The Tricarboxylic Acid Cycle

Background (why are eight enzymes necessary?)
In principle, acetyl-CoA could be converted to carbon dioxide very simply. However, doing so has three potential problems:

1) A single reaction would **not** allow the recovery of much energy in usable form (a single reaction might result in one NADH molecule, or one ATP, or at most, one of each, while the TCA cycle results in several reduced cofactors and therefore in much more conservation of the energy in the acetyl-CoA molecule).
2) Two-carbon compounds are a little difficult to work with, because they are small enough to readily diffuse away from the enzyme.
3) Enzymes lack magical powers; they must obey the laws of chemistry, and therefore can only catalyze some types of reactions.

Cleaving carbon-carbon bonds in a controlled manner is not always possible. For carbonyl-containing compounds, two types of cleavage events are typically possible: β-cleavage (cleaving the bond between the carbons α and β to the carbonyl carbon) and α-cleavage (cleaving the bond between the carbonyl carbon and the α-carbon).

Neither of these reactions is possible for acetate: the two-carbon molecule acetate does not have a β-carbon, and adding a hydroxyl to acetate would be difficult. The series of reactions that oxidize acetyl-CoA involve first attaching the two carbon unit to a larger compound, which avoids some problems with the direct cleavage of acetyl-CoA.
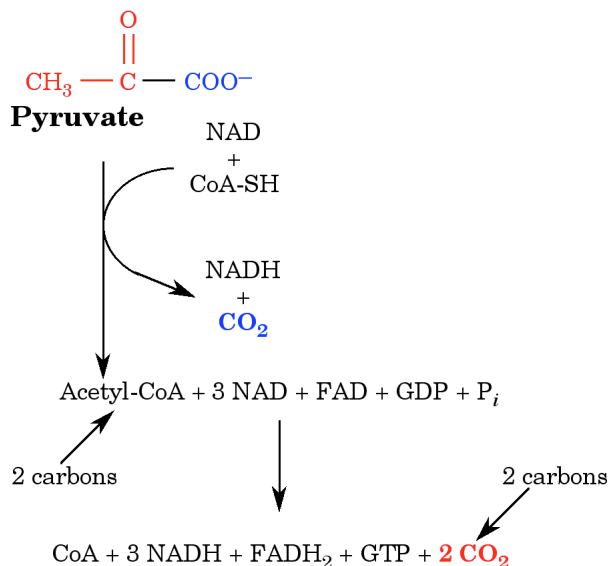
The tricarboxylic acid cycle (usually abbreviated as "TCA cycle") results in the conversion of acetate to carbon dioxide in a controlled and efficient manner that retains much of the energy inherent in the acetyl-CoA molecule, although doing so requires a total of eight enzymes.

The original name for the pathway proposed by Hans Krebs was "citric acid cycle"; for a while there was some doubt as to whether citrate was actually used in the cycle, and the name was altered to tricarboxylic acid cycle. Both names are still used. The cycle is sometimes called the Krebs cycle in honor of Hans Krebs, although it is actually the *second* Krebs cycle (his discovery of the urea cycle was several years earlier).

Net reaction for the TCA cycle
Note that, while running the TCA cycle, there is no change in the amount of carbon in the cycle. For each two-carbon unit that enters in the form of acetyl-CoA, two

carbons are lost in the form of CO_2. The only change is the generation of one GTP, one reduced flavin, and three reduced nicotinamide cofactors. In effect, the **TCA cycle intermediates act as coenzymes** in the oxidation of acetyl-CoA, and are neither produced nor consumed in the process.

$$O$$
$$\|$$
$$CH_3 \text{---} C \text{---} COO^-$$

Pyruvate

NAD
+
CoA-SH

NADH
+
CO_2

Acetyl-CoA + 3 NAD + FAD + GDP + P$_i$

2 carbons   2 carbons

CoA + 3 NADH + FADH$_2$ + GTP + **2 CO_2**

The catalytic nature of the TCA cycle means that acetyl-CoA cannot be used to synthesize TCA cycle intermediates. If more TCA cycle intermediates are necessary, they can be obtained from amino acid breakdown or from pyruvate via the pyruvate carboxylase reaction. (This latter reaction explains in part why acetyl-CoA concentration regulates pyruvate carboxylase.)
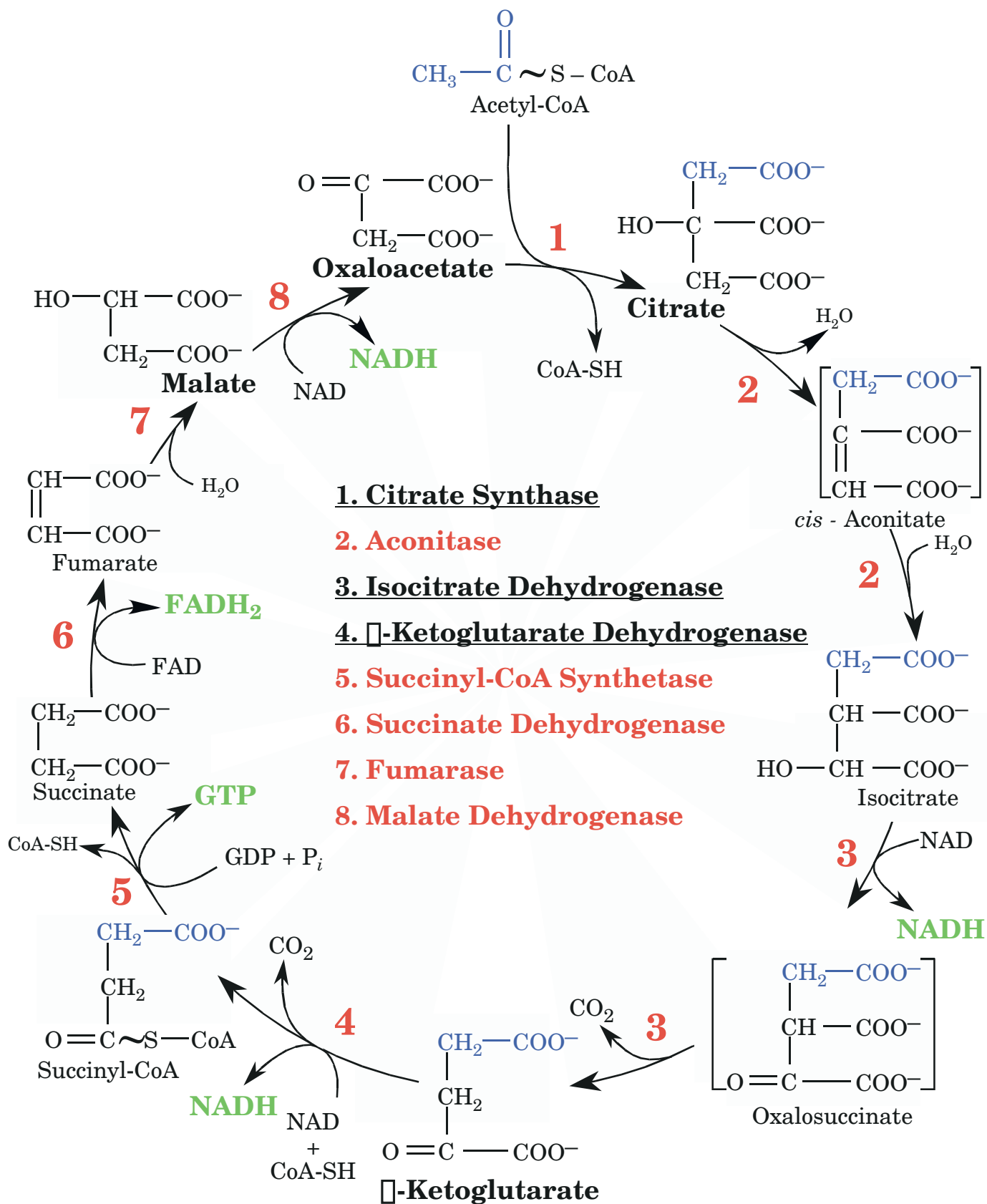
Background (discovery)
Albert Szent-Györgyi and Hans Krebs and a number of other biochemists worked on the pathway for oxidation of pyruvate. Szent-Györgyi found that adding certain compounds stimulated the oxidation process in an apparently catalytic manner. Krebs had invented the cycle concept in explaining the urea cycle in 1932; in 1937, he proposed that pyruvate oxidative metabolism also involved a cycle.

Some details required additional work; Coenzyme A was not discovered until 1945, and acetyl-CoA was not established to be the major starting material until the work of Severo Ochoa and Feodor Lynen in 1951.

Reactions and enzymes
The diagram below shows the reactions of the tricarboxylic acid cycle. The enzymes are numbered, and listed in the center of the cycle (note that aconitase and isocitrate dehydrogenase each catalyze two of the steps shown in the diagram). The enzymes in underlined black text (citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase) catalyze physiologically irreversible steps and function as regulatory control points for the cycle.

Tricarboxylic Acid Cycle

$CH_3-\overset{\overset{\displaystyle O}{\|}}{C}\sim S-CoA$
Acetyl-CoA

$O=C-COO^-$
CH_2-COO^-
Oxaloacetate

CH_2-COO^-
$HO-C-COO^-$
CH_2-COO^-
Citrate

1

CoA-SH

NADH

NAD

H_2O

2

$\left[\begin{array}{l} CH_2-COO^- \\ C-COO^- \\ CH-COO^- \end{array}\right]$
cis - Aconitate

$HO-CH-COO^-$
CH_2-COO^-
Malate

8

7

$CH-COO^-$
$CH-COO^-$
Fumarate

H_2O

H_2O

2

CH_2-COO^-
$CH-COO^-$
$HO-CH-COO^-$
Isocitrate

FADH$_2$

6

FAD

CH_2-COO^-
CH_2-COO^-
Succinate

1. **Citrate Synthase**
2. **Aconitase**
3. **Isocitrate Dehydrogenase**
4. **α-Ketoglutarate Dehydrogenase**
5. **Succinyl-CoA Synthetase**
6. **Succinate Dehydrogenase**
7. **Fumarase**
8. **Malate Dehydrogenase**

GTP

GDP + P$_i$

5

CoA-SH

CH_2-COO^-
CH_2
$O=C\sim S-CoA$
Succinyl-CoA

4

NADH

NAD
+
CoA-SH

CO_2

CH_2-COO^-
CH_2
$O=C-COO^-$
α-Ketoglutarate

CO_2

3

3

NAD

NADH

$\left[\begin{array}{l} CH_2-COO^- \\ CH-COO^- \\ O=C-COO^- \end{array}\right]$
Oxalosuccinate

1. Citrate synthase

Citrate synthase catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate. This reaction has a large $-\Delta G'^\circ$ ($\Delta G'^\circ = -31.5$ kJ/mol), because it uses the energy from the high-energy bond between the acetate and the CoA to drive the condensation reaction.

Citrate synthase is **one of three irreversible enzymes in the TCA cycle**, and is **one of the control points for the cycle**. The product, citrate, is an important molecule: it is used to regulate glycolysis (it inhibits phosphofructokinase), and it is required for fatty acid synthesis (it stimulates the activity of acetyl-CoA carboxylase, the control enzyme for that pathway, and also acts as a source of the cytoplasmic acetyl-CoA required as a substrate for the fatty acid synthesis pathway).

The reaction mechanism for citrate synthase has been extensively studied. It has been used as a model for understanding reactions involving carbonyls, and as a system for studying general catalytic principles. The citrate synthase from pigs is the only mammalian enzyme to be crystallized, and it is the basis of some the discussion below.

Oxaloacetate binds the enzyme first (if oxaloacetate does not bind first, the presence of acetyl-CoA will prevent oxaloacetate binding). The binding of the substrate is thought to result in a conformational change that is required for catalysis, based on analysis of crystal structures solved in the presence and absence of various substrates, products, and substrate analogs. The conformational change upon substrate binding is evident in the structures below. The blue structure was crystallized in the absence of Coenzyme A, and has the open conformation. The yellow structure has Coenzyme A bound, and is thought to reflect the catalytically active conformation of the enzyme. (Although citrate synthase is a dimer, for clarity, the structure shown is of one monomer.) The close-up view (on the right) shows the active site of the enzyme. The two catalytically important histidine residues are highlighted. Note that although His^{274} remains in a similar location, His^{320} moves significantly following binding of the Coenzyme A molecule.

62
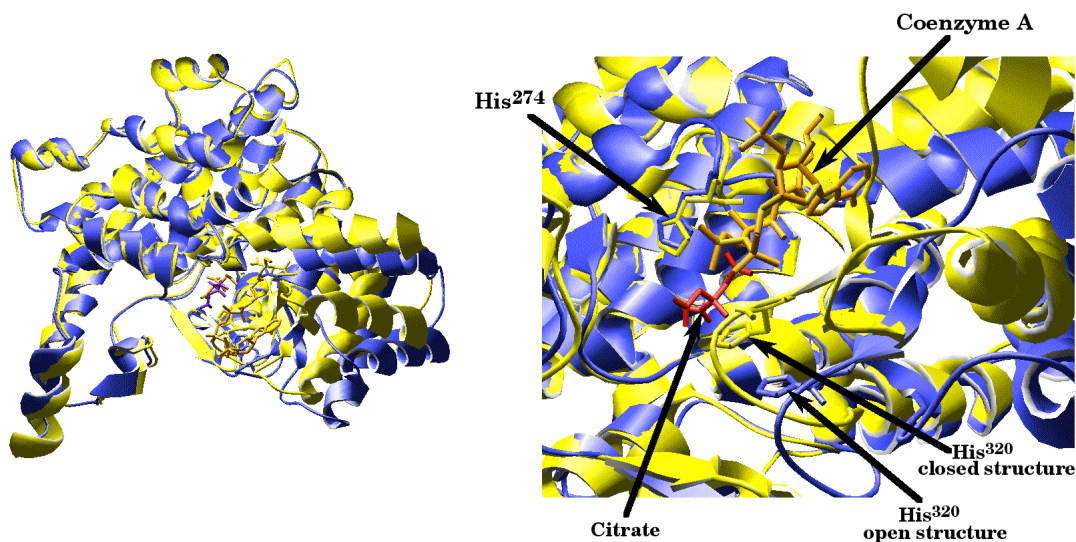
Once both substrates have bound, an essential aspartate (Asp^{375} in the porcine enzyme) abstracts a proton from the acetyl-CoA methyl group, allowing the methyl group to perform a nucleophilic attack on the oxaloacetate carbonyl carbon. Some studies have suggested that the acetyl-CoA forms an enol intermediate, with the thioester carbonyl being protonated; more recent studies, however, have shown that the process is probably concerted, and that His^{274} does not actually donate a proton to the substrate. Note that the citryl-CoA intermediate product must obtain a proton for its hydroxyl group; it abstracts a proton from an uncharged histidine (His^{320}), resulting in an unusual negatively charged histidine. The protonation of the aspartate and the deprotonation of the neutral histidine illustrate some of the pK$_a$ changes that are possible in the enclosed environment of an enzyme active site.

Once formed, the citryl-CoA is hydrolyzed to release free citrate and Coenzyme A. Most studies suggest that the hydrolysis step is rate limiting for the overall reaction; this step is also the physiologically irreversible portion of the reaction.
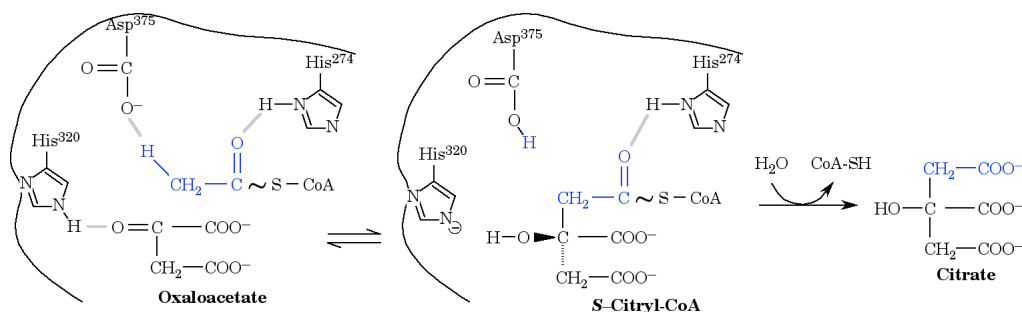
Citrate synthase is an allosterically regulated enzyme. Its regulation will be discussed below.

Side note: Conformational changes and catalysis
The conformational flexibility shown above is thought to have a role in the actual catalytic process (in addition to merely placing the active site residues in the correct orientation). The bacterium *Thermoplasma acidophilum* prefers temperatures of 70°C. Its citrate synthase (unlike that of pigs or humans) must therefore be stable at high temperatures. Studies on the *T. acidophilum* citrate synthase suggest that it achieves its high thermostability by having a more rigid structure. The rigid structure appears to have some drawbacks, however. The porcine enzyme, tested at 37°C, has a roughly 4-fold higher k_{cat} than the *T. acidophilum* citrate synthase has at 70°C. Normal chemical reaction rates increase by about 2-fold for every 10°C; the fact that the bacterial enzyme exhibits a lower rate in spite of the higher temperature suggests that it is less efficient than the porcine enzyme. The bacterium therefore appears to have traded catalytic efficiency for enhanced thermostability.

2. Aconitase
Aconitase catalyzes the reversible interconversion of citrate and isocitrate via an intermediate, *cis*-aconitate, which does not appear to be released from the enzyme.

However, the *cis*-aconitate appears to rotate within the active site without dissociating. Aconitase contains an iron-sulfur center prosthetic group. Iron-sulfur centers are usually found in proteins involved in electron transfer processes; aconitase is therefore unusual in that its reaction does not involve a redox mechanism.

3. Isocitrate dehydrogenase

Isocitrate dehydrogenase catalyzes the second irreversible step in the pathway, the decarboxylation of isocitrate to yield α-ketoglutarate. The process occurs in two steps. The first step is the dehydrogenase reaction, which forms the reduced nicotinamide cofactor and a six-carbon intermediate, oxalosuccinate. The oxalosuccinate is then converted to α-ketoglutarate, in an irreversible reaction driven by the loss of carbon dioxide. The isocitrate dehydrogenase reaction results in **formation of the first NADH**. Isocitrate dehydrogenase is probably the most important **regulated step** in the TCA cycle; its control allows the diversion of citrate to other purposes.

The oxidation reaction of isocitrate dehydrogenase results in formation of a ketone β to one of the carboxylates of the product oxalosuccinate. The as is true for many α-keto- and β-ketoacids, oxalosuccinate is relatively unstable, and is readily decarboxylated to produce α-ketoglutarate.
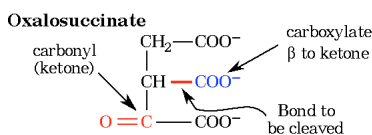
Most species have two isozymes of isocitrate dehydrogenase. The NAD-dependent isozyme is normally considered to be the TCA cycle enzyme, while the NADP-dependent enzyme is largely found in the cytoplasm, and is involved in production of NADPH for biosynthetic reactions.

4. α-Ketoglutarate dehydrogenase complex

The α-ketoglutarate dehydrogenase complex is an enzyme complex similar to the pyruvate dehydrogenase complex (it uses an identical E_3, and homologous E_1 and E_2, and uses all of the same coenzymes as the pyruvate dehydrogenase complex).

The α-ketoglutarate dehydrogenase complex catalyzes the oxidative decarboxylation of α-ketoglutarate, and both **forms the second NADH** and forms the high-energy bond present in succinyl-CoA. The reaction catalyzed by α-ketoglutarate dehydrogenase is the third irreversible step in the TCA cycle. It is also the **third control point of the TCA cycle**. The control of α-ketoglutarate dehydrogenase allows the diversion of α-ketoglutarate to other purposes; α-ketoglutarate is used for the synthesis of glutamate, and is crucial for both amino acid metabolism and synthesis of a number of other biologically relevant molecules.

5. Succinyl-CoA synthetase

Succinyl-CoA synthetase catalyzes the conversion of succinyl-CoA to succinate and

free CoA-SH. The energy of the high-energy bond in the succinyl-CoA is conserved in the substrate-level phosphorylation of GDP to form GTP. Because the reaction **GTP + ADP ⇌ GDP + ATP** has a $\Delta G'^{\circ}$ of zero, the formation of GTP is equivalent to forming an ATP.

Succinyl-CoA synthetase catalyzes a reversible reaction. The reaction mechanism involves the transfer of high-energy bonds from one compound to another. This transfer mechanism is necessary. If the first reaction, the loss of the coenzyme A, occurred as a simple hydrolysis step, the formation of GTP from GDP would be thermodynamically unfavorable. Instead, the energy of the succinyl-CoA bond is conserved by replacing the thioester with an acyl phosphate.

Like thioesters, acyl phosphates are high-energy compounds. A comparison of the structure of 1,3-bisphosphoglycerate (which is involved in the first substrate-level phosphorylation step in the glycolytic pathway) and succinyl phosphate reveals some significant similarities. The reaction mechanism of succinyl-CoA synthetase, however, differs from that of phosphoglycerate kinase. Instead of directly transferring the phosphate from the acyl phosphate to ADP, succinyl-CoA synthetase first transfers the phosphate to an active site histidine side-chain, releasing the succinate product, and forming another high-energy intermediate. It is this phosphohistidine residue that acts as the phosphate donor for GTP formation.
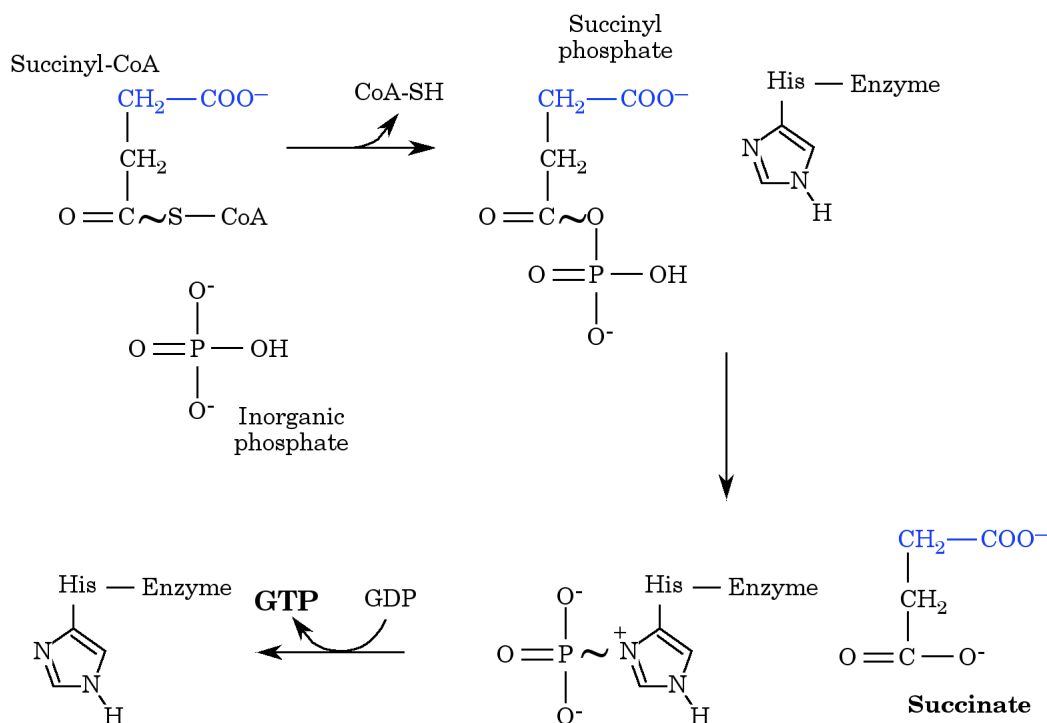
6. Succinate dehydrogenase
Succinate dehydrogenase contains a covalently bound FAD (this is somewhat unusual; although FAD is normally a prosthetic group, it is typically non-covalently associated with the enzyme). Succinate dehydrogenase is imbedded in the

mitochondrial inner membrane, and is the only one of the TCA cycle enzymes that is not located in the mitochondrial matrix. Succinate dehydrogenase oxidizes succinate to fumarate and **reduces FAD to FADH$_2$**. The succinate dehydrogenase FADH$_2$ transfers electrons directly to the electron transport chain. Succinate dehydrogenase will be discussed further in the section on electron transport.

The term "fumarate" refers to the *trans*-form of the four-carbon double-bond containing dicarboxylic acid. Maleate (the *cis*-form of the compound) is not a product of the reaction.

7. Fumarase
Fumarase adds a water molecule across the double bond in fumarate to produce L-malate. The precise mechanism for the fumarase reaction is still a matter for debate.

8. Malate Dehydrogenase
Malate dehydrogenase catalyzes the "last step" of the TCA cycle, the conversion of malate to oxaloacetate, with the production of the **final NADH**. This reaction has a large +ΔG´° (+29.7 kJ/mol), but operates at a ΔG close to zero under physiological conditions. The malate dehydrogenase reaction in "reverse" allows the direct conversion of oxaloacetate to malate; this is important because malate can be transported out of the mitochondria, while oxaloacetate cannot.

The reactions catalyzed by malate dehydrogenase, fumarase, succinate dehydrogenase, and succinyl-CoA synthetase are reversible and function at ΔG close to zero under physiological conditions. This means that, depending on other concurrent processes, these reactions may proceed clockwise (as drawn in the diagram at the beginning of this section) or may proceed counterclockwise, resulting in an energy-requiring conversion of oxaloacetate to succinyl-CoA. In many cases, it is only the large negative ΔG of the citrate synthase reaction that allows the pathway to proceed in the clockwise direction.
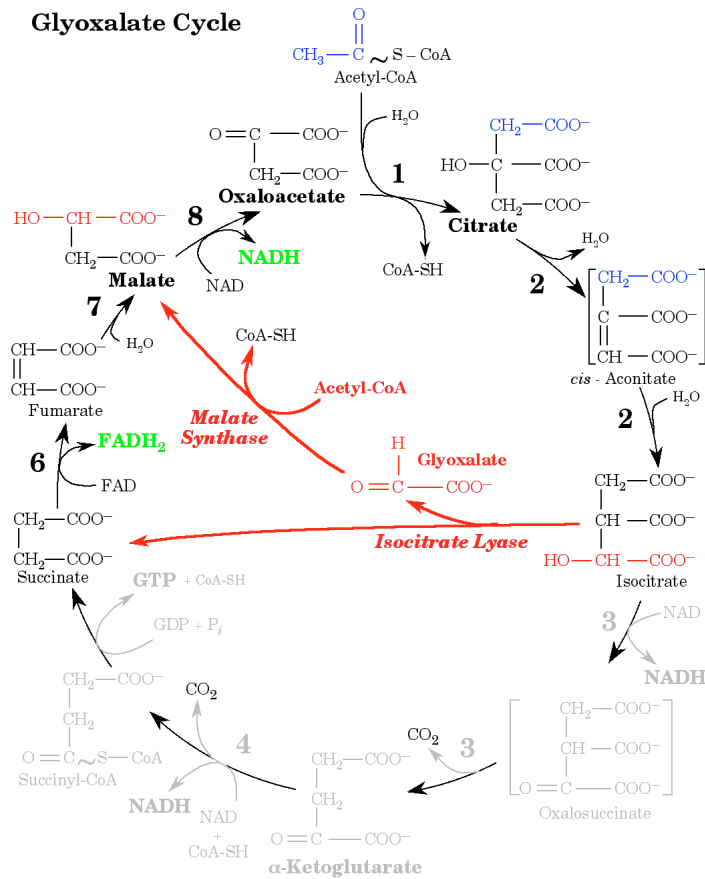

Glyoxalate cycle
Plants and some microorganisms can carry out a modified form of the TCA cycle. This pathway, the glyoxalate cycle, allows the use of acetyl-CoA to form oxaloacetate because it avoids the steps in which carbon dioxide is lost. The glyoxalate cycle must also bypass two of the NADH-producing steps and the substrate-level phosphorylation (Reactions 3-5 of the TCA cycle, shown in grey in the diagram below).

To achieve the net synthesis of TCA cycle intermediates from acetyl-CoA, the glyoxalate cycle uses two enzymes not present in the normal TCA cycle: **isocitrate lyase** and **malate synthase**. Isocitrate lyase splits isocitrate to form succinate and glyoxalate. Malate synthase then combines the glyoxalate with another molecule of acetyl-CoA to form malate. Note that isocitrate is located prior to the CO$_2$ loss steps, while both succinate and malate are after those steps in the pathway; the glyoxalate cycle therefore conserves both of the carbons in the acetyl-CoA that participates in the citrate synthase reaction.

The net reaction of the complete glyoxalate cycle is:

$$2 \text{ Acetyl-CoA} + 2 \text{ NAD} + \text{FAD} \rightarrow \text{Oxaloacetate} + 2 \text{ CoA} + 2 \text{ NADH} + \text{FADH}_2$$
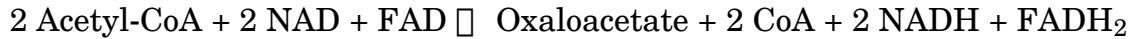
Note that this net reaction assumes that the succinate and malate formed both go on to oxaloacetate. The glyoxalate pathway therefore generates some energy in the form of reduced cofactors, although the amount of recovered energy is considerably smaller than would have been true if the acetyl-CoA molecule had been processed in the standard TCA cycle.

Animals cannot perform the glyoxalate cycle because they lack isocitrate lyase and malate synthase. As a result, **animals cannot use acetyl-CoA to synthesize TCA intermediates or pyruvate**.

Regulation of the TCA cycle

Substrate availability is a major regulator of the TCA cycle. Substrate availability includes availability of acetyl-CoA, availability of NAD, and availability of TCA cycle intermediates, especially oxaloacetate. Oxaloacetate and acetyl-CoA are normally present at concentrations at or below their citrate synthase K_m, and

therefore increases in the concentrations of these compounds directly affects the rate of the TCA cycle.

Acetyl-CoA can be derived from several sources: breakdown of carbohydrates, breakdown of amino acids, and breakdown of fatty acids. TCA cycle intermediates have two main sources: breakdown of amino acids, and the conversion of pyruvate to oxaloacetate by pyruvate carboxylase.

Pyruvate carboxylase

Reactions that increase the amount of TCA cycle intermediates are called anaplerotic reactions ("anaplerotic" comes from the Greek for "filling up"; these reactions "fill up" the cycle with intermediates). In the liver, the most important of these reactions is catalyzed by pyruvate carboxylase.
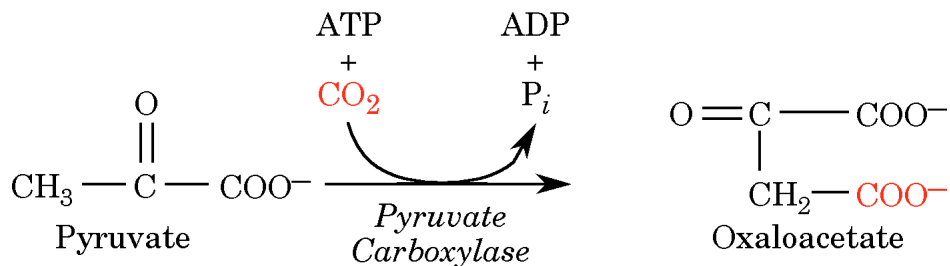
As mentioned in the section on gluconeogenesis, pyruvate carboxylase is a biotin-dependent enzyme; the biotin prosthetic group is covalently bound to the amino group of a pyruvate carboxylase lysine side chain. The enzyme bonds carbon dioxide (in the form of carbonate) to the indicated nitrogen on the biotin in an ATP-dependent reaction; pyruvate carboxylase then transfers the carbonate to the pyruvate to produce oxaloacetate.
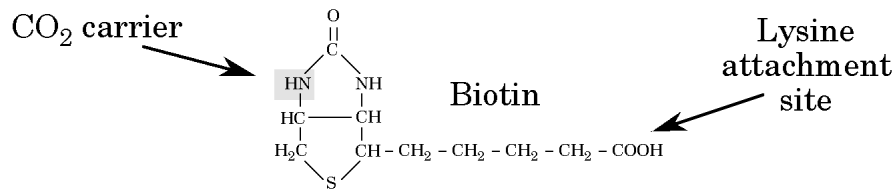

Pyruvate carboxylase is activated by acetyl-CoA. The enzyme acts as a mechanism for increasing the capacity of TCA cycle to metabolize acetyl-CoA. The reaction catalyzed by **pyruvate carboxylase therefore indirectly acts as an accelerator of the TCA cycle** by producing more oxaloacetate; the higher concentration of oxaloacetate allows the TCA cycle to run at an enhanced rate. Note, however, that if high levels of NADH inhibit other steps in the TCA cycle, the oxaloacetate formed is converted to malate, which is transported out of the mitochondria, and can be used for other purposes (primarily for glucose or NADPH synthesis).

Phosphoenolpyruvate carboxylase

Many plants use a different enzyme, phosphoenolpyruvate carboxylase, to

68

synthesize oxaloacetate from glycolytic intermediates. This enzyme uses the large amount of energy inherent in the phosphoenolpyruvate to drive the carboxylase reaction. **Animals lack phosphoenolpyruvate carboxylase**. While the phosphoenolpyruvate carboxykinase reaction could, in principle, result in oxaloacetate synthesis, in most species, phosphoenolpyruvate carboxykinase has too low an affinity for carbon dioxide to allow significant net synthesis of oxaloacetate.

Phosphoenolpyruvate

Oxaloacetate
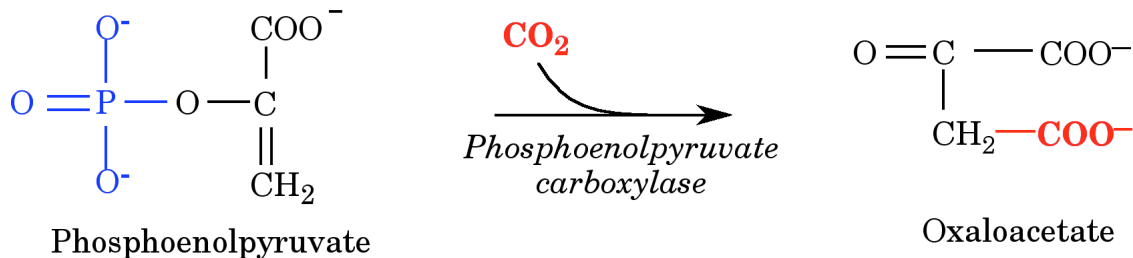
Amino acid breakdown: glutamate dehydrogenase

Amino acid breakdown is a major source of both TCA cycle intermediates and energy, especially in carnivores. In humans, amino acid breakdown is important under some conditions, and is used by most tissues (other than the liver) to produce TCA intermediates. Humans can convert all of the amino acids except lysine and leucine to either pyruvate or a TCA cycle intermediate. One of the most important reactions used for TCA cycle intermediate production is catalyzed by glutamate dehydrogenase, which produces α-ketoglutarate from glutamate.
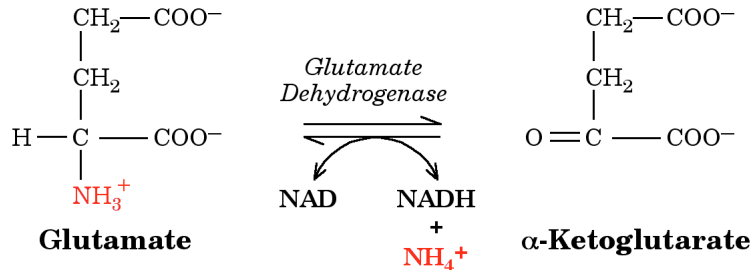
The glutamate dehydrogenase reaction is reversible, and in the reverse direction is extremely important in amino acid synthesis.

Amino acid breakdown: AMP deaminase

Muscle cells lack pyruvate carboxylase, and have only small amounts of glutamate dehydrogenase. Muscle cells tend to use a different pathway to generate TCA cycle intermediates from amino acids.

The reaction catalyzed by AMP deaminase is ordinarily involved in nucleotide breakdown. However muscle cells use the AMP deaminase reaction, followed by re-synthesis of AMP to result in a net conversion of aspartate to the TCA cycle intermediate fumarate.

The process requires aspartate and a GTP, and releases the excess nitrogen in the form of ammonium (although ammonium is toxic, in most cases, the amounts

released are relatively small). The major advantage of this process is that it is directly regulated by AMP. In muscle cells, if AMP levels are high, the cell is using ATP, and will need to generate more via the TCA cycle. Since AMP acts as a direct substrate for this pathway, it, in the presence of aspartate, allows the production of increased amounts of TCA intermediates, and therefore, allows increased ATP production.
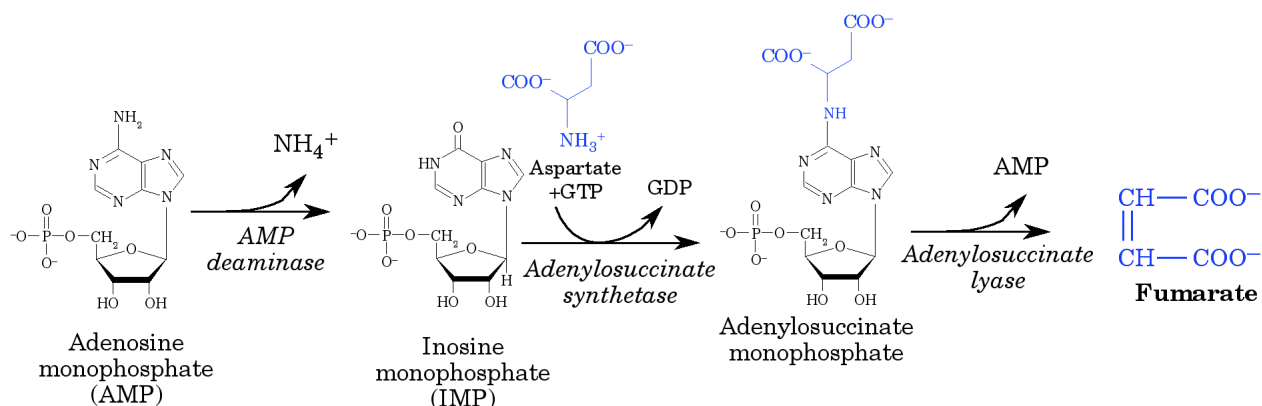
Allosteric regulation of the TCA cycle
In addition to regulation by substrate availability, three of the TCA cycle enzymes are regulated allosterically. Citrate synthase is commonly thought of as the gatekeeper for the TCA cycle, because acetyl-CoA enters via the citrate synthase reaction. Because the TCA cycle is circular, and because compounds enter and leave the cycle at various points, the citrate synthase reaction is not the only regulatory step. In fact, it is clear that all three of the irreversible enzymes play important roles in regulating the cycle.

Citrate synthase:
Citrate synthase is competitively **inhibited** by citrate, and by succinyl-CoA (the product of the α-ketoglutarate dehydrogenase reaction). Citrate synthase is also inhibited by long-chain acyl-CoA, because high concentrations in the absence of acetyl-CoA indicate decreased levels of fatty acid breakdown. In addition to its regulation by competitive inhibitors, citrate synthase is also **inhibited by ATP and NADH acting at allosteric sites**.

Citrate synthase is **stimulated** by NAD and ADP, and, as already mentioned, by increased **substrate concentration**.

Isocitrate dehydrogenase:
Isocitrate dehydrogenase is **inhibited** by ATP and NADH. NADH exhibits cooperative binding, so its effect changes dramatically over a fairly narrow range of concentrations. Isocitrate dehydrogenase is usually the first of the TCA cycle regulated enzymes to respond to elevated NADH levels, leading to an accumulation of citrate.

Isocitrate dehydrogenase is **stimulated** by ADP and NAD.

α-Ketoglutarate dehydrogenase complex:

The α-ketoglutarate dehydrogenase complex is **inhibited** by NADH, ATP, GTP, and succinyl-CoA. Note that, unlike the related pyruvate dehydrogenase complex, the α-ketoglutarate dehydrogenase complex is not regulated by phosphorylation.

The α-ketoglutarate dehydrogenase complex is **stimulated** by calcium (at least in some cell types).

Rationale for the TCA Cycle regulatory effectors

NADH and ATP inhibit all the control point enzymes for the TCA cycle. This is logical: these compounds are produced directly (in the case of the three NADH and the single nucleotide triphosphate produced in the TCA cycle) or indirectly (in the case of the ATP derived from the NADH-dependent electron transport/oxidative phosphorylation pathway). Thus, if ATP and NADH are present in high concentrations, making more of these compounds is probably unnecessary. In addition, producing excessive amounts of NADH may result in inhibition of other processes due to lack of NAD.

Citrate synthase and α-ketoglutarate dehydrogenase are both inhibited by succinyl-CoA, the product of the α-ketoglutarate dehydrogenase reaction. Succinyl-CoA is a product of other reactions besides that catalyzed by the α-ketoglutarate dehydrogenase complex; high levels of this compound is also an indicator that the cell does not need to use up additional acetyl-CoA in the TCA cycle. In addition, citrate synthase is inhibited by its product, citrate. Citrate builds up if NADH and ATP levels rise due to inhibition of isocitrate dehydrogenase; the citrate can then be transported to the cytoplasm where can interact with phosphofructokinase to inhibit glycolysis and where it can act as the carbon source for fatty acid synthesis.

The TCA cycle intermediates α-ketoglutarate and oxaloacetate are used for other purposes; utilization of both of these compounds is controlled by one of the regulated enzymes (α-ketoglutarate dehydrogenase and citrate synthase, respectively).

The cycle is stimulated by high levels of NAD and ADP, which act as signals of energy depletion. In addition, both α-ketoglutarate dehydrogenase and isocitrate dehydrogenase are stimulated by calcium. Calcium is often used as a signal for increased cellular energy requirements (especially in muscle, where it is a signal for muscle contraction). It is therefore logical that calcium will also increase the ability of the cell to produce the ATP required to deal with the increased energy requirements.

Finally, the pyruvate dehydrogenase reaction is tightly regulated, and contributes to the regulation of the TCA cycle. If the pyruvate dehydrogenase reaction is the only source of acetyl-CoA, its regulation will directly regulate the TCA cycle. In most cells, however, the inhibition of pyruvate dehydrogenase simply functions to force utilization of acetyl-CoA from sources other than pyruvate. One of the most important regulators of pyruvate dehydrogenase is acetyl-CoA; high levels of this compound indicate that other catabolic pathways are active and that pyruvate should be conserved for gluconeogenesis.

TCA cycle regulatory enzymes

Enzyme	Stimulators	Inhibitors	Comments
Pyruvate carboxylase	Acetyl-CoA	ADP	Several hormones alter the amount of this enzyme: Insulin ↓ Cortisol ↑ Glucagon ↑
Pyruvate dehydrogenase	CoA, NAD, Ca^{2+}, Mg^{2+}, pyruvate, insulin	acetyl-CoA, NADH, phosphorylation and ATP	Effect of Mg^{2+}: ATP binds Mg^{2+} with higher affinity than ADP; high free Mg^{2+} means low ATP concentration. Mg and Ca activate the phosphatase
Citrate synthase	NAD, CoA, ADP High [oxaloacetate] and [acetyl-CoA],	Citrate, Long chain acyl-CoA, ATP, NADH, succinyl-CoA	
Isocitrate dehydrogenase	Ca^{2+}, ADP	ATP, NADH	Isocitrate dehydrogenase is probably the most important regulatory enzyme in the TCA cycle.
α-Ketoglutarate dehydrogenase	Ca^{2+}	NADH, succinyl-CoA	

Entry and exit of carbon
Carbon removal

Malate is shuttled out of the mitochondria. One reason is to synthesize glucose via the gluconeogenic pathway; another is to remove carbon from the TCA cycle. Fatty acid synthesis requires citrate, although this citrate may not actually use up TCA intermediates, because only acetyl-CoA is actually used in lipid synthesis (in the cytoplasm, the citrate is cleaved to release acetyl-CoA and oxaloacetate; the oxaloacetate can be used for more than one purpose, and may indirectly return to the mitochondrion). Amino acids are produced from several of the TCA cycle intermediates, including α-ketoglutarate and oxaloacetate. Succinyl-CoA is used for heme biosynthesis.

All of these processes tend to decrease the amount of carbon present in the TCA cycle. Unchecked, they could prevent the TCA cycle from occurring at all; under normal conditions, these processes are regulated, and can act as a method for modulating the activity of the TCA cycle.

Carbon entry

Carbon units can also enter the TCA cycle. Pyruvate carboxylase, which converts pyruvate into oxaloacetate, was discussed above. The breakdown of amino acids (also mentioned above) results in the formation of several TCA cycle intermediates. Depending on the amino acid, degradation of amino acids can yield α-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, and acetyl-CoA. All except the last result in an increase in the amount of carbon in the TCA cycle.

Although the vast majority of fatty acids present in animals have an even number of carbons, small amounts of odd-chain fatty acids are sometimes present (these are found in some plants and microorganisms). The breakdown of odd-chain fatty acids results in the production of one molecule of succinyl-CoA from each odd-chain fatty acid.

Production of TCA cycle intermediates can increase the capacity to perform the TCA cycle, or can act as a mechanism for converting one TCA cycle intermediate to another to allow interchange of biosynthetic intermediates from one pathway to another.

Summary

Pyruvate derived from glycolysis, and acetyl-CoA derived from a variety of sources can be converted into energy and carbon dioxide in a mitochondrial pathway called the citric acid cycle, the Krebs cycle, or the tricarboxylic acid cycle (TCA cycle). The enzymes in the cycle combine the two carbons from acetyl-CoA with the four carbon compound oxaloacetate; the citrate thus formed is converted back to oxaloacetate in a series of steps that result in the formation of two carbon dioxide molecules, three NADH, an $FADH_2$, and a GTP.

The TCA cycle essentially uses oxaloacetate as a coenzyme; the cycle does not change the amount of oxaloacetate present, but runs faster when the levels of oxaloacetate are higher.

A modified form of the TCA cycle, the glyoxalate cycle, is performed by some plants and microorganisms, but does not exist in animals. The glyoxalate cycle allows the formation of oxaloacetate from acetyl-CoA, a reaction that animals cannot perform.

The TCA cycle is regulated at several steps: 1) the formation of acetyl-CoA (from pyruvate or from other sources) is regulated in several ways depending on the pathway, 2) three of the enzymes in the TCA cycle are allosterically regulated, and 3) the amount of oxaloacetate in the TCA cycle is regulated by either increased synthesis, or increased utilization of TCA intermediates by other processes.