❑ Citric acid cycle

- ❑ Also known as the **tricarboxylic acid (TCA) cycle or the Krebs cycle**
- ❑ A series of chemical reactions used by all aerobic organisms to release stored energy through the **oxidation of acetyl-CoA** derived from **carbohydrates**, **fats**, and **proteins** into **carbon dioxide** and chemical energy in the form of **adenosine triphosphate (ATP).**
- ❑ The cycle consumes **acetate** (in the form of acetyl-CoA) and **water,** reduces NAD^+ to NADH, and produces **carbon dioxide** as a waste byproduct.

- The NADH generated by the citric acid cycle is fed into the **oxidative phosphorylation (electron transport) pathway**. The net result of these two closely linked pathways is the oxidation of nutrients to produce usable chemical energy in the form of ATP.

- In **eukaryotic cells**, the citric acid cycle occurs in the **matrix of the mitochondrion.**

- In **prokaryotic cells**, such as **bacteria**, which lack mitochondria, the citric acid cycle reaction sequence is performed in the **cytosol with the proton gradient for ATP production** being across the cell's surface (plasma membrane) rather than the inner membrane of the mitochondrion.

Discovery

- Several of the components and reactions of the citric acid cycle were established in the 1930s by the research of Albert Szent-Györgyi, who received the Nobel Prize in Physiology or Medicine in 1937 specifically for his discoveries pertaining to fumaric acid, a key component of the cycle. He was able to make this discovery successful with the help of pigeon breast muscle

- The citric acid cycle itself was finally identified in **1937** by **Hans Adolf Krebs** and William Arthur Johnson while at the **University of Sheffield**, for which the former received the **Nobel Prize for Physiology or Medicine** **in 1953**, and for whom the cycle is sometimes named (Krebs cycle).

Evolution

- It is believed that components of the citric acid cycle were derived from anaerobic bacteria, and that the TCA cycle itself may have evolved more than once. Theoretically, several alternatives to the TCA cycle exist; however, the TCA cycle appears to be the most efficient. If several TCA alternatives had evolved independently, they all appear to have converged to the TCA cycle.

Overview

- The citric acid cycle is a key metabolic pathway that connects carbohydrate, fat, and protein metabolism.
- The reactions of the cycle are carried out by **eight enzymes** that completely oxidize acetate, in the form of acetyl-CoA, **into two molecules each of carbon dioxide and water**. Through catabolism of sugars, fats, and proteins, the two-carbon organic product acetyl-CoA (a form of acetate) is produced which enters the citric acid cycle.

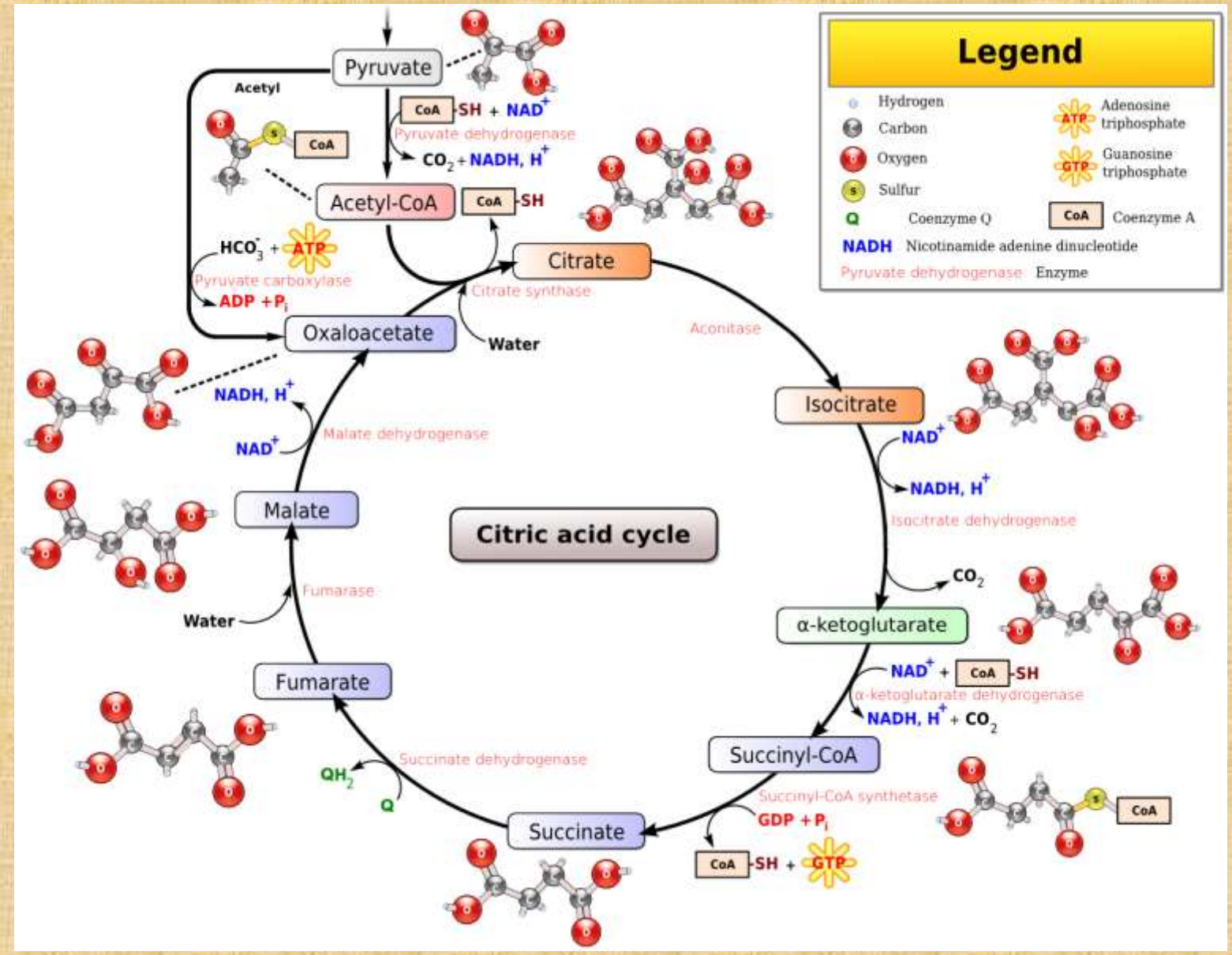
- The reactions of the cycle also convert three equivalents of **nicotinamide adenine dinucleotide (NAD$^+$)** into three equivalents of reduced **NAD$^+$ (NADH**), one equivalent of **flavin adenine dinucleotide (FAD)** into one equivalent of **$FADH_2$**, and one equivalent each of **guanosine diphosphate(GDP)** and **inorganic phosphate (P_i)** into one equivalent of **guanosine triphosphate (GTP).**

- The **NADH and $FADH_2$**generated by the citric acid cycle are, in turn, used by the **oxidative phosphorylation** pathway to generate energy-rich ATP.

- One of the primary sources of acetyl-CoA is from the breakdown of sugars by **glycolysis** which yield **pyruvate** that in turn is decarboxylated by the enzyme **pyruvate dehydrogenase** generating acetyl-CoA according to the following reaction

$$\underbrace{CH_3C(=O)C(=O)O^-}_{\text{pyruvate}} + HSCoA + NAD^+ \rightarrow \underbrace{CH_3C(=O)SCoA}_{\text{acetyl-CoA}} + NADH + CO_2$$

Citric acid cycle



- The citric acid cycle begins with the transfer of a two-carbon acetyl group from acetyl-CoA to the four-carbon acceptor compound (oxaloacetate) to form a six-carbon compound (citrate).

- The citrate then goes through a series of chemical transformations**, losing two carboxyl groups as CO_2.**

- The carbons lost as CO_2 originate from what was oxaloacetate, not directly from acetyl-CoA.

- The carbons donated by acetyl-CoA become part of the oxaloacetate carbon backbone after the first turn of the citric acid cycle.

- Loss of the acetyl-CoA-donated carbons as CO_2 requires several turns of the citric acid cycle. However, because of the role of the citric acid cycle in anabolism, they might not be lost, since many citric acid cycle intermediates are also used as precursors for the biosynthesis of other molecules.

- Most of the energy made available by the oxidative steps of the cycle is transferred as energy-rich electrons to NAD^+, forming NADH. For each acetyl group that enters the citric acid cycle, three molecules of NADH are produced.

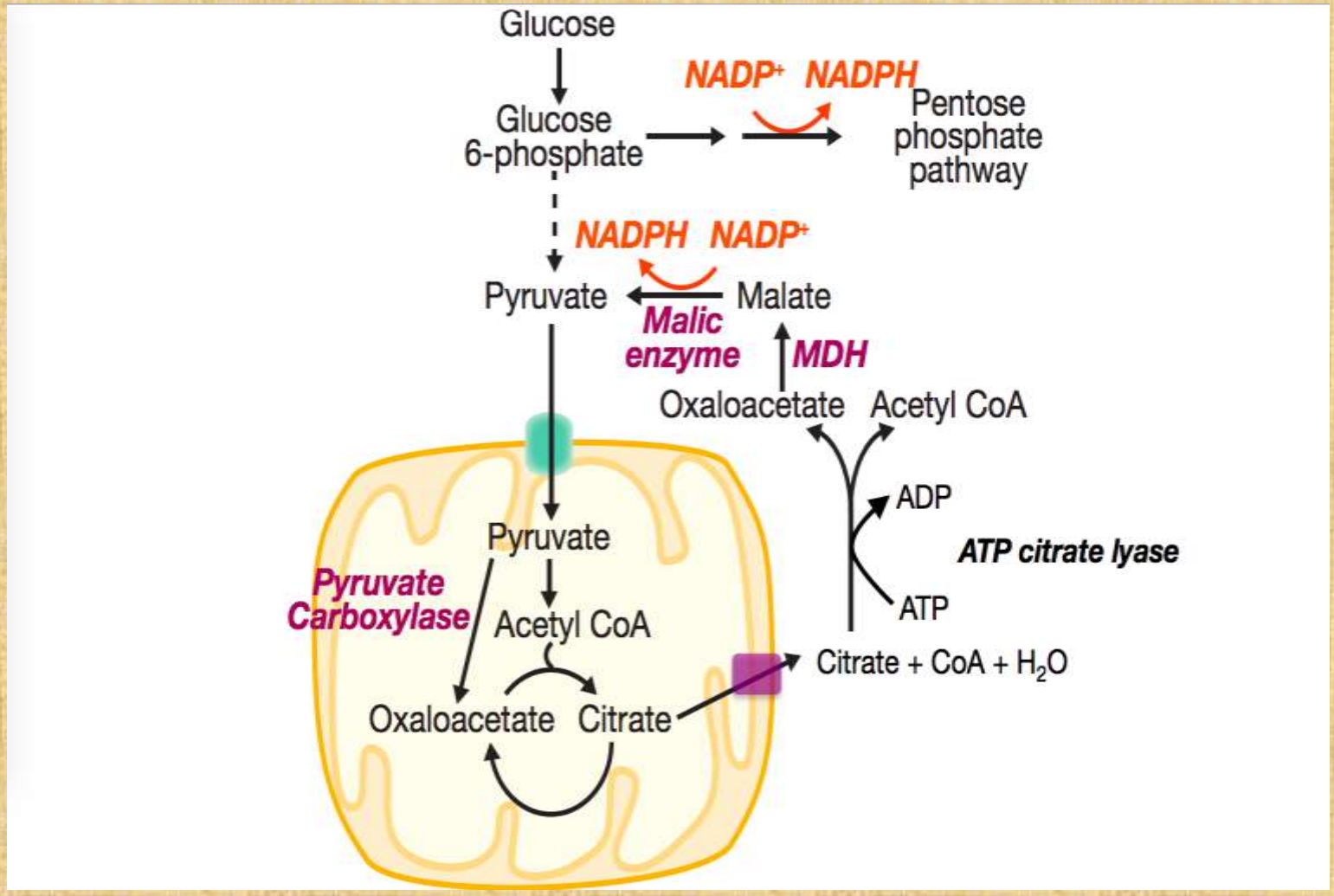
- In addition, electrons from the succinate oxidation step are transferred first to the **FAD cofactor of succinate dehydrogenase, reducing it to $FADH_2$, and eventually to ubiquinone(Q) in the mitochondrial membrane, reducing it to ubiquinol (QH_2) which is a substrate of the electron transfer chain at the level of Complex III.**

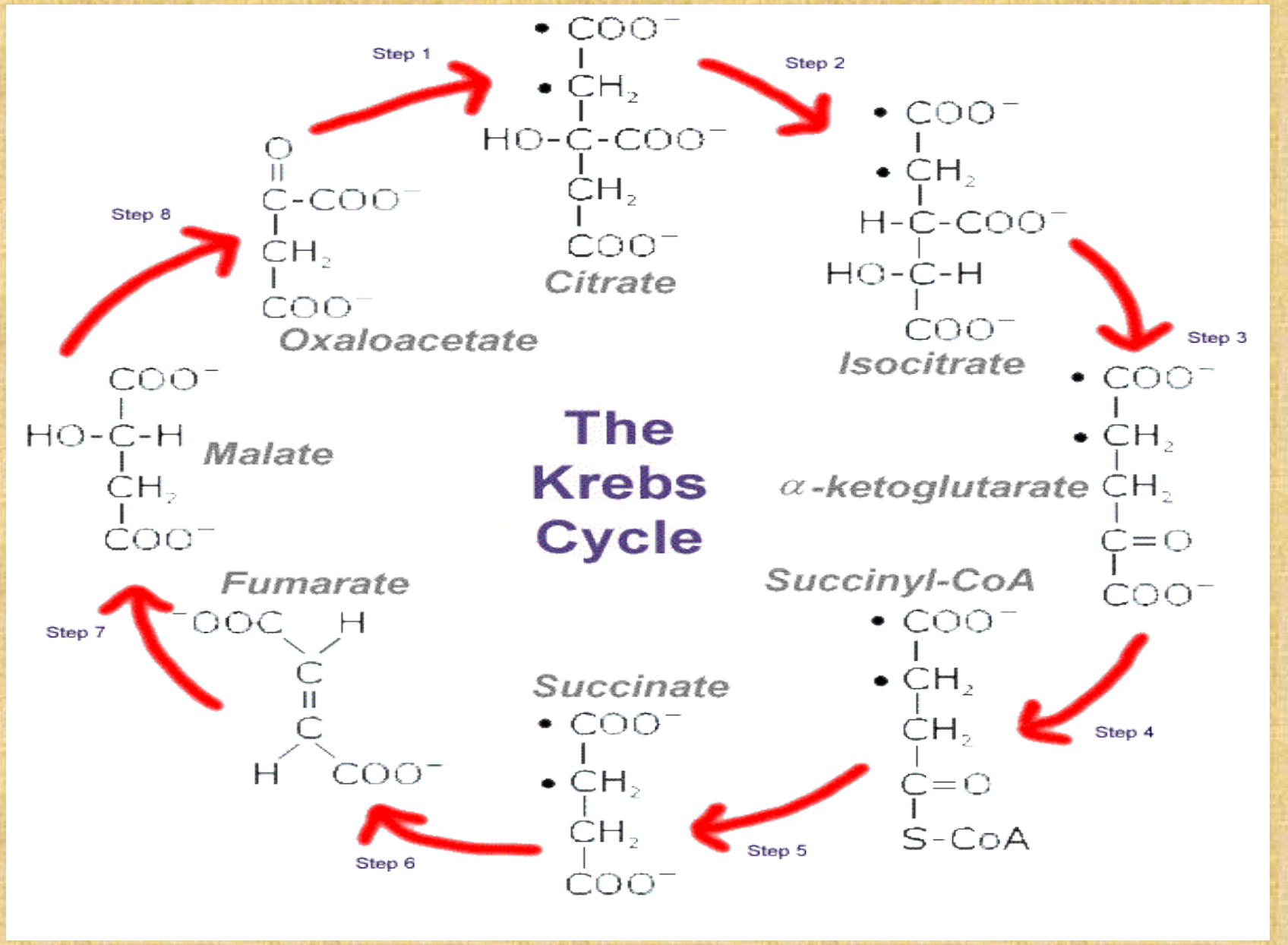
- For every **NADH and $FADH_2$** that are produced in the citric acid cycle, **2.5 and 1.5 ATP molecules are generated in oxidative phosphorylation**, respectively.

- At the end of each cycle, **the four-carbon oxaloacetate** has been regenerated, and the cycle continues

- **Overview**

Production OF Acetyl-COA




The Krebs Cycle
Step 1
Step 2
Step 3
Step 4
Step 5
Step 6
Step 7
Step 8
Citrate
Isocitrate
α-ketoglutarate
Succinyl-CoA
Succinate
Fumarate
Malate
Oxaloacetate

Efficiency

- The theoretical maximum yield of ATP through oxidation of one molecule of glucose in glycolysis, citric acid cycle, and oxidative phosphorylation is 38 (assuming 3 molar equivalentsof ATP per equivalent NADH and 2 ATP per $FADH_2$).
- In eukaryotes, two equivalents of NADH are generated in glycolysis, which takes place in the cytoplasm.
- Transport of these two equivalents into the mitochondria consumes two equivalents of ATP, thus reducing the net production of ATP to 36.
- Furthermore, inefficiencies in oxidative phosphorylation due to leakage of protons across the mitochondrial membrane and slippage of the ATP synthase/proton pump commonly reduces the ATP yield from NADH and $FADH_2$ to less than the theoretical maximum yield.
- The observed yields are, therefore, closer to ~2.5 ATP per NADH and ~1.5 ATP per $FADH_2$, further reducing the total net production of ATP to approximately 30.
- An assessment of the total ATP yield with newly revised proton-to-ATP ratios provides an estimate of 29.85 ATP per glucose molecule.

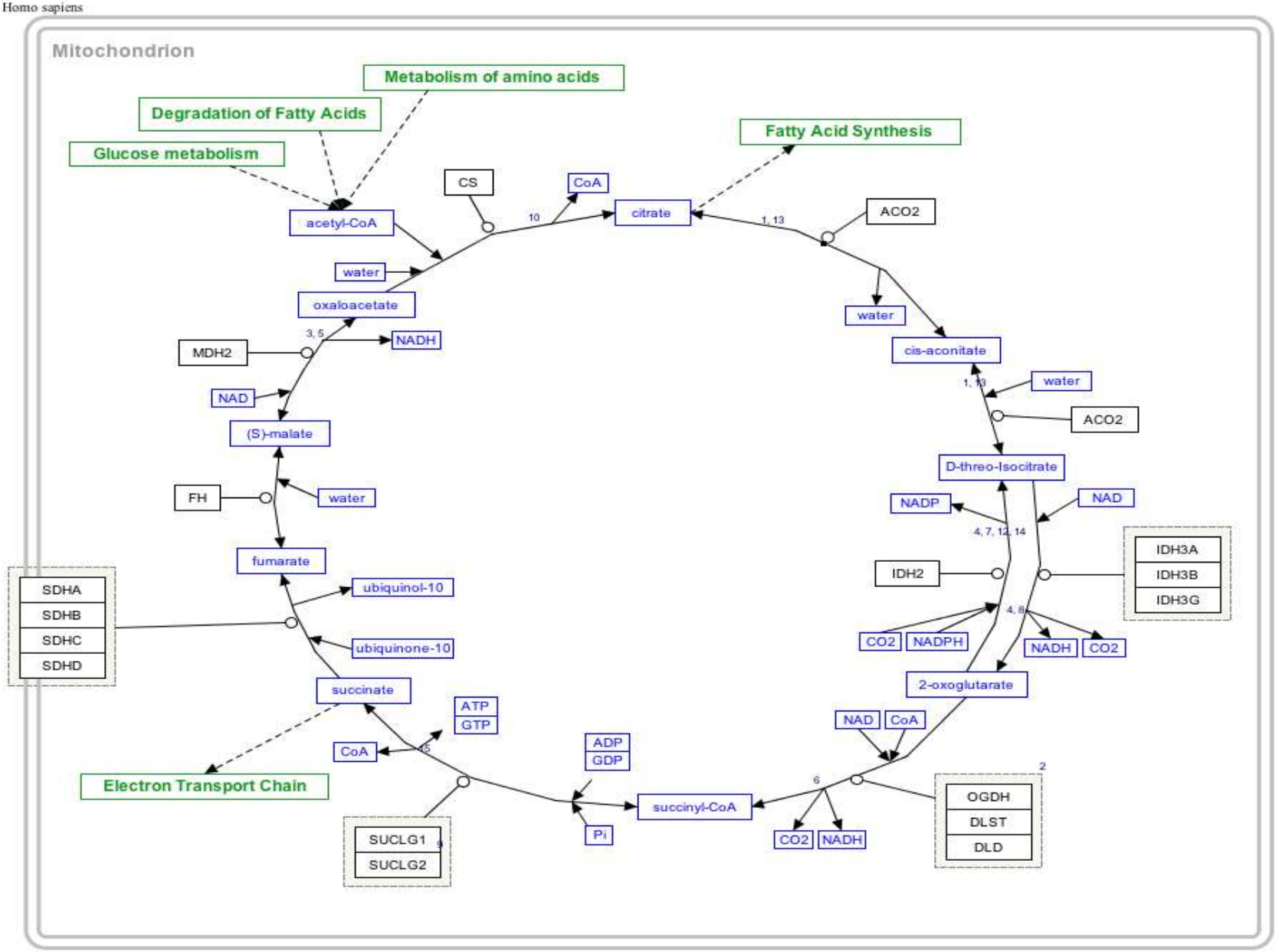
❑ Regulation

- The regulation of the citric acid cycle is largely determined by product inhibition and substrate availability. If the cycle were permitted to run unchecked, large amounts of metabolic energy could be wasted in overproduction of reduced coenzyme such as NADH and ATP.
- The major eventual substrate of the cycle is ADP which gets converted to ATP. A reduced amount of ADP causes accumulation of precursor NADH which in turn can inhibit a number of enzymes.
- NADH, a product of all dehydrogenases in the citric acid cycle with the exception of succinate dehydrogenase, inhibits pyruvate dehydrogenase, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and also citrate synthase.
- Acetyl-coA inhibits pyruvate dehydrogenase, while succinyl-CoA inhibits alpha-ketoglutarate dehydrogenase and citrate synthase.
- When tested in vitro with TCA enzymes, ATP inhibits citrate synthase and α-ketoglutarate dehydrogenase; however, ATP levels do not change more than 10% in vivo between rest and vigorous exercise. There is no known allosteric mechanism that can account for large changes in reaction rate from an allosteric effector whose concentration changes less than 10%.

- Calcium is also used as a regulator in the citric acid cycle.
- Calcium levels in the mitochondrial matrix can reach up to the tens of micromolar levels during cellular activation.
- It activates pyruvate dehydrogenase phosphatase which in turn activates the pyruvate dehydrogenase complex.
- Calcium also activates isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.[32]
- This increases the reaction rate of many of the steps in the cycle, and therefore increases flux throughout the pathway.
- Citrate is used for feedback inhibition, as it inhibits phosphofructokinase, an enzyme involved in glycolysis that catalyses formation of fructose 1,6-bisphosphate, a precursor of pyruvate.
- This prevents a constant high rate of flux when there is an accumulation of citrate and a decrease in substrate for the enzyme.
- Recent work has demonstrated an important link between intermediates of the citric acid cycle and the regulation of hypoxia-inducible factors (HIF).

- HIF plays a role in the regulation of oxygen homeostasis, and is a transcription factor that targets angiogenesis, vascular remodeling, glucose utilization, iron transport and apoptosis.
- HIF is synthesized consititutively, and hydroxylation of at least one of two critical proline residues mediates their interaction with the von Hippel Lindau E3 ubiquitin ligase complex, which targets them for rapid degradation.
- This reaction is catalysed by prolyl 4-hydroxylases. Fumarate and succinate have been identified as potent inhibitors of prolyl hydroxylases, thus leading to the stabilisation of HIF.

Title: TCA Cycle
Availability: CC BY 2.0 [11]
Organism: Homo sapiens



Citric acid cycle intermediates serve as substrates for biosynthetic processes

In this subheading, as in the previous one, the TCA intermediates are identified by italics.
Several of the citric acid cycle intermediates are used for the synthesis of important compounds, which will have significant cataplerotic effects on the cycle. Acetyl-CoA cannot be transported out of the mitochondrion. To obtain cytosolic acetyl-CoA, citrate is removed from the citric acid cycle and carried across the inner mitochondrial membrane into the cytosol. There it is cleaved by ATP citrate lyase into acetyl-CoA and oxaloacetate. The oxaloacetate is returned to mitochondrion as malate (and then converted back into oxaloacetate to transfer more acetyl-CoA out of the mitochondrion). The cytosolic acetyl-CoA is used for fatty acid synthesis and the production of cholesterol. Cholesterol can, in turn, be used to synthesize the steroid hormones, bile salts, and vitamin D.

- To turn them into amino acids the alpha keto-acids formed from the citric acid cycle intermediates have to acquire their amino groups from glutamate in a transamination reaction, in which pyridoxal phosphate is a cofactor.
- In this reaction the glutamate is converted into alpha-ketoglutarate, which is a citric acid cycle intermediate. The intermediates that can provide the carbon skeletons for amino acid synthesis are oxaloacetate which forms aspartate and asparagine; and alpha-ketoglutarate which forms glutamine, proline, and arginine.
- Of these amino acids, aspartate and glutamine are used, together with carbon and nitrogen atoms from other sources, to form the purines that are used as the bases in DNA and RNA, as well as in ATP, AMP, GTP, NAD, FAD and CoA.
- The pyrimidines are partly assembled from aspartate (derived from oxaloacetate). The pyrimidines, thymine, cytosine and uracil, form the complementary bases to the purine bases in DNA and RNA, and are also components of CTP, UMP, UDP and UTP.
- The majority of the carbon atoms in the porphyrins come from the citric acid cycle intermediate, succinyl-CoA. These molecules are an important component of the hemoproteins, such as hemoglobin, myoglobin and various cytochromes.

- During gluconeogenesis mitochondrial *oxaloacetate* is reduced to *malate* which is then transported out of the mitochondrion, to be oxidized back to oxaloacetate in the cytosol.
- Cytosolic oxaloacetate is then decarboxylated to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase, which is the rate limiting step in the conversion of nearly all the gluconeogenic precursors (such as the glucogenic amino acids and lactate) into glucose by the liver and kidney.
- Because the citric acid cycle is involved in both catabolic and anabolic processes, it is known as an amphibolic pathway.