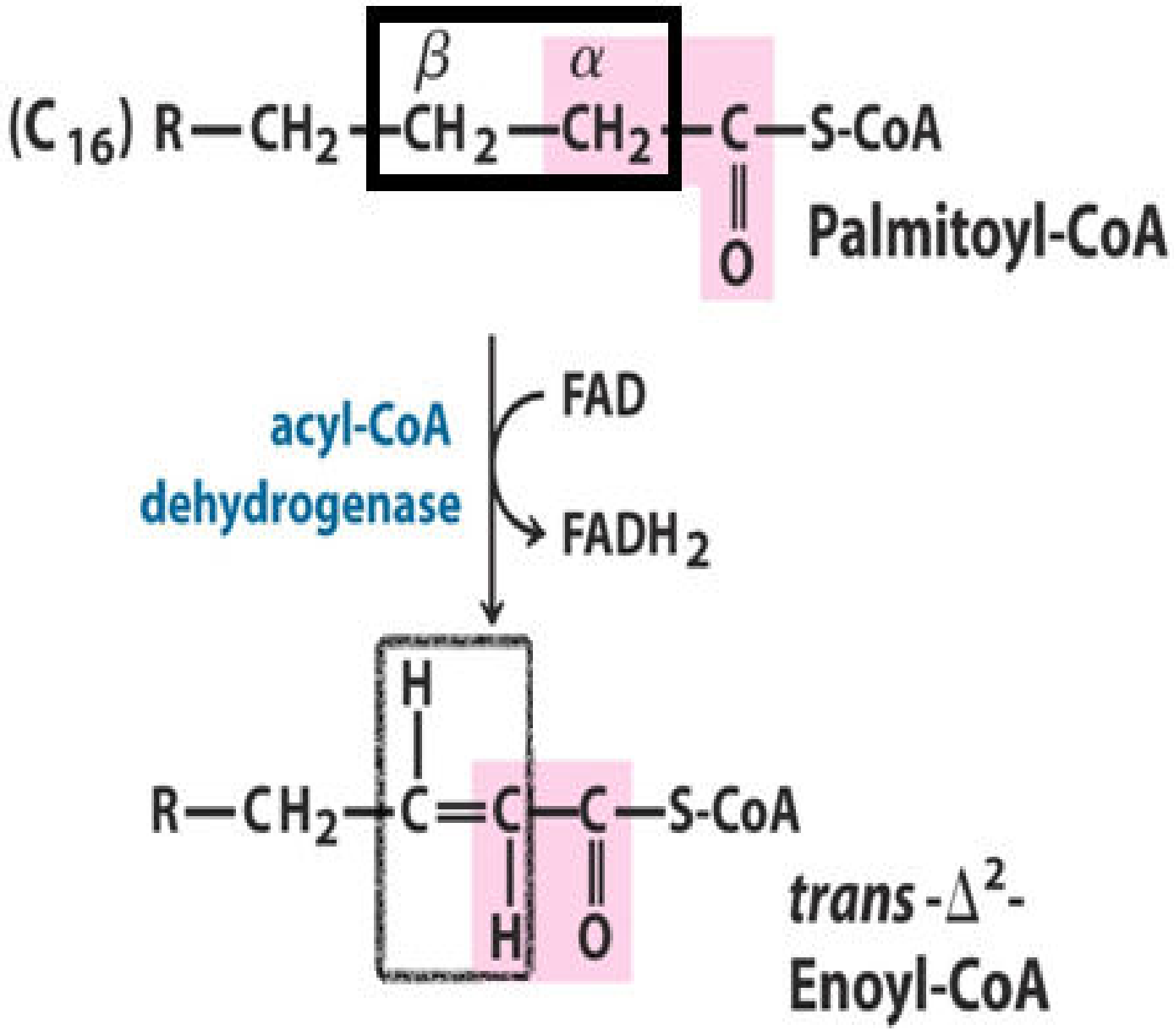
Acyl CoA Dehydrogenase is a
FAD linked Enzyme
(Flavoprotein)

- Acyl CoA Dehydrogenase **catalyzes Oxidation reaction**
- Where there is a **removal of Hydrogen** from **alpha and beta carbon atoms** of Acyl-CoA.

- There **forms a double bond** between **C α-C β / C2 and C3** of Fatty Acid.
- The product of this oxidation reaction is **α-β Unsaturated Acyl CoA /Trans Enoyl CoA.**

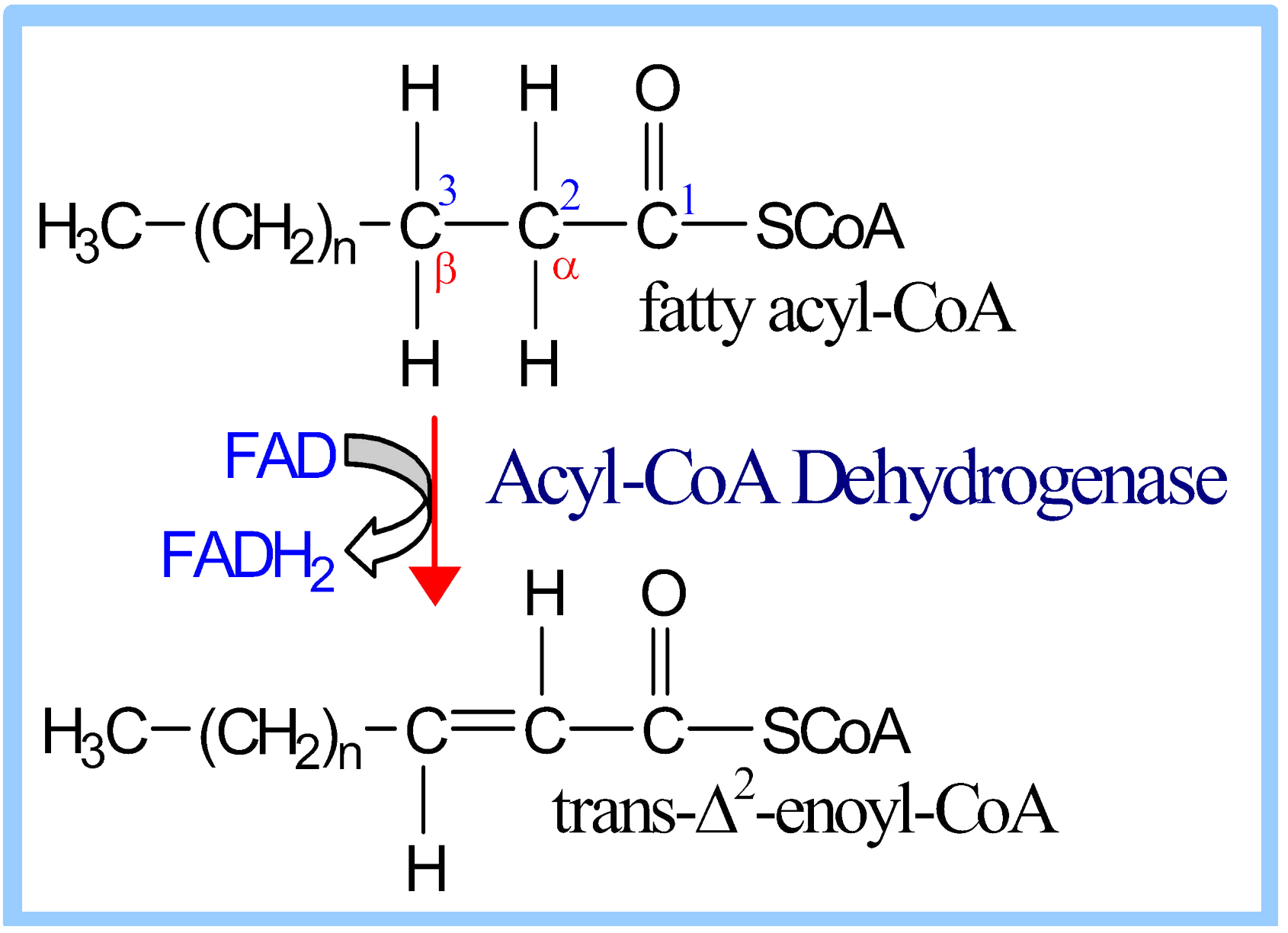
- Coenzyme **FAD** is the **temporary hydrogen acceptor** in this oxidation reaction .
- The **reduced FADH2** is generated by oxidation reaction of Acyl CoA Dehydrogenase.
- **FADH2** is then **reoxidized**, after its enter into **Electron Transport Chain**

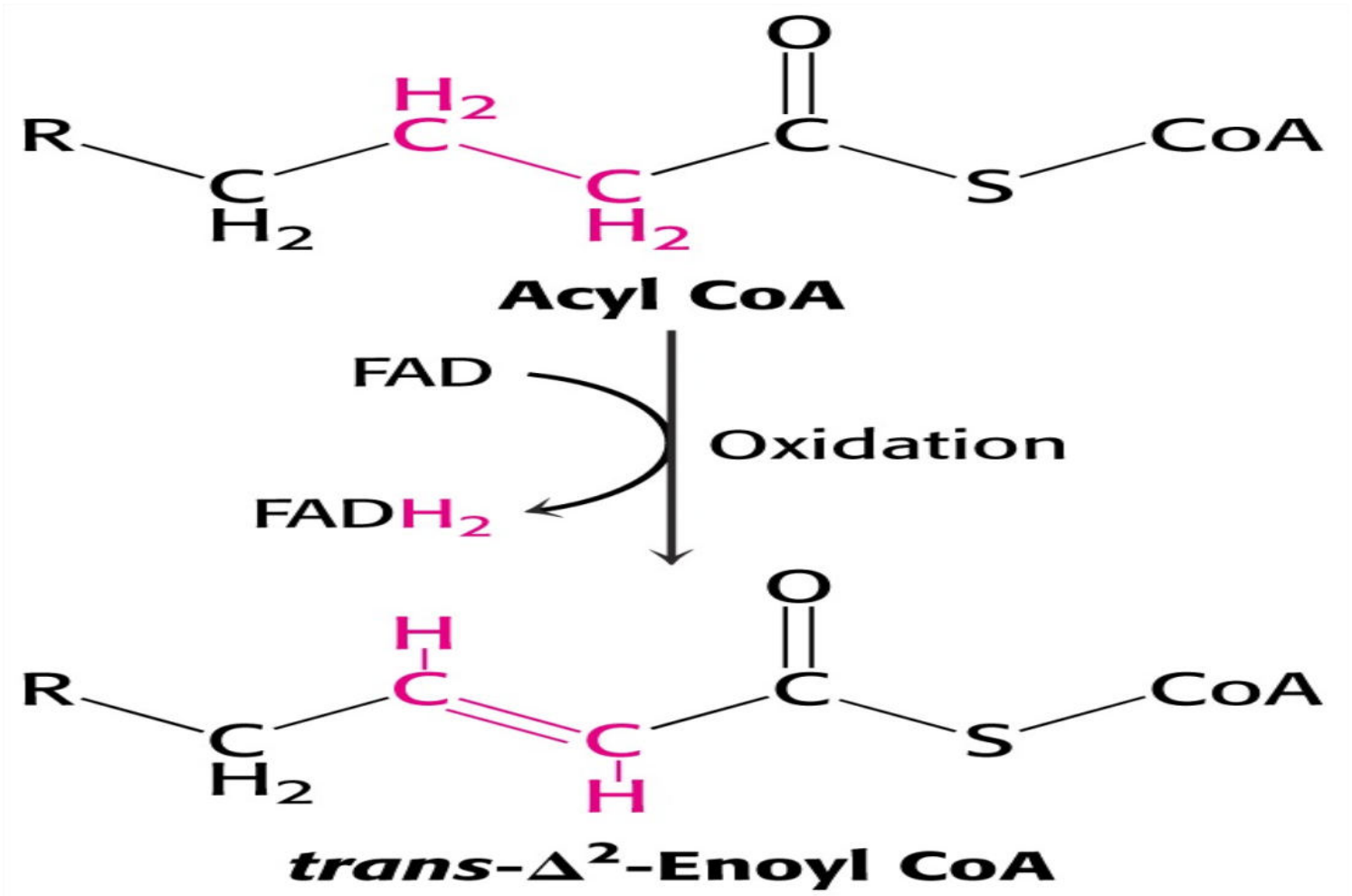
- **Mechanism of Acyl CoA Dehydrogenase involves :**
  - Proton **Abstraction/Removes Hydrogen**
  - **Double bond formation**
  - **Hydride removal by FAD**
  - Generation of **reduced FADH2**



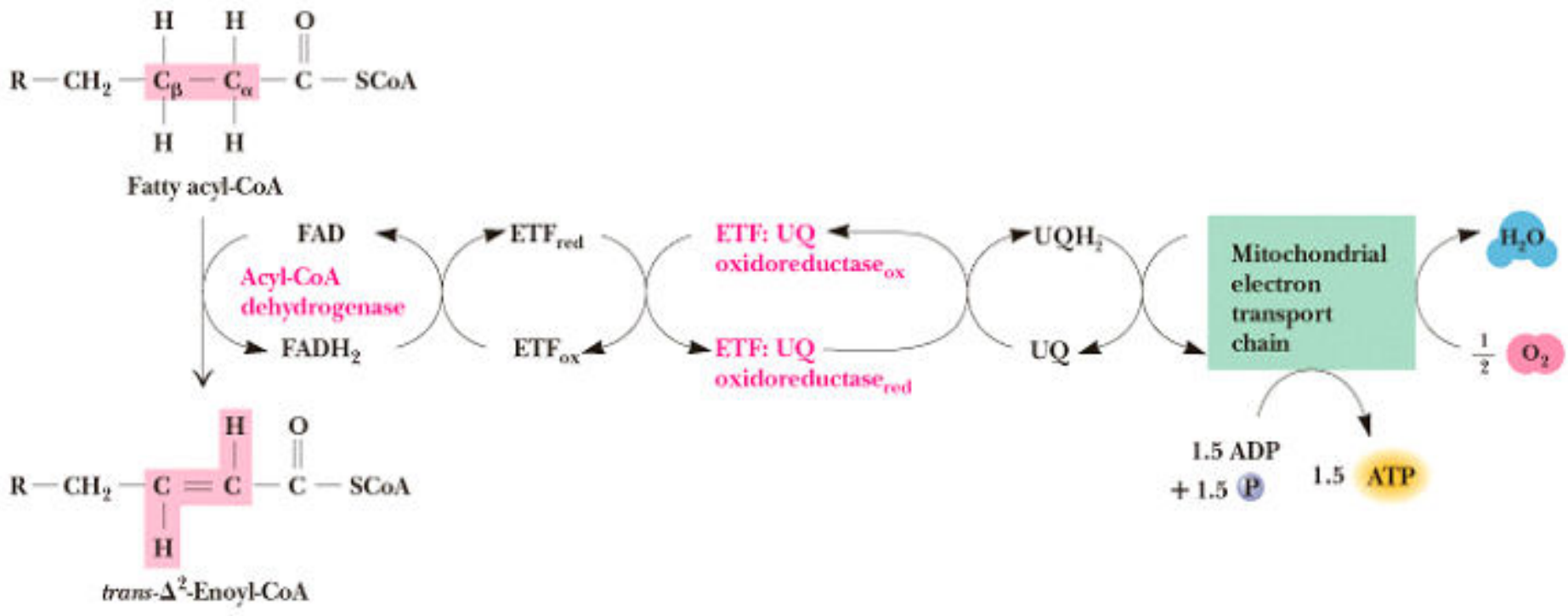
- FADH2 is oxidized by entering into **ETC.**
- Electrons from **FADH2 are passed to Electron transport chain components**,
- Coupled with phosphorylation to generate **1.5 ATP**

(By Oxidative Phosphorylation).





Acyl-CoA Dehydrogenase



- There are **different Acyl-CoA Dehydrogenases :**
  - Short Chain Fatty acids (4-6 C),
  - Medium Chain Fatty Acids (6-10 C),
  - Long (12-18 C) and very long (22 and more)chain Fatty acids.

Inhibitor Of
Acyl CoA Dehydrogenase

- Acyl CoA Dehydrogenase is **inhibited by** a **Hypoglycin** (from **Akee fruit**)

Step 2
Role Of
Enoyl CoA Hydratase

To add water across the double bond

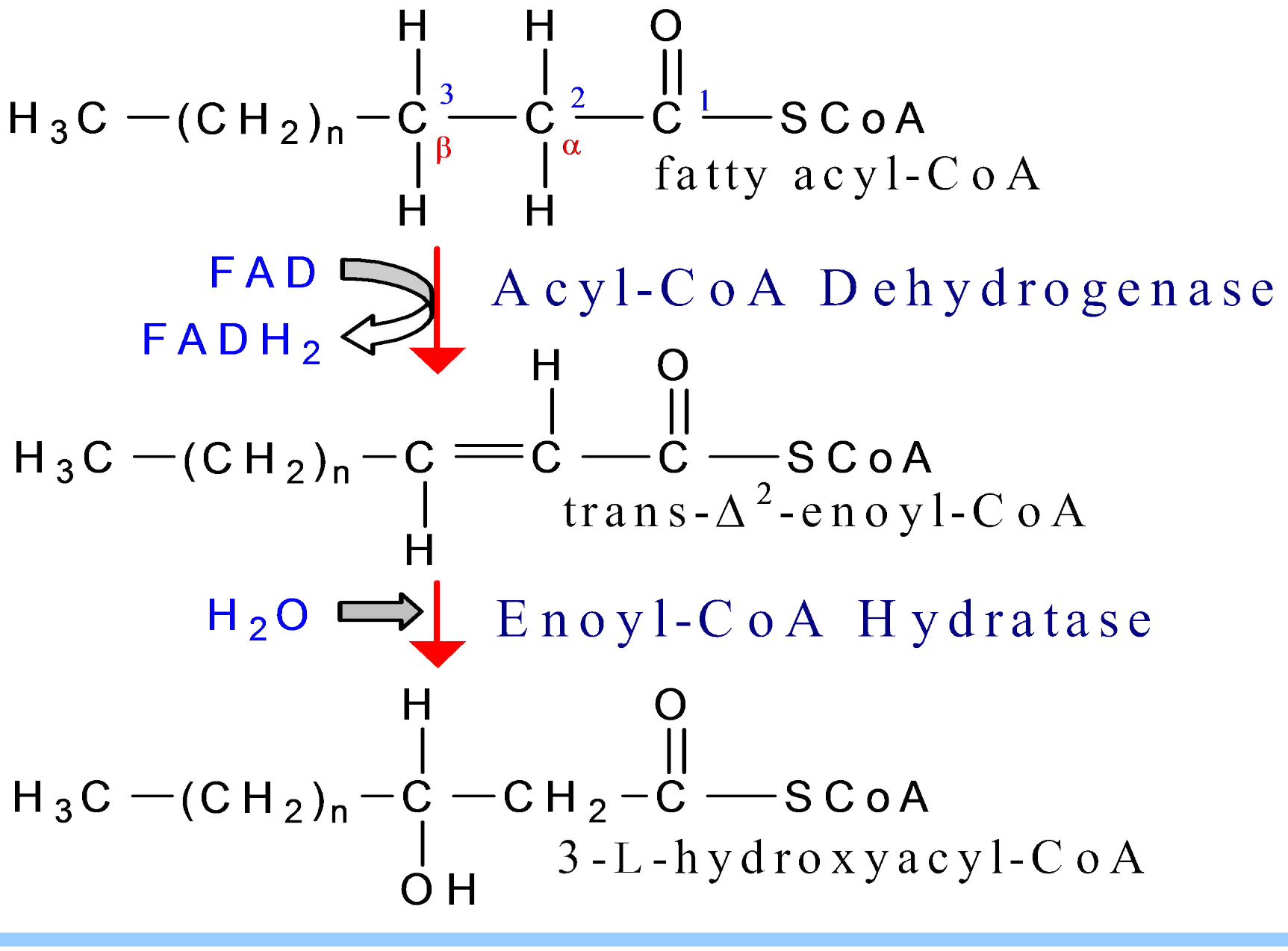
Cα = Cβ of Trans-Enoyl-CoA

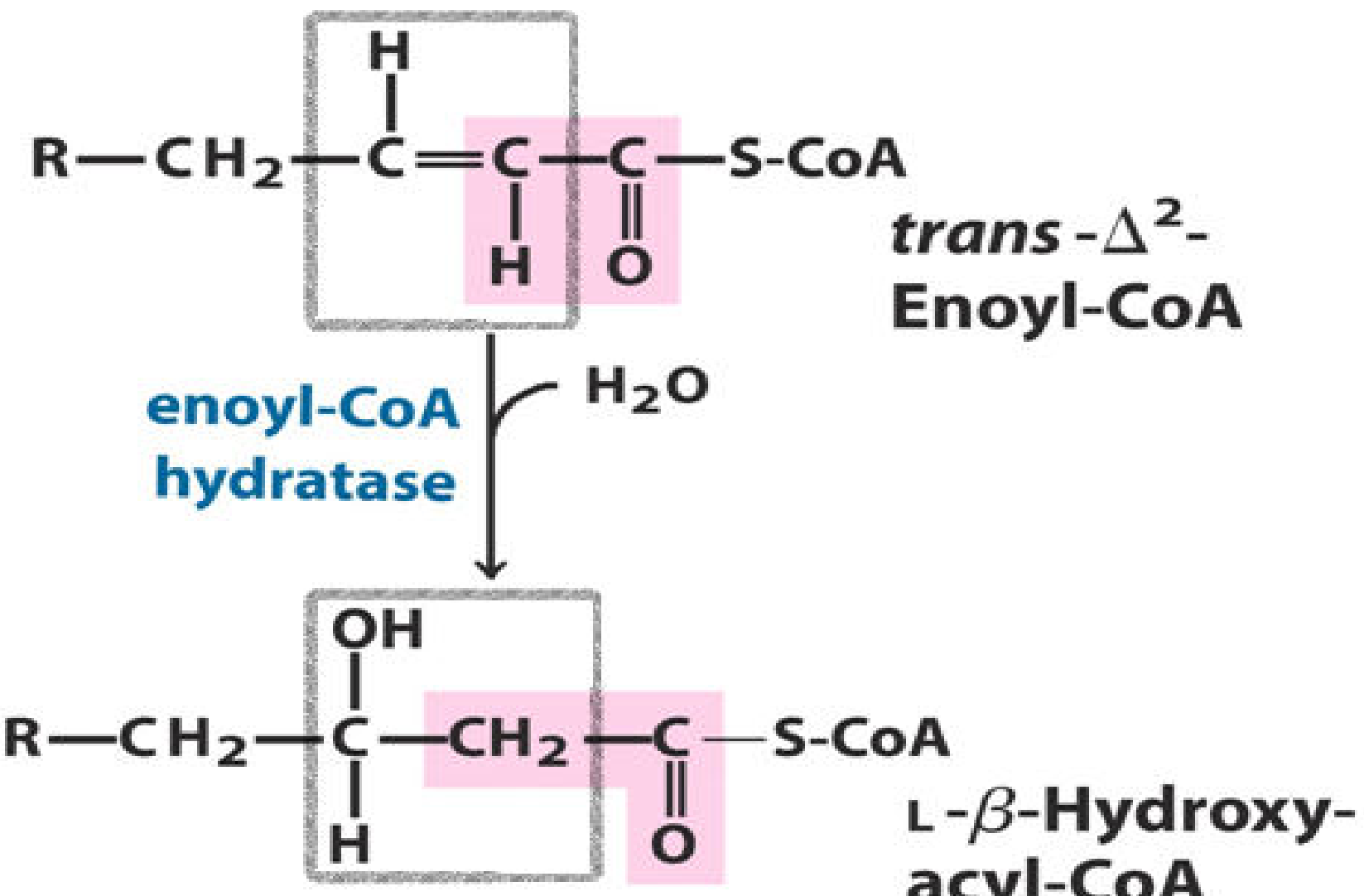
Saturate the double bond of Enoyl-CoA

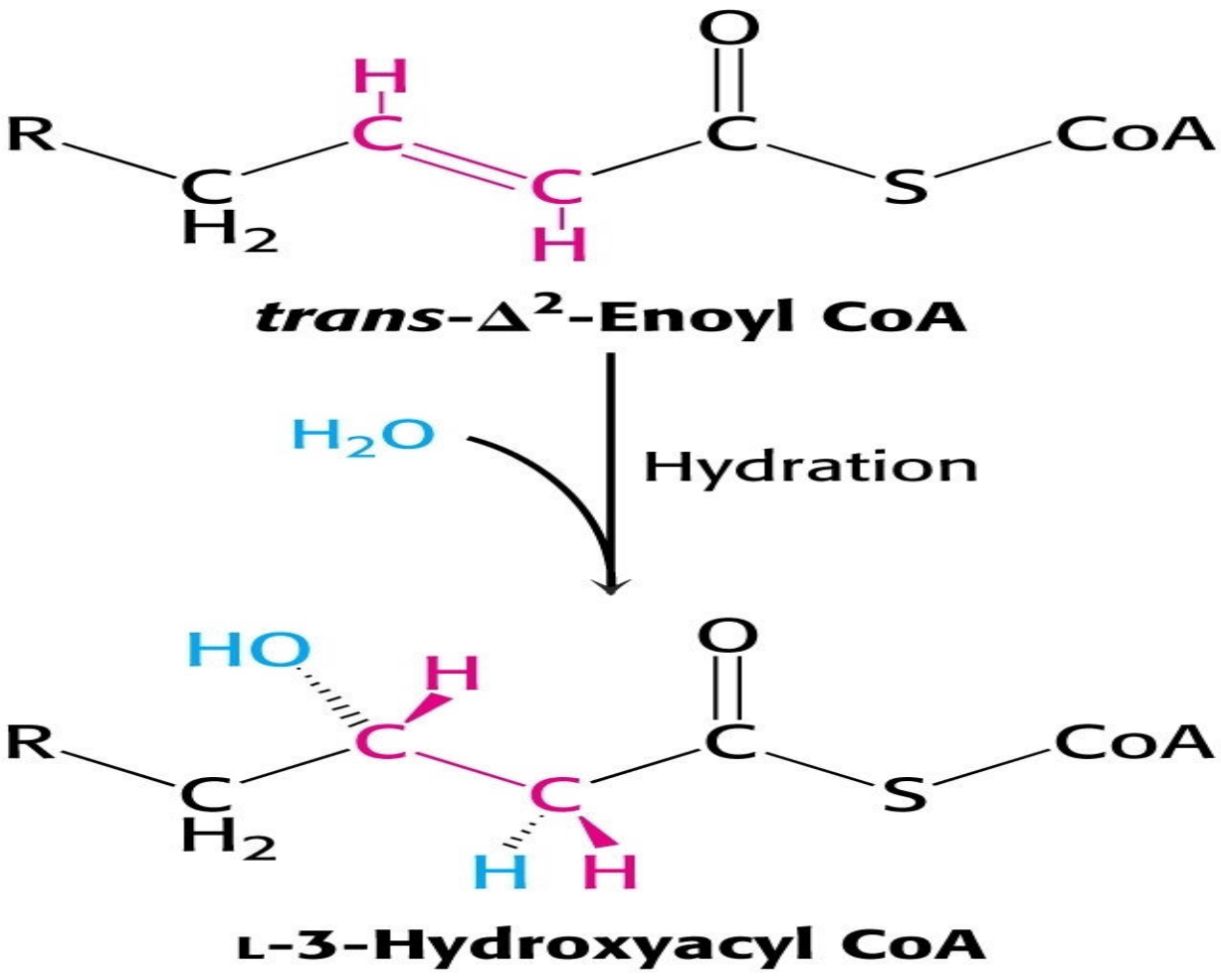
Generate Hydroxyl group at beta carbon

- **Enoyl-CoA Hydratase** catalyzes stereospecific **hydration** of the trans double bond
- It adds water across the double bond **at C2 and C3** of **Trans Enoyl CoA**

- This hydration reaction **generates Hydroxyl (OH) group** at beta carbon atom of FA
- **Converts Trans-Enoyl-CoA** to L **β-Hydroxyacyl-CoA**







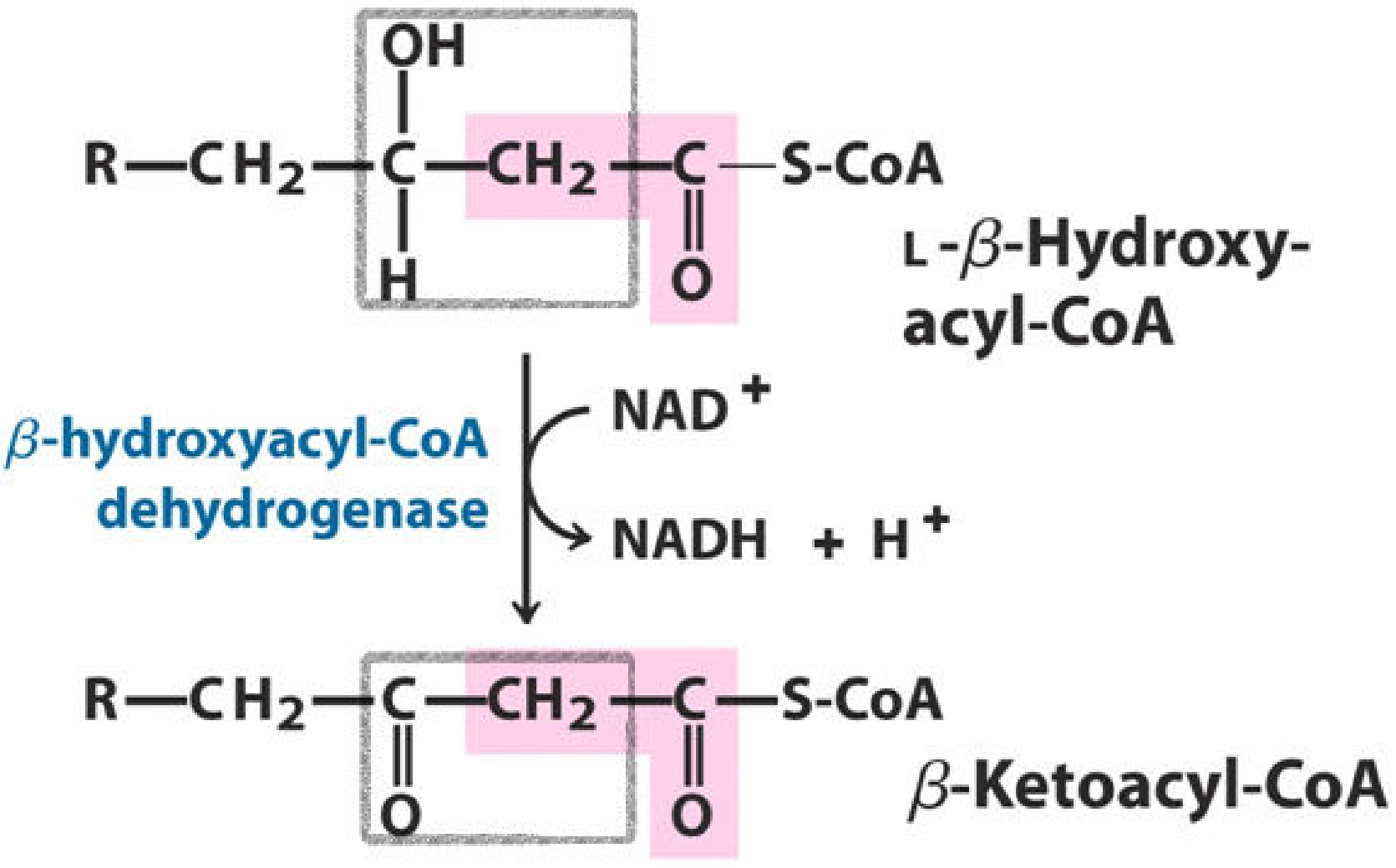
Step 3
Role Of
Hydroxyacyl-CoA Dehydrogenase

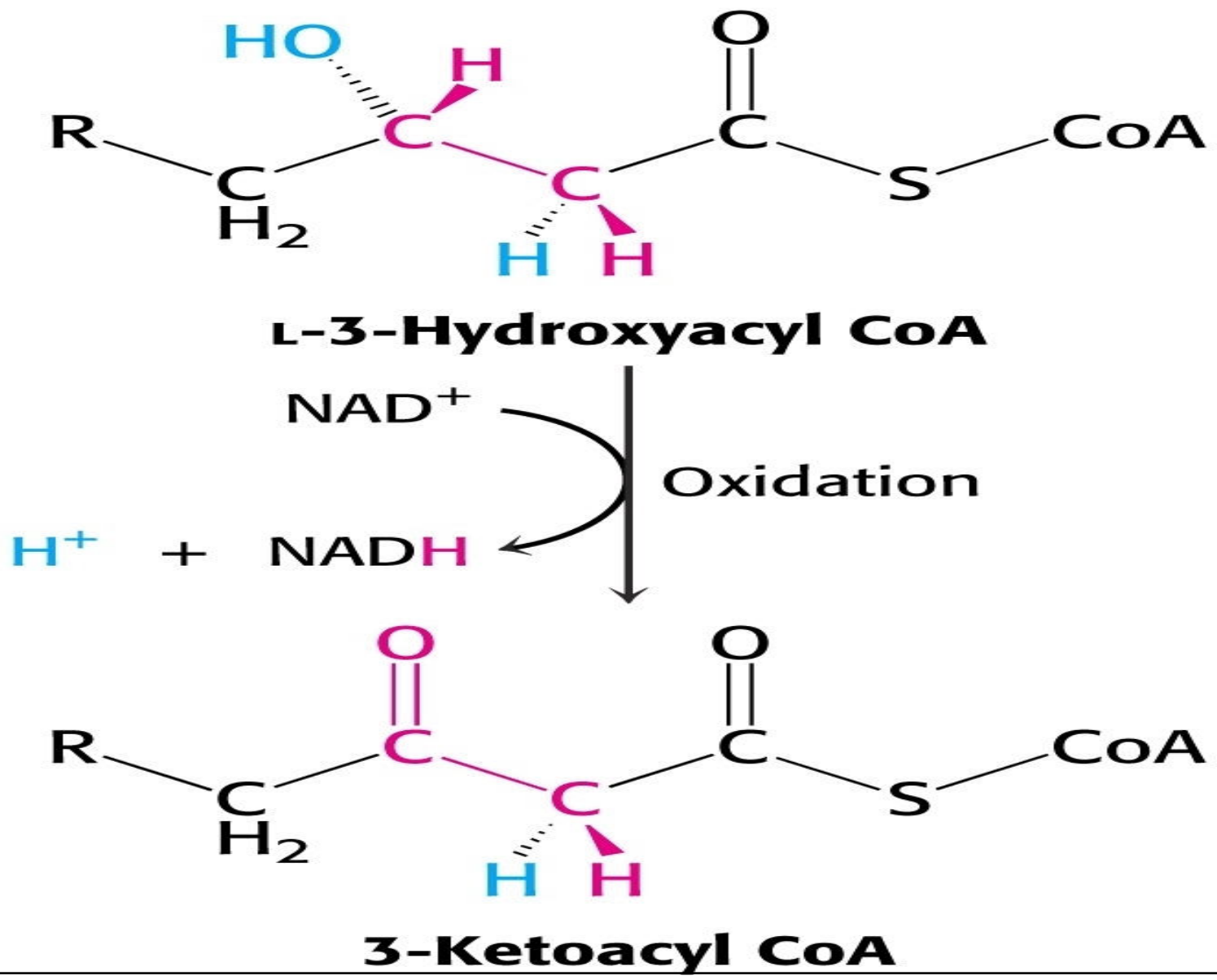
To Oxidizes the β-Hydroxyl Group of β-Hydroxyacyl-CoA
And
Transform it into β-Ketoacyl-CoA

- **β-Hydroxyacyl-CoA Dehydrogenase is NAD^+ dependent**
- It catalyzes specific oxidation of the Hydroxyl group in the β position (C3) to form a ketone group.
- **NAD^+** is the temporary electron acceptor for this step which generates reduced form **$NADH+H^+$**

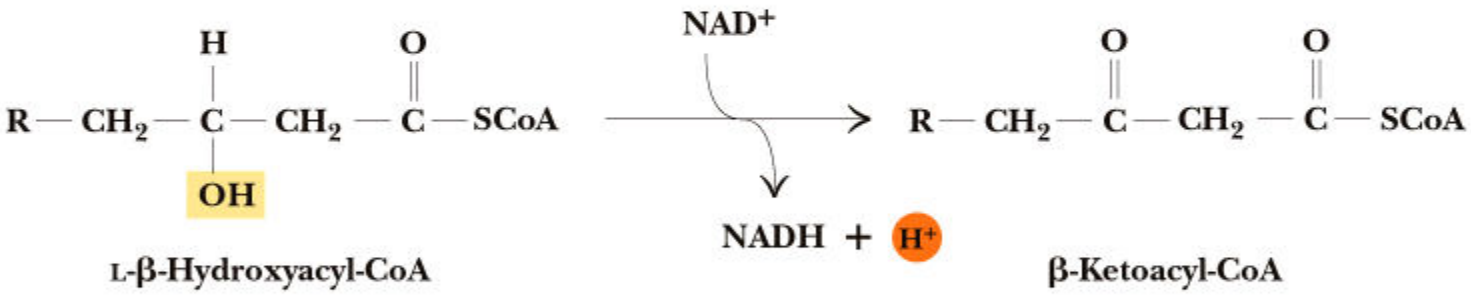
- **The oxidation** of

β-Hydroxyacyl CoA produces a product **β- Ketoacyl-CoA.**





Garrett & Grisham: Biochemistry, 2/e
Figure 24.16



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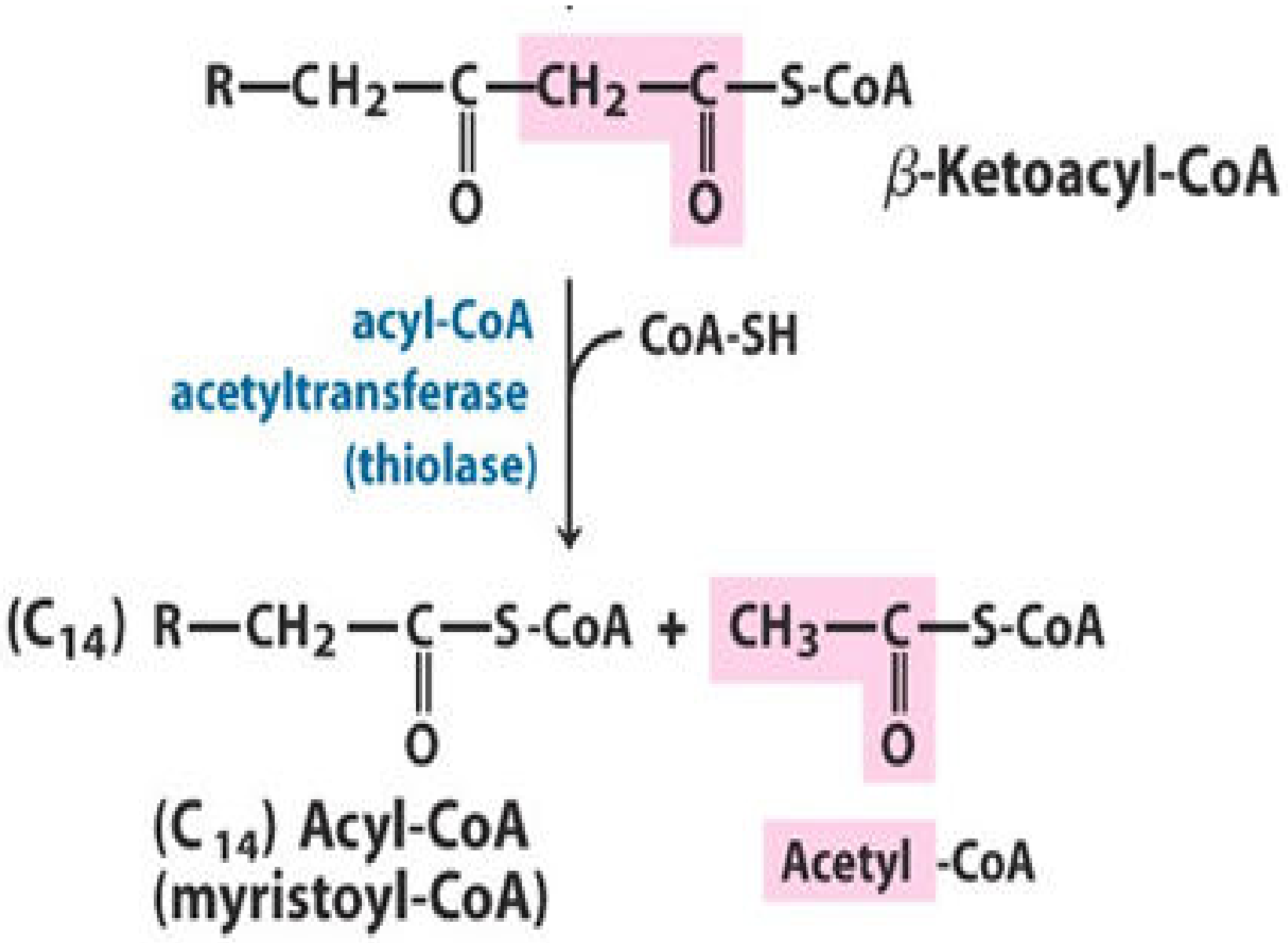
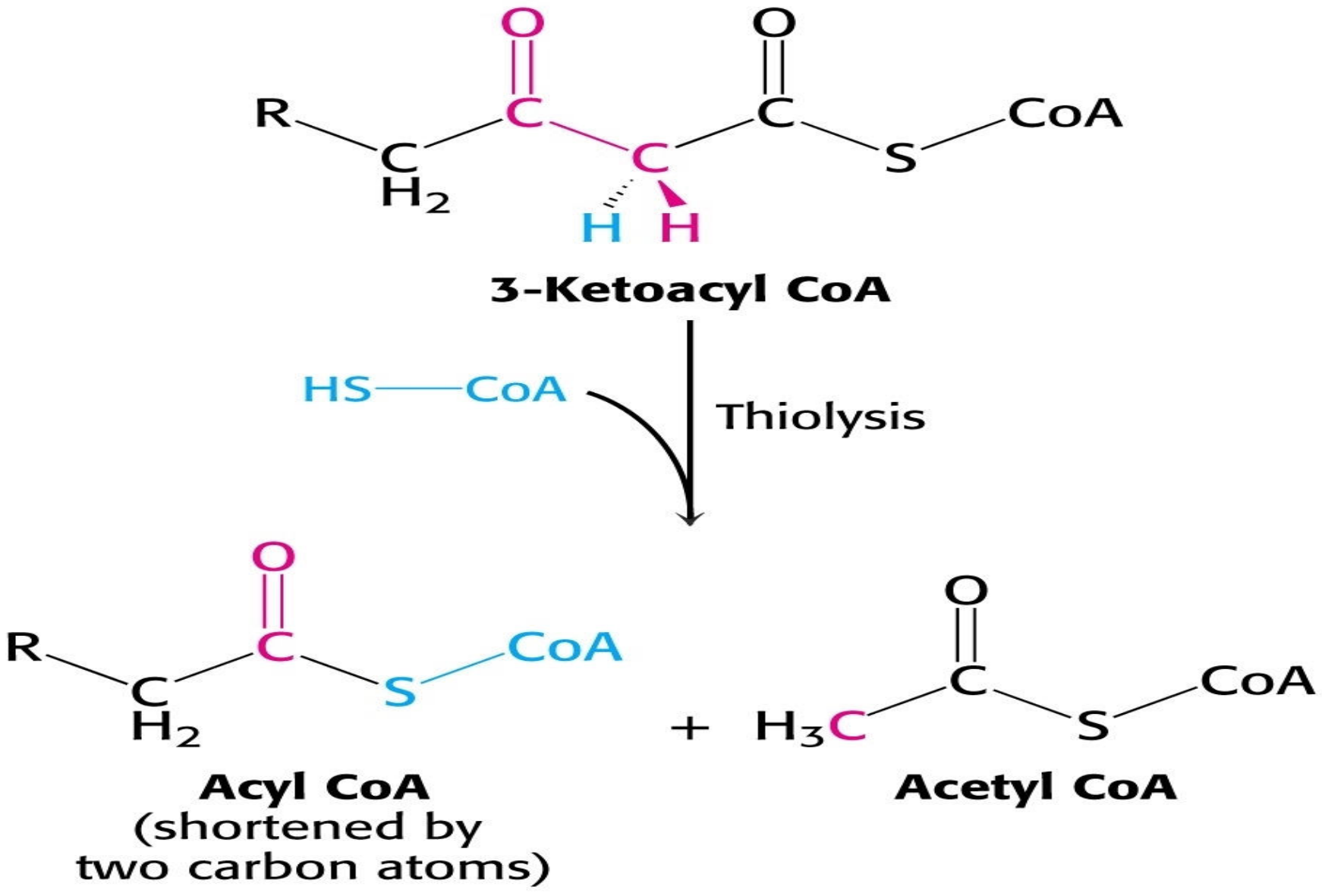
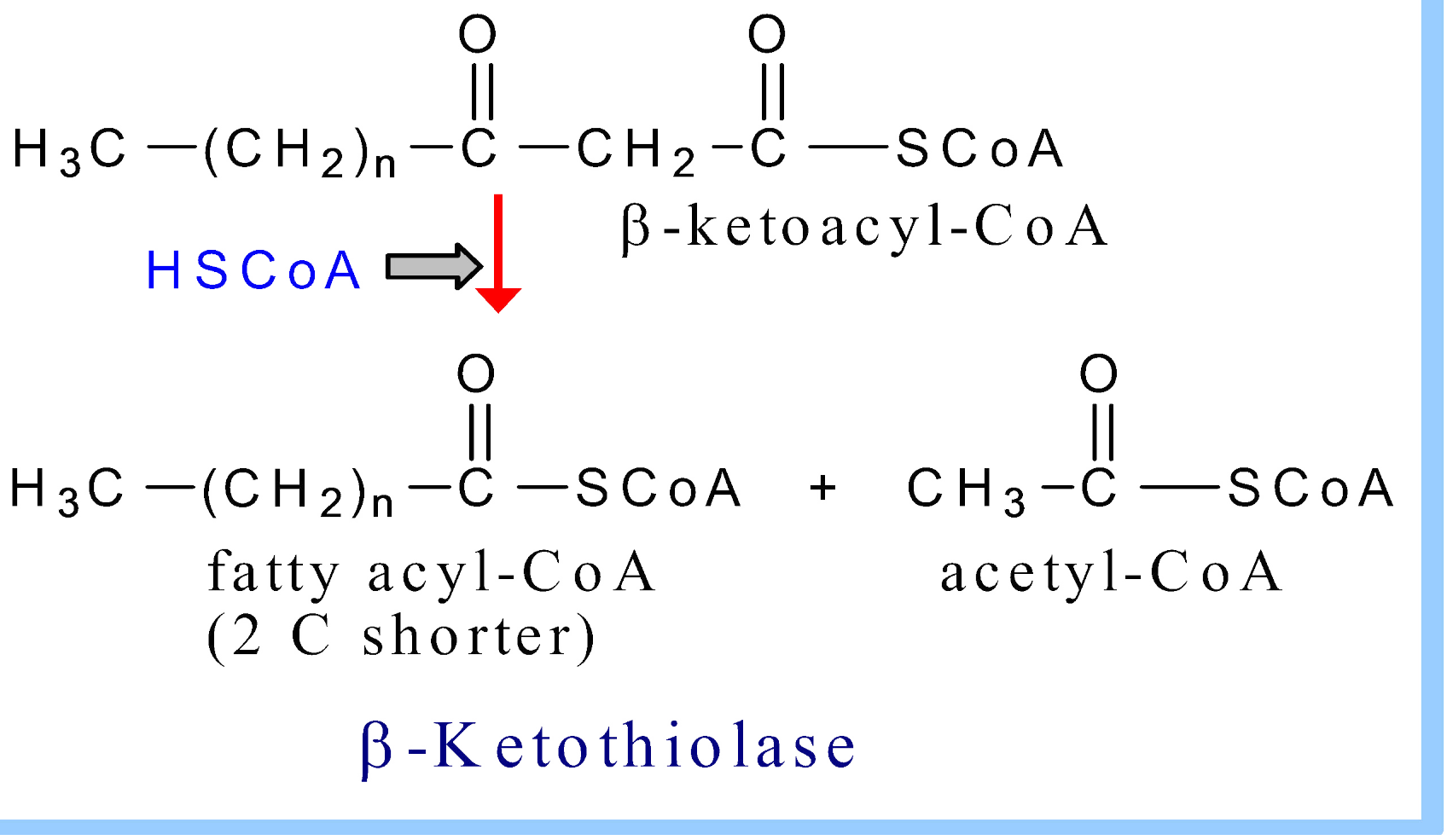
Step 4
Role Of β- Ketothiolase /Thiolase

Catalyzes Thiolytic cleavage of the two carbon fragment by splitting the bond between α and β carbons

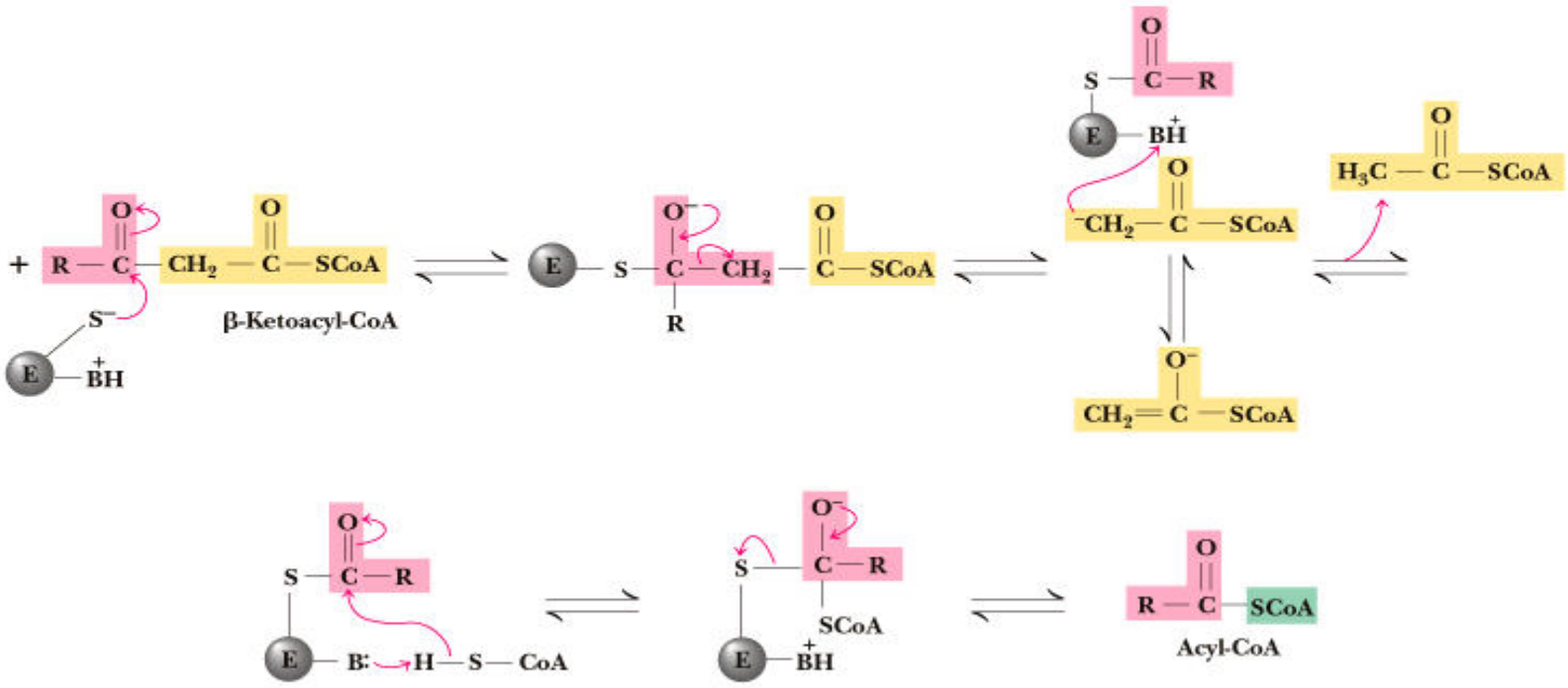
- An enzyme β–Keto Thiolase attacks the β-carbonyl group of β-Ketoacyl-CoA.
- This results in the **cleavage of the C_α-C_β bond.**
- Releases **Acetyl-CoA**(2C) and an **Acyl-CoA** (-2carbons shorter).







Garrett & Grisham: Biochemistry, 2/e
Figure 24.17



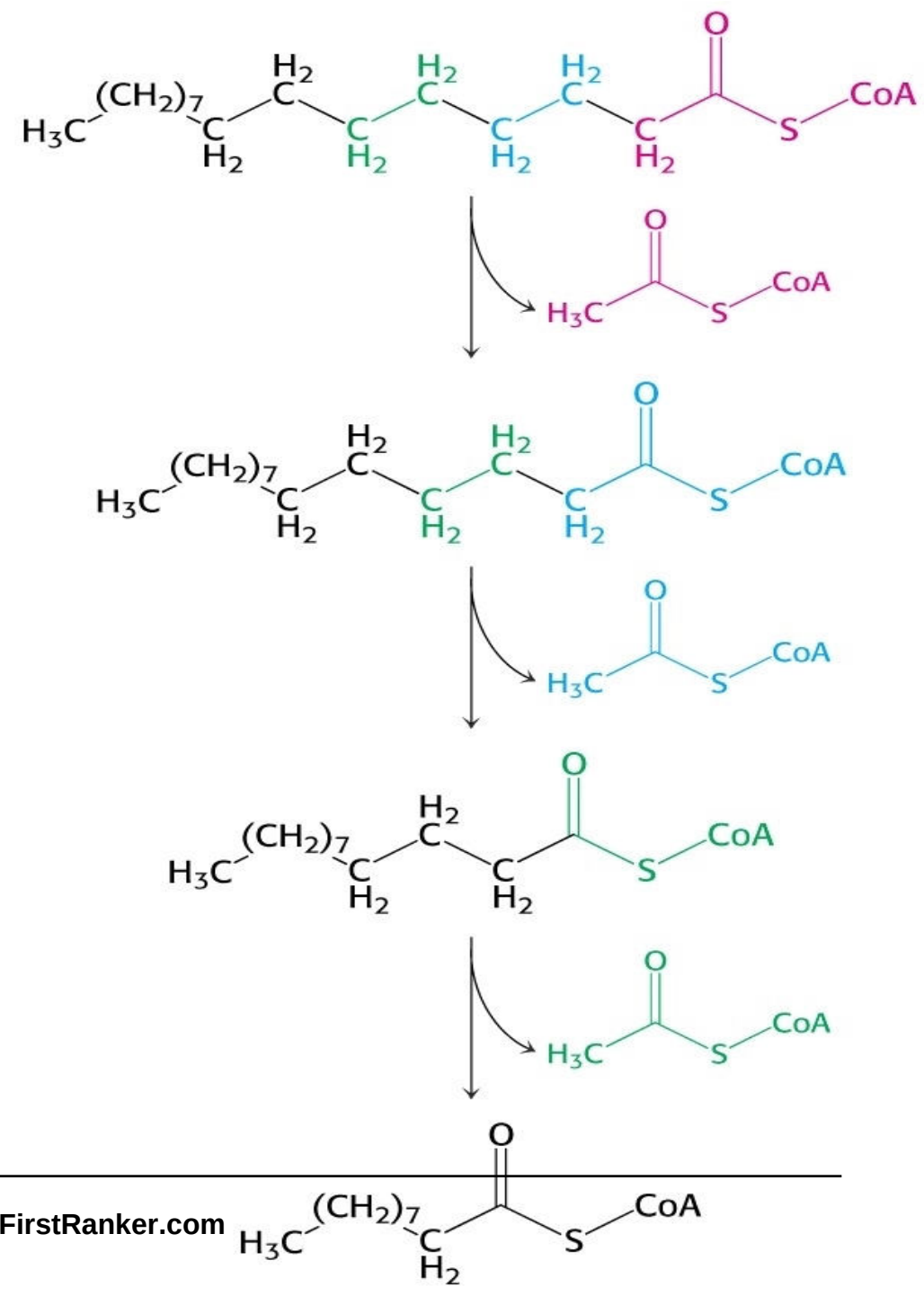
Repetitions Of 4 Steps Of Beta Oxidation Proper

- The β-oxidation proper pathway is **cyclic**.
- 4 Steps of Beta Oxidation proper are repeated
- **Till whole chain of Fatty acid is oxidized completely.**

- Product, **2 carbons shorter Acyl -CoA**,
- **Is input to another round/turn** of the beta oxidation proper pathway.

- **Acyl CoA molecule released at end of Beta Oxidation**
- Is the **substrate for the next round** of oxidation starting with Acyl CoA Dehydrogenase.
- Repetition continues until all the carbon atoms of the original Fatty acyl CoA are converted to Acetyl CoA.

The shortened Acyl CoA then undergoes another cycle of beta oxidation



The number of beta oxidation cycles:

n/2-1, where n – the number of carbon atoms

Products Of Each Turn Of Beta Oxidation Proper

- **Each turn/cycle** of **β oxidation proper** generates **one molecule each of:**
  - **$FADH_2$**
  - **$NADH+H^+$**
  - **Acetyl CoA**
  - **Fatty Acyl CoA (with 2 carbons shorter each round)**

Steps Of β-Oxidation Proper of Fatty Acids Continues With

A Repeated Sequence of 4 Reactions

Till

A Long Fatty Acyl Chain Is Completely Oxidized

- For an **oxidation of Palmitic acid** through beta oxidation
- **7 turns/cycles** of beta oxidation proper steps occur.

Cycles of β-Oxidation

The length of a fatty acid:

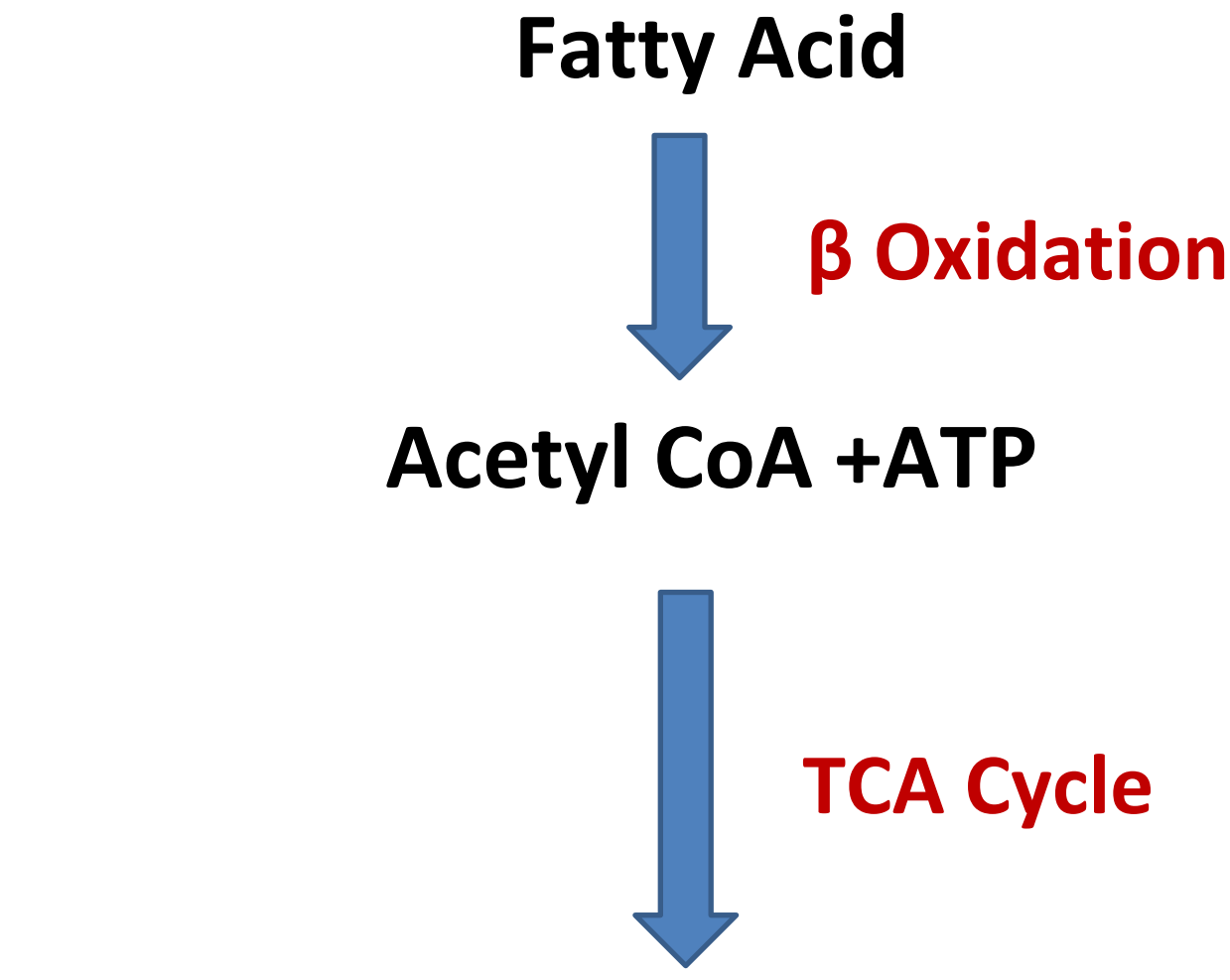
- Determines the number of oxidations and
- The total number of acetyl CoA groups.

Carbons in Fatty Acid	Acetyl CoA (C/2)	β-Oxidation Cycles (C/2 –1)
12	6	5
14	7	6
16	8	7
18	9	8

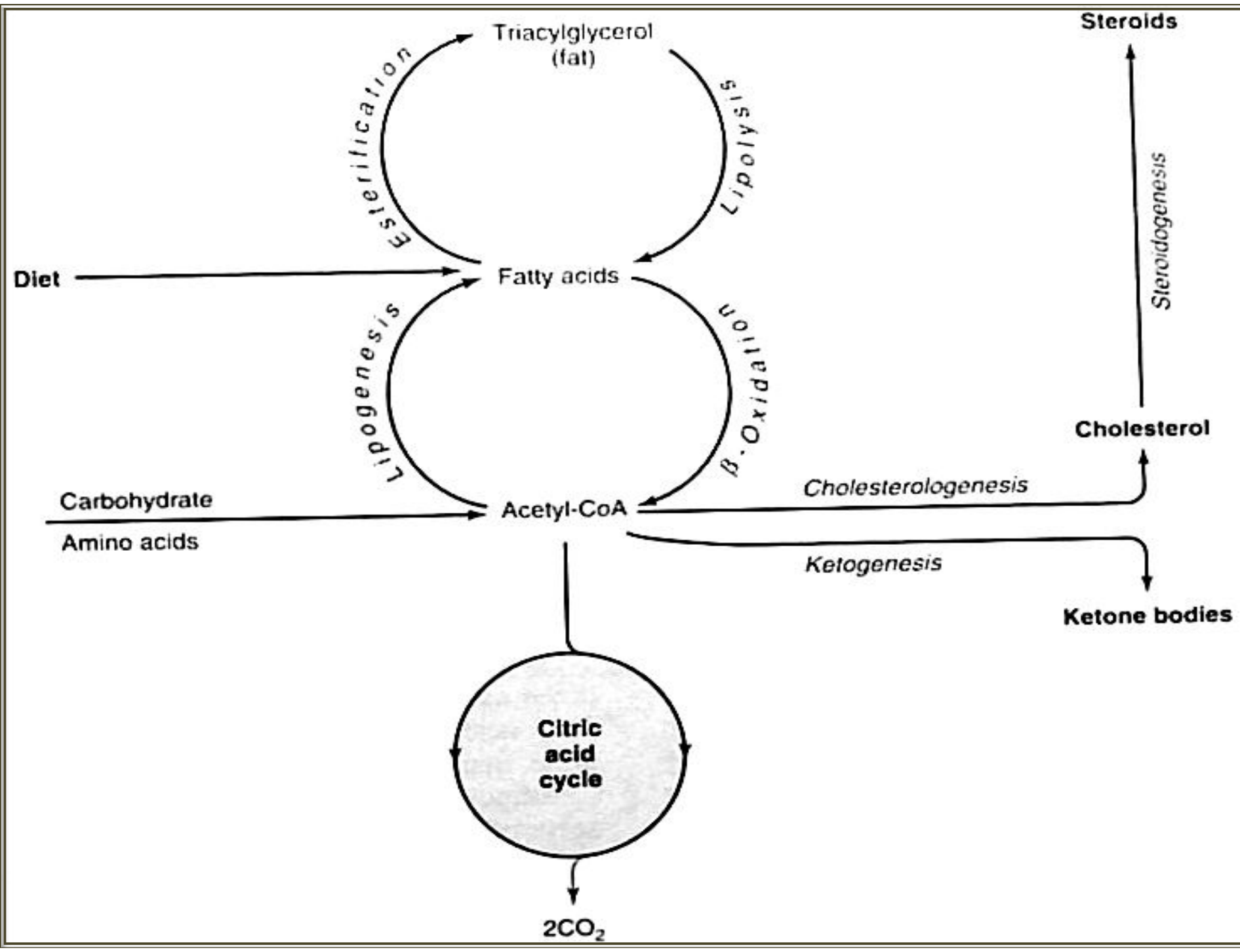
Fates of the products of β-oxidation of Fatty Acid

- **$NADH+H^+$ and $FADH_2$** - are reoxidized in **ETC** to **generate ATP**
- **Acetyl CoA** - Enters the **Citric acid cycle(TCA cycle)** for its **complete oxidation.**
- **Acyl CoA** – Undergoes the **next turn/cycle of β oxidation proper**.

Complete Oxidation Of Fatty Acids



- Fatty acid is activated and oxidized via **Beta Oxidation** in specific number of cycles depending upon chain length.
- **Acetyl CoA** an **end product** of Beta oxidation of Fatty acid
- Is further **completely oxidized** via **TCA cycle**.



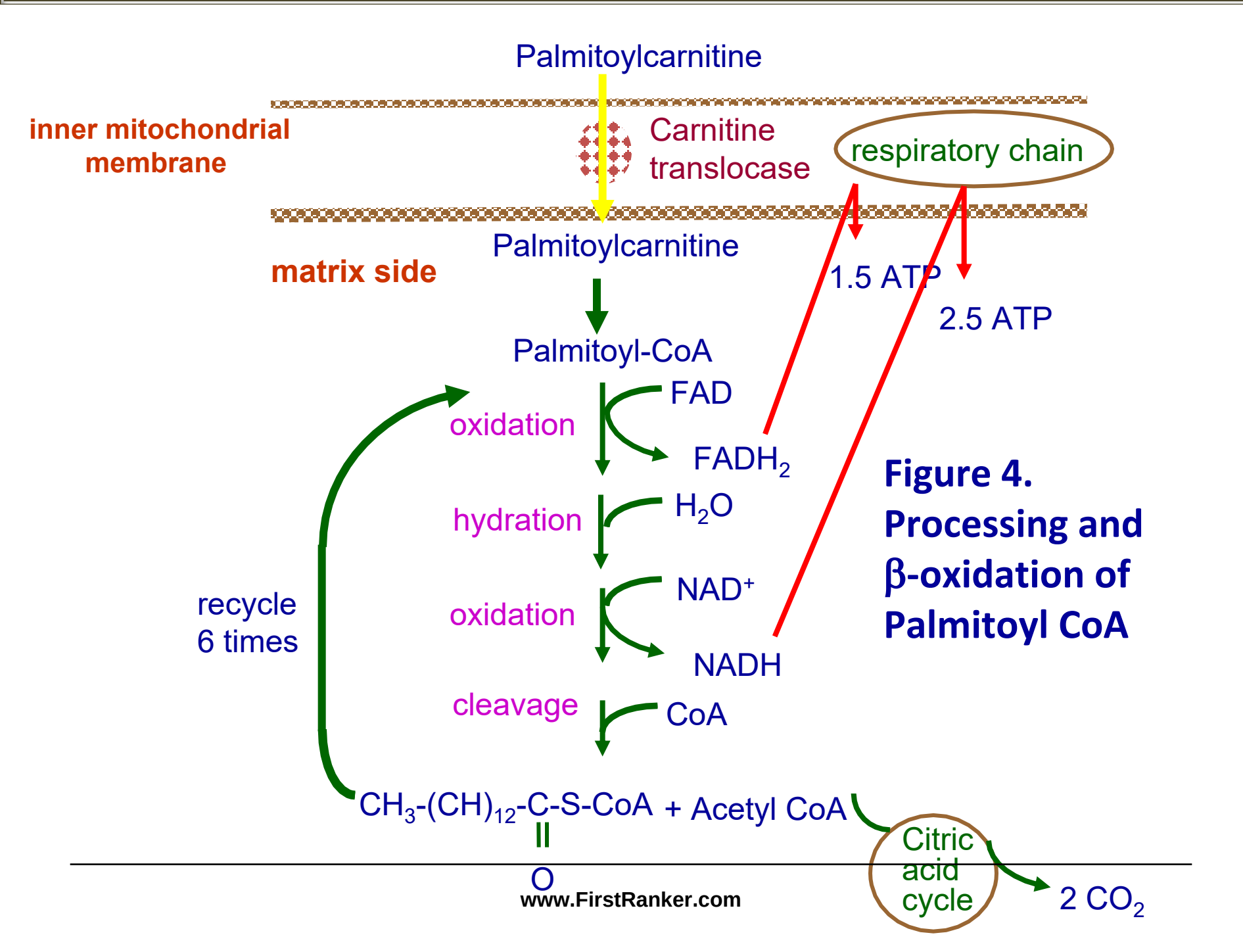
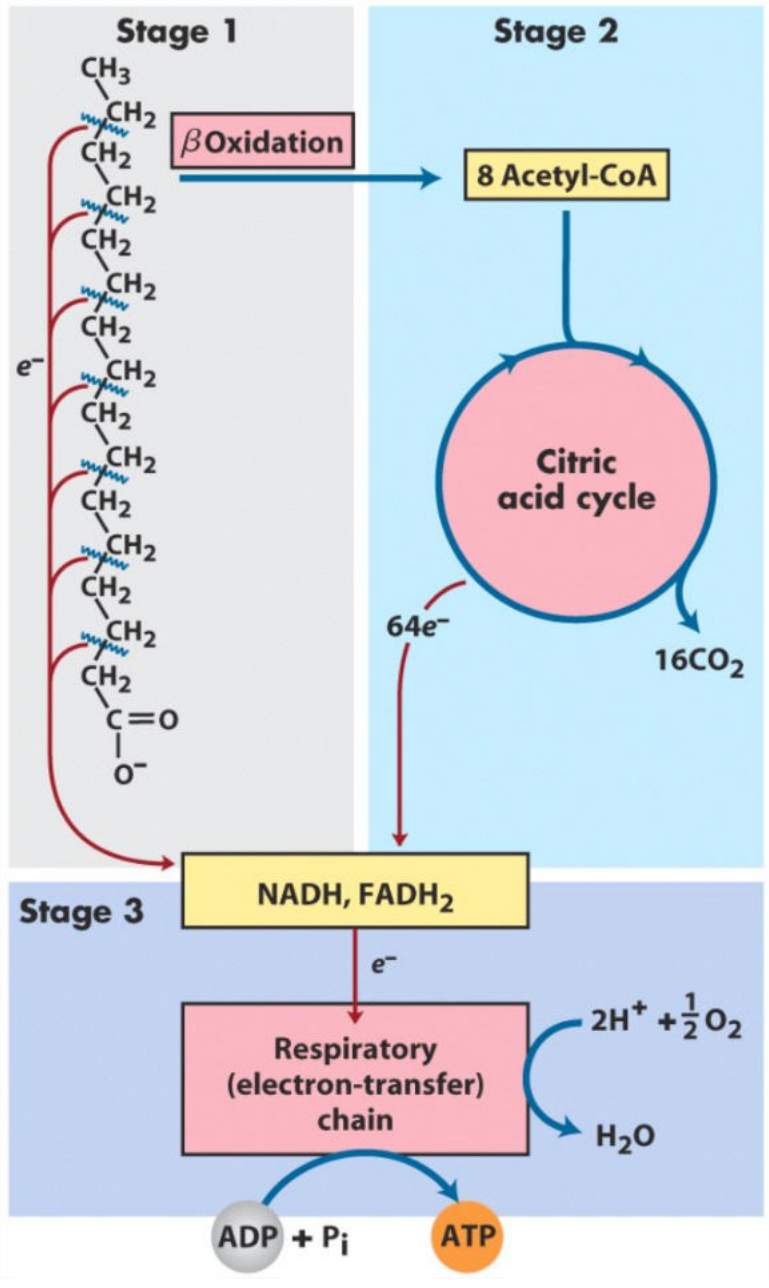


Figure 4. Processing and β-oxidation of Palmitoyl CoA

B-Oxidation Overall Flow



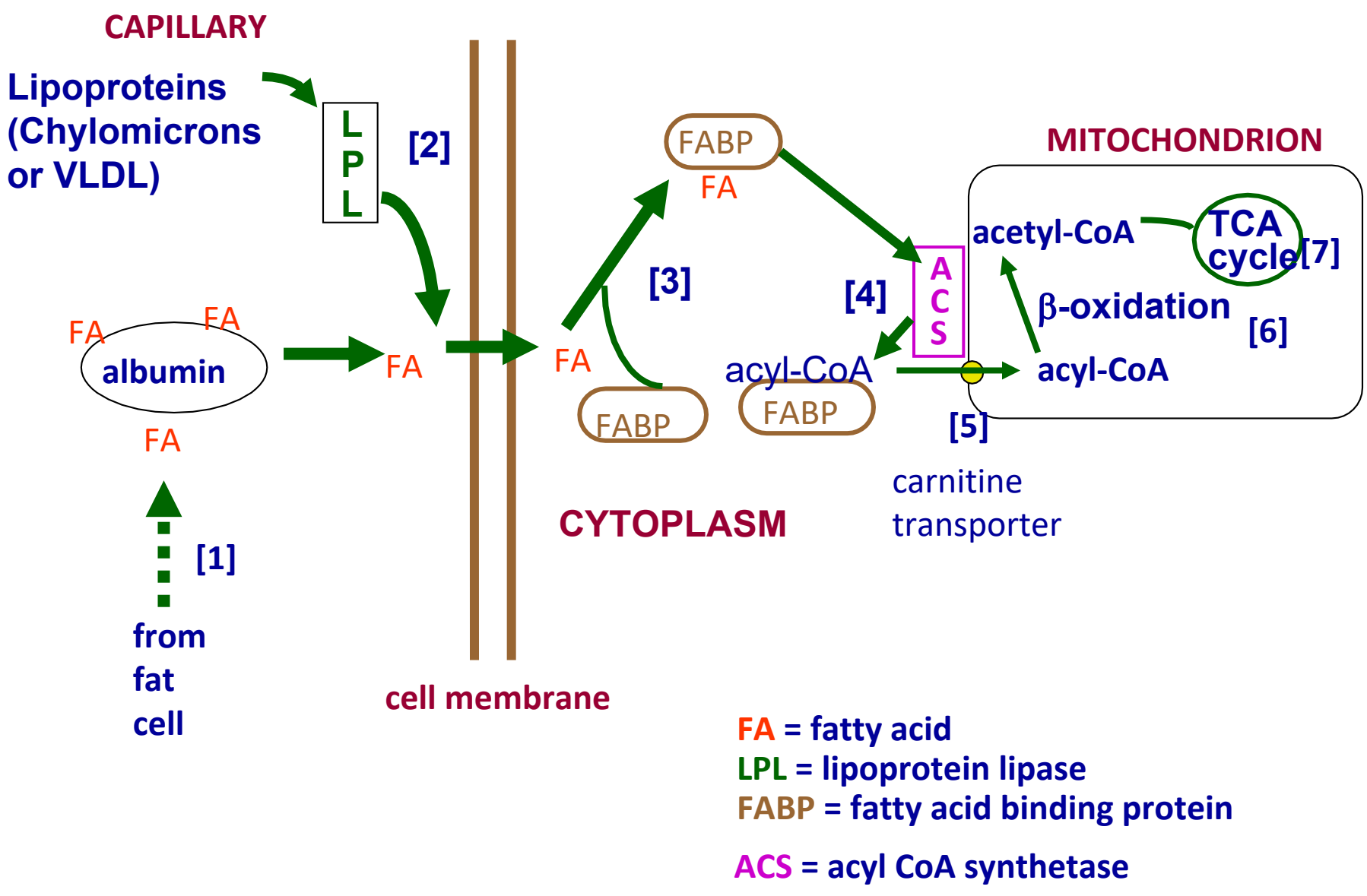


Figure 2. Overview of fatty acid degradation

Energetics Of Beta oxidation Of Palmitate

- Oxidation of Palmitic Acid **C16** Number of turns of fatty acid spiral = **8-1 = 7 Cycles** of beta oxidation proper.
- **Generates 8 Acetyl CoA**

During Electron Transport and Oxidative Phosphorylation Each FADH2 yield 1.5 ATP and NADH 2.5 ATP

Energetics of Fatty Acid Beta Oxidation e.g. Palmitic (16C):

1. β-oxidation of Palmitic acid will be repeated in **7 cycles** producing ***8 molecules of Acetyl COA*****.**

2. In each cycle 1 FADH2 and 1 $NADH+H^+$ is produced and will be transported to the respiratory chain/ETC
   - $FADH_2$ 1.5 ATP
   - $NADH + H^+$ 2.5 ATP

   - Thus Each cycle of β-oxidation **04 ATP**

- So 7 cycles of β-oxidation **4 x 7 = 28 ATP**

1 Acetyl CoA Yields 10 ATPs via TCA Cycle

- **Review ATP Generation –TCA/ Citric Acid Cycle which start with Acetyl CoA**
- Step ATP produced
- Step 4 (NADH+H to ETC) 2.5 ATP
- Step 6 (NADH+H to E.T.C.) 2.5 ATP
- Step 10 (NADH+H to ETC) 2.5 ATP
- Step 8 (FADH2 to E.T.C.) 1.5 ATP
- 1 GTP 01 ATP
- **NET per turn of TCA Cycle 10 ATP**

1 ATP converted to AMP during activation of Palmitic acid to Palmitoyl-CoA is equivalent to 2ATPs utilized

3. Each Acetyl COA which is oxidized completely in citric cycle/TCA cycle gives 10 ATP
4. Hence 8 Acetyl CoA via TCA cycle (**8 x 10 = 80 ATP)**

5. 2 ATP are utilized in the activation of Fatty acid
6. Energy gain = Energy produced - Energy utilized
7. **28 ATP + 80 ATP - 2 ATP = 106 ATP**

Thus On Complete Oxidation of One molecule of Palmitate

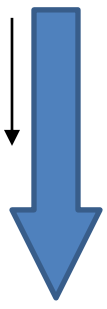
106 molecules of ATP are generated

ATP Generation from Palmitate Oxidation

Net yield of ATP per one oxidized Palmitate

Palmitate ($C_{15}H_{31}COOH$) - 7 cycles - n/2-1

Palmitoyl CoA + 7 HS-CoA + 7 FAD^+ + 7 NAD^+ + 7 H_2O



8 Acetyl CoA + 7$FADH_2$ + 7 NADH + 7 H^+

	ATP generated
8 Acetyl CoA(TCA)	10x8=80
7 $FADH_2$	7x1.5=10.5
7 NADH	7x2.5=17.5
	108 ATP

ATP expended to activate Palmitate **-2 ATP**

Net yield of ATPs with Palmitate Oxidation: 106 ATP

Total End Products
Of
Beta Oxidation
Of
1 molecule of a Palmitic Acid

Palmitic acid
With 7 Turns of
Beta Oxidation Proper
Generates
8 Molecules Of Acetyl-CoA
7 $FADH_2$+7 $NADH+H^+$

BETA OXIDATION

- The fatty acyl chain is shortened by two carbon atoms as a result of these reactions,
- FADH2, NADH, and acetyl Co A are generated.
- Because oxidation is on the β carbon and the chain is broken between the α (2)- and β (3)-carbon atoms—hence the name – β oxidation .

Summary of one round/turn/cycle of the β-oxidation pathway:

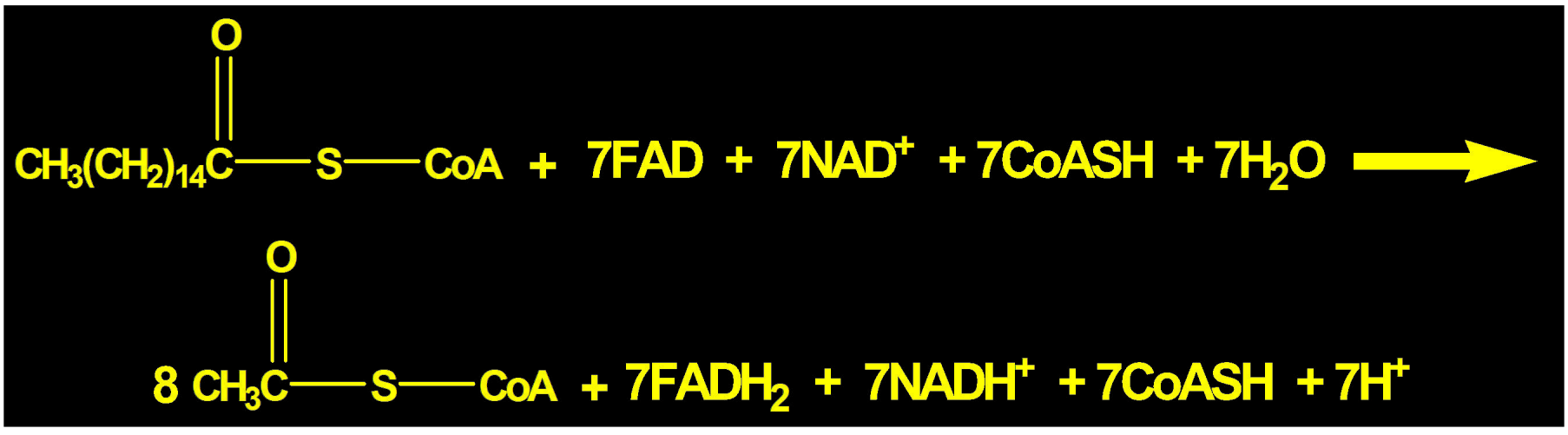
Fatty Acyl-CoA + FAD + NAD^+ + HS-CoA

↓

+Acetyl-CoA

Fatty Acyl-CoA (2 C less) **+ $FADH_2$ + NADH + H^+**

Stoichiometry for Palmitic Acid Oxidation



Fatty acyl-CoA

acyl-CoA dehydrogenase

FAD

$FADH_2$

trans-Δ^2-enoyl-CoA

enoyl-CoA hydratase

H_2O

3-L-hydroxyacyl-CoA

3-hydroxyacyl-CoA dehydrogenase

NAD^+

$NADH + H^+$

MTP

β-ketoacyl-CoA

β-ketothiolase

CoA-SH

Fatty acyl-CoA (-2C)

Acetyl-CoA

β-Oxidation Proper of Acyl-CoA

Acyl-S-CoA

Acyl-CoA dehydrogenase

FAD

$FADH_2$

Enoyl-CoA

Enoyl-CoA hydrase

H_2O

L-β-Hydroxyacyl-CoA

L-β-Hydroxyacyl-CoA dehydrogenase

NAD^+

NADH + H^+

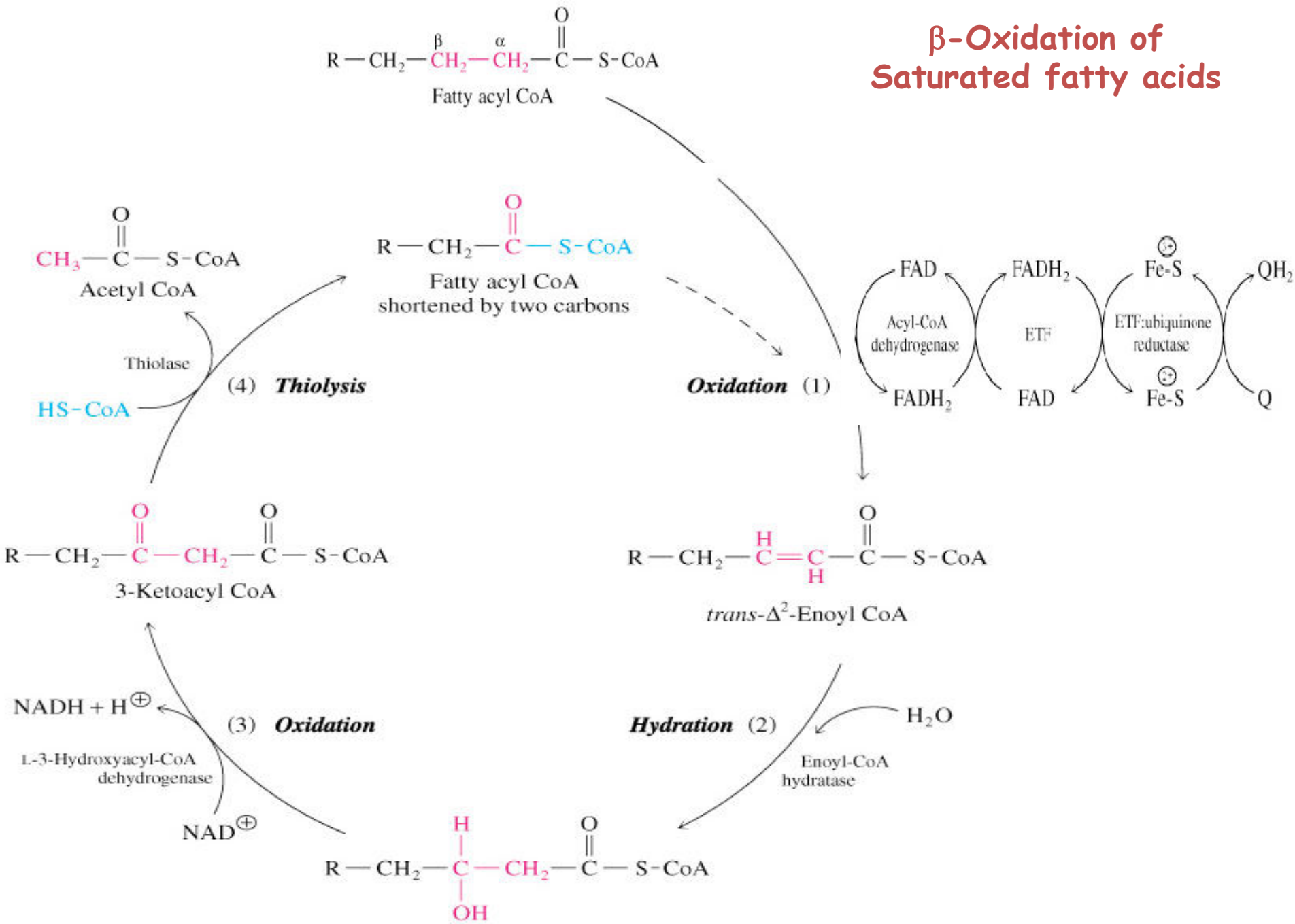
β-Ketoacyl-CoA

Thiolase

CoASH

Acyl-S-CoA + Acetyl-S-CoA

β-Oxidation of Saturated fatty acids



Regulation Of Beta Oxidation Of Fatty Acids

- Lipolysis and

β Oxidation of Fatty acids are well **regulated under Hormonal influence**.

Insulin secretion is In Well Fed Condition

- **Insulin inhibits Lipolysis** of Adipose Fat (TAG) and mobilization of Free Fatty acids.

- **Insulin decreases β Oxidation of Fatty acids.**

Glucagon In Emergency Condition

- When Cellular or Blood Glucose lowers down there is secretion of Glucagon.
- **Glucagon and Epinephrine stimulates Lipolysis** in emergency condition.
- **Glucagon stimulates the Enzyme Hormone sensitive Lipase** and hydrolyzes depot Fat(TAG).
- Glucagon mobilizes Free fatty acids out into blood circulation
- **Increases β Oxidation of Fatty acids.**

Regulation Of
Beta Oxidation Of Fatty Acid
At Two Levels

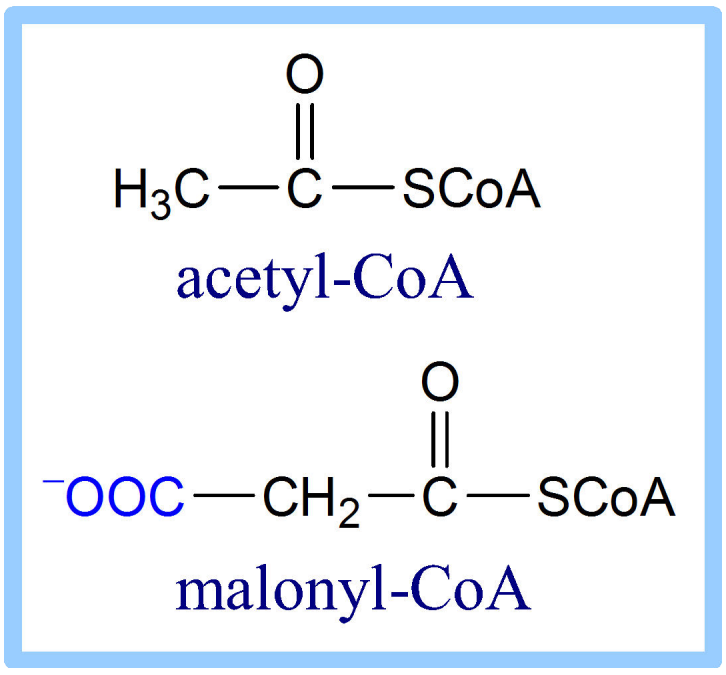
- **Carnitine Shuttle**
- **Beta Oxidation Proper**

Transport of Fatty Acyl CoA from Cytosol into Via Carnitine Shuttle Mitochondrial Matrix

Is a Rate-limiting step

Malonyl-CoA Regulates Beta Oxidation At Carnitine Transport Level

Malonyl-CoA an intermediate of Lipogenesis Is an Inhibitor of Carnitine Acyl Transferase I



Malonyl-CoA is produced from Acetyl-CoA by the enzyme **Acetyl-CoA Carboxylase during Fatty acid biosynthesis.**

Malonyl-CoA (which is a precursor for fatty acid synthesis) **inhibits Carnitine Palmitoyl Transferase I**.

This Control of Fatty acid oxidation is exerted mainly at the step of ~~**Fatty acid entry** into mitochondria.~~

Acyl-CoA Dehydrogenase is Regulatory or Key Enzyme of Beta Oxidation Of Fatty Acids

Significance Of Beta oxidation of a Fatty acid

- Beta oxidation cycles helps in **cleaving and shortening of a long chain Fatty acid**

- Oxidation of Beta carbon atom of a Fatty acid **transforms stronger bond between alpha and beta carbon atom to a weaker bond**.

- Transformation to **a weaker bond helps in easy cleavage between alpha and beta carbon**

- During β oxidation there is **dehydrogenation of beta carbon atom (CH2 to C=O)**

- **Hydrogen atoms removed during beta oxidation** are
- Temporarily accepted by the oxidized coenzymes (FAD and NAD^+) to **form reduced coenzymes**
- Reduced coenzymes then **finally enter ETC and get reoxidized**
- The byproduct of ETC is **ATP**

- Thus **Beta oxidation** of Fatty acid
- Metabolizes a long chain fatty acid with **liberation of** chemical form of energy **ATP** for cellular activities.

Summary of β-Oxidation

Repetition of the β-Oxidation Cycle yields a succession of Acetate units

- Palmitic acid yields **eight Acetyl-CoAs**
- Complete β-oxidation of one Palmitic acid yields **106 molecules of ATP**
- Large energy yield is consequence of the **highly reduced state of the carbon in fatty acids**
- This makes **fatty acid the fuel of choice for ~~migratory birds and many other animals~~**

Disorders OF Beta Oxidation Of Fatty Acids

Deficiencies of Carnitine OR Carnitine Transferase OR Translocase Activity Are Related to Disease State

Biochemical Consequences of Carnitine Shuttle Defect

- Defect in Carnitine shuttle system
- No Beta Oxidation of Fatty acids
- No ATP generation
- All ATP dependent processes will be ceased
- Cell deaths
- Organ failures

Carnitine Shuttle Defects

- Affects normal function of **Muscles, Kidney, and Heart**.

- **Symptoms** include **Muscle cramping,** during exercise, **severe weakness** and death.

- **Muscle weakness occurs** since they are related with **Fatty acid oxidation** for **long term energy source.**

Management Of Individuals with Carnitine Shuttle Defects

- **Note people with the Carnitine Transporter Defect**

  – Should be **supplemented with a diet with medium chain fatty acids**

  – Since **MCFAs** do **not require Carnitine shuttle to enter Mitochondria**.

Sudden Infant Death Syndrome (SIDS)

SIDS

- SIDS is a **congenital rare disorder** with an incidence of **1 in 10,000 births**.

- **Biochemical Defect:** Due to **congenital defect** of Enzyme **Acyl-CoA Dehydrogenase** a regulatory enzyme of β Oxidation of Fatty acid.

- **Biochemical Consequences Of SIDS**

- Deficiency of Acyl-CoA Dehydrogenase
- **Blocks β Oxidation** of Fatty acid.
- **Stops liberation** and supply of energy in form **of ATPs in fasting condition**
- Leads to **unexpected death of an infant.**

Symptoms in defective Beta Oxidation of Fatty acids include:

- **Hypoglycemia**
- **Low Ketone body** production during fasting
- **Fatty Liver**
- **Heart and/or Skeletal muscle defects**
- **Complications of pregnancy**
- **Sudden infant death** (SID).

- Hereditary deficiency of **Medium Chain Acyl-CoA Dehydrogenase** (MCAD)

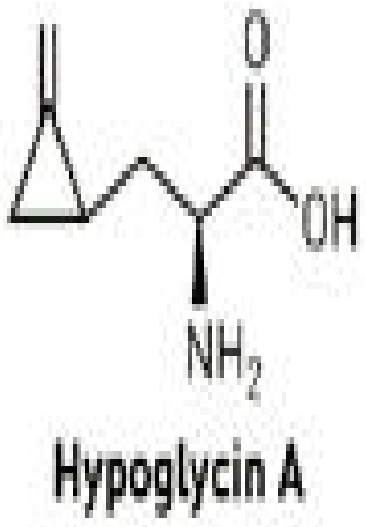
- **Most common genetic disease** relating to fatty acid catabolism, has been **linked to SIDS.**

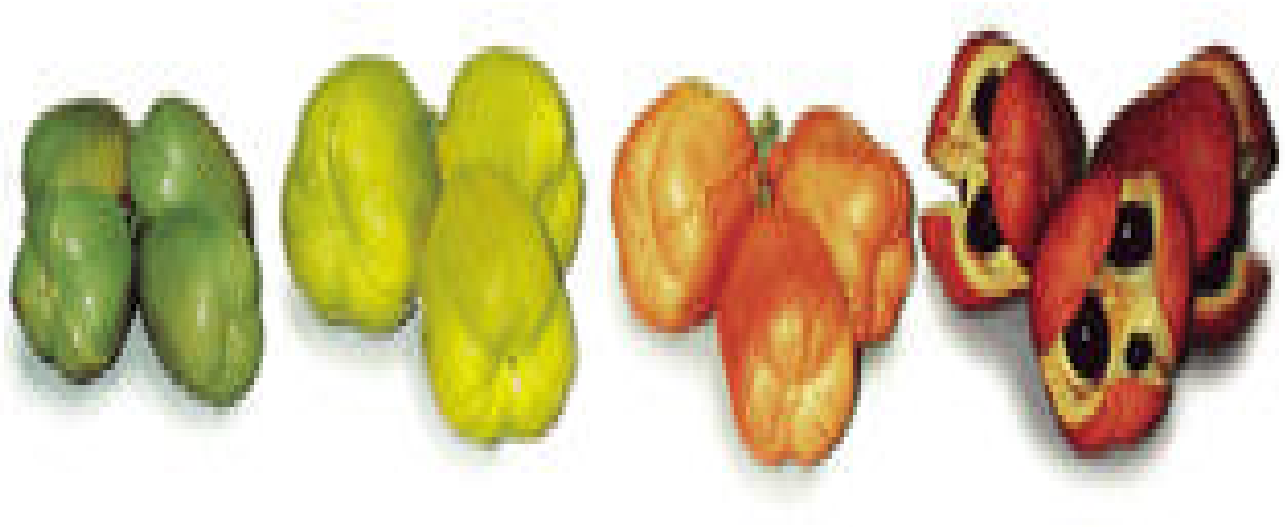
Jamaican Vomiting Sickness

- Jamaican Vomiting Syndrome is due to **ingestion of unripe Ackee fruit by people in Jamaica**

(Jamaica-Country of Caribbean**)**

Ackee Fruit





- Ackee fruit is rich in **Hypoglycin –A**

- **Hypoglycin is an inhibitor of** regulatory Enzyme β Oxidation Proper **Acyl-CoA Dehydrogenase.**

- **Jamaican Vomiting Disease** leads to **complications** characterized by :
  - **–Severe Vomiting (throwing out)**
  - **–Hypoglycemia**
  - **–Water Electrolyte Imbalance**
  - **–Convulsions**
  - **–Coma**
  - **–Death**

Beta Oxidation
Of
Odd Chain Saturated Fatty Acids

- Ingestion of Odd chain carbon Fatty acids are **less common in human body.**

- Odd chain Fatty acids are formed by **some bacteria in the stomachs** of ruminants and the **human colon.**

- β-oxidation of odd chain Saturated Fatty acid occurs **same as even chain Fatty acid oxidation**
- **Releasing Acetyl CoA (2C) in every turn.**
- **Until the final Thiolase cleavage**
- Which results in a **3 Carbon Acyl-CoA /Propionyl-CoA in last cycle and last step of beta oxidation.**

End Products Of Odd Chain Fatty Acid Oxidation

- **End products of β-oxidation of an odd-number** Fatty acid is :
  - **Acetyl-CoA(C2)**
  - **Propionyl-CoA(C3)**

Fate Of Acetyl-CoA

- Acetyl CoA released from beta oxidation of odd chain fatty acid
- **Enter in TCA cycle** and get **completely oxidized**.

Fate Of Propionyl-CoA

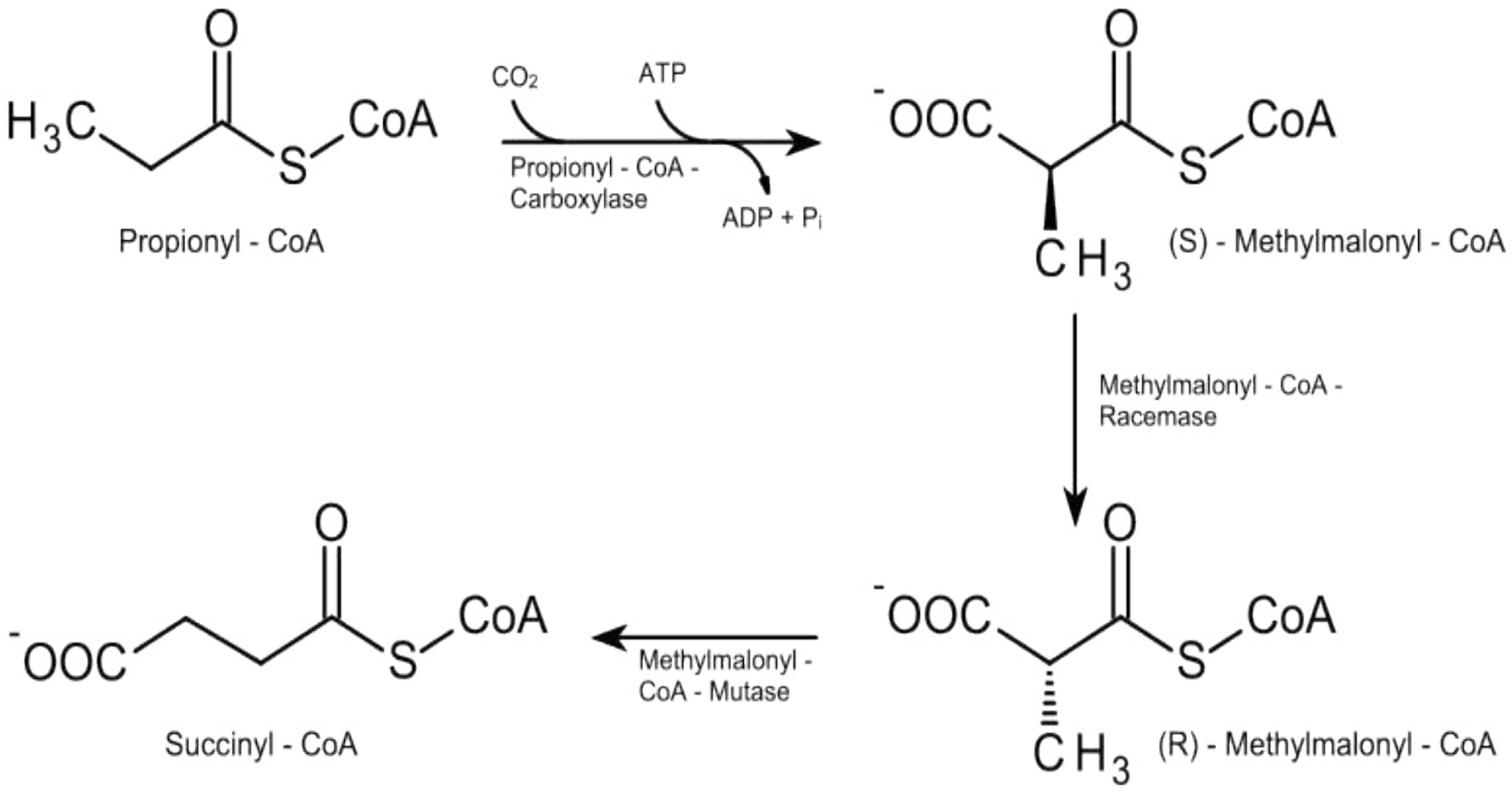
OR

Metabolism Of Propionyl CoA

Propionyl CoA (3C)
An End Product Of Odd Chain Fatty Acid

Is Converted into
Succinyl CoA (4C)
A TCA intermediate

Metabolism Of Propionyl-CoA



- **Metabolism of Propionyl-CoA**

- The Propionyl-CoA is **converted** to **Succinyl-CoA.**

- Which is **an intermediate of TCA/Citric acid cycle**

- Propionyl CoA metabolism is **dependent on Two Vitamin B complex members:**
  - **Biotin**
  - **Vitamin B_{12}**

- Special **set of 3 Enzymes are required** to further metabolize Propionyl-CoA to Succinyl -CoA.

- Final Product **Succinyl-CoA enters TCA cycle and get metabolized.**

- **Three Enzymes convert Propionyl-CoA to Succinyl-CoA:**

1. **Carboxylase**
2. **Epimerase**
3. **Mutase**

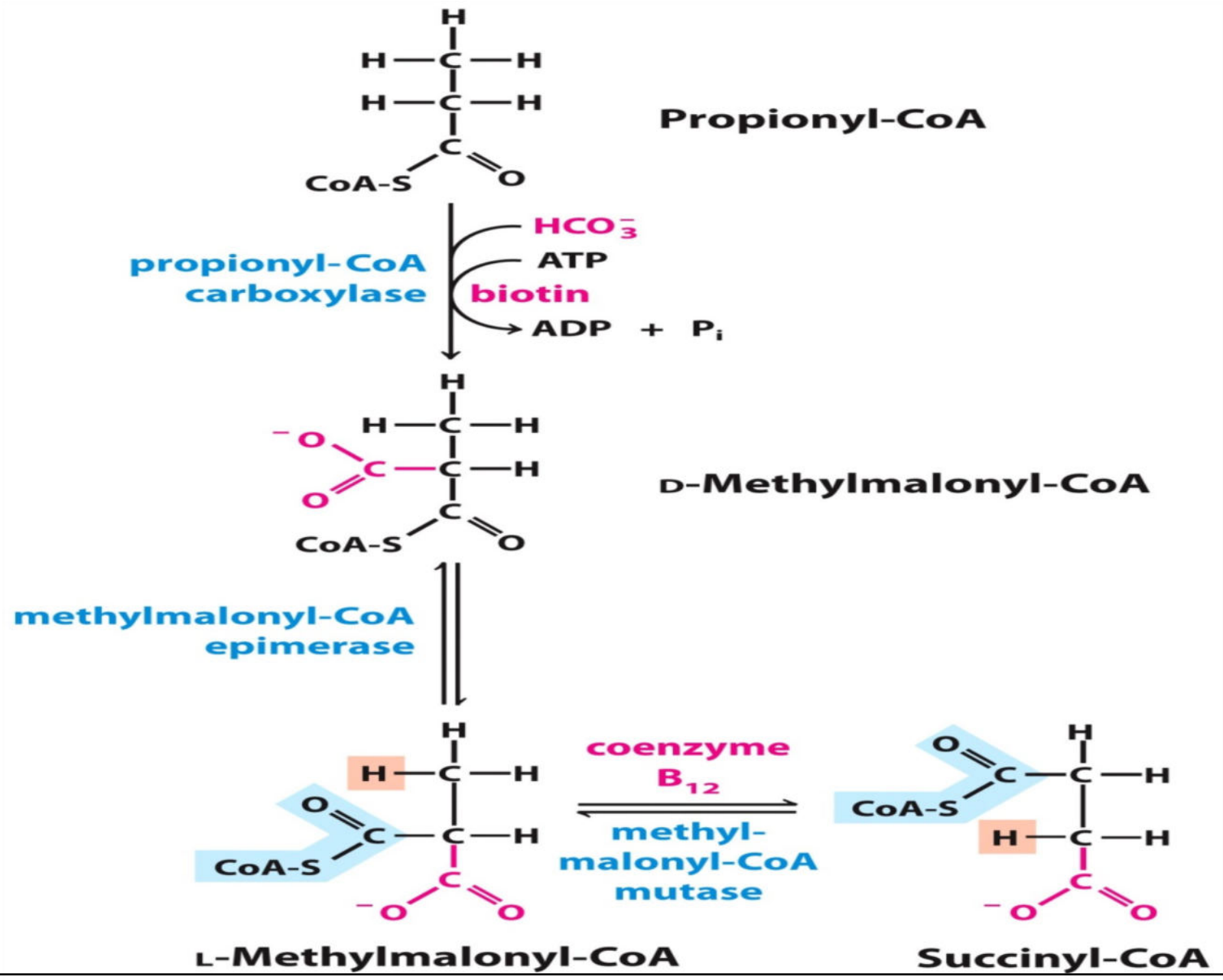


Figure 17-12
Lehninger Principles of Biochemistry, Sixth Edition
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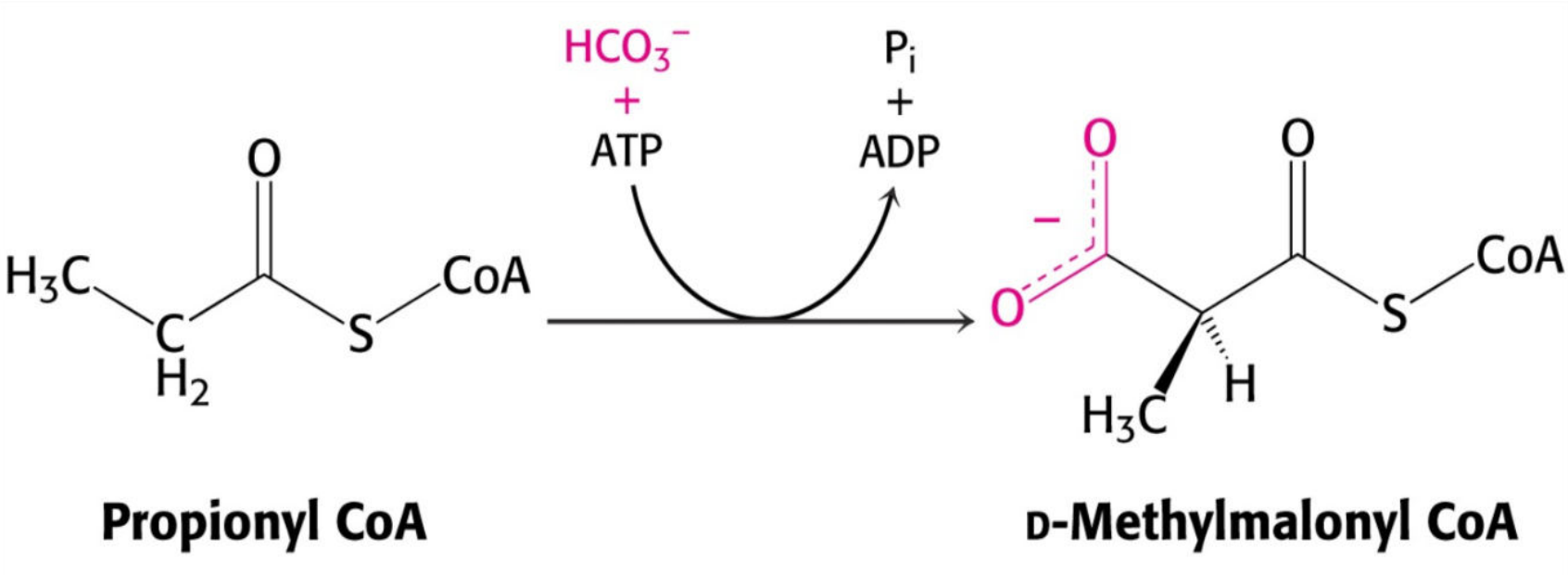
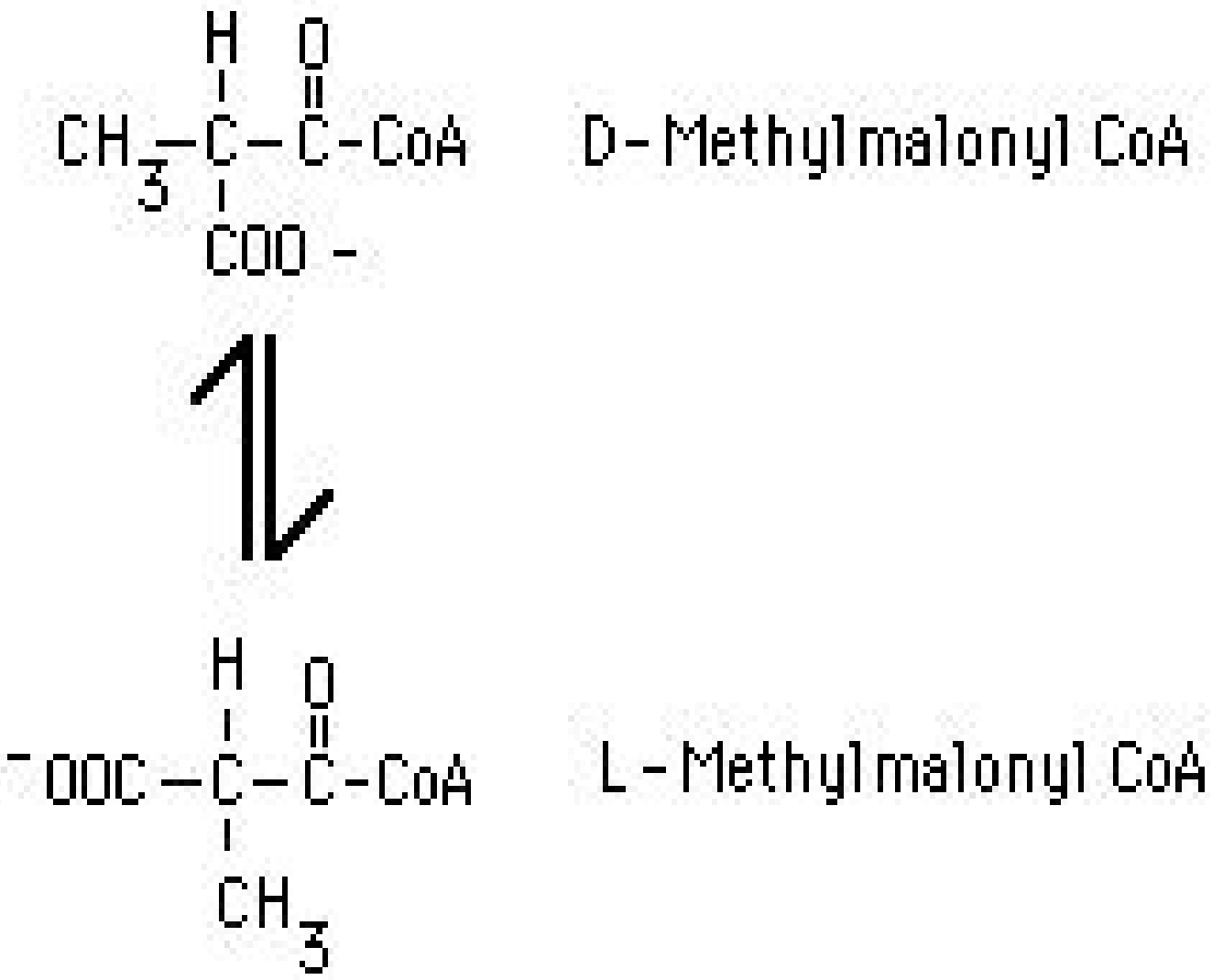
Step1

- **Propionyl CoA** is **Carboxylated** to yield **D Methylmalonyl CoA.**
- Enzyme: **Propionyl CoA Carboxylase**
- Coenzyme: **Cyto Biotin**
- An **ATP is required**



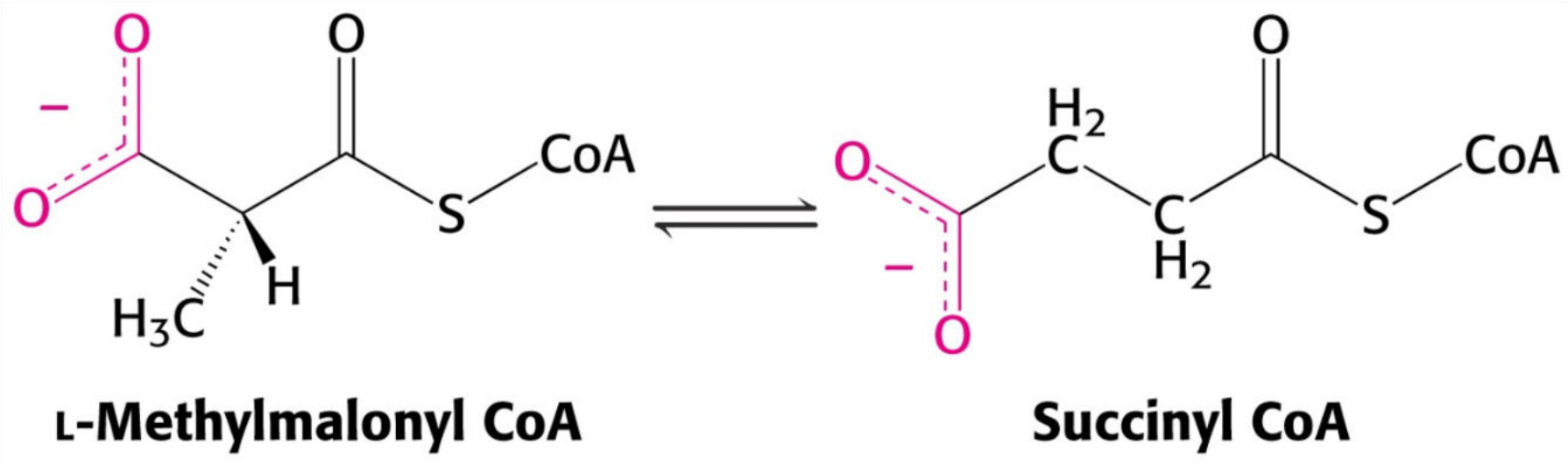
Step2

- The D Methylmalonyl CoA is racemized to the L Methylmalonyl CoA.
- Enzyme: Methylmalonyl-CoA Racemase/ Epimerase



Step 3

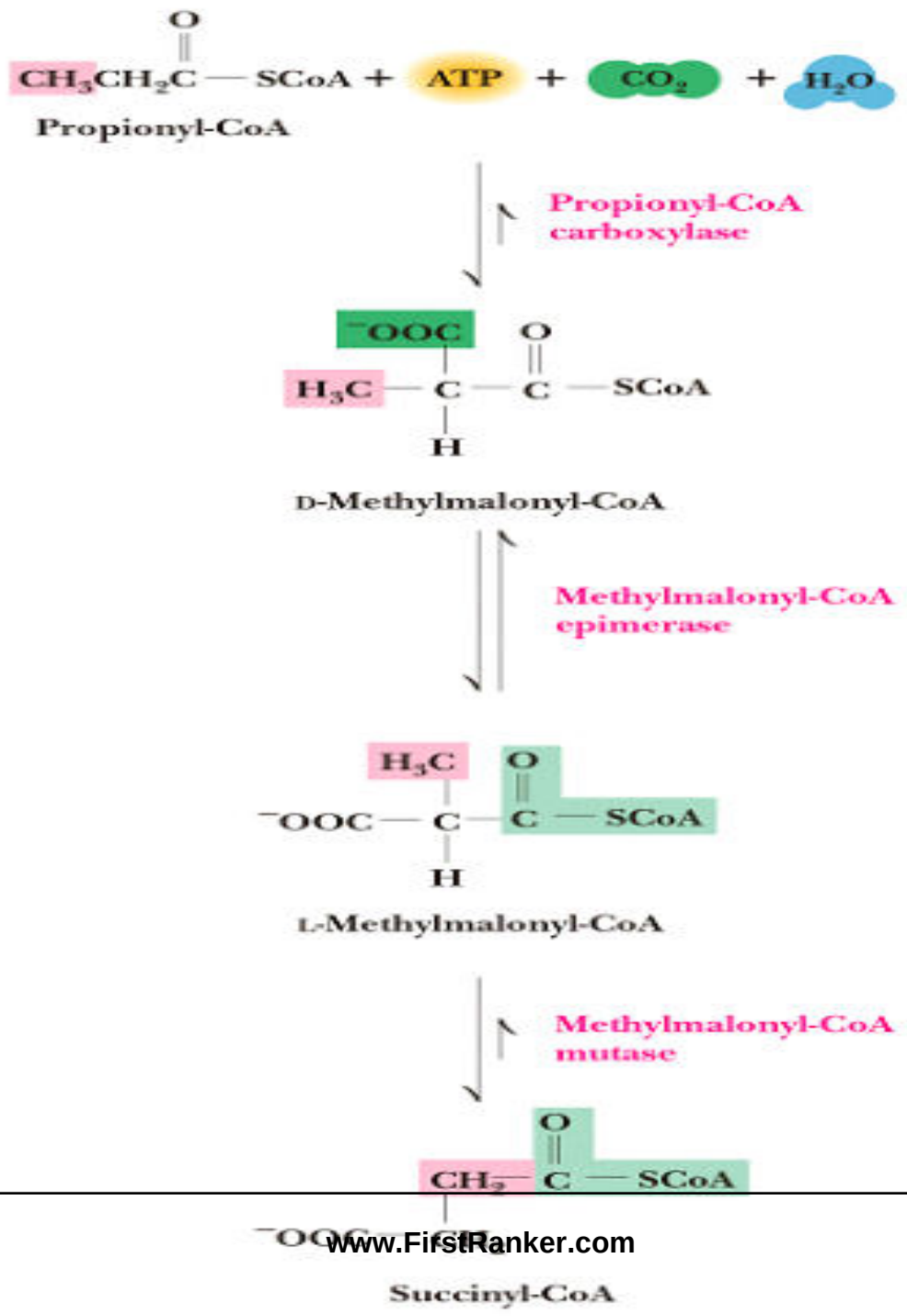
- **L Methylmalonyl CoA is converted into Succinyl CoA by an intramolecular rearrangement**
- **Enzyme: Methylmalonyl CoA Mutase**
- **Coenzyme of Vitamin B12 :Deoxy Adenosyl Cobalamin**



Fates Of Succinyl CoA

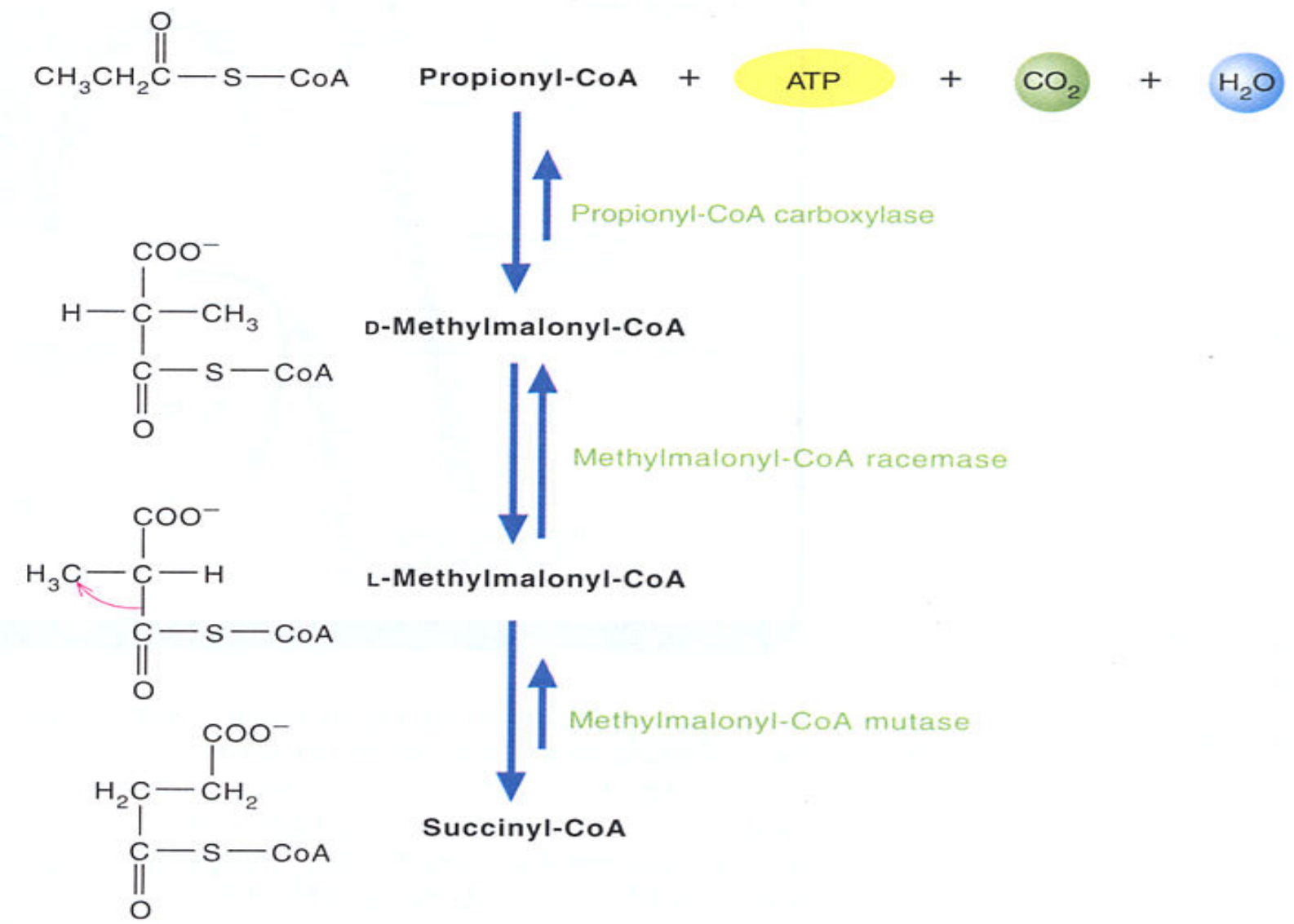
- **Succinyl CoA**

- **Enters TCA cycle** and get metabolized
- Serve as **Glucogenic precursor** for Glucose biosynthesis in emergency condition
- Used as a **precursor for Heme biosynthesis**
- Involves in **Thiophorase reaction** of **Ketolysis**.



Oxidation of Odd-chain Fatty Acids

Conversion of Propionyl-CoA to Succinyl-CoA



Defects In Propionyl CoA Metabolism

- Deficiency of **Enzyme Propionyl-CoA Carboxylase** will block the metabolism of Propionyl-CoA.

- Accumulates Propionyl-CoA in blood leading to **Propionicacidemia.**

- **Deficiency** of **Vitamin B Complex members** affects Propionyl CoA metabolism to Succinyl –CoA.
- **Vitamin B12** deficiency **blocks** the **Mutase reaction**
- **Accumulates L-Methyl Malonyl-CoA** leading to **Methyl Malonylaciduria.**

Alpha Oxidation Of Fatty Acid

OR

Oxidation Of Branched-Chain Fatty Acid

OR

Phytanic Acid Oxidation

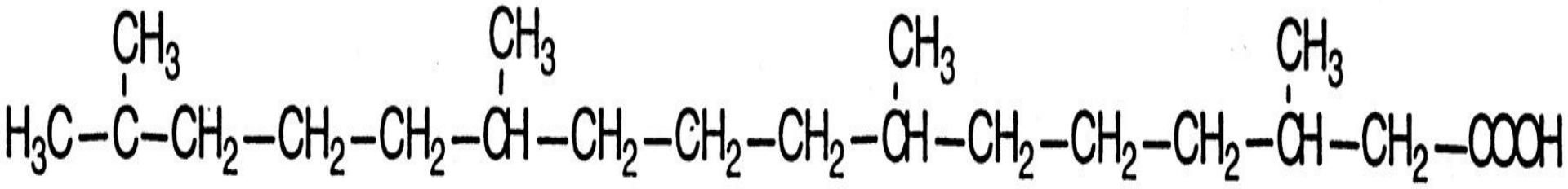
3,7,11,15-tetramethyl Hexadecanoic acid

- Source of **Phytanic acid in human body** is through ingestion of **animal Foods.**

- **Phytanic acid** is a **breakdown product of Phytol component of plant chlorophyll**.

Why Phytanic Acid Does Not Initiate With Beta Oxidation Process?

- **Phytanic acid is a 16 Carbon Branched chain Fatty Acid.**
- **Has Four Methyl branches at odd-number carbons 3,7,11 and 15.**
- Which is not **good substrates for β-oxidation**.



Phytanic acid

- Branched chain Phytanic acid **contains Methyl (CH3)** group **at β Carbon atom.**

- Hence it **cannot get oxidized initially via β oxidation** pathway

- Thus initially Phytanic acid **follows α Oxidation**
- **Modify Phytanic acid to Pristanic acid** and
- **Further present it for Beta Oxidation process.**

Occurrence Of Alpha Oxidation Of Phytanic Acid

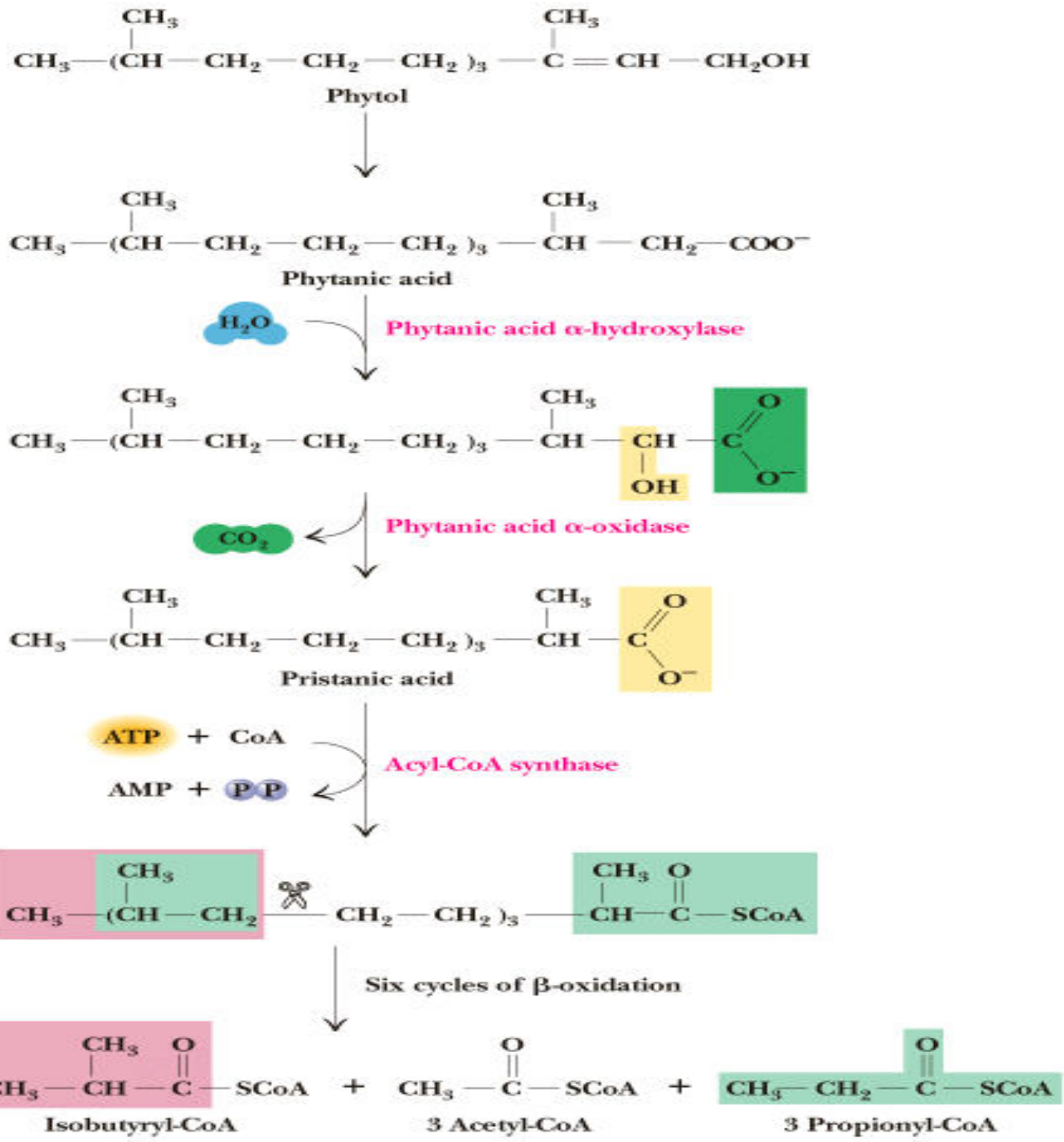
Predominantly Alpha Oxidation Of Phytanic Acid Takes Place in

Endoplasmic Reticulum of Brain Cells

Also In Peroxisomes

Mechanism Of Alpha Oxidation Of Phytanic Acid

- Phytanic acid **3,7,11,15-Tetramethyl Hexadecanoic acid**
- Alpha oxidation **removes Methyl groups** at beta carbon.
- **Later** making Fatty acid ready for **beta oxidation process**.



- **During α Oxidation there occurs:**

- **Hydroxylation at α Carbon** in presence of Enzyme **Hydroxylase or Monoxygenase .**

- This reaction is **Vitamin C dependent** forming **α Hydroxy Acyl-CoA.**

- α Hydroxy Acyl-CoA is then oxidized to **α Keto Acyl-CoA**.

- **Ketonic group at α Carbon** atom is decarboxylated

- **Yielding CO2 molecule** and a Fatty acid with one Carbon atom less.

- **Phytanic acid** on alpha oxidation is **converted to Pristanic acid**

- Which is **further metabolized via beta oxidation process** to generate Propionyl-CoA.

Products of Phytanic Acid Oxidation

- **Alpha oxidation of Phytanic acid Generates**

–Acetyl-CoA

–Propionyl-CoA

–Isobutryl-CoA

Disorders Associated With Defective α Oxidation Of Phytanic Acid

Refsums Disease

- **Refsums disease** is a **rare** but **severe neurological disorder.**
- Caused due to **defect in α Oxidation** of Phytanic acid

The Enzyme Defects

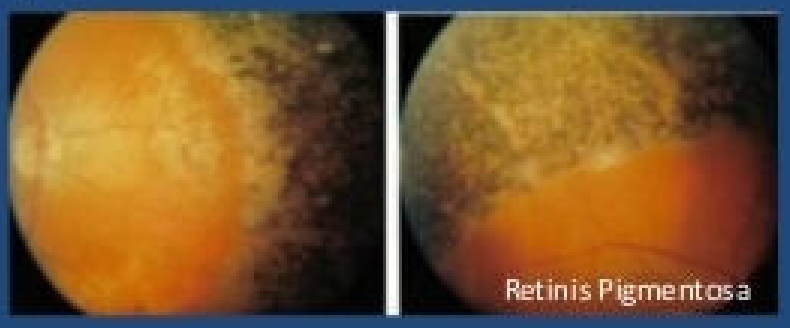
- **Deficiency** of **Enzyme Phytanic acid α Oxidase/ Phytanol-CoA Dioxygenase** leads to Refsum's disease.
- **Autosomal Recessive**

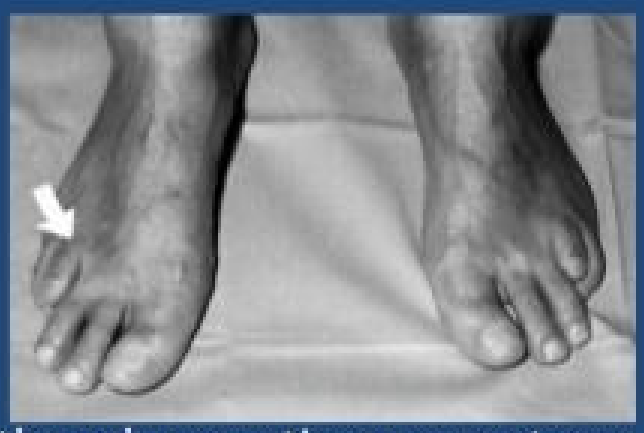
- **Biochemical Consequence Of Refsums disease Is:**
- No Oxidation of Phytanic acid
- **Accumulation of Phytanic acid in Brain cells and Other Tissues**
- **Dysfunction of Brain**
- **Manifesting Neurological disorder**

Symptoms

How do I know if I have Refsum Disease?

- **Impaired eyesight** (Retinitis Pigmentosa)
  - Loss of night vision in childhood → disrupted peripheral vision → blindness
  - Most apparent and serious symptom
- **Deafness**
  - ✧ Apparent later in life
- **Loss of smell** (Anosmia)
  - Always apparent in patient
- **Balance or coordination problems** (Ataxia)
  - ✧ Apparent later in life
- **Dry, scaly skin** (Ichthyosis)
  - ✧ Apparent later in life
- **Heartbeat abnormalities** (Cardiac arrhythmias)
- **Shortened fingers or toes**





✧ Disease usually apparent in childhood, although sometimes symptoms may not develop until 40s or 50s

- Management Of Refsums disease is :
- **Avoid eating diet containing Phytol /Phytanic acid.**

Omega Oxidation Of Fatty Acids

- Omega Oxidation of Fatty acid is:

- **Oxidation of Omega Carbon atom (CH3) of a Fatty acid.**

When Does Omega Oxidation Of Fatty Acid Occurs?

- Omega Oxidation takes place **when there is defect in β Oxidation of fatty acid.**

- **During ω Oxidation of Fatty acid**
- **ω Carbon atom (CH3) of a Fatty acid is** transformed to -**COOH**

- Omega oxidation forms **Dicarboxylic acid**
- Which **further undergo oxidation**
- **Form more short Dicarboxylic acids Adipic acid and Succinic acid**
- **Which are more polar excreted out in Urine.**

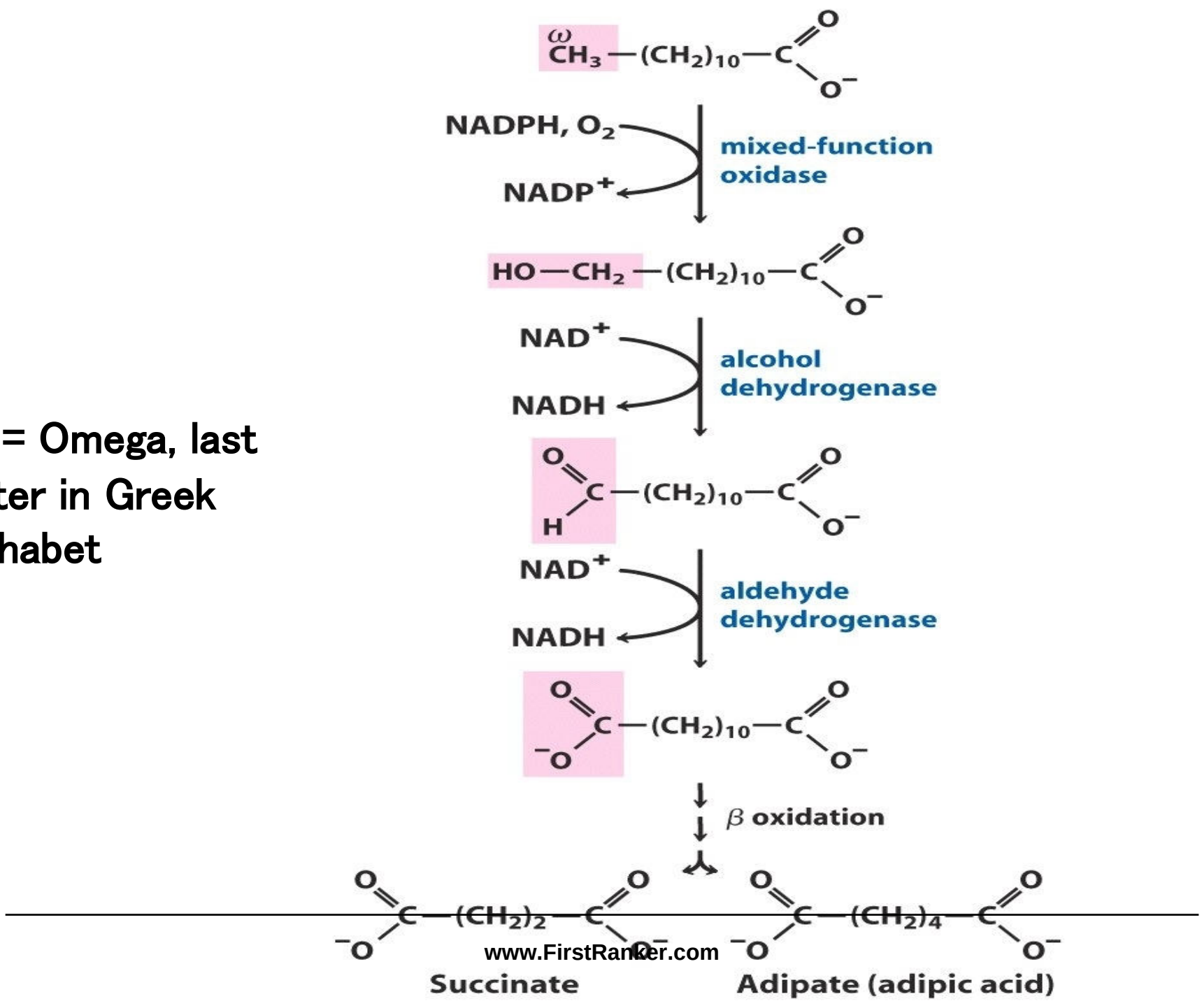
ω-Oxidation of Fatty acids Occur in Endoplasmic Reticulum of Liver Cells

Mechanism Of ω Oxidation

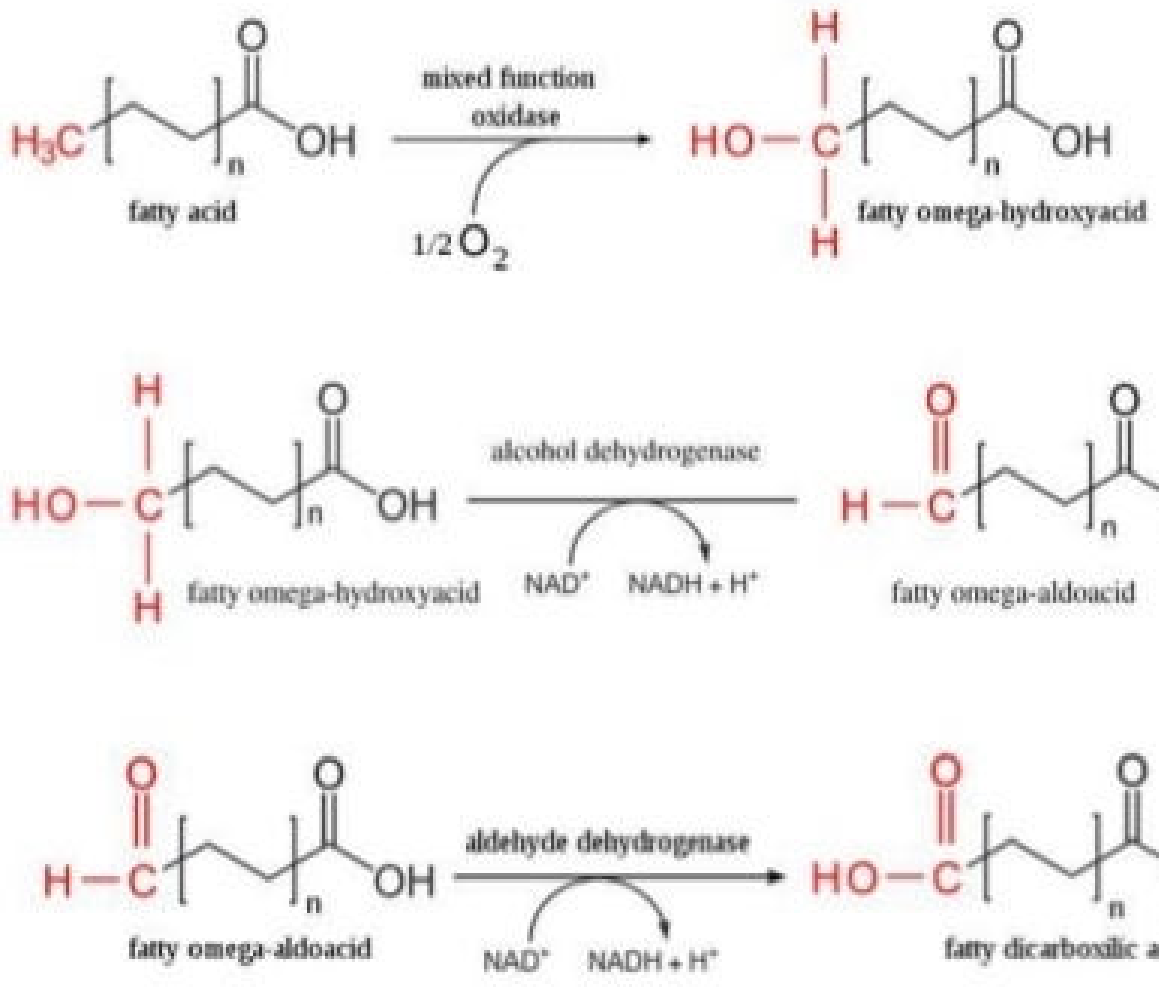
- ω Oxidation of Fatty acid is a **minor alternative oxidative Pathway.**

- Omega Oxidation of a Fatty acid takes place with:
  - **–Hydroxylation Reaction**
  - **–Oxidation Reaction**

ω = Omega, last letter in Greek alphabet



OMEGA OXIDATION OF FATTY ACIDS



Dicarboxylic acids so formed can undergo beta oxidation to produce shorter chain dicarboxylic acids such as Adipic acids(C6) and succinic acid (C4).

- In ω Oxidation of Fatty acid there occurs **Hydroxylation at ω Carbon atom**
- **Converting** into **Primary terminal Alcohol (-CH2OH) group.**
- This reaction is catalyzed by NADPH+H$^+$ dependent **Cytochrome P450 system**
- **Next** primary terminal Alcohol group is **oxidized to form -COOH group** .

- Further **Dicarboxylic acid generated through Omega Oxidation** undergoes beta oxidation
- To **produce short chain Dicarboxylic acids as Adipic acid and Succinic acid**
- Which are **polar and excreted out through Urine.**

Significance Of Omega Oxidation

- Omega Oxidation **transforms a non polar Fatty acid to polar** Dicarboxylic fatty acid.
- Omega Oxidation of fatty acid **facilitates excretion of accumulated fatty acids** due to defective normal β Oxidation in cells.

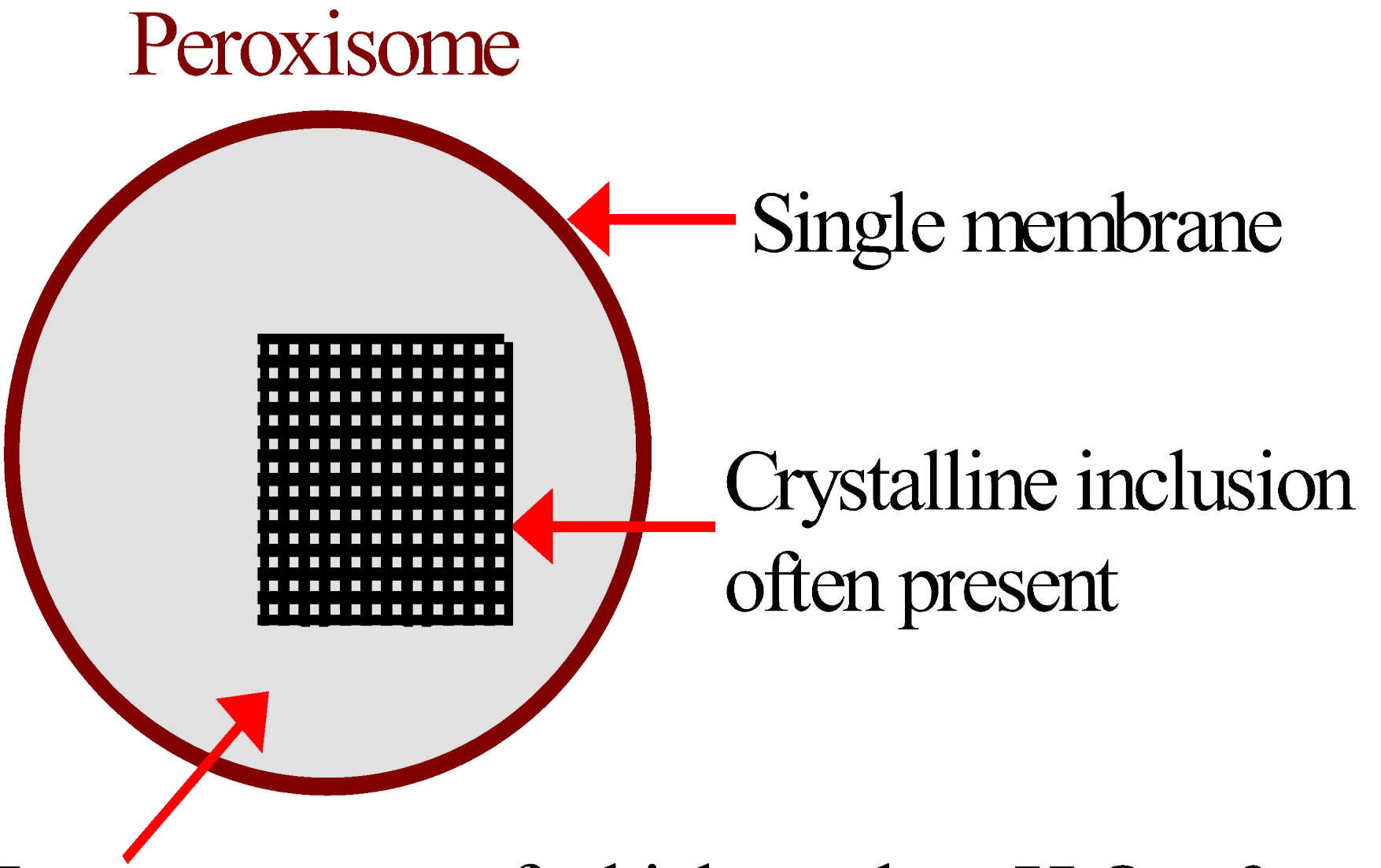
Peroxisomal Oxidation Of Fatty Acids

OXIDATION OF FATTY ACIDS IN PEROXISOMES

- **Peroxisomes** – Cell organelles containing Enzymes ***Peroxidase*** and ***Catalase***

- These Enzymes catalyzes dismutation of **Hydrogen peroxide** into **water and molecular oxygen**

When ? Why? How? Does Peroxisomal Oxidation OF Fatty Acid Occurs?



- **β-Oxidation of very long-chain fatty acids(>C22)** occurs within **Peroxisomes initially**
- **Later undergoes Mitochondrial β Oxidation .**

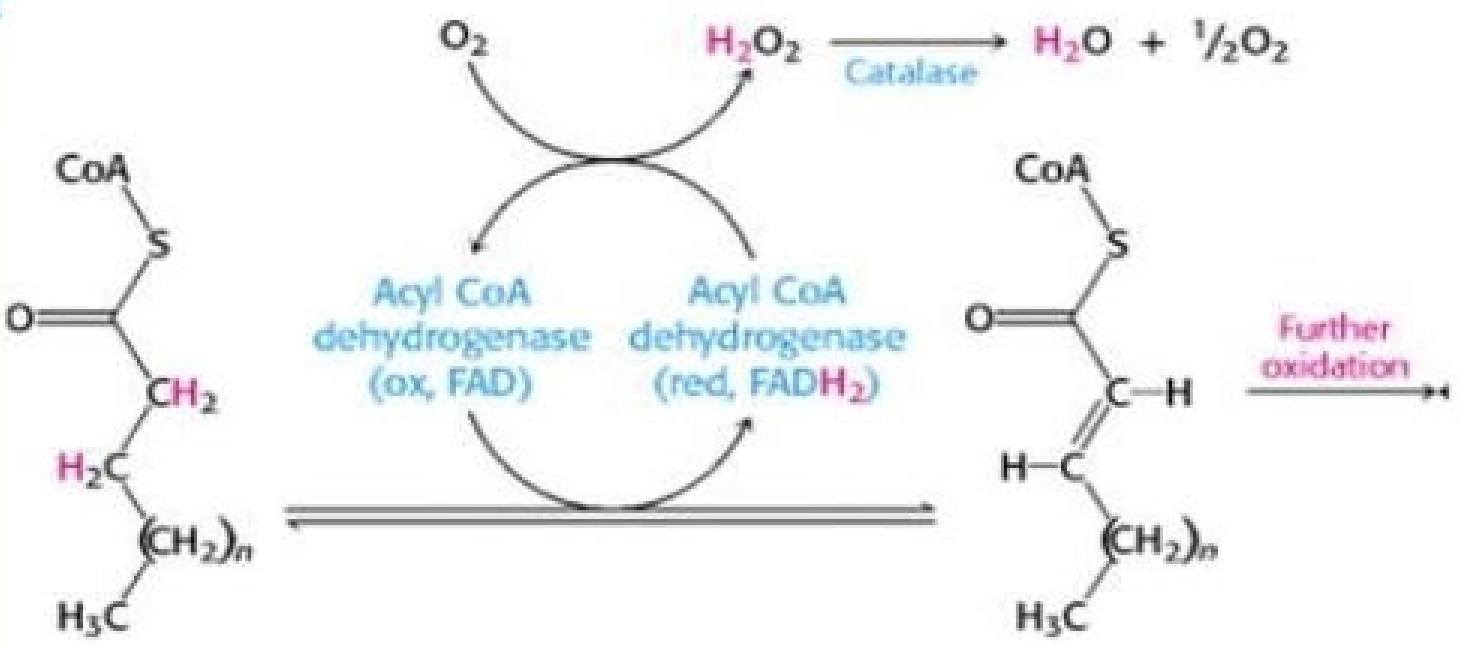
- **Carnitine** is involved in transfer of Very long Chain Fatty acids (**VLCFAS >C22**) **into and out of Peroxisomes.**

- Peroxisomal Fatty acid oxidation is **induced by a high Fat diet with VLCFAs.**
- **To shortens VLCFAs into LCFAs**
- Which are **further degraded by Beta oxidation process**.

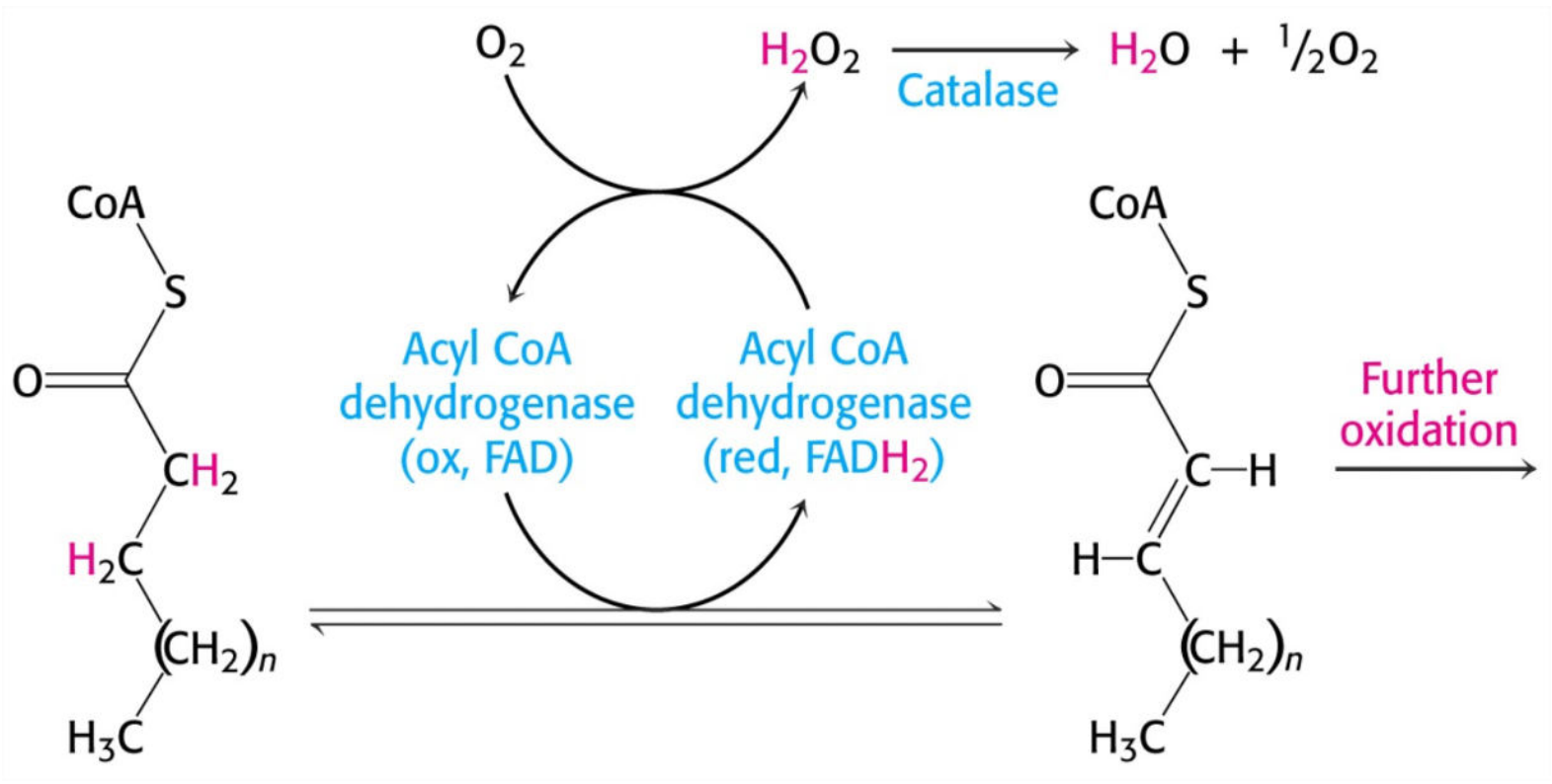
Peroxisomal β-Oxidation

- Similar to Mitochondrial β-oxidation,
- Initial double bond formation is catalyzed by **Flavoprotein Acyl-CoA Oxidase**

PEROXISOMAL OXIDATION OF VERY LONG CHAIN FATTY ACIDS



The specificity of the peroxisomal enzymes is for longer chain fatty acids. Thus peroxisomal enzymes function to shorten the chain length of relatively long chain fatty acids to a point at which beta oxidation can be completed in mitochondria.



Acyl CoA Oxidase–FAD transfers electrons to O_2 to yield H_2O_2.

- **Coenzyme FAD** is e^- acceptor for Peroxisomal **Acyl-CoA Oxidase,** which catalyzes 1^{st} oxidative step of pathway.

- **FADH2 generated** at this step instead of transferring high-energy electrons to ETC, as occurs in Mitochondrial beta-oxidation.
- Electrons of **FADH2 directly go to O_2 at reaction level to generate H2O2** in **Peroxisomes.**

- **Thus FADH2** generated **in Peroxisomes** by Fatty acid oxidation **do not enter ETC to liberate ATPs.**

- **Instead** peroxisome, **$FADH_2$** generated by fatty acid oxidation by **Acyl CoA Oxidase** is **reoxidized producing Hydrogen peroxide**.

$FADH_2 + O_2 \rightarrow FAD + H_2O_2$

Peroxisomal enzyme **Catalase** degrades H_2O_2:

$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$

These reactions produce **No ATP**.

- Once Very Long Chain Fatty acids are reduced in length within the Peroxisomes

- They may shift to Mitochondrial beta oxidation for further catabolism of fatty acids.

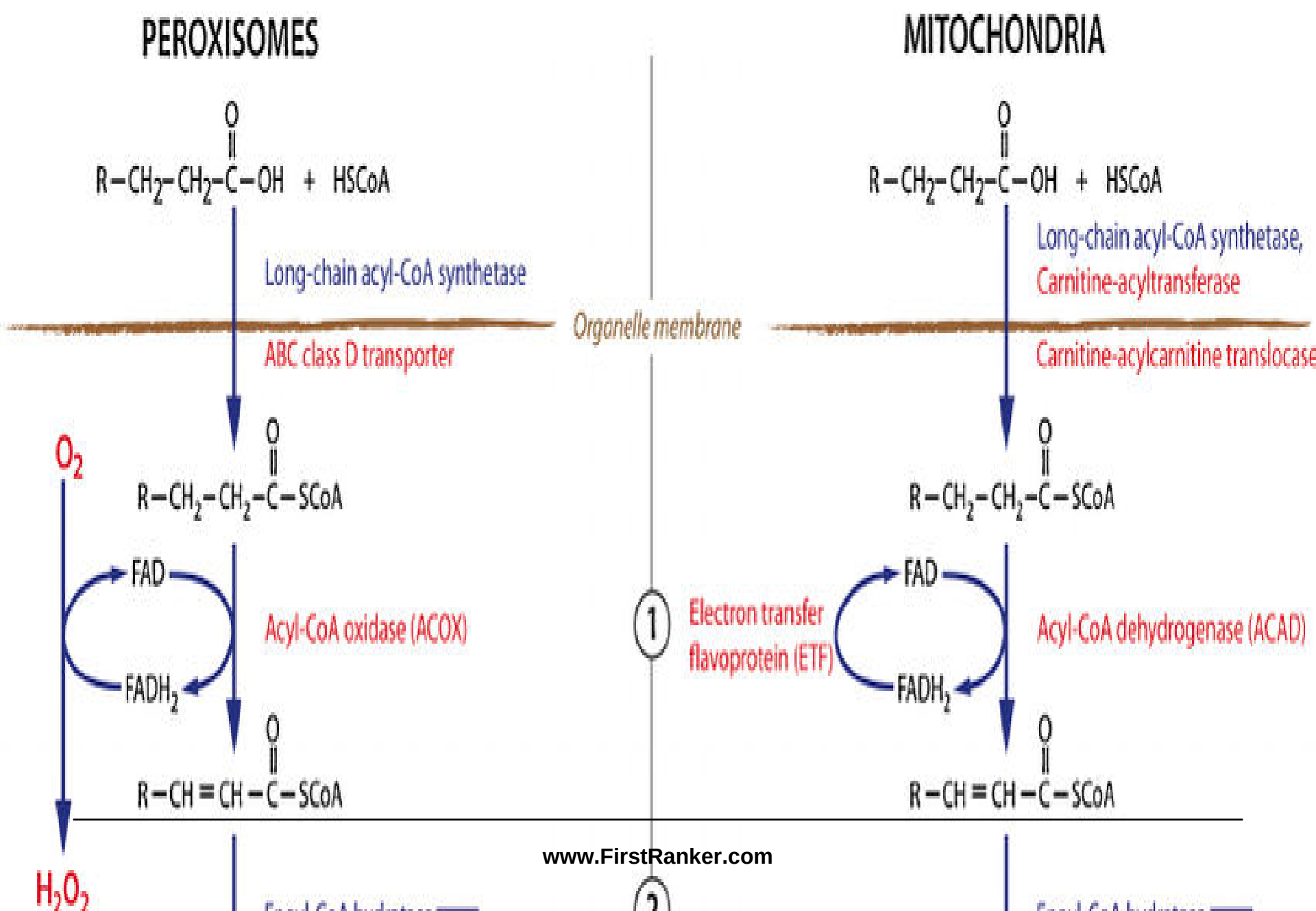
- No ATPs result from steps of Peroxisomal oxidation of VLCFAs.

- Steps of Peroxisomal Oxidation of Fatty acid **does not generate ATPs**

- Instead **energy dissipated** in **form of heat.**

- Many **drugs** commercially available in market for **reducing obesity**
- **Stimulate Peroxisomal beta oxidation**
- Where **Fatty acids are oxidized without much liberation of calories (ATPs).**

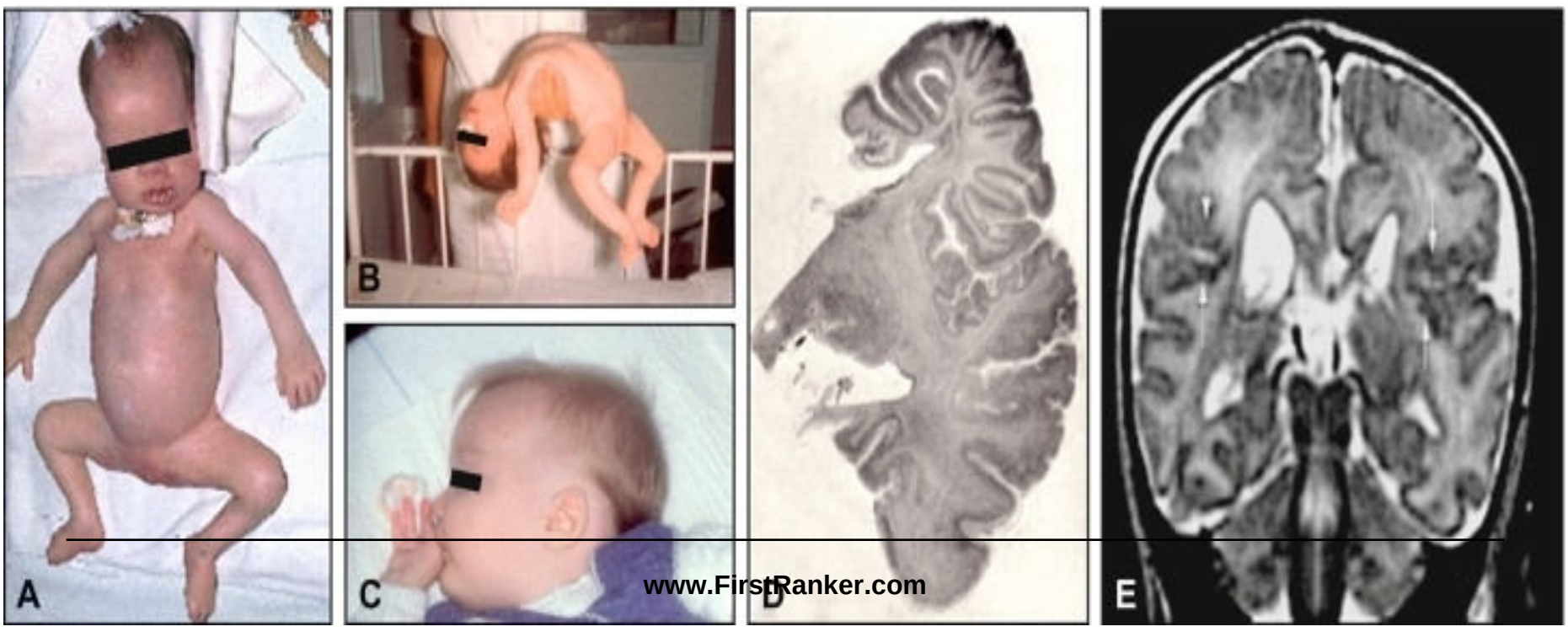
- Peroxisomal Oxidation of Fatty acid efficiently takes place in:
  - –Obese persons
  - –Persons taking Hypolipidemic drugs(Clofibrate).



Peroxisomal β-Oxidation

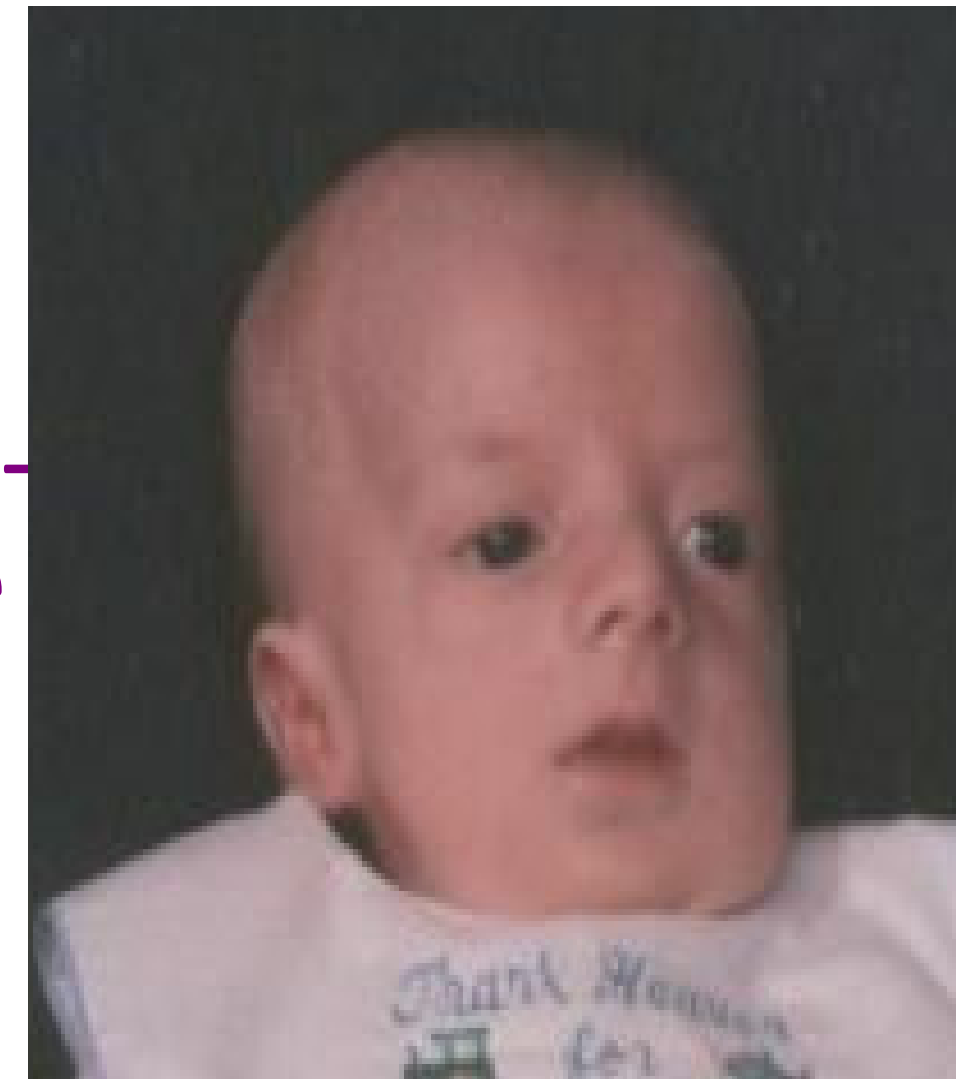
- A second important difference between mitochondrial and peroxisomal β oxidation in mammals is in the **specificity for fatty acyl–CoAs**;
- The peroxisomal system is much more active on **very-long chain fatty acids such as hexacosanoic acid (26:0) and on branched-chain fatty acids such as phytanic acid and pristanic acid**
- The **inability of cells/patients** to make peroxisomes leads to neurological disorder called as **Zellweger syndrome**; and therefore lack all the metabolism unique to that organelle

Zellwegers Syndrome
OR
Cerebrohepatorenal Syndrome



Peroxisomal Disorder

- Zellweger Syndrome
- Cerebro-Hepato-Renal Syndrome



Biochemical Causes

- **Rare genetic autosomal recessive disorder.**
- Characterized by **absence of functional Peroxisomes.**

- Gene mutations in **PEX Genes** leads to Zellwegers Syndrome.

Biochemical Alterations

- **No oxidation** of **very long chain Fatty acids** and **branched chain fatty acids** in **Peroxisomes**

- **Accumulation** of large abnormal amounts of **VLCFAs** in Peroxisomes of tissues.

- **No normal function of Peroxisomes**.

- Progressive degeneration of **Brain/Liver/Kidney,** with **death ~6 month after onset.**

Signs and Symptoms

- **Defect** in normal function of **multiple organ system.**
- **Impaired** neuronal migration, positioning and **brain development.**
- Hypomyelination **affecting nerve impulse transmission.**
- **Hepatomegaly**
- **Renal Cysts**
- Typical **Dysmorphic facies.**

Diagnosis

- **Detection of Increased levels of Serum Very Long Chain Fatty Acids-VLCFAs**

ZELLWEGER SYNDROME

- The **abnormally high levels of VLCFA(Very long chain fatty acids),** are most diagnostic.
- There is no cure for Zellweger syndrome, nor is there a standard course of treatment.
- Most treatments are **symptomatic and supportive.**
- Most infants do not survive past the first 6 months, and usually succumb to **respiratory distress, gastrointestinal bleeding, or liver failure.**

Oxidation Of Unsaturated Fatty Acids

- **PUFAs** having **double bonds** in their structure **are unstable**.
- **Double bonds** are hydrolyzed and **metabolized faster** than **saturated bonds**.
- Thus dietary **intake of PUFA get readily metabolized**
- **Which reduces risk of ~~Atherosclerosis.~~**

- PUFAs are less reduced than SFAs

- Hence PUFAs are less energetic than SFAs

Mechanism Of Oxidation Of Unsaturated Fatty Acids

- **Initial and later Oxidation of PUFAs** is by

- **Similar steps of β Oxidation** in parts, of **saturated bonds**.

- **Double bonds of UFAs are cleaved by action of Extra Enzymes:**
  - **Isomerase (Enoyl CoA Isomerase)**

    (For odd numbered double bonds MUFAs)
  - **Reductase (2,4 Dienoyl CoA Reductase)**

    (For even numbered double bonds PUFAS)
  - **Epimerase**

    (Converts D-Isomer to L-Isomer)

- **Enoyl CoA Isomerase** handles **odd numbered double bonds** in MUFAs.
- **2,4 Dienoyl CoA Reductase** handles **even numbered double bonds** in PUFAs.

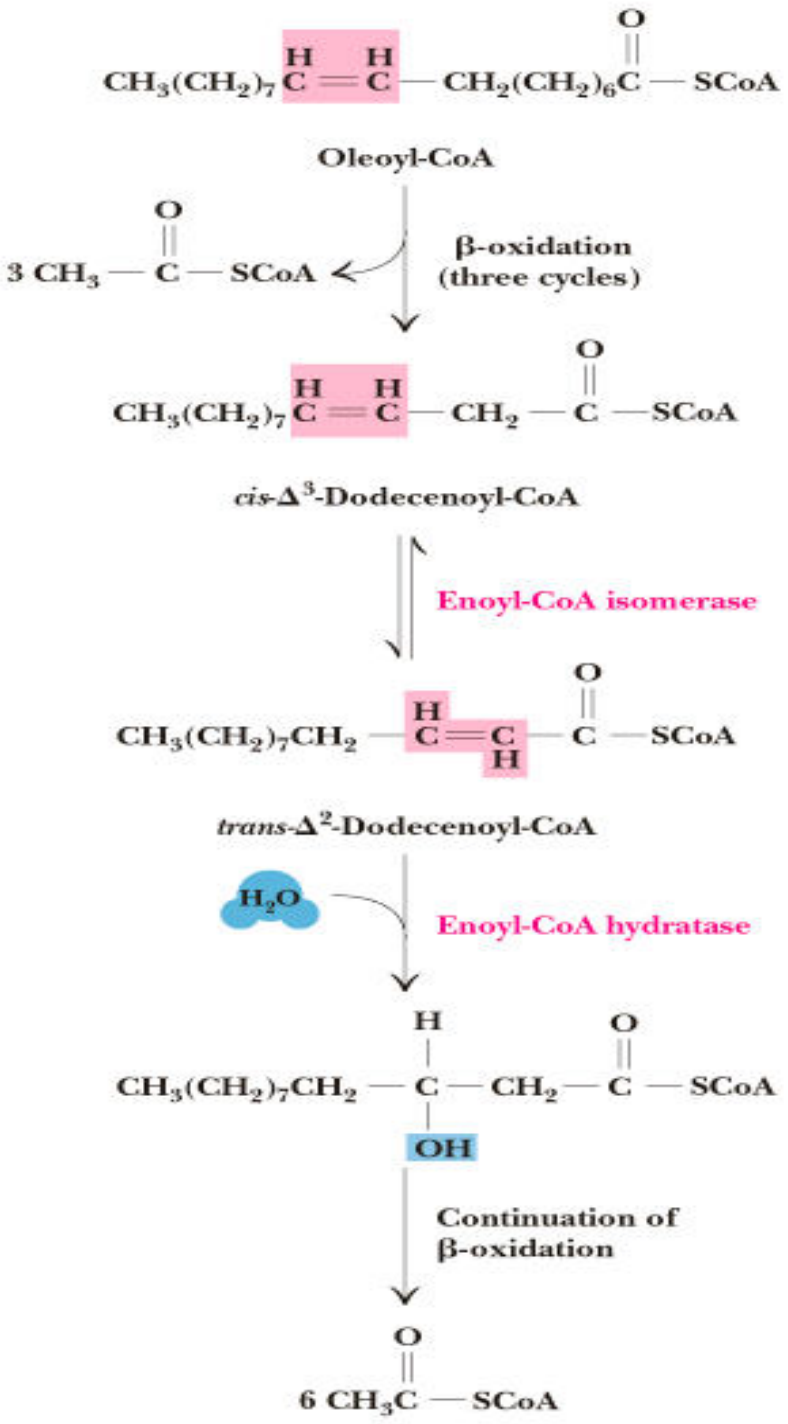
- Usually natural unsaturated fatty acids have **cis double bonds.**
- Which is **transformed to *trans* *double bonds*** by the action of an **Isomerase .**
- As next enzyme to act is **Enoyl Hydratase ,which acts only on *trans* double bonds.**

- **Enoyl-CoA Isomerase** converts Cis unsaturated Fatty acids to **Trans- Δ^2 Enoyl-CoA**

- Now β-oxidation can continue with hydration of trans-Δ^2-Enoyl-CoA by Enoyl CoA Hydratase

Oxidation Of Monounsaturated Fatty Acids

- Oleic acid, Palmitoleic acid
- Normal β-oxidation for three cycles
- Cis-Δ^3 Acyl-CoA **cannot be utilized by Acyl-CoA dehydrogenase**
- **Enoyl-CoA Isomerase** converts this to trans- Δ^2 Acyl CoA
- β-oxidation continues from this point



Oxidation Of Polyunsaturated Fatty Acids

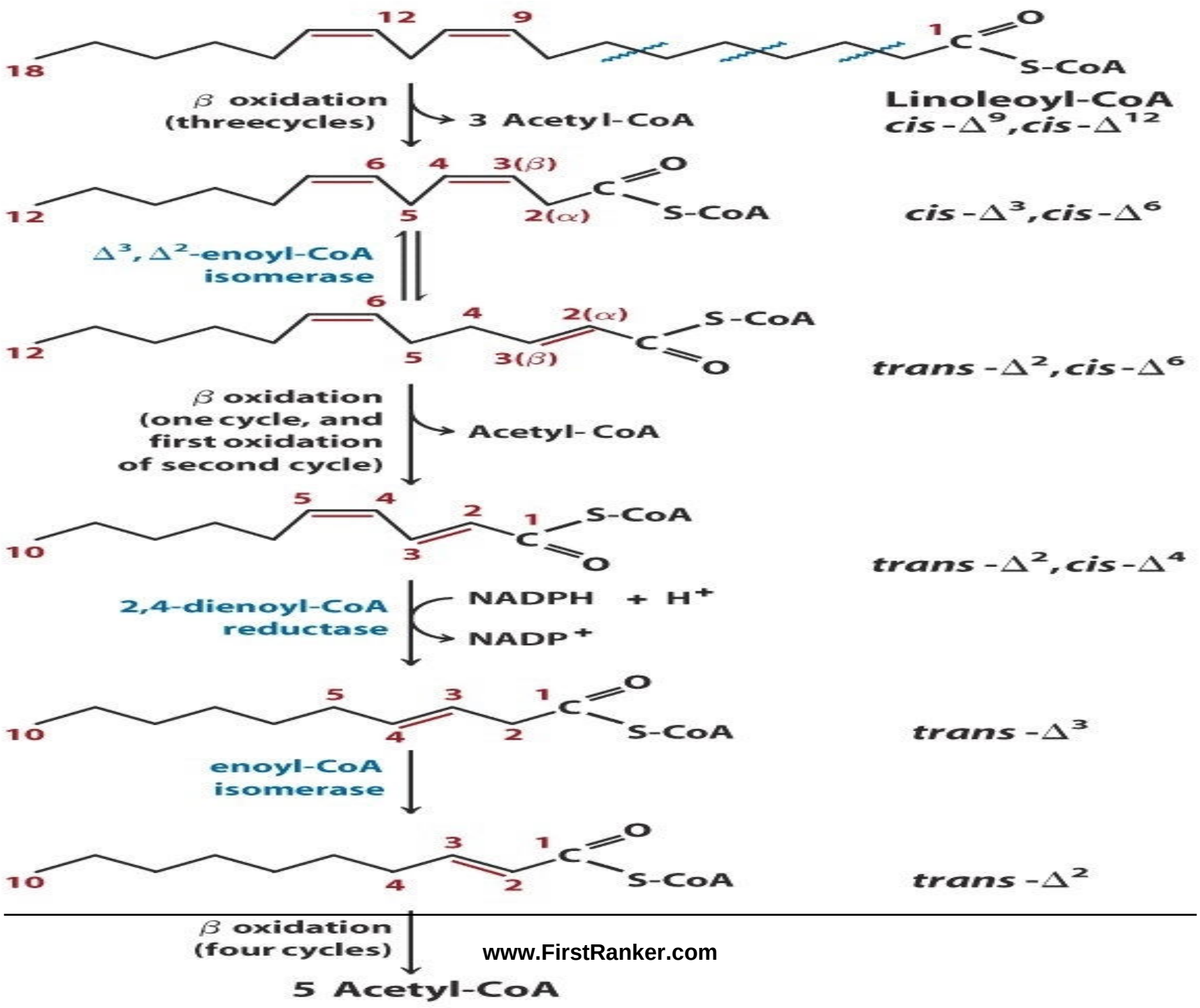
Slightly more complicated

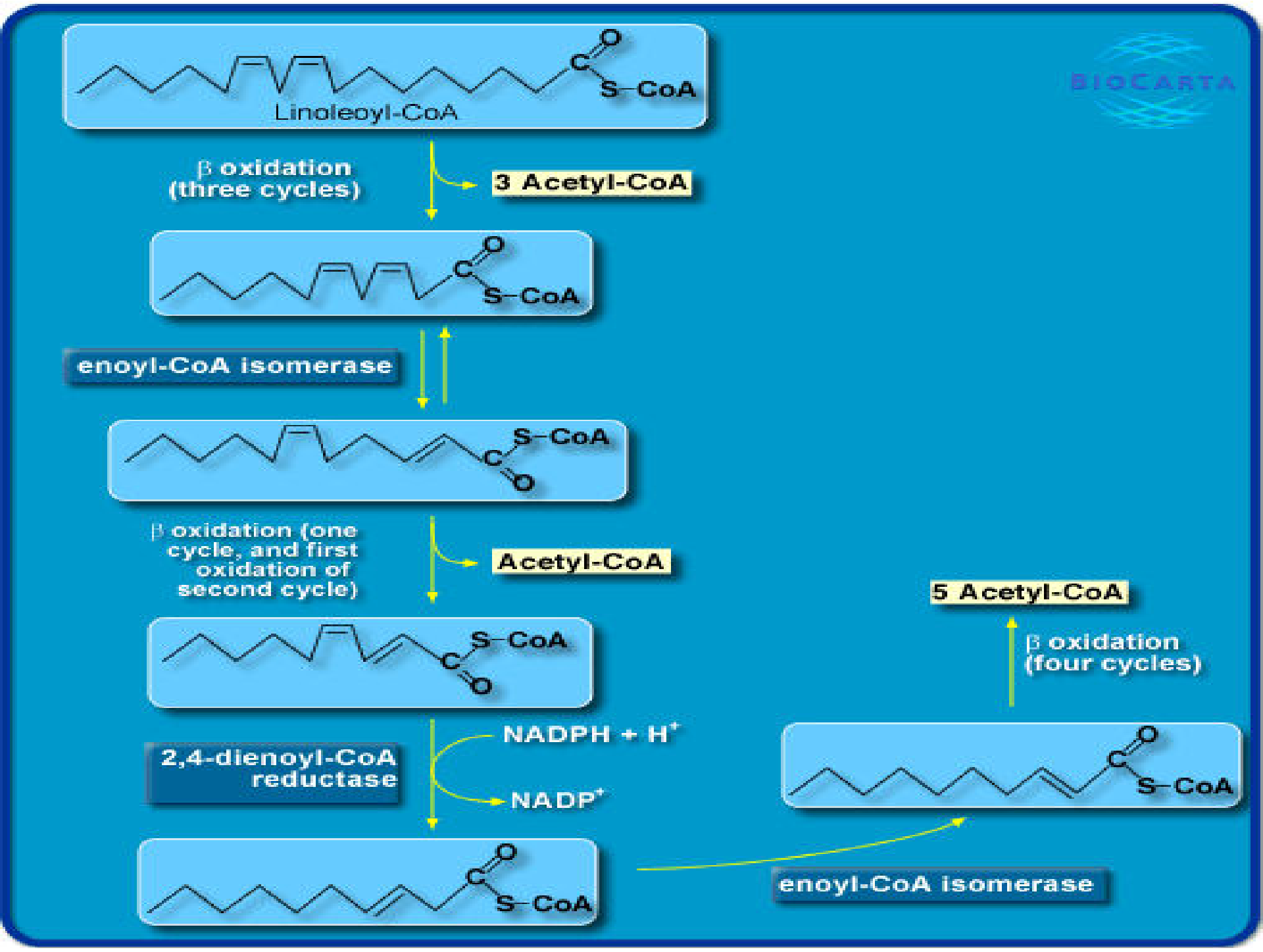
- Same as for Oleic acid, but only up to a point:
  - 3 cycles of β-oxidation
  - Enoyl-CoA Isomerase
  - 1 more round of β-oxidation
  - ~~Trans- Δ^2, cis- Δ^4 structure is a problem.~~

- 2,4-Dienoyl-CoA **Reductase** transform it to odd numbered.

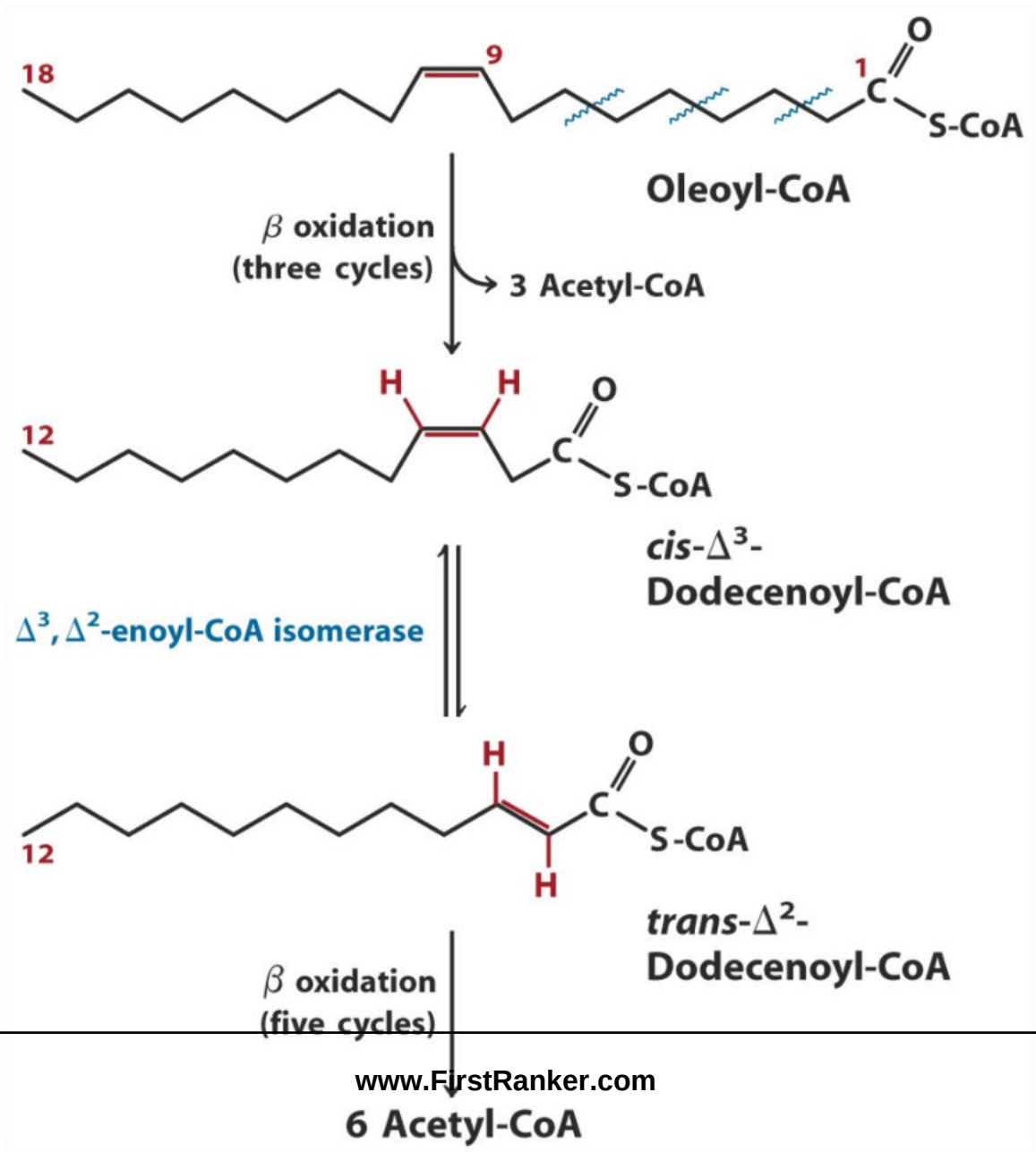
- **NADPH dependent 2,4-Dienoyl- Co A Reductase reduces and merges two double bonds** to form **one Trans at C3**

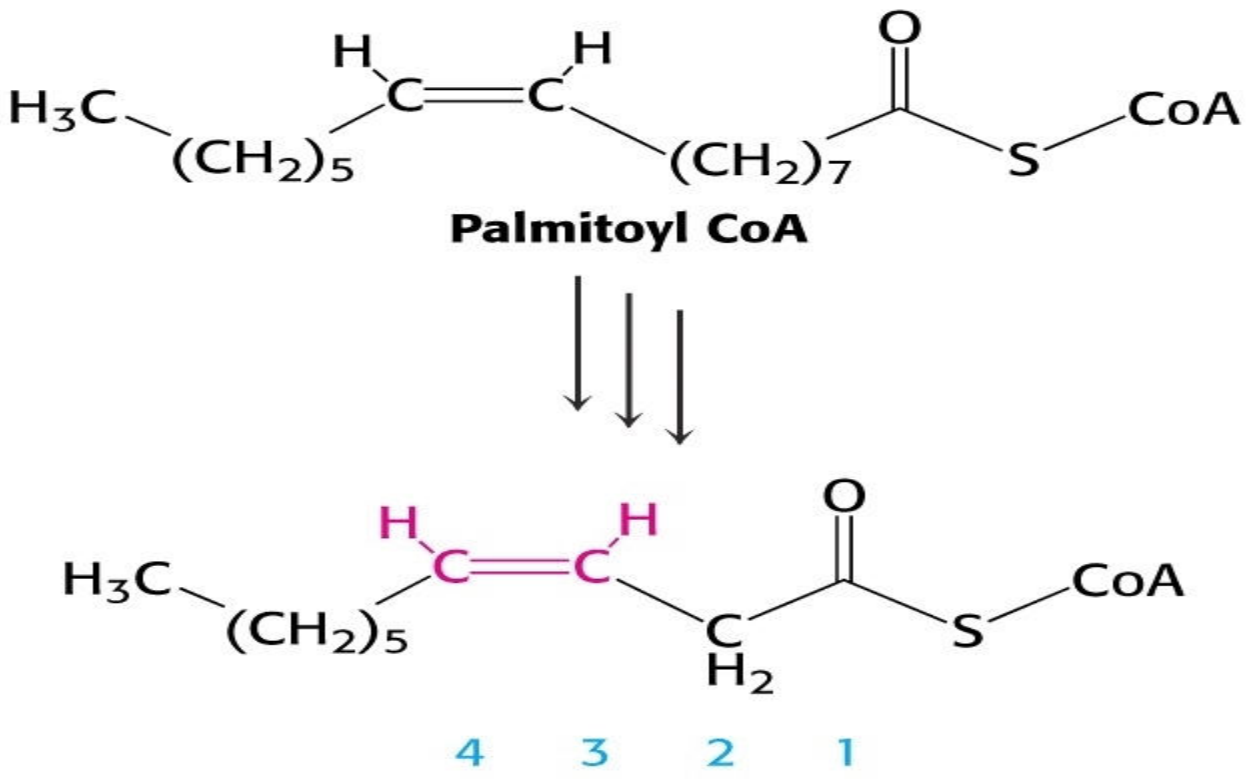
- That is then isomerized by Enoyl CoA Isomerase to C2- Trans double bond.



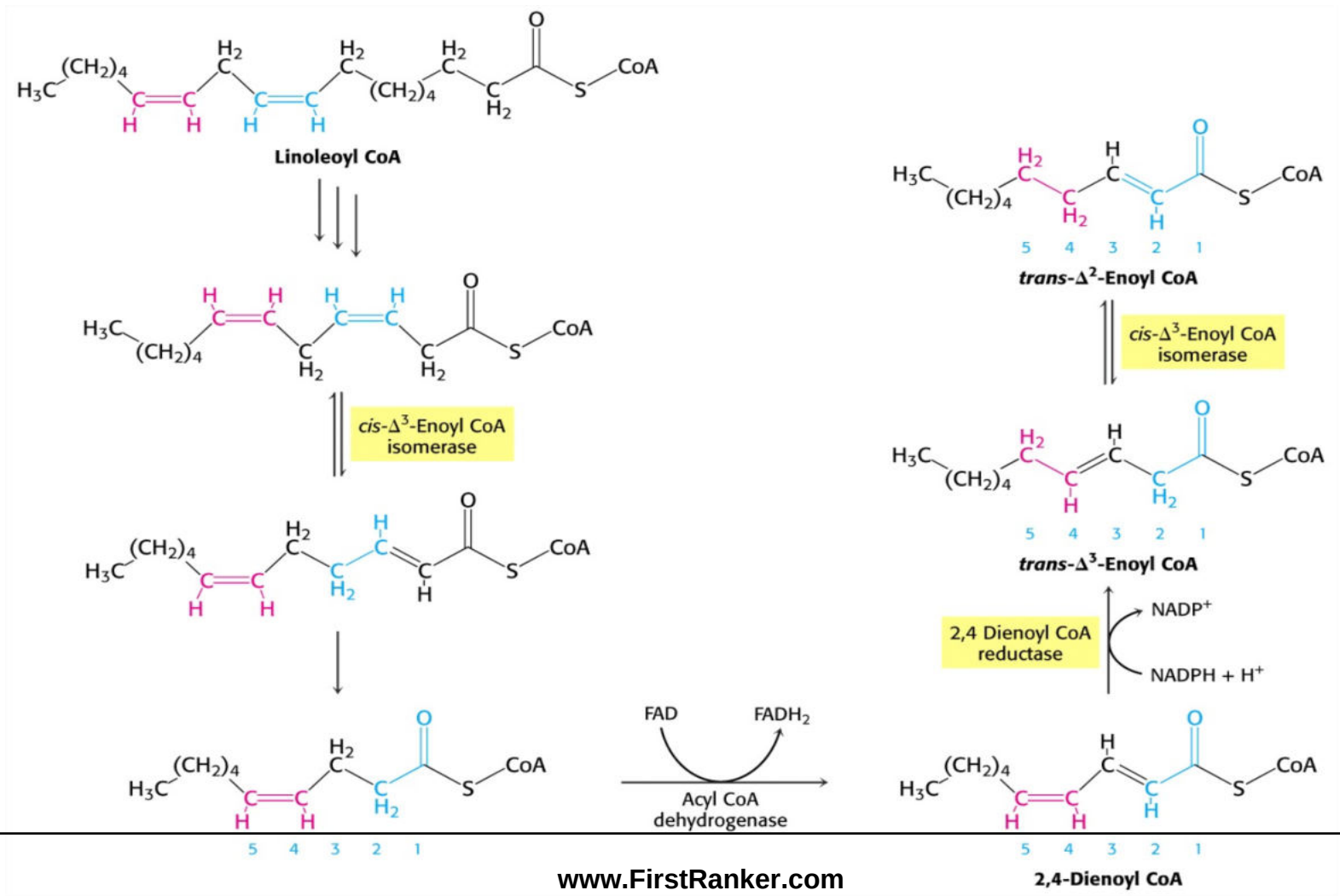


Oxidation of Unsaturated Fatty Acids (Remember they are cis!)





β-oxidation of fatty acids with even numbered double bonds



- The Oxidation of PUFAs **provide less energy** than saturated Fatty acids as they are **less reduced compounds**.
- At double bonds the Isomerase act and convert it into **Trans –Enoyl CoA.**
- This **bypasses the Acyl-CoA Dehydrogenase –FAD linked beta oxidation reaction**.
- 1.5 ATP less per double bond.