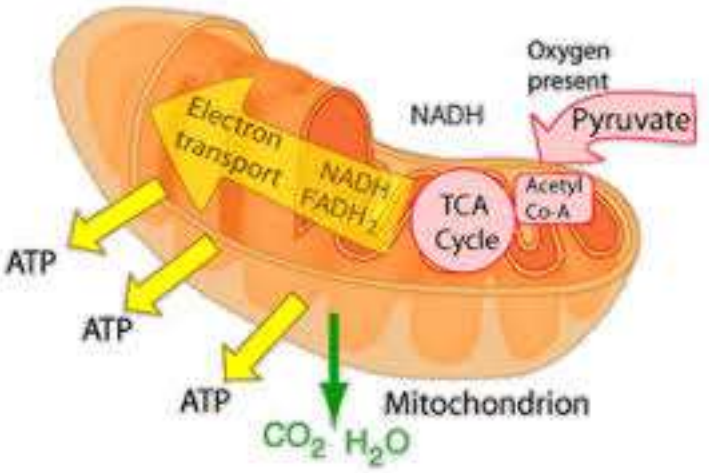
Citric Acid Cycle



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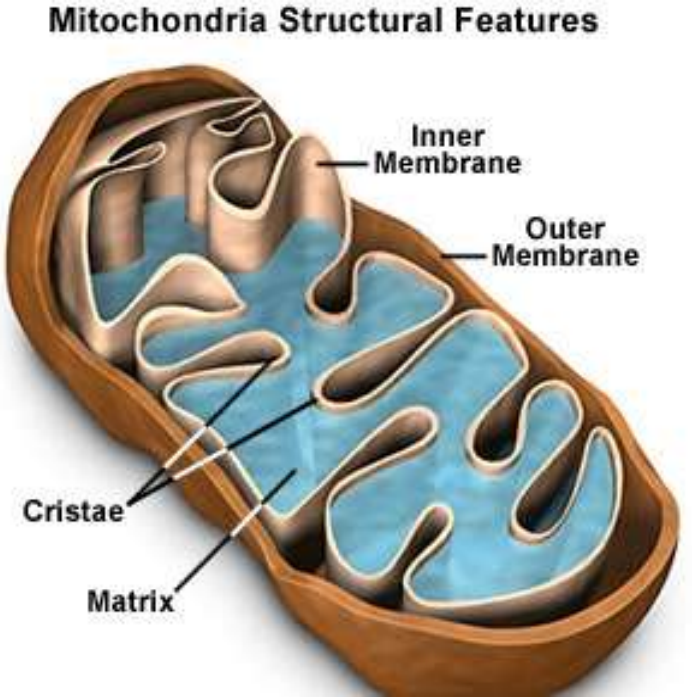
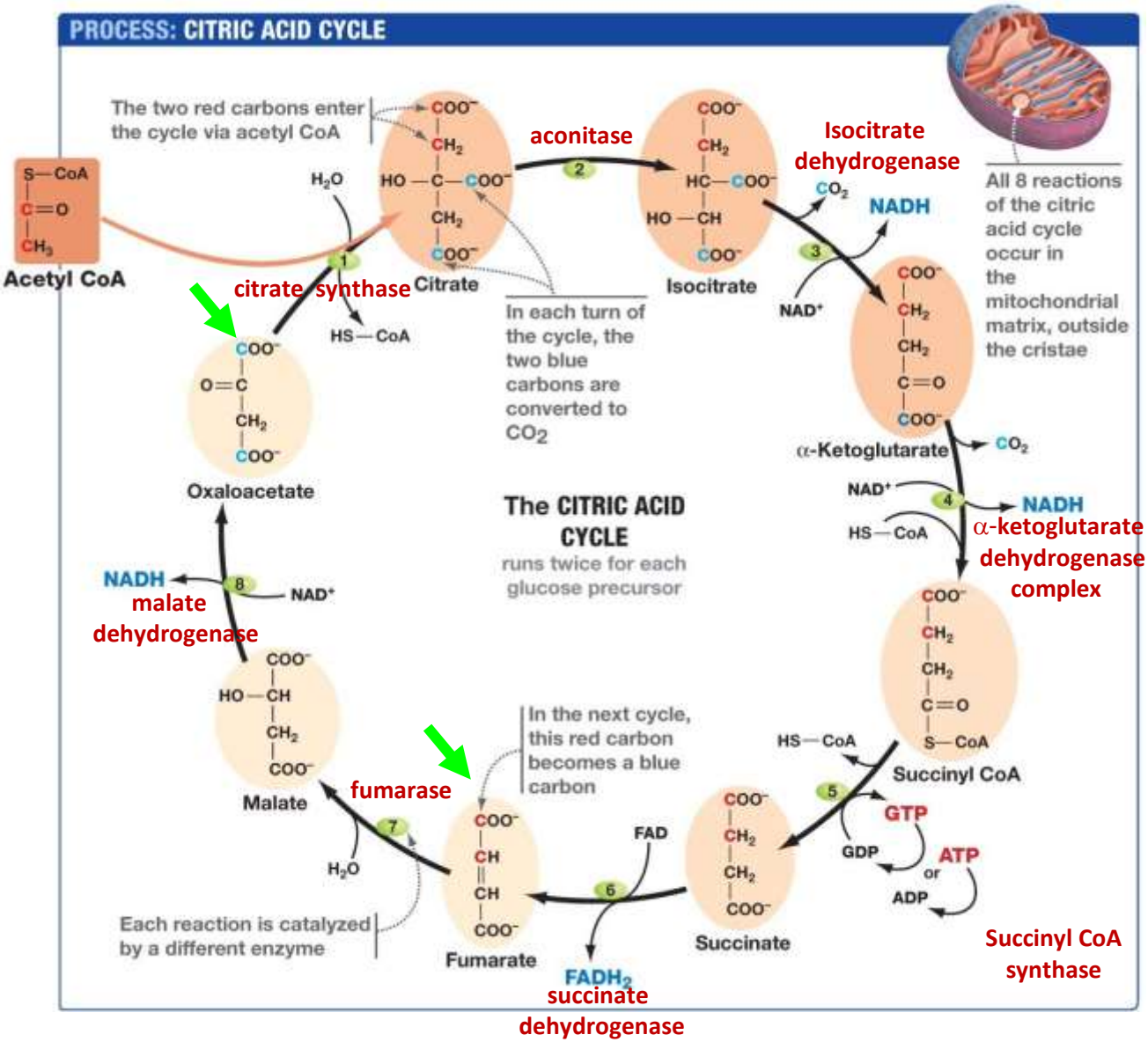
Citric Acid Cycle

- 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 enzyme is found in inner mitochondrial membrane (it is the only transmembrane protein in Krebs cycle)

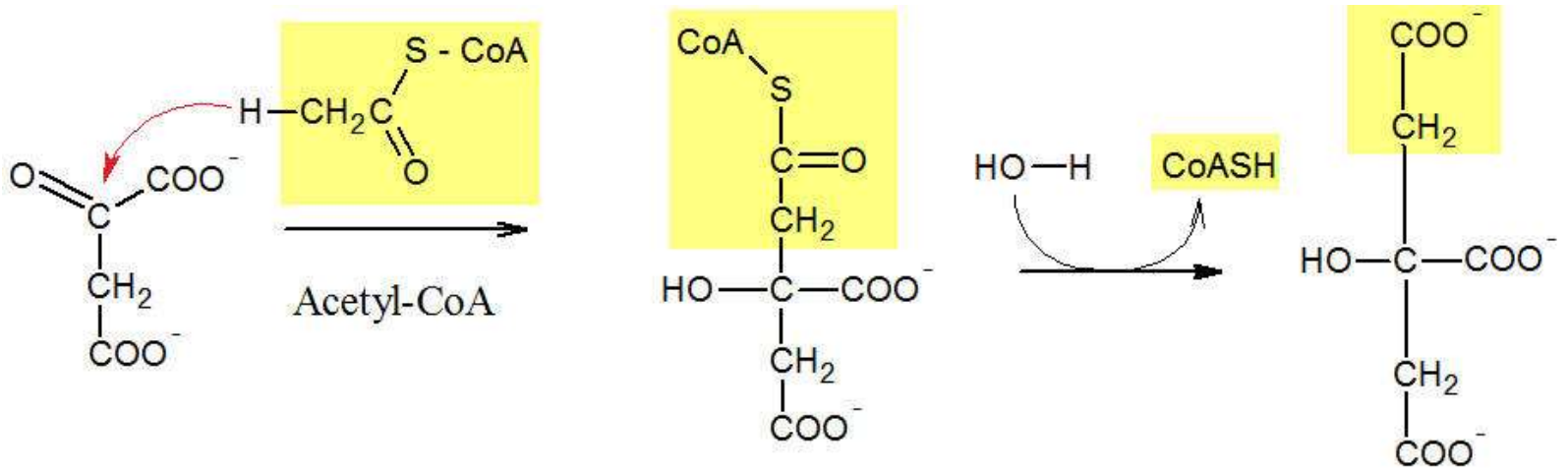


Citric Acid Cycle



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)

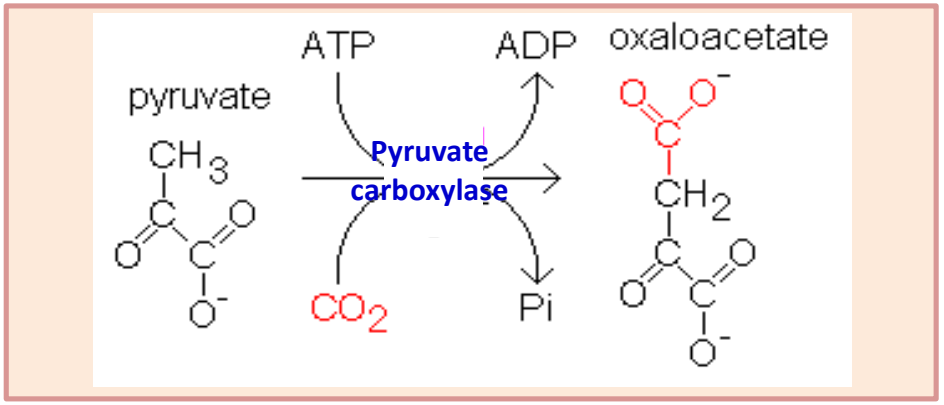


Oxaloacetate

Citrate

Citric Acid Cycle

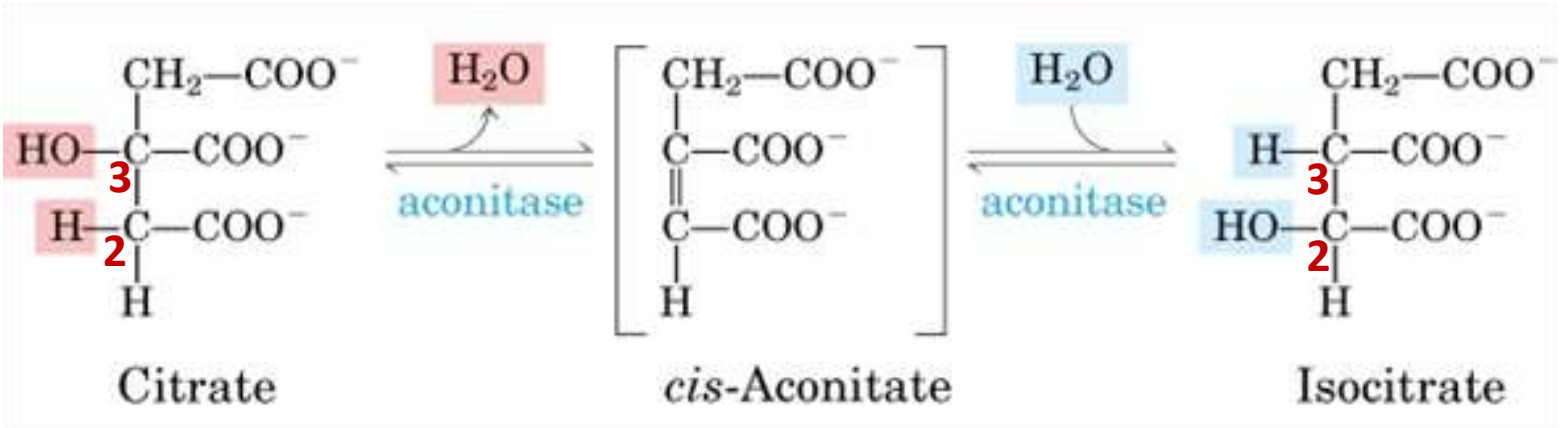
- Oxaloacetate is already found in matrix. It can be produced in several ways in nature. For example, it is generated from an ATP-dependent carboxylation of pyruvate catalyzed by pyruvate carboxylase. This reaction occurs in the matrix



- **Step 2:** Aconitase enzyme catalyzes the reversible isomerization of citrate to isocitrate (isomers differ in the position of OH group from C3 to C2)

Citric Acid Cycle

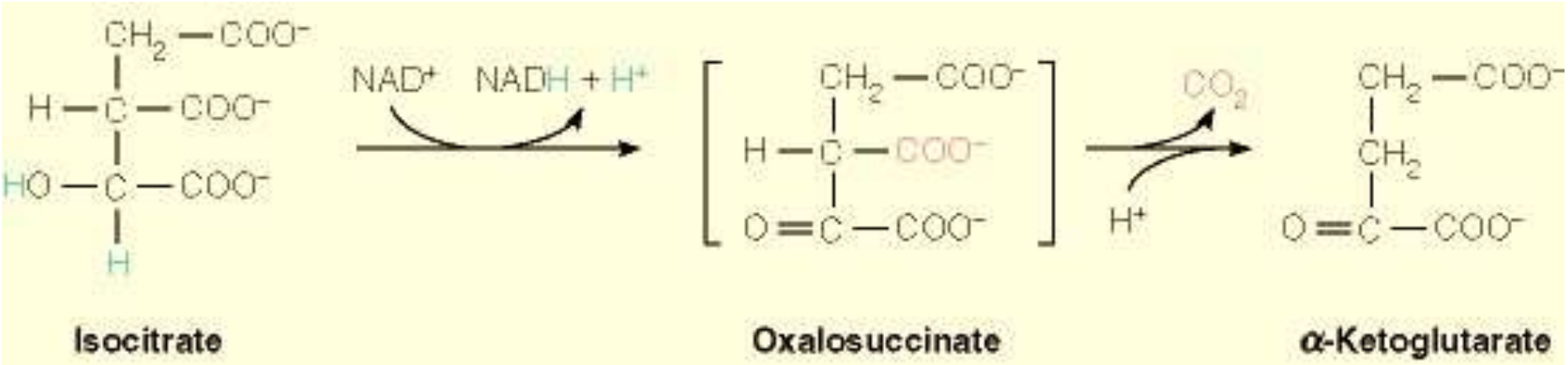
- This isomerization reaction is pre-required step to prepare substrates for decarboxylation reaction



- It involves successive dehydration and hydration reactions

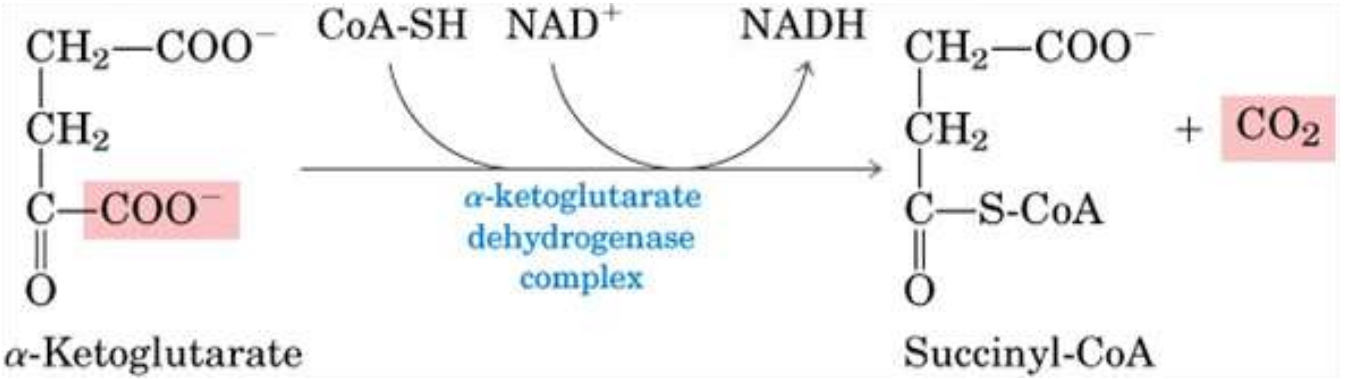
Citric Acid Cycle

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



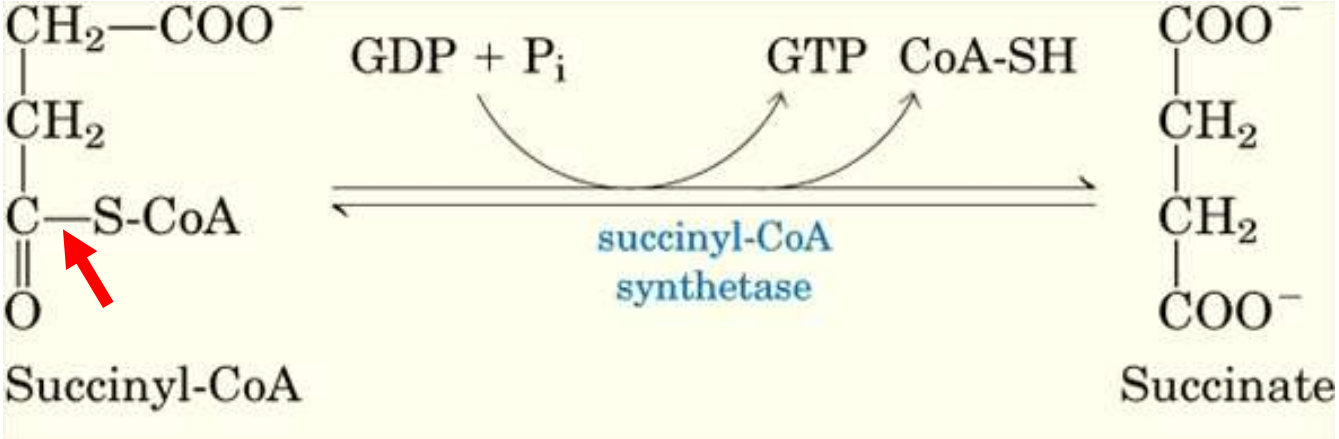
Citric Acid Cycle

- **Step 4:** α-ketoglutarate dehydrogenase complex catalyzes the oxidative decarboxylation of α-ketoglutarate (5C) to succinyl CoA (4C) releasing the second CO_2 and producing the second NADH molecule



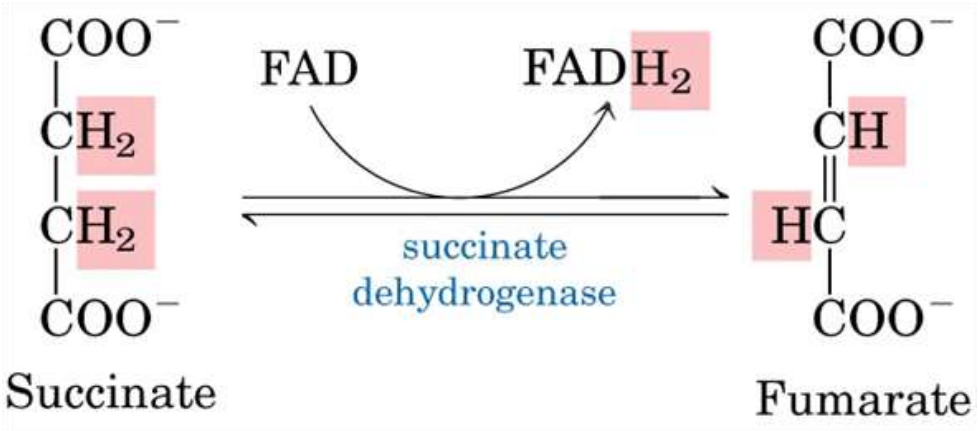
Citric Acid Cycle

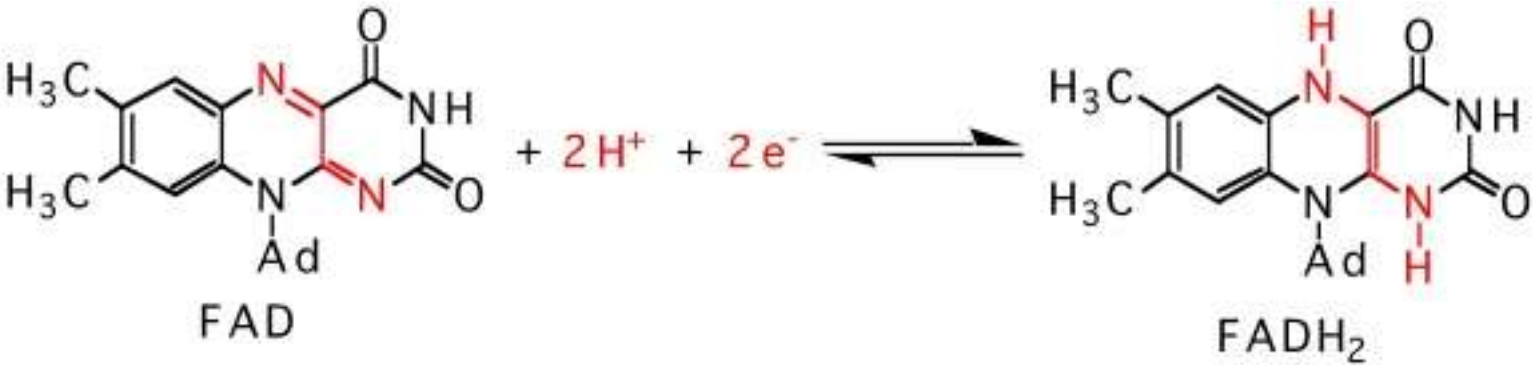
- **Step 5:** Succinyl CoA synthetase generates the first ATP (e.g. brain & heart tissues) or GTP (e.g. liver tissues) by the substrate-level phosphorylation mechanism. The thioester bond of succinyl-CoA is energy-rich and can drive the phosphorylation of ADP or GDP



Citric Acid Cycle

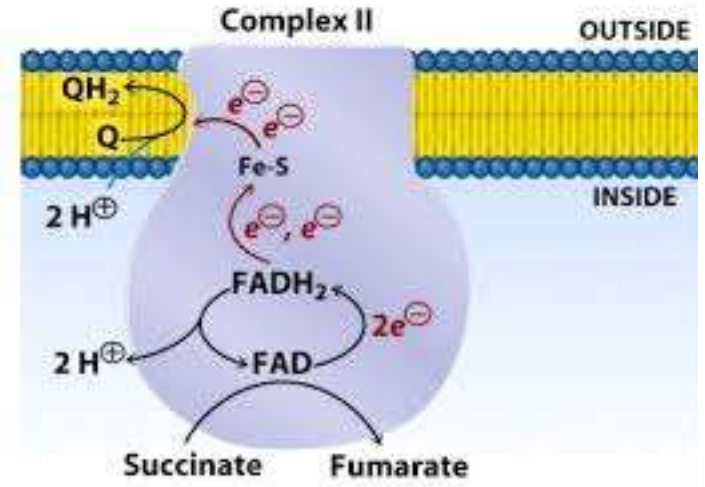
- **Step 6**: Succinate dehydrogenase catalyzes the oxidation of succinate to fumarate and consequently, the reduction of prosthetic group FAD into $FADH_2$

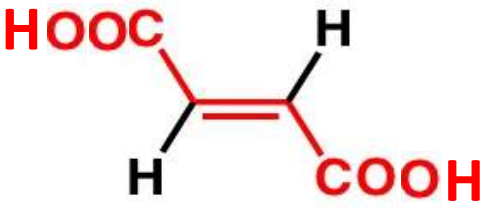




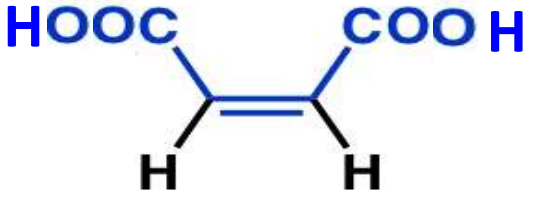
Citric Acid Cycle

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





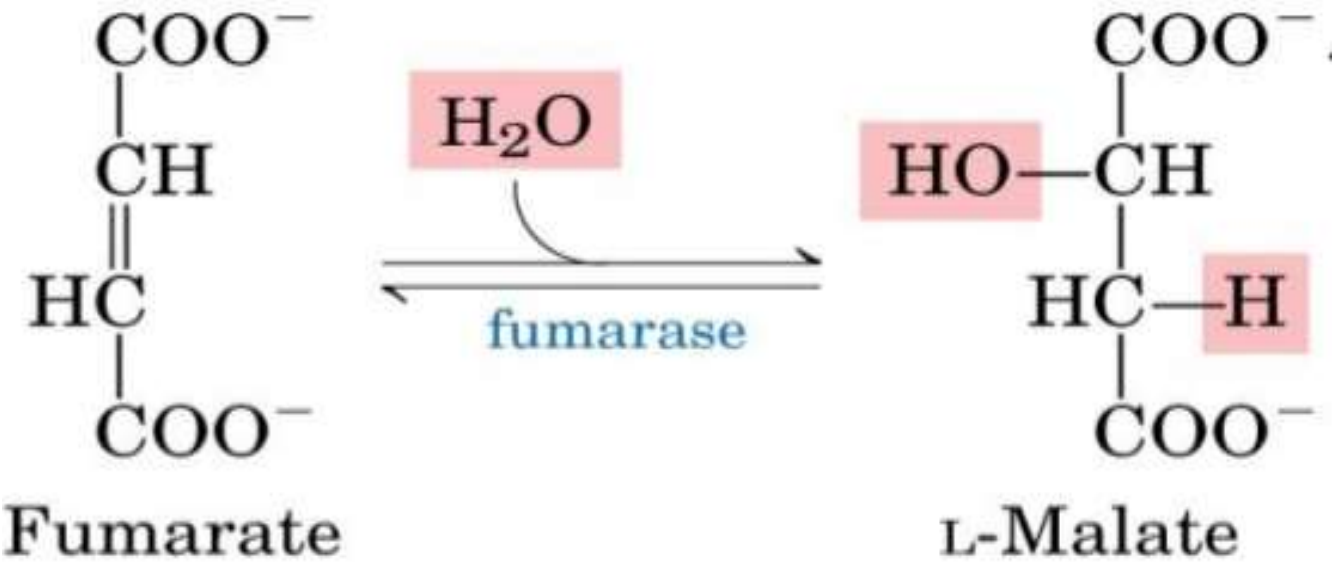
trans fumarate



cis fumarate(maleate)

Citric Acid Cycle

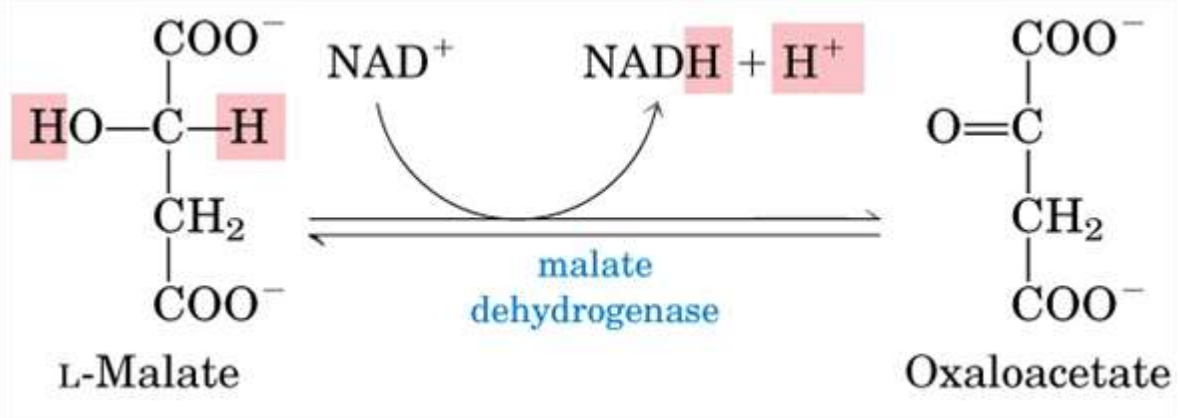
- **Step 7**: Fumarate is converted to L-malate in a hydration reaction catalyzed by fumarase (reversible reaction)



- Fumarase is a 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

ATP Yield per one Glucose

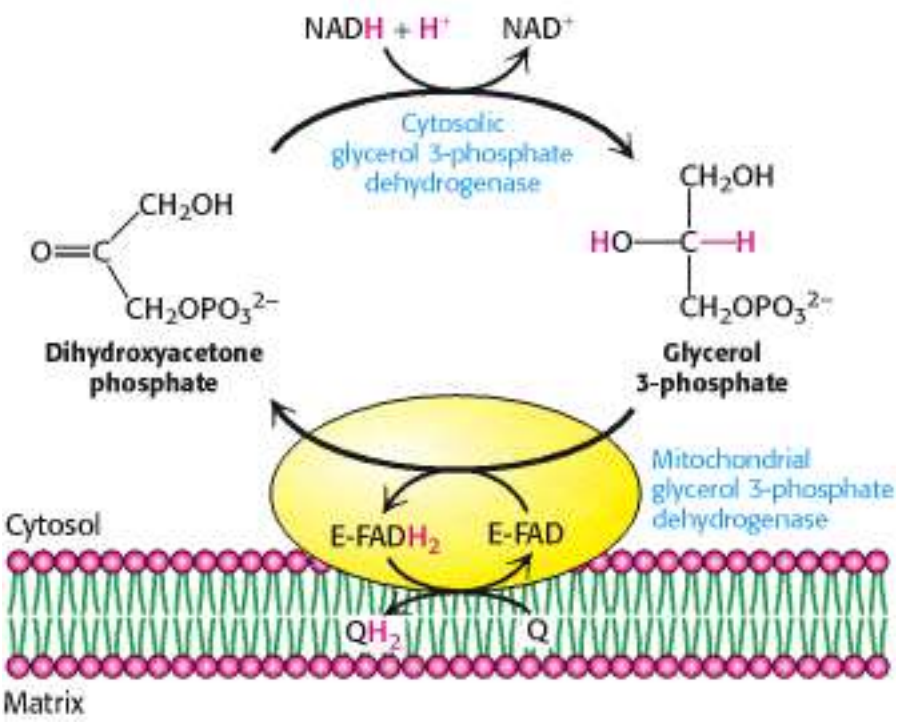
Stage	ATP produced by substrate-level phosphorylation
Glycolysis	2 ATP
Acetyl CoA production	none
Krebs Cycle	2 ATP
Total/glucose 4 ATP molecules	

Stage	Electron-carrier molecule	Total H^+ pumped	ATP synthase $4H^+ \longrightarrow$ 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

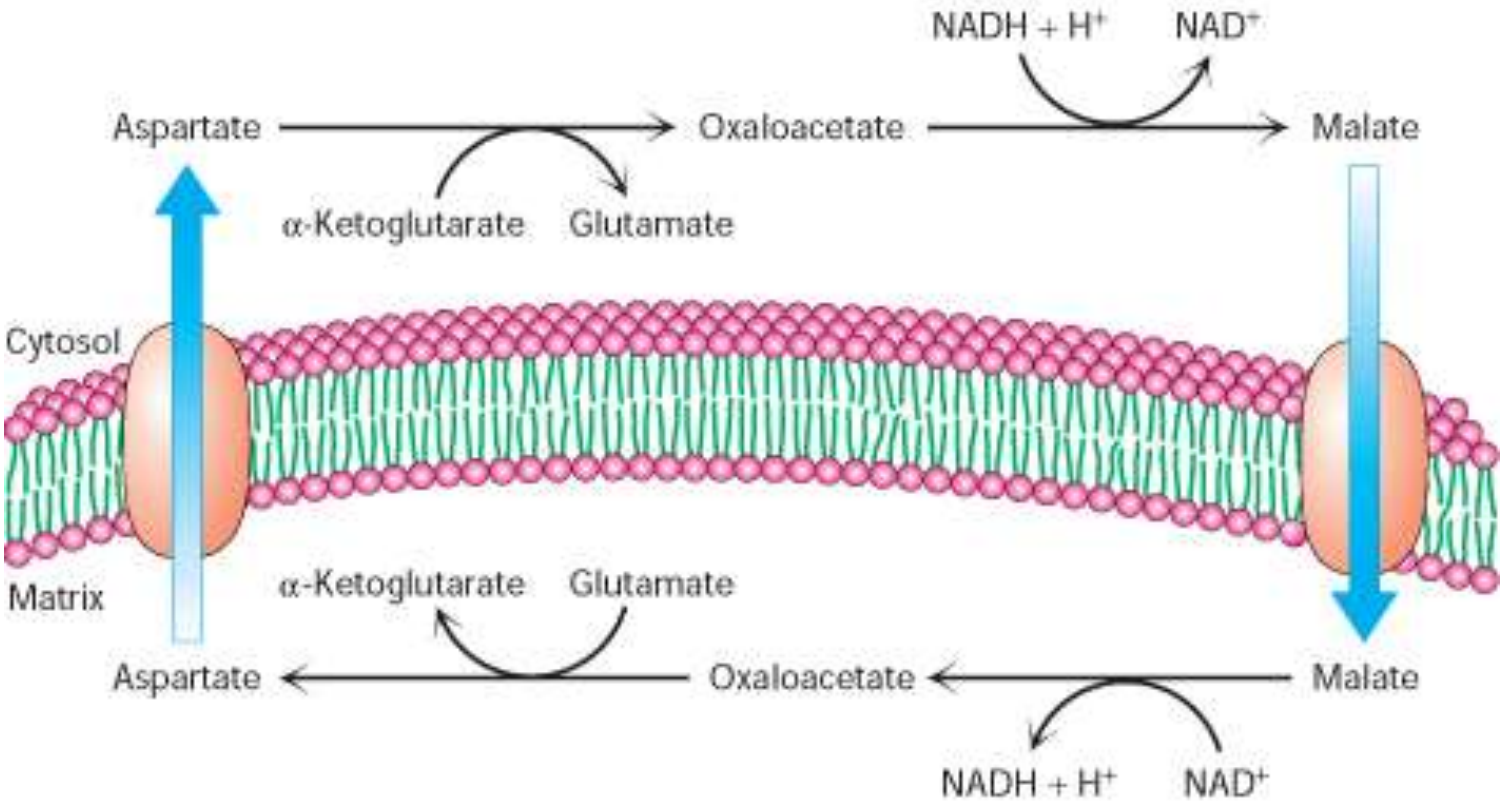
- The electrons carried by cytosolic NADH (i.e. NADH generated by glycolysis) will be shuttled to the matrix by one of two mechanisms:

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

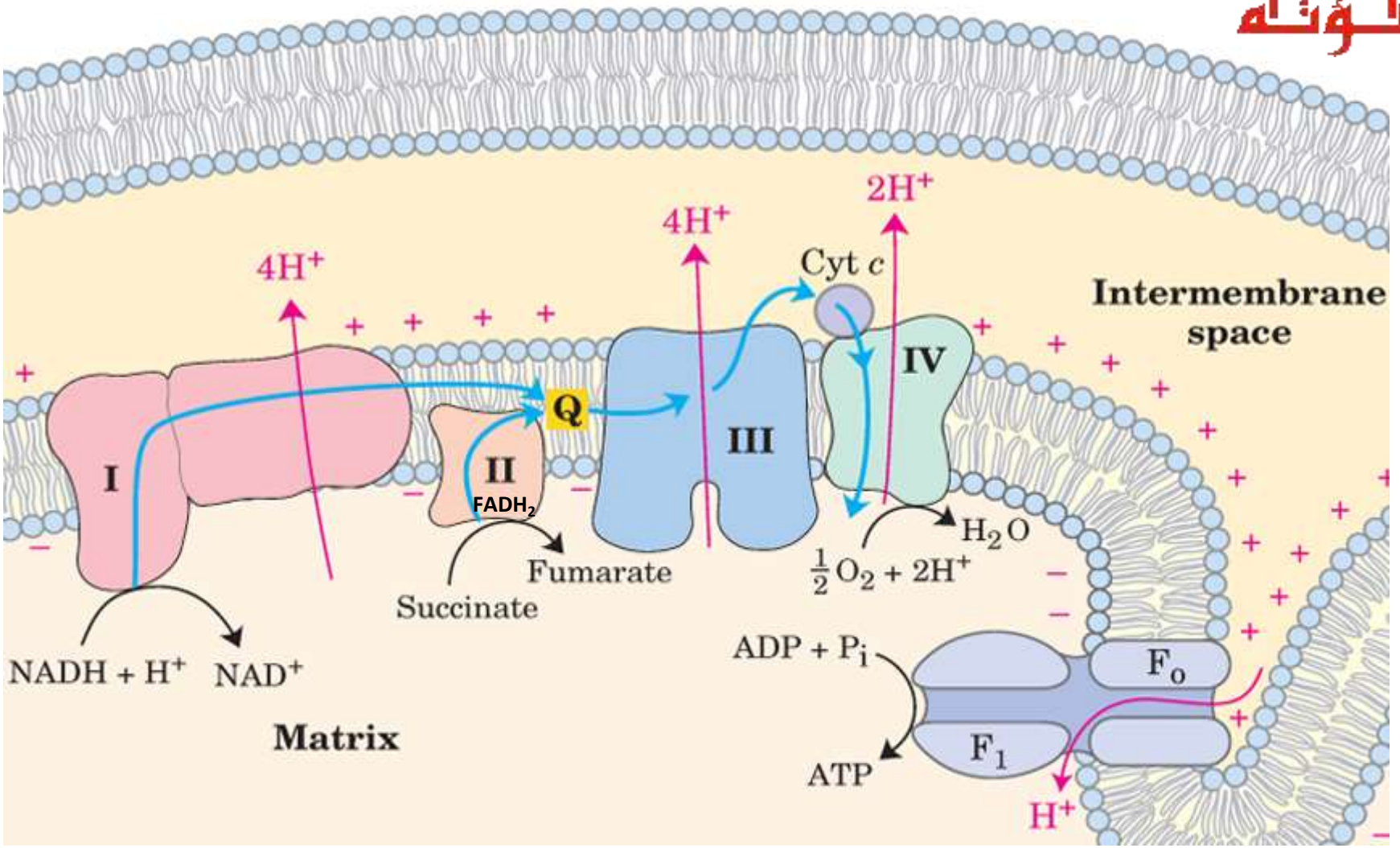


NADH Shuttling

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)



Oxidative Phosphorylation

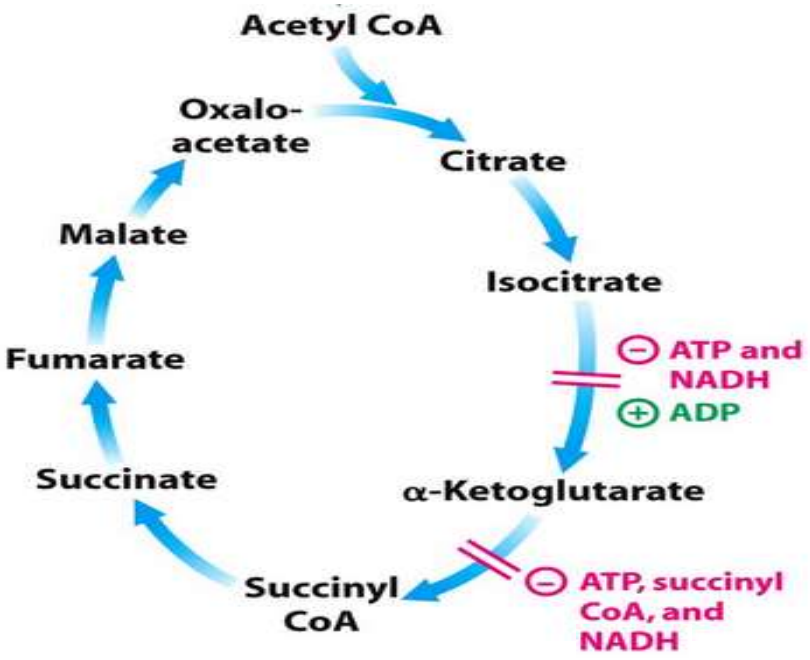


- TCA is considered as a part of aerobic metabolism although it does not use O_2 in any of its reaction ??

TCA Cycle Regulation

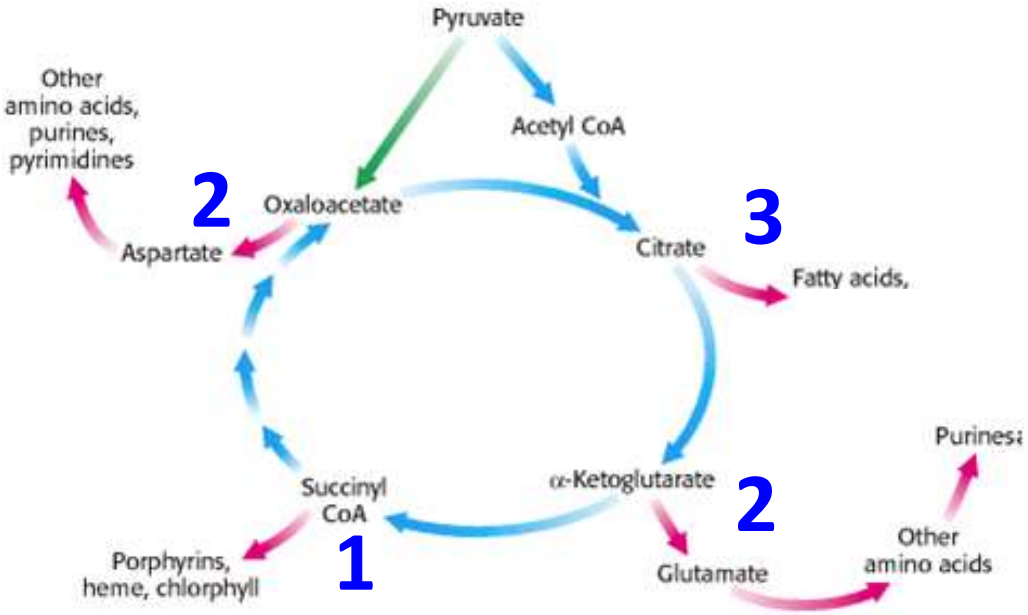
- Reactions 3 and 4 are key sites for allosteric regulation:

1. Isocitrate dehydrogenase is activated by ADP and directly inhibited by NADH and ATP
2. α-ketoglutarate dehydrogenase activity is inhibited by NADH, ATP and succinyl-CoA



Biosynthetic Role of TCA Intermediates

- In addition to its role in catabolism and energy generation, TCA intermediates have anabolic role in biosynthesis of other molecules:



Biosynthetic Role of TCA Intermediates

1. Succinyl CoA is used in synthesis of heme and other porphyrins
2. Oxaloacetate and α-ketoglutarate are converted by transamination to the corresponding amino acids aspartate and glutamate, respectively
3. Citrate in some tissues is transported to cytosol where it is converted back to acetyl CoA for fatty acids biosynthesis

Anaplerotic Pathway

- Anaplerotic pathways (from Greek word meaning filling up) are the processes that replenish TCA intermediates so that the flow of carbon out of the cycle is balanced by these reactions

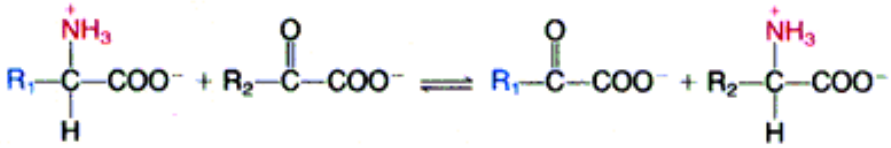
- Reactions that replenish oxaloacetate:

1. Pyruvate carboxylase (PC) catalyzes the irreversible ATP-dependent carboxylation of pyruvate to generate oxaloacetate (**occurs in the matrix**)

Anaplerotic Pathway

- Transamination replenish TCA intermediates:

Transamination are reversible reaction in which an amino acid loses an amino group thereby converted itself to a keto acid



- For example glutamate and aspartate undergo transamination to generate the TCA intermediates α-ketoglutarate and oxaloacetate, respectively