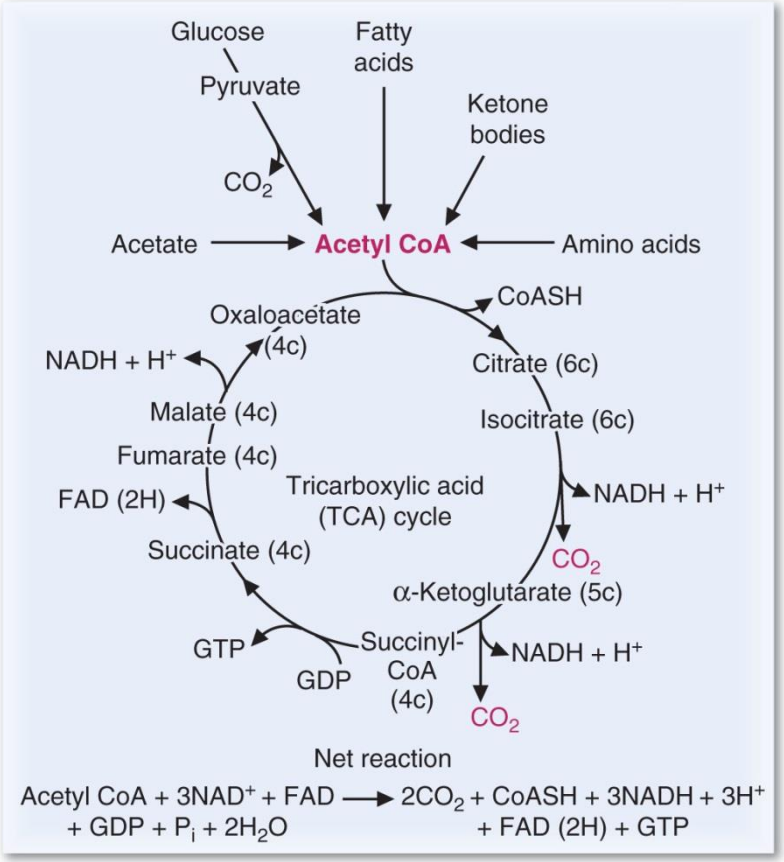
Chapter 23

Tricarboxylic Acid Cycle

Human Biochemistry

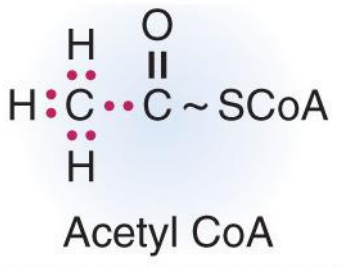


Summary of the tricarboxylic acid (TCA) cycle. The number of carbons in each intermediate of the cycle is indicated in *parentheses* by the name of the compound.

- As the activated two-carbon acetyl group is oxidized to two molecules of **CO_2**, energy is conserved as reduced **nicotinamide adenine dinucleotide (NADH)** and **flavin adenine dinucleotide (FAD[2H])**, and guanosine triphosphate (**GTP**).

- Within the TCA cycle, the oxidative decarboxylation of α-ketoglutarate is catabolyzed by the multisubunit **α-ketoglutarate dehydrogenase complex**, which contains the coenzymes **thiamin pyrophosphate (TPP)**, **lipoate**, and **FAD**.

- A similar complex, the **pyruvate dehydrogenase complex (PDC)**, catalyzes the oxidation of pyruvate to acetyl-CoA, thereby providing a link between the pathways of glycolysis and the TCA cycle.



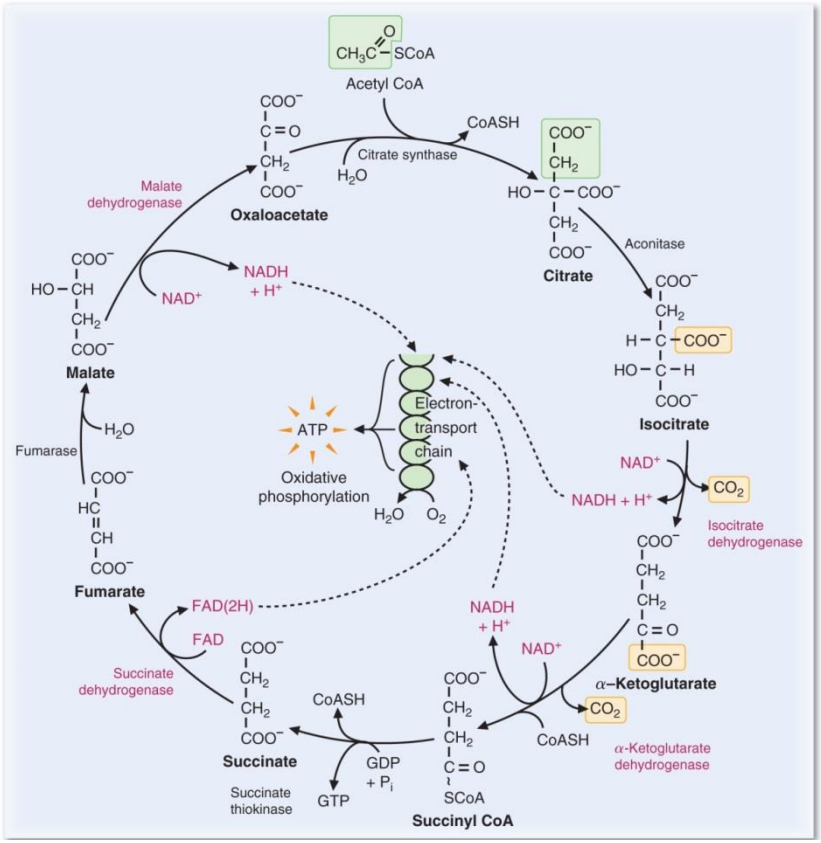
Acetyl CoA

The acetyl group of acetyl-CoA.
Acetyl-CoA donates eight electrons to the TCA cycle, which are shown in *red*, and two carbons. The high-energy bond is shown by a. The acetyl group is the ultimate source of the carbons in the two molecules of CO_2 that are produced and the source of electrons in the one molecule of FAD(2H) and three molecules of NADH, which have each accepted two electrons. However, the same carbon atoms and electrons that enter from one molecule of acetyl-CoA do not leave as CO_2, NADH, or FAD(2H) within the same turn of the cycle.

- In the TCA cycle, the two-carbon acetyl group of acetyl-CoA is oxidized to two CO_2 molecules. The function of the cycle is to conserve the energy from this oxidation, which it accomplishes principally by transferring electrons from intermediates of the cycle to NAD^+ and FAD.

- The eight electrons donated by the acetyl group (four from each carbon) eventually end up in three molecules of NADH and one of FAD(2H).

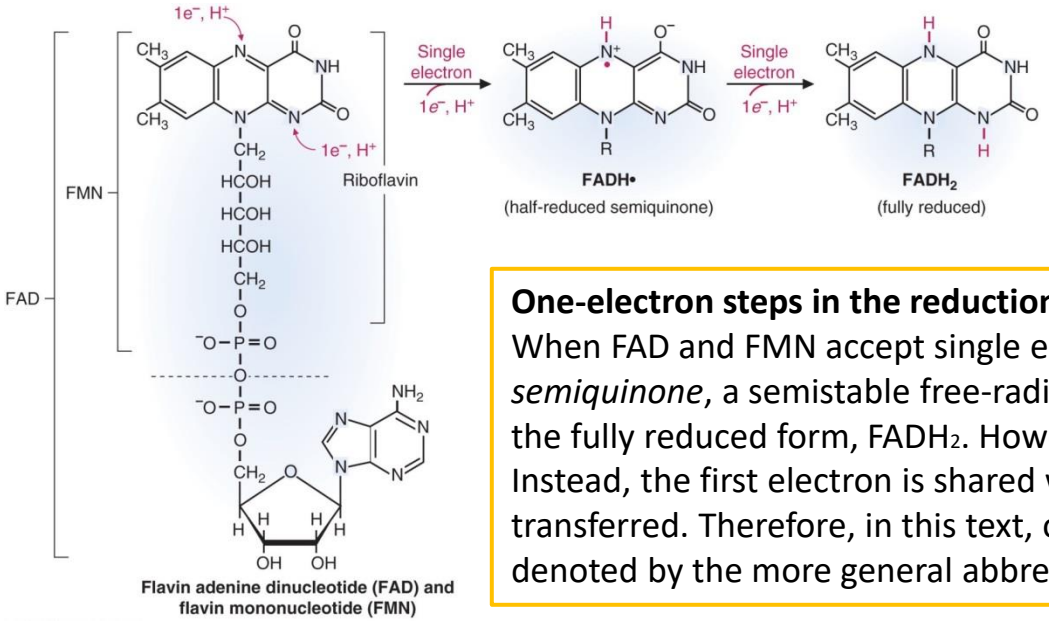
- As a consequence, ATP can be generated from oxidative phosphorylation when NADH and FAD(2H) donate these electrons to O_2 via the ETC.



Reactions of the TCA cycle. The oxidation–reduction enzymes and coenzymes are shown in *red*. Entry of the two carbons of acetyl-CoA into the TCA cycle are indicated with the *green box*. The carbons released as CO_2 are shown with *yellow boxes*.

- Initially, the acetyl group is incorporated into citrate, an intermediate of the TCA cycle. As citrate progresses through the cycle to oxaloacetate, it is oxidized by four dehydrogenases (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase), which remove electron-containing hydrogen or hydride atoms from a substrate and transfer them to electron-accepting coenzymes such as NAD^+ or FAD.

- The isomerase aconitase rearranges electrons in citrate, thereby forming isocitrate, to facilitate an electron transfer to NAD^+. An iron cofactor in aconitase facilitates the isomerization.



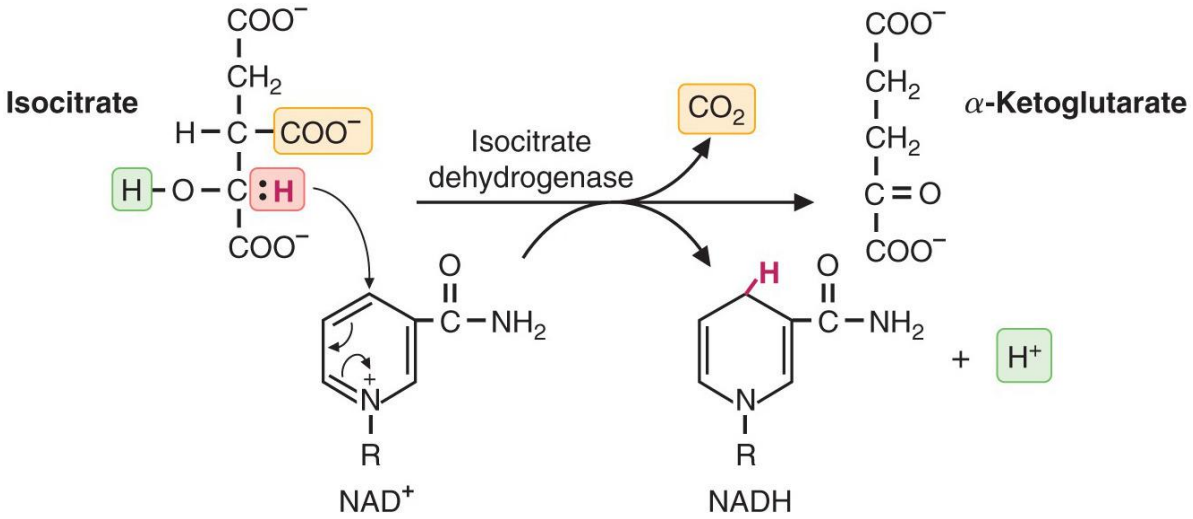
Flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN)

One-electron steps in the reduction of FAD.
When FAD and FMN accept single electrons, they are converted to the half-reduced *semiquinone*, a semistable free-radical form. They can also accept two electrons to form the fully reduced form, $FADH_2$. However, in most dehydrogenases, $FADH_2$ is never formed. Instead, the first electron is shared with a group on the protein as the next electron is transferred. Therefore, in this text, overall acceptance of two electrons by FAD has been denoted by the more general abbreviation, FAD(2H).

- Both FAD and NAD^+ are electron-accepting coenzymes. Why is FAD used in some reactions and NAD^+ in others? Their unique structural features enable FAD and NAD^+ to act as electron acceptors in different types of reactions and to play different physiologic roles in the cell.

- FAD is able to accept single electrons ($H\cdot$) and forms a half-reduced single-electron intermediate.

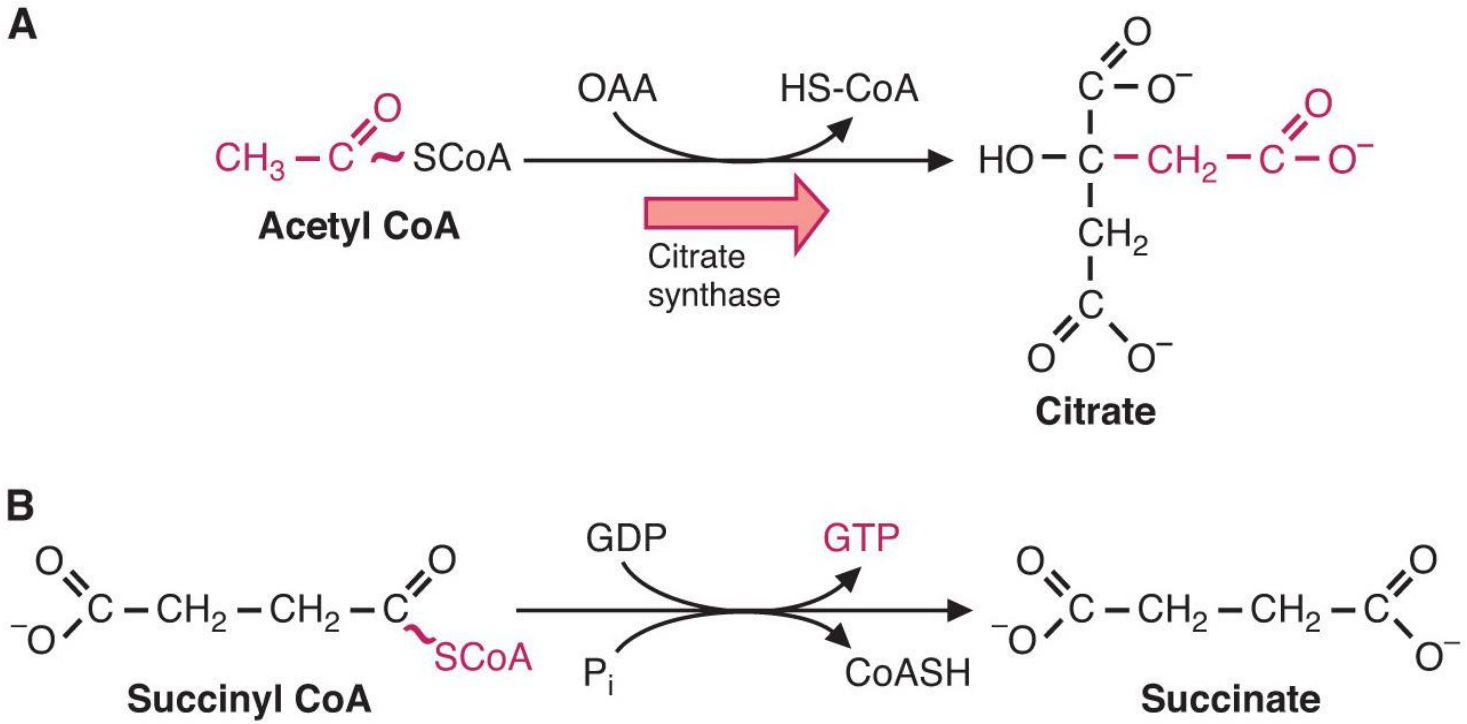
- It thus participates in reactions in which single electrons are transferred independently from two different atoms, which occurs in double-bond formation (e.g., succinate to fumarate) and disulfide bond formation (e.g., lipoate to lipoate disulfide in the α-ketoglutarate dehydrogenase reaction).



Oxidation and decarboxylation of isocitrate.
The alcohol group (C–OH) is oxidized to a ketone, with the C–H electrons donated to NAD as the hydride ion. Subsequent electron shifts in the pyridine ring remove the positive charge. The H of the OH group dissociates into water as a proton, H. NAD, the electron acceptor, is reduced.

- In contrast, NAD^+ accepts a pair of electrons as the hydride ion (H^-), which is attracted to the carbon opposite the positively charged pyridine ring.

- This occurs, for example, in the oxidation of alcohols to ketones by malate dehydrogenase and isocitrate dehydrogenase. The nicotinamide ring accepts a hydride ion from the C-H bond, and the alcoholic hydrogen is released into the medium as a positively charged proton, H^+.



Utilization of the high-energy thioester bond of acyl-CoAs.
Energy transformations are shown in *red*. **A.** The energy released by hydrolysis of the thioester bond of acetyl coenzyme A (acetyl-CoA) in the citrate synthase reaction contributes a large negative G0 to the forward direction of the TCA cycle. **B.** The energy of the succinyl-CoA thioester bond is used for the synthesis of the high-energy phosphate bond of GTP.

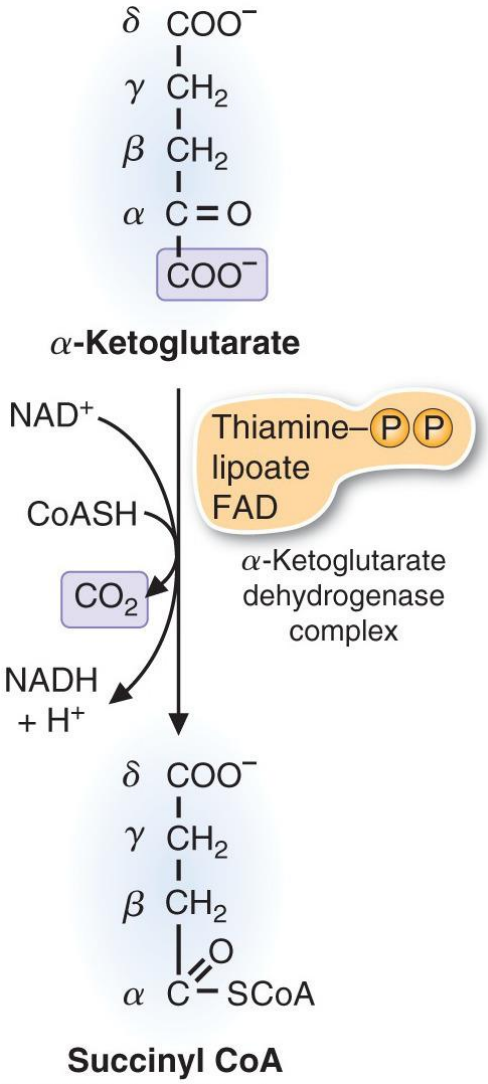
- CoASH, the acylation coenzyme, participates in reactions through the formation of a thioester bond between the sulfure (S) of CoASH and an acyl group (e.g., acetyl-CoA, syccinyl-CoA).

- A thioester bond differs from a typical oxygen ester bond because sulfur, unlike oxygen, does not share its electrons and participates in resonance formations.

- One of the consequences of this feature of sulfur chemistry is that the carbonyl carbon, the α-carbon, and the β-carbon of the acyl group in a CoA thioester can be activated for participation in different types of reactions (e.g., in the citrate synthase reaction, the α-carbon methyl group is activated for condensation with oxaloacetate.

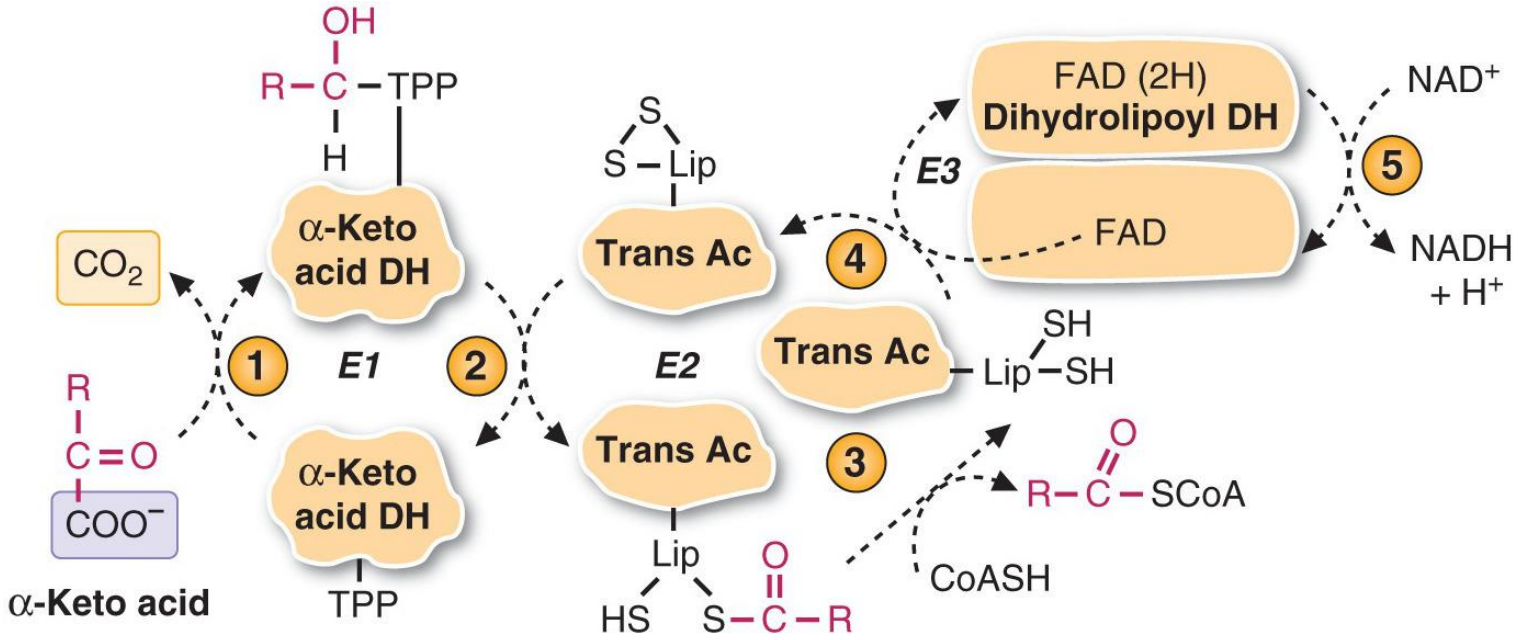
- The energy from cleavage of the high-energy thioester bonds of succinyl-CoA and acetyl-CoA is used in two different ways in the TCA cycle.

- When the succinyl-CoA thioester bond is cleaved by succinate thiokinase, the energy is used directly for activating an enzyme-bound phosphate that is transferred to GDP.



- The α-ketoglutarate dehydrogenase complex is one of a three-member family of similar α-keto acid dehydrogenase complexes. The other members of this family are the PDC and the branched-chain amino acid α-keto acid dehydrogenase complex.
- Each of these complexes is specific for a different α-keto acid structure. In the sequence of reactions catalyzed by the complexes, the α-keto acid is decarboxylated (i.e., releases the carboxyl group as CO_2).
- The keto group is oxidized to the level of a carboxylic acid and then combined with CoASH to form an acyl-CoA thioester (e.g., succinyl-CoA).

Oxidative decarboxylation of α-ketoglutarate. The α-ketoglutarate dehydrogenase complex oxidizes α-ketoglutarate to succinyl-CoA. The carboxyl group is released as CO2. The keto group on the α-carbon is oxidized and then forms the acyl-CoA thioester, succinyl-CoA. The α,β,γ, and δ on succinyl-CoA refer to the sequence of atoms in α-ketoglutarate.



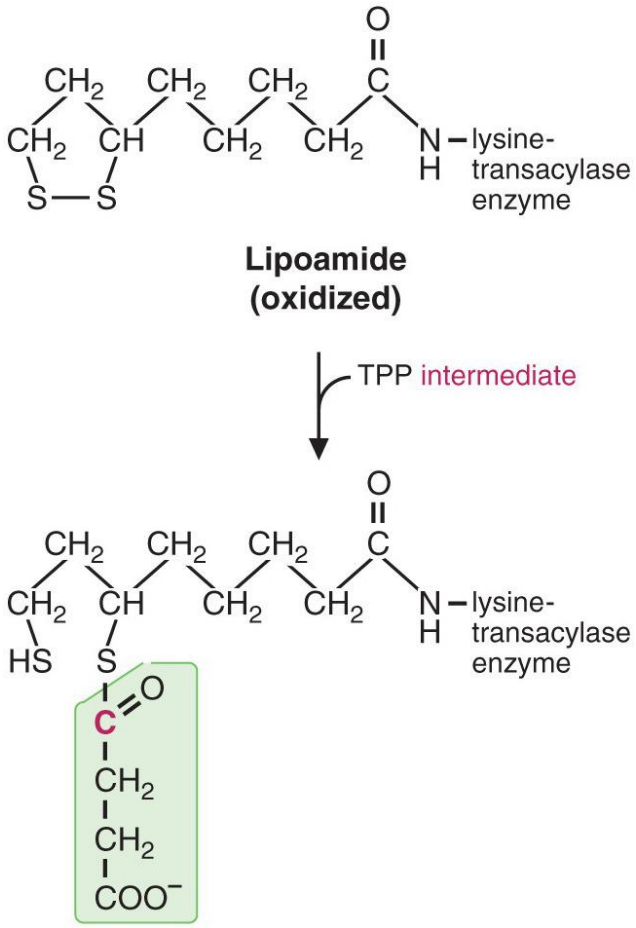
Mechanism of -keto acid dehydrogenase complexes (including α–ketoglutarate dehydrogenase, pyruvate dehydrogenase, and the branched-chain α-keto acid dehydrogenase complex).
R represents the portion of the α-keto acid that begins with the β-carbon. In α-ketoglutarate, R is CH2–CH2–COOH. In pyruvate, R is CH3. The individual steps in the oxidative decarboxylation of α-keto acids are catalyzed by three different subunits: *E1*, α-keto acid decarboxylase (α-ketoglutarate decarboxylase); *E2*, transacylase (*trans*succinylase); and *E3*, dihydrolipoyl dehydrogenase. *(1)* Thiamine pyrophosphate (TPP) on E1 decarboxylates the α-keto acid and forms a covalent intermediate with the remaining portion. *(2)* The acyl portion of the α-keto acid is transferred by TPP on E1 to lipoate on E2, which is a transacylase. *(3)* E2 transfers the acyl group from lipoate to CoASH. Note how lipoate is reduced during this conversion. The lipoyl disulfide bond has been reduced to sulfhydral groups (dihydrolipoate). *(4)* E3, dihydrolipoyl dehydrogenase (DH) transfers the electrons from reduced lipoate to its tightly bound FAD molecule, thereby oxidizing lipoate back to its original disulfide form. *(5)* The electrons are then transferred from FAD(2H) to NAD
to form NADH.

- All of the α-keto acid dehydrogenase complexes are huge enzyme complexes composed of multiple subunits of three different enzymes, designated as E_1, E_2, and E_3.

- E_1 is an α-keto acid decarboxylase that contains TPP; it cleaves off the carboxyl group of the α-keto acid.
- E_2 is a transacylase-containing lipoate; it transfers the acyl portion of the α-keto acid from thiamin to CoASH.
- E_3 is dihydrolipoyl dehydrogenase, which contains FAD; it transfers electrons from reduced lipoate to NAD^+.

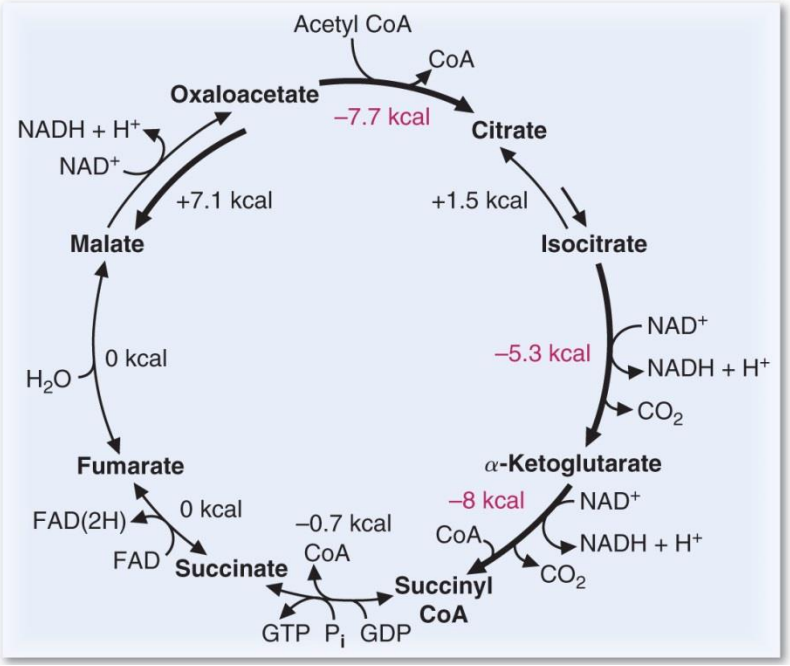
- The collection of three enzyme activities into one huge complex enables the product of one enzyme to be transferred to the next enzyme without loss of energy.

- Complex formation also increases the rate of catalysis because the substrates for E_2 and E_3 remain bound to the enzyme complex.



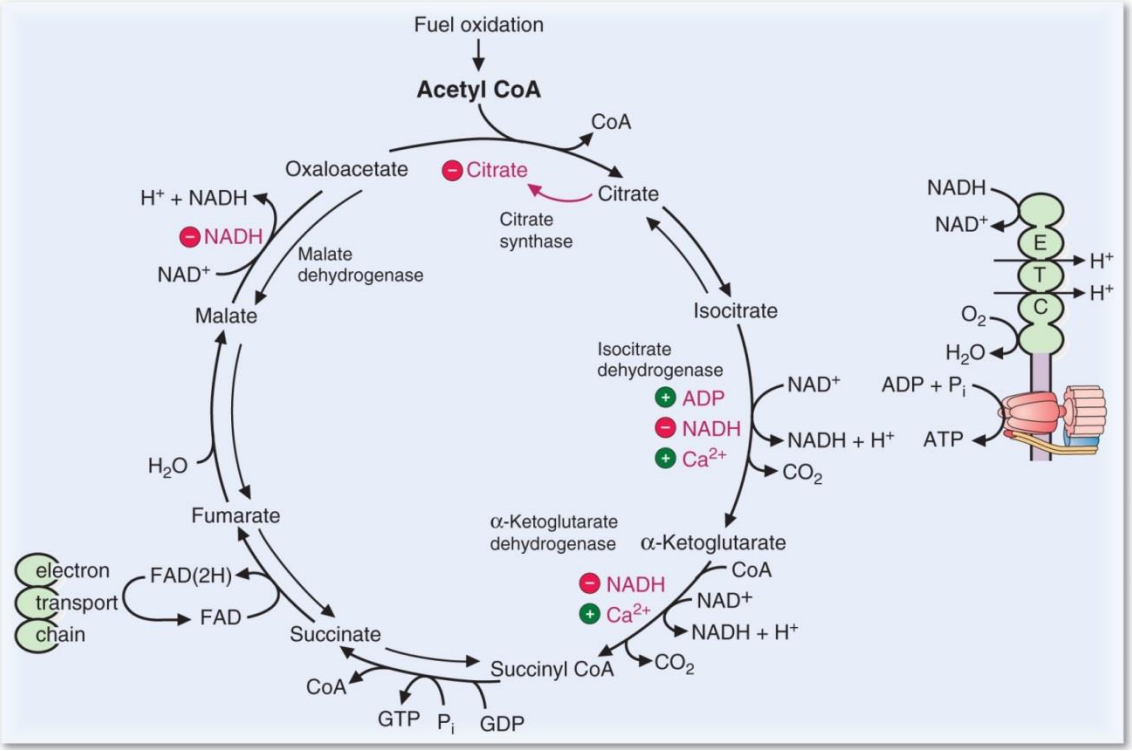
- Lipoate is a coenzyme found only in α-keto acid dehydrogenase complexes. It is synthesized in humans from carbohydrate and amino acids, and it does not require a vitamin precursor.
- Lipoate is attached to the transacylase enzyme through its carboxyl group, which is covalently bound to the terminal $-NH_2$ of a lysine in the protein.

Function of lipoate.
Lipoate is attached to the ε-amino group of a lysine side chain of the transacylase enzyme (E2). The oxidized lipoate disulfide form is reduced as it accepts the acyl group from thiamine pyrophosphate (TPP) attached to E1. The example shown is for the α-ketoglutarate dehydrogenase complex.



Approximate ΔG_0 values for the reactions in the TCA cycle, given for the forward direction.
The reactions with *large* negative ΔG_0 values are shown in *red*. See Chapter 19 for a discussion of the meaning of ΔG_0 values

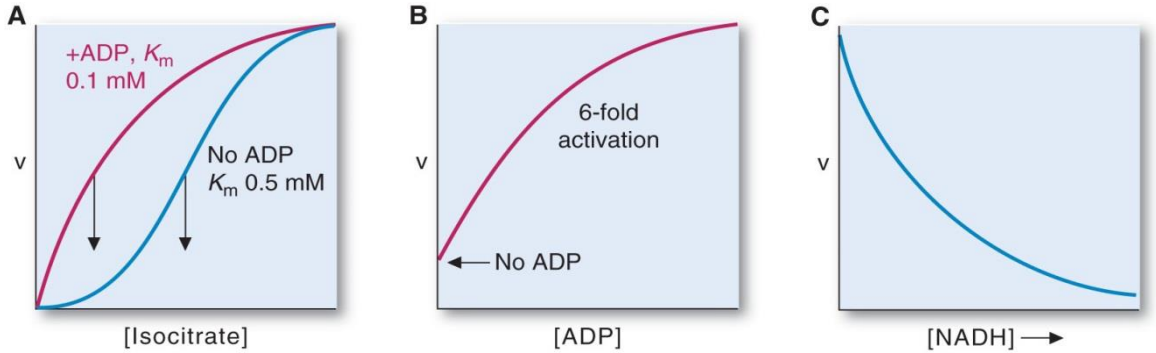
- Like all metabolic pathways, the TCA cycle operates with an overall net negative $\Delta G^{0'}$. The conversion of substrates to products is, therefore, energetically favorable. However, some of the reactions, such as the malate dehydrogenase reaction, have a positive value.
- The TCA cycle reactions are able to conserve about 90% of the energy available from the oxidation of acetyl-CoA.
- Three reactions in the TCA cycle have large negative values for $\Delta G^{0'}$ that strongly favor the forward direction: the reactions catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.



- Two major messengers feed information on the rate of ATP use back to the TCA cycle: (1) the phosphorylation state of ATP, as reflected in ATP and ADP levels: and (2) the reduction state of NAD^+, as reflected in the ratio of $NADH/NAD^+$.
- Citrate synthase, which is the first enzyme of the TCA cycle, is a simple enzyme that has no allosteric regulators. Its rate is controlled principally by the concentration of oxaloacetate, its substrate, and the concentration of citrate, a product inhibitor, that is competitive with oxaloacetate.

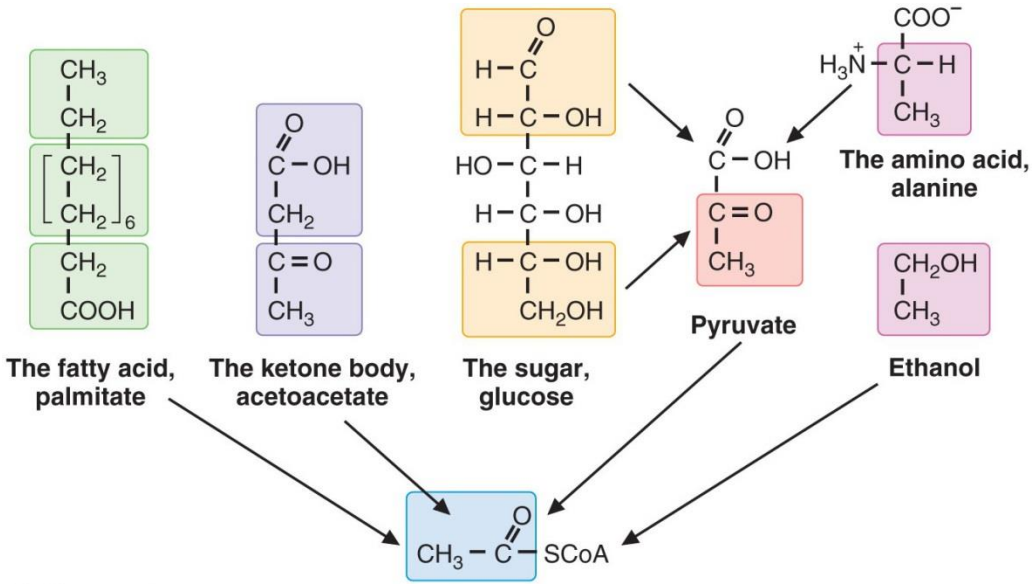
Major regulatory interactions in the TCA cycle.

The rate of ATP hydrolysis controls the rate of ATP synthesis, which controls the rate of NADH oxidation in the electron-transport chain (ETC). All NADH and FAD(2H) produced by the cycle donate electrons to this chain (shown on the **right**). Thus, oxidation of acetyl-CoA in the TCA cycle can go only as fast as electrons from NADH enter the electron-transport chain, which is controlled by the ATP and ADP content of the cells. The ADP and NADH concentrations feed information on the rate of oxidative phosphorylation back to the TCA cycle. Isocitrate dehydrogenase (DH), α-ketoglutarate DH, and malate DH are inhibited by increased NADH concentration. The $NADH/NAD^+$ ratio changes the concentration of oxaloacetate. Citrate is a product inhibitor of citrate synthase. ADP is an allosteric activator of isocitrate DH. During muscular contraction, increased Ca^{2+} concentrations activate isocitrate DH and α-ketoglutarate DH (as well as pyruvate DH).



Allosteric regulation of isocitrate dehydrogenase (ICDH).
Isocitrate, NAD^+, and NADH bind in the active site; ADP and Ca^{2+} are activators and bind to separate allosteric sites. **A.** A graph of velocity versus isocitrate concentration shows positive cooperativity (sigmoid curve) in the absence of ADP. The allosteric activator ADP changes the curve into one closer to a rectangular hyperbola and decreases the K_m ($S_{0.5}$) for isocitrate. **B.** The allosteric activation by ADP is not an all-or-nothing response. The extent of activation by ADP depends on its concentration. **C.** Increases in the concentration of product, NADH, decrease the velocity of the enzyme through effects on the allosteric activation.

- Isocitrate dehydrogenase, which consists of eight subunits, is considered one of the rate-limiting steps of the TCA cycle, and it is allosterically activated by ADP and inhibited by NADH.
- In the presence of ADP, all of the subunits are in their active conformation, and isocitrate binds more readily. Consequently, the apparent $K_{m,app}$ (the $S_{0.5}$) shifts to a much lower value.
- Thus, at the concentration of isocitrate found in the mitochondrial matrix, a small change in the concentration of ADP can produce a large change in the rate of the isocitrate dehydrogenase reaction.
- Small changes in the concentration of the product, NADH, and of the cosubstrate, NAD^+, also affect the rate of the enzyme more than they would a nonallosteric enzyme.



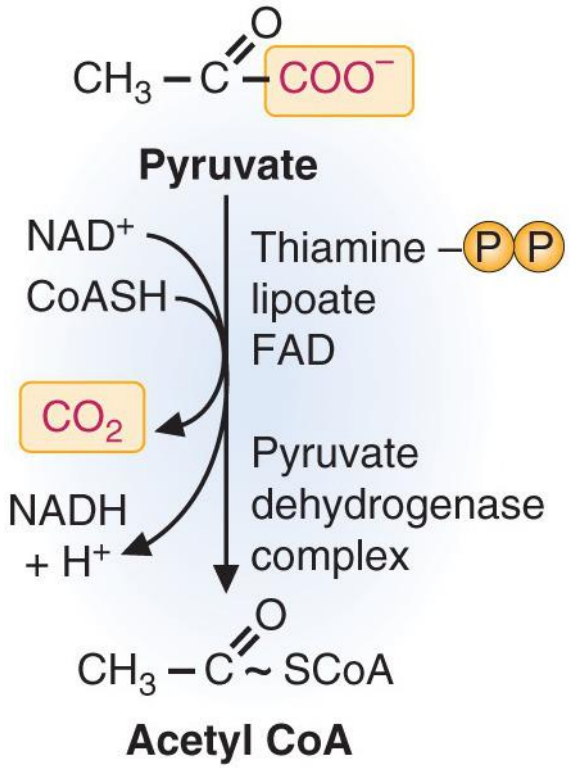
Origin of the acetyl group from various fuels. Acetyl coenzyme A (acetyl-CoA) is derived from the oxidation of fuels. The portions of fatty acids, ketone bodies, glucose, pyruvate, the amino acid alanine, and ethanol that are converted to the acetyl group of acetyl coenzyme A (acetyl-CoA) are shown in *boxes*.

- Acetyl-CoA serves as a common point of convergence for the major pathways of fuel oxidation. It is generated directly from the β-oxidation of fatty acids and degradation of the ketone bodies β-hydroxybutyrate and acetoacetate.

- It is also formed from acetate, which can arise from the diet or from ethanol oxidation. Glucose and other carbohydrates enter glycolysis, a pathway common to all cells, and are oxidized to pyruvate.

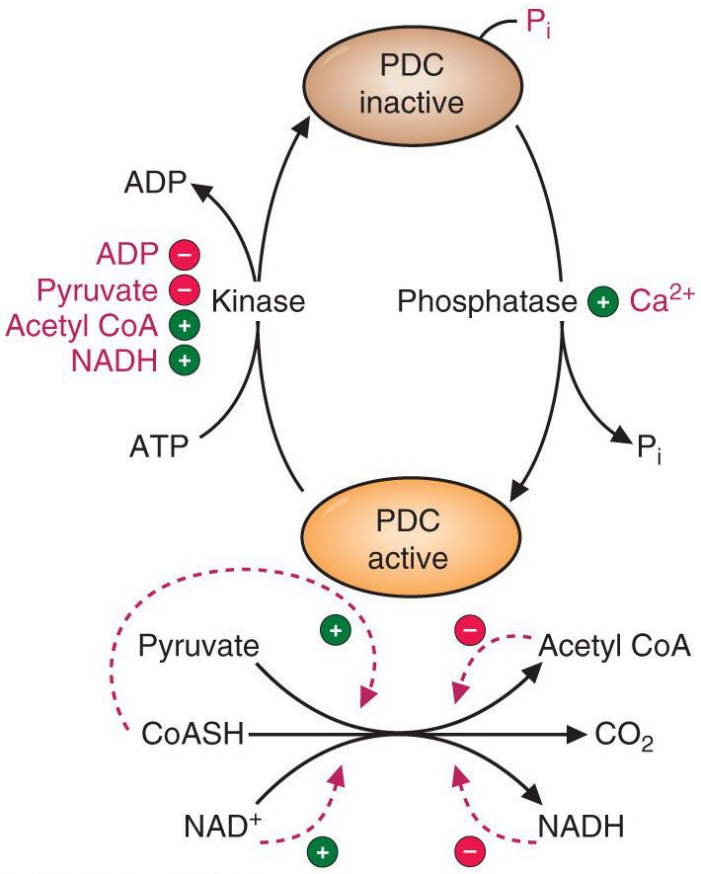
- The amino acids alanine and serine are also converted to pyruvate. Pyruvate is oxidized to acetyl-CoA by the PDC complex.

- Several amino acids, such as leucine and isoleucine, are also oxidized to acetyl-CoA. Thus, the final oxidation of acetyl-CoA to CO_2 in the TCA cycle is the last step in all the major pathways of fuel oxidation.



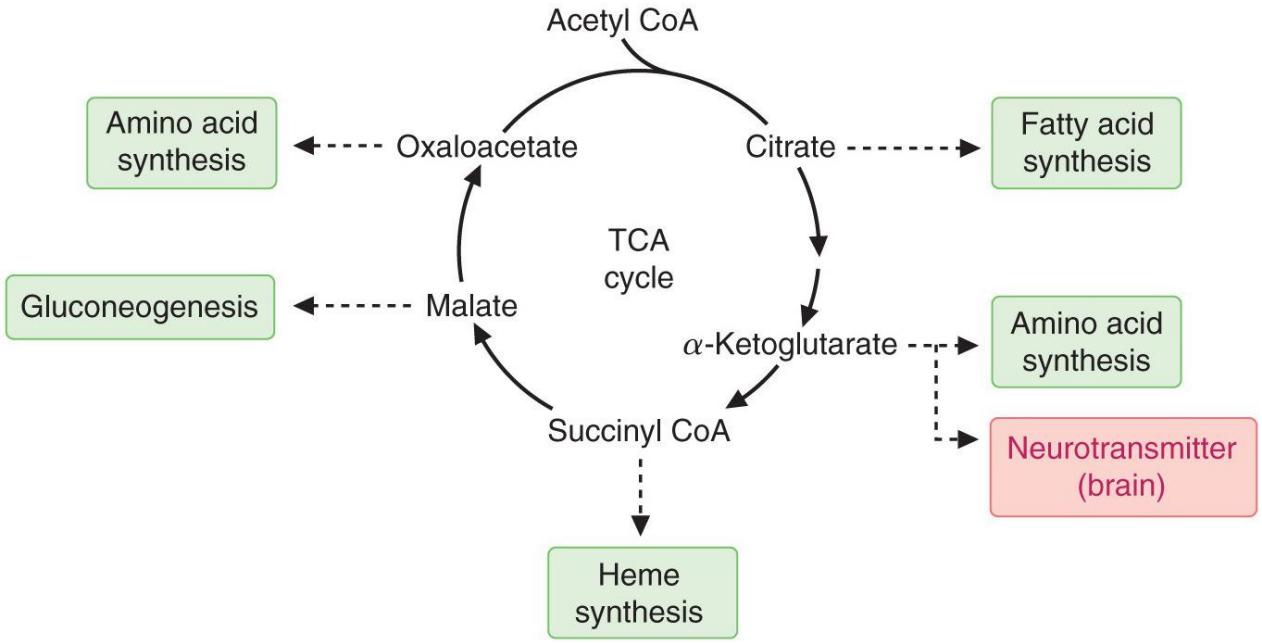
PDC catalyzes oxidation of the α-keto acid pyruvate to acetyl-CoA.

- The PDC belongs to the α-ketoacid dehydrogenase complex family and thus shares structural and catalytic features with the α-ketoglutarate dehydrogenase complex and the branched-chain α-ketoacid dehydrogenase complex.



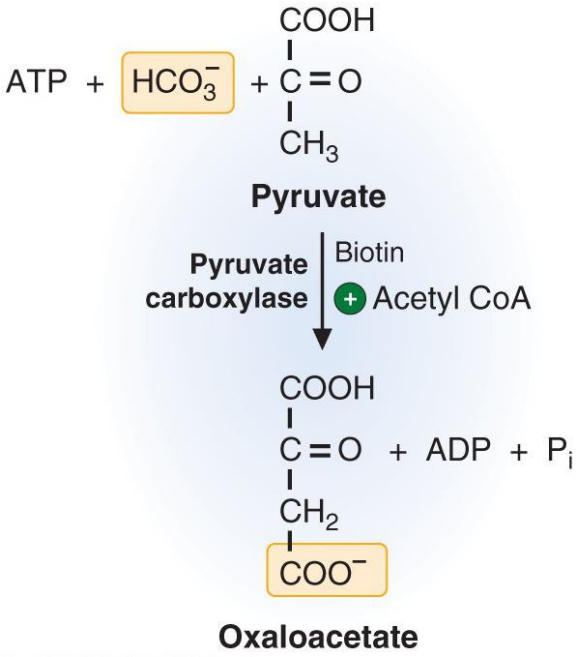
- PDC activity is controlled principally through phosphorylation by pyruvate dehydrogenase kinase, which inhibits the enzyme, and dephosphorylation by pyruvate dehydrogenase phosphatase, which activates it.
- PDC kinase is itself inhibited by ADP and pyruvate. Thus, when rapid ATP use results in an increase of ADP, or when activation of glycolysis increases pyruvate levels, PDC kinase is inhibited and PDC remains in an active, nonphosphorylated form.
- PDC is also regulated principally by the rate of ATP use through rapid phosphorylation to an inactive form. Thus, in a normally respiring cell, with an adequate supply of oxygen, glycolysis and the TCA cycle are activated together, and glucose can be completely oxidized to carbon dioxide.

Regulation of PDC.
PDC kinase, a subunit of the enzyme, phosphorylates PDC at a specific serine residue, thereby converting PDC to an inactive form. The kinase is inhibited by adenosine diphosphate (ADP) and pyruvate. PDC phosphatase, another subunit of the enzyme, removes the phosphate, thereby activating PDC. The phosphatase is activated by Ca^{2+}. When the substrates pyruvate and CoASH are bound to PDC, the kinase activity is inhibited and PDC is active. When the products acetyl-CoA and NADH bind to PDC, the kinase activity is stimulated, and the enzyme is phosphorylated to the inactive form. E_1 and the kinase exist as tissue-specific isozymes with overlapping tissue specificity and somewhat different regulatory properties.



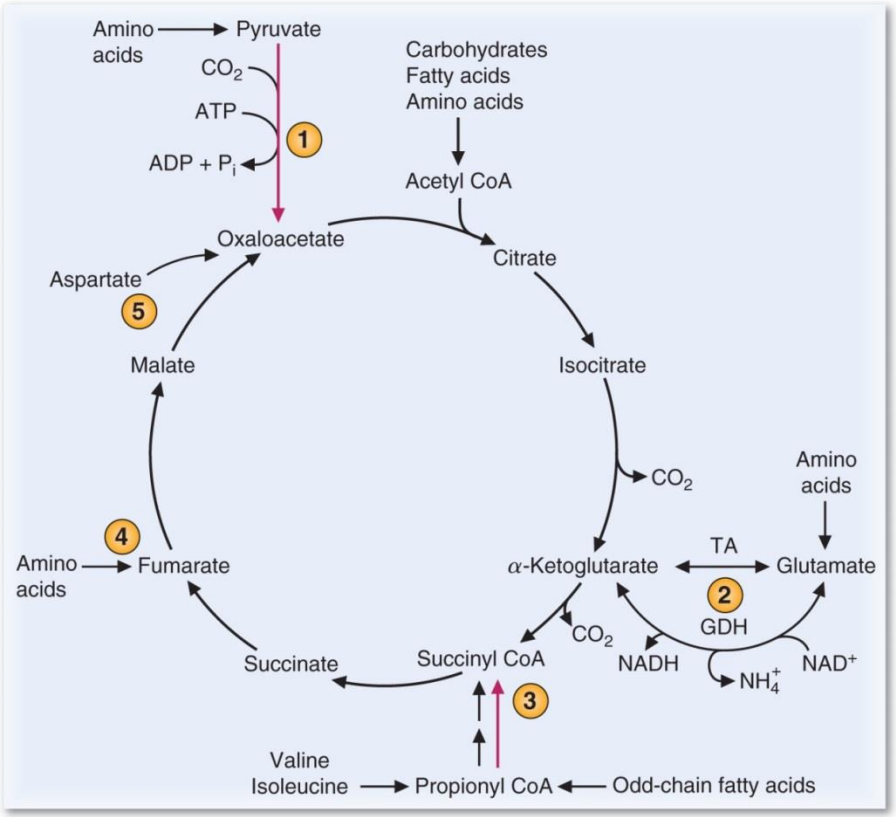
Efflux of intermediates from the tricarboxylic acid (TCA) cycle. In the liver, TCA cycle intermediates are continuously withdrawn into the pathways of fatty acid synthesis, amino acid synthesis, gluconeogenesis, and heme synthesis. In the brain, α-ketoglutarate is converted to glutamate and γ-aminobutyric acid (GABA), both of which are neurotransmitters

- The intermediates of the TCA cycle serve as precursors for a variety of different pathways present in different cell types.
- This is particularly important in the central metabolic role of the liver. The TCA cycle in the liver is often called an *open cycle* because there is such a high efflux of intermediates.
- After a high-carbohydrate meal, citrate efflux and cleavage to acetyl-CoA provides acetyl units for cytosolic fatty acid synthesis. During fasting, gluconeogenic precursors are converted to malate, which leaves the mitochondria for cytosolic gluconeogenesis.
- In the brain, α-ketoglutarate is converted to glutamate and then to γ-aminobutyric acid (GABA), a neurotransmitter.



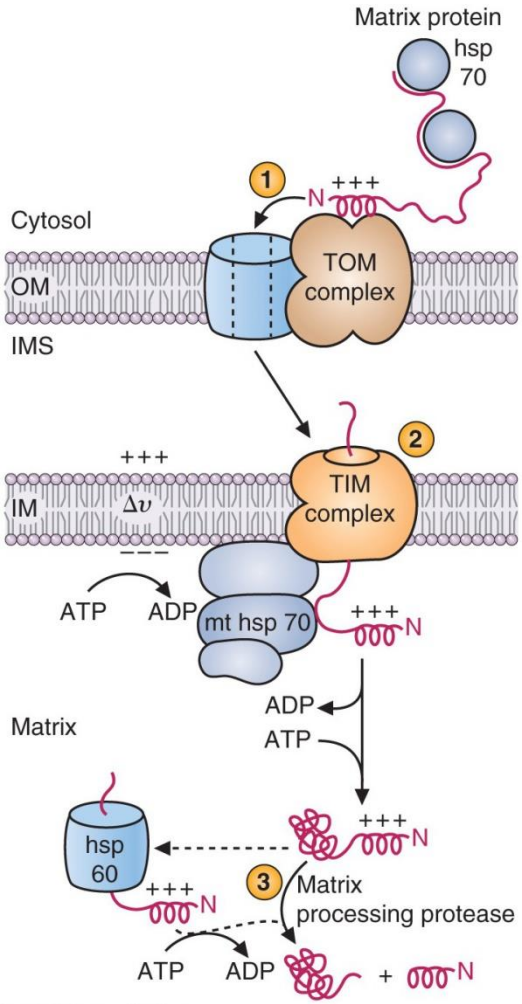
Pyruvate carboxylase reaction. Pyruvate carboxylase adds a carboxyl group from bicarbonate (which is in equilibrium with CO_2) to pyruvate to form oxaloacetate. Biotin is used to activate and transfer the CO_2. The energy to form the covalent biotin–CO_2 complex is provided by the high-energy phosphate bond of ATP, which is cleaved in the reaction. The enzyme is activated by acetyl coenzyme A (acetyl-CoA).

- Pyruvate carboxylase is one of the major anaplerotic enzymes in the cell. Like most carboxylases, pyruvate carboxylase contains biotin (a vitamin), which forms a covalent intermediate with CO_2 in a reaction that requires ATP and MG^{2+}.
- The activated CO_2 is then transferred to pyruvate to form the carboxyl group of oxaloacetate.
- Pyruvate carboxylase is found in many tissues, such as liver, brain, adipocytes, and fibroblasts.
- Pyruvate carboxylase is activated by acetyl-CoA and inhibited by high concentrations of many acyl-CoA derivatives. As the concentration of oxaloacetate is depleted through the efflux of TCA cycle intermediates, the rate of the citrate synthase reaction decreases and acetyl-CoA concentration rises.
- The acetyl-CoA then activates pyruvate carboxylase to synthesize more oxaloacetate.



Major anaplerotic pathways of the tricarboxylic acid (TCA) cycle.
(1) and *(3) (red arrows)* are the two major anaplerotic pathways. *(1)* Pyruvate carboxylase. *(2)* Glutamate is reversibly converted to α-ketoglutarate by transaminases (TA) and glutamate dehydrogenase (GDH) in many tissues. *(3)* The carbon skeletons of valine and isoleucine, a three-carbon unit from odd-chain fatty acid oxidation, and a number of other compounds enter the TCA cycle at the level of succinyl-CoA. Other amino acids are also degraded to fumarate *(4)* and oxaloacetate *(5),* principally in the liver.

- The pathways for oxidation of many amino acids convert their carbon skeletons into five- and four-carbon intermediates of the TCA cycle that can regenerate oxaloacetate.
- Alanine and serine carbons can enter through pyruvate carboxylase (circle 1).
- In all tissues with mitochondria (except for, surprisingly, the liver), oxidation of the two branched-chain amino acids isoleucine and valine to succinyl-CoA forms a major anaplerotic route (circle 3).
- In most tissues, glutamine is taken up from the blood, converted to glutamate, and then oxidized to α-ketoglutarate, forming another major anaplerotic route (circle 2).



- All mitochondrial matrix proteins, such as the TCA cycle enzymes, are encoded by the nuclear genome. They are imported into the mitochondrial matrix as unfolded proteins that are pushed and pulled through channels in the outer and inner mitochondrial membranes.
- The mitochondrial matrix proteins are synthesized on free ribosomes in the cytosol and maintain an unfolded conformation by binding to heat-shock protein 70 (hsp 70) chaperonins. This basic presequence binds to a receptor in a *t*ranslocase of the *o*uther *m*embrane (TOM) complex (circle 1).
- The matrix preprotein is translocated across the inner membrane through a *t*ranslocases of the *i*nner *m*embrane (TIM) complex (circle 2).
- The final step in the import process is cleavage of the signal sequence by a matrix-processing protease (circle 3).

Model for the import of nuclearencoded proteins into the mitochondrial matrix. The matrix preprotein with its positively charged N-terminal presequence is shown in *red*. *hsp*, heat-shock protein; *OM*, outer mitochondrial membrane; *IMS*, intermembrane space; *IM*, inner mitochondrial membrane; *TOM*, translocases of the outer mitochondrial membrane; *TIM*, translocases of the inner mitochondrial membrane; *mthsp70*, mitochondrial heat-shock protein 70.