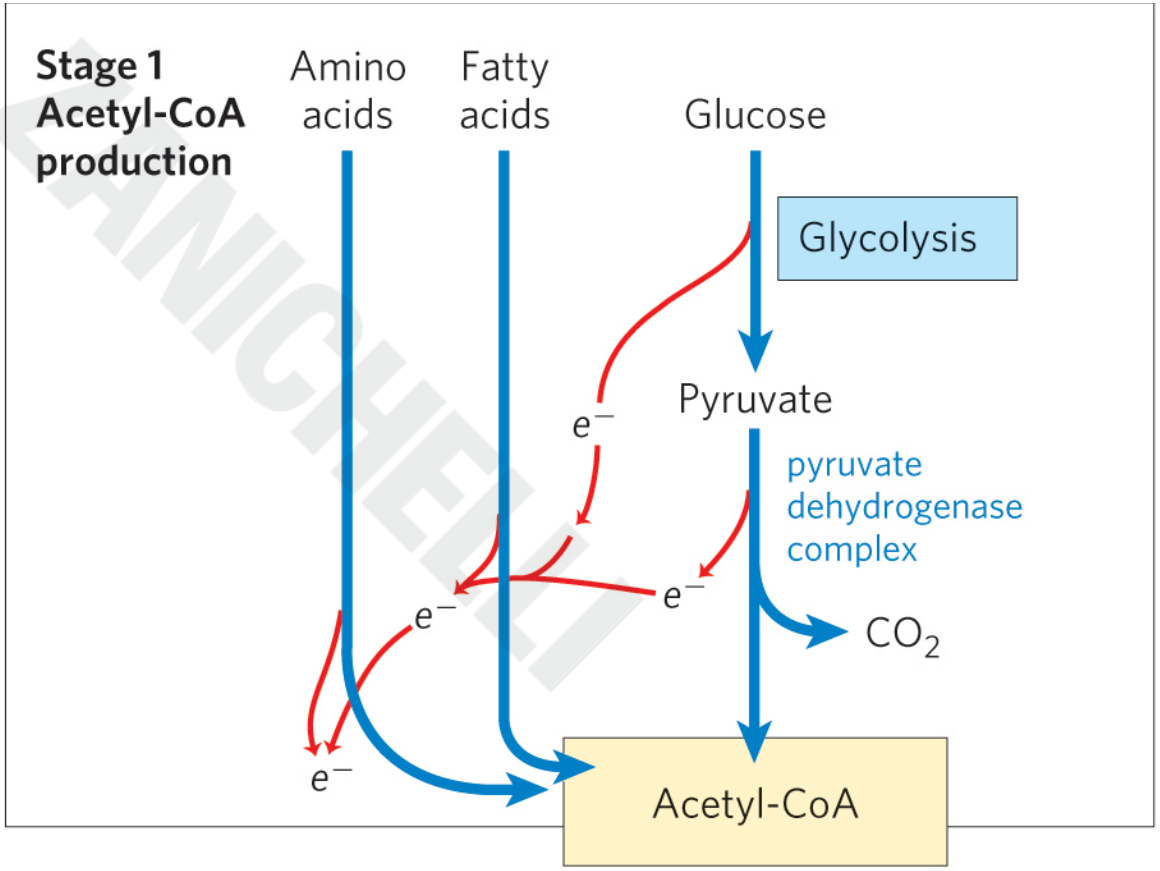
16 The Citric Acid Cycle

Cellular Respiration

- **cellular respiration** = process by which the pyruvate produced by glycolysis is further oxidized to H_2O and CO_2

Stage 1 of Cellular Respiration

- Stage 1: oxidation of fuels to acetyl-CoA
  - generates ATP, NADH, $FADH_2$



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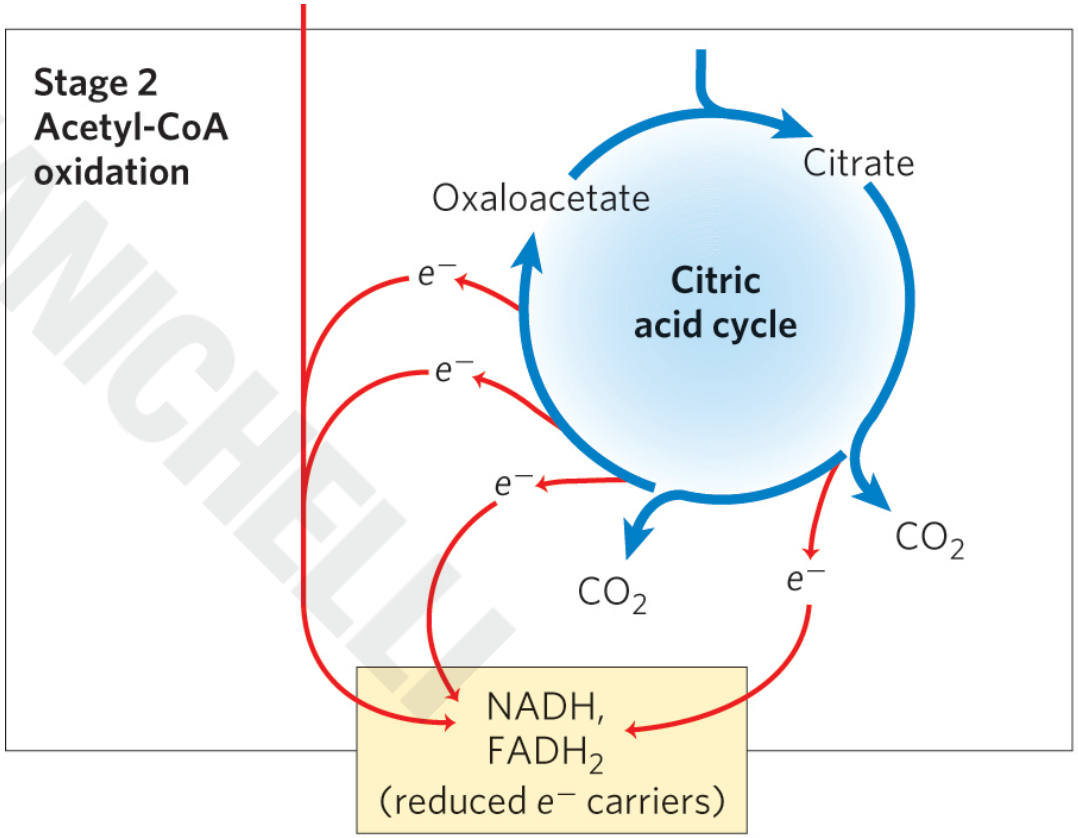
Stage 2 of Cellular Respiration

- Stage 2: oxidation of acetyl groups to CO_2 in the **citric acid cycle (tricarboxylic acid (TCA) cycle, Krebs cycle)**
  - nearly universal pathway
  - generates NADH, $FADH_2$, and one GTP



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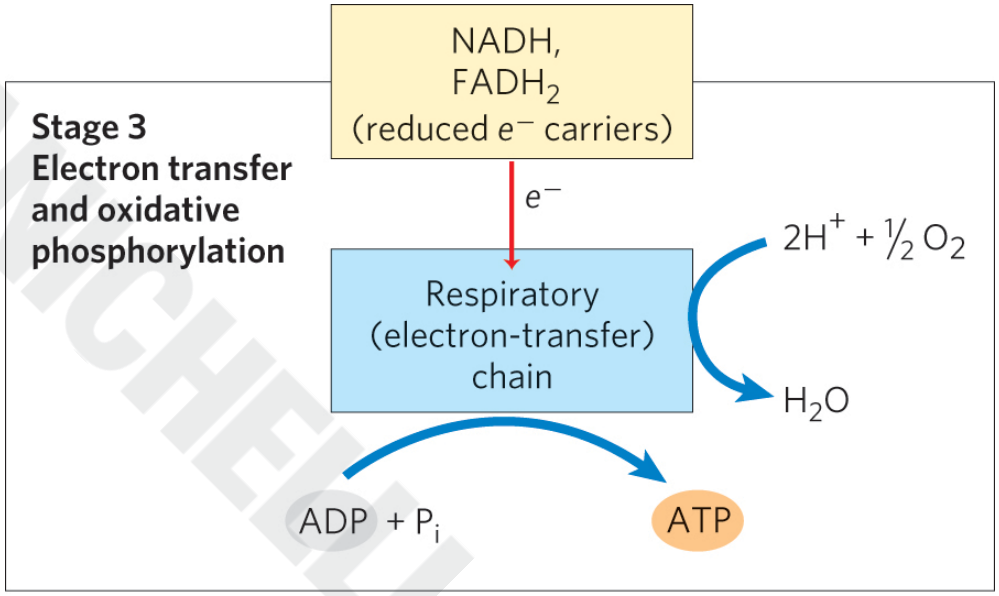
Stage 3 of Cellular Respiration

- Stage 3: electron transfer chain and oxidative phosphorylation
  - generates the vast majority of ATP from catabolism



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P1 Principle 1 (1 of 4)

Pyruvate is the metabolite that links two central catabolic pathways, glycolysis and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

P2 Principle 2 (1 of 7)

The reactions of the citric acid cycle follow a chemical logic. In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO_2 and H_2O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways.

P3 Principle 3 (1 of 4)

The citric acid cycle is a hub of metabolism, with catabolic pathways leading in and anabolic pathways leading out. Acetate groups (acetyl-CoA) from the catabolism of various fuels are used in the synthesis of such metabolites as amino acids, fatty acids, and sterols. The breakdown products of many amino acids and nucleotides are intermediates of the cycle, and they can be fed in or siphoned off as needed by the cell.

P4 Principle 4 (1 of 5)

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis. Some mutations that affect the reactions of the citric acid cycle lead to tumor formation.

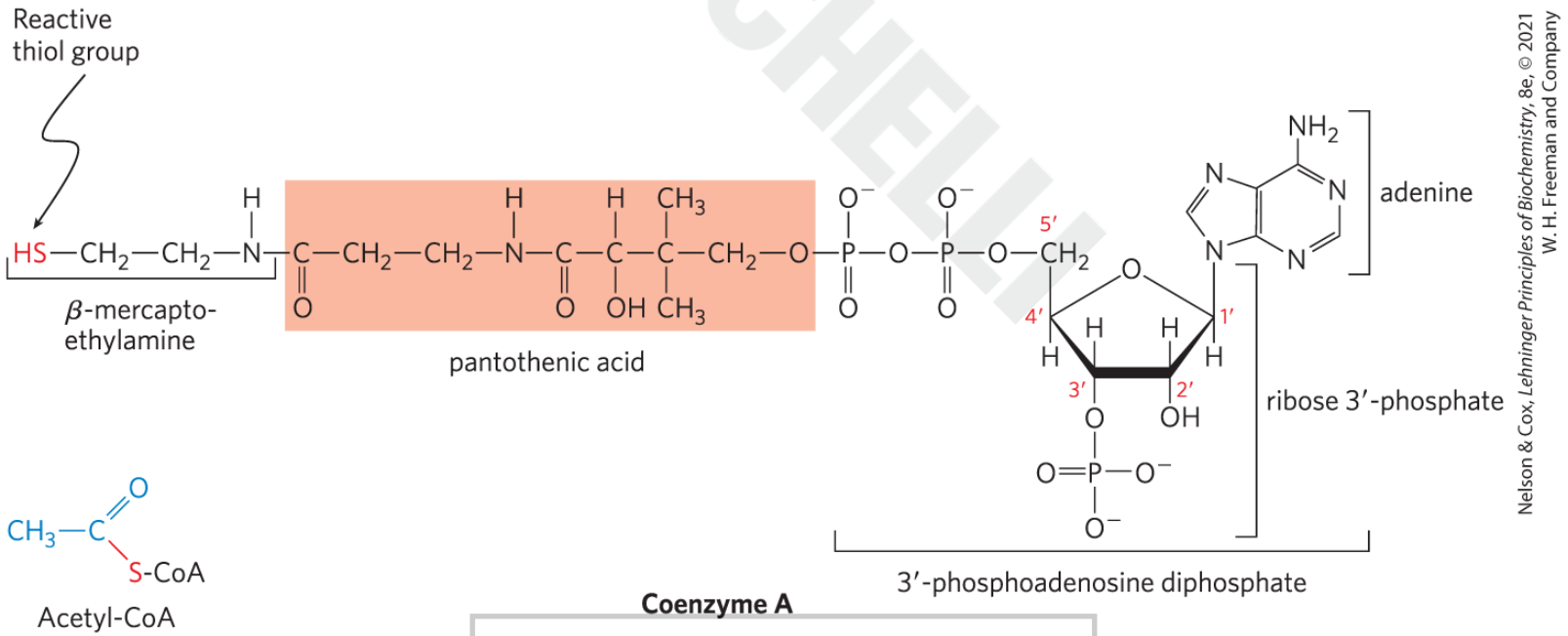
P5 Principle 5 (1 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

16.1 Production of Acetyl-CoA (Activated Acetate)

Coenzyme A (CoA-SH)

- coenzyme A has a reactive thiol (–SH) group that is critical to its role as an acyl carrier
  - the –SH group forms a **thioester** with acetate in acetyl-CoA



P1 Principle 1 (2 of 4)

Pyruvate is the metabolite that links two central catabolic pathways, glycolysis and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

P5 Principle 5 (2 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

P4 Principle 4 (2 of 5)

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis. Some mutations that affect the reactions of the citric acid cycle lead to tumor formation.

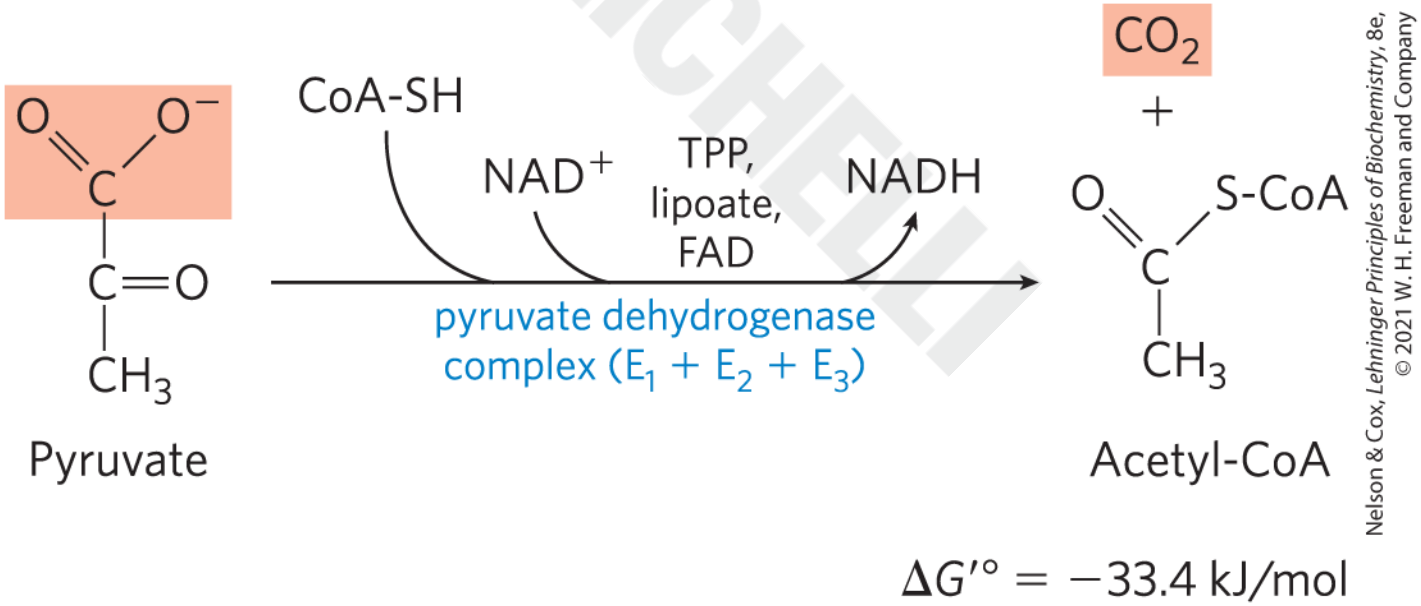
Pyruvate Is Oxidized to Acetyl-CoA and CO_2

- **mitochondrial pyruvate carrier (MPC)** = an H^+-coupled pyruvate-specific symporter in the inner mitochondrial membrane

- **pyruvate dehydrogenase (PDH) complex** = highly ordered cluster of enzymes and cofactors that oxidizes pyruvate in the mitochondrial matrix to acetyl-CoA and CO_2
  - the series of chemical intermediates remain bound to the enzyme subunits
  - regulation results in precisely regulated flux

The PDH Complex Catalyzes an Oxidative Decarboxylation

- **oxidative decarboxylation** = an irreversible oxidation process in which the carboxyl group is removed, forming CO_2



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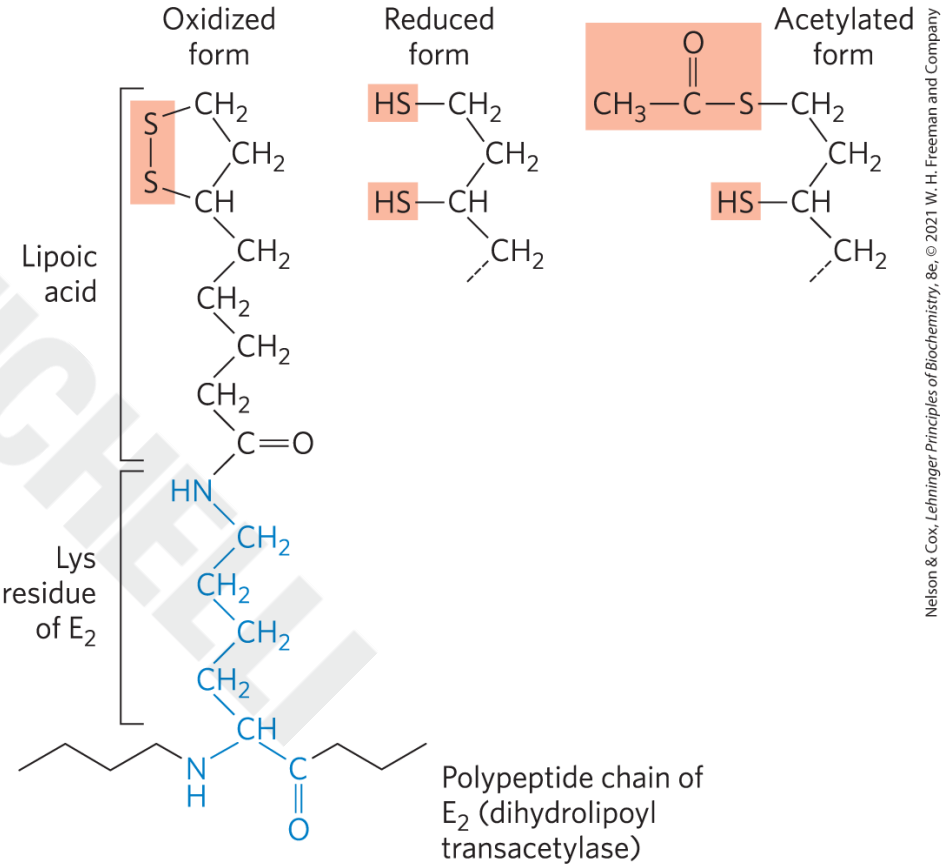
The PDH Complex Employs Three Enzymes and Five Coenzymes to Oxidize Pyruvate

- three enzymes:
  - pyruvate dehydrogenase, E_1
  - dihydrolipoyl transacetylase, E_2
  - dihydrolipoyl dehydrogenase, E_3
- five coenzymes:
  - thiamine pyrophosphate (TPP)
  - lipoate
  - coenzyme A (CoA, CoA-SH)
  - flavin adenine dinucleotide (FAD)
  - nicotinamide adenine dinucleotide (NAD)

Lipoate

- **lipoate** = coenzyme with two thiol groups that can undergo reversible oxidation to a disulfide bond (–S–S–)
  - serves as an electron (hydrogen) carrier and an acyl carrier
  - covalently linked to E_2 via a lysine residue



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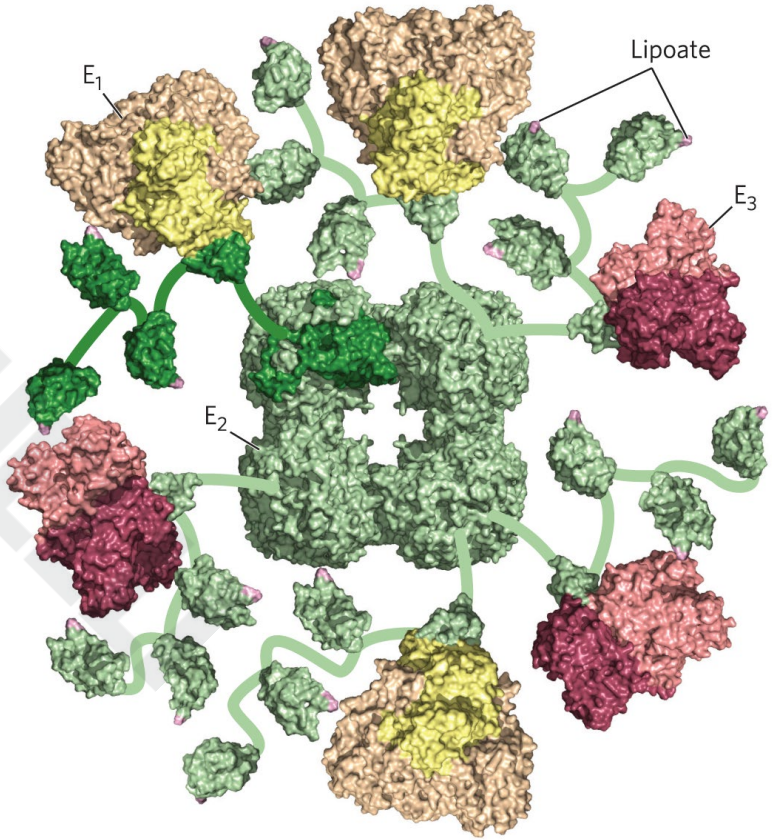
The PDH Complex Enzymes

- the PDH complex contains multiple copies of:
  - **pyruvate dehydrogenase** (E_1)
  - **dihydrolipoyl transacetylase** (E_2)
  - **dihydrolipoyl dehydrogenase** (E_3)



Information from D. Goodsell, doi:10.2210/rcsb_pdb/mom_2012_9. Data from PDB ID 1LAC F. Dardel et al., *J. Mol. Biol.* 229:1037, 1993; PDB ID 1W85 R. A. Frank et al., *Science* 306:872, 2004; PDB ID 1EBD S. S. Mande et al., *Structure* 4:277, 1996.

- an E_2 core (of 24-60 copies) is surrounded by multiple and variable numbers of E_1 and E_3 copies

P5 Principle 5 (3 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

The E_1-E_2-E_3 Structure of the PDH Complex

- the structure of the PDH complex is similar to other enzymes that catalyze oxidations:
  - α-ketoglutarate dehydrogenase
  - branched-chain α-keto acid dehydrogenase

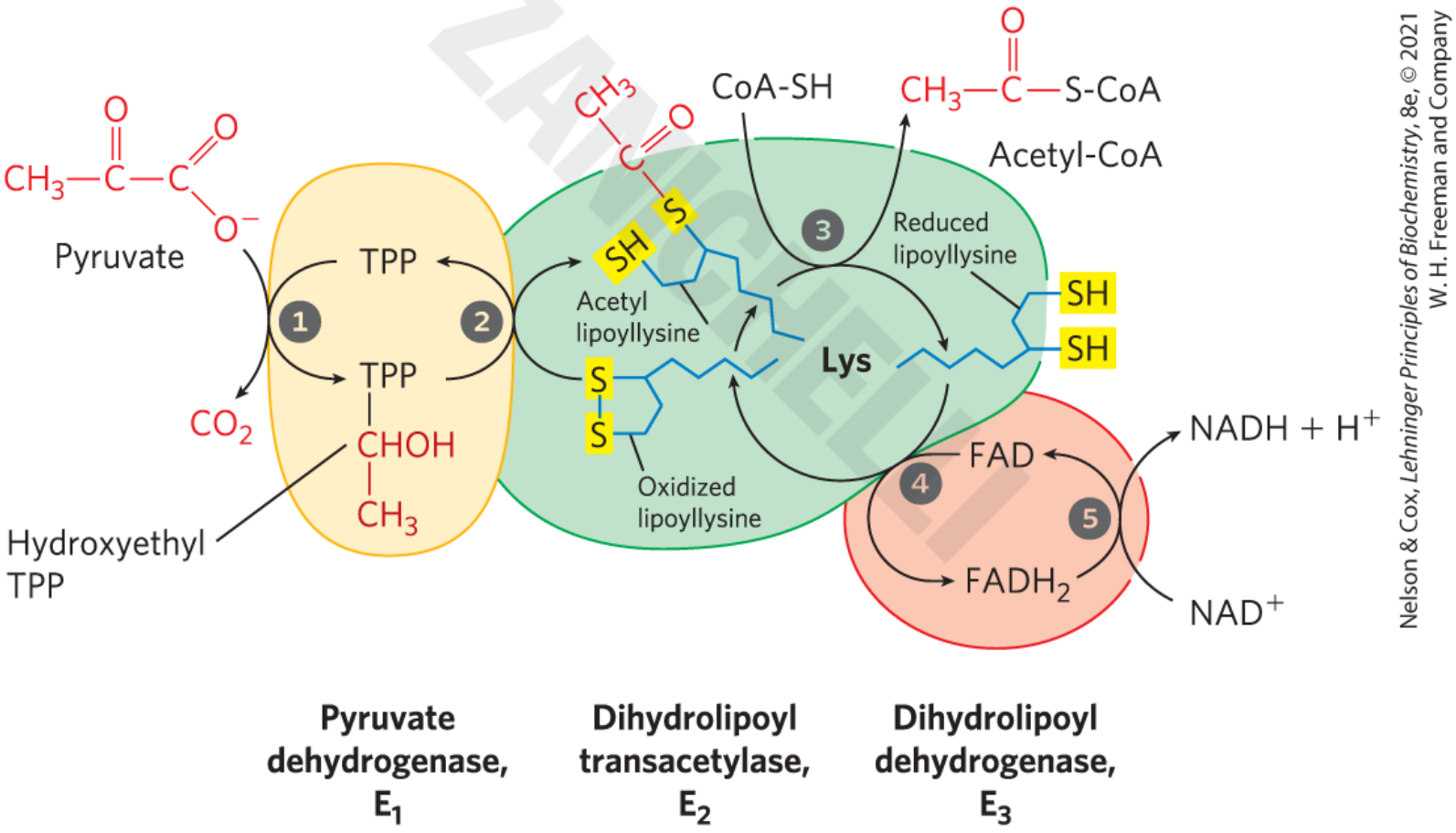
- in a given species, E_3 is identical in all three complexes

- similarities reflect a common evolutionary origin
  - they are paralogs

P5 Principle 5 (4 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

The PDH Complex Channels Its Intermediates through Five Reactions



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Oxidative Decarboxylation of Pyruvate - Steps 1 and 2

- pyruvate dehydrogenase, E_1, with bound TPP catalyzes:
  - step 1: decarboxylation of pyruvate to the hydroethyl derivate
    - rate-limiting step
  - step 2: oxidation of the hydroethyl derivate to an acetyl group
    - electrons and the acetyl group are transferred from TPP to the lipoyllysyl group of E_2

Oxidative Decarboxylation of Pyruvate - Steps 3-5

- dihydrolipoyl transacetylase, E_2, catalyzes:
  - step 3: esterification of the acetyl moiety to one of the lipoyl –SH groups, followed by transesterification to CoA to form acetyl-CoA

- dihydrolipoyl dehydrogenase, E_3, catalyzes:
  - step 4: electron transfer to regenerate the oxidized form of the lipoyllysyl group
  - step 5: electron transfer to regenerate the oxidized FAD cofactor, forming NADH

P5 Principle 5 (5 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

The Five-Reaction Sequence of the PDH Complex Is An Example of Substrate Channeling

- **substrate channeling** = the passage of intermediates from one enzyme directly to another enzyme without release

- the long lipoyllysyl arm of E_2 channels the substrate from the active site of E_1 to E_2 to E_3
  - tethers intermediates to the enzyme complex
  - increases the efficiency of the overall reaction
  - minimizes side reactions

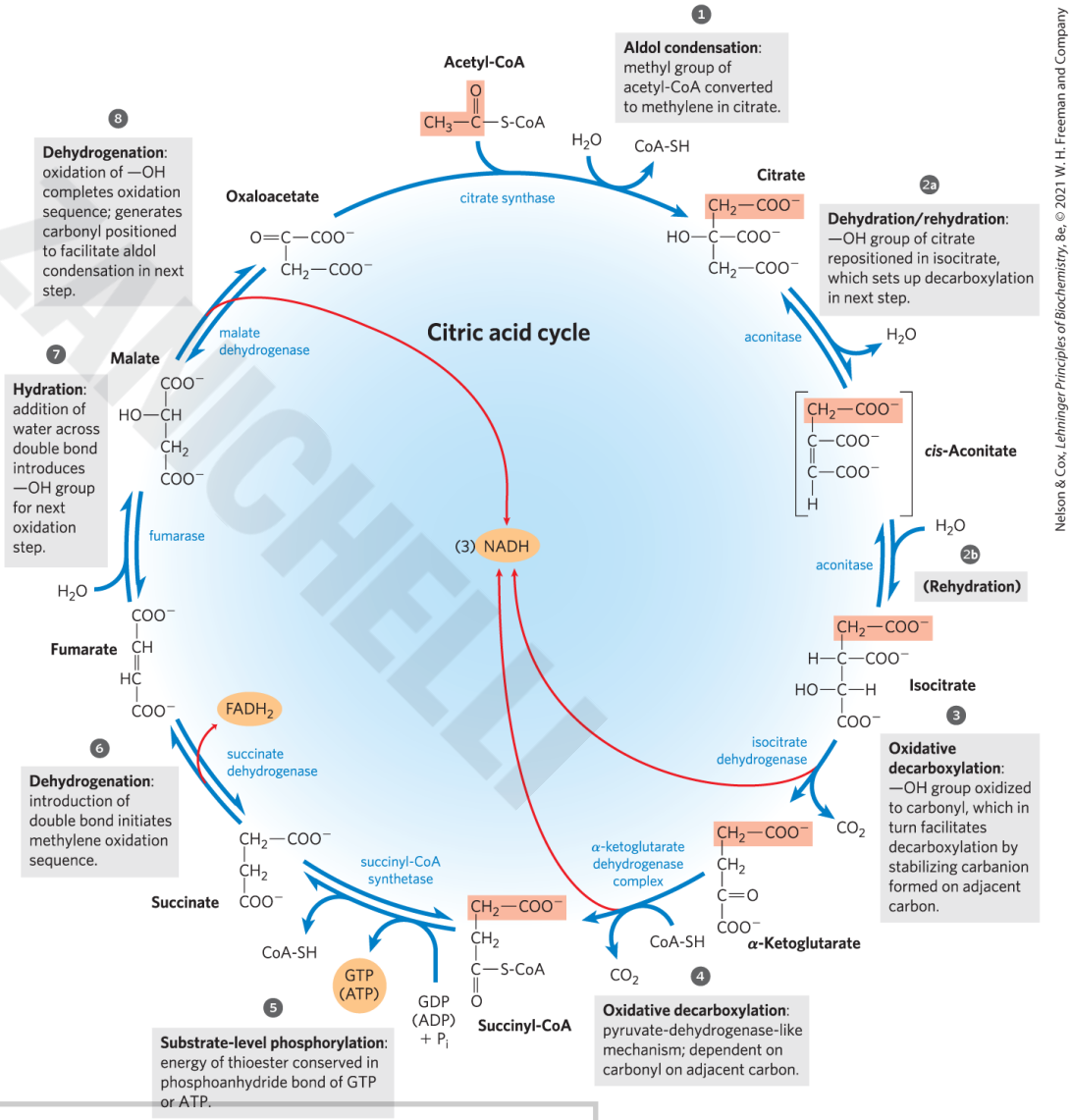
16.2 Reactions of the Citric Acid Cycle

P2 Principle 2 (2 of 7)

The reactions of the citric acid cycle follow a chemical logic. In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO_2 and H_2O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways.

Reactions of the Citric Acid Cycle

- one oxaloacetate molecule can theoretically oxidize an infinite number of acetyl groups
- energy from the four oxidations is conserved as NADH and $FADH_2$



In Eukaryotes, the Mitochondrion Is the Site of Energy-Yielding Oxidative Reactions and ATP Synthesis

- isolated mitochondria contain all enzyme, coenzymes, and proteins needed for:
  - the citric acid cycle
  - electron transfer and ATP synthesis by oxidative phosphorylation
  - oxidation of fatty acids and amino acids to acetyl-CoA
  - oxidative degradation of amino acids to citric acid cycle intermediates

P2 Principle 2 (3 of 7)

The reactions of the citric acid cycle follow a chemical logic. In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO_2 and H_2O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways.

The Sequence of Reactions in the Citric Acid Cycle Makes Chemical Sense

- complete oxidation of acetyl-CoA to CO_2 extracts the maximum potential energy
- direct oxidation to yield CO_2 and CH_4 is not biochemically feasible because organisms cannot oxidize CH_4

P2 Principle 2 (4 of 7)

The reactions of the citric acid cycle follow a chemical logic. In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO_2 and H_2O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways.

The Chemical Logic of the Citric Acid Cycle

- carbonyl groups are more chemically reactive than a methylene group or methane

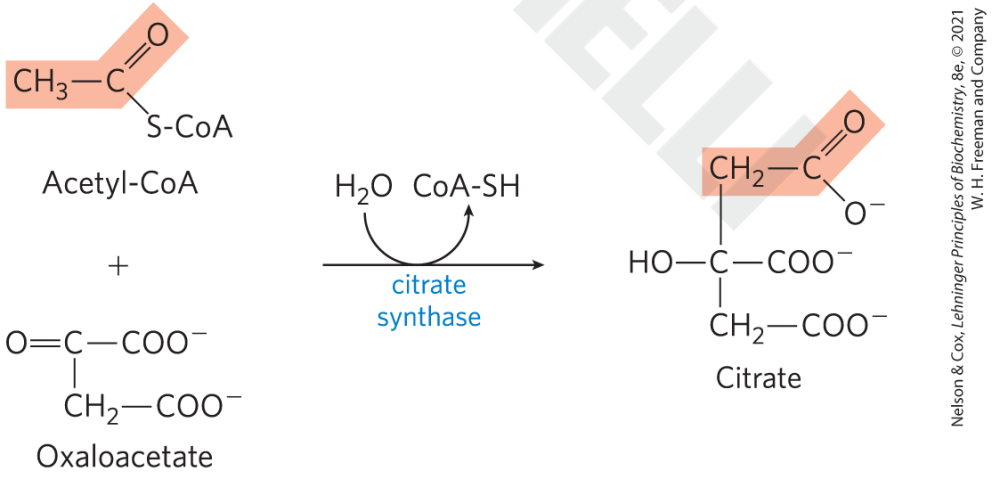
- each step of the cycle involves either:
  - an energy-conserving oxidation
  - placing functional groups in position to facilitate oxidation or oxidative decarboxylation

The Citric Acid Cycle Has Eight Steps

- citrate formed from acetyl-CoA and oxaloacetate is oxidized to yield:
  - CO_2
  - NADH
  - $FADH_2$
  - GTP or ATP

Formation of Citrate

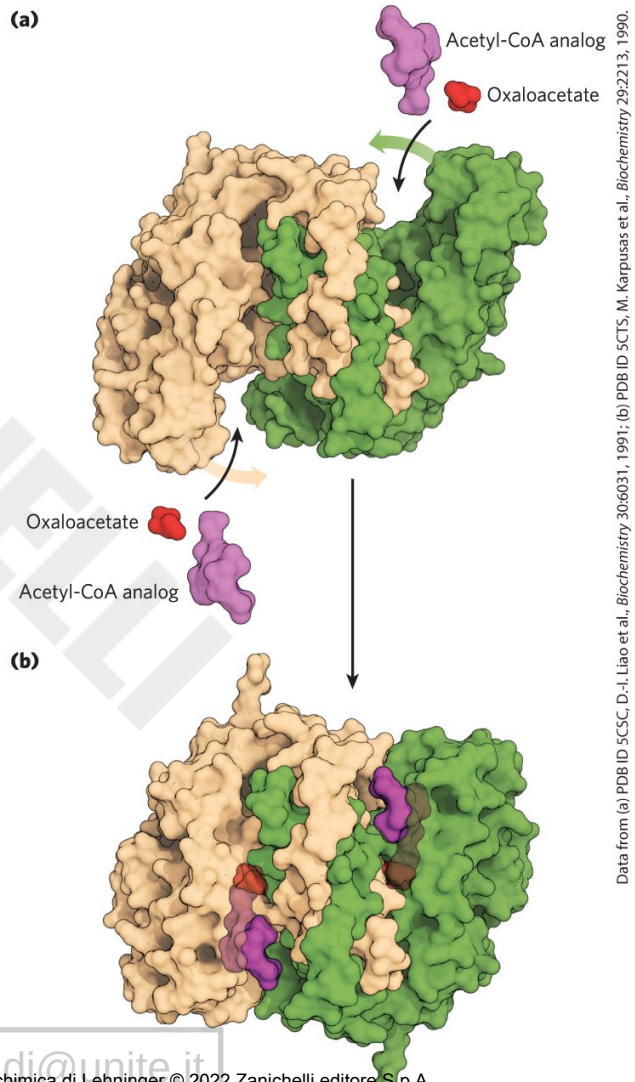
- **citrate synthase** = catalyzes the condensation of acetyl-CoA with **oxaloacetate** to form **citrate**
  - involves the formation of a transient intermediate, citroyl-CoA
  - large, negative $\Delta G'°$ is needed because [oxaloacetate] is normally very low



$\Delta G'° = -32.2$ kJ/mol

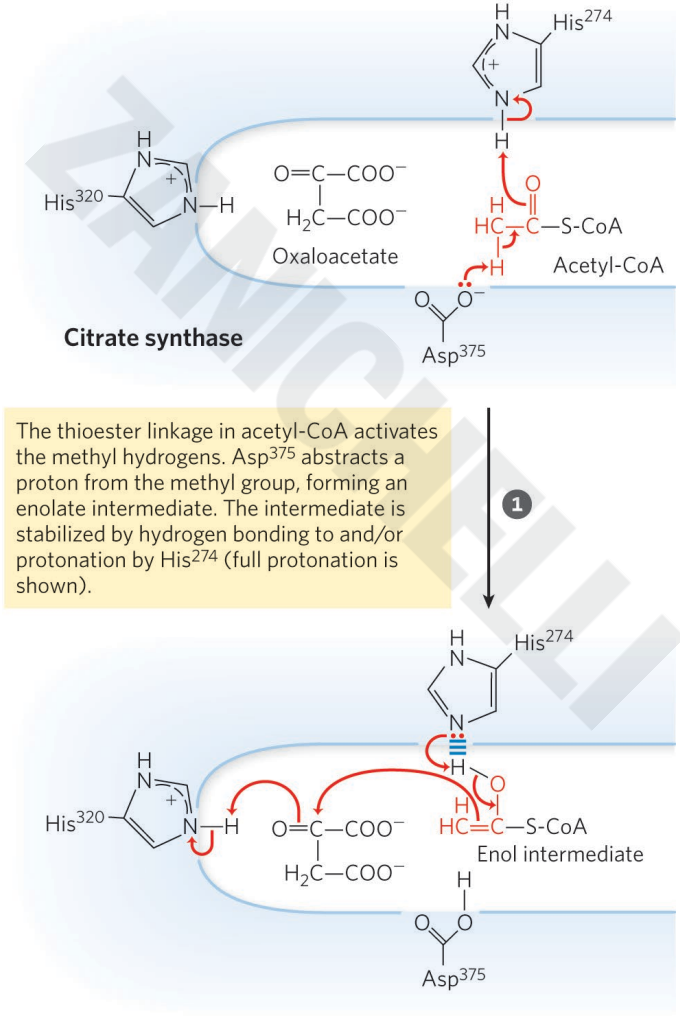
Structure of Citrate Synthase

- binding of oxaloacetate creates a binding site for acetyl-CoA
- induced fit decreases the likelihood of premature cleavage of the thioester bond of acetyl-CoA



Data from (a) PDB ID 5CSC, D.-I. Liao et al., *Biochemistry* 30:6031, 1991; (b) PDB ID 5CTS, M. Karpusas et al., *Biochemistry* 29:2213, 1990.

Mechanism of Citrate Synthase: Acid/Base Catalysis



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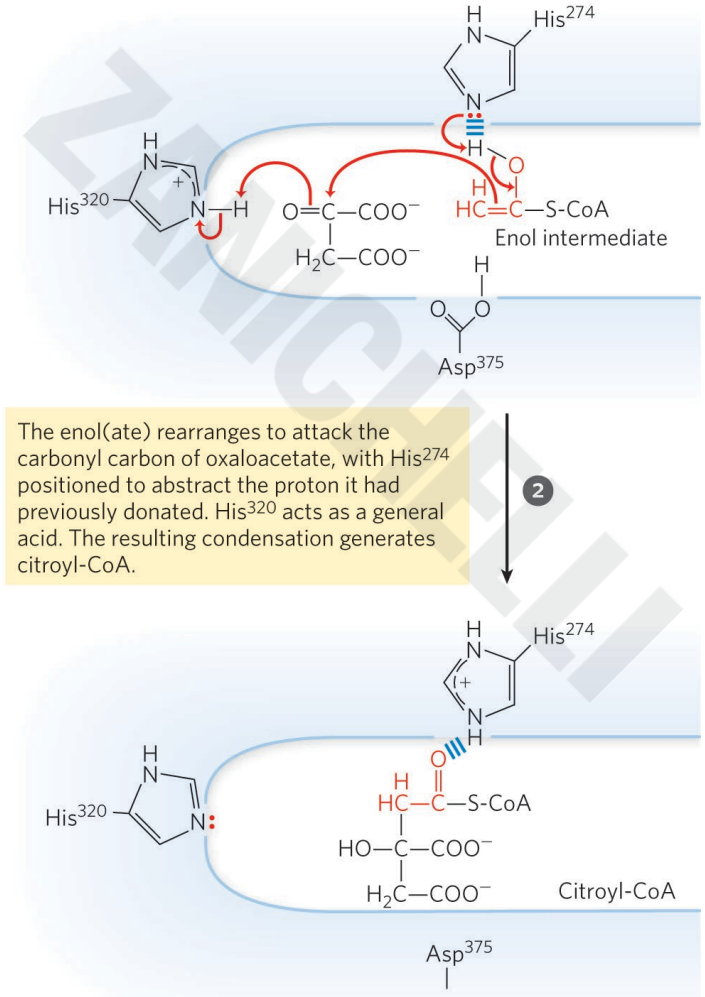
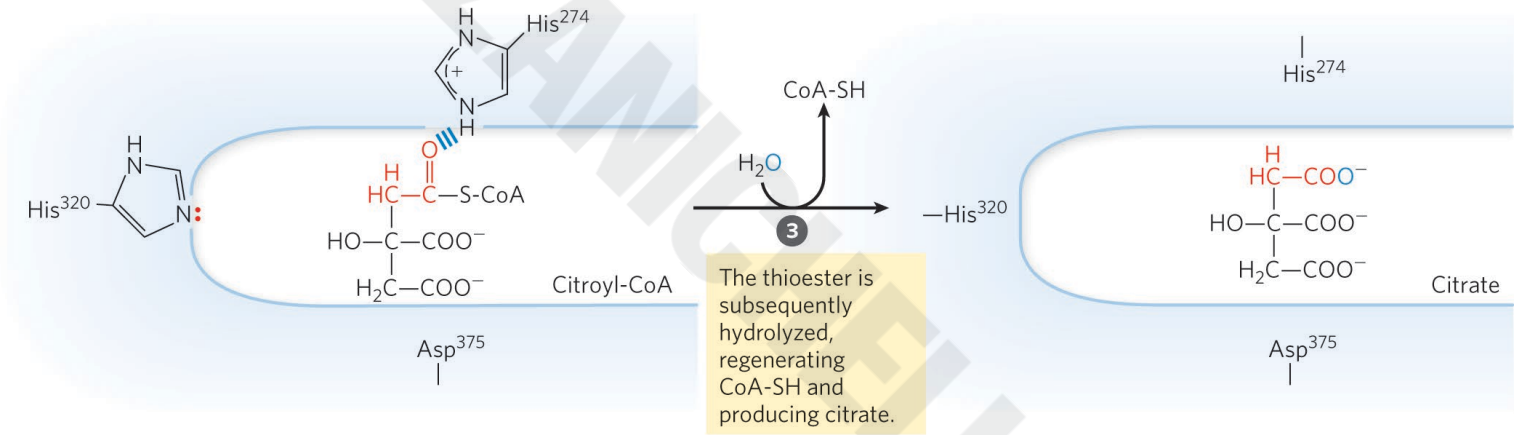
Mechanism of Citrate Synthase: Acid/Base Catalysis, Continued



Mechanism of Citrate Synthase: Thioester Hydrolysis



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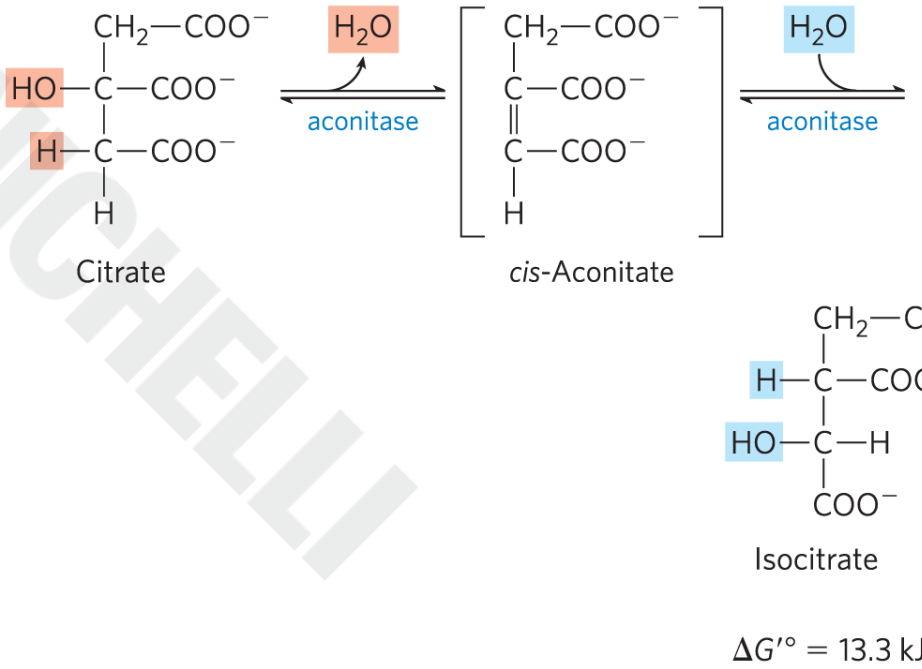
Formation of Isocitrate via *Cis*-Aconitate

- **aconitase (aconitate hydratase)** = catalyzes the reversible transformation of citrate to **isocitrate** through the intermediate ***cis*-aconitate**
  - addition of H_2O to *cis*-aconitate is stereospecific
  - low [isocitrate] pulls the reaction forward



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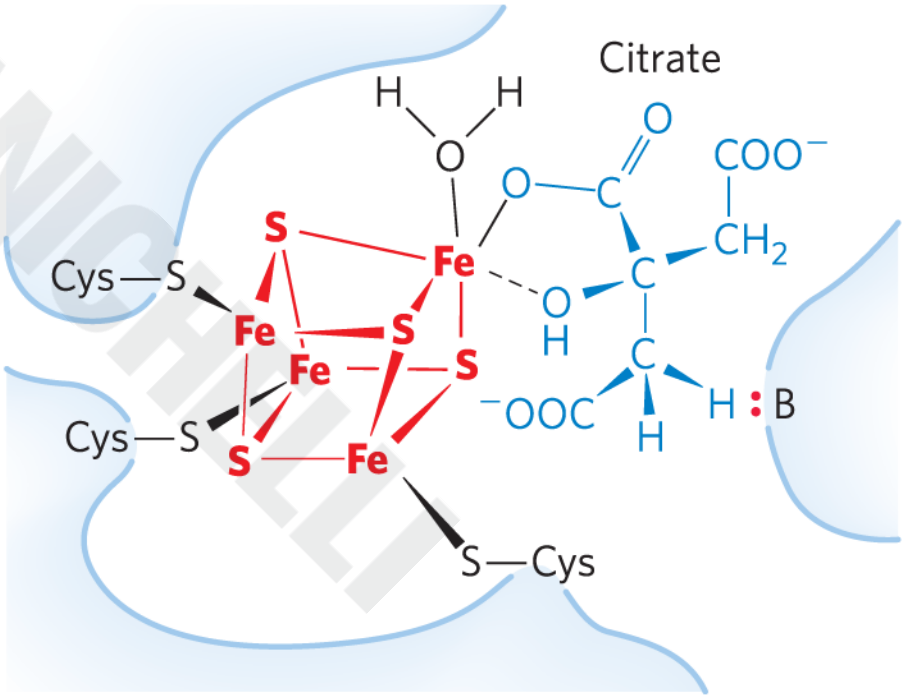
Iron-Sulfur Center in Aconitase

- **iron-sulfur center** = acts both in the binding of the substrate to the active site and in the catalytic addition or removal of H_2O



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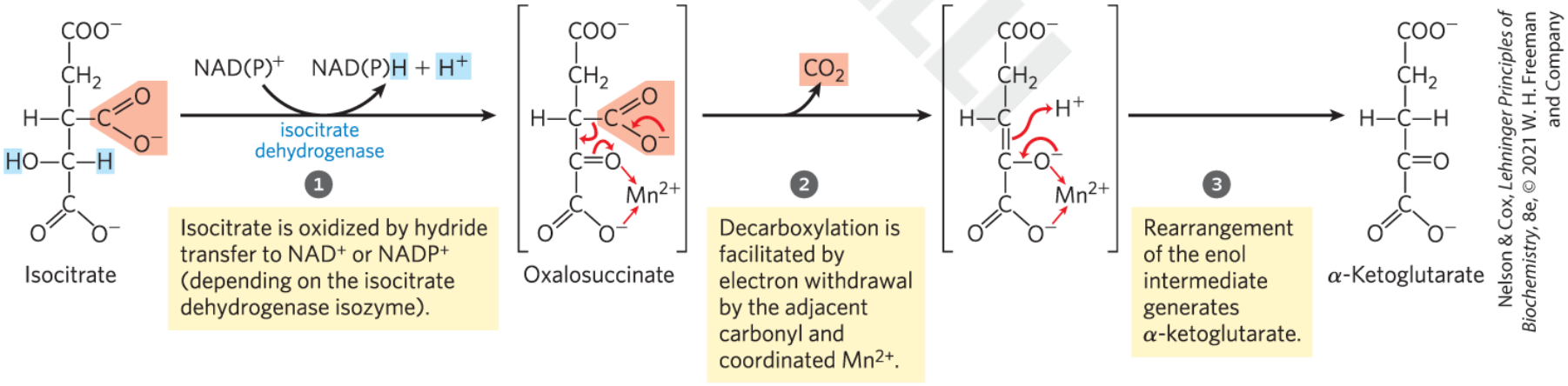
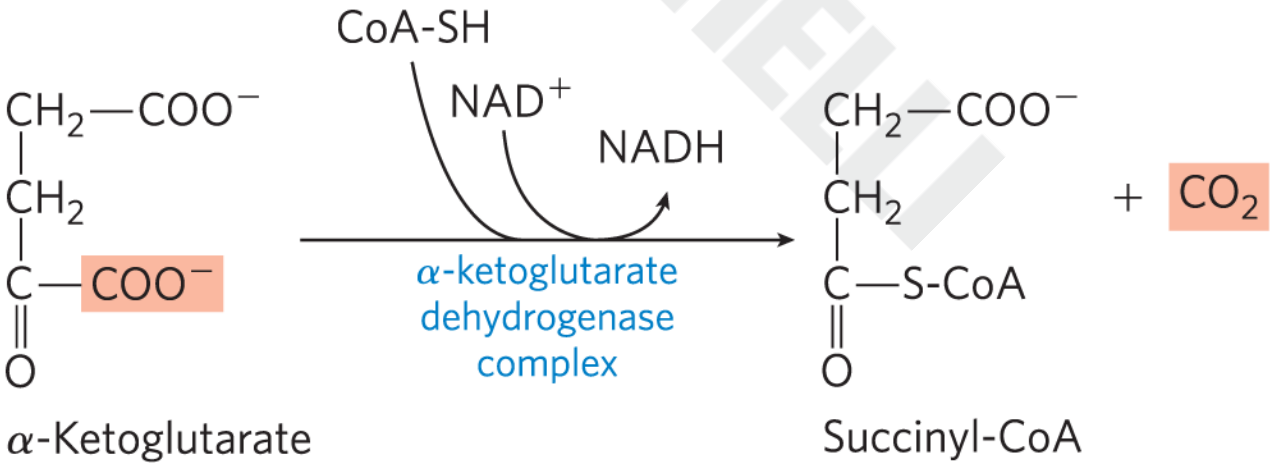
Oxidation of Isocitrate to α-Ketoglutarate and CO_2

- **isocitrate dehydrogenase** = catalyzes the oxidative decarboxylation of isocitrate to α-ketoglutarate
  - Mn^{2+} interacts with the carbonyl group of the oxalosuccinate and stabilizes the transiently-formed enol
  - specific isozymes for $NADP^+$ (cytosolic and mitochondrial) or NAD^+ (mitochondrial)



Oxidation of α-Ketoglutarate to Succinyl-CoA and CO_2

- **α-ketoglutarate dehydrogenase complex** = catalyzes the oxidative decarboxylation of α-ketoglutarate to **succinyl-CoA** and CO_2
  - energy of oxidation is conserved in the thioester bond of succinyl-CoA



$\Delta G'° = -33.5$ kJ/mol

P2 Principle 2 (5 of 7)

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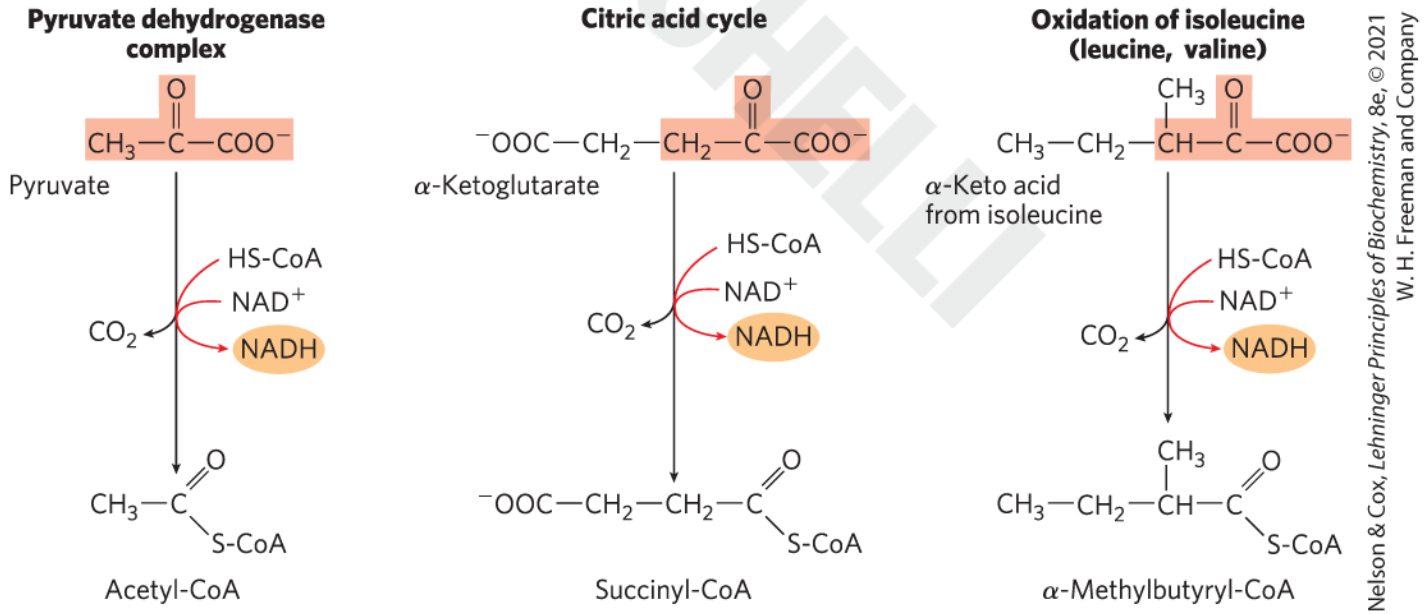
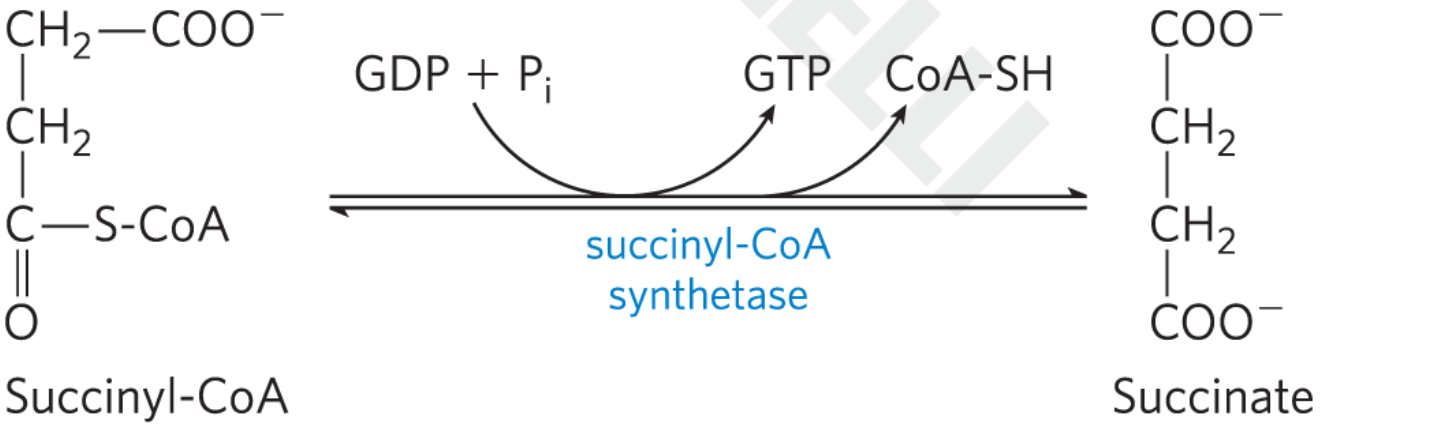
A Conserved Mechanism for Oxidative Decarboxylation

- pathways use the same five cofactors, similar multienzyme complexes, and the same enzymatic mechanism
  - have homologous E_1 and E_2 and identical E_3
  - example of gene duplication and **divergent evolution**



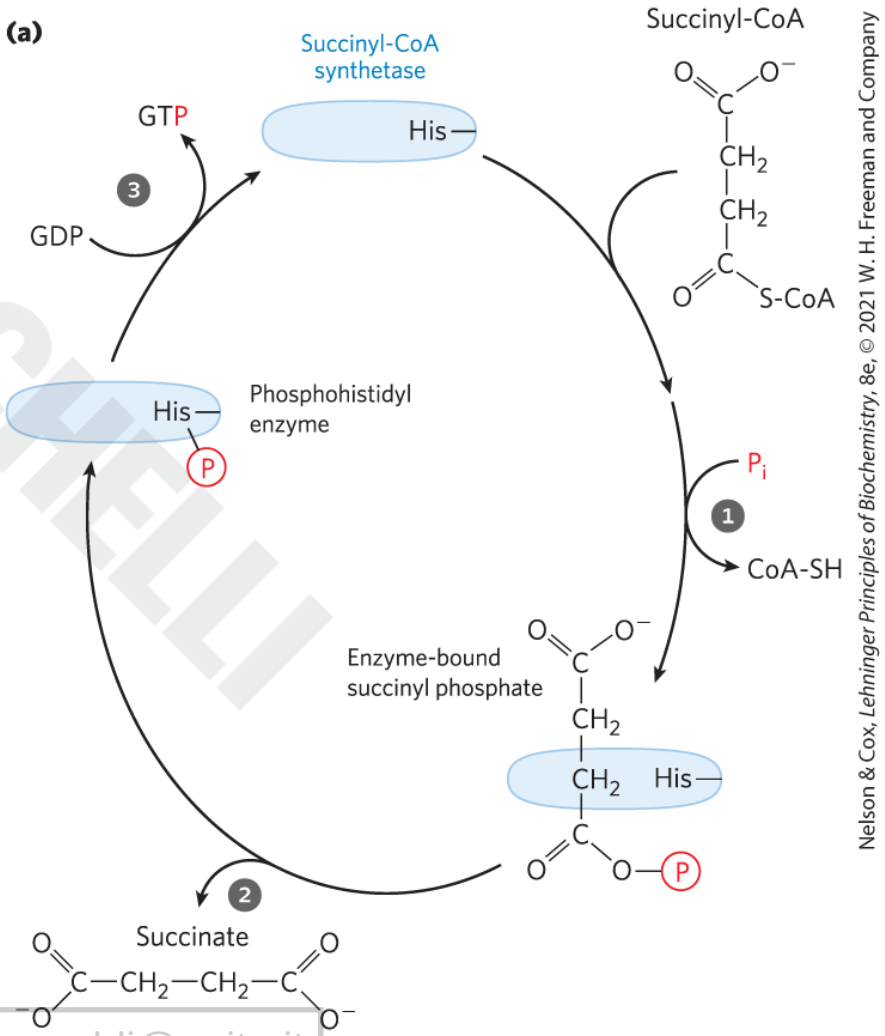
Conversion of Succinyl-CoA to Succinate

- **succinyl-CoA synthetase (succinic thiokinase)** = catalyzes the breakage of the thioester bond of succinyl-CoA to form **succinate**
  - energy released drives the synthesis of a phosphoanhydride bond in either GTP or ATP (substrate level phosphorylation)



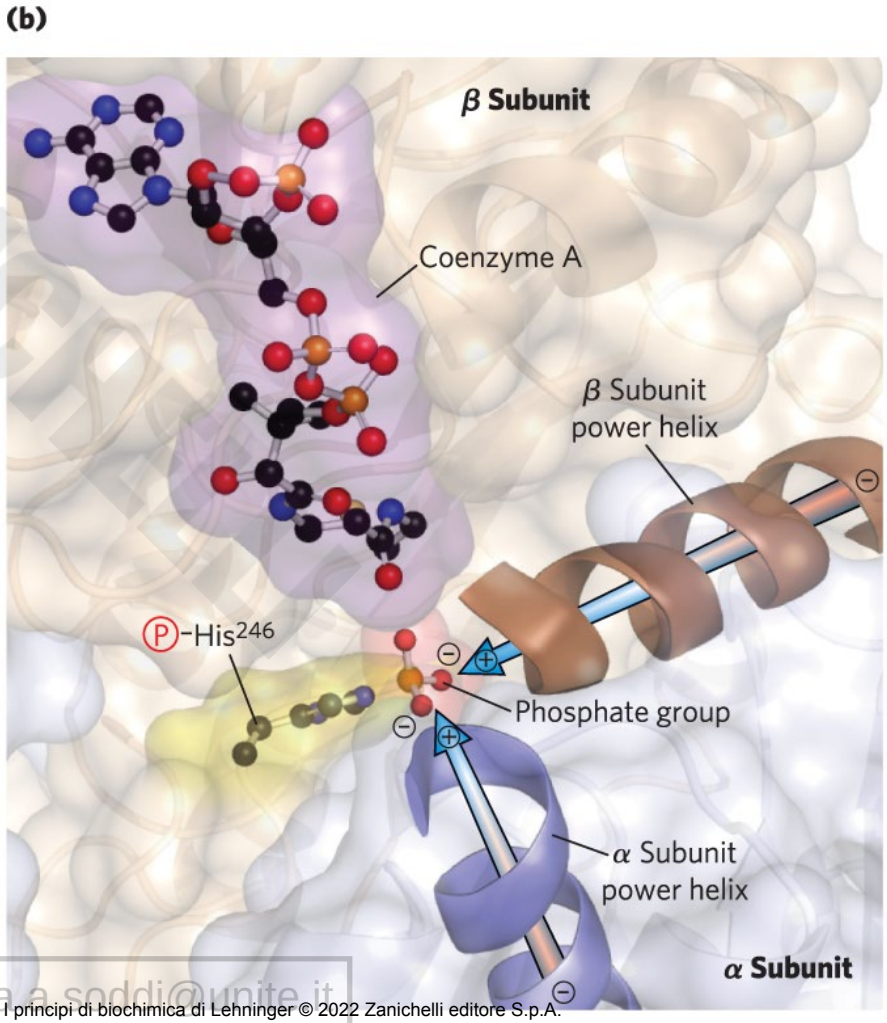
The Succinyl-CoA Synthetase Reaction (1 of 2)

- enzyme molecule becomes phosphorylated at a His residue in the active site
- phosphoryl group is then transferred to ADP or GDP to form ATP or GTP
  - animal cells have specific isozymes for ADP and GDP



The Succinyl-CoA Synthetase Reaction (2 of 2)

- power helices place the partial positive charges of the helix dipole near the phosphate group of the α chain phosphorylated His^{246} to stabilize the phosphoenzyme intermediate



Nucleoside Diphosphate Kinase

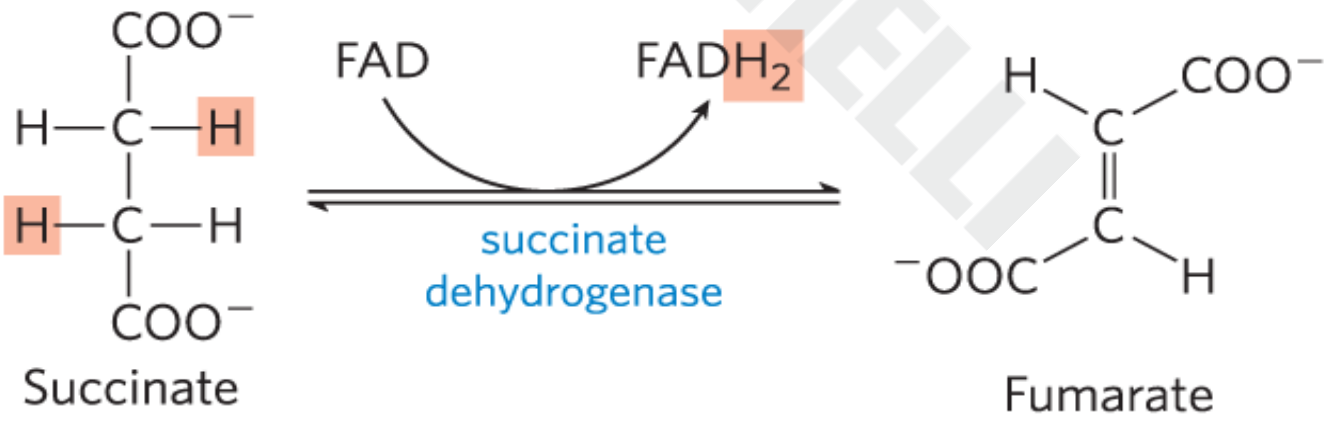
- **nucleoside diphosphate kinase** = catalyzes the reversible conversion of GTP and ATP

$$GTP + ADP \rightleftharpoons GDP + ATP \qquad \Delta G'^{\circ} = 0 \text{ kJ/mol}$$

- net result of the activity of either isozyme of succinyl-CoA synthetase is the conservation of energy as ATP

Oxidation of Succinate to Fumarate

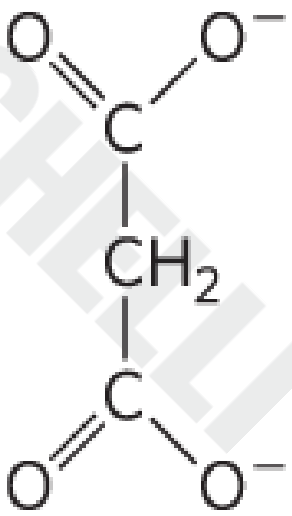
- **succinate dehydrogenase** = flavoprotein that catalyzes the reversible oxidation of succinate to **fumarate**
  - integral protein of the mitochondrial inner membrane in eukaryotes
  - contains three iron-sulfur clusters and covalently bound FAD



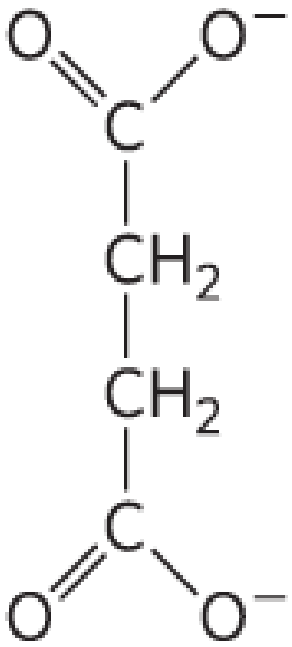
$\Delta G'° = 0$ kJ/mol

Malonate Is a Strong Competitive Inhibitor of Succinate Dehydrogenase

- malonate = an analog of succinate
  - not normally present in cells
  - addition to mitochondria in vitro blocks citric acid cycle activity



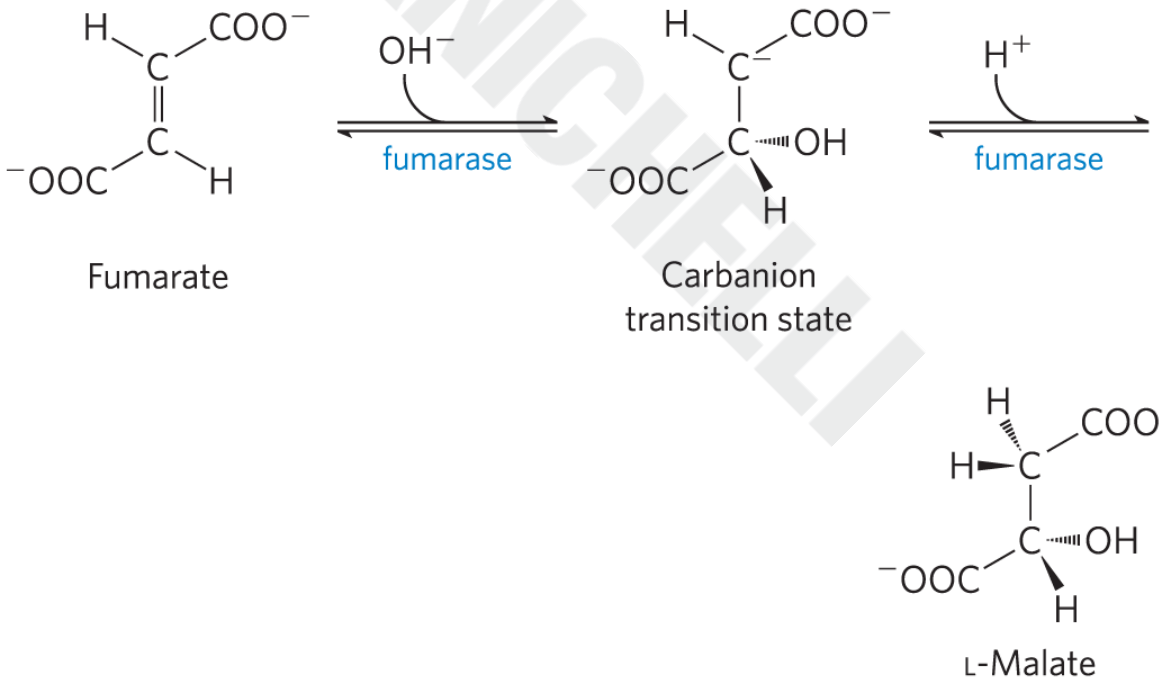
Malonate



Succinate

Hydration of Fumarate to Malate

- **fumarase** = catalyzes the reversible hydration of fumarate to **L-malate**
  - transition state is a carbanion

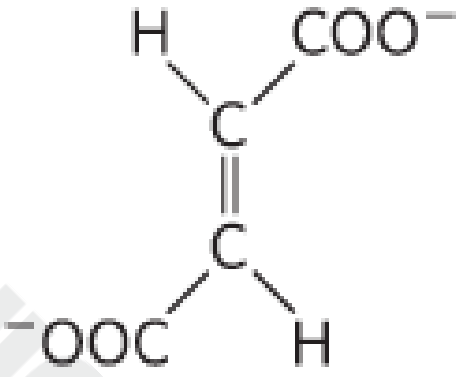


$\Delta G'° = -3.8$ kJ/mol

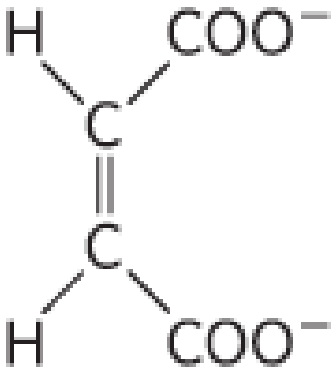
Fumarase Is Highly Stereospecific

- in the forward direction, fumarase catalyzes hydration of the trans double bond of fumarate but not the cis double bond of maleate

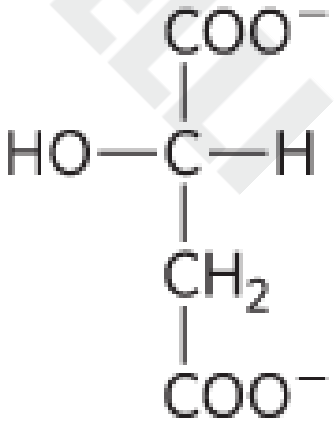
- in the reverse direction, fumarase is equally stereospecific



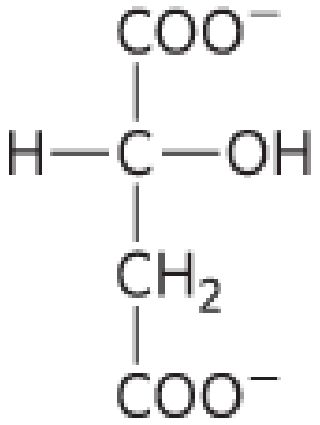
Fumarate



Maleate



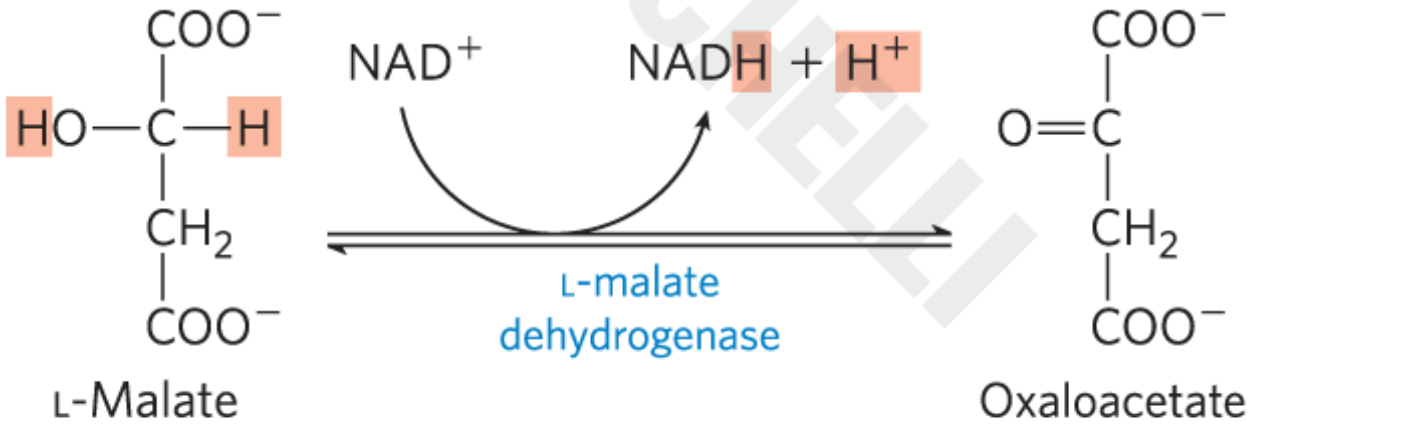
L-Malate



D-Malate

Oxidation of Malate to Oxaloacetate

- **L-malate dehydrogenase** = catalyzes the oxidation of **L-malate** to oxaloacetate, coupled to the reduction of NAD^+
  - low [oxaloacetate] pulls the reaction forward
  - regenerates oxaloacetate for citrate synthesis



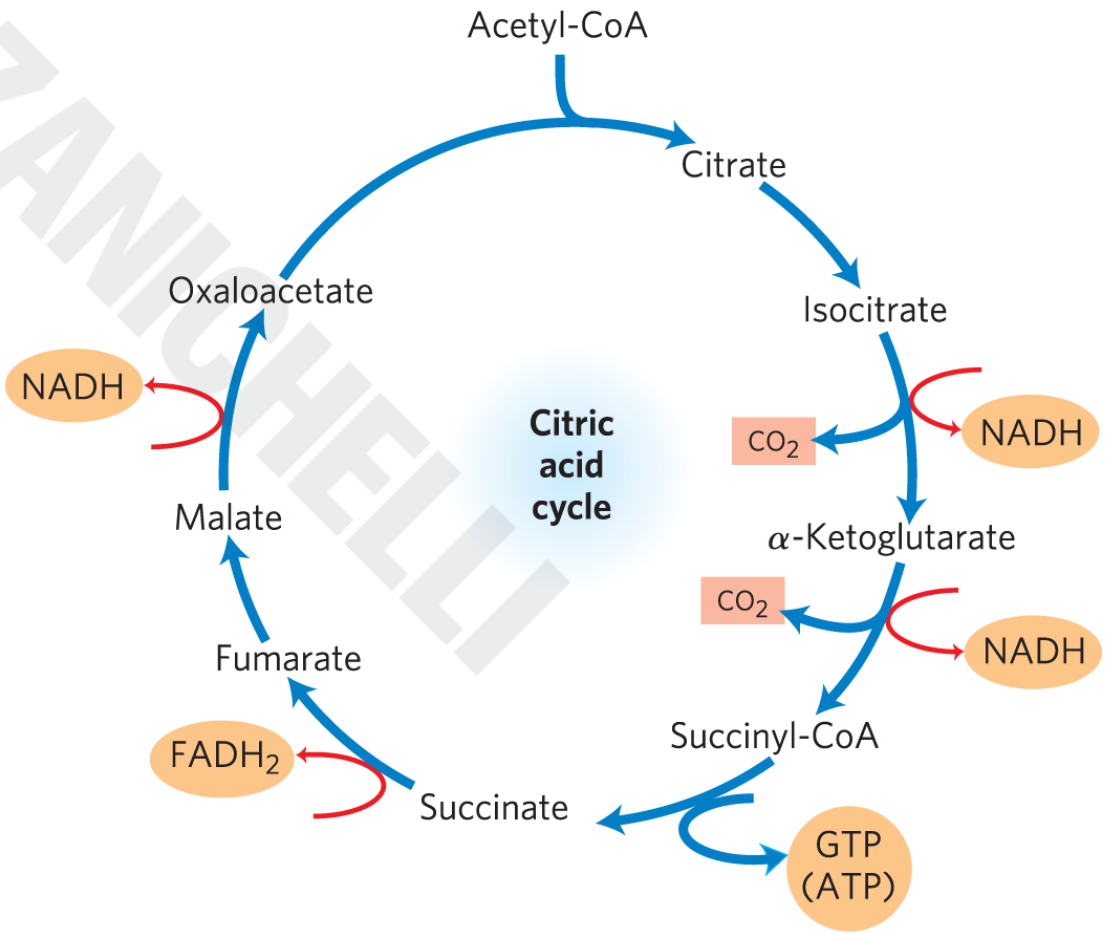
$\Delta G'° = 29.7$ kJ/mol

P2 Principle 2 (6 of 7)

The reactions of the citric acid cycle follow a chemical logic. In its catabolic role, the citric acid cycle oxidizes acetyl-CoA to CO_2 and H_2O. Energy from the oxidations in the cycle drives the synthesis of ATP. The chemical strategies for activating groups for oxidation and for conserving energy in the form of reducing power and high-energy compounds are used in many other biochemical pathways.

The Energy of Oxidations in the Cycle Is Efficiently Conserved

- energy released by oxidation is conserved in the production of:
  - 3 NADH
  - 1 $FADH_2$
  - 1 GTP (or ATP)



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P2 Principle 2 (7 of 7)

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Electrons from NADH and $FADH_2$ Enter the Respiratory Chain

- the citric acid cycle directly generates only one ATP per turn

- the large flow of electrons into the respiratory chain via NADH and $FADH_2$ leads to formation of almost 10 times more ATP during oxidative phosphorylation
  - each NADH drives formation of ~2.5 ATP
  - each $FADH_2$ drives formation of ~1.5 ATP

Stoichiometry of Coenzyme Reduction and ATP Formation in Aerobic Oxidation of Glucose

Table 16-1 Stoichiometry of Coenzyme Reduction and ATP Formation in the Aerobic Oxidation of Glucose via Glycolysis, the Pyruvate Dehydrogenase Complex Reaction, the Citric Acid Cycle, and Oxidative Phosphorylation

Reaction	Number of ATP or reduced coenzyme directly formed	Number of ATP ultimately formed
Glucose → glucose 6-phosphate	−1 ATP	−1
Fructose 6-phosphate → fructose 1,6-bisphosphate	−1 ATP	−1
2 Glyceraldehyde 3-phosphate → 2 1,3-biphosphoglycerate	2 NADH	3 or 5
2 1,3-Bisphosphoglycerate → 2 3-phosphoglycerate	2 ATP	2
2 Phosphoenolpyruvate → 2 pyruvate	2 ATP	2
2 Pyruvate → 2 acetyl-CoA	2 NADH	5
2 Isocitrate → 2 α-ketoglutarate	2 NADH	5
2 α-Ketoglutarate → 2 succinyl-CoA	2 NADH	5
2 Succinyl-CoA → 2 succinate	2 ATP (or 2 GTP)	2
2 Succinate → 2 fumarate	2 $FADH_2$	3
2 Malate → 2 oxaloacetate	2 NADH	5
Total		30-32

16.3 The Hub of Intermediary Metabolism

P3 Principle 3 (2 of 4)

The citric acid cycle is a hub of metabolism, with catabolic pathways leading in and anabolic pathways leading out. Acetate groups (acetyl-CoA) from the catabolism of various fuels are used in the synthesis of such metabolites as amino acids, fatty acids, and sterols. The breakdown products of many amino acids and nucleotides are intermediates of the cycle, and they can be fed in or siphoned off as needed by the cell.

The Citric Acid Cycle Accepts Carbon Skeletons

- the cycle accepts 3-, 4-, and 5-carbon skeletons

- the **breakdown of amino acids** yields carbon skeletons:
  - deaminated aspartate yields oxaloacetate
  - deaminated glutamate yields *α*-ketoglutarate

P3 Principle 3 (3 of 4)

The citric acid cycle is a hub of metabolism, with catabolic pathways leading in and anabolic pathways leading out. Acetate groups (acetyl-CoA) from the catabolism of various fuels are used in the synthesis of such metabolites as amino acids, fatty acids, and sterols. The breakdown products of many amino acids and nucleotides are intermediates of the cycle, and they can be fed in or siphoned off as needed by the cell.

The Citric Acid Cycle Serves in Both Catabolic and Anabolic Processes

- **amphibolic pathway** = one that serves in both catabolic and anabolic processes

- animals cannot convert acetate or acetyl-CoA to glucose
  - in the citric acid cycle, there is no *net* conversion of acetate to oxaloacetate

- **glyoxylate cycle** = reaction sequence that converts acetate to carbohydrate
  - present in bacteria, plants, fungi, and protists

P3 Principle 3 (4 of 4)

The citric acid cycle is a hub of metabolism, with catabolic pathways leading in and anabolic pathways leading out. Acetate groups (acetyl-CoA) from the catabolism of various fuels are used in the synthesis of such metabolites as amino acids, fatty acids, and sterols. The breakdown products of many amino acids and nucleotides are intermediates of the cycle, and they can be fed in or siphoned off as needed by the cell.

Anaplerotic Reactions Replenish Citric Acid Cycle Intermediates

- when intermediates are shunted from the citric acid cycle to other pathways, they are replenished
- **anaplerotic reactions** = chemical reactions that replenish intermediates

TABLE 16-2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney
$\text{Phosphoenolpyruvate} + CO_2 + \text{GDP} \xrightleftharpoons{\text{PEP carboxykinase}} \text{oxaloacetate} + \text{GTP}$	Heart, skeletal muscle
$\text{Phosphoenolpyruvate} + HCO_3^- \xrightleftharpoons{\text{PEP carboxylase}} \text{oxaloacetate} + P_i$	Higher plants, yeast, bacteria
$\text{Pyruvate} + HCO_3^- + \text{NAD(P)H} \xrightleftharpoons{\text{malic enzyme}} \text{malate} + \text{NAD(P)}^+$	Widely distributed in eukaryotes and bacteria

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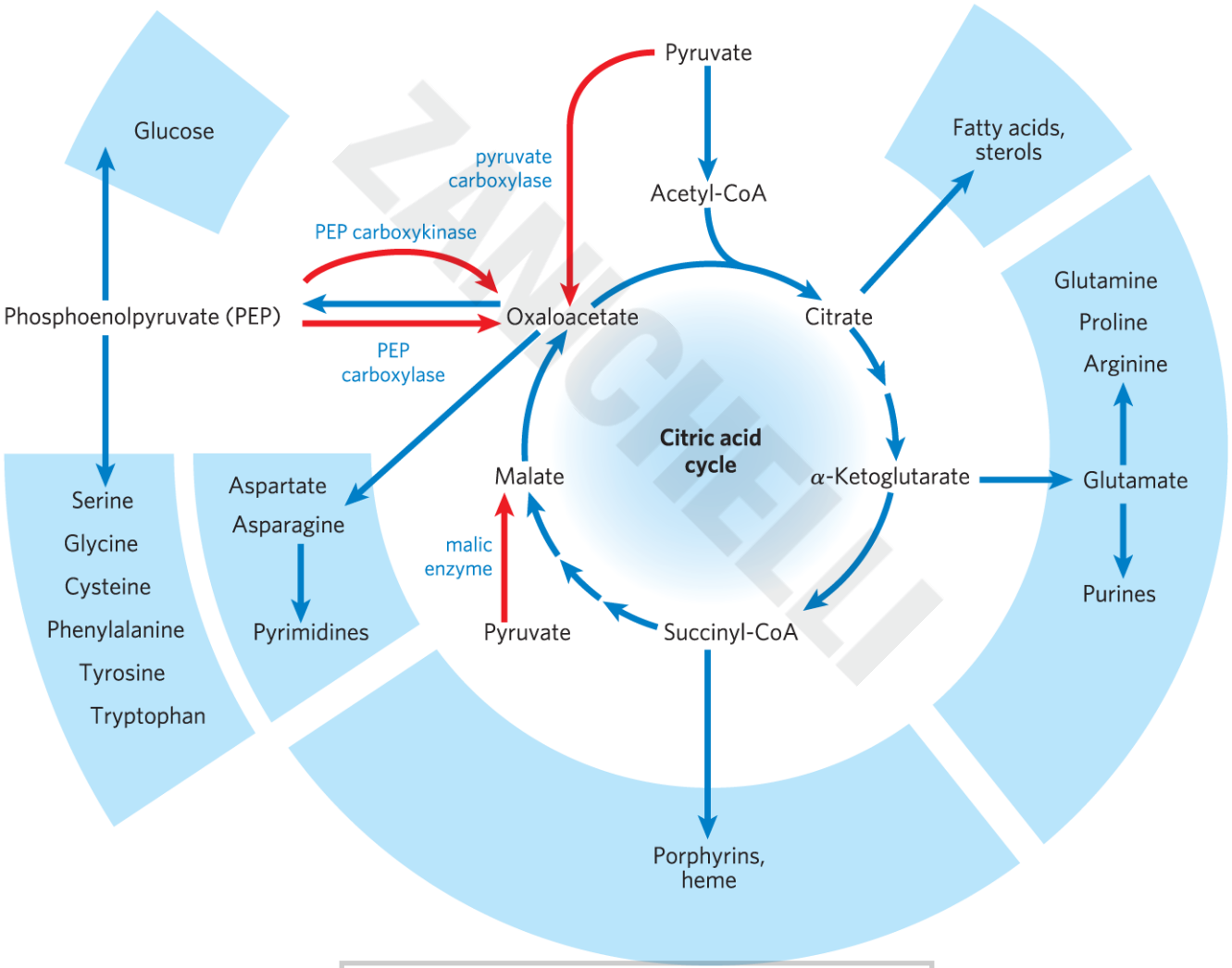
Role of the Citric Acid Cycle in Anabolism



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Pyruvate Carboxylase

- **pyruvate carboxylase** = catalyzes the reversible carboxylation of pyruvate by HCO_3^- to form oxaloacetate
  - most important anaplerotic reaction in mammalian liver, kidney, and brown adipose tissue
  - requires energy from ATP
  - allosterically activated by acetyl-CoA

Biotin in Pyruvate Carboxylase Carries One-Carbon (CO_2) Groups

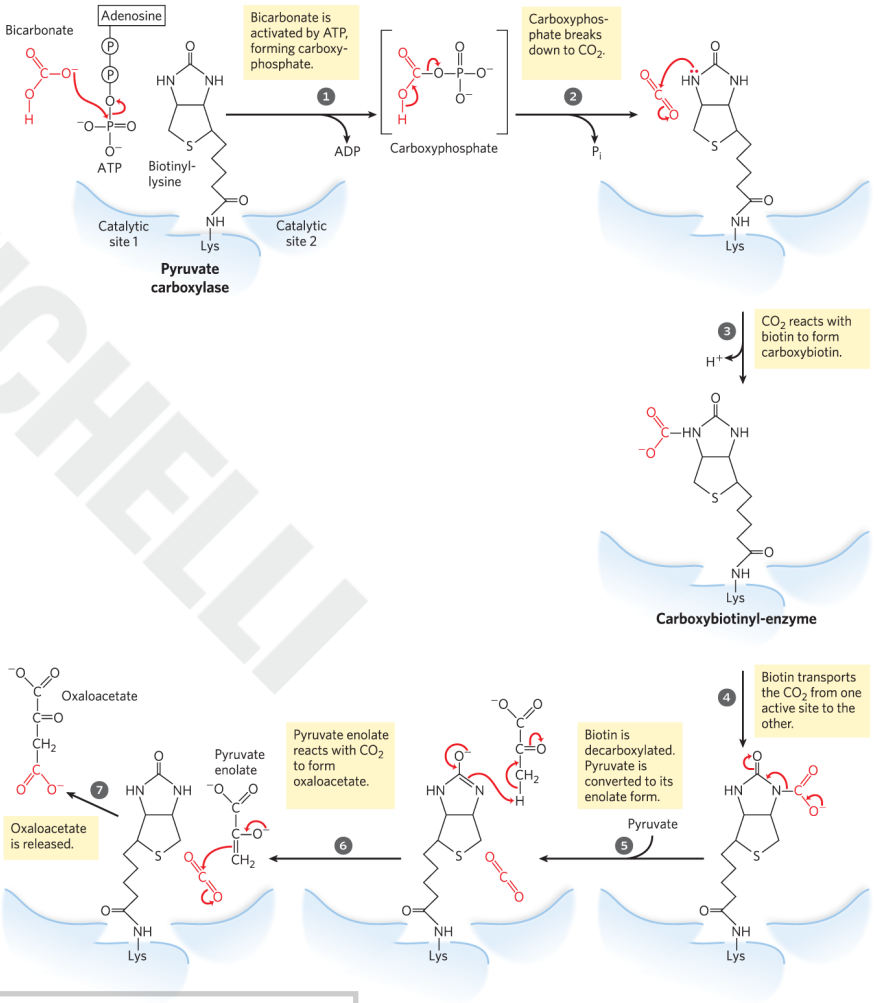
- **biotin** = vitamin that acts as a specialized carrier of one-carbon groups as CO_2 in many carboxylation reactions
  - serves as the prosthetic group of pyruvate carboxylase

P5 Principle 5 (6 of 6)

Enzymes have evolved to form complexes to efficiently achieve a series of chemical transformations without releasing the intermediates into the bulk solvent. This strategy, seen in the pyruvate dehydrogenase complex and the metabolons of the citric acid cycle, is ubiquitous in other pathways of metabolism, in respiration, and in the many “ — somes” that assemble and disassemble informational macromolecules.

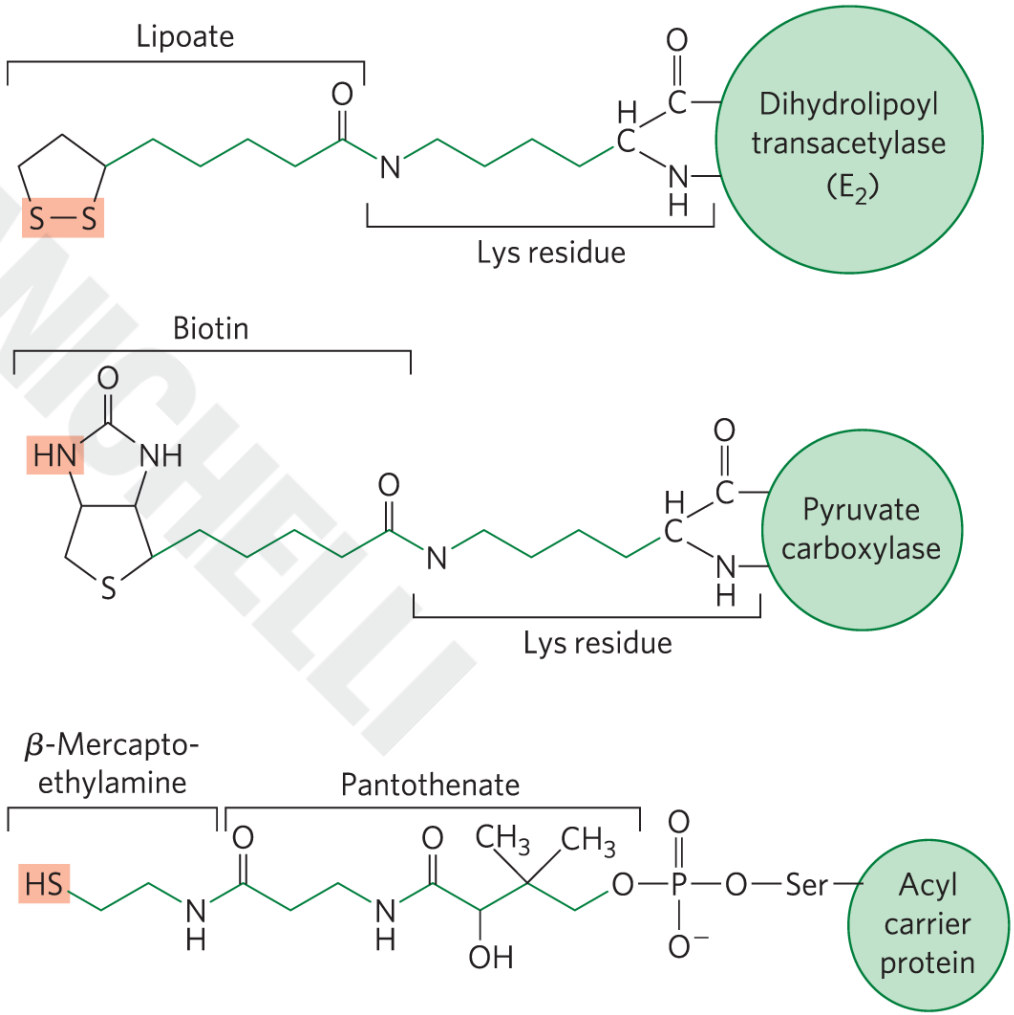
The Role of Biotin in the Pyruvate Carboxylase Reaction

- the two steps in carboxylation of pyruvate occur at separate active sites
  - the arm of biotin transfers activated carboxyl groups from the first active site to the second



Biological Tethers Allow Flexibility

- all participate in substrate channeling through the flexible tethers that move intermediates from one active site to the next



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16.4 Regulation of the Citric Acid Cycle

P4 Principle 4 (3 of 5)

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis. Some mutations that affect the reactions of the citric acid cycle lead to tumor formation.

Citric Acid Cycle Regulation

- regulation balances the supply of key intermediates with the demands of energy production and biosynthetic processes

- regulation occurs at several points:
  - PDH complex
  - citrate synthase
  - isocitrate dehydrogenase complex
  - *α*-ketoglutarate dehydrogenase complex

P1 Principle 1 (3 of 4)

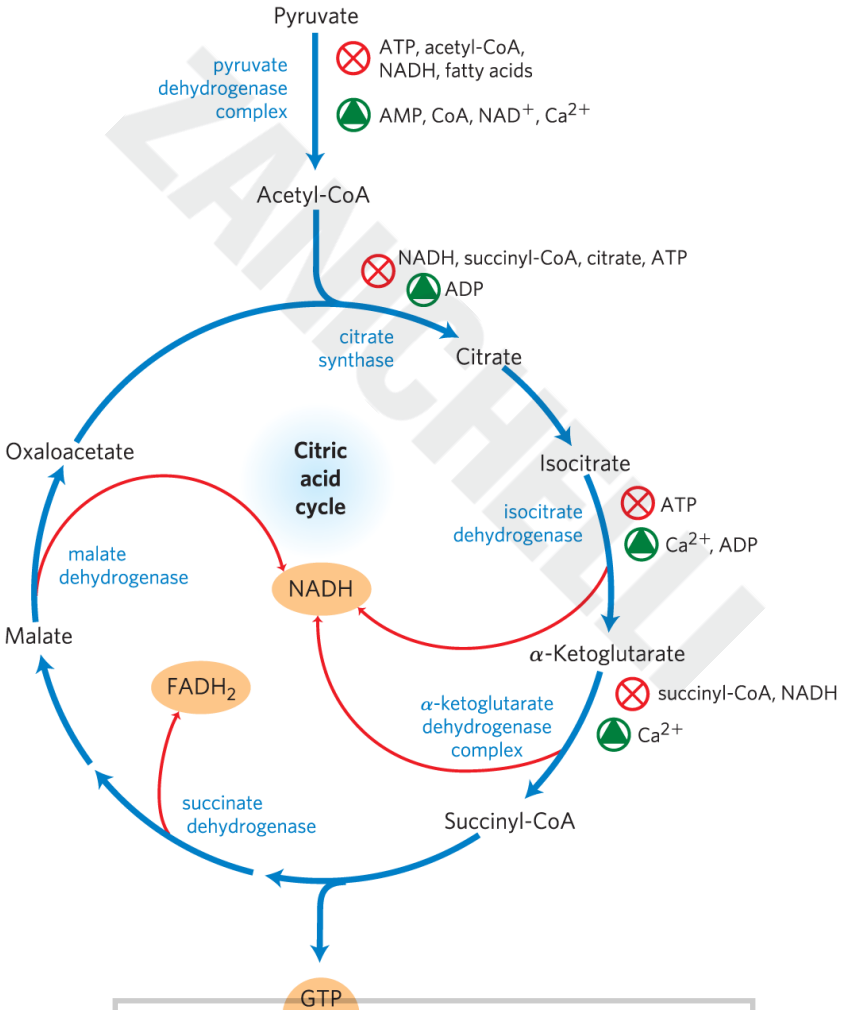
Pyruvate is the metabolite that links two central catabolic pathways, glycolysis and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

Production of Acetyl-CoA by the PDH Complex Is Regulated by Allosteric and Covalent Mechanisms

- PDH complex activity is turned off when:
  - ample fatty acids and acetyl-CoA are available as fuel
  - [ATP]/[ADP] and [NADH]/[NAD^+] ratios are high

- PDH complex activity is turned on when:
  - energy demands are high
  - the cell requires greater flux of acetyl-CoA into the citric acid cycle

Regulation of Metabolite Flow Through the Citric Acid Cycle

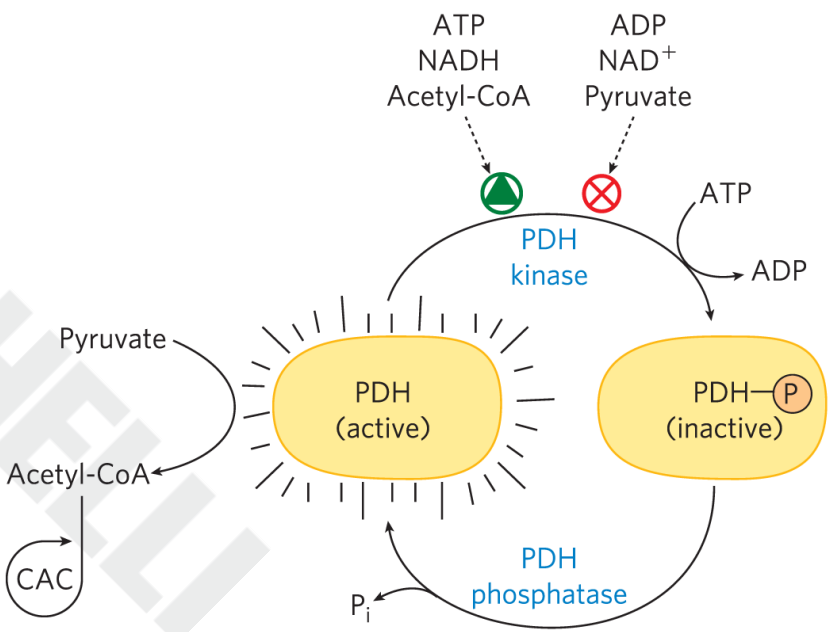


P1 Principle 1 (4 of 4)

Pyruvate is the metabolite that links two central catabolic pathways, glycolysis and the citric acid cycle. It is therefore a logical point for regulation that determines the rate of catabolic activity and the partitioning of pyruvate among its possible uses.

Covalent Modification of the PDH Complex

- **PDH kinase** = inhibits the PDH complex by phosphorylation
  - allosterically activated by products of the complex
  - inhibited by substrates of the complex
- **PDH phosphatase** = reverses the inhibition by PDH kinase



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P4 Principle 4 (4 of 5)

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis. Some mutations that affect the reactions of the citric acid cycle lead to tumor formation.

The Citric Acid Cycle Is Also Regulated at Three Exergonic Steps

- regulation occurs at strongly exergonic steps catalyzed by:
  - citrate synthase
  - isocitrate dehydrogenase complex
  - *α*-ketoglutarate dehydrogenase complex

- fluxes are affected by the concentrations of substrates and products:
  - end products ATP and NADH are inhibitory
  - NAD^+ and ADP are stimulatory
  - long-chain fatty acids are inhibitory

Citric Acid Cycle Activity Changes in Tumors

- some mutations that affect the PDH complex or citric acid cycle enzymes are oncogenic:
  - downregulation of mitochondrial pyruvate carrier (MPC)
  - inactivation of PDH complex
  - inactivation of succinate dehydrogenase

- **oncometabolites** = stimulate tumor growth by acting though specific GPCRs in the plasma membrane

Lactate and Succinate Are Oncometabolites

- tumor cells accumulate lactate and succinate
- **oncometabolites** = stimulate tumor growth by acting though specific GPCRs in the plasma membrane

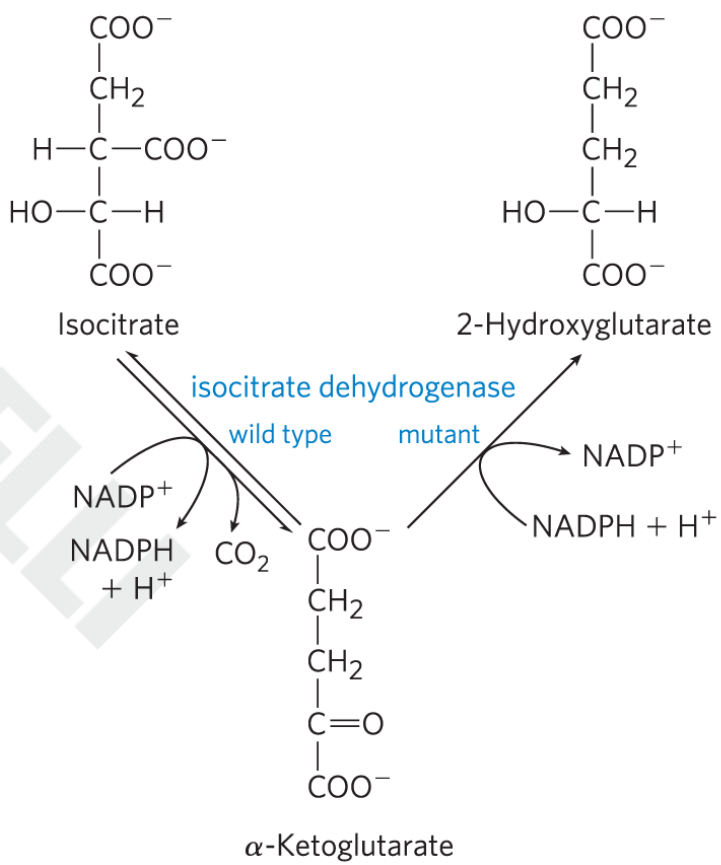
P4 Principle 4 (5 of 5)

The central role of the citric acid cycle in metabolism requires that it be regulated in coordination with many other pathways. Regulation occurs by both allosteric and covalent mechanisms that overlap and interact to achieve homeostasis. Some mutations that affect the reactions of the citric acid cycle lead to tumor formation.

Mutations in Citric Acid Cycle Enzymes

- mutations in succinate dehydrogenase and fumarase cause tumors, defining them as tumor suppressors

- many glial cell tumors have mutant NADPH-dependent isocitrate dehydrogenase
  - lose ability to convert isocitrate to *α*-ketoglutarate
  - gain ability to convert *α*-ketoglutarate to 2-hydroxyglutarate



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Certain Intermediates Are Channeled through Metabolons

- **metabolons** = integrated multienzyme complexes that are held together by noncovalent interactions

- malate dehydrogenase, citrate synthase, and aconitase likely constitute a metabolon