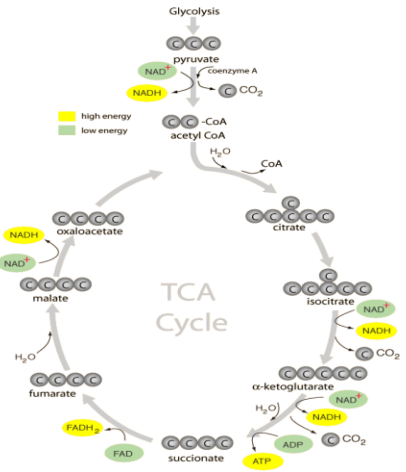
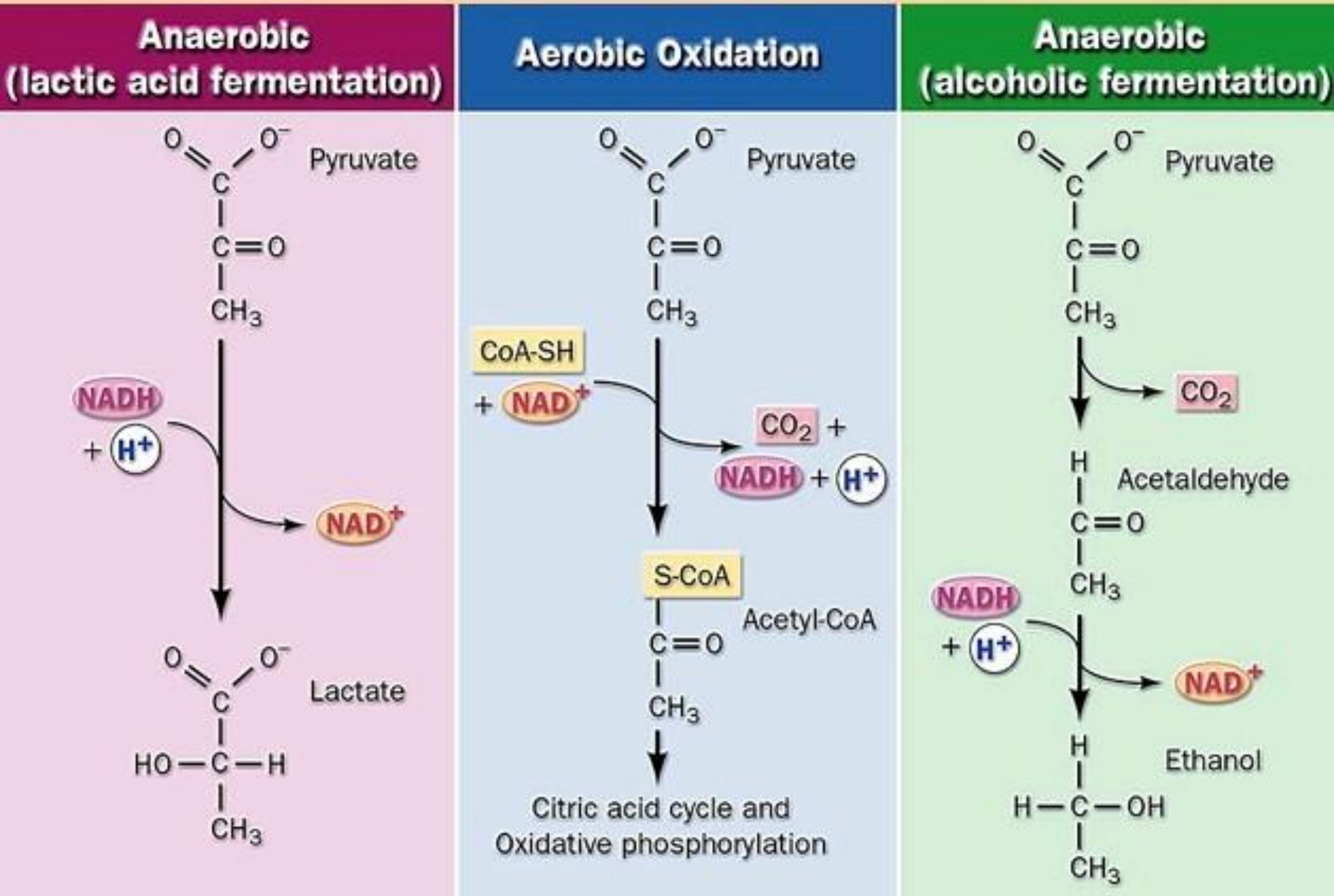
TCA CYCLE
STEPS
REGULATION AND SIGNIFICANCE



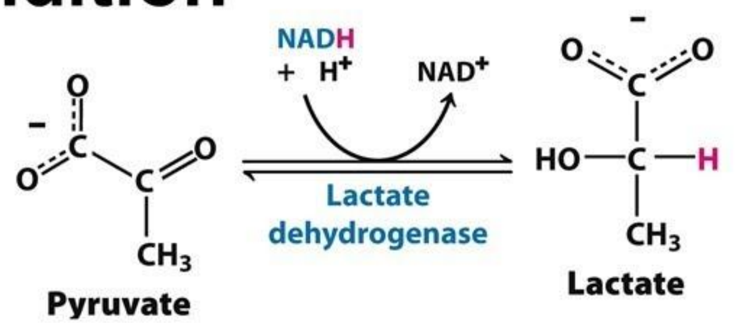
Introduction

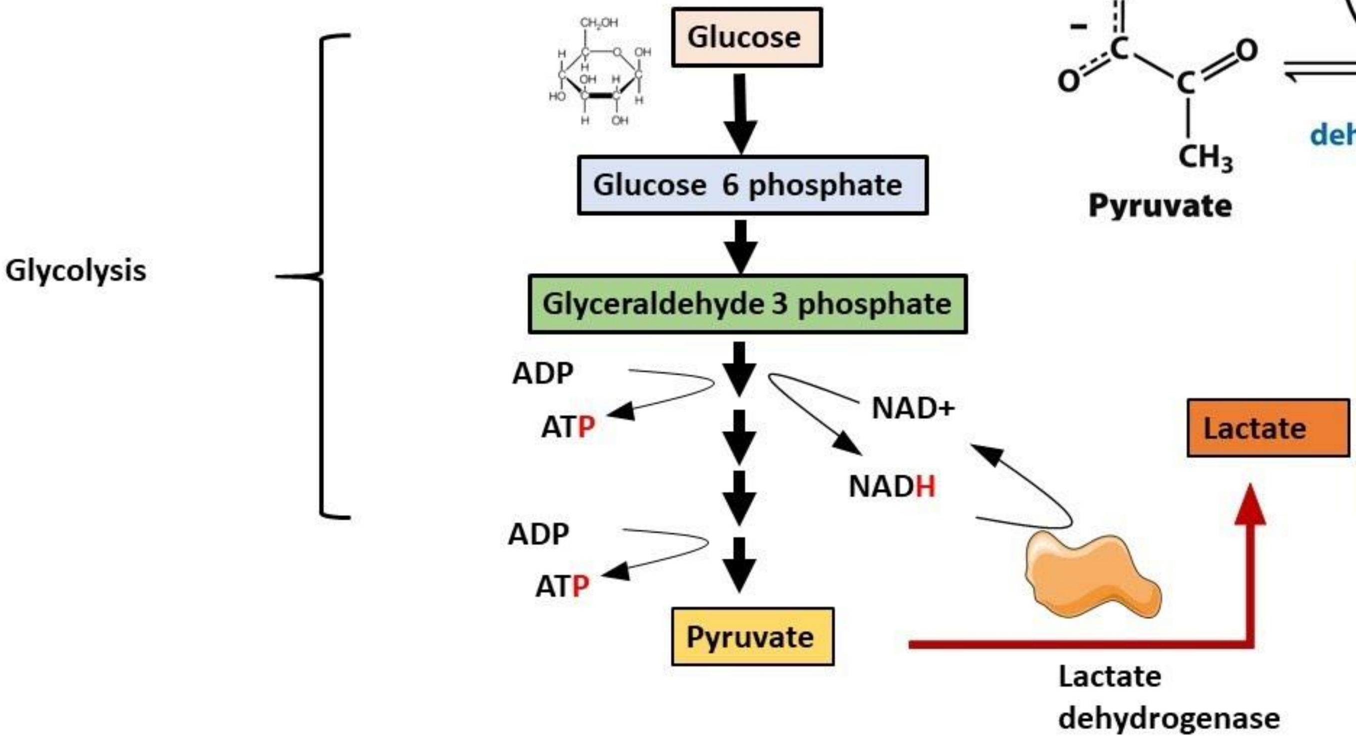
- The citric acid cycle is the central metabolic hub of the cell.
- It is the *final common pathway for the oxidation of fuel molecule such as* amino acids, fatty acids, and carbohydrates.
- In eukaryotes, the reactions of the citric acid cycle take place inside mitochondria, in contrast with those of glycolysis, which take place in the cytosol.

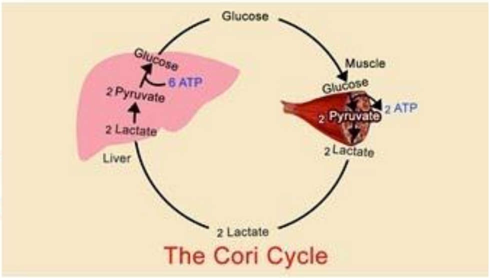
Three fates of pyruvate produced by glycolysis



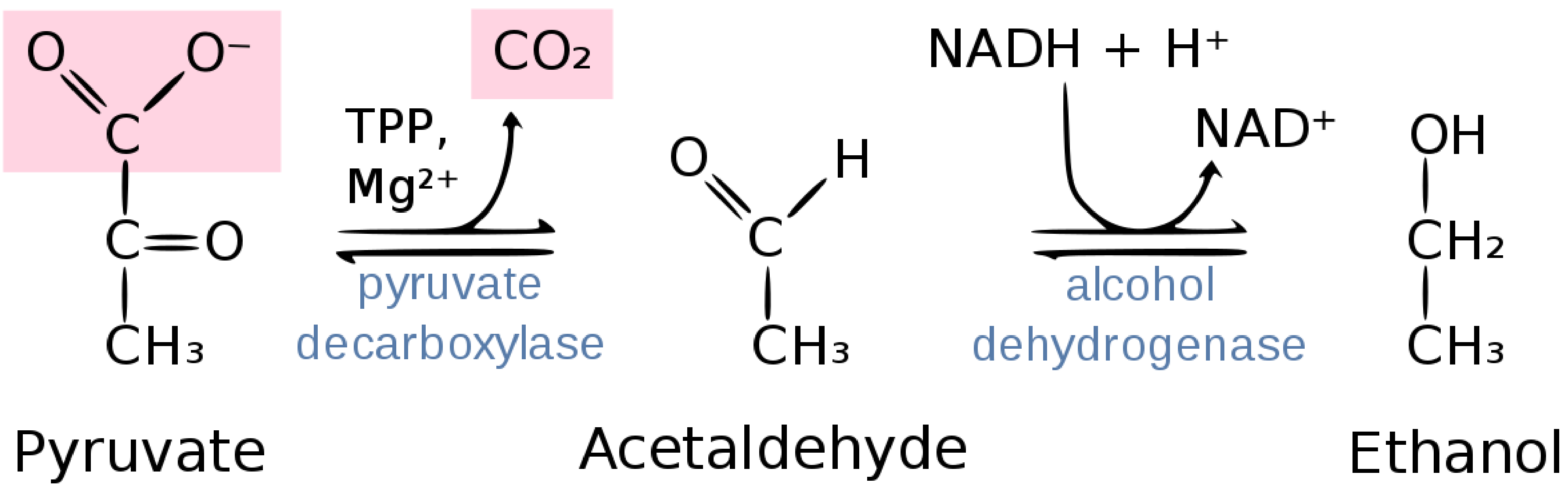
Lactic acid fermentation : fate of pyruvate under anaerobic condition



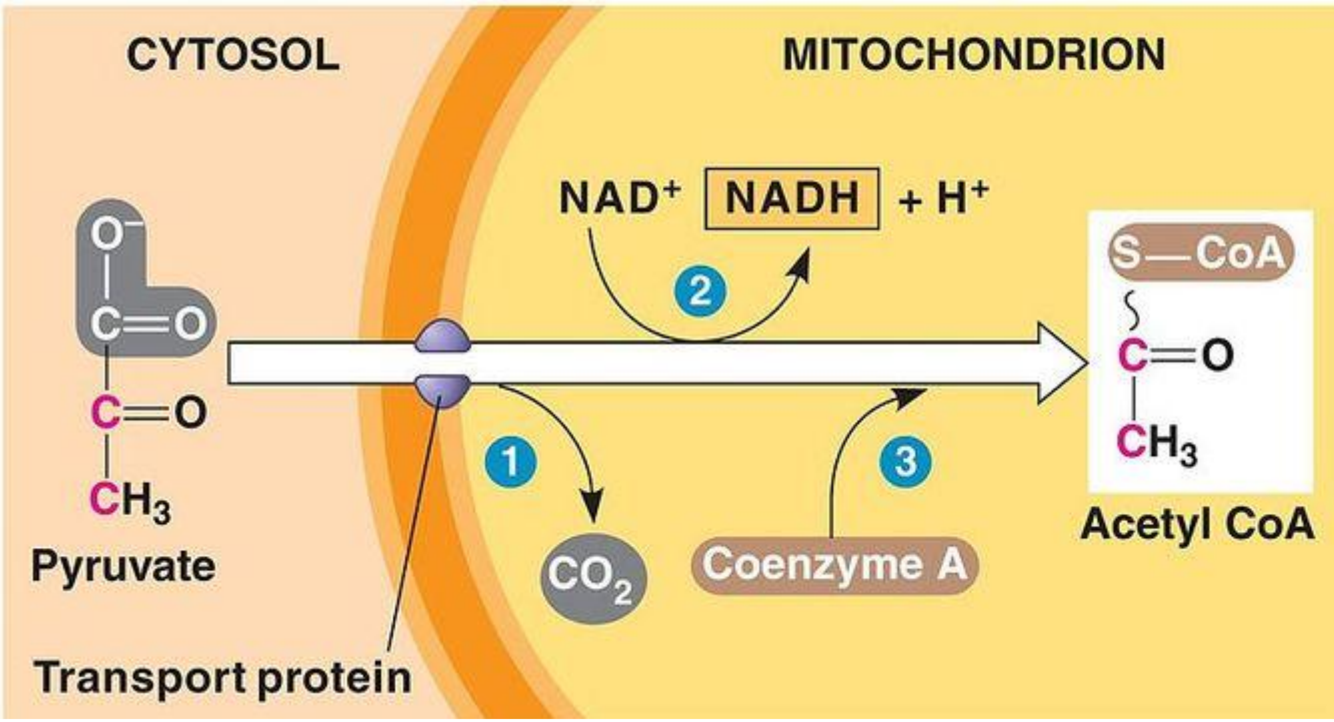


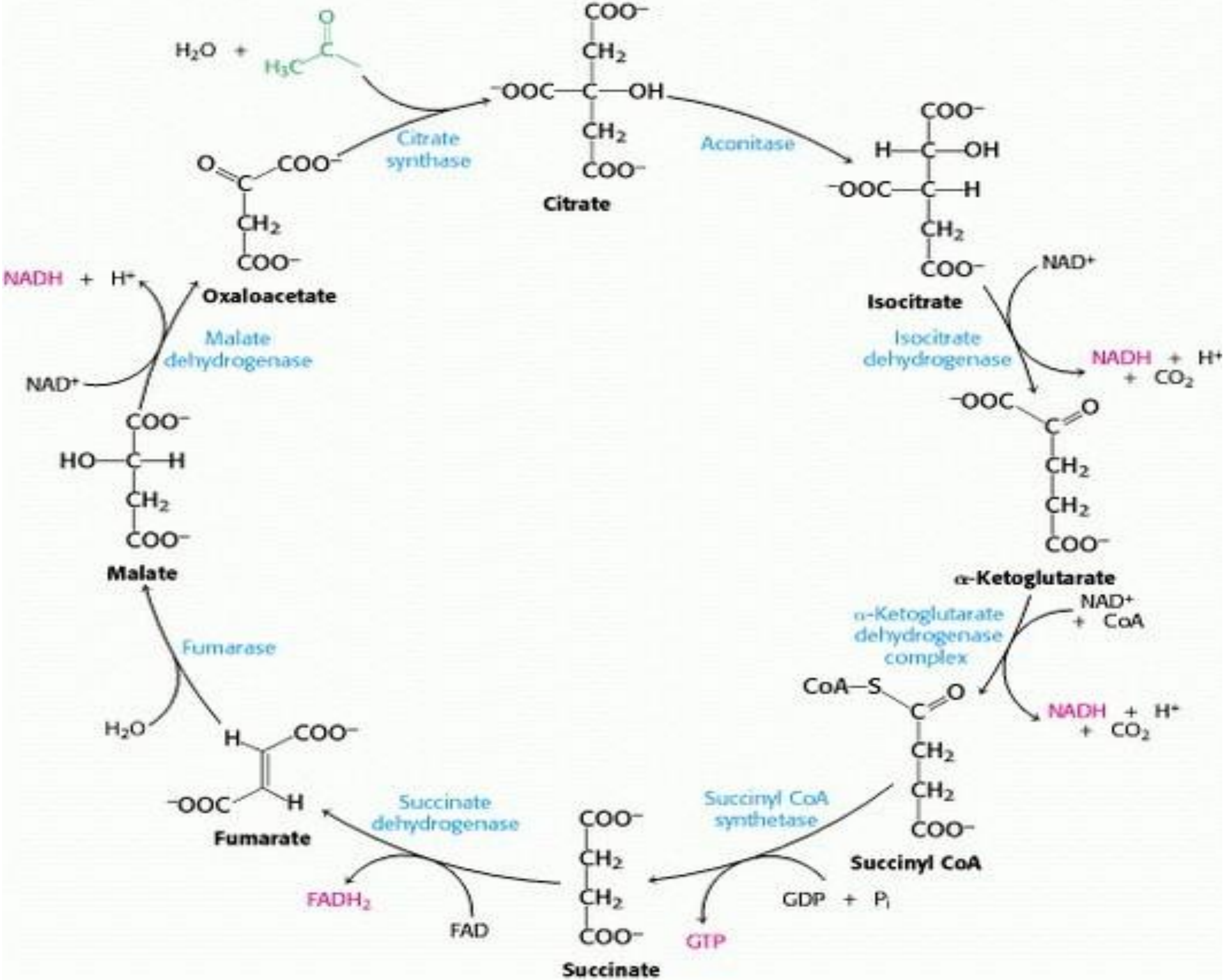


Alcoholic Fermentation



Acetyl CoA from Pyruvate





H2O +
Citrate synthase
Citrate
Aconitase
Isocitrate
NAD+
Isocitrate dehydrogenase
NADH + H+ + CO2
α-Ketoglutarate
NAD+ + CoA
α-Ketoglutarate dehydrogenase complex
NADH + H+ + CO2
CoA—S
Succinyl CoA
Succinyl CoA synthetase
GDP + Pi
GTP
Succinate
Succinate dehydrogenase
FAD
FADH2
Fumarate
Fumarase
H2O
Malate
NAD+
Malate dehydrogenase
NADH + H+
Oxaloacetate

Overview of the Citric Acid Cycle

The citric acid cycle (Krebs cycle, tricarboxylic acid cycle) includes a series of oxidation-reduction reactions in mitochondria that result in the oxidation of an acetyl group to two molecules of carbon dioxide and reduce the coenzymes that are reoxidized through the electron transport chain, linked to the formation of ATP.

Overview of the Citric Acid Cycle

A four- carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid (citrate).

An isomer of citrate is then oxidatively decarboxylated.

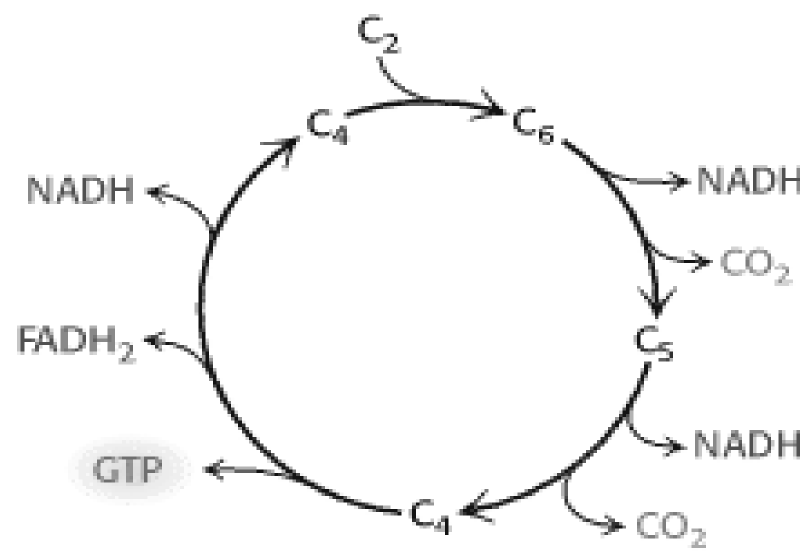
The resulting five-carbon compound (α-ketoglutarate) also is oxidatively decarboxylated to yield a four carbon compound (succinate).

Oxaloacetate is then regenerated from succinate.

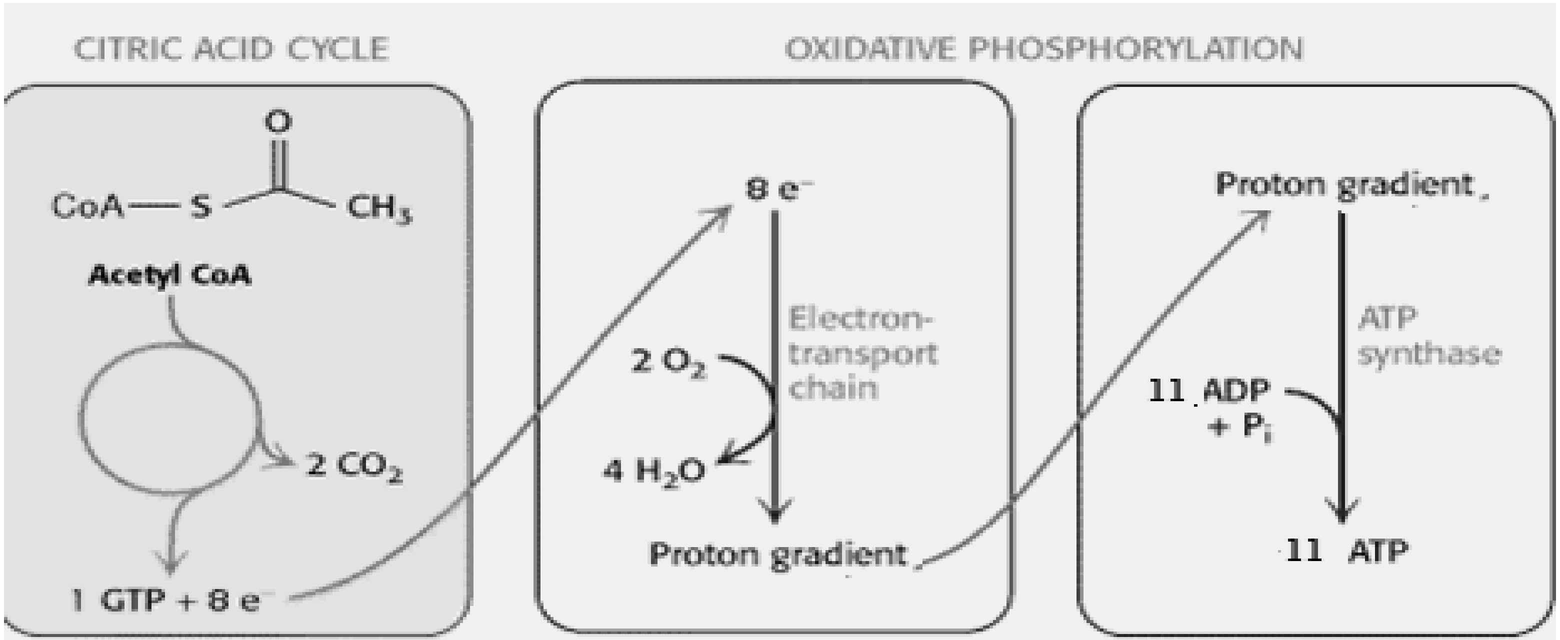
Two carbon atoms enter the cycle as an acetyl unit and two carbon atoms leave the cycle in the form of two molecules of carbon dioxide.

Overview of the Citric Acid Cycle

- Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD+), whereas one pair of hydrogen atoms (hence, two electrons) are transferred to one molecule of flavin adenine dinucleotide (FAD) .
- The function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.



Citric acid cycle and requirement of oxygen



Oxygen is required for the citric acid cycle indirectly in as much as it is the electron acceptor at the end of the electron-transport chain, necessary to regenerate NAD+ and FAD.

Citric acid cycle and requirement of oxygen (contd.)

- The citric acid cycle itself neither generates a large amount of ATP nor includes oxygen as a reactant.
- Instead, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to form NADH and FADH2 .
- In *oxidative phosphorylation*, electrons released in the reoxidation of NADH and FADH2 flow through a series of membrane proteins (referred to as the *electron-transport chain*) to generate a proton gradient across the membrane
- The citric acid cycle, in conjunction with oxidative phosphorylation, provides the vast majority of energy used by aerobic cells in human beings, greater than 95%.

Role of oxaloacetate in citric acid cycle

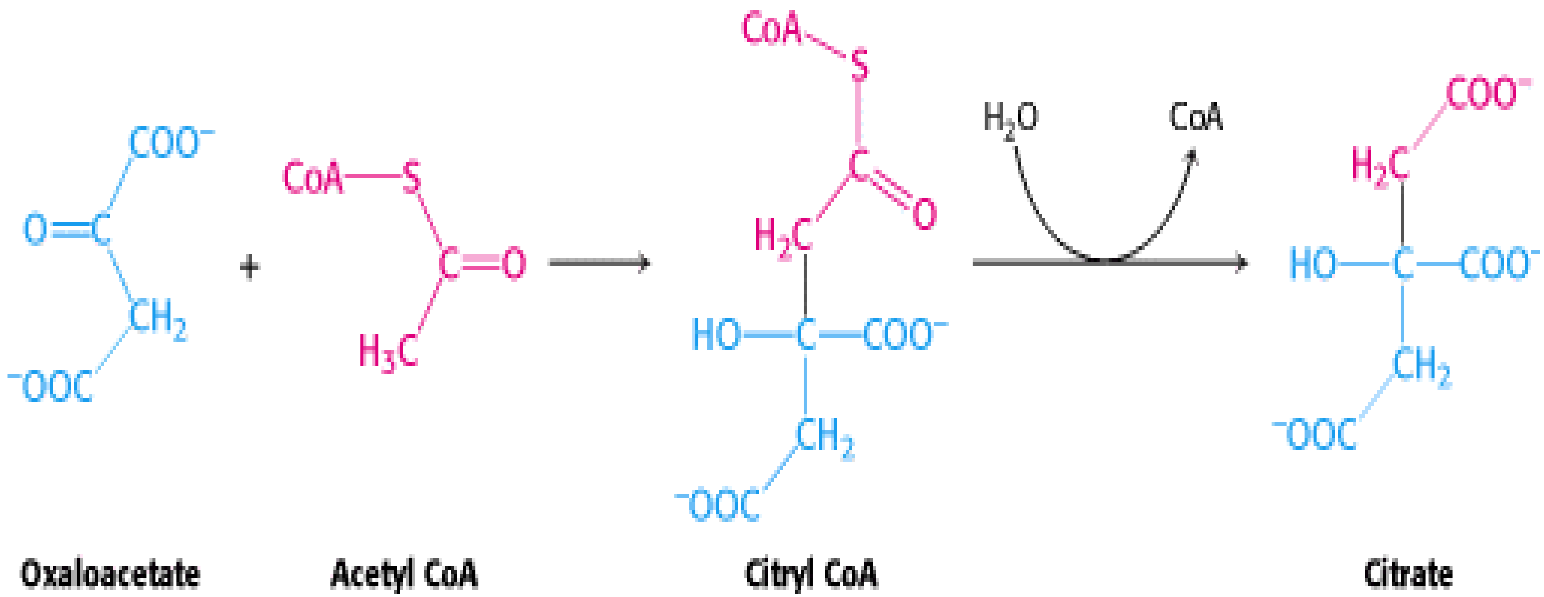
- The four-carbon molecule, oxaloacetate that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle.
- The oxaloacetate acts catalytically: it participates in the oxidation of the acetyl group but is itself regenerated.
- Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many

  acetyl molecules.

Reactions of the Citric Acid Cycle

- **Step-1 Formation of Citrate-** The citric acid cycle begins with the condensation of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA. Oxaloacetate reacts with acetyl CoA and H2O to yield citrate and CoA.

Step-1-Formation of Citrate



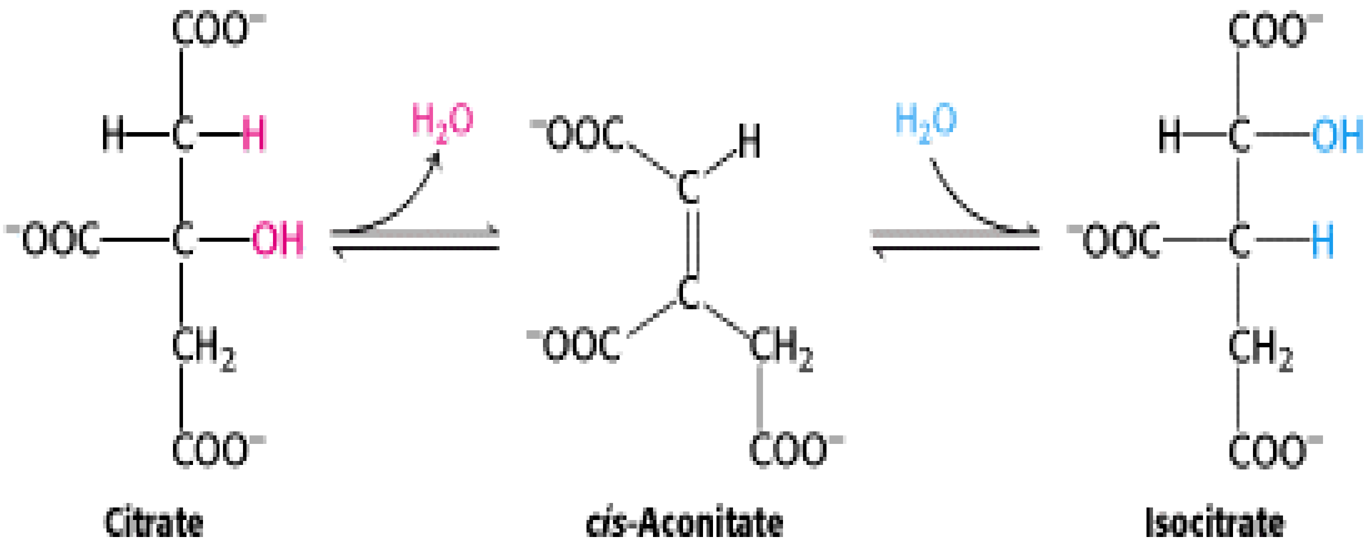
Oxaloacetate first condenses with acetyl CoA to form citryl CoA, which is then hydrolyzed to citrate and CoA.

Step-2-Formation of Isocitrate

- Citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation.

  The isomerization of citrate is accomplished by a dehydration step followed by a hydration step.

Step-2-Formation of Isocitrate



Step-3- Formation of α- Keto Glutarate

Isocitrate undergoes dehydrogenation catalyzed by **isocitrate dehydrogenase** to form, initially, Oxalo succinate, which remains enzyme-bound and undergoes decarboxylation to **α** -ketoglutarate.

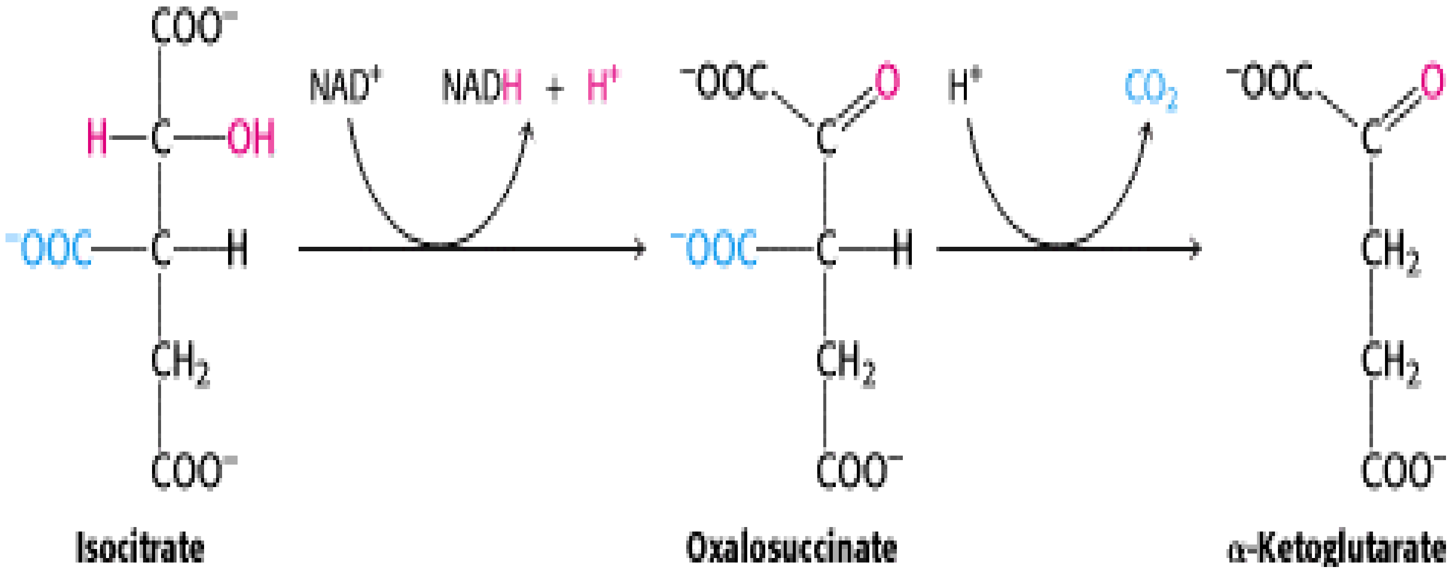
The decarboxylation requires Mg^{++} or Mn^{++} ions.

There are three isoenzymes of isocitrate dehydrogenase.

One, which uses NAD^{+}, is found only in mitochondria.

The other two use $NADP^{+}$ and are found in mitochondria and the cytosol.

Step-3- Formation of α- Keto Glutarate

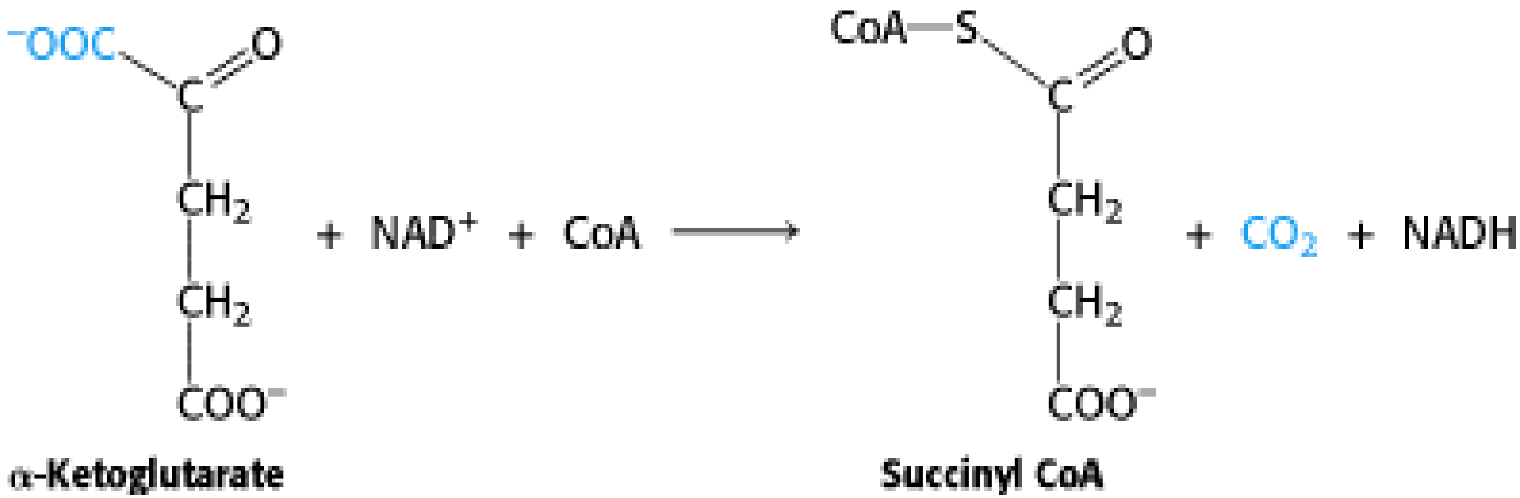


Respiratory chain-linked oxidation of isocitrate proceeds almost completely through the NAD^+-dependent enzyme.

Step-4-Formation of Succinyl Co A

- α-Ketoglutarate undergoes **oxidative decarboxylation** in a reaction catalyzed by a multi-enzyme complex similar to that involved in the oxidative decarboxylation of pyruvate.
- The α-**-ketoglutarate dehydrogenase complex** requires the same cofactors as the pyruvate dehydrogenase complex—thiamine diphosphate, lipoate, NAD^+, FAD, and CoA—and results in the formation of succinyl-CoA.

Step-4-Formation of Succinyl Co A

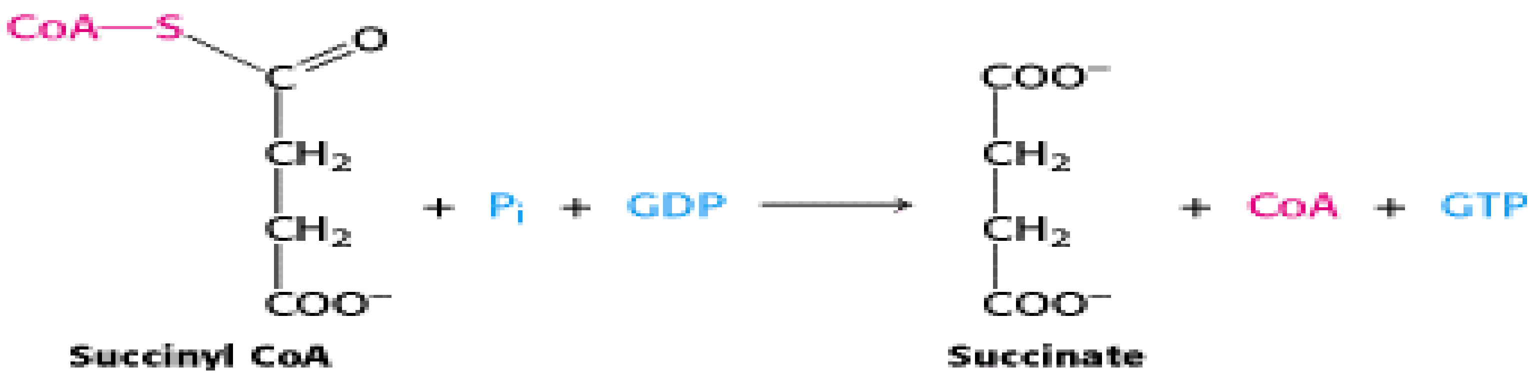


The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of Succinyl CoA from α-ketoglutarate.

Step-5- Formation of Succinate

- Succinyl CoA is an energy-rich thioester compound
- The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually GDP.
- This reaction is catalyzed by succinyl CoA synthetase (succinate thiokinase).

Step-5- Formation of Succinate



- This is the only example in the citric acid cycle of substrate level phosphorylation.

  Tissues in which gluconeogenesis occurs (the liver and kidney) contain two isoenzymes of succinate thiokinase, one specific for GDP and the other for

Step-5- Formation of Succinate

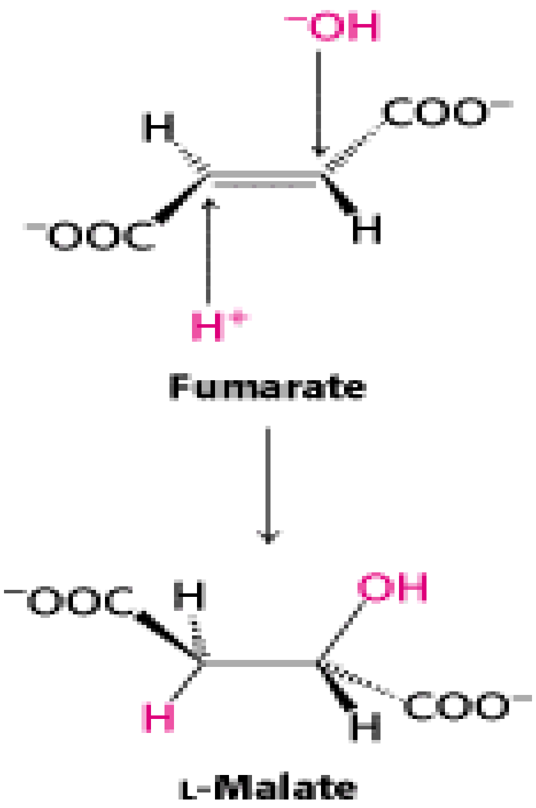
- The GTP formed is used for the decarboxylation of oxaloacetate to phosphoenolpyruvate in gluconeogenesis, and provides a regulatory link between citric acid cycle activity and the withdrawal of oxaloacetate for gluconeogenesis.
- Nongluconeogenic tissues have only the isoenzyme that uses ADP.

Step-6- Formation of Fumarate

- The first dehydrogenation reaction, forming fumarate, is catalyzed by **succinate dehydrogenase,** which is bound to the inner surface of the inner mitochondrial membrane.
- The enzyme contains FAD and iron-sulfur (Fe:S) protein, and directly reduces ubiquinone in the electron transport chain.

$$\text{E-FAD} + \text{succinate} \rightleftharpoons \text{E-FADH}_2 + \text{fumarate}$$

Step-7- Formation of Malate



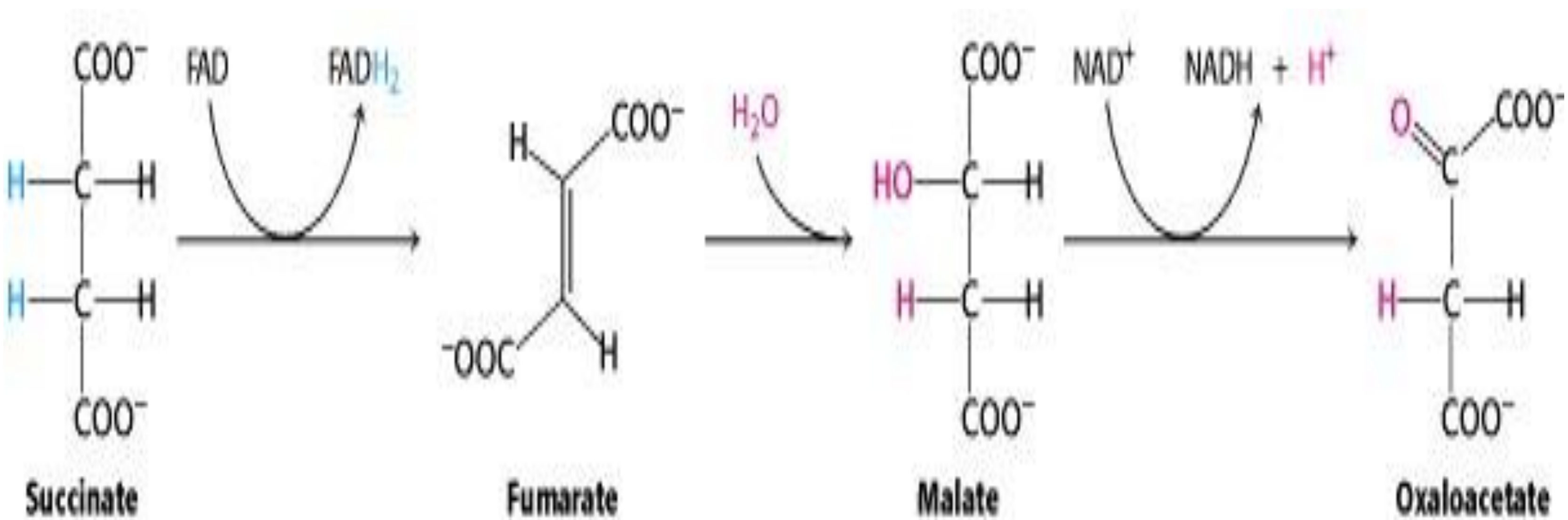
Fumarase (fumarate hydratase) catalyzes the addition of water across the double bond of fumarate, yielding malate.

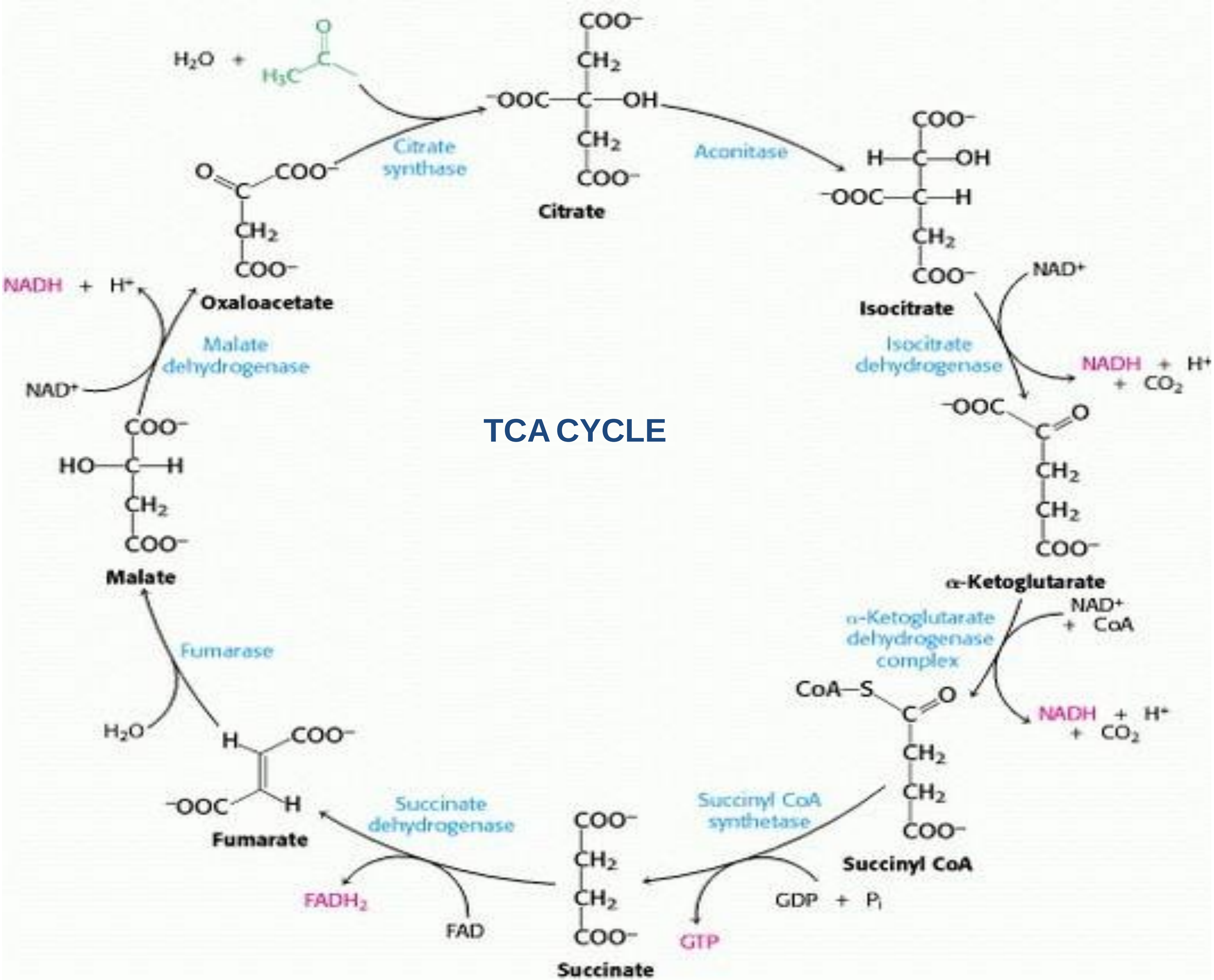
Step-8- Regeneration of oxaloacetate

- Malate is converted to oxaloacetate by **malate dehydrogenase,** a reaction requiring NAD^+.
- Although the equilibrium of this reaction strongly favors malate, the net flux is to oxaloacetate because of the continual removal of oxaloacetate (to form citrate, as a substrate for gluconeogenesis, or to undergo transamination to aspartate) and also the

Step-8- Regeneration of oxaloacetate

$$\text{Malate} + NAD^+ \rightleftharpoons \text{oxaloacetate} + NADH + H^+$$




TCA CYCLE
Citrate
Citrate synthase
Aconitase
Isocitrate
Isocitrate dehydrogenase
α-Ketoglutarate
α-Ketoglutarate dehydrogenase complex
Succinyl CoA
Succinyl CoA synthetase
Succinate
Succinate dehydrogenase
Fumarate
Fumarase
Malate
Malate dehydrogenase
Oxaloacetate
H2O +
NAD+
NADH + H+
NADH + H+ + CO2
NAD+ + CoA
GDP + Pi
GTP
FAD
FADH2
H2O
NAD+
NADH + H+