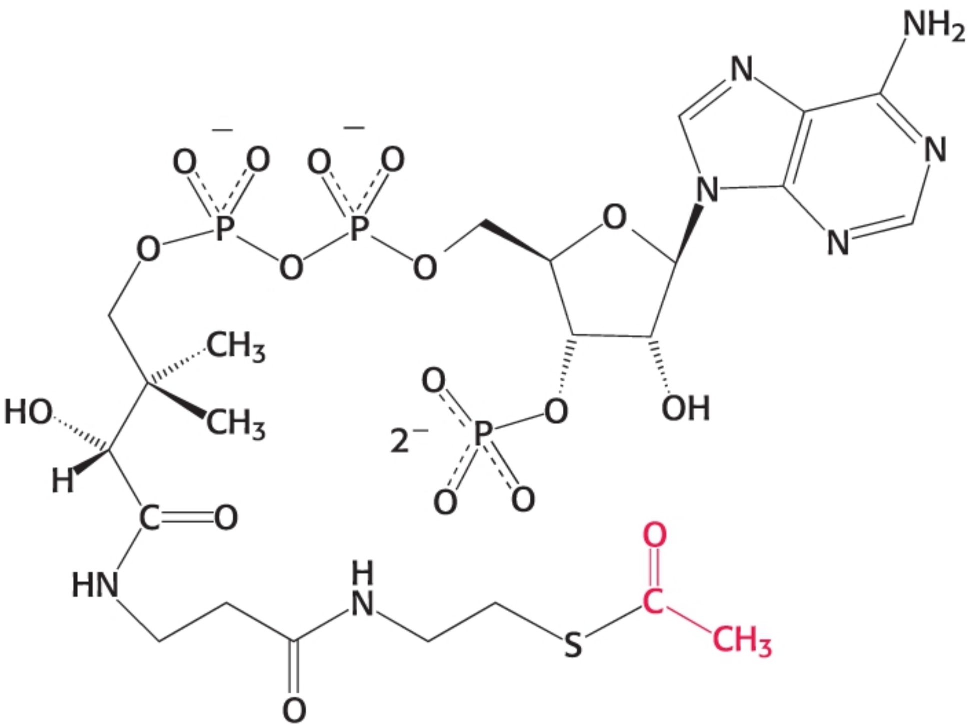
17.1 The Citric Acid Cycle Harvests High-Energy Electrons

The **citric acid cycle (CAC)**, also known as the **tricarboxylic acid (TCA) cycle** or the **Krebs cycle**, is the final pathway for the oxidation of fuel molecules — carbohydrates, fatty acids, and amino acids. Most fuel molecules enter the cycle as **acetyl CoA** (acetyl coenzyme A).



Acetyl coenzyme A (Acetyl CoA)

Under aerobic conditions, the pyruvate generated from glucose is first oxidatively decarboxylated to form acetyl CoA by a large enzyme

complex called the **pyruvate dehydrogenase complex**. The acetyl CoA then enters the citric acid cycle, where all remaining carbons originally derived from glucose are completely oxidized to CO_2. In eukaryotes, the reactions of the pyruvate dehydrogenase complex and the citric acid cycle take place in the matrix of the mitochondria (**Figure 17.1**), in contrast with those of glycolysis, which take place in the cytoplasm.

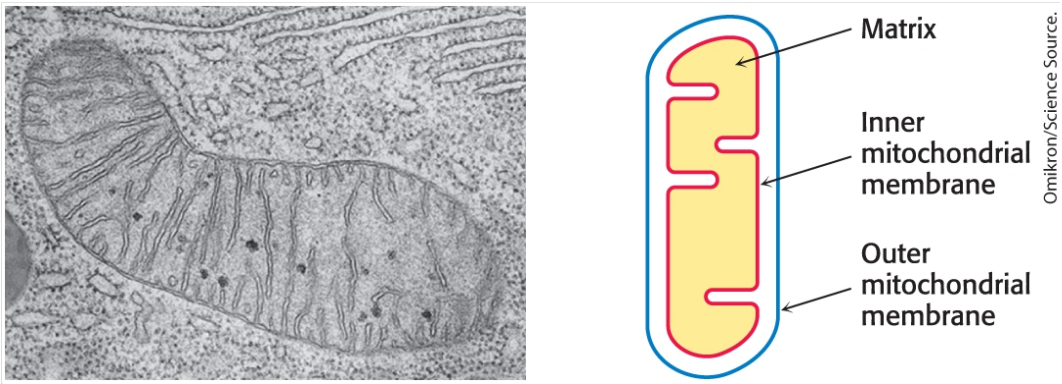


FIGURE 17.1 Mitochondria have distinct compartments defined by two membranes. The double membrane of a single mitochondrion is evident in this electron micrograph. The numerous invaginations of the inner mitochondrial membrane are called *cristae*. The oxidative decarboxylation of pyruvate and the sequence of reactions in the citric acid cycle take place within the matrix.

What is the function of the citric acid cycle in transforming fuel molecules into ATP? Recall that fuel molecules are carbon compounds that are capable of being oxidized — that is, of losing electrons (Section 15.3). The citric acid cycle includes a series of oxidation–reduction reactions that result in the oxidation of an acetyl group to two molecules of carbon dioxide. This oxidation generates high-energy electrons that will be used to power the synthesis of ATP. The catabolic function of the citric acid cycle is the harvesting of high-energy electrons from carbon fuels.

The citric acid cycle is also the central metabolic hub of the cell, providing a link that connects both catabolic and anabolic pathways. It is the gateway to the aerobic metabolism of any molecule that can be transformed into an acetyl group or a component of the citric acid cycle. The cycle is also an important source of precursors for the building blocks of many other molecules such as amino acids, nucleotide bases, and porphyrin (the organic component of heme). The citric acid cycle component, oxaloacetate, is also an important precursor to glucose (Section 16.4).

The overall pattern of the citric acid cycle is shown in **Figure 17.2**. A four-carbon compound (oxaloacetate) condenses with a two-carbon acetyl unit to yield a six-carbon tricarboxylic acid. The six-carbon compound releases CO_2 twice in two successive oxidative decarboxylations that yield high-energy electrons. What remains is a four-carbon compound that is further processed to regenerate oxaloacetate, which can initiate another round of the cycle. Two carbon atoms enter the cycle as an acetyl unit, and two carbon atoms leave the cycle in the form of two molecules of CO_2.

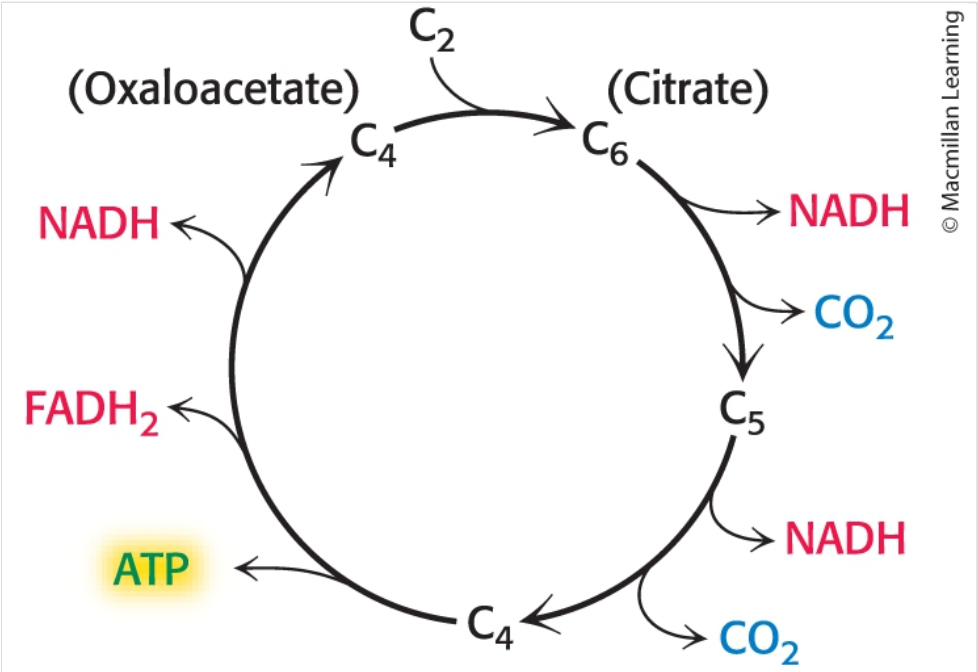


FIGURE 17.2 An overview of the citric acid cycle reveals the flow of carbon atoms and the major products of the cycle. A two-carbon group (C_2) combines with a four-carbon molecule (C_4, oxaloacetate) to form a six-carbon molecule (C_6, citrate). Two carbons are lost as CO_2 in the cycle, ending with the regeneration of oxaloacetate for another round. As the result, each round of the citric acid cycle oxidizes two-carbon units, producing two molecules of CO_2, one molecule of ATP, and high-energy electrons in the form of NADH and $FADH_2$.

Note that the citric acid cycle itself neither generates much ATP nor includes oxygen as a reactant (Figure 17.3). Instead, the citric acid cycle removes electrons from acetyl CoA and uses these electrons to reduce NAD^+ and FAD to form NADH and $FADH_2$. Three hydride ions (hence, six electrons) are transferred to three molecules of nicotinamide adenine dinucleotide (NAD^+), and one pair of hydrogen atoms (hence,

two electrons) is transferred to one molecule of flavin adenine dinucleotide (FAD) each time an acetyl CoA is processed by the cycle. Electrons released in the reoxidation of NADH and $FADH_2$ flow through a series of membrane proteins (referred to as the *electron-transport chain*) to generate a proton gradient across the inner mitochondrial membrane. These protons then flow through ATP synthase to generate ATP from ADP and inorganic phosphate. These electron carriers yield nine molecules of ATP when they are oxidized by O_2 through the process of oxidative phosphorylation (Chapter 18).

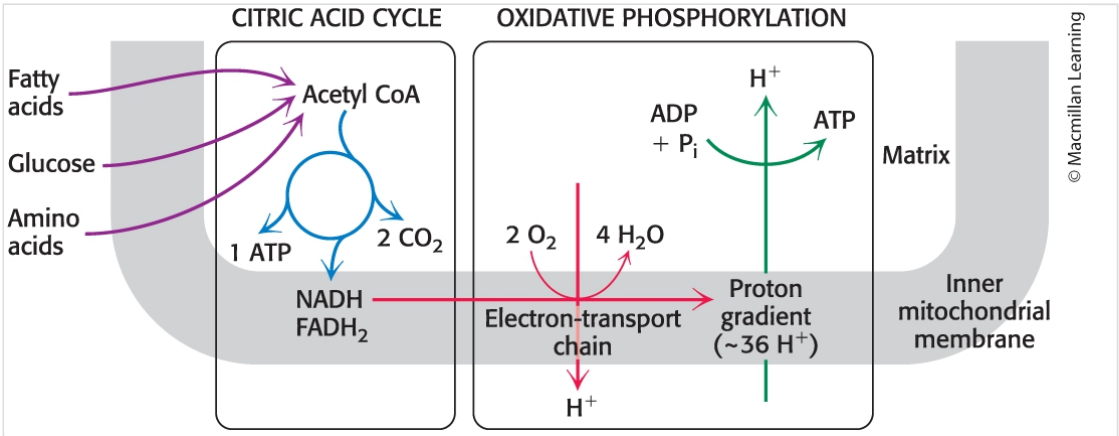


FIGURE 17.3 Cellular respiration removes high-energy electrons from carbon fuel molecules to generate ATP. The citric acid cycle constitutes the first stage in cellular respiration, the removal of high-energy electrons from carbon fuels in the form of NADH and $FADH_2$ (blue pathway). These electrons reduce O_2 to generate a proton gradient (red pathway), which is used to synthesize ATP (green pathway). The reduction of O_2 and the synthesis of ATP constitute oxidative phosphorylation.

The citric acid cycle, in conjunction with oxidative phosphorylation, provides the bulk of energy used by aerobic cells — in human beings, greater than 90%. It is highly efficient because the oxidation of a limited number of citric acid cycle molecules can generate large amounts of

NADH and $FADH_2$. Note in Figure 17.2 that the four-carbon molecule, oxaloacetate, that initiates the first step in the citric acid cycle is regenerated at the end of one passage through the cycle. Thus, one molecule of oxaloacetate is capable of participating in the oxidation of many acetyl groups.

17.2 The Pyruvate Dehydrogenase Complex Links Glycolysis to the Citric Acid Cycle

Carbohydrates, most notably glucose, are processed by glycolysis into pyruvate (Chapter 16). Under anaerobic conditions, the pyruvate is converted into lactate or ethanol, depending on the organism. Under aerobic conditions, the pyruvate is transported into mitochondria by a specific carrier protein embedded in the mitochondrial membrane. In the mitochondrial matrix, pyruvate is oxidatively decarboxylated by the pyruvate dehydrogenase complex — a highly integrated unit of three distinct enzymes — to form acetyl CoA.

$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \rightarrow \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

This irreversible reaction is the link between glycolysis and the citric acid cycle (**Figure 17.4**). Note that the pyruvate dehydrogenase complex produces CO_2 and captures high-transfer-potential electrons in the form of NADH. Thus, the pyruvate dehydrogenase reaction has many of the key features of the reactions of the citric acid cycle itself.

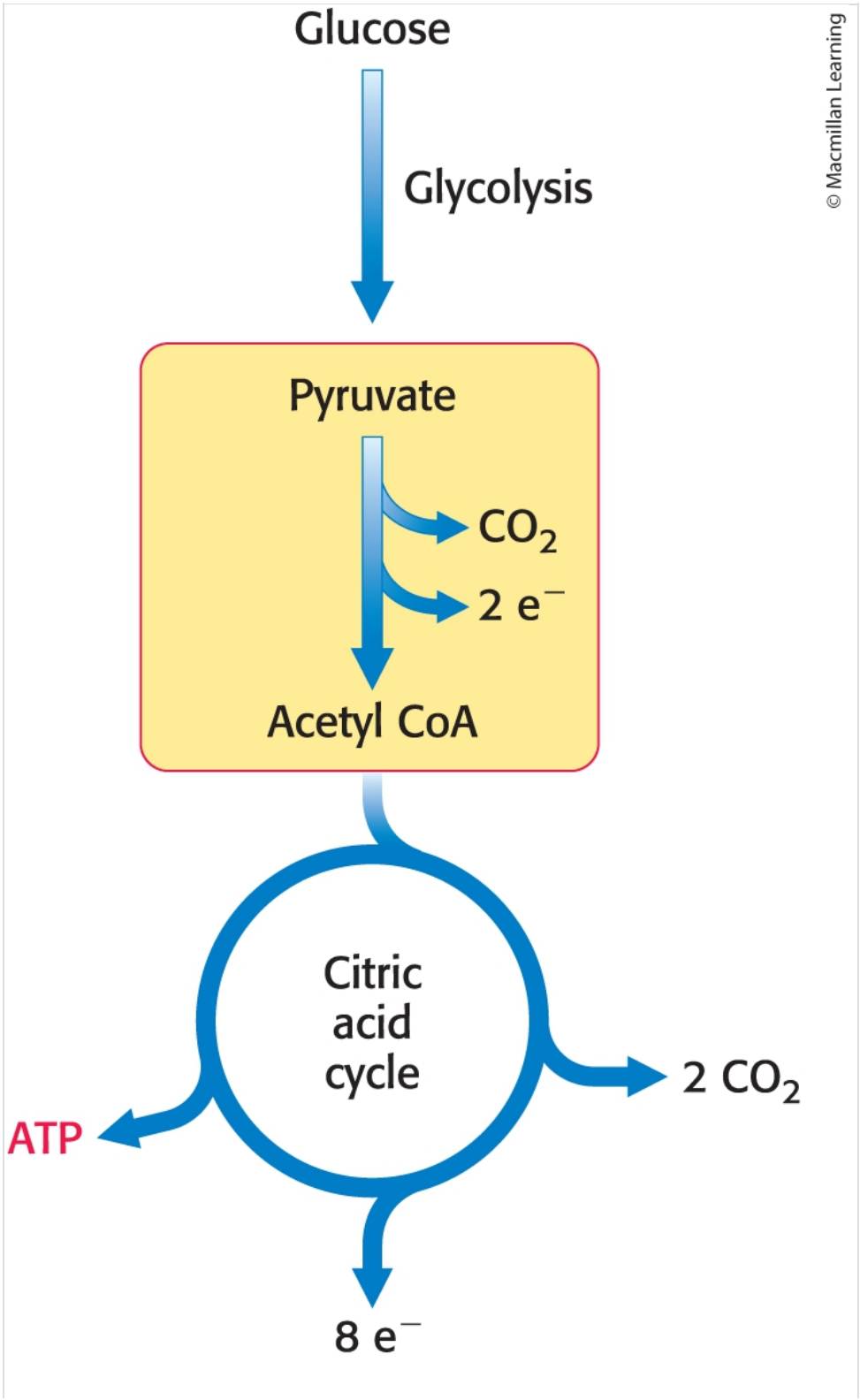

Glucose
Glycolysis
Pyruvate
CO_2
2 e^-
Acetyl CoA
Citric acid cycle
2 CO_2
ATP
8 e^-
© Macmillan Learning

FIGURE 17.4 The pyruvate dehydro-genase complex connects glycolysis and the citric acid cycle. Pyruvate produced by glycolysis is converted into acetyl CoA, the fuel of the citric acid cycle.

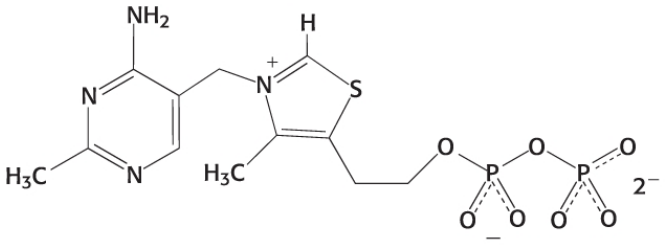
The pyruvate dehydrogenase complex, the components of which are detailed in **Table 17.1**, is another example of the organization of enzymes into supramolecular structures (Section 16.3). Pyruvate dehydrogenase complex is a member of a family of homologous complexes that include the citric acid cycle enzyme α-ketoglutarate dehydrogenase complex (Section 17.3). These complexes are giant, larger than ribosomes, with molecular masses ranging from 4 million to 10 million Da. As we will see, their elaborate structures allow groups to travel from one active site to another, connected by tethers to the core of the structure.

TABLE 17.1 Pyruvate dehydrogenase complex of *E. coli*

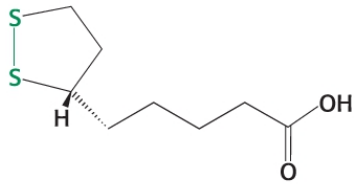
Enzyme	Abbreviation	Prosthetic group	Reaction catalyzed
Pyruvate dehydrogenase component	E_1	TPP	Oxidative decarboxylation of pyruvate
Dihydrolipoyl transacetylase	E_2	Lipoamide	Transfer of acetyl group to CoA
Dihydrolipoyl dehydrogenase	E_3	FAD	Regeneration of the oxidized form of lipoamide

Mechanism: The synthesis of acetyl coenzyme A from pyruvate requires three enzymes and five coenzymes

The mechanism of the pyruvate dehydrogenase reaction is wonderfully complex, more so than is suggested by its simple stoichiometry. The reaction requires the participation of the three enzymes of the pyruvate dehydrogenase complex and five coenzymes. The coenzymes thiamine pyrophosphate (TPP), lipoic acid, and FAD serve as catalytic cofactors, and CoA and NAD^+ are stoichiometric cofactors, cofactors that function as substrates.

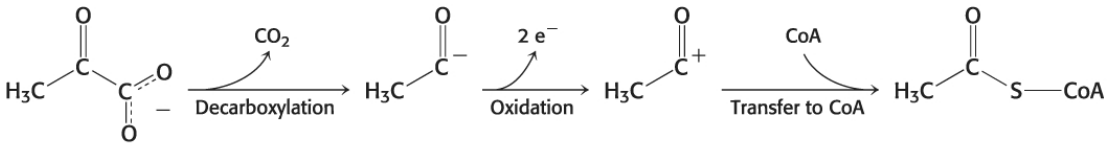


Thiamine pyrophosphate (TPP)



Lipoic acid

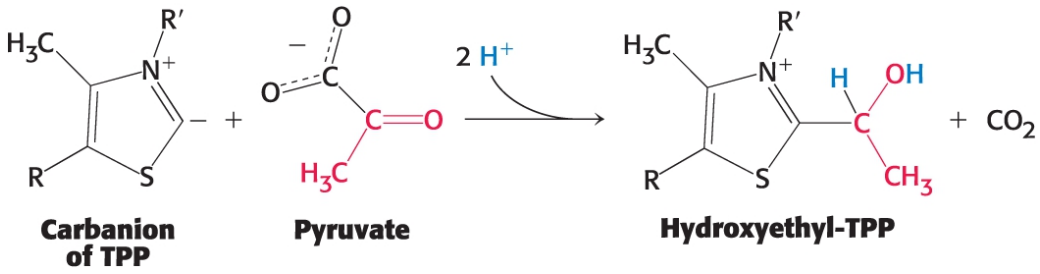
The conversion of pyruvate into acetyl CoA consists of decarboxylation, oxidation, and transfer of the resultant acetyl group to CoA. A fourth step is required to regenerate the active enzyme.



Pyruvate **Acetyl CoA**

These steps must be coupled to preserve the free energy derived from the decarboxylation step to drive the formation of NADH and acetyl CoA. Let us look into each step in more detail:

1. *Decarboxylation*. Pyruvate combines with TPP and is then decarboxylated to yield hydroxyethyl-TPP.



This reaction, the rate-limiting step in acetyl CoA synthesis, is catalyzed by the pyruvate dehydrogenase component (E_1) of the multienzyme complex. TPP is the prosthetic group of the pyruvate dehydrogenase component (Figure 17.5).

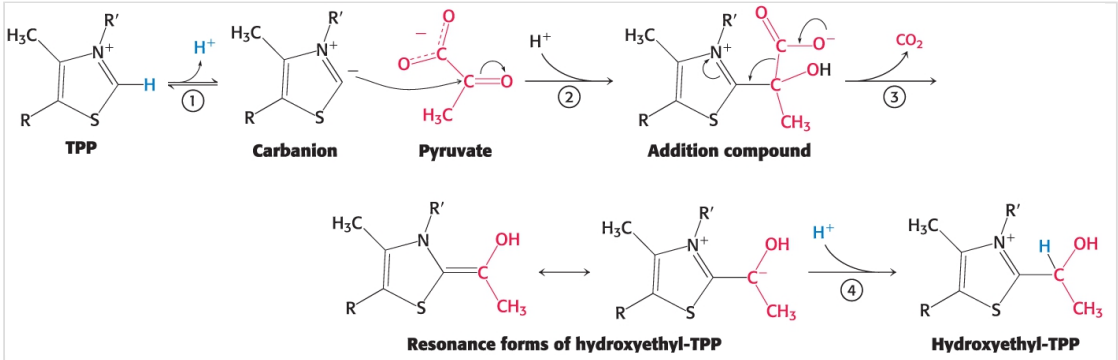
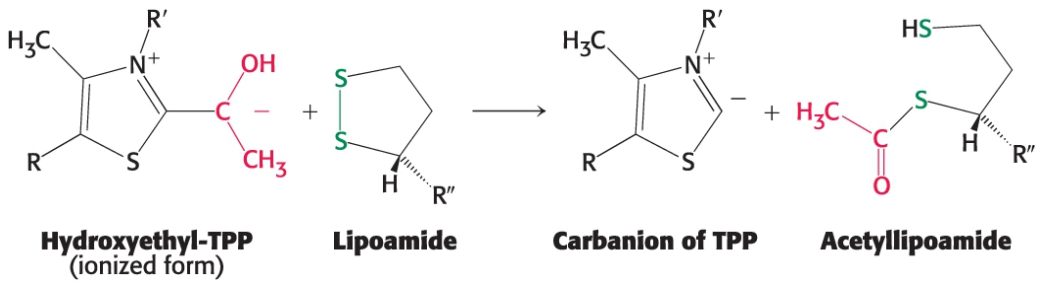


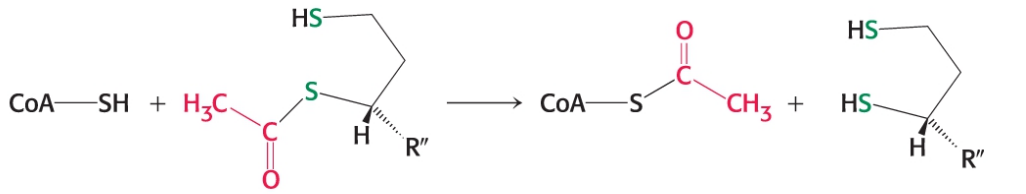
FIGURE 17.5 The mechanism of the E_1 decarboxylation reaction uses a critical thiamine-derived prosthetic group. E_1 is the pyruvate dehydrogenase component of the pyruvate dehydrogenase complex. A key feature of the prosthetic group, TPP, is that the carbon atom between the nitrogen and sulfur atoms in the thiazole ring is much more acidic than most $=C-$ groups, with a pK_a value near 10. (1) This carbon center ionizes to form a *carbanion.* (2) The carbanion readily adds to the carbonyl group of pyruvate. (3) This addition is followed by the decarboxylation of pyruvate. The positively charged ring of TPP acts as an electron sink that stabilizes the negative charge that is transferred to the ring as part of the decarboxylation. (4) Protonation yields hydroxyethyl-TPP.

2. *Oxidation*. The hydroxyethyl group attached to TPP is oxidized to form an acetyl group while being simultaneously transferred to lipoamide, a derivative of lipoic acid that is linked to the side chain of a lysine residue by an amide linkage. Note that this transfer results in the formation of an energy-rich thioester bond.



The oxidant in this reaction is the disulfide group of lipoamide, which is reduced to its disulfhydryl form. This reaction, also catalyzed by the pyruvate dehydrogenase component E_1, yields acetyllipoamide.

3. *Formation of acetyl CoA*. The acetyl group is transferred from acetyllipoamide to CoA to form acetyl CoA.

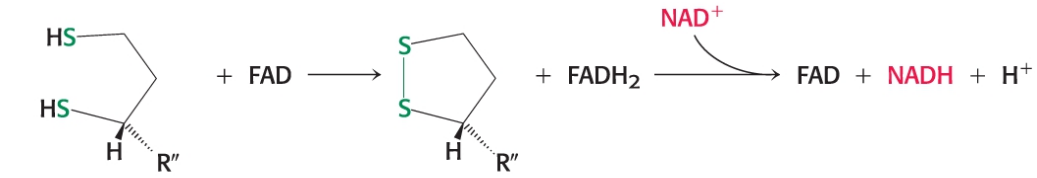


Coenzyme A **Acetyllipoamide** **Acetyl CoA** **Dihydrolipoamide**

Dihydrolipoyl transacetylase (E_2) catalyzes this reaction. The energy-rich thioester bond is preserved as the acetyl group is transferred to CoA. Recall that CoA serves as a carrier of many activated acyl groups, of which acetyl is the simplest (Section 15.4). Acetyl CoA, the fuel for the citric acid cycle, has now been generated from pyruvate.

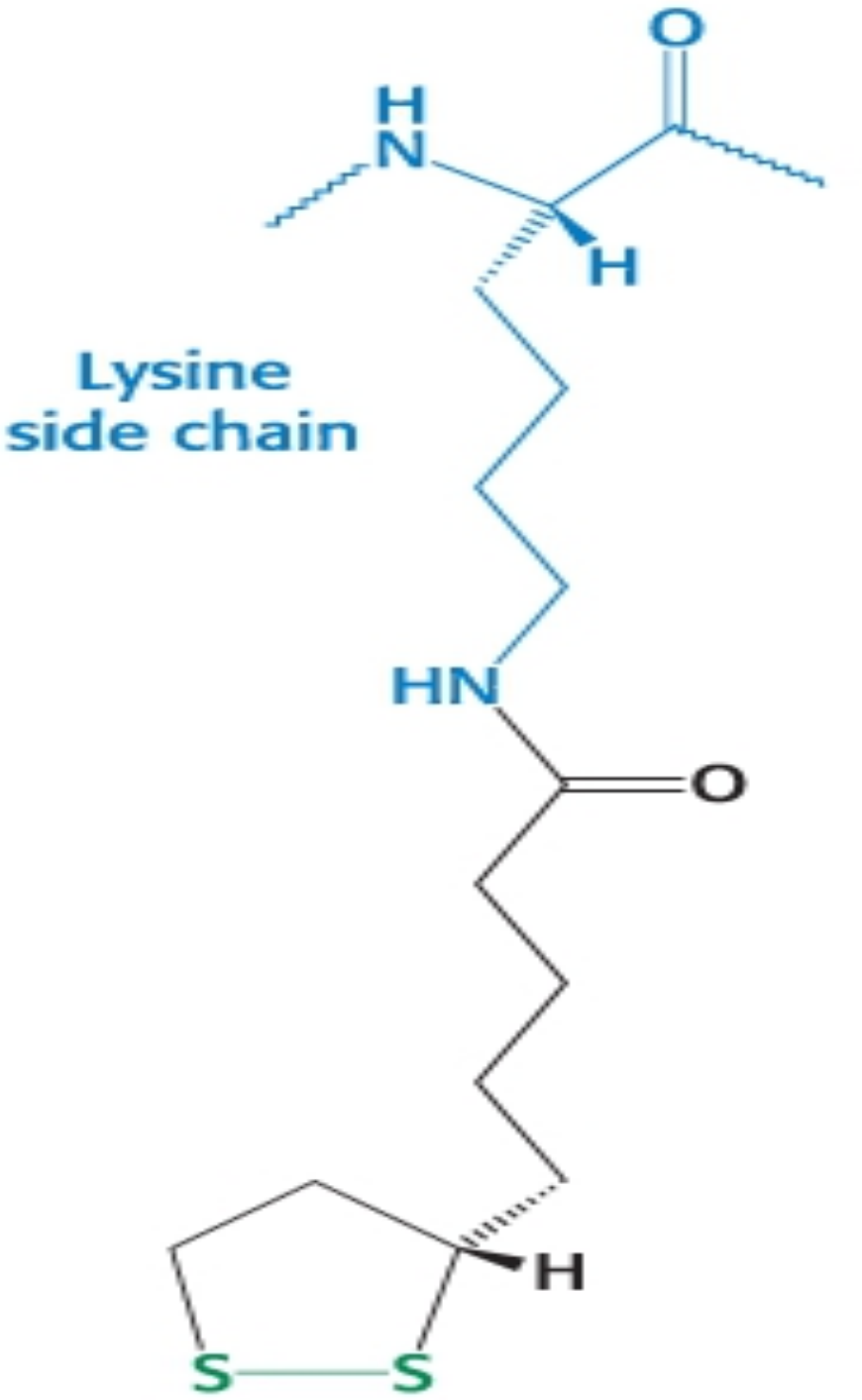
4. *Regeneration of oxidized lipoamide*. The pyruvate dehydrogenase complex cannot complete another catalytic cycle until the dihydrolipoamide is oxidized to lipoamide. In the fourth step,

the oxidized form of lipoamide is regenerated by dihydrolipoyl dehydrogenase (E_3). Two electrons are transferred to an FAD prosthetic group of the enzyme and then to NAD^+.



Dihydrolipoamide **Lipoamide**

This electron transfer from FAD to NAD^+ is unusual because the common role for FAD is to receive electrons from NADH. The electron-transfer potential of FAD is increased by its chemical environment within the enzyme, enabling it to transfer electrons to NAD^+. Proteins tightly associated with FAD or flavin mononucleotide (FMN) are called **flavoproteins**.



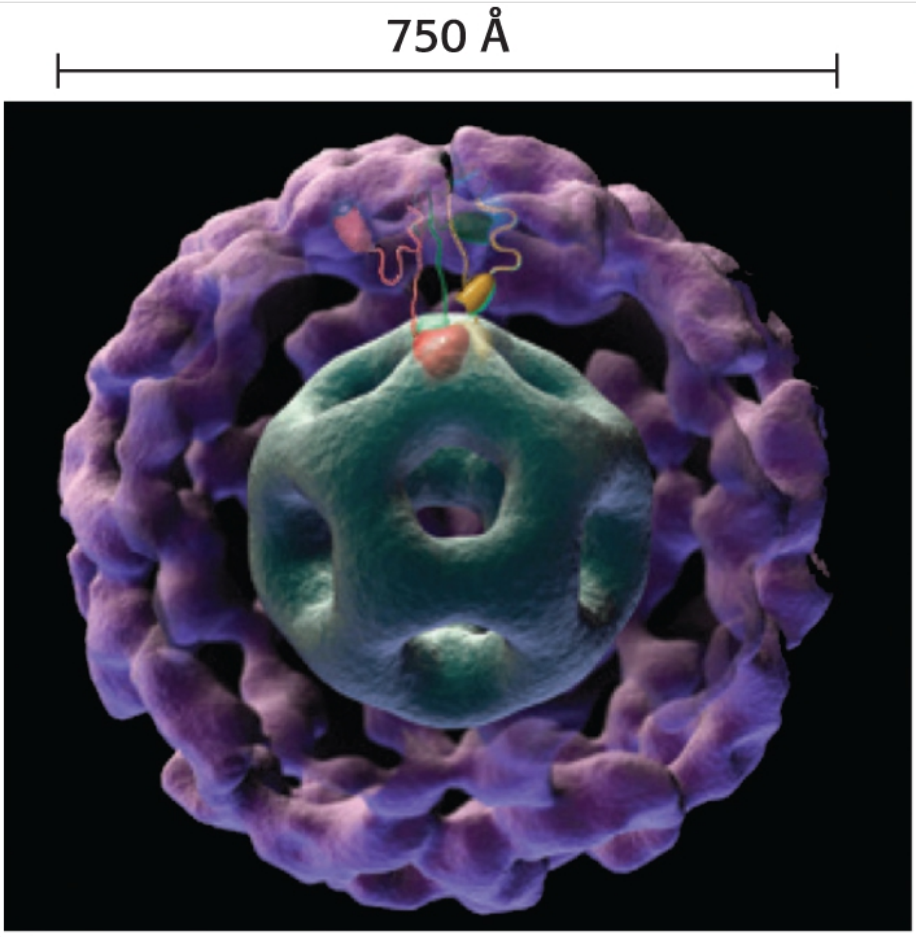
Reactive disulfide bond

Lipoamide

Flexible linkages allow lipoamide to move between different active sites

The structures and precise composition of the component enzymes of the pyruvate dehydrogenase complex vary among species. However, there are common features.

The core of the complex is formed by 60 molecules of the transacetylase component E_2 (**Figure 17.6**). Transacetylase consists of 20 catalytic trimers assembled to form a hollow cube. Each of the three subunits forming a trimer has three major domains (**Figure 17.7**). At the amino terminus is a small domain that contains a bound flexible lipoamide cofactor attached to a lysine residue. This domain is homologous to biotin-binding domains such as that of pyruvate carboxylase (Figure 16.29). The lipoamide domain is followed by a small domain that interacts with E_3 within the complex. A larger transacetylase domain completes an E_2 subunit.



Donald Bliss, National Library of Medicine.

FIGURE 17.6 The structure of the pyruvate dehydrogenase complex from bacteria reveals a massive protein complex. The image of the complex from *B. stearothermophilus*, which was derived from cryo-electron microscopic data (Section 4.5), shows an inner core consisting of the E_2 enzyme. The shell surrounding the core consists of E_1 and E_3 enzymes, although only the E_1 enzymes are shown in this structure. Two of the 60 lipoamide arms are shown (red and yellow).

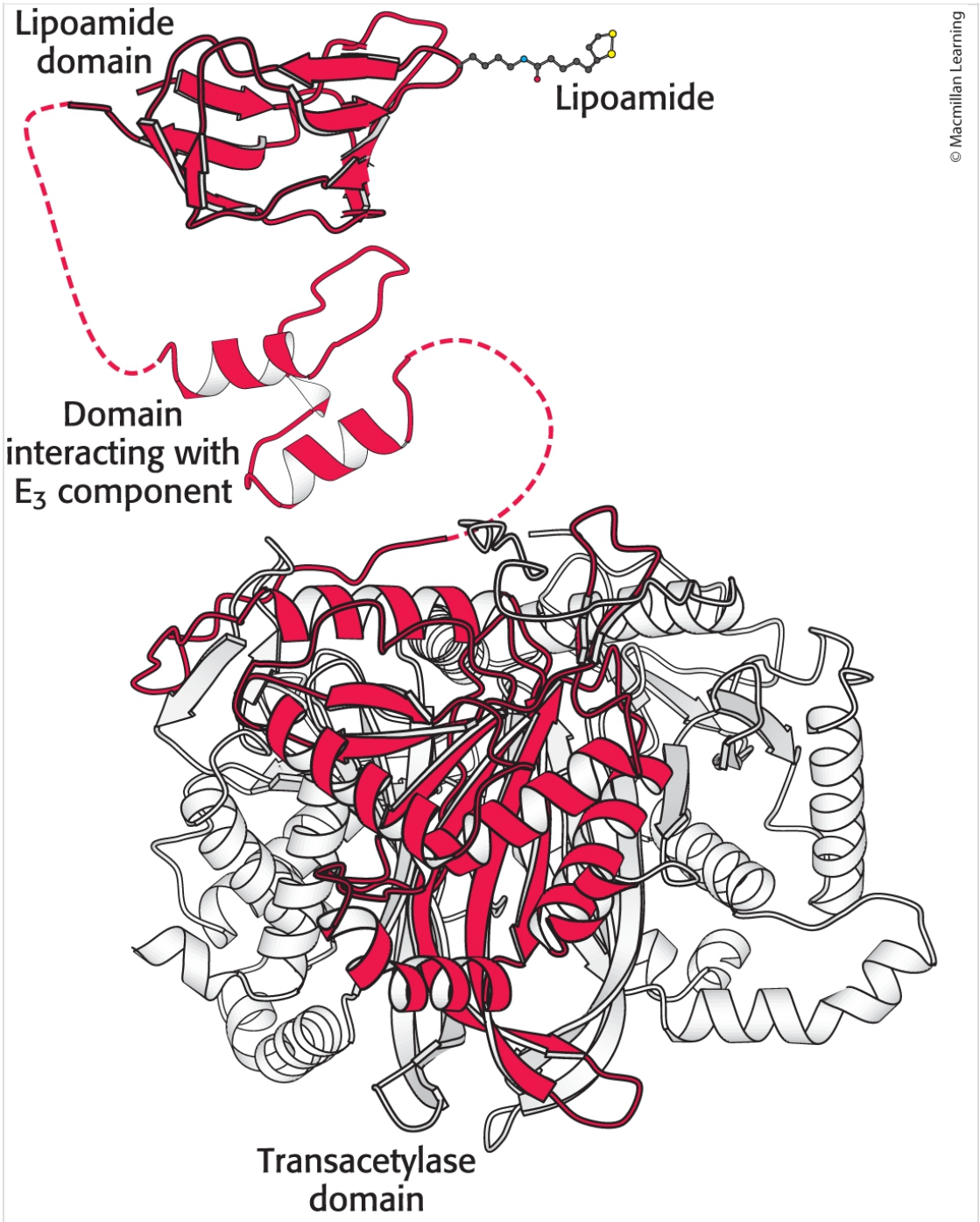


FIGURE 17.7 The transacetylase (E_2) core is made up of three distinct domains. The figure shows one subunit of the transacetylase trimer. *Notice* that each subunit consists of three domains: a lipoamide-binding domain, a small domain that interacts with E_3, and a

large transacetylase catalytic domain. The catalytic domains interact with one another to form the catalytic trimer. Transacetylase domains of three identical subunits are shown, with one depicted in red and the others in white in the ribbon representation.

Surrounding the core transacetylase is a shell composed of ~ 45 copies of the E_1 and ~ 10 copies of E_3 enzymes. In mammals, E_1 is an $\alpha_2\beta_2$ tetramer, and E_3 is an $\alpha\beta$ dimer, and this core contains another protein, E_3-binding protein (E_3-BP), which facilitates the interaction between E_2 and E_3. If E_3-BP is missing, the complex has greatly reduced activity. The gap between the outer shell and the transacetylase core allows the lipoamide arms to visit the various active sites (Figure 17.6).

How do the three distinct active sites work in concert? The key is the long, flexible lipoamide arm of the E_2 subunit, which carries substrate from active site to active site (**Figure 17.8**):

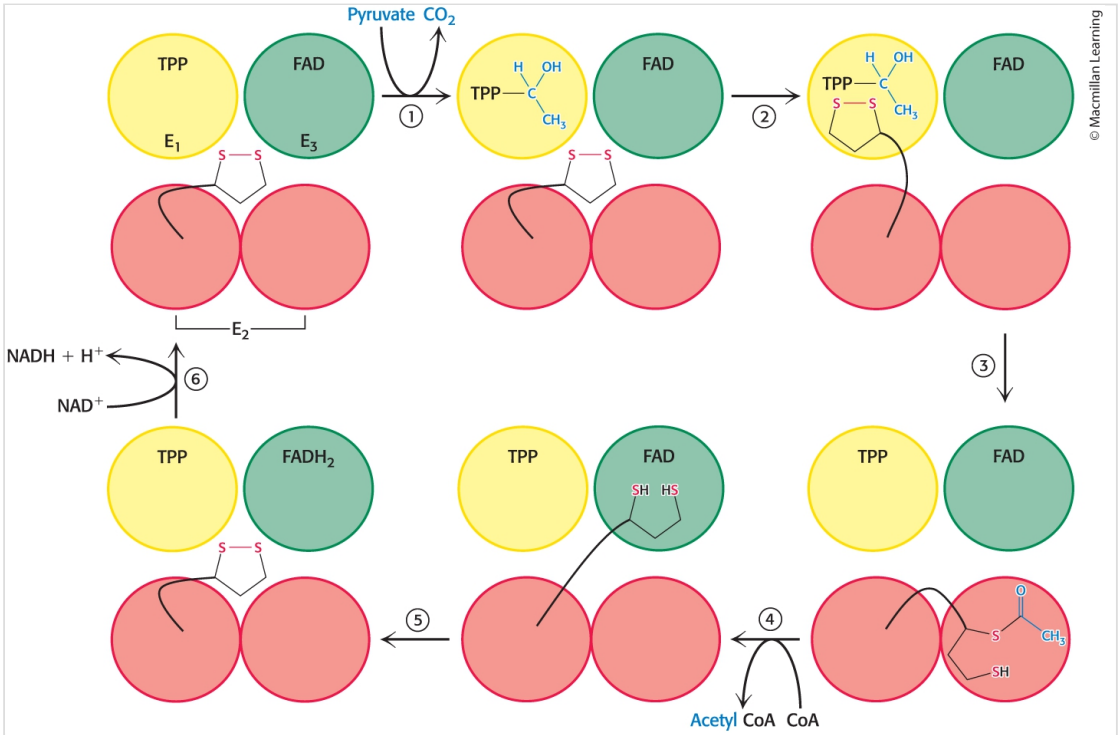


FIGURE 17.8 Three enzymes cooperate in the full reactions of the pyruvate dehydrogenase complex. At the top left, the enzyme (represented by a yellow, a green, and two red spheres) is unmodified and ready for a catalytic cycle. (1) Pyruvate is decarboxylated to form hydroxyethyl-TPP. (2) The lipoamide arm of E_2 moves into the active site of E_1. (3) E_1 catalyzes the transfer of the two-carbon group to the lipoamide group to form the acetyl–lipoamide complex. (4) E_2 catalyzes the transfer of the acetyl moiety to CoA to form the product acetyl CoA. The dihydrolipoamide arm then swings to the active site of E_3. E_3 catalyzes (5) the oxidation of the dihydrolipoamide and (6) the transfer of the protons and electrons to NAD^+ to complete the reaction cycle.

1. Pyruvate is decarboxylated at the active site of E_1, forming the hydroxyethyl-TPP intermediate, and CO_2 leaves as the first product. This active site lies deep within the E_1 complex,

connected to the enzyme surface by a 20-Å-long hydrophobic channel.

2. E_2 inserts the lipoamide arm of the lipoamide domain into the deep channel in E_1 leading to the active site.

3. E_1 catalyzes the transfer of the acetyl group to the lipoamide. The acetylated arm then leaves E_1 and enters the E_2 cube to visit the active site of E_2, located deep in the cube at the subunit interface.

4. The acetyl moiety is then transferred to CoA, and the second product, acetyl CoA, leaves the cube. The reduced lipoamide arm then swings to the active site of the E_3 flavoprotein.

5. At the E_3 active site, the lipoamide is oxidized by coenzyme FAD. The reactivated lipoamide is ready to begin another reaction cycle.

6. The final product, NADH, is produced with the reoxidation of $FADH_2$ to FAD.

The structural integration of three kinds of enzymes and the long, flexible lipoamide arm make the coordinated catalysis of a complex reaction possible. The proximity of one enzyme to another increases the overall reaction rate and minimizes side reactions. All the intermediates in the oxidative decarboxylation of pyruvate remain bound to the complex throughout the reaction sequence and are readily transferred as the flexible arm of E_2 calls on each active site in turn.

Self-Check Question

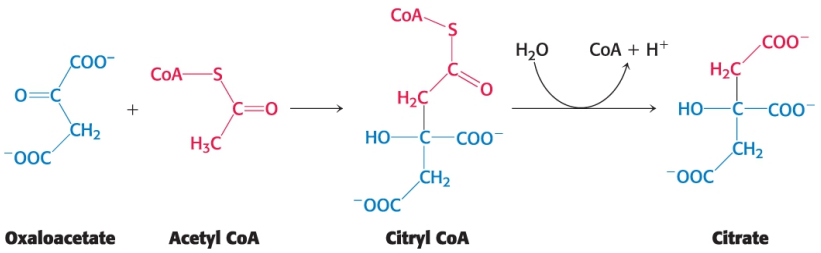
A key product of pyruvate dehydrogenase complex reaction, acetyl CoA, is released after the fourth step. What is the purpose of the remaining steps?

17.3 The Citric Acid Cycle Oxidizes Two-Carbon Units

The conversion of pyruvate into acetyl CoA by the pyruvate dehydrogenase complex is the link between glycolysis and cellular respiration because acetyl CoA is the fuel for the citric acid cycle. Indeed, under aerobic conditions, all fuels are ultimately metabolized to acetyl CoA or components of the citric acid cycle.

Citrate synthase forms citrate from oxaloacetate and the acetyl group from acetyl coenzyme A

The citric acid cycle begins with the addition of a four-carbon unit, oxaloacetate, and a two-carbon unit, the acetyl group of acetyl CoA. Oxaloacetate reacts with acetyl CoA and H_2O to yield citrate and CoA.



This reaction, which is an aldol addition followed by a hydrolysis, is catalyzed by citrate synthase. Oxaloacetate first combines with acetyl CoA to form citryl CoA, a molecule that is energy rich because it contains the thioester bond that originated in acetyl CoA. The hydrolysis of citryl CoA thioester to citrate and CoA drives the overall reaction far in the direction of the synthesis of citrate. In essence, the hydrolysis of the thioester powers the synthesis of a new molecule from two precursors.

Mechanism: The mechanism of citrate synthase prevents undesirable reactions

Because the condensation of acetyl CoA and oxaloacetate initiates the citric acid cycle, it is very important that side reactions, notably the hydrolysis of acetyl CoA to acetate and CoA, be

minimized. Let us briefly consider how citrate synthase prevents the wasteful hydrolysis of acetyl CoA.

Mammalian citrate synthase is a dimer of identical 49-kDa subunits. Each active site is located in a cleft between the large and the small domains of a subunit, adjacent to the subunit interface. X-ray crystallographic studies of citrate synthase and its complexes with several substrates and inhibitors have revealed that the enzyme undergoes large conformational changes in the course of catalysis. Citrate synthase exhibits sequential, ordered kinetics: oxaloacetate binds first, followed by acetyl CoA.

The reason for the ordered binding is that oxaloacetate induces a major structural rearrangement leading to the creation of a binding site for acetyl CoA. The binding of oxaloacetate converts the open form of the enzyme into a more closed form (Figure 17.9). In each subunit, the small domain rotates 19 degrees relative to the large domain. Movements as large as 15 Å are produced by the rotation of α helices elicited by quite small shifts of side chains around bound oxaloacetate. These structural changes create a binding site for acetyl CoA.

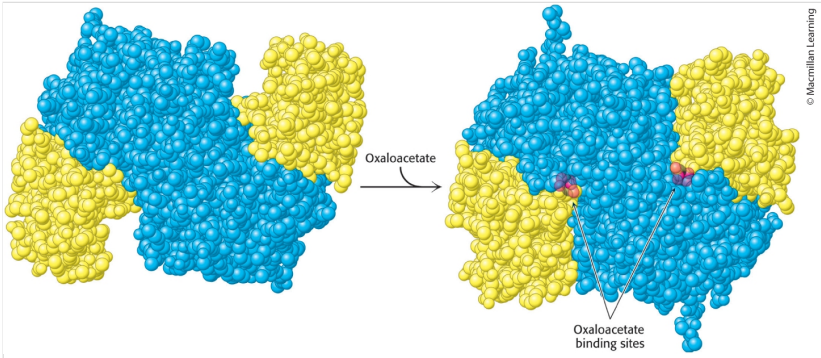


FIGURE 17.9 The ordered binding of substrates by citrate synthase is explained by conformational changes upon binding oxaloacetate. The small domain of each subunit of the homodimer is shown in yellow; the large domains are shown in blue. (Left) The open form of enzyme alone is shown. (Right) The closed form of the oxaloacetate-enzyme complex reveals significant structural changes upon oxaloacetate binding that create a binding site with high affinity for acetyl CoA, the second substrate to bind.

[Drawn from 5CSC.pdb and 4CTS.pdb.]

INTERACT with this model in Achieve

Citrate synthase catalyzes the condensation reaction by bringing the substrates into close proximity, orienting them, and polarizing certain bonds (Figure 17.10). The donation and removal of protons transforms acetyl CoA into an enol intermediate. The enol attacks oxaloacetate to form a carbon–carbon double bond linking acetyl CoA and oxaloacetate. The newly formed citryl CoA induces additional structural changes in the enzyme, causing the active site to become completely enclosed. The enzyme cleaves the citryl CoA thioester by hydrolysis.

CoA leaves the enzyme, followed by citrate, and the enzyme returns to the initial open conformation.

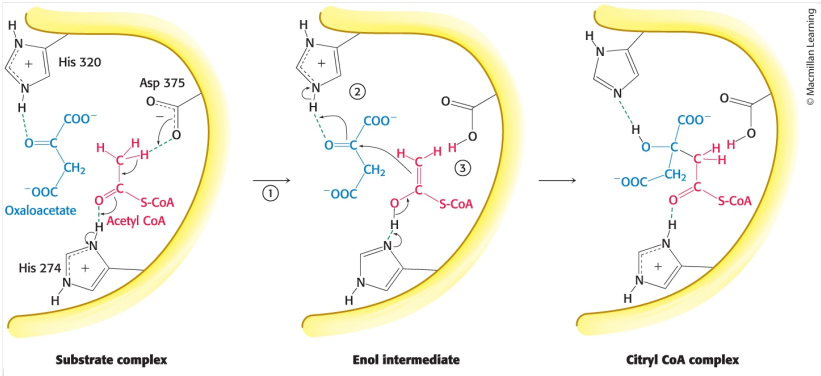
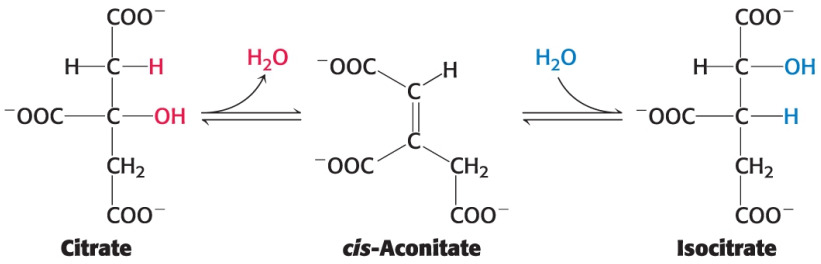


FIGURE 17.10 The first part of the citrate synthase mechanism forms citryl CoA. (1) In the substrate complex (left), His 274 donates a proton to the carbonyl oxygen of acetyl CoA to promote the removal of a methyl proton by Asp 375 to form the enol intermediate (center). (2) Oxaloacetate is activated by the transfer of a proton from His 320 to its carbonyl carbon atom. (3) Simultaneously, the enol of acetyl CoA attacks the carbonyl carbon of oxaloacetate to form a carbon–carbon bond linking acetyl CoA and oxaloacetate. His 274 is reprotonated, and citryl CoA is formed. His 274 participates again as a proton donor to hydrolyze the thioester (not shown), yielding citrate and CoA.

We can now understand how the wasteful hydrolysis of acetyl CoA is prevented. Citrate synthase is well suited to hydrolyze *citryl* CoA but not *acetyl* CoA. How is this discrimination accomplished? First, acetyl CoA does not bind to the enzyme until oxaloacetate is bound and ready for condensation. Second, the catalytic residues crucial for the hydrolysis of the thioester linkage are not appropriately positioned until citryl CoA is formed. As with hexokinase and triose phosphate isomerase (Section 16.2), induced fit prevents an undesirable side reaction.

Citrate is isomerized into isocitrate

The hydroxyl group is not properly located in the citrate molecule for the oxidative decarboxylations that follow. Thus, citrate is isomerized into isocitrate to enable the six-carbon unit to undergo oxidative decarboxylation. The isomerization of citrate is accomplished by a dehydration step followed by a hydration step. The result is an interchange of an H and an OH. The enzyme catalyzing both steps is called *aconitase* because *cis*-aconitate is an intermediate.



Citrate ***cis*-Aconitate** **Isocitrate**

Aconitase is an iron–sulfur protein, or nonheme iron protein, in that it contains iron that is not bonded to heme. Rather, its four iron atoms are complexed to four inorganic sulfides and three cysteine sulfur atoms, leaving one iron atom available to bind citrate through one of its COO^- groups and an OH group (Figure 17.11). This Fe–S cluster participates in dehydrating and rehydrating the bound substrate.

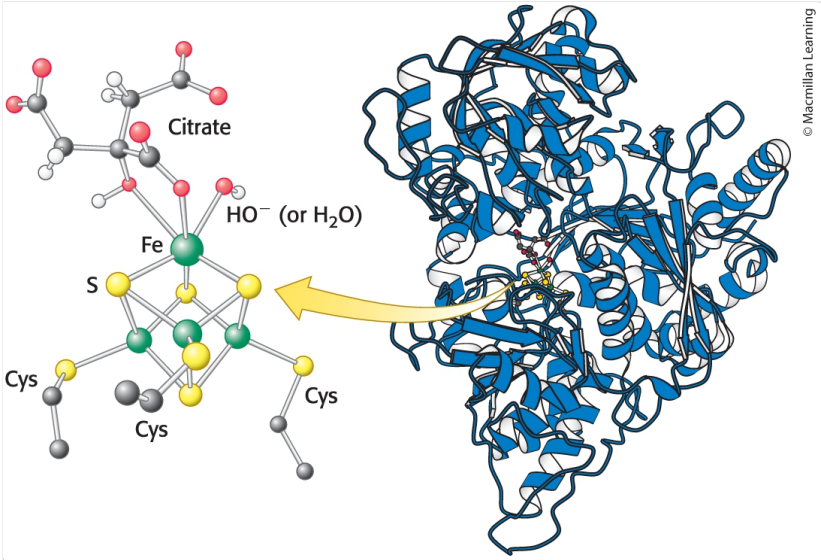


FIGURE 17.11 Citrate binds directly to the iron–sulfur complex of aconitase. A 4Fe–4S iron–sulfur cluster is a component of the active site of aconitase. Notice that one of the iron atoms of the cluster binds to a COO^- group and an OH group of citrate.

[Drawn from 1C96.pdb.]

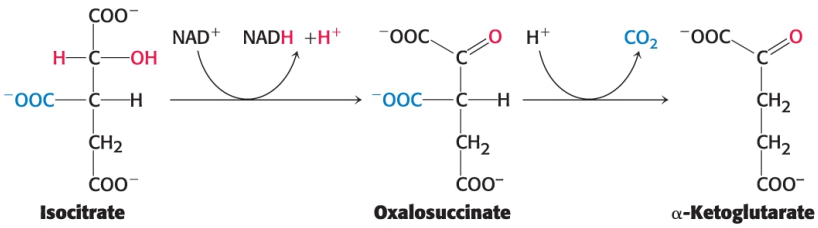
INTERACT with this model in Achieve

Isocitrate is oxidized and decarboxylated to alpha-ketoglutarate

We come now to the first of four oxidation–reduction reactions in the citric acid cycle. The oxidative decarboxylation of isocitrate is catalyzed by isocitrate dehydrogenase.

$$\text{Isocitrate} + NAD^+ \rightarrow \alpha\text{–ketoglutarate} + CO_2 + NADH$$

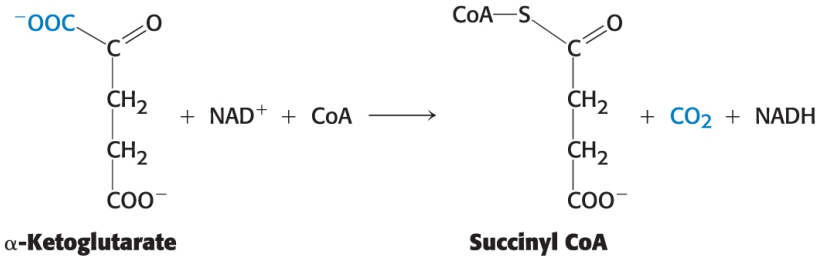
The intermediate in this reaction is oxalosuccinate, an unstable β-ketoacid. While bound to the enzyme, it loses CO_2 to form α-ketoglutarate.



This oxidation generates the first high-transfer-potential electron carrier, NADH, in the cycle.

Succinyl coenzyme A is formed by the oxidative decarboxylation of alpha-ketoglutarate

The conversion of isocitrate into α-ketoglutarate is followed by a second oxidative decarboxylation reaction, the formation of succinyl CoA from α-ketoglutarate.



This reaction is catalyzed by the **α-ketoglutarate dehydrogenase complex**, an organized assembly of three kinds of enzymes that is homologous to the pyruvate dehydrogenase complex. In fact, the E_3 component is identical in both enzymes. The oxidative decarboxylation of α-ketoglutarate closely resembles that of pyruvate (Section 17.2), also an α-ketoacid.

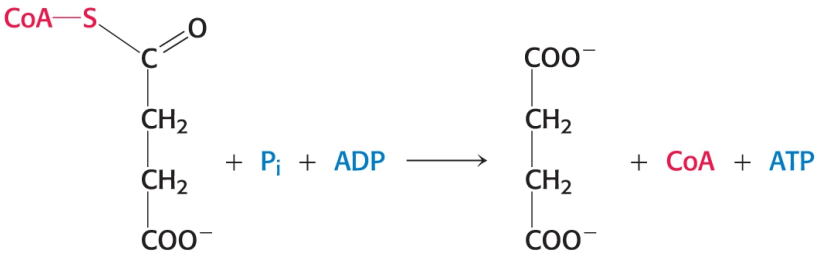
$$\text{Pyruvate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\text{Pyruvate dehydrogenase complex}} \text{acetyl CoA} + \text{CO}_2 + \text{NADH} + \text{H}^+$$

$$\alpha\text{-Ketoglutarate} + \text{CoA} + \text{NAD}^+ \xrightarrow{\alpha\text{-Ketoglutarate dehydrogenase complex}} \text{succinyl CoA} + \text{CO}_2 + \text{NADH}$$

Both reactions include the decarboxylation of an α-ketoacid and the subsequent formation of a thioester linkage with CoA that has a high transfer potential. The reaction mechanisms are entirely analogous.

A compound with high phosphoryl-transfer potential is generated from succinyl coenzyme A

Succinyl CoA is an energy-rich thioester compound. The $\Delta G^{\circ\prime}$ for the hydrolysis of succinyl CoA is about $-33.5\ \text{kJ mol}^{-1}(-8.0\ \text{kcal mol}^{-1})$, which is comparable to that of ATP $(-30.5\ \text{kJ mol}^{-1}$, or $-7.3\ \text{kcal mol}^{-1})$. In the citrate synthase reaction, the cleavage of the thioester bond powers the synthesis of the six-carbon citrate from the four-carbon oxaloacetate and the two-carbon fragment. The cleavage of the thioester bond of succinyl CoA is coupled to the phosphorylation of a purine nucleoside diphosphate, usually ADP. This reaction, which is readily reversible, is catalyzed by succinyl CoA synthetase.



This reaction is the only step in the citric acid cycle that directly yields a compound with high phosphoryl-transfer potential. In mammals, there are two isozymic forms of the enzyme, one specific for ADP and one for GDP. In tissues that perform large amounts of cellular respiration, such as skeletal and heart muscle, the ADP-requiring isozyme predominates. In tissues that perform many anabolic reactions, such as the liver, the GDP-requiring enzyme is common. The GDP-requiring enzyme is believed to work in reverse of the direction observed in the TCA cycle; that is, GTP is used to power the synthesis of succinyl CoA, which is a precursor for heme synthesis (Section 25.4). The specificity and nucleoside used varies also by species. For example, the enzyme in *E. coli* can use either GDP or ADP as the phosphoryl-group acceptor.

Note that the enzyme **nucleoside diphosphokinase**, which catalyzes the following reaction,

$$\mathrm{XTP + YDP \rightleftharpoons XDP + YTP}$$

allows the γ phosphoryl group to be readily transferred from any nucleotide triphosphate (XTP) to any other nucleotide diphosphate (YDP); for example, from GTP to form ATP from ADP. This reaction thereby allows the adjustment of the concentration of GTP or ATP to meet the cell's need and keeps the concentrations of all the various nucleoside triphosphates in the cell near equilibrium with one another.

Mechanism: Succinyl coenzyme A synthetase transforms types of biochemical energy

The mechanism of this reaction is a clear example of an energy transformation: energy inherent in the thioester molecule is transformed into phosphoryl-group-transfer potential (**Figure 17.12**). First, coenzyme A is displaced by orthophosphate (step 1), which generates another energy-rich compound, succinyl phosphate. A histidine residue plays a key role as a moving arm that detaches the phosphoryl group (step 2), then swings over to a bound ADP (step 3) and transfers the group to form ATP (step 4). The participation of high-energy compounds in all the steps is attested to by the fact that the reaction is readily reversible:
$\Delta G^{\circ\prime} = -3.4\ \mathrm{kJ\ mol^{-1}} (-0.8\ \mathrm{kcal\ mol^{-1}})$. The formation of ATP at the expense of succinyl CoA is an example of substrate-level phosphorylation (Section 16.2).

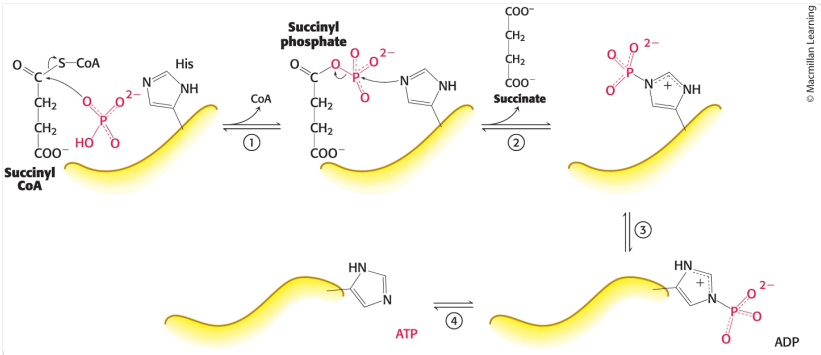
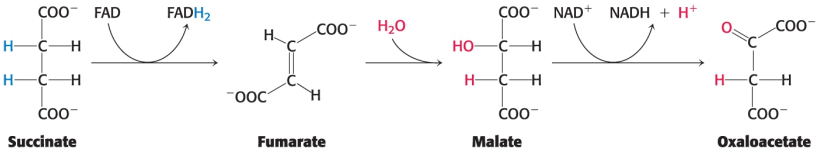


FIGURE 17.12 The mechanism of succinyl CoA synthetase allows the formation of a phosphoanhydride through a phosphorylated enzyme intermediate. (1) Orthophosphate displaces coenzyme A, which generates another energy-rich compound, succinyl phosphate. (2) A histidine residue removes the phosphoryl group with the concomitant generation of succinate and phosphohistidine. (3) The phosphohistidine residue then swings over to a bound ADP, and (4) the phosphoryl group is transferred to form ATP.

Oxaloacetate is regenerated by the oxidation of succinate

Reactions of four-carbon compounds constitute the final stage of the citric acid cycle: the regeneration of oxaloacetate.



The reactions constitute a metabolic motif that we will see again in fatty acid synthesis and degradation as well as in the degradation of some amino acids. A methylene group (CH_2) is converted into a carbonyl group $C{=}O$ in three steps: an oxidation, a hydration, and a second oxidation reaction. Oxaloacetate is thereby regenerated for another round of the cycle, and more energy is extracted in the form of $FADH_2$ and NADH.

Succinate is oxidized to fumarate by succinate dehydrogenase. The hydrogen acceptor is FAD rather than NAD^+, which is used in the other three oxidation reactions in the cycle. FAD is the electron acceptor in this reaction because the free-energy change is insufficient to reduce

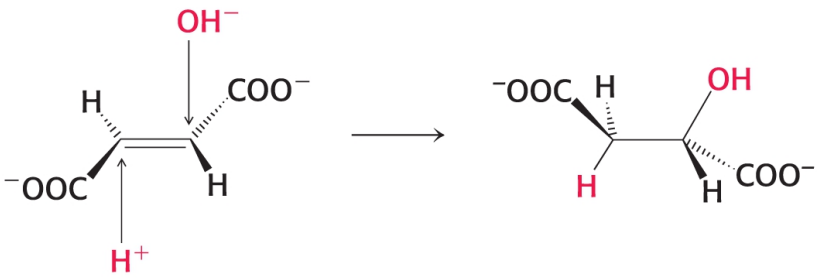
NAD^+. FAD is nearly always the electron acceptor in oxidations that remove two hydrogen atoms from a substrate. In succinate dehydrogenase, the isoalloxazine ring of FAD is covalently attached to a histidine side chain of the enzyme (denoted E-FAD).

$$\text{E-FAD} + \text{succinate} \rightleftharpoons \text{E-FADH}_2 + \text{fumarate}$$

Succinate dehydrogenase, like aconitase, is an iron–sulfur protein. Indeed, succinate dehydrogenase contains three different kinds of iron–sulfur clusters: 2Fe–2S (two iron atoms bonded to two inorganic sulfides), 3Fe–4S, and 4Fe–4S. Succinate dehydrogenase differs from other enzymes in the citric acid cycle because it is embedded in the inner mitochondrial membrane. In fact, succinate dehydrogenase is directly associated with the electron-transport chain, the link between the citric acid cycle and ATP formation.

$FADH_2$ produced by the oxidation of succinate does not dissociate from the enzyme, in contrast with NADH produced in other oxidation–reduction reactions. Rather, two electrons are transferred from $FADH_2$ directly to iron–sulfur clusters of the enzyme, which in turn passes the electrons to coenzyme Q. Coenzyme Q, an important member of the electron-transport chain, passes electrons ultimately to the final acceptor, molecular oxygen, as we shall see in Chapter 18.

The next step is the hydration of fumarate to form L-malate. Fumarase catalyzes a stereospecific trans addition of H^+ and OH^-. The OH^- group adds to only one side of the double bond of fumarate; hence, only the L isomer of malate is formed.



Fumarate **L-Malate**

Finally, malate is oxidized to form oxaloacetate. This reaction is catalyzed by malate dehydrogenase, and NAD^+ is again the hydrogen acceptor.

$$\text{Malate} + \text{NAD}^+ \rightleftharpoons \text{oxaloacetate} + \text{NADH} + \text{H}^+$$

The standard free energy for this reaction, unlike that for the other steps in the citric acid cycle, is significantly positive ($\Delta G^{\circ\prime} = +29.7\ \text{kJ mol}^{-1}$, or $+7.1\ \text{kcal mol}^{-1}$). The oxidation of malate is driven by the use of the products — oxaloacetate by citrate synthase and NADH by the electron-transport chain.

Self-Check Question

Fluoroacetate is a toxic molecule that enters the citric acid cycle (CAC) due to its similarity to an acetyl group, but ultimately it inhibits the CAC. When fluoroacetate is added to mitochondria, fluorocitrate builds up. What step of the CAC is inhibited by fluoroacetate?

The citric acid cycle produces high-transfer-potential electrons, ATP, and CO_2

The net reaction of the citric acid cycle is

$$\text{Acetyl CoA} + 3\ \text{NAD}^+ + \text{FAD} + \text{ADP} + \text{P}_i + 2\ \text{H}_2\text{O} \rightarrow 2\ \text{CO}_2 + 3\ \text{NADH} + \text{FADH}_2 + \text{ATP} + 2\text{H}^+ + \text{CoA}$$

Let us recapitulate the reactions that give this stoichiometry (Figure 17.13 and Table 17.2) and summarize.

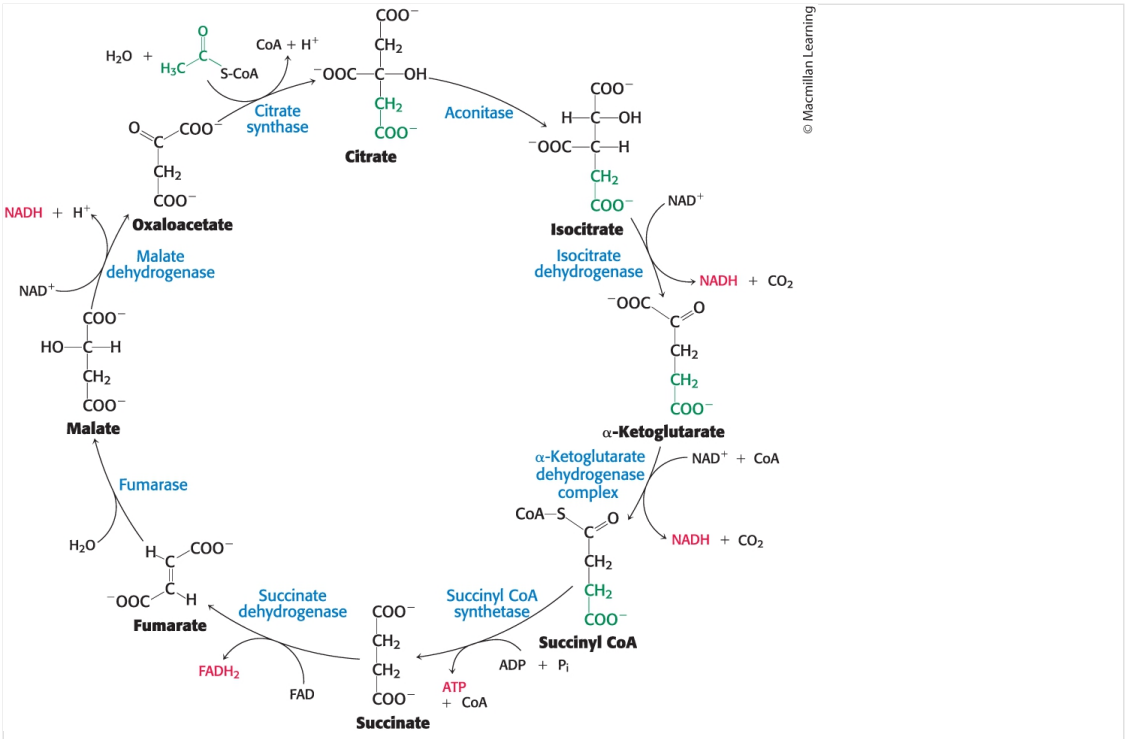


FIGURE 17.13 Eight enzyme-catalyzed reactions make up the full citric acid cycle. Notice that since succinate is a symmetric molecule, the identity of the carbons from the acetyl unit is lost.

TABLE 17.2 Citric acid cycle

Step	Reaction	Enzyme	Prosthetic group	Type*	ΔG°′ kJ mol⁻¹	ΔG°′ kcal mol⁻¹
1	Acetyl CoA + oxaloacetate + H_2O → citrate + CoA + H^+	Citrate synthase		a	−31.4	−7.5
2a	Citrate ⇌ *cis*-aconitate + H_2O	Aconitase	Fe–S	b	+8.4	+2.0

2b	*cis*-Aconitate + $H_2O \rightleftharpoons$ isocitrate	Aconitase	Fe–S	c	−2.1	−0.5
3	Isocitrate + $NAD^- \rightleftharpoons$ α-ketoglutarate + CO_2 + NADH	Isocitrate dehydrogenase		d+e	−8.4	−2.0
4	α-Ketoglutarate + NAD^+ + CoA $\rightleftharpoons$ succinyl CoA + CO_2 + NADH	α-Ketoglutarate dehydrogenase complex	Lipoic acid, FAD, TPP	d+e	−30.1	−7.2

TABLE 17.2 Citric acid cycle

					$\Delta G^{\circ\prime}$	
Step	**Reaction**	**Enzyme**	**Prosthetic group**	**Type***	**kJ mol^{-1}**	**kcal mol^{-1}**
5	Succinyl CoA + P_i + ADP $\rightleftharpoons$ succinate + ATP + CoA	Succinyl CoA synthetase		f	−3.3	−0.8
6	Succinate + FAD (enzyme-bound) $\rightleftharpoons$ fumarate + $FADH_2$ (enzyme-bound)	Succinate dehydrogenase	FAD, Fe–S	e	0	0
7	Fumarate + $H_2O \rightleftharpoons$ L-malate	Fumarase		c	−3.8	−0.9
8	L-Malate + $NAD^+ \rightleftharpoons$ oxaloacetate + NADH + H^+	Malate dehydrogenase		e	+29.7	+7.1

*Reaction type: (a) condensation; (b) dehydration; (c) hydration; (d) decarboxylation; (e) oxidation; (f) substrate-level phosphorylation.

- Two carbon atoms enter the cycle in the condensation of an acetyl unit (from acetyl CoA) with oxaloacetate. Two carbon atoms leave the cycle in the form of CO_2 in the successive decarboxylations catalyzed by isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

- Four pairs of hydrogen atoms leave the cycle in four oxidation reactions. Two NAD^+ molecules are reduced in the oxidative decarboxylations of isocitrate and α-ketoglutarate, one FAD molecule is reduced in the oxidation of succinate, and one NAD^+ molecule is reduced in the oxidation of malate. Recall also that one NAD^+ molecule is reduced in the oxidative decarboxylation of pyruvate to form acetyl CoA.
- One compound with high phosphoryl-transfer potential, usually ATP, is generated from the cleavage of the thioester linkage in succinyl CoA.
- Two water molecules are consumed: one in the synthesis of citrate by the hydrolysis of citryl CoA and the other in the hydration of fumarate.

Isotope-labeling studies have revealed that the two carbon atoms that enter each cycle are not the ones that leave. The two carbon atoms that enter the cycle as the acetyl group are retained during the initial two decarboxylation reactions (Figure 17.13) and then remain incorporated in the four-carbon acids of the cycle. Note that succinate is a symmetric molecule. Consequently, the two carbon atoms that enter the cycle can occupy any of the carbon positions in the subsequent metabolism of the four-carbon acids. The two carbons that enter the cycle as the acetyl group will be released as CO_2 in later rounds of the cycle.

Various techniques, such as fluorescence recovery after photobleaching (FRAP, Section 12.5) and tandem mass spectroscopy analysis (Section 4.3), have established that there is a physical association of all of the enzymes of the citric acid cycle into a supramolecular complex. Recall that this close arrangement of enzymes allows for substrate channeling (Chapter 16), which enhances the efficiency of the citric acid cycle because a reaction product can pass directly from one active site to the next through connecting channels.

As will be considered in Chapter 18, the electron-transport chain oxidizes the NADH and $FADH_2$ formed in the citric acid cycle. The transfer of electrons from these carriers to O_2, the final electron acceptor, leads to the generation of a proton gradient across the inner mitochondrial membrane. This proton-motive force then powers the generation of ATP; the net stoichiometry is about 2.5 ATP per NADH, and 1.5 ATP per $FADH_2$. Consequently, nine high-transfer-potential phosphoryl groups are generated when the electron-transport chain oxidizes 3 NADH molecules and $1\ FADH_2$ molecule, and one ATP is directly formed in one round of the citric acid cycle. Thus, one acetyl unit generates approximately 10 molecules of ATP. In dramatic contrast, the anaerobic glycolysis of an entire glucose molecule generates only 2 molecules of ATP (and 2 molecules of lactate).

Recall that molecular oxygen does not participate directly in the citric acid cycle. However, the cycle operates only under aerobic conditions because NAD^+ and FAD can be regenerated in the mitochondrion only by the transfer of electrons to molecular oxygen. In contrast, glycolysis can proceed under anaerobic conditions because NAD^+ is regenerated in the conversion of pyruvate into lactate. Glycolysis has both an aerobic and an anaerobic mode, whereas the citric acid cycle is strictly aerobic.

Self-Check Question

The nucleoside trisphosphate produced directly in the citric acid cycle is sometimes GTP. Explain why a GTP molecule, or another nucleoside triphosphate, is energetically equivalent to an ATP molecule in metabolism.

17.4 Entry to the Citric Acid Cycle and Metabolism Through It Are Controlled

The citric acid cycle is the final common pathway for the aerobic oxidation of fuel molecules. Moreover, as we will see shortly (Section 17.5) and repeatedly elsewhere in our study of biochemistry, the cycle is an important source of building blocks for a host of important biomolecules. As befits its role as the metabolic hub of the cell, entry into the cycle and the rate of the cycle itself are controlled at several stages.

The pyruvate dehydrogenase complex is regulated allosterically and by reversible phosphorylation

As stated earlier, glucose can be formed from pyruvate. However, the formation of acetyl CoA from pyruvate is an irreversible step in animals and thus they are unable to convert acetyl CoA back into glucose. The oxidative decarboxylation of pyruvate to acetyl CoA commits the carbon atoms of glucose to one of two principal fates: oxidation to CO_2 by the citric acid cycle, with the concomitant generation of energy, or incorporation into lipids (**Figure 17.14**).

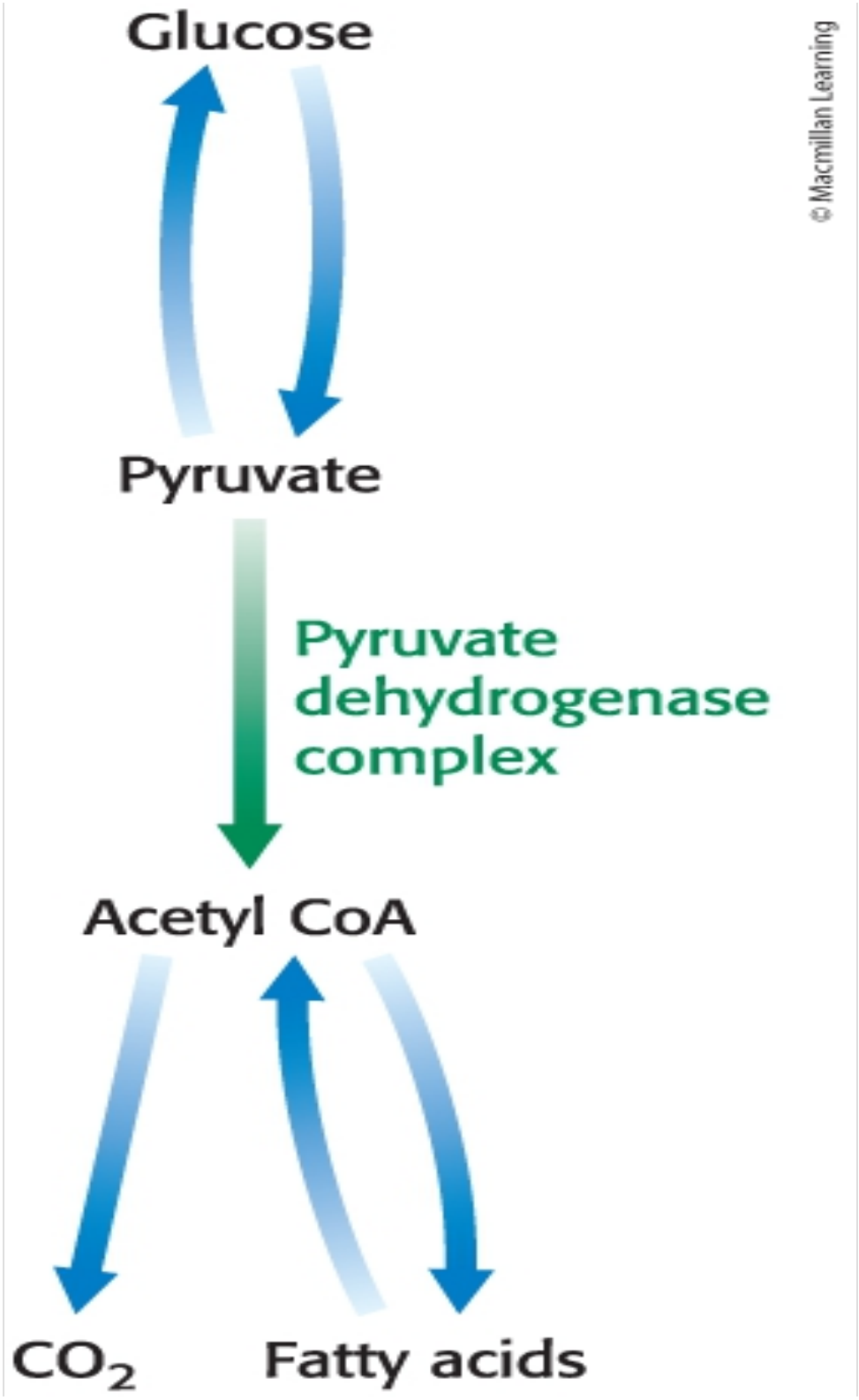

Glucose
Pyruvate
Pyruvate dehydrogenase complex
Acetyl CoA
CO_2
Fatty acids
© Macmillan Learning

FIGURE 17.14 The synthesis of acetyl CoA by the pyruvate dehydrogenase complex is a key irreversible step in glucose metabolism.

EXPLORE this pathway further in the Metabolic Map in Achieve

As expected of an enzyme at a critical branch point in metabolism, the activity of the pyruvate dehydrogenase complex is tightly regulated. High concentrations of reaction products inhibit the reaction: acetyl CoA inhibits the transacetylase component (E_2) by binding directly, whereas NADH inhibits the dihydrolipoyl dehydrogenase (E_3). High concentrations of NADH and acetyl CoA inform the enzyme that the energy needs of the cell have been met or that fatty acids are being degraded to produce acetyl CoA and NADH. In either case, there is no need to metabolize pyruvate to acetyl CoA. This inhibition has the effect of conserving glucose, because most pyruvate is derived from glucose by glycolysis (Section 16.2).

The key means of regulation of the complex in eukaryotes is reversible covalent modification (**Figure 17.15**). Phosphorylation of the pyruvate dehydrogenase component (E_1) by one of four tissue-specific isozymes of pyruvate dehydrogenase kinase (PDK) switches off the activity of the complex. Deactivation is then reversed by one of two isozymes of pyruvate dehydrogenase phosphatase (PDP). In mammals, both the kinase and phosphatase are associated with the E_2-E_3-BP core complex, and the activities of both are regulated and subject to tissue-specific hormonal control.

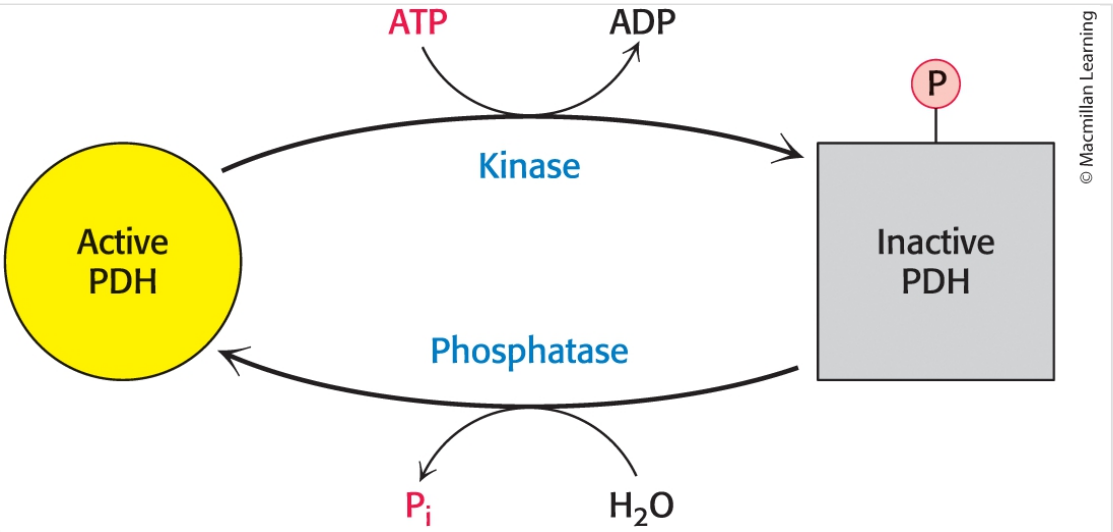


FIGURE 17.15 The activity of the pyruvate dehydrogenase complex is regulated by reversible phosphorylation. A specific kinase phosphorylates and inactivates pyruvate dehydrogenase (PDH), and a phosphatase activates the dehydrogenase by removing the phosphoryl group. The kinase and the phosphatase also are highly regulated enzymes.

To see how this regulation works in biological conditions, consider muscle that is becoming active after a period of rest (Figure 17.16). At rest, the muscle will not have significant energy demands. Consequently, the $NADH/NAD^+$, acetyl CoA/CoA, and ATP/ADP ratios will be high. These high ratios promote phosphorylation and inactivation of the complex by activating PDK. In other words, high concentrations of immediate products of the reaction (acetyl CoA and NADH) and a final product of the pathway (ATP) inhibit the activity. Thus, pyruvate dehydrogenase is switched off when the energy charge is high.

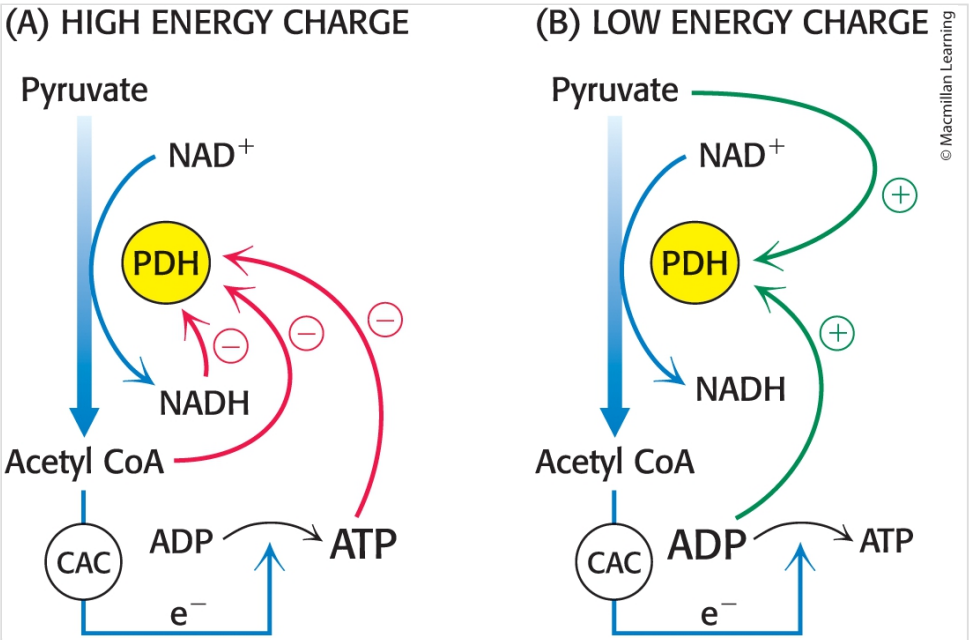


FIGURE 17.16 The pyruvate dehydrogenase complex responds to changes in the energy charge of the cell. (A) The complex (PDH) is inhibited by its immediate products, NADH and acetyl CoA, as well as by the ultimate product of the citric acid cycle (CAC) and oxidative phosphorylation, ATP. (B) PDH is activated by pyruvate and ADP, which inhibit the kinase that phosphorylates PDH.

As exercise begins, the concentrations of ADP and pyruvate will increase as muscle contraction consumes ATP and glucose is converted into pyruvate to meet the energy demands. Both ADP and pyruvate activate the dehydrogenase by inhibiting the kinase. Moreover, the phosphatase is stimulated by Ca^{2+}, the same signal that initiates muscle contraction. A rise in the cytoplasmic Ca^{2+} concentration increases the mitochondrial Ca^{2+} concentration. The rise in mitochondrial Ca^{2+} activates the phosphatase, enhancing pyruvate dehydrogenase activity.

In some tissues, the phosphatase is also controlled by hormones. For example, in liver tissue, epinephrine binds to the α-adrenergic receptor to initiate the phosphatidylinositol pathway (Section 14.2), causing an increase in Ca^{2+} concentration that activates the phosphatase. In tissues capable of fatty acid synthesis, such as liver and adipose tissue, insulin — the hormone that signifies the fed state — stimulates the phosphatase, increasing the conversion of pyruvate into acetyl CoA. Acetyl CoA is the precursor for fatty acid synthesis (Section 22.5). In these tissues, the pyruvate dehydrogenase complex is activated to funnel glucose to pyruvate and then to acetyl CoA and ultimately to fatty acids. In people with a pyruvate dehydrogenase phosphatase deficiency, pyruvate dehydrogenase is always phosphorylated and thus inactive. Consequently, glucose is processed to lactate rather than acetyl CoA, resulting in lactic acidosis. Lactic acidosis causes many tissues to malfunction, including the central nervous system.

Diabetic neuropathy may be due to inhibition of the pyruvate dehydrogenase complex

Diabetic neuropathy — a numbness, tingling, or pain in the hands, arm, fingers, toes, feet, and legs — is a common complication of both type 1 and type 2 diabetes, affecting approximately 50% of patients. There is no cure for the condition, and treatment relies on painkillers. Recent research in mice and tissue culture suggests that overproduction of lactic acid by cells in the dorsal root ganglion, a

part of the nervous system responsible for pain perception, may be a significant contributor.

Lactate, which is produced by some cells of the nervous system, is a common fuel for neurons, which import and convert it to pyruvate for use in cellular respiration. However, it appears that hyperglycemia (high glucose concentration), the defining feature of diabetes, increases pyruvate dehydrogenase kinase activity in the cells of the dorsal root ganglion, leading to inhibition of the pyruvate dehydrogenase complex. Glycolytically produced pyruvate is then processed to lactate. The overabundance of lactate leads to an increase in acid-sensing nociceptors (pain receptors), a type of G-protein-coupled receptor (Section 14.2), resulting in diabetic neuropathy. In experimental systems, three approaches — pharmacological inhibition of either pyruvate dehydrogenase kinase or lactic acid dehydrogenase, or genetic elimination of the kinase gene — greatly reduce, but do not eliminate, diabetic neuropathy. Thus, the search for good therapeutics shows promise but must continue.

The citric acid cycle is regulated at several points

The rate of the citric acid cycle is precisely adjusted to meet an animal cell's needs for ATP (**Figure 17.17**). Two allosteric enzymes primarily regulate the rate of cycling: isocitrate dehydrogenase and α-ketoglutarate dehydrogenase, the first two enzymes in the cycle to generate high-energy electrons.

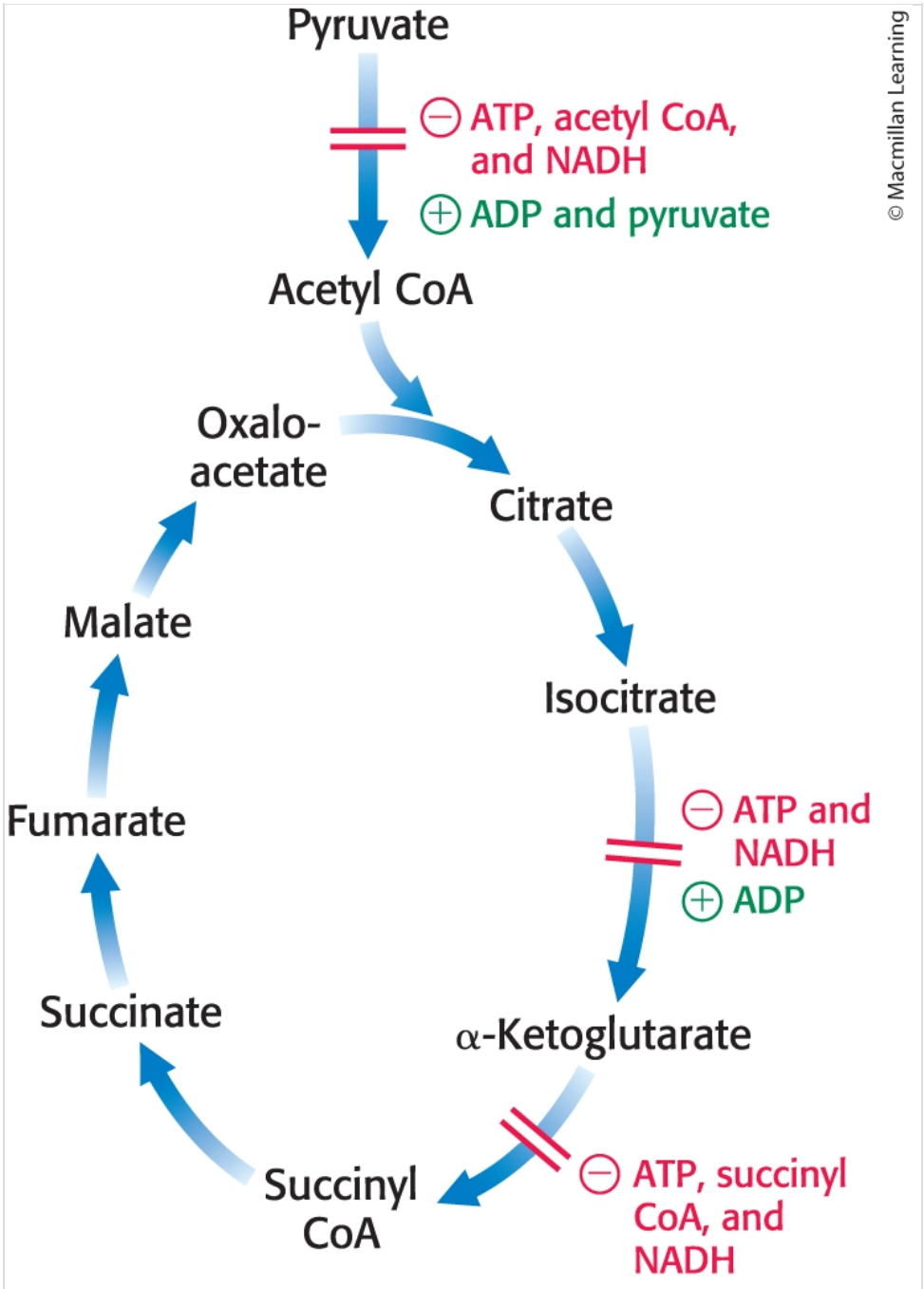


FIGURE 17.17 The citric acid cycle is regulated primarily by the concentrations of ATP and NADH. The key control points are the enzymes isocitrate dehydrogenase and α-ketoglutarate dehydrogenase.

1. *Isocitrate dehydrogenase is allosterically stimulated by ADP, which enhances the enzyme's affinity for substrates.* The binding of isocitrate, NAD^+, Mg^{2+}, and ADP are all mutually synergistic, whereas ATP is inhibitory. The reaction product NADH also inhibits isocitrate dehydrogenase by directly displacing NAD^+. It is important to note that several steps in the cycle require NAD^+ or FAD, which are abundant only when the energy charge is low.

2. The α-*ketoglutarate dehydrogenase complex catalyzes the rate-limiting step.* Some aspects of this enzyme complex's regulation are like those of the pyruvate dehydrogenase complex, as might be expected from the homology of the two enzymes. α-Ketoglutarate dehydrogenase is inhibited by succinyl CoA and NADH, the products of the reaction that it catalyzes. In addition, α-ketoglutarate dehydrogenase is inhibited by ATP, so that the rate of the citric acid cycle is reduced when cellular ATP is abundant. *α*-Ketoglutarate dehydrogenase deficiency is observed in a number of neurological disorders, including Alzheimer's disease.

In many bacteria, the synthesis of citrate from oxaloacetate and acetyl CoA carbon units is another important control point. In these organisms, ATP is an allosteric inhibitor of citrate synthase, causing an increase in the apparent value of K_M for acetyl CoA. The result is that as the level of ATP increases, less citrate synthase is bound to acetyl CoA and so less citrate is formed.

The use of isocitrate dehydrogenase and α-ketoglutarate dehydrogenase as points of regulation in human metabolism integrates the citric acid cycle with other pathways and highlights the central role of the citric acid cycle in metabolism. For instance, the inhibition of isocitrate dehydrogenase leads to a buildup of citrate, because the interconversion of isocitrate and citrate is readily reversible under intracellular conditions. Citrate can be transported to the cytoplasm, where it signals phosphofructokinase to halt glycolysis (Section 16.3) and where it can serve as a source of acetyl CoA for fatty acid synthesis (Section 22.5). The α-ketoglutarate that accumulates when α-ketoglutarate dehydrogenase is inhibited can be used as a precursor for several amino acids and the purine bases (Chapters 25 and 26). Finally, as we will explore in Chapter 24, defects in the citric acid cycle and related enzymes can lead to the formation of cancer.

Self-Check Question

The citric acid cycle is part of aerobic respiration, but no O_2 is required for the cycle. Explain this apparent paradox.

17.5 The Citric Acid Cycle Is a Source of Biosynthetic Precursors

Thus far, discussion has focused on the citric acid cycle as the major degradative pathway for the generation of ATP. As a major metabolic hub of the cell, the citric acid cycle also integrates many of the cell's other metabolic pathways, including those of carbohydrates, fats, amino acids, and porphyrins. This integration is aided by the fact that the cytoplasmic and mitochondrial pools of citric acid cycle components are shared, allowing the use of citric acid intermediates for biosyntheses taking place throughout the cell (**Figure 17.18**). For example, most of the carbon atoms in porphyrins come from succinyl CoA in a pathway that occurs both in the cytoplasm and mitochondria; fats are synthesized in the cytoplasm from mitochondrial citrate; and many of the amino acids, used throughout the cell, are derived from α-ketoglutarate and oxaloacetate. The details of these biosynthetic processes will be considered in subsequent chapters.

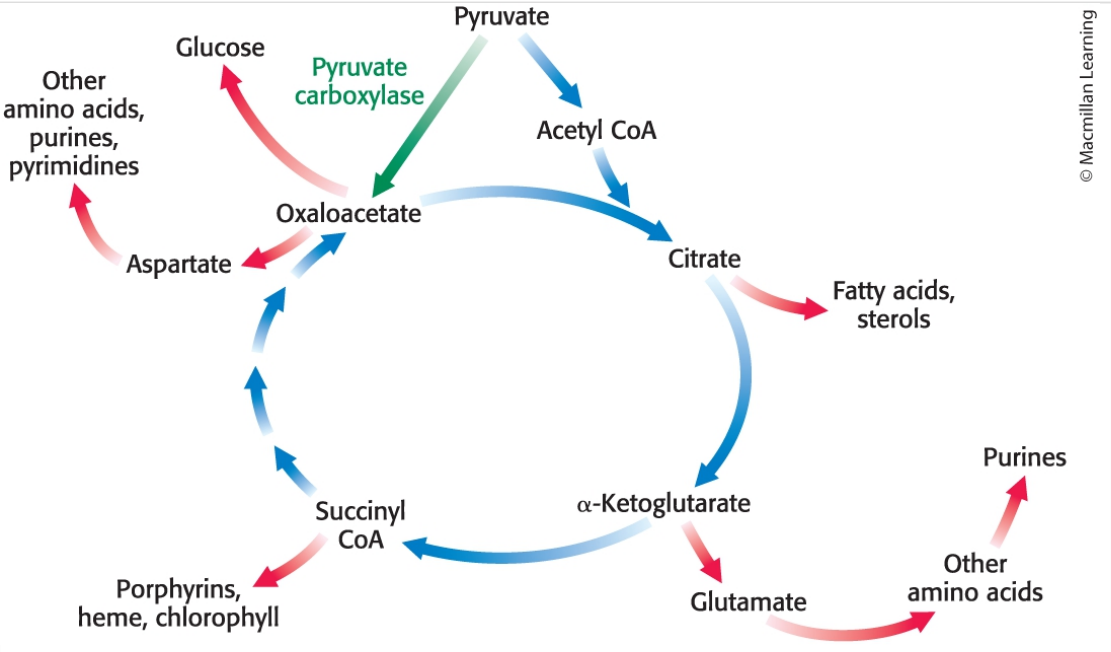


FIGURE 17.18 The citric acid cycle plays an important role in biosynthesis. Intermediates are drawn off for biosyntheses (shown by red arrows) when the energy needs of the cell are met. Intermediates are replenished by a variety of anaplerotic reactions, the most important of which is the formation of oxaloacetate from pyruvate by pyruvate carboxylase.

EXPLORE this pathway further in the Metabolic Map in Achieve

The citric acid cycle must be capable of being rapidly replenished

An important consideration is that citric acid cycle intermediates must be replenished if any are drawn off for biosyntheses. Suppose that much oxaloacetate is converted into amino acids for protein synthesis and, subsequently, the energy needs of the cell rise. The citric acid cycle will operate to a reduced extent unless new oxaloacetate is formed, because acetyl CoA cannot enter the cycle

unless it condenses with oxaloacetate. Even though oxaloacetate is recycled, a minimal level must be maintained to allow the cycle to function.

How is oxaloacetate replenished? Mammals lack the enzymes for the net conversion of acetyl CoA into oxaloacetate or any other citric acid cycle intermediate, so no matter how much acetyl CoA enters the cycle, there is never any *net* increase in the number of oxaloacetate molecules. Rather, oxaloacetate is formed by the carboxylation of pyruvate, in a reaction catalyzed by the biotin-dependent enzyme pyruvate carboxylase (Figure 17.18).

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i + 2H^+$$

Recall that this enzyme plays a crucial role in gluconeogenesis (Section 16.4). It is active only in the presence of acetyl CoA, which signifies the need for more oxaloacetate. If the energy charge is high, oxaloacetate is converted into glucose. If the energy charge is low, oxaloacetate replenishes the citric acid cycle. The synthesis of oxaloacetate by the carboxylation of pyruvate is an example of an **anaplerotic reaction** (*anaplerotic* is of Greek origin, meaning to "fill up"), a reaction that leads to the net synthesis, or replenishment, of pathway components. Note that because the citric acid cycle is a cycle, it can be replenished by the generation of any of the intermediates. For example, aspartate can be deaminated to also generate oxaloacetate. Glutamine is an especially important source of citric acid cycle intermediates in rapidly growing cells, including cancer cells. Glutamine is converted into glutamate and then into α-ketoglutarate (Section 23.5).

The disruption of pyruvate metabolism is the cause of beriberi and poisoning by mercury and arsenic

A neurologic and cardiovascular disorder known as **beriberi** is caused by a dietary deficiency of thiamine (also called *vitamin* B_1). The disease is prevalent in areas of the world where rice is the major food staple, because white rice has a rather low content of thiamine. This deficiency is partly overcome if the whole rice grain is soaked in water before milling, because some of the thiamine in the husk then leaches into the rice kernel. The problem is worsened if the rice is polished to remove the outer layer (that is, converted from brown to white rice), because only the outer layer contains significant amounts of thiamine. A common form of beriberi called *wet beriberi* is characterized by swelling in the abdomen and legs due to cardiac abnormalities.

Another form of beriberi, called *Wernicke's encephalopathy*, is also occasionally seen in alcoholics who are severely malnourished and thus thiamine deficient. The disease is characterized by neurologic and cardiac symptoms. Damage to the peripheral nervous system is expressed as pain in the limbs, weakness of the musculature, and distorted skin sensation. The heart may be enlarged and the cardiac output inadequate.

Which biochemical processes might be affected by a deficiency of thiamine? Thiamine is the precursor of the cofactor thiamine pyrophosphate (TPP). This cofactor is the prosthetic group of three important enzymes: pyruvate dehydrogenase, α-ketoglutarate

dehydrogenase, and transketolase. (Transketolase functions in the pentose phosphate pathway, which will be considered in Chapter 20.) The common feature of enzymatic reactions using TPP is the transfer of an activated aldehyde unit.

In patients with beriberi, the levels of pyruvate and α-ketoglutarate in the blood are higher than normal, and the increase in the level of pyruvate in the blood is especially pronounced after the ingestion of glucose. A related finding is that the activities of the pyruvate and α-ketoglutarate dehydrogenase complexes are abnormally low. The low transketolase activity of red blood cells in beriberi is an easily measured and reliable diagnostic indicator of the disease.

Why does TPP deficiency lead primarily to cardiac and neurological disorders? Heart tissue is highly sensitive to metabolic disruptions, as its need for ATP is never-ending and it has no appreciable stores of energy. The nervous system relies essentially on glucose as its only fuel. The product of glycolysis, pyruvate, can enter the citric acid cycle only through the pyruvate dehydrogenase complex. With that enzyme deactivated, the nervous system has no source of fuel. In contrast, most other tissues can use fats as a source of fuel for the citric acid cycle.

Symptoms similar to those of beriberi appear in organisms exposed to mercury or arsenite (AsO_3^{3-}). Both materials have a high affinity for neighboring (vicinal) sulfhydryls, such as those in the reduced dihydrolipoyl groups of the E_3 component of the pyruvate dehydrogenase complex (**Figure 17.19**). The binding of mercury or arsenite to the dihydrolipoyl groups inhibits the complex and leads to central nervous system pathologies. The proverbial phrase “mad as a hatter” refers to the strange behavior of poisoned hatmakers who used mercury nitrate, which is absorbed through the skin, to soften and shape animal furs. Similar symptoms afflicted the early

photographers, who used vaporized mercury to create daguerreotypes.

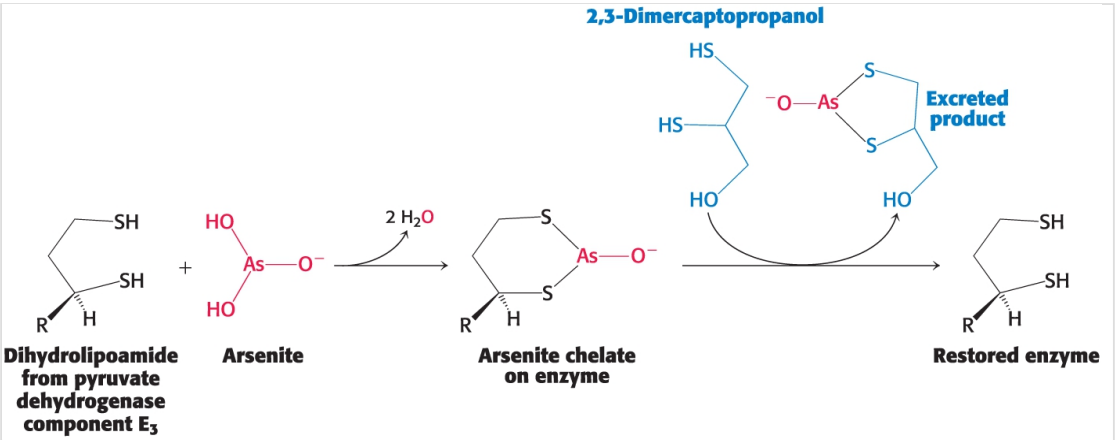


FIGURE 17.19 Arsenite inhibits the pyruvate dehydrogenase complex by inactivating the dihydrolipoamide component of the transacetylase. Some sulfhydryl reagents, such as 2,3-dimercaptoethanol, relieve the inhibition by forming a complex with the arsenite that can be excreted.

Treatment for these poisons is the administration of sulfhydryl reagents with adjacent sulfhydryl groups to compete with the dihydrolipoyl residues for binding with the metal ion. The reagent–metal complex is then excreted in the urine. Indeed, 2,3-dimercaptopropanol (Figure 17.19) was developed after World War I as an antidote to an arsenic-based chemical weapon.

The citric acid cycle likely evolved from preexisting pathways

How did the citric acid cycle come into being? Although definitive answers are elusive, informed speculation is possible. We can hypothesize how evolution might work at the level of biochemical pathways.

The citric acid cycle was most likely assembled from preexisting reaction pathways. As noted earlier, many of the intermediates formed in the citric acid cycle are used in metabolic pathways for amino acids and porphyrins. Thus, compounds such as pyruvate, α-ketoglutarate, and oxaloacetate were likely present early in evolution for biosynthetic purposes. The oxidative decarboxylation of these α-ketoacids is quite favorable thermodynamically and can be used to drive the synthesis of both acyl CoA derivatives and NADH. These reactions almost certainly formed the core of processes that preceded the citric acid cycle evolutionarily. Interestingly, α-ketoglutarate and oxaloacetate can be interconverted by transamination of the respective amino acids by aspartate aminotransferase, another key biosynthetic enzyme. Thus, cycles comprising smaller numbers of intermediates used for a variety of biochemical purposes could have existed before the present form evolved.