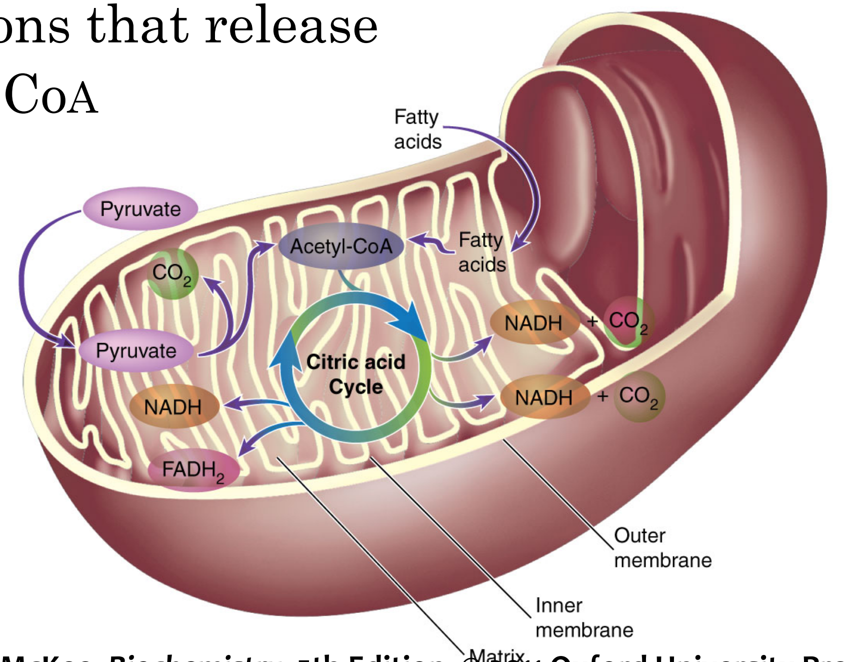
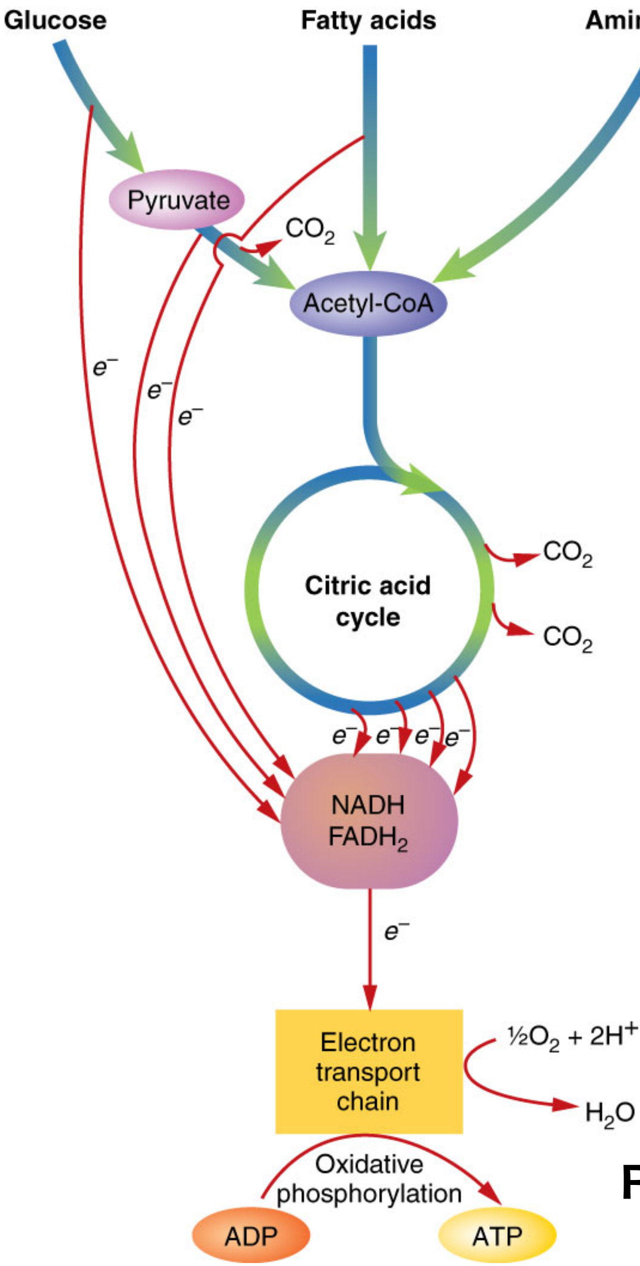
Chapter 9

Overview

- **Live processes** - series of oxidation-reduction reactions
  - Ingestion of proteins, carbohydrates, lipids
  - Provide basic building blocks for major molecules
  - Produces energy
- **Aerobic metabolism I**
  - Citric Acid Cycle – series of reactions that release chemical energy stored in acetyl-CoA
    - Acetyl-CoA derived from pyruvate

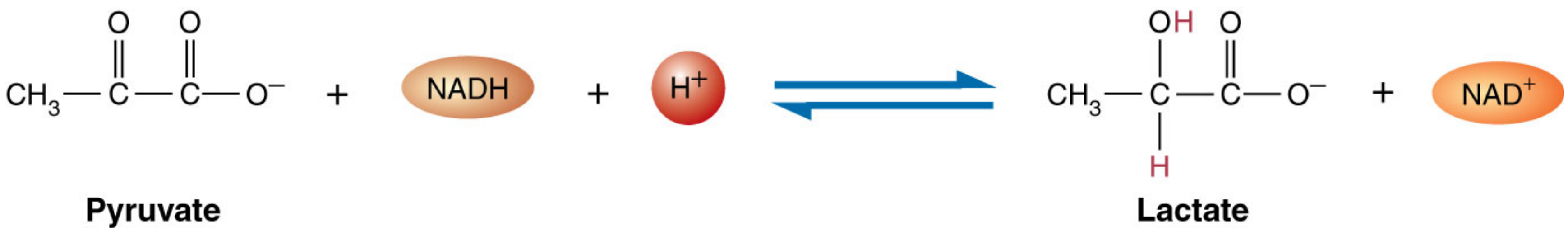




- **Citric acid cycle**
  - Two-carbon fragments oxidized to form CO_2
  - NAD^+/FAD reduced to $NADH/FADH_2$
- **Electron transport chain**
  - Transfer of electrons from $NADH/FADH_2$ to electron carriers
  - Terminal acceptor O_2
- **Oxidative phosphorylation**
  - Energy released forms proton gradient
  - Drives ATP synthesis

Figure 9.1 Overview of Aerobic Metabolism

Figure 9.3 Reduction of Pyruvate by NADH



- Redox reactions – electron transfer between an electron donor (reducing agent) & electron acceptor (oxidizing agent)
    - Many redox reactions have both an electron (e^-) and a proton (H^+) transferred
    - Conversion of pyruvate and NADH to lactate and NAD^+ (shown above) is under anaerobic conditions

- Half-reactions of redox reactions

Cu loses e$^-$, electron donor
$Cu^+ \leftarrow \rightarrow Cu^{2+} + e^-$

Fe gains e$^-$, electron acceptor
$Fe^{3+} + e^- \leftarrow \rightarrow Fe^{2+}$

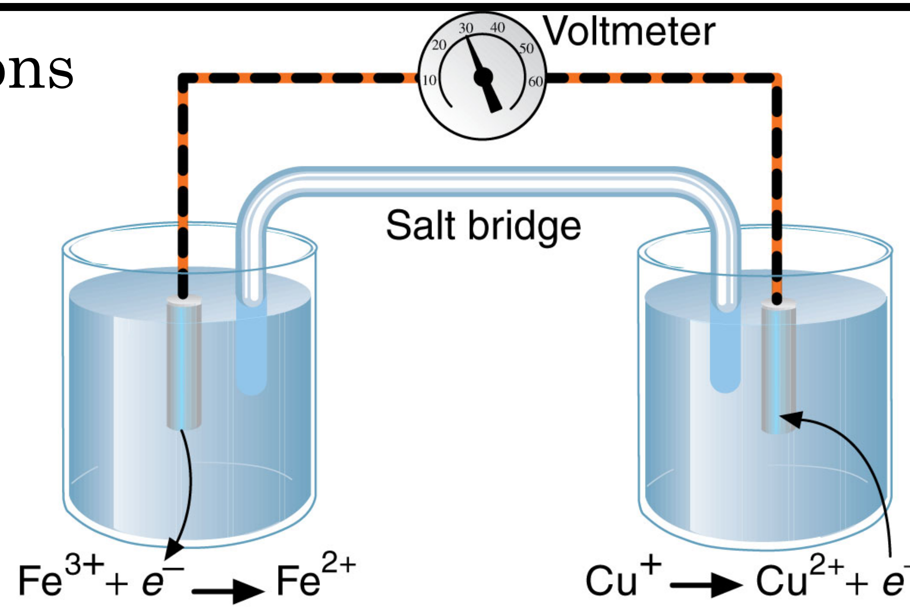


Figure 9.4 An Electrochemical Cell

- **Reduction potential** – tendency for gaining electrons
  - **Standard reduction potentials** (E^o) relative to standard hydrogen electrode; $2H^+ + 2e^- \rightarrow H_2$ $E^o = 0$
  - Biochemical reference half-reaction is $2H^+ + 2e^- \rightarrow H_2$ $E^o = -0.42$
- Lower E^o - lower affinity for electrons; higher E^o - greater affinity

- $\Delta E^{o\prime}$ - difference in reduction potential between donor and acceptor under standard conditions
- Relationship between standard reduction potentials ($\Delta E^{o\prime}$) and standard free energy ($\Delta G^{o\prime}$) is:

$$\Delta G^{o\prime} = -nF\,\Delta E^{o\prime}$$

  - *n* - # electrons transferred,
  - *F* - Faraday constant (96,485 J/V•mol)
- NADH and $FADH_2$ - redox coenzymes, high-energy electron carriers

Nicotinic Acid

- nicotinamide adenine dinucleotide (NAD)
  - Oxidized NAD^+; reduced NADH
  - NAD^+ involved in catabolic reactions
- nicotinamide adenine dinucleotide phosphate (NADP)
  - Oxidized $NADP^+$; reduced NADPH)
  - $NADP^+$ involved in biosynthetic reactions

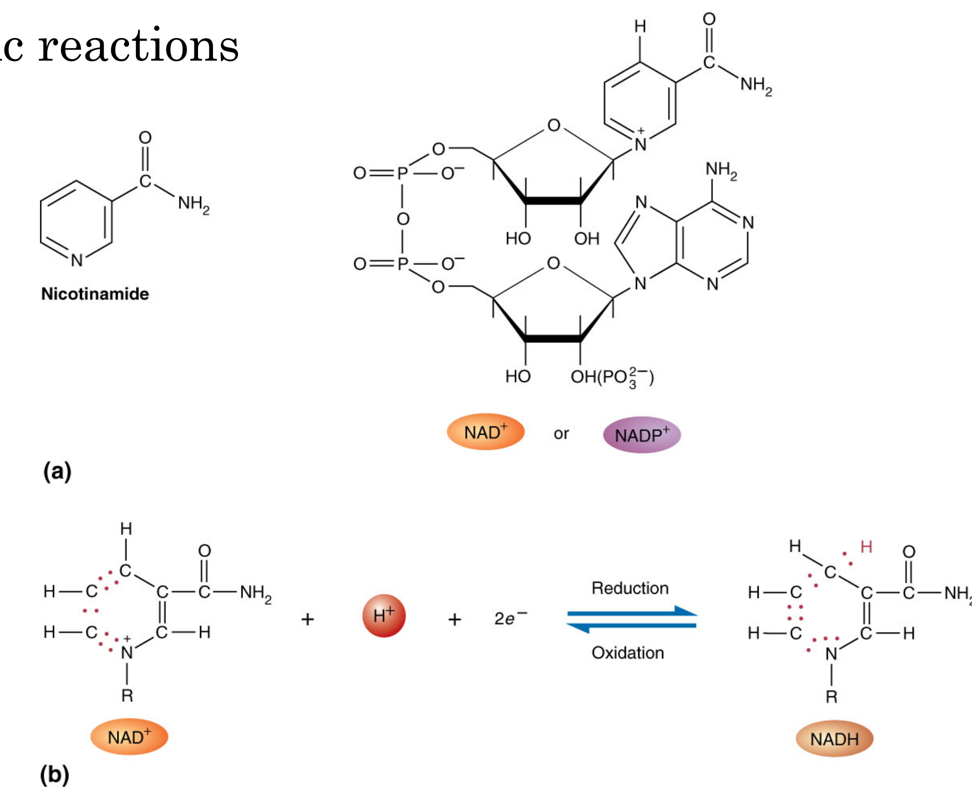
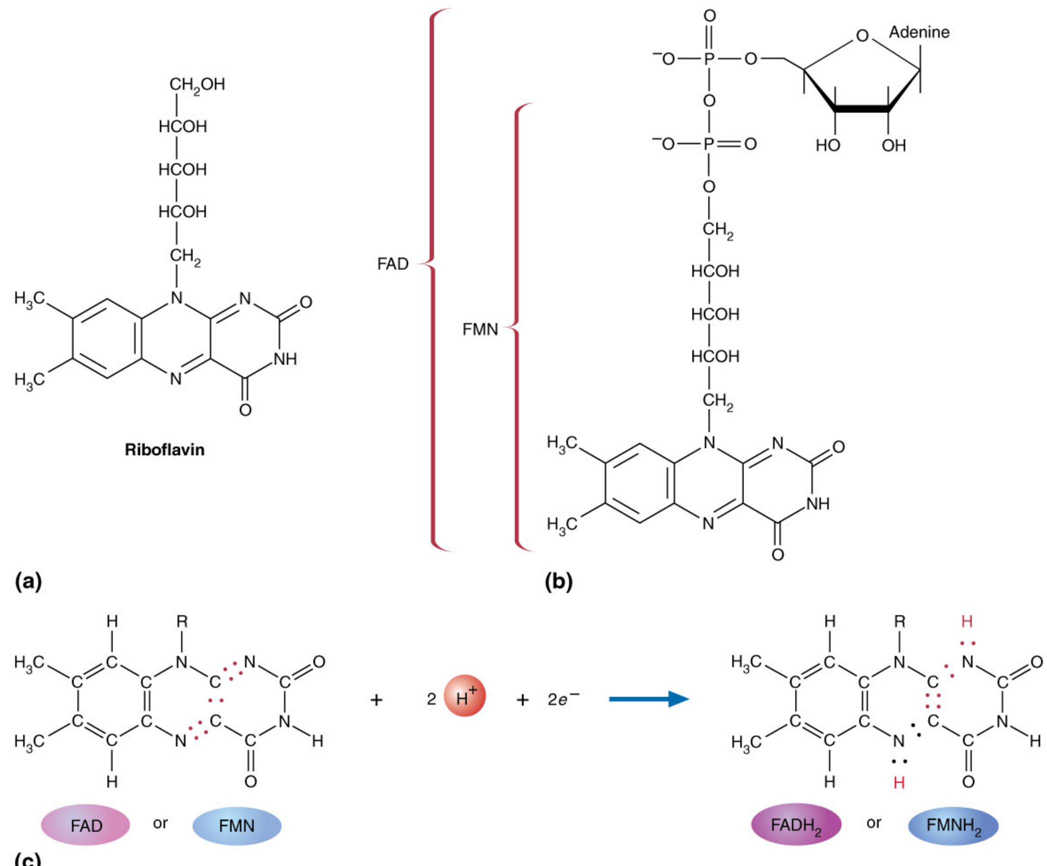


Figure 9.5 Nicotinamide Adenine Dinucleotide (NAD)

- **Redox Coenzymes: Riboflavin** (vitamin B_2)
  - flavin mononucleotide (FMN)
  - flavin adenine dinucleotide (FAD)
  - **Flavoproteins** – FMN/FAD tightly bound prosthetic groups to enzymes
    - function in a diverse class of redox enzymes; dehydrogenases, oxidases, hydroxylases

Figure 9.6 Flavin Coenzymes



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Section 9.1: Oxidation-Reduction Reactions

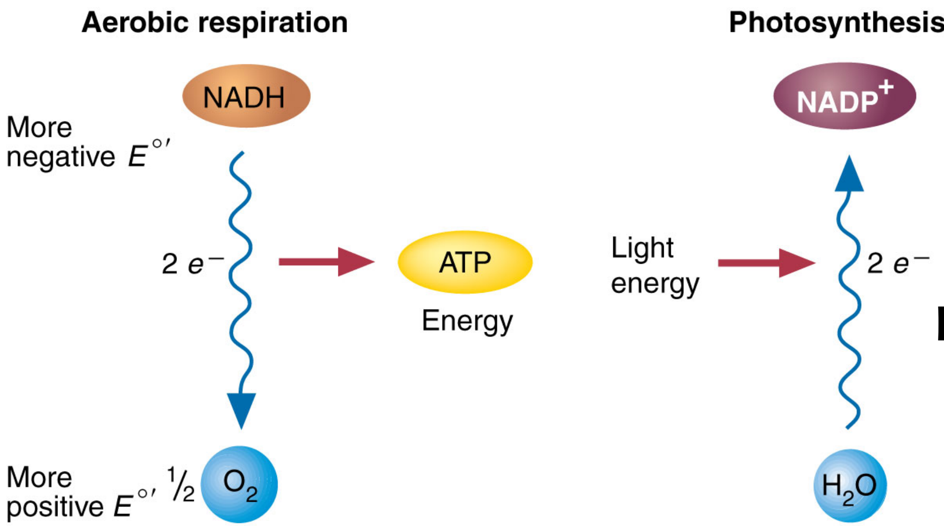
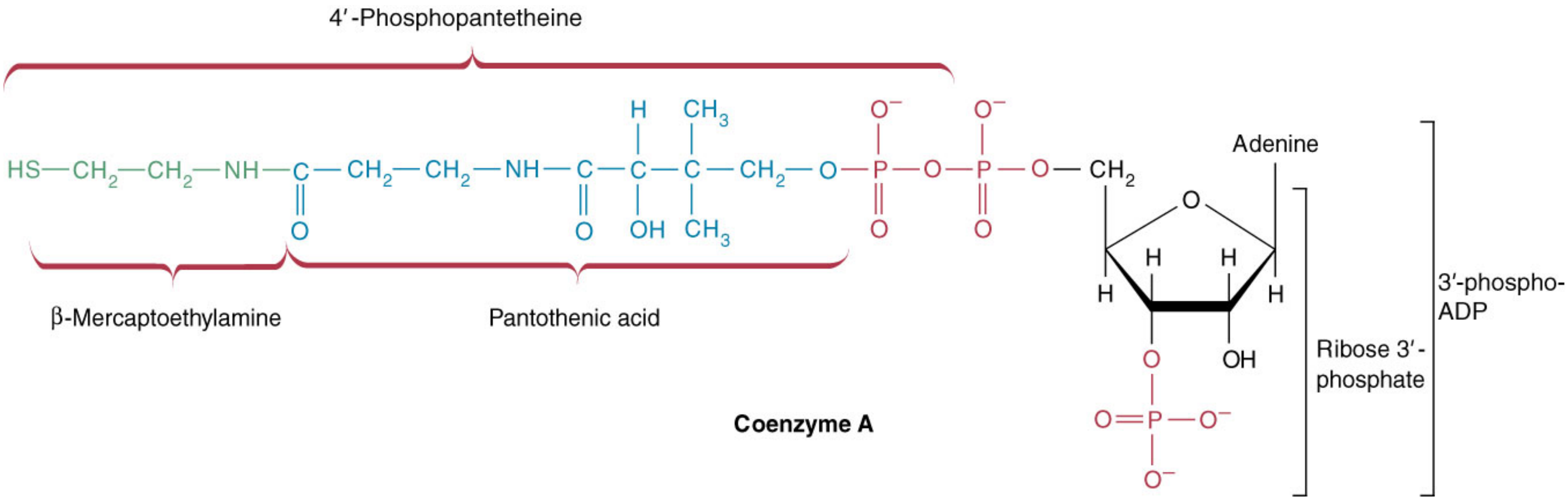


Figure 9.7 Electron Flow and Energy

- **Photosynthesis to Aerobic Metabolism**
  - Photosynthesis captures energy in chemical bonds
  - Aerobic respiration releases bond energy
  - Energy captured by mitochondrial ETC
    - Energy transferred from NADH to O_2

$$\tfrac{1}{2}O_2 + NADH + H^+ \rightarrow H_20 + NAD^+ \ (-220\ kJ/mol)$$



- Citric acid cycle -harvests energy from acetyl group of acetyl- CoA
  - Acetyl is derived from catabolism of carbohydrates (e.g., pyruvate), lipids, and some amino acids
  - **Coenzyme A** is an acyl carrier molecule
- CAC net reaction:

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

- Coenzyme roles in a variety of biosynthetic reactions

TABLE 9.2 Summary of the Coenzymes in the Citric Acid Cycle

Coenzyme	Functions
Thiamine pyrophosphate (TPP)	Decarboxylation and aldehyde group transfer
Lipoic acid	Carrier of hydrogens or acetyl groups
NADH	Electron carrier
$FADH_2$	Electron carrier
Coenzyme A (CoASH)	Acetyl group carrier

Conversion of Pyruvate to Acetyl-CoA

- **Pyruvate dehydrogenase complex** converts pyruvate to acetyl-CoA
  - Highly exergonic ($\Delta G^{\circ\prime}$ = -33.5 kJ/mol)
  - Net reaction:

$$\text{Pyruvate} + NAD^+ + \text{CoASH} \rightarrow \text{acetyl-CoA} + \text{NADH} + CO_2 + H^+$$

TABLE 9.3 *E. coli* Pyruvate Dehydrogenase Complex

Enzyme Activity	Function	Copies per Complex*	Coenzymes
Pyruvate dehydrogenase (E_1)	Decarboxylates pyruvate	24 (20–30)	TPP
Dihydrolipoyl transacetylase (E_2)	Catalyzes transfer of acetyl group to CoASH	24 (60)	Lipoic acid, CoASH
Dihydrolipoyl dehydrogenase (E_3)	Reoxidizes dihydrolipoamide	12 (20–30)	NAD^+, FAD

* The number of molecules of each enzyme activity found in mammalian pyruvate dehydrogenase is shown in parentheses.

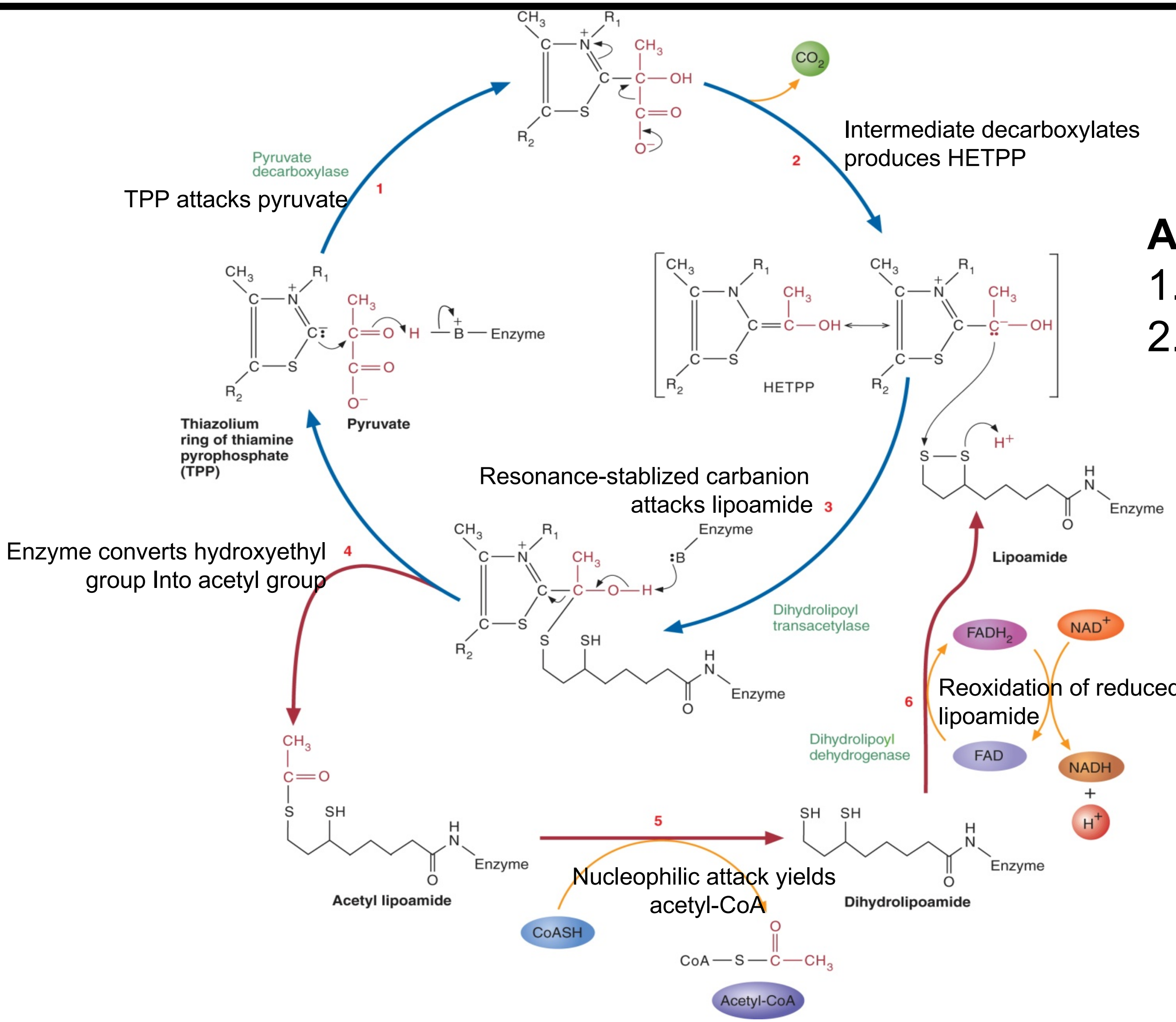
Pyruvate dehydrogenase complex

- ***pyruvate dehydrogenase*** - thiamine pyrophosphate (TPP) coenzyme, decarboxylation
- ***dihydrolipoyl transacetylase*** – lipoic acid coenzyme; converts HETPP into acetyl-CoA
- ***dihydrolipoyl dehydrogenase*** – reoxidizes reduced lipoamide

Conversion Steps:

1. Pyruvate loses CO_2. *hydroxyethylTPP* formed; coenzyme – *thiamine pyrophosphate*
2. Active lipoic acid bound to *dihydrolipoyl transacetylase* forming HETPP
3. Hydroxyethyl group oxidized & transferred to reduced lipoamide
4. Hydroxyethyl group converted to acetyl group forming *acetyl lipoamide*
5. Acetyl lipoamide reduced to dihyrolipoamide; acetyl group transferred to sulfhydryl group of CoA – ***acetyl-CoA***
6. Dihyrolipoamide oxidized to lipamide

Section 9.2: Citric Acid Cycle



Advantages:

1. Reaction efficiency
2. Regulatory control efficiency

Regulatory control:

- Allosteric effectors & covalent modification
- Inhibited: hi ATP, pyruvate, CoASH, NAD^+

- Eight reactions in two stages:

Stage 1. Liberation of two CO_2 from acetyl-CoA, 1-4

1. Introduction of two carbons as acetyl-CoA-forming citrate

2. Citrate isomerization

3. Isocitrate is oxidized to form NADH and CO_2

4. α-Ketoglutarate is oxidized; forms NADH and CO_2

- Reactions 3 and 4 are oxidative decarboxylation reactions
- **Products**: succinyl-CoA, 2 NADH, $2CO_2$, H^+

Stage 2. Regeneration of oxaloacetate. 5 – 8

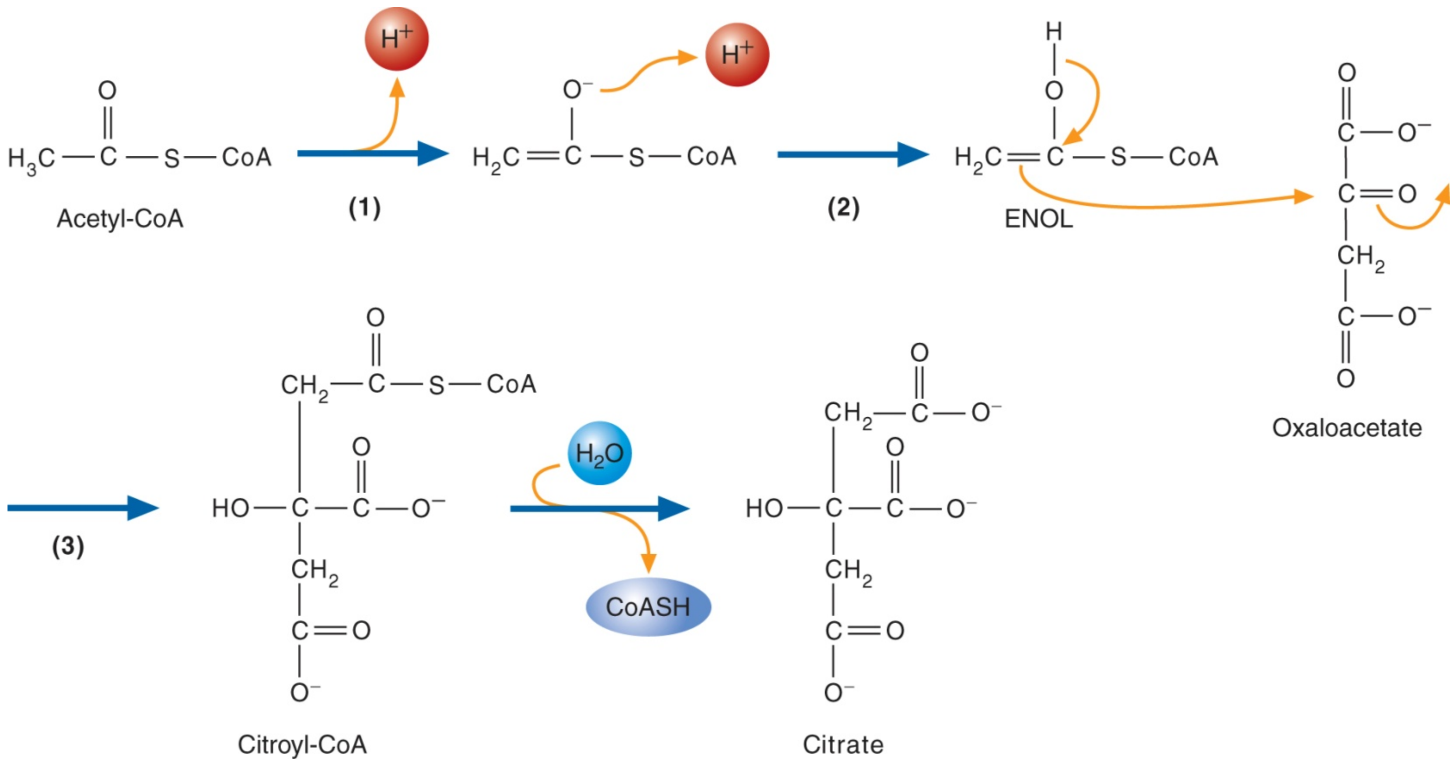
5. Cleavage of Succinyl-CoA leads to substrate-level phosphorylation

6. Succinate is oxidized to form fumarate and $FADH_2$

7. Fumarate is hydrated and forms L-malate

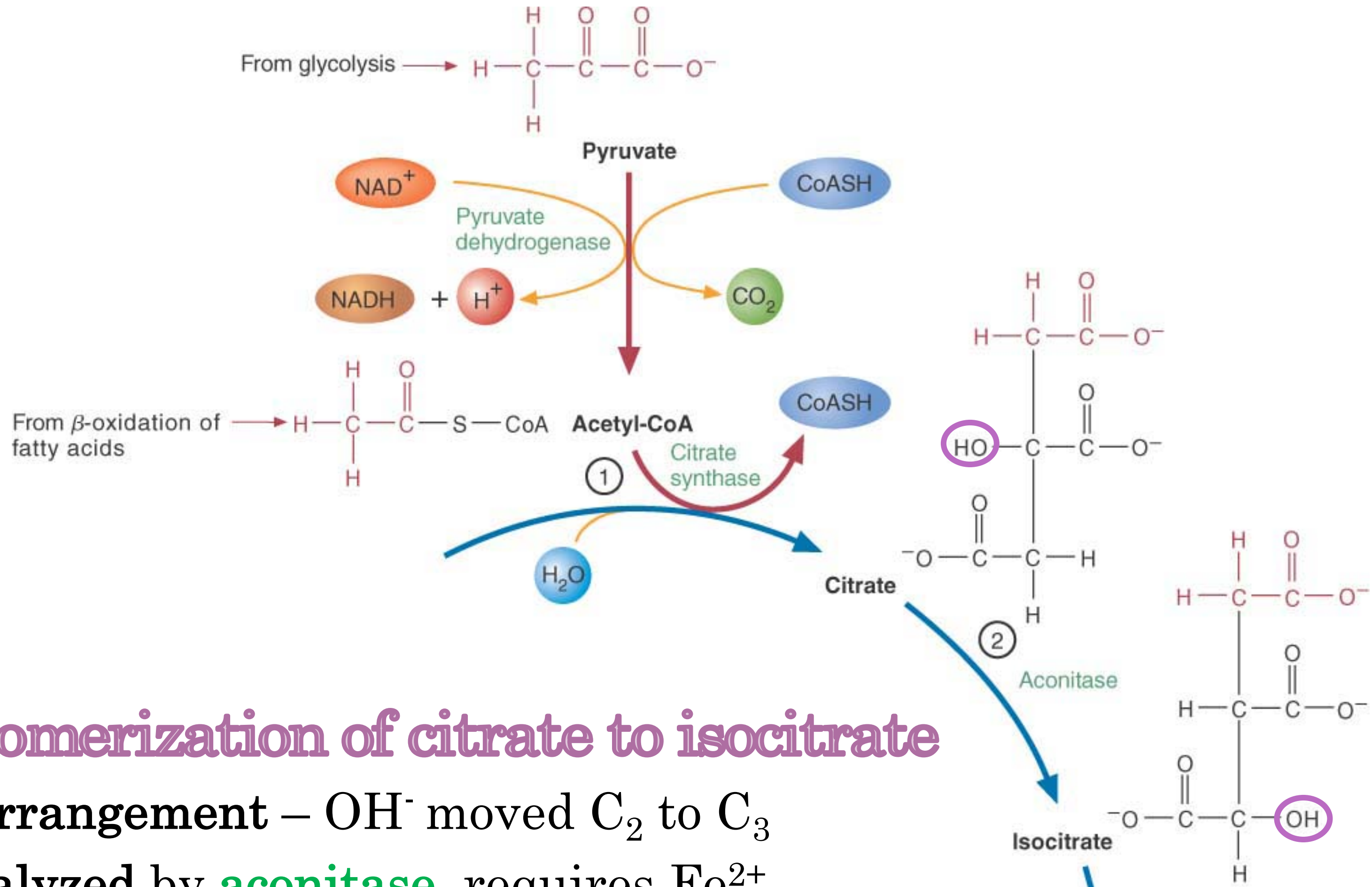
8. Malate is oxidized to form oxaloacetate and a third NADH

- **Products**: L-oxaloacetate, CoASH, $FADH_2$, NADH, H^+, ATP or GTP



Rxn 1: Formation of citrate

- **Condensation reaction** – new C-C bond
- **Catalyzed** by citrate synthase
- **Exergonic** – releases energy from hydrolysis of thioester
- **Inhibited** - NADH, ATP, succinyl-CoA

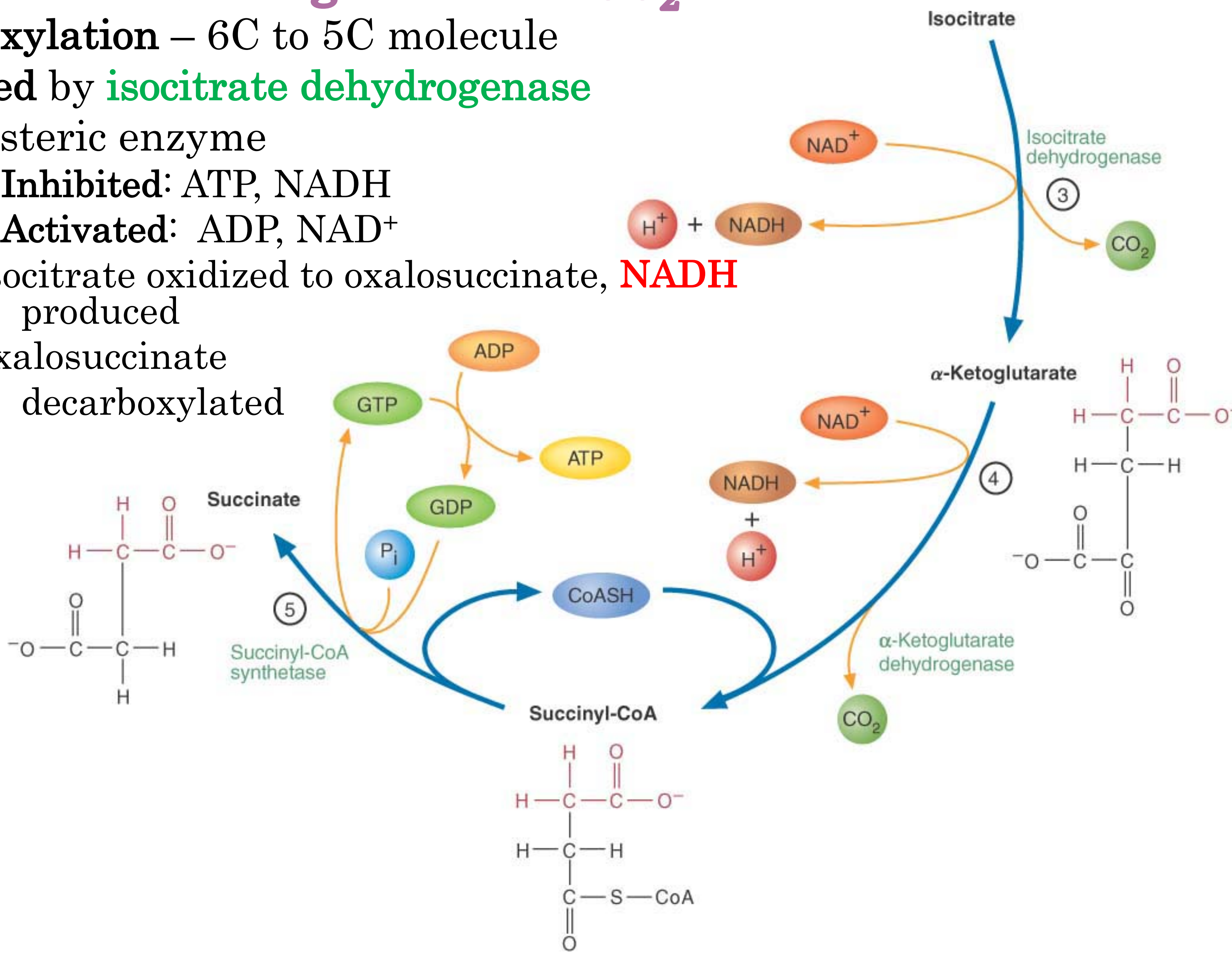


Rxn 2: Isomerization of citrate to isocitrate

- **Rearrangement** – OH^- moved C_2 to C_3
- **Catalyzed** by aconitase, requires Fe^{2+}

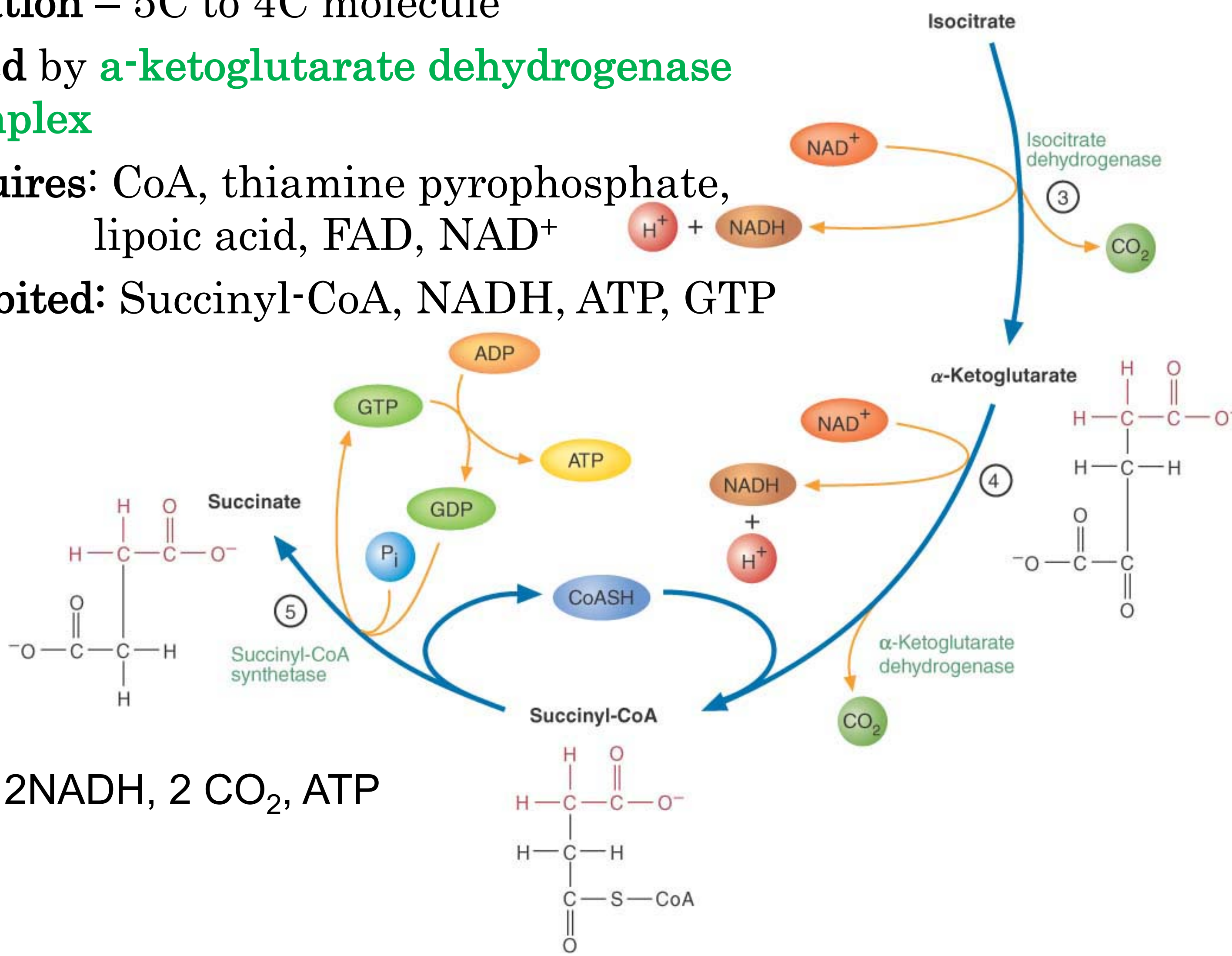
Rxn 3: Formation of α-ketoglutarate & CO_2

- **Decarboxylation** – 6C to 5C molecule
- **Catalyzed** by isocitrate dehydrogenase
  - Allosteric enzyme
    - **Inhibited**: ATP, NADH
    - **Activated**: ADP, NAD^+
- **Step 1**: isocitrate oxidized to oxalosuccinate, **NADH** produced
- **Step 2**: oxalosuccinate decarboxylated



Rxn 4: Formation of succinyl-CoA & CO_2

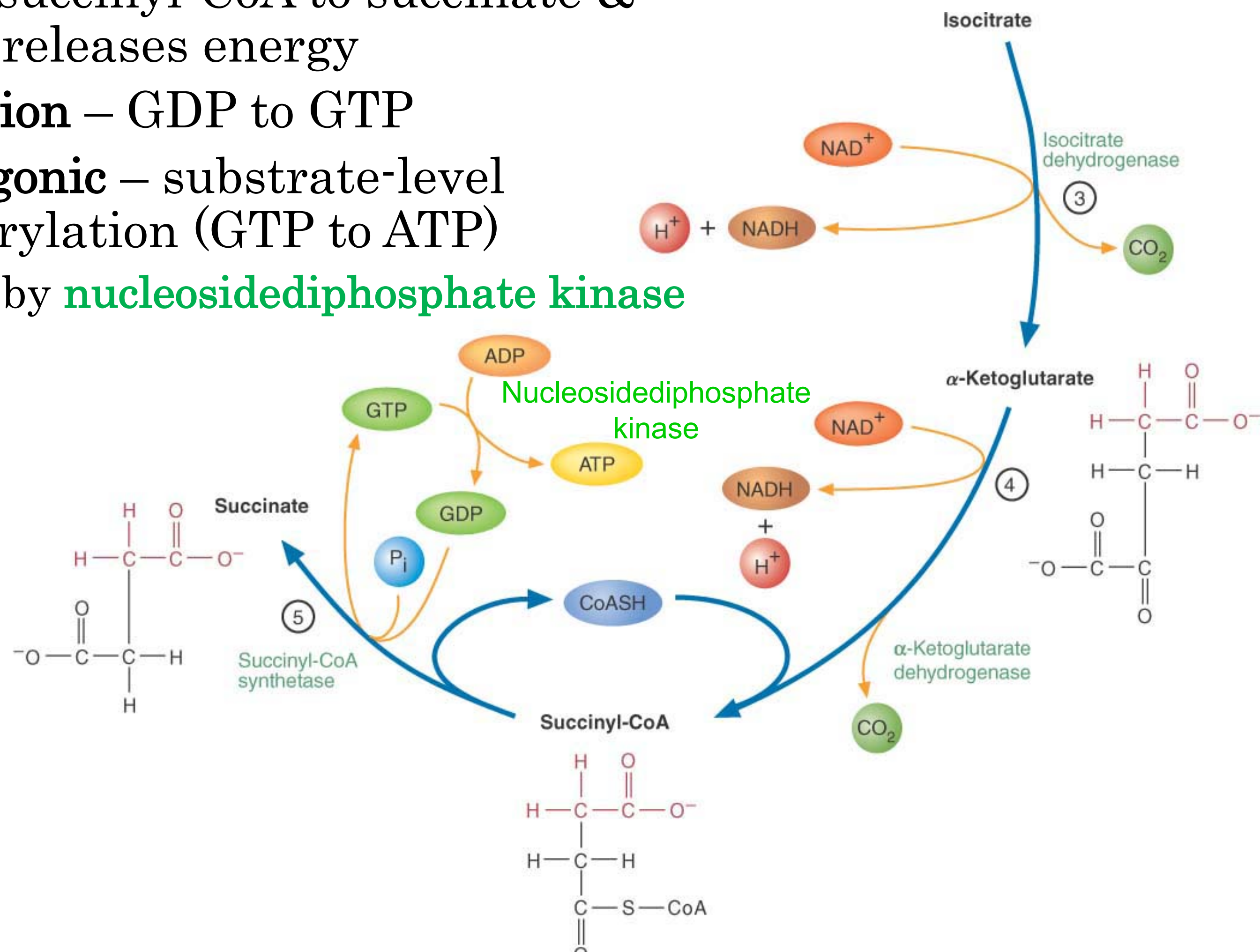
- **2nd oxidation** – 5C to 4C molecule
- **Catalyzed** by a-ketoglutarate dehydrogenase complex
  - **Requires**: CoA, thiamine pyrophosphate, lipoic acid, FAD, NAD^+
  - **Inhibited:** Succinyl-CoA, NADH, ATP, GTP



End of 1st Stage: 2NADH, 2 CO_2, ATP

Rxn 5: Formation of succinate

- **Catalyzed** by succinyl-CoA synthetase
- **Hydrolysis** – succinyl-CoA to succinate & CoASH, releases energy
- **Phosphorylation** – GDP to GTP
- **Slightly exergonic** – substrate-level phosphorylation (GTP to ATP)
  - **Catalyzed** by nucleosidediphosphate kinase



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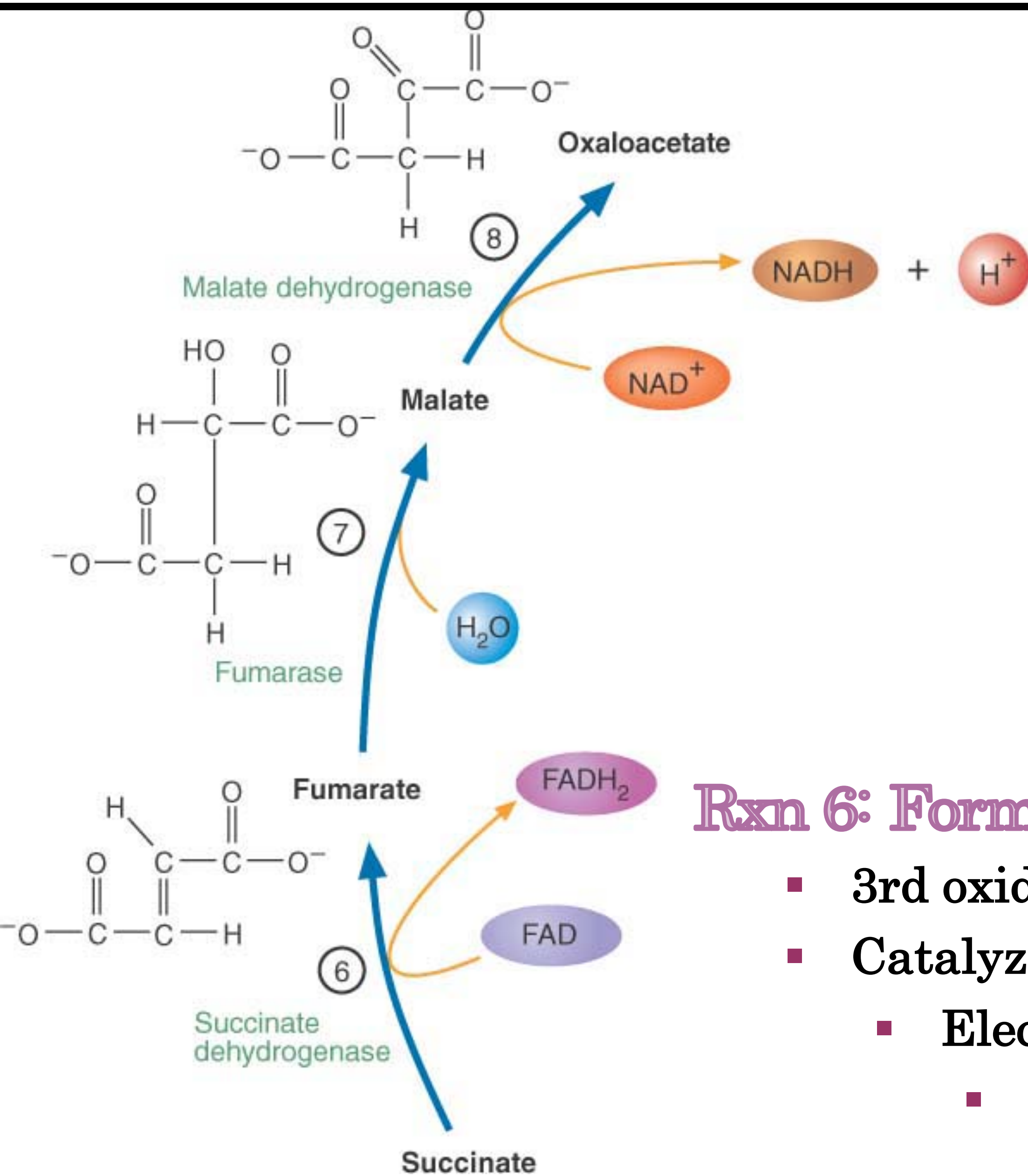
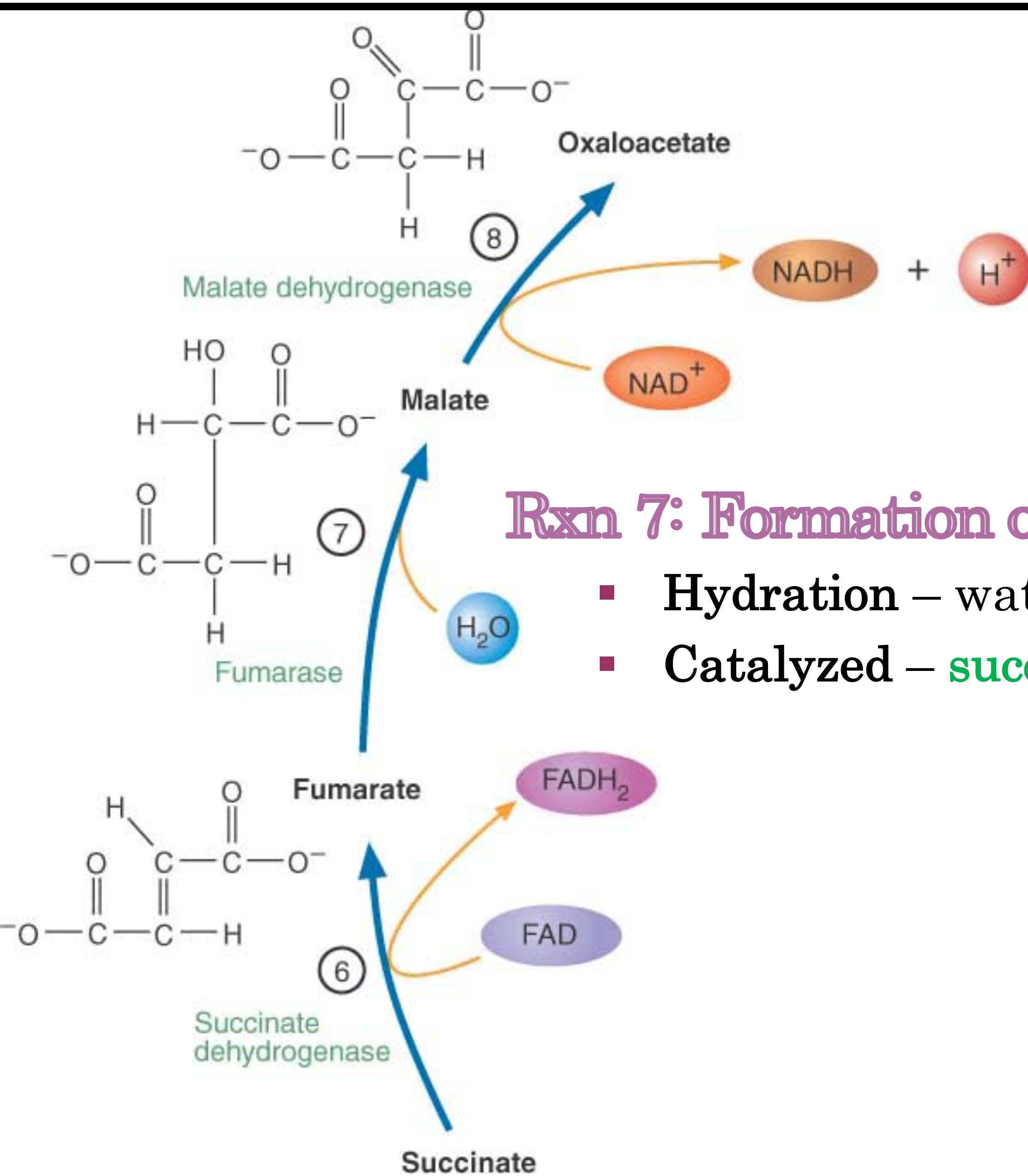


Rxn 6: Formation of fumarate

- **3rd oxidation** – FAD linked
- **Catalyzed** – succinate dehydrogenase
  - **Electron acceptor**: FAD
    - **FAD reduced to $FADH_2$**



Rxn 7: Formation of L-malate

- **Hydration** – water added
- **Catalyzed** – succinate dehydrogenase

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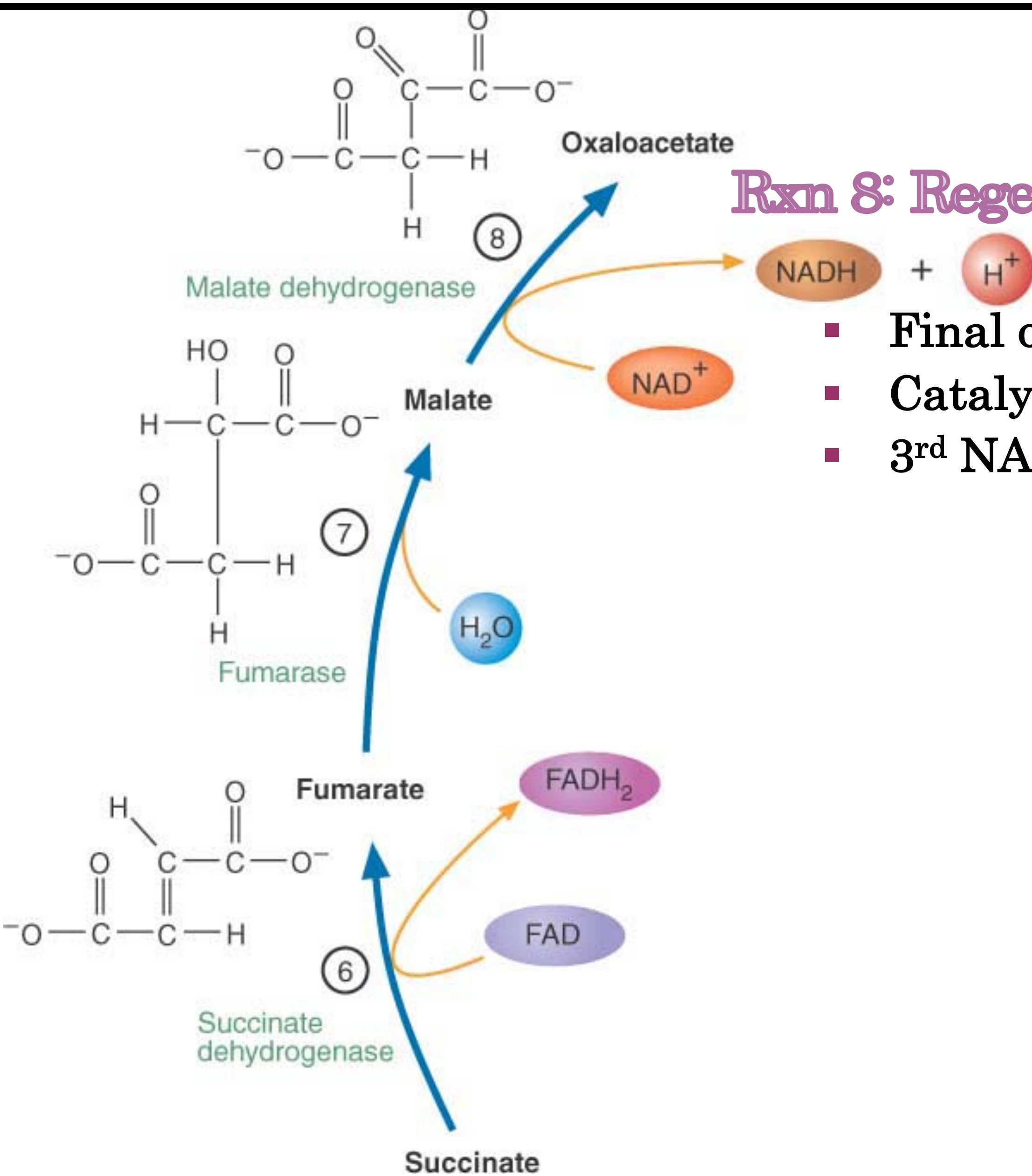
Rxn 8: Regeneration of oxaloacetate



- Final oxidation
- Catalyzed – succinate dehydrogenase
- 3rd NAD^+ reduced to NADH

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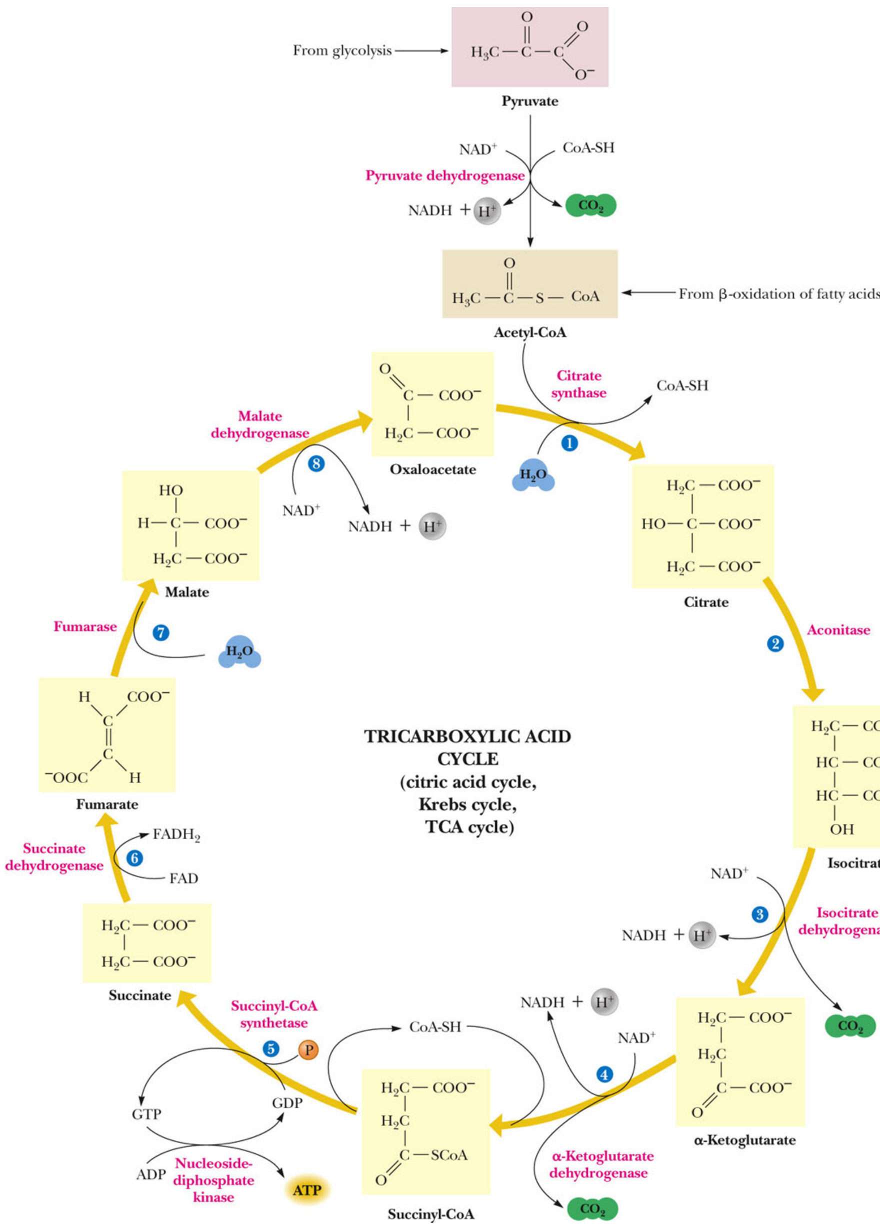
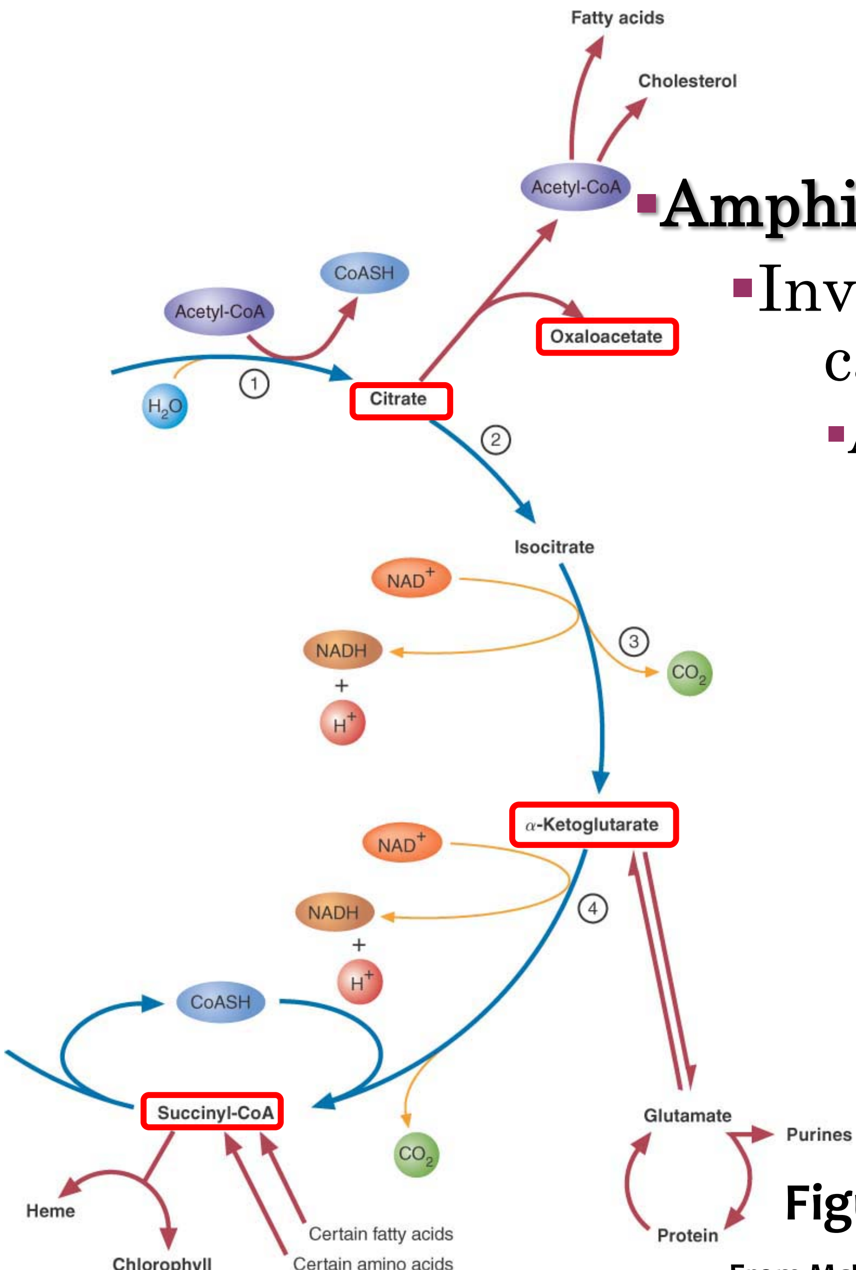
Section 9.2: Reactions of the **CAC**





Amphibolic Citric Acid Cycle

- Involved in both anabolic & catabolic processes
  - Anabolic – intermediates are precurors to biosynthetic pathways
    - OAA – gluconeogenesis substrate; precursor for lysine, threonine, isoleuine, methionine
    - α-ketoglutarate – precursor for glutamate, glutamine, proline, arginine
    - Succinyl-CoA – synthesis of porphyrins; heme & chlorophyll
    - Excess citrate – transported to cytoplasm; forms OAA & acetyl-CoA

Figure 9.13 Amphibolic Citric Acid Cycle

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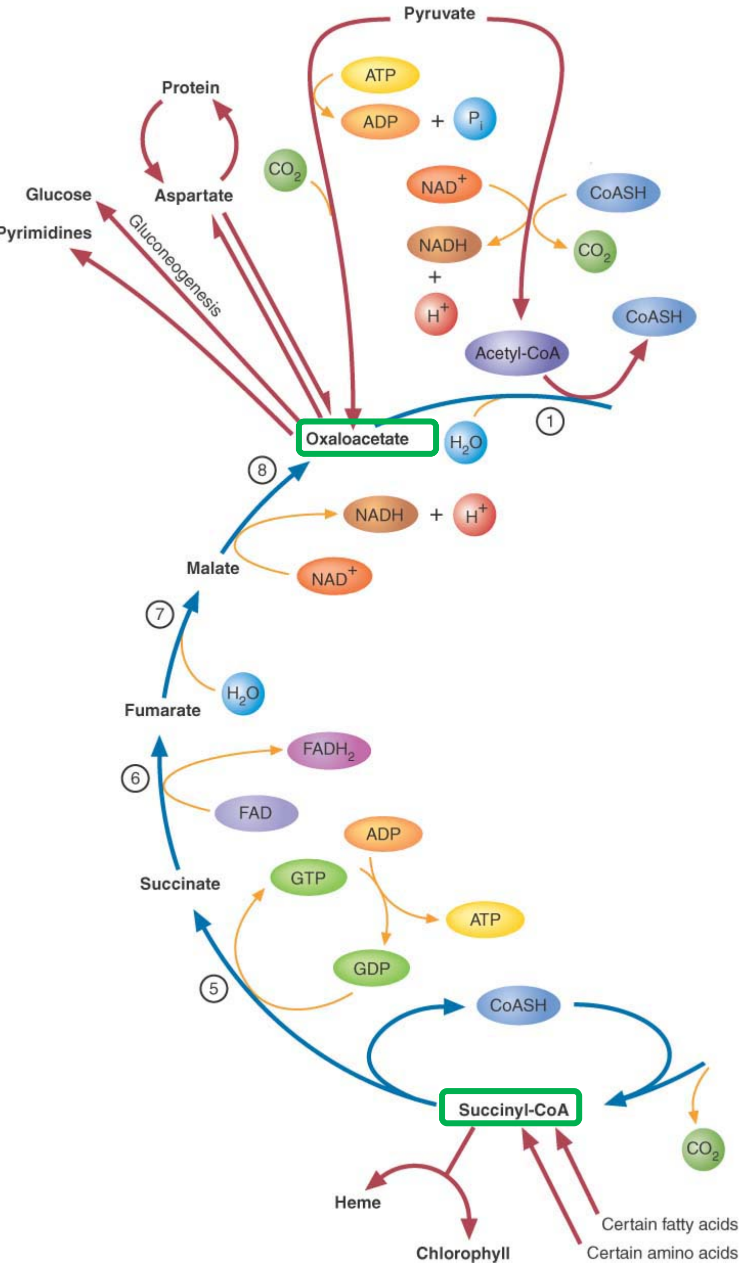

- **Amphibolic Citric Acid Cycle**
  - **Anaplerotic reactions** contribute intermediates into the cycle
    - Oxaloacetate from pyruvate or aspartate
    - Succinyl-CoA from fatty acids

Figure 9.13 Amphibolic Citric Acid Cycle

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Section 9.2: CAC Regulation

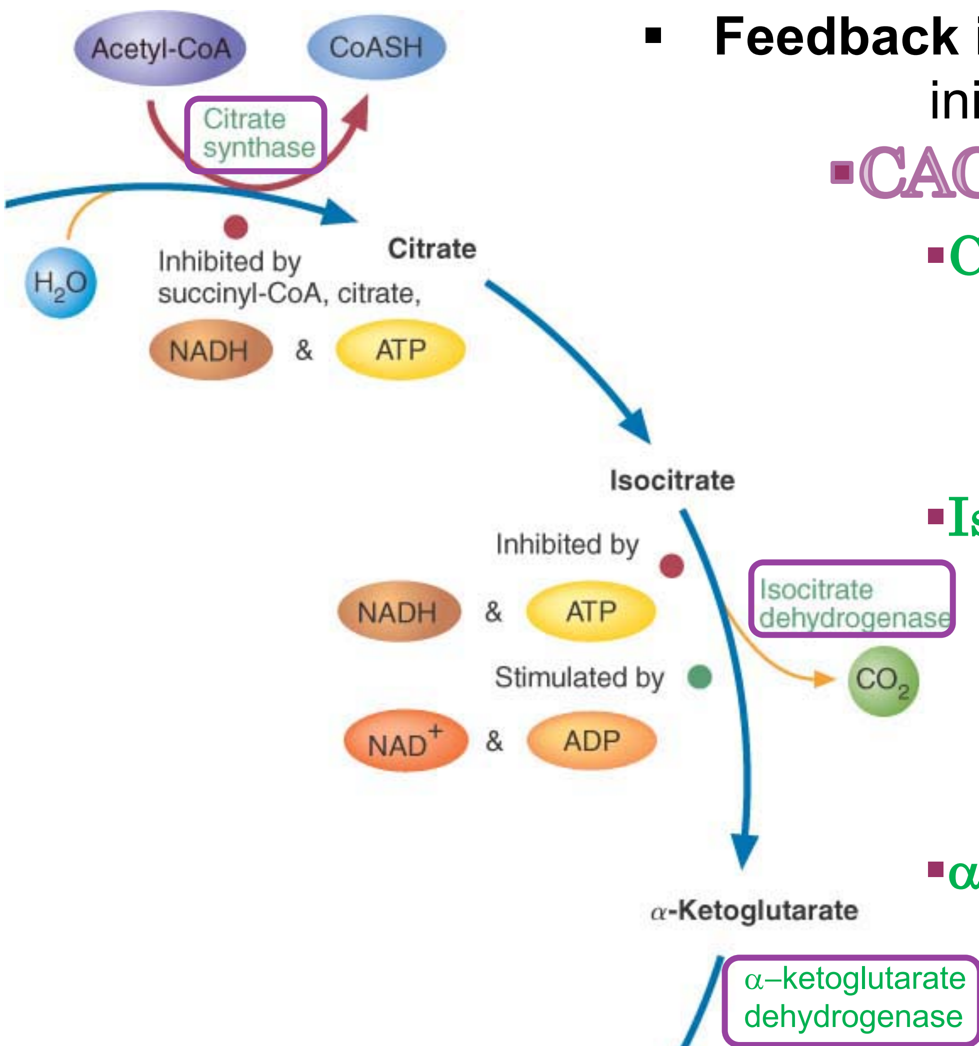

Figure 9.14 Control of Citric Acid Cycle

Pyruvate dehydrogenase – controls access to CAC

- **Inihibited**: ATP, NADH
- **Feedback inhibition:** final product inhibits initial reaction

- CAC control points:
  - Citrate synthase
    - **Inhibited**: ATP, NADH, succinyl-CoA
    - Substrate inhibition by citrate
  - Isocitrate dehydrogenase
    - **Activated**: ADP, NAD^+
    - **Inhibited**: ATP, NADH
  - α-Ketoglutarate dehydrogenase
    - **Activated**: ADP, NAD^+
    - **Inhibited**: ATP, NADH, succinyl-CoA

CAC control via signal transduction

- **Calcium regulation:** cytoplasmic [Ca^{2+}] increase is followed rapidly by [Ca^{2+}] increase in the matrix
  - Cytoplasmic increase - increases ATP production by stimulating enzymes that regulate the pace of the citric acid cycle
    - Stimulates PDHC activity
    - Activates isocitrate dehydrogenase, α-ketoglutarate dehydrogenase directly
  - Signal driven response in matrix - matches energy demand with energy production

Figure 9.15 Citrate Metabolism

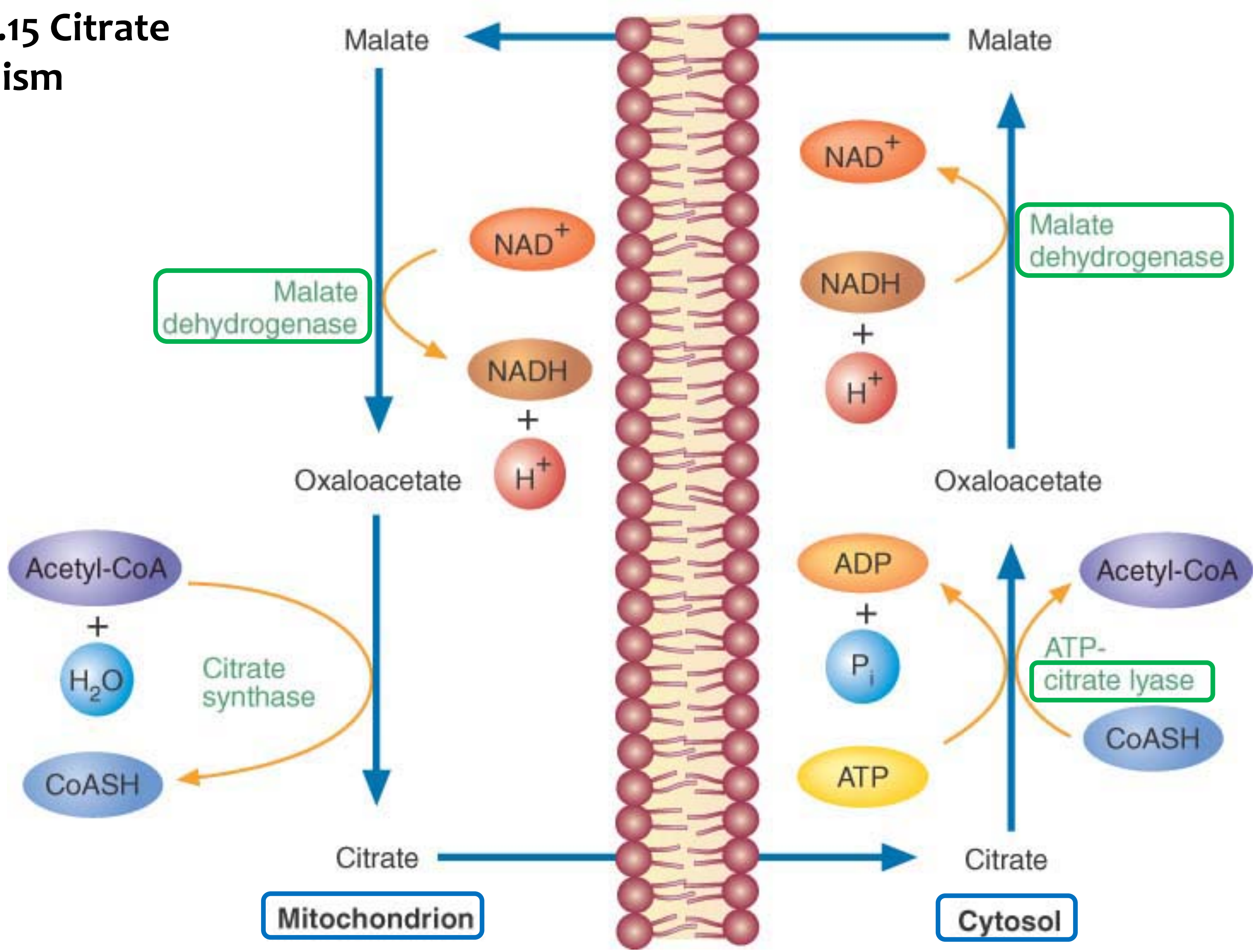

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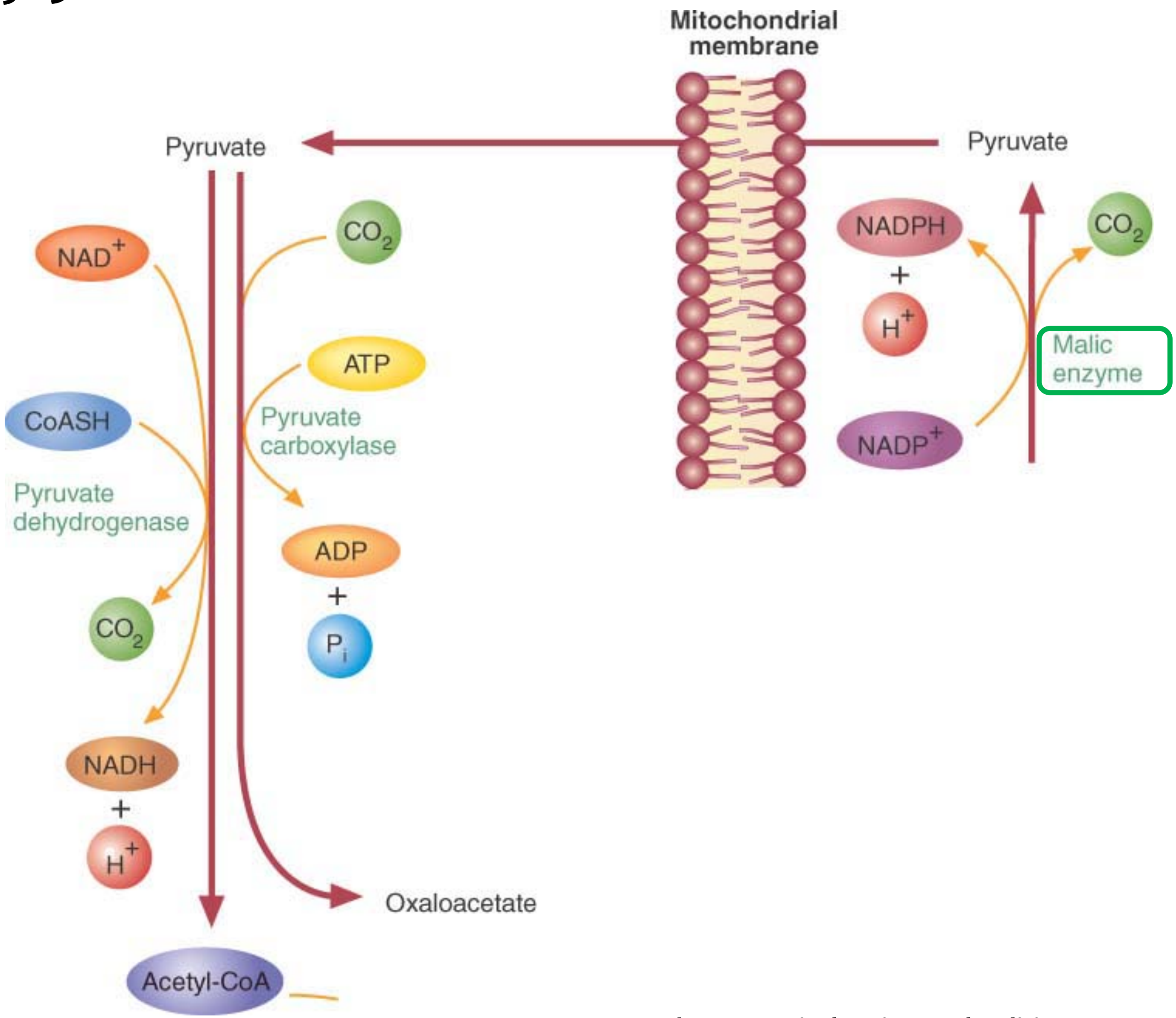
Figure 9.15 Citrate Metabolism



Glyoxylate Cycle

- Plants, some fungi, algae, protozoans, bacteria
- Modified citric acid cycle

- Five reactions – 2C cmpds
  1. Citrate synthesis
  2. Isocitrate synthesis
  3. Split isocitrate to glyoxylate & succinate
     isocitratelyase
  4. Malate formation
     malate synthases
  5. Oxaloacetate formation
     malate dehydrogenase

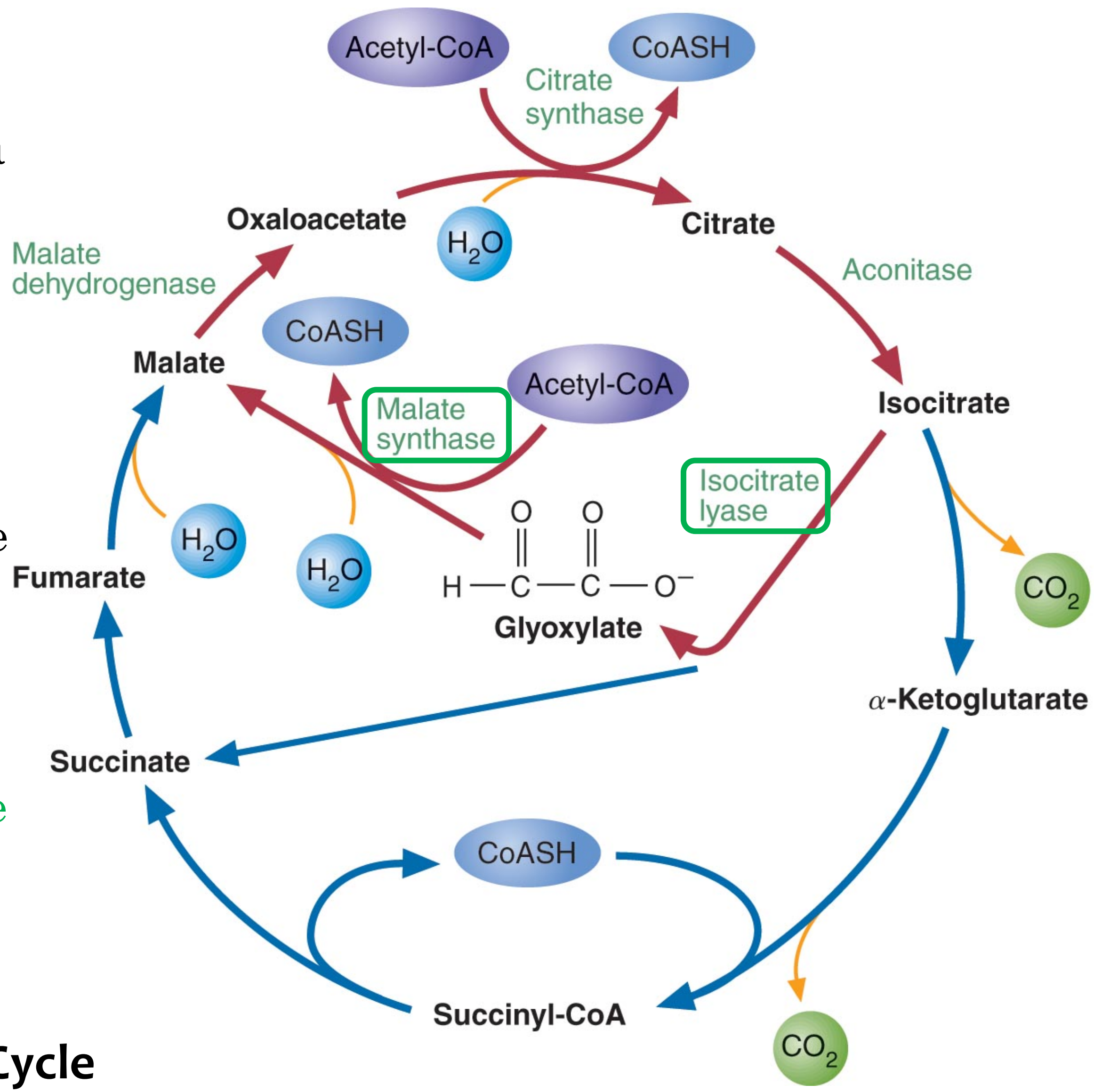


Figure 9.16 The Glyoxylate Cycle

- **Citric Acid Cycle and Human Disease**
  - Most common diseases are severe forms of encephalopathy
    - Encephalopathies have been linked to mutations in a-ketoglutarate dehydrogenase, succinate dehydrogenase, fumarase, and succinyl-CoA synthetase

- **Carcinogenesis: The Warburg Effect and Metabolic Reprogramming**
  - Emerging research reveals that “aerobic” glycolysis is closely associated with altered cell signaling pathways that cause a reprograming of metabolic pathways.
  - Aerobic Glycolysis is a process in tumor cells in which there is rapid glycolysis-generated ATP synthesis that occurs even when O_2 is present.
  - Loss of glycolysis regulation is one facet of carcinogenesis, the set of mechanisms whereby normal cells gradually transform into cancerous cells.