III. Metabolism
The Citric Acid Cycle
The Eight Steps of the Citric Acid Cycle

Enzymes:
- 4 dehydrogenases (2 decarboxylation)
- 3 hydration/dehydration
- 1 substrate level phosphorylation
Overall Reaction (TCA cycle)

Overall reaction

\[
\text{Acetyl-CoA} + 3\text{NAD}^+ + \text{FAD} + \text{GDP} + P_i \rightarrow 2\text{CO}_2 + \text{CoA} + 3\text{NADH} + \text{FADH} + \text{GTP}
\]

Citric acid cycle is central to the energy-yielding metabolism, but it also produces 4- and 5-carbon precursors for other metabolic pathways.

Replenishing (anaplerotic) reactions are needed to keep the cycle going!
TCA Cycle – Citrate Synthase

**Rxn 1**  Formation of Citrate by condensation of oxaloacetate and acetyl-CoA, catalyzed by citrate synthase.

\[
\begin{align*}
\text{Acetyl-CoA} & \quad + \quad \text{Oxaloacetate} \\
\text{Citroyl-CoA} & \quad \text{Citrate}
\end{align*}
\]

\[\Delta G' = -32.2 \text{ kJ/mol}\]

\[\Delta G'\] has to be large to overcome the low oxaloacetate concentration

Citroyl-CoA is formed as an intermediate
TCA Cycle – Citrate Synthase

**Rxn 1** Structure of citrate synthase from *G. galus* mitochondria

- Oxaloacetate (yellow) binds first and induces large conformational change
- Creates binding site for Acetyl-CoA
Structure of Citrate Synthase

Conformational change upon OAA binding creates Acetyl CoA site

Ordered sequential mechanism
TCA Cycle – Citrate Synthase Mechanism

**Rxn 1** Mechanism of citrate synthase reaction (1st step)

**Formation of Enol Intermediate**

The thioester linkage in acetyl-CoA activates the methyl hydrogens, and Asp<sup>375</sup> abstracts a proton from the methyl group, forming an enolate intermediate.
**TCA Cycle – Citrate Synthase Mechanism**

*Rxn 1*  
Mechanism of citrate synthase reaction (2\textsuperscript{nd} step)

\(\alpha\)-keto addition (condensation)

The intermediate is stabilized by hydrogen bonding to and/or protonation by His\textsuperscript{274} (full protonation is shown).

The resulting condensation generates citroyl-CoA.
TCA Cycle – Citrate Synthase Mechanism

**Rxn 1**  Mechanism of citrate synthase reaction (3rd step)

**Thioester hydrolysis**

The thioester is subsequently hydrolyzed, regenerating CoA-SH and producing citrate.
**TCA Cycle – Citrate Synthase Mechanism**

**Rxn 1**  Mechanism of citrate synthase reaction

"Stabilized" enol intermediate of acetyl CoA attacks α-keto group of oxaloacetate.

Hydrolysis of citroyl-CoA intermediate drives reaction.
TCA Cycle – Aconitase reaction

**Rxn 2**  Formation Isocitrate via cis-Aconitate

Aconitase **dehydrates** citrate to cis-aconitate ... then **hydrates** cis-aconitate to isocitrate

Equilibrium mixture at pH 7.4 and 25°C contains <10% Isocitrate.

\[ \Delta G^\circ = 13.3 \text{ kJ/mol} \]

Hydroxyl moved from C3 to C2
Rxn 2  Mechanism of aconitase

Aconitase contains an iron-sulfur center

4Fe:4S center aids substrate binding and is required for catalytic addition / removal of H$_2$O.

Cis-aconitate intermediate does not (typically) dissociate from enzyme.

PDBid 1B0J
**TCA Cycle – isocitrate dehydrogenase**

**Rxn 3** Oxidation decarboxylation of Isocitrate to $\alpha$-ketoglutarate

1. Isocitrate $\rightarrow$ Oxalosuccinate
   - **Mn$^{2+}$** in the active site interacts with the carbonyl group of intermediate oxalosuccinate and stabilizes the transiently formed enol.

Two different isocitrate DH$_2$ases: a NAD$^+$ and a NADP$^+$ dependent form.

- An NAD$^+$ dependent form in the mitochondrial matrix
- An NADP$^+$ dependent form in both the mitochondria and cytosol
**TCA Cycle – α-ketoglutarate dehydrogenase complex**

**Rxn 4** Oxidation of α-ketoglutarate to succinyl-CoA and CO$_2$

![Chemical reaction diagram]

\[ \Delta G^\circ = -33.5 \text{ kJ/mol} \]

The mechanism is identical to the pyruvate dehydrogenase reaction.

α-ketoglutarate dehydrogenase complex is very similar to the pyruvate dehydrogenase complex (homologs of E$_1$, E$_2$, and E$_3$). It also contains TPP, E$_2$ bound lipoate, FAD, NAD and CoA. E$_3$ is identical in both complexes.

Specificity due to E$_1$
TCA Cycle – succinyl-CoA synthetase

Rxn 5 Conversion of Succinyl-CoA to Succinate

\[
\begin{align*}
\text{CH}_2\text{—COO}^- \\
\text{CH}_2 \\
\text{C—S-CoA} \\
\text{O} \\
\text{Succinyl-CoA}
\end{align*}
\quad
\xrightarrow{\text{succinyl-CoA synthetase}}
\quad
\begin{align*}
\text{CH}_2 \\
\text{COO}^- \\
\text{Succinate}
\end{align*}
\quad
\begin{align*}
\text{GDP + P_i} \\
\text{GTP CoA-SH}
\end{align*}
\]

\[\Delta G^{\circ} = -2.9 \text{ kJ/mol}\]

Energy of the **thioester** bond cleavage drives the formation of a **phosphoanhydride** bond in GTP.

- (another) substrate level phosphorylation

- conversion of one high energy bond to another
**Rxn 5**  Mechanism of succinyl-CoA synthetase

**Step 1**
Succinyl-CoA binds to the enzyme and a phosphoryl group replaces the CoA of succinyl-CoA

(substrate level phosphorylation)

Consume a high-energy thioester and create a high-energy acyl phosphate.
TCA Cycle – succinyl-CoA synthetase

**Rxn 5** Mechanism of succinyl-CoA synthetase

**Step 2**
Succinyl phosphate transfers phosphoryl group to His residue on the enzyme.

Consume a high-energy acyl phosphate and create a high-energy phosphohistidyl.

**Step 3**
Phosphohistidyl enzyme transfers phosphoryl group to GDP

Consume a high-energy phosphohistidyl and create a high-energy phosphoanhydride.

GTP can be:
1) converted to ATP by nucleoside diphosphate kinase.
2) utilized by G-proteins and other GTPases.
TCA Cycle – succinyl-CoA synthetase

Rxn 5 Mechanism of succinyl-CoA synthetase

1\textsuperscript{st} step
\[
\text{ Succinyl-CoA } + \text{ P}_i \xrightleftharpoons{1} \text{ Succinyl-phosphate } + \text{ CoASH }
\]

2\textsuperscript{nd} step
\[
\text{ Enzyme-His } + \text{ Succinyl-phosphate } \xrightarrow{2} \text{ 3-Phospho-His } + \text{ Succinate }
\]

3\textsuperscript{rd} step
\[
\text{ GDP } + \text{ Enzyme-His } \xrightarrow{3} \text{ GTP } + \text{ 3-Phospho-His }
\]

Three successive phosphoryl group transfers
**Succinyl-CoA synthetase Structure**

**Rxn 5**  
Structure of succinyl-CoA synthetase

Two subunits:

α Subunit (32 kDa)  
His$^{246}$ is phosphorylated

β Subunit (42 kDa)  
confers ATP/GTP specificity

Active site is at the Subunit interface  
→ “power helices” facilitate phosphoryl group transfers
TCA Cycle – Succinate dehydrogenase (a.k.a Complex II)

**Rxn 6** Oxidation of Succinate to Fumarate – Succinate dehydrogenase

Eukaryotic succinate dehydrogenase is tightly bound to the inner mitochondrial membrane; prokaryotes $\rightarrow$ plasma membrane.

Succinate dehydrogenase is Complex II of Electron Transfer Chain

$$\Delta G^\circ = 0 \text{ kJ/mol}$$
TCA Cycle – Succinate dehydrogenase (a.k.a Complex II)

**Rxn 6** Oxidation of Succinate to Fumarate – **Succinate dehydrogenase**

**Succinate dehydrogenase** (or Complex II) contains several iron-sulfur centers that mediate the flow of electrons from succinate (via a covalently bound FAD to enzyme) to the electron transfer chain and finally to \( \text{O}_2 \).

![Malonate and Succinate structures]

**Malonate**, an analog of succinate strongly **inhibits succinate dehydrogenase** → not normally present in cells
TCA Cycle - Fumarase

**Step 7** Hydration of Fumarate to Malate – Fumarase

Highly stereospecific → only catalyzes trans double bond hydration and vice versa.

\[ \Delta G^\circ = -3.8 \text{ kJ/mol} \]

Fumarate → Maleate

\[ \text{Fumarate} \quad \text{OH}^- \quad \text{fumarase} \quad \text{Malate} \]

Not substrates
TCA Cycle - Malate Dehydrogenase

**Step 8** Oxidation of Malate to Oxaloacetate – *L*-malate dehydrogenase

$L$-malate is oxidized to oxaloacetate

$\rightarrow$ equilibrium strongly favours substrate (*L*-malate)

$\Delta G^\circ = 29.7 \text{ kJ/mol}$

*Couple reaction with strongly favourable Citrate Synthase reaction to overcome unfavourable energetics*
**Energy Yields**

Yield = \(~32\) ATP/glucose

**Standard Conditions:**

\[32 \times 30.5 \text{ kJ/mol} = 976 \text{ kJ/mol}\]

Combustion of glucose = 2,840 kJ/mol

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**Table of ATP Yields**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Number of ATP or reduced coenzyme directly formed</th>
<th>Number of ATP ultimately formed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose → glucose 6-phosphate</td>
<td>−1 ATP</td>
<td>−1</td>
</tr>
<tr>
<td>Fructose 6-phosphate → fructose 1,6-bisphosphate</td>
<td>−1 ATP</td>
<td>−1</td>
</tr>
<tr>
<td>2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate</td>
<td>−2 NADH</td>
<td>3 or 5†</td>
</tr>
<tr>
<td>2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate</td>
<td>2 ATP</td>
<td>2</td>
</tr>
<tr>
<td>2 Phosphoenolpyruvate → 2 pyruvate</td>
<td>2 ATP</td>
<td>2</td>
</tr>
<tr>
<td>2 Pyruvate → 2 acetyl-CoA</td>
<td>2 ATP</td>
<td>2</td>
</tr>
<tr>
<td>2 Isocitrate → 2 α-ketoglutarate</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td>2 α-Ketoglutarate → 2 succinyl-CoA</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td>2 Succinyl-CoA → 2 succinate</td>
<td>2 ATP (or 2 GTP)</td>
<td>2</td>
</tr>
<tr>
<td>2 Succinate → 2 fumarate</td>
<td>2 FADH₂</td>
<td>3</td>
</tr>
<tr>
<td>2 Malate → 2 oxaloacetate</td>
<td>2 NADH</td>
<td>5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>30–32</strong></td>
</tr>
</tbody>
</table>

*This is calculated as 2.5 ATP per NADH and 1.5 ATP per FADH₂. A negative value indicates consumption.

† This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19-27 and 19-28.
## Summary of Enzyme Properties

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>EC Class</th>
<th>Mechanism</th>
<th>Inter</th>
<th>$\Delta G^\circ$</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate Dehydrogenase Complex</td>
<td>Oxidoreductase(E1)</td>
<td>TPP; $\alpha$-keto deCO$_2$</td>
<td>ylid; enol</td>
<td>-33</td>
<td>Acetyl CoA; NADH; CO$_2$</td>
</tr>
<tr>
<td>Transferase (E2)</td>
<td>Transferase</td>
<td>Lipoate</td>
<td>Acyl lipoate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidoreductase(E3)</td>
<td>Oxidoreductase(E3)</td>
<td>NAD+ $\rightarrow$ NADH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate Synthase</td>
<td>Transferase</td>
<td>Condensation</td>
<td>enol; citroyl-CoA</td>
<td>-32</td>
<td>2C + 4C sugars $\rightarrow$ 6C sugar</td>
</tr>
<tr>
<td>Aconitase</td>
<td>Lyase</td>
<td>Dehydration; Hydration</td>
<td>cis-aconitate; 4Fe$\bullet$4S</td>
<td>+13</td>
<td></td>
</tr>
<tr>
<td>Isocitrate Dehydrogenase</td>
<td>Oxidoreductase</td>
<td>Mn$^{2+}$; oxidative deCO$_2$</td>
<td>$\beta$-keto CO$_2$; enol</td>
<td>-8</td>
<td>NADH; CO$_2$</td>
</tr>
<tr>
<td>$\alpha$-ketoglutarate Dehydrogenase Complex</td>
<td>See PDC.</td>
<td>See PDC.</td>
<td>See PDC</td>
<td>-34</td>
<td>Succinyl CoA; NADH; CO$_2$</td>
</tr>
<tr>
<td>Succinyl-CoA synthetase</td>
<td>Ligase</td>
<td>Phosphoryl transfer; SLP</td>
<td>Succinyl phosphate; phosho-His</td>
<td>-3</td>
<td>GTP(ATP)</td>
</tr>
<tr>
<td>Succinate Dehydrogenase</td>
<td>Oxidoreductase</td>
<td>FAD $\rightarrow$ FADH$_2$</td>
<td>4Fe$\bullet$4S</td>
<td>0</td>
<td>FADH$_2$</td>
</tr>
<tr>
<td>Fumarase</td>
<td>Lyase</td>
<td>Hydration</td>
<td>carbanion; ???</td>
<td>-4</td>
<td></td>
</tr>
<tr>
<td>Malate Dehydrogenase</td>
<td>Oxidoreductase</td>
<td>NAD+ $\rightarrow$ NADH</td>
<td></td>
<td>+30</td>
<td>NADH</td>
</tr>
</tbody>
</table>
Why is Oxidation of Acetate so Complicated?

It is a hub of intermediary metabolism; in aerobic organisms it serves in catabolic and anabolic processes. →amphibolic pathway
- oxidative catabolism
- production of biosynthetic precursors.

Intermediates removed from the Cycle are replenished by anaplerotic reactions.

Under steady state conditions (normal) intermediate concentrations remain constant

Anaerobic bacteria (above) have an 'incomplete' TCA → lack α-ketoglutarate dehydrogenase complex
Citric Acid Cycle in Anabolism

Glucose
- Phosphoenolpyruvate (PEP)
  - Serine
  - Glycine
  - Cysteine
  - Phenylalanine
  - Tyrosine
  - Tryptophan

Pyruvate
- PEP carboxykinase
- Pyruvate carboxylase
- Oxaloacetate
  - Aspartate
  - Asparagine
  - Pyrimidines

Acetyl-CoA
- Citrate
  - α-Ketoglutarate
  - Malate

Succinyl-CoA
- Pyruvate
  - PEP carboxykinase

Fatty acids, sterols
- Glutamine
  - Proline
  - Arginine

Glutamate
- Purines

Porphyrrins, heme
Regulation of the Citric Acid Cycle

Mammals, allosteric regulation is complemented by covalent protein modification.

E₁ of PDH complex can be inactivated by phosphorylation - kinase that inactivates E1 is a subunit of the mammalian PDH complex

PDH kinase is allosterically activated by ATP

TCA Cycle is regulated at its 3 exergonic steps. Citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase complex
In many organisms other than vertebrates, the glyoxylate cycle serves as mechanism for converting acetate to carbohydrate.

The glyoxylate cycle produces four-carbon compounds from acetate.

In plants, glyoxylate cycle enzymes are found in organelles → Glyoxysomes

Found in lipid rich seeds during germination, before glucose from photosynthesis is available.
The Glyoxylate Cycle

Citrate synthase, aconitase, and malate dehydrogenase are **isozymes** of the TCA cycle enzymes.

Isocitrate lyase and malate synthase are **unique** to the cycle.
Relationship Between the Glyoxylate And the Citric Acid Cycle

Glyoxylate cycle produces **succinate** which enters TCA cycle (anabolism)
Coordinated Regulation

Sharing common intermediates requires coordinated regulation.

*Isocitrate* is at the branch point between the glyoxylate and TCA cycle.

Isocitrate DH is also regulated by covalent modification (specific protein kinase).

E. coli has the full complement of enzymes and therefore grows on acetate as the sole carbon source.