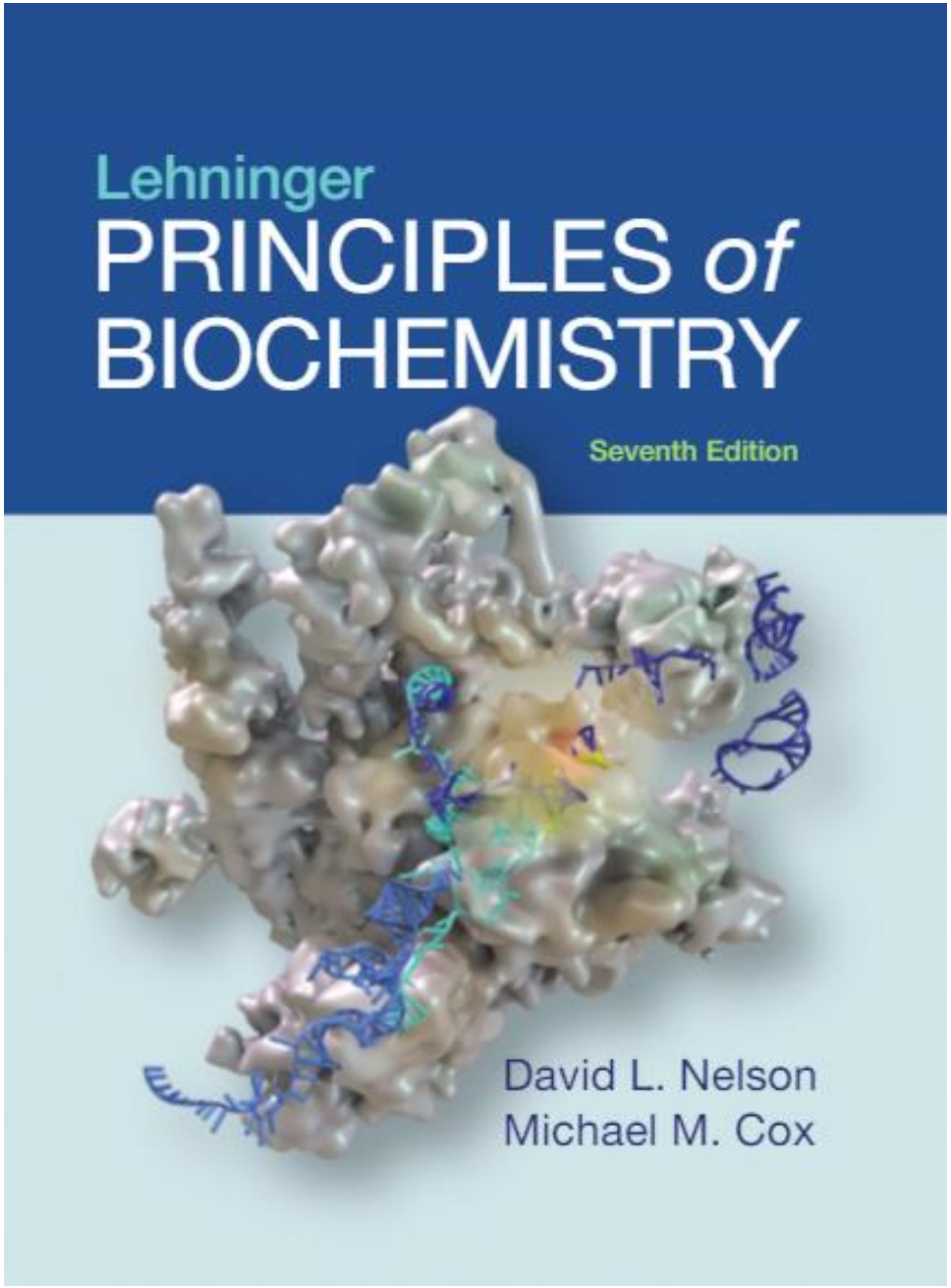
16| The Citric Acid Cycle



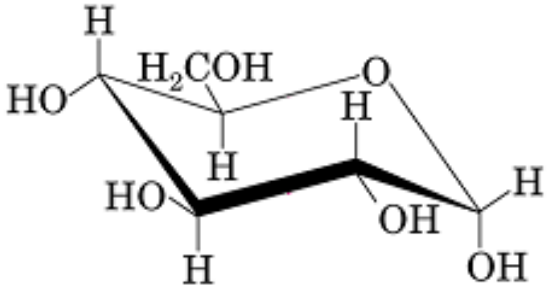
CHAPTER 16:
The Citric Acid Cycle

Learning goals:

- Cellular respiration
- Conversion of pyruvate to activated acetate
- Reactions of the citric acid cycle
- Regulation of the citric acid cycle
- Amphibolic nature of citric acid cycle intermediates
- Mechanisms of replenishing citric acid cycle intermediates

Only a small amount of energy available in glucose is captured in glycolysis

Only the 1st stage of Glucose oxidation

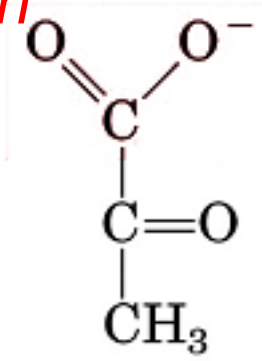


Glucose

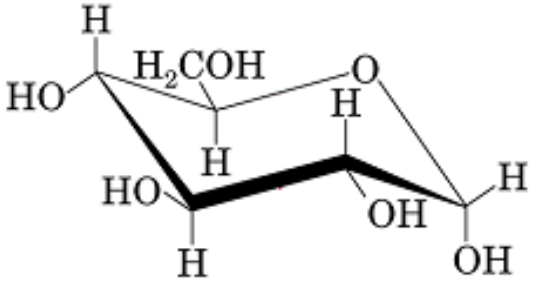
Glycolysis

$\Delta G'° = -146$ kJ/mol

2



Pyruvate



Full oxidation (+ 6 O_2)

$\Delta G'° = -2{,}840$ kJ/mol

6 CO_2 + 6 H_2O

Cellular Respiration

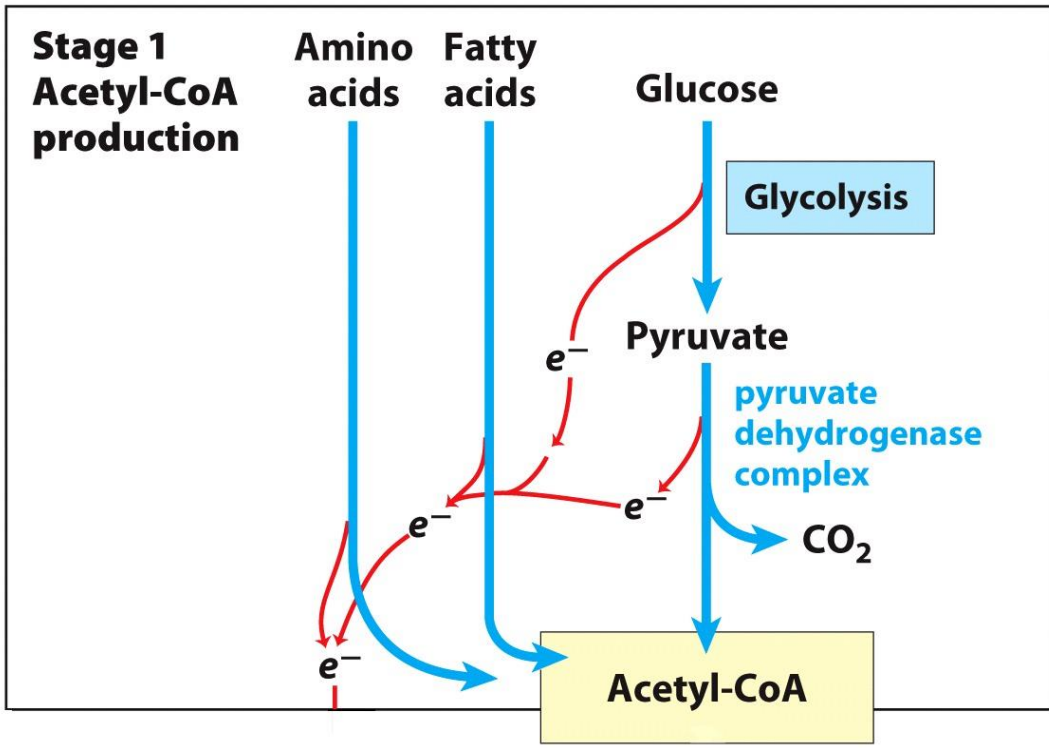
- Process in which ***cells*** consume O_2 and produce CO_2
- Provides more energy (ATP) from glucose than glycolysis
- Also captures energy stored in lipids and amino acids
- Used by animals, plants, and many microorganisms

- Occurs in three major stages:
  - acetyl CoA production (from organic fuel molecules)
  - acetyl CoA oxidation (in the CAC to produce CO_2)
  - electron transfer and oxidative phosphorylation (reduced coenzymes from CAC give their e^-'s to O_2 forming ATP in the process)

Respiration: Stage 1
Acetyl-CoA Production

- Activated form of acetate
- C-skeleton of sugars and fatty acids are converted to acetyl-CoA before entering the CAC

some a.a. enter CAC via other intermediates



- **Pyruvate dehydrogenase complex (PDH)**
- Multiple copies of 3 enzymes
- 5 reactions by 3 enzymes, whereby the intermediates remain bound to the enzyme molecule until forming the final product
- 5 cofactors (4 derived from vitamins)

Respiration: Stage 2
Acetyl-CoA oxidation

Generates NADH, $FADH_2$, and one GTP

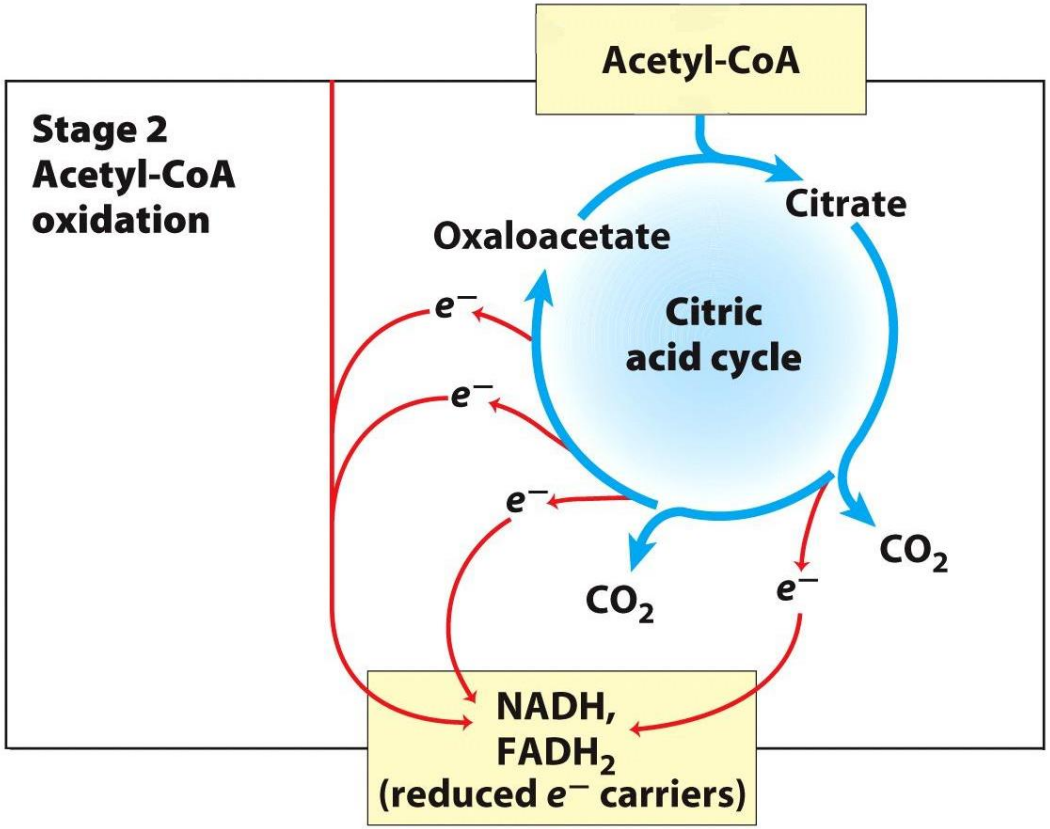


Figure 16-1
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Respiration: Stage 3
Oxidative Phosphorylation

Generates
a lot of ATP

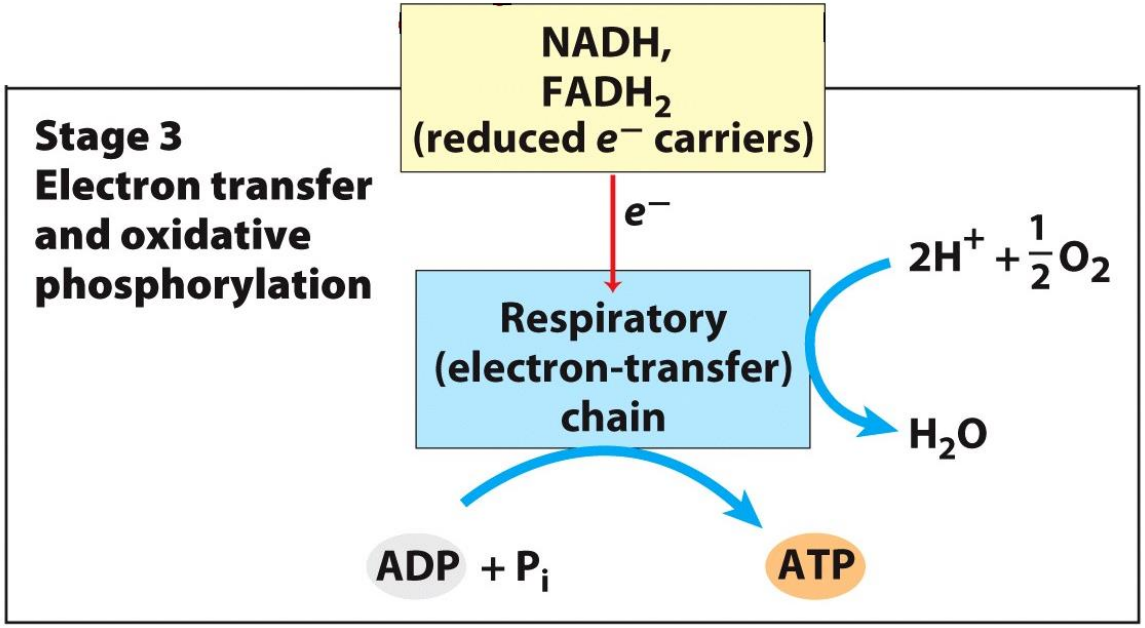

Figure 16-1
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In eukaryotes, citric acid cycle occurs in mitochondria

- Glycolysis occurs in the cytoplasm
- Citric acid cycle occurs in the mitochondrial matrix[†]
- Oxidative phosphorylation occurs on and in the inner membrane

[†]Except succinate dehydrogenase, which is located in the mitochondrial inner membrane

Conversion of Pyruvate to Acetyl-CoA

- Net Reaction:
  - ***Oxidative decarboxylation*** of pyruvate
  - First carbons of glucose to be fully oxidized (remember: 2 pyr/glc)
- Catalyzed by PDH
  - Requires 5 coenzymes
  - TPP, lipoate, and FAD are prosthetic groups
  - NAD^+ and CoA-SH are co-substrates

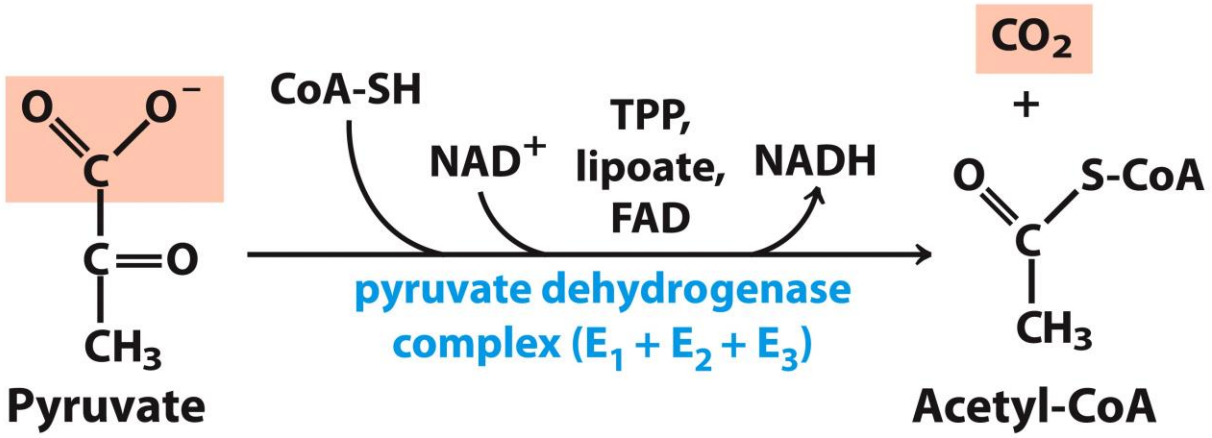
Derived from:

TPP – thiamine (B1)

FAD – riboflavin (B2)

NAD – niacin (B3)

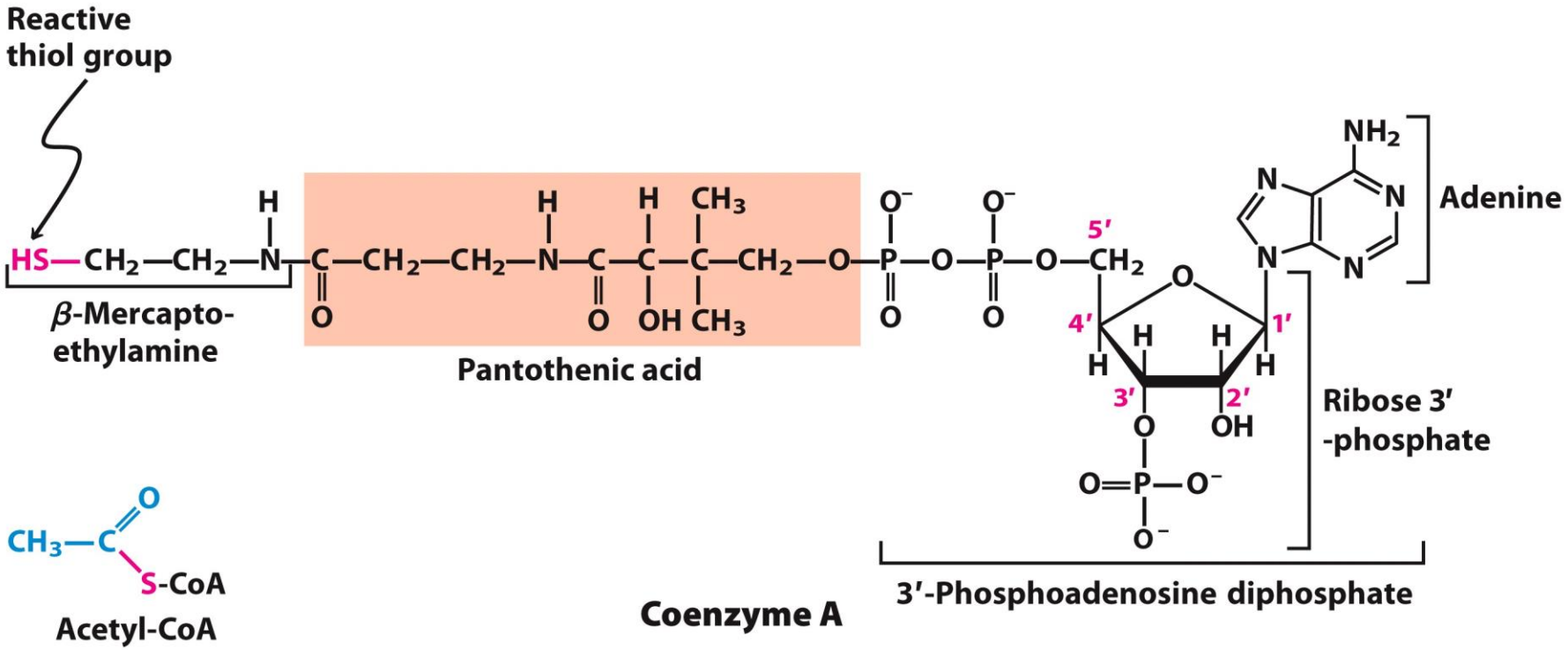
CoA – panthothenic acid (B5)



$\Delta G'^{\circ} = -33.4$ kJ/mol

Structure of Coenzyme A

- Coenzymes are not a permanent part of the enzymes' structure.
  - They associate, fulfill a function, and dissociate
- The function of CoA is to accept and carry acetyl groups



- **Thioesters** have a high acyl group transfer potential (donate their acyl groups to different groups)

Structure of Lipoyllysine

- Prosthetic groups are strongly bound to the protein
  - The lipoic acid is covalently linked to the enzyme via a lysine residue (lipoyllysine)
  - Undergo reversible redox reactions between thiols and disulfides; hence can serve as an electron (Hydrogen) carrier and an acyl carrier

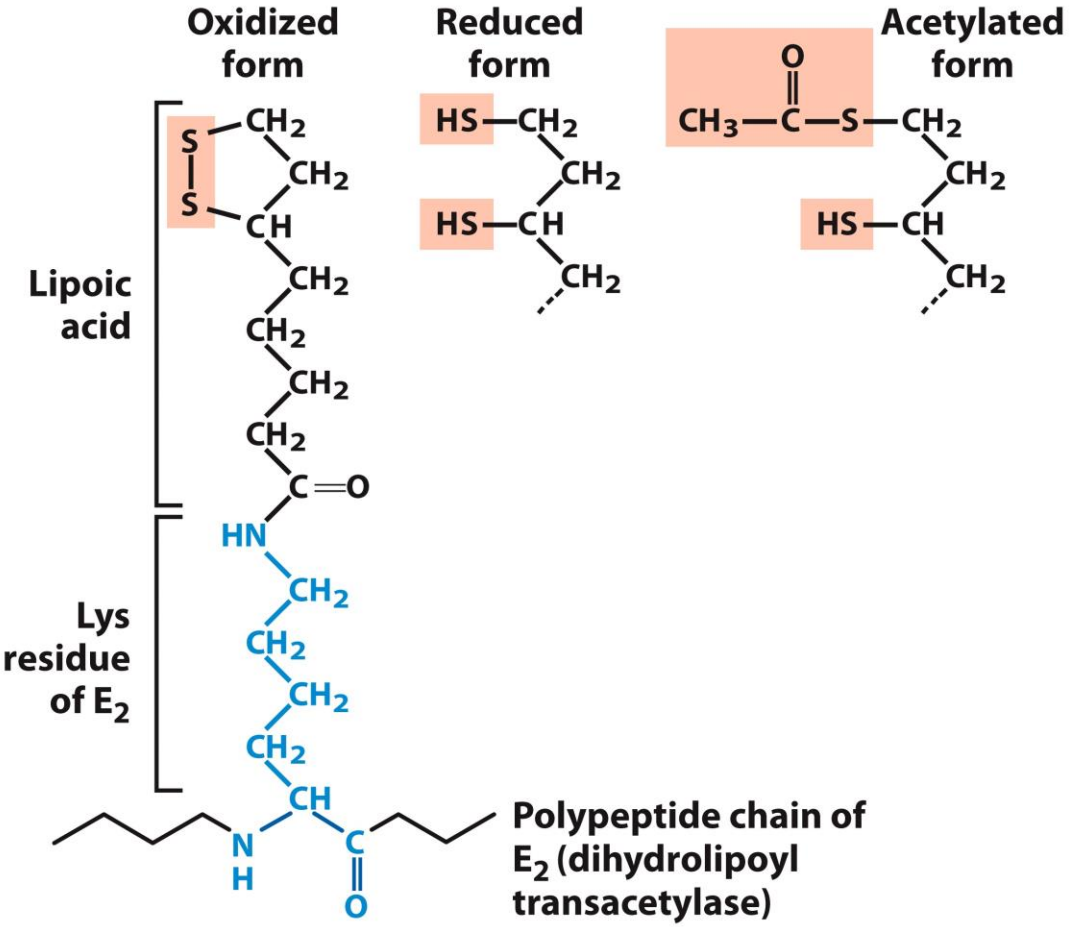


Figure 16-4
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Pyruvate Dehydrogenase Complex (PDC)

- Large (up to **10 MDa**) multienzyme complex
  - large enough to be seen with cryoEM
  - pyruvate dehydrogenase (E_1)
  - dihydrolipoyl transacetylase (E_2)
  - dihydrolipoyl dehydrogenase (E_3)
  - each present in *multiple copies*

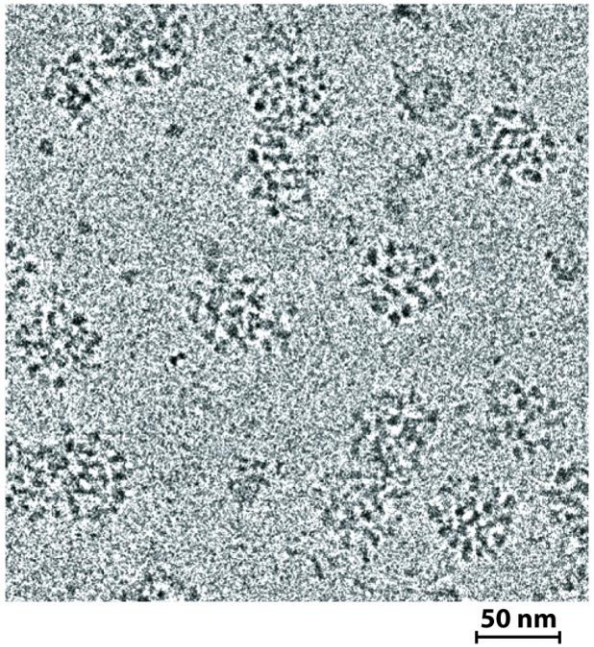

Figure 16-5a
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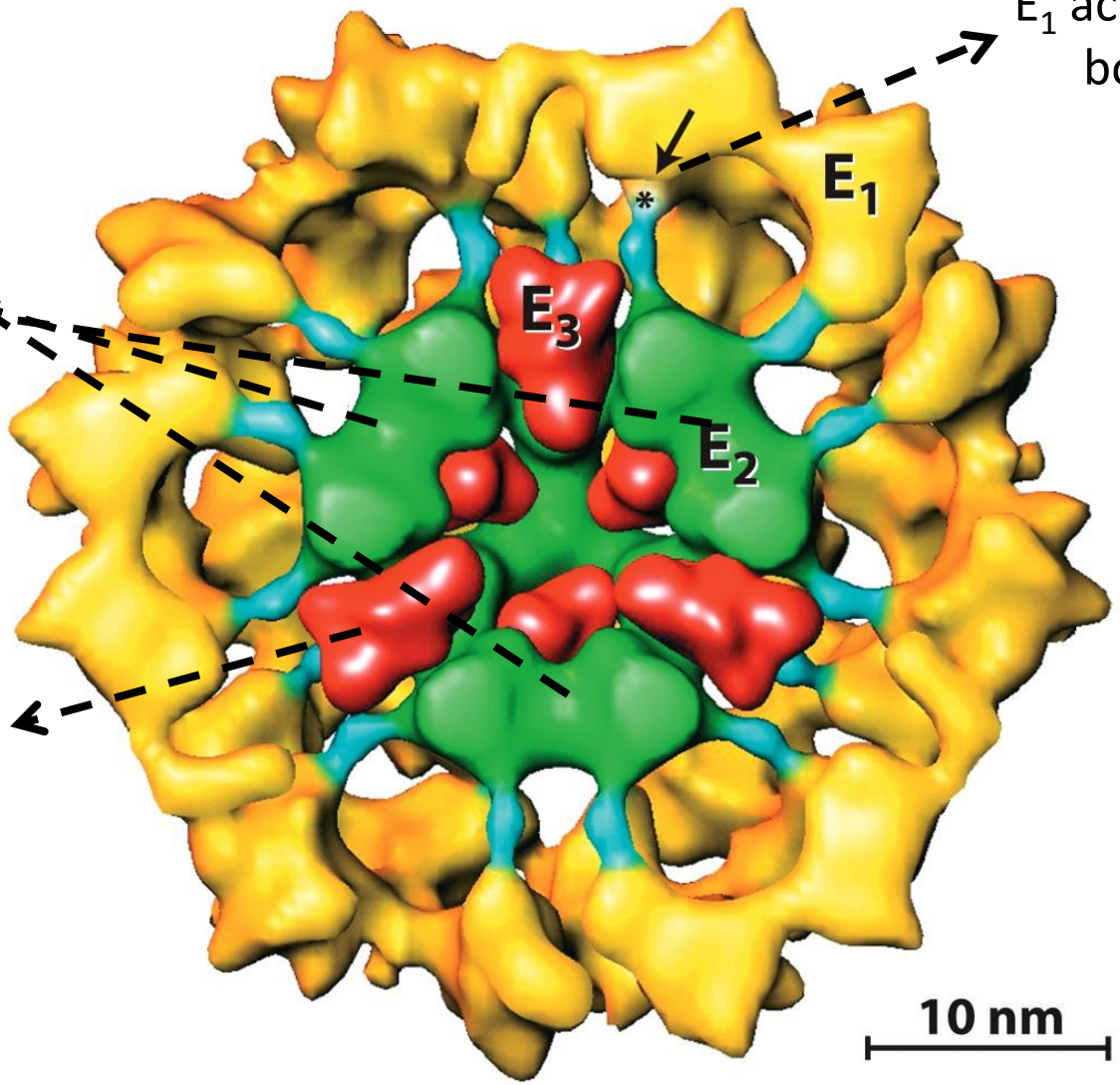
- Advantages of multienzyme complexes:
  - –short distance between catalytic sites allows channeling of substrates from one catalytic site to another
  - –channeling minimizes side reactions
  - – regulation of activity of one subunit affects the entire complex

3D Reconstruction from Cryo-EM data

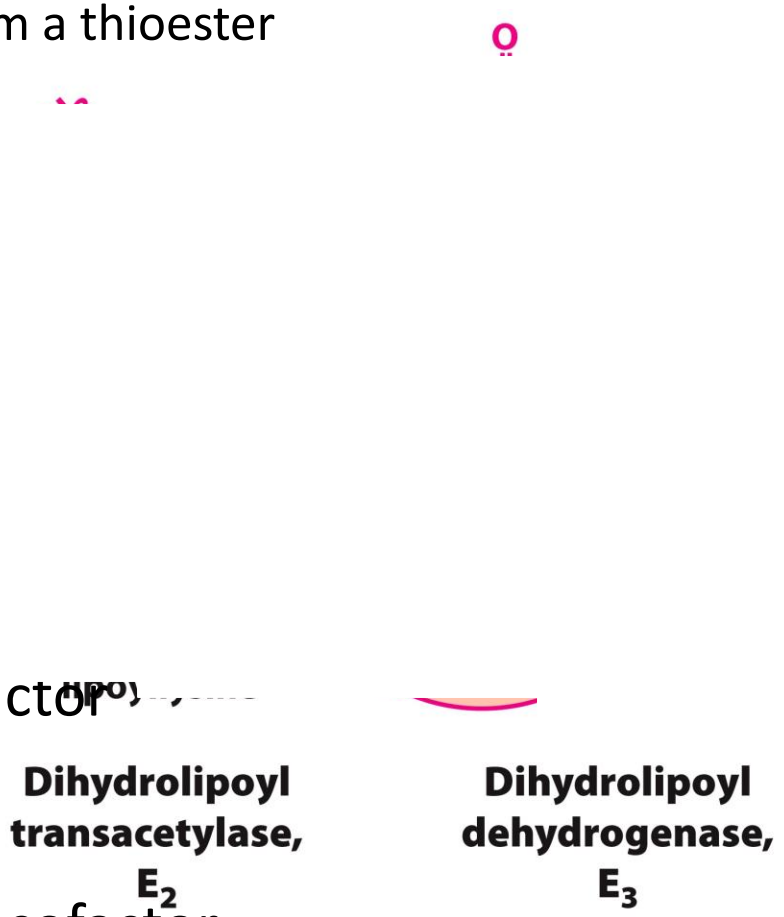
The bovine enzyme



Protein kinase and phosphoprotein phosphatase are part of the complex

Overall Reaction of PDC

- **Step 1:** Decarboxylation of pyruvate to an aldehyde forming CO_2 (E_1) (product 1)
- **Step 2:** Oxidation of aldehyde to a carboxylic acid
  - Electrons reduce lipoamide and form a thioester (E_2)
- **Step 3:** Formatic of acetyl (product (E_3)
- **Step 4:** Reoxidat cofactor
- **Step 5:** Regeneration of the oxidized FAD cofactor
  - Forming NADH (product 3)



Overall Reaction of PDC

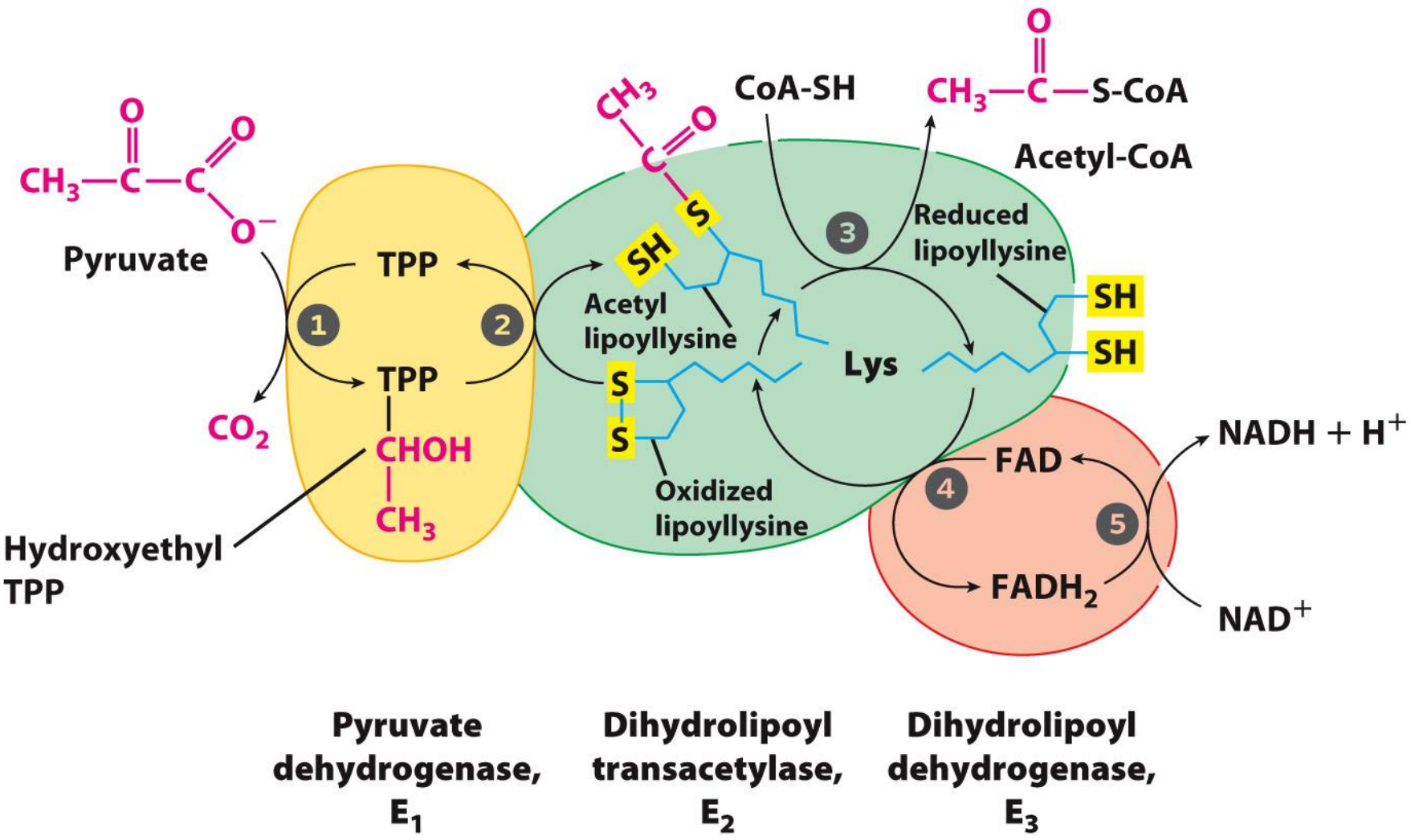


Figure 16-6
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Sequence of Events in Oxidative Decarboxylation of Pyruvate

Enzyme 1

- **Step 1:** Decarboxylation of pyruvate to an aldehyde forming CO_2 (product 1)
- **Step 2:** Oxidation of aldehyde to a carboxylic acid
  - Electrons reduce lipoamide and form a thioester.

Enzyme 2

- **Step 3:** Formation of acetyl-CoA (product 2)

Enzyme 3

- **Step 4:** Reoxidation of the lipoamide cofactor
- **Step 5:** Regeneration of the oxidized FAD cofactor
  - forming NADH (product 3)

The Citric Acid Cycle (CAC)

C-C bond formation to make citrate

Dehydrogenation to give **1 NADH**

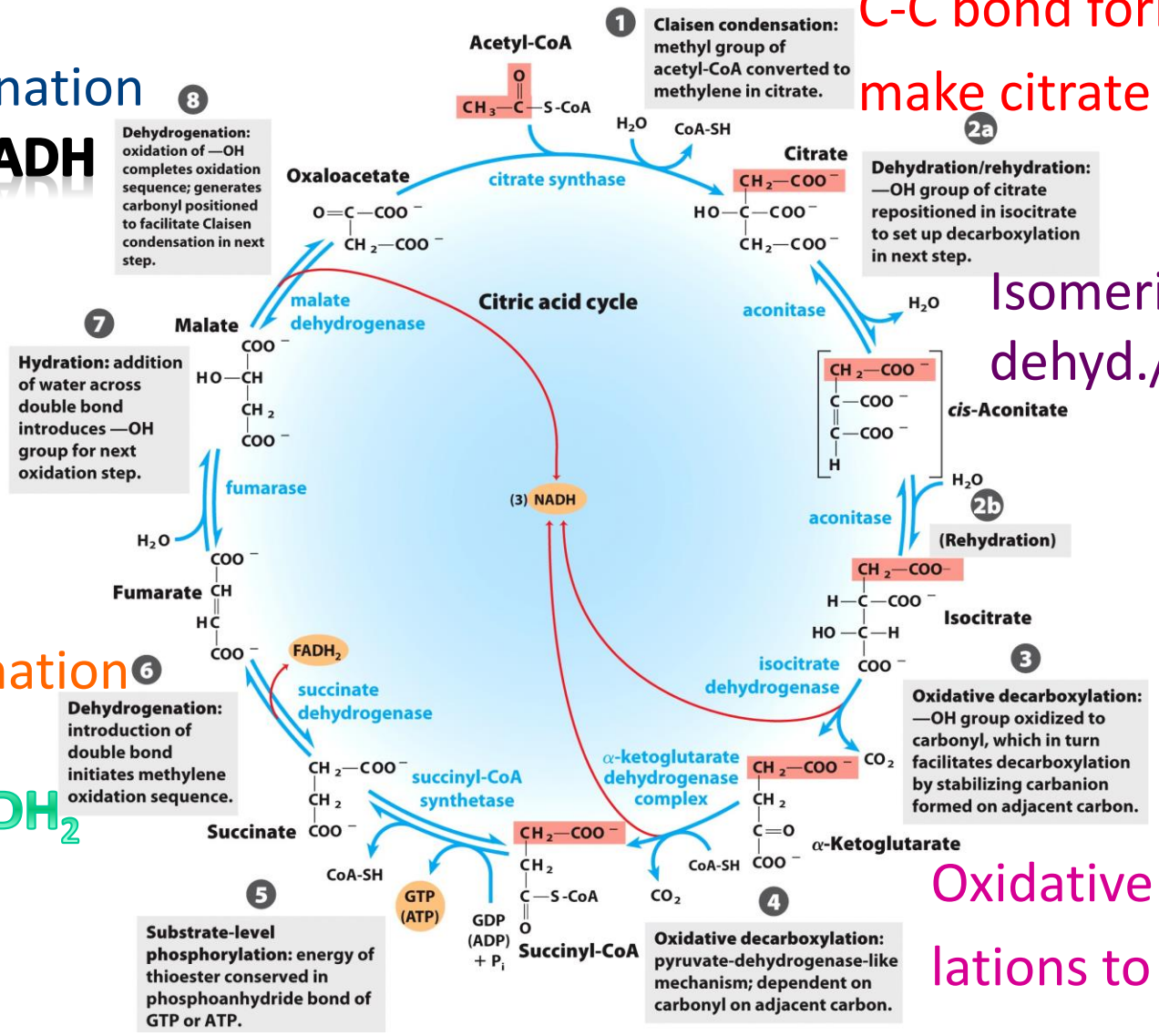
Isomerization via dehyd./rehydration

Hydration

Dehydrogenation to give reduced **$FADH_2$**

Oxidative decarboxy-lations to give **2 NADH**

Substrate-level phosphorylation to give **1 GTP** (= 1ATP)



Sequence of Events in the Citric Acid Cycle

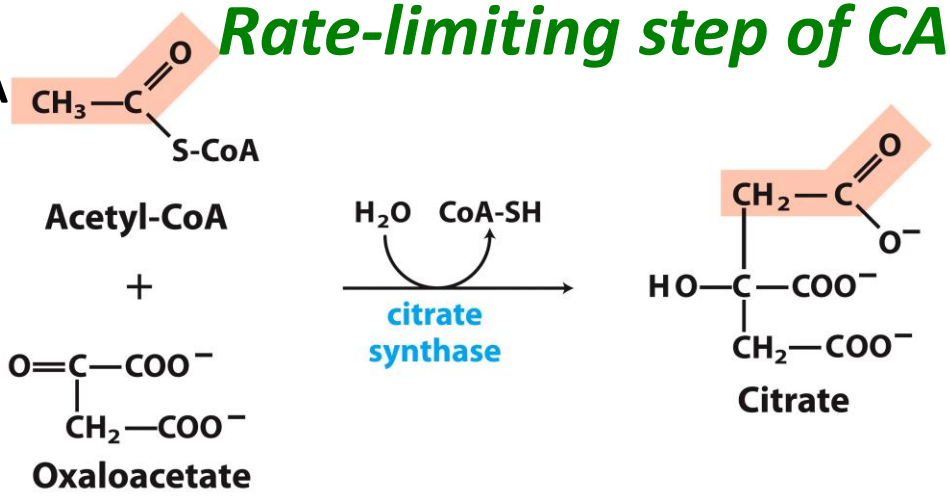
- Step 1: C-C bond formation between acetate (2C) and oxaloacetate (4C) to make citrate (6C)
- Step 2: Isomerization via dehydration/rehydration
- Steps 3–4: Oxidative decarboxylations to give 2 NADH
- Step 5: Substrate-level phosphorylation to give GTP
- Step 6: Dehydrogenation to give $FADH_2$
- Step 7: Hydration
- Step 8: Dehydrogenation to give NADH

The Citric Acid Cycle

- **Per each turn of the cycle:**
  - One acetyl group enters (2 C) and 2 CO_2 leave
  - One molecule of oxaloacetate is used to make citrate and one molecule is regenerated (no net change in OA concentration; which is very low)
  - 4 of the 8 steps are oxidations (the energy of oxidation is conserved in NADH and $FADH_2$)
- **Not limited to energy production**
  - 4- and 5-C intermediates serve as precursors for different products
  - To replace these intermediates, cells use ***anaplerotic*** (replenishing) reactions

C-C Bond Formation by Condensation of Acetyl-CoA and Oxaloacetate (step 1)

Rate-limiting step of CAC

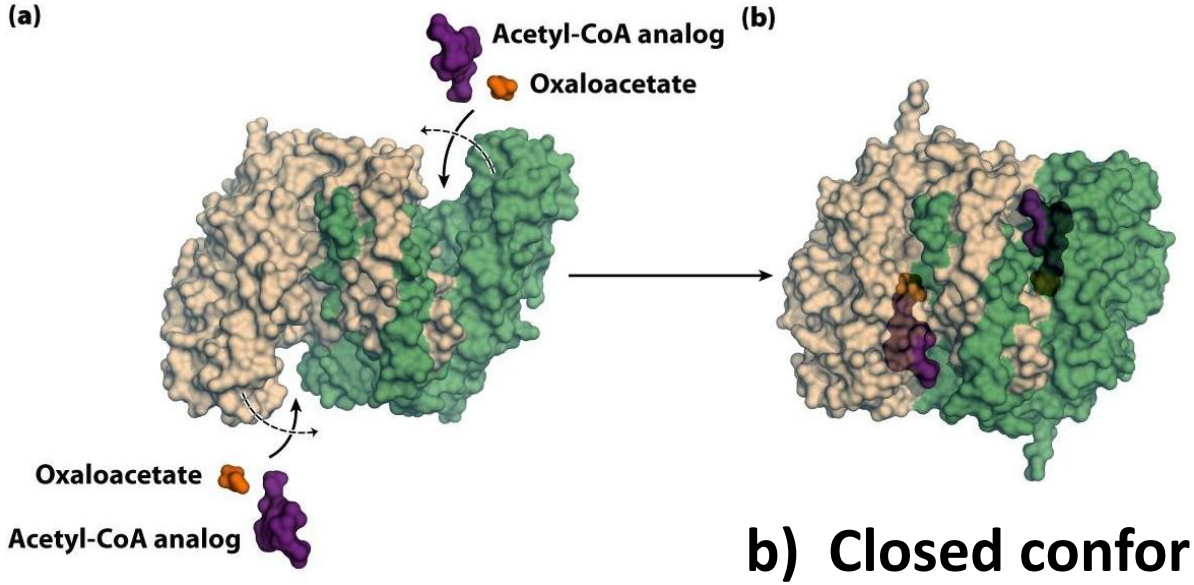


$\Delta G'^{\circ} = -32.2$ kJ/mol

- Condensation of acetyl-CoA and oxaloacetate
- The only reaction with C-C bond formation
- Uses Acid/Base Catalysis
  - Carbonyl of oxaloacetate is a good electrophile (stabilization of carbanions)
  - Methyl of acetyl-CoA is not a good nucleophile unless activated by deprotonation
- Activity largely depends on [oxaloacetate]
- Highly thermodynamically favorable/irreversible
  - Regulated by substrate availability and product inhibition

Induced Fit in the Citrate Synthase

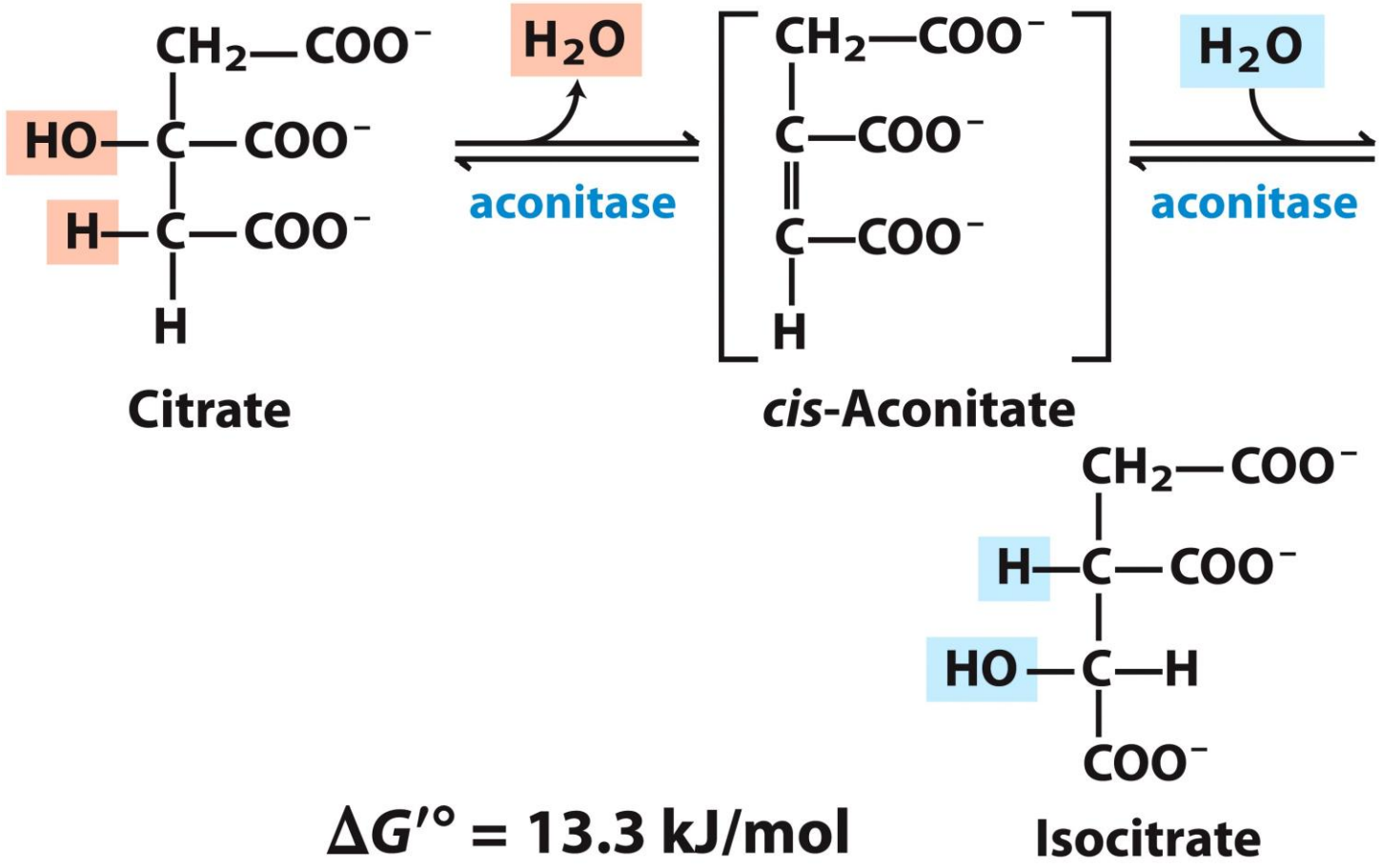
- oxaloacetate binds first → creating a binding site for acetyl-CoA
- Avoids unnecessary hydrolysis of thioester in acetyl-CoA



a) **Open conformation:**
Free enzyme does not have a binding site for acetyl-CoA

b) **Closed conformation:**
Binding of OAA creates binding for acetyl-CoA
Reactive carbanion is protected

Isomerization by Dehydration/Rehydration (step 2)

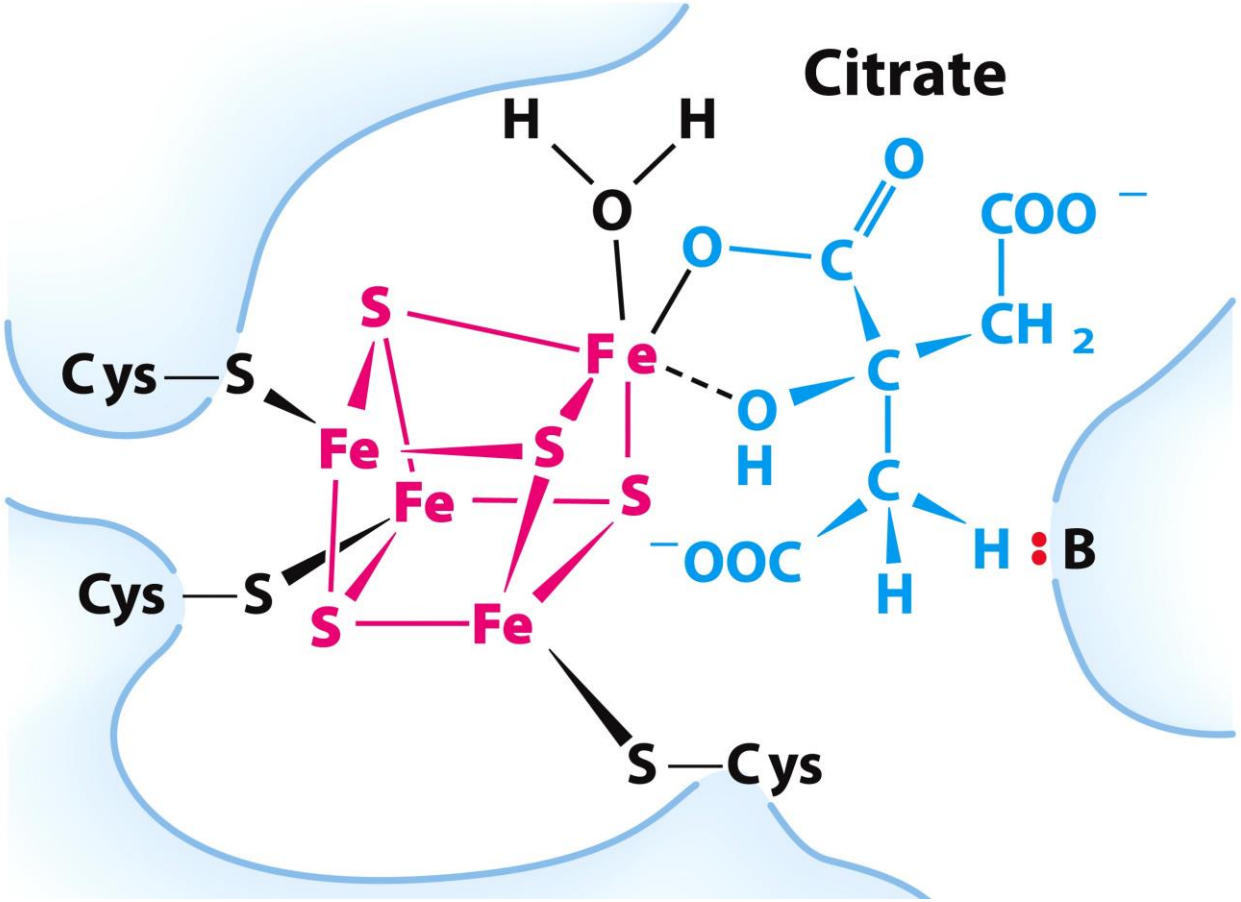


Aconitase

- Elimination of H_2O from citrate gives a cis C=C bond
  - Lyase
- Citrate, a tertiary alcohol, is a poor substrate for oxidation
- Isocitrate, a secondary alcohol, is a good substrate for oxidation
- Addition of H_2O to *cis*-aconitate is stereospecific (either to form isocitrate or citrate)
- Cytosolic isozyme uses $NADP^+$ as a cofactor
- Thermodynamically unfavorable/reversible
  - Product is consumed rapidly by the next step (concentration kept low) to pull reaction forward

Iron-Sulfur Center in Aconitase

- Water removal from citrate and subsequent addition to *cis*-aconitate are catalyzed by the iron-sulfur center: sensitive to oxidative stress.
- The iron-sulfur center acts in both substrate binding and catalysis.



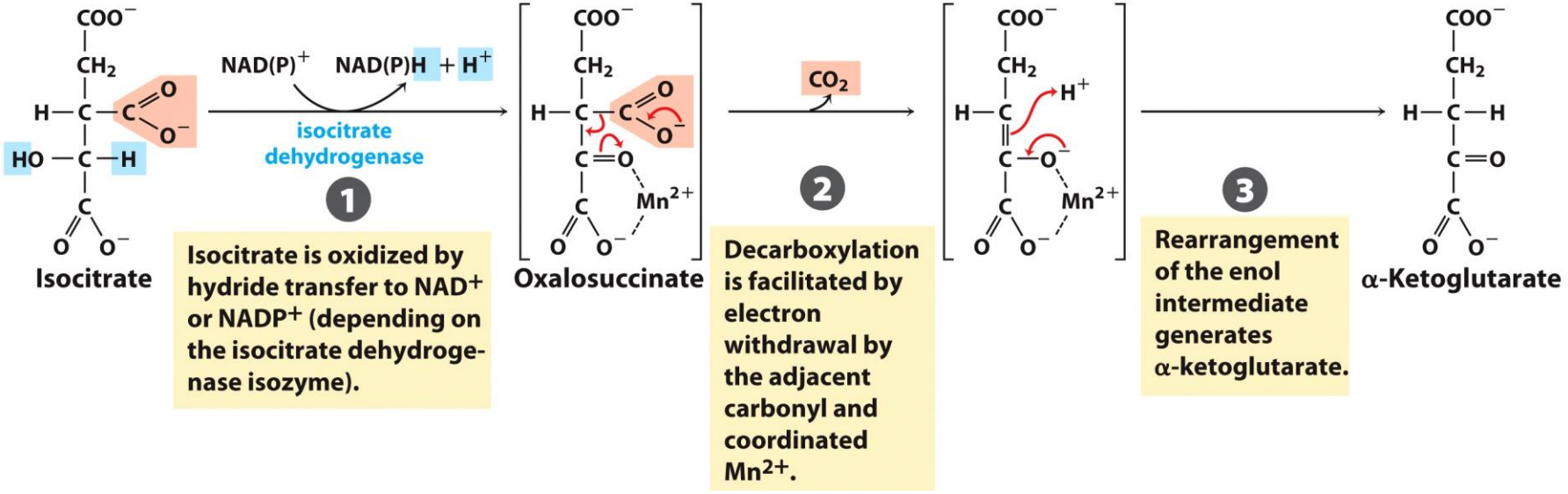
Aconitase is a "moonlighting" enzyme

- When Fe is deficient, aconitase loses its Fe-S center and acquires a *new role* in Fe homeostasis
- Cytosolic Aconitase is an enzyme (with Fe-S) and a regulator of protein synthesis (– Fe)

- In humans Fe levels must be regulated: too little → anemia; too much → liver damage
- ***Transferrin***: carries Fe in the blood
- ***Transferrin receptor***: receives and endocytoses Fe
- ***Ferritin***: stores excess Fe inside the cells

- Apoaconitase (– Fe) regulates protein levels by stabilizing or destabilizing the mRNA of transferrin receptor or ferritin
- **Apoaconitase → ↓ferritin and ↑ TfR synthesis (the results would be an increase in cellular [Fe])**

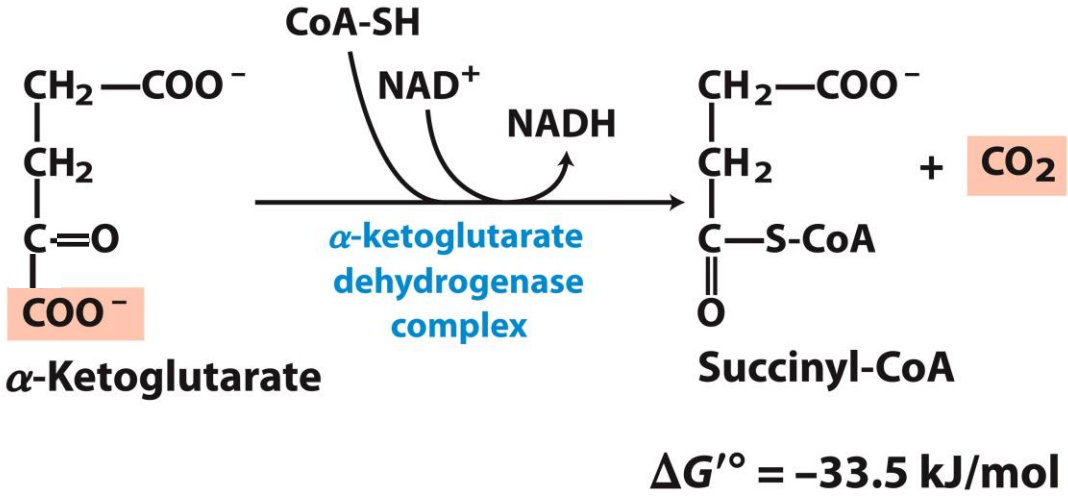
Oxidative Decarboxylation #2 (step 3)



- Carbon is lost as CO_2 and NADH is generated
  - Carbon lost as CO_2 did NOT come from acetyl-CoA
- Oxidation of the alcohol to a ketone
  - Transfers a hydride to NAD^+
- Cytosolic isozyme uses $NADP^+$ as a cofactor
- Highly thermodynamically favorable/irreversible
  - Regulated by product inhibition and ATP

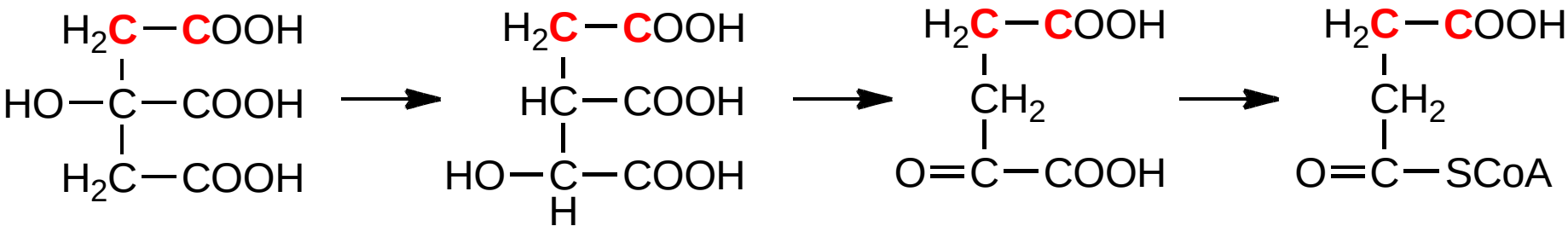
Final Oxidative Decarboxylation (step 4)

- Last oxidative decarboxylation
  - Net full oxidation of all carbons of glucose



- Succinyl-CoA is another higher-energy thioester bond
- Highly thermodynamically favorable/irreversible
  - Regulated by product inhibition

Origin of C-atoms in CO_2



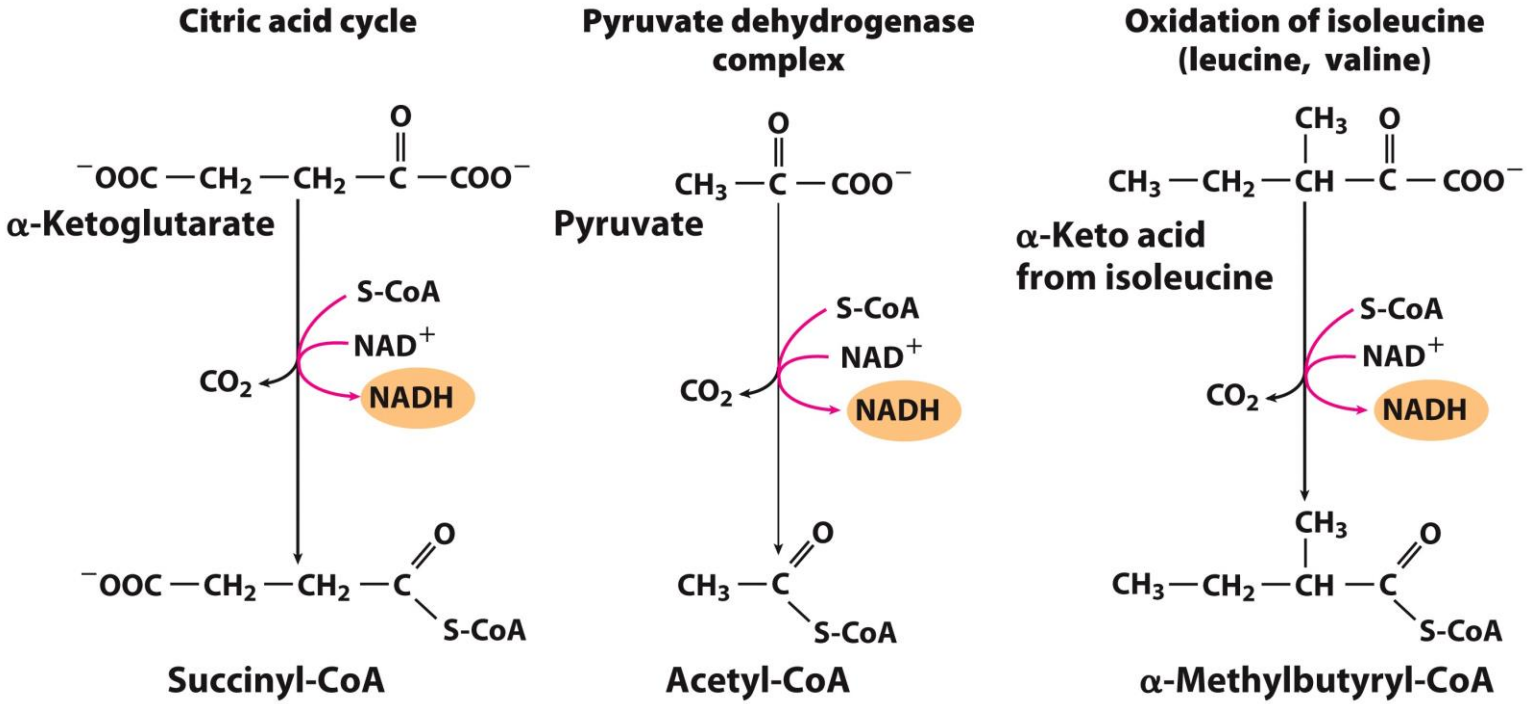
We have lost 2 CO_2 already, so we have a net complete oxidation of glucose after two pyruvates go through the CAC.

But its not the actual carbons from pyruvate (in red) in each cycle.

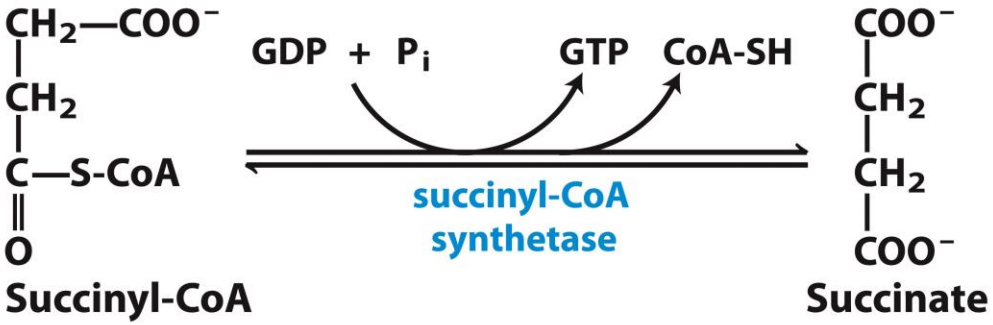
Both CO_2 carbon atoms are derived from oxaloacetate

α-Ketoglutarate Dehydrogenase

- Complex similar to pyruvate dehydrogenase
  - Same coenzymes, identical mechanisms
  - Active sites different to accommodate different-sized substrates
  - E_1 aa sequences differ (and specificity)
    E_2 are very similar
    E_3 are identical



Generation of GTP through Thioester (step 5)



- Substrate level phosphorylation
- Energy of thioester allows for incorporation of inorganic phosphate into ADP or GDP to make ATP or GTP

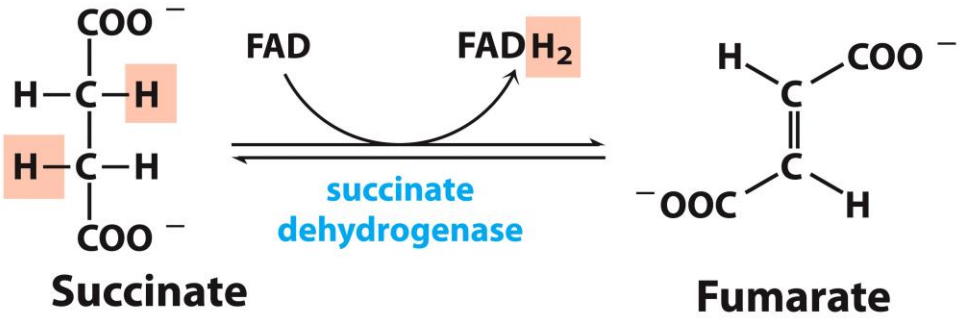
$\Delta G'^{\circ} = -2.9$ kJ/mol

- Goes through a phospho-enzyme intermediate
- Produces GTP, which can be converted to ATP, or ATP directly (2 isozymes in animal cells, specific for GDP or ADP)

- Slightly thermodynamically favorable/reversible
  - Product concentration kept low to pull forward

Oxidation of an Alkane to Alkene (step 6)

- Bound to mitochondrial inner membrane
  - Complex II in the ETC



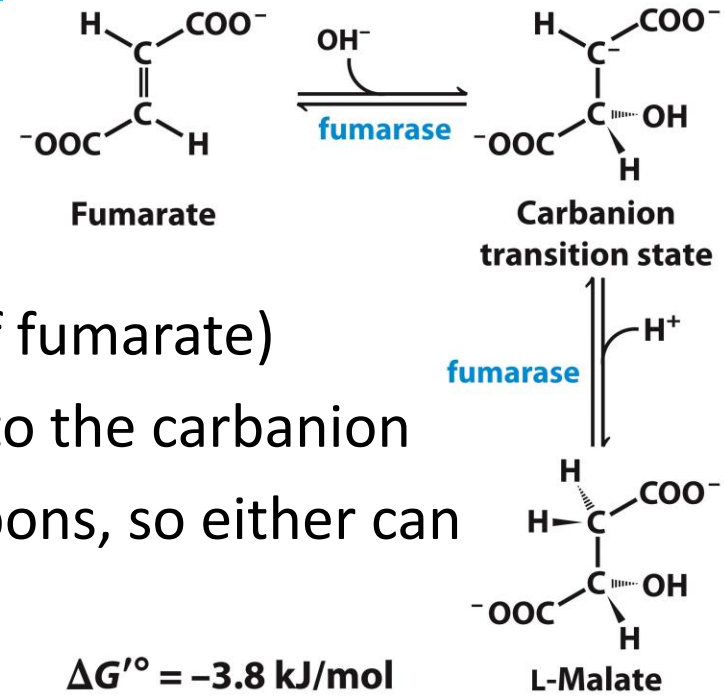
- Oxidation of the alkane to alkene requires FAD

$\Delta G'^{\circ} = 0$ kJ/mol

- FAD is covalently bound
- 3 Fe-S clusters
- Near equilibrium/reversible
  - Product concentration kept low to pull forward

Hydration Across a Double Bond (step 7)

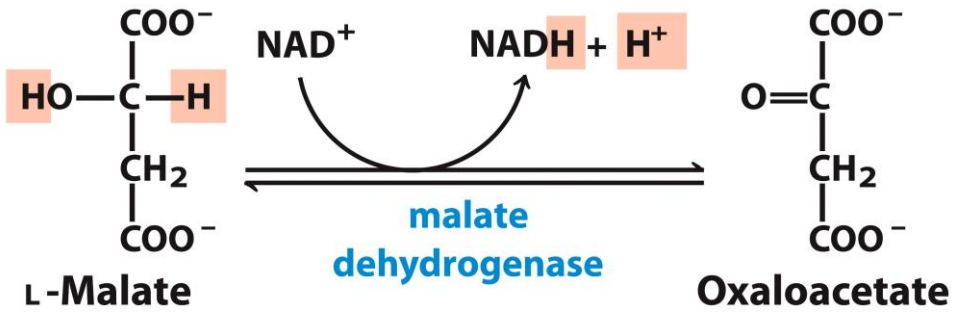
- Highly stereospecific
  - Addition of water is always *trans* and forms L-malate
  - Cannot work on maleate (cis isomer of fumarate)
  - OH- adds to fumarate… then H+ adds to the carbanion
  - Cannot distinguish between inner carbons, so either can gain –OH



- Slightly thermodynamically favorable/reversible
  - Product concentration kept low to pull reaction forward

Oxidation of Alcohol to a Ketone (step 8)

- Final step of the cycle
- Regenerates oxaloacetate for citrate synthase



$\Delta G'^{\circ} = 29.7$ kJ/mol

- Highly thermodynamically **UN**favorable/reversible
  - Oxaloacetate concentration kept VERY low by citrate synthase ($< 10^{-6}$ M)
    - Pulls the reaction forward

One Turn of the Citric Acid Cycle

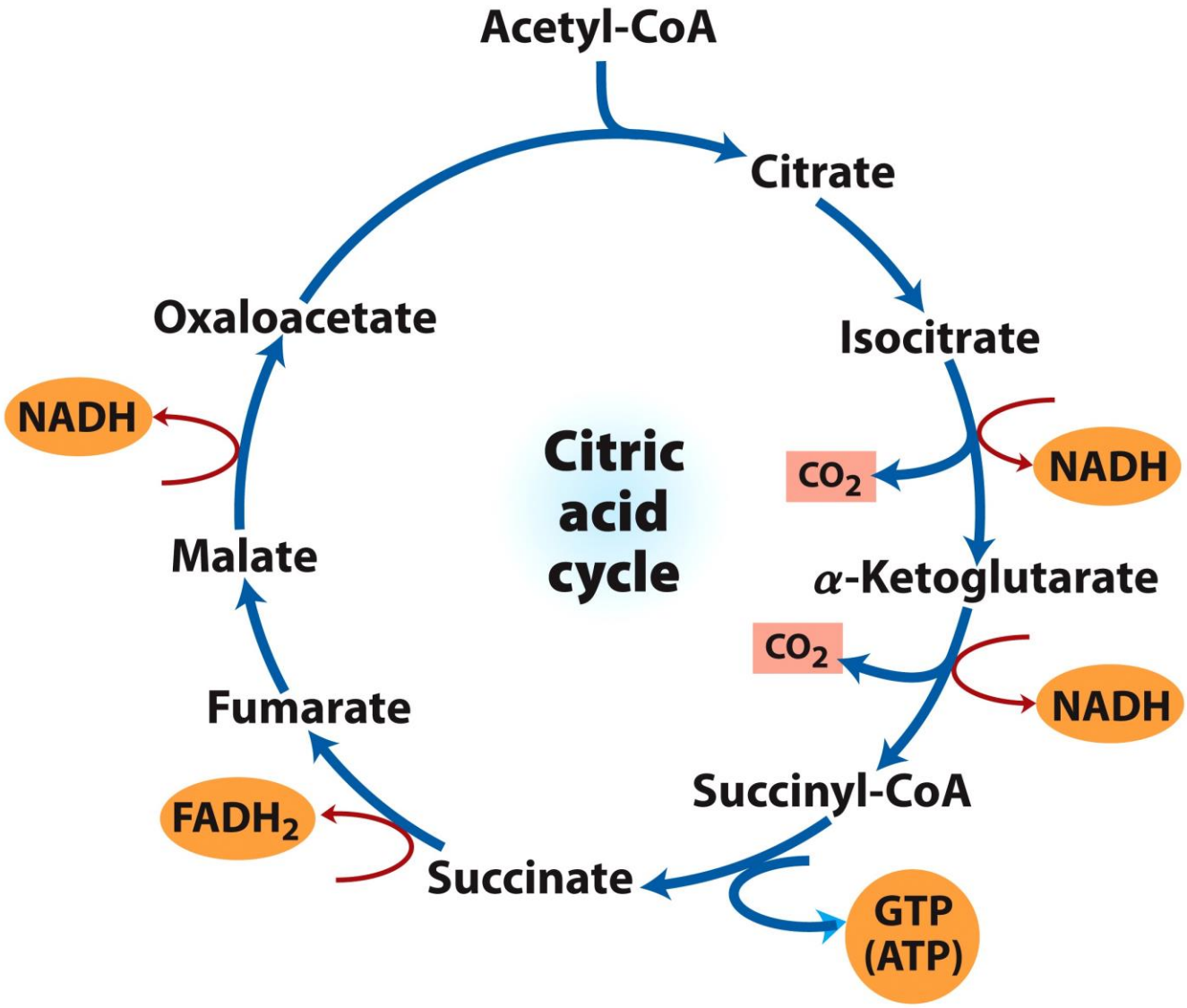


Figure 16-14
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Net Result of the Citric Acid Cycle

$$\text{Acetyl-CoA} + 3NAD^+ + FAD + GDP + P_i + 2\,H_2O \rightarrow$$

$$2CO_2 + 3NADH + FADH_2 + GTP + CoA + 3H^+$$

- Net oxidation of two carbons to CO_2
  - Equivalent to two carbons of acetyl-CoA
  - but NOT the exact same carbons
- Energy captured by electron transfer to NADH and $FADH_2$
- Generates 1 GTP, which can be converted to ATP

Direct and Indirect ATP Yield

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[a]
Glucose → glucose 6-phosphate	–1 ATP	–1
Fructose 6-phosphate → fructose 1,6-bisphosphate	–1 ATP	–1
2 Glyceraldehyde 3-phosphate → 2 1,3-bisphosphoglycerate	2 NADH	3 or 5[b]
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

[a]This is calculated as 2.5 ATP per NADH and 1.5 ATP per $FADH_2$. A negative value indicates consumption.
[b]This number is either 3 or 5, depending on the mechanism used to shuttle NADH equivalents from the cytosol to the mitochondrial matrix; see Figures 19-30 and 19-31.

CAC is an amphibolic pathway

- ***Amphibolic-*** serves in both catabolism and anabolism
- Precursors for many molecules
- Needs to be replenished (by anaplerotic reactions) *Red arrows*

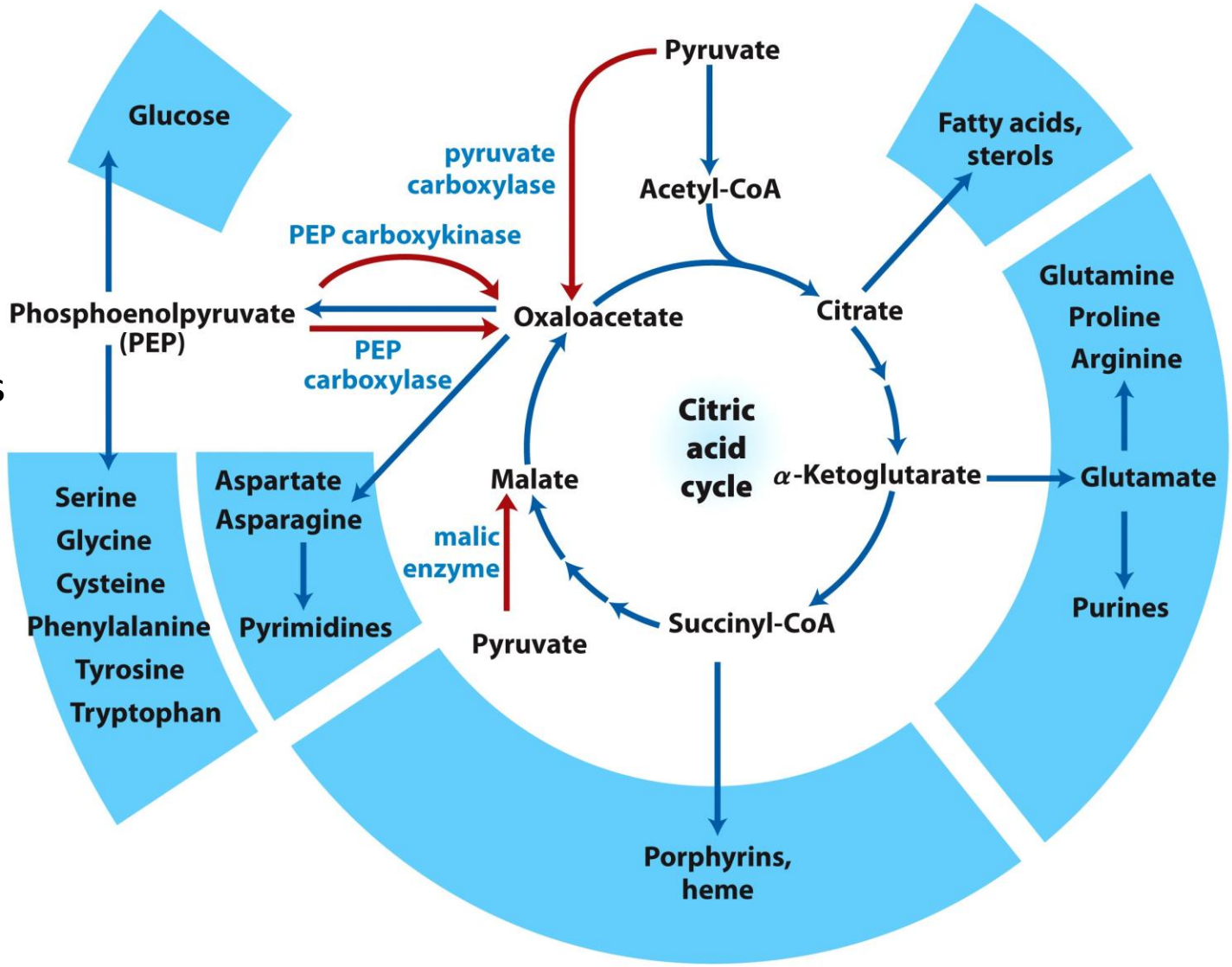

Figure 16-16
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Anaplerotic Reactions

- Must replenish the intermediates in order for the cycle and central metabolic pathway to continue
- 4-carbon intermediates are formed by carboxylation of 3-carbon precursors
- The replenishing and consuming reactions are in dynamic balance ([CAC intermediates] is ~ constant)

TABLE 16-2 Anaplerotic Reactions

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

Table 16-2
Lehninger Principles of Biochemistry, Seventh Edition

Anaplerotic Reactions

- Must replenish the intermediates in order for the cycle and central metabolic pathway to continue
- 4-carbon intermediates are formed by carboxylation of 3-carbon precursors
- The replenishing and consuming reactions are in dynamic balance ([CAC intermediates] is ~ constant)

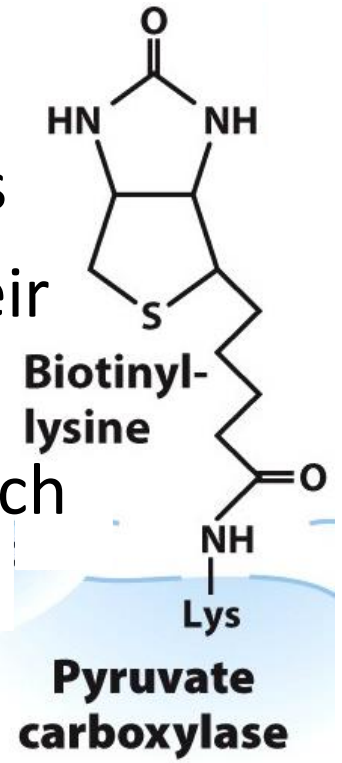
TABLE 16–2 Anaplerotic Reactions

Reaction	Tissue(s)/organism(s)
$\text{Pyruvate} + HCO_3^- + \text{ATP} \xrightleftharpoons{\text{pyruvate carboxylase}} \text{oxaloacetate} + \text{ADP} + P_i$	Liver, kidney

- Regulatory enzyme – ***inactive*** in the absence of acetyl-CoA
- More acetyl-CoA, more activity ➔ more OAA to react with acetyl-CoA to start the cycle

Biotin is a CO_2 carrier

- Vitamin B7 (biotin) is required in our food
- It is a cofactor (prosthetic group) in carboxylases
- Biotin is a specialized carrier of 1-C groups in their most OXIDIZED state (CO_2)
- Pyruvate carboxylase has 4 identical subunits each carrying a molecule of biotin
- It is present in many foods and intestinal bacteria are able to synthesize it, hence biotin deficiency is rare
- Consumption of raw eggs in large quantities leads to biotin deficiency since egg white have the protein **avidin** which binds biotin very tightly and prevents its absorption in the intestine



Biological tethers allow flexibility

- All enter the cells on the same transporter
- All are covalently attached to proteins
- All provide flexible arms on the enzymes to which they are covalently bound
- All act as tethers that move intermediates from one active site to the next

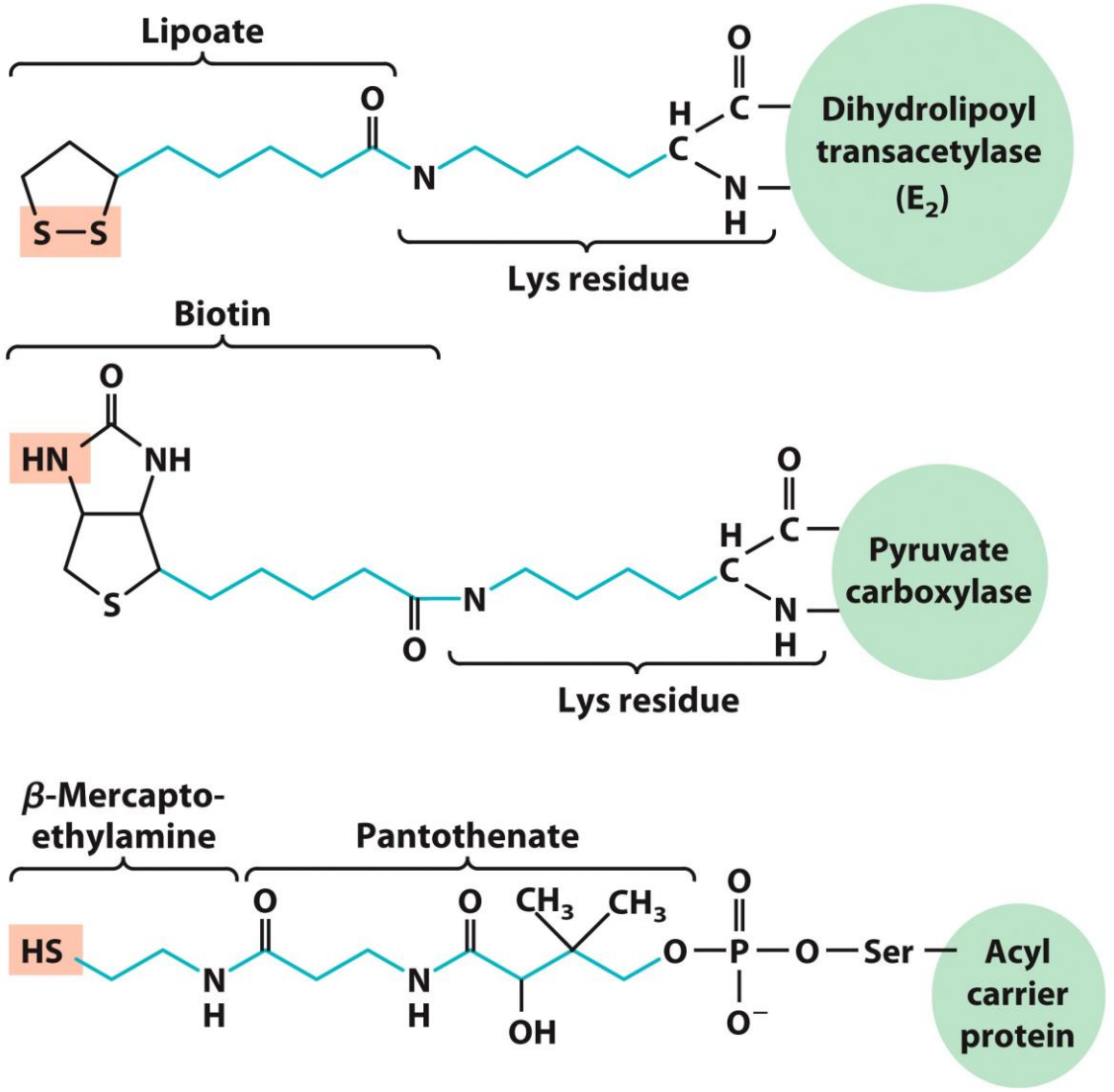


Figure 16-18
Lehninger Principles of Biochemistry, Sixth Edition

Regulation of the Citric Acid Cycle

Cell has high energy demands!

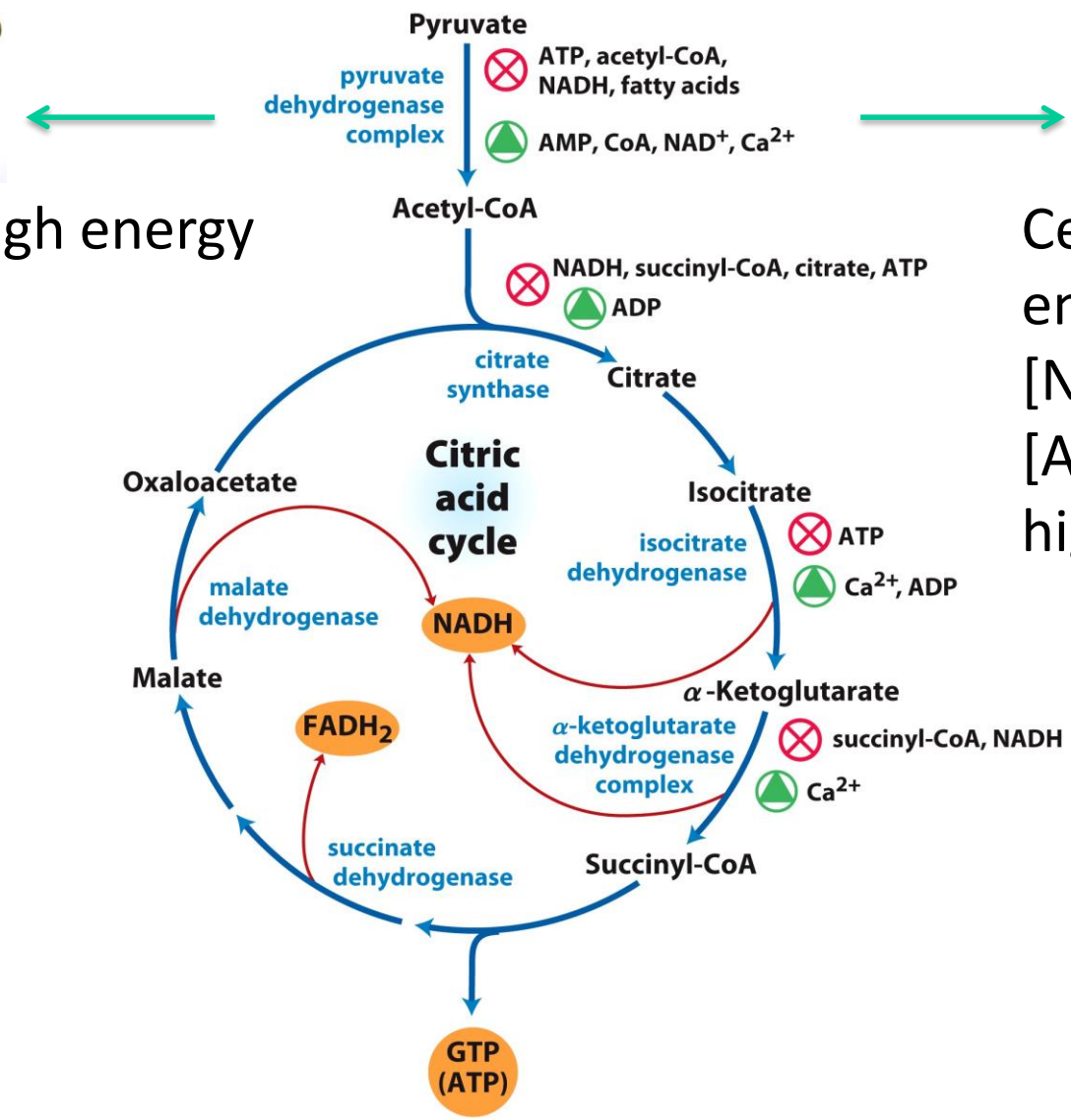


Figure 16-19
Lehninger Principles of Biochemistry, Sixth Edition

Cell is supplied with enough energy [NADH]/[NAD$^+$] and [ATP]/[ADP] are high

Regulation of the Citric Acid Cycle

- Regulated at highly thermodynamically favorable and irreversible steps
  - PDH, citrate synthase, IDH, and αKDH

- General regulatory mechanism
  - Activated by substrate availability
  - Inhibited by product accumulation
  - Overall products of the pathway are NADH and ATP
    - Affect all regulated enzymes in the cycle
    - Inhibitors: NADH and ATP
    - Activators: NAD^+ and AMP
    - Ca^{2+} in muscles activates the cycle (Ca^{2+} signals muscle contraction → need for energy)

Regulation of Pyruvate Dehydrogenase

- Also regulated by reversible phosphorylation of E1
  - Phosphorylation: inactive
  - Dephosphorylation: active
- PDH kinase and PDH phosphatase are part of mammalian PDH complex
  - Kinase is activated by ATP
    - High ATP → phosphorylated PDH → less acetyl-CoA
    - Low ATP → kinase is less active and phosphatase removes phosphate from PDH → more acetyl-CoA
  - Phosphatase is activated by insulin, PEP, and AMP, and inhibited by ATP, NADH, and Acetyl-CoA

Additional Regulatory Mechanisms

- Citrate synthase is also inhibited by succinyl-CoA
  - α-ketoglutarate is an important branch point for amino acid metabolism
  - Succinyl-CoA communicates flow at this branch point to the start of the cycle

- Regulation of isocitrate dehydrogenase controls citrate levels
  - Aconitase is reversible
  - Inhibition of IDH leads to accumulation of isocitrate and reverses aconitase
  - Accumulated citrate leaves mitochondria and inhibits PFK-1 in glycolysis

CAC mutations lead to cancer

- Mutations in CAC enzymes are very rare in man
- Genetic defects in fumarase → smooth muscle and kidney cancer
- Mutations in succinate DH → tumors of the adrenal glands
- Both enzymes are defined as tumor suppressor genes
- IDH mutation leads to a new function of the enzyme the net result of which is the development of glial cell tumors in the brain

Question 5 (Take home exam)
Due: NEXT WEEK (jstiban@birzeit.edu)

- **Please solve questions:**

1. **5 (NAD redox carriers)**
2. **10 (OAA in mito)**
3. **18 (^{14}C-glucose)**
4. **19 (Beriberi)**

For written answers, I prefer to have them typed in Word. I can accept the assignment in one file sent to my email. For answers that require solving mathematically, you can either type them or write them down and scan them.