The Citric Acid Cycle
Overview

• Acetyl CoA is
  – formed by the oxidative decarboxylation of pyruvate
    • with the release of CO$_2$
  – one of the key intermediates in the interconversion of small organic acids

• The citric acid cycle (CAC) begins with
  – The transfer of acetyl group
    • from acetyl CoA
    • to the four-carbon dicarboxylic acid oxaloacetate to
    • form a new six-carbon tricarboxylic acid known as citrate

• Citrate can then be oxidized in a
  – seven-step pathway to
  – regenerate oxaloacetate and
  – release two molecules of CO$_2$

• The pathway is known as
  – the **citric acid cycle**
  – the tricarboxylic acid cycle (TCA cycle), or
  – the Krebs cycle
Overview of the citric acid cycle
The citric acid cycle
the hub of energy metabolism in eukaryotic cells

- The energy released in the cycle is largely
  - conserved as reducing power in
  - coenzyme NADH and
  - reduced ubiquinone (QH$_2$)

- NADH and QH$_2$ are substrates in
  - membrane-associated electron transport that
  - lead to the formation of a proton gradient which
  - drives synthesis of ATP
Conversion of Pyruvate to Acetyl CoA

- catalyzed by
  - the pyruvate dehydrogenase complex
  - a large complex of enzymes and cofactors
  - need coenzyme A (HS–CoA)
  - Two electrons are transferred to NADH from pyruvate
The pyruvate dehydrogenase complex

- a multienzyme complex containing
  - multiple copies of three distinct enzymatic activities:
    - pyruvate dehydrogenase ($E_1$)
    - dihydrolipoamide acetyltransferase ($E_2$), and
    - dihydrolipoamide dehydrogenase ($E_3$)
- There are 5 steps in the oxidative decarboxylation of pyruvate
1. Formation of a hydroxyethyl—TPP intermediate and the release of CO2

The E₁ component contains the prosthetic group thiamine pyrophosphate (TPP, vitamin B₁)
2. Two-carbon hydroxyethyl group is transferred to the lipoamide group of E₂.

oxidation of hydroxyethyl—TPP is coupled to the reduction of the disulfide of lipoamide.
The lipoamide group consists of:
- lipoic acid covalently bound by an amide linkage
- to a lysine residue of an subunit E$_2$

This particular coenzyme is only found in pyruvate dehydrogenase and related enzymes.
3. Transfer of the acetyl group to HS—CoA, forming acetyl CoA and leaving the lipoamide in the reduced dithiol form.
4. The reduced lipoamide of E$_2$ must be reoxidized in order to regenerate the prosthetic group.

FAD (also a coenzyme) is the prosthetic group of E3.
5. $E_3$—FADH$_2$ is reoxidized to FAD

- $E_3$—FADH$_2$ + NAD$^+$ $\rightarrow$ $E_3$—FAD + NADH + H$^+$
- regenerates the original pyruvate dehydrogenase complex,
  - completing the catalytic cycle
The interplay of five coenzymes in the pyruvate dehydrogenase complex

• Two are cosubstrates
  – HS—CoA and NAD+
• Three are prosthetic groups
  – TPP,
  – lipoamide, and
  – FAD
• illustrates the importance of coenzymes in metabolic reactions
Lipoamide prosthetic group acts as a swinging arm that visits the three active sites in the enzyme complex.
The swinging arm mechanism of lipoamide

- The various subunits of the complex are arranged in a way
  - to ensure that
    - the product of one reaction
    - does not diffuse into the medium but is
    - immediately acted on by the next component

**This arrangement is called**

- the
- swinging arm mechanism of lipoamide
The eukaryotic pyruvate dehydrogenase complex is the largest multienzyme complex known.
The inner core:
• 60 $E_2$ enzymes
• in the shape of a pentagonal Dodecahedron
• with one $E_2$ trimer at each of the 20 vertices

A single trimer is outlined by a yellow box.

The center of the pentagon shape is indicated by the red pentagon.
Cutaway view of outer $E_1$ enzymes (yellow) E3 enzymes (red) located in the space between the E2 enzymes
Electron micrograph of pyruvate dehydrogenase complexes from *E. coli*. 
2-oxo acid dehydrogenase family

- A family of multi-enzyme complexes
  - Pyruvate dehydrogenase is a member
  - Pyruvate is the smallest 2-oxo organic acid
- Two other 2-oxo (or \(\alpha\)-keto) acid dehydrogenases
  - A citric acid cycle enzyme
    - \(\alpha\)-ketoglutarate dehydrogenase
  - branched-chain \(\alpha\)-keto acid dehydrogenase,
    - used in amino acid metabolism
- catalyze essentially irreversible reactions in which
  - an organic acid is oxidized to \(\text{CO}_2\) and
  - an energy-rich coenzyme A derivative is formed
In eukaryotic cells the enzymes of the citric acid cycle are located in the mitochondria.
Transporting pyruvate into mitochondria

- In bacterial cells,
  - pyruvate is converted to acetyl CoA in the cytosol
- In eukaryotic cells the pyruvate dehydrogenase complex is located in mitochondria
  - enclosed by a double membrane
  - Small molecules pass through the outer membrane via
    - aqueous channels formed by transmembrane proteins called porins
    - Pyruvate translocase
      - a specific transport protein
      - transports pyruvate in symport with H⁺
Please note two general features of the pathway:

- flow of carbon
- production of energy-rich molecules

The two carbon atoms that enter the cycle as the acetyl group on acetyl CoA are not the same carbon atoms that are lost as CO₂.
Entry of substrate by condensation with oxaloacetate

Oxaloacetate

Acetyl CoA

Citrate synthase

Citrate
Figure 13-3 part 2 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
Figure 13-3 part 3 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
Substrate-level phosphorylation

\[ \text{Succinate} \]

- GTP (or ATP)
- GDP (or ADP)
- \( P_i \)

\[ \text{Succinyl-CoA synthetase} \]

\[ \text{Succinyl CoA} \]
Oxidation

Fumarate

Succinate dehydrogenase complex

Figure 13-3 part 6 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
Hydration

L-Malate

Fumarase

Fumarate

Figure 13-3 part 7 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
Oxidation

NADH + H⁺  →  NAD⁺

Malate dehydrogenase

L-Malate

COO⁻

C = O

CH₂

COO⁻

Oxaloacetate

Figure 13-3 part 8 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
The citric acid cycle is equivalent to the

- oxidation of an acetyl CoA molecule with release of 8 electrons

\[
\begin{align*}
\text{S-CoA} \\
\text{C} &= \text{O} + 2 \text{H}_2\text{O} + \text{OH}^- \quad \longrightarrow \quad 2 \text{CO}_2 + \text{HS-CoA} + 7 \text{H}^+ + 8\text{e}^- \\
\text{CH}_3
\end{align*}
\]

(13.8)

6 electrons are transferred to 3 NAD+ and
2 electrons to one ubiquinone (Q)
The oxidation of an acetyl CoA equivalent by the citric acid cycle showing the valence electrons in the reactants and products.

Most of the time, electrons are released when double bonds are formed.
Fates of the carbon atoms during one turn of the citric acid cycle.

Carbon atoms from acetyl CoA (green) are uniformly distributed in the four-carbon intermediates leading to oxaloacetate.
Citrate is the first of two tricarboxylic acids in the cycle.

\[ \Delta G^\circ' = -31.5 \text{ kJ mol}^{-1} \]

The large negative free energy ensures that
• the reaction proceeds in the direction of citrate synthesis
• when the concentration of oxaloacetate is very low
Citrate synthase

• a lyase
  – catalyze cleavage of substrates in simple elimination reactions that are
    • *not* oxidation–reduction reactions and are
    • *not* coupled to ATP hydrolysis

• “Synthase” is used
  – for lyases whose primary role is the reverse reaction
    • where two substrates are combined to form a larger molecule

• “Synthetases,” are members of the ligase category of enzymes
  – The reactions must be coupled to ATP (or GTP) hydrolysis
Citrate synthase from chicken

The two identical subunits are colored blue and purple. Each is composed of a small and a large domain.
A near-equilibrium conversion of citrate to isocitrate.

Citrate is a tertiary alcohol and thus
• cannot be oxidized directly to a keto acid
• The formation of a keto acid intermediate
• is required for the oxidative decarboxylation
There are four different stereoisomers, but only one of these is produced: 2R,3S-isocitrate.
Three-point Attachment of Prochiral Substrates to Enzymes (Box 13.2)

- In the citrate-to-isocitrate reaction
  - a chiral molecule was produced from a non-chiral molecule
  - But only one of the two possible forms of $2R,3S$-isocitrate was produced
- Why formation of the double bond of $cis$-aconitate, and
  - Subsequent addition of water to form isocitrate, occurred
  - only in the moiety contributed originally by oxaloacetate
  - not in the group derived from acetyl CoA
- Remember that citrate is symmetric
Two forms of isocitrate. The green carbon atoms represent the group originally derived from acetyl CoA.

Only this form was produced.

The reaction was expected to yield two forms of isocitrate in equal quantities.
The substrate itself is symmetric.

However, when there are 3 binding sites, the way of binding is not symmetric.
3. Isocitrate Dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to Form $\alpha$-ketoglutarate

Unnumbered figure pg 398 Principles of Biochemistry, 4/e © 2006 Pearson Prentice Hall, Inc.
4. The α-Ketoglutarate Dehydrogenase Complex

\[
\begin{align*}
\alpha\text{-Ketoglutarate} & \quad \text{COO}^- \\
& \quad \bigg| \quad \text{CH}_2 \\
& \quad \bigg| \quad \text{C} = \text{O} \\
& \quad \bigg| \quad \text{COO}^- \\
\end{align*}
\]

\[
\begin{align*}
\text{CH}_2 + \text{HS-CoA} + \text{NAD}^+ & \quad \rightarrow \quad \text{COO}^- \\
& \quad \bigg| \quad \text{CH}_2 \\
& \quad \bigg| \quad \text{C} = \text{O} \\
& \quad \bigg| \quad \text{S-CoA} \\
\end{align*}
\]

analogous to the reaction catalyzed by pyruvate dehydrogenase. The reactants are an α-keto acid and HS–CoA and the products are CO\(_2\) and a high-energy thioester compound.
5. Succinyl–CoA synthetase (a.k.a. succinate thiokinase)

Proposed mechanism will be described in detail in the next 2 slides.
Phosphate displaces CoA, forming the mixed acid anhydride succinyl phosphate.
1. Forming covalent phosphoenzyme intermediate

2. Transferring phosphoryl group to GDP (or ADP, depending on the organism)
The overall stoichiometry of the succinyl–CoA synthetase reaction

\[
\text{Succinyl CoA} + P_i + GDP \rightarrow \text{Succinate} + \text{HS-CoA} + \text{GTP} \quad (13.13)
\]

the enzyme is named for the reverse reaction where succinyl CoA is synthesized from succinate at the expense of GTP or ATP

It is called a \textit{synthetase} because the reaction combines two molecules and it is coupled to the hydrolysis of a nucleoside triphosphate
6. Succinate Dehydrogenase Complex

catalyzes the oxidation of succinate to fumarate, forming a carbon–carbon double bond with the loss of two protons and two electrons. Electrons are passed to a quinone.

Unnumbered figure pg 401a Principles of Biochemistry, 4/e © 2006 Pearson Prentice Hall, Inc.
a competitive inhibitor of the succinate dehydrogenase complex

Succinate

Malonate
7. Fumarase (fumarate hydratase)

Fumarate, like citrate, is a prochiral molecule. Product is L stereoisomer of the hydroxy acid malate.

Near-equilibrium conversion of fumarate to malate through the stereospecific trans addition of water to the double bond of fumarate.

Fumarate, like citrate, is a prochiral molecule. Product is L stereoisomer of the hydroxy acid malate.
8. Malate Dehydrogenase

The reaction catalyzed by malate dehydrogenase is analogous to the reversible reaction catalyzed by lactate dehydrogenase. Lactate dehydrogenase and malate dehydrogenase share a common ancestor.
L-Lactate

L-Malate

NAD\(^{+}\)  NADH, H\(^{+}\)

Pyruvate

Oxaloacetate

Lactate dehydrogenase

Malate dehydrogenase

Box 13-3 figure 1  Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.
BOX 13.3 Converting One Enzyme into Another

Lactate dehydrogenase

Malate dehydrogenase

Conversion of Gln-102 to Arg-102

positively charged arginine forms an ion pair with 3-carboxylate group
Reduced Coenzymes Can Fuel the Production of ATP

**net reaction of the citric acid cycle**

\[
\text{Acetyl CoA} + 3 \text{NAD}^{\oplus} + Q + \text{GDP (or ADP)} + P_i + 2 \text{H}_2\text{O} \rightarrow \\
\text{HS-CoA} + 3 \text{NADH} + \text{QH}_2 + \text{GTP (or ATP)} + 2 \text{CO}_2 + 2 \text{H}^{\oplus} \quad (13.17)
\]

NADH and QH$_2$ can be oxidized by the membrane associated electron-transport chain that is coupled to the production of ATP.
The complete oxidation of 1 molecule of acetyl CoA by the citric acid cycle and subsequent reactions is associated with the production of approximately 10 ATP.
The catabolism of 1 glucose by glycolysis, the citric acid cycle, and reoxidation of NADH and QH$_2$

The complete oxidation of glucose produces up to 32 ATP.
Regulation of the Citric Acid Cycle

- The citric acid cycle occupies a central position in cellular metabolism
  - The pathway is stringently controlled
- Regulation is mediated by
  - allosteric modulators and by
  - covalent modification of the citric acid cycle enzymes
  - supply of acetyl CoA
The activity of the pyruvate dehydrogenase complex controls the supply of acetyl CoA.

Substrates activate the complex.

Accumulation of the products acetyl CoA and NADH decreases flux.
Regulation of the mammalian pyruvate dehydrogenase complex by covalent modification.

NAD\(^+\), HS-CoA
ADP, Pyruvate

NADH, Acetyl CoA

ATP
ADP

Pyruvate dehydrogenase kinase
Pyruvate dehydrogenase phosphatase

Pyruvate dehydrogenase
E\(_1\)

E\(_2\)

E\(_3\)

active

Dephosphorylation

inactive

\(\text{P}\)

\(\text{Ca}^{2+}\)

\(\text{H}_2\text{O}\)

\(\text{P}_i\)
Regulation of the CAC

• Three reactions are regulated
  – citrate synthase,
  – isocitrate dehydrogenase, and
  – α-ketoglutarate dehydrogenase complex
The Citric Acid Cycle Isn’t Always a “Cycle”

• not exclusively a catabolic pathway for the oxidation of acetyl CoA
• It also plays a central role in metabolism at the intersection of several other pathways
• Some intermediates are important metabolic precursors in biosynthesis
• Some catabolic pathways produce citric acid cycle intermediates.
Routes leading to and from the CAC.

Intermediates of the CAC are precursors of carbohydrates, lipids, and amino acids, as well as nucleotides and porphyrins.

Reactions feeding into the cycle replenish the cycle intermediates.
The production of oxaloacetate by pyruvate carboxylase

- The rate at which the citric acid cycle metabolizes acetyl CoA is extremely sensitive to changes in the concentrations of its intermediates.
- Metabolites removed by entry into biosynthetic pathways must be replenished by anaplerotic (Greek for “filling up”) reactions.
- An important regulated replenishment reaction is

\[
\text{Pyruvate} + \text{CO}_2 + \text{ATP} + \text{H}_2\text{O} \longrightarrow \text{Oxaloacetate} + \text{ADP} + \text{P}_i \quad (13.19)
\]
The Glyoxylate Pathway

- bypasses some of the reactions of the CAC
- named after the two-carbon molecule glyoxylate
- There are only two reactions
  - In the first reaction,
    - a six-carbon tricarboxylic acid (isocitrate) is split into
    - a two-carbon molecule (glyoxylate) and
    - a four-carbon dicarboxylic acid (succinate)
    - catalyzed by isocitrate lyase
  - In the second reaction,
    - the two-carbon glyoxylate molecule combines with
    - a two-carbon acetyl CoA molecule
    - to make a four-carbon dicarboxylic acid (malate)
    - catalyzed by malate synthase
The steps which produce CO$_2$ are bypassed.

When the pathway is functioning, the acetyl carbon atoms of acetyl CoA are converted to malate rather than oxidized to CO$_2$. 
Why glyoxylate pathway?

• provides an anabolic alternative for the metabolism of acetyl CoA
  – leading to the formation of glucose
• Cells that contain glyoxylate pathway enzymes can
  – synthesize all their required carbohydrates
  – from any substrate that is a precursor of acetyl CoA
• Yeast can grow on ethanol
  – oxidize ethanol to form acetyl CoA
• Similarly, many bacteria use the glyoxylate pathway to sustain growth on acetate

\[
\begin{align*}
\text{H}_3\text{C} - \text{COO}^{-} + \text{HS-CoA} & \xrightarrow{\text{AMP, PP}_{i}} \text{H}_3\text{C} - \text{C} \equiv \text{S} - \text{CoA} \\
\text{Acetate} & \quad \text{Acetyl CoA synthetase} & \quad \text{Acetyl CoA}
\end{align*}
\]
The glyoxylate pathway in plants

• especially active in oily seed plants
• stored seed oils (triacylglycerols) are converted to carbohydrates that
  – Sustain the plant during germination.
The net effect of the glyoxylate pathway

- can be regarded as a shunt within the CAC
- bypass around the \( \text{CO}_2 \)-producing reactions of the CAC
- net formation of a four-carbon molecule from two molecules of acetyl CoA supplies — a precursor that can be converted to glucose

\[
\begin{align*}
2 \text{Acetyl CoA} + 2 \text{NAD}^+ + \text{Q} + 3 \text{H}_2\text{O} & \rightarrow \text{Oxaloacetate} + 2 \text{HS-CoA} + 2 \text{NADH} + \text{QH}_2 + 4 \text{H}^+ \\
\text{(13.22)}
\end{align*}
\]
Regulation of the glyoxylate pathway in bacteria

- often used to replenish citric acid cycle metabolites
  - that are diverted into a number of biosynthesis pathways
- The key regulated enzyme is isocitrate dehydrogenase
  - covalent modification
- Kinase-catalyzed phosphorylation of
  - a serine residue
  - abolishes isocitrate dehydrogenase activity
- In the dephosphorylated form of
  - the serine residue forms
  - a hydrogen bond with a carboxylate group of isocitrate
(a) The dephosphorylated enzyme is active; isocitrate binds to the active site.
(b) The phosphorylated enzyme is inactive because the negatively charged phosphoryl group (red) electrostatically repels the substrate, preventing it from binding.
A bifunctional enzyme catalyzes both phosphorylation and dephosphorylation. The two activities of the bifunctional enzyme are reciprocally regulated allosterically by intermediates of glycolysis and the citric acid cycle.
Evolution of the Citric Acid Cycle

• probably evolved from the more primitive forked pathway
  – found in many modern species of bacteria
• The evolution involved several of the pathway evolution mechanisms
  – discussed in Chapter 10
• There is evidence for
  – gene duplication,
  – pathway extension,
  – retro-evolution,
  – pathway reversal, and
  – enzyme theft
The left-hand side: a reductive pathway leading to the synthesis of succinate or \( \alpha \)-ketoglutarate

The right-hand branch: an oxidative pathway similar to the first few reactions of the CAC