RAMSADAY COLLEGE

Semester-3
Core Course
CC-3/GE3: MICROBIAL METABOLISM
(THEORY)
MCB-G-CC-3-3-TH

Unit 3 Chemoheterotrophic Metabolism - Aerobic Respiration: Tri Carboxylic Acid (TCA) Cycle

Pyruvate to Carbon Dioxide Is Accomplished by the Tricarboxylic Acid Cycle

- In the glycolytic pathways, glucose is oxidized to pyruvate. During aerobic respiration, the catabolic process continues by oxidizing pyruvate to three CO_2.

- The **first step of this process employs a multienzyme system called the pyruvate dehydrogenase complex**. It oxidizes and cleaves pyruvate to form one CO_2 and the 2-carbon molecule **acetyl-coenzyme A** (**acetyl-CoA**) (**figure 11.8**).

- Acetyl-CoA is energy rich because hydrolysis of the bond that links acetic acid to coenzyme A (a thioester bond) has a large negative change in free energy.

Hans Krebs, 1900–1981

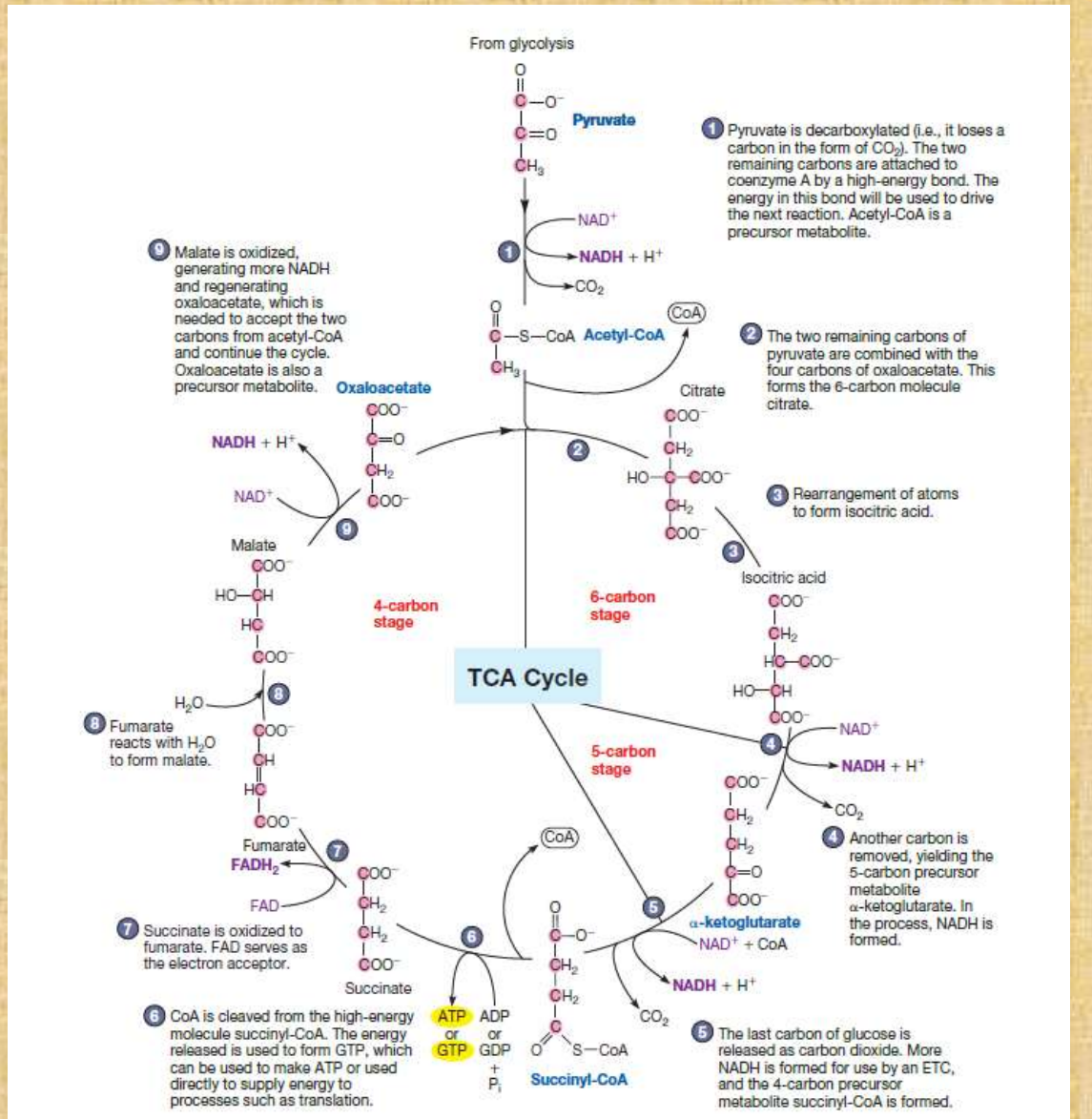


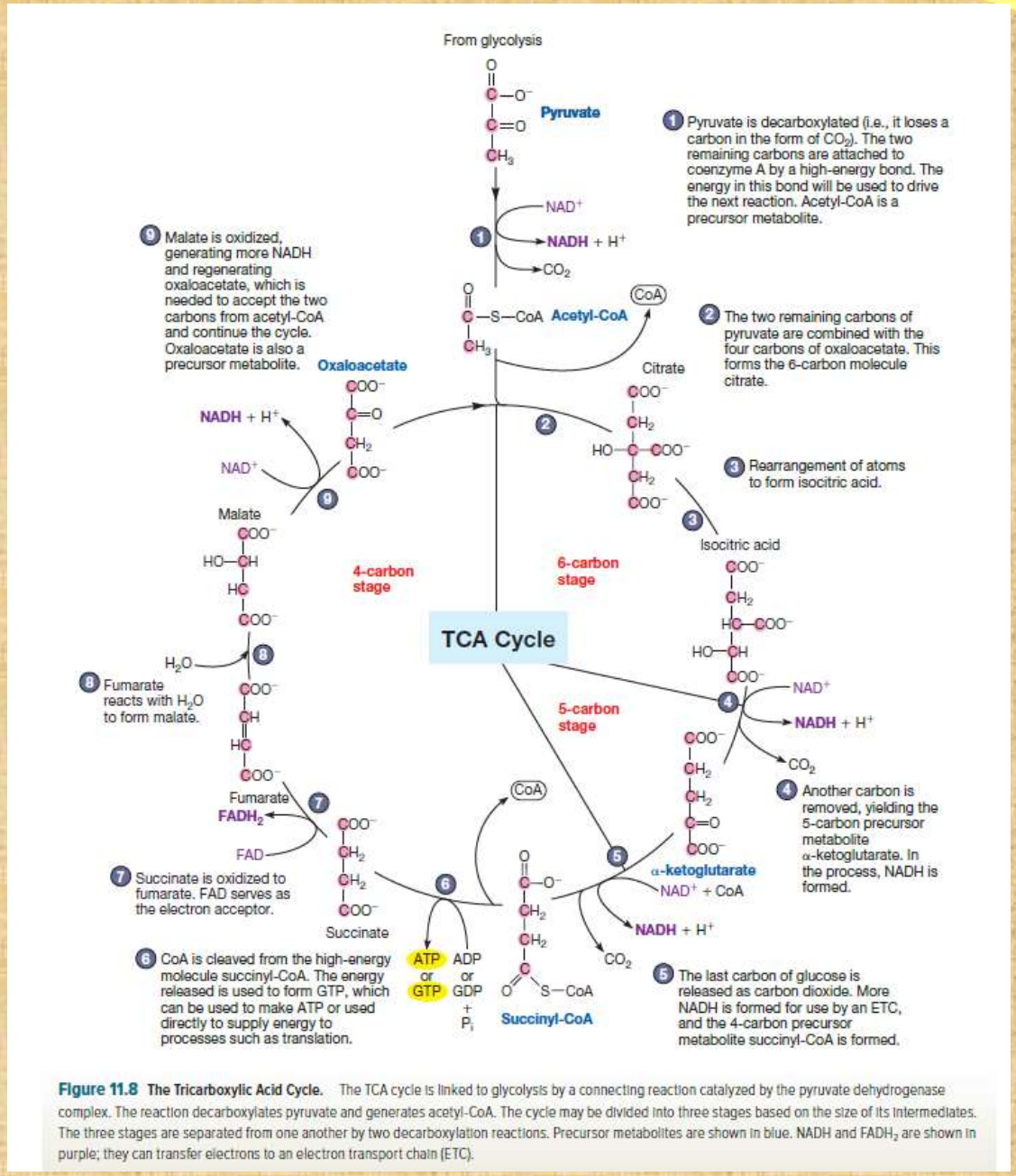
Figure 11.8 The Tricarboxylic Acid Cycle. The TCA cycle is linked to glycolysis by a connecting reaction catalyzed by the pyruvate dehydrogenase complex. The reaction decarboxylates pyruvate and generates acetyl-CoA. The cycle may be divided into three stages based on the size of its intermediates. The three stages are separated from one another by two decarboxylation reactions. Precursor metabolites are shown in blue. NADH and $FADH_2$ are shown in purple; they can transfer electrons to an electron transport chain (ETC).

Acetyl-CoA then enters the **tricarboxylic acid** (**TCA**) **cycle,** which is also called the **citric acid cycle** or the **Krebs cycle** (after its discoverer, Hans Krebs) (figure 11.8).

1) In the first reaction, acetyl-CoA is condensed with (i.e., added to) the 4-carbon intermediate oxaloacetate to form citrate, a molecule with six carbons.

2) Citrate is rearranged to give isocitrate, a more readily oxidized alcohol. Isocitrate is subsequently oxidized and decarboxylated twice to yield a-ketoglutarate (five carbons) and then succinyl-CoA (four carbons), another high-energy molecule containing a thioester bond.

3) At this point, two NADH molecules have been formed and two carbons lost from the cycle as CO_2. The cycle continues when succinyl-CoA is converted to succinate. This involves hydrolysis of the thioester bond in succinyl-CoA and using the large amount of energy released to form either one ATP or one GTP by substrate-level phosphorylation.



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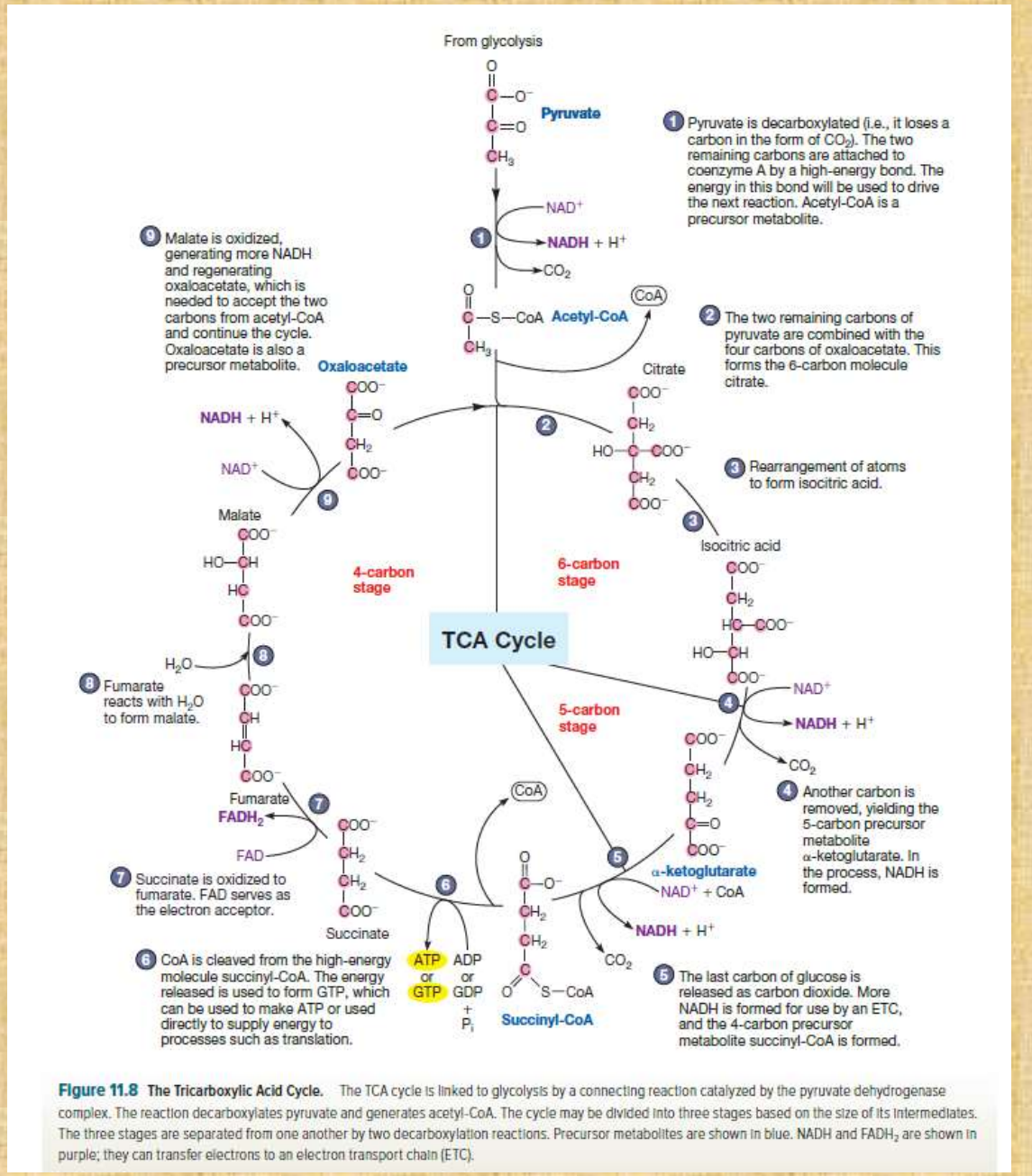
GTP is also a high-energy molecule, and it is functionally equivalent to ATP. It is used in protein synthesis and to make other nucleoside triphosphates, including ATP.

4) Two oxidation steps follow, yielding one $FADH_2$ and one NADH. The last oxidation step regenerates oxaloacetate, and as long as there is a supply of acetyl-CoA, the cycle can repeat itself.

TCA cycle generates two CO_2 molecules, three NADH molecules, one $FADH_2$, and either one ATP or GTP for each acetyl-CoA molecule oxidized.



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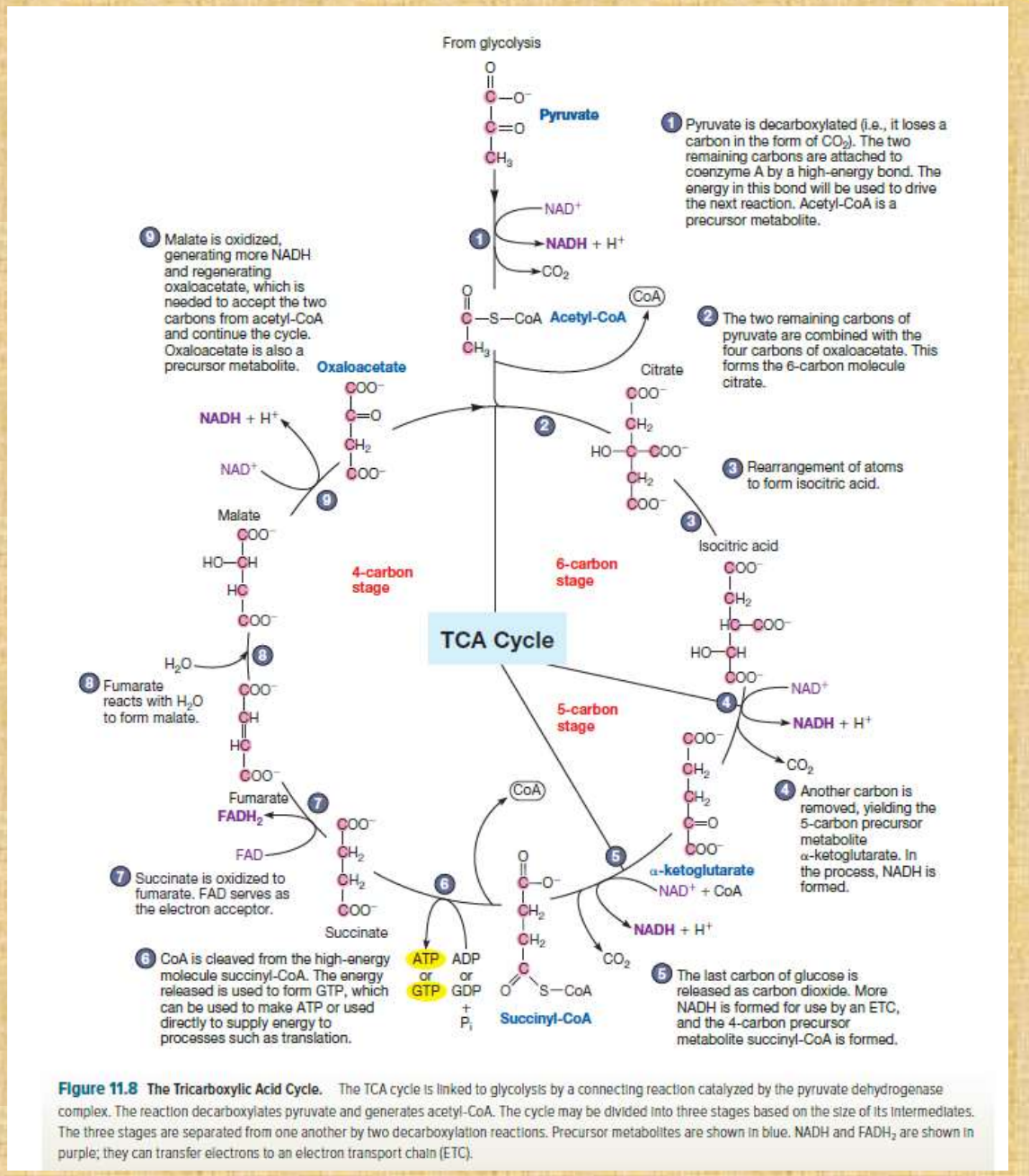
TCA cycle enzymes are widely distributed among micro-organisms. **In bacteria and archaea, they are located in the cytoplasm. In eukaryotes, they are found in the mitochondrial matrix**.

The complete cycle appears to be functional in many aerobic bacteria, free-living protists, and fungi.

This cycle plays an important role in energy conservation by producing numerous NADH and $FADH_2$.



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In aerobic organisms, glucose and other sugars, fatty acids, and most amino acids are ultimately oxidized to CO_2 and H_2O via the citric acid cycle and the respiratory chain.

Pyruvate Is Oxidized to Acetyl-CoA and CO_2 :

The overall reaction catalyzed by the pyruvate dehydrogenase complex is an **oxidative decarboxylation,** an irreversible oxidation process in which the carboxyl group is removed from pyruvate as a molecule of CO_2 and the two remaining carbons become the acetyl group of acetyl-CoA (Fig. 16–2).
The transfer of electrons from NADH to oxygen ultimately generates 2.5 molecules of ATP per pair of electrons.

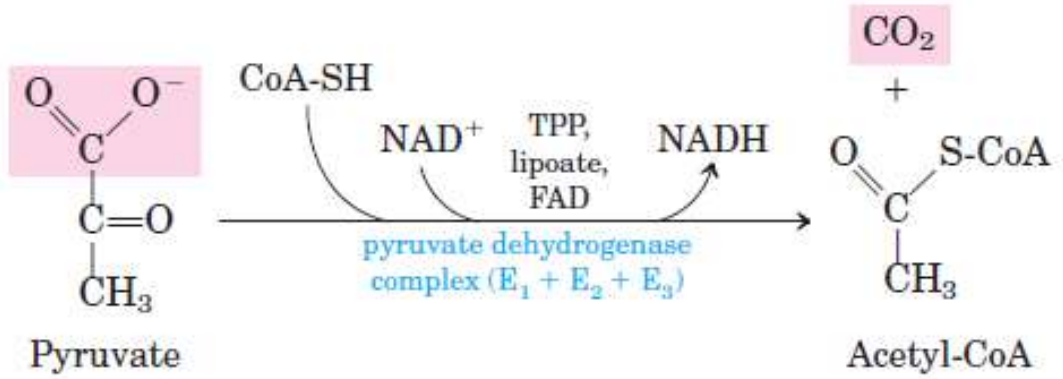


FIGURE 16–2 **Overall reaction catalyzed by the pyruvate dehydrogenase complex.** The five coenzymes participating in this reaction, and the three enzymes that make up the enzyme complex, are discussed in the text.

The Pyruvate Dehydrogenase Complex Requires Five Coenzymes

The combined dehydrogenation and decarboxylation of pyruvate to the acetyl group of acetyl-CoA (Fig. 16–2) requires the sequential action of **three different enzymes and five different coenzymes or prosthetic groups**—

✓thiamine pyrophosphate (TPP),
✓flavin adenine dinucleotide (FAD),
✓coenzyme A (CoA, sometimes denoted CoA-SH),
✓nicotinamide adenine dinucleotide (NAD), and
✓lipoate.

The Pyruvate Dehydrogenase Complex Consists of Three Distinct Enzymes

The PDH complex contains three enzymes—
Pyruvate dehydrogenase (E1),
Dihydrolipoyl transacetylase (E2), and
Dihydrolipoyl dehydrogenase (E3)—each present in multiple copies. The number of copies of each enzyme and therefore the size of the complex varies among species. The PDH complex isolated from mammals is about 50 nm in diameter—more than five times the size of an entire ribosome and big enough to be visualized with the electron microscope.

The PDH complex is composed of multiple copies of three enzymes:
Pyruvate dehydrogenase, E_1 (with its bound cofactor TPP);
Dihydrolipoyl transacetylase, E_2 (with its covalently bound lipoyl group); and
Dihydrolipoyl dehydrogenase, E_3 (with its cofactors FAD and NAD).

■ E_1 catalyzes first the decarboxylation of pyruvate, producing hydroxyethyl-TPP, and then the oxidation of the hydroxyethyl group to an acetyl group. The electrons from this oxidation reduce the disulfide of lipoate bound to E_2, and the acetyl group is transferred into thioester linkage with one -SH group of reduced lipoate.

■ E_2 catalyzes the transfer of the acetyl group to coenzyme A, forming acetyl-CoA.

■ E_3 catalyzes the regeneration of the disulfide (oxidized) form of lipoate; electrons pass first to FAD, then to NAD^+.

■ The long lipoyllysine arm swings from the active site of E_1 to E_2 to E_3, tethering the intermediates to the enzyme complex to allow substrate channeling.

All these enzymes and coenzymes are clustered, allowing the intermediates to react quickly without diffusing away from the surface of the enzyme complex.

The five-reaction sequence shown in Figure 16–6 is thus an example of **substrate channeling. The intermediates of the** multistep sequence never leave the complex, and the local concentration of the substrate of E_2 is kept very high. Channeling also prevents theft of the activated acetyl group by other enzymes that use this group as substrate.

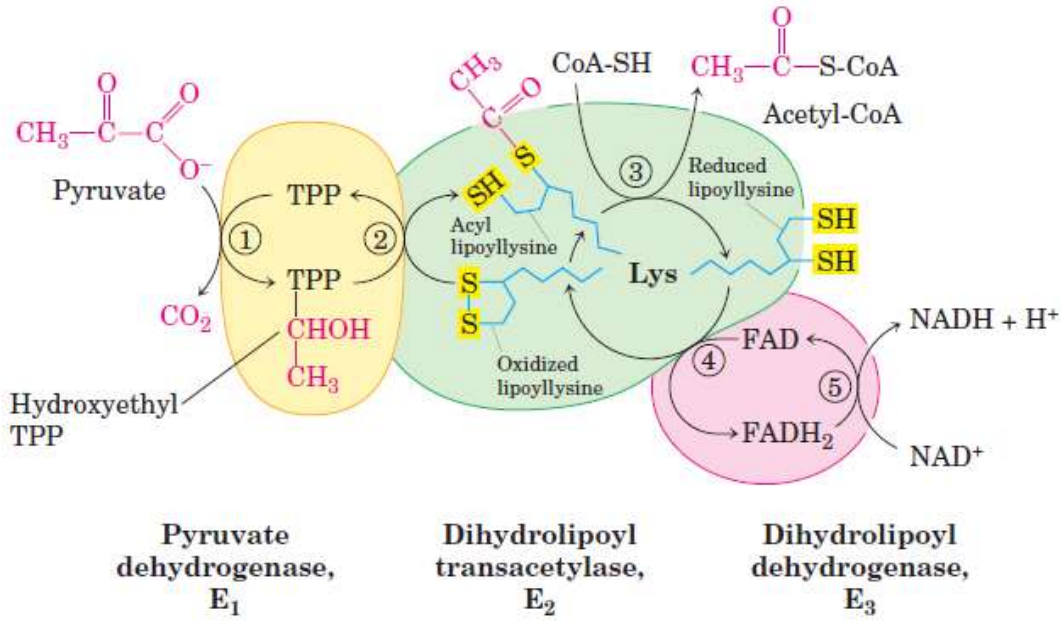


FIGURE 16–6 Oxidative decarboxylation of pyruvate to acetyl-CoA by the PDH complex. The fate of pyruvate is traced in red. In step ① pyruvate reacts with the bound thiamine pyrophosphate (TPP) of pyruvate dehydrogenase (E_1), undergoing decarboxylation to the hydroxyethyl derivative (see Fig. 14–13). Pyruvate dehydrogenase also carries out step ②, the transfer of two electrons and the acetyl group from TPP to the oxidized form of the lipoyllysyl group of the core enzyme, dihydrolipoyl transacetylase (E_2), to form the acetyl thioester of the reduced lipoyl group. Step ③ is a transesterification in which the —SH group of CoA replaces the —SH group of E_2 to yield acetyl-CoA and the fully reduced (dithiol) form of the lipoyl group. In step ④ dihydrolipoyl dehydrogenase (E_3) promotes transfer of two hydrogen atoms from the reduced lipoyl groups of E_2 to the FAD prosthetic group of E_3, restoring the oxidized form of the lipoyllysyl group of E_2. In step ⑤ the reduced $FADH_2$ of E_3 transfers a hydride ion to NAD^+, forming NADH. The enzyme complex is now ready for another catalytic cycle. (Subunit colors correspond to those in Fig. 16–5b.)

Mutations in the genes for the subunits of the PDH complex, or a dietary thiamine deficiency, can have severe consequences.
Thiamine-deficient animals are unable to oxidize pyruvate normally. This is of particular importance to the brain, which usually obtains all its energy from the aerobic oxidation of glucose in a pathway that necessarily includes the oxidation of pyruvate.

Beriberi, a disease that results from thiamine deficiency, is characterized by loss of neural function. This disease occurs primarily in populations that rely on a diet consisting mainly of white (polished) rice, which lacks the hulls in which most of the thiamine of rice is found.

People who habitually consume large amounts of alcohol can also develop thiamine deficiency, because much of their dietary intake consists of the vitamin-free “empty calories” of distilled spirits. An elevated level of pyruvate in the blood is often an indicator of defects in pyruvate oxidation due to one of these causes.

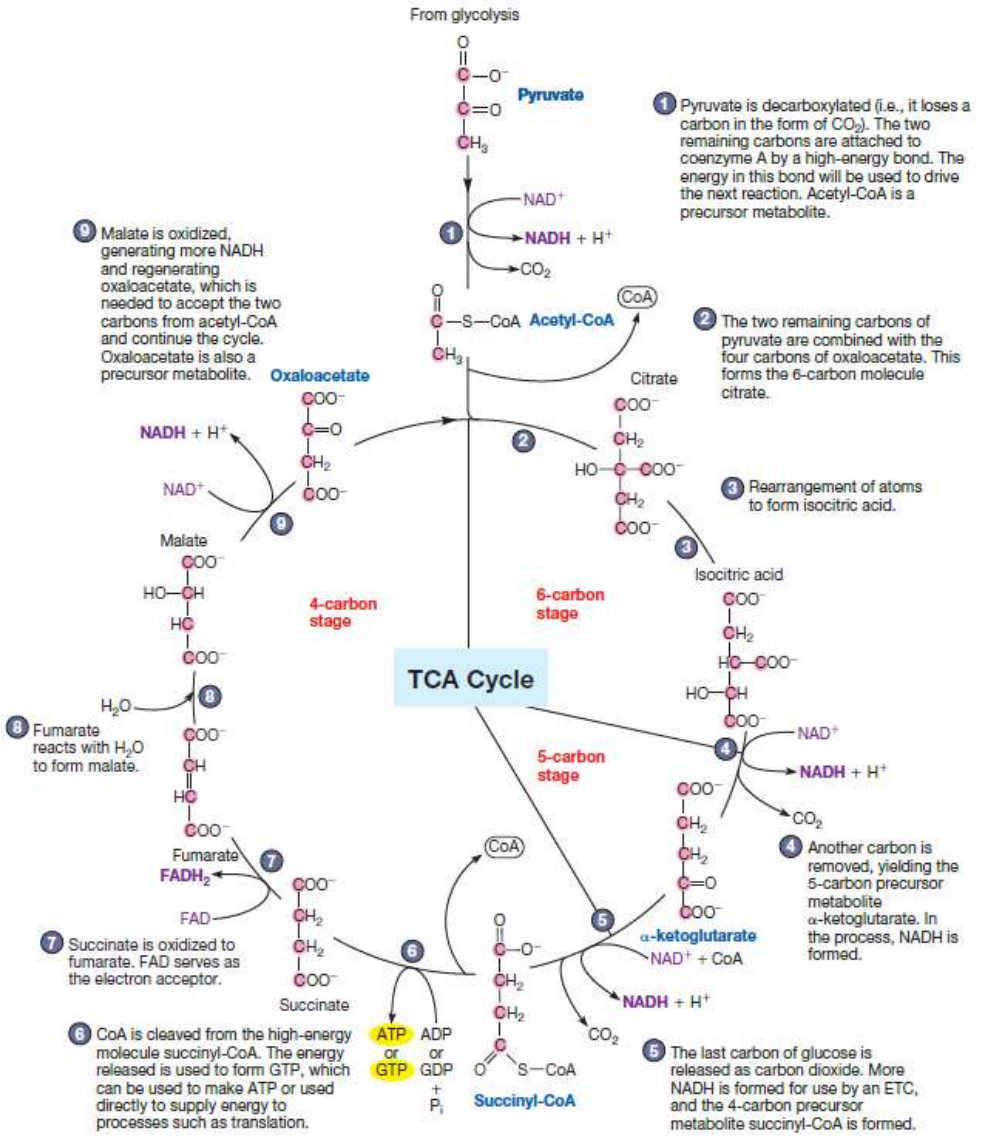
❖Eugene Kennedy and Albert Lehninger showed in 1948 that, **in eukaryotes, the entire set of reactions of the citric acid cycle takes place in mitochondria.**

❖Isolated mitochondria were found to contain not only all the enzymes and coenzymes required for the citric acid cycle, but also all the enzymes and proteins necessary for the last stage of respiration—electron transfer and ATP synthesis by oxidative phosphorylation.

❖Mitochondria also contain the enzymes for the oxidation of fatty acids and some amino acids to acetyl-CoA, and the oxidative degradation of other amino acids to α-ketoglutarate, succinyl-CoA, or oxaloacetate. Thus, **in nonphotosynthetic eukaryotes, the mitochondrion is the site of most energy-yielding oxidative reactions and of the coupled synthesis of ATP.**

❖**In photosynthetic eukaryotes, mitochondria are the major site of ATP production in the dark, but in daylight chloroplasts produce most of the organism's ATP.**

❖**In most prokaryotes, the enzymes of the citric acid cycle are in the cytosol, and the plasma membrane plays a role analogous to that of the inner mitochondrial membrane in ATP synthesis.**



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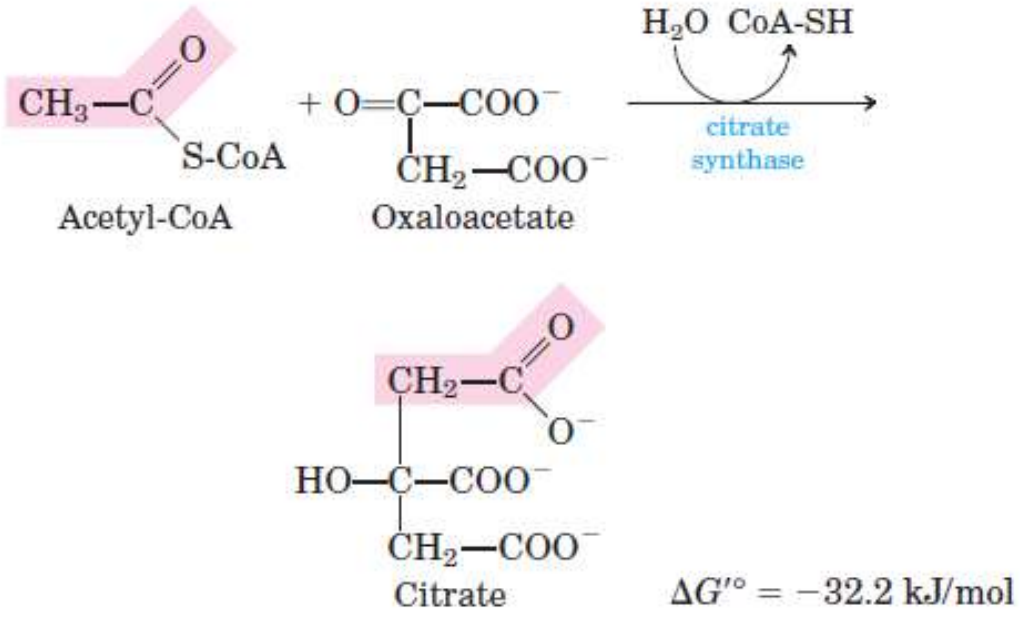
The eight successive reaction steps of the citric acid cycle, we place special emphasis on the chemical transformations taking place as citrate formed from acetyl-CoA and oxaloacetate is oxidized to yield CO_2 and the energy of this oxidation is conserved in the reduced coenzymes NADH and $FADH_2$.

The Citric Acid Cycle Has Eight Steps

(1) Formation of Citrate The first reaction of the cycle is the condensation of acetyl-CoA with **oxaloacetate** to form **citrate,** catalyzed by **citrate synthase:**



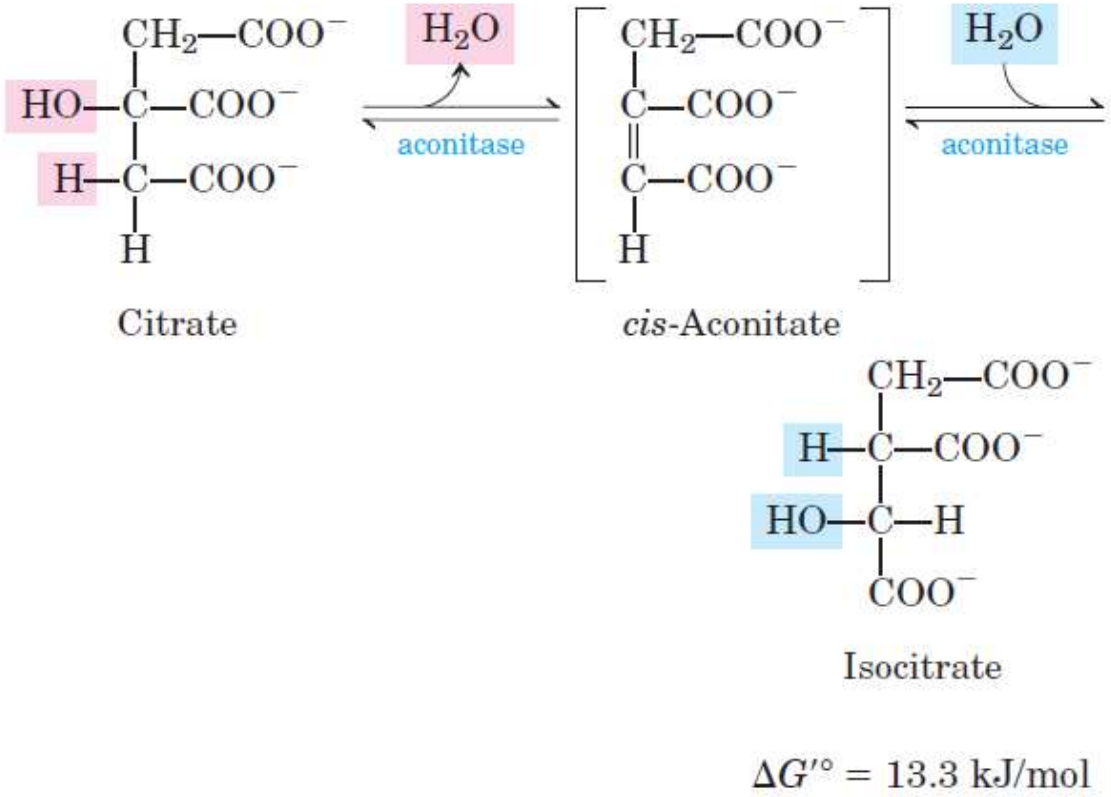
The hydrolysis of high-energy thioester intermediate makes the **forward reaction highly exergonic**. The large, negative standard free-energy change of the citrate synthase reaction is essential to the operation of the cycle because, the concentration of oxaloacetate is normally very low.

The **CoA liberated in this reaction is recycled to participate in the oxidative decarboxylation of another molecule of pyruvate by the PDH complex.**

Citrate synthase, homodimeric enzyme is a single polypeptide with two domains, one large and rigid, the other smaller and more flexible, with the active site between them. **Oxaloacetate, the first substrate to bind to the enzyme, induces a large conformational change in the flexible domain, creating a binding site for the second substrate, acetyl-CoA.**

2) Formation of Isocitrate via cis-Aconitate

The enzyme aconitase (more formally, aconitate hydratase) catalyzes the reversible transformation of citrate to **isocitrate, through the intermediary formation of the** tricarboxylic acid **cis-aconitate, which normally does** not dissociate from the active site. Aconitase can promote the reversible addition of H_2O to the double bond of enzyme-bound cis-aconitate in two different ways, one leading to citrate and the other to isocitrate:



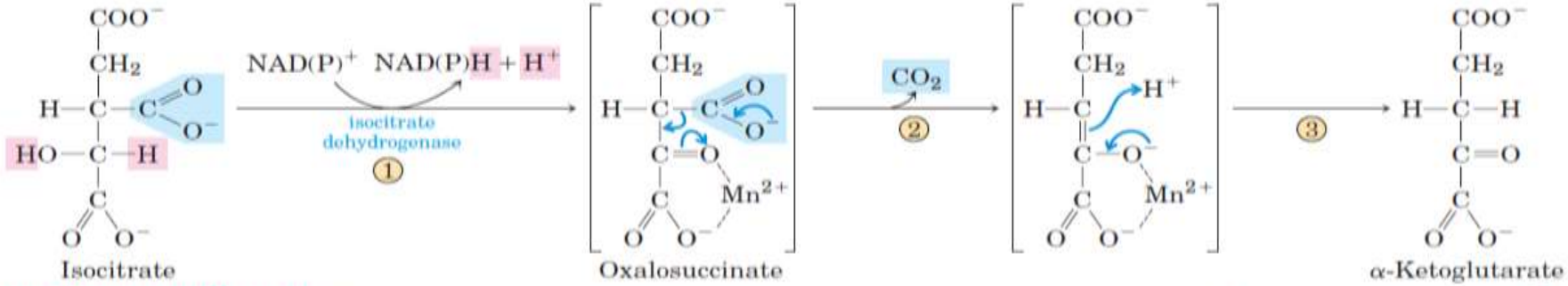
Although the equilibrium mixture at pH 7.4 and 25° C contains less than 10% isocitrate, in the cell the reaction is pulled to the right because isocitrate is rapidly consumed in the next step of the cycle, lowering its steady-state concentration.

Aconitase contains an **iron-sulfur center, which acts both in the binding** of the substrate at the active site and in the catalytic addition or removal of H_2O.

3) Oxidation of Isocitrate to α-Ketoglutarate and CO_2 :

In the next step, **isocitrate dehydrogenase catalyzes oxidative decarboxylation of isocitrate to form α–ketoglutarate** (Fig. 16–11). Mn^{2+} in the active site interacts with the carbonyl group of the intermediate oxalosuccinate, which is formed transiently but does not leave the binding site until decarboxylation converts it to α-ketoglutarate. Mn^{2+} also stabilizes the enol formed transiently by decarboxylation.

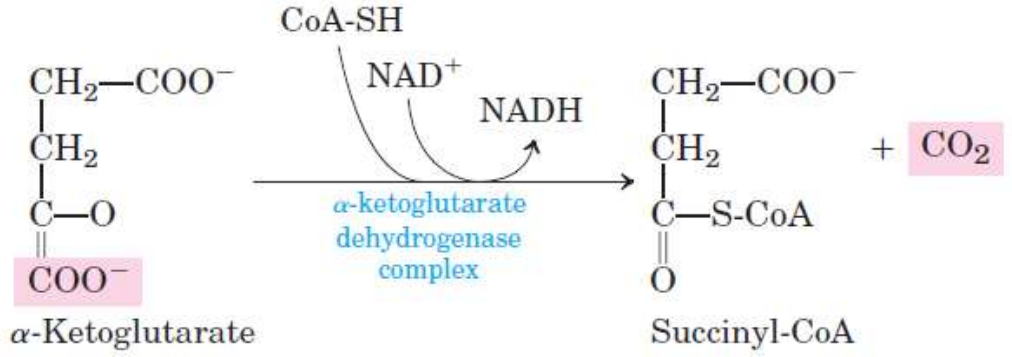
There **are two different forms of isocitrate dehydrogenase in all cells, one requiring NAD^+ as electron acceptor and the other requiring $NADP^+$.** The overall reactions are otherwise identical. **In eukaryotic cells, the NAD-dependent enzyme occurs in the mitochondrial matrix and serves in the citric acid cycle. The main function of the NADP-dependent enzyme, found in both the mitochondrial matrix and the cytosol, may be the generation of NADPH, which is essential for reductive anabolic reactions.**



MECHANISM FIGURE 16–11 **Isocitrate dehydrogenase.** In this reaction, the substrate, isocitrate, loses one carbon by oxidative decarboxylation. In step (1), isocitrate binds to the enzyme and is oxidized by hydride transfer to NAD^+ or $NADP^+$, depending on the isocitrate dehydrogenase isozyme. (See Fig. 14–12 for more information on hydride transfer reactions involving NAD^+ and $NADP^+$.) The resulting carbonyl group sets up the molecule for decarboxylation in step (2). Interaction of the carbonyl oxygen with a bound Mn^{2+} ion increases the electron-withdrawing capacity of the carbonyl group and facilitates the decarboxylation step. The reaction is completed in step (3) by rearrangement of the enol intermediate to generate α-ketoglutarate.

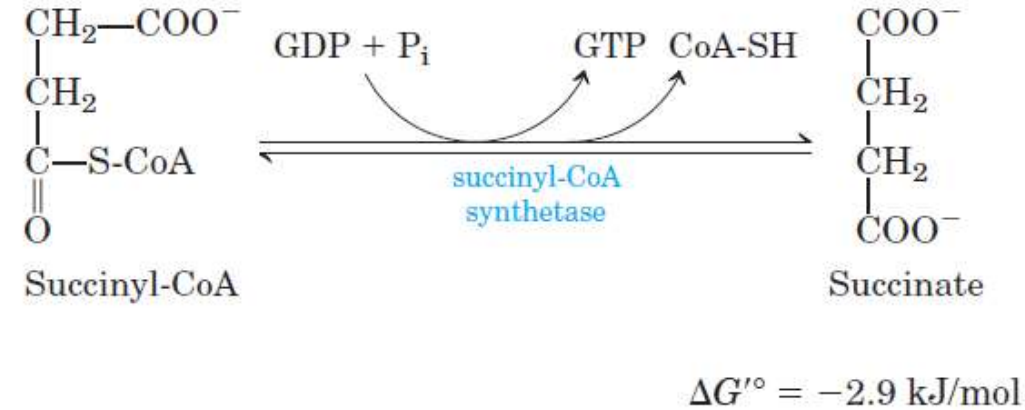
4) Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2 :

The next step is another oxidative decarboxylation, in which α-ketoglutarate is converted to **succinyl-CoA** and CO_2 by the action of the **α-ketoglutarate dehydrogenase complex; NAD+ serves as electron acceptor** and CoA as the carrier of the succinyl group. The energy of oxidation of α-ketoglutarate is conserved in the formation of the thioester bond of succinyl-CoA:

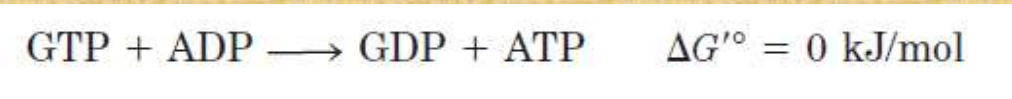


5) Conversion of Succinyl-CoA to Succinate

Succinyl-CoA, like acetyl-CoA, has a thioester bond with a strongly negative standard free energy of hydrolysis ($G'° \approx$ -36 kJ/mol). In the next step of the citric acid cycle, energy released in the breakage of this bond is used to drive the synthesis of a phosphoanhydride bond in either GTP or ATP, with a net G′° of only -2.9 kJ/mol. **Succinate is formed in the process:**



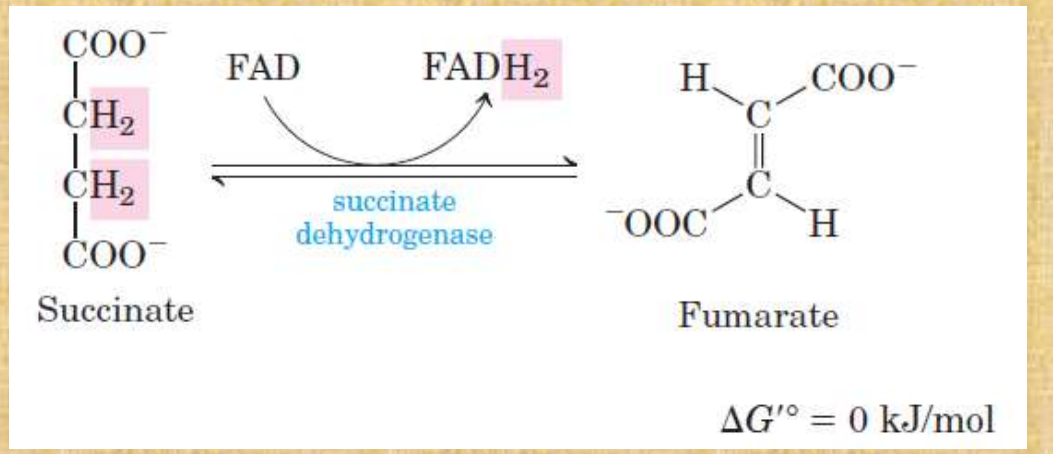
The GTP formed by succinyl-CoA synthetase can donate its terminal phosphoryl group to ADP to form ATP, in a reversible reaction catalyzed by **nucleoside diphosphate kinase.**



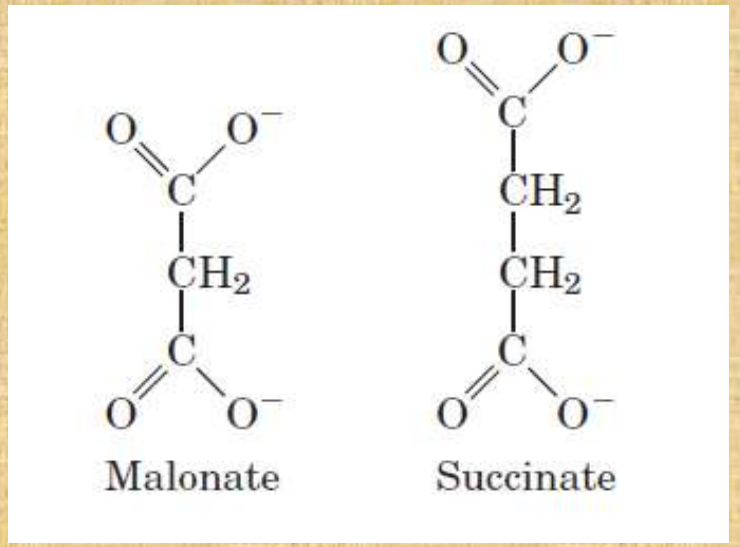
6) Oxidation of Succinate to Fumarate:

The succinate formed from succinyl-CoA is oxidized to **fumarate by** the flavoprotein **succinate dehydrogenase:**

In eukaryotes, succinate dehydrogenase is tightly bound to the inner mitochondrial membrane; in prokaryotes, to the plasma membrane.

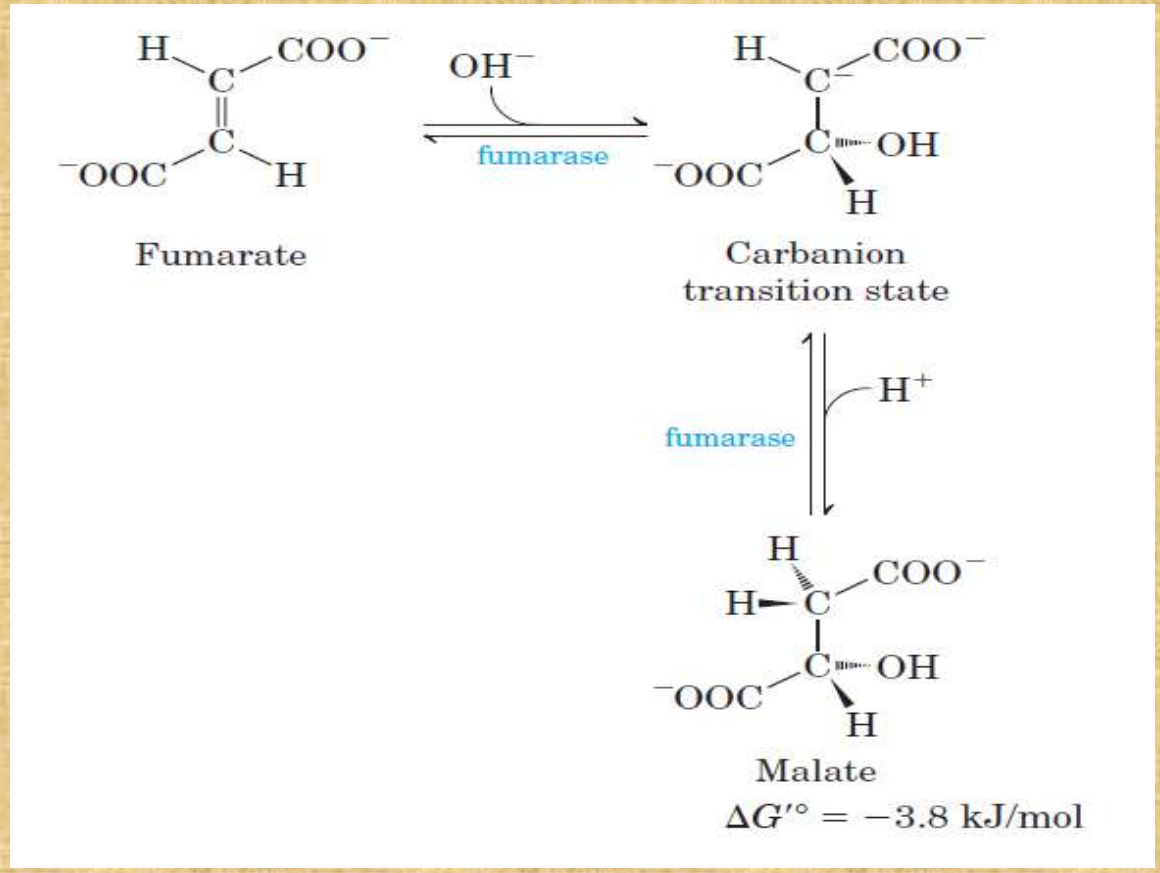


<u>Malonate, an analog of succinate not normally present in cells, is a strong competitive inhibitor of succinate dehydrogenase and its addition to mitochondria blocks the activity of the citric acid cycle.</u>



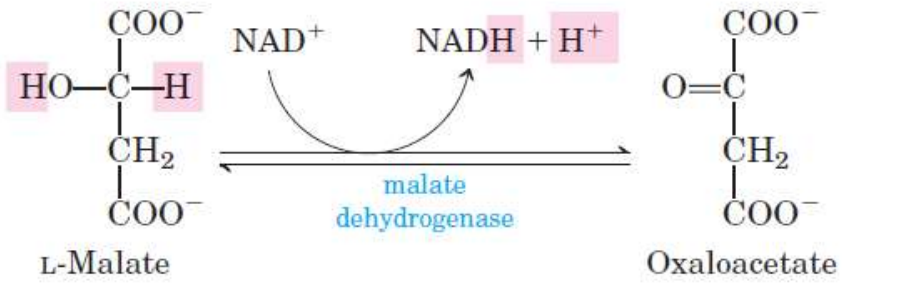
7) Hydration of Fumarate to Malate:

The reversible hydration of fumarate to **L-malate is catalyzed by fumarase** (formally, **fumarate hydratase). The transition state** in this reaction is a carbanion:



8) Oxidation of Malate to Oxaloacetate:

In the last reaction of the citric acid cycle, NAD-linked **L-malate dehydrogenase** catalyzes the oxidation of L-malate to oxaloacetate:



<u>The equilibrium of this reaction lies far to the left under standard thermodynamic conditions, but in intact cells oxaloacetate is continually removed by the highly exergonic citrate synthase reaction (step 1 of Fig. 16–7). This keeps the concentration of oxaloacetate in the cell extremely low (<10^{-6} M), pulling the malate dehydrogenase reaction toward the formation of oxaloacetate.</u>

THANK YOU