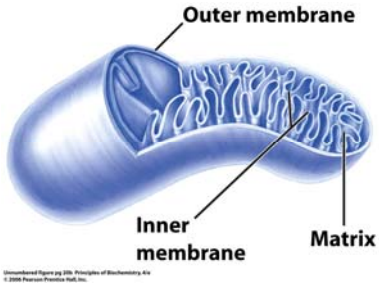
Chapter 13 - TCA Cycle

The third fate of glucose/pyruvate is complete oxidation to CO_2 + H_2O in the matrix of the mitochondrion.



The outer membrane is leaky and lets pyruvate from glycolysis pass through.

The inner membrane contains a transporter to move pyruvate into the matrix.

Mitochondria are unusual organelles. They act as the power plants of the cell, are surrounded by two membranes, and have their own genome. They also divide independently of the cell in which they reside, meaning mitochondrial replication is not coupled to cell division. Some of these features are holdovers from the ancient ancestors of mitochondria, which were likely free-living prokaryotes.

What Is the Origin of Mitochondria?

Mitochondria are thought to have originated from an ancient symbiosis that resulted when a nucleated cell engulfed an aerobic prokaryote. The engulfed cell came to rely on the protective environment of the host cell, and, conversely, the host cell came to rely on the engulfed prokaryote for energy production. Over time, the descendants of the engulfed prokaryote developed into mitochondria, and the work of these organelles — using oxygen to create energy — became critical to eukaryotic evolution (Figure 1).

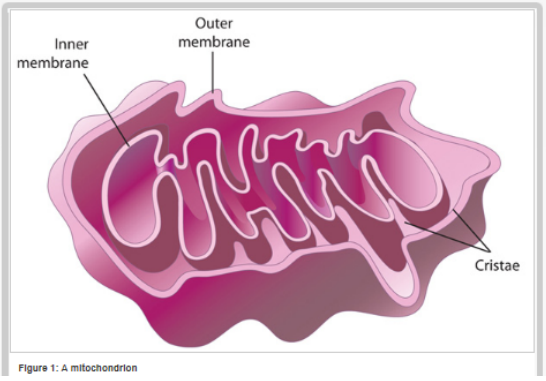


Figure 1: A mitochondrion

http://www.nature.com/scitable/topicpage/mitochondria-14053590

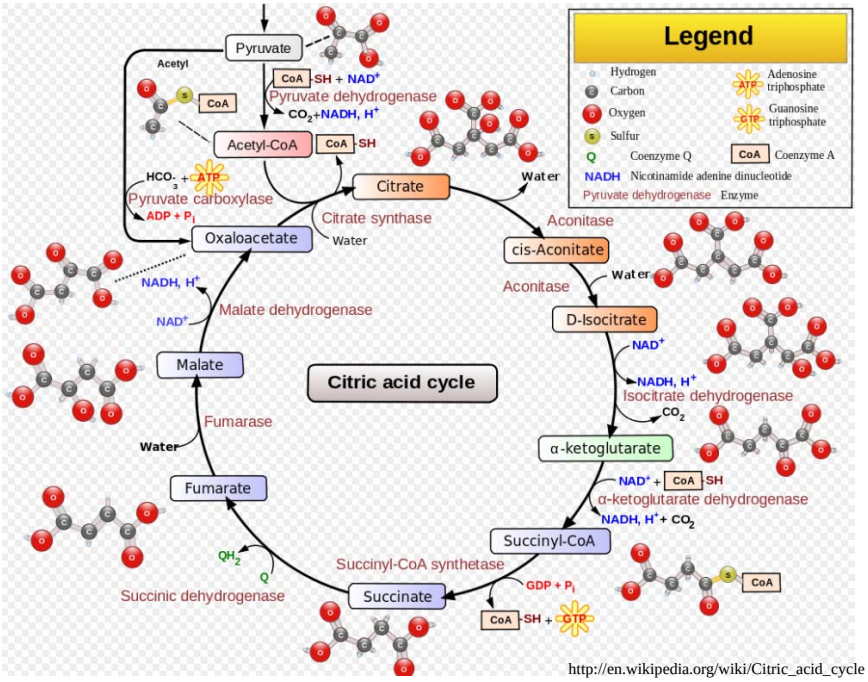
The TCA cycle

The citric acid cycle, aka the tricarboxylic acid cycle (TCA), or the Krebs cycle:

Series of chemical reactions used by all aerobic organisms to generate energy. It works by the oxidation of acetate derived from carbohydrates, fats and proteins into CO_2 and G in the form of ATP.

The cycle also provides precursors of certain amino acids and of NADH that is used in numerous other biochemical reactions.

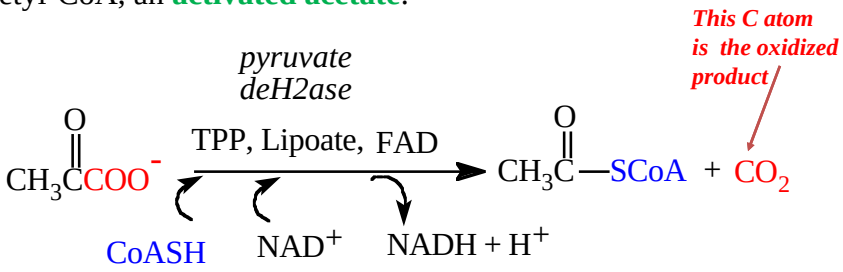
Its central importance to many biochemical pathways suggests that it was one of the earliest established components of cellular metabolism.



http://en.wikipedia.org/wiki/Citric_acid_cycle

Step joining the glycolysis and Krebs cycles:

Oxidation and **decarboxylation** of pyruvate to form Acetyl-CoA, an **activated acetate**:



$\Delta G'^{o} = -33.4$ kJ/mol $K_{eq} = 7x10^5$

Remember, there are 2 pyruvates from each glucose so 2 CO_2 are are released.

***Pyruvate dehydrogenase* is a large complex of 3 enzymes:**

E_1 = *pyruvatedeH$_2$ase*
E_2 = *dihydrolipoyltransacetylase*
E_3 = *dihydrolipoyldeH$_2$ase*

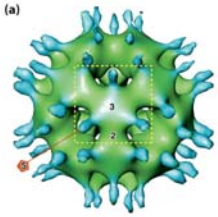
It uses 5 co-enzymes; 4 are derived from Vitamins:

TPP → Thiamin = Vitamin B_1 FAD → Riboflavin = Vitamin B_2

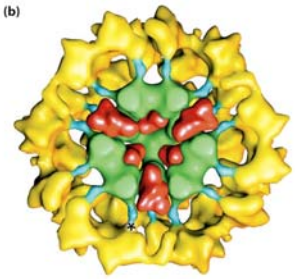
NAD → Niacin = Vitamin B_3 CoA → Pantothenate = Vitamin B_5

Lipoate → on lysine of E_2

Enzyme	Abbrev.	Cofactor(s)	# subunits prokaryotes	# subunits eukaryotes
pyruvate dehydrogenase	E1	TPP (thiamine pyrophosphate)	24	30
dihydrolipoyl transacetylase	E2	lipoate coenzyme A	24	60
dihydrolipoyl dehydrogenase	E3	FAD NAD^+	12	12

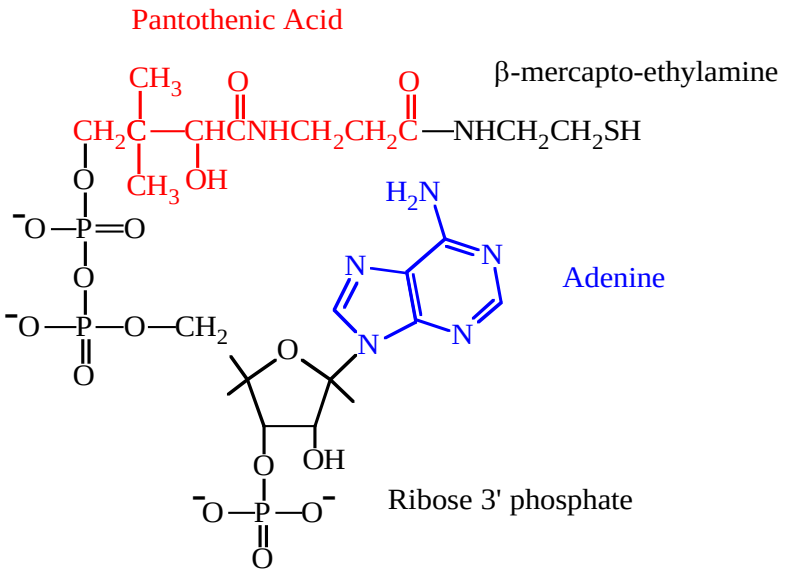


Inner core: 60 E2 enzymes (20 trimers)

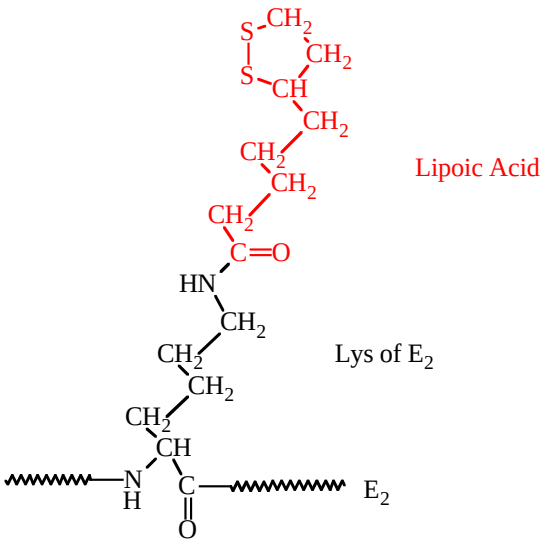


Cutaway view of complete complex
Yellow: E3 Red: E2

Coenzyme A



Lipoic Acid

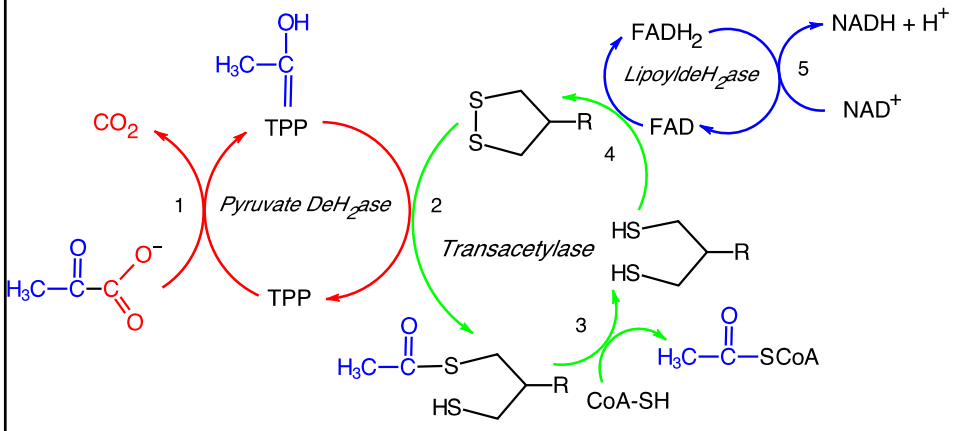


E_1 uses TPP to decarboxylate pyruvate exactly as for *pyruvate decarboxylase*.

Next, the flexible arm of lipoic acid on E_2 transfers the acetate from E_1 to CoA.

Then, the lipoic acid is re-oxidized by the FAD on E_3.

Finally, the $FADH_2$ is re-oxidized by NAD^+ and NADH carries the electrons away.



The reaction is irreversible and an important control point linking glycolysis and the TCA Cycle.

It is **inhibited** by **ATP, acetyl-CoA, NADH, fatty acids, CO_2** - "*high-energy signals*"

It is **activated** by **Pyruvate, AMP, CoA, NAD^+** - "*low-energy signals*"

In eukaryotes, the **Citric Acid Cycle / Krebs Cycle / Tricarboxylic Acid Cycle** acetate is oxidized to CO_2 and H_2O.

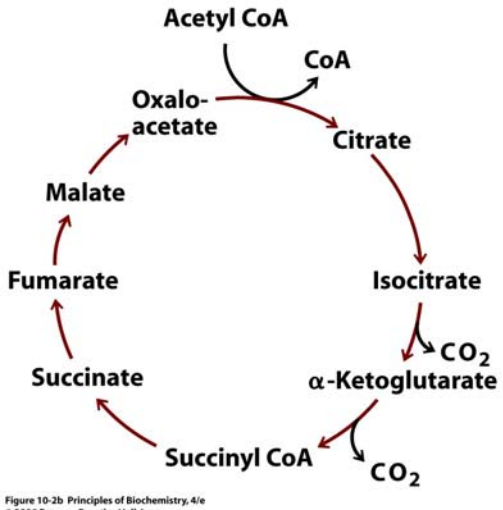
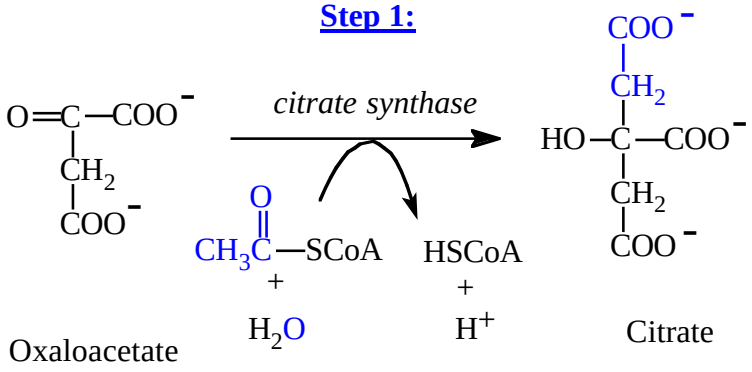


Figure 10-2b Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

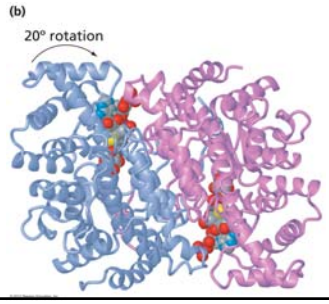
The acetate may come from oxidation of glucose, amino acids, or lipids.

The intermediates are used in AA, carbohydrate, pyrimidine nucleotide and lipid synthesis.

Step 1:



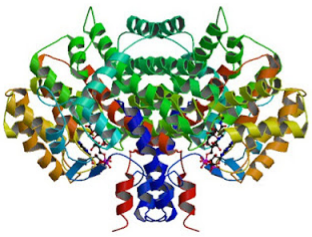
$\Delta G'^{o} = -32.2$ kJ/mol $K_{eq} = 4 \times 10^5$



Citrate is a **tricarboxylic acid**.

Citrate synthase is **inhibited** by **ATP**, **NADH**, **Acetyl-CoA**, **Succinyl-CoA and Citrate**.

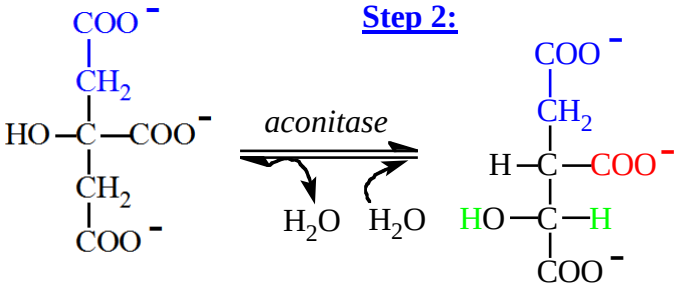
Citrate synthase



The citrate synthase is an enzyme widely used as a biomarker for the presence of intact mitochondria in cell cultures or organelle preparations. Despite being a mitochondrial enzyme it is encoded by nuclear DNA and synthesized in the cytosol.
This enzyme is the first regulatory enzyme in the Krebs cycle. It uses two different substrates, the acetyl-CoA and oxaloacetate. The oxaloacetate firstly binds to the enzyme, which induces conformational changes that create the binding site for the acetyl-CoA molecule.

http://worldofbiochemistry.blogspot.ca/2012/04/krebs-cycle-enzymes-part-1.html

Step 2:



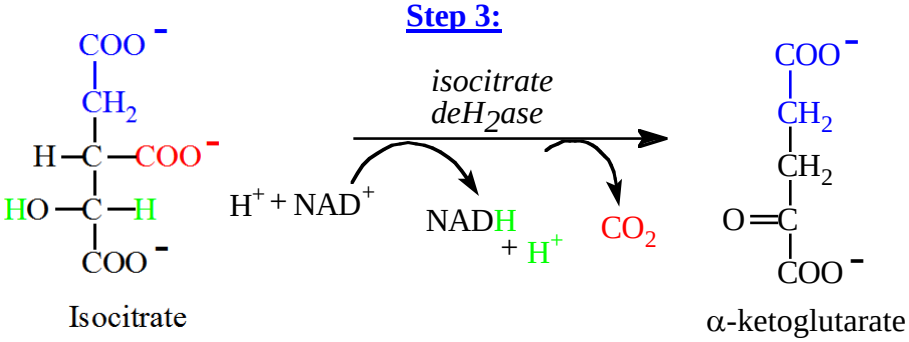
Citrate Isocitrate

$\Delta G'^{o}$ = +13.3 kJ/mol K_{eq} = 5x10^{-3}

Aconitase catalyses 2 reactions that result in the **isomerization** of citrate to isocitrate: $C_6H_5O_7 \rightarrow C_6H_5O_7$

The reaction is pulled forward by the following exergonic steps which consume isocitrate.

Step 3:

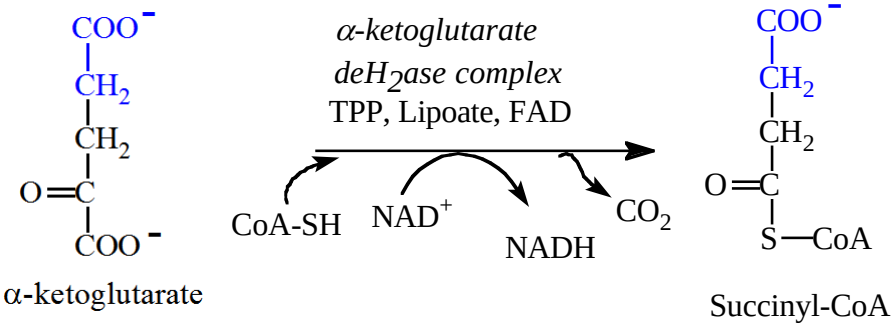


$\Delta G'^{o} = -20.9$ kJ/mol $K_{eq} = 5x10^3$

This reaction is an **oxidation** and a **decarboxylation** utilizing NAD^+ or $NADP^+$.

It is **inhibited** by **ATP** and **activated** by **ADP**.

Step 4:



$\Delta G'^{o} = -33.5$ kJ/mol $K_{eq} = 7x10^5$

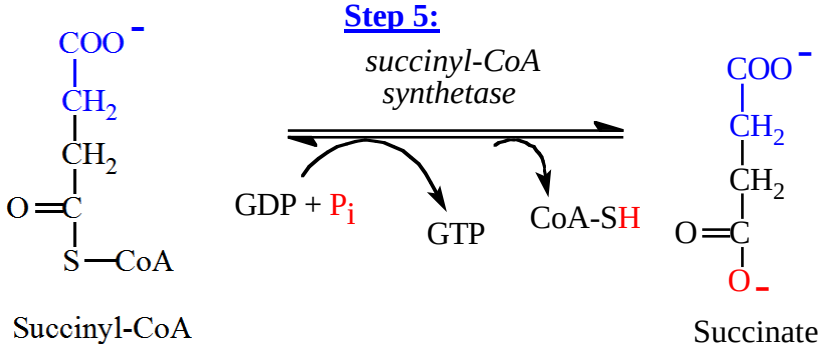
The mechanism is identical to the *pyruvate dehydrogenase* reaction.

This reaction is an **oxidation** and a **decarboxylation**.

Some of the G of oxidation is conserved in the formation of a thioester bond of succinyl-CoA.

This enzyme is **inhibited** by **NADH** and **succinyl-CoA**.

Step 5:



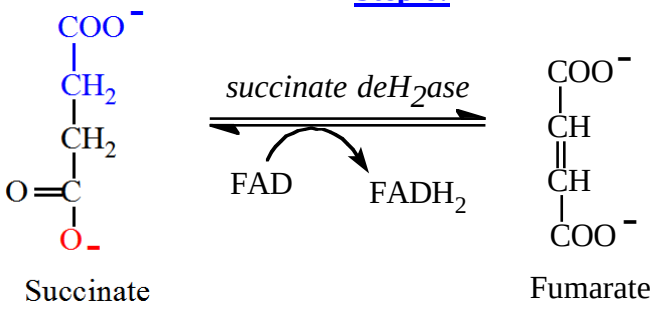
Succinyl-CoA Succinate

$\Delta G'^{o} = -2.9$ kJ/mol $K_{eq} = 3$

The *G* released when the high-energy thioester is hydrolysed is conserved in the formation of GTP.

"Substrate Level Phosphorylation" Remember, GTP and ATP are energetically equivalent.

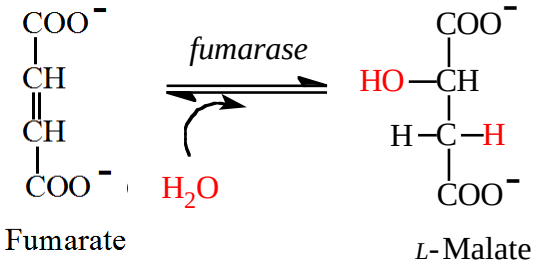
Step 6:



$\Delta G'^{o}$ = 0 kJ/mol K_{eq} = 1

The *G* of oxidation is stored in reduced FAD which is covalently attached to the enzyme.

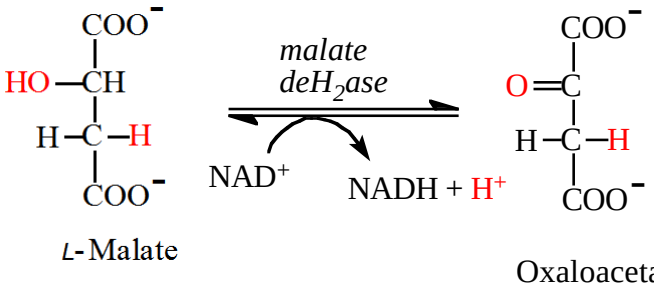
Step 7:



$\Delta G'^{o}$ = –3.8 kJ/mol K_{eq} = 5

Fumarase stereospecifically adds water across the C=C bond.

Step 8:



L-Malate

Oxaloacetate

$\Delta G'^{o}$ = +29.7 kJ/mol K_{eq} = 6x10^{-6}

This oxidation is highly endergonic so [OAA] is always low. Exergonic reaction 1 pulls this forward.

About –50 kJ /mol of *G* is released by the cycle and drives it in the direction of products.

Energy and Mass Balance

Acetyl-CoA + $2H_2O$ + $3NAD^+$ + FAD + GDP + P_i → $2CO_2$ + CoA-SH + 3NADH + $2H^+$ + $FADH_2$ + GTP

2 pyruvates from 1 glucose produce 2 acetyl-CoA which are oxidized to 4 CO_2 by turns of the cycle.

4 steps involve oxidations that conserve *G* by reducing electron carriers (3 NADH + 1 $FADH_2$) plus 1 high energy phosphate is formed (GTP).

Note that reactions 6 to 8 regenerate OAA so there is no net consumption or production of intermediates. The cycle functions as a **catalyst**.

Why is O_2 required?

The cycle would stop if NAD^+ were not regenerated:

$$NADH + H^+ + \tfrac{1}{2} O_2 \rightarrow H_2O + NAD^+$$

The transport of electrons from NADH to O_2 is coupled to ATP formation.

$$ADP + P_i + H^+ \longrightarrow ATP + H_2O$$

The process is called **oxidative phosphorylation** and is the subject of Chapter 14.

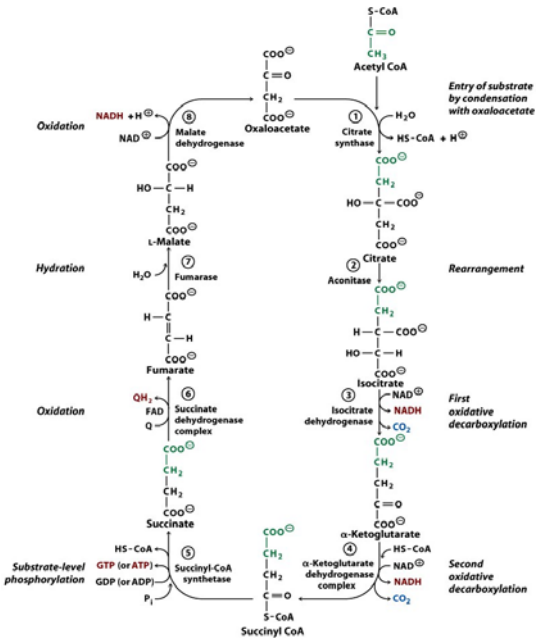


Figure 13-3 Principles of Biochemistry, 4/e