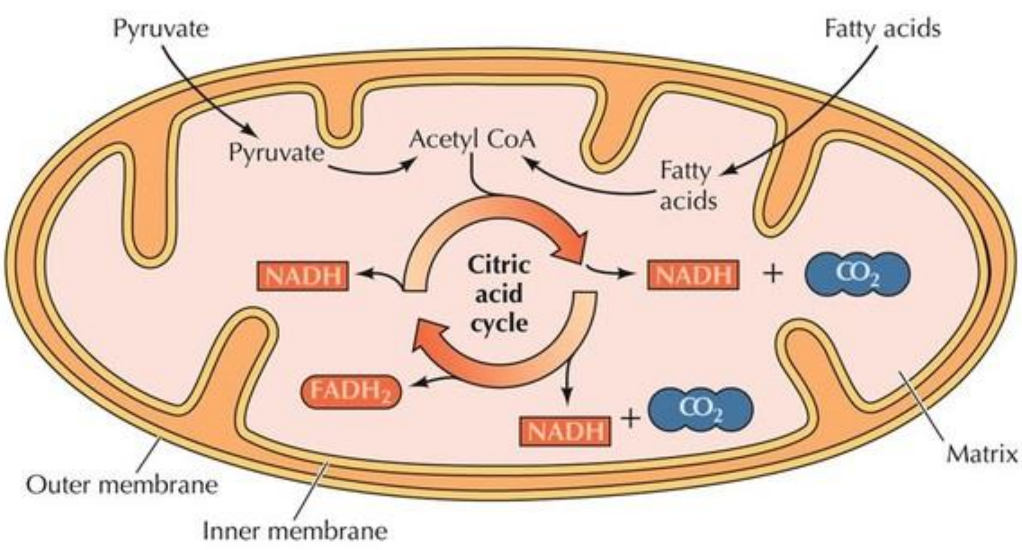
Citric Acid Cycle



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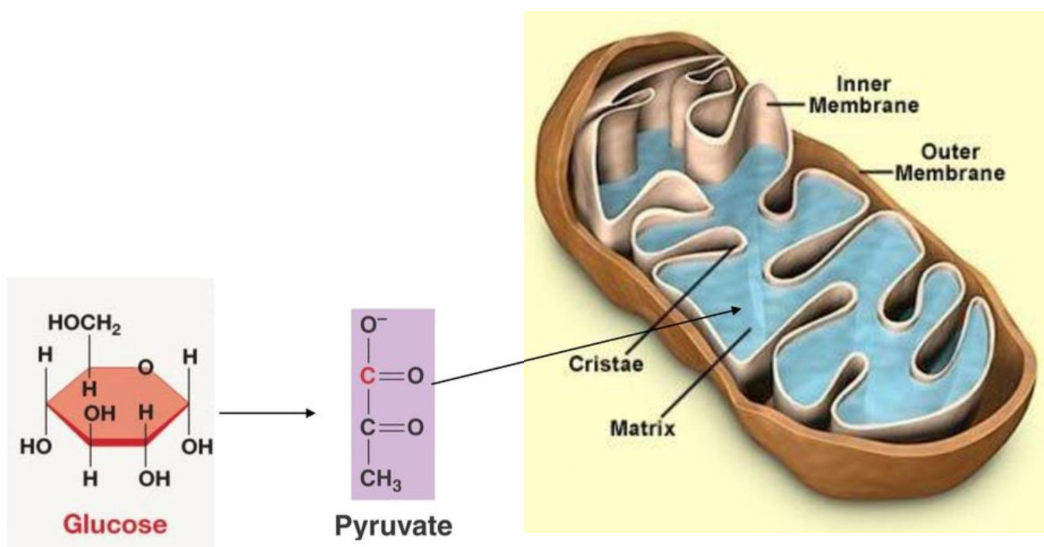
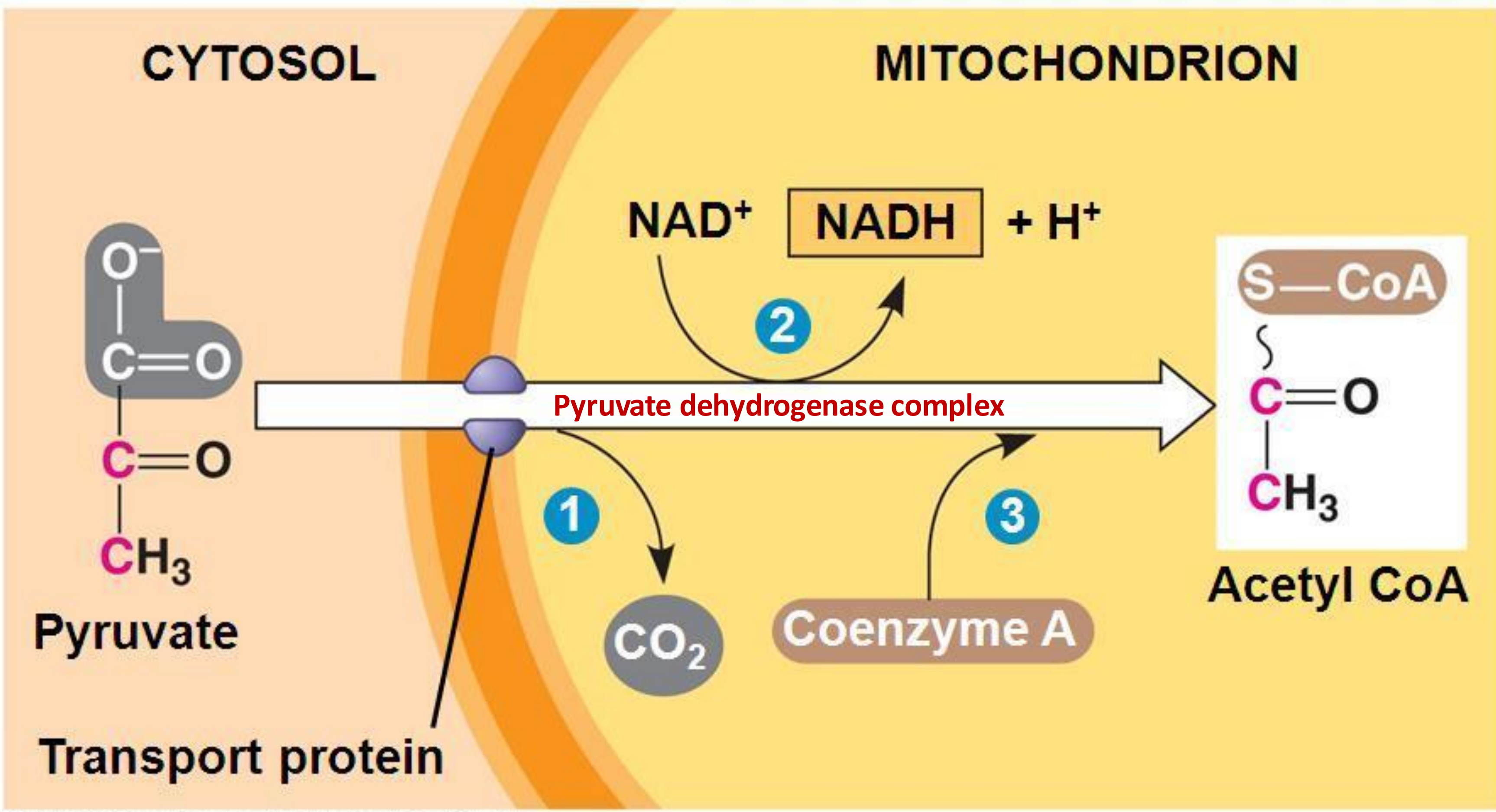
Acetyl CoA Formation

- In aerobic respiration, pyruvate (3C) joins the citric acid cycle after its conversion to acetyl CoA (2C)
- Citric acid cycle occurs in the mitochondrial matrix. Shuttling of pyruvate from the cytosol is facilitated by a transporter protein embedded in the inner mitochondrial membrane called pyruvate translocase



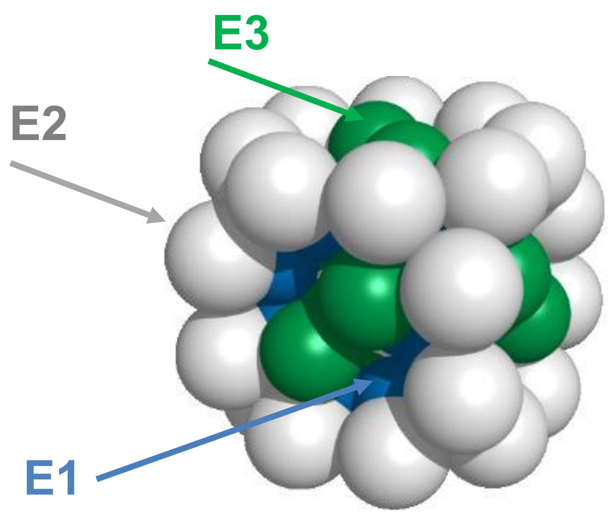
Acetyl CoA Formation



Acetyl CoA Formation

- Pyruvate dehydrogenase complex (PDC) catalyzes the **irreversible** oxidative decarboxylation of pyruvate into Acetyl CoA with the release of CO_2
- Energy-rich molecule "NADH" is also produced from reduction of NAD^+ **(the oxidizing agent in this reaction)**
- Coenzyme A (CoA) acts as acetyl group carrier due to its free sulfhydryl (–SH) end capable of forming **thioester bond**
- PDC is a multi-enzyme system consists of three catalytic enzymes and five coenzymes (three of them are prosthetic groups as they are tightly bound to their corresponding enzymes)

Pyruvate Dehydrogenase Complex

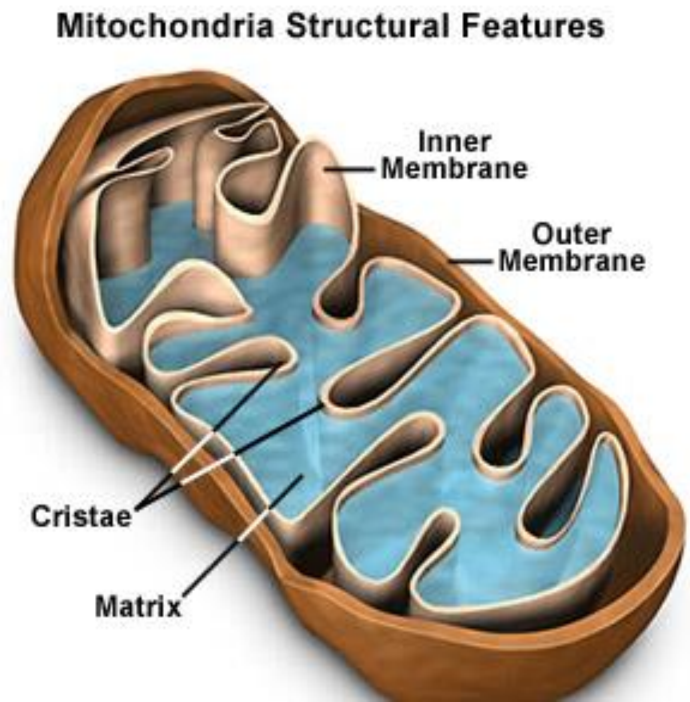


Coenzymes

- Thiamine pyrophosphate (TPP) a prosthetic group of **E1**
- Lipoic acid (lipoamide) a prosthetic group of **E2**
- Flavin adenine dinucleotide (FAD) a prosthetic group of **E3**
- Coenzyme A (CoA or CoA-SH)
- Nicotinamide adenine dinucleotide (NAD^{+})

Citric Acid Cycle

- Citric acid, Tricarboxylic acid cycle (TCA) or Krebs cycle is a central pathway used by all aerobic organisms to generate energy through the oxidation of acetate (in the form of acetyl CoA) into CO_2 and ATP. Also it releases the energy-rich molecules: NADH and FADH2
- It occurs in mitochondrial matrix except reaction 6 in which succinate dehydrogenase (complex II in ETC) enzyme is found in inner mitochondrial membrane (it is the only transmembrane protein in Krebs cycle).

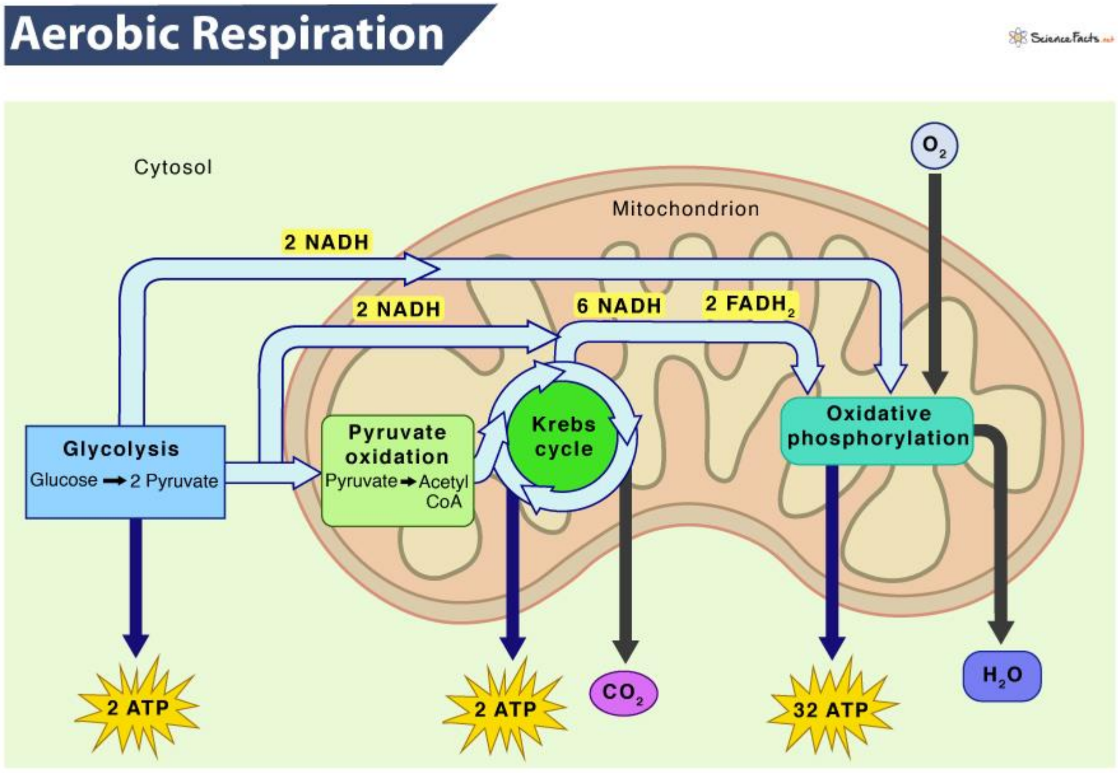


Citric Acid Cycle

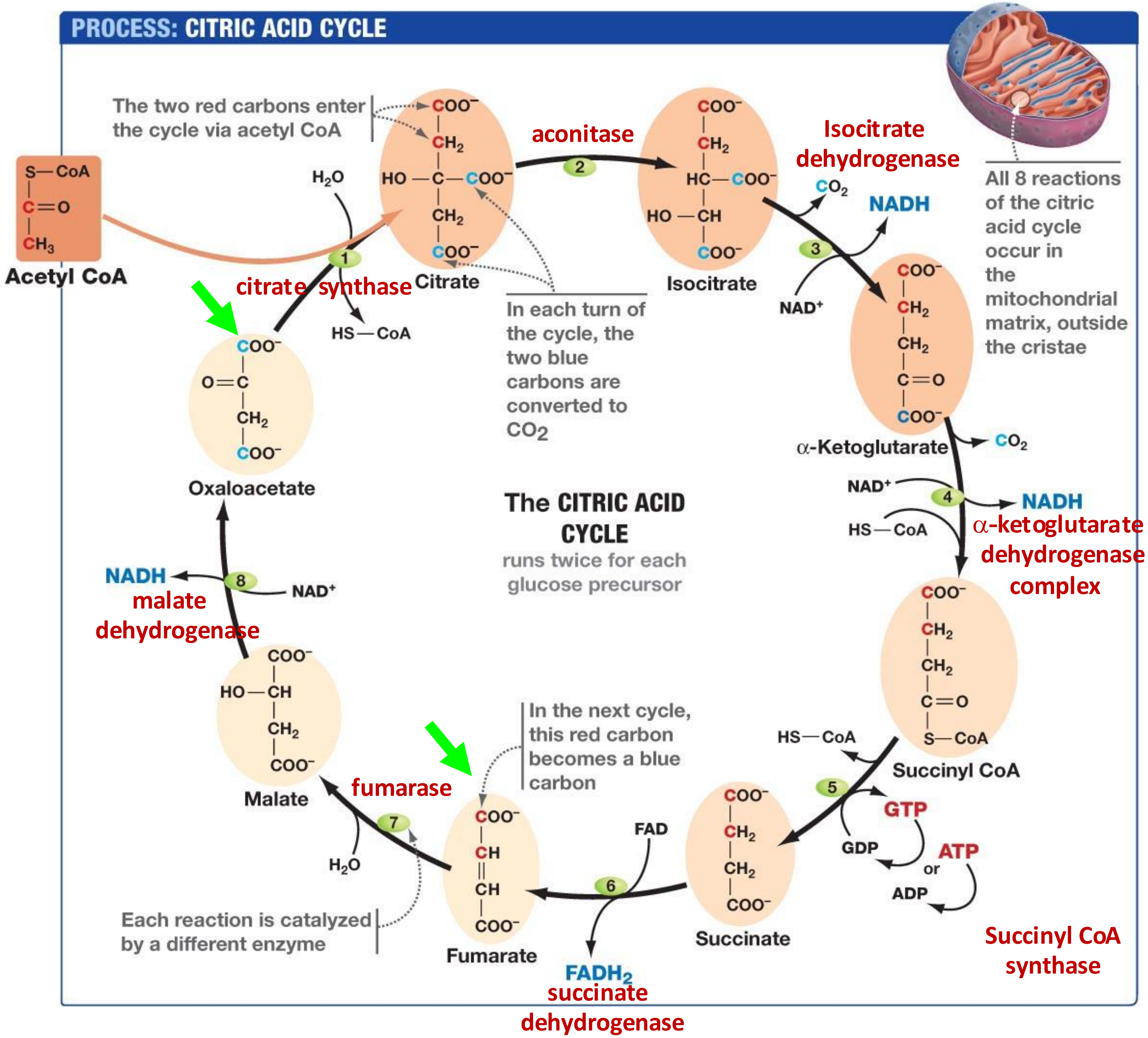
- To proceed in Krebs cycle we need the presence of:

  1. Mitochondria

  2. O_2



Citric Acid Cycle

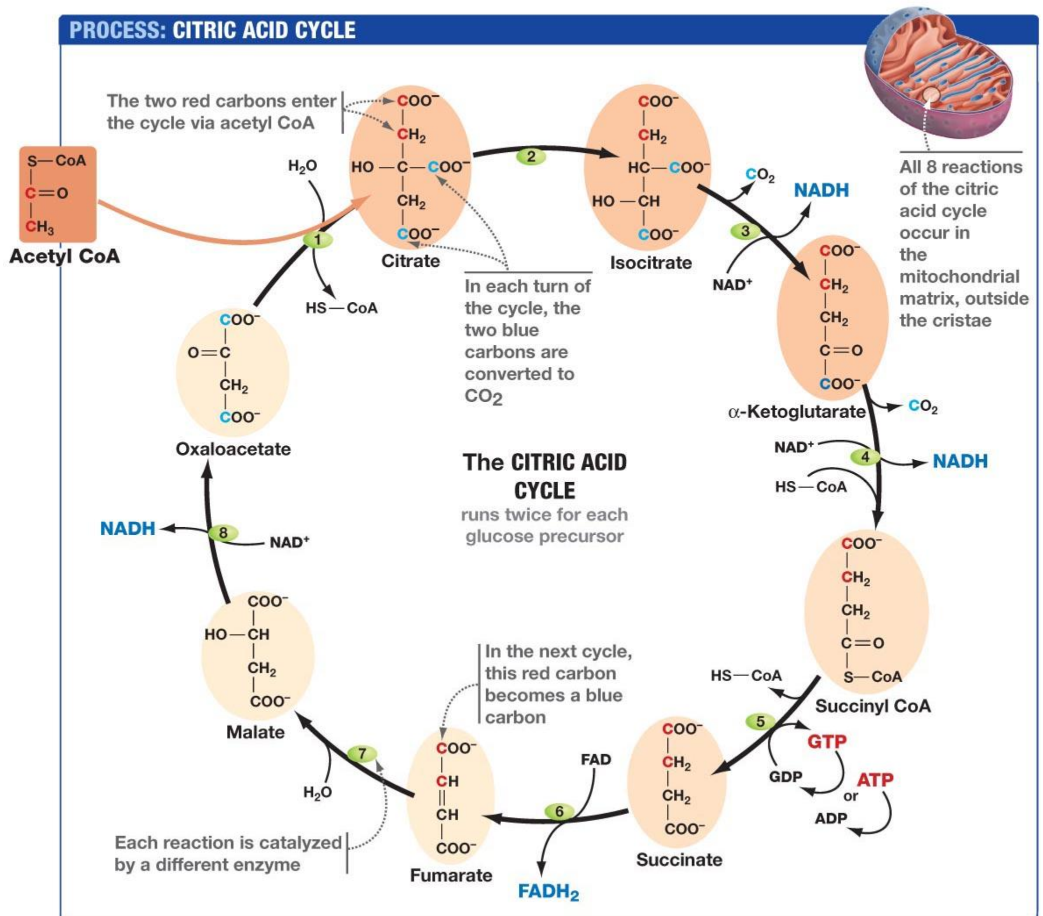


Citric Acid Cycle

- Two phases in Krebs cycle:

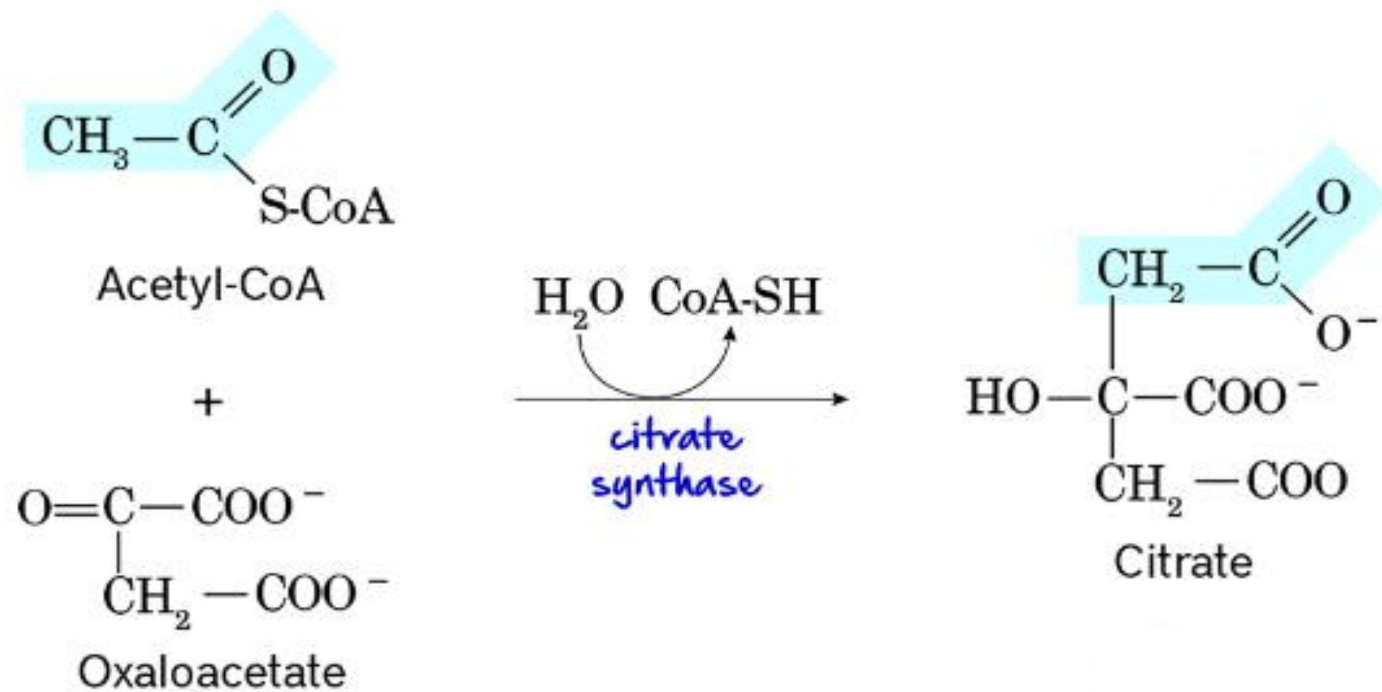
1. Phase I : the release of 2 CO_2 molecules (mostly irreversible steps: 1,3 & 4 so act as regulatory steps)

2. Phase II : regeneration of oxaloacetate (reversible steps)



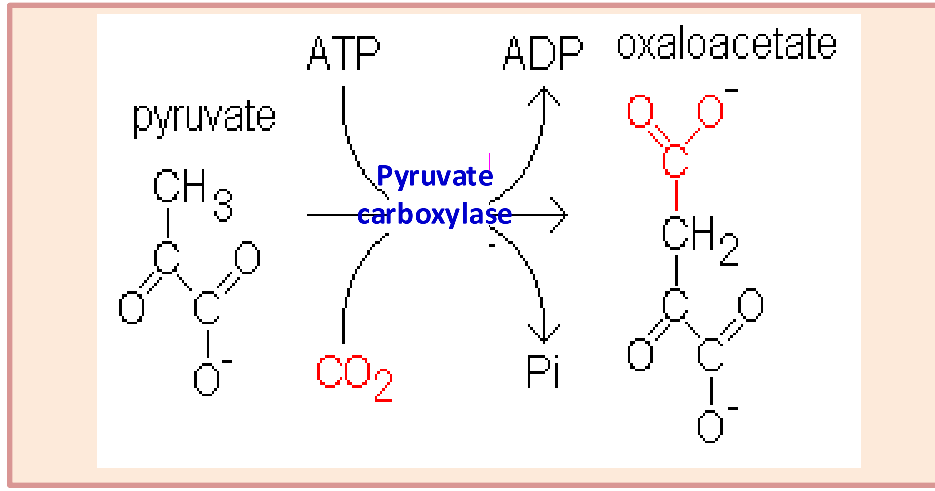
Citric Acid Cycle

- Krebs cycle is a series of 8 reactions run twice / glucose molecule:
- **Step 1:** The irreversible condensation of acetyl CoA (2C) and oxaloacetate (4C) via citrate synthase to form citrate (6C)



Citric Acid Cycle

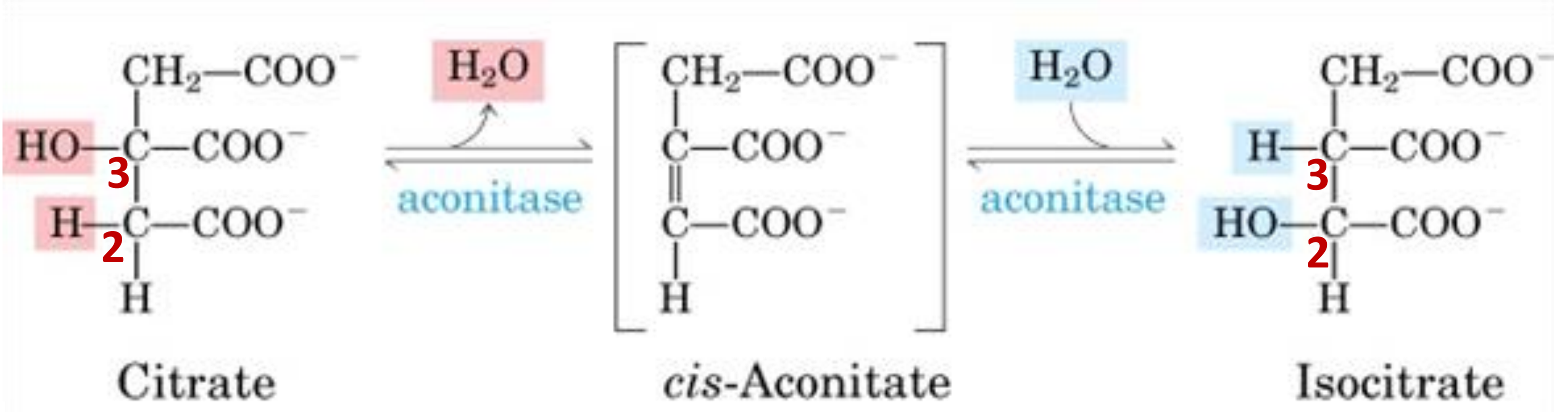
- Oxaloacetate is already found in matrix. It can be produced in several ways in nature. For example, it is generated from an ATP-dependent carboxylation of pyruvate catalyzed by pyruvate carboxylase. This reaction occurs in the matrix



- **Step 2:** Aconitase enzyme catalyzes the reversible isomerization of citrate (tertiary alcohol) to isocitrate (secondary alcohol). Both are structural isomers differ in the position of OH group from C3 to C2)

Citric Acid Cycle

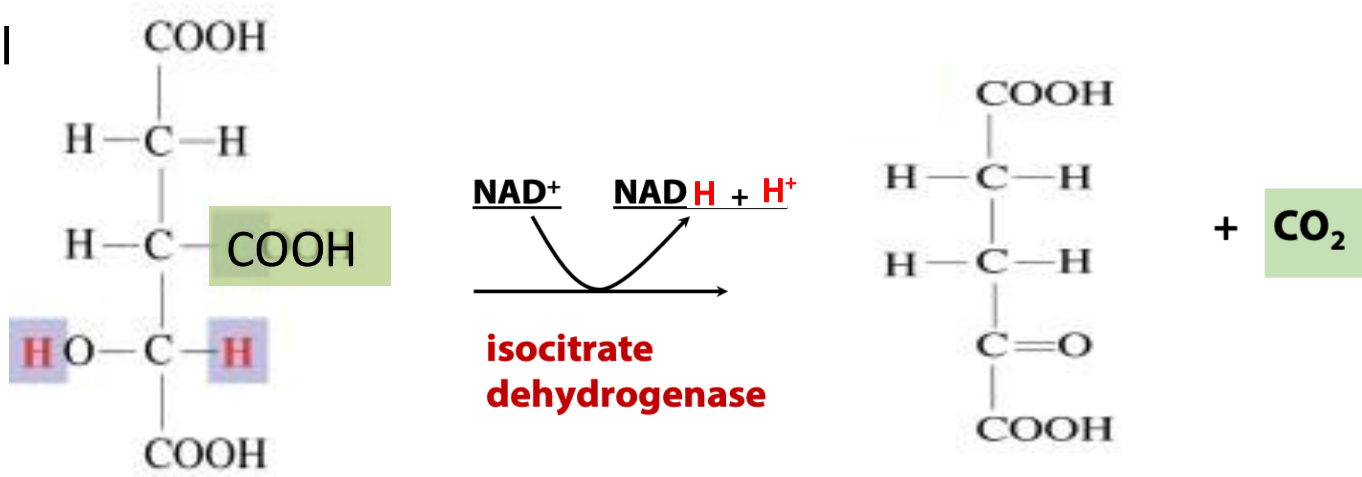
- This isomerization reaction is pre-required step to prepare substrates for decarboxylation reaction



- It involves successive dehydration and hydration reactions

Citric Acid Cycle

- **Step 3:** Isocitrate dehydrogenase catalyzes the first oxidative decarboxylation of isocitrate (6C) to α-ketoglutarate (5C) irreversibly resulting in the release of first CO_2 and the formation of first NADH molecule
- It involves successive oxidation and decarboxylation reactio

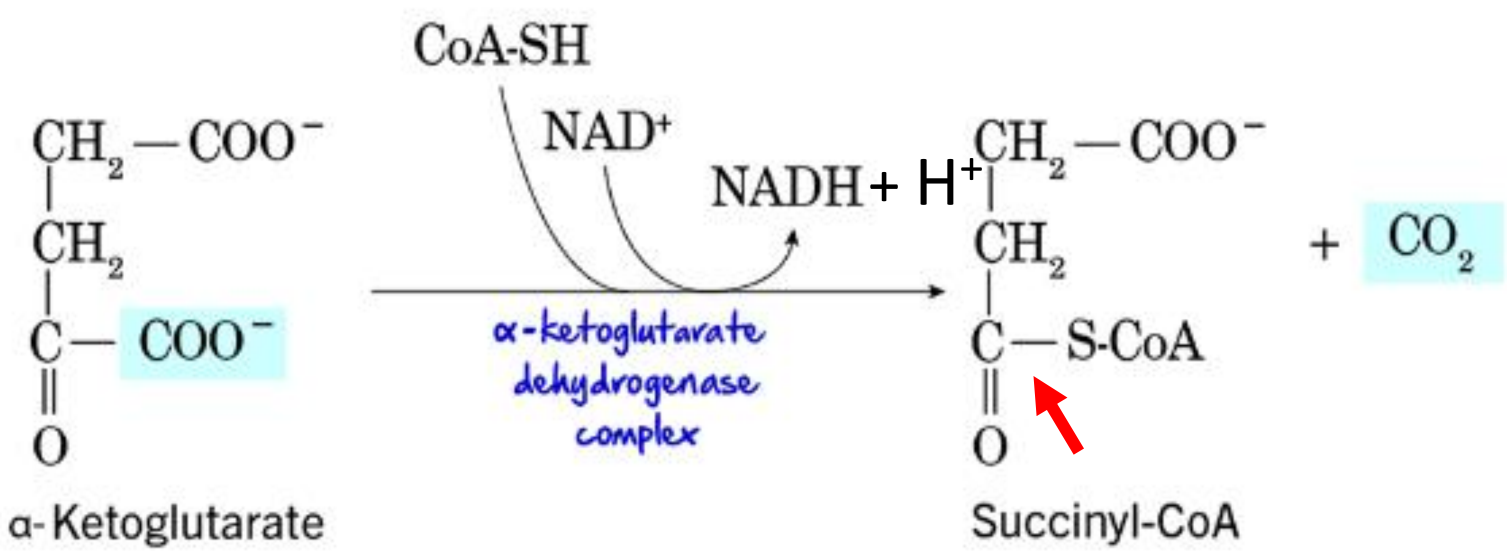


isocitrate

α-ketoglutarate

Citric Acid Cycle

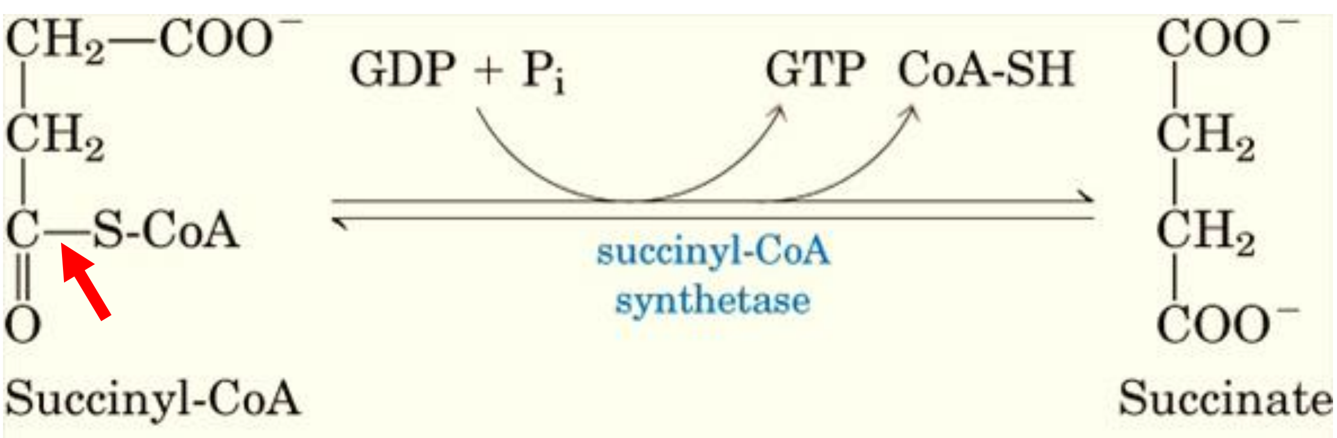
- **Step 4:** α-ketoglutarate dehydrogenase complex catalyzes the irreversible oxidative decarboxylation of α-ketoglutarate (5C) to succinyl CoA (4C) releasing the second CO_2 and producing the second NADH molecule. Succinyl-CoA is super high energy molecule storing the energy in thioester



Citric Acid Cycle

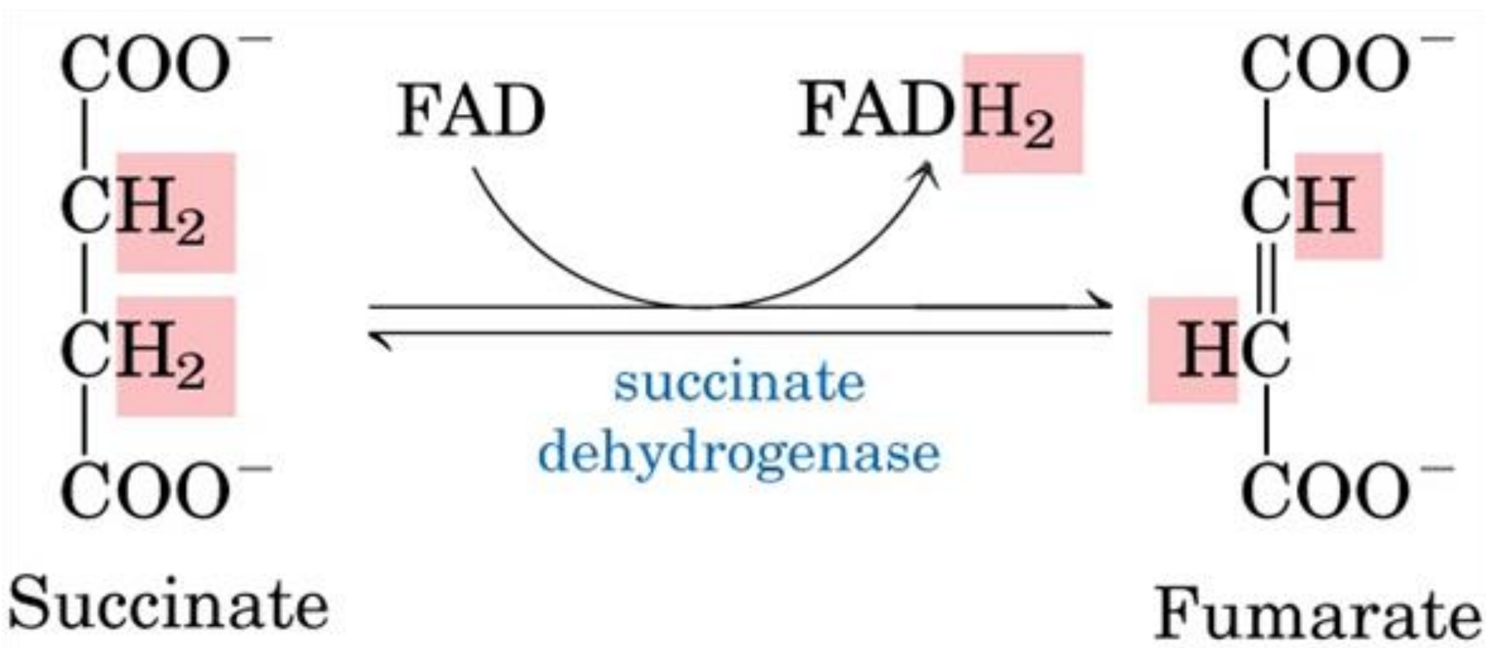
- **Step 5:** Succinyl CoA synthetase generates the first energy substrate which is ATP (in brain & heart tissues) or GTP (in liver tissues). The thioester bond of succinyl-CoA is energy-rich and can drive the phosphorylation of ADP or GDP

(substrate-level phosphorylation)



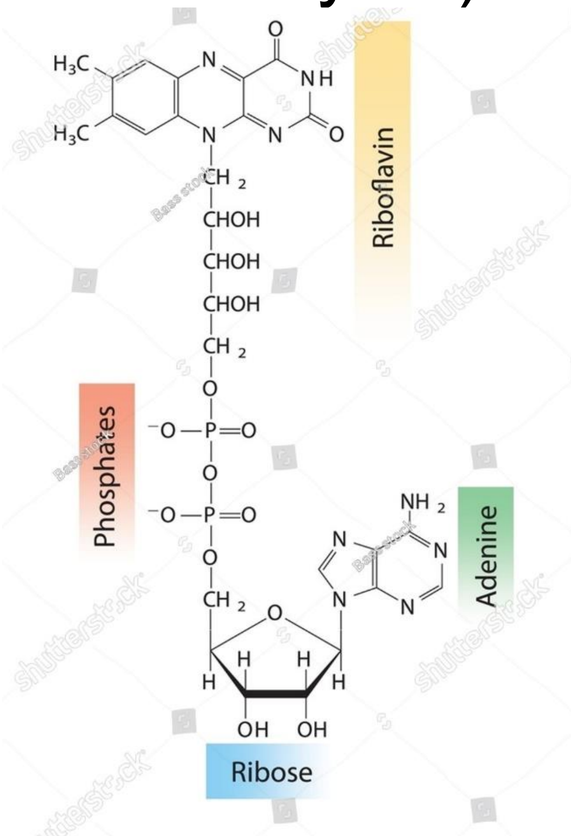
Citric Acid Cycle

- **Step 6**: Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate and consequently, the reduction of prosthetic group FAD into $FADH_2$



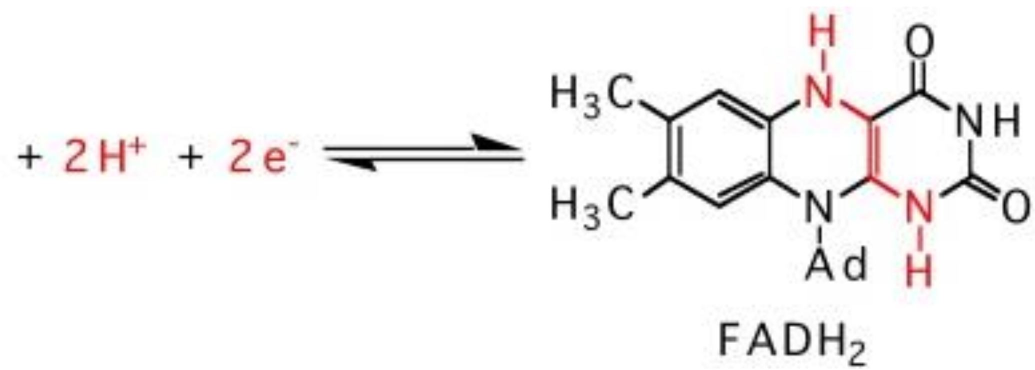
Citric Acid Cycle

FAD: Flavin adenine dinucleotide is prosthetic group (coenzyme which is tightly or covalently bound to its enzyme). It is derived from riboflavin vitamin B2



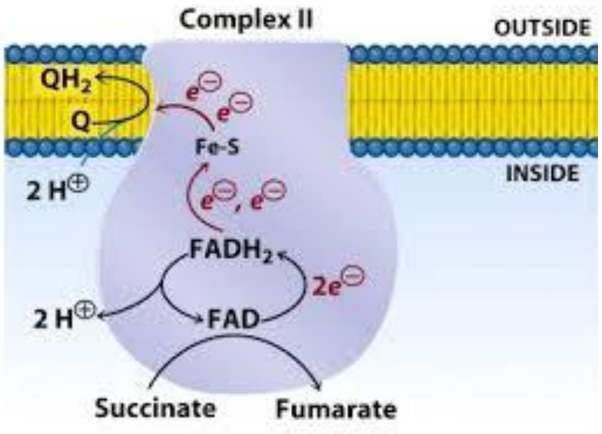
Flavin adenine dinucleotide (FAD)

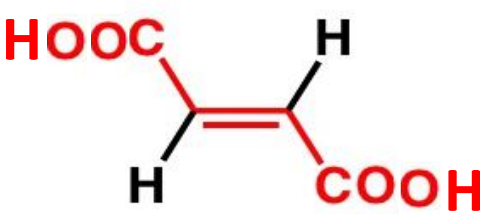
unlike NAD^+, FAD has a binding for two H atoms



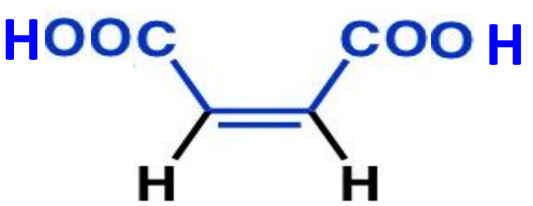
Citric Acid Cycle

- Succinate dehydrogenase is the only enzyme found in the inner membrane of mitochondria
- FAD is more powerful oxidizing agent than NAD^+ (oxidation of C-C is more difficult than C-O)
- It is stereoselective enzyme and only the trans isomer "fumarate" is formed but not the cis isomer "maleate"





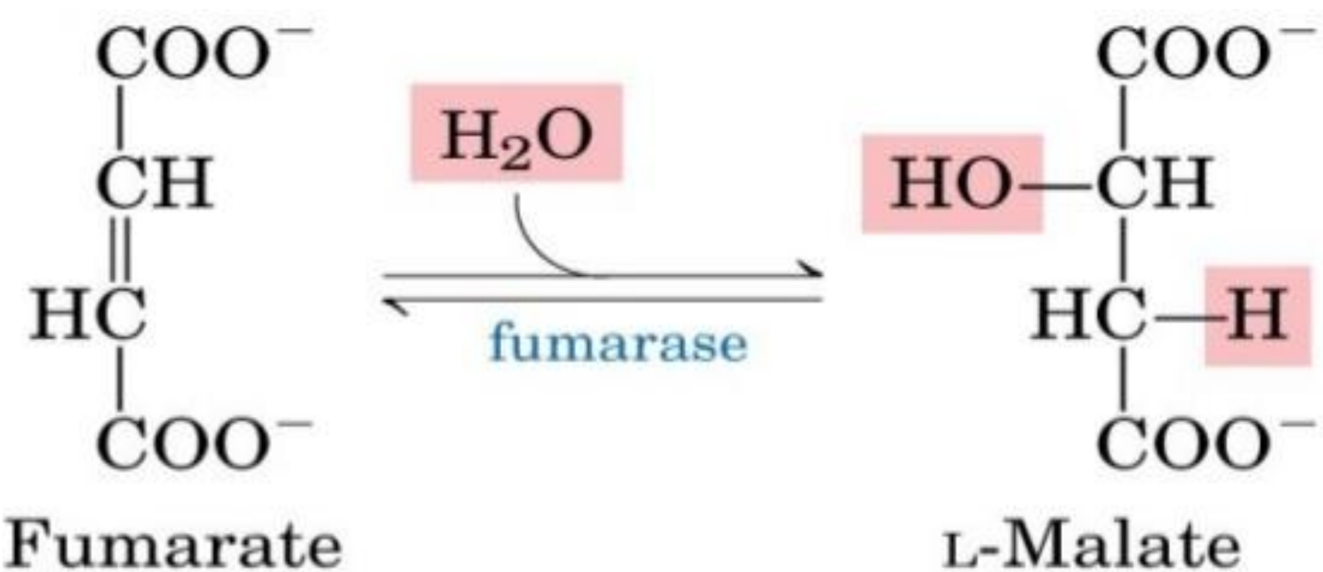
trans fumarate



cis fumarate(maleate)

Citric Acid Cycle

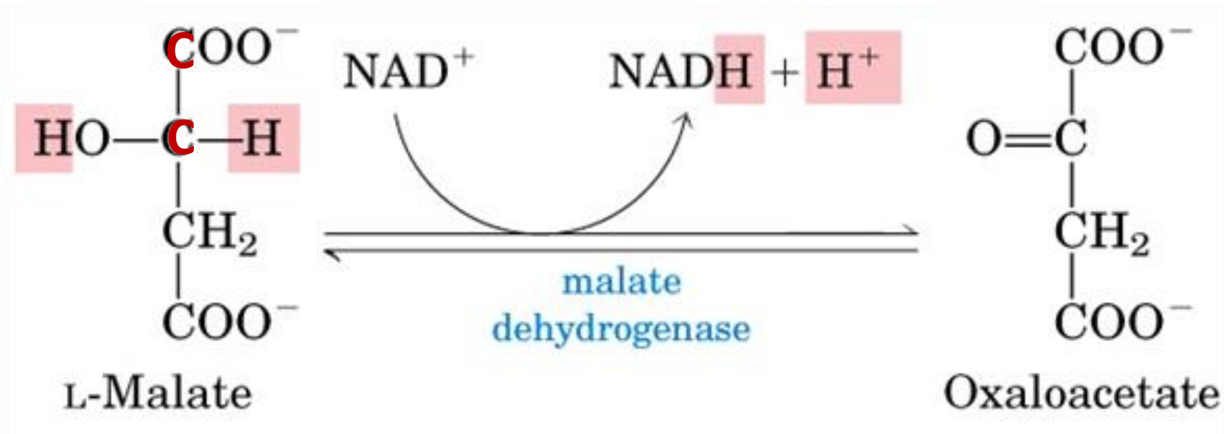
- **Step 7**: Fumarate is converted to L-malate in a hydration reaction catalyzed by fumarase (reversible reaction)



- Fumarase is another stereospecific enzyme

Citric Acid Cycle

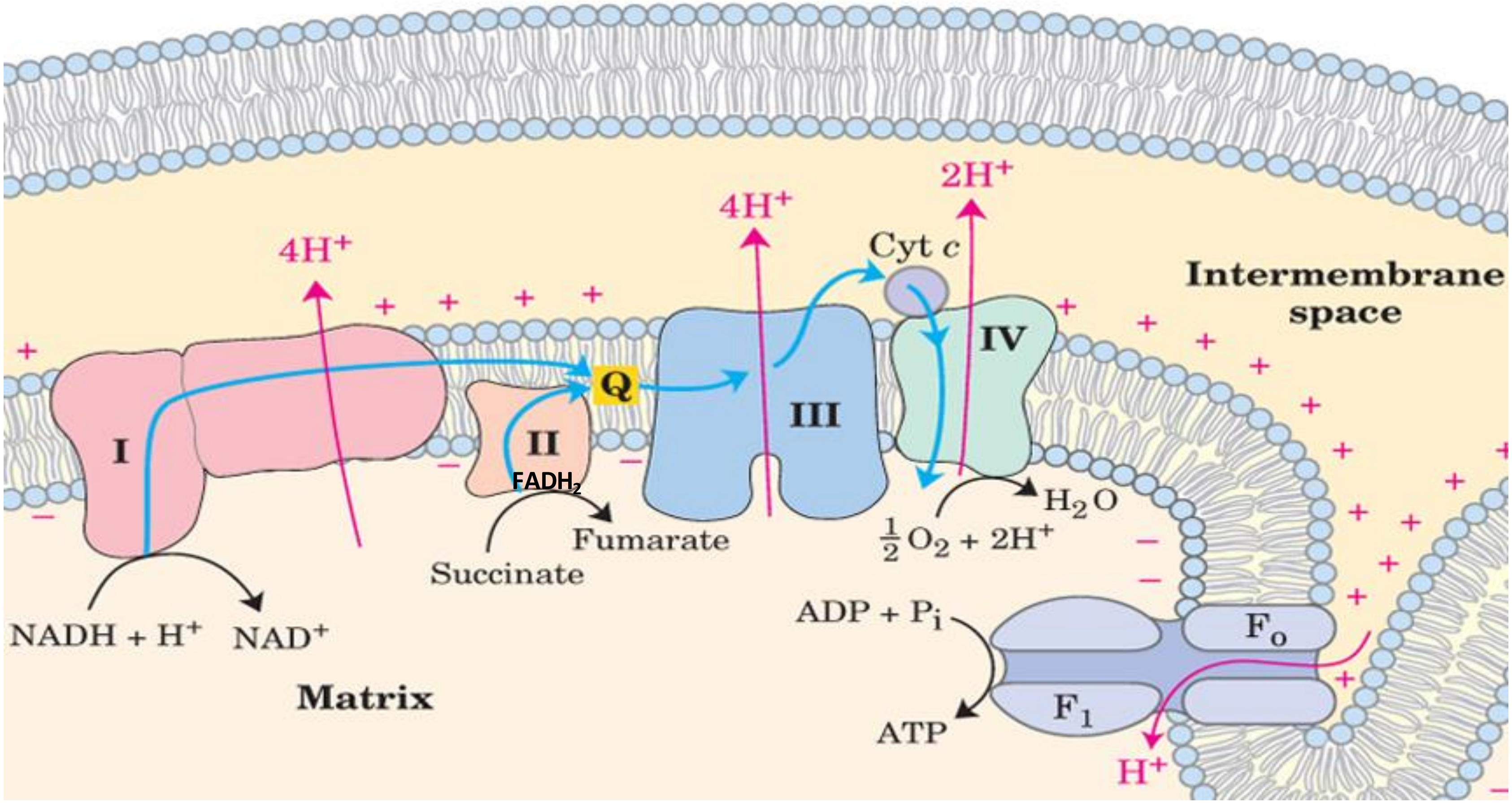
- **Step 8**: L-malate is oxidized to regenerate oxaloacetate via malate dehydrogenase enzyme thus generating the third NADH (reversible)



- At the end of krebs cycle, the products of oxidation of one glucose via glycolysis and TCA are:

  4 ATP + **6** CO_2 + **10** NADH + **2** FADH2

Oxidative Phosphorylation
Electron Transport Chain



- TCA is considered as a part of aerobic metabolism although it does not use O_2 in any of its reaction ??

Cytosolic NADH Shuttling

- Oxygen molecule acts as the final destination (acceptor) of electrons extracted from the oxidation of glucose. At the end water molecules will be generated
- The equation of complete oxidation of one glucose molecule:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 30/32\ ATP$$

ATP Yield per one Glucose

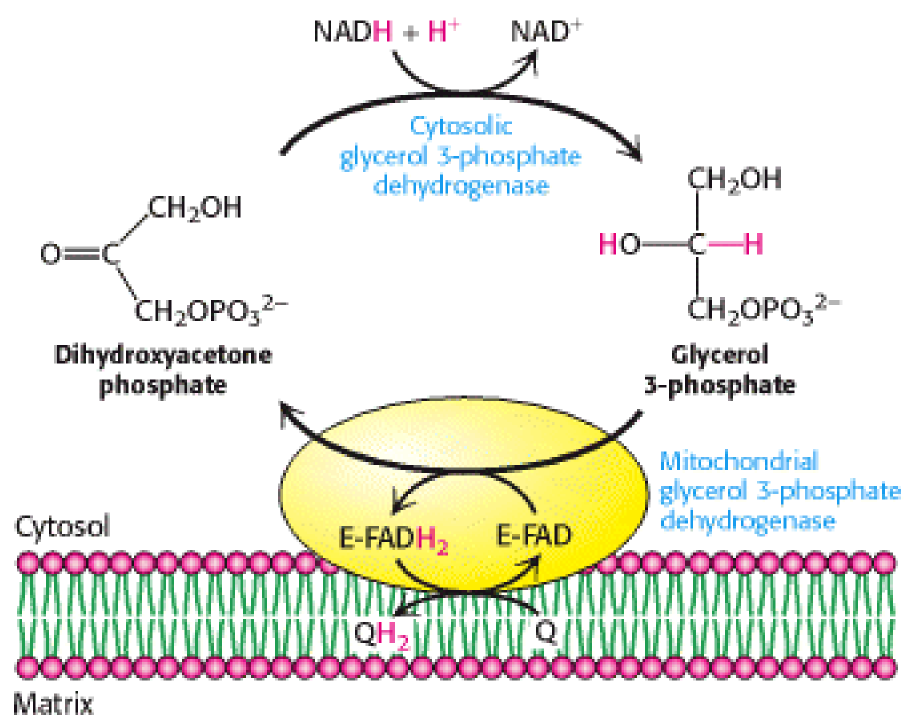
Stage	ATP produced by substrate-level phosphorylation
Glycolysis	2 ATP
Acetyl CoA production	none
Krebs Cycle	2 ATP
Total/glucose 4 ATP molecules	

Stage	Electron-carrier molecule	Total H^+ pumped	ATP synthase $4H^+ \rightarrow 1$ ATP
Glycolysis	2 NADH	12-20	3-5 ATP
Acetyl CoA production	2 NADH	20	5 ATP
Krebs Cycle	6 NADH 2 $FADH_2$	60 12	15 ATP 3 ATP
Total/glucose 26-28 ATP produced by oxidative phosphorylation			

Cytosolic NADH Shuttling

- The electrons carried by cytosolic NADH (i.e. NADH generated by glycolysis) will be shuttled to the matrix by one of two mechanisms:

1. DHAP/G3P shuttle: it is active in brain and skeletal muscle. This pathway delivers the 2e from cytosolic NADH to mitochondrial FAD



NADH Shuttling

2. Aspartate/malate shuttle: it is active in liver and heart. This pathway delivers the 2e from cytosolic NADH to mitochondrial NAD^+ (found in the matrix)