The citric acid cycle

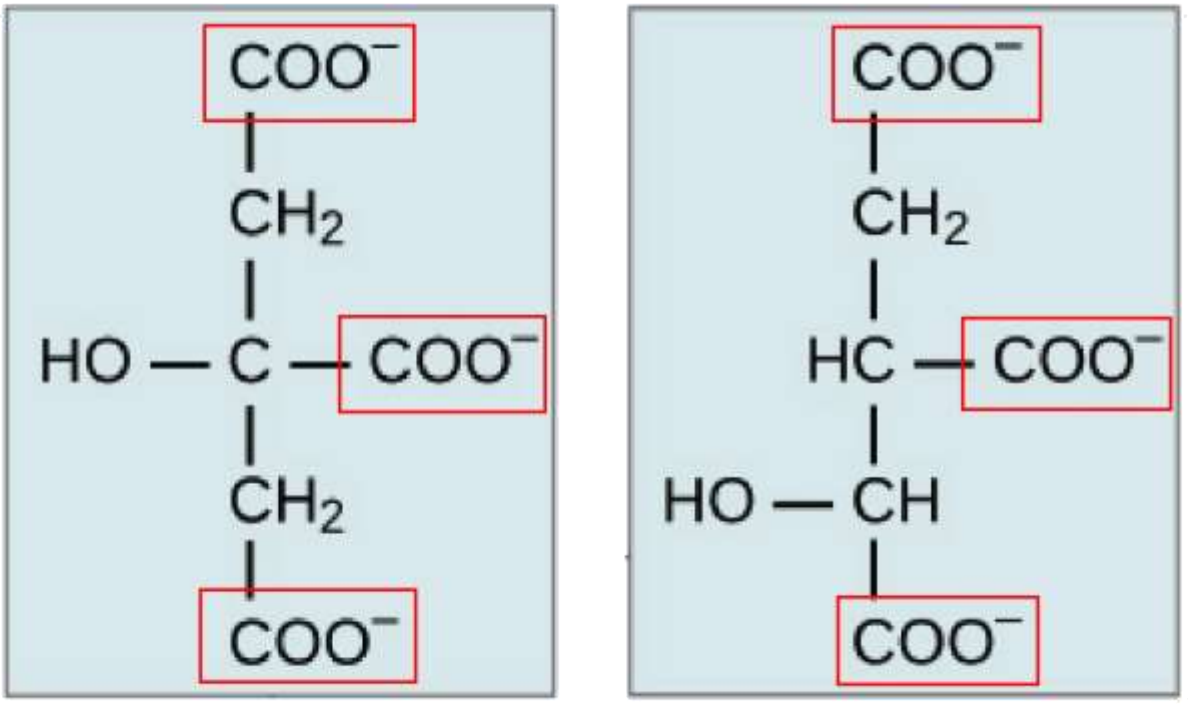
Overview and steps of the citric acid cycle, also known as the Krebs cycle or tricarboxylic acid (TCA) cycle.

Introduction

How important is the citric acid cycle? So important that it has not one, not two, but three different names in common usage today!

The name we'll primarily use here, the citric acid cycle, refers to the first molecule that forms during the cycle's reactions—citrate, or, in its protonated form, citric acid. However, you may also hear this series of reactions called the tricarboxylic acid (TCA) cycle, for the three carboxyl groups on its first two intermediates, or the Krebs cycle, after its discoverer, Hans Krebs.

The first two intermediates of the citric acid cycle are shown below. Each has three carboxyl groups, marked with red boxes. When citrate gains three $H+$ ions, so that it no longer has a negative charge, it is called citric acid.



Citrate   Isocitrate

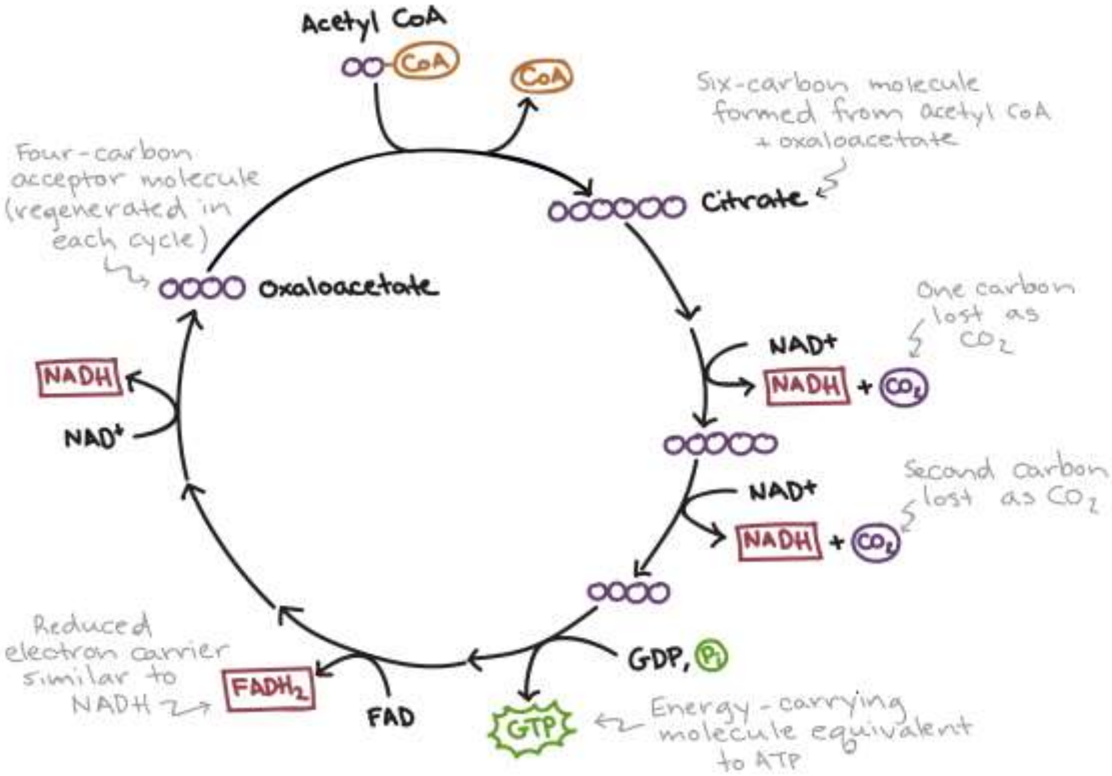
image credit: modified from "Oxidation of pyruvate and citric acid cycle: Figure 2" by OpenStax College, Biology, CC BY 3.0

Whatever you prefer to call it, the citric cycle is a central driver of cellular respiration. It takes acetyl CoA—produced by the oxidation of pyruvate and originally derived from glucose—as its starting material and, in a series of redox reactions, harvests much of its bond energy in the form of $NADH$, $FADH_2$, and ATP molecules. The reduced electron carriers—$NADH$ and $FADH_2$ generated in the TCA cycle will pass their electrons into the electron transport chain and, through oxidative phosphorylation, will generate most of the ATP produced in cellular respiration.

Below, we'll look in more detail at how this remarkable cycle works.

Overview of the citric acid cycle

In eukaryotes, the citric acid cycle takes place in the matrix of the mitochondria, just like the conversion of pyruvate to acetyl CoA. In prokaryotes, these steps both take place in the cytoplasm. The citric acid cycle is a closed loop; the last part of the pathway reforms the molecule used in the first step. The cycle includes eight major steps.



Simplified diagram of the citric acid cycle. First, acetyl CoA combines with oxaloacetate, a four-carbon molecule, losing the CoA group and forming the six-carbon molecule citrate. After citrate undergoes a rearrangement step, it undergoes an oxidation reaction, transferring electrons to NAD^+ to form NADH and releasing a molecule of carbon dioxide. The five-carbon molecule left behind then undergoes a second, similar reaction, transferring electrons to NAD^+ to form NADH and releasing a carbon dioxide molecule. The four-carbon molecule remaining then undergoes a series of transformations, in the course of which GDP and inorganic phosphate are converted into GTP—or, in some organisms, ADP and inorganic phosphate are converted into ATP—an FAD molecule is reduced to $FADH_2$, and another NAD^+ is reduced to NADH. At the end of this series of reactions, the four-carbon starting molecule, oxaloacetate, is regenerated, allowing the cycle to begin again.

In the first step of the cycle, acetyl CoA combines with a four-carbon acceptor molecule, oxaloacetate, to form a six-carbon molecule called citrate. After a quick rearrangement, this six-carbon molecule releases two of its carbons as carbon dioxide molecules in a pair of similar reactions, producing a molecule of NADH each time. The enzymes that catalyse these reactions are key regulators of the citric acid cycle, speeding it up or slowing it down based on the cell's energy needs.

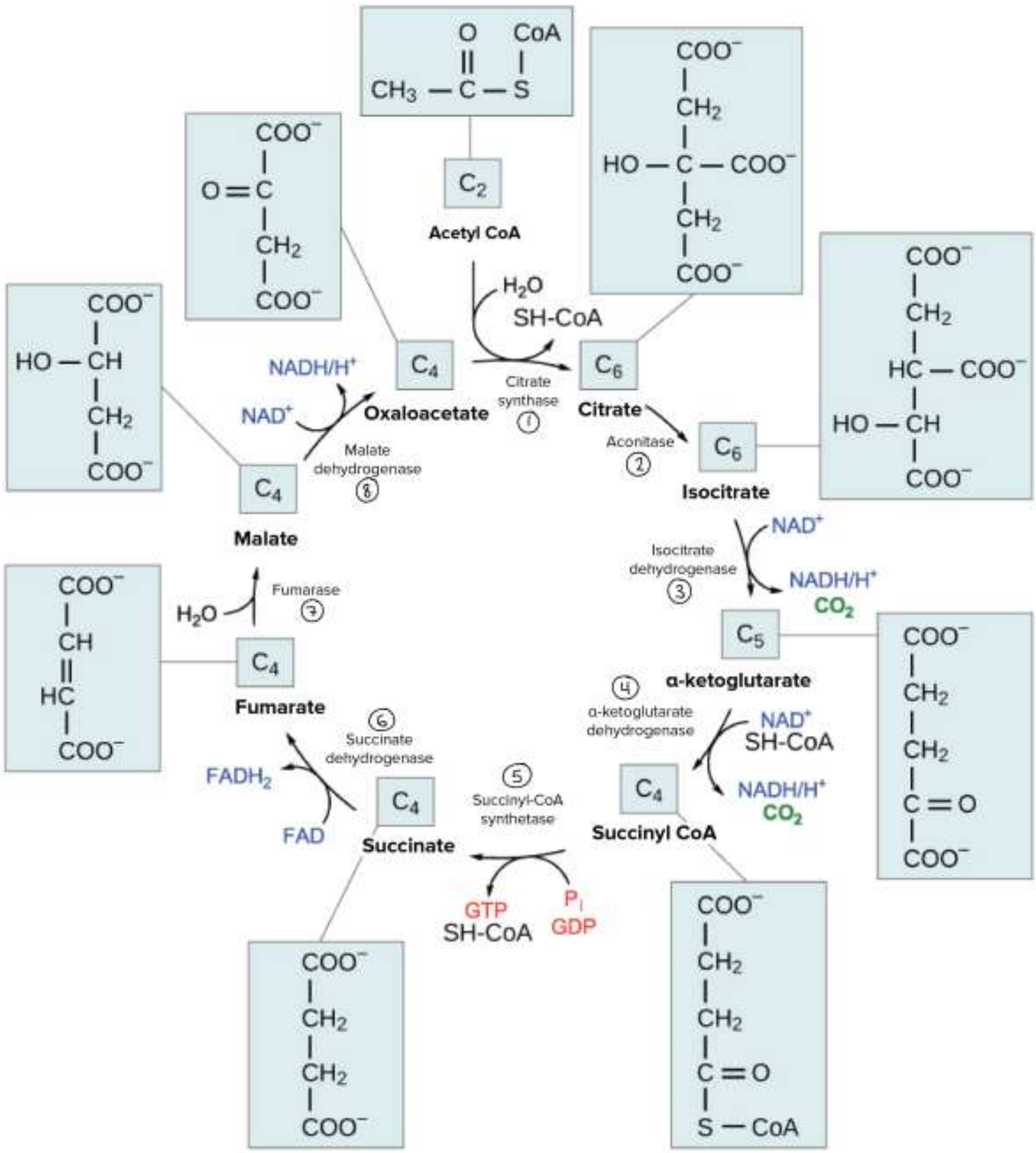
The remaining four-carbon molecule undergoes a series of additional reactions, first making an ATP molecule—or, in some cells, a similar molecule called GTP—then reducing the electron carrier FAD to $FADH_2$ and finally generating another NADH. This set of reactions regenerates the starting molecule, oxaloacetate, so the cycle can repeat.

Overall, one turn of the citric acid cycle releases two carbon dioxide molecules and produces three NADH one $FADH_2$and one ATP or GTP. The citric acid cycle goes around twice for each molecule of glucose that enters cellular respiration because there are two pyruvates—and thus, two acetyl CoA's—made per glucose.

Steps of the citric acid cycle

You've already gotten a preview of the molecules produced during the citric acid cycle. But how, exactly, are those molecules made? We'll walk through the cycle step by step, seeing how NADH, $FADH_2$ and ATP / GTP are produced and where carbon dioxide molecules are released.

Step 1. In the first step of the citric acid cycle, acetyl CoA joins with a four-carbon molecule, oxaloacetate, releasing the CoA group and forming a six-carbon molecule called citrate.
Step 2. In the second step, citrate is converted into its isomer, isocitrate. This is actually a two-step process, involving first the removal and then the addition of a water molecule, which is why the citric acid cycle is sometimes described as having nine steps—rather than the eight listed here.
Step 3. In the third step, isocitrate is oxidized and releases a molecule of carbon dioxide, leaving behind a five-carbon molecule—α-ketoglutarate. During this step, NAD^+ is reduced to form NADH. The enzyme catalysing this step, **isocitrate dehydrogenase**, is important in regulating the speed of the citric acid cycle.
Step 4. The fourth step is similar to the third. In this case, it's α-ketoglutarate that's oxidized, reducing NAD^+ to NADH and releasing a molecule of carbon dioxide in the process. The remaining four-carbon molecule picks up Coenzyme A, forming the unstable compound succinyl CoA. The enzyme catalysing this step, **α-ketoglutarate dehydrogenase**, is also important in regulation of the citric acid cycle.



Detailed diagram of the citric acid cycle, showing the structures of the various cycle intermediates and the enzymes catalysing each step.

Step 1. Acetyl CoA combines with oxaloacetate in a reaction catalysed by citrate synthase. This reaction also takes a water molecule as a reactant, and it releases a SH-CoA molecule as a product.

Step 2. Citrate is converted into isocitrate in a reaction catalysed by aconitase.

Step 3. Isocitrate is converted into α-ketoglutarate in a reaction catalysed by isocitrate dehydrogenase. An NAD^+ molecule is reduced to NADH + H^+ in this reaction, and a carbon dioxide molecule is released as a product.

Step 4. α-ketoglutarate is converted to succinyl CoA in a reaction catalysed by α-ketoglutarate dehydrogenase. An NAD^+ molecule is reduced to NADH + H^+ in this reaction, which also takes a SH-CoA molecule as reactant. A carbon dioxide molecule is released as a product.

Step 5. Succinyl CoA is converted to succinate in a reaction catalysed by the enzyme succinyl-CoA synthetase. This reaction converts inorganic phosphate, Pi, and GDP to GTP and also releases a SH-CoA group.

Step 6. Succinate is converted to fumarate in a reaction catalyzed by succinate dehydrogenase. FAD is reduced to $FADH_2$ in this reaction.
Step 7. Fumarate is converted to malate in a reaction catalyzed by the enzyme fumarase. This reaction requires a water molecule as a reactant.
Step 8. Malate is converted to oxaloacetate in a reaction catalyzed by malate dehydrogenase. This reaction reduces an NAD^+ molecule to NADH + H^+.

Step 5. In step five, the CoA of succinyl CoA is replaced by a phosphate group, which is then transferred to ADP to make ATP. In some cells, GDP—guanine diphosphate—is used instead of ADP forming GTP—guanine triphosphate—as a product. The four-carbon molecule produced in this step is called succinate.

GTP is similar to ATP: both serve as energy sources, and the two can be readily interconverted. Which of the two molecules is produced during the citric acid cycle depends on the organism and cell type. For example, ATP is made in human heart cells, but GTP is made in liver cells.
Step 6. In step six, succinate is oxidized, forming another four-carbon molecule called fumarate. In this reaction, two hydrogen atoms—with their electrons—are transferred to FAD producing $FADH_2$ start subscript, 2, end subscript. The enzyme that carries out this step is embedded in the inner membrane of the mitochondrion, so $FADH_2$ can transfer its electrons directly into the electron transport chain.

FAD is a better electron acceptor than NAD+N, A, D, start superscript, plus, end superscript, meaning that it has a higher affinity, or "hunger", for electrons. Succinate is not a great electron donor, meaning that it has a fairly high affinity for electrons itself and is not eager to give them up. NAD^+ is not electron-hungry enough to pull electrons away from succinate, but FAD is[4,5].
Step 7. In step seven, water is added to the four-carbon molecule fumarate, converting it into another four-carbon molecule called malate.

Step 8. In the last step of the citric acid cycle, oxaloacetate—the starting four-carbon compound—is regenerated by oxidation of malate. Another molecule of NAD^+ is reduced to NADH in the process.

Products of the citric acid cycle

Let's take a step back and do some accounting, tracing the fate of the carbons that enter the citric acid cycle and counting the reduced electron carriers—NADH and $FADH_2$—and ATP produced.

In a single turn of the cycle,

- two carbons enter from acetyl CoA, and two molecules of carbon dioxide are released;
- three molecules of NADH and one molecule of $FADH_2$ are generated; and
- one molecule of ATP is produced.

These figures are for one turn of the cycle, corresponding to one molecule of acetyl CoA. Each glucose produces two acetyl CoA molecules, so we need to multiply these numbers by 2 if we want the per-glucose yield.

Two carbons—from acetyl CoA—enter the citric acid cycle in each turn, and two carbon dioxide molecules are released. However, the carbon dioxide molecules don't actually contain carbon atoms from the acetyl CoA that just entered the cycle. Instead, the carbons from acetyl CoA are initially incorporated into the intermediates of the cycle and are released as carbon dioxide only during later turns. After enough turns, all the carbon atoms from the acetyl group of acetyl CoA will be released as carbon dioxide.

Where's all the ATP?

You may be thinking that the ATP output of the citric acid cycle seems pretty unimpressive. All that work for just one ATP or GTP?

It's true that the citric acid cycle doesn't produce much ATP directly. However, it can make a lot of ATP *indirectly*, by way of the NADH and FADH_2 , it generates. These electron carriers will connect with the last portion of cellular respiration, depositing their electrons into the electron transport chain to drive synthesis of ATP molecules through oxidative phosphorylation.